AGEING-RELATED SYMPTOMS, KAMPO MEDICINE AND TREATMENT

EDITED BY: Akio Inui, Masahiro Ohsawa and Yasuhito Uezono
PUBLISHED IN: Frontiers in Nutrition

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-925-9
DOI 10.3389/978-2-88971-925-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

AGEING-RELATED SYMPTOMS, KAMPO MEDICINE AND TREATMENT

Topic Editors:
Akio Inui, Kagoshima University, Japan
Masahiro Ohsawa, Nagoya City University, Japan
Yasuhito Uezono, Jikei University School of Medicine, Japan

Prof. Akio Inui is employed by Kagoshima University Graduate School of Medical and Dental Services, and belongs to a laboratory with funds donated by Kracie Pharmacautical, Ltd. All other Topic Editors declare no competing interests with regards to the Research Topic subject.

Citation: Inui, A., Ohsawa, M., Uezono, Y., eds. (2021). Ageing-Related Symptoms, Kampo Medicine and Treatment. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-925-9

Table of Contents

frontiers in Nutrition

EDITORIAL
published: 01 November 2021
doi: 10.3389/fnut.2021.749320

Editorial: Ageing-Related Symptoms, Kampo Medicine, and Treatment

Masahiro Ohsawa[1]*, *Yasuhito Uezono*[2] and *Akio Inui*[3]

[1] Department of Neuropharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan, [2] Department of Pain Control Research, Jikei University School of Medicine, Tokyo, Japan, [3] Pharmacological Department of Herbal Medicine, Kagoshima University Graduate School of Medical & Dental Sciences, Kagoshima, Japan

Keywords: herbal medicine, Japanese Kampo medicine, aging-related disease, longevity, frail

Editorial on the Research Topic

Ageing-Related Symptoms, Kampo Medicine, and Treatment

AN AGING POPULATION IS A CRITICAL PROBLEM FACING THE ENTIRE WORLD

The number of individuals aged 60 years and older in the population is increasing worldwide, and was reported by the World Health Organization to be one billion worldwide in 2019 (1). This number will increase to 1.4 billion by 2030 and 2.1 billion by 2050, meaning that the aging of the population will progress rapidly over the next half a century. According to the estimated rate of aging in each region, the aging population is expected to increase rapidly, not only in developed countries, but also in developing countries.

Concurrent with an aging population, birth rate has also been declining significantly. To maintain economic activities in the face of a declining future population, ensuring the health and activities of older adults will become increasingly important. In particular, the aging of the population accompanied by a low birth rate is expected to continue in developed countries, such as the United States, China, and Europe, as well as in some developing countries. Facilitating an age-friendly world requires an essential and urgent response to our changing demographics, for example, by including health and social care, transportation, housing, and urban planning.

THE IMPORTANCE OF KAMPO MEDICINE FOR RESOLVING AGE-RELATED SYMPTOMS

Kampo medicine utilizes herbal formulas derived from classical Chinese medicine that originated ∼3000 years ago and developed in Japan (2). Classical Chinese medicine was brought to Japan in the fifth and sixth centuries and was modified to fit the Japanese climate and the characteristics of Japanese people. Different formulations of Kampo medicine are prescribed based on the International Classification of Diseases-10 diagnosis, similar to Western medicine that uses prescribed drugs for symptoms. Importantly, Kampo medicine formulations are composed of multiple herbs, and the ratio of each herb is calculated based on experience accumulated over 1000 years.

The beneficial effects of Kampo medicine have been identified in basic and clinical research. For example, as published in our previous research topic, Kampo medicine is effective in treating anorexia, loss of skeletal muscle mass, and decreased activity in older adults (3). This research topic follows on from our previous one titled "Frailty and herbal medicine—-from molecular mechanisms to clinical efficacy" (3). In this Research Topic, Takayama et al. summarized the recent advances in Kampo medicine for the management of symptoms related to functional decline of

OPEN ACCESS

Edited and reviewed by:
Maurizio Muscaritoli,
Sapienza Università di Roma, Italy

***Correspondence:**
Masahiro Ohsawa
ohsawa@phar.nagoya-cu.ac.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *29 July 2021*
Accepted: *07 October 2021*
Published: *01 November 2021*

Citation:
Ohsawa M, Uezono Y and Inui A (2021) Editorial: Ageing-Related Symptoms, Kampo Medicine, and Treatment. Front. Nutr. 8:749320. doi: 10.3389/fnut.2021.749320

the cardiovascular, respiratory, and digestive systems, cognitive impairment and related disorders, pain, and other sensory issues, among others.

THE QUALITY OF KAMPO MEDICINE

The main problem using Kampo medicine is the misunderstanding relating to its validity. Differences in the validity of different Kampo medicine formulations result from the manufacturing variations because there is no gold standard as a reference to control the quality of Kampo medicinal formulations. In Japan, the amounts of active components in Kampo medicine are strictly regulated. Wang et al. characterized the ingredients of various commercially available traditional crude drugs, *shinkiku,* which are prepared by microbial fermentation of wheat and herbs. They showed that digestive enzymes, organic acids, and 39 volatile compounds were commonly found in the products. However, the chemical and microbial characteristics differed according to the manufacture process. Interestingly, the dominant ingredient in Korean products differs from that in Chinese products. There are both commonalities and diversities among the commercially available *shinkiku*. The commonalities may serve as reference standards for quality control of *shinkiku*. In contrast, the diversities suggest the importance of microbial management in stabilizing the quality of *shinkiku*.

BENEFICIAL EFFECTS OF KAMPO MEDICINE FOR AGE-RELATED SYMPTOMS

Kampo medicine has been shown to improve various bodily functions. For geriatric syndromes, ninjin'yoeito (NYT) and yokukansan-ka-chimpihange (YKSCH) are often prescribed to improve symptoms such as fatigue, anemia, anorexia, night sweats, cold limbs, slight fever, chills, persistent cough, malaise, mental disequilibrium, and insomnia. NYT activates the hypothalamic neuropeptide Y (NPY) neurons through the activation of voltage-gated Ca^{2+} channels (Goswami et al.). Interestingly, NYT preferentially affects the N-type and L-type Ca^{2+} channels in ghrelin-sensitive and ghrelin-insensitive neurons, respectively. Moreover, NYT activates the orexin 1 receptors (Miyano et al.). These two important basic studies implicate the orexigenic effects of NYT. NYT also alleviates neuropathic pain (Takemoto et al.) and suppresses the progression of age-related hearing loss (Kawashima et al.), suggesting that NYT could improve the undesirable symptoms of aging. In addition to NYT, YKSCH improves sleep disruption and reduces the levels of allopregnanolone, a positive allosteric modulator of $GABA_A$ receptor (Murata et al.). Taken together, these results strongly suggest that Kampo medicine has the potential to improve geriatric syndrome.

CLINICAL STUDIES SUPPORT THE EFFECTIVENESS OF KAMPO MEDICINE FOR AGE-RELATED SYMPTOMS

The clinical case reports in this research topic demonstrate many beneficial effects of Kampo medicine, especially NYT and YKSCH. NYT has been shown to improve the quality of life of frail patients after hospitalization (Kashima). NYT also increased body weight and muscle mass in patients with dementia (Matsui and Matsui). Furthermore, NYT has demonstrated great outcomes in terms of muscle mass, appetite, and body weight (Morinaga et al.). Notably, rikkunshito was ineffective in NYT-sensitive patients with hip fracture and sarcopenia. These results suggest that the appropriate choice of Kampo medicine formulation based on the patient's condition is important for improving geriatric symptoms. NYT also improved the subjective symptoms and nutritional status of patients with idiopathic pulmonary fibrosis (Kushima et al.). In the case of adjuvant chemotherapy regimen, NYT reduced the adverse effects of anticancer therapy, particularly in older patients (Aomatsu et al.) Therefore, NYT can improve several geriatric syndromes in several disease conditions.

In addition to NYT, YKSHC also improved REM sleep behavior disorder in Lewy body dementia (Manabe), aggressive disorder, and sleep disorder in patients with dementia (Katsumoto et al.). Hachimijiogan (HJG) also improved irregular menstruation in young to middle-aged women (Hirabayashi). This effect of HJG is more prominent in crude drug preparations than in HJG extract preparations.

FURTHER CONSIDERATIONS OF THE EFFECTIVENESS OF KAMPO MEDICINE FOR AGE-RELATED SYMPTOMS

This research topic contains many clinical case reports supporting the beneficial effects of several different Kampo herbs and preparations across patients of different ages. The key limitations of these research studies include the small sample sizes and the lack of a double-blind randomized controlled trial (RCT). A double-blind RCT for NYT is currently underway in Japan. In the future, we hope that a large RCT will be conducted based on the findings reported in this research topic. Our previously published research topic may also be informative for readers interested in herbal medicines (3). The past and present research topics can provide further insights into the use of Kampo medicine for improving the quality of life in older adults.

AUTHOR CONTRIBUTIONS

MO wrote the manuscript. AI conceived and organized the structure of the editorial. MO, AI, and YU contributed to the critical reading of the first draft version, revision, and approved the final manuscript for publication. All authors contributed to the article and approved the submitted version.

REFERENCES

1. WHO. *WHO Decade of Healthy Ageing: Baseline Report*: *Summary*. Geneva: World Health Organization (2021).
2. Motoo Y, Seki T, Tsutani K. Traditional Japanese medicine, kampo: its history and current status. *Chin J Integr Med.* (2011) 17:85–7. doi: 10.1007/s11655-011-0653-y
3. Ataka K, Takahashi R, Li JB, Iizuka N, Inui A. Editorial: frailty and herbal medicines-from molecular mechanisms to clinical efficacy. *Front Nutr.* (2020) 7:41. doi: 10.3389/fnut.2020.00041

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 11 February 2020
doi: 10.3389/fnut.2020.00008

Yokukansankachimpihange Improves the Social Isolation-Induced Sleep Disruption and Allopregnanolone Reduction in Mice

*Kenta Murata, Feng Li, Kanako Shinguchi, Misaki Ogata, Nina Fujita and Ryuji Takahashi**

Kampo Research Laboratories, Kracie Pharma, Ltd., Takaoka, Japan

Yokukansankachimpihange (YKSCH), a traditional Japanese medicine composed of 9 crude drugs, is designed to improve neurosis, insomnia in adults, and night crying in children. YKSCH has been reported to improve diurnal rhythm in patients with Alzheimer's disease and prolong the total sleeping time in healthy subjects. However, little is known about how YKSCH alleviates sleep disorders. Here, we investigated whether and how YKSCH treatment affected sleep latency and duration in group-housed and socially isolated mice. Male ddy mice were treated with YKSCH [1,500 mg/kg, per os (p.o.)] in group-housed or socially isolated conditions for 3–4 weeks. After the last injection, mice were intraperitoneally (i.p.) administered with pentobarbital (60 mg/kg) and the sleep latency and duration was evaluated. The results show that pretreatment with YKSCH had no effect on sleep latency or duration in group-housed mice. However, YKSCH treatment significantly improved the reduced sleep duration in socially isolated mice. This effect of YKSCH was inhibited by the administration of bicuculline (3 mg/kg, i.p.), a $GABA_A$ receptor antagonist. Furthermore, we showed that YKSCH treatment improved the decrease in allopregnanolone content and its synthase expression levels in the olfactory bulb. These results suggest that YKSCH treatment improved social isolation stress-induced insomnia via the GABAergic pathway and that the mechanism of action of YKSCH is partly due to improvement of allopregnanolone levels of expression.

Keywords: Yokukansankachimpihange, insomnia, social isolation stress, GABA, allopregnanolone

OPEN ACCESS

Edited by:
Masahiro Ohsawa,
Nagoya City University, Japan

Reviewed by:
Charikleia Stefanaki,
National and Kapodistrian University of Athens, Greece
Yukio Ago,
Osaka University, Japan

****Correspondence:***
Ryuji Takahashi
r_takahashi@phm.kracie.co.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *17 September 2019*
Accepted: *20 January 2020*
Published: *11 February 2020*

Citation:
Murata K, Li F, Shinguchi K, Ogata M, Fujita N and Takahashi R (2020) Yokukansankachimpihange Improves the Social Isolation-Induced Sleep Disruption and Allopregnanolone Reduction in Mice. Front. Nutr. 7:8. doi: 10.3389/fnut.2020.00008

INTRODUCTION

Yokukansankachimpihange (YKSCH), a traditional Japanese medicine, is designed to improve neurosis, insomnia in adults, and night crying in children. YKSCH is composed of 9 herbs: Pinellia tuber, Atractylodes rhizome, Poria sclerotium, Cnidium rhizome, Citrus unshiu peel, Japanese Angelica root, Bupleurum root, Uncaria hook, and Glycyrrhiza. In addition to traditional use, YKSCH has been approved for administration to patients with dementia in Japan to treat behavioral and psychological symptoms of dementia (BPSD). A recent clinical trial revealed that YKSCH treatment improves the Neuropsychiatric Inventory scores of "agitation," "delusion," and "sleep and night-time behavior change" in patients with Alzheimer's disease (1). Other research revealed that a combination treatment of YKSCH with donepezil improved diurnal rhythm in patients with Alzheimer's disease (2). Furthermore, YKSCH treatment prolongs the total sleeping time and tends to increase sleep efficacy based on polysomnography recordings in normal young healthy subjects

(3). YKSCH treatment has also been reported to reduce memory impairment and BPSD-like behavior, such as hallucinations and aggressive behavior, in several rodent models (4–7). Although some research using animal models have begun to elucidate the action of YKSCH in the central nervous system, little is known about how YKSCH alleviates insomnia observed in clinical practice.

Social isolation stress is reported to have negative effects on longevity as well as physical and mental conditions such as depression, fatigue, and sleep disruption (8, 9). Sleep disruption leads to a reduction in work productivity and personal quality of life, such as working deficits, day time sleepiness, depression, and attention and learning problems. In addition, a reduction of sleep quality is also reported to trigger a reduction in social interactions (10). On the other hand, social isolation stress in rodent models also leads to the same abnormal behaviors observed in humans. Mice housed in social isolation for 3–4 weeks develop increased anxiety-like and aggressive behavior and display decreased responsiveness to drugs that stimulate aminobutyric acid type A (GABAA) receptors such as pentobarbital (11, 12). Hence, socially isolated animal have been used as a model to evaluate the effect of sedative drugs. Changes in various neurotransmitter systems were reported in socially isolated stress animals, including dopaminergic (13), serotonergic (14), and noradrenergic systems (15, 16). Among these neurotransmitter systems, several reports showed that the reduction of responsiveness to pentobarbital in this model was related to the dysfunction of the GABAergic neurotransmitter system and the reduction of neurosteroid biosynthesis in the brain. Allopregnanolone (ALLO) is the cholesterol-derived neurosteroid in the brain; it declines with age and because of neurodegenerative diseases (17, 18). ALLO acts as a positive allosteric modulator of the $GABA_A$ receptor, and ALLO content in the olfactory bulb (OB) and prefrontal cortex (PFC) is reduced by long-term social isolation (19).

The purpose of this study was to investigate whether and how YKSCH enhances pentobarbital-induced sleep in group-housed and socially isolated mice.

MATERIALS AND METHODS

Animals

Six-week-old male Slc;ddY mice, weighing 32.1–35.5 g (mean ± SD = 33.6 ± 0.9 g), were purchased from SLC (Shizuoka, Japan). Animals were housed in sterilized polypropylene cages (4 mice/cage) and provided laboratory pellet chow (CE-2, Clea Japan Inc., Tokyo, Japan) and water *ad libitum* at 24 ± 2°C under a 12 h light–dark cycle (lights on from 8:00 to 20:00). Before experimental procedures, they were acclimatized to the room for 1 week. Behavioral experiments were performed between 9:00 and 18:00, except for the locomotor activity test. All efforts were made to minimize both the suffering of and the number of animals used. This study was carried out in accordance with the principles of the Basel Declaration and recommendations of guidelines for Proper Conduct of Animal Experiments, the Experimental Animal Care Committee of Kracie Pharma, Ltd. (Toyama, Japan). The protocol was approved by the Experimental Animal Care Committee of Kracie Pharma, Ltd.

TABLE 1 | Medical herb composition of YKSCH.

Common name	Weight (g)
Pinellia tuber	5
Atractylodes rhizome	4
Poria sclerotium	4
Cnidium rhizome	3
Citrus unshiu peel	3
Japanese Angelica root	3
Bupleurum root	2
Glycyrrhiza	1.5
Uncaria hook	3

Plant Materials and Preparation of the Extract

YKSCH is composed of nine dried medical herbs, including Pinellia tuber, Atractylodes rhizome, Poria sclerotium, Cnidium rhizome, Citrus unshiu peel, Japanese Angelica root, Bupleurum root, Uncaria hook, and Glycyrrhiza (**Table 1**), and is supplied by Kracie Pharma, Ltd. as a formulation (EK-83). Each plant material was identified based on its external morphology and was authenticated by compound markers of plant specimens according to the method of the Japanese Pharmacopeia and our company's standard. EK-83 (lot No.06MH) was suspended in distilled water immediately before use and was administered orally at a dose of 1,500 mg/kg body-weight/day. A dose of YKSCH was determined by following the formula for the FDA's human equivalent dose guidance (20). Human equivalent volume (mg/kg) = animal dose (mg/kg) × $[\text{Weight}_{\text{animal}}\ (\text{kg})/\text{Weight}_{\text{human}}\ (\text{kg})]^{(1-0.67)}$. The human weight was calculated as 60 kg, and the weight of mice was calculated as 0.03 kg. The YKSCH dosage prescribed clinically is 7,500 mg/day. According to the above formula, the dosage in mice is calculated as 1535.5 mg/kg body-weight/day.

High-Performance Liquid Chromatography Analysis of YKSCH

YKSCH extract was mixed and shaken with 50% MeOH, and the supernatant was subjected to high-performance liquid chromatography (HPLC) analysis. The HPLC profile of YKSCH was obtained using a Shimazu LC-20AD liquid chromatography equipped with a SPD-M30A detector with a scanning range of 245 nm and a reversed-phase column (YMC-pack ProC18, 2.0 mm i.d. × 150 mm, 12 nm, column temperature: 20°C). The column was equipped with solvent A (0.1% formic acid in acetonitrile) and solvent B (0.1% formic solution), and the ratio of solvent A was increased from 5% to 70% over 90 min, and remained at 70% over 10 min, with a flow rate of 0.2 mL/min.

Pentobarbital-Induced Sleeping Model

Male ddy mice were divided into three groups so that their average body weight is almost the same in each group: control

group, YKSCH (1,500 mg/kg)-treated group, and diazepam-treated group. Mice were administered distilled water or 1,500 mg/kg YKSCH for 3 weeks. One hour after the last injection, mice were treated with pentobarbital (60 mg/kg, intraperitoneally (i.p.); Wako industry, Osaka, Japan). As a positive control, diazepam (1 mg/kg, i.p.) was administered once 1 h before pentobarbital injection (**Figure 1A**). The mice were considered asleep if they stayed immobile and lost their righting reflex when positioned on their back. The time interval between injection of pentobarbital and the start of sleep was noted as sleep latency. Sleep latency and total sleeping time were determined for each mouse. The mice were considered awake if they returned to the upright position. In the test, an equal number of animals from each group were examined once, and the test was performed two times. We checked whether the pentobarbital solution leaked from the mice or not, and the mice the pentobarbital solution leaked from were excluded from analysis. The observer was blind to the treatment.

Social Isolation Stress-Induced Insomnia Model

Mice were divided into 4 groups so that their average body weight is almost the same in each group: control group ($n = 8$), social isolation stress group ($n = 8$), social isolation stress + YKSCH (1,500 mg/kg)-treated group ($n = 8$), and social isolation stress + diazepam-treated group ($n = 8$). YKSCH was orally administered once daily for 4 weeks. One hour after the last injection, mice were treated with pentobarbital (60 mg/kg, i.p.). Sleep latency and total sleeping time were determined for each mouse. As a positive control, diazepam was administered 1 h before pentobarbital injection (**Figure 1B**).

To examine whether the effect of YKSCH was mediated by the GABAergic neurotransmitter system, we performed an antagonist study in this model. After 30 min of administration of YKSCH, mice were treated once with 3 mg/kg of bicuculline (Sigma Aldrich, MO, USA), a $GABA_A$ receptor antagonist. Then, 30 min later, mice were injected with 60 mg/kg of pentobarbital (**Figure 1C**). In the test, an equal number of animals from each group was examined once, and the test was performed twice. We checked whether the pentobarbital solution leaked from the mice or not, and the mice the pentobarbital solution leaked from were excluded from the analysis. The observer was blind to the treatment.

Locomotor Activity Test

For the measurement of locomotor activity, a given mouse was placed in a cage [plastic cage (175 × 245 × 125 mm), with wood-chips, food, and water], and locomotion was measured every 30 min for 2 days using a digital counter with an infrared sensor (Muromachi kikai Co., Ltd, Tokyo, Japan). Animals were placed in the cages at 8:00 a.m. for a 48 h period. To evaluate the effect on locomotor activity, we used the last 24 h of data for analysis.

Measurement of Allopregnanolone Content

To clarify the mechanisms of YKSCH, mice were randomly divided into 3 groups: control group ($n = 5$), social isolation stress group ($n = 5$), social isolation stress + YKSCH (1,500 mg/kg)-treated group ($n = 5$). Mice were subjected to social isolation stress for 4 weeks with or without YKSCH treatment. One hour after the last treatment with YKSCH, mice were decapitated, and brains quickly removed from the skull, briefly washed in ice-cold saline, laid on a cooled (4°C) metal plate, and both the OB and PFC were rapidly dissected out (**Figure 1D**). The tissues (10 mg) were added to 100 μL ethanol and shaken vigorously for 30 min, and then centrifuged at 5,000 rpm for 15 min. Supernatants were removed and transferred to new clean tubes and evaporated to dryness under nitrogen. Then, 100 μL ethanol and 400 μL assay buffer were added to dissolve the sample and vortexed well. The extracted samples were used for immunoassays. The ALLO content in the OB and PFC was assayed using ELISA (Arbor Assays, MI, USA).

Western Blot Analysis

Mice OB and PFC were homogenized in 10 mL/g RIPA buffer (WAKO) supplemented with protease inhibitor cocktail (Sigma Aldrich) and phosphate inhibitor cocktails 2 and 3 (Sigma Aldrich). Lysates were centrifuged at 15,000 g for 20 min at 4°C. An aliquot of 10 μg of protein was subjected to 10–20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, with the separated protein being transferred onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore, MA, USA). For immunoblotting, the following primary antibodies were used: rabbit anti-SRD5A1 polyclonal antibody (1:1,000; ABclonal technology, MA, USA) and mouse anti-β-actin monoclonal antibody (1:1,000; Cell Signaling Technology (CST), MA, USA). Secondary antibodies were as follows: horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:5,000; CST) or HRP-conjugated goat anti-mouse IgG (1:5,000; CST). Immunoreactive bands were visualized using LAS-2,000. Band intensity was measured using Image J (NIH, MD, USA).

Statistical Analysis

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics (21). All data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by Peritz's *F*-test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

HPLC Analysis of YKSCH

Figure 2 shows a HPLC profile of YKSCH along with a chemical analysis at 254 nm wavelength. Chemical markers, such as saikosaponin b_2, hesperidin and glycyrrhizic acid, were used for quality control.

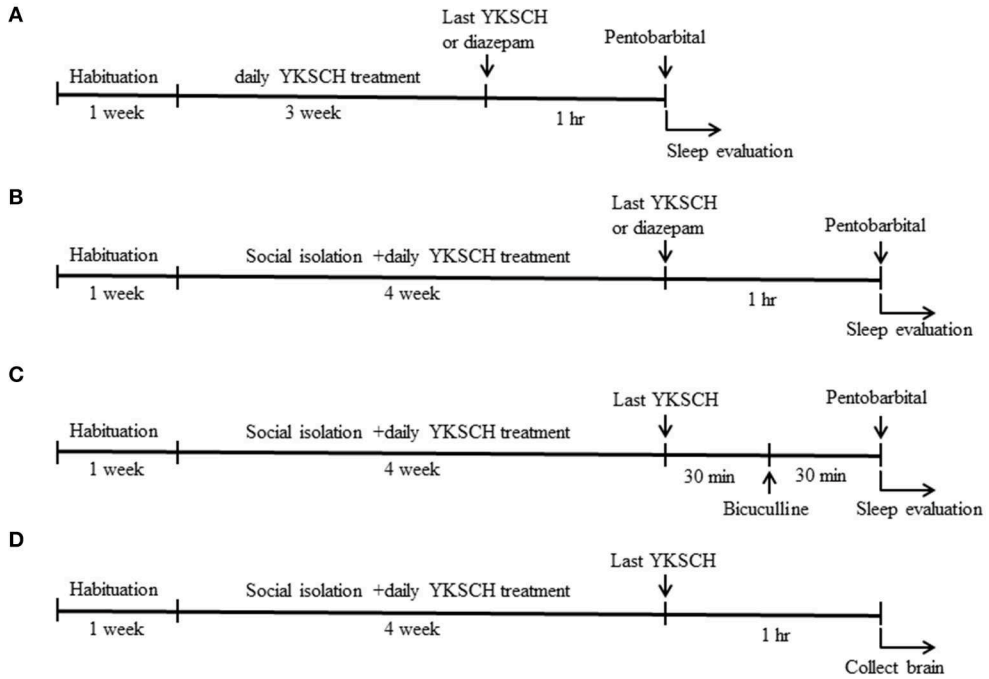

FIGURE 1 | Time schedule of the experiment. Six-week-old male ddy mice were used in this study. Mice were pretreated with 1,500 mg/kg YKSCH for 3 weeks in a group-housed mouse study **(A)** or for 4 weeks in a social isolation stress study **(B)**. One hour after the last treatment, mice were treated with 60 mg/kg pentobarbital. For the GABA receptor antagonist study, mice were treated with 3 mg/kg bicuculline 30 min after the last YKSCH treatment in socially isolated mice **(C)**. For the ELISA and western blot analysis, socially isolated mice were treated with YKSCH for 4 weeks, and mice were sacrificed 1 h after the last YKSCH treatment **(D)**.

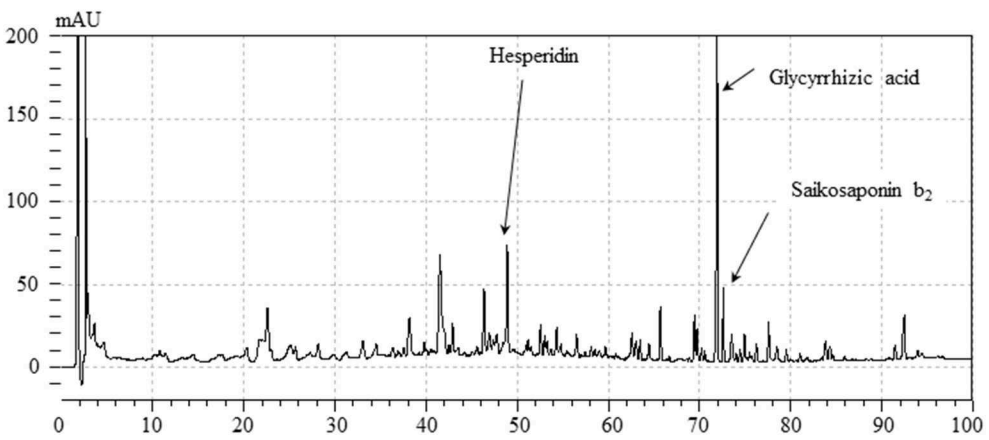

FIGURE 2 | HPLC profile of YKSCH.Each chemical marker (Saikosaponin b_2, hesperidin, glycyrrhizic acid) in the HPLC profile was identified by comparison with retention times and UV spectra (245 nm) of their reference standards.

Effect of YKSCH on Pentobarbital-Induced Sleep Behavior in Group-Housed or Socially Isolated Mice

We first evaluated the effect of YKSCH on a pentobarbital-induced sleep model that is widely used to evaluate sedative drugs. Mice were treated with 1,500 mg/kg YKSCH for 3 weeks before pentobarbital injection. As a result, both the latency and duration of sleep were not affected by YKSCH treatment [sleep latency; $F_{(2, 15)} = 0.913$, $p = 0.422$, sleep duration; $F_{(2, 15)} = 4.959$, $p = 0.022$]. Conversely, treatment with diazepam prolonged the duration of sleep significantly (**Figure 3**).

Next, we evaluated the effect of YKSCH using a social isolation-induced insomnia model. In this experiment, social isolation stress decreased the sleep duration but had no effect on sleep latency. Treatment with YKSCH significantly improved the decrease in sleep duration [sleep latency; $F_{(3, 26)} = 1.524$, $p = 0.232$, sleep duration; $F_{(3, 26)} = 17.73$, $p < 0.001$]. Conversely, treatment with diazepam also affected sleep duration significantly

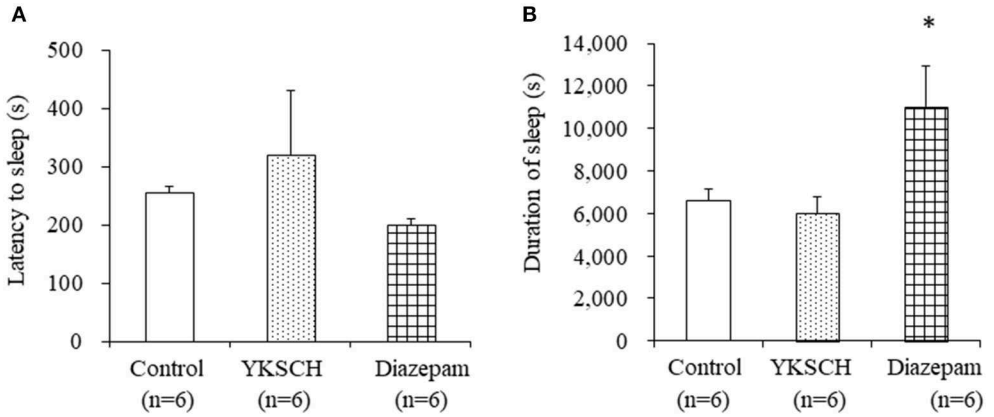

FIGURE 3 | Effect of 3-week treatment with YKSCH on pentobarbital-induced sleeping behavior in group housed mice. One hour after the last injection, mice were treated with pentobarbital. Sleep latency **(A)** and duration **(B)** were measured. Diazepam was administered once, 1 h before pentobarbital injection. Data are expressed as the mean ± SEM ($n = 6$). $^{*}p < 0.05$ vs. the control group, Peritz's F-test.

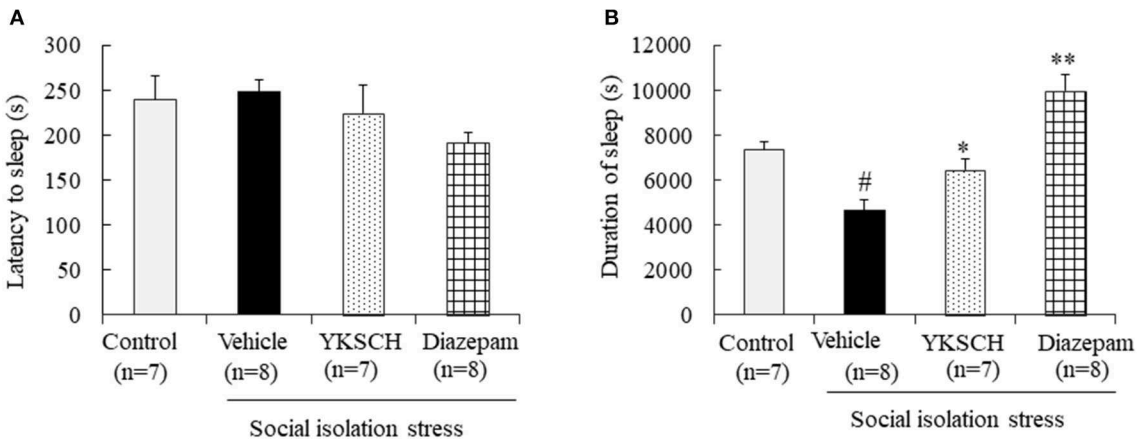

FIGURE 4 | Effect of YKSCH on pentobarbital-induced sleeping behavior in socially isolated mice. Mice were housed individually and treated with YKSCH or vehicle for 4 weeks. One hour after the last injection, mice were treated with pentobarbital. Sleep latency **(A)** and duration **(B)** were measured. Diazepam was treated once 1 h before pentobarbital injection. Data are expressed as the mean ± SEM ($n = 7$–8). $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the vehicle-treated group, Peritz's F-test.

(**Figure 4**). However, YKSCH has no effect on 24 h locomotor activity in the social isolation model (**Supplemental Figure 1**).

According to previous reports, social isolation stress disrupts many kinds of brain networks, including the GABAergic neuron system. In this study, we investigated whether the effect of YKSCH is related to the GABAergic neuron system by using bicuculline, a GABA receptor antagonist. Treatment with bicuculline did not change either sleep latency or sleep duration in socially isolated mice. On the other hand, prolonged sleep duration with YKSCH treatment was blocked by bicuculline treatment (**Figure 5**) [sleep latency; $F_{(4, 29)} = 3.701$, $p = 0.0149$, sleep duration; $F_{(4, 29)} = 4.378$, $p = 0.0068$].

Effect of YKSCH on ALLO and SRD5A1 Expression in the OB and PFC

A previous report have showed that social isolation stress-induced behavioral abnormalities are related to a reduction in ALLO contents in the OB and PFC (22). In this context, we evaluated the ALLO content in both brain regions. We showed that 4 weeks of isolation-induced stress reduced the ALLO content in both brain regions. Importantly, YKSCH treatment inhibited the decrease in ALLO content in the OB, but not in the PFC (**Figure 6**) [OB; $F_{(2, 10)} = 7.083$, $p = 0.0121$, PFC; $F_{(2, 12)} = 11.93$, $p = 0.0014$].

Brain cells synthesize ALLO from progesterone by two types enzymes, SRD5A1 and 3α-hydroxysteroid oxidoreductase. In socially isolated mice, the expression level of SRD5A1 is reduced in the OB and PFC, but 3α-hydroxysteroid oxidoreductase is unaffected (19). This report indicates that SRD5A1 is responsible for producing ALLO in this model. In this study, we investigated whether YKSCH treatment affected the SRD5A1 expression level in the OB and PFC. As a result, YKSCH treatment improved the reduction of SRD5A1 expression levels in the OB. However, YKSCH treatment had no effect in the PFC (**Figure 7**) [OB; $F_{(2, 12)} = 5.503$, $p = 0.0201$, PFC; $F_{(2, 12)} = 2.786$ $p = 0.101$].

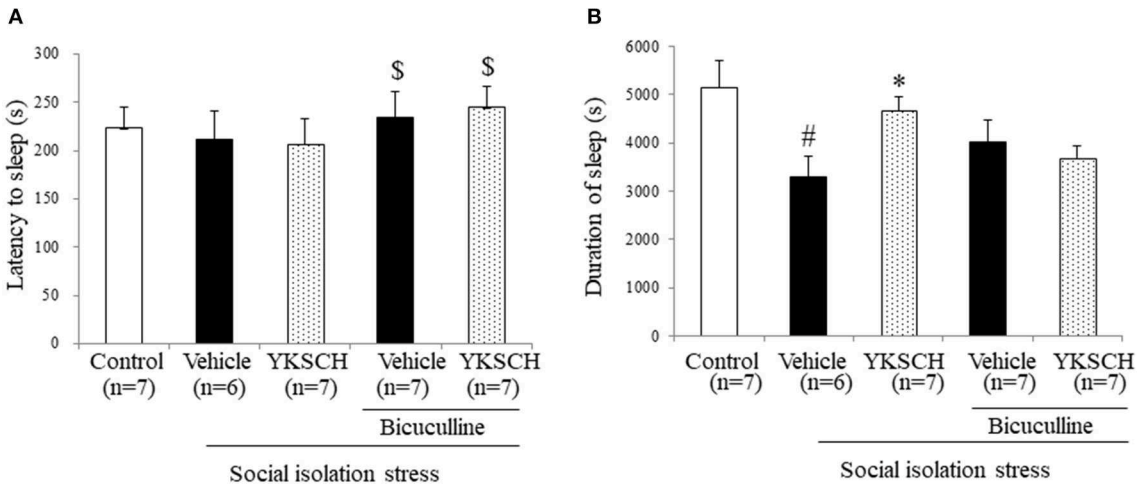

FIGURE 5 | Effect of YKSCH was mediated by GABAergic neuron system in socially isolated mice. Mice were housed individually and treated with YKSCH or vehicle for 4 weeks. Thirty minutes after the last injection, mice were treated with 3 mg/kg bicuculline (i.p.). Mice were treated with 60 mg/kg pentobarbital 30 min after bicuculline injection. The sleep latency **(A)** and duration **(B)** were measured. Diazepam was administered once 1 h before pentobarbital injection. Data are expressed as the mean ± SEM ($n = 6$–7). $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$, vs. the vehicle-treated group; $^{\$}p < 0.05$, vs. the YKSCH-treated group, Peritz's F-test.

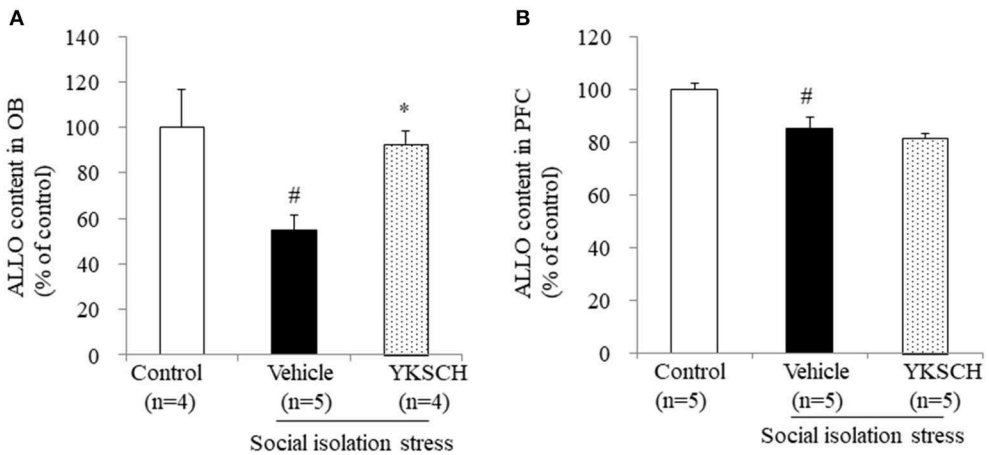

FIGURE 6 | Effect of YKSCH on allopregnanolone content in the olfactory bulb and prefrontal cortex. Allopregnanolone content in the OB **(A)** and PFC **(B)**. Data are expressed as the mean ± SEM ($n = 4$–5). $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$ vs. the vehicle-treated group, Peritz's F-test.

DISCUSSION

Here, we showed YKSCH could improve social isolation stress-induced insomnia via GABAergic actions. In addition, YKSCH treatment improved the ALLO content and SRD5A1 expression level in the mice, especially in the OB.

Benzodiazepines are widely used to treat insomnia, however, benzodiazepines often cause side effects such as sleepiness, amnesia, and dizziness. Therefore, treatment with benzodiazepines is not recommended for elderly people in Japan because of the potential for abuse, dependence, and adverse effects. For that reason, there is a need to develop new drugs with a different mechanism of action than benzodiazepines. Diazepam, a member of the benzodiazepine family, is a positive allosteric modulator of the $GABA_A$ receptor. Diazepam works by binding to the $GABA_A$ receptor subunit directly and enhancing the affinity of GABA for its binding site. In a previous clinical trial, Aizawa et al. demonstrated that YKSCH treatment in healthy subjects extended the total sleeping time significantly, had no influence on rapid eye movement (REM) sleep, tended to increase stage 2 sleep, and decreased stage 3+4 sleep compared with Anchu-san as the control drug (3). Benzodiazepine treatment was also reported to affect the same sleep stage as that observed with YKSCH treatment. Therefore, this report concluded that the effect of YKSCH may have a similar mechanism of action to that of benzodiazepines in terms of non-REM sleep. However, in the present study, we showed that diazepam treatment significantly prolonged sleep duration

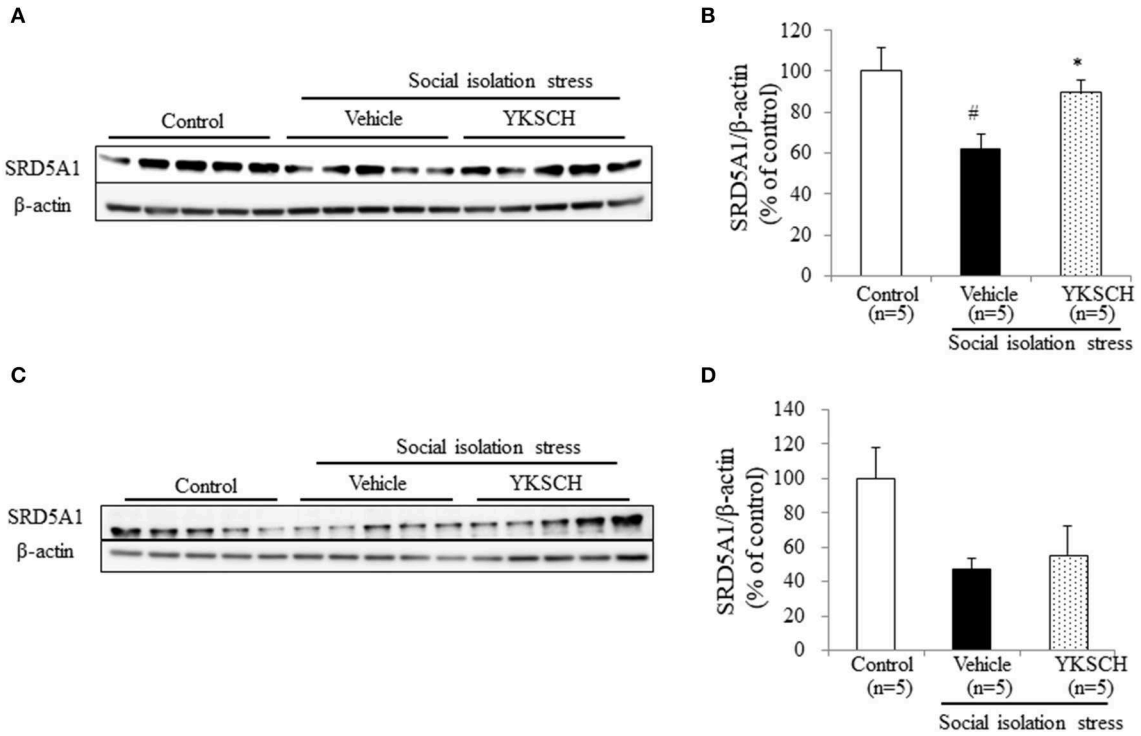

FIGURE 7 | Effect of YKSCH on SRD5A1 expression in the olfactory bulb and prefrontal cortex. **(A,B)** Western blot analyses of SRD5A1 expression in the OB. **(A)** Representative images of immunoblots obtained using antibodies against SRD5A1 and β-actin. **(B)** Quantitative analyses of OB SRD5A1 in socially isolated mice. **(C,D)** Western blot analyses of SRD5A1 expression in the PFC. **(C)** Representative images of immunoblots obtained using antibodies against SRD5A1 and β-actin. **(D)** Quantitative analyses of PFC SRD5A1 in socially isolated mice. Data are expressed as mean % of control ± SEM ($n = 5$). $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$ vs. the vehicle-treated group, Peritz's F-test.

in both models. On the other hand, YKSCH treatment improved sleep duration in socially isolated mice only (**Figures 2**, **3**). Among the components of YKSCH, Japanese Angelica root extract improves pentobarbital-induced sleep in socially isolated mice without affecting pentobarbital-induced sleep behavior in group-housed mice (23). Here, we did not investigate which component is responsible for the effect of YKSCH on sleep; however, Japanese Angelica root might partly contribute to the effects of YKSCH.

The abnormal behavior induced by social isolation involves functional changes in various brain neurotransmitter systems, including dopaminergic (13), serotonergic (14), and noradrenergic systems (15, 16). Yokukansan, which is the Chinese medicine that excludes both C. unshiu peel and pinellia tuber from YKSCH, also improves sleeping duration but only in socially isolated mice and not in group-housed mice. Furthermore, Yokukansan improved the shortening of sleeping duration via the GABAergic and not the serotonergic neurotransmitter system in a GABA or 5-HT receptor antagonist study (24). In the present study, we also demonstrated that the effect of YKSCH on a social isolation stress model was blocked by bicuculline injection (**Figure 4**). This result indicates that long-term YKSCH treatment improved GABAergic neuron disruption induced by social isolation stress and that this mechanism may be similar to that of Yokukansan. And this result also indicates that YKSCH and diazepam have similar mechanisms in that both drugs affect the GABAergic neuron system to improvement social isolation-induced insomnia. Some crude drugs contained in YKSCH (Bupleurum root, Japanese angelica root, and Cnidium rhizome) are reported to have compounds that bind to the GABA binding sites of the $GABA_A$ receptor in a receptor binding assay (25). According to previous studies, oral administration of GABA prolonged pentobarbital-induced sleep duration in group-housed mice (26). Though Yokukansan includes water-soluble botanic GABA (27), orally administered GABA does not cross the blood-brain barrier efficiently, and YKSCH treatment could not prolong the sleeping time in group-housed mice (**Figure 2**). Therefore, we hypothesized that YKSCH might potentiate the GABAergic neuron system through an indirect pathway, such as ALLO.

ALLO is a neuroactive steroid derived from progesterone, and acts as positive allosteric modulator of $GABA_A$ receptor action. As a positive allosteric modulator of the $GABA_A$ receptor, ALLO has been reported to be an anxiolytic, anesthetic, and anticonvulsant agent (28–30). In addition, ALLO treatment has been reported to improve memory impairment and BPSD-like behavior in Alzheimer's disease mouse models (22, 31, 32). Both ALLO and benzodiazepines are positive allosteric

modulators of the $GABA_A$ receptor and enhance the GABAergic neurotransmitter system, however, there may differ in terms of side effects, such as tolerance development and abuse liability (33). Several reports have shown that social isolation stress-induced behavioral abnormalities are related to the reduction in ALLO contents in the OB and PFC (22). The ALLO content is reported to be about twice higher in the OB than that in PFC (22). Olfactory dysfunction is frequently observed in patients with REM sleep behavior disorders, such as dementia and Parkinson's disease (34, 35). In addition, some reports have shown that olfactory bulbectomy in rats induces a reduction in REM sleep duration and frequency (36). These reports suggest that the OB has an important role in sleep-wakefulness patterns. Biosynthesis of ALLO in the brain is regulated by two specific enzymes. It starts with the conversion of progesterone into 5α-dihydroprogesterone by the SRD5A1 enzyme. Next, 5α-dihydroprogesterone is converted into ALLO by the 3α-HSD enzyme. Social isolation stress does not change the brain's content of progesterone and pregnenolone (29). In socially isolated mice, brain expression of SRD5A1 mRNA and protein was approximately 50% less than in group-housed mice, whereas the expression of 3α-HSD mRNA was unchanged (19). These reports suggest SRD5A1 is the enzyme responsible for producing the brain ALLO in this model. Here, we demonstrated that YKSCH treatment inhibited the decrease in both ALLO content and SRD5A1 expression levels in the OB (**Figures 5**, **6**). On the other hand, Yokukansan administered once just before pentobarbital injection had the ability to improve the reduced sleep duration in socially isolated mice (24). Although YKSCH could have other rapid mechanisms to improve sleep disruption, the present study suggests that the increase in ALLO content and SRD5A1 expression might be involved in the mechanism of YKSCH in part. However, further studies are required to clarify the mechanisms mediating the action of YKSCH.

There are some limitations to this study. The first is in **Figures 3**, **4**. In the group-housed and social isolation stress model animals, mice were treated with YKSCH for different periods. A previous report showed that social isolation stress for 4 weeks not 3 weeks reduced the sleep duration induced by pentobarbital, so we set the experimental condition based on that study in the social isolation stress model (37). While we performed YKSCH treatment for 3 weeks in group-housed mice, it is possible that YKSCH treatment for 4 weeks might have a similar effect to the effect observed in the social isolation stress model. The second is in **Figure 5**. There was no significant difference between the YKSCH group and bicuculline + YKSCH group with regard to sleep duration. In the GABA receptor antagonist study, we administered bicuculline once just before pentobarbital injection. This might be the reason why bicuculline treatment did not inhibit the effect of YKSCH completely.

In conclusion, this study demonstrates for the first time that YKSCH treatment improves social isolation stress-induced insomnia via the GABAergic nervous system. In addition, YKSCH treatment increased the ALLO content and SRD5Al expression level in the mouse brain. Moreover, these results suggest that YKSCH may be a good candidate drug to treat patients with insomnia.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Experimental Animal Care Committee of Kracie Pharma, Ltd.

AUTHOR'S NOTE

This is a Japanese language translation/reprint of 不眠モデルマウスに対する抑肝散加陳皮半夏の効果 originally published in phil 漢方 (phil Kampo) 70, 26–27, 2018. Permission was granted by Medical Publisher Inc. (Japan).

AUTHOR CONTRIBUTIONS

KM, NF, and RT contributed to the conception and design of the study. KM conducted all experiments, analyzed the data, and wrote the manuscript. FL, KS, and MO conducted part of the experiments with KM. NF and RT revised the manuscript. All authors gave final approval of the version to be published.

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.jp) for English language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.00008/full#supplementary-material

Supplemental Figure 1 | Effect of YKSCH on locomotor activity in socially isolated mice. **(A,B)** Locomotor activity test. **(A)** Number of beam-breaks throughout the 24 h period. **(B)** Number of beam breaks analyzed separately for day and night. Data are expressed as mean number of beam-breaks ± SEM ($n = 8$).

REFERENCES

1. Arita R, Yoshino T, Ohgishi M, Hamaguchi T, Horiba Y, Sawai K, et al. The efficacy and safety of Yokukansankachimpihange for treating behavioral and psychological symptoms of dementia in patients with Alzheimer's disease: an open-label pilot study. *Adv Alzheimers Dis.* (2018) 7:141–52. doi: 10.4236/aad.2018.74010
2. Meguro K, Yamaguchi S. Decreased behavioral abnormalities after treatment with combined donepezil and Yokukansankachimpihange in Alzheimer disease: an observational study. the osaki-tajiri project. *Neurol Ther.* (2018) 7:333–40. doi: 10.1007/s40120-018-0109-9

3. Aizawa R, Kanbayashi T, Saito Y, Ogawa Y, Sugiyama T, Kitajima T, et al. Effects of Yoku-kan-san-ka-chimpi-hange on the sleep of normal healthy adult subjects. *Psychiatry Clin Neurosci.* (2002) 56:303–4. doi: 10.1046/j.1440-1819.2002.01006.x
4. Izumi H, Sasaki Y, Yabuki Y, Shinoda Y, Fujita N, Yomoda S, et al. Memory improvement by Yokukansankachimpihange and Atractylenolide III in the olfactory bulbectomized mice. *Adv Alzheimers Dis.* (2016) 5:35–45. doi: 10.4236/aad.2016.52003
5. Murayama C, Wang C-C, Michihara S, Norimoto H. Pharmacological effects of "Jutsu" (Atractylodis rhizome and Atractylodis lanceae rhizome) on 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head twitch response in mice (I). *Molecules.* (2014) 19:14979–86. doi: 10.3390/molecules190914979
6. Tabuchi M, Mizuno K, Mizoguchi K, Hattori T, Kase Y. Yokukansan and Yokukansankachimpihange ameliorate aggressive behaviors in rats with cholinergic degeneration in the nucleus basalis of meynert. *Front Pharmacol.* (2017) 8:235. doi: 10.3389/fphar.2017.00235
7. Tamano H, Yusuke E, Ide K, Takeda A. Influences of yokukansankachimpihange on aggressive behavior of zinc-deficient mice and actions of the ingredients on excessive neural exocytosis in the hippocampus of zinc-deficient rats. *Exp Anim.* (2016) 65:353–61. doi: 10.1538/expanim.16-0028
8. Cho JH, Olmstead R, Choi H, Carrillo C, Seeman TE, Irwin MR. Associations of objective versus subjective social isolation with sleep disturbance, depression, and fatigue in community-dwelling older adults. *Aging Ment Health.* (2018) 23:1130–8. doi: 10.1080/13607863.2018.1481928
9. Olsen RB, Olsen J, Gunner-Svensson F, Waldstrøm B. Social networks and longevity. A 14 year follow-up study among elderly in Denmark. *Soc Sci Med.* (1991) 33:1189–95. doi: 10.1016/0277-9536(91)90235-5
10. Ben Simon E, Walker MP. Sleep loss causes social withdrawal and loneliness. *Nat Commun.* (2018) 9:3146. doi: 10.1038/s41467-018-05377-0
11. Guo M, Wu CF, Liu W, Yang JY, Chen D. Sex difference in psychological behavior changes induced by long-term social isolation in mice. *Prog Neuropsychopharmacol Biol Psychiatry.* (2004) 28:115–21. doi: 10.1016/j.pnpbp.2003.09.027
12. Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. *Stress Amst Neth.* (2005) 8:85–93. doi: 10.1080/10253890500159022
13. Bell R, Hepper PG. Catecholamines and aggression in animals. *Behav Brain Res.* (1987) 23:1–21. doi: 10.1016/0166-4328(87)90238-5
14. Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and *in vivo* neurochemistry. *Pharmacol Biochem Behav.* (1992) 43:17–35. doi: 10.1016/0091-3057(92)90635-S
15. Matsumoto K, Ojima K, Watanabe H. Noradrenergic denervation attenuates desipramine enhancement of aggressive behavior in isolated mice. *Pharmacol Biochem Behav.* (1995) 50:481–4. doi: 10.1016/0091-3057(94)00300-8
16. Ojima K, Matsumoto K, Tohda M, Watanabe H. Hyperactivity of central noradrenergic and CRF systems is involved in social isolation-induced decrease in pentobarbital sleep. *Brain Res.* (1995) 684:87–94. doi: 10.1016/0006-8993(95)00388-7
17. George O, Vallée M, Vitiello S, Le Moal M, Piazza P-V, Mayo W. Low brain allopregnanolone levels mediate flattened circadian activity associated with memory impairments in aged rats. *Biol Psychiatry.* (2010) 68:956–63. doi: 10.1016/j.biopsych.2010.03.022
18. Marx CE, Trost WT, Shampine LJ, Stevens RD, Hulette CM, Steffens DC, et al. The neurosteroid allopregnanolone is reduced in prefrontal cortex in Alzheimer's disease. *Biol Psychiatry.* (2006) 60:1287–94. doi: 10.1016/j.biopsych.2006.06.017
19. Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, et al. Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. *Proc Natl Acad Sci USA.* (2001) 98:2849–54. doi: 10.1073/pnas.051628598
20. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm.* (2016) 7:27–31. doi: 10.4103/0976-0105.177703
21. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transp.* (2013) 48:452–8. doi: 10.1038/bmt.2012.244
22. Pibiri F, Nelson M, Guidotti A, Costa E, Pinna G. Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: a model relevant for posttraumatic stress disorder. *Proc Natl Acad Sci USA.* (2008) 105:5567–72. doi: 10.1073/pnas.0801853105
23. Matsumoto K, Kohno S, Tezuka Y, Kadota S, Watanabe H. Effect of Japanese angelica root extract on pentobarbital-induced sleep in group-housed and socially isolated mice: evidence for the central action. *Jpn J Pharmacol.* (1997) 73:353–6. doi: 10.1254/jjp.73.353
24. Egashira N, Nogami A, Iwasaki K, Ishibashi A, Uchida N, Takasaki K, et al. Yokukansan enhances pentobarbital-induced sleep in socially isolated mice: possible involvement of GABA(A) - benzodiazepine receptor complex. *J Pharmacol Sci.* (2011) 116:316–20. doi: 10.1254/jphs.11079SC
25. Liao JF, Jan YM, Huang SY, Wang HH, Yu LL, Chen CF. Evaluation with receptor binding assay on the water extracts of ten CNS-active Chinese herbal drugs. *Proc Natl Sci Counc Repub China B.* (1995) 19:151–8.
26. Mabunga DFN, Gonzales ELT, Kim HJ, Choung SY. Treatment of GABA from fermented rice germ ameliorates caffeine-induced sleep disturbance in mice. *Biomol Ther.* (2015) 23:268–74. doi: 10.4062/biomolther.2015.022
27. Nishi A, Yamaguchi T, Sekiguchi K, Imamura S, Tabuchi M, Kanno H, et al. Geissoschizine methyl ether, an alkaloid in Uncaria hook, is a potent serotonin 1a receptor agonist and candidate for amelioration of aggressiveness and sociality by yokukansan. *Neuroscience.* (2012) 207:124–36. doi: 10.1016/j.neuroscience.2012.01.037
28. Frye CA. The neurosteroid 3 alpha, 5 apha-THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res.* (1995) 696:113–20. doi: 10.1016/0006-8993(95)00793-P
29. Matsumoto K, Uzunova V, Pinna G, Taki K, Uzunov DP, Watanabe H, et al. Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. *Neuropharmacology.* (1999) 38:955–63. doi: 10.1016/S0028-3908(99)00018-0
30. Yoshizawa K, Okumura A, Nakashima K, Sato T, Higashi T. Role of allopregnanolone biosynthesis in acute stress-induced anxiety-like behaviors in mice. *Synapse.* (2017) 71:e21978. doi: 10.1002/syn.21978
31. Pinna G, Costa E, Guidotti A. Changes in brain testosterone and allopregnanolone biosynthesis elicit aggressive behavior. *Proc Natl Acad Sci USA.* (2005) 102:2135–40. doi: 10.1073/pnas.0409643102
32. Singh C, Liu L, Wang JM, Irwin RW, Yao J, Chen S, et al. Allopregnanolone restores hippocampal-dependent learning and memory and neural progenitor survival in aging 3xTgAD and nonTg mice. *Neurobiol Aging.* (2012) 33:1493–506. doi: 10.1016/j.neurobiolaging.2011.06.008
33. Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, et al. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science.* (2009) 325:490–3. doi: 10.1126/science.1175055
34. Fantini ML, Postuma RB, Montplaisir J, Ferini-Strambi L. Olfactory deficit in idiopathic rapid eye movements sleep behavior disorder. *Brain Res Bull.* (2006) 70:386–90. doi: 10.1016/j.brainresbull.2006.07.008
35. Miyamoto T, Miyamoto M, Iwanami M, Hirata K, Kobayashi M, Nakamura M, et al. Olfactory dysfunction in idiopathic REM sleep behavior disorder. *Sleep Med.* (2010) 11:458–61. doi: 10.1016/j.sleep.2009.09.013
36. Araki H, Yamamoto T, Watanabe S, Ueki S. Changes in sleep-wakefulness pattern following bilateral olfactory bulbectomy in rats. *Physiol Behav.* (1980) 24:73–8. doi: 10.1016/0031-9384(80)90016-5
37. Koga N, Yamaguchi T, Lee KK, Kobayashi H. Kososan, a standardized traditional Japanese herbal medicine, reverses sleep disturbance in socially isolated mice via GABAA-benzodiazepine receptor complex activation. *Phytomedicine Int J Phytother Phytopharm.* (2014) 21:697–03. doi: 10.1016/j.phymed.2013.10.008

Conflict of Interest: All authors are employees of Kracie Pharma, Ltd.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 27 February 2020
doi: 10.3389/fnut.2020.00005

Japanese Herbal Medicine Ninjinyoeito Mediates Its Orexigenic Properties Partially by Activating Orexin 1 Receptors

Kanako Miyano[1], *Kaori Ohshima*[1,2], *Nozomi Suzuki*[1,3], *Saho Furuya*[1,3], *Yuki Yoshida*[1,4], *Miki Nonaka*[1], *Yoshikazu Higami*[4], *Kazumi Yoshizawa*[2], *Hideaki Fujii*[3] and *Yasuhito Uezono*[1,5,6*]

[1] *Division of Cancer Pathophysiology, National Cancer Center Research Institute, Tokyo, Japan,* [2] *Laboratory of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan,* [3] *Department of Medicinal Chemistry, School of Pharmacy, Kitasato University, Tokyo, Japan,* [4] *Laboratory of Molecular Pathology and Metabolic Disease, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan,* [5] *Division of Supportive Care Research, National Cancer Center Exploratory Oncology Research and Clinical Trial Center, Tokyo, Japan,* [6] *Innovation Center for Supportive, Palliative and Psychosocial Care, National Cancer Center Hospital, Tokyo, Japan*

Cancer cachexia is highly prevalent in patients with progressive cancer and is characterized by decreased food consumption, and body weight. Japanese herbal medicine Ninjinyoeito (NYT), composed of 12 herbal crude drugs, is prescribed in Asian countries to improve several symptoms such as anorexia and fatigue, which are commonly observed in patients with cancer cachexia. However, the action mechanisms of NYT in improving anorexia or fatigue in patients with cancer are not clear. Therefore, in the present study, we examined the effects of NYT on the activities of several G-protein-coupled receptors (GPCRs), which activate hyperphagia signaling in the central nervous system, using an *in vitro* assay with the CellKey^{TM} system, which detects the activation of GPCRs as a change in intracellular impedance (ΔZ). NYT increased the ΔZ of human embryonic kidney 293 (HEK293) cells expressing orexin 1 receptor (OX1R) and those expressing neuropeptide Y1 receptor (NPY1R) in a dose-dependent manner. On the contrary, NYT did not significantly increase the ΔZ of HEK293A cells expressing growth hormone secretagogue receptor (GHSR) and those expressing NPY5R. The selective OX1R antagonist SB674042 significantly decreased the NYT-induced increase in ΔZ in OX1R-expressing cells. Contrarily, the selective NPY1R antagonist BIBO3340 failed to inhibit the NPY-induced increase in ΔZ in NPY1R-expressing cells. Additionally, we prepared modified NYT excluding each one of the 12 herbal crude drugs in NYT and investigated the effects on the activity of OX1R. Among the 12 modified NYT formulations, the one without *citrus unshiu* peel failed to activate OX1R. A screening of each of the 12 herbal crude drugs showed that *citrus unshiu* peel significantly activated OX1R, which was significantly suppressed by SB674042. These finding suggest that NYT and *citrus unshiu* peel could increase food intake via activation of orexigenic OX1R-expressing neurons in the hypothalamus. This study provides scientific evidence to support the potential of NYT for cancer patients with anorexia.

Keywords: anorexia, *citrus unshiu* peel, kampo medicine, ninjinyoeito, orexin 1 receptor

OPEN ACCESS

Edited by:
Sergueï O. Fetissov,
Université de Rouen, France

Reviewed by:
Koji Ataka,
Kagoshima University, Japan
Lila Oyama,
Federal University of São Paulo, Brazil

***Correspondence:**
Yasuhito Uezono
yuezono@ncc.go.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *15 November 2019*
Accepted: *10 January 2020*
Published: *27 February 2020*

Citation:
Miyano K, Ohshima K, Suzuki N, Furuya S, Yoshida Y, Nonaka M, Higami Y, Yoshizawa K, Fujii H and Uezono Y (2020) Japanese Herbal Medicine Ninjinyoeito Mediates Its Orexigenic Properties Partially by Activating Orexin 1 Receptors. Front. Nutr. 7:5. doi: 10.3389/fnut.2020.00005

INTRODUCTION

Cancer cachexia, which is characterized by a decrease in body weight and food consumption, occurs in 80% of patients with progressive cancer, causing at least 20% of cancer-related deaths (1–3). This syndrome not only decreases the quality of life (QOL) but also attenuates the efficacy of chemotherapy (4–7). Studies suggest that cancer cachexia is caused by complicated interrelation among several mediators in the hypothalamus, such as hormones (e.g., leptin and ghrelin), and neuropeptides (e.g., neuropeptide Y and orexin), which regulate food intake (8–10). However, the mechanisms underlying this syndrome are not fully understood, and appropriate therapies for the treatment of cancer cachexia have not been established. The current treatment options for cancer cachexia are far from being satisfactory because of the lack of effective drugs currently available (6).

Ninjinyoeito (NYT), a traditional Japanese kampo medicine that contains extracted ingredients of 12 herbal crude drugs, is approved by Japan's Ministry of Health, Labor, and Welfare as a prescribed medicine in clinical practice. Since the 16th century, NYT has been prescribed in Japan and other Asian countries to ameliorate diseases and improve several symptoms such as anorexia and fatigue (11). In addition, several studies have shown that some herbal crude drugs of NYT improved appetite in a cancer cachexia model of animals or patients with cancer (12–16). However, the action mechanisms of NYT in improving anorexia and/or fatigue in cancer cachexia–anorexia syndrome are not clear.

Therefore, in the present study, we examined the effects of NYT on the activities of several G-protein-coupled receptors (GPCRs), which activate hyperphagia signaling in the central nervous system (CNS). With respect to GPCR-activating hyperphagia signaling, we focused on activated appetite-stimulating receptors, such as growth hormone secretagogue receptor 1a (GHSR), neuropeptide Y1 receptor (NPY1R), neuropeptide Y5 receptor (NPY5R), and orexin 1 receptor (OX1R) (17–32). First, we analyzed the effects of NYT on the activities of these receptors. Second, we identified active medicinal herbs contained in NYT by screening both modified NYT excluding 1 of the 12 herbal crude drugs and each of the 12 herbal crude drugs contained in NYT.

MATERIALS AND METHODS

Chemicals and Reagents

The following reagents and medium were used in the present study: poly-D-lysine and bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA); fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and geneticin (Gibco, Carlsbad, CA, USA); penicillin/streptomycin and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Nacalai Tesque, Kyoto, Japan); and DMEM (Fujifilm Wako Pure Chemical, Osaka, Japan).

NYT extract powder (lot no. 15112017), the base powder of NYT without excipients, was obtained from Kracie Pharma, Ltd. (Tokyo, Japan), as an aqueous extract of the following 12 medicinal herbs (percentage): atractylodes rhizome (12.9), Japanese angelica root (12.9), *poria sclerotium* (12.9), *Rehmannia* root (12.9), ginseng (9.7), cinnamon bark (8.1), *citrus unshiu* peel (6.5), peony root (6.5), polygala root (6.5), astragalus root (4.8), *Glycyrrhiza* (3.2), and schisandra fruit (3.2). NYT formulations excluding each one of the 12 herbal crude drugs were also obtained from Kracie Pharma, Ltd. The dried powdered extract of NYT and its crude drugs were suspended in sterile water at 100 mg/ml concentration, diluted 100-fold with Hanks' balanced salt solution (in mM: 1.3 $CaCl_2 \cdot 2H_2O$, 0.81 $MgSO_4$, 5.4 KCl, 0.44 KH_2PO_4, 4.2 $NaHCO_3$, 136.9 NaCl, 0.34 Na_2HPO_4, and 5.6 D-glucose) containing 20 mM HEPES and 0.1% BSA, and filtered through a 0.2 μm membrane (KURABO Industry Ltd., Osaka, Japan). The solution was used to treat cells at final concentrations of 3, 10, 30, and 100 μg/ml. All other reagents were of the highest purity available from commercial sources.

Generation of Stable Cell Lines

GHSR-expressing cells were cultured as described previously (33). The expression vector C-terminal FLAG-tagged human GHS-R1a was transfected into human embryonic kidney 293A (HEK293A) cells using PEI Max (Polysciences, Inc., Warrington, PA, USA). For human NPY1R and NPY5R clones, we synthesized the combined fragment of the NruI restriction site, human EF1 promoter (34), N-terminal cleavable hemagglutinin secretion signal (MKTIIALSYIFCLVFA) (35), and NheI site and inserted it into the pIRESpuro3 expression vector (Takara, Shiga, Japan) using its recognition site (pEF1-IRESpuro). Subsequently, we amplified the cDNA encoding hNPY1R (NM_000909.6) ORF and hNPY5R (NM_001317091.1) ORF with the primers tagged with the NheI (N′) and BamHI (C′) sites from a full-length cDNA clone (Genscript, Piscataway, NJ, USA), and it was transferred into the pEF1-IRESpuro vector. The expression constructs were transfected into HEK293T cells according to the manufacturers' instructions, and 48 h after transfection, cells stably expressing either NPY1R or NPY5R were selected. The human OX1R clone (GenBank accession: AB463762; Kazusa DNA Research Institute, Chiba, Japan) was amplified according to the manufacturer's instructions. HEK293 cells (American Type Culture Collection, Manassas, VA, USA) stably expressing OX1R were generated through transfection of plasmids using ScreenFect™ (Fujifilm Wako Pure Chemical) and selected based on the OX1R activity measured using the CellKey™ assay. Ethical approval of the experimental procedures was obtained from National Cancer Center Research Institute (approval no. B85M1-13).

Cell Culture

All cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2. GHSR-expressing HEK293A cells and OX1R-expressing HEK293 cells were maintained in DMEM (Gibco or Fujifilm Wako Pure Chemical) supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 mg/ml), and geneticin (800 μg/ml). HEK293T cells expressing NPY1R or NPY5R were maintained in DMEM (Gibco) supplemented with sodium pyruvate (1 mM), 10% FBS, penicillin (100 U/ml), and streptomycin (100 mg/ml).

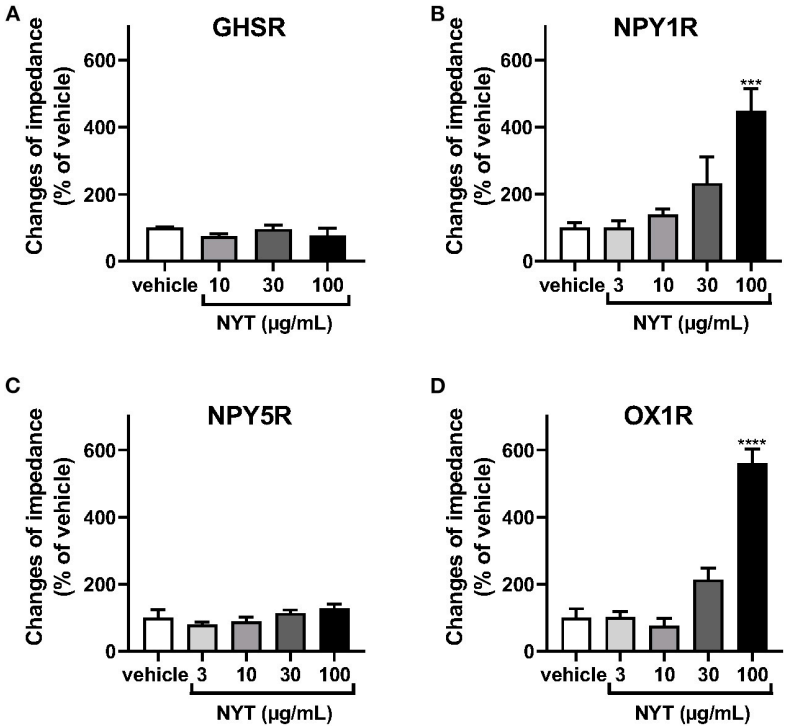

FIGURE 1 | Effects of Ninjinyoeito (NYT) on impedance changes in cells expressing several G-protein-coupled receptors, which activate hyperphagia signaling in the central nervous system using the CellKey™ assay. The cells stably expressing growth hormone secretagogue receptor 1a (GHSR) (**A**, $n = 6$–8), neuropeptide Y1 receptor (NPY1R) (**B**, $n = 6$), neuropeptide Y5 receptor (NPY5R) (**C**, $n = 6$), or orexin 1 receptor (OX1R) (**D**, $n = 6$) were treated with NYT (3–100 μg/kg) or its vehicle (control). The rate of change in impedance was measured using the CellKey™ system and expressed as the difference of the minimum impedance and maximum impedance after drug injection. The data are expressed as mean ± S.E.M. *** and **** indicate $p < 0.001$ and $p < 0.0001$, respectively, compared with the control; Bonferroni's multiple comparison test following one-way ANOVA.

Measurement of GPCR Activity Using the CellKey™ System

The assay with the CellKey™ system was conducted as described previously (36–40). Briefly, the cells were cultured at a density of 4.0×10^4 (GHSR-expressing HEK293A), 6.0×10^4 (NPY1R-expressing HEK293T), 5.0×10^4 (NPY5R-expressing HEK293T), and 6.0×10^4 cells/well (OX1R-expressing HEK293) in CellKey™ 96-well microplates. After incubating at 37°C for 24 h, the cells were washed with Hanks' balanced salt solution containing 20 mM HEPES and 0.1% BSA, and allowed to equilibrate in the assay buffer for 30 min before the assay. The CellKey™ instrument applies small voltages to the electrodes every 10 s and measures impedance of the cell layer. In this study, we recorded at 5 min baseline, added drugs, and measured changes in impedance (ΔZ) for 25 min. The rate of change in impedance is expressed as the difference of the minimum impedance and maximum impedance after drug injection as previously reported (39).

Statistical Analysis

The data are presented as mean ± S.E.M. The statistical analyses were performed using the one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (GraphPad Prism 8, GraphPad Software, San Diego, CA, USA). The results with a probability value $p < 0.05$ were considered statistically significant.

RESULTS

NYT Activated OX1R but Not GHSR, NPY1R, and NPY5R

We examined the effects of NYT on the activation of GHSR, NPY1R, NPY5R, and OX1R using the CellKey™ system. As shown in **Figures 1A,C**, NYT (3–100 μg/ml) did not significantly change the ΔZ of GHSR-expressing HEK293A cells and NPY5R-expressing HEK293T cells. On the contrary, the GHSR agonist ghrelin (10^{-7} M) and NPYR agonist neuropeptide Y (NPY, 10^{-6} M) increased the ΔZ of these cells, respectively, in GHSR-expressing cells: control vs. ghrelin (10^{-7} M) (% of control, mean ± S.E.M.), 100 ± 7.81 vs. 2,227.5 ± 288.4; in NPY5R-expressing cells: control vs. NPY (10^{-6} M), 100 ± 10.24 vs. 172.8 ± 9.68. NYT significantly increased the ΔZ of cells expressing NPY1R or OX1R in a dose-dependent manner (**Figures 1B–D**). However, the NYT-induced increase in ΔZ of NPY1R-expressing HEK293T cells was not significantly attenuated by BIBO3340 (BIBO, 10^{-5} or 10^{-4} M), at concentrations that completely inhibited the increase in ΔZ induced by NPY (10^{-8} M) (**Figures 2A,B**). Contrarily, SB676042 (SB, 10^{-6} or 10^{-5} M)

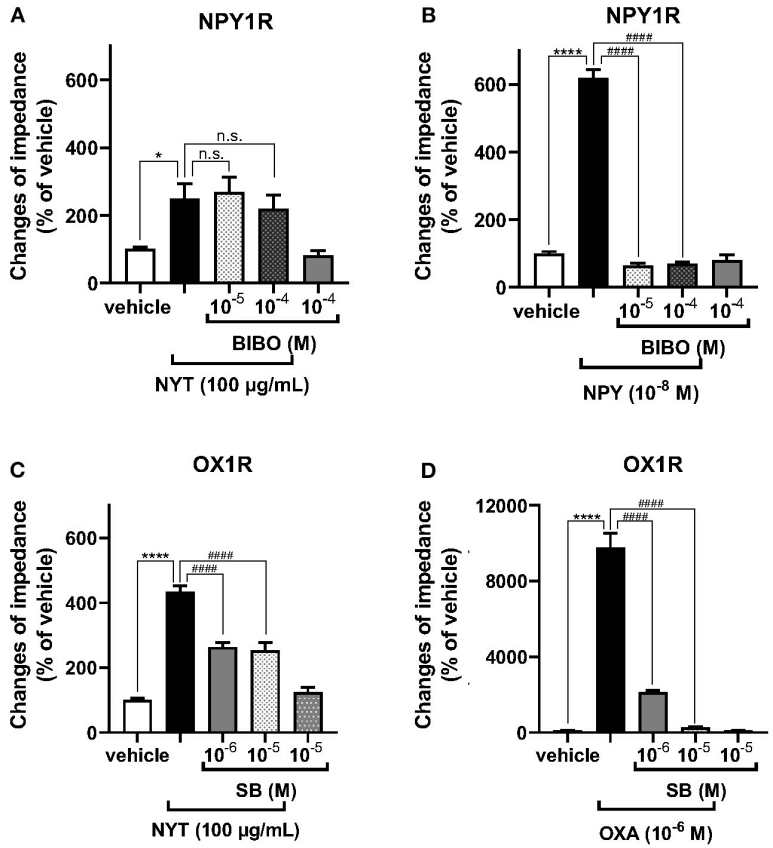

FIGURE 2 | Effects of the antagonists of NPY1R and OX1R on the NYT-induced increase in impedance of cells expressing NPY1R or OX1R. The cells expressing NPY1R **(A,B)** were pretreated with or without BIBO3340 (BIBO, 10^{-5} or 10^{-4} M), a selective NPY1R antagonist, for 30 min and then treated with NYT (**A**, 100 μg/ml) or NPY (**B**, 10^{-8} M), respectively. After treatment with the vehicle or selective OX1R antagonist SB676042 (SB, 10^{-6} or 10^{-5} M) for 30 min, OX1R-expressing HEK293 cells were treated with NYT **(C)** or orexin A (**D**, OXA). The rate of change in impedance is expressed as the difference of the minimum and maximum impedance after drug injection. The data are expressed as mean ± S.E.M. ($n = 6$–12). *$p < 0.05$, and ****$p < 0.0001$, respectively, compared with the vehicle (control); ####$p <$ 0.0001, compared with NYT or each selective agonist; Bonferroni's multiple comparison test following one-way ANOVA.

significantly suppressed the NYT-induced increase in ΔZ of OX1R-expressing HEK293 cells (**Figure 2C**). Pretreatment with SB676042 (SB, 10^{-6}, or 10^{-5} M) significantly inhibited the increase in ΔZ induced by the OX1R agonist orexin A (OXA) in a dose-dependent manner (**Figure 2D**).

Only Citrus Unshiu Peel, One of the Herbal Crude Drugs in NYT, Elicited OX1R Activation

To clarify the medical herbal crude drugs involved in NYT-induced OX1R activation, we investigated the effects of modified NYT excluding 1 of the 12 herbal drugs composing NYT on the increase in ΔZ of OX1R-expressing HEK293 cells. As shown in **Figure 3**, only the modified NYT without *citrus unshiu* peel (100 μg/ml) failed to significantly increase the ΔZ of OX1R-expressing cells; compared with the NPY-induced increase in ΔZ, the responses were significantly low (**Figure 3**).

We further examined the effects of each of the medical herbal crude drugs composing NYT on the activity of OX1R. NYT contains 12 medicinal herbs in the following ratio: atractylodes rhizome (4), Japanese angelica root (4), *poria sclerotium* (4), *Rehmannia* root (4), ginseng (3), cinnamon bark (2.5), *citrus unshiu* peel (2), peony root (2), polygala root (2), astragalus root (1.5), *Glycyrrhiza* (1), and schisandra fruit (1). Thus, the major components of the herbal drugs were atractylodes rhizome, Japanese angelica root, poria sclerotium, and *Rehmannia* root, each accounting for 13% of NYT. We therefore analyzed the effects of 20 μg/ml of the 12 individual medicinal herbs (20% of the volume of NYT) on the activity of OX1R to reveal the medicinal herbs in NYT that activate OX1R. As shown in **Figure 4A**, only *citrus unshiu* peel (20 μg/ml) significantly increased the ΔZ (**Figure 4A**), and the responses were suppressed by pretreatment with SB676042 (SB, 10^{-6} and 10^{-5} M) in a dose-dependent manner (**Figure 4B**).

DISCUSSION

The present study, to the best of our knowledge, for the first time, revealed that NYT activated OX1R but not GHSR, NPY1R, and NPY5R, which are receptors of various hypothalamic peptides

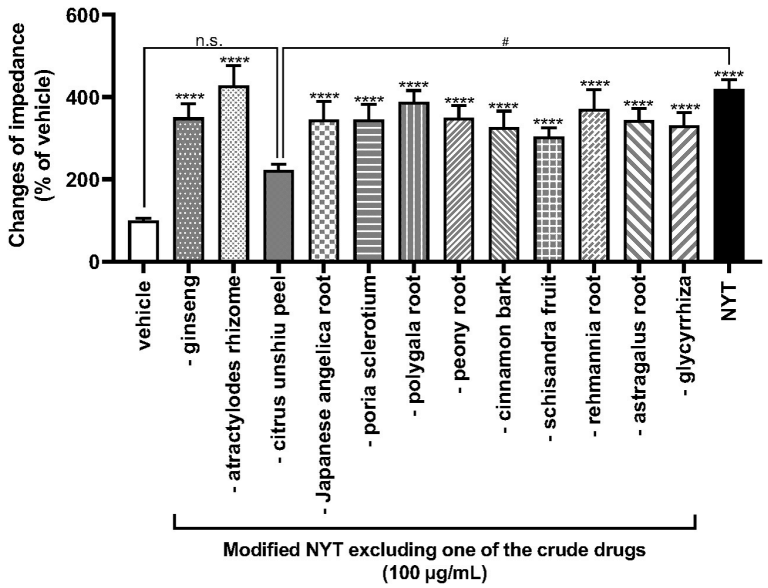

FIGURE 3 | Effects of modified NYT excluding each one of the 12 herbal crude drugs on the increase in impedance of OX1R-expressing HEK293 cells. HEK293 cells stably expressing OX1R were treated with modified NYT excluding 1 herbal crude drug (100 μg/ml) from 12 ingredients of NYT or its vehicle (control). The rate of change in impedance is expressed as the difference of the minimum and maximum impedance after drug injection. The data are expressed as mean ± S.E.M. ($n = 6$). ****$p < 0.0001$, compared with the control; #$p < 0.05$, compared with the NYT; Bonferroni's multiple comparison test following one-way ANOVA.

regulating feeding behavior. Moreover, we found that *citrus unshiu* peel in NYT induced the activation of OX1R. These data suggest that *citrus unshiu* peel in NYT is an activator of feeding behavior.

NPY is a 36-amino-acid neuropeptide and abundantly distributed in the arcuate nucleus of the hypothalamus, which integrates signals for energy homeostasis (18). NPYR is classified into five subtypes (NPY1, NPY2, NPY3, NPY5, and NPY6), and NPY1R and NPY5R play roles in appetite control (30). The present results showed that NYT induced an increase of ΔZ in HEK293T cells expressing NPY1R but not NPY5R (**Figure 1**). However, the selective NPY1R antagonist BIBO3340 did not significantly decrease NPY1R-induced responses (**Figure 2A**). Recently, it has been reported that NPYR forms heterodimers with other GPCRs (41, 42). In some cases, the responses of GPCR heterodimers were not notably inhibited by the antagonist of each GPCR monomer (43). Overall, our present data suggest that NYT might activate NPY1R/endogenous unidentified GPCRs expressed in HEK293 cells as heterodimers.

Orexin (orexin A and orexin B), one of the neuropeptides, was initially recognized as a regulator of feeding behavior, because of its exclusive production in the lateral hypothalamic area (LHA), a region known as the feeding center (17, 20, 29, 31). The orexin receptor is classified into two subtypes (OX1R and OX2R), and OX1R is mainly involved in the increase in food intake (20, 22, 31). In this study, NYT-induced OX1R activities were significantly attenuated by SB676042 (**Figure 2C**), suggesting that NYT could be an agonist of OX1R. Modified NYT without *citrus unshiu* peel (100 μg/ml) failed to increase OX1R activities in the cells (**Figure 3**), and the increase was completely suppressed by SB676042 (**Figure 4**). However, the level of increase in ΔZ induced by NYT or *citrus unshiu* peel [NYT (% of control): 433.3 ± 19.5, *citrus unshiu* peel: 207.4 ± 28.0] was considerably smaller than that caused by OXA (% of control: 9,781.5 ± 252.9). Theoretically, kampo medicine comprises several medical herbal crude drugs; thus, it is considered to exert multiple actions (11, 44–51). Furthermore, each action of kampo medicine is considered milder than that of a drug composed of only one component, such as western drugs. Thus, kampo medicines are known to cause fewer adverse effects (52). Taken together, these results suggest that NYT might have other actions, besides the activation of OX1R, to improve appetite and fatigue.

In the present study, modified NYT excluding *citrus unshiu* peel (100 μg/ml) did not significantly activate OX1R (**Figure 3**). In addition, the OX1R activities were induced by *citrus unshiu* peel alone, and this was suppressed by SB676042 (**Figure 4**). These data suggest that *citrus unshiu* peel is an agonist of OX1R. In an aqueous extract mixture of NYT, the main ingredients of *citrus unshiu* peel were hesperidine, nobiletin, tangeretin, heptamethoxyflavone, naringin, and synephrine (53–55). Some studies on blood pharmacokinetics indicated that these ingredients are absorbed into the blood in humans after oral administration (56, 57). In addition, some reports have shown permeation of polymethoxyflavones and nobiletin into the brain using animal models (58, 59). Therefore, these data suggest that ingredients derived from *citrus unshiu* peel could pass the blood–brain barrier (BBB), and reach the OX1R on neurons. The facts suggest that these ingredients in *citrus unshiu* peel could pass the BBB and act as agonists of OX1R in neurons. However, further studies are required, and we will

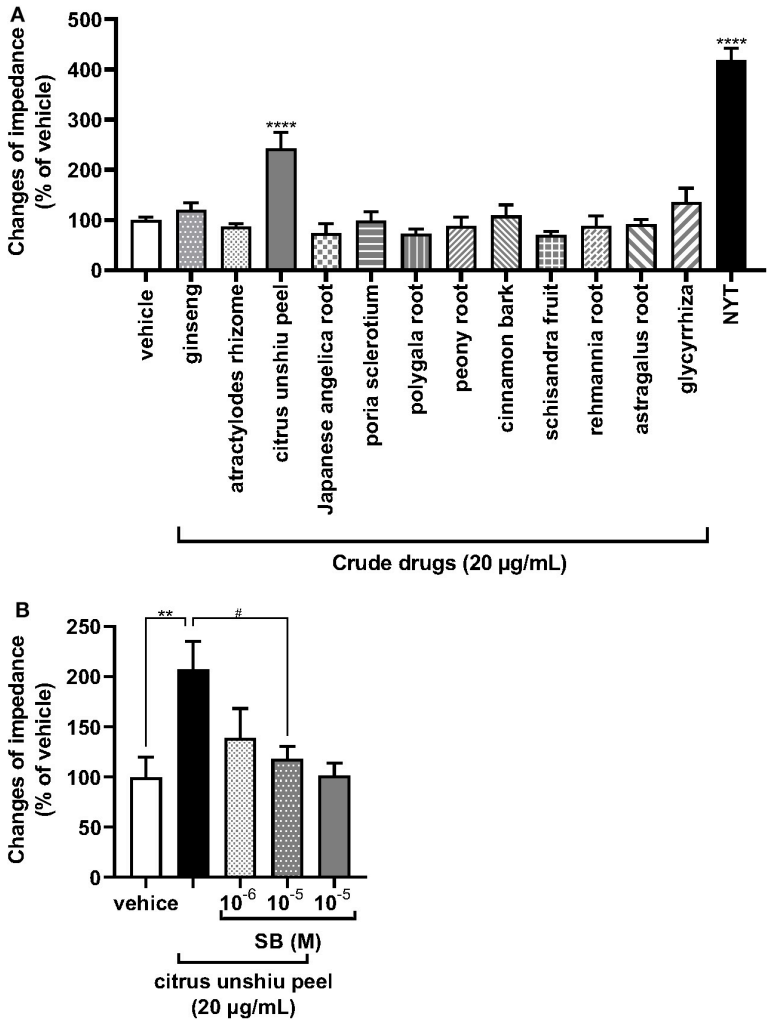

FIGURE 4 | Effects of each herbal crude drug containing NYT on the increase in impedance of OX1R-expressing HEK293 cells. **(A)** HEK293 cells stably expressing OX1R were treated with each herbal crude drug alone contained in NYT (20 μg/ml) or its vehicle (control) ($n = 6$–12). **(B)** The cells expressing OX1R were pretreated with or without the selective OX1R antagonist SB676042 (SB, 10^{-6} or 10^{-5} M) for 30 min and then treated with *citrus unshiu* peel (20 μg/ml) ($n = 5$–6). The rate of change in impedance is expressed as the difference of the minimum and maximum impedance after drug injection. The data are expressed as mean ± S.E.M ($n = 6$). **< 0.001, and ****$p < 0.0001$, respectively, compared with the vehicle (control); #$p < 0.05$, compared with *citrus unshiu* peel; Bonferroni's multiple comparison test following one-way ANOVA.

elucidate the ingredients that induce the activation of OX1R in the future.

Several studies have shown that cancer cachexia induced a decrease in food intake in accordance with the changes in orexigenic/anorexigenic neuropeptides such as orexin. This suggests that modulating orexigenic/anorexigenic neuropeptide expression is important for patients with cancer to improve cachexia (10, 18). In this study, we revealed that NYT and *citrus unshiu* peel activated OX1R using an *in vitro* assay. Kim et al. have shown that *citrus unshiu* peel extract alleviates cancer-induced weight loss in mice bearing CT-26 adenocarcinoma (60). Although further studies using cancer cachexia–anorexia model animals are required, these previous and our present data suggest that NYT might improve cancer cachexia–anorexia via the activation of OX1R.

Ghrelin, a 28-amino-acid peptide, is mainly secreted from X/A-like cells in the stomach as an orexigenic peptide (19, 28, 32). The ghrelin receptor GHSR is primarily located in NPY and agouti-related protein (AGRP) containing neurons of the hypothalamus–pituitary unit (21–28). The plasma ghrelin levels increase in response to prolonged fasting and rapidly decrease after feeding, suggesting that peripheral ghrelin is significantly important for appetite regulation (26). We previously reported that rikkunshito (RKT), a Japanese herbal kampo medicine, improved appetite as assessed using a visual analog scale (VAS) in a randomized phase II study (61).

In addition, we previously revealed the mechanism through which RKT ameliorated anorexia in cancer cachexia model rats (32, 62). It has been demonstrated that RKT alleviated ghrelin resistance by enhancement of ghrelin signaling (32). We also previously reported that atractylodin, an ingredient in *Atractylodes lancea* rhizome, an herbal drug composing RKT, enhanced ghrelin-induced GHSR activation via an increase in the ghrelin/GHSR-binding activity (33). However, NYT does not contain *Atractylodes lancea* rhizome, and the present study showed that NYT neither activated GHSR (**Figure 1A**) nor enhanced the ghrelin-induced GHSR activation (**Supplementary Figure 1**). These data suggest that although both RKT and NYT are involved in the improvement of anorexia, they may ameliorate cancer cachexia–related anorexia via different mechanisms.

In conclusion, the present results suggest that NYT and its ingredient *citrus unshiu* peel activated OX1R. Although further studies using animal models with cancer cachexia–anorexia are needed, these data suggest that NYT might improve cancer cachexia–anorexia partially via activation of OX1R. This study provides scientific evidence supporting the use of NYT in patients with cancer cachexia–anorexia.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

KM and YU: conceptualization and writing—review and editing. KM, KO, and YY: methodology: MN: validation. KO, NS, and SF: investigation. KM, KO, and NS: data curation. KM: writing—original draft preparation. YH, KY, and HF: supervision. YU: project administration and funding acquisition.

FUNDING

This work was supported by a grant from Kracie Pharma, Ltd. This funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.jp) for English language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.00005/full#supplementary-material

REFERENCES

1. Laviano A, Meguid MM, Inui A, Muscaritoli M, Rossi-Fanelli F. Therapy insight: cancer anorexia-cachexia syndrome–when all you can eat is yourself. *Nat Clin Pract Oncol.* (2005) 2:158–65. doi: 10.1038/ncponc0112
2. Muscaritoli M, Bossola M, Aversa Z, Bellantone R, Rossi Fanelli F. Prevention and treatment of cancer cachexia: new insights into an old problem. *Eur J Cancer.* (2006) 42:31–41. doi: 10.1016/j.ejca.2005.07.026
3. Evans WJ, Morley JE, Argiles J, Bales C, Baracos V, Guttridge D, et al. Cachexia: a new definition. *Clin Nutr.* (2008) 27:793–9. doi: 10.1016/j.clnu.2008.06.013
4. Theologides A. Cancer cachexia. *Cancer.* (1979) 43:2004–12. doi: 10.1002/1097-0142(197905)43:5+<2004::AID-CNCR2820430708>3.0.CO;2-%23
5. Tisdale MJ. Cachexia in cancer patients. *Nat Rev Cancer.* (2002) 2:862–71. doi: 10.1038/nrc927
6. Topkan E, Yavuz AA, Ozyilkan O. Cancer cachexia: pathophysiologic aspects and treatment options. *Asian Pac J Cancer Prev.* (2007) 8:445–51.
7. Sudo Y, Otsuka H, Miyakawa R, Goto A, Kashiwase Y, Terawaki K, et al. Differential metabolic responses to adipose atrophy associated with cancer cachexia and caloric restriction in rats and the effect of rikkunshito in cancer cachexia. *Int J Mol Sci.* (2018) 19:3852. doi: 10.3390/ijms19123852
8. Hanada T, Toshinai K, Kajimura N, Nara-Ashizawa N, Tsukada T, Hayashi Y, et al. Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells. *Biochem Biophys Res Commun.* (2003) 301:275–9. doi: 10.1016/S0006-291X(02)03028-0
9. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab.* (2004) 89:2832–6. doi: 10.1210/jc.2003-031768
10. Ramos EJ, Suzuki S, Marks D, Inui A, Asakawa A, Meguid MM. Cancer anorexia-cachexia syndrome: cytokines and neuropeptides. *Curr Opin Clin Nutr Metab Care.* (2004) 7:427–34. doi: 10.1097/01.mco.0000134363.53782.cb
11. Miyano K, Nonaka M, Uzu M, Ohshima K, Uezono Y. Multifunctional actions of ninjinyoeito, a Japanese kampo medicine: accumulated scientific evidence based on experiments with cells and animal models, and clinical studies. *Front Nutr.* (2018) 5:93. doi: 10.3389/fnut.2018.00093
12. Liu Y, Jia Z, Dong L, Wang R, Qiu G. A randomized pilot study of atractylenolide I on gastric cancer cachexia patients. *Evid Based Complement Alternat Med.* (2008) 5:337–44. doi: 10.1093/ecam/nem031
13. Lobina C, Carai MA, Loi B, Gessa GL, Riva A, Cabri W, et al. Protective effect of Panax ginseng in cisplatin-induced cachexia in rats. *Future Oncol.* (2014) 10:1203–14. doi: 10.2217/fon.13.276
14. Unlu A, Nayir E, Kirca O, Ay H, Ozdogan M. Ginseng and cancer. *J BUON.* (2016) 21:1383–7.
15. Tahaghoghi-Hajghorbani S, Ebrahimzadeh MA, Rafiei A, Golpour M, Hosseini-Khah Z, Akhtari J. Improvement of chemotherapy through reducing of cachexia by using *Citrus unshiu* peel extract. *J Ethnopharmacol.* (2019) 242:111929. doi: 10.1016/j.jep.2019.111929
16. Bae T, Jang J, Lee H, Song J, Chae S, Park M, et al. *Paeonia lactiflora* root extract suppresses cancer cachexia by down-regulating muscular NF-kappaB signalling and muscle-specific E3 ubiquitin ligases in cancer-bearing mice. *J. Ethnopharmacol.* (2020) 246:112222. doi: 10.1016/j.jep.2019.112222
17. Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol.* (1999) 160:R7–12. doi: 10.1677/joe.0.160r007
18. Inui A. Cancer anorexia-cachexia syndrome: are neuropeptides the key? *Cancer Res.* (1999) 59:4493–501.
19. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature.* (1999) 402:656–60. doi: 10.1038/45230
20. Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, et al. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept.* (2000) 96:45–51. doi: 10.1016/S0167-0115(00)00199-3
21. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on

hypothalamic peptide gene expression. *Endocrinology.* (2000) 141:4797–800. doi: 10.1210/endo.141.12.7920
22. Yamada H, Okumura T, Motomura W, Kobayashi Y, Kohgo Y. Inhibition of food intake by central injection of anti-orexin antibody in fasted rats. *Biochem Biophys Res Commun.* (2000) 267:527–31. doi: 10.1006/bbrc.1999.1998
23. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology.* (2001) 120:337–45. doi: 10.1053/gast.2001.22158
24. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature.* (2001) 409:194–8. doi: 10.1038/35051587
25. Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes.* (2001) 50:227–32. doi: 10.2337/diabetes.50.2.227
26. Tschop M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, et al. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest.* (2001) 24:Rc19–21. doi: 10.1007/BF03351037
27. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab.* (2001) 86:5992. doi: 10.1210/jcem.86.12.8111
28. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology.* (2002) 123:1120–8. doi: 10.1053/gast.2002.35954
29. Haynes AC, Chapman H, Taylor C, Moore GB, Cawthorne MA, Tadayyon M, et al. Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept.* (2002) 104:153–9. doi: 10.1016/S0167-0115(01)00358-5
30. Lin S, Boey D, Herzog H. NPY and Y receptors: lessons from transgenic and knockout models. *Neuropeptides.* (2004) 38:189–200. doi: 10.1016/j.npep.2004.05.005
31. Sakurai T. Roles of orexins in the regulation of body weight homeostasis. *Obes Res Clin Pract.* (2014) 8:e414–20. doi: 10.1016/j.orcp.2013.12.001
32. Terawaki K, Kashiwase Y, Sawada Y, Hashimoto H, Yoshimura M, Ohbuchi K, et al. Development of ghrelin resistance in a cancer cachexia rat model using human gastric cancer-derived 85As2 cells and the palliative effects of the Kampo medicine rikkunshito on the model. *PLoS ONE.* (2017) 12:e0173113. doi: 10.1371/journal.pone.0173113
33. Fujitsuka N, Asakawa A, Uezono Y, Minami K, Yamaguchi T, Niijima A, et al. Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. *Transl Psychiatry.* (2011) 1:e23. doi: 10.1038/tp.2011.25
34. Mizushima S, Nagata S. pEF-BOS, a powerful mammalian expression vector. *Nucleic Acids Res.* (1990) 18:5322. doi: 10.1093/nar/18.17.5322
35. Quitterer U, Pohl A, Langer A, Koller S, Abdalla S. A cleavable signal peptide enhances cell surface delivery and heterodimerization of Cerulean-tagged angiotensin II AT1 and bradykinin B2 receptor. *Biochem Biophys Res Commun.* (2011) 409:544–9. doi: 10.1016/j.bbrc.2011.05.041
36. Miyano K, Sudo Y, Yokoyama A, Hisaoka-Nakashima K, Morioka N, Takebayashi M, et al. History of the G protein-coupled receptor (GPCR) assays from traditional to a state-of-the-art biosensor assay. *J Pharmacol Sci.* (2014) 126:302–9. doi: 10.1254/jphs.14R13CP
37. Hisaoka-Nakashima K, Miyano K, Matsumoto C, Kajitani N, Abe H, Okada-Tsuchioka M, et al. Tricyclic antidepressant amitriptyline-induced glial cell line-derived neurotrophic factor production involves pertussis toxin-sensitive galphai/o activation in astroglial cells. *J Biol Chem.* (2015) 290:13678–91. doi: 10.1074/jbc.M114.622415
38. Kajitani N, Miyano K, Okada-Tsuchioka M, Abe H, Itagaki K, Hisaoka-Nakashima K, et al. Identification of lysophosphatidic acid receptor 1 in astroglial cells as a target for glial cell line-derived neurotrophic factor expression induced by antidepressants. *J Biol Chem.* (2016) 291:27364–70. doi: 10.1074/jbc.M116.753871
39. Meguro Y, Miyano K, Hirayama S, Yoshida Y, Ishibashi N, Ogino T, et al. Neuropeptide oxytocin enhances mu opioid receptor signaling as a positive allosteric modulator. *J Pharmacol Sci.* (2018) 137:67–75. doi: 10.1016/j.jphs.2018.04.002
40. Manabe S, Miyano K, Fujii Y, Ohshima K, Yoshida Y, Nonaka M, et al. Possible biased analgesic of hydromorphone through the G protein-over beta-arrestin-mediated pathway: cAMP, CellKey, and receptor internalization analyses. *J Pharmacol Sci.* (2019) 140:171–7. doi: 10.1016/j.jphs.2019.06.005
41. Parker MS, Sah R, Balasubramaniam A, Park EA, Sallee FR, Parker SL. Dimers of G-protein coupled receptors as versatile storage and response units. *Int J Mol Sci.* (2014) 15:4856–77. doi: 10.3390/ijms15034856
42. Kilpatrick LE, Humphrys LJ, Holliday ND. A G protein-coupled receptor dimer imaging assay reveals selectively modified pharmacology of neuropeptide Y Y1/Y5 receptor heterodimers. *Mol Pharmacol.* (2015) 87:718–32. doi: 10.1124/mol.114.095356
43. Hubner H, Schellhorn T, Gienger M, Schaab C, Kaindl J, Leeb L, et al. Structure-guided development of heterodimer-selective GPCR ligands. *Nat Commun.* (2016) 7:12298. doi: 10.1038/ncomms12298
44. Kono T, Kaneko A, Matsumoto C, Miyagi C, Ohbuchi K, Mizuhara Y, et al. Multitargeted effects of hangeshashinto for treatment of chemotherapy-induced oral mucositis on inducible prostaglandin E2 production in human oral keratinocytes. *Integr Cancer Ther.* (2014) 13:435–45. doi: 10.1177/1534735413520035
45. Fukamachi H, Matsumoto C, Omiya Y, Arimoto T, Morisaki H, Kataoka H, et al. Effects of Hangeshashinto on growth of oral microorganisms. *Evid Based Complement Alternat Med.* (2015) 2015:512947. doi: 10.1155/2015/512947
46. Matsumoto C, Sekine-Suzuki E, Nyui M, Ueno M, Nakanishi I, Omiya Y, et al. Analysis of the antioxidative function of the radioprotective Japanese traditional (Kampo) medicine, hangeshashinto, in an aqueous phase. *J Radiat Res.* (2015) 56:669–77. doi: 10.1093/jrr/rrv023
47. Hiroshima Y, Bando M, Inagaki Y, Kido R, Kataoka M, Nagata T, et al. Effect of Hangeshashinto on calprotectin expression in human oral epithelial cells. *Odontology.* (2016) 104:152–62. doi: 10.1007/s10266-015-0196-3
48. Hitomi S, Ono K, Yamaguchi K, Terawaki K, Imai R, Kubota K, et al. The traditional Japanese medicine hangeshashinto alleviates oral ulcer-induced pain in a rat model. *Arch Oral Biol.* (2016) 66:30–7. doi: 10.1016/j.archoralbio.2016.02.002
49. Hitomi S, Ono K, Terawaki K, Matsumoto C, Mizuno K, Yamaguchi K, et al. [6]-gingerol and [6]-shogaol, active ingredients of the traditional Japanese medicine hangeshashinto, relief oral ulcerative mucositis-induced pain via action on Na(+) channels. *Pharmacol Res.* (2017) 117:288–302. doi: 10.1016/j.phrs.2016.12.026
50. Miyashita T, Kono T, Matsui D, Yamazaki Y, Sadatomi D, Fujitsuka N, et al. Preventive effect of oral hangeshashinto (TJ-14) on the development of reflux-induced esophageal cancer. *Surgery.* (2018) 164:49–55. doi: 10.1016/j.surg.2018.02.003
51. Hitomi S, Ujihara I, Ono K. Pain mechanism of oral ulcerative mucositis and the therapeutic traditional herbal medicine hangeshashinto. *J Oral Biosci.* (2019) 61:12–5. doi: 10.1016/j.job.2019.01.004
52. Tatsumi L, Suzuki T, Yamada K, Mimura M, Uchida H. Kampo, A Japanese traditional medicinal system for psychiatric conditions: a narrative review. *Pharmacopsychiatry.* (2019) 52:251–60. doi: 10.1055/a-0637-9760
53. Zhang C, Lu Y, Tao L, Tao X, Su X, Wei D. Tyrosinase inhibitory effects and inhibition mechanisms of nobiletin and hesperidin from citrus peel crude extracts. *J Enzyme Inhib Med Chem.* (2007) 22:83–90. doi: 10.1080/14756360600953876
54. Matsumoto T, Nishikawa T, Furukawa A, Itano S, Tamura Y, Hasei T, et al. Antimutagenic effects of polymethoxy flavonoids of *Citrus unshiu. Nat Prod Commun.* (2017) 12:23–6. doi: 10.1177/1934578X1701200108
55. Kim JJ, Kim K, Jung YR, Bian Y, Ngo T, Bae ON, et al. Co-existence of hypertensive and anti-hypertensive constituents, synephrine, and nobiletin in *Citrus unshiu* Peel. *Molecules.* (2019) 24:1197. doi: 10.3390/molecules24071197
56. Kanaze FI, Bounartzi MI, Georgarakis M, Niopas I. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *Eur J Clin Nutr.* (2007) 61:472–7. doi: 10.1038/sj.ejcn.1602543
57. Kitagawa H, Munekage M, Matsumoto T, Sadakane C, Fukutake M, Aoki K, et al. Pharmacokinetic profiles of active ingredients and its

metabolites derived from rikkunshito, a ghrelin enhancer, in healthy Japanese volunteers: a cross-over, randomized study. *PLoS ONE.* (2015) 10:e0133159. doi: 10.1371/journal.pone.0133159
58. Singh SP, Wahajuddin Tewari D, Patel K, Jain GK. Permeability determination and pharmacokinetic study of nobiletin in rat plasma and brain by validated high-performance liquid chromatography method. *Fitoterapia.* (2011) 82:1206–14. doi: 10.1016/j.fitote.2011.08.010
59. Okuyama S, Miyazaki K, Yamada R, Amakura Y, Yoshimura M, Sawamoto A, et al. Permeation of Polymethoxyflavones into the mouse brain and their effect on MK-801-induced locomotive hyperactivity. *Int J Mol Sci.* (2017) 18:489. doi: 10.3390/ijms18030489
60. Kim A, Im M, Gu MJ, Ma JY. *Citrus unshiu* peel extract alleviates cancer-induced weight loss in mice bearing CT-26 adenocarcinoma. *Sci Rep.* (2016) 6:24214. doi: 10.1038/srep24214
61. Inoue T, Takagi H, Owada Y, Watanabe Y, Yamaura T, Fukuhara M, et al. The efficacy of the Kampo medicine rikkunshito for chemotherapy-induced anorexia (RICH trial): study protocol for a randomized controlled trial. *Trials.* (2017) 18:485. doi: 10.1186/s13063-017-2227-6
62. Fujitsuka N, Uezono Y. Rikkunshito, a ghrelin potentiator, ameliorates anorexia-cachexia syndrome. *Front Pharmacol.* (2014) 5:271. doi: 10.3389/fphar.2014.00271

Conflict of Interest: YU received grant support from Kracie Pharma, Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

CASE REPORT
published: 30 April 2020
doi: 10.3389/fnut.2020.00057

Postoperative Adjuvant Chemotherapy Regimen of CAPOX Combined With Ninjin'yoeito in an Elderly Patient With Stage III Colon Cancer: A Case Report

Naoki Aomatsu[1,2], Yasutake Uchima[2], Gen Tsujio[2], Yoshinari Miyamoto[2], Takuma Okada[2], Shigeaki Kurihara[2], Shinji Matsutani[2], Toshiki Hirakawa[2], Takehiko Iwauchi[2], Junya Morimoto[2], Shigehito Yamagata[2], Kazunori Nakazawa[2], Takafumi Nishii[1], Akiko Tachimori[1], Kiyoshi Maeda[1], Katsumi Ikeda[3] and Kazuhiro Takeuchi[2]*

[1] Department of Gastroenterological Surgery, Osaka City General Hospital, Osaka, Japan, [2] Department of Surgery Center, Fuchu Hospital, Osaka, Japan, [3] Department of Breast Surgical Oncology, Osaka City General Hospital, Osaka, Japan

OPEN ACCESS

Edited by:
Akio Inui,
Kagoshima University, Japan

Reviewed by:
Masatoshi Kanno,
Nara Medical University, Japan
Kanako Miyano,
National Cancer Center Research Institute, Japan

***Correspondence:**
Naoki Aomatsu
hong_shim@yahoo.co.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: 25 January 2020
Accepted: 08 April 2020
Published: 30 April 2020

Citation:
Aomatsu N, Uchima Y, Tsujio G, Miyamoto Y, Okada T, Kurihara S, Matsutani S, Hirakawa T, Iwauchi T, Morimoto J, Yamagata S, Nakazawa K, Nishii T, Tachimori A, Maeda K, Ikeda K and Takeuchi K (2020) Postoperative Adjuvant Chemotherapy Regimen of CAPOX Combined With Ninjin'yoeito in an Elderly Patient With Stage III Colon Cancer: A Case Report. Front. Nutr. 7:57. doi: 10.3389/fnut.2020.00057

We report the successful management of stage III colon cancer in an elderly patient who received an adjuvant chemotherapy regimen of capecitabine plus oxaliplatin (CAPOX) with the Japanese kampo medicine ninjin'yoeito (NYT). A 75-year-old woman with a medical history of hypertension presented at another institution with fecal occult blood, and a colonoscopy that showed a type II tumor in the sigmoid colon. She was referred to our hospital for tumor resection, where colonoscopy confirmed the location of the type II tumor in the sigmoid colon. Histopathology of the biopsy specimen indicated a moderately differentiated tubular adenocarcinoma. Enhanced computed tomography of the thorax and abdomen indicated thickening of the sigmoid colon wall. Regional lymph node metastasis was suspected, but distant metastasis was not indicated. A blood examination revealed an elevated carcinoembryonic antigen (CEA) concentration (32.7 ng/ml). Following a diagnosis of cancer of the sigmoid colon, clinical stage IIIb [cT4a, N1b, M0], a laparoscopic sigmoid colectomy was performed without complications. The postoperative histopathological examination revealed a moderately differentiated to mucinous adenocarcinoma. Three of 16 retrieved lymph nodes contained malignant cells. The final tumor classification was Stage IIIb [pT4a, pN1b, M0]. The patient recovered uneventfully, and was discharged 10 days after surgery with a recommendation for adjuvant chemotherapy with CAPOX starting 4 weeks after surgery. The patient also received 7.5 g of NYT daily throughout the adjuvant chemotherapy course. She did not report any loss of appetite, general fatigue, peripheral neuropathy, neutropenia, or febrile neutropenia. During a 1-year postoperative follow-up, she has not experienced any recurrence. We conclude that NYT might be useful for reducing the adverse effects of anticancer therapy, particularly in elderly patients.

Keywords: CAPOX, ninjin'yoeito, colon cancer, adjuvant chemotherapy, kampo medicine

INTRODUCTION

Combinations of oxaliplatin (L-OHP) with fluoropyrimidine drugs have been used as standard adjuvant chemotherapy regimens for the treatment of stage III colon cancer since 2004. Although elderly patients reap the same benefits from these regimens as younger patients, the main adverse effect of L-OHP is peripheral neuropathy. In previous reports, ninjin'yoeito (NYT) was reported to be useful for reducing adverse effects such as anticancer anemia, peripheral neuropathy, and cancer cachexia (1–4). We report a case of postoperative adjuvant chemotherapy comprising capecitabine plus L-OHP (CAPOX) combined with NYT for the treatment of stage III colon cancer in an elderly patient.

CASE PRESENTATION

A 75-year-old woman with a medical history of hypertension presented at another institution with fecal occult blood, and a colonoscopy showed a type II tumor in the sigmoid colon. She was referred to our hospital for tumor resection, where colonoscopy determined that the tumor was located 23 cm from the anal verge. Histopathology of a biopsy specimen revealed a moderately differentiated tubular adenocarcinoma. Enhanced computed tomography of the thorax and abdomen showed sigmoid colon wall thickening. Regional lymph node metastasis was suspected, but no evidence of distant metastasis was observed. A blood examination revealed an elevated carcinoembryonic antigen (CEA) concentration (32.7 ng/ml). Following a diagnosis of cancer of the sigmoid colon, clinical stage IIIb [cT4a, N1b, M0], a laparoscopic sigmoid colectomy was performed without complications. The postoperative histopathological examination revealed a moderately differentiated to mucinous adenocarcinoma. Three of the 16 retrieved lymph nodes contained malignant cells. Finally, the cancer was classified as stage IIIb [pT4a, pN1b, M0]. The patient recovered uneventfully and was discharged 10 days after the surgery. Following the diagnosis of stage III colorectal cancer, the patient was recommended to receive adjuvant chemotherapy with CAPOX starting 4 weeks after surgery. The selected regimen consisted of capecitabine (1,000 mg/m^2 orally twice daily) for 14 days and L-OHP (130 mg/m^2 intravenous infusion) on the first day of each cycle, with a periodicity of 3 weeks over 3 months (four cycles). The anticancer drug dosage was reduced to 80% because of the patient's age. The patient had postoperative physical weakness and appetite loss, and also received 7.5 g of NYT daily throughout the course of adjuvant chemotherapy. She did not report any events of peripheral loss of appetite, general fatigue, peripheral neuropathy, neutropenia, or febrile neutropenia. Adverse effects were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. She has not experienced any recurrence during a 1-year postoperative follow-up.

DISCUSSION

Herein, we report the successful management of stage III colon cancer in an elderly patient via adjuvant CAPOX chemotherapy combined with NYT. Adjuvant chemotherapy improves overall survival in patients with resected stage III colorectal cancer (5). The MOSAIC Multicenter International Study (6) revealed that a regimen of FOLFOX [fluorouracil (FU) bolus and continuous infusion combined with leucovorin (LV) and L-OHP] significantly improved 3-year disease-free survival outcomes when compared with fluorouracil and leucovorin alone. Additionally, CAPOX improved overall survival outcomes in patients with resected stage III colon cancer when compared with bolus FU after a median follow-up of almost 7 years (7). CAPOX allows for central venous (CV)-port-free administration. Therefore, this regimen might be considered a standard adjuvant treatment option for patients with stage III colon cancer.

Elderly patients receive the same survival benefit from these regimens as younger patients. In both older (≥70 years) and younger patients, adjuvant 5-FU therapy has been significantly associated with reduced mortality in randomized controlled trials (8). Haller reported that in a pooled analysis of individual patient data from four randomized controlled trials of CAPOX/FOLFOX vs. LV/5-FU [NSABP C-08 (9), XELOXA (7), X-ACT (10), and AVANT (11)], disease-free survival benefits were observed regardless of age or medical comorbidity. However, the benefits were modestly attenuated in patients aged ≥70 years, with a hazard ratio of 0.77 ($P < 0.014$) vs. 0.68 ($P < 0.0001$) among patients aged <70 years (12).

Peripheral neuropathy is the main adverse effect of L-OHP therapy. In the MOSAIC study, grade 2 and 3 peripheral sensory neuropathy was observed during treatment in 31.4 and 12.5% patients in the FOLFOX group (13). During treatment with L-OHP chemotherapy, patients have experienced acute and chronic mechanical hyperalgesia and cold allodynia. The dose and duration of therapy are limited once a patient develops peripheral neuropathy, leading to a reduced quality of life. The cumulative administered dose of L-OHP increases the risk of associated sensory neurotoxicity (13, 14). In the MOSAIC trial (13), the frequency of grade 3 peripheral sensory neuropathy among patients receiving L-OHP persisted over time (1.3% at 12 months and 0.7% at 48 months after treatment). This toxic adverse effect may be severe and can persist long after treatment is completed, leading to potentially life-long effects on the patients' activities of daily living (15).

To date, various integrative approaches, including Japanese kampo medicine, have been used in an attempt to prevent the adverse effects of chemotherapy. Kampo medicines are currently used to treat several types of diseases, and are also used to improve the quality of life of patients throughout the world and especially in Asian countries (3). Of the traditional medicine components prescribed to prevent L-OHP-induced peripheral neuropathy, goshajinkigan has been the most popular with animal experiments (16–18). However, goshajinkigan could not prevent L-OHP-induced peripheral neurotoxicity in colorectal

cancer patients treated with FOLFOX regimens in a randomized phase III clinical trial (19).

NYT is a Japanese kampo medicine composed of 12 herbal plants that is used to facilitate disease recovery and improve several symptoms, such as anemia, anorexia, and fatigue. In previous reports, NYT appeared to be useful for reducing the adverse effects of anticancer anemia, peripheral neuropathy and cancer cachexia (1–4). NYT should be administered at the same time as adjuvant chemotherapy. At that time, the indicating disease, such as postoperative physical weakness, general fatigue, appetite loss, and anemia, must be present. Some references indicate that more human data, both younger and elderly patients, is needed to validate the efficacy of NYT in managing adverse effects of a systemic chemotherapy regimen (20, 21). In mice, NYT improved 5-FU-induced anemia and increased the populations of burst-forming unit-erythroid cells and colony-forming unit-erythroid cells in bone marrow (22).

In an *in vitro* study, Suzuki reported that an extract of NYT prevented L-OHP-induced neurodegeneration in PC12 cells (1). Particularly, ginseng extract appeared to exert the strongest protective effect against neurodegeneration among the 12 herbal components of NYT. In a mouse model experiment, NYT and ginseng reduced L-OHP-induced neurite damage and neuropathic pain. In an *in vitro* study, L-OHP treatment suppressed neurite outgrowths from primary dorsal root ganglion cells. NYT extract blocked this suppression in a concentration-dependent manner. Ginseng showed a protective effect against neurite damage induced by L-OHP, and one of its active ingredients was identified as ginsenoside Rg3 (4). In addition to NYT, other kampo medicines, such as ginseng and hochuekkito also reduce the adverse effects of anticancer therapy. Hochuekkito is not expected to improve peripheral neuropathy, but has been noted to improve gastrointestinal conditions and increase physical strength, and is expected to improve immune function (23). Consistent with those earlier observations, the administration of NYT reduced the adverse effects associated with adjuvant CAPOX chemotherapy, such as peripheral neuropathy, neutropenia, and febrile neutropenia in our elderly patient with stage III colorectal cancer.

In conclusion, our observations suggest that NYT might be useful for reducing the adverse effects of anticancer therapy in elderly patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the Fuchu Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NA made a substantial contribution to the study conception, conducted a literature search, and drafted the manuscript. NA and GT contributed to the acquisition of data. NA, SK, TO, and YU performed the surgery. NA, YU, GT, YM, TO, SK, SM, TH, TI, JM, SY, KN, TN, AT, KM, KI, and KT reviewed the manuscript and gave final approval for publication. All authors read and approved the final manuscript.

REFERENCES

1. Suzuki T, Yamamoto A, Ohsawa M, Motoo Y, Mizukami H, Makino T. Ninjin'yoeito and ginseng extract prevent oxaliplatin-induced neurodegeneration in PC12 cells. *J Nat Med.* (2015) 69:531–7. doi: 10.1007/s11418-015-0921-9
2. Ohsawa M, Maruoka J, Inami C, Iwaki A, Murakami T, Ishikura, K. Effect of ninjin'yoeito on the loss of skeletal muscle function in cancer-bearing mice. *Front Pharmacol.* (2018) 9:1400. doi: 10.3389/fphar.2018.01400
3. Miyano K, Nonaka M, Uzu M, Ohshima K, Uezono Y. Multifunctional actions of Ninjinyoeito, a Japanese kampo medicine: accumulated scientific evidence based on experiments with cells and animal models, and clinical studies. *Front Nutr.* (2018) 5:93. doi: 10.3389/fnut.2018.00093
4. Suzuki T, Yamamoto A, Ohsawa M, Motoo Y, Mizukami H, Makino T. Effect of ninjin'yoeito and ginseng extracts on oxaliplatin-induced neuropathies in mice. *J Nat Med.* (2017) 71:757–64. doi: 10.1007/s11418-017-1113-6
5. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *N Engl J Med.* (1990) 322:352–8. doi: 10.1056/NEJM199002083220602
6. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med.* (2004) 350:2343–51. doi: 10.1056/NEJMoa032709
7. Schmoll HJ, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, et al. Capecitabine plus oxaliplatin compared with fluorouracil/folinic acid as adjuvant therapy for stage iii colon cancer: final results of the NO16968 Randomized Controlled Phase III Trial. *J Clin Oncol.* (2015) 33:3733–40. doi: 10.1200/JCO.2015.60.9107
8. Sundararajan V, Mitra N, Jacobson JS, Grann VR, Heitjan DF, Neugut AI. Survival associated with 5-fluorouracil-based adjuvant chemotherapy among elderly patients with node-positive colon cancer. *Ann Intern Med.* (2002) 136:349–57. doi: 10.7326/0003-4819-136-5-200203050-00007
9. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Petrelli NJ, Colangelo LH, et al. Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: results of NSABP protocol C-08. *J Clin Oncol.* (2011) 29:11–6. doi: 10.1200/JCO.2010.30.0855
10. Twelves C, Wong A, Nowacki MP, Abt M, Burris H 3rd, Carrato A, et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med.* (2005) 352:2696–704. doi: 10.1056/NEJMoa043116
11. de Gramont A, Van Cutsem E, Schmoll HJ, Tabernero J, Clarke S, Moore, MJ, et al. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial. *Lancet Oncol.* (2012) 13:1225–33. doi: 10.1016/S1470-2045(12)70509-0
12. HallerDG, O'Connell MJ, Cartwright TH, Twelves CJ, McKenna EF, Sun W, et al. (2015). Impact of age and medical comorbidity on adjuvant treatment outcomes for stage III colon cancer: a pooled analysis of individual patient data from four randomized, controlled trials. *Ann Oncol.* (2015) 26:715–24. doi: 10.1093/annonc/mdv003

13. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, Taieb J, et al. Duration of adjuvant chemotherapy for stage iii colon cancer. *N Engl J Med.* (2018) 378:1177–88. doi: 10.1056/NEJMoa1713709
14. Andre T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol.* (2009) 27:3109–16. doi: 10.1200/JCO.2008.20.6771
15. Grothey, A. Oxaliplatin-safety profile: neurotoxicity. *Semin Oncol.* (2003) 30:5–13. doi: 10.1016/S0093-7754(03)00399-3
16. Ushio S, Egashira N, Sada H, Kawashiri T, Shirahama M, Masuguchi, K, et al. Goshajinkigan reduces oxaliplatin-induced peripheral neuropathy without affecting anti-tumour efficacy in rodents. *Eur J. Cancer.* (2012) 48:1407–13. doi: 10.1016/j.ejca.2011.08.009
17. Kitamura R, Andoh T, Fushimi H, Komatsu K, Shibahara N, Kuraishi Y. Involvement of descending monoaminergic systems in antiallodynic effect of goshajinkigan in oxaliplatin-treated mice. *J Trad Med.* (2013) 30:183–9.
18. Mizuno K, Kono T, Suzuki Y, Miyagi C, Omiya Y, Miyano K, et al. Goshajinkigan, a traditional Japanese medicine, prevents oxaliplatin-induced acute peripheral neuropathy by suppressing functional alteration of TRP channels in rat. *J Pharmacol Sci.* (2014) 13244FP. doi: 10.1254/jphs.13244FP
19. Oki, E, Emi Y, Kojima H, Higashijima J, Kato T, Miyake Y, et al. Preventive effect of Goshajinkigan on peripheral neurotoxicity of FOLFOX therapy (GENIUS trial): a placebo-controlled, double-blind, randomized phase III study. *Int J Clin Oncol.* (2015) 20:767–75. doi: 10.1007/s10147-015-0784-9
20. Sugimachi K. Igan Jutsugo hojokagakuryouhou ni okeru Ninjin'yoeito no yuuyouseini kannsuru kennkyuu (Study of efficacy of Ninjin Yoei-to for adjuvant chemotherapy after gastric cancer surgery). *Jpn J Clin Exp Med.* (1995) 72:454–8.
21. Abe K. Jutsugo hojokagakuryouhou ni okeru Ninjin Yoei-to no yuuyousei ni kannsurukennkyuu (Experience of use of Ninjin Yoei-to in postoperative maintenance chemotherapy). *Progress Med.* (1990) 10:2855–63.
22. Takano F, Ohta Y, Tanaka T, Sasaki K, Kobayashi K, Takahashi T, et al. Oral administration of Ren-shen-yang-rong-tang 'ninjin'yoeito'protects against hematotoxicity and induces immature erythroid progenitor cells in 5-fluorouracil-induced anemia. *Evid Based Complement Alternat Med.* (2009) 6:247–56. doi: 10.1093/ecam/nem080
23. Kono T, Takeda H, Uezono Y. Kampo medicine for the treatment of adverse effects caused by anticancer drugs. *Nihon Geka Gakkai Zasshi.* (2013) 114:251–5.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

CASE REPORT
published: 26 June 2020
doi: 10.3389/fnut.2020.00085

Good Rehabilitation Outcomes and Improved Nutritional Status After Treatment With the Japanese Herbal Medicine Ninjin'yoeito in an Elderly Patient With Hip Fracture and Sarcopenia: A Case Report

Akinori Morinaga[1], Hiroki Nakamura[2], Kenji Hattanmaru[2], Natasya Trivena Rokot[3], Yoko Kimura[1] and Takashi Ito[1]*

[1] Institute of Oriental Medicine, Tokyo Women's Medical University, Tokyo, Japan, [2] Department of Rehabilitation, Hattanmaru Rehabilitation Hospital, Kagoshima, Japan, [3] Pharmacological Department of Herbal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

OPEN ACCESS

Edited by:
Masahiro Ohsawa, Nagoya City University, Japan

Reviewed by:
Ulkan Kilic, University of Health Sciences, Turkey
Meghit Boumediene Khaled, University of Sidi-Bel-Abbès, Algeria

***Correspondence:**
Akinori Morinaga
morinaga.akinori@twmu.ac.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *29 February 2020*
Accepted: *11 May 2020*
Published: *26 June 2020*

Citation:
Morinaga A, Nakamura H, Hattanmaru K, Rokot NT, Kimura Y and Ito T (2020) Good Rehabilitation Outcomes and Improved Nutritional Status After Treatment With the Japanese Herbal Medicine Ninjin'yoeito in an Elderly Patient With Hip Fracture and Sarcopenia: A Case Report. Front. Nutr. 7:85. doi: 10.3389/fnut.2020.00085

We report a case involving a 92-year-old man who successfully received treatment with ninjin'yoeito, a Japanese herbal medicine, during the rehabilitation phase after hip fracture surgery. The patient was diagnosed with a left femoral neck fracture and underwent surgery. Two weeks after surgery, he was admitted to a rehabilitation hospital. At that time, his height, weight, body fat percentage, muscle mass, and Functional Independence Measure (FIM) score were 167 cm, 61 kg, 34.1%, 38.2 kg, and 49, respectively. For 1 month after surgery (i.e., 2 weeks after admission to the rehabilitation facility), he received rikkunshito, a traditional Japanese herbal medicine also known as Kampo medicine, for appetite loss and underwent rehabilitation. However, his appetite loss showed no improvement, and rikkunshito (7.5 g/d) was replaced with ninjin'yoeito (7.5 g/d). Two months later, although the patient's body weight and body fat percentage decreased to 56.5 kg and 21.1%, respectively, his muscle mass increased to 38.9 kg. Nutritional status evaluation indicated an improvement in the level of proteins such as transferrin, prealbumin, and retinol-binding protein, which reflected an increase in food intake. The FIM score improved from 49 to 105. No side effects were observed. The findings from this case suggest that ninjin'yoeito, which includes *Astragalus* root and *Schisandra* fruit, may be an effective treatment option for sarcopenia or frailty with appetite loss and impaired activities of daily living in aged patients.

Keywords: ninjin'yoeito, sarcopenia, frailty, rehabilitation, body composition, hip fracture, malnutrition

INTRODUCTION

Japan is becoming a super-aged society, and this trend has been accompanied by a substantial increase in the incidence of age-related disease in recent decades. Frailty due to sarcopenia associated with aging and underlying diseases has received particular attention because it impairs the quality of life (QOL) of the elderly and shortens the healthy life span. Fracture resulting from

sarcopenia or frailty is one of the main reasons why elderly individuals become bedridden, which markedly impairs their QOL. Thus, rehabilitation after treatment of the fracture is important. However, many elderly individuals with fractures exhibit a state of malnutrition, which affects the outcome of rehabilitation (1–3). Although it has been shown that nutritional management is an important part of rehabilitation, some patients exhibit appetite loss, and decreased food intake and show no progress during rehabilitation.

Mosapride and acotiamide, which are indicated for the treatment of functional dyspepsia (4), are generally used to treat appetite loss. Japanese traditional herbal medicines (Kampo medicine) are also used as appetite stimulants when pharmacological treatment is ineffective in Japan. Kampo medicine, which is derived from Chinese medicine and developed independently (5), has been integrated into modern medicine owing to its high quality and safety (6). The choice of treatment is guided according to the patient's constitution and symptoms (7). Ninjin'yoeito is used for treating serious illnesses and has been approved for use in the treatment of systemic malaise, appetite loss, and anemia by the Japanese Ministry of Health, Labor and Welfare. Ninjin'yoeito is a multicomponent formulation comprising 12 crude drugs: ginseng 3 g, *Astragalus* root 1.5 g, *Atractylodes* rhizome 4 g, *Poria* sclerotium 4 g, Japanese angelica root 4 g, *Rehmannia* root 4 g, cinnamon bark 2.5 g, peony root 2 g, *Citrus unshiu* peel 2 g, polygala root 2 g, *Schisandra* fruit 1 g, and *Glycyrrhiza* 1 g. However, the effects of ninjin'yoeito on nutritional status and rehabilitation outcomes have not been reported to date.

Herein, we report a case involving an elderly man with appetite loss and decreased motivation levels during the rehabilitation phase after surgery for a femoral neck fracture. Although his rehabilitation did not progress well initially, the outcomes improved with the introduction of ninjin'yoeito as part of the treatment. Specifically, he exhibited an improvement in nutritional status and activities of daily living (ADLs), with a notable increase in lean body mass.

CASE PRESENTATION

A 92-year-old man who experienced a fall while walking presented at an acute care hospital with left hip pain. The patient's medical history included hypertension, dyslipidemia, and a previous myocardial infarction. The patient's current medications included amlodipine besylate, pravastatin sodium, ethyl icosapentate, aspirin, and nicorandil. Prior to his falling, he was independent with his ADLs and had a good appetite. Examination revealed a left femoral neck fracture, and the patient underwent surgery as a result. Although rehabilitation was initiated immediately after surgery, the patient's motivation level was low, because of which his rehabilitation did not progress well. Moreover, he did not eat sufficiently because of appetite loss. Accordingly, treatment was initiated with rikkunshito (7.5 g/d; Tsumura & Co., Tokyo, Japan), a Japanese herbal medicine used for appetite loss and functional dyspepsia. Two weeks after surgery, the patient was transferred to our rehabilitation hospital. At that time, his height, weight, body fat percentage, muscle mass, and Functional Independence Measure (FIM) score were 167 cm, 61 kg, 34.1%, 38.2 kg, and 49, respectively. His general condition and nutritional status from admission to discharge are described in **Table 1**, whereas findings of body composition assessments and FIM scores during that period are described in **Tables 2**, **3**, respectively. InBody S10 (InBody, Tokyo, Japan) was used to measure the body composition. The patient's hand grip (HG) strength and skeletal muscle mass index (SMI) were 19.6 kg and 6.42 kg/m^2, respectively. He was diagnosed with sarcopenia according to the Asian Working Group for Sarcopenia criteria as follows: HG strength of <26 kg and SMI of <7.0 kg/m^2 (8). He also exhibited weakness, poor endurance, and low activity, thus meeting the criteria for frailty (9).

Prior to the fracture, the patient was ambulatory; however, when he was transferred to our rehabilitation hospital, he initially used a wheelchair. In addition, his total FIM score was 49 (**Table 3**); he showed independence only while eating meals and required assistance with all other activities such as dressing, defecating, and moving around. Furthermore, he felt like eating only occasionally and ingested a variable amount of food, with an average daily calorie intake of 992 kcal. Although conventional rehabilitation (joint range-of-motion training and ADL training) was ongoing, he typically remained in bed. Considering the constant appetite loss and decreased motivation levels, rikkunshito treatment was replaced with ninjin'yoeito treatment (7.5 g/d; Kracie Pharma, Ltd., Tokyo, Japan) twice a day before meals according to the package insert from day 15. There was no restriction on other drugs during the ninjin'yoeito treatment. Subsequently, his food intake gradually stabilized, and the average daily calorie intake during the period of ninjin'yoeito treatment increased to 1,159 kcal. In particular, the average daily calorie intake increased to 1,234 kcal during the last month of hospitalization. Moreover, there was an increase in the serum levels of transferrin, prealbumin, and retinol-binding protein, which are typical indices of nutritional status. The duration of ninjin'yoeito treatment was 2 months. Although the patient's HG strength and SMI still indicated sarcopenia, he became almost fully independent with his lifestyle activities and exhibited a total FIM score of 105, with markedly improved motor subscores, at the time of discharge 2.5 months after admission. Despite weight loss and decreased body fat percentage relative to the time of admission, there was an increase in lean body mass. During the period of hospitalization, the patient had good adherence and reported no adverse events related to the ninjin'yoeito treatment.

The treatment protocol for this case was approved by the ethics committee of Hattanmaru Rehabilitation Hospital, and

Abbreviations: FIM, Functional Independence Measure; QOL, quality of life; ADLs, activities of daily living; HG, hand grip; SMI, skeletal muscle mass index; NPY, neuropeptide Y.

TABLE 1 | Findings and laboratory data from admission to discharge for an elderly patient who received ninjin'yoeito, a Japanese herbal medicine, during the rehabilitation phase after hip fracture surgery.

		At admission		At discharge
Height, cm		167		167
BW, kg		61.0		56.5
BMI, kg/m2		21.9		20.3
HS, kg (right)		19.6		22.6
HS, kg (left)		23.3		20.7
Nutritional status	**At admission**	**Ninjin'yoeito treatment initiation**	**1 month after treatment initiation**	**2 months after treatment initiation**
TP, g/dL	6.4	6.8	6.7	7.1
ALB, g/dL	3.0	3.5	3.3	3.5
Tf, mg/dL		227	213	238
PreALB, mg/dL		24.7	21.9	27.9
RBP, mg/dL		2.9	2.6	3.6

BW, body weight; BMI, body mass index; HS, handgrip strength; TP, total protein; ALB, albumin; Tf, transferrin; PreALB, prealbumin; RBP, retinol-binding protein.

TABLE 2 | Body composition of an elderly patient who received ninjin'yoeito, a Japanese herbal medicine, during the rehabilitation phase after hip fracture surgery.

	At admission	1 month after admission	2 months after admission	At discharge after 2.5 months
BW, kg	61.0	59.1	57.5	56.5
Lean mass, kg	38.2	37.5	42.0	42.2
Fat mass, kg	20.8	19.6	13.1	11.9
PBF, %	34.1	33.2	22.7	21.1
LEAN MASS				
Right arm, kg	2.22	2.04	2.38	2.45
Left arm, kg	2.14	2.02	2.33	2.46
Trunk, kg	19.3	18.5	20.1	20.6
Right leg, kg	6.64	6.82	6.71	6.62
Left leg, kg	6.90	6.93	6.98	6.78
SMI, kg/m^2	6.42	6.39	6.60	6.57
BME, kcal	1,239	1,223	1,330	1,333

PBF, percent body fat; SMI, skeletal muscle mass index; BME, basal metabolic expenditure.

the patient provided written informed consent for publication of this report.

DISCUSSION

In this report, we describe the successful rehabilitation after surgery for a femoral neck fracture in an elderly patient who was associated with improvement of average daily calorie intake from 992 to 1,159 kcal, muscle mass from 38.2 to 38.9 kg, and FIM score from 49 to 105. Because of appetite loss and decreased motivation levels, the patient's rehabilitation did not progress well. Even after 2-week administration of rikkunshito, used for the management of appetite loss and functional dyspepsia in Japan (10), there was no improvement in the amount of food ingested by the patient. Therefore, his treatment was switched to ninjin'yoeito, which is used to manage appetite loss, sarcopenia, mild depression, and fatigue during cancer treatment (11). The initiation of ninjin'yoeito treatment increased the patient's daily calorie intake, thus resulting in good progress of rehabilitation and an improvement in ADLs. In addition, there was an increase in the serum levels of transferrin, prealbumin, and retinol-binding protein, which are typical indices of nutritional status. Ohsawa et al. (12) reported that ninjin'yoeito maintained the skeletal muscle mass through the improvement of amino acid metabolism and might improve the capacity of amino acid storage in the skeletal muscle in tumor-bearing mice. In the present case, muscle mass increased from 38.2 to 38.9 kg with ninjin'yoeito treatment. However, with no cancer-induced sarcopenia, ninjin'yoeito might improve the amino acid metabolism and storage in the skeletal muscle. Whereas the body fat percentage decreased (it exceeded 30% at the time of admission), the trunk and appendicular skeletal muscle mass increased. Malafarina and colleagues (13) evaluated elderly patients with hip fracture and found that intake of oral nutritional supplements in addition to a standard diet helped in maintaining body weight, fat mass, and muscle mass, all of which exhibited a decrease

TABLE 3 | Functional Independence Measure scores at admission and discharge for an elderly patient who received ninjin'yoeito, a Japanese herbal medicine, during the rehabilitation phase after hip fracture surgery.

FIM item	At admission	At discharge	Maximum	Improvement
Self-care	18	38	42	20
Sphincter control	2	14	14	12
Transfer	9	18	21	9
Locomotion	2	10	14	8
FIM-M total	31	80	91	49
Communication	9	13	14	4
Social cognition	9	12	21	3
FIM-C total	18	25	35	7
Total	49	105	126	56

FIM, functional independence measure; FIM-M, FIM motor subscores; FIM-C, FIM cognitive subscores.

in the control group. The increase in skeletal muscle mass and decrease in body weight and fat mass in the present patient can be attributed to improved ADLs and increased calorie consumption.

To date, clinical studies on the use of ninjin'yoeito have demonstrated efficacy in patients with lung cancer (11), pulmonary non-tuberculous mycobacteriosis (14), pharyngeal vascular malformation (15), anemia associated with chronic hepatitis C (16), chronic inflammation and poor QOL due to hemodialysis (17), depression associated with Alzheimer-type dementia (when used in combination with donepezil) (18), and frailty (19–22). Rikkunshito is a formulation comprising eight crude drugs, five of which are also included in ninjin'yoeito: ginseng, *Atractylodes* rhizome, *Poria* sclerotium, *C. unshiu* peel, and *Glycyrrhiza*. Rikkunshito has been reported to enhance ghrelin signaling and consequently improve appetite loss (23). Hesperidin, a component of *C. unshiu* peel, has been reported to inhibit the binding of serotonin to serotonin receptors, thereby increasing the secretion of ghrelin, an orexigenic hormone, from the stomach (23). Goswami et al. (24) reported that ninjin'yoeito activates the orexigenic peptide neuropeptide Y (NPY) in the arcuate nucleus of the hypothalamus, independent of ghrelin, and has a therapeutic potential to improve appetite in the elderly patients with ghrelin resistance. This suggests a difference in efficacy between rikkunshito and ninjin'yoeito with regard to appetite stimulation. *Astragalus* root, which is not included in rikkunshito but is a constituent of ninjin'yoeito, has been reported to improve cancer-associated anorexia in patients with advanced cancer (25). In addition, ogikenchuto, a Kampo formula that includes *Astragalus* root, has been reported to improve ADLs in bedridden elderly patients in a Japanese study (26). Conversely, there are no similar published reports regarding rikkunshito. Kawamata et al. (26) reported that ogikenchuto improved motivation levels for rehabilitation and ADLs in two elderly bedridden patients, one of which received rikkunshito before ogikenchuto and showed a poor response. In the present case, the beneficial effects of ninjin'yoeito on appetite loss and decreased ADLs could be attributed to *Astragalus* root, which is not present in rikkunshito. *Schisandra* fruit, another ingredient of ninjin'yoeito, has also been reported to enhance exercise-induced adaptive muscle strengthening in aged mice (27) and improve endurance and metabolism in the skeletal muscle of exercised rats (28). Thus, it may be responsible for the improvement in ADLs and increase in muscle mass in our patient.

CONCLUSION

We described herein the successful use of ninjin'yoeito for the management of appetite loss, malnutrition, and decreased ADLs during the rehabilitation phase after surgery for femoral neck fracture in an elderly patient. Our findings suggest that Kampo medicine containing *Astragalus* root and *Schisandra* fruit, such as ninjin'yoeito, may be an effective treatment option for sarcopenia or frailty with malnutrition due to appetite loss. Furthermore, ninjin'yoeito may be applied to other types of fracture due to sarcopenia. Further large clinical trials on the efficacy of ninjin'yoeito treatment for sarcopenia or frailty are warranted.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Hattanmaru Rehabilitation Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM designed the study and wrote the initial draft of the manuscript. All other authors have contributed to data collection and interpretation and critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work, while ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Wakabayashi H, Sashika H. Malnutrition is associated with poor rehabilitation outcome in elderly inpatients with hospital-associated deconditioning a prospective cohort study. *J Rehabil Med.* (2014) 46:277–82. doi: 10.2340/16501977-1258
2. Niitsu M, Ichinose D, Hirooka T, Mitsutomi K, Morimoto Y, Sarukawa J, et al. Effects of combination of whey protein intake and rehabilitation on muscle strength and daily movements in patients with hip fracture in the early postoperative period. *Clin Nutr.* (2016) 35:943–9. doi: 10.1016/j.clnu.2015.07.006
3. Nishioka S, Wakabayashi H, Momosaki R. Nutritional status changes and activities of daily living after hip fracture in convalescent rehabilitation units: a retrospective observational cohort study from the Japan rehabilitation nutrition database. *J Acad Nutr Diet.* (2018) 118:1270–6. doi: 10.1016/j.jand.2018.02.012
4. Miwa H, Kusano M, Arisawa T, Oshima T, Kato M, Joh T, et al. Evidence-based clinical practice guidelines for functional dyspepsia. *J Gastroenterol.* (2015) 50:125–39. doi: 10.1007/s00535-014-1022-3
5. Kuchta K. Traditional Japanese kampo medicine - History of ideas and practice; Part 1: from ancient shamanic practice to the medical academies of Edo. *Tradit Kampo Med.* (2019) 6:49–56. doi: 10.1002/tkm2.1209
6. Motoo Y, Seki T, Tsutani K. Traditional Japanese medicine, Kampo: its history and current status. *Chin J Integr Med.* (2011) 17:85–7. doi: 10.1007/s11655-011-0653-y
7. Satoh H. Kampo pharmacology: integration and fusion between Kampo medicine and Western Medicine–the efficacy of Kampo medicine. *Nihon Yakurigaku Zasshi.* (2014) 143:56–60.
8. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc.* (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025
9. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* (2001) 56:M146–56. doi: 10.1093/gerona/56.3.M146
10. Tominaga K, Sakata Y, Kusunoki H, Odaka T, Sakurai K, Kawamura O, et al. Rikkunshito simultaneously improves dyspepsia correlated with anxiety in patients with functional dyspepsia: a randomized clinical trial (the DREAM study). *Neurogastroenterol Motil.* (2018) 30:e13319. doi: 10.1111/nmo.13319
11. Kamei T, Kumano H, Iwata K, Nariai Y, Matsumoto T. The effect of a traditional Chinese prescription for a case of lung carcinoma. *J Altern Complement Med.* (2000) 6:557–9. doi: 10.1089/acm.2000.6.557
12. Ohsawa M, Maruoka J, Inami C, Iwaki A, Murakami T, Ishikura KI. Effect of ninjin'yoeito on the loss of skeletal muscle function in cancer-bearing mice. *Front Pharmacol.* (2018) 9:1400. doi: 10.3389/fphar.2018.01400
13. Malafarina V, Uriz-Otano F, Malafarina C, Martinez JA, Zulet MA. Effectiveness of nutritional supplementation on sarcopenia and recovery in hip fracture patients. A multi-centre randomized trial. *Maturitas.* (2017) 101:42–50. doi: 10.1016/j.maturitas.2017.04.010
14. Nogami T, Sekiya N, Mitsuma T, Yamaguchi T. A case of pulmonary *Mycobacterium fortuitum* infection successfully treated with Kampo treatments. *Kekkaku.* (2006) 81:525–9.
15. Ogawa-Ochiai K, Osuga K, Hidaka K, Nakahata K, Tazuke Y, Yamamoto Y, et al. A case of extensive pharyngeal vascular malformation successfully treated with Kampo medicine. *Auris Nasus Larynx.* (2018) 45:190–3. doi: 10.1016/j.anl.2017.03.008
16. Motoo Y, Mouri H, Ohtsubo K, Yamaguchi Y, Watanabe H, Sawabu N. Herbal medicine Ninjinyoeito ameliorates ribavirin-induced anemia in chronic hepatitis C: a randomized controlled trial. *World J Gastroenterol.* (2005) 11:4013–7. doi: 10.3748/wjg.v11.i26.4013
17. Hsiao PJ, Lin KS, Chiu CC, Chen HW, Huang JS, Kao SY, et al. Use of traditional Chinese medicine (Ren Shen Yang Rong Tang) against microinflammation in hemodialysis patients: an open-label trial. *Complement Ther Med.* (2015) 23:363–71. doi: 10.1016/j.ctim.2015.03.002
18. Kudoh C, Arita R, Honda M, Kishi T, Komatsu Y, Asou H, et al. Effect of ninjin'yoeito, a Kampo (traditional Japanese) medicine, on cognitive impairment and depression in patients with Alzheimer's disease: 2 years of observation. *Psychogeriatrics.* (2016) 16:85–92. doi: 10.1111/psyg.12125
19. Ohsawa M, Tanaka Y, Ehara Y, Makita S, Onaka KA Possibility of simultaneous treatment with the multicomponent drug, ninjin'yoeito, for anorexia, apathy, and cognitive dysfunction in frail Alzheimer's disease patients: an open-label pilot study. *J Alzheimers Dis Rep.* (2017) 1:229–35. doi: 10.3233/ADR-170026
20. Kuniaki H, Akihiko T, Tetsuya H, Hatsuko M, Tomoko K, Shin O, et al. Improvement in frailty in a patient with severe chronic obstructive pulmonary disease after ninjin'yoeito therapy: a case report. *Front Nutr.* (2018) 5:71. doi: 10.3389/fnut.2018.00071
21. Sakisaka N, Mitani K, Sempuku S, Imai T, Takemoto Y, Shimomura H, et al. A clinical study of ninjin'yoeito with regard to frailty. *Front Nutr.* (2018) 5:73. doi: 10.3389/fnut.2018.00073
22. Kimura Y, Ito T. Ninjin'yoeito for symptoms of frailty: successful treatment of three cases. *Tradit Kampo Med.* (2019) 6:37–40. doi: 10.1002/tkm2.1206
23. Suzuki H, Asakawa A, Amitani H, Fujitsuka N, Nakamura N, Inui A. Cancer cachexia pathophysiology and translational aspect of herbal medicine. *Jpn J Clin Oncol.* (2013) 43:695–705. doi: 10.1093/jjco/hyt075
24. Goswami C, Dezaki K, Wang L, Inui A, Seino Y, Yada T. Ninjin-yoeito activates ghrelin-responsive and unresponsive NPY neurons in the arcuate nucleus and counteracts cisplatin-induced anorexia. *Neuropeptides.* (2019) 75:58–64. doi: 10.1016/j.npep.2019.03.001
25. Lee JJ, Lee JJ. A phase II study of an herbal decoction that includes *Astragali radix* for cancer-associated anorexia in patients with advanced cancer. *Integr Cancer Ther.* (2010) 9:24–31. doi: 10.1177/1534735409359180
26. Kawamata H, Tosa H, Terasawa K. Two cases of report of bedridden aged patients effectively treated with a kampo prescription ogi-kenchu-to. *Kampo Med.* (1996) 47:253–60. doi: 10.3937/kampomed.47.253
27. Kim KY, Ku SK, Lee KW, Song CH, An WG. Muscle-protective effects of *Schisandrae* Fructus extracts in old mice after chronic forced exercise. *J Ethnopharmacol.* (2018) 212:175–87. doi: 10.1016/j.jep.2017.10.022
28. Kim YJ, Yoo SR, Chae CK, Jung UJ, Choi MS. Omija fruit extract improves endurance and energy metabolism by upregulating PGC-1α expression in the skeletal muscle of exercised rats. *J Med Food.* (2014) 17:28–35. doi: 10.1089/jmf.2013.3071

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

REVIEW
published: 15 July 2020
doi: 10.3389/fnut.2020.00086

Kampo Medicine for Various Aging-Related Symptoms: A Review of Geriatric Syndrome

Shin Takayama[1,2,3]*, *Naoki Tomita*[4], *Ryutaro Arita*[2,3], *Rie Ono*[2,3], *Akiko Kikuchi*[1,2,3] and *Tadashi Ishii*[1,2,3]

[1] Department of Kampo and Integrative Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan, [2] Department of Education and Support for Regional Medicine, Tohoku University Hospital, Sendai, Japan, [3] Department of Kampo Medicine, Tohoku University Hospital, Sendai, Japan, [4] Department of Geriatrics and Gerontology, Institue of Development, Aging and Cancer, Tohoku University, Sendai, Japan

With the continued growth of the aging population in Japan, geriatric syndrome (GS), which is associated with aging-related symptoms, has become a social problem. GS is caused by physiological and pathological aging and may manifest various symptoms. Physicians use multidisciplinary approaches to provide treatment for individual GS symptoms. Kampo medicine, a Japanese traditional medicine that uses multiple pharmacologically active substances, is useful for many syndromes, conditions, disorders, and diseases associated with GS. Evidence of the effectiveness of Kampo medicine for GS has accumulated in recent years. The effects of Kampo treatment for symptoms related to functional decline of the cardiovascular, respiratory, and digestive systems, cognitive impairment and related disorders, pain and other sensory issues, among others, support the use of Kampo medicine for the management of GS. The role of Kampo medicine for GS is summarized in this review.

Keywords: aging, Kampo medicine, geriatric syndrome, elderly, evidence

OPEN ACCESS

Edited by:
Akio Inui,
Kagoshima University, Japan

Reviewed by:
Dario Coletti,
Sapienza University of Rome, Italy
Irena Keser,
University of Zagreb, Croatia

***Correspondence:**
Shin Takayama
takayama@med.tohoku.ac.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *22 March 2020*
Accepted: *12 May 2020*
Published: *15 July 2020*

Citation:
Takayama S, Tomita N, Arita R, Ono R, Kikuchi A and Ishii T (2020) Kampo Medicine for Various Aging-Related Symptoms: A Review of Geriatric Syndrome. Front. Nutr. 7:86. doi: 10.3389/fnut.2020.00086

INTRODUCTION

Geriatric syndrome (GS) is a well-known clinical entity characterized by symptoms highly prevalent in old age. It presents with multiple contributing factors, including physiological aging, and requires a multidisciplinary approach. When compared to the "disease" entity, the differences in features are unknown etiology and inclusion of physiological aging as a cause (1). There is no universal definition of GS; there are some variations in the features included in GS definitions. These variations in definition lead to ambiguity regarding the included symptoms. Originally, GS symptoms were expressed as the 3Ms (mentality, micturition, and mobility) or 4Is (immobility, instability, impaired cognition, and incontinence—also named the "geriatric giants") (2). Today, 20 or more symptoms are listed (3). The majority of GS symptoms emerge slowly and are chronic, with a low risk of mortality by themselves. Consequently, they tend to be overlooked as physiological changes, resulting in increased dependency. GS is clinically significant as a warning sign for the risk of increased care dependency. When determining countermeasures for GS, we must exclude the possibility of a single cause before considering multifactorial etiology (3, 4) (e.g., endocrine disorders) (5). Then, we must exclude medication-related side effects (6).

Kampo medicine is effective in many cases of GS with multiple causes. Original GS symptoms (such as symptoms related to functional decline of the cardiovascular, respiratory, and digestive systems; cognitive impairment and related disorders; and pain and other sensory issues) are considered treatment targets. Because of the multifactorial nature of GS and specialization in medicine, care for those with GS tends to be fragmented. Kampo medicine could prevent fragmentation of patient care.

On the other hand, medical expenses amounted to more than one third of social security expenses in 2018 (7). With the growing "super-aging" society and the declining birth rate in Japan, medical expenses are only expected to increase. In light of these points, we have herein summarized the efficacy, safety, and social economic advantage associated with the use of Kampo medicine for GS.

In this review, we summarize randomized controlled trials (RCTs) for GS. When no RCT was available for specific conditions or disorders, some observational studies were described. Details of each Kampo medication are listed on the Standards of Reporting Kampo Products (STORK) website (http://mpdb.nibiohn.go.jp/stork/) (8). The names of Kampo medicines were abbreviated according to the Japan Society of Oriental Medicine (9).

KAMPO MEDICINE FOR GS

Kampo Medicine for Cardiovascular Disease and Related Symptoms

Generally, cardiovascular disease and related disorders increase with age. The Japanese lifestyle has shifted toward a Western lifestyle; thus, cardiovascular disease has increased in the last half century. Western medications are commonly used to control hypertension and related diseases; furthermore, they have been shown to effectively suppress cardiovascular events. A few clinical trials have been conducted on Kampo medicine for the treatment of cardiovascular disease and related symptoms. Overall, these RCTs were conducted to manage symptoms difficult to control in Western medicine. Soft endpoints were the improvement of accessory symptoms of hypertension, orthopedic hypotension related to diabetes mellitus, and edema according to deep vein thrombosis of the lower limb (**Table 1**).

Hypertension

Arakawa et al. conducted a double-blinded (DB) RCT on orengedokuto (OGT) for the treatment of accessory symptoms of hypertension; the study included elderly subjects (10). Efficacy was significantly higher in the OGT group based on the total score for the accessory symptoms of hypertension; sub-analysis showed the efficacy to be higher for hot flashes and facial suffusion in the OGT group. However, there were no significant differences between the OGT and placebo groups regarding the decrease of blood pressure or the antihypertensive effect.

Hypotension

Nakamura et al. reported the efficacy of goreisan (GRS) for orthopedic hypotension related with diabetes mellitus in an RCT that included elderly subjects (11). The change in orthopedic systolic and diastolic pressure was significantly lower in the GRS group than in the placebo group. All patients complained of dizziness in the placebo group, but only 10% complained of the symptom in the GRS group.

Disorders Related to Vein Dysfunction

Uchida reported the effect of keishibukuryogan (KBG) for edema according to deep vein thrombosis of the lower limb in an RCT of elderly subjects (12). The improvement rates of circumference difference between the affected and the normal limbs were significantly higher in the KBG group than in the conventional treatment group.

Kampo Medicine for Aspiration Pneumonia and Chronic Obstructive Pulmonary Disease

Respiratory disease is increasing with the aging of society. Kampo medicine has been effective at treating acute respiratory infection, and there are some reports that Kampo medicine has a prophylactic effect in aspiration pneumonia and acute exacerbation of chronic obstructive pulmonary disease (COPD) (**Table 2**).

Kampo Medicine for Aspiration Pneumonia

Pneumonia is one of the leading causes of death in the elderly. Therefore, preventing pneumonia, including aspiration pneumonia, is very important. Aspiration pneumonia occurs frequently in patients with cerebrovascular disease, patients with neurodegenerative disease, and bedridden patients with dysphagia and depression of swallowing and cough reflex. Patients with swallowing or coughing impairment have low levels of substance-P in their saliva (13, 14). Substance-P is a neuropeptide that plays an important role in swallowing and cough reflexes (13). **Table 2A** shows studies of Kampo medicine for aspiration pneumonia.

Iwasaki et al. reported that hangekobokuto (HKT) improves swallowing reflex and increases salivary levels of substance-P in patients who had a stroke (15). They also reported that HKT improves swallowing reflex in patients with Parkinson's disease despite no significant changes in their salivary levels of substance-P (16). Iwasaki et al. also showed that HKT improves cough reflex of patients with cerebral atrophy and lacunar infarction (17), reduces the risk of aspiration pneumonia in the elderly, and maintains self-feeding capacity better than the control (18). Additionally, Kawago et al. reported that HKT prevents aspiration pneumonia in patients after cardiovascular surgery (19). HKT is thought to act via regulation of the cerebral levels of 5-hydroxytryptamine, noradrenaline, and dopamine

Abbreviations: BAK, Bakumondoto; BOT, Boiogito; CTS, Chotosan; DKT, Daikenchuto; DKZT, Daiokanzoto; GRS, Goreisan; GJG, Goshajinkigan; HJG, Hachimijiogan; HKT, Hangekobokuto; HET, Hochuekkito; ICKT, Inchinkoto; JDI, Jidabokuippo; KBG, Keishibukuryogan; KHT, Kihito; NYT, Ninjinyoeito; OGT, Orengedokuto; RKT, Rikkunshito; SBT, Saibokuto; SHT, Seihaito; YKS, Yokukansan.

TABLE 1 | RCT of Kampo medicine in cardiovascular and related conditions.

References	Study design	Subjects (*n*)	Age, years (mean ± SD)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Uchida (12)	RCT	12	65.8 ± 16.3	Acute symptomatic proximal deep vein thrombosis	KBG added to conventional treatment	Conventional treatment with thrombolysis and anticoagulant	The improvement rates of circumference difference between the affected and the normal limbs were significantly higher in the KBG group.	N/A
Arakawa et al. (10)	DB-RCT	204	52.3 ± 11	Accessory symptoms of hypertension	OGT	Placebo	Efficacy was significantly higher in the OGT group based on the total score for the accessory symptoms of hypertension, especially hot flushes and facial suffusion.	None
Nakamura et al. (11)	Crossover RCT	10	57.6 ± 8.1	Orthopedic hypotension related with diabetes mellitus	GRS	Placebo	The change of orthopedic systolic and diastolic pressure was significantly lower in the GRS group than in the placebo group. All patients complained of dizziness in the placebo group, but only 10% complained of the symptom in the GRS group.	N/A

DB, double-blinded; GRS, goreisan; KBG, keishibukuryogan; OGT, orengedokuto; RCT, randomized controlled trial; SD, standard deviation; N/A, not assigned.

(20). Impairment of the swallowing reflex correlates strongly with decreased dopamine levels in the basal ganglia (21). Therefore, HKT-induced improvement of swallowing reflex may be associated with HKT-induced increase in brain dopamine levels. Hochuekkito (HET) is another Kampo formula for prevention of aspiration pneumonia. Tamano et al. reported that administration of HET, alone or in combination with rehabilitation, reduces the number of hospitalizations due to aspiration pneumonia (22, 23). HET also improves clinical symptoms such as appetite loss and general malaise, increases body weight and serum albumin, and increases temperature in patients with low body temperature. Mantani et al. reported that seihaito (SHT), added to conventional treatment, decreases the mean values of fever, C-reactive protein (CRP) levels, and antibiotics use compared with conventional therapy alone (24). However, SHT does not improve the latency of swallowing reflex. This study indicated that SHT has an anti-inflammatory effect in patients with recurrent aspiration pneumonia but does not improve swallowing reflex. Iwasaki et al. reported that xanthine oxidase activity in lung tissues is elevated in a mouse model of aspiration pneumonia and that SHT is able to reverse this elevation (25). The authors speculated that SHT pretreatment can reduce oxygen radical production in inflamed lungs. Dysphagia is also considered to relate to gastroesophageal reflux disease (GERD).

Kampo Medicine for COPD

COPD does not affect solely the airways; it is considered a systemic inflammation. The treatment guidelines for COPD recommend the use of bronchodilators, inhaled corticosteroids, and rehabilitation. One of the main goals of COPD treatment is to prevent acute exacerbation, which is known to affect patient prognosis. **Table 2B** shows an RCT of Kampo medicine for COPD.

Among Kampo medications, bakumondoto (BAK) and SHT have been shown to improve the symptoms of COPD. Sasaki et al. reported that BAK significantly helps loosen phlegm of patients with chronic respiratory disease (26). Mukaida et al. showed that BAK significantly improves visual analog scale (VAS) scores for cough frequency, but not for cough intensity (27). Kato et al. reported that administration of SHT improves the clinical symptoms of COPD (28). BAK is thought to exert a peripheral antitussive effect by inhibiting the synthesis or release of nitric oxide (29). According to the traditional theory, BAK should be used for patients with dry cough and SHT for patients with productive cough.

Shinozuka et al. and Tatsumi et al. reported that HET reduces the number of common cold and acute exacerbation episodes in patients with COPD (30, 31). HET decreased serum CRP, tumor necrosis factor (TNF)-α, and interleukin-6 levels and increased serum prealbumin levels. Furthermore, HET resulted in a significant increase in body weight over 6 months and a decrease in St. George's Respiratory Questionnaire score, indicating an improvement in quality of life (QOL). HET has antiviral and anti-inflammatory effects, thus contributing to preventing exacerbation (32). Jo et al. reported that daikenchuto (DKT) reduces exacerbation in patients with COPD (33). Patients treated with DKT had

TABLE 2A | Studies of Kampo medicine for aspiration pneumonia.

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range) Kampo group/control group	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Iwasaki et al. (15)	Controlled clinical trial	32	76.4 ± 3.1/70.5 ± 5.6	Aspiration pneumonia	HKT	Placebo	The swallowing reflex was significantly improved and substance-P in saliva increased significantly in the Kampo group.	N/A
Iwasaki et al. (17)	RCT	16	79.6 ± 4.3/70.5 ± 5.6	Aspiration pneumonia, lacunar infarction, or brain atrophy	HKT	Placebo	Cough reflex was significantly improved after HKT administration.	N/A
Mantani et al. (24)	RCT	15	78.7 (65–96)/80.5 (72–93)	Aspiration pneumonia	SHT	No Kampo, only conventional therapy	The mean values of fever index, CRP, and antibiotics use were decreased significantly in the SHT group. The latency of the swallowing reflex was not significantly changed.	None
Iwasaki et al. (18)	RCT	95 (92)	84.5 ± 6.8/83.1 ± 7.2	Aspiration pneumonia in dementia	HKT	Placebo	HKT reduced pneumonia onset and tended to reduce pneumonia-related mortality. The relative risk of pneumonia in the Kampo group compared with the control group was 0.51, and that of death from pneumonia was 0.41.	None
Kawago et al. (19)	DB-RCT (envelope)	34 (30)	65.2 ± 13.9/69.2 ± 13.0	Aspiration pneumonia after cardiovascular surgery	HKT	Placebo	The rate of postoperative aspiration pneumonia was significantly lower in the Kampo group than in the placebo group. White blood cell counts and CRP levels on postoperative day 3 were significantly lower in the Kampo group.	N/A

CRP, C-reactive protein; DB, double-blinded; HET, hochuekkito; HKT, hangekobokuto; RCT, randomized controlled trial; SD, standard deviation; SHT, seihaito; N/A, not assigned.

TABLE 2B | RCT of Kampo medicine for COPD.

References	Study design	Subjects (*n*)	Age, years (range or mean ± SD) Kampo group/control group	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Sasaki et al. (26)	RCT (envelope)	19	62–83/65–88	Chronic respiratory disease	BAK	Bromhexine hydrochloride	The BAK group showed improved loosening of phlegm after 2 and 4 weeks.	None
Kato et al. (28)	RCT (envelope)	31	66.7 ± 7.1/66.7 ± 6.4	COPD	SHT + smoking cessation	No Kampo, only smoking cessation	SHT improved the clinical symptoms of patients with COPD for 6 months, and chest X-ray or CT findings at 24 months.	N/A
Shinozuka et al. (30)	RCT	35	73 ± 1	COPD	HET + bronchodilators	Bronchodilators	In the HET group, serum CRP and TNF-α significantly decreased, and serum albumin level was significantly increased.	N/A
Tatsumi et al. (31)	RCT (envelope)	71	Elderly	COPD	HET + conventional therapy	Conventional therapy	In the HET group, body weight significantly increased for 6 months, and St. George's Respiratory Questionnaire score decreased, indicating that quality of life improved. The number of common cold and acute exacerbations was significantly lower. CRP, TNF-α, and IL-6 decreased, and serum prealbumin increased.	None
Mukaida et al. (27)	Crossover RCT	24 (23)	Group A: 76.2 ± 8.5/group B: 79.2 ± 2.6	COPD	BAK	No Kampo	BAK significantly improved VAS scores for cough frequency in group A. VAS scores for cough intensity in each group tended to improve. BAK improved scores of cough severity significantly.	Serum ALP elevation in two participants

ALP, alkaline phosphatase; BAK, bakumondoto; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CT, computed tomography; HET, hochuekkito; IL, interleukin; SHT, seihaito; TNF-α, tumor necrosis factor-α; VAS, visual analog scale; N/A, not assigned.

a significantly lower risk of rehospitalization or death after discharge. By improving bowel movements and tolerance to muscarinic antagonists, DKT improves the respiratory status of patients with COPD. Recently, there have been a few DB-RCTs on traditional Chinese medicine for patients with COPD (34–36). In the majority of the studies, crude drugs added to the conventional therapy prevented recurrence of acute exacerbation.

Kampo Medicine for the Digestive System

Kampo medicine was developed to control and maintain the function of the digestive system. It is used to enhance motility in the gastrointestinal tract and promote digestion. Kampo medicine has recently been used for early recovery from surgical intervention, especially for elderly patients receiving cancer treatment. RCTs have explored the use of Kampo medicine for constipation, perioperative symptoms, and conditions in the gut, functional dyspepsia (FD), GERD, and nonerosive reflux disease (NERD) (**Table 3**). After development of a placebo of these Kampo medicines, DB-RCTs using DKT or rikkunshito (RKT) were conducted. RCTs showed that DKT can be used for preventing postoperative ileus, improving bowel movement in the early days, improving QOL, having anti-inflammatory effects, improving early oral intake, enhancing total oral/enteral caloric intake and portal venous flow volume, and minimizing weight loss after abdominal surgery in the perioperative stage. RCTs showed that RKT can be used for improving upper gastric symptoms (globus sensation, delayed gastric emptying, abdominal bloating, heavy feeling in the stomach, sick feeling after meals, heartburn after meals, and epigastric pain), psychological symptoms, appetite loss, acyl ghrelin levels, and a low body mass index in FD, GERD, and NERD. **Table 3A** shows an RCT of Kampo medicine for FD and GERD.

FD and GERD

Tominaga et al. reported the effects of RKT administration on FD and its correlation with anxiety (37). RKT increased the overall treatment efficacy and improved upper gastrointestinal symptoms, especially postprandial fullness/early satiety and bloating. Improvement of the Hospital Anxiety and Depression Scale was correlated with that of the Patient Assessment of Upper Gastrointestinal Disorders–Symptom Severity Index, the Global Overall Symptom scale, and the modified Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease (postprandial fullness/early satiety, dyspepsia, and postprandial distress syndrome). This suggests that RKT may be beneficial for patients with FD to simultaneously treat gastrointestinal and psychological symptoms. Tominaga et al. also studied the use of RKT for patients with NERD refractory to proton-pump inhibitors (PPIs) in a DB-RCT that included elderly subjects (38). The mental component summary scores improved more in the RKT group than in the control group, especially in patients with a low body mass index, and significantly improved the acid-related dysmotility symptoms in female and elderly patients. Sakata et al. reported additional analysis of this study (39). Especially in the elderly, the degrees of improvement in the total and acid-related dysmotility symptom scores were significantly greater in the RKT group. Combination therapy with RKT led to significant improvement in abdominal bloating, heavy feeling in the stomach, sick feeling after meals, and heartburn after meals. Kato et al. reported that HKT significantly improved respiratory symptoms associated with GERD (40). Suzuki et al. reported the efficacy and safety of RKT for FD in a DB-RCT that included elderly subjects (41). Administration of RKT reduced dyspepsia, particularly the symptoms of epigastric pain and postprandial fullness. Among the patients positive for *Helicobacter pylori*, RKT improved acyl ghrelin levels. In a DB-RCT, Hayakawa et al. reported the effects of RKT on enteral feeding and plasma ghrelin levels in critically ill elderly patients (42). The RKT group reached 50% of the target amount of enteral feeding significantly earlier than the metoclopramide group. RKT increased the plasma level of active ghrelin. Tokashiki et al. reported the effects of RKT on the globus sensation in patients with PPI-refractory laryngopharyngeal reflux in an RCT that included elderly subjects (43). RKT or RKT with PPI significantly decreased the globus sensation and improved delayed gastric emptying. A significant positive correlation was found between improvements in the globus sensation and gastric emptying. Tominaga et al. reported the efficacy of RKT for patients with GERD refractory to treatment with PPI in a DB-RCT that included patients from 20 to 90 years of age (44). RKT combined with PPI significantly decreased the frequency scale of the GERD symptoms' score, similar to the decrease seen after treatment with a double dose of PPI. Subgroup analysis showed that the improvement rate of male NERD patients in the RKT group was significantly greater. Subgroup analysis also showed that the patients of male sex or with a low body mass index showed more improvement than other subgroups. Furthermore, no adverse events were observed in this study. Additional RKT therapy for patients with GERD refractory to PPI treatment seemed to be more effective for NERD, male, or low-body mass index patients, and the therapy was shown to be safe. Arai et al. reported a significant improvement in dyspeptic symptoms in patients treated with either RKT or domperidone (45). The improvements of reflux and indigestion symptoms in patients treated with RKT showed good correlations with the increased levels of acylated ghrelin. Harasawa et al. conducted a DB-RCT on RKT for the treatment of dysmotility-like dyspepsia in elderly subjects (46). The regular dose of RKT improved dysmotility-like dyspepsia significantly more than the low dose of RKT. Tatsuta et al. reported the effects of RKT on gastric emptying and gastrointestinal symptoms in dyspeptic patients (47). Gastric emptying was significantly accelerated, and gastrointestinal symptoms were significantly reduced in patients treated with RKT. Miyoshi et al. examined the effects of RKT for complaints related to gastrointestinal function in an RCT that included elderly subjects (48). RKT significantly improved the symptoms of appetite loss, epigastric pain, abdominal discomfort, cold feelings of the limb, and dazzling when compared with cisapride. In a subanalysis, RKT was more effective in patients over 60 years of age, with a thin type body, and with water retention.

TABLE 3A | RCT of Kampo medicine for FD and GERD.

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Tominaga et al. (37)	DB-RCT	125	50.4 ± 13.7 (23–76)/50.4 ± 14.9 (23–83)	Functional dyspepsia	RKT	Placebo	RKT increased overall treatment efficacy. RKT improved upper gastrointestinal symptoms, especially postprandial fullness/early satiety and bloating. Improvement of the Hospital Anxiety and Depression Scale correlated with those of the Patient Assessment of Upper Gastrointestinal Disorders-Symptom Severity Index, the Global Overall Symptom scale, and the modified Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease, postprandial fullness/early satiety, dyspepsia, and postprandial distress syndrome. RKT may be beneficial for patients with FD to simultaneously treat gastrointestinal and psychological symptoms.	None
Tominaga et al. (38)	DB-RCT	242	62.1 (25–85)/59.4 (22–83)	Patients with PPI-refractory non-erosive reflux disease	RKT with PPI	Placebo with PPI	The mental component summary scores improved more in RKT administration, especially more effective in patients with a low body mass index and significantly improved the acid-related dysmotility symptoms in female and elderly patients.	Nausea, mild cough, dizziness, diarrhea, etc. (low level)
Sakata et al. (39)	DB-RCT	95	72.1 (65–85)/73.4 (65–83)	Patients with PPI-refractory non-erosive reflux disease	RKT with PPI	Placebo with PPI	The degree of improvement of total and acid-related dysmotility symptoms scores was significantly greater in the RKT group. Combination therapy with RKT showed significant improvement in abdominal bloating, heavy feeling in stomach, sick feeling after meals, and heartburn after meals.	N/A
Suzuki et al. (41)	DB- RCT	247	54.5 ± 16.2 (22–85)/53.6 ± 16.0 (21–85)	Functional dyspepsia	RKT	Placebo	Administration of RKT reduced dyspepsia, particularly symptoms of epigastric pain and postprandial fullness. Among *Helicobacter pylori*-positive individuals, RKT improved acyl ghrelin levels.	None
Hayakawa et al. (42)	DB- RCT	23	75 ± 11/70 ± 13	Patients who were projected to require intragastric tube feeding	RKT	Metoclopramide	RKT group reached 50% of the target amount of enteral feeding significantly earlier than the metoclopramide group. RKT increased the plasma level of active ghrelin.	N/A

(Continued)

TABLE 3A | Continued

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Tokashiki et al. (43)	RCT	22	55.9 (39–76)/56.6 (25–76)	PPI-refractory laryngopharyngeal reflux	RKT	RKT with PPI	RKT significantly decreased the globus sensation. It also improved delayed gastric emptying. A significant positive correlation between improvements in globus sensation and in gastric emptying were shown.	N/A
Tominaga et al. (44)	DB-RCT	101	63.6 (25–86)/64.5 (25–90)	Refractory GERD with PPI	RKT with a standard dose of PPI	Double dose of PPI	RKT addition significantly decreased the frequency scale of the GERD symptom score similar to treatment with a double dose of PPI.	None
Arai et al. (45)	RCT	27	56.5 ± 15.0/59.0 ± 14.0	FD	RKT	Domperidone	A significant improvement was shown in dyspeptic symptoms treated with either RKT or domperidone. RCT increased acylated ghrelin. The symptom improvement of reflex and ingestion were well correlated with the increase of acylated ghrelin.	N/A
Kato et al. (40)	RCT	19	74.1 ± 6.4/71.7 ± 5.2	GERD with cough, sputum, pharyngolaryngeal discomfort, or mild dyspnea	HKT	no HKT	HKT significantly improved respiratory symptoms related with GERD.	N/A
Harasawa et al. (46)	DB-RCT	235	53.5 (23–79)/52.3 (21–78)	Dysmotility-like dyspepsia	Regular dose of RKT	Low dose of RKT	Regular dose of RKT improved dysmotility-like dyspepsia.	Gastrointestinal symptom, liver dysfunction, or pseudo aldosteronism were shown, the frequency and severity were low
Tatsuta et al. (47)	RCT	42		Chronic dyspepsia	RKT	Placebo	Gastric emptying was significantly accelerated and gastrointestinal symptoms were significantly reduced in patients treated with RKT.	N/A
Miyoshi et al. (48)	RCT	246	Under 20 to over 80 years	Non-ulcer dyspepsia	RKT	Cisapride	RKT significantly improved symptom of appetite loss, epigastric pain, abdominal discomfort, cold feeling of limb, dazzling compared with cisapride. In subanalysis, RKT was more effective in patients over 60 years old, thin, and with water retention.	N/A

HKT, hangekobokuto; PPI, proton-pump inhibitor; RKT, rikkunshito; N/A, not assigned.

Constipation

Table 3B shows an RCT of Kampo medicine for constipation. Numata et al. reported the efficacy of DKT for functional constipation in elderly patients after stroke (49). Constipation scoring system points, especially the frequency of bowel movements, feeling of incomplete evacuation, and need for an enema/disimpaction, improved significantly with the addition of DKT. The gas volume score also significantly reduced with the addition of DKT. Arita et al. performed a responder analysis of DKT treatment for constipation in poststroke patients (50). The total neurogenic bowel dysfunction score and Gastrointestinal Symptom Rating Scale (GSRS)-constipation subscale score were significantly reduced after DKT administration. The total neurogenic bowel dysfunction score, GSRS-constipation subscale score, and gas volume score at baseline were significantly correlated with the change in these scores, suggesting that higher scores in these categories and a higher gas volume in the gut may be predictors of response to DKT. Horiuchi et al. reported the effect of DKT in patients with chronic constipation in an RCT that included elderly subjects (51). The addition of DKT to sennoside resulted in a significant improvement in bloating and abdominal pain and a significant decrease in the gas volume score comparing a regular dose and a half dose of DKT. Miyoshi et al. reported the effect of daiokanzoto (DKZT) in patients with chronic constipation in an RCT that included elderly subjects (52). DKZT was significantly more effective for constipation than the placebo. A regular dose of DKZT has a strong effect on some patients; as such, the dose should be determined considering the patient's condition.

Perioperative Symptoms and Conditions in the Gut

Table 3C shows an RCT of Kampo medicine for the perioperative period. Nishino et al. reported the effects of DKT after esophageal cancer resection in an RCT that included elderly subjects (53). The rate of weight loss at postoperative day 21 was significantly suppressed in the DKT group. Postoperative bowel symptoms tended to be rare, and the serum CRP level at postoperative day 3 tended to be lower in the DKT group. This suggests that DKT treatment after esophageal cancer resection may promote the recovery of gastrointestinal motility and minimize weight loss; it may also suppress the excess inflammatory reaction related to surgery. In a DB-RCT, Katsuno et al. reported the effect of DKT on elderly patients with colon cancer undergoing open surgery by transit analysis using radiopaque markers (54). The number of radiopaque markers in the anal side of the small intestine at 6 h was significantly greater in the DKT group. This suggests that DKT may contribute to early oral intake in the postoperative course. Okada et al. examined the efficacy of DKT for the prevention of paralytic ileus after pancreaticoduodenectomy in a DB-RCT of elderly patients (55). Perioperative treatment with DKT neither decreased the incidence of clinically relevant postoperative paralytic ileus nor shortened the time to the first postoperative flatus, suggesting that DKT may preclude the routine use of DKT in clinical practice after this operation. Akamatsu et al. reported the effects of DKT on intestinal motility after total gastrectomy in an RCT that included elderly subjects (56). DKT significantly improved the number of stools per day, stool consistency, and gas volume scores. This suggests that DKT promoted early postoperative bowel functions after total gastrectomy. In a DB-RCT, Katsuno et al. reported the clinical efficacy of DKT for gastrointestinal dysfunction following colon surgery in elderly patients (57). Bowel movement frequency in the DKT group at postoperative day 8 was significantly lower than that in the placebo group, suggesting that the moderate effects of DKT were observed in the early days after the operation. In a DB-RCT, Yoshikawa reported the effects of DKT after total gastrectomy for gastric cancer in elderly patients (58). DKT administration shortened the median time to the first bowel movement and resulted in fewer gastrointestinal dysfunctions on postoperative day 12. This suggests that DKT administration in the immediate postoperative period after total gastrectomy promotes early recovery of postoperative bowel function. Yaegashi et al. reported the effects of DKT on colonic motility after laparoscopic-assisted colectomy in elderly colon cancer patients (59). The DKT group had a significantly faster time until the first flatus and bowel movement and colonic transit time. This suggests DKT accelerates colonic motility in patients undergoing laparoscopic-assisted colectomy for colon cancer. Yoshikawa et al. reported the effects of DKT on the surgical inflammatory response following laparoscopic colorectal resection in an RCT that included elderly subjects (60). Postoperative DKT administration significantly suppressed the CRP level and shortened the time until first flatus. This suggests that DKT has anti-inflammatory effects and may help patients recover following surgery. Takahashi et al. reported the effects of RKT on the stasis of patients after pylorus-preserving gastrectomy in a crossover RCT that included elderly patients (61). RKT significantly reduced gastric stasis-related symptoms and improved emptying of solid meals from the remnant stomach. Endo et al. reported the effect of DKT on the stasis of patients with total gastrectomy and jejunal pouch interposition in a crossover RCT that included elderly subjects (62). DKT significantly reduced stasis-related symptoms. In the emptying test, DKT significantly accelerated emptying of both liquid and solid meals from the pouch. The pouch showed bursts of contractions, which were increased significantly by oral intake of DKT. This suggests that DKT increased intestinal motility and improved the QOL of patients with this condition. Itoh et al. reported the effects of DKT on postoperative ileus in an RCT that included elderly subjects (63). The need for further surgery was significantly reduced in patients receiving DKT. Patients receiving DKT also showed a lower tendency for recurrent ileus than those receiving the placebo. Takagi et al. reported the effects of DKT on paralytic ileus after repair of abdominal aortic aneurysm in elderly subjects (64). DKT administration significantly reduced intestinal gas. Kubo et al. reported the effects of DKT on ileus in an RCT that included elderly subjects (65). The duration to defecation, exhaust gas, and ileus tube removal did not differ significantly between the DKT and control groups. However, DKT administration reduced abdominal boating, nausea, and vomiting.

Kaido et al. reported the effect of DKT on oral and enteral caloric intake after liver transplantation in a DB-RCT that

TABLE 3B | RCT of Kampo medicine for constipation.

References	Study design	Subjects (*n*)	Age, years (mean ± SD)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Arita et al. (50)	RCT subanalysis	34	77.5 ± 11.9 78.7 ± 12.1	Functional constipation	DKT added to conventional therapy	Conventional therapy	The total neurogenic bowel dysfunction score, Gastrointestinal Symptom Rating Scale-constipation subscale score, and gas volume score at baseline were significantly correlated with the change in these scores.	N/A
Numata et al. (49)	RCT	34	78.1 ± 11.6	Functional constipation	DKT added to conventional therapy	Conventional therapy	The frequency of bowel movements, feeling of incomplete evacuation, and need for enema/disimpaction were significantly improved by DKT. The gas volume score was also significantly reduced by DKT.	Liver dysfunction (low level)
Horiuchi et al. (51)	RCT	22	69.2 ± 13/68.9 ± 16	Chronic constipation	Regular dose of DKT added to sennoside	Half of regular dose of DKT added to sennoside	The addition of DKT reduced abdominal bloating and pain in chronic constipation patients receiving stimulant laxatives with decreasing the bowel gas volume.	None
Miyoshi et al. (52)	DB-RCT	146	65 patients over 60 years	Constipation	Regular dose of DKZT	Low dose of DKZT or placebo	DKZT was significantly effective for constipation compared to the placebo.	No significant difference between groups.

DKT, daikenchuto; DKZT, daiokanzoto; N/A, not assigned.

TABLE 3C | RCT of Kampo medicine for the perioperative period.

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse event related with Kampo
Kaido et al. (66)	DB-RCT	104	56 (22–69)/57 (30–67)	Patients undergoing liver transplantation	DKT	Placebo	Postoperative total oral/enteral caloric intake was significantly accelerated in the DKT group. Portal venous flow volume and velocity were significantly higher in the DKT.	None
Nishino et al. (53)	RCT	39	68.0 (61.0–74.0)/60.5 (55.0–67.0)	Patients planned of subtotal esophageal resection for esophageal cancer	DKT	Non-DKT	The rate of body weight decreased at postoperative day 21 was significantly suppressed in the DKT group. Postoperative bowel symptoms tended to be rare in the DKT group. The serum CRP level at postoperative day 3 showing a tendency of a suppressed serum CRP level in the DKT group.	N/A
Katsuno et al. (54)	DB-RCT	71	67.7 (39–88)/68.2 (51–85)	Patients who were scheduled to undergo open surgery for sigmoid or rectosigmoid cancer	DKT	Placebo	The number of radiopaque markers in the anal side of the small intestine at 6 h was significantly greater in the DKT group.	None
Okada et al. (55)	DB-RCT	207	68.9 ± 8.4/64.9 ± 11.3	Patients who were scheduled to undergo pancreaticoduodenectomy for periampullary tumors and tumors of the head of the pancreas	DKT	Placebo	Perioperative treatment with DKT neither decreased the incidence of clinically relevant postoperative paralytic ileus nor shortened the time to first postoperative flatus.	N/A
Shimada et al. (67)	RCT	209	68(36–87)/69(31–84)	Primary and metastatic liver cancer patients who underwent hepatic resection	DKT	Placebo	DKT improve gastrointestinal dysmotility and reduce serum CRP levels in patients with grade B liver damage after hepatectomy.	None
Akamaru et al. (56)	RCT	81	63.4 ± 8.9 (32–77)/63.7 ± 9.2 (40–78)	Patients with gastric cancer scheduled for a total gastrectomy	DKT	Non-DKT	DKT significantly improved the number of stools per day, stool consistencies, and gas volume scores.	None
Katsuno et al. (57)	DB-RCT	336	68 (28–88)/69 (35–91)	Patients scheduled to undergo colectomy for colon cancer	DKT	Placebo	The frequency of bowel movement in the DKT group at postoperative day 8 was significantly lower than that in the placebo group. The moderate effects of DKT were observed early days after the operation.	None
Yoshikawa et al. (58)	DB-RCT	195	68 (33–83)/67 (28–84)	Gastric cancer patients who underwent total gastrectomy	DKT	Placebo	DKT shorter median time to first bowel movement and made fewer gastrointestinal dysfunction on postoperative day 12.	None
Yaegashi et al. (59)	RCT	51	69 (51–83)/68 (43–89)	Colon cancer patients who underwent colectomy	DKT	*Lactobacillus* preparation	DKT group had significantly faster time until first flatus and bowel movement and colonic transit time. DKT accelerated colonic motility in patients undergoing laparoscopy-assisted colectomy for colon cancer.	None

(Continued)

TABLE 3C | Continued

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse event related with Kampo
Nishi M et al. (68)	RCT	32	68.8 ± 8.7/64.3 ± 7.3	Patients who underwent hepatic resection	DKT	No DKT	DKT significantly decreased the levels of c-reactive protein and beta-(1–3)-d-glucan on postoperative day 3. DKT significantly shortened postoperative periods for the first flatus, bowel movement, and full recovery of oral intake.	None
Yoshikawa et al. (60)	RCT	30	62 ± 12 (41–80)/70 ± 5 (61–86)	Patients who underwent laparoscopic colectomy for colorectal carcinoma	DKT	Non-DKT	Postoperative DKT administration significantly suppressed CRP level and shortened the time until first flatus.	N/A
Takahashi et al. (61)	Crossover RCT	11	60 (46–70)	Pylorus-preserving gastrectomy for early gastric cancer	Rikkunshito	No rikkunshito administration	Rikkunshito significantly reduced gastric stasis-related symptoms and improved emptying of solid meals from the remnant stomach.	N/A
Endo et al. (62)	Crossover RCT	17	62 ± 10	Patients who underwent total gastrectomy with jejunal pouch interposition for gastric cancer	DKT	Non-DKT	DKT significantly reduced stasis-related symptoms. In the emptying test, DKT significantly accelerated emptying of both liquid and solid meals from the pouch. The pouch showed bursts of contractions, which were increased significantly by oral intake of DKT.	N/A
Kaiho et al. (69)	RCT	43	61.6 ± 8.1/62.4 ± 19.3/63.8 ± 10.0	Patients with liver resection	DKT	Lactulose or no administration of DKT/lactulose	DKT significantly lower postoperative serum ammonia levels with low occurrence of diarrhea.	N/A
Itoh et al. (63)	RCT	24	58 ± 10/60 ± 11	Postoperative ileus after abdominal surgery	DKT	Placebo	The need for further surgery was significantly lower in patients receiving DKT. DKT also showed a lower tendency in recurrent ileus than those receiving placebo.	N/A
Takagi et al. (64)	RCT	21	72 ± 5	Patients who underwent an aortic replacement for intrarenal abdominal aortic aneurysm with transperitoneal approach	DKT from nasogastric tube	Panthenol and/or lukewarm water from nasogastric tube	DKT significantly improved the timing and disappearance of intestinal gas.	No
Kubo et al. (65)	RCT	30	56.1 ± 22.6/53.3 ± 21.5	Simple adhesive ileus	DKT from ileus tube	Lukewarm water from ileus tube	The duration to defecation, exhaust gas, and ileus tube removal were not significantly different between DKT administration and control. However, DKT reduced abdominal bloating, nausea, and vomiting.	N/A

DKT, daikenchuto; RCT, randomized controlled trial; N/A, not assigned.

included elderly subjects (66). Postoperative total oral/enteral caloric intake was significantly accelerated in the DKT group. Portal venous flow volume and velocity were significantly higher in the DKT group. This suggests that postoperative administration of DKT may enhance total oral/enteral caloric intake and portal venous flow volume and velocity after liver transplantation and favorably contribute to the performance of the Enhanced Recovery After Surgery protocol. Shimada et al. reported the effect of DKT administered after hepatic resection in elderly patients with liver cancer (67). DKT improved gastrointestinal dysmotility and reduced serum CRP levels in patients with grade B liver damage after hepatectomy. This suggests that DKT is an effective and safe treatment option after hepatic resection in patients with liver cancer. Masaki et al. also reported the effect of DKT in patients who underwent hepatic resection. DKT significantly decreased the levels of CRP and beta-(1–3)-D-glucan on postoperative day 3. DKT significantly shortened postoperative periods for the first flatus, bowel movement, and full recovery of oral intake (68). Takahashi et al. reported the effect of DKT in patients with liver resection. DKT significantly lowered postoperative serum ammonia levels with low occurrence of diarrhea (69).

Other Diseases and Conditions Related to the Digestive System

Table 3D shows an RCT of Kampo medicine for other conditions and symptoms related with the digestive system. Bessho et al. reported the effectiveness of saibokuto (SBT) for patients with glossodynia in an RCT that included elderly subjects (70). When compared with diazepam with vitamin B complex, SBT significantly reduced the symptoms of pain, burning sensation, and discomfort.

In an RCT, Okabayashi et al. reported the effects of inchinkoto (ICKT) on the bilirubin reduction rate after biliary drainage in elderly patients with obstructive jaundice (71). ICKT significantly improved jaundice following biliary drainage and also improved subjective symptoms such as loss of appetite and general fatigue. Miyazaki et al. reported the efficacy of ninjinyoeito (NYT) for dry mouth induced by oxybutynin hydrochloride to treat psychogenic frequency or unstable bladder in an RCT that included elderly subjects (72). The addition of NYT reduced the symptom of dry mouth in 75% of patients. Saliva secretion also improved after NYT addition.

Kampo Medicine for Symptoms of Dementia

Dementia has become a global health issue. The number of people living with dementia in the world was estimated to be 46.8 million in 2015 (73). Japan has one of the most rapidly aging societies; the prevalence of dementia was already beyond 3% (five million) in 2015, and it has been increasing, even though the Japanese population has begun to decline (74). This situation might drive doctors to conduct a number of clinical studies using Kampo medicine for the symptoms of dementia. Dementia is a syndrome associated with declines in memory, thinking, behavior, and the ability to perform daily activities. Cognitive disorders and noncognitive symptoms, that is, behavioral and psychological symptoms of dementia (BPSD), are equally important clinical manifestations. Kampo medications are composed of multiple herbal ingredients and have different target symptoms. Therefore, based on the current clinical evidence, we herein introduce some Kampo medications and their target symptoms (**Table 4**).

Cognitive Disorders

Chotosan (CTS) was originally used for headache, tinnitus, and dizziness. In 1994, Shimada et al. conducted a multicenter placebo-controlled RCT using CTS (75). After 12 weeks of CTS treatment, patients with vascular dementia had a decrease in the score of cognitive dysfunctions (Hasegawa's Dementia Scale-Revised) when compared to baseline. CTS was superior to the placebo in the global improvement rating, utility rating, global rating for subjective symptoms, subjective symptoms (shoulder stiffness and palpations), global rating for psychiatric symptoms, psychiatric symptoms (decline in interest in television or books and lack of facial expression), and activities of daily living (ADLs). Terasawa et al. conducted another placebo-controlled RCT using CTS for vascular dementia (76). When compared with the placebo, the global improvement rating, global rating for subjective symptoms, psychiatric symptoms (decline in simple arithmetic ability, global intellectual ability, sleep disturbance, hallucination, and delusion), and ADLs significantly improved after a 12-week administration of CTS. In the study, cognitive dysfunctions did not improve. The overall safety rating did not differ significantly between the chitosan treatment group and the placebo group. Suzuki et al. reported that an 8-week treatment of CTS improved cognitive dysfunction [assessed by the Mini-Mental State Examination (MMSE)] and ADL when compared to the baseline in patients with Alzheimer's disease; goshajinkigan (GJG) treatment and the placebo did not improve these symptoms (77). In 2017, Imai et al. conducted a meta-analysis of the three above-mentioned RCTs to assess the effectiveness and acceptability of CTS (78). CTS was more effective than the placebo for short-term improvement of cognitive function. The acceptability, measured in terms of the number of dropouts due to adverse effects, did not differ between the CTS treatment group and the placebo group. However, the results are considered imprecise, partly because of the small number of participants. Iwasaki et al. conducted a placebo-controlled DB-RCT using hachimijiogan (HJG), a pill made with herbs and honey, for the treatment of dementia (79). Administration of HJG for 8 weeks significantly improved cognitive dysfunction (assessed by MMSE) and ADL (assessed by the Barthel index) when compared to baseline, while the placebo did not change those scores. No adverse events were observed. Maruyama et al. reported the effectiveness of a combination of donepezil and kamiuntanto on cognitive function and brain perfusion in patients with Alzheimer's disease (80). A 12-week observer-blinded RCT revealed that combination treatment with a donepezil and kamiuntanto decoction significantly improved cognitive function (MMSE and ADAS-cog) when compared with treatment with donepezil alone. Furthermore, cerebral blood flow in the frontal region (measured by single photon emission computed tomography) significantly increased in the

TABLE 3D | RCT of Kampo medicine for other conditions and symptoms related with the digestive system.

References	Study design	Subjects (*n*)	Age, years (mean ± SD or range)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse event related with Kampo
Bessho et al. (70)	RCT	200	61.3 (28–85)	Glossodynia	Saibokuto	Diazepam with vitamin B complex	Symptom of pain, burning sensation, and discomfort were significantly reduced compared with control.	N/A
Okabayashi et al. (71)	RCT	24	68.3 ± 7.5/68.1 ± 7.6	Patients with obstructive jaundice who received percutaneous transhepatic cholangio-drainage	Inchinkoto with drainage	Drainage	Inchinkoto significantly improved jaundice following biliary drainage and also improved subjective symptoms such as loss of appetite and general fatigue.	N/A
Miyazaki et al. (72)	RCT	16	52.3 (31–72)	Dry mouth patients who received oxybutynin hydrochloride	Ninjinyoeito added to oxybutynin hydrochloride	Oxybutynin hydrochloride	Ninjinyoeito addition reduced the symptoms of dry mouth. Gum test also showed improvement of saliva symptoms with ninjinyoeito administration.	N/A

RCT, randomized controlled trial; N/A, not assigned.

TABLE 4 | RCT of Kampo medicine for symptoms of dementia.

References	Study design	Subjects (n)	Age, years (mean ± SD)	Disease/ symptom	Kampo formulation	Comparator	Outcome	Adverse event related with Kampo
Shimada et al. (75)	RCT	57	78.9 ± 7.6	VD and subarachnoid hemorrhage	CTS	Placebo	The scores of cognitive dysfunction (Hasegawa's Dementia Scale-Revised), the global improvement rating, utility rating, global rating for subjective symptoms, subjective symptoms (shoulder stiffness and palpations), global rating for psychiatric symptoms, psychiatric symptoms (decline in interest in television or books, lack of facial expression), and ADL were decreased after 12-week administration of CTS compared to placebo.	1 liver dysfunction in 1 case
Terasawa et al. (76)	DB-RCT	139	76.6 ± 8.4	VD	CTS	Placebo	The global improvement rating, global rating for subjective symptoms, psychiatric symptoms (decline in simple arithmetic ability, global intellectual ability, sleep disturbance, hallucination, and delusion), and ADL were alleviated after 12-week administration of CTS compared to placebo.	Urtic aria in one, diarrhea in one, appetite, in one loss, oral bitterness in one, and liver dysfunction in one case (no information of intervention group)
Suzuki et al. (78)	DB-RCT	30	84.4 ± 6.3	AD and VD	CTS/GJG	Placebo	CTS improved the scores of cognitive dysfunction (MMSE), and ADL, compared to placebo.	N/A
Iwasaki et al. (79)	DB-RCT	33	84.4 ± 7.8	Mixed dementia and AD	HJG	Placebo	Cognitive dysfunction (MMSE), and ADL were improved after treatment of HJG compared to placebo.	No
Maruyama et al. (77)	Observer blind RCT	38	73.7 ± 5.6 74.6 ± 3.9	AD	Kamiuntanto add to donepezil	Donepezil	Kamiuntanto added to donepezil improved the scores of MMSE, and Alzheimer's Disease Assessment Scale, compared to donepezil alone.	No
Higashi et al. (81)	RCT	75	82.8 ± 8.1 84.2 ± 6.4 86.1 ± 5.0	AD	KHT/GJG	No treatment	The scores of MMSE improved only in the KHT treatment group. The orientation and attention subscale scores of the MMSE improved significantly in the KHT-treatment group.	N/A
Iwasaki et al. (82)	Observer blind RCT	52	80.3 ± 9.0	AD, VD, mixed dementia and DLB	YKS	No treatment	Four-week administration of YKS significantly improved BPSD, especially in hallucinations, agitation/aggression, irritability/lability, and aberrant motor activity, and ADL.	No

(Continued)

TABLE 4 | Continued

References	Study design	Subjects (n)	Age, years (mean ± SD)	Disease/ symptom	Kampo formulation	Comparator	Outcome	Adverse event related with Kampo
Mizukami et al. (83)	RCT- cross over	*N* = 88	78.7 ± 5.4	AD and DLB	YKS	No treatment	BPSD, especially in agitation/aggression and irritability/lability subscale scores, were improved after treatment of YKS.	Gastrointestinal distress in 3 cases
Monji et al. (84)	RCT	*N* = 15	80.2 ± 4.0	AD	YKS add to sulpiride	Sulpiride	The average dose of sulpiride tened to be less in the YKS treatment group than the control group.	Hypokalemia in 2 cases
Okahara et al. (85)	RCT	*N* = 61	76.1 ± 8.1 77.1 ± 6.8	AD	YKS add to donepezil	Donepezil	The scores of BPSD decreased in the YKS treatment group. The subscale score of agitation and irritability decreased significantly.	N/A
Teranishi et al. (86)	Rater blind RCT	*N* = 76	83.5 ± 5.8 80.7 ± 8.8 83.2 ± 5.4	AD, VD, and DLB	YKS	Risperidone/fluvoxamine	The three intervention significantly alleviated BPSD. The adverse events were more frequent in the risperidone-treatment group.	No
Fukuhara et al. (87)	DB-RCT	*N* = 145	78.3 ± 5.4 78.5 ± 5.1	AD	YKS	Placebo	The BPSD scores did not change in both YKS and placebo intervention. The subgroup scoring below 20 points on the MMSE at baseline showed a greater improvement in BPSD, especially in agitation/aggregation in the YKS-treatment group, compared to the placebo group. In the subgroup younger than 74 years of age, a significant decrease in the score for agitation/aggression was shown in the YKS-treatment group.	Hypokalemia in 3 cases

VD, Vascular dementia; CTS, chotosan; ADL, activity of daily living; AD, Alzheimer's disease; GJG, goshajinkigan; HJG hachimijiogan; MMSE, Mini-Mental State Examination; KHT, kihito; DLB, dementia with Lewy body; YKS, yokukansan; BPSD, behavioral and psychological symptoms of dementia; N/A, not assigned; SD, standard deviaton.

combination treatment group. In 2007, Higashi et al. reported the effectiveness of kihito (KHT) extract granules on the cognitive function of patients with Alzheimer's disease (81). The MMSE showed significant improvement 3 months after treatment with KHT, but not in the nontreated or GJG-treated groups. The orientation and attention subscale scores of the MMSE improved significantly in the KHT treatment group when compared with those of the nontreatment group. No adverse events were observed in any of the groups.

BPSD

Yokukansan (YKS) was originally used in children for the treatment of agitation and crying at night. Starting in the 1980s, when the Japanese society shifted to an aging society, YKS began to be used for the treatment of BPSD. Five RCTs and one meta-analysis have shown the efficacy of YKS for BPSD, especially for delusions, hallucinations, and agitation/aggression. In 2005, Iwasaki et al. firstly conducted a multicenter RCT using YKS for dementia patients (82). A 4-week administration of YKS significantly improved BPSD [assessed by the Neuropsychiatric Inventory (NPI)], especially hallucinations, agitation/aggression, irritability/lability, and aberrant motor activity. YKS also improved ADL (assessed by the Barthel index). In an RCT conducted by Mizukami et al., 88 dementia patients received 4 weeks of YKS treatment and spent another 4 weeks under observation (no treatment) in a crossover design (83). BPSD improved in the YKS treatment period, and no rebound phenomenon was observed in the following observation period. Monji et al. reported that 12 weeks of YKS treatment significantly improved BPSD in patients with Alzheimer's disease (84). The average dose of antipsychotics (sulpiride) tended to be less in the YKS treatment group than in the control group. The Barthel index did not change in the YKS treatment group or the control group. In 2010, Okahara et al. reported the efficacy of 4 weeks of treatment with YKS and donepezil for BPSD in patients with Alzheimer's disease (85). Among the NPI subscales, the agitation and irritability scores decreased significantly. Cognitive dysfunction, ADL, and caregiver burden scores did not change in the YKS treatment group or in the control group. Teranishi et al. reported the efficacy and safety of YKS compared with risperidone and fluvoxamine for BPSD in patients with dementia (86). All three drugs significantly alleviated BPSD, with no significant intergroup differences. The tolerability analysis revealed that adverse effects (constipation, muscle rigidity, and extrapyramidal symptoms) were more frequent in the risperidone treatment group. In 2016, Furukawa et al. conducted a placebo-controlled DB-RCT on patients with Alzheimer's disease (87). Both 4 weeks of YKS treatment and the placebo improved BPSD, with no significant intergroup differences. The subgroup scoring below 20 points on the MMSE at baseline showed a greater improvement in BPSD, especially in agitation/aggregation in the YKS treatment group, when compared to the placebo group. In the subgroup younger than 74 years of age, a significant decrease in the subcategory score for agitation/aggression was shown in the YKS treatment group when compared with the placebo group. In 2016, Matsunaga et al. conducted a meta-analysis of the above-mentioned RCTs using YKS for BPSD in dementia patients (88). YKS significantly decreased total BPSD scores when compared with the controls (placebo or usual care), especially the subscale scores for delusions, hallucinations, and agitation/aggression. However, only in the Alzheimer's disease patients, YKS was not superior to the controls for BPSD. YKS treatment significantly improved ADL when compared with the controls. MMSE scores did not improve in the YKS treatment group or in the control group. Incidence of adverse effects did not differ significantly between the YKS treatment and control groups. Various Kampo formulations are clinically effective for the treatment of dementia. A Kampo medicine may be selected according to the patients' symptoms. Adverse events due to Kampo medicine are not frequent. Therefore, Kampo medicine may be a treatment option for both cognitive dysfunction and BPSD.

Kampo Medicine for Pain Control

In elderly individuals, physical, psychological, and social changes cause various types of chronic pain. Western analgesic medications are used as the basic approach for pain relief; however, modulation of organ systems and pharmacokinetics often induce adverse effects in aging patients. Furthermore, chronic pain often accompanies various symptoms such as coldness, fatigue, and depression. These conditions exacerbate pain and hinder the physical exercise needed to control pain.

Kampo medicine balances the equilibrium of mind and body disturbed due to external and internal factors. As a result, it is possible to relieve pain as well as multiple coexisting symptoms. In Japan, Kampo medicine is empirically assumed to be effective and widely applied for the treatment of pain. However, the suitable formula often differs depending on the patient's personality. This inhibits the performance of large clinical trials; most studies are case reports or case series (**Table 5**). However, animal studies have recently begun to elucidate the mechanisms of Kampo formulae.

Musculoskeletal Pain

Back Pain

Back pain has a prevalence of 24.4% in the Japanese population over 70 years of age (89). Degenerative spine conditions (spondylosis, spinal stenosis, interval disc disease, etc.) and osteoporosis (OP) induce skeletal deformities, joint imbalance, and tension in muscular structures (90), which lead to chronic musculoskeletal pain.

GJG is used to alleviate symptoms in the lower part of the body associated with aging. Hamaguchi et al. reported the efficacy of routine GJG administration for low back pain (LBP) (91). In a retrospective observational study, LBP improved within 6 months in 10 out of 28 patients. Patients with spinal stenosis were less likely to respond to GJG than those without spinal disease. GJG is expected to relieve LBP in patients without spinal disease. In a retrospective cohort study, Oohata et al. reported the efficacy of Kampo medicine in patients with lumbar spinal stenosis (92). Patients received routine medication with or without Kampo treatment. The frequently used Kampo medicines were GJG, HJG, and shakuyakukanzoto.

TABLE 5 | RCT of Kampo medicine for pain.

References	Study design	Subjects (*n*)	Age, years (mean ± SD)	Disease/symptom	Kampo formulation	Comparator	Outcome	Adverse reaction
Nakae et al. (101)	RCT	162	60(16–90) 66(23–90)	Rib fracture	JDI	NSAIDs	Shorten the duration, Lower healthcare expenditure	No adverse reaction in JDI, gastrointestinal symptoms in 2.5% of the NSAIDs group
Majima et al. (104)	RCT	47	68.3 ± 10.0 71.5 ± 6.0	Osterarthritis of knee	BOT	Loxoprofen	Improve the Knee Society Rating System and SF-36	Dry month in a patient
Watanabe et al. (114)	RCT	116	59.4 ± 7.8 60.9 ± 7.4	Diabetes mellitus type 2	GJG	No GJG	Decrease progression neuropathy	None

JDI, jidabokuippo; BOT, boiogito; GJG, goshajinkigan; NSAIDs, non-steroidal anti-inflammatory drugs; SD, standard deviaton.

The rate of reduced and discontinued use of pregabalin and opioid was significantly greater in the Kampo treatment group than in the non-Kampo treatment group. Side effects were observed in 6.3% of patients treated with Kampo medicine and in 62.5% of patients treated without Kampo medicine. Hamaguchi et al. reported the efficacy of Kampo medicine for symptoms in the lower extremities caused by various lumbar spinal diseases (93). In a retrospective observational study, the addition of Kampo medicine to Western medications relieved pain in 53% of patients and relieved coldness in 50% of patients. On the other hand, numbness was improved in only 21% of patients. The effective formulae included shimbuto, keishikajutsubuto (KSTJB), ryokeijutsukanto, and GJG. Coldness is an uncomfortable symptom that exaggerates chronic pain. Takahashi et al. reported the effectiveness and safety of tokishigyakukagoshuyushokyoto (TSGS) for improving coldness with LBP (94). This retrospective observation study showed that 74% of patients were satisfied with the relief from coldness. The VAS significantly decreased from 57.7 ± 11.4 to 43.7 ± 14.1. Routine treatment combined with Kampo medicine may be safer and more effective than treatment using only Western medicines. There are some case reports on back pain. In four case reports, Western treatment involving nerve block could not relieve the pain of geriatric patients with lumbar spinal stenosis. However, Kampo treatment was successful (95–98). In these case reports, some patients were able to avoid surgery. Patients often suffer from residual symptoms after operations for spinal diseases. Ogawa et al. reported a case of postoperative residual pain, coldness, and numbness treated with Kampo medicine (99).

OP-induced fractures result in severe pain. Tetsumura et al. reported a case of multiple OP-induced fractures treated with Kampo medicine (100). KSTJB and other Kampo formulae diminished the suffering of a bedridden patient. The patient was able to stand 11 months after treatment. Nakae et al. performed an RCT to compare the efficacies of jidabokuippo (JDI) and nonsteroidal anti-inflammatory drugs (NSAIDs) in young and old patients with rib fracture (101). The treatment duration was significantly shorter in the JDI group than in the NSAID group. Furthermore, healthcare expenditure was significantly lower in the JDI group than in the NSAID group.

Osteoarthritis

Osteoarthritis (OA) has a prevalence of 32.5% in the Japanese population over 60 years of age (102). The pain of OA is attributed to unstable joint structure, anatomical degeneration, and inflammation (103). Though standard pharmacotherapy is used for nociceptive pain, it is sometimes ineffective. Boiohito (BOT) is often used for arthritis of the knee. In an RCT, Majima et al. reported the clinical efficacy of BOT on OA of the knee (104). Patients were assigned to the concomitant-use group (both loxoprofen and BOT) and the loxoprofen group (loxoprofen alone). The knee score, based on the Knee Society Rating System and the 36-item short form from the Medical Outcome Study Questionnaire (SF-36), improved in both groups. However, the score for the ability to climb up and down a staircase, based on the Knee Society Rating System functional score and joint fluid, was significantly improved in the concomitant-use group compared to the loxoprofen group.

Bushi is a crude drug with an analgesic effect. In a nonrandomized prospective study, Nakae reported the efficacy and safety of bushimatu (powdered processed aconite root) for the treatment of pain associated with orthopedic disease (105). OA of the knee was the most common orthopedic disease. Patients were administered bushimatu (1.5–8 g/day) with other Kampo formulae without NSAIDs. Patients with ≥50% and ≥25% reductions in VAS accounted 102 and 84 out of 257 patients, respectively, 4 weeks after treatment. Three patients (1.2%) experienced side effects.

Neuropathic Pain

Neuropathic pain develops after difficult-to-treat injury of neurons along nociceptive pathways. YKS has a variety of neuropharmacological actions, such as neuroprotection, anti-stress effect, promotion of neuroplasticity, and anti-inflammatory effect (106). Therefore, YKS is sometimes used to treat neuropathic pain. Nakamura et al. reported 11 cases (36–85 years old) of successful treatment of neuropathic pain [postherpetic neuralgia (PHN), central pain, complex regional pain syndrome, and trigeminal neuralgia] using YKS (107). The patients had VAS scores of 17–81 despite Western conventional

treatment. The VAS scores decreased to 0–22 after YKS administration for 2 days to 2 months.

PHN is a persisting neuropathic pain syndrome that occurs after resolution of a herpes zoster (HZ) rash. The frequency increases with age, occurring in 20% of people aged 60–65 years and in more than 30% of people aged >80 years who had acute HZ (108). Nakabayashi et al. published a case series on medication combined with KBG and bushimatsu for patients with PHN (2–92 months after HZ onset) (109). The VAS score improvement rate was 76.5 ± 27.7%. However, three of 15 patients could not continue the study due to hot flashes and gastric discomfort. There are some reports of successful PHN treatment with Kampo medicine (110–112). In these reports, patients had suffered from PHN from 2 months to 2 years. From 8 weeks to 4 months after treatment, their symptoms disappeared with Kampo medicine. However, in some cases, pain worsened again when the Kampo medication was discontinued. Radical treatment of PHN may be difficult, but it may be effective if Kampo medication is started during the acute stage of herpes infection.

In elderly people, the prevalence of diabetes increases due to glucose intolerance. Diabetic neuropathy is the most common chronic complication, with an estimated lifetime prevalence exceeding 50% (113). In an RCT, Watanabe et al. reported the efficacy of GJG on the progression of type 2 diabetes complications in middle-aged and older people (114). GJG significantly decreased glycated hemoglobin and progression of neuropathy (ankle reflex) when compared with the control. GJG is also used to prevent and relieve peripheral neuropathy due to chemotherapy (115).

Kampo Medicine for Others

Peripheral Arterial Disease

Peripheral arterial disease (PAD) represents atherosclerotic disease associated with aging. PAD has a prevalence of 15–20% in the Japanese population over 70 years of age (116). The clinical presentation of a reduction in limb blood flow includes peripheral coldness, atypical leg pain, or intermittent claudication; as it progresses, it may present with ischemic ulcer or critical limb ischemia.

In a prospective study, Kawago et al. reported the efficacy of HJG for improvement of the QOL in patients with PAD (117). The patients were administered HJG for 6 months without any new interventions. The pain score on the Japanese version of the Walking Impairment Questionnaire (WIQ) improved from 25.0 (0.0–50.0) at baseline to 75.0 (68.8–100.0). The absolute change was 37.5 (25.0–75.0). TSGS improved peripheral blood flow and perception of peripheral coldness (118). In a nonrandomized prospective study, Jojima reported the efficacy of TSGS for arteriosclerosis obliterans (ASO) (119). TSGS and cilostazol improved the absolute claudication distance 1 and 3 months after treatment. However, side effects were observed in 4% of patients treated with TSGS, while they were observed in 38% of patients treated without Kampo. One case report has been published regarding Kampo treatment for severe limb pain with ASO (120). A decoction of KBG and daisaikoto relieved pain, coldness, and ischemic ulcers and eliminated the need for limb amputation.

Rehabilitation

Physical exercise is necessary to improve pain and prevent secondary injuries. However, elderly individuals often cannot take exercise sufficiently due to frailty or sarcopenia.

Hozai is one group of Kampo formulations that restore vitality to patients who have lost psychological and physical energy due to various diseases or aging (121). These formulations improve pain in various conditions induced by sarcopenia and frailty, such as fatigue, anorexia, and mental problems. Sakamoto et al. reported their experience of using Kampo, mainly Hozai formulae (RKT, HET, NYT, etc.) for rehabilitation (122). In a prospective non-RCT, Sakisake et al. reported the efficacy of NYT against frailty (123). Administration of NYT for 24 weeks prevented deterioration of muscle mass and muscle quality score when compared to the control group. Furthermore, the NYT group significantly improved grip strength, whereas there was no change in the control group.

DISCUSSION

Here, we reviewed RCTs on the efficacy of Kampo medicine for GS. **Figure 1** shows the relationship between Kampo medicines and organs and physiological systems. One of the characteristics of Kampo medicine is the use of multiple crude drugs (**Table 6**). Therefore, Kampo medicine can act upon multiple organs and physiological systems. HET is effective for COPD, nutrition, anti-inflammation, and QOL; RKT for GERD, NERD, FD, and appetite; and DKT for constipation and perioperative conditions. As GS symptoms are expressed by the 3Ms or 4Is, Kampo medicine can contribute to GS.

The possible mechanisms of Kampo medicines have been reported in recent years. For example, YKS is composed of seven crude drugs (**Table 6**) and has been used to improve irritation, insomnia, muscle twitching, and pain. Several studies reported various neuropharmacological actions of YKS, namely, on serotonergic, glutamatergic, cholinergic, dopaminergic, adrenergic, and gamma-aminobutyric acidergic neural systems (124). These actions maintain neural signal conduction and neuronal function of neurons as well as glial cells (125). GJG is composed of 10 crude drugs and has been used to alleviate various types of age-related conditions, including muscle weakness of the lower limbs, dysuria, foot edema, and cold sensation of the lower limbs. Recently, GJG is used to prevent and relieve various types of peripheral neuropathy. GJG has antinociceptive effects via increasing produced nitric oxide (126), reduces hypersensitivity by suppressing the overexpression of TRPM8 and TRPA1 mRNA (127), and ameliorates allodynia via the suppression of TNF-α expression in the spinal cord (128). Furthermore, GJG has also been reported to suppress sarcopenia via the insulin growth factor-1/insulin pathway, maintains the expression of mitochondrial-related transcription factors, and suppresses the expression of TNF-α (129).

DKT is composed of four crude drugs and has been used to treat abdominal pain and abdominal bloating with abdominal coldness. DKT treats abdominal symptoms by enhancing the secretions of motilin (130), substance-P, calcitonin gene-related

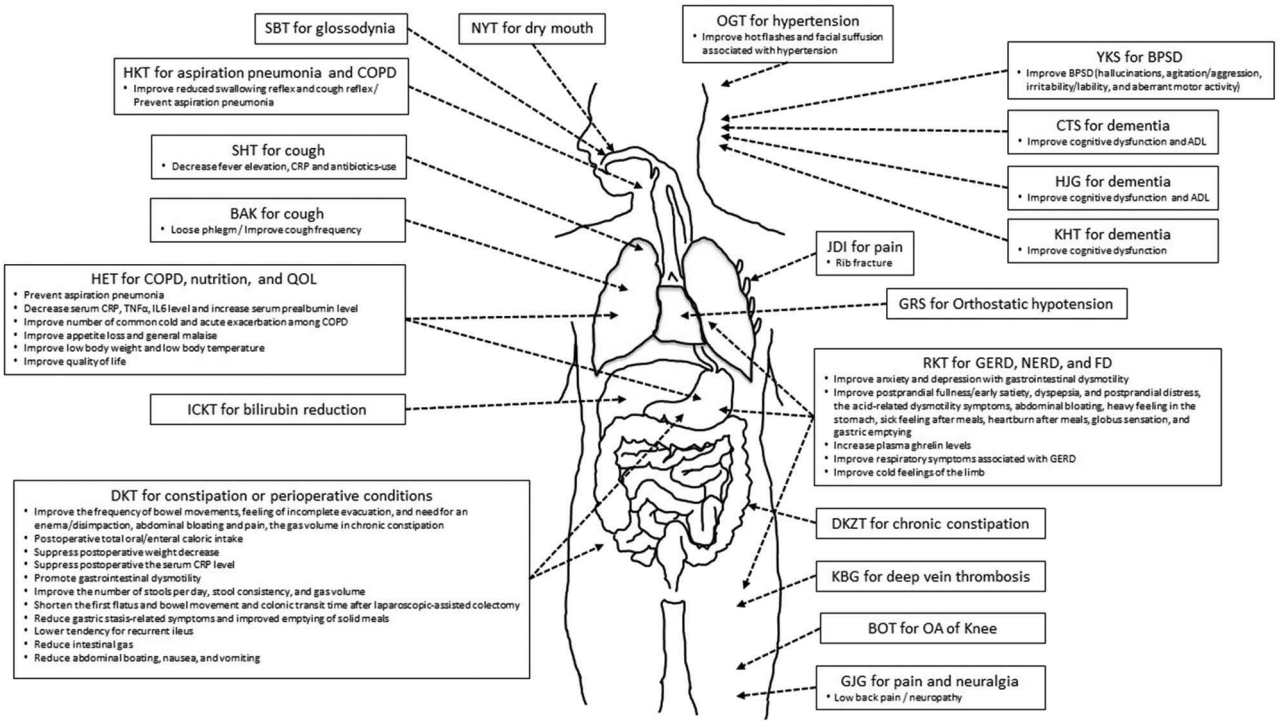

FIGURE 1 | The relationship between Kampo medicines and symptome/condition/disorder in organs and viscera.

peptide, and adrenomedullin (131–133) and activating the transient receptor potential of the vanilloid receptor complex (134). RKT is composed of eight crude drugs and has been used to treat appetite loss, upper abdominal discomfort, and indigestion. A recent study reported that RKT increases plasma ghrelin levels in humans and mice (135) and restores decreased plasma ghrelin levels induced by serotonin release in rats. HKT is composed of five crude drugs and has been used for pharyngeal discomfort. It has been reported that HKT modulates cerebral levels of 5-hydroxytryptamine, noradrenaline, and dopamine in mice (20).

The efficacy and safety of Kampo medicine were investigated in several clinical studies. Based on these reports, clinical practice guidelines have recommended Kampo medicines for symptoms in geriatrics. Our previous study (136), conducted between January 1, 2012, and October 31, 2017, showed that the Clinical Practice Guideline for the Pruritus Cutaneous Universalis (137), Practical Guideline for the Management of Allergic Rhinitis in Japan (138), the Japanese Respiratory Society Guidelines for the Management of Cough (139), Evidence-Based Clinical Practice Guidelines for GERD (140), Evidence-Based Clinical Practice Guidelines for Functional Dyspepsia (141), Evidence-Based Clinical Practice Guidelines for Irritable Bowel Syndrome (142), Evidence-Based Clinical Practice Guidelines for Chronic Constipation (143), Clinical Guidelines for Overactive Bladder Syndrome (144), and Practice Guideline for Dementia (145) have recommended the use of Kampo medicines for skin symptoms, allergy, cough, gastrointestinal dysfunction, urinary dysfunction, and dementia.

In recent years, the usefulness of Kampo medicine in the clinical setting has been investigated using the diagnosis procedure combination (DPC) inpatient database in Japan (**Table 7**). A propensity score analysis using DPC is a retrospective investigation; however, the groups of patients with or without intervention can be matched, and the subject number is large. Thus, this method can show the intervention's effect and influence on the social economy. Jo et al. reported a reduction in the exacerbation of COPD in patients of advanced age using DKT (33). DKT users had a significantly lower risk of rehospitalization or death after discharge. Subgroup analysis of long-acting muscarinic receptor antagonist users showed a significant difference in rehospitalization or death, while subgroup analysis of long-acting muscarinic receptor antagonist nonusers showed no significant difference. Yasunaga reported the effects of GRS on reoperation rates after burr-hole surgery for chronic subdural hematoma (146). GRS use was significantly associated with a lower reoperation rate when compared with nonuse. These results suggest that GRS use reduced the need for reoperation after burr-hole surgery for chronic subdural hematoma. Yasunaga et al. also reported effects of DKT on postoperative adhesive small bowel obstruction requiring long-tube decompression (LTD) (147). DKT use was associated with a significantly shorter duration of LTD, a shorter duration between long-tube insertion and discharge, and

TABLE 6 | Kampo medicines and their crude drugs by RCT.

Kampo medicine	Indication as per the Japanese Pharmacopeia (i.e.,)	Included crude drugs						
Orengedokuto (OGT)	Patients with ruddy face with comparatively strong constitution, a touch of hot flushes, and a tendency to irritability: nose bleeding, hypertension, insomnia, neurosis, gastritis, alcoholic hangover, climacteric disturbance and automatic imbalance syndrome peculiar to women resembling climacteric disturbance, dizziness, palpitation, eczema or dermatitis, and pruritus cutaneous	JP Scutellaria Root	JP Coptis Rhizome	JP Gardenia Fruit	JP Phellodendron Bark			
Goreisan (GRS)	Patients with oral dryness and decreased urine volume: edema, nephrosis, alcoholic hangover, acute gastrointestinal catarrh, diarrhea, nausea, vomiting, dizziness, water retention in the stomach, headache, uremia, heat-stroke, and diabetes mellitus	JP Alisma Rhizome	JP Atractylodes Lancea Rhizome	JP Polyporus Sclerotium	JP Poria Sclerotium	JP Cinnamon Bark		
Keishibukuryogan (KBG)	Patients with solid constitution who have ruddy face and generally solid abdomen with resistance in the lower abdomen: inflammation in the uterus and its adnexa, endometritis, menstrual irregularity, dysmenorrhea, leukorrhea, climacteric disturbance (headache, dizziness, feeling of hot flushes, shoulder stiffness, etc.), oversensitivity to cold, peritonitis, contusion, hemorrhoid, and orchitis	JP Cinnamon Bark	JP Peony Root	JP Peach Kernel	JP Poria Sclerotium	JP Moutan Bark		
Rikkunshito (RKT)	Patients with weak stomach, loss of appetite and full stomach pit, and those who are easily fatigued, anemic and likely to have cold limbs: gastritis, gastric atony, gastroptosis, maldigestion, anorexia, gastric pain, and vomiting	JP Atractylodes Lancea Rhizome	JP Ginseng	JP Pinellia Tuber	JP Poria Sclerotium	JP Jujube	JP Citrus Unshiu Peel	JP Glycyrrhiza	JP Ginger

(Continued)

TABLE 6 | Continued

Kampo medicine	Indication as per the Japanese Pharmacopeia (i.e.,)	Included crude drugs											
Daikenchuto (DKT)	Abdominal cold feeling and pain accompanied by abdominal flatulence	JP Processed Ginger	JP Ginseng	JP Zanthoxylum Fruit	JP Koi								
Daiokanzoto (DKZT)	Constipation	JP Rhubarb	JP Glycyrrhiza										
Saibokuto (SBT)	Patients who have depressed feelings and a feeling of foreign body in the throat and esophagus and who sometimes have palpitation, dizziness, nausea, etc.: infantile asthma, bronchial asthma, bronchitis, coughing, and anxiety neurosis	JP Bupleurum Root	JP Pinellia Tuber	JP Poria Sclerotium	JP Scutellaria Root	JP Magnolia Bark	JP Jujube	JP Ginseng	JP Glycyrrhiza	JP Perilla Herb	JP Ginger		
Ninjinyoeito (NYT)	Declined constitution after recovery from disease, fatigue and malaise, anorexia, perspiration during sleep, cold limbs, and anemia	JP Rehmannia Root	JP Japanese Angelica Root	JP Atractylodes Rhizome	JP Poria Sclerotium	JP Ginseng	JP Cinnamon Bark	JP Polygala Root	JP Peony Root	JP Citrus Unshiu Peel	JP Astragalus Root	JP Glycyrrhiza	JP Schisandra Fruit
Inchinkoto (ICKT)	Patients with a comparatively strong constitution and decreased urine volume who are somewhat likely to have constipation: jaundice, hepatic cirrhosis, nephrosis, urticaria, and stomatitis	JP Artemisia Capillaris Flower	JP Gardenia Fruit	JP Rhubarb									
Chotosan (CTS)	Chronic headache with hypertension in those middle-aged or elderly	JP Gypsum	JP Uncaria Hook	JP Citrus Unshiu Peel	JP Ophiopogon Tuber	JP Pinellia Tuber	JP Poria Sclerotium	JP Chrysanthemum Flower	JP Ginseng	JP Saposhnikovia Root	JP Glycyrrhiza	JP Ginger	
Hachimijiogan (HJG)	Patients with severe fatigue or malaise, decreased urinary output or increased urinary frequency, dry mouth, and alternate cold and hot feeling in the extremities: nephritis, diabetes mellitus, impotence, sciatica, low back pain, beriberi, cystorrhea, prostatic hypertrophy, and hypertension	JP Rehmannia Root	JP Cornus Fruit	JP Dioscorea Rhizome	JP Alisma Rhizome	JP Poria Sclerotium	P Moutan Bark	JP Cinnamon Bark	JP Powdered Processed Aconite Root				
Kihito (KHT)	patients with a delicate constitution and a poor complexion: anemia and insomnia	JP Astragalus Root	JP Jujube Seed	JP Ginseng	JP Atractylodes Rhizome	JP Poria Sclerotium	JP Longan Aril	JP Polygala Root	JP Jujube	JP Japanese Angelica Root	JP Glycyrrhiza	JP Ginger	JP Saussurea Root
Yokukansan (YKS)	Patients with delicate constitution and nervousness: neurosis, insomnia, night cry in children, and peevishness in children	JP Atractylodes Lancea Rhizome	JP Poria Sclerotium	JP Cnidium Rhizome	JP Uncaria Hook	JP Japanese Angelica Root	JP Bupleurum Root	JP Glycyrrhiza					

(Continued)

TABLE 6 | Continued

Kampo medicine	Indication as per the Japanese Pharmacopeia (i.e.,)	Included crude drugs									
Hangekobokuto (HKT)	Patients who have depressed feelings and a feeling of foreign body in the throat and esophagus and who sometimes have palpitation, dizziness, nausea, etc.: anxiety neurosis, nervous gastritis, hyperemesis gravidarum, coughing, hoarseness, nervous esophageal stricture, and insomnia	JP Pinellia Tuber	JP Poria Sclerotium	JP Magnolia Bark	JP Perilla Herb	JP Ginger					
Bakumondoto (BAK)	Coughing with a hard, obstructive sputum, bronchitis, and bronchial asthma	JP Ophiopogon Tuber	JP Brown Rice	JP Pinellia Tuber	JP Jujube	JP Glycyrrhiza	JP Ginseng				
Goshajinkigan (GJG)	Patients with decreased urine volume or polyuria sometimes having dry mouth who are easily fatigued and easily feel cold in the extremities: leg pain, low back pain, numbness, blurred vision in old patients, pruritus, dysuria, frequent urination, and edema	JP Rehmannia Root	JP Achyranthes Root	JP Cornus Fruit	JP Dioscorea Rhizome	JP Plantago Seed	JP Alisma Tuber	JP Poria Sclerotium	JP Moutan Bark	JP Cinnamon Bark	JP Powdered Processed Aconite Root
Jidabokuippo (JDI)	Swelling and pain caused by contusion	JP Cnidium Rhizome	JP Atractylodes Lancea Rhizome	JP Forsythia Fruit	JP Lonicera Leaf and Stem	JP Saposhnikovia Root	JP Glycyrrhiza	JP Schizonepeta Spike	JP Safflower	JP Rhubarb	
Boiogito (BOT)	Patients with a white-complexion, soft muscles, and a flabby constitution who are easily fatigued, perspire profusely, do not excrete enough urine, and develop edema in the lower limbs and swelling and pain of the knee joint: nephritis, nephrosis, nephropathy of pregnancy, hydrocele testis, obesity, arthritis, carbuncle, furuncle, myositis, edema, dermatosis, hyperhidrosis, and menstrual irregularity	JP Astragalus Root	JP Sinomenium Stem	JP Atractylodes Lancea Rhizome	JP Jujube	JP Glycyrrhiza	JP Ginger				

TABLE 7 | Propensity score analysis of Kampo medicine.

References	Study design	Subjects (*n*)	Age, years (mean ± SD)	Disease/ symptom	Kampo formulation	Comparator	Outcome
Jo et al. (146)	Propensity score analysis	2385	82.1 ± 4.8 82.1 ± 4.8	Chronic obstructive pulmonary disease exacerbation	DKT	No DKT	DKT users had a significantly lower risk of re-hospitalization or death after discharge. Subgroup analysis of long-acting muscarinic receptor antagonists users showed a significant difference in re-hospitalization or death, while subgroup analysis of long-acting muscarinic receptor antagonists non-users showed no significant difference.
Yasunaga (147)	Propensity score analysis	7758	76.2 (10.7) 76.2 (10.7)	Chronic subdural hematoma	GRS	No GRS	GRS use was significantly associated with a lower reoperation rate compared with non-use.
Yasunaga et al. (148)	Propensity score analysis	288	68.4 ± 10.1 67.9 ± 9.1	Postoperative adhesive small bowel obstruction requiring long-tube decompression	DKT	No DKT	Patients who received DKT showed significant shorter duration of long-tube decompression (LTD), shorter duration between long-tube insertion and discharge, and lower hospital charges compared with patients without DKT. It suggested that DKT is effective for reducing the duration of LTD and saving costs.

DKT, daikenchuto; GRS, goreisan; SD, standard deviation.

lower hospital charges when compared with DKT nonuse. This suggests that DKT effectively reduces the duration of LTD and saves costs.

Not only the efficacy but also adverse drug reactions (ADRs) were reported in RCTs of Kampo medicine (148). The total incidence of ADRs was 2.47%, and those of pseudoaldosteronism and liver disorders caused by Kampo medicine were 0.02 and 0.16%, respectively. In our previous study, the incidence of ADRs was 0.09% for BAK, 0.44% for DKT, 2.04% for RKT, 1.7% for GJG, 3.45% for HET, 3.34% for CTS, 4.41% for NYT, and 5.17% for YKS. Many of the ADRs were gastrointestinal disorders.

Due to an increase in Japan's "super-aging population" and a decline in the country's birth rate, medical expenses are expected to increase and pose an important problem. Furthermore, medical expenses have grown every year. This review has shown the efficacy, safety, and the social and economic advantages associated with Kampo treatment.

AUTHOR CONTRIBUTIONS

ST designed this report. ST, NT, RO, RA, and AK collected and selected the articles and wrote the manuscript. TI revised the manuscript.

REFERENCES

1. Inouye SK, Studenski S, Tinetti ME, Kuchel GA. Geriatric syndromes: clinical, research, and policy implications of a core geriatric concept. *J Am Geriatr Soc.* (2007) 55:780–91. doi: 10.1111/j.1532-5415.2007.01156.x
2. Morley JE. A brief history of geriatrics. *J Gerontol A Biol Sci Med Sci.* (2004) 59:1132–52. doi: 10.1093/gerona/59.11.1132
3. Walston JD. Geriatric syndromes in clinical practice. In: Michel JP, Beattie L, Martin FC, Walston JD, editors. *Oxford Textbook of Geriatric Medicine, Third Edition*. Oxford: Oxford University Press (2018).
4. Schwab WS. Geriatric syndromes. *J Am Geriatr Soc.* (2008) 56:363–4. doi: 10.1111/j.1532-5415.2007.01488.x
5. Nanao M, Kojima T, Yamaguchi Y, Ogawa S, Akishita M. An elderly man with rapidly progressive depression and activities of daily living decline: case report of late-onset hypogonadism syndrome. *Geriatr Gerontol Int.* (2015) 15:1098–9. doi: 10.1111/ggi.12502
6. Stevenson JM, Davies JG, Martin FC. Medication-related harm: a geriatric syndrome. *Age Ageing.* (2019) 49:7–11. doi: 10.1093/ageing/afz121
7. Ministry of Health, Labour and Welfare in Japan. *Trends in Medical Expenses*. Tokyo: Ministry of Health, Labour and Welfare (2020). Available online at: https://www.mhlw.go.jp/topics/medias/year/18/index.html. Japanese.
8. STORK (2018). Available online at: http://mpdb.nibiohn.go.jp/stork/
9. Hagihara K, Abbreviation of Kampo Formulations Group, Yakubo S, Committee for Vocabulary in the Japan Society for Oriental Medicine, Namiki T, Makino T, et al. Abbreviation of kampo formulations and basic terminology in kampo medicine. *Trad Kampo Med.* (2017) 4:65–88. doi: 10.1002/tkm2.1078
10. Arakawa K, Saruta T, Abe K, Iimura O, Ishii M, Ogihara T, et al. Improvement of accessory symptoms of hypertension by TSUMURA Orengedokuto Extract, a four herbal drugs containing Kampo-Medicine Granules for ethical use: a double-blind, placebo-controlled study. *Phytomedicine.* (2006) 13:1–10. doi: 10.1016/j.phymed.2004.02.012
11. Nakamura H, Nakamura T, Nakagawa S, Aizawa Y. Efficacy of goreisan in treatment of orthostatic hypotension in patients with diabetes mellitus. *Diabetes Frontier.* (2000) 11:561–3.
12. Uchida N. A randomized controlled trial of the Chinese herbal medicine keisi-bukuryo-gan (gui-zhi-fu-ling-wan) in the treatment of deep vein thrombosis. *Jpn J Phlebol.* (2009) 20:1–6.

13. Nakagawa T, Ohrui T, Sekizawa K, Sasaki H. Sputum substance P in aspiration pneumonia. *Lancet.* (1995) 345:1447. doi: 10.1016/S0140-6736(95)92638-0
14. Sasaki H, Sekizawa K, Yanai M, Arai H, Yamaya M, Ohrui T. New strategies for aspiration pneumonia. *lntern Med*. (1997) 36:851–5. doi: 10.2169/internalmedicine.36.851
15. Iwasaki K, Wang Q, Nakagawa T, Suzuki T, Sasaki H. The traditional Chinese medicine banxia houpo tang improves swallowing reflex. *Phytomedicine.* (1999) 6:103–6. doi: 10.1016/S0944-7113(99)80043-9
16. Iwasaki K, Wang Q, Seki H, Satoh K, Takeda A, Arai H, et al. The effects of the traditional Chinese medicine, "Banxia Houpo Tang (Hange-Koboku To)" on the swallowing reflex in Parkinson's disease. *Phytomedicine.* (2000) 7:259–63. doi: 10.1016/S0944-7113(00)80042-2
17. Iwasaki K, Cyong JC, Kitada S, Kitamura H, Ozeki J, Satoh Y, et al. A traditional Chinese herbal medicine, banxia houpo tang, improves cough reflex of patients with aspiration pneumonia. *J Am Geriatr Soc.* (2002) 50:1751–2. doi: 10.1046/j.1532-5415.2002.50479.x
18. Iwasaki K, Kato S, Monma Y, Niu K, Ohrui T, Okitsu R, et al. A pilot study of banxia houpu tang, a traditional Chinese medicine, for reducing pneumonia risk in older adults with dementia. *J Am Geriatr Soc.* (2007) 55:2035–40. doi: 10.1111/j.1532-5415.2007.01448.x
19. Kawago K, Nishibe T, Shindo S, Inoue H, Motohashi S, Akasaka J, et al. A double-blind randomized controlled trial to determine the preventive effect of hangekobokuto on aspiration pneumonia in patients undergoing cardiovascular surgery. *Ann Thorac Cardiovasc Surg.* (2019) 25:318–25. doi: 10.5761/atcs.oa.19-00128
20. Kaneko A, Cho S, Hirai K, Okabe T, Iwasaki K, Nanba Y, et al. Hange-koboku-to, a Kampo medicine, modulates cerebral levels of 5-HT (5-hydroxytryptamine), NA (noradrenaline) and DA (dopamine) in mice. *Phytother Res.* (2005) 19:491–5. doi: 10.1002/ptr.1669
21. Nakagawa T, Sekizawa K, Arai H, Kikuchi R, Manabe K, Sasaki H. High incidence of pneumonia in elderly patients with basal ganglia infarction. *Arch Intern Med.* (1997) 157:321–4. doi: 10.1001/archinte.157.3.321
22. Tamano M, Kato S, Okamura A, Ozone S, Hoshino T, Takahashi S. Clinical investigation of hochuekkito for prevention of aspiration pneumonia and improvement of QOL in the elderly. *Sci Kampo Med.* (2016) 40:238–41. Japanese.
23. Tamano M, Kato S, Okamura A, Hoshino T, Takahashi S. Clinical investigation on prevention of aspiration pneumonia and improvement of QOL in the elderly by combined use of hochuekkito and rehabilitation. *Kampo and the Newest Study.* (2017) 26:53–8. Japanese.
24. Mantani N, Kasahara Y, Kamata T, Sekiya N, Shimada Y, Usuda K, et al. Effect of Seihai-to, a Kampo medicine, in relapsing aspiration pneumonia–an open-label pilot study. *Phytomedicine.* (2002) 9:195–201. doi: 10.1078/0944-7113-00111
25. Iwasaki K, Wang Q, Satoh N, Yoshida S, Akaike T, Sekizawa K, et al. Effects of qing fei tang (TJ-90) on aspiration pneumonia in mice. *Phytomedicine.* (1999) 6:95–101. doi: 10.1016/S0944-7113(99)80042-7
26. Sasaki H, Satoh K, Sasaki M, Miyano M, Fujii M, Tezuka M, et al. Usefulness of bakumondoto in senile chronic respiratory disease patients having difficulty in expectoration: comparison with bromhexine hydrochloride preparations. *Kampo and Immuno-allergy.* (1993) 7:139–45. Japanese.
27. Mukaida K, Hattori N, Kondo K, Morita N, Murakami I, Haruta Y, et al. A pilot study of the multiherb Kampo medicine bakumondoto for cough in patients with chronic obstructive pulmonary disease. *Phytomedicine.* (2011) 18:625–9. doi: 10.1016/j.phymed.2010.11.006
28. Kato S, Matsuda T, Nakajima T, Kaneko N, Iwasaki K. Clinical significance of combination therapy of smoking cessation and the traditional Kampo medicine, "qing fei tang (seihaito)" in chronic obstructive pulmonary disease. *Kampo New Ther.* (2005) 14:260–5.
29. Kamei J, Yoshikawa Y, Saito A. Antitussive effect of bakumondoto (mai-men-dong-tang) in guinea-pigs exposed to cigarette smoke. *J Tradit Med.* (2005) 22:44–8. doi: 10.11339/jtm.22.44
30. Shinozuka N, Tatsumi K, Nakamura A, Terada J, Kuriyama T. The traditional herbal medicine hochuekkito improves systemic inflammation in patients with chronic obstructive pulmonary disease. *J Am Geriatr Soc.* (2007) 55:313–4. doi: 10.1111/j.1532-5415.2007.01057.x
31. Tatsumi K, Shinozuka N, Nakayama K, Sekiya N, Kuriyama T, Fukuchi Y. Hochuekkito improves systemic inflammation and nutritional status in elderly patients with chronic obstructive pulmonary disease. *J Am Geriatr Soc.* (2009) 57:169–70. doi: 10.1111/j.1532-5415.2009.02034.x
32. Yamaya M, Sasaki T, Yasuda H, Inoue D, Suzuki T, Asada M, et al. Hochu-ekki-to inhibits rhinovirus infection in human tracheal epithelial cells. *Br J Pharmacol.* (2007) 150:702–10. doi: 10.1038/sj.bjp.0707135
33. Jo T, Michihata N, Yamana H, Sasabuchi Y, Matsui H, Urushiyama H, et al. Reduction in exacerbation of COPD in patients of advanced age using the Japanese Kampo medicine Dai-kenchu-to: a retrospective cohort study. *Int J Chron Obstruct Pulmon Dis.* (2019) 14:129–39. doi: 10.2147/COPD.S181916
34. Hong M, Hong C, Chen H, Ke G, Huang J, Huang X, et al. Effects of the Chinese herb formula Yufeining on stable chronic obstructive pulmonary disease: a randomized, double-blind, placebo-controlled trial. *Medicine (Baltimore).* (2018) 97:e12461. doi: 10.1097/MD.0000000000012461
35. Ma J, Zheng J, Zhong N, Bai C, Wang H, Du J, et al. Effects of YuPingFeng granules on acute exacerbations of COPD: a randomized, placebo-controlled study. *Int J Chron Obstruct Pulmon Dis.* (2018) 13:3107–14. doi: 10.2147/COPD.S170555
36. Guo S, Sun Z, Liu E, Feng J, Fu M, Li Y, et al. Effect of bufei granule on stable chronic obstructive pulmonary disease: a randomized, double blinded, placebo-controlled, and multicenter clinical study. *J Tradit Chin Med.* (2014) 34:437–44. doi: 10.1016/S0254-6272(15)30043-1
37. Tominaga K, Sakata Y, Kusunoki H, Odaka T, Sakurai K, Kawamura O, et al. Rikkunshito simultaneously improves dyspepsia correlated with anxiety in patients with functional dyspepsia: a randomized clinical trial (the DREAM study). *Neurogastroenterol Motil.* (2018) 30:e13319. doi: 10.1111/nmo.13319
38. Tominaga K, Kato M, Takeda H, Shimoyama Y, Umegaki E, Iwakiri R, et al. A randomized, placebo-controlled, double-blind clinical trial of rikkunshito for patients with non-erosive reflux disease refractory to proton-pump inhibitor: the G-PRIDE study. *J Gastroenterol.* (2014) 49:1392–405. doi: 10.1007/s00535-013-0896-9
39. Sakata Y, Tominaga K, Kato M, Takeda H, Shimoyama Y, Takeuchi T, et al. Clinical characteristics of elderly patients with proton pump inhibitor-refractory non-erosive reflux disease from the G-PRIDE study who responded to rikkunshito. *BMC Gastroenterology.* (2014) 14:116. doi: 10.1186/1471-230X-14-116
40. Kato S, Nakajima T, Matsuda T. Efficacy of hangekobokuto for respiratory symptom related with reflex esophagitis. *Kampo and newest theray.* (2005) 14:333–8. [in Japanese]
41. Suzuki H, Matsuzaki J, Fukushima Y, Suzaki F, Kasugai K, Nishizawa T, et al. Randomized clinical trial: rikkunshito in the treatment of functional dyspepsia–a multicenter, double-blind, randomized, placebo-controlled study. *Neurogastroenterol Motil.* (2014) 26:950–61. doi: 10.1111/nmo.12348
42. Hayakawa M, Ono Y, Wada T, Yanagida Y, Sawamura A, Takeda H, et al. Effects of Rikkunshito (traditional Japanese medicine) on enteral feeding and the plasma ghrelin level in critically ill patients: a pilot study. *J Intensive Care.* (2014) 2:53. doi: 10.1186/s40560-014-0053-4
43. Tokashiki R, Okamoto I, Funato N, Suzuki M. Rikkunshito improves globus sensation in patients with proton-pump inhibitor-refractory laryngopharyngeal reflux. *World J Gastroenterol.* (2013) 19:5118–24. doi: 10.3748/wjg.v19.i31.5118
44. Tominaga K, Iwakiri R, Fujimoto K, Fujiwara Y, Tanaka M, Shimoyama Y, et al. Rikkunshito improves symptoms in PPI-refractory GERD patients: a prospective, randomized, multicenter trial in Japan. *J Gastroenterol.* (2012) 47:284–92. doi: 10.1007/s00535-011-0488-5
45. Arai M, Matsumura T, Tsuchiya N, Sadakane C, Inami R, Suzuki T, et al. Rikkunshito improves the symptoms in patients with functional dyspepsia, accompanied by an increase in the level of plasma ghrelin. *Hepatogastroenterology.* (2012) 59:62–6. doi: 10.5754/hge11246
46. Harasawa S, Miwa T, Miyoshi A, Masamune O, Matsuo Y, Mori H, et al. Double-blind multicenter post-marketing clinical trial of TJ-43 TSUMURA Rikkunshi-to for the treatment of dysmotility-like dyspepsia. *Igaku Ayumi.* (1998) 187:207–29. Japanese. Available online at: http://mol.medicalonline.jp/library/journal/download?GoodsID=aa7ayuma/1998/018703/013&name=0207-0229j&UserID=130.34.173.69
47. Tatsuta M, Iishi H. Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients. *Aliment Pharmacol Ther.* (1993) 7:459–62. doi: 10.1111/j.1365-2036.1993.tb00120.x
48. Miyoshi A, Taniuchi A, Masamune K. Clinical evaluation of rikkunshito for complains related with gastrointestinal function, A randomized multi-center study using cisapride as control. *Prog Med.* (1991) 11:1605–31.

49. Numata T, Takayama S, Tobita M, Ishida S, Katayose D, Shinkawa M, et al. Traditional Japanese medicine daikenchuto improves functional constipation in poststroke patients. *Evid Based Complement Alternat Med.* (2014) 2014:231258. doi: 10.1155/2014/231258
50. Arita R, Numata T, Takayama S, Obara T, Kikuchi A, Ohsawa M, et al. Responder analysis of daikenchuto treatment for constipation in poststroke patients: a subanalysis of a randomized control trial. *J Evid Based Integr Med.* (2019) 24:2515690X19889271. doi: 10.1177/2515690X19889271
51. Horiuchi A, Nakayama Y, Tanaka N. Effect of traditional Japanese medicine, daikenchuto (TJ-100) in patients with chronic constipation. *Gastroenterology Res.* (2010) 3:151–5. doi: 10.4021/gr219w
52. Miyoshi A, Masamune O, Fukutomi H, Mori H, Miwa T, Kojima K, et al. Clinical effect of Tsumura Daiokanzoto (TJ-84) for constipation, a Double-blinded study. *Gastrienterology.* (1994) 18:299-312. [in Japanese]. Available online at: https://www.jstage.jst.go.jp/article/bpb/39/3/39_b15-00815/_article/-char/ja/
53. Nishino T, Yoshida T, Goto M, Inoue S, Minato T, Fujiwara S, et al. The effects of the herbal medicine Daikenchuto (TJ 100) after esophageal cancer resection, open label, randomized controlled trial. *Esophagus.* (2018) 15:75–82. doi: 10.1007/s10388-017-0601-9
54. Katsuno H, Maeda K, Ohya M, Yoshioka K, Tsunoda A, Koda K, et al. Clinical pharmacology of daikenchuto assessed by transit analysis using radiopaque markers in patients with colon cancer undergoing open surgery: a multicenter double-blind randomized placebo-controlled study (JFMC39-0902 additional study). *J Gastroenterol.* (2016) 51:222–229. doi: 10.1007/s00535-015-1100-1
55. Okada K, Kawai M, Hirono S, Yoshioka K, Tsunoda A, Koda K, et al. Evaluation of the efficacy of daikenchuto (TJ−100) for the prevention of paralytic ileus after pancreaticoduodenectomy: a multicenter, double-blind, randomized, placebo-controlled trial. *Surgery.* (2016) 159:1333–41. doi: 10.1016/j.surg.2015.11.019
56. Akamaru Y, Takahashi T, Nishida T, Omori T, Nishikawa K, Mikata S, et al. Effects of daikenchuto, a Japanese herb, on intestinal motility after total gastrectomy: a prospective randomized trial. *J Gastrointest Surg.* (2015) 19:467–72. doi: 10.1007/s11605-014-2730-y
57. Katsuno H, Maeda K, Kaiho T, Kunieda K, Funahashi K, Sakamoto J, et al. Clinical efficacy of Daikenchuto for gastrointestinal dysfunction following colon surgery: a randomized, double-blind, multicenter, placebo-controlled study (JFMC39-0902). *Jpn J Clin Oncol.* (2015) 45:650–6. doi: 10.1093/jjco/hyv056
58. Yoshikawa K, Shimada M, Wakabayashi G, Ishida K, Kaiho T, Kitagawa Y, et al. Effect of daikenchuto, a traditional Japanese herbal medicine, after total gastrectomy for gastric cancer: a multicenter, randomized, double-blind, placebo-controlled, phase II trial. *J Am Coll Surg.* (2015) 221:571–8. doi: 10.1016/j.jamcollsurg.2015.03.004
59. Yaegashi M, Otsuka K, Itabashi T, Kimura T, Kato K, Fujii H, et al. Daikenchuto stimulates colonic motility after laparoscopic-assisted colectomy. *Hepatogastroenterology.* (2014) 61:85–9. Available online at: https://pubmed.ncbi.nlm.nih.gov/24895799/
60. Yoshikawa K, Shimada M, Nishioka M, Kurita N, Iwata T, Morimoto S, et al. The effects of the Kampo medicine (Japanese herbal medicine) "Daikenchuto" on the surgical inflammatory response following laparoscopic colorectal resection. *Surg Today.* (2012) 42:646–51. doi: 10.1007/s00595-011-0094-4
61. Takahashi T, Endo S, Nakajima K, Souma Y, Nishida T. Effect of rikkunshito, a chinese herbal medicine, on stasis in patients after pylorus-preserving gastrectomy. *World J Surg.* (2009) 33:296–302. doi: 10.1007/s00268-008-9854-8
62. Endo S, Nishida T, Nishikawa K, Nakajima K, Hasegawa J, Kitagawa T, et al. Dai-kenchu-to, a Chinese herbal medicine, improves stasis of patients with total gastrectomy and jejunal pouch interposition. *Am J Surg.* (2006) 192:9–13. doi: 10.1016/j.amjsurg.2006.01.022
63. Itoh T, Yamakawa J, Mai M, Yamaguchi N, Kanda T. The effect of the herbal medicine dai-kenchu-to on post-operative ileus. *J Int Med Res.* (2002) 30:428–32. doi: 10.1177/14732300020300 0410
64. Takagi Y, Kawasaki S, Komai H, Fujiwara K, Naito Y. The effect of Chinese herbal medicine (DAI-KENCHU-TO) on paralytic ileus after repair of abdominal aortic aneurysm. *Nihon Rinsho Geka Gakkai Zasshi.* (2000) 61:325–8. doi: 10.3919/jjsa.61.325
65. Kubo N, Uchida Y, Akiyoshi T. The effect of Daikenchuto on ileus, a multicenter trial. *Prog Med.* (1995) 15:1962–7.
66. Kaido T, Shinoda M, Inomata Y, Yagi T, Akamatsu N, Takada Y, et al. Effect of herbal medicine daikenchuto on oral and enteral caloric intake after liver transplantation: a multicenter, randomized controlled trial. *Nutrition.* (2018) 54:68–75. doi: 10.1016/j.nut.2018.02.022
67. Shimada M, Morine Y, Nagano H, Hatano E, Kaiho T, Miyazaki M, et al. Effect of TU-100, a traditional Japanese medicine, administered after hepatic resection in patients with liver cancer: a multicenter, phase III trial (JFMC40-1001). *Int J Clin Oncol.* (2015) 20:95–104. doi: 10.1007/s10147-014-0678-2
68. Nishi M, Shimada M, Uchiyama H, Ikegami T, Arakawa Y, Hanaoka J, et al. The beneficial effects of Kampo medicine Dai-Ken-Chu-To after hepatic resection: a prospective randomized control study. *Hepato-gastroenterology.* (2012) 59:2290–4. Available online at: https://pubmed.ncbi.nlm.nih.gov/23435143/
69. Kaiho T, Tanaka T, Tsuchiya S, Yanagisawa S, Takeuchi O, Miura M, et al. Effect of the herbal medicine Dai-kenchu-to for serum ammonia in hepatectomized patients. *Hepatogastroenterology.* (2005) 52:161–5. Available online at: https://pubmed.ncbi.nlm.nih.gov/15783019/
70. Bessho K, Okubo Y, Hori S, Murakami KI, Iizuka T. Effectiveness of kampo medicine (sai-boku-to) in treatment of patients with glossodynia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* (1998) 86:682–6. doi: 10.1016/S1079-2104(98)90204-9
71. Okabayashi T, Tanaka N, Orita K. The effect of a Kampo medicine, Inchinkoto for the bilirubin reduction rate after biliary drainage on the patients with obstructive jaundice. *J Jpn Surg Assoc.* (1998) 59:2495–500. doi: 10.3919/jjsa.59.2495
72. Miyazaki Y, Yamada A, Saitou M. Effect of Ninjin-Youei-tou on xerostomia induced by oxybutynin hydrochloride, Shinyaku to Rinsho. *J N Rem Clin.* (1994) 43:2613–7. Japanese.
73. Alzheimer's Disease International. *World Alzheimer Report 2015. The Global Impact of dementia. An Analysis of Prevalence, Incidence, Cost & Trends*. London: Alzheimer's Disease International (2015). Available online at: https://www.alz.co.uk/research/WorldAlzheimerReport2015.pdf
74. Cabinet Office. *Annual Report in the Aging Society: 2016 (Summary).* Available online at: https://www8.cao.go.jp/kourei/english/annualreport/2016/pdf/c1-2-1.pdf
75. Shimada Y, Terasawa K, Yamamoto T. A well-controlled study of Choto-san and placebo in the treatment of vascular dementia. *J Tradit Med.* (1995) 11:246-55.
76. Terasawa K, Shimada Y, Kita T, Yamamoto T, Tosa H, Tanaka N, et al. Choto-san in the treatment of vascular dementia: a double-blind, placebo-controlled study. *Phytomedicine.* (1997) 4:15–22. doi: 10.1016/S0944-7113(97)80022-0
77. Suzuki T, Futami S, Igari Y, Matsumura N, Watanabe K, Nakano H, et al. A Chinese herbal medicine, choto-san, improves cognitive function and activities of daily living of patients with dementia: a double-blind, randomized, placebo-controlled study. *J Am Geriatr Soc.* (2005) 53:2238–40. doi: 10.1111/j.1532-5415.2005.00512_7.x
78. Imai H, Takeshima N, Oda H, Chen P, Sawada E, Furukawa TA. Choto-san versus placebo for patients with dementia: systematic review and meta-analysis. *Psychogeriatrics.* (2017) 17:466–78. doi: 10.1111/psyg.12275
79. Iwasaki K, Kobayashi S, Chimura Y, Taguchi M, Inoue K, Cho S, et al. A randomized, double-blind, placebo-controlled clinical trial of the Chinese herbal medicine "ba wei di huang wan" in the treatment of dementia. *J Am Geriatr Soc.* (2004) 52:1518–21. doi: 10.1111/j.1532-5415.2004.52415.x
80. Maruyama M, Tomita N, Iwasaki K, Ootsuki M, Matsui T, Nemoto M, et al. Benefits of combining donepezil plus traditional Japanese herbal medicine on cognition and brain perfusion in Alzheimer's disease: a 12-week observer-blind, donepezil monotherapy controlled trial. *J Am Geriatr Soc.* (2006) 54:869–71. doi: 10.1111/j.1532-5415.2006.00722.x
81. Higashi K, Rakugi H, Yu H, Moriguchi A, Shintani T, Ogihara T. Effect of kihito extract granules on cognitive function in patients with Alzheimer's-type dementia. *Geriatr Gerontol Int.* (2007) 7:245–51. doi: 10.1111/j.1447-0594.2007.00407.x
82. Iwasaki K, Satoh-Nakagawa T, Maruyama M, Monma Y, Nemoto M, Tomita N, et al. A randomized, observer-blind, controlled trial of the

traditional Chinese medicine Yi-Gan San for improvement of behavioral and psychological symptoms and activities of daily living in dementia patients. *J Clin Psychiat.* (2005) 66:248–52. doi: 10.4088/JCP.v66n0214
83. Mizukami K, Asada T, Kinoshita T, Tanaka K, Sonohara K, Nakai R, et al. A randomized cross-over study of a traditional Japanese medicine (kampo), yokukansan, in the treatment of the behavioural and psychological symptoms of dementia. *Int J Neuropsychopharmacol.* (2009) 12:191–9. doi: 10.1017/S146114570800970X
84. Monji A, Takita M, Samejima T, Takaishi T, Hashimoto K, Matsunaga H, et al. Effect of yokukansan on the behavioral and psychological symptoms of dementia in elderly patients with Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry.* (2009) 33:308–11. doi: 10.1016/j.pnpbp.2008.12.008
85. Okahara K, Ishida Y, Hayashi Y, Inoue T, Tsuruta K, Takeuchi K et al. Effects of Yokukansan on behavioral and psychological symptoms of dementia in regular treatment for Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry.* (2010) 34:532–6. doi: 10.1016/j.pnpbp.2010.02.013
86. Teranishi M, Kurita M, Nishino S, Takeyoshi K, Numata Y, Sato T, et al. Efficacy and tolerability of risperidone, yokukansan, and fluvoxamine for the treatment of behavioral and psychological symptoms of dementia: a blinded, randomized trial. *J Clin Psychopharmacol.* (2013) 33:600–7. doi: 10.1097/JCP.0b013e31829798d5
87. Furukawa K, Tomita N, Uematsu D, Okahara K, Shimada H, Ikeda M, et al. Randomized double-blind placebo-controlled multicenter trial of Yokukansan for neuropsychiatric symptoms in Alzheimer's disease. *Geriatr Gerontol Int.* (2017) 17:211–8. doi: 10.1111/ggi.12696
88. Matsunaga S, Kishi T, Iwata N. Yokukansan in the treatment of behavioral and psychological symptoms of dementia: an updated meta-analysis of randomized controlled trials. *J Alzheimers Dis.* (2016) 54:635–43. doi: 10.3233/JAD-160418
89. Takahashi A, Kitamura K, Watanabe Y, Kobayashi R, Saito T, Takachi R, et al. Epidemiological profiles of chronic low back and knee pain in middle-aged and elderly Japanese from the Murakami cohort. *J Pain Res.* (2018) 11:3161–9. doi: 10.2147/JPR.S184746
90. Paolucci T, Saraceni VM, Piccinini G. Management of chronic pain in osteoporosis: challenges and solutions. *J Pain Res.* (2016) 9:177–86. doi: 10.2147/JPR.S83574
91. Hamaguchi T, Yoshino T, Horiba Y, Watanabe K. Goshajinkigan for low back pain: an observational study. *J Alten Complement Med.* (2017) 23:208–13. doi: 10.1089/acm.2016.0276
92. Oohata M, Aoki Y, Miyata M, Mizobe H, Suzuki KS. Japanese traditional herbal medicine reduces use of pregabalin and opioids for pain in patients with lumbar spinal canal stenosis: a retrospective cohort study. *JA Clin Rep.* (2017) 3:60. doi: 10.1186/s40981-017-0130-5
93. Hamaguchi S, Komatsuzaki M, Kitajima T, Egawa H. A retrospective study assessing kampo medicine for the treatment of lower extremity symptoms caused by lumbar spinal diseases. *Kampo Med.* (2017) 68:366–71. Japanese. doi: 10.3937/kampomed.68.366
94. Takahashi Y, Mitsuhata H, Kamiyama Y. The effectiveness of tokishigyakukagosyuyusyokyoto in lower back pain patients with coldness. *Kampo Med.* (2016) 67:390–3. Japanese. doi: 10.3937/kampomed.67.390
95. Takahashi H, Sakaki T, Koike Y, Ono M, Nanbu T, Koike Y, et al. Two cases of lumbar spinal cord stenosis successfully treated with Kampo medicine. *J Japanese Society Study Chronic Pain.* (2015) 34:170–4. Japanese.
96. Yoshinaga R, Goto Y, Inoue H, Yano H, Nabeshima S, Tahara E. A case of lower back with extremity pain successfully treated with kanzobushito. *Kampo Med.* (2019) 70:146–50. Japanese. doi: 10.3937/kampomed.70.146
97. Han C, Hirasaki Y, Okamoto H, Ueda K, Yagi A, Shimada H, et al. A case of prolonged lumbago with severe cold intolerance successfully treated with keppuchikuoto and uzushakusekishigan. *Kampo Med.* (2015) 66:112–8. Japanese. doi: 10.3937/kampomed.66.112
98. Nagasaka K, Hikiami H, Tatsumi T, Tosa H, Terasawa K. Effect of ryokyo-jutukanto ka bushi on low back pain. *J Traditional Med.* (1999) 16:83–9. Japanese.
99. Ogawa K, Namiki T, Sekiya N, Kasahara Y, Chino A, Raimura M, et al. A case with lumbar spinal canal stenosis successfully treated with kigikenchutokauzu. *Kampo Med.* (2009) 60:167–70. Japanese. doi: 10.3937/kampomed.60.167
100. Tetsumura S, Kimira H, Sato M, Kota K, Mitsuma T. A case of painful bedridden patient with multiple fractures due to osteoporosis successfully treated with keishi-bushi-to. *Kampo Med.* (2005) 56:103–8. Japanese. doi: 10.3937/kampomed.56.103
101. Nakae H, Yokoi A, Kodama H, Horikawa A. Comparison of the effects on rib fracture between the traditional Japanese medicine jidabokuippo and nonsteroal anti-inflammatory drugs: a randomized controlled trial. *Evid Based Complement Alternat Med.* (2012) 2012:837958. doi: 10.1155/2012/837958
102. Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, et al. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. *J Bone Miner Metab.* (2009) 27:620–8. doi: 10.1007/s00774-009-0080-8
103. Skou ST, Roos E. Physical therapy for patients with knee and hip osteoarthritis: supervised, active treatment is current best practice. *Clin Exp Rheumatol.* (2019) 120:112–7. Available online at: https://pubmed.ncbi.nlm.nih.gov/31621559/
104. Majima T, Inoue M, Kasahara Y, Onodera T, Takahashi D, Minami A. Effect of the Japanese herbal medicine, boiogito, on the osteoarthritis of the knee with joint effusion. *Sports Med Arthrosc Rehabil Ther Technol.* (2012) 4:1–6. doi: 10.1186/1758-2555-4-3
105. Nakae H. Efficacy of aconite tuber powder in patients with arthralgia and somatic pain. *Kampo Med.* (2009) 60:81–5. Japanese. doi: 10.3937/kampomed.60.81
106. Mizoguchi K, Ikarashi Y. Multiple psychopharmacological effects of the traditional Japanese Kampo medicine yokukansan, and the brain regions it affects. *Front Pharmacol.* (2017) 8:149 doi: 10.3389/fphar.2017.00149
107. Nakamura Y, Tajima K, Kawagoe I, Kanai M, Mitsuhata H. Efficacy of traditional herbal medicine yokukansan on patients with neuropathic pain. *Masui.* (2009) 58:1248–55. Available online at: https://pubmed.ncbi.nlm.nih.gov/19860227/
108. Mallick-Searle T, Snodgrass B, Brant JM. Postherpetic neuralgia: epidemiology pathophysiology, and pain management pharmacology. *Multidiscip Healthc.* (2016) 9:447–54. doi: 10.2147/JMDH.S106340
109. Nakanishi M, Arimitsu J, Kageyama M, Otsuka S, Inoue T, Nishida S, et al. Efficacy of traditional Japanese herbal medicines - Keishikajutsubuto (TJ-18) and Bushi-matsu (TJ-3022) - against postherpetic neuralgia aggravated by self-reported cold stimulation: a case series. *J Altern Complement Med.* (2012) 18:686–92. doi: 10.1089/acm.2010.0745
110. Kogure T, Harada N, Yamamoto K, Tatsumi T. Positive effects of Uyakujunkisan therapy (Kampo medicine) for postherpetic neuralgia with concomitant pregabalin: two case reports with a literature review. *Altern Ther Health Med.* (2016) 22:40–4. Available online at: https://pubmed.ncbi.nlm.nih.gov/27866180/
111. Fukuda S, Nambu T, Takahashi H, Kuroki K, Nishiyama H, Mitsuma T. Two patients with ophthalmic symptoms due to post herpetic infection dramatically improve with chitosan. *Kampo Med.* (2010) 61:912–6. Japanese. doi: 10.3937/kampomed.61.912
112. Nogami T, Oka H, Fujimoto M, Hikiami H, Goto H, Shibahara N, et al. Two cases of postherpetic neuralgia recurring after withdrawal of Kampo medicine including Uzu. *Kampo Med.* (2011) 62:369–73. Japanese. doi: 10.3937/kampomed.62.369
113. Albers JW, Pop-Busui R. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. *Curr Neurol Neurosci Rep.* (2014) 14:473. doi: 10.1007/s11910-014-0473-5
114. Watanabe K, Shimada A, Miyaki K, Hirakata A, Matsuoka K, Omae K, et al. Long-term effects of goshajinkigan in prevention of diabetic complications: a randomized open-labeled clinical trial. *Evid Based Complement Alternat Med.* (2014) 2014:128726. doi: 10.1155/2014/128726
115. Hoshino N, Ganeko, Hida K, Sakai Y. Goshajinkigan for reducing chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis. *Int J Clin Oncol.* (2018) 23:434–42. doi: 10.1007/s10147-017-1229-4
116. Dua A, Lee CJ. Epidemiology of peripheral arterial disease and critical limb ischemia. *Tech Vasc Interv Radiol.* (2016) 19:91–5. doi: 10.1053/j.tvir.2016.04.001

117. Kawago K, Shindo S, Inoue H, Akasaka J, Motohashi S, Urabe G, et al. The effect of hachimi-jio-gan (Ba-Wei-Di-Huang-Wan) on the quality of life in patients with peripheral arterial disease - a prospective study using Kampo Medicine. *Ann Vasc.* (2016) 9:289–94. doi: 10.3400/avd.oa.15-00133
118. Nishida S, Eguchi E, Ohira T, Kitamura A, Kato YH, Hagihara K, et al. Effects of a traditional herbal medicine on peripheral blood flow in women experiencing peripheral coldness: a randomized controlled trial. *BMC Complement Altern Med.* (2015) 15:105. doi: 10.1186/s12906-015-0617-4
119. Jojima K. The clinical effectiveness of traditional Japanese herbal-medicine, tokishigyakukagosyuyusyokyoto for vascular intermittent claudication. *Kampo Med.* (2011) 62:529–36. Japanese. doi: 10.3937/kampomed.62.529
120. Raimura M, Terasawa K, Sekiya N, Chino A, Hashimoto S, Namiki T, et al. A case of intermittent claudication and skin ulcers of lower limb due to arteriosclerosis obliterans successfully treated with prepared in the hospital keisibukuryogan and daisaikoto. *Kampo Med.* (2009) 60:365–9. Japanese. doi: 10.3937/kampomed.60.365
121. Uto NS, Amitani H, Atobe Y, Sameshima Y, Sakaki M, Rokot N, et al. Herbal medicine ninjin'yoeito in the treatment of sarcopenia and frailty. *Front Nutr.* (2018) 5:126. doi: 10.3389/fnut.2018.00126
122. Sakamoto R, Asari H. Benefit of Kampo medicine for stroke rehabilitation. *Jpn Rehabi Med.* (2018) 55:968–77. Japanese. doi: 10.2490/jjrmc.55.968
123. Sakisaka N, Mitani K, Sempuku S, Imai T, Takemoto Y, Shimomura H, et al. A clinical study of ninjin'yoeito with regard to frailty. *Front Nutr.* (2018) 5:73. doi: 10.3389/fnut.2018.00073
124. Ikarashi Y, Mizoguchi. Neuropharmacological efficacy of the traditional Japanese Kampo medicine yokukansan and its active ingredients. *Phamacol Ther.* (2016) 166:84–95. doi: 10.1016/j.pharmthera.2016.06.018
125. Mizoguchi K, Ikarashi Y. Cillular pharmacological effects of the traditional Japanese Kampo medicine yokukansan on brain cells. *Front Pharmacol.* (2017) 20:655. doi: 10.3389/fphar.2017.00655
126. Suzuki Y, Goto K, Ishida A, Komura Y, Kamei J. Antinociceptive mechanism of Gosha-jinki-gan in streptozotocin-induced diabetic animals: role of nitric oxide in theperiphery. *Jpn J Phamacol.* (1999) 79:387–91. doi: 10.1254/jjp.79.387
127. Kato Y, Tateai Y, Ohkubo M, Saito Y, AmagaiSY, Kimura YS, et al. Gosha-jinki-gan reduced oxaliplatin-induced hypersensitivity to cold sensation and its effect would be related to suppression of the expression of TRPM8 and TRPA1 in rats. *Anticancer Drugs.* (2014) 25:39–43. doi: 10.1097/CAD.0000000000000022
128. Matsumura Y, Yokoyama Y, Hirakawa H, Shigeto T, Futagami M, Mizumura H. The proohylactic effects of a traditional Japanese medicine, goshoajinkigan, on paclitaxel-induced peripheral neuropathy and its mechanism of action. *Mol Pain.* (2014) 10:61. doi: 10.1186/1744-8069-10-61
129. Kishida Y, Kagawa S, Arimitsu J, Nakanishi M, Sakashita N, Otsuka S, et al. Go-sha-jinki-Gan (GJG), a traditional Japanese herbal medicine, protects against sarcopenia in senescence-accelerated mice. *Phytomedicine.* (2015) 22:16–22. doi: 10.1016/j.phymed.2014.11.005
130. Nagano T, Itoh H, Takeyama M. Effect of Dai-kenchu-to on levels of 3 brain-gut peptides (motilin, gastrin and somatostatin) in human plasma. *Biol Pharm Bull.* (1999) 22:1131–3. doi: 10.1248/bpb.22.1131
131. Kono T, Koseki T. Chiba S, Ebisawa Y, Chisato N, Iwamoto J, et al. Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide receptoractivity modifying protein 1. *J Surg Res.* (2008) 150:78–84. doi: 10.1016/j.jss.2008.02.057
132. Kono T, Kaneko A, Hira Y, Suzuki T, Chisato N, Ohtake N, et al. Anticolitis -adhesion effects of Daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model. *J Crohns Colitis.* (2010) 4: 161–70. doi: 10.1016/j.crohns.2009.09.006
133. Kono T, Omiya Y, Hira Y, Kaneko A, Chiba S, Suzuki T, et al. Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model. *J Gastroenterol.* (2011) 46:1187–96. doi: 10.1007/s00535-011-0438-2
134. Kikuchi D, Shibata C, Imoto H, Naitoh T, Miura K, Unno M. Intragastric Dai-Kenchu-To, a Japanese herbal medicine, stimulates colonic motility via transient receptor potential cation channel subfamily V member 1 in dogs. *Tohoku J Exp Med.* (2013) 230:197–204. doi: 10.1620/tjem.230.197
135. Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T, et al. The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. *J Gastroenterol.* (2010) 45:300–7. doi: 10.1007/s00535-009-0166-z
136. Takayama S, Arita R, Kikuchi A, Ohsawa M, Kaneko S, Ishii T. Clinical practice guidelines and evidence for the efficacy of traditional japanese herbal medicine (kampo) in treating geriatric patients. *Front Nutr.* (2018) 5:66. doi: 10.3389/fnut.2018.00066
137. Satoh T, Yokozeki H, Katayama I, Murota H, Tokura Y, Boku N, et al. Clinical practice guideline for the Pruritus cutaneus universalis [in Japanese]. *Jpn J Dermatol.* (2012) 122:267–80. Available online at: https://www.jstage.jst.go.jp/article/dermatol/122/2/122_267/_article/-char/ja
138. Okubo K, Kurono Y, Ichimura K, Enomoto T, Okamoto Y, Kawauchi H, et al. *Practical Guideline for the Management of Allergic Rhinitis in Japan*. Tokyo: Life Science Press [in Japanese]. (2015).
139. Kohno S, Okada K, Kadota J, Shioya T, Tanaka H, Tokuyama K, et al. *The Japanese Respiratory Society Guidelines for Management of Cough*. Tokyo: Medical Revew Co., Ltd. [in Japanese]. (2012).
140. Kinoshita Y, Iwakiri K, Ashida K, Iwakiri R, Oshima T, Ohara S, et al. *Evidence-Based Clinical Practice Guidelines for GERD 2015*. 2nd ed. Tokyo: Nankodo Press [in Japanese] (2015).
141. Miwa H, Kusano M, Arisawa T, Oshima T, Kato T, Joh T, et al. *Evidence-based Clinical Practice Guidelines for Functional Dyspepsia*. Tokyo: Nankodo Press [in Japanese]. (2014).
142. Fukudo S, Kaneko H, Akiho H, Inamori M, Endo Y, Okumura T, et al. *Evidence-based Clinical Practice Guidelines for Irritable Bowel Syndrome*. Tokyo: Nankodo Press [in Japanese]. (2014). doi: 10.1007/s00535-014-1017-0
143. Miwa H, Torii A, Maeda K, Akiho H, Araki Y, Iijima H, et al. *Evidence-based Clinical Practice Guidelines for Chronic Constipation 2017*. Tokyo: Nankodo Press [in Japanese]. (2017).
144. Takeda M, Yokoyama O, Goto M, Homma Y, Asakura H, Yamanishi T, et al. *Clinical Guidelines for Overactive Bladder Syndrome*. 2nd ed. Tokyo: RichHill Medical Inc. [in Japanese] (2015).
145. Nakashima K, Tomimoto H, Aiba I, Akishita M, Kurita S, Iijima S, et al. *Practice Guideline for Dementia 2017*. Tokyo: Igaku-Shoin Ltd. [in Japanese]. (2017).
146. Yasunaga H. Effect of japanese herbal kampo medicine goreisan on reoperation rates after burr-hole surgery for chronic subdural hematoma: analysis of a national inpatient database. *Evid Based Complement Alternat Med.* (2015) 2015:817616. doi: 10.1155/2015/817616
147. Yasunaga H, Miyata H, Horiguchi H, Kuwabara K, Hashimoto H, Matsuda S.Effect of the Japanese herbal kampo medicine dai-kenchu-to on postoperative adhesive small bowel obstruction requiring long-tube decompression: a propensity score analysis. *Evid Based Complement Alternat Med.* (2011) 2011:264289. doi: 10.1155/2011/264289
148. Arai I, Hagiwara Y, Motoo Y. Estimated incidence of adverse reactions to Kampo medicines in randomized controlled clinical trials. *Tradi Kampo Med.* (2018) 5:106–12. doi: 10.1002/tkm2.1200

Conflict of Interest: ST, AK, and TI belong to the Department of Kampo and Integrative Medicine at Tohoku University School of Medicine. The department received a grant from Tsumura, a Japanese manufacturer of Kampo medicine; however, the grant was used as per Tohoku University rules. Potential conflicts of interests were addressed by the Tohoku University Benefit Reciprocity Committee and were managed appropriately.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 17 July 2020
doi: 10.3389/fnut.2020.00104

Ninjin'yoeito Targets Distinct Ca^{2+} Channels to Activate Ghrelin-Responsive vs. Unresponsive NPY Neurons in the Arcuate Nucleus

Chayon Goswami[1,2,3,4†], *Katsuya Dezaki*[3,5†], *Lei Wang*[1,2,3], *Akio Inui*[6], *Yutaka Seino*[1,7] *and Toshihiko Yada*[1,2,3,6*]

[1] Division of Integrative Physiology, Center for Integrative Physiology, Kansai Electric Power Medical Research Institute, Kobe, Japan, [2] Division of Diabetes, Metabolism and Endocrinology, Kobe University Graduate School of Medicine, Kobe, Japan, [3] Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Tochigi, Japan, [4] Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh, Bangladesh, [5] Faculty of Pharmacy, Iryo Sosei University, Iwaki, Japan, [6] Pharmacological Department of Herbal Medicine, Kagoshima University Graduate School of Medical & Dental Sciences, Kagoshima, Japan, [7] Center for Diabetes Research, Division of Diabetes and Endocrinology, Kansai Electric Power Medical Research Institute, Kobe, Japan

OPEN ACCESS

Edited by:
Sergueï O. Fetissov, Université de Rouen, France

Reviewed by:
Mario Perello, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
Jeffrey Zigman, University of Texas Southwestern Medical Center, United States

***Correspondence:**
Toshihiko Yada
toshihiko.yada@kepmri.org

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *02 April 2020*
Accepted: *08 June 2020*
Published: *17 July 2020*

Citation:
Goswami C, Dezaki K, Wang L, Inui A, Seino Y and Yada T (2020) Ninjin'yoeito Targets Distinct Ca^{2+} Channels to Activate Ghrelin-Responsive vs. Unresponsive NPY Neurons in the Arcuate Nucleus. Front. Nutr. 7:104. doi: 10.3389/fnut.2020.00104

Appetite loss or anorexia substantially deteriorates quality of life in various diseases, and stand upstream of frailty. Neuropeptide Y (NPY) in the hypothalamic arcuate nucleus (ARC) and ghrelin released from stomach are potent inducers of appetite. We previously reported that Ninjin'yoeito, a Japanese kampo medicine comprising twelve herbs, restores food intake, and body weight in cisplatin-treated anorectic mice. Furthermore, Ninjin'yoeito increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in not only ghrelin-responsive but ghrelin-unresponsive NPY neurons in ARC. The cellular lineage/differentiation of ghrelin-unresponsive neuron is less defined but might alter along with aging and diet. This study examined the occupancy of ghrelin-unresponsive neurons among ARC NPY neurons in adult mice fed normal chow, and explored the mechanisms underlying Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases in ghrelin-unresponsive vs. ghrelin-responsive NPY neurons. Single ARC neurons were subjected to $[Ca^{2+}]_i$ measurement and subsequent immunostaining for NPY. Ghrelin failed to increase $[Ca^{2+}]_i$ in 42% of ARC NPY neurons. Ninjin'yoeito (10 μg/ml)-induced increases in $[Ca^{2+}]_i$ were abolished in Ca^{2+} free condition in ghrelin-responsive and ghrelin-unresponsive ARC NPY neurons. Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited by N-type Ca^{2+} channel blocker ω-conotoxin in the majority (17 of 20), while by L-type Ca^{2+} channel blocker nitrendipine in the minority (2 of 23), of ghrelin-responsive neurons. In contrast, Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited by nitrendipine in the majority (14 of 17), while by ω-conotoxin in the minority (8 of 24), of ghrelin-unresponsive neurons. These results indicate that ghrelin-unresponsive neurons occur substantially among NPY neurons of ARC in adult mice fed normal chow. Ninjin'yoeito preferentially target N-type and L-type Ca^{2+} channels in the majority of ghrelin-responsive and ghrelin-unresponsive neurons, respectively, to increase $[Ca^{2+}]_i$. We suggest ARC N- and L-type Ca^{2+} channels as potential targets for activating, respectively, ghrelin-responsive, and unresponsive NPY neurons to treat anorexia.

Keywords: Ninjin-yoeito, anorexia, arcuate nucleus, neuropeptide Y, ghrelin, N-type Ca^{2+} channel, L-type Ca^{2+} channel

TABLE 1 | Composition (daily dose*) of kampo formula Ninjin'yoeito.

Ingredient		Contents (g)
English name	**Latin name**	
Rehmannia root	*Rehmanniae radix*	4.0
Japanese angelica root	*Angelicae acutilobae*	4.0
Atractylodes rhizome	*Atractylodis rhizoma*	4.0
Poria sclerotium	*Poria*	4.0
Ginseng	*Ginseng radix*	3.0
Cinnamon bark	*Cinnamomi cortex*	2.5
Polygala root	*Polygalae radix*	2.0
Peony root	*Paeoniae radix*	2.0
Citrus unshiu peel	*Citri unshiu pericarpium*	2.0
Astragalus root	*Astragali radix*	1.5
Glycyrrhiza	*Glycyrrhizae radix*	1.0
Schisandra fruit	*Schisandrae fructus*	1.0

**Approximate 6,700 mg of dried water extract of Ninjin'yoeito was prepared in GMP-standardized factory of Kracie Pharma, Ltd. (Japan) on the basis of above described composition.*

INTRODUCTION

Reduced appetite and body weight are associated with cancer, sarcopenia and frailty (1, 2), and deteriorate the quality of life (QOL) (3, 4). Hence, effective means to promote appetite have been awaited. Ninjin'yoeito, a Japanese traditional Kampo medicine, has been used clinically and demonstrated to be effective to treat anorexia, fatigue, anemia, cold limbs, persistent cough, mental disequilibrium, and to promote recovery from disease (5–7). Its ability to ameliorate this variety of symptoms is considered to result partly from counteraction of anorexia. Ninjin'yoeito comprises 12 crude drugs (**Table 1**). Some of these crude drugs are known to pass through the blood brain barrier (BBB) and hence possibly access to the hypothalamus including the arcuate nucleus (ARC), while ghrelin, an orexigenic gut hormone, freely accesses to the ARC neurons without crossing the BBB (8). Hence, oral administration of Ninjin'yoeito could act on ARC via one or both of these routes.

We previously reported that Ninjin'yoeito counteracted the anorexigenic and body weight-lowering effects of cisplatin, a chemo-therapy drug, in mice (9). In parallel, Ninjin'yoeito increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single neurons isolated from ARC, and the majority (79%) of the Ninjin'yoeito-responsive neurons were immunoreactive (IR) to neuropeptide Y (NPY). The neuron coexpressing NPY and agouti-related protein (AgRP) (NPY/AgRP neuron) in ARC of hypothalamus is considered as the principal neuron for initiating feeding behavior (10–13). Selective activation of ARC NPY/AgRP neurons acutely and robustly triggers feeding (14, 15), while their selective deletion in adult mice markedly reduces feeding (16, 17), placing the ARC NPY/AgRP neuron as the necessary and adequate inducer of feeding.

Abbreviations: VDCC, voltage-dependent Ca^{2+} channel; NPY, Neuropeptide Y; ARC, arcuate nucleus.

We reported previously that Ninjin'yoeito increased $[Ca^{2+}]_i$ in the ghrelin-responsive and ghrelin-unresponsive NPY neurons in ARC. Ghrelin, released from gut under fasted condition, stimulates feeding via activating NPY neurons (18–20). Hence, the ghrelin-responsive NPY neurons in ARC is considered to play a central role in stimulating physiological feeding. Compared to ghrelin-responsive NPY neurons, the role of the ghrelin-unresponsive NPY neurons is less defined. A possibility exists that ghrelin-responsive and ghrelin-unresponsive NPY neurons could be converted to each other depending on the diet/metabolic states and aging, including ghrelin resistance (21–23). The present study firstly investigated the occupancy (percentage) of the ghrelin-unresponsive neurons among ARC NPY neurons in adult mice fed normal chow. Notably, it has been documented that ghrelin-resistance occurs with aging and likely contributes to appetite reduction and resultant BW decrease and frailty. In this line, our previous finding that Ninjin'yoeito interacts with and recruits ghrelin-unresponsive NPY neurons to $[Ca^{2+}]_i$ increases suggests its potential to restore appetite and counteract frailty. Hence, it is of relevance to elucidate the mechanisms underlying the $[Ca^{2+}]_i$ responses to Ninjin'yoeito in ghrelin-unresponsive NPY neurons. The present study aimed to elucidate the Ca^{2+} channel type implicated in the $[Ca^{2+}]_i$ response to Ninjin'yoeito in ghrelin-unresponsive NPY neurons in comparison with that in ghrelin-responsive NPY neurons.

It has been reported that N-type (CaV2.2) and L-type (CaV1.3) voltage-dependent Ca^{2+} channels (VDCCs) participate in depolarization-induced release of NPY in rat medium eminence-ARC preparation (24). In this report, 23 mM KCl-induced NPY release was blunted by nitrendipine, a blocker of L-type Ca^{2+} channels, and 45 mM KCl-induced NPY release was markedly attenuated by ω-conotoxin, a blocker of N-type Ca^{2+} channels, but not by nitrendipine. These data may indicate differential roles of N-type and L-type Ca^{2+} channels depending on the magnitude of depolarization by KCl. In addition, N-type and/or L-type Ca^{2+} channels in ARC neurons are involved in the $[Ca^{2+}]_i$ responses to several substances including ghrelin (25, 26). Hence, the present study explored the link of Ninjin'yoeito to N-type and/or L-type VDCCs in ghrelin-responsive vs. unresponsive neurons. Single neurons were isolated from ARC of adult mice fed normal chow, subjected to Ca^{2+} imaging to determine their responsiveness to ghrelin and Ninjin'yoeito, and subsequently immunostained for NPY. In the ghrelin-responsive and ghrelin-unresponsive NPY neurons, the effects of Ninjin'yoeito on $[Ca^{2+}]_i$ and their modulation by N-type and L-type Ca^{2+} channel blockers were examined.

MATERIALS AND METHODS

Ninjin'yoeito Extract

Ninjin'yoeito is an herbal supplement composed of 12 crude drugs (**Table 1**). Ninjin'yoeito extract was supplied by Kracie Co. (Tokyo, Japan). Ninjin'yoeito extract was mixed with distilled water to prepare the stock solution.

Materials and Solution

For $[Ca^{2+}]_i$ imaging, Ninjin'yoeito solution was diluted at the concentrations used for superfusion in HEPES-buffered Krebs-Ringer bicarbonate buffer (HKRB) solution composed of (in mM) 129 NaCl, 5.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.8 $CaCl_2$, 1.2 $MgSO_4$, and 10 HEPES with pH adjusted at 7.4 using NaOH. Ghrelin was purchased from Peptide Institute (Osaka, Japan). Fresh solution of Ninjin'yoeito and ghrelin were prepared before each experiment.

Animals

Male C57BL/6J mice aged 4–6 weeks were obtained from Japan SLC (Shizuoka, Japan) and housed for at least 1 week under conditions of controlled temperature (23 ± 1°C), humidity (55 ± 5%) and lighting (light phase 7:30–19:30). Food and water were available ad libitum. Animal experiments were carried out after receiving approval from the Institutional Animal Experiment Committee and in accordance with the Institutional Regulation for Animal Experiments at Jichi Medical University (IACUC approval number; 17-229) and Kobe University (IACUC approval number; 30-10-06-R1).

Preparation of Single Neurons From ARC

The ARC was isolated from the brain of mice aged 5–7 weeks and single neurons were prepared as reported previously (25). Briefly, mice were anesthetized with intraperitoneal injection of urethane (ethyl carbamate; 1 g/kg, ip) or inhalation administration of isoflurane and decapitated, and their brain was removed. Brain slices containing ARC were prepared, and the whole ARC of the left and right sides was punched out. The dissected tissues were incubated in HKRB supplemented with 20 units/ml papain (Sigma Aldrich, St. Louis, MO), 0.015 mg/ml deoxyribonuclease, and 0.75 mg/ml BSA for 16 min at 36°C in a shaking water bath, followed by gentle mechanical trituration for 5–10 min. The cell suspension was centrifuged at 100 × g for 5 min. The pellet was resuspended in HKRB and distributed onto coverslips. The cells were kept at 30°C in moisture-saturated dishes till $[Ca^{2+}]_i$ measurements for up to 6 h.

Measurements of $[Ca^{2+}]$ in Single ARC Neurons

At 2–10 h after cell preparation, $[Ca^{2+}]_i$ was measured by ratiometric fura-2 fluorescence imaging as previously reported (25). Briefly, following incubation with 2 μM fura-2AM (DOJINDO, Kumamoto, Japan) for 30 min at 30°C, the cells were mounted in a chamber and superfused at 1 ml/min with HKRB containing 2.5 mM glucose at 30°C. Data were taken from the single cells that were identified as neurons by the criteria reported previously (25); relatively large diameter (≥10 μm), clear and round cell bodies on phase-contrast microscopy. Ninjin'yoeito (10 μg/ml) and ghrelin (10^{-8} M) were administered under superfusion conditions. Fluorescence ratio (F340/F380) images were produced by Aquacosmos ver. 2.5 (Hamamatsu Photonics, Shizuoka, Japan). When $[Ca^{2+}]_i$ increases took place within 10 min of superfusion with agents and their amplitudes were at least twice larger than fluctuations of baseline, they were considered responses. In all experiments, neurons from at least three separate preparations were analyzed.

Immunocytochemistry and Identification of NPY Neurons

After $[Ca^{2+}]_i$ measurements, cells were fixed with 4% paraformaldehyde, pretreated with 3% H_2O_2 for 1 h, and blocked in 10% normal goat serum and in 0.1 M PBS for 1 h at room temperature. Cells were incubated overnight at 4°C with primary antiserum to NPY (DiaSorin, Stillwater, MN) diluted 1:10,000 in PBS containing 1.5% normal goat serum. After rinsing, cells were incubated with biotinylated secondary antibody raised against rabbit IgG (Vector Laboratories Inc., Burlingame, CA; diluted 400-fold) for 1 h at room temperature. After rinsing, the sections were labeled with avidin-peroxidase complex (ABC kit, Vector) for 1 h and color-developed with 3, 3'-diaminobenzidine (DAB). $[Ca^{2+}]_i$ and immunocytochemical data were correlated to each other, based on the photographs of the single neurons subjected to $[Ca^{2+}]_i$ measurements in the microscopic field (25).

RESULTS

Effect of Ghrelin on $[Ca^{2+}]_i$ in ARC NPY Neurons

Single neurons isolated from ARC were superfused with HKRB containing 2.5 mM glucose for $[Ca^{2+}]_i$ imaging. After $[Ca^{2+}]_i$ was stabilized at baseline, administration of ghrelin (10^{-8} M) increased $[Ca^{2+}]_i$ in some single neurons that were subsequently shown to be immunoreactive (IR) to NPY by immunocytochemistry (**Figure 1A**). In contrast, administration of ghrelin failed to increase $[Ca^{2+}]_i$ in other single neurons that were IR to NPY (**Figure 1B**). Among 43 ARC NPY neurons, 25 (58%) neurons responded to ghrelin and 18 (42%) neurons did not respond to ghrelin (**Figure 1C**). These results indicated that ghrelin-unresponsive neurons occur substantially among NPY neurons of ARC in adult mice fed normal chow.

Ninjin-yoeito Increases $[Ca^{2+}]_i$ via Ca^{2+} Influx Preferentially Through N-Type VDCC in the Minority of Ghrelin-Responsive Neurons

The effect of Ninjin'yoeito (10 μg/ml) on $[Ca^{2+}]_i$ in ARC NPY neurons that responded to ghrelin were examined. Under superfusion with HKRB without added Ca^{2+} and with 0.1 mM EGTA (Ca^{2+}-free HKRB), administration of Ninjin'yoeito (10 μg/ml) for 10–12 min did not increase $[Ca^{2+}]_i$, while it subsequently increased $[Ca^{2+}]_i$ in HKRB with 2 mM Ca^{2+} (normal HKRB) in a single neuron that subsequently responded to ghrelin with $[Ca^{2+}]_i$ increase (**Figure 2A**). In nine ghrelin-responsive neurons, none responded to Ninjin'yoeito in Ca^{2+}-free HKRB (**Figure 2A**, Right). This result indicated that the $[Ca^{2+}]_i$ response to Ninjin'yoeito in ghrelin-responsive neurons requires the presence of extracellular Ca^{2+}.

We next examined whether particular type of VDCCs could be implicated in the $[Ca^{2+}]_i$ response to Ninjin'yoeito

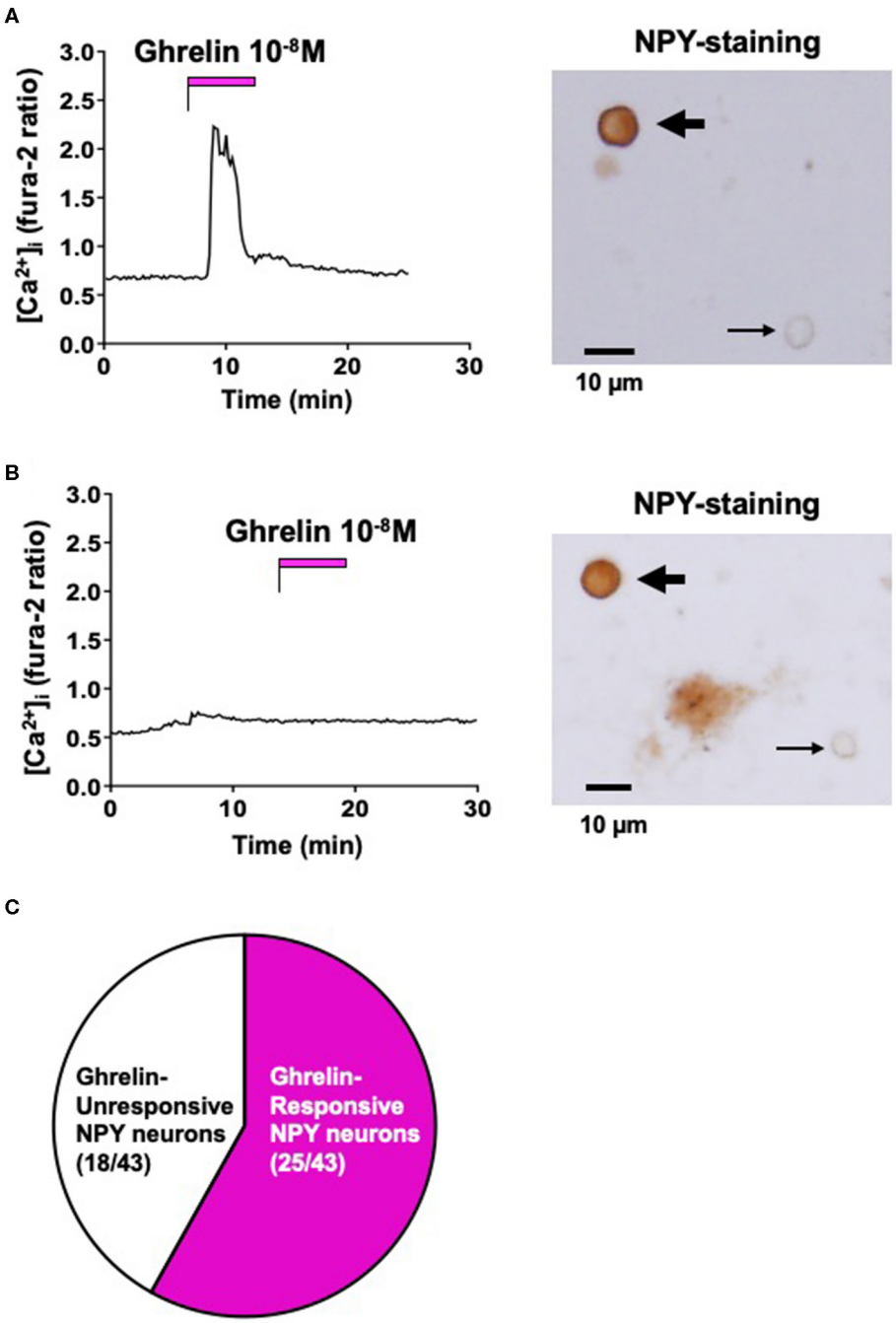

FIGURE 1 | Effect of ghrelin on $[Ca^{2+}]_i$ in ARC NPY neurons. **(A)** Ghrelin (10^{-8} M) increased $[Ca^{2+}]_i$ in a single ARC neuron that was subsequently shown to be IR to NPY, indicated by the thick arrow in right. **(B)** Ghrelin (10^{-8} M) failed to increase $[Ca^{2+}]_i$ in a single ARC neuron that was subsequently shown to be IR to NPY, indicated by the thick arrow in right. Thin arrows in right of **(A,B)** indicate neurons that were not IR to NPY. Scale bars in right of **(A,B)** show 10 µm. **(C)** Incidence of ghrelin-responsive and ghrelin-unresponsive ARC NPY neurons: 25 of 43 (58%) neurons responded to ghrelin and 18 of 43 (42%) neurons did not respond to ghrelin. Glucose concentration was 2.5 mM. These data were obtained from 3 preparations of single neurons from 3 mice.

in ghrelin-responsive neurons. The effects of N-type Ca^{2+} channel blocker, ω-conotoxin, and L-type Ca^{2+} channel blocker, nitrendipine, were examined. In the presence of ω-conotoxin (100 nM) Ninjin'yoeito did not increase $[Ca^{2+}]_i$, while it subsequently increased $[Ca^{2+}]_i$ after washing out ω-conotoxin in the majority of single neurons (**Figure 2B**). In the presence of ω-conotoxin Ninjin'yoeito increased $[Ca^{2+}]_i$ in only 3 of 20 (15.0%) ghrelin-responsive neurons (**Figure 2B**, Right). By

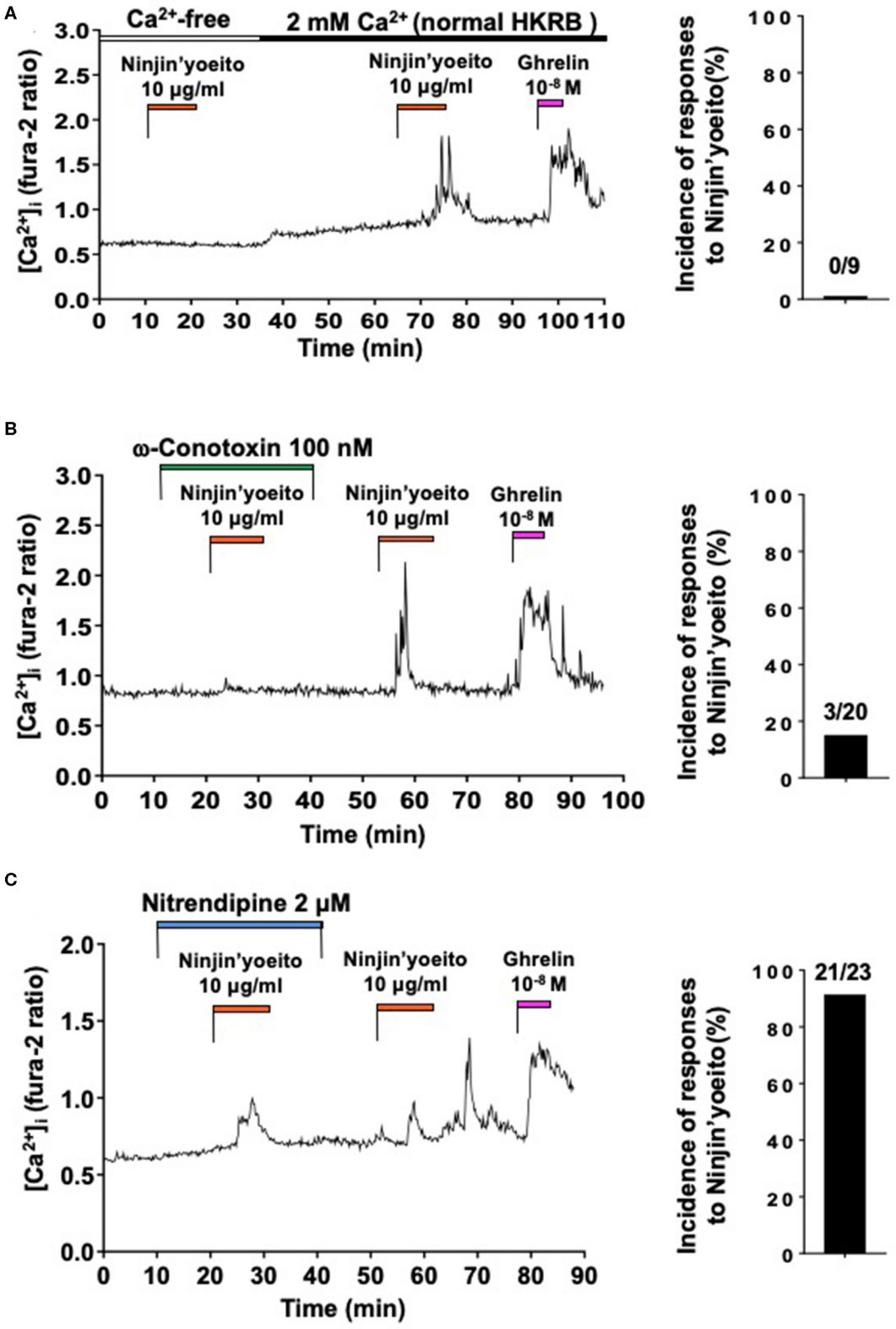

FIGURE 2 | Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited in Ca^{2+} free condition and preferentially by N-type Ca^{2+} channel blocker in the majority of ghrelin-responsive ARC NPY neurons. ARC NPY neurons that responded to Ninjin'yoeito (10 µg/ml) and ghrelin (10^{-8} M) with increases in $[Ca^{2+}]_i$ were studied. Glucose concentration was 2.5 mM. **(A)** The $[Ca^{2+}]_i$ response to Ninjin'yoeito was inhibited under superfusion with Ca^{2+} free HKRB. Right: incidence (%) of

(Continued)

FIGURE 2 | responses to Ninjin'yoeito in Ca^{2+} free HKRB. Numbers above bar indicate number of neurons responding to Ninjin'yoeito over that examined. Data were from five preparations of single neurons from four mice. **(B)** An N-type Ca^{2+} channel blocker, ω-conotoxin, inhibited the $[Ca^{2+}]_i$ response to Ninjin'yoeito. Right: incidence of responses to Ninjin'yoeito in the presence of ω-conotoxin. Numbers above bar indicate number of neurons responding over examined. Data were from six preparations of single neurons from three mice. **(C)** An L-type Ca^{2+} channel blocker, nitrendipine, failed to inhibit the $[Ca^{2+}]_i$ response to Ninjin'yoeito. Right: incidence of responses to Ninjin'yoeito in the presence of nitrendipine. Numbers above bar indicate number of neurons responding over examined. Data were from 9 preparations of single neurons from four mice.

contrast, in the presence of nitrendipine (2 μM) Ninjin'yoeito increased $[Ca^{2+}]_i$ in the majority of single neurons (**Figure 2C**), and this response occurred in 21 of 23 (91.3%) of ghrelin-responsive neurons (**Figure 2C**, Right). These results indicated that Ninjin'yoeito increases $[Ca^{2+}]_i$ in the majority of ghrelin-responsive neurons via Ca^{2+} influx to which N-type Ca^{2+} channel has greater contribution than L-type Ca^{2+} channel.

Ninjin-yoeito Increases $[Ca^{2+}]_i$ via Ca^{2+} Influx Preferentially Through L-Type VDCC in the Minority of Ghrelin-Unresponsive Neurons

The effect of Ninjin'yoeito (10 μg/ml) on $[Ca^{2+}]_i$ in ARC NPY neurons that did not respond to ghrelin were examined. Under superfusion with Ca^{2+} free KRB, Ninjin'yoeito did not increase $[Ca^{2+}]_i$ while it subsequently increased $[Ca^{2+}]_i$ in 2 mM Ca^{2+} KRB in a single neuron that subsequently failed to respond to ghrelin with $[Ca^{2+}]_i$ increase (**Figure 3A**). In nine ghrelin-unresponsive neurons, none responded to Ninjin'yoeito in Ca^{2+}-free HKRB (**Figure 3A**, Right). This result indicated that the $[Ca^{2+}]_i$ response to Ninjin'yoeito in ghrelin-unresponsive neurons requires the presence of extracellular Ca^{2+}.

We examined involvement of particular type of VDCC in the $[Ca^{2+}]_i$ response to Ninjin'yoeito in ghrelin-unresponsive neurons. Ninjin'yoeito induced increases in $[Ca^{2+}]_i$ both in the presence of and after washing out ω-conotoxin (100 nM) in the majority of single neurons (**Figure 3B**). The pattern and amplitude of $[Ca^{2+}]_i$ increases in response to Ninjin'yoeito in the presence and absence of ω-conotoxin were comparable (**Figure 3B**). In the presence of ω-conotoxin, Ninjin'yoeito increased $[Ca^{2+}]_i$ in 16 of 24 ghrelin-unresponsive neurons (66.7%) (**Figure 3B**, Right). By contrast, in the presence of nitrendipine (2 μM) Ninjin'yoeito failed to increase $[Ca^{2+}]_i$ in the majority of single neurons (**Figure 3C**), while it subsequently increased $[Ca^{2+}]_i$ after washing out this drug, showing a reversible inhibition. In the presence of nitrendipine Ninjin'yoeito increased $[Ca^{2+}]_i$ in only 3 of 17 ghrelin-unresponsive neurons (17.6%) (**Figure 3C**, Right). These results indicated that Ninjin'yoeito increases $[Ca^{2+}]_i$ in ghrelin-unresponsive neurons via Ca^{2+} influx to which L-type Ca^{2+} channel has greater contribution than N-type Ca^{2+} channel.

DISCUSSION

The present study employed $[Ca^{2+}]_i$ measurement in ARC single neurons combined with immunocytochemistry and found that among 43 ARC NPY neurons, 25 (58%) neurons responded to ghrelin and 18 (42%) neurons did not respond to ghrelin. This incidence of ghrelin-responsive neurons (58%) is not far from that in previous reports, considering different experimental conditions used among studies. The extracellular single unit recordings from *in vitro* slices indicated that ghrelin excited 73% of neurons in the ventromedial ARC, where NPY neurons are dominant, in adult rats (27). *Ex vivo* whole-cell patch-clamp recordings showed that ghrelin depolarized 40% of GFP-labeled arcuate NPY neurons in brain slices from 8–12 week-old male NPY-humanized Renilla reniformis green fluorescent protein transgenic mice (28). Ghrelin increased $[Ca^{2+}]_i$ in 59% of single ARC NPY neurons (9) and in 21–41% of single ARC neurons (12, 25) in 5–7 week-old male mice. The present study demonstrated that ghrelin-unresponsive neurons occur substantially among NPY neurons of ARC in 5–7 week-old male mice fed normal chow. Ninjin'yoeito-induced $[Ca^{2+}]_i$ increase was blunted in Ca^{2+}-free condition in both ghrelin-responsive and ghrelin-unresponsive neurons. Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited by N-type Ca^{2+} channel blocker ω-conotoxin in the majority (17 of 20), while by L-type Ca^{2+} channel blocker nitrendipine in the minority (2 of 23), of ghrelin-responsive neurons. In contrast, Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited by nitrendipine in the majority (14 of 17), while by ω-conotoxin in the minority (8 of 24), of ghrelin-unresponsive neurons. Both N- and L-type Ca^{2+} channels are reportedly expressed and functioning in ARC (24, 25, 29). Our results demonstrate that Ninjin'yoeito increases $[Ca^{2+}]_i$ via Ca^{2+} influx, to which N-type Ca^{2+} channels have greater contribution in ghrelin-responsive neurons and L-type Ca^{2+} channels have greater contribution in ghrelin-unresponsive neurons. The $[Ca^{2+}]_i$ increase often results from membrane excitation and/or stimulated signal transduction and results in exocytosis, transport, gene expression, and/or protein regulation. Thus, the $[Ca^{2+}]_i$ increase, in general, reflects the neuronal activation. The present findings place N-type and L-type Ca^{2+} channels in ARC as potential molecular targets for Ninjin'yoeito to preferentially activate ghrelin-responsive and ghrelin-unresponsive NPY neurons, respectively, in ARC.

The mechanisms underlying the link of Ninjin'yoeito to distinct VDCCs in ghrelin-responsive and ghrelin-unresponsive neurons remain to be elucidated. It has been documented that ghrelin and/or GHSR influence the activities of VDCCs (30–36). Since ghrelin increases $[Ca^{2+}]_i$ via Ca^{2+} influx primarily through N-type Ca^{2+} channels in NPY neurons (25), it is speculated that Ninjin'yoeito could interact with the GHSR and/or downstream signaling linked to N-type Ca^{2+} channels in ghrelin-responsive NPY neurons. In consistent with this, it was previously reported that GHSR coexpression with dopamine type 2 receptor (D2R)

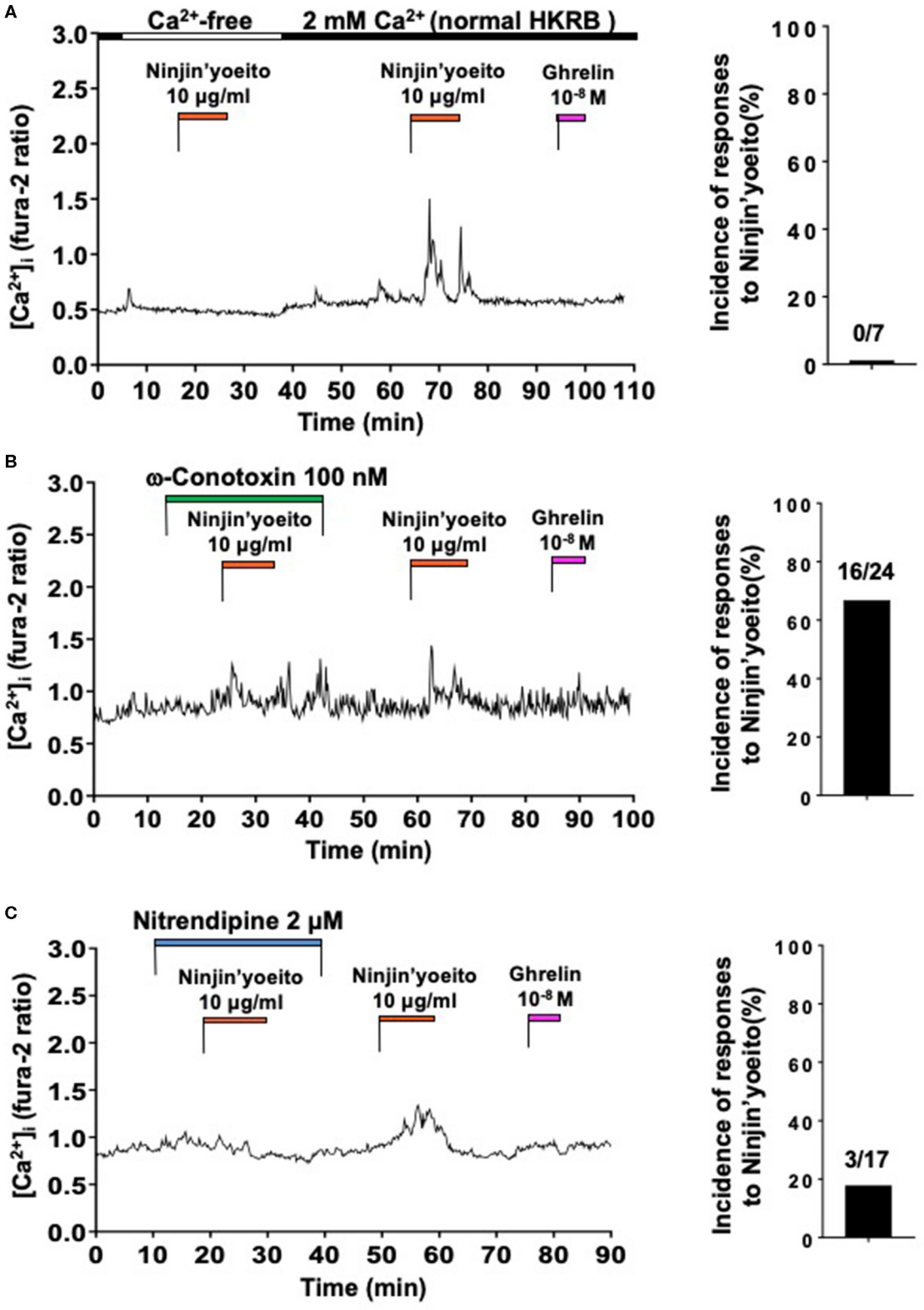

FIGURE 3 | Ninjin'yoeito-induced $[Ca^{2+}]_i$ increases were inhibited in Ca^{2+} free condition and preferentially by L-type Ca^{2+} channel blocker in the majority of ghrelin-unresponsive ARC NPY neurons. ARC NPY neurons that responded to Ninjin'yoeito (10 µg/ml) but not to ghrelin (10^{-8} M) with increases in $[Ca^{2+}]_i$ were studied. Glucose concentration was 2.5 mM. **(A)** The $[Ca^{2+}]_i$ response to Ninjin'yoeito was inhibited under superfusion with Ca^{2+} free HKRB. Right: incidence (%) of

(Continued)

FIGURE 3 | responses to Ninjin'yoeito in Ca^{2+} free HKRB. Numbers above bar indicate number of neurons responding to Ninjin'yoeito over that examined. Data were from five preparations of single neurons from four mice. **(B)** An N-type Ca^{2+} channel blocker, ω-conotoxin, failed to inhibit the $[Ca^{2+}]_i$ response to Ninjin'yoeito. Right: incidence of responses to Ninjin'yoeito in the presence of ω-conotoxin. Numbers above bar indicate number of neurons responding over examined. Data were from six preparations of single neurons from three mice. **(C)** An L-type Ca^{2+} channel blocker, nitrendipine, inhibited the $[Ca^{2+}]_i$ response to Ninjin'yoeito. Right: incidence of responses to Ninjin'yoeito in the presence of nitrendipine. Numbers above bar indicate number of neurons responding over examined. Data were from nine preparations of single neurons from four mice.

reduces the inhibition of N-type VDCC currents by D2R activation (35). However, different results were also reported that N-type VDCC currents is inhibited by constitutive GHSR activity in mouse hippocampal cultures (34) and hypothalamic neurons (33). Though the cause for the apparent discrepancy among previous documents and our finding remains unknown, we investigated the effect of short-term (∼5 min) administration of ghrelin in the current and previous studies (25), while some of previous documents observed the effect of constitutive GHSR activation (33, 34). Hence, ghrelin and GHSR signaling may have dual, acute stimulatory and chronic inhibitory, actions on N-type Ca^{2+} channel activity. Compared to ghrelin-responsive NPY neurons, properties of ghrelin-unresponsive ARC NPY neurons are less characterized. However, it was reported that a ligand for the taste receptor T1R2/T1R3 increased $[Ca^{2+}]_i$ in ARC neurons, the majority of which did not respond to ghrelin (26), and that this $[Ca^{2+}]_i$ increase was inhibited by nitrendipine, but not ω-conotoxin. Hence, T1R2/T1R3 is possibly linked to L-type Ca^{2+} channels in ghrelin-unresponsive ARC neurons and this pathway could be involved in the action of Ninjin'yoeito.

Though the Ninjin'yoeito's cellular signaling is less defined, it could interact with the excitatory signaling pathways in NPY neurons, which include the orexin—OX1R—phospholipase C pathway and low glucose—Na^+,K^+-ATPase suppression—depolarization pathway (37, 38). Notably, it was shown that AMPK activator AICAR increased $[Ca^{2+}]_i$ in two types of ARC NPY neurons, one with and the other without $[Ca^{2+}]_i$ responses to ghrelin (39), and that the AICAR-induced $[Ca^{2+}]_i$ increases were blunted in Ca^{2+}-free conditions (40). Thus, Ninjin'yoeito and AICAR share the common properties: they stimulate Ca^{2+} influx in both ghrelin-responsive and unresponsive NPY neurons in ARC. In line with this, expression of carnitine palmitoyltransferase 1 (CPT1), a signaling molecule of AMPK, is regulated by metabolic conditions (41). Taken together, Ninjin'yoeito may elicit intracellular AMPK signaling pathway for activating ghrelin-responsive and ghrelin-unresponsive NPY neurons. However, further studies are definitely required to elucidate intracellular signaling mechanisms of Ninjin'yoeito.

The functional role of the Ninjin'yoeito-regulated VDCCs in ARC NPY neurons remains to be clarified. In ghrelin-responsive neurons, administration of Ninjin'yoeito interacts with N-type Ca^{2+} channels to enhance and/or cooperate with the action of ghrelin, possibly leading to efficacious activation of ghrelin-responsive NPY neurons and consequent stimulation of appetite. Our study places ARC N-type Ca^{2+} channel as a potential mediator and integrator of the actions of Ninjin'yoeito and ghrelin in ghrelin-responsive NPY neurons. On the other hand, the ghrelin-unresponsive ARC NPY neuron has been less defined for its physiological property and cellular lineage/differentiation. It has been documented that metabolic/feeding conditions induce dynamic remodeling of NPY/AgRP neurons and differentiation of feeding related neurons in ARC (42). Hence, the ghrelin-responsive neurons and ghrelin-unresponsive neurons could be converted to each other, depending on metabolic/feeding conditions and aging. Our finding that the type of VDCC correlates with ghrelin responsiveness in NPY neurons suggests that expression of specific VDCC type may be related to remodeling of NPY neurons. In this line, the ghrelin resistance, the phenomenon that ghrelin administration cannot stimulate feeding, occurs in association with aging and diet-induced obesity (21–23). This ghrelin resistance reportedly takes place in ARC NPY neurons (21), which could result in reductions in NPY neuronal activity and appetite. The transformation of ghrelin-responsive to ghrelin-unresponsive NPY neurons may underly ghrelin resistance. Of note, we found that Ninjin'yoeito preferentially target L-type VDCC to activate ghrelin-unresponsive NPY neurons in ARC. This action of Ninjin'yoeito could serve to compensate for the ghrelin resistance and restore appetite.

The present study demonstrated that Ninjin'yoeito activates the majority of ghrelin-responsive ARC NPY neurons preferentially via N-type VDCC while the majority of ghrelin-unresponsive NPY neurons preferentially via L-type VDCCs. We suggest ARC N-type VDCC as a target for activating ghrelin-responsive NPY neurons and promoting feeding while L-type VDCC as a target for activating ghrelin-unresponsive NPY neurons and possibly compensating for ghrelin-resistance to restore appetite.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Animal experiments were carried out after receiving approval from the Institutional Animal Experiment Committee and in accordance with the Institutional Regulation for Animal Experiments at Jichi Medical University and Kobe University.

AUTHOR CONTRIBUTIONS

TY designed the study. CG, KD, and LW conducted experiments. AI and YS participated in discussion. TY and CG wrote the manuscript. TY supervised the work. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Grant-in-Aid for Scientific Research (B) (19H04045) and Challenging Exploratory Research (19K22611) from Japan Society for the Promotion of Science (JSPS) to TY. TY was supported by the Advanced Research and Development Programs for Medical Innovation (AMED-CREST) 2015-2020 from Japan Agency for Medical Research and development (AMED).

ACKNOWLEDGMENTS

We thank Ryuji Takahashi and Nina Fujita for valuable discussion, Ms. Maya Ikeda for secretarial assistance, and Drs. Wanxin Han and Yanan Zhao for technical support.

REFERENCES

1. Raghavendran HR, Rekha S, Shin JW, Kim HG, Wang JH, Park HJ, et al. Effects of Korean ginseng root extract on cisplatin-induced emesis in a rat-pica model. *Food Chem Toxicol*. (2011) 49:215–21. doi: 10.1016/j.fct.2010.10.019
2. Morley JE. Pathophysiology of the anorexia of aging. *Curr Opin Clin Nutr Metab Care*. (2013) 16:27–32. doi: 10.1097/MCO.0b013e328359efd7
3. Hiura Y, Takiguchi S, Yamamoto K, Takahashi T, Kurokawa Y, Yamasaki M, et al. Effects of ghrelin administration during chemotherapy with advanced esophageal cancer patients: a prospective, randomized, placebo-controlled phase 2 study. *Cancer*. (2012) 118:4785–94. doi: 10.1002/cncr.27430
4. Landi F, Calvani R, Tosato M, Martone AM, Ortolani E, Savera G, et al. Anorexia of aging: risk factors, consequences, and potential treatments. *Nutrients*. (2016) 8:69. doi: 10.3390/nu8020069
5. Yamasaki A, Sugahara K, Takemoto T, Ikeda T, Yamashita H. Effect of ninjin-yoei-to (Rensheng-Yangrong-Tang) on olfactory behavior after olfactory nerve transection. *Phytomedicine*. (2008) 15:358–66. doi: 10.1016/j.phymed.2007.08.006
6. Suzuki T, Yamamoto A, Ohsawa M, Motoo Y, Mizukami H, Makino T. Ninjin'yoeito and ginseng extract prevent oxaliplatin-induced neurodegeneration in PC12 cells. *J Nat Med*. (2015) 69:531–7. doi: 10.1007/s11418-015-0921-9
7. Takaku S, Shimizu M, Takahashi H. Japanese kampo medicine ninjin'yoeito synergistically enhances tumor vaccine effects mediated by CD8(+) T cells. *Oncol Lett*. (2017) 13:3471–8. doi: 10.3892/ol.2017.5937
8. Perello M, Cabral A, Cornejo MP, De Francesco PN, Fernandez G, Uriarte M. Brain accessibility delineates the central effects of circulating ghrelin. *J Neuroendocrinol*. (2019) 31:e12677. doi: 10.1111/jne.12677
9. Goswami C, Dezaki K, Wang L, Inui A, Seino Y, Yada T. Ninjin-yoeito activates ghrelin-responsive and unresponsive NPY neurons in the arcuate nucleus and counteracts cisplatin-induced anorexia. *Neuropeptides*. (2019) 75:58–64. doi: 10.1016/j.npep.2019.03.001
10. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. (2000) 404:661–71. doi: 10.1038/35007534
11. Zhang L, Bijker MS, Herzog H. The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer. *Pharmacol Ther*. (2011) 131:91–113. doi: 10.1016/j.pharmthera.2011.03.011
12. Kohno D, Yada T. Arcuate NPY neurons sense and integrate peripheral metabolic signals to control feeding. *Neuropeptides*. (2012) 46:315–9. doi: 10.1016/j.npep.2012.09.004
13. Yada T, Kohno D, Maejima Y, Sedbazar U, Arai T, Toriya M, et al. Neurohormones, rikkunshito and hypothalamic neurons interactively control appetite and anorexia. *Curr Pharm Des*. (2012) 18:4854–64. doi: 10.2174/138161212803216898
14. Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nat Neurosci*. (2011) 14:351–5. doi: 10.1038/nn.2739
15. Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, et al. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest*. (2011) 121:1424–8. doi: 10.1172/JCI46229
16. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, et al. Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci*. (2005) 8:1289–91. doi: 10.1038/nn1548
17. Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science*. (2005) 310:683–5. doi: 10.1126/science.1115524
18. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature*. (2001) 409:194–8. doi: 10.1038/35051587
19. Kohno D, Nakata M, Maekawa F, Fujiwara K, Maejima Y, Kuramochi M, et al. Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway. *Endocrinology*. (2007) 148:2251–63. doi: 10.1210/en.2006-1240
20. Zhang L, Yagi M, Herzog H. The role of NPY and ghrelin in anorexia nervosa. *Curr Pharm Des*. (2012) 18:4766–78. doi: 10.2174/138161212803216988
21. Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology*. (2010) 151:4745–55. doi: 10.1210/en.2010-0556
22. Miyazaki Y, Kaneko K, Iguchi S, Mizushige T, Kanamoto R, Yoshikawa M, et al. Orally administered delta opioid agonist peptide rubiscolin-6 stimulates food intake in aged mice with ghrelin resistance. *Mol Nutr Food Res*. (2014) 58:2046–52. doi: 10.1002/mnfr.201400100
23. Amitani M, Amitani H, Cheng KC, Kairupan TS, Sameshima N, Shimoshikiryo I, et al. The role of ghrelin and ghrelin signaling in aging. *Int J Mol Sci*. (2017) 18:1511. doi: 10.3390/ijms18071511
24. Sahu A, Crowley WR, Kalra SP, Kalra PS. Role of multiple voltage-sensitive calcium channels in depolarization-induced release of neuropeptide y and luteinizing hormone-releasing hormone from rat median eminence-arcuate nucleus. *Mol Cell Neurosci*. (1993) 4:492–8. doi: 10.1006/mcne.1993.1061
25. Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca^{2+} signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes*. (2003) 52:948–56. doi: 10.2337/diabetes.52.4.948
26. Kohno D, Koike M, Ninomiya Y, Kojima I, Kitamura T, Yada T. Sweet taste receptor serves to activate glucose- and leptin-responsive neurons in the hypothalamic arcuate nucleus and participates in glucose responsiveness. *Front Neurosci*. (2016) 10:502. doi: 10.3389/fnins.2016.00502
27. Riediger T, Traebert M, Schmid HA, Scheel C, Lutz TA, Scharrer E. Site-specific effects of ghrelin on the neuronal activity in the hypothalamic arcuate nucleus. *Neurosci Lett*. (2003) 341:151–5. doi: 10.1016/S0304-3940(02)01381-2
28. Torz LJ, Osborne-Lawrence S, Rodriguez J, He Z, Cornejo MP, Mustafá ER, et al. Metabolic insights from a GHSR-A203E mutant mouse model. *Mol Metab*. (2020) 39:101004. doi: 10.1016/j.molmet.2020.101004
29. Ishii M, Hiller AJ, Pham L, McGuire MJ, Iadecola C, Wang G. Amyloid-beta modulates low-threshold activated voltage-gated L-type calcium channels of arcuate neuropeptide Y neurons leading to calcium dysregulation and hypothalamic dysfunction. *J Neurosci*. (2019) 39:8816–25. doi: 10.1523/JNEUROSCI.0617-19.2019

30. Falls HD, Dayton BD, Fry DG, Ogiela CA, Schaefer VG, Brodjian S, et al. Characterization of ghrelin receptor activity in a rat pituitary cell line RC-4B/C. *J Mol Endocrinol*. (2006) 37:51–62. doi: 10.1677/jme.1.01943
31. Erriquez J, Bernascone S, Ciarletta M, Filigheddu N, Graziani A, Distasi C. Calcium signals activated by ghrelin and D-Lys(3)-GHRP-6 ghrelin antagonist in developing dorsal root ganglion glial cells. *Cell Calcium*. (2009) 46:197–208. doi: 10.1016/j.ceca.2009.07.003
32. Hashiguchi H, Sheng Z, Routh V, Gerzanich V, Simard JM, Bryan J. Direct versus indirect actions of ghrelin on hypothalamic NPY neurons. *PLoS ONE*. (2017) 12:e0184261. doi: 10.1371/journal.pone.0184261
33. Mustafá ER, López Soto EJ, Martínez Damonte V, Rodríguez SS, Lipscombe D, Raingo J. Constitutive activity of the ghrelin receptor reduces surface expression of voltage-gated Ca^{2+} channels in a $Ca_V\beta$-dependent manner. *J Cell Sci*. (2017) 130:3907–17. doi: 10.1242/jcs.207886
34. Martínez Damonte V, Rodríguez SS, Raingo J. Growth hormone secretagogue receptor constitutive activity impairs voltage-gated calcium channel-dependent inhibitory neurotransmission in hippocampal neurons. *J Physiol*. (2018) 596:5415–28. doi: 10.1113/JP276256
35. Cordisco Gonzalez S, Mustafá ER, Rodriguez SS, Perello M, Raingo J. Dopamine receptor type 2 and ghrelin receptor coexpression alters $Ca_V2.2$ modulation by G protein signaling cascades. *ACS Chem Neurosci*. (2020) 11:3–13. doi: 10.1021/acschemneuro.9b00426
36. Mustafá ER, Cordisco Gonzalez S, Raingo J. Ghrelin selectively inhibits $Ca_V3.3$ subtype of low-voltage-gated calcium channels. *Mol Neurobiol*. (2020) 57:722–35. doi: 10.1007/s12035-019-01738-y
37. Muroya S, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, et al. Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca^{2+} signaling in a reciprocal manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. *Eur J Neurosci*. (2004) 19:1524–34. doi: 10.1111/j.1460-9568.2004.03255.x
38. Kurita H, Xu KY, Maejima Y, Nakata M, Dezaki K, Santoso P, et al. Arcuate Na^+,K^+-ATPase senses systemic energy states and regulates feeding behavior through glucose-inhibited neurons. *Am J Physiol Endocrinol Metab*. (2015) 309:E320–33. doi: 10.1152/ajpendo.00446.2014
39. Kohno D, Sone H, Minokoshi Y, Yada T. Ghrelin raises $[Ca^{2+}]_i$ via AMPK in hypothalamic arcuate nucleus NPY neurons. *Biochem Biophys Res Commun*. (2008) 366:388–92. doi: 10.1016/j.bbrc.2007.11.166
40. Kohno D, Sone H, Tanaka S, Kurita H, Gantulga D, Yada T. AMP-activated protein kinase activates neuropeptide Y neurons in the hypothalamic arcuate nucleus to increase food intake in rats. *Neurosci Lett*. (2011) 499:194–8. doi: 10.1016/j.neulet.2011.05.060
41. Yasrebi A, Hsieh A, Mamounis KJ, Krumm EA, Yang JA, Magby J, et al. Differential gene regulation of GHSR signaling pathway in the arcuate nucleus and NPY neurons by fasting, diet-induced obesity, and 17beta-estradiol. *Mol Cell Endocrinol*. (2016) 422:42–56. doi: 10.1016/j.mce.2015.11.007
42. Cabral A, Fernandez G, Tolosa MJ, Rey Moggia A, Calfa G, De Francesco PN, et al. Fasting induces remodeling of the orexigenic projections from the arcuate nucleus to the hypothalamic paraventricular nucleus, in a growth hormone secretagogue receptor-dependent manner. *Mol Metab*. (2020) 32:69–84. doi: 10.1016/j.molmet.2019.11.014

Conflict of Interest: TY received grant support from Kracie Pharma Ltd. provided Ninjin-yoeito but was not involved in the conducting of the current study at any stage.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 31 July 2020
doi: 10.3389/fnut.2020.00115

Microbial Community Structure and Chemical Constituents in *Shinkiku*, a Fermented Crude Drug Used in Kampo Medicine

Zitai Wang[1], *Kayu Okutsu*[2*], *Taiki Futagami*[1,2], *Yumiko Yoshizaki*[1,2], *Hisanori Tamaki*[1,2], *Takuro Maruyama*[3], *Kazufumi Toume*[4], *Katsuko Komatsu*[4], *Fumio Hashimoto*[1,5] and *Kazunori Takamine*[1,2]

[1] The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan, [2] Faculty of Agriculture, Education and Research Center for Fermentation Studies, Kagoshima University, Kagoshima, Japan, [3] Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, Kawasaki, Japan, [4] Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama, Toyama, Japan, [5] Department of Horticultural Science, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan

OPEN ACCESS

Edited by:
Masahiro Ohsawa,
Nagoya City University, Japan

Reviewed by:
Lila Oyama,
Federal University of São Paulo, Brazil
Mitsuhiko Nose,
Meijo University, Japan
Yohei Sasaki,
Kanazawa University, Japan

***Correspondence:**
Kayu Okutsu
k8673711@kadai.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: 17 February 2020
Accepted: 19 June 2020
Published: 31 July 2020

Citation:
Wang Z, Okutsu K, Futagami T, Yoshizaki Y, Tamaki H, Maruyama T, Toume K, Komatsu K, Hashimoto F and Takamine K (2020) Microbial Community Structure and Chemical Constituents in Shinkiku, a Fermented Crude Drug Used in Kampo Medicine. Front. Nutr. 7:115. doi: 10.3389/fnut.2020.00115

Shinkiku (Massa Medicata Fermentata) is a traditional crude drug used to treat anorexia and dyspepsia of elder patients in east Asia. *Shinkiku* is generally prepared by the microbial fermentation of wheat and herbs. *Shinkiku* is also used in Japanese Kampo medicine as a component of 半夏白朮天麻湯 (*Hangebyakujutsutemmato*). However, the quality of *shinkiku* varies by manufacture because there are no reference standards to control the quality of medicinal *shinkiku.* Thus, we aim to characterize the quality of various commercially available *shinkiku* by chemical and microbial analysis. We collected 13 *shinkiku* products manufactured in China and Korea and investigated the microbial structure and chemical constituents. Amplicon sequence analysis revealed that *Aspergillus* sp. was common microorganism in *shinkiku* products. Digestive enzymes (α-amylase, protease, and lipase), organic acids (ferulic acid, citric acid, lactic acid, and acetic acid), and 39 volatile compounds were commonly found in *shinkiku* products. Although there were some commonalities in *shinkiku* products, microbial and chemical characteristic considerably differed as per the manufacturer. *Aspergillus* sp. was predominant in Korean products, and Korean products showed higher enzyme activities than Chinese products. Meanwhile, *Bacillus* sp. was commonly detected in Chinese *shinkiku*, and ferulic acid was higher in Chinese products. Principal component analysis based on the GC-MS peak area of the volatiles also clearly distinguished *shinkiku* products manufactured in China from those in Korea. Chinese products contained higher amounts of benzaldehyde and anethole than Korean ones. Korean products were further separated into two groups: one with relatively higher linalool and terpinen-4-ol and another with higher hexanoic acid and 1-octen-3-ol. Thus, our study revealed the commonality and diversity of commercial *shinkiku* products, in which the commonalities can possibly be the reference standard for quality control of *shinkiku,* and the diversity suggested the importance of microbial management to stabilize the quality of *shinkiku*.

Keywords: *shinkiku*, *Hangebyakujutsutemmato*, *Aspergillus* sp., *Rhizopus* sp., ferulic acid

INTRODUCTION

Massa Medicata Fermentata is a traditional crude drug used for treatment of anorexia and dyspepsia in elders in East Asia, called *shinkiku* in Japan, *shenqu* in China, and *singug* in Korea. *Shinkiku* is prepared from wheat (*Triticum sativum*), apricot kernel (*Prunus armeniaca*), red beans (*Phaseolus angularis*), polygonum (*Polygonum hydropiper*), sweet wormwood (*Artemisia apiacea*), and cocklebur (*Xanthium strumarium*). After mixing these materials, the mixture is fermented for a few days and subsequently dried to obtain *shinkiku* (1). Although *shinkiku* is manufactured in only China or Korea, it is also used in Japanese Kampo medicine as a component of "*Hangebyakujutsutemmato* (半夏白朮天麻湯)," which is used to treat dizziness and nausea (2, 3). Along with the increase in the consumption of *Hangebyakujutsutemmato*, the demand for *shinkiku* has also increased in recent years in Japan (4). However, the quality of *shinkiku* considerably differs with respect to the manufacture (5). This is because there are no reference standards for quality control of *shinkiku*. The voluntary standard in Japan stated that *shinkiku* should contain appreciable starch and reducing sugar, while that in Korea or China focused on the maximum ash or water content (1, 6, 7). The quality instability of *shinkiku* is a serious problem among the distributers of crude drugs in Japan. Thus, the characterization and standardization of commercial *shinkiku* are strongly needed to stabilize its medicinal grade quality.

A characteristic of *shinkiku* is the unique microbial fermentation process during its manufacture. "*Qu*" is sometimes mixed as a fermentative starter in China. *Qu* is the same as "*Koji*" in Japan, which is a solid-state culture of filamentous fungi (*Aspergillus* sp., *Rhizopus* sp., or *Mucor* sp.) on cereal grains, and is used to manufacture soy sauce, vinegar, and liquor. As these filamentous fungi produce various enzymes during growth (8–10), *qu* serves as an exogenous enzyme source during the production of fermented foods. Enzymes purified from *Aspergillus* sp. are also applied for medical industries. α-Amylase and lipase produced by *Aspergillus oryzae* have been used as digestive agents to treat dyspepsia (11). Furthermore, *Aspergillus* sp. produces ferulic acid esterase and releases free ferulic acid from the cell wall of plants (12, 13). Ferulic acid has been reported to show anti-inflammatory effects and accelerate gastrointestinal motility (14, 15). It has also been reported that free ferulic acid increased by fermentation with *qu* (16). Thus, the fermentation process by those filamentous fungi would add the stomachic function to the unfermented materials.

Chen et al. (17) has reported that *Aspergillus* sp. and *Rhizopus* sp. also exist in Chinese *shinkiku* products. Our previous study showed that *shinkiku* obtained from local markets in China and Korea contained digestive enzymes and ferulic acid (5). Therefore, digestive enzymes and ferulic acid derived by filamentous fungi possibly contribute to the stomachic property of *shinkiku*. However, in the previous study, neither *Aspergillus* sp. nor *Rhizopus* sp. could be detected using denaturing gradient gel electrophoresis (DGGE). It seems that DGGE is not appropriate for investigating the microbial structures of *shinkiku*. In addition, it was possible that *shinkiku* was stored in unsuitable environments in local markets, which altered the microbial conditions during storage. As *shinkiku* is prepared by microbial fermentation, characterization of microorganisms is required for quality control. In the present study, we obtained new commercial *shinkiku* products from a Japanese trading company specializing in crude drugs and investigated their chemical constituents and microbial structures using amplicon sequencing to establish reference standards for quality control of *shinkiku* in Japan.

MATERIALS AND METHODS

Chemicals and Strains

Chemicals used for the analysis were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and Wako Pure Industries Ltd. (Osaka, Japan). Folin and Ciocalteu's phenol reagent were purchased from MP Biomedicals (CA, USA), and ethylenediaminetetraacetic acid (EDTA) was obtained from DOJINDO (Kumamoto, Japan).

Crude Drug Materials

Thirteen commercial *shinkiku* products, imported from Korea or China, were kindly provided by Tochimoto Tenkaido Co., Ltd. (Osaka, Japan), Kotaro pharmaceutical Co., Ltd. (Osaka, Japan), and UCHIDAWAKANYAKU Ltd. (Tokyo, Japan). *Shinkiku* products used in this study are shown in **Figure 1**, and their production country and year of import are shown in **Table 1**. All the samples were maintained at −20°C until analysis.

DNA Extraction

DNA extraction was performed using a ZR Fecal DNA Miniprep kit (Zymo Research, CA, USA) according to the manufacturer's instructions. Mechanical disruption was carried out for each 100 mg of *shinkiku* at 6.0 m/s for 30 s by bead beating using a FastPrep 120 Cell Disrupter System (Thermo Savant; Carlsbad, CA, USA) and subjected to DNA extraction. The extracted DNA was quantified using a NanoDrop-8000 Spectrophotometer (Thermo Fisher Scientific K.K., Japan) and stored at −20°C until amplicon sequencing.

Amplicon Sequencing Analysis

Amplicon sequencing analysis was performed at Bioengineering Lab. Co., Ltd. (Kanagawa, Japan). PCR amplification was performed using primer sets V3V4f and V3V4r for bacterial 16S rDNA V3/V4 regions and ITS1F_KYO1 and ITS2_KYO2 for the fungal ITS1 region (18, 19). For each sample, a library was prepared using the two-step tailed PCR method. The obtained amplicons were subjected to a 2 × 300 bp paired end run using a MiSeq system (Illumina K.K., Tokyo, Japan). The data were deposited to the DNA data Bank of Japan (DDBJ) under the accession numbers DRR205793-DRR205818. After quality filtering and chimera check of sequencing reads, the operational taxonomic units (OTU) were predicted using QIIME (Quantitative Insight Into Microbial Ecology) (20). The OTUs that accounted for more than 1% of the total sequence number were classified, whereas taxa with abundance <1% were summarized as "others."

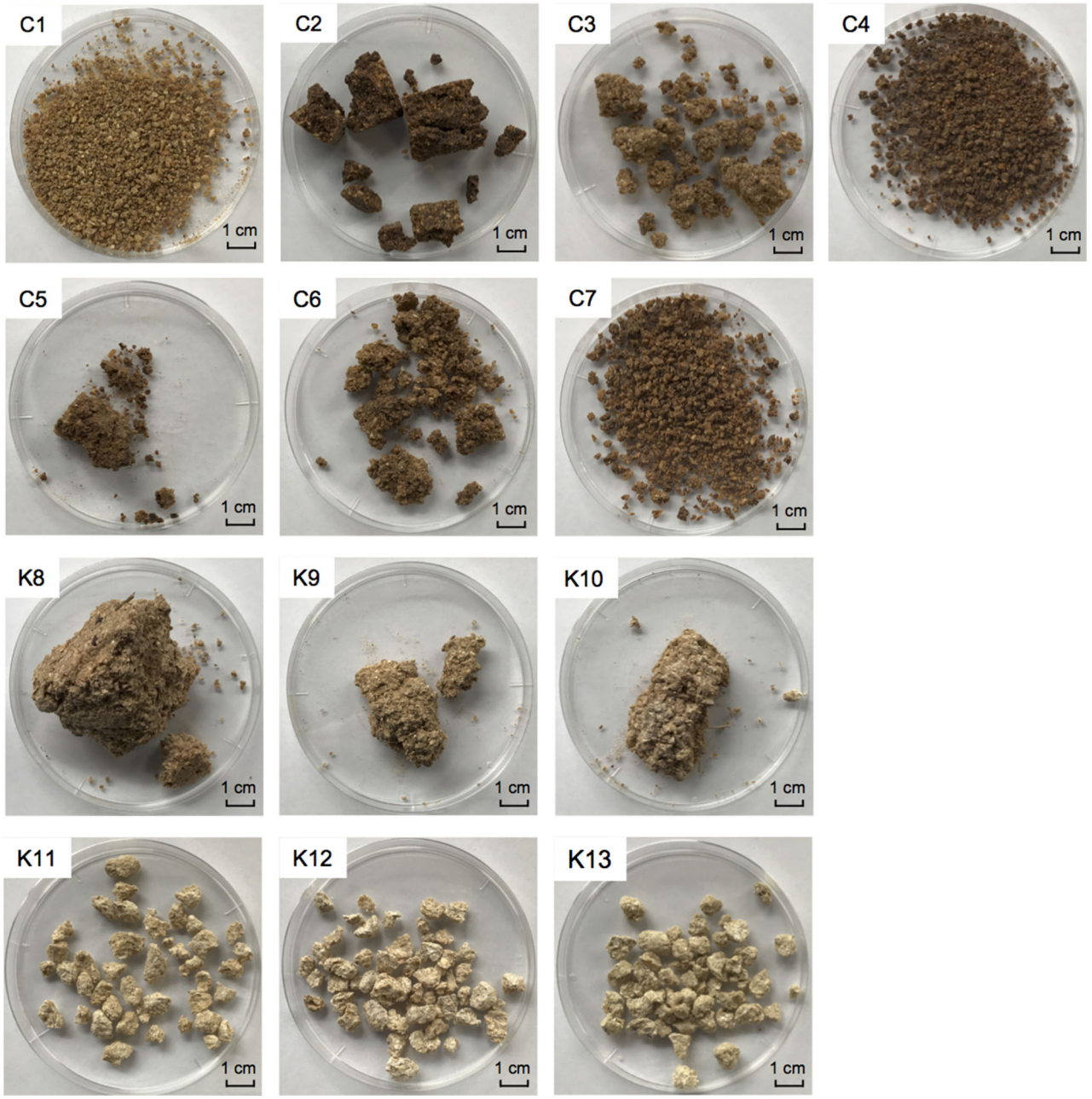

FIGURE 1 | Commercial *shinkiku* products used in this study. Chinese products for C1–C7, and Korean products for K8–K13.

Enzymatic Activity Assays

α-Amylase Assay

Crude enzyme extract was prepared according to the Official Analysis Method of National Tax Agency of Japan. *Shinkiku* (2 g) was suspended in 10 mL of 0.1 M acetate buffer solution (pH 5.0) containing 0.5% NaCl and homogenized at 11,000 rpm for 2 min on ice. Then, the homogenate was centrifuged at 22,300 × g at 4°C for 3 min. α-Amylase activity was measured using an α-amylase assay kit (Kikkoman Biochemifa, Tokyo, Japan) following the manufacturer's instructions, and 2-chloro-4-nitrophenyl-6^5-azido-6^5-deoxy-β-maltopentaoside (N3-G5-β-CNP) was used as a substrate, which was cleaved to 2-chloro-4-nitrophenol (CNP) on action of α-amylase. The increment of CNP was measured at 400 nm using a spectrophotometer. One unit of α-amylase activity was referred to the quantity of α-amylase required to release 1 μmol of CNP from N3-G5-β-CNP per minute at 37°C.

TABLE 1 | Commercial *shinkiku* products used in this study.

Sample no.	Production area	Import year
C1	China	2016
C2	China	2015
C3	China	2013
C4	China	2013
C5	China	2012
C6	China	2009
C7	China	2008
K8	Korea	2000
K9	Korea	1998
K10	Korea	1993
K11	Korea	2013
K12	Korea	2015
K13	Korea	2017

Protease Assay

Crude enzyme extract (10 mL) was dialyzed in a cellulose tube (Dialysis membrane 27/32, Viskase Companies Inc., USA) for 15 h in 1 L of 10 mM of acetate buffer (pH 5.0) and then filled up to 20 mL with water for the protease activity assay. A casein solution (2%, w/v) was used as a substrate, and the derived tyrosin by protease was quantified by a chromogenic reaction which used Folin and Ciocalteu's phenol reagent. Tyrosine was quantified using the standard curve obtained from absorbance of the known concentration of tyrosine standard. One unit of protease activity was defined as the quantity of protease required to release 1 μg of tyrosine from casein per hour at 40°C.

Lipase Assay

Lipase solution from *shinkiku* was prepared according to the method of Rahayu et al. (21) with slight modification. *Shinkiku* (5 g) was added to 15 mL of 0.1 M acetate buffer solution (pH 5.0) containing 0.5% NaCl and homogenized at 11,000 rpm for 1 min, and then, 10 mL of ethanol (99.5%) was added to the mixture and held for 1 h to extract. The supernatant obtained by centrifugation at 890 × g for 5 min was used as a lipase solution. The lipase activity was determined by Lipase Activity Assay Kit (Cayman Chemical, MI, USA) following the assay protocol. An arachidonoyl-1-thioglycerol was used as substrate, and thioglycerol derived by lipase was quantified using a fluorescence detector (ex. 380 nm, em. 510 nm) (Mithras LB940, Berthold, GmbH & co. KG., Germany) after reaction with the thiol detector. The thioglycerol concentration was determined using the calibration curve obtained from the fluorescence intensity of the known concentration of thioglycerol standard. One unit was defined as the amount of lipase that was required to release 1 nmol of thioglycerol from arachidonoyl-1-thioglycerol per minute at 37°C.

Quantification of Ferulic Acid

Ferulic acid (4-hydroxy-methoxycinnamic acid) was quantified according to the method of Okutsu et al. (5) with slight modifications. *Shinkiku* (2 g) was added to 10 mL of a water/methanol mixture (3:2) and was homogenized at 11,000 rpm for 1 min. The mixture was then centrifuged at 4,800 × g for 5 min. The supernatant was filtered through a 0.45-μm cellulose acetate membrane filter (Toyo Roshi Kaisha Ltd., Tokyo, Japan), and 10 μL of the sample was injected into the High Performance Liquid Chromatography (HPLC) system (Shimadzu LC system 20A, Shimadzu Corp., Kyoto, Japan). The ferulic acid concentration was determined using the calibration curve obtained from the peak area of the known concentration of ferulic acid standard.

Quantification of Other Organic Acids

Organic acids except for ferulic acid were determined using a Prominence HPLC system and an electroconductivity detection (Shimadzu CDD-10AVP). Each *shinkiku* (2 g) sample was mixed with 20 mL of deionized water, homogenized at 11,000 rpm for 1 min, and then, centrifuged at 4,800 × g for 5 min to obtain a supernatant. The supernatant was filtered through a 0.45 μm pore-size cellulose acetate membrane filter. The separation of organic acids was performed using an ion-exclusion chromatography column (Shim-pack SCR-102H, 8 mm I.D. × 300 mm ×2) equipped with a Guard column (SCR-102H, 6 mm I.D. × 50 mm) at 50°C using 4 mM *p*-toluenesulfonic acid as the mobile phase at a flow rate of 0.8 mL/min. The buffer solution containing 16 mM Bis-Tris, 4 mM *p*-toluenesulfonic acid, and 80 μM EDTA was pumped into the column at 0.8 mL/min flow rate.

Volatile Compounds Analysis

The supernatant used for organic acid analysis was also used to analyze the volatile compounds in *shinkiku* products. The volatile compounds were evaluated with the method described by Rahayu et al. (21). In brief, 10 mL supernatant, described in the quantification of organic acid, was transferred to a 10 mL sample vial with a 15-mm stir bar coated with 0.5 mm polydimethylsiloxane (Twister, GERSTEL K.K., Japan). The sample was stirred using a stir bar at 1,200 rpm for 60 min at 25–30°C to adsorb volatile components. Then, the stir bar was removed from the sample, washed with deionized water, and placed into a glass insert. The volatile compounds were desorbed from the SBSE stir bar using the temperature program of thermal desorption system (GERSTEL TDS 3, GERSTEL CIS 4, GERSTEL K.K., Japan) and transferred to a gas chromatograph (6890N Network GC System) equipped with a mass spectrometer (5975B insert MSD) (Agilent Technologies, Palo Alto, CA). The volatile compounds were separated on a Pure-WAX column (0.25 mm I.D. × 60 m length × 0.25 μm film thickness, J&W Scientific, Folsom, CA), and the oven temperature was held at 50°C for 5 min at 3°C/min till the temperature reached 240°C (hold for 5 min). The carrier gas was helium at a flow rate of 2 mL/min. The detected compounds were identified by comparison of their mass spectra with the National Institute of Standards and Technology (Gaithersburg, MD, USA) mass spectral library (NIST 05) and by comparison of their retention index (RI) with the database of Aroma Office software (Nishikawa Keisoku/Gerstel K.K., Japan).

RESULTS

Microbial Structure in Shinkiku Products

Preliminary, we investigated the viable fungi in *shinkiku* products using a plating assay to detect *Aspergillus* sp., *Rhizopus* sp., or *Mucor* sp.; however, no filamentous fungus like colonies were detected except for C1, K11, and K13 (**Table S1**). Thus, amplicon sequencing analysis was applied to investigate the community structure of microorganism that included filamentous fungi in *shinkiku* products.

Aspergillus sp. was detected in all *shinkiku* products, and *Rhizopus* sp. was found in more than half of *shinkiku* products (7/13) (**Figure 2A**). *Aspergillus* sp. and *Rhizopus* sp. were totally accounted for more than 50% in C3, K11, K12, and K13. *Rhizopus* sp. was predominant in C1, C3, and C4 compared with *Aspergillus* sp., but *Aspergillus* sp. was predominant in C2, K11, K12, and K13. In particular, *Aspergillus* sp. accounted for more than 70% in K11, K12, and K13. Meanwhile, *Mucor* sp. was detected only in K11 at 3%, and *Saccharomyces* sp. was detected in K11 and K12 at 4–7%. *Xeromyces* sp. was detected in C5, K8, and K9, and accounted for more than 40%. *Wallemia* sp. was detected in K10 and accounted for more than 80%. In addition, *Fusarium* sp. was detected in Chinese products such as C1, C2, C3, C4, C6, and C7, and accounted for 36% in sample C4. *Botrytis* sp. was characteristically detected in C7 with an abundance rate of 55%. *Wickerhamomyces* sp. was detected in C1, C3, C6, C7, K9, and K11 and accounted for 52% in C6.

For bacterial community structure, *Bacillus* sp. accounted for more than 60% in all Chinese products, but chloroplast and mitochondria DNA accounted for more than 50% in Korean products such as K11, K12, and K13 (**Figure 2B**). Multiple lactic acid bacteria were detected in Korean products. *Pediococcus* sp. and *Lactobacillus* sp. were detected in K12, and *Weissella* sp. was detected in K9 and K10. In contrast, *Erwinia* sp. was detected in K8.

Enzyme Activities in *Shinkiku* Products

As *shinkiku* is used to treat anorexia and dyspepsia, digestive enzymes derived by microorganisms in *shinkiku* work as a digestive promotor (22). Thus, we measured the digestive enzyme activities in *shinkiku* products. All *shinkiku* products showed α-amylase (8.3–51.3 U/g), protease (86.0–7892.8 U/g), and lipase (0.1–5805.5 U/g) activity, and Korean products tended to have higher enzyme activity than Chinese products (**Table 2**). K8 and K10 showed higher amylase activity compared to other *shinkiku* products. K13 showed the highest protease activity, and K11 showed the highest lipase activity among all *shinkiku* products.

Ferulic Acid in *Shinkiku* Products

Ferulic acid could contribute to efficacies of *shinkiku* because it accelerates gastrointestinal motility (15). Because some microorganisms secrete ferulic acid esterase (13, 23), free ferulic acid could be released from wheat materials during *shinkiku* fermentation. All *shinkiku* products were confirmed to contain free ferulic acid, and the content ranged from 25.8 to 91.4 nmol/g in Korean products and from 99.9 to 554.6 nmol/g in Chinese products (**Table 3**).

Other Organic Acids in *Shinkiku* Products

Organic acids are important metabolites of fermentative microorganisms. As each microorganism secretes different organic acids, we quantified the organic acids to investigate the relationship with microbial structure in *shinkiku* products. Citric acid, pyruvic acid, lactic acid, formic acid, and acetic acid were detected in *shinkiku* products (**Table 3**). Although K11, K12, and K13 contained remarkably high citric acid (71.9–88.8 μmol/g), the total organic acid contents tended to be higher in Chinese products compared to Korean products. Especially C2, C4, and C5 contained higher lactic acid compared to other *shinkiku* products.

Volatiles in *Shinkiku* Products

Herb materials in *shinkiku* contain various essential oils, and they act as appetizers (stomachic) (24). We measured the volatile content in *shinkiku* products exhaustively using GC-MS. A total of 146 compounds were detected, and among them, 39 compounds were common in all Chinese and Korean products: 7 acids, 5 alcohols, 8 aldehydes, 7 esters, 5 ketones, 3 lactones, 3 phenols, and 1 polycyclic aromatic hydrocarbon (**Table 4**). Principal component analysis (PCA) were used to analyze datasets of peak area of 39 common volatiles to differentiate *shinkiku* products. The PCA score plot indicated a clear variance for different volatiles based on different manufacture across PC1 (42.98%) and PC2 (16.91%) (**Figure 3**).

A clustered pattern was observed in Chinese products with a higher content of some benzenoid aromatic hydrocarbons such as naphthalene, ethyl benzoate, benzaldehyde, and benzyl alcohol. The Korean products were further separated into two groups. Samples K8, K9, and K10 were classified into the same group (Korea A) because of the higher content of terpenoids such as linalool, paeonol, and terpinen-4-ol. Meanwhile, samples K11, K12, and K13 were differentiated (Korea B) from other groups with higher contents of fatty acid (hexanoic acid, capric acid, and caprylic acid), aldehydes (decanal and hexanal), and alcohols (β-phenylethyl alcohol and 1-octen-3-ol). In addition to the common volatiles, 2,3-butanediol and pyrazines were characteristically detected in all Chinese products. Vanillin and 4-vinylguaiacol, which are metabolites of ferulic acid, were also detected in all Chinese products. Meanwhile, *d*-limonene, myristic acid, toluene, 2-pentylfuran, 2-heptanone, hinesol, and methylguaiacol were only detected in all Korean products (data not shown).

DISCUSSION

Amplicon sequencing analysis revealed that *Aspergillus* sp. was common microorganism in all *shinkiku* products, and no common bacterial types was detected in all products. Thus, *shinkiku* was manufactured with microbial fermentation with such filamentous fungi. As these fungi were difficult to be detected as viable cells, they possibly died during the production or storage process of *shinkiku*. Thus, amplicon sequencing analysis seems appropriate for clarifying the microbial structure in *shinkiku*. In addition, digestive enzymes (α-amylase, protease, and lipase), organic acids (ferulic acid,

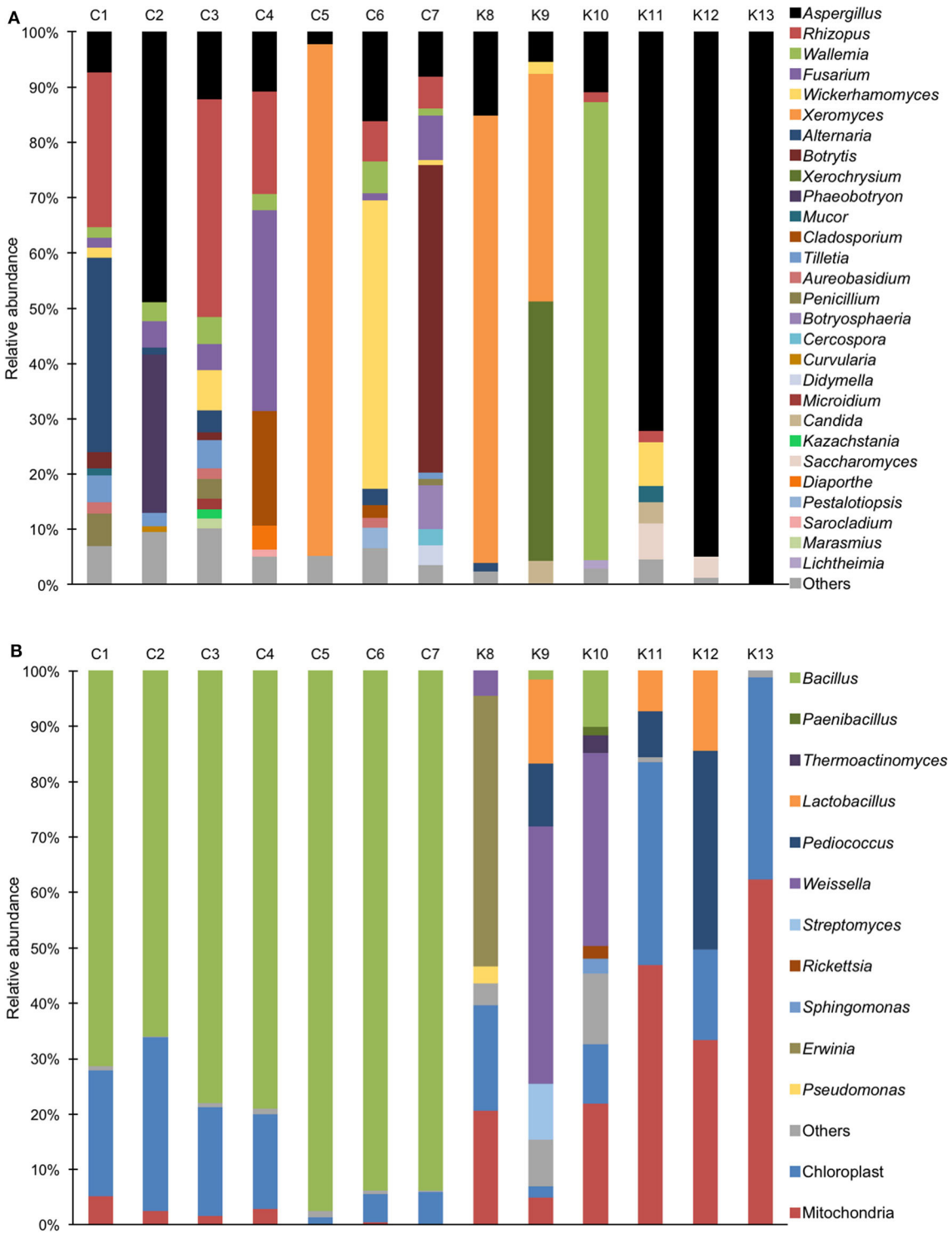

FIGURE 2 | Relative abundance of bacterial and fungal sequence reads at the genus level in *shinkiku* products. **(A)** Fungi detected by ITS1F_KYO1-ITS2_KYO2 primer set; **(B)** Bacteria detected by V3/V4f-V3/V4r primer set.

citric acid, lactic acid, and acetic acid), and 39 volatiles were commonly found in *shinkiku* products. These microbial and chemical characteristics of *shinkiku* is fundamental data for the quality control of *shinkiku* in the market, and the commonalities would contribute to normalize the quality of *shinkiku*.

However, microbial and chemical characteristics of *shinkiku* were considerably different among the *shinkiku* products. Various fungi were detected in Chinese products. Not only *Aspergillus* sp. and *Rhizopus* sp. but also *Mucor* sp. and *Saccharomyces* sp. were detected with a small abundance. These microorganisms have been reported to exist in Chinese *qu* (25, 26). *Qu* is mixed as a fermentation starter for *shinkiku* manufacture in China. The microbial community of *qu* was reported to be different among the products because it was prepared by natural fermentation (8, 27). Thus, the microbial diversity of *qu* possibly affects the microbial structures of *shinkiku* in China.

For bacteria communities, *Bacillus* sp. was predominant in all Chinese products. As *Bacillus* sp. is generally found in soil and grass hay, it possibly comes from wheat bran or herb materials in *shinkiku*. *Bacillus* sp. is reported to secret considerable ferulic acid esterase (23). It is expected that the higher ferulic acid in Chinese products was caused by ferulic acid esterase secreted by *Bacillus* sp. Higher levels of benzenoid compounds such as benzaldehyde, and anethole in Chinese products were also derived by *Bacillus* sp. because these volatiles have been reported to be synthesized by *Bacillus* sp. (28, 29).

In the case of Korean products, *Aspergillus* sp. was more predominant than *Rhizopus* sp., and microbial composition was simpler compared to Chinese products. It suggests that pure cultivated *Aspergillus* sp. might be inoculated in the materials to ferment *shinkiku* in Korea. In addition, a relatively high abundance of chloroplast DNA from herb materials and that from mitochondria were detected in Korean products. This result suggests that the amount of DNA derived from bacteria was extremely low in Korean products. The number of viable bacteria in Korean products was also considerably lower than that in Chinese products (**Table S1**). It was reported that the number of viable bacteria in *qu* was increased at the late stage of fermentation (30). Therefore, it was expected that Korean *shinkiku* was short-fermented with simple microorganisms.

Lactic acid bacteria such as *Weissella* sp., *Lactobacillus* sp., and *Pediococcus* were detected in Korean products. As these lactic acid bacteria are known to have potential to promote digestion for humans (31, 32), they possibly contribute to the efficacies of Korean *shinkiku* for anorexia and dyspepsia. However, the relative abundances of lactic acid bacteria were inconsistent with lactic acid contents in *shinkiku*. In addition, *Rhizopus* sp. in *qu* was also reported to secrete lactic acid (33), but the relative abundance of *Rhizopus* sp. was inconsistent with lactic acid content in *shinkiku* products. It possible that the microbial structure changed during fermentation, and the microorganisms in the early stage of the fermentation process affect the lactic acid contents in *shinkiku* products. Although our study revealed the microbial structure only in the final product of *shinkiku*, further

TABLE 2 | Digestive enzyme activities of *shinkiku* products.

Sample	U/g		
	α-Amylase	Protease	Lipase
C1	10.2	560.8	2.6
C2	10.3	86.0	0.1
C3	8.3	591.7	11.4
C4	35.4	435.9	1.9
C5	9.8	328.3	25.8
C6	10.0	116.1	5.5
C7	8.3	260.6	11.9
K8	51.3	2,029.8	1,673.8
K9	35.8	1,858.2	696.7
K10	49.6	2,814.6	2,656.5
K11	19.7	1,552.7	5,805.5
K12	11.8	1,762.6	4,025.0
K13	21.1	7,892.8	2,639.7

TABLE 3 | Organic acid contents of *shinkiku* products.

Sample	nmol/g	μmol/g					
	Ferulic acid	Citric acid	Pyruvic acid	Lactic acid	Formic acid	Acetic acid	Total
C1	99.9	8.0	4.6	9.5	4.3	5.6	032.1
C2	321.0	7.8	8.4	80.3	7.0	7.7	111.2
C3	87.8	7.7	10.7	16.3	5.0	5.9	045.7
C4	554.6	8.6	10.5	38.3	6.9	8.2	072.6
C5	140.7	8.0	13.5	23.8	7.3	3.3	056.0
C6	109.8	9.7	5.4	11.4	6.8	6.9	040.2
C7	176.5	9.6	14.3	9.2	8.7	5.7	047.6
K8	60.7	8.1	6.0	0.7	n.d.	0.5	015.3
K9	42.3	3.7	0.2	2.3	n.d.	0.5	006.6
K10	91.4	3.8	n.d.	1.0	0.8	0.4	005.9
K11	75.4	72.6	n.d.	11.5	1.9	3.1	089.2
K12	59.9	88.8	n.d.	7.0	2.8	2.0	100.6
K13	25.8	71.9	n.d.	0.9	4.5	1.1	078.5

n.d., not detected.

TABLE 4 | Volatile compounds commonly detected in shinkiku products.

Name	RI[a]	m/z	Identification	Peak area (1/1.0 × 10^6)[b]		
				minimum	maximum	mean[c]
Acids						
Non-anoic acid	2,147	73	MS, RI	1.8	107.8	24.5
Capric acid	2,253	73	MS, RI	3.2	23.5	9.8
Lauric acid	2,464	73	MS, RI	8.7	22.9	12.3
Acetic acid	1,439	60	MS, RI	1.4	10.6	2.8
Hexanoic acid	1,829	60	MS, RI	1.0	407.7	70.3
Palmitic acid	2,881	73	MS, RI	131.0	479.5	268.3
Caprylic acid	2,041	60	MS, RI	1.4	105.3	24.6
Alcohols						
Linalool	1,538	93	MS, RI	3.3	48.0	15.9
Terpinen-4-ol	1,592	93	MS, RI	1.8	16.9	8.1
Benzyl alcohol	1,858	108	MS, RI	3.6	117.3	51.1
1-Octen-3-ol	1,442	57	MS, RI	5.3	158.7	33.7
β-Phenylethyl Alcohol	1,893	91	MS, RI	4.0	37.4	10.7
Aldehydes						
(*E*)-2-Heptenal	1,315	83	MS, RI	1.0	15.4	4.9
Benzaldehyde	1,509	106	MS, RI	11.1	4,610.7	1,013.1
2-Phenyl-2-butenal	1,912	146	MS, RI	0.8	13.2	6.1
5-Methyl-2-phenyl-2-hexenal	2,056	117	MS, RI	0.7	36.1	11.6
Hexanal	1,085	56	MS, RI	6.3	427.1	96.2
Nonanal	1,384	57	MS, RI	2.7	64.8	17.2
Decanal	1,489	57	MS, RI	2.4	18.9	7.4
Furfural	1,451	96	MS, RI	1.4	89.5	15.3
Esters						
Ethyl benzoate	1,655	105	MS, RI	1.0	60.8	13.9
Dibutyl phthalate	2,676	149	MS, RI	11.8	129.3	49.3
Benzyl acetate	1,715	108	MS, RI	1.0	46.7	19.5
Ethyl caproate	1,229	88	MS, RI	0.5	480.2	75.9
Ethyl caprylate	1,428	88	MS, RI	1.0	33.7	9.1
Methyl salicylate	1,760	120	MS, RI	1.9	22.1	7.4
2-Phenylethyl acetate	1,801	104	MS, RI	2.1	37.1	12.9
Ketones						
Pulegone	1,635	152	MS, RI	2.7	66.1	14.9
Piperitone	1,713	110	MS, RI	1.9	181.8	23.4
2-Octanone	1,279	58	MS, RI	1.6	71.0	11.1
2-Nonanone	1,381	58	MS, RI	0.8	30.2	5.7
Geranylacetone	1,842	69	MS, RI	3.9	14.9	7.0
Lactones						
γ-Octalactone	1,897	85	MS, RI	0.8	32.4	10.8
γ-Nonalactone	2,009	85	MS, RI	13.6	203.5	94.8
Dihydroactinolide	2,319	111	MS, RI	2.8	13.3	6.0
Phenols						
Anethole	1,811	148	MS, RI	3.8	100.5	28.5
Paeonol	2,237	151	MS, RI	35.6	5,626.8	807.8
Methyleugenol	1,994	178	MS, RI	3.6	5,499.0	452.4
PAH*						
Naphthalene	1,724	128	MS, RI	3.7	45.8	16.4

**PAH, polycyclic aromatic hydrocarbon.*

[a]*RI, retention index calculated on a Pure-WAX column.*

[b]*Peak area was shown as a relative value with 1 × 10^6.*

[c]*Mean value of component peak area was calculated on the average of peak areas in 13 shinkiku.*

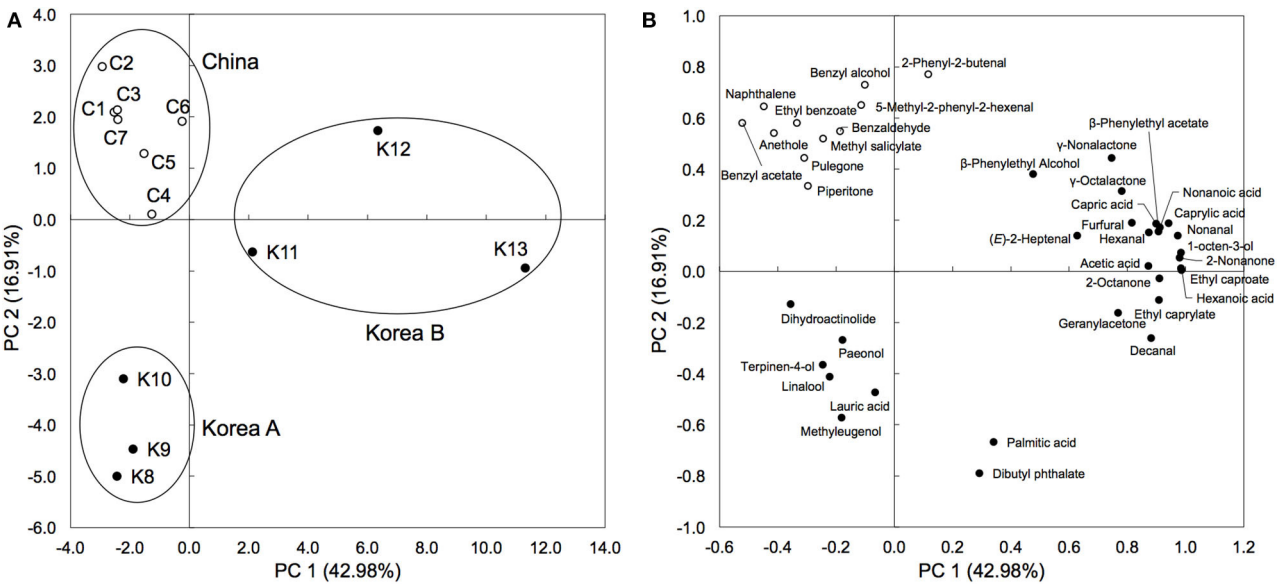

FIGURE 3 | Principal component analysis based on peak area of common volatile components in *shinkiku* products. **(A)** is for Score plot, and **(B)** is for Loading plot.

studies are needed to investigate the changes in microbes and their metabolites during *shinkiku* fermentation.

PCA based on the volatile components differentiated *shinkiku* products into three groups, among them two groups were composed of Korean *shinkiku* (Korea A and Korea B). The relative abundance of *Aspergillus* sp. in Korea B was considerably higher than that in Korea A. High levels of fatty acids in Korea B were possibly caused by lipase because of their higher lipase activity. The higher citric acid content in Korea B was also caused by its high abundance of *Aspergillus* sp. because *Aspergillus* sp. is known to secrete considerable digestive enzymes and citric acid (34). Therefore, the microbial characteristics were found to affect the chemical composition of *shinkiku*.

In contrast, some contaminating microorganisms were also detected in several *shinkiku* products. *Xeromyces* sp. and *Wallemia* sp. are known as food-contaminating fungi, and they were reported to have drought-resistance (35, 36). *Fusarium* sp. is a type of plant pathogenic fungi that produces mycotoxin (37). *Erwinia* sp. is known as a pathogenic bacteria of plants (38), and thus, *Erwinia* sp. detected in *shinkiku* products was possibly derived from infected herb materials. This result suggests that some samples in the market were possibly contaminated with poisonous microorganisms, and thus, microbial control would be critically important in *shinkiku* production.

In addition to the microbial structure, manufacturing conditions seemed to be different among manufacturers. *Bacillus* sp., which is predominant in Chinese products, forms heat-resistant spores (39), and thermal treatment caused the selective survival of *Bacillus* sp. (40). The lower enzyme activities in Chinese products indicated the possibility of enzyme deactivation by heat treatment. In addition, pyrazines, which are produced during heating (41), were also detected in Chinese products. Thus, Chinese products should be thermally treated for dryness after fermentation. In contrast, heat treatment should not be carried out for Korean products because enzyme activities in Korean products are higher than those in Chinese products. Korean products, especially Korea A, contained higher terpenoids such as linalool and terpinen-4-ol. They were derived from essential oils in herbal materials in *shinkiku*: wormwood (42), cocklebur (43), and polygonum (44). As these terpenoids are volatile, Korean *shinkiku* is possibly dried at lower temperature than Chinese *shinkiku*. In addition, ferulic acid content was relatively lower in Korean products in spite of the higher abundance of *Aspergillus* sp., which is also reported to secret ferulic acid esterase (12, 13). As ferulic acid is an enzymatic product, the production temperature and duration possibly affected the ferulic acid content in *shinkiku*. Further studies are needed to investigate the relationships between manufacturing processes and chemical constituents in *shinkiku*.

Although there was no relationship between the import year and quality of *shinkiku* (**Table 1**), some *shinkiku* products contained vanillin, which is possibly formed during storage. Vanillin is derived from 4-vinylguaiacol by the maturation of liquors such as *awamori* (45). As vanillin formation is affected by temperature, the storage condition of *shinkiku* would affect the vanillin content. The effects of storage and transportation were also confirmed by comparing the effects observed in the previous study. Digestive enzyme activities and ferulic acid contents of some *shinkiku* obtained from local markets were significantly lower than those in the present study (5). As *shinkiku* seems to be stored for a long time in unsuitable environment in the local markets, the enzyme activities or ferulic acid content were possibly altered depending on the storage condition. Further studies are required to investigate the aging effects on the quality of *shinkiku*.

In conclusion, our study revealed that the commonality and diversity of commercial *shinkiku* products. The commonalities were possibly the reference standard for quality control of *shinkiku*. Furthermore, amplicon sequence analysis showed a clearly different microbial characteristic among the products. As a result of chemical analysis partially corresponding to microbial structure, the microorganisms in *shinkiku* were found to affect its chemical composition. Thus, microbial management was suggested to be important to stabilize the quality of *shinkiku* products. In addition, the results indicated that manufacturing conditions such as heating temperature seemed to be different among manufacturers. To standardize the *shinkiku* quality, further studies are needed to elucidate the effects of microbes or manufacturing conditions on chemical constituents of *shinkiku*.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the DNA data Bank of Japan (DDBJ)/DRR205793-DRR205818.

AUTHOR CONTRIBUTIONS

ZW and KO performed all chemical analyses and wrote the original draft. YY, KK, FH, and KTa contributed to the conception and design of the experiment and paper preparation. TF and HT supervised microbial analysis and reviewed the drafts of the paper. TM and KTo performed sample collection and reviewed drafts of the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a Grant-in-Aid for the Cooperative research Project from Institute of Natural Medicine, University of Toyama in 2017 and Grant No. (19K02374) of JSPS KAKENHI.

ACKNOWLEDGMENTS

The institute of Natural Medicine, University of Toyama, is gratefully acknowledged for having provided the financial support for this study. We appreciate Dr. Yohei Shiraishi in Bio'c Co., Ltd. for viable cell analysis of *shinkiku* products. We wish to thank Kotaro pharmaceutical Co., Ltd., Tochimoto Tenkaido Co., Ltd. and UCHIDAWAKANYAKU Ltd. for kindly providing us the *shinkiku* products. We would like to thank Editage (www.editage.jp) for English language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.00115/full#supplementary-material

REFERENCES

1. Ministry of Health, Labour, and Welfare Evaluation, and Licensing Division of Pharmaceutical and Food Safety Bureau. *Self-Imposed Standards for the Crude Drugs Without Official Standards* (Provisional translation). (2012).
2. Watanabe K, Mori K, Tokumine J, Yorozu T. Effect of hangebyakujutsutemmato on pregabalin-induced dizziness in a rat model of neuropathic pain. *Tradit Kampo Med*. (2019) 6:88–95. doi: 10.1002/tkm2.1218
3. Suzuki H, Sone H, Watanabe K. Japanese kampo medicines for diabetes mellitus. In: Arumugam S, Watanabe K, editors. *Japanese Kampo Medicines for the Treatment of Common Diseases: Focus on Inflammation*. Elsevier: Academic Press (2017). p. 69–73. doi: 10.1016/B978-0-12-809398-6.00008-1
4. Yamamoto Y, Ko H, Sasaki H, Takeda O, Higuchi Y, Mukaida Y, et al. Survey on crude drug in Japan. *Japan J Pharmacog*. (2019) 73:16–35. doi: 10.24684/jspharm.73.1_16
5. Okutsu K, Kadooka C, Kojo A, Yoshizaki Y, Futagami T, Tamaki H, et al. Microbial diversity and chemical constituents in "*Shinkiku*", a fermented crude drug. *Japan J Pharmacog*. (2017) 71:41–8. doi: 10.24684/jspharm.71.1_41
6. National Health Commission of the People's Republic, China. *National Standard for Chinese Patent Drugs (Provisional Translation)*. (1989).
7. Korea Food and Drug Administration. *The Korean Herbal Pharmacopoeia Fourth edition (KHP IV)*. (2002). p. 162.
8. Huang FX, Cai TD, Nip W. Chinese wines: jiu. In: Hui YY, editor. *Handbook of Food Science, Technology, and Engineering*. Boca Raton, FL: CRC press (2006). p. 3–15.
9. Ushijima S. Improvement of industrial *Aspergillus* fungi. In: Smith JE, editor. *Aspergillus*. New York, NY: Springer Science & Business Media, LLC (2012). p. 41–64.
10. Londoño-Hernándeza L, Ramírez-Torob C, Ruiza AH, Ascacio-Valdésa AJ, Aguilar-Gonzalezc AM, Rodríguez-Herreraa R, et al. *Rhizopus oryzae* – ancient microbial resource with importance in modern food industry. *Int J Food Microbiol*. (2017) 257:110–27. doi: 10.1016/j.ijfoodmicro.2017.06.012
11. Zhu Y, Tramper J. Koji - where East meets West in fermentation. *Biotechnol Adv*. (2013) 31:1448–57. doi: 10.1016/j.biotechadv.2013.07.001
12. Faulds CB, Williamson G. Release of ferulic acid from wheat bran by a ferulic acid esterase (FAE-III) from *Aspergillus niger*. *Appl Microbiol Biotechnol*. (1995) 43:1082–7. doi: 10.1007/BF00166929
13. Koseki T, Hori A, Seki S, Murayama T, Shiono Y. Characterization of two distinct feruloyl esterases, AoFaeB d AoFaeC, from *Aspergillus oryzae*. *Appl Microbiol Biotechnol*. (2009) 83:689–96. doi: 10.1007/s00253-009-1913-z<underline
14. Ou SY, Kwok KC. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J Sci Food Agric*. (2004) 84:1261–9. doi: 10.1002/jsfa.1873
15. Badary OA, Awad AS, Sherief MA, Hamada FM. *In vitro* and *vivo* effects of ferulic acid on gastrointestinal motility: inhibition of cisplatin-induced delay in gastric emptying in rats. *World J Gastroenterol*. (2006) 12:5363–7. doi: 10.3748/wjg.v12.i33.5363
16. Mo X, Xu Y. Ferulic acid release and 4-vinylguaiacol formation during Chinese rice wine brewing and fermentation. *J Inst Brew*. (2012) 116:304–11. doi: 10.1002/j.2050-0416.2010.tb00435.x<underline
17. Chen J, Jiao XL, Yang CY, Song MF, Gao WW. Fungal composition in massa medicata fermentata based on culture dependent method and independent PCR-SSCP technique. *Zhongguo Zhong Yao Za Zhi*. (2014) 39:4169–73. doi: 10.4268/cjcmm20142117
18. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE*. (2012) 7:e40863. doi: 10.1371/journal.pone.0040863
19. Herlemann D, Labrenz M, Jürgens K, Bertilsson S, Waniek J, Anderssonet A. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J*. (2011) 5:1571–9. doi: 10.1038/ismej.2011.41

20. Caporaso J, Kuczynski J, Stombaugh J, Bittinger K, Bushman F, Costello E, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. (2010) 7:335–6. doi: 10.1038/nmeth.f.303
21. Rahayu YYS, Yoshizaki Y, Yamaguchi K, Okutsu K, Futagami T, Tamaki H, et al. Key volatile compounds in red *koji-shochu*, a *Monascus*-fermented product, and their formation steps during fermentation. *Food Chem*. (2017) 224:398–406. doi: 10.1016/j.foodchem.2016.12.005
22. Whitcomb DC, Lowe ME. Human pancreatic digestive enzymes. *Dig Dis Sci*. (2007) 52:1. doi: 10.1007/s10620-006-9589-z
23. Donaghy J, Kelly PF, McKay AM. Detection of ferulic acid esterase production by *Bacillus* spp and lactobacilli. *Appl Microbiol Biotechnol*. (1998) 50:257–60. doi: 10.1007/s002530051286
24. Skalicka-Wozniak K, Walasek M, Ludwiczuk A, Glowniak K. Isolation of terpenoids from *Pimpinella anisum* essential oil by high-performance counter-current chromatography. *J Sep Sci*. (2013) 36:2611–4. doi: 10.1002/jssc.201300407
25. Gou M, Wang H, Yuan H, Zhang W, Tang Y, Kida K. Characterization of the microbial community in three types of fermentation starters used for Chinese liquor production. *J. Inst. Brew*. (2015) 121:620–7. doi: 10.1002/jib.272
26. Wu H, Zhang S, Ma Y, Zhou J, Luo H, Yang J. Comparison of microbial communities in the fermentation starter used to brew *Xiaoqu* liquor. *J Inst Brew*. (2017) 123:113–20. doi: 10.1002/jib.388<underline
27. Tang Q, He G, Huang J, Wu C, Jin Y, Zhou R. Characterizing relationship of microbial diversity and metabolite in Sichuan Xiaoqu. *Front Microbiol*. (2019) 10:696. doi: 10.3389/fmicb.2019.00696
28. Fujita K, Fujita T, Kubo I. Anethole, a potential antimicrobial synergist, converts a fungistatic dodecanol to a fungicidal agent. *Phytother. Res*. (2007) 21:47–51. doi: 10.1002/ptr.2016
29. Chukeatirote E, Eungwanichayapant PD, Kanghae A. Determination of volatile components in fermented soybean prepared by a co-culture of *Bacillus subtilis* and *Rhizopus oligosporus*. *Food Res*. (2017) 1:225–33. doi: 10.26656/fr.2017.6.066
30. Hu Y, Dun Y, Li S, Fu B, Xiong X, Peng N, et al. Changes in microbial community during fermentation of high-temperature *Daqu* used in the production of Chinese 'Baiyunbian' liquor. *J Inst Brew*. (2017) 123:594–9. doi: 10.1002/jib.455
31. Gilliland SE. Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol Rev*. (1990) 7:175–88. doi: 10.1111/j.1574-6968.1990.tb04887.x
32. Fessard A, Remize F. Why are *Weissella* spp. not used as commercial starter cultures for food fermentation? *Fermentation*. (2017) 3:38. doi: 10.3390/fermentation3030038
33. Yin X, Yoshizaki Y, Ikenaga M, Han LX, Okutsu K, Futagami T, et al. Manufactural impact of the solid-state saccharification process in rice-flavor *baijiu* production. *J Biosci Bioeng*. (2019) 129:315–21. doi: 10.1016/j.jbiosc.2019.09.017
34. Hong SB, Lee M, Kim DH, Varga J, Frisvad JC, Perrone G. *Aspergillus luchuensis*, an industrially important black *Aspergillus* in East Asia. *PLoS ONE*. (2013) 8:e63769. doi: 10.1371/journal.pone.0063769
35. Leong SL, Pettersson OV, Rice T, Hocking AD, Schnurer J. The extreme xerophilic mould *Xeromyces bisporus* – growth and competition at various water activities. *Int J Food Microbiol*. (2011) 145:57–63. doi: 10.1016/j.ijfoodmicro.2010.11.025
36. Zajc J, Gunde-Cimerman N. The genus *Wallemia*—from contamination of food to health threat. *Microorganisms*. (2018) 6:46. doi: 10.3390/microorganisms6020046
37. Munkvold GP. Fusarium species and their associated mycotoxins. In: Moretti A, Susca A, editors. *Mycotoxigenic Fungi*. New York, NY: Humana Press (2017). p. 51–106.
38. Chatterjee AK, Starr MP. Genetics of *Erwinia* species. *Ann Rev Microbiol*. (1980) 34:645–76. doi: 10.1146/annurev.mi.34.100180.003241
39. Setlow P. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *J Appl Microbiol*. (2006) 101:514–25. doi: 10.1111/j.1365-2672.2005.02736.x
40. Luu S, Cruz-Mora J, Setlow B, Feeherry FE, Doona CJ, Setlow P. The effects of heat activation on *Bacillus* spore germination, with nutrients or under high pressure, with or without various germination proteins. *Appl Environ Microbiol*. (2015) 81:2927–38. doi: 10.1128/AEM.00193-15
41. Maga JA, Sizer CE, Myhre DV. Pyrazines in foods. *Crit Rev Food Sci Nutr*. (1973) 4:39–115. doi: 10.1080/10408397309527153
42. Zhang N, Tang L, Hu W, Wang K, Zhou Y, Li H, et al. Insecticidal, fumigant, and repellent activities of sweet wormwood oil and its individual components against red imported fire ant workers (Hymenoptera: Formicidae). *J Insect Sci*. (2014) 14:241. doi: 10.1093/jisesa/ieu103
43. Esmaeili A, Rustaiyan A, Akbari TM, Moazami N, Masoudi S, Amiri H. Composition of the essential oils of *Xanthium strumarium L* and *Cetaurea solstitialis L* from Iran. *J Essent Oil Res*. (2006) 18:427–9. doi: 10.1080/10412905.2006.9699131<underline
44. Lee TK, Vairappan CS. Antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of selected South East Asian herbs. *J Med Plant Res*. (2011) 5:5284–90.
45. Koseki T, Ito Y, Furuse S, Ito K. Conversion of ferulic acid into 4-vinylguaiacol, vanillin and vanillic acid in model solutions of shochu. *J Ferment Bioeng*. (1996) 82:46–50. doi: 10.1016/0922-338X(96)89453-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

CASE REPORT
published: 14 August 2020
doi: 10.3389/fnut.2020.00119

A Preliminary Trial in the Efficacy of Yokukansankachimpihange on REM Sleep Behavior Disorder in Dementia With Lewy Bodies

Yuta Manabe [1,2,3]*

[1] Department of Dementia and Geriatric Internal Medicine, Kanagawa Dental University, Yokosuka, Japan, [2] Department of Emergency and General Internal Medicine, School of Medicine, Fujita Health University, Toyoake, Japan, [3] Department of Internal Medicine, Dementia Diagnosis Center, Yokohama Shintoshi Neurosurgical Hospital, Yokohama, Japan

OPEN ACCESS

Edited by:
Akio Inui,
Kagoshima University, Japan

Reviewed by:
Masayuki Nakamura,
Graduate School of Medical and Dental Sciences, Kagoshima University, Japan
Yoshiyuki Takimoto,
The University of Tokyo, Japan

***Correspondence:**
Yuta Manabe
manabe.epikuros@gmail.com

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *24 March 2020*
Accepted: *24 June 2020*
Published: *14 August 2020*

Citation:
Manabe Y (2020) A Preliminary Trial in the Efficacy of Yokukansankachimpihange on REM Sleep Behavior Disorder in Dementia With Lewy Bodies. Front. Nutr. 7:119. doi: 10.3389/fnut.2020.00119

Background: Clonazepam (CNZP) is effective in ∼90% of patients with rapid eye movement sleep behavior disorder (RBD) but has risks of oversedation, muscular relaxation, and adverse effects on cognitive function when used to treat RBD associated with dementia with Lewy bodies (DLB). Yokukansankachimpihange (YKSCH), a traditional herbal medicine, decreases sleep latency and increases sleep stage 2, like benzodiazepines (BZPs), but does not cause adverse events such as oversedation, muscular relaxation, and adverse effects on cognitive function. Given these pharmacological properties, YKSCH was studied as a potential alternative to CNZP.

Methods: Of patients who were diagnosed with DLB according to the criteria for the clinical diagnosis of DLB established by the Consortium on Dementia with Lewy Bodies (CDLB) in 2017, 13 consecutive patients with the cutoff score (5 points) or more in a REM sleep behavior disorder screening questionnaire and polysomnographic evidence of REM without atonia were observed using the Neuropsychiatric Inventory (NPI) night-time behavior disturbance, visual analog scale (VAS) frequency, and VAS severity as the co-primary endpoints. Data from 11 patients who completed the study were statistically analyzed.

Results: Statistically significant improvements were observed in the NPI night-time behavior disturbance, VAS frequency, and VAS severity. No notable adverse events were reported.

Conclusion: The results indicated that YKSCH, which does not cause oversedation, muscular relaxation, or adverse effects on cognitive function, may provide a new therapeutic option for RBD associated with DLB as an alternative to CNZP.

Keywords: clonazepam, Lewy body disease, polysomnography, REM sleep behavior disorder, traditional herbal medicine

INTRODUCTION

Rapid eye movement sleep behavior disorder (REM sleep behavior disorder; RBD) is a parasomnia involving dream enactment behavior (DEB). Among neurodegenerative diseases, this disease attracts special interest because of its close relationship with α-synucleinopathy, including Lewy body diseases (LBD) such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), as evidenced by a report that half of patients with RBD develop PD-related disease within 10 years (1). Schenck et al. reported that ∼38% of 29 patients with idiopathic RBD developed PD after a mean time of 3.7 years since diagnosis (2), with a 16 year follow-up report that RBD progressed to PD-related disease or dementia in ∼81% of patients (3). In addition to these clinical studies, Boeve et al. conducted a neuropathological study in 172 patients with RBD and reported that 93% of the patients had α-synucleinopathy, including 136 patients with LBD (and Alzheimer's disease in 59 patients) (4). Based on many findings, including the aforementioned, the criteria for the clinical diagnosis of DLB in 2017 have raised RBD to a core feature of DLB and added polysomnographic (PSG) evidence of REM without atonia (RWA) as a new biomarker indicative of DLB (5).

In the treatment of RBD, clonazepam (CNZP) is effective in ∼90% of patients with RBD (6), and it was reported that CNZP was as effective for RBD associated with PD as for idiopathic RBD, leading to the use of CNZP as the standard of care (7), although no randomized comparative study in LBD has been conducted to evaluate the usefulness of CNZP. While RBD, which poses a risk of injury to patients and their bed partners, requires aggressive therapeutic intervention, patients with LBD, especially DLB, are hypersensitive to drugs and vulnerable to the side effects of CNZP, including oversedation, muscular relaxation, and adverse effects on cognitive function. Hence, drugs that improve RBD by acting on the sleep architecture without muscular relaxation are awaited, and potential alternative treatments include Yokukansankachimpihange (YKSCH), a traditional herbal medicine, which has been reported to be effective for sleep disorder, including RBD associated with PD (8). To the best of our knowledge, however, the position of YKSCH in the treatment of sleep disorder, including RBD associated with DLB, has not been studied or reported. YKSCH, which decreases sleep latency and increases sleep stage 2 (9), is approved for the treatment of sleep disorder in Japan and is widely used in clinical settings, partly due to its large safety margin, a feature common to traditional herbal medicines.

We studied YKSCH as a potential alternative to CNZP for RBD associated with DLB based on our experience, previous reports, and pharmacological features of YKSCH.

METHODS

Participants

Thirteen new patients who visited our hospitals from September 1, 2017, to March 31, 2019, and met all of the inclusion criteria listed below were included in the study.

Inclusion and Exclusion Criteria

1. Patients who were diagnosed with DLB as evidenced by ^{123}I-MIBG myocardial scintigraphy and/or ^{123}I-ioflupane SPECT according to the criteria for the clinical diagnosis of DLB established by the Consortium on Dementia with Lewy Bodies (CDLB) in 2017. Patients who met the diagnostic criteria but were not diagnosed with dementia but had a Mini-Mental State Examination (MMSE) score of ≥24 were included as candidate subjects with pre-dementia stage of DLB.
2. Aforementioned candidate subjects who were definitively diagnosed with RBD based on the cut-off score (5 points) or more on the REM sleep behavior disorder screening questionnaire (REM sleep behavior disorder screening questionnaire—Japanese version; RBDSQ-J) and had polysomnographic (PSG; PSG-1100, Nihon Kohden Corp., Tokyo, Japan) evidence of RWA. The cut-off score was based on the results of a validation study of the RBDSQ-J conducted by Miyamoto et al. (10).
3. Patients who had not received YKSCH or Yokukansan of the same class, and they were drug-naïve about psychotropics.
4. Patients who met all of the aforementioned criteria and provided oral and written informed consent to participate in the study after receiving an explanation of the study.

Preexisting psychotropic drugs were allowed at stable doses, with dose modification prohibited throughout the observation period. Starting treatment with psychotropic drugs, including acetylcholinesterase inhibitors, anti-parkinsonian drugs, and hypnotics, was prohibited during the observation period.

Those who, in the opinion of the clinician, had disease or comorbidity inadequate for participation in the study such as severe cardiac failure, assisted-living residents, those living alone, and those who had no consistent bed partner were excluded from the study.

Procedures

Thirteen patients meeting all of the aforementioned criteria orally received YKSCH at a dose of 3.75 g before dinner and at bedtime. Symptoms were assessed in terms of the endpoints described below before (Week 0) and 4 weeks after the start of treatment.

RBD was assessed by bed partners using the Neuropsychiatric Inventory (NPI) night-time behavior disturbance and the visual analog scale (VAS) for frequency and severity. The VAS for frequency was defined as follows: 0: neither talking nor shouting during sleep, nor intense movements of limbs are observed; 1: talking or shouting during sleep and/or intense movements of limbs are observed less than once a week; 2: talking or shouting during sleep and/or intense movements of limbs are observed at least once a week; and 3: talking or shouting during sleep and/or intense movements of limbs are observed every day. The VAS for severity was defined as follows: 0: neither talking nor shouting during sleep nor intense movements of limbs are observed; 1: talking or shouting during sleep and/or intense movements of limbs are observed but do not bother the bed partner; and 2: talking or shouting during sleep

and/or intense movements of limbs are observed and bother the bed partner.

The cognitive function of subjects was assessed using the MMSE, the revised Hasegawa's dementia rating scale (HDS-R), and the Japanese version of Montreal Cognitive Assessment (MoCA-J). The motor symptoms were assessed using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III).

In addition, adverse events, including hypokalemia, edema, and weakness, were monitored at Weeks 0 and 4 by means of general physical examination and blood biochemical tests.

Statistical Analysis

Collected data were statistically analyzed using Wilcoxon's signed-rank test using the statistical analysis software EZR Version 1.37 (Easy R, The R Foundation for Statistical Computing, Vienna, Austria) (11).

This study was conducted after being reviewed and approved by the institutional review board of Yokohama Shintoshi

TABLE 1 | The baseline data for individual subjects.

Sex	Age	Diagnosis	MMSE	HDS-R	MoCA-J	RBDSQ-J	MDS UPDRSIII	NPI-night time behavior disturbance	VAS frequency	VAS severity	Serum potassium (mEq/l)	Concomitant drugs
1 M	85	DLB	23	24	18	6	1	8	3	2	4.7	
2 M	76	Pre-dementia stage of DLB	25	26	22	8	0	8	3	2	4	
3 M	66	Pre-dementia stage of DLB	30	29	26	8	1	4	3	2	4.6	
4 F	74	DLB	25	22	19	7	2	4	3	2	4.1	
5 M	81	DLB	24	21	17	9	6	8	3	2	4.5	Pitavastatin, Clopidogrel
6 M	76	Pre-dementia stage of DLB	26	26	25	5	0	4	2	1	4.5	
7 F	81	DLB	16	14	11	5	10	6	2	1	5.1	
8 M	71	Pre-dementia stage of DLB	30	28	24	6	0	3	1	1	4.2	Topiroxostat, Zinc
9 F	89	DLB	20	23	14	6	3	4	3	1	5.1	
10 M	66	Pre-dementia stage of DLB	30	30	27	8	0	8	3	2	4.7	
11 M	76	Pre-dementia stage of DLB	29	29	24	10	0	8	3	2	4.4	Telmisartan, Benidipine Atrorvastatin, Famotidine

DLB, dementia with Lewy bodies; HDS-R, the revised Hasegawa's dementia rating scale; MoCA-J, Japanese version of Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; MDS-UPDRSIII, Movement Disorder Society- Unified Parkinson's Disease Rating Scale part III; NPI, Neuropsychiatric Inventory; VAS, visual analog scale.

TABLE 2 | The changes after 4 weeks from baseline of NPI night-time behavior disturbance, VAS for frequency and severity, and serum potassium level.

Sex	Age	Diagnosis	NPI night time behavior disturbance		VAS frequency		VAS severity		Serum potassium (mEq/l)	
			0 w	4 w	0 w	4 w	0 w	4 w	0 w	4 w
1 M	85	DLB	8	2	3	1	2	1	4.7	4.4
2 M	76	Pre-dementia stage of DLB	8	2	3	1	2	1	4	3.9
3 M	66	Pre-dementia stage of DLB	4	4	3	3	2	2	4.6	5.1
4 F	74	DLB	4	1	3	1	2	1	4.1	4
5 M	81	DLB	8	6	3	2	2	1	4.5	4.5
6 M	76	Pre-dementia stage of DLB	4	4	2	2	1	1	4.5	4.2
7 F	81	DLB	6	3	2	1	1	1	5.1	4.1
8 M	71	Pre-dementia stage of DLB	3	0	1	0	1	0	4.2	4.7
9 F	89	DLB	4	3	3	2	1	1	5.1	4.7
10 M	66	Pre-dementia stage of DLB	8	0	3	0	2	0	4.7	4
11 M	76	Pre-dementia stage of DLB	8	3	3	2	2	1	4.4	4.5

DLB, dementia with Lewy bodies; NPI, Neuropsychiatric Inventory; VAS, visual analog scale.

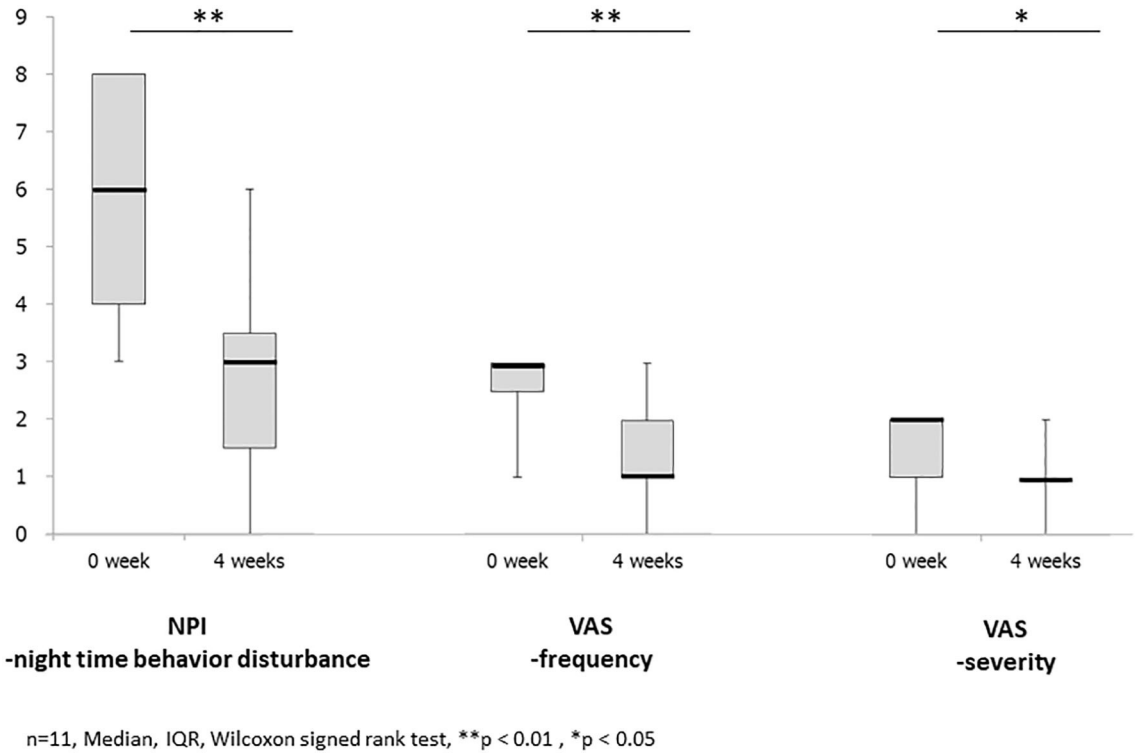

FIGURE 1 | It shows the results of changes before and after treatment with Yokukansankachimpihange in statistical analysis. The mean and median scores improved in NPI night-time behavior disturbance, VAS, frequency, and severity with statistically significant differences observed between the two time points. There is no statistically significant difference in the serum potassium level.

Neurosurgical Hospital. The author declares no conflict of interest.

RESULTS

Of 13 subjects, 2 dropped out of the study; therefore, the remaining 11 subjects were included in the analysis. The reasons for dropout were as follows: one subject who was found to have breast cancer after informed consent withdrew consent to receive treatment for breast cancer, and the other subject voluntarily dropped out of the study due to poor treatment compliance.

Baseline data for individual subjects are presented in **Table 1**, and the changes after for weeks from baseline of NPI night-time behavior disturbance, VAS for frequency and severity, and serum potassium level after 4 weeks are shown in **Table 2**. Furthermore, the results of statistical analysis are presented in **Figure 1**.

Of the 11 subjects, 8 were male, and 3 were female, with a mean age of 76.5 ± 2.3 years; 5 had DLB, and 6 had pre-dementia stage of DLB. All female subjects had DLB. These demographic characteristics of subjects are presented in **Table 3**.

On behalf of subjects, the polysomnogram and polysomnographic findings obtained from PSG in Case 11 are presented in **Figure 2**. The polysomnogram revealed REM sleep from 22:03 to 23:03, whereas the concurrent electromyogram (EMG) revealed RWA.

TABLE 3 | The demographic characteristics of subjects.

Total participants	11
Men	8
Women	3
Age	76.5 ± 2.3
The number of patients with DLB	5
The number of patients with pre-dementia stage of DLB	6
MMSE	25.3 ± 4.5 median: 25 (16–30)
HDS-R	24.7 ± 4.7 median: 26 (14–30)
MoCA-J	20.6 ± 5.2 median: 22 (11–27)
MDS-UPDRS III	2.1 ± 3.2 median: 1 (0–16)

DLB, dementia with Lewy bodies; HDS-R, the revised Hasegawa's dementia rating scale; MoCA-J, Japanese version of Montreal Cognitive Assessment; MMSE, Mini-Mental State Examination; MDS-UPDRSIII, Movement Disorder Society- Unified Parkinson's Disease Rating Scale part III.

NPI Night-Time Behavior Disturbance

The mean and median scores improved from 5.9 ± 2.1 and 6 (3–8) at baseline to 2.5 ± 1.8 and 3 (0–6) after treatment with YKSCH, respectively, with statistically

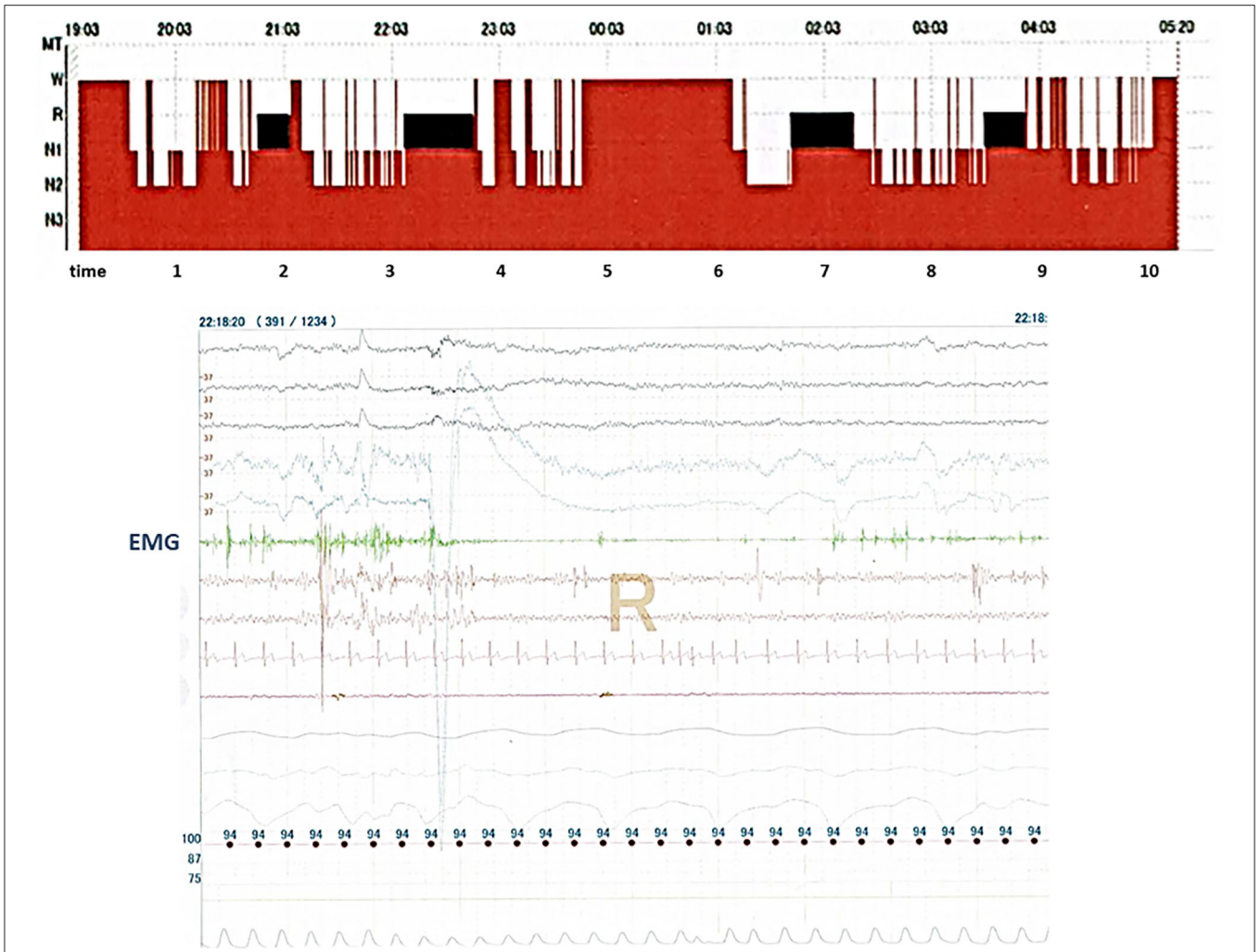

FIGURE 2 | It shows records of polysomnography (PSG) in Case 11. The top part of the figure shows sleep stages in Case 11, and the bottom part of the figure shows records from many physiological signals including electroencephalograms, electrooculograms, electromyograms (EMG), nasal–oral flow, chest and abdominal movements, electrocardiograms, and arterial oxygen saturation which obtained PSG in Case 11. REM sleep without atonia is seen on the record of EMG channels during polysomnography.

significant differences observed between the two time points ($p < 0.01$) (**Figure 1**).

Individually, there were no changes in score from baseline after treatment with YKSCH in Cases 3 and 6.

Visual Analog Scale—Frequency

The mean and median scores improved from 2.6 ± 0.7 and 3 (1–3) at baseline to 1.4 ± 0.9 and 1 (0–3) after treatment with YKSCH, respectively, with statistically significant differences observed between the two time points ($p < 0.01$) (**Figure 1**).

Individually, there were no changes in score from baseline after treatment with YKSCH in Cases 3 and 6.

Visual Analog Scale—Severity

The mean and median scores improved from 1.6 ± 0.5 and 2 (1, 2) at baseline to 0.9 ± 0.5 and 1 (0–2) after treatment with YKSCH, respectively, with statistically significant differences observed between the two time points ($p < 0.05$) (**Figure 1**).

Individually, there were no changes in score from baseline after treatment with YKSCH in Cases 3 and 6.

MDS-UPDRS III

There were no changes from mean score of 2.1 ± 3.2 or median score of 1.0 (0–10) at baseline after treatment with YKSCH; therefore, data were not statistically analyzed.

Adverse Events

During the observation period, no serious adverse events, including edema, increased blood pressure, and acute cardiac failure, were reported. In addition, hearing of subjects or their bed partners revealed no episodes of fall associated with oversedation or muscular relaxation.

The mean serum potassium level was 4.5 ± 0.4 mEq/L at Week 0 before treatment with YKSCH and 4.4 ± 0.4 mEq/L at Week 4 after the start of treatment. There was no statistically significant decrease in serum K level from baseline after treatment with YKSCH ($p = 0.260$).

DISCUSSION

CNZP is the standard of care for RBD associated with LBD as well as for idiopathic RBD. On the other hand, many patients with DLB have fall and subsequent facture, as evidenced by a report that DLB had a greater risk of admission to hospital (or death) because of most commonly fall-related injuries than AD (12). For the treatment of RBD associated with DLB, therefore, drugs that improve symptoms without muscular relaxation are awaited. YKSCH, a traditional herbal medicine used in the treatment of sleep disorder, decreases sleep latency and increases the total duration of sleep and sleep stage 2 (9). These actions are based on pharmacological mechanisms similar to those of benzodiazepines (BZPs), which are widely used in the treatment of sleep disorder (13). In addition, YKSCH contains Citrus Unshiu Peel as equal to Chimpi as a constituent crude drug, and hesperidin, an ingredient of Citrus Unshiu Peel, is metabolized to hesperetin, which has anxiolytic effects through the serotonergic system (14). Angelica root, another constituent crude drug, has been demonstrated to act on the GABA receptor, as a partial agonist at the 5-HT1A receptor, and to downregulate the 5-HT2A receptor (15, 16), and has been reported to act on the BZP receptor to have anxiolytic effects (15). Geissoschizine methyl ether, which is contained in Uncaria hook, also acts as an agonist at the 5-HT1A receptor to have anxiolytic and antidepressant effects (17). Taken together, YKSCH, like CNZP, may alleviate the symptoms of RBD directly by acting as an agonist at the BZP binding site of the GABAA receptor, but the ingredients of various constituent crude drugs may also play a role in alleviating nightmare and DEB by acting on serotonergic neurons.

Regardless of the above, there are limits to discussion of potential mechanisms of action of YKSCH against RBD. More specifically, since REM sleep may be generated in the brain stem, lesions responsible for RBD are suspected to be damaged to neuronal nuclei involved in regulating REM sleep, including the locus coeruleus, pedunculopontine tegmental nucleus, and medullary gigantocellular reticular nucleus, but have not yet been identified; therefore, the reason why DEB occurs, that is, the mechanism underlying the development of RBD, is unknown. In addition, the mechanism of action of CNZP, the gold standard treatment for RBD, has not been elucidated, including whether CNZP produces radical cure by acting directly on the etiology of RBD or only alleviates symptoms by acting on the sleep architecture or reducing dream-induced anxiety. Since neither the pathophysiology of RBD nor the mechanism of action of CNZP is clear, the mechanism of action of YKSCH can be hypothesized or assumed but unfortunately cannot be determined. It is absolutely crucial to elucidate the mechanism underlying the development of RBD, including essential neuropathological findings. In addition, one of roles of REM sleep is memory retention. Therefore, it is necessary to consider what kind of influence occurs for a memory function by controlling REM sleep. Similarly, Matsui et al. pointed out in his paper about efficacy of Yokukansan (YKS) for the treatment of RBD (18). YKS is a herbal medicine with the same indications of YKSCH. By the way, the authors said that a mixture of various ingredients derived from seven medical herbs in YKS makes it more difficult to identify the specific positive mechanism of action for RBD symptoms in that paper. However, the glutamate uptake function of YKS suggests that the drug possibly reduces oneiric behavior through the suppression of phasic muscle activity as one of hypothesis with a possibility.

While the pathophysiology of RBD and the mechanism of action of CNZP remain unclear, the present study demonstrated that YKSCH improved RBD associated with DLB without causing fall due to oversedation or muscular relaxation or impairing cognitive function, indicating that YKSCH may be a potential alternative to CNZP. In addition, since patients with a definitive diagnosis of RBD as determined by PSG were enrolled in the study, the usefulness of YKSCH in the treatment of RBD was evaluated in subjects with an accurate diagnosis. This is particularly important in evaluation of the potential of YKSCH for RBD.

Finally, I discuss two cases that did not respond to a treatment and several limitations in this study.

The total number of pre-dementia stage of DLB was six cases and all patients were male. Two cases of those, Case 3 and Case 6, were not improved in clinical measures by using YKSCH. They had no significant differences in age, sex, level of cognitive function, and MDS-UPDRS III, among others. In an elemental analysis of PSG, Arousal Index (AI: awakening more than 3 s per 1 h) was 20.7% in Case 3 and 6.4% in Case 6. The mean AI was 15.8% in total subjects with pre-dementia stage of DLB. Hence, a low or high percentage of AI cannot be the cause of ineffective results.

One of possible causes is a ratio of REM sleep during the sleep (REM%). The mean REM% was 17.4% in total subjects, and the mean REM% in cases except Cases 3 and 6 was 24.9%. By contrast, REM% of Case 3 was 5.0 and 13.2% in Case 6. REM% in these two cases showed a significantly low frequency of REM sleep during the total sleep. One of the mechanisms of YKSCH against RBD is the increase in the total duration of sleep and sleep stage 2; therefore, REM sleep is relatively decreased. The patients had a few REM% in the first place; thereby, it is suggested that YKSCH may be ineffective against RBD in these two cases.

Second, since the subjects had DLB, the NPI night-time behavior disturbance was used as the measure of RBD in view of behavioral and psychological symptoms of dementia. The Parkinson's Disease Sleep Scale (PDSS), the recommended scale for sleep disorder associated with PD, was not employed in the study. In view of the neuropathological connection between PD and the target disease, including pre-dementia stage of DLB, however, the PDSS perhaps should have been selected as another assessment tool, although the disease of subjects was not pure PD.

To determine the pathophysiology of RBD or the pharmacological mechanisms of YKSCH, furthermore, PSG should be used to investigate how YKSCH changes the onset of RWA and the sleep architecture itself, including REM sleep, and whether YKSCH treats RBD fundamentally. Since the present study was conducted under the regulations of Japanese

health insurance law, PSG could not be used after therapeutic intervention because of medical economic or legal constraints. Furthermore, because of the strict inclusion and exclusion criteria and the shortness of the entry period, the sample size in this study became small. These factors might carry a high risk of causing false-positive results. Therefore, the results in this study should be regarded as reference value.

Hence, the aforementioned problems should be addressed in future studies. With the limitations due to the preliminary nature of the current study in mind, we would like to collect further evidence.

CONCLUSION

The potential of YKSCH, which, like CNZP, acts on the sleep architecture but does not cause oversedation or muscular relaxation, was studied for the treatment of RBD associated with DLB. The results of this study, although there were limitations due to the preliminary nature of the study, verified the hypothesis, indicating that YKSCH may provide a new therapeutic option for RBD associated with DLB.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Yokohama Shintoshi Neurosurgical Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YM made the conception or design of this work, the acquisition, analysis, interpretation of data for this study, described draft of this study, revised this manuscript, decided final approval of the version to be published, and will be responsible for that to the end.

REFERENCES

1. Howell MJ, Schenck CH. Rapid eye movement sleep behavior disorder and neurodegenerative disease. *JAMA Neurol.* (2015) 72:707–12. doi: 10.1001/jamaneurol.2014.4563
2. Schenck CH, Bundlie SR, Mahowald MW. Delayed emergence of a Parkinsonian disorder in 38% of 29 older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder. *Neurology.* (1996) 46:388–93. doi: 10.1212/WNL.46.2.388
3. Schenck CH, Boeve BF, Mahowald MW. Delayed emergence of Parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16 year update on a previously reported series. *Sleep Med.* (2013) 14:744–8. doi: 10.1016/j.sleep.2012.10.009
4. Boeve BF, Silber MH, Ferman TJ, Lin SC, Benarroch EE, Schmeichel AM, et al. Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. *Sleep Med.* (2013) 14:754–62. doi: 10.1016/j.sleep.2012.10.015
5. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology.* (2017) 89:88–100. doi: 10.1212/WNL.0000000000004058
6. Olson EJ, Boeve BF, Silber MH. Rapid eye movement sleep behaviour disorder: demographic, clinical and laboratory findings in 93 cases. *Brain.* (2000) 123:331–9. doi: 10.1093/brain/123.2.331
7. Iranzo A, Santamaría J, Rye DB, Valldeoriola F, Martí MJ, Muñoz E, et al. Characteristics of idiopathic REM sleep behavior disorder and that associated with MSA and PD. *Neurology.* (2005) 65:247–52. doi: 10.1212/01.wnl.0000168864.97813.e0
8. Jang JH, Lee J, Jung I, Yoo H. Efficacy of Yokukansankachimpihange on sleep disturbance in Parkinson's disease: A study protocol of a randomized, double blind, placebo-controlled pilot trial. *Medicine.* (2018) 97:e11298. doi: 10.1097/MD.0000000000011298
9. Aizawa R, Kanbayashi T, Saito Y, Ogawa Y, Sugiyama T, Kitajima T, et al. Effects of Yoku-kan-san-ka-chimpi-hange on the sleep of normal healthy adult subjects. *Psychiatry Clin Neurosci.* (2002) 56:303–4. doi: 10.1046/j.1440-1819.2002.01006.x
10. Miyamoto T, Miyamoto M, Iwanami M, Kobayashi M, Nakamura M, Inoue Y, et al. The REM sleep behavior disorder screening questionnaire: validation study of a Japanese version. *Sleep Med.* (2009) 10:1151–4. doi: 10.1016/j.sleep.2009.05.007
11. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* (2013) 48:452–8. doi: 10.1038/bmt.2012.244
12. Hanyu H, Sato T, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Differences in clinical course between dementia with Lewy bodies and Alzheimer's disease. *Eur J Neurol.* (2009) 16:212–7. doi: 10.1111/j.1468-1331.2008.02388.x
13. Ito A, Shin N, Tsuchida T, Okubo T, Norimoto H. Anxiet-like effects of Chimp (dried citrus peels) in the elevated open-platform test. *Molecules.* (2013) 18:10014–23. doi: 10.3390/molecules180810014
14. Nishio S, Mignot E, Dement WC. Sedative hypnotics. In: Schatzberg AF, Nemeroff CB, editors. *The American Psychiatric Press Textbook of Psychopharmacology*. Washington, DC: American Psychiatric Press (1995). p. 405–6.
15. Liao JF, Jan YM, Huang SY, Wang HH, Yu LL, Chen CF. Evaluation with receptor binding assay on the water extracts of ten CNS-active Chinese herbal drugs. *Proc Natl Sci Counc Repub China B.* (1995) 19:151–8.
16. Kanno H, Sekiguchi K, Yamaguchi T, Terawaki K, Yuzurihara M, Kase Y, et al. Effect of yokukansan, a traditional Japanese medicine, on social and aggressive behaviour of para-chloroamphetamine-induced rats. *J Pharm Pharmacol.* (2009) 61:1249–56. doi: 10.1211/jpp/61.09.0016
17. Nishi A, Yamaguchi T, Sekiguchi K, Imamura S, Tabuchi M, Kanno H, et al. Geissoschizine methyl ether, an alkaloid in Uncaria hook, is a potent serotonin 1A receptor agonist and candidate for amelioration of aggressiveness and sociality by yokukansan. *Neuroscience.* (2012) 207:124–36. doi: 10.1016/j.neuroscience.2012.01.037
18. Matsui K, Sasai-Sakuma T, Ishigooka J, Nishimura K, Inoue Y. Effect of Yokukansan for the treatment of idiopathic rapid eye movement sleep behavior disorder: a retrospective analysis of consecutive patients. *J Clin Sleep Med.* (2019) 15:1173–8. doi: 10.5664/jcsm.7816

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

CASE REPORT
published: 11 September 2020
doi: 10.3389/fnut.2020.00146

Two Cases of Successful Treatment With Hachimijiogan for Irregular Menstruation

*Tatsuji Hirabayashi**

Tokorozawa Akitsu Clinic, Saitama, Japan

Hachimijiogan (HJG), a Kampo prescription medication composed of eight crude drugs, has been used for treatment of climacteric disturbance and irregular menstruation. In the Japanese pharmaceutical market, HJG pills consisting of a powdered mixture of these crude drugs are available, as well as a water extract preparation. In this study, two cases of successful treatment with HJG pills are reported. Case 1 was a 37-years-old woman who had irregular menstruation that had previously been treated with HJG extract granules for 3 months; however, her symptoms were not improved. Subsequent treatment with HJG pills at a dose of 40 pills/day for 10 months led to slight improvement of her menstrual cycle, and at a dose of 60 pills/day, her menstrual cycle was normalized. Case 2 was a 29-years-old woman who had irregular menstruation for more than 5 years and was previously treated with HJG extract granules, which led to slight improvement of her symptoms. Her menstrual cycle improved slightly after 9-months treatment with HJG pills at a dose of 40 pills/day and was normalized at a dose of 60 pills/day. This study suggests that the HJG crude drug preparation is more effective than the HJG extract preparation in some cases.

Keywords: Hachimijiogan, Kampo prescription medication, crude drug preparation, case report, irregular menstruation

OPEN ACCESS

Edited by:
Masahiro Ohsawa,
Nagoya City University, Japan

Reviewed by:
Kenji Watanabe,
Yokohama College of Pharmacy, Japan
Lila Oyama,
Federal University of São Paulo, Brazil

***Correspondence:**
Tatsuji Hirabayashi
t-akitsu.clinic@tbm.t-com.ne.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *31 March 2020*
Accepted: *23 July 2020*
Published: *11 September 2020*

Citation:
Hirabayashi T (2020) Two Cases of Successful Treatment With Hachimijiogan for Irregular Menstruation. Front. Nutr. 7:146. doi: 10.3389/fnut.2020.00146

INTRODUCTION

Hachimijiogan (HJG) is described in the Chinese classical medical book "Jin Gui Yao Lue" (Kinkiyouryaku in Japanese), and it is known as one of the most useful Kampo medications in Japan. HJG granules made from water-based extract preparations are widely used; however, tablets (HJG pills) consisting of a mixture of the powdered crude drug constituents are also commercially available. This study reports two cases of patients treated with HJG pills after providing written consent.

CASE ONE

Patient 1 was a 37-years-old single female (height, 155 cm; weight, 49.1 kg; blood pressure, 108/48 mmHg) with chief complaints of cold sensitivity, tinnitus, dizziness, headache, and irregular menstruation. The patient had no noteworthy family medical history or genetic information. She had no yellowing of the conjunctiva bulbi; no conjunctival anemia; no cervical, thoracic, or abdominal abnormalities; and no edema on the anterior tibia. Considering that the patient was somewhat ectomorphic and tended to

experience worsening of symptoms during menstruation, tokishakuyakusan extract granules (5.0 g/day), which are effective for treating cold sensitivity and headaches, were prescribed (**Figure 1**). After taking tokishakuyakusan extract granules, symptoms including cold sensitivity, tinnitus, dizziness, and headache were alleviated; however, her menstrual cycle had remained irregular. Three years after the initiation of the Kampo treatment she had no menstruation for more than 2 months, so Kampo treatment was started anew for the irregular menstruation.

Subjective symptoms included cold sensitivity of the lower body, tinnitus, and lower back pain, in addition to irregular menstruation; thus, the prescription was changed from tokishakuyakusan extract granules to HJG extract granules (5.0 g/day). Two months later, she reported no menstruation despite continuous administration of HJG extract granules.

The patient's chief complaint was irregular menstruation with subjective symptoms including cold sensitivity of the lower body and lower back pain. In addition, she was somewhat thin and had weakness of the lower abdominal region. Thus, treatment with HJG was expected to be effective for this patient, and as a result, treatment was changed to HJG pills (40 pills/day) instead of simply increasing the dose of HJG extract granules.

One month after the administration of HJG pills, the patient had menstruation for 5 days. She continued taking the medication and menstruated again, but her cycle was abnormally long at around 40 days. Hence, the dose of HJG pills was increased from 40 to 60 pills/day. Since then, her menstrual cycle has become ∼30 days.

CASE TWO

Patient 2 was a 29-years-old single female pharmacist working at a hospital (height, 158 cm; weight, 48.0 kg; blood pressure, 102/54 mmHg) with menstrual irregularities. The patient had no noteworthy family medical history or genetic background. She had no yellowing of the conjunctiva bulbi; no conjunctival anemia; no abnormalities in the neck, chest, or abdomen; and no edema on the anterior tibia. Her subjective symptoms were as follows; slightly dark skin, somewhat ectomorphic, normal food intake, no sleep disorder, no cold or heat sensitivity, no chills, no headache, no shoulder tension, no dizziness, lower back pain due to work in a standing position, tendency to have swelling of the lower limbs, no urination during the night, and defecation once per day.

The patient had her first menstruation at age 12, and had a menstruation cycle of 35–42 days until the age of 18. Since the age of 19, her menstrual cycle became longer, and her menstruation stopped briefly. She visited a gynecologist and found out that her basal body temperature was irregular rather than biphasic. Hence, she underwent hormone therapy. She continued the treatment on a regular basis and maintained a normal menstrual cycle; however, she experienced an absence of menstruation whenever the treatment was interrupted. Treatment with Kampo medicine had been considered for the improvement of her symptoms, and she visited the medical institution previously indicated, where she was prescribed and started treatment with 5.0 g/day keishibukuryogan extract granules and 5.0 g/day rokumigan extract granules (**Figure 2**).

She first visited our hospital with the same chief complaint. At first, the prescription from her previous doctor was continued, but menstruation was irregular: therefore, she was switched from 5.0 g/day rokumigan extract granules to 5.0 g/day HJG extract granules to normalize her unstable menstrual cycle. She continued taking the prescribed drug for roughly 2 years, during which her menstruation was on a 35–42-days cycle without hormonal therapy. Although the cycle was relatively long, she considered that the cycle had stabilized and stopped taking the extract granules at her own discretion. After ceasing to take the drug, her menstruation cycle remained the same for 3 years. Subsequently, she had not menstruated for the past 2 months: therefore, she visited our hospital.

Her symptoms were lower back pain related to work and swelling of the lower limbs without any major complaints other than the chief complaint of menstrual irregularity. She had been taking keishibukuryogan extract granules and HJG extract granules until a few years prior, observing that it positively affected her menstrual cycle. Thus, HJG was expected to be effective considering that she had only a few subjective symptoms of lower back pain and swelling of the lower limbs. However, because her menstrual cycle was longer and she showed a benefit at the previous dose of the extract granules, treatment was initially started with HJG pills (40 pills/day) (**Figure 3**). Approximately 1 month after the initial administration of the pills, she menstruated for 5 days. Subsequently, her menstrual cycle became more regular at ∼1 month intervals. However, 5 months after the initial administration of the pills, she started to have a somewhat longer menstrual cycle and missed her menstrual period for 49 days during the subsequent period. Thus, the dose of HJG pills was subsequently increased from 40 to 60 pills/day. Consequently, she started having a regular menstrual cycle (e.g., 34 days after and 32 days after). Since then, she has had regular menstruation occurring in nearly 30-days cycles.

DISCUSSION

HJG is composed of eight crude drug powders: Rehmanniae Radix, Corni Fructus, Dioscoreae Rhizoma, Alismatis Tuber, PORIA, Moutan Cortex, Cinnamomi Cortex and Aconiti Radix, and is a Kampo medication used for the treatment of the diseases referred to in Kampo medicine as being characterized by reduced function of tissues that control growth, development, and overall fertility (1). HJG has been found to be effective for many diseases and symptoms including diabetes, hypertension, back pain, edema, nephritis, bronchial asthma and dementia, and many successful cases of its use have been reported (2–5).

In particular, there have been many reports of using HJG for infertility. In a study by Usuki et al. oral administration of HJG granules (5.0–10.0 g/day) in 27 patients, aged 24–38 years, with hyperprolactinemia infertility improved their blood prolactin levels and led 12 cases (44%) to pregnancy (6).

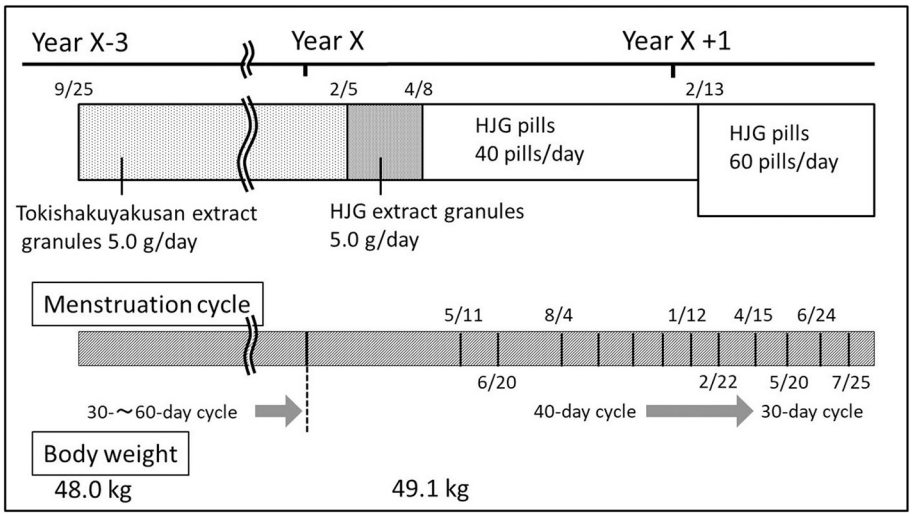

FIGURE 1 | Case 1. Progress chart.

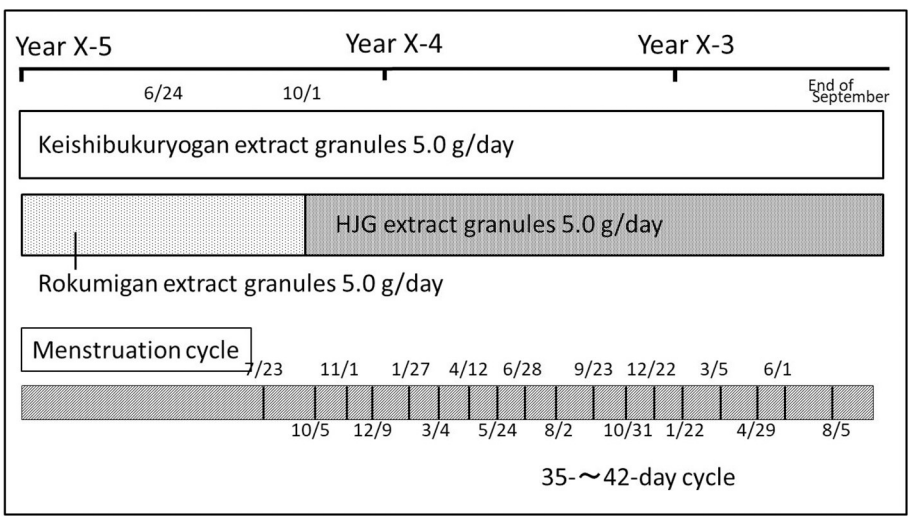

FIGURE 2 | Case 2. Progress chart 1.

Furthermore, Shima et al. reported that administration of HJG extract granules (7.5 g/day) or HJG pills (60 pills/day) in 50 intractable infertility cases resulted in pregnancy in a total of 45 cases (90%) within 6 months (7). Among the cases in which pregnancy was not possible during treatment with HJG extract granules, there were many cases in which pregnancy could be achieved by switching the prescription to HJG pills. With respect to gynecologic diseases, there have been many reports on tokishakuyakusan, keishibukuryogan, unkeito, kamishoyosan, and other Kampo medications. In contrast, there have been relatively few reports on the use of HJG for gynecologic diseases. One of the reasons could be that HJG contains aconite root, which is used to relieve pain in people with weak constitutions, often including "after middle age," "pain," and "sharp pain" in the descriptions. Thus, there has been reluctance to use it in young women.

The two cases in the present study had reported experiencing menstrual irregularity observed for a relatively long time. In the first case, there was no significant effect on the menstrual cycle after several years of taking tokishakuyakusan extract granules, which are frequently used to treat irregular menstruation. As a result, she was switched to HJG, taking into account her symptoms of cold sensitivity in the lower back and lower limbs, and lower back pain. She experienced no improvement during treatment with extract granules, while her symptoms improved after switching to the pill formulation.

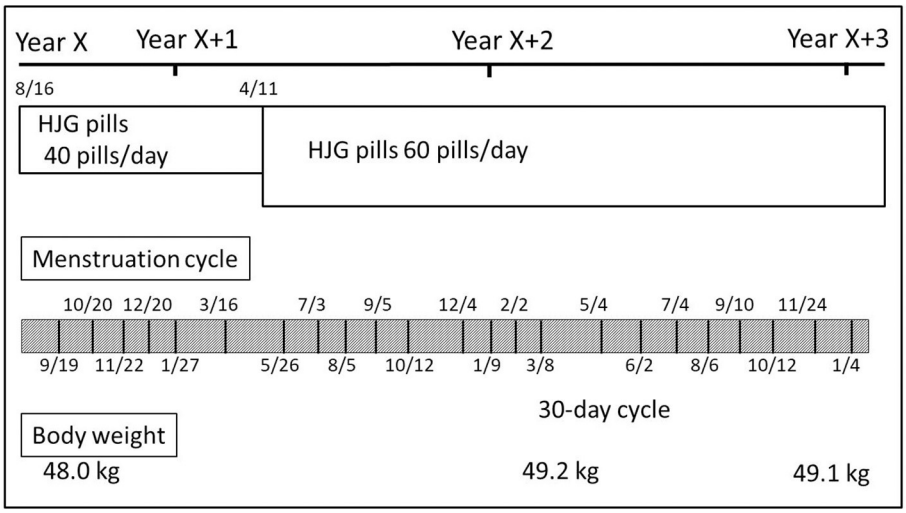

FIGURE 3 | Case 2. Progress chart 2.

The second case had a history of taking both rokumigan extract granules and HJG extract granules, and the extract granules were also effective. However, considering her back pain and edema of the lower limbs, which often occurred in the evening, she was treated with the crude drug HJG alone instead of the extract granules. In both cases, a dose-dependent effect was observed. Both patients were satisfied with the treatment due to the improved menstrual cycle.

A study by Toriizuka et al. compared the difference in effect between keishibukuryogan as a decoction and in pill form with regard to (8) and a clear difference in their medicinal effects was reported (9). They noted that the constituents of keishibukuryogan decoctions and pills differed and hypothesized that the differences may have occurred because the extraction efficiency of the essential oil component is poor in the water-based extract preparation, while the pill contains all the components as a solidified crude drug powder.

Hachimijiogan contains essential oils such as paeonol and paeoniflorin, a fat-soluble ingredients, which are considered to be effective in improving blood flow (10).

Since pills are made by powdered crude herbs without the process of extracting, both water-soluble and fat-soluble ingredients are contained. On the other hand, the extract granules are mainly composed by water-soluble ingredients. Based on this fact, pills might contain a large amount of essential oil ingredients such as paeonol and paeoniflorin due to the difference in manufacturing method compared to the extract granules. In our cases, the reason why the menstrual cycle was improved might be that the fat-soluble ingredients composed in the pills improved the disturbance of blood flow due to pelvic congestion.

Regarding studies on HJG, Shima et al. reported that the use of powdered crude drug tablets improved the effect of infertility treatment, as described above. Similarly, the cases in this study indicate that the crude drug preparation had enhanced efficacy compared with the extract preparation. However, this study is an inference based on two case reports, and it is necessary to consider many cases in the future.

TRANSLATION

This paper was submitted to a journal written in Japanese (philkampo No. 55, 25–27, 2015). It was rewritten in English with permission from the magazine so as to reach a global audience.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the individuals for the publication of any potentially identifiable data included in this article.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

This is a Japanese language translation of ウチダの八味丸Mと生理不順の2症例 originally published in Phil Kampo. TH prepared this translation. Permission was granted by Medical Publisher.

REFERENCES

1. Terasawa K. *Learning Japanese Oriental Medicine With Cases (in Japanese)*. Tokyo: Igaku-shoin (1990).
2. Ito T, Kita T, Shimada Y, Shibahara N, Terasawa K. The effect of Hachimijiogan on chronic asthma (the first report) (in Japanese). *Japan J Oriental Med.* (1996) 47:433–41. doi: 10.3937/kampomed.47.433
3. Ito T, Shibahara N, Shintani T, Kita T, Terasawa K. The effect of Hachimijiogan on chronic asthma (the second report) (in Japanese). *Japan J Oriental Med.* (1996) 47:443–9. doi: 10.3937/kampomed.47.443
4. Kato S, Kageyama M, Tokura M, Kikuchi A. Three cases of bronchial asthma associated with irregular menstruation in which renal supplementary drugs were effective (in Japanese). *Kampo Novel Treat.* (2008) 17:304–9.
5. Haruta M. Application of Hachimijiogan in neuropsychiatry (in Japanese). *Kampo Novel Treat.* (2000) 9:249–52.
6. Usuki S, Hosokawa A, Ichikawa Y, Kubota S, Okane M, Saitou M, et al. Clinical application of Hachimijiogan for patients with hyperprolactinemia (in Japanese). *History Obstetr Gynecol Kampo Res.* (1998) 5:43–54.
7. Shima C, Kageyama M, Shima H, Konno A, Koshida M, Nishida S. Investigation of 50 cases of intractable infertility that led to pregnancy due to "Hachimijiogan" for the purpose of anti-aging (in Japanese). *History Obstetr Gynecol Kampo Res.* (2008) 25:99–105.
8. Toriizuka K, Terasawa K, Honma S, Adachi I, Nakagawa T, Horikoshi I. Pharmaceutical study of Keishibukuryogan: comparison between the ingredients of decoction and pills (in Japanese). *Japan J Oriental Med.* (1984) 35:185–9. doi: 10.3937/kampomed.35.185
9. Terasawa K, Matsuda H, Imadaya A, Tosa H, Mitsuma T, Toriizuka K, et al. Study on clinical effect of homemade Keishibukuryogan (in Japanese). *Japan J Oriental Med.* (1984) 35:131–6. doi: 10.3937/kampomed.35.131
10. Gai Z, Wang Z, Zhang L, Ma J, Zhu Q. Paeonol protects against hypertension in spontaneously hypertensive rats by restoring vascular endothelium. *Biosci Biotechnol Biochem*. (2019) 83:1992–9. doi: 10.1080/09168451.2019.1648203

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 09 October 2020
doi: 10.3389/fnut.2020.528864

Ninjinyoeito Has a Protective Effect on the Auditory Nerve and Suppresses the Progression of Age-Related Hearing Loss in Mice

Takanori Kawashima, Kenji Harai, Nina Fujita and Ryuji Takahashi*

Kampo Research Laboratories, Kracie Pharma, Ltd., Tokyo, Japan

Currently, there are limited reports available regarding the treatment and prevention of progressive age-related hearing loss. This is because age-related hearing loss is not a critical disease with direct fatalities and has several well-established countermeasures such as hearing aids and cochlear implants. This study evaluated the efficacy of Ninjinyoeito (NYT) in the treatment of age-related hearing loss. C57BL/6J mice were divided into three groups: baseline group, untreated group, and NYT-treated group, with the latter receiving NYT treatment for 2 months. The mice were fed with NYT extract mixed with 4% mouse normal chow. Hearing loss was confirmed by a reduction in intact cell density of the auditory nerve from the age of 5–7 months. The suppression of hearing loss with aging and decrease in the intact cell density of the auditory nerve were significant in mice fed with NYT for 2 months. NYT has been reported to improve blood flow and enhance mitochondrial activity and may exert its protective effects on spiral neurons through these mechanisms. There was no decrease in the size of the stria vascularis from the age of 5–7 months in C57BL/6J mice. The present model failed to reveal the effect of NYT on atrophy of the stria vascularis of the cochlear duct. In conclusion, NYT appears to have a protective effect on the auditory nerve and suppress the progression of age-related hearing loss by reducing age-related auditory nerve degeneration.

Keywords: age-related hearing loss, cochlear, Ninjinyoeito (NYT), auditory nerve, stria vascularis, auditory brainstem response

OPEN ACCESS

Edited by:
Akio Inui,
Kagoshima University, Japan

Reviewed by:
Taro Yamaguchi,
Setsunan University, Japan
Yasuhiro Manabe,
Saiseikai Toyama Hospital, Japan

****Correspondence:***
Takanori Kawashima
kawashima_takanori@phm.kracie.co.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *22 January 2020*
Accepted: *01 September 2020*
Published: *09 October 2020*

Citation:
Kawashima T, Harai K, Fujita N and Takahashi R (2020) Ninjinyoeito Has a Protective Effect on the Auditory Nerve and Suppresses the Progression of Age-Related Hearing Loss in Mice. Front. Nutr. 7:528864. doi: 10.3389/fnut.2020.528864

INTRODUCTION

The World Health Organization estimates that 466 million persons worldwide have disabling hearing loss (6.1% of the world's population); among them, 432 million (93%) are adults (242 million men and 190 million women), whereas 34 million (7%) are children; approximately one-third of persons older than 65 years have disabling hearing loss (https://www.who.int/deafness/estimates/en/). In Japan, more than 15 million people older than 65 years have age-related hearing loss (1). There has been limited research on the treatment and prevention of progressive age-related hearing loss because deafness is not perceived as a critical disease directly associated with mortality. Moreover, there are several countermeasures available (hearing aids and cochlear implants) for managing diagnosed cases of deafness.

However, deafness not only leads to a reduced communication ability but also causes other symptoms, such as isolation, depression, dementia, and other comorbidities associated with age-related deafness (2, 3). Moreover, the Japan Hearing Instrument Manufacturers Association has reported an extremely low proportion of hearing aid users for deafness in Japan. This could be attributed to the inability to purchase a hearing aid because of the high cost (24%). Even if they have a hearing aid, they do not use it because of factors, such as discomfort (46%), a feeling of unnecessity (25%), and shame (19%). In addition to hearing aid use and cochlear implant placement, controlling deafness progression with age is equally necessary for the management of age-related hearing loss. These pathological changes are reportedly caused by mitochondria-derived reactive oxygen species that reduce cochlear blood flow and cause abnormal accumulation of mitochondrial DNA (4). In Oriental medicine (*Shang Han Lun*), reduced cochlear blood flow in patients with age-related deafness is attributed to declined kidney function; moreover, the blood flow can be restored with kidney supplements, which are similarly considered to improve deafness and tinnitus. A previous study demonstrated the beneficial effects of Ninjinyoeito (NYT) on aging and enhancement of the general well-being of elderly patients (5). However, no study has analyzed the effect of NYT on age-related hearing loss. Therefore, this study aimed to evaluate the usefulness of NYT for the treatment of age-related hearing loss through improved cochlear blood flow in mice.

MATERIALS AND METHODS

Plant Materials

NYT is produced by Kracie Pharma (Toyama, Japan) as a powder of the dried *Rehmannia* root (4 g), Japanese angelica root (4 g), *Atractylodes* rhizome (4 g), *Poria sclerotium* (4 g), ginseng (3 g), cinnamon bark (2.5 g), polygala root (2 g), peony root (2 g), *Citrus unshiu* peel (2 g), astragalus root (1.5 g), *Glycyrrhiza* (1 g), and schisandra fruit (1 g) (**Figure 1A**). These plants are identified based on their external morphology and are authenticated based on marker compounds according to the Japanese Pharmacopeia as well as using our company's standards. The powder (lot no. E1712111A0) was mixed at 4% (w/w) with normal chow.

High-Performance Liquid Chromatography

NYT extract (0.5 g) was mixed and shaken with 50% methanol (50 mL) followed by extraction through ultrasonication for 30 min. The supernatant was filtered using a membrane filter (0.22 μm) and subjected to three-dimensional high-performance liquid chromatography (HPLC) fingerprint analysis. The HPLC system comprised an LC-30AD pump, an SPD-M30A diode array detector (Shimadzu, Kyoto, Japan), a YMC-Triart C18 column (ϕ3.0 × 150 mm; YMC Co., Ltd., Kyoto, Japan), and 0.2% phosphoric acid in water/0.2% phosphoric acid in CH_3CN (6:4) or 0.2% phosphoric acid in water/0.2% phosphoric acid in CH_3CN (8:2) as solvent. In HPLC analyses, the flow rate was controlled by the LC-30AD at 0.5 mL/min. The eluent from the

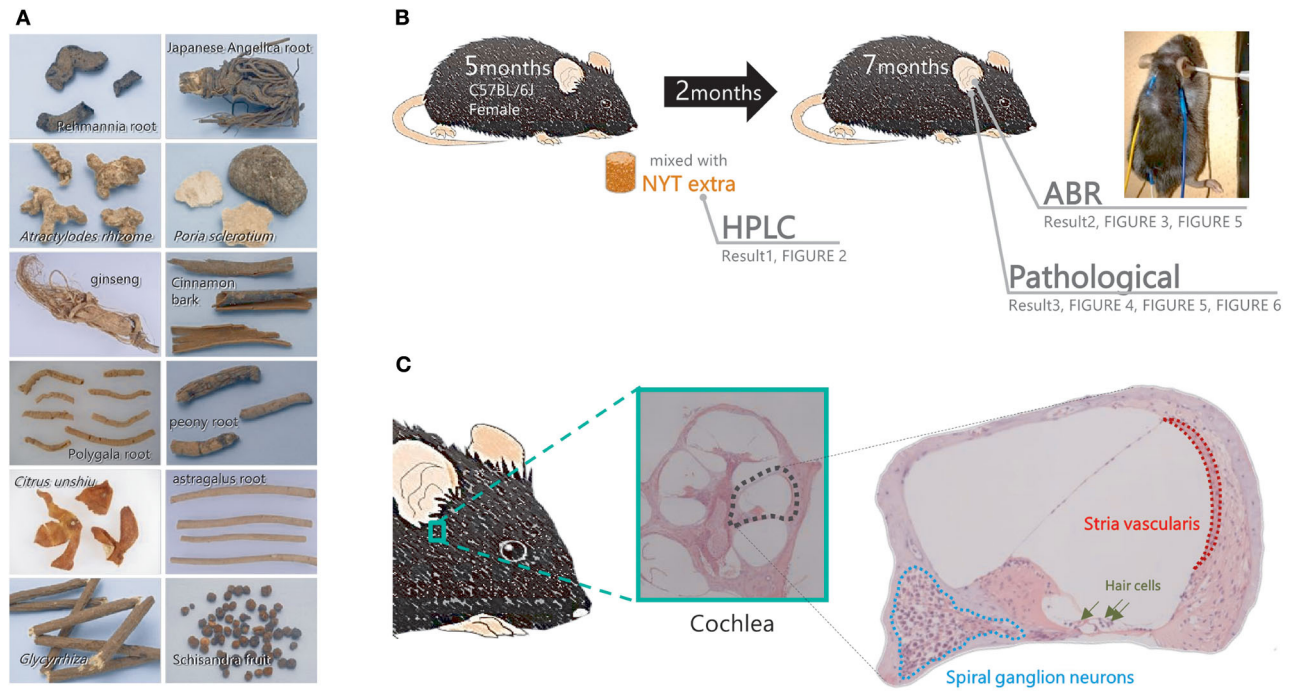

FIGURE 1 | Flow of the experiment. **(A)** The component profile of the Ninjinyoeito (NYT) extract was evaluated using high-performance liquid chromatography. **(B,C)** Mice in the NYT-treated group were fed normal chow supplemented with 4% NYT, whereas those in the untreated group were fed normal chow for 2 months. After 2 months of treatment, the auditory brain response and pathological changes in the cochlea (spiral ganglion neurons and stria vascularis) were evaluated.

column was monitored, and the three-dimensional data were processed by the SPD-M30A diode array detector.

Animals

Five-month-old female C57BL/6J mice were purchased from the Oriental Yeast Company, Ltd. (Shiga, Japan) and were acclimated for 1 week at 23 ± 3°C under a 12-h light/dark cycle (lights on from 08:00 to 20:00), with *ad libitum* access to chow and water. All efforts were made to minimize the suffering and the number of animals used. All mice were treated following the guidelines presented in the Standards for Human Care and Use of Laboratory Animals of Tohoku University and Guidelines for Proper Conduct of Animal Experiments by the Ministry of Education, Culture, Sports, Science, and Technology of Japan. All animal experiments were approved by the Ethics Committee for Animal Experiments of Tohoku University Graduate School of Medicine and the Experimental Animal Care Committee of Kracie Pharma. The approval number for the animal experiments was 2018MdA-189-1.

Reagents and Mouse Diets

Ketamine was purchased from the Daiichi Sankyo Company Limited (Tokyo, Japan), and xylazine was purchased from the Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Mouse normal chow (MF) was purchased from CLEA Japan Inc. (Tokyo, Japan). Five-month-old female C57BL/6J mice were treated for 2 months with NYT powder mixed at 4% (w/w) with normal chow until 7 months of age ($n = 10$). The non-treated mice were fed MF.

Model for Age-Related Hearing Loss

Five-month-old female C57BL/6J mice were administered NYT powder mixed at 4% (w/w) with normal chow. As controls, the non-treated mice received a regular diet of MF. After 2 months of NYT treatment, a hearing assessment was performed under anesthesia (7-month-old mice). The female C57BL/6J mice were classified into three groups. A hearing assessment was conducted in 10 mice at 5 months of age to establish a baseline. The control group of non-treated mice received a regular diet of MF for 2 months ($n = 10$) before the hearing assessment. The remaining 10 mice were administered NYT powder mixed at 4% (w/w) with normal chow from the age of 5–7 months. After 2 months of NYT treatment, a hearing assessment was performed under anesthesia. After undergoing the auditory brainstem response (ABR) assessment described below, the mice were sacrificed, and their cochlear tissues were collected for analysis (**Figure 1B**).

Hearing Assessment

ABR hearing assessment was conducted as previously described (6). The mice were anesthetized by an intraperitoneal administration of ketamine (100 mg/kg body weight) and xylazine (20 mg/kg body weight). ABR was assessed in a soundproof room. For hearing threshold evaluation, three subdermal electrodes (ground, reference, and active electrodes) were placed 2–3 mm under the skin. The active electrode was subdermally inserted on the forehead (**Figure 1B**: yellow). The reference and ground electrodes were inserted below the pinna of the right ear (**Figure 1B**: blue) and back (**Figure 1B**: white). ABR recordings were obtained using a TDT System 3 auditory-evoked potential workstation and analyzed using the BioSigRP software (Tucker-Davis Technologies, Alachua, FL, USA). The ABR responses were evoked using bursts of pure tones at frequencies of 4, 8, 12, 16, and 32 kHz. Evoked responses were averaged across 1,000 sweeps. The responses were collected for stimulus levels in 5-dB steps from 100 to 10 dB SPL. The threshold shift was defined as the lowest sound intensity that could elicit at least one peak in the averaged ABR. In this experiment, we used wave V. Under blinded conditions, the ABR threshold was determined with a clear confirmation of wave V from the raw data. This was performed through double-checks by the person in charge and the blinded caregiver.

Cochlear Tissue Analysis

After the ABR hearing assessment, cochlear tissues were collected from the mice (**Figure 1C**). After specimen collection, the perilymph was replaced with 4% paraformaldehyde, which was injected from the oval or round window with a syringe; subsequently, the cochlea was fixed. Thereafter, decalcification treatment using 10% ethylenediaminetetraacetic acid was performed, followed by stepwise dehydration with 70–100% ethanol. Subsequently, a paraffin block was prepared, and thin histological sections were obtained. Deparaffinization was performed with xylene, which was subsequently removed with ethanol. The slide was washed with water. The cells were stained with Meier hematoxylin for 5 min and washed with water for 30 s. The cells were soaked in a phosphate-buffered saline for 1 min and washed with water for 2 min. Eosin staining was performed for 1 min followed by washing of the cells with water and encapsulated. The intact cell density and area of the stria vascularis of the auditory nerve in the basal turn of the cochlea were measured.

Statistical Analysis

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is the graphical user interface for R-2.3-0 (The R Foundation for Statistical Computing, Vienna, Austria). All data are expressed as mean ± standard error of the mean.

Statistical comparisons were performed using one-way analysis of variance, followed by Tukey's test. Steel–Dwass test was used for data for which the p-value of the Bartlett test was <0.01 or for which the p-value of the Kolmogorov–Smirnov test (Shapiro–Wilk normality test) was <0.05.

Differences with $p < 0.05$ were considered statistically significant.

RESULTS

High-Performance Liquid Chromatography of NYT Extract

Figure 2 shows the chromatographic profile and composition of NYT. Chemical compounds were identified in the chromatographic profile according to their retention times and ultraviolet spectra, based on the corresponding reference

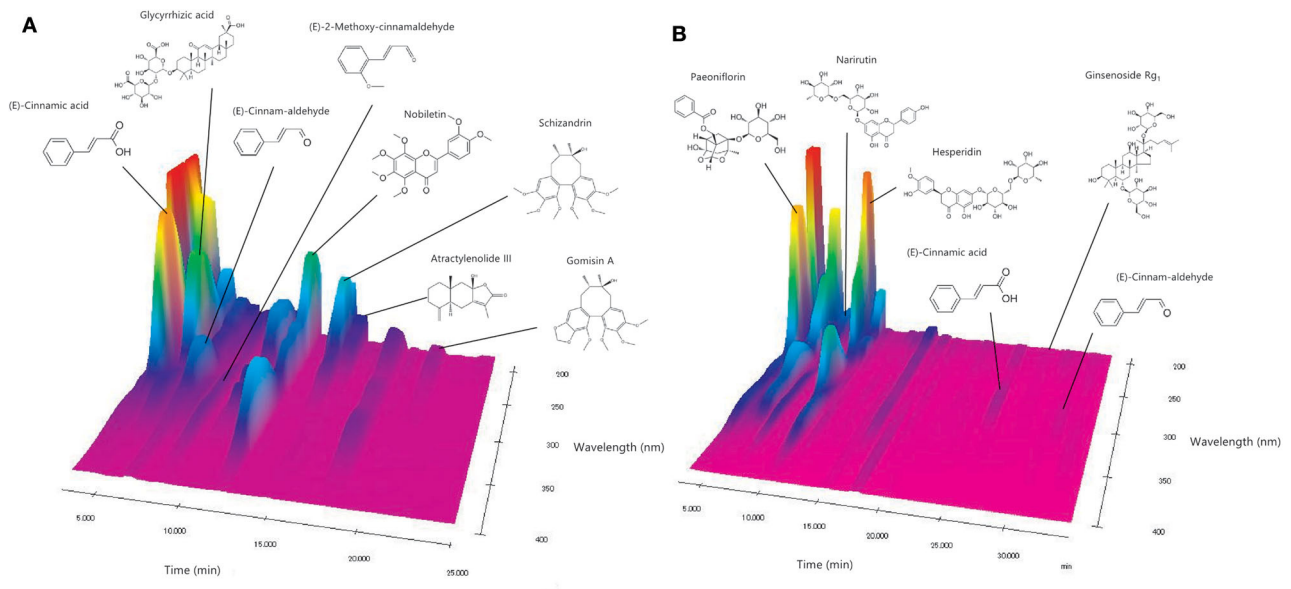

FIGURE 2 | Analysis of NYT components. **(A,B)** The chemical compounds were identified in the chromatograms based on the comparison of their retention times and ultraviolet spectra (240–380 nm) with corresponding reference standards.

standards. Specifically, (*E*)-cinnamic acid, glycyrrhizic acid, (*E*)-cinnam-aldehyde, (*E*)-2-methoxy-cinnamaldehyde, nobiletin, schizandrin, atractylenolide III, and gomisin A were identified when 0.2% phosphoric acid in water/0.2% phosphoric acid in CH_3CN (6:4) was used as solvent (**Figure 2A**). Contrastingly, paeoniflorin, narirutin, hesperidin, (*E*)-cinnamic acid, ginsenoside Rg_1, and (*E*)-cinnam-aldehyde were identified when 0.2% phosphoric acid in water/0.2% phosphoric acid in CH_3CN (8:2) was used as solvent (**Figure 2B**).

Hearing Assessment

In the ABR evaluation of female C57BL/6J mice after NYT administration, the threshold shift increased from 5 to 7 months of age, which confirmed decreased hearing. NYT was administered for 2 months from the age of 5 months; moreover, the hearing acuity was evaluated (**Figure 3**). Hearing acuity remained unchanged from the initiation of NYT administration at 5 months of age. Meanwhile, the hearing loss did not progress, and the hearing acuity at the age of 7 months was significantly higher in the NYT-treated mice than that in the untreated mice. Moreover, evaluation of the ABR results for each frequency revealed that hearing acuity was significantly higher in the NYT-treated mice than that in the untreated mice at 8, 12, and 32 kHz. The raw ABR data are presented in **Supplementary Table 1**.

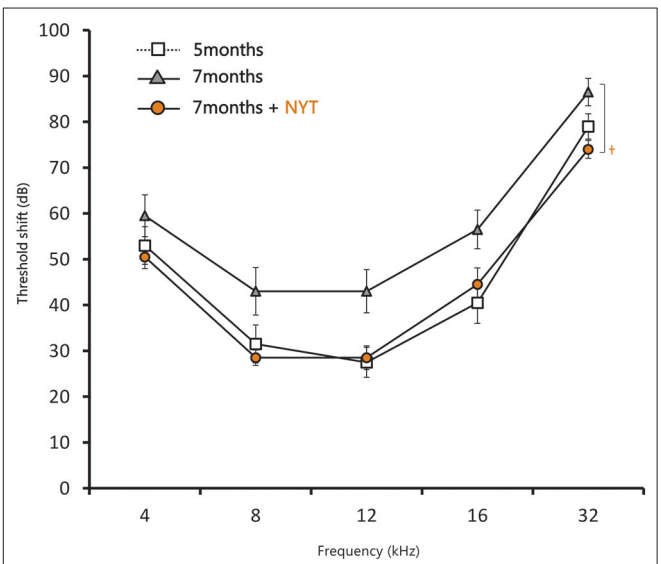

FIGURE 3 | Results of the auditory brain response assessment after 2 months of NYT treatment. Data are expressed as mean ± standard error of the mean ($n = 10$). $^{\dagger}p < 0.05$ vs. group of mice treated for 7 months (Steel–Dwass test).

Analysis of Cochlear Tissue

The intact cell density in the spiral ganglion was significantly reduced from 5 to 7 months of age. The intact cell density was significantly higher in the NYT-treated group than in the untreated group (**Figures 4A,B**). NYT seems to have a protective effect against atrophy and decreased auditory nerve excitation with aging. The spiral ganglion cell analyzed in this study was located in the basal turn of the cochlea and is associated with hearing acuity in the high-frequency auditory region. There was a marginally positive correlation between hearing acuity at a high frequency (16 kHz) and intact cell density of the spiral ganglion cells (cells/mm^2) ($r = 0.5$; **Figure 5**). There was no decrease in the size of the stria vascularis from the age of 5–7 months (**Figure 6**).

DISCUSSION

This study reveals that NYT appears to suppress the progression of age-related hearing loss and protect the auditory nerve. Evaluation of the ABR results for each frequency revealed that the hearing acuity was significantly higher in the NYT-treated mice than that in the untreated mice at 32 kHz, which implies that NYT was effective in the high-frequency region characteristic of age-related hearing loss (**Figure 3**). The suppression of age-related hearing loss in NYT-treated mice could be attributed to a protective effect on the spiral ganglion. Age-related hearing loss is caused by the production of excess reactive oxygen species by the mitochondria due to age-related reductions in cochlear blood flow as well as the accumulation of abnormal mitochondrial DNA. NYT, which seemingly suppresses age-related hearing loss, is effective in improving blood flow. Additionally, it reportedly increases the mitochondrial activity and has a protective effect on the spiral ganglion cells. There was no decrease in the size of the stria vascularis from the age of 5–7 months (**Figure 6**). The present model failed to reveal the effect of NYT on atrophy of the stria vascularis of the cochlear duct. Various functional and structural changes that occur peripherally and centrally contribute to the development of age-related hearing loss, including degeneration of the stria vascularis, loss of hair cells and primary afferent neurons, and alterations in the central auditory pathways, including a reduction in the neurons of the cochlear nucleus and changes in neurotransmitter release (7).

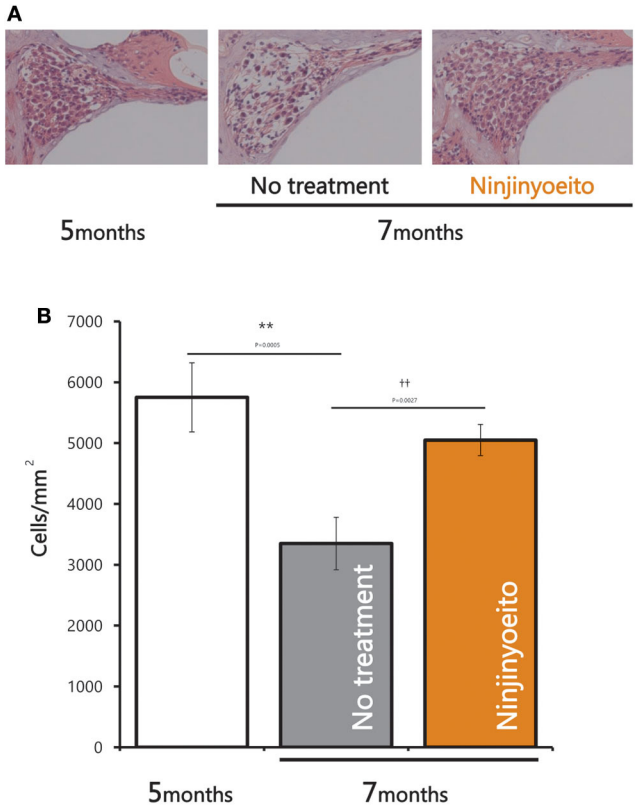

FIGURE 4 | Pathological changes in spiral ganglion neurons after 2 months of NYT treatment. **(A)** Histological examination (hematoxylin and eosin stain) of specimens from each group. **(B)** Nerve cell viability (cells/mm). Data are represented as mean ± standard error of the mean ($n = 7$). $^{**}p < 0.01$ vs. group of mice treated for 5 months; $^{††}p < 0.01$ vs. group of mice treated for 7 months by Tukey's test.

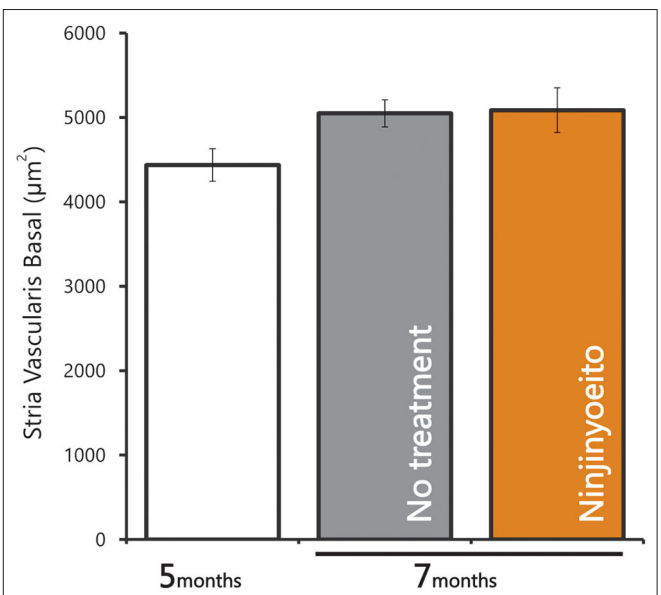

FIGURE 6 | Area of the stria vascularis in the basal turn of cochlea. Data are represented as mean ± standard error of the mean ($n = 7$).

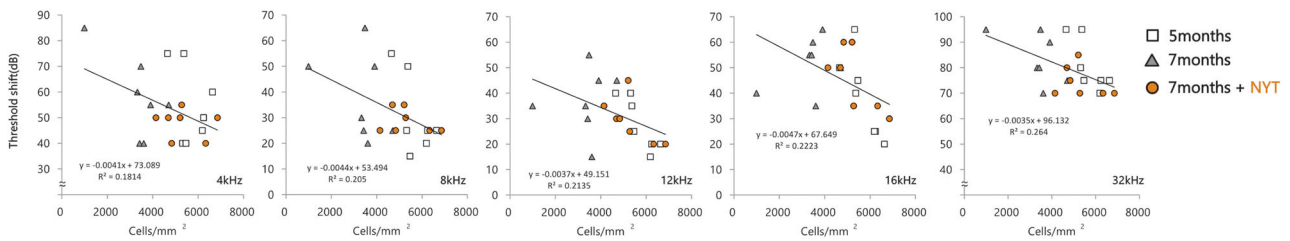

FIGURE 5 | Correlation between hearing and nerve cell viability at each frequency (kHz).

Age-related hearing loss supposedly results from aging, oxidative damage, mitochondrial impairment, and environmental factors (8, 9). Oxidative damage caused by reactive oxygen species has equally been postulated to play a causal role in age-related hearing loss (9–13). Since NYT is designed to improve fatigue, cold limbs, anorexia, night sweats, and anemia, it has been used for elderly people in traditional Oriental medicine. Specifically, NYT reportedly contributes to the improvement of peripheral circulation disorders (14, 15). Regarding ear disease, the Manabe group administered NYT to patients with acute, low-tone, sensorineural hearing loss. They hypothesized that NYT would improve the impaired blood supply to the stria vascularis and therefore be effective in patients with intractable acute, low-tone, sensorineural hearing loss (16). The C57BL/6J mice used in this study were reported to develop age-related hearing loss at 6 months (17) and have been widely used as an evaluation model for age-relating hearing loss (13, 18–23). In this study, we observed that NYT administration to 5-month-old C57BL/6J mice suppressed the progression of age-related hearing loss. The ABR results and the assessment of the association of intact cells suggest that NYT maintains hearing by protecting neurons in the cochlear nucleus that degenerate or die with age (**Figure 5**). It is speculated that NYT may have suppressed the progression of age-related hearing loss by improving the blood flow.

This study has several limitations. First, changes in cochlear blood flow could not be confirmed as being age-related. Currently, blood flow in the cochlea is evaluated based on a previous report by Kong's team (24). Second, the C57BL/6J mouse is an incomplete model for accurately reflecting the human age-related hearing loss. It is empirically known that men experience more rapid age-related hearing loss than women. However, in C57BL/6J mice, female mice develop age-related hearing loss much earlier and at a younger age than male mice. Another limitation is the short observation period, which was 2 months, as well as the administration of NYT before the increase in symptom severity. In the future, we plan to use CBA mice, which significantly correspond to the model of human age-related hearing loss (25). Third, in this experiment, it was technically impossible to evaluate changes in hair cells. Finally, this was only an observational study, and the molecular mechanism was not elucidated. HPLC results indicate that NYT contains cinnamaldehyde from cinnamon bark (**Figure 2B**). Cinnamaldehyde reportedly contributes to Nrf2 activation; however, this could not be verified in this study (26). In the future, we plan to evaluate whether NYT can cause Nrf2 activation and other underlying mechanisms.

Several components have been reported to be useful in preventing age-related hearing loss (11, 27). To our knowledge, food, or medicine that is useful for age-related diseases, such as fatigue, cold limbs, anorexia, night sweats, and anemia, has never been reported to suppress age-related hearing loss. NYT is covered by insurance. Since age-related hearing loss gradually progresses in all patients, NYT supplements for various geriatric problems, including progressive age-related hearing loss, can help enhance the general quality of life in the elderly population. In conclusion, NYT appears to have a protective effect on the auditory nerve and suppress the progression of age-related hearing loss by reducing age-related auditory nerve degeneration.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This animal study was reviewed and approved by the Ethics Committee for Animal Experiments of Tohoku University Graduate School of Medicine the Experimental Animal Care Committee of Kracie Pharma.

AUTHOR CONTRIBUTIONS

TK designed the study and wrote the manuscript. TK and KH performed the experiments and analyzed the data. NF and RT revised the manuscript. All authors discussed the results and contributed to the final manuscript.

ACKNOWLEDGMENTS

We thank Dr. Yukio Katori for providing important advice. We also thank Dr. Yosuke Honkura for providing important advice and special skills during the entire study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.528864/full#supplementary-material

REFERENCES

1. Uchida Y, Sugiura S, Nakashima T, Ando F, Shimokata H. [Estimates of the size of the hearing-impaired elderly population in Japan and 10-year incidence of hearing loss by age, based on data from the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA)]. *Nihon Ronen Igakkai Zasshi*. (2012) 49:222–7. doi: 10.3143/geriatrics.49.222
2. Gates GA, Mills JH. Presbycusis. *Lancet*. (2005) 366:1111–20. doi: 10.1016/S0140-6736(05)67423-5
3. Lin FR, Metter EJ, O'Brien RJ, Resnick SM, Zonderman AB, Ferrucci L. Hearing loss and incident dementia. *Arch Neurol*. (2011) 68:214–20. doi: 10.1001/archneurol.2010.362
4. Lyu AR, Kim TH, Park SJ, Shin SA, Jeong SH, Yu Y, et al. Mitochondrial damage and necroptosis in aging cochlea. *Int J Mol Sci*. (2020) 21:2505. doi: 10.3390/ijms21072505
5. Suzuki S, Aihara F, Shibahara M, Sakai K. Safety and effectiveness of Ninjin'yoeito: a utilization study in elderly patients. *Front Nutr*. (2019) 6:14. doi: 10.3389/fnut.2019.00014

6. Honkura Y, Matsuo H, Murakami S, Sakiyama M, Mizutari K, Shiotani A, et al. NRF2 Is a key target for prevention of noise-induced hearing loss by reducing oxidative damage of cochlea. *Sci Rep*. (2016) 6:19329. doi: 10.1038/srep19329
7. Tavanai E, Mohammadkhani G. Role of antioxidants in prevention of age-related hearing loss: a review of literature. *Eur Arch Otorhinolaryngol*. (2017) 274:1821–34. doi: 10.1007/s00405-016-4378-6
8. Kokotas H, Petersen MB, Willems PJ. Mitochondrial deafness. *Clin Genet*. (2007) 71:379–91. doi: 10.1111/j.1399-0004.2007.00800.x
9. Liu XZ, Yan D. Ageing and hearing loss. *J Pathol*. (2007) 211:188–97. doi: 10.1002/path.2102
10. Seidman MD, Khan MJ, Bai U, Shirwany N, Quirk WS. Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. *Am J Otol*. (2000) 21:161–7. doi: 10.1016/s0196-0709(00)80003-4
11. Darrat I, Ahmad N, Seidman K, Seidman MD. Auditory research involving antioxidants. *Curr Opin Otolaryngol Head Neck Surg*. (2007) 15:358–63. doi: 10.1097/MOO.0b013e3282efa641
12. Yamasoba T, Someya S, Yamada C, Weindruch R, Prolla TA, Tanokura M. Role of mitochondrial dysfunction and mitochondrial DNA mutations in age-related hearing loss. *Hear Res*. (2007) 226:185–93. doi: 10.1016/j.heares.2006.06.004
13. Someya S, Xu J, Kondo K, Ding D, Salvi RJ, Yamasoba T, et al. Age-related hearing loss in C57BL/6J mice is mediated by Bak-dependent mitochondrial apoptosis. *Proc Natl Acad Sci USA*. (2009) 106:19432–7. doi: 10.1073/pnas.0908786106
14. Fujinami M, Aotsuka S, Okawa M, Kinoshita M, Sumiya M. Thermographic assessment of Renshen-yangrong-tang (Ninjin-youei-tou) for the treatment of peripheral circulatory disturbance in patients with collagen disease [*In Japanese*]. *Prog Med*. (1980) 17:393–408.
15. Takemiya T. Clinical effects of ninjin-yoei-to in patients with peripheral circulation disturbance [*In Japanese*]. *Jpn Pharmacol Ther*. (1991) 19:409–16.
16. Manabe Y, Tokunaga T. The effectiveness of Kampo medicines in the treatment of intractable acute, low-tone, sensorineural hearing loss. *Otology Jpn*. (2018) 28:139–43. doi: 10.11289/otoljpn.28.139
17. Kane KL, Longo-Guess CM, Gagnon LH, Ding D, Salvi RJ, Johnson KR. Genetic background effects on age-related hearing loss associated with Cdh23 variants in mice. *Hear Res*. (2012) 283:80–8. doi: 10.1016/j.heares.2011.11.007
18. Carlson S, Willott JF. The behavioral salience of tones as indicated by prepulse inhibition of the startle response: relationship to hearing loss and central neural plasticity in C57BL/6J mice. *Hear Res*. (1996) 99:168–75. doi: 10.1016/s0378-5955(96)00098-6
19. Willott JF, Bross LS. Morphological changes in the anteroventral cochlear nucleus that accompany sensorineural hearing loss in DBA/2J and C57BL/6J mice. *Brain Res Dev Brain Res*. (1996) 91:218–26. doi: 10.1016/0165-3806(95)00188-3
20. Johnson KR, Erway LC, Cook SA, Willott JF, Zheng QY. A major gene affecting age-related hearing loss in C57BL/6J mice. *Hear Res*. (1997) 114:83–92. doi: 10.1016/s0378-5955(97)00155-x
21. Ison JR, Allen PD, O'Neill WE. Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex. *J Assoc Res Otolaryngol*. (2007) 8:539–50. doi: 10.1007/s10162-007-0098-3
22. Vlajkovic SM, Guo CX, Telang R, Wong ACY, Paramananthasivam V, Boison D, et al. Adenosine kinase inhibition in the cochlea delays the onset of age-related hearing loss. *Exp Gerontol*. (2011) 46:905–14. doi: 10.1016/j.exger.2011.08.001
23. Oike H, Aoki-Yoshida A, Kimoto-Nira H, Yamagishi N, Tomita S, Sekiyama Y, et al. Dietary intake of heat-killed *Lactococcus lactis* H61 delays age-related hearing loss in C57BL/6J mice. *Sci Rep*. (2016) 6:23556. doi: 10.1038/srep23556
24. Kong TH, Yu S, Jung B, Choi JS, Seo YJ. Monitoring blood-flow in the mouse cochlea using an endoscopic laser speckle contrast imaging system. *PLoS ONE*. (2018) 13:e0191978. doi: 10.1371/journal.pone.0191978
25. Sha S-H, Kanicki A, Dootz G, Talaska AE, Halsey K, Dolan D, et al. Age-related auditory pathology in the CBA/J mouse. *Hear Res*. (2008) 243:87–94. doi: 10.1016/j.heares.2008.06.001
26. Uchi H, Yasumatsu M, Morino-Koga S, Mitoma C, Furue M. Inhibition of aryl hydrocarbon receptor signaling and induction of NRF2-mediated antioxidant activity by cinnamaldehyde in human keratinocytes. *J Dermatol Sci*. (2017) 85:36–43. doi: 10.1016/j.jdermsci.2016.10.003
27. Seidman MD. Effects of dietary restriction and antioxidants on presbyacusis. *Laryngoscope*. (2000) 110:727–38. doi: 10.1097/00005537-200005000-00003

Conflict of Interest: All authors are employees of Kracie Pharma (Toyama, Japan).

frontiers in Nutrition

ORIGINAL RESEARCH
published: 21 January 2021
doi: 10.3389/fnut.2020.529390

Yokukansankachimpihange Is Useful to Treat Behavioral/Psychological Symptoms of Dementia

Eiichi Katsumoto [1,2], Toru Ishida [1,3], Kenji Kinoshita [1,4], Miho Shimizu [1,5], Toshihito Tsutsumi [1,6], Yoko Nagai [1,7], Masakazu Nishimura [1,8], Toshio Yokouchi [1,9] and Yasushi Yoshida [1,10]*

[1] Osaka Association of Psychiatric Clinics, Osaka, Japan, [2] Katsumoto Mental Clinic, Osaka, Japan, [3] Ishida Clinic, Osaka, Japan, [4] Kinoshita Clinic, Osaka, Japan, [5] Shimizu Clinic, Osaka, Japan, [6] Tsutsumi Clinic, Osaka, Japan, [7] Nagai Clinic, Osaka, Japan, [8] Nishimura Clinic, Osaka, Japan, [9] Yokouchi Clinic, Osaka, Japan, [10] Yoshida Clinic, Osaka, Japan

Yokukansankachimpihange is a Japanese herbal medicine reported to benefit anxiety and sleep disorders, and it has recently been introduced to treat behavioral and psychological symptoms of dementia. There are no multicenter studies of its effectiveness regarding dementia in Japan, and this study's main objective was to clarify the effects of Yokukansankachimpihange on behavioral and psychological symptoms of dementia in a sample of patients from multiple healthcare centers. Nine facilities affiliated with Osaka Association of Psychiatric Clinics participated in November 2013 through April 2015 and provided 32 Alzheimer's disease patients to whom Yokukansankachimpihange was orally administered for 8 weeks. During the study, the patients continued their regular medication regimens. Behavioral and psychological symptoms of dementia (Behavioral Pathology in Alzheimer's Disease Rating Scale [Behave-AD]), core symptoms [Mini-Mental State Examination (MMSE)], activities of daily living [Nishimura Activity of Daily Living Scale (N-ADL)], and gastrointestinal symptoms (nausea/vomiting, loss of appetite, gastric discomfort, constipation, and diarrhea) were measured at baseline, after 4 weeks of treatment and after 8 weeks of treatment. Yokukansankachimpihange was orally administered at a dosage of 7.5 g twice daily before or between meals for 8 weeks. The Behave-AD mean score significantly improved after 8 weeks of treatment. There were no significant changes in MMSE, N-ADL, or gastrointestinal symptoms; however, decreased gastrointestinal scores were observed after 8 weeks. There were no side effects related to Yokukansankachinpihange. Pharmaceutical treatments are important for treating behavioral and psychological symptoms of dementia, and this study confirmed Yokukansankachimpihange's efficacy for treating Alzheimer's disease. Because the aggressiveness and sleep disorder components of the Behave-AD construct were the symptoms most improved and those symptoms are known to significantly burden dementia patients' caregivers, Yokukansankachimpihange's efficacy might indirectly relieve these caregivers' burden of care.

Keywords: multi-center research, behavioral dementia symptoms, psychological dementia symptoms, dementia, insomnia, Alzheimer's disease

OPEN ACCESS

Edited by:
Yasuhito Uezono, National Cancer Centre (Japan), Japan

Reviewed by:
Kanako Miyano, National Cancer Center Research Institute, Japan
Miki Nonaka, Jikei University School of Medicine, Japan

****Correspondence:***
Eiichi Katsumoto
ekatsumoto@kmc2007.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *30 January 2020*
Accepted: *08 December 2020*
Published: *21 January 2021*

Citation:
Katsumoto E, Ishida T, Kinoshita K, Shimizu M, Tsutsumi T, Nagai Y, Nishimura M, Yokouchi T and Yoshida Y (2021) Yokukansankachimpihange Is Useful to Treat Behavioral/Psychological Symptoms of Dementia. Front. Nutr. 7:529390. doi: 10.3389/fnut.2020.529390

INTRODUCTION

Antipsychotics used to treat behavioral and psychological symptoms of dementia (BPSD) require particularly careful administration because of their potential side effects. Their main side effects are extrapyramidal symptoms, oversedation, cognitive decline, increased cerebrovascular disorders, and increased risk of mortality. A risk of increased blood sugar levels needs to be considered in the use of atypical antipsychotics. Avoiding typical antipsychotics as much as possible and limited treatment with atypical antipsychotics have been recommended (1).

Recently, Yokukansan (YKS) (2), a Japanese herbal medicine, has been used to treat BPSD. Yokukansankachimpihange (YKSCH) is a Japanese herbal medicine made up of *Citrus unshiu* peel and *Pinellia tuber* combined with YKS. *Citrus unshiu* peel and *Pinellia tuber* were formulated to improve gastrointestinal symptoms (GIS), such as loss of appetite, nausea, and vomiting (3). The blend is expected to favorably influence anxiety and sleep disorders because of the influence that *Citrus unshiu* peel has on serotoninergic neural pathways (4).

Several previous clinical studies have examined the effects of YKSCH on BPSD (5–9); however, most of them were single-setting studies and none involved multiple healthcare centers. Further, the only cholinesterase inhibitor (ChEI), which is the only class of medications on the market currently being used to treat dementia, evaluated by the previous studies was donepezil. This study aimed to clarify the effects of YKSCH on BPSD in a nine-center joint clinical study under conditions similar to those in which Alzheimer's disease (AD) medications are used in real-world settings without specifying the type and route of ChEI administration.

METHODS AND MATERIALS

Setting and Participants

This multi-center prospective clinical study initially recruited 53 patients diagnosed with AD who met the inclusion and exclusion criteria. It was conducted between November 2013 and April 2015 at nine facilities affiliated with the Osaka Association of Psychiatric Clinics, Osaka, Japan.

The inclusion criteria were AD patients (1) currently on a ChEI that lacked efficacy for their BPSD, (2) who had difficulty continuing the ChEI treatment because of gastrointestinal side effects, (3) who had no ChEI currently administered with a demonstrated need for BPSD treatment, or (4) on continuous ChEI treatment for more than 4 weeks without a change in medication type or dosage.

The exclusion criteria were (1) patients currently on memantine or YKS or (2) patients who had recently started or changed the dosage of any medication that might influence the efficacy of YKSCH, such as ChEI, antipsychotics, or Japanese herbal medicines, within 4 weeks before the study began.

Ethics

The Ethics Committee of the Osaka Association of Psychiatric Clinics approved this study. Written informed consent was obtained from all the enrolled patients.

Procedure

YKSCH was orally administered at a dosage of 7.5 g twice daily before or between meals for 8 weeks. All other medications were continuously administered without changing the dosages, and no additional medications were administered during the study period. Four outcome variables were assessed at baseline, 4 weeks into the YKSCH treatment, and 8 weeks into the YKSCH treatment.

Variables

BPSD was measured by responses to the Behavioral Pathology in Alzheimer's Disease Rating Scale (Behave-AD) using the total Behave-AD score, which was based on responses to 25 items, with a maximum score of 75 points. The item measuring "care burden" was excluded from the calculation of Behave-AD score, because it was an overall indicator of BPSD. The 11-item Mini-Mental State Examination (MMSE) was used to measure core symptoms on a 30-point scale. Activities of daily living were evaluated using the N-type older adult living activities scale (Nishimura Activity of Daily Living Scale; N-ADL), a five-item 50-point scale. Four levels of GIS regarding nausea and vomiting, loss of appetite, stomach discomfort, constipation, and diarrhea were identified where 0 = no symptoms, 1 = some untroublesome symptoms, 2 = some symptoms that do not interfere with activities of daily living, and 3 = symptoms that interfere with activities of daily living. The patients assessed the severity of their symptoms on a questionnaire survey using a 15-point scale. Higher scores on the Behave-AD and GIS and lower scores on the MMSE and N-ADL indicated more severe symptoms/disabilities. The four variables (Behave-AD, MMSE, N-ADL, and GIS) were measured before YKSCH administration (baseline), 4 weeks into the YKSCH treatment, and 8 weeks into the YKSCH treatment.

Statistical Analysis

Multiple comparison tests were performed using one-way analysis of variance (ANOVA) and Tukey's HSD test, with a statistical significance cutoff level of $p < 0.05$, to assess the influences of YKSCH on the four outcomes after 4 weeks and after 8 weeks.

RESULTS

At baseline, 53 cases at nine institutions were enrolled in the study. Two patients did not visit the hospital after the baseline, 12 patients violated the protocol, and the safety analysis of the influence of YKSCH was assessed for the remaining 39 patients. The 12 protocol violations (some cases had more than one violation) were two cases of memantine use, six cases of concomitant use of a ChEI initiated at the start of the study, six cases of concomitant use of non-ChEI medications started within 1 month of the study, and three cases of additional use of concomitant medications during the study period. The efficacy analysis was performed on 32 patients because of the loss of two patients who refused to take the medication, four patients who did not participate in the 8-week follow-up, and one patient

TABLE 1 | Sample characteristics.

Characteristic	**Mean ± SD**
Age (years)	76.9 ± 9.4
Height (cm)	156.7 ± 6.5
Weight (kg) ($n = 13$)[a]	53.1 ± 10
Dementia history (months)	49.4 ± 40.5
Gender	**Number of cases (%)**
Male	9 (28)
Female	23 (72)
Total	32 (100)
Medical history	**Number of cases (%)**
None	21 (66)
Existing	11 (34)
Total	32 (100)
Disease[b]	
Hypertension	1
Hyperlipidemia	1
Glaucoma	1
Cholecystitis	1
Alcoholism	1
Cerebral infarction	1
Epidural hematoma	1
Lumbar compression fracture	1
Previous spinal canal stenosis surgery	1
Breast cancer	1
Previous breast cancer surgery	1
Previous stomach cancer surgery	1
Medical complications	**Number of cases (%)**
None	16 (50)
Existing	16 (50)
Total	32 (100)
Complication[b]	
Hypertension	8
Diabetes	3
Hyperlipidemia	2
Prostatic hypertrophy	2
Depressive state	2
Hyperuricemia	1
Chronic gastritis	1
Chronic nephritis	1
Primary osteoporosis	1
Hypothyroidism	1
Tension headache	1
Depression	1
Suspected Parkinsonism	1
Concomitant AD medication	**Number of cases (%)**
Yes	25 (78)
No	7 (22)
Total	32 (100)
Concomitant ChEI medication	**Number of cases (%)**
None	12 (38)
Existing[c]	20 (62)
Total	32 (100)
Medication[b]	
Donepezil (5 mg)	9
Donepezil (10 mg)	2
Galantamine (8 mg)	1
Galantamine (16 mg)	3
Galantamine (24 mg)	1
Rivastigmine (18 mg)	4
Other concomitant medications for BPSD	**Number of cases (%)**
None	22 (69)
Existing	10 (31)
Total	32 (100)
Medication[b]	
Antipsychotic	7
Antidepressant	5
Antianxiety	4
Hypnotic	1

[a] *Missing data = 19.*
[b] *There are duplicate cases.*
[c] *Administration period until start of study, 9.9 ± 9.7 months [n = 16].*

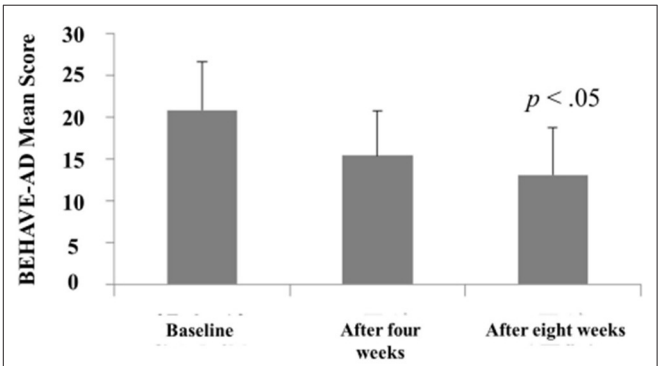

FIGURE 1 | The effects of YKSCH on BPSD ($n = 29$). Data were Behave-AD scores (means ± SD and ranges) after YKSCH administration. Three cases with missing data were dropped from the analysis. *Post hoc* Tukey's HSD tests were compared to baseline.

who independently discontinued treatment due to perceived ineffectiveness. Of the 32 patients, nine were male and 23 were female. The average age was 76.9 ± 9.4 years. **Table 1** describes the personal characteristics of the 32 patients in the efficacy analysis. No side effects deemed attributable to the treatment were observed.

YKSCH Results

The Behave-AD mean score was 20.8 ± 11.4 at baseline, 15.4 ± 10.4 4 weeks later, and 13.1 ± 9.1 after 8 treatment weeks. An improvement was observed between baseline and 8 weeks (**Figure 1**). Of the construct's 25 items, "aggression" significantly improved after 4 and after 8 weeks of treatment, and "circadian rhythm disorder" was significantly improved after 4 weeks of treatment. The "care burden" indicator also was significantly reduced after 8 weeks of treatment (**Figure 2**). Core symptoms (MMSE) and activities of daily living (N-ADL) did not significantly change during the treatment period (**Figure 3**). Regarding GIS, 15 patients reported symptoms at baseline [mean 2.0 ± 2.6 (range: 1–8)]. The score decreased to 0.9 ± 1.4 (range:

1–9) after 4 weeks of treatment and to 0.7 ± 1.4 after 8 weeks of treatment, but there was no significant difference from baseline (**Figure 4**). Seven of 15 patients had symptoms at the start of the study that disappeared after 8 weeks of treatment.

DISCUSSION

YKSCH was administered for 8 weeks to 32 AD patients who were not experiencing sufficient efficacy with conventional BPSD treatments or had difficulties with continuous use of a ChEI due to gastrointestinal side effects. Because improvement was observed in 8 weeks in the previous study (6), the administration period was set to 8 weeks in this study as well. The results indicated significant improvement in two Behave-AD items (aggression and circadian rhythm disorders) and in the Behave-AD overall care burden score. MMSE and N-ADL mean scores were not significantly influenced.

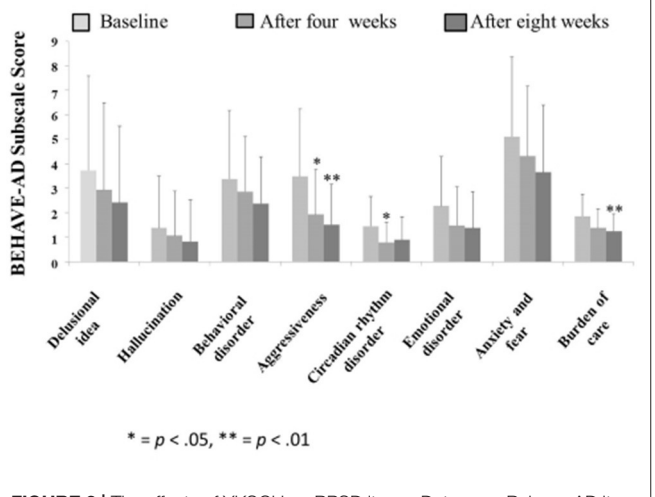

FIGURE 2 | The effects of YKSCH on BPSD items. Data were Behave-AD item scores (means ± SD and ranges) after YKSCH administration. Results were on 29 cases regarding "burden of care" and 28 cases otherwise. All excluded cases were dropped because of missing data. *Ad hoc* multiple comparison Tukey's HSD tests (*$p < 0.05$, **$p < 0.01$) were compared to baseline.

YKSCH is a blend of *Citrus unshiu* peel and *Pinellia tuber* with YKS. YKS is a prescription medication developed to cure oversensitivity conditions, including children crying at night. *Citrus unshiu* peel and *Pinellia tuber* are used to improve GIS, such as loss of appetite and nausea/vomiting (3); thus, it is suitable for older adults with reduced digestive function. In this study's analysis of GIS, the mean score improved, but the differences were not statistically significant. YKSCH might have contributed to improving GIS because *Citrus unshiu* peel and *Pinellia tuber* have been reported to improve loss of appetite (10) caused by suppression of cisplatin-induced anorexia via 5-HT_2 receptor antagonism.

The "aggression" item in Behave-AD aims to assess the extent of rants, threats, violence, and restlessness. This BPSD symptom was the most improved in response to YKSCH treatment. Basic research has investigated the mechanism by which YKS diminishes aggression (11). It has been hypothesized that YKS affects glutamatergic neural pathways related to aggressiveness and serotoninergic (5-HT) pathways related to impulsivity (11). It also has been reported that Uncaria hook, a component of YKSCH, has a 5-HT_{1A} receptor-stimulating action and a 5-HT_{2A} receptor-blocking action as well as an ability to normalize the extracellular fluid concentration of glutamate (11), all of which might be expected to contribute to reductions in aggression.

The "circadian rhythm disorder" Behave-AD item is focused on sleep-wake disorders (insomnia). High rates of insomnia are observed in patients with dementia, which not only significantly affects patients' quality of life, but also increases the burden of care because it causes physical and mental stress for the caregiver. Regarding YKSCH's mechanism of action on insomnia, basic research has suggested that Uncaria hook (11) and *Citrus unshiu* peel (4) have effects on nervous system serotonin and that YKS influences the $GABA_A$-benzodiazepine receptor complex (12). We found that YKSCH treatment alleviated the severity of circadian rhythm disorder, i.e., sleep-wake disorder, implying that YKSCH might be effective for nighttime insomnia and improving quality of life, which, in turn, suggests that the mental and physical burden on caregivers might be reduced by suppressing patients' nighttime activity levels.

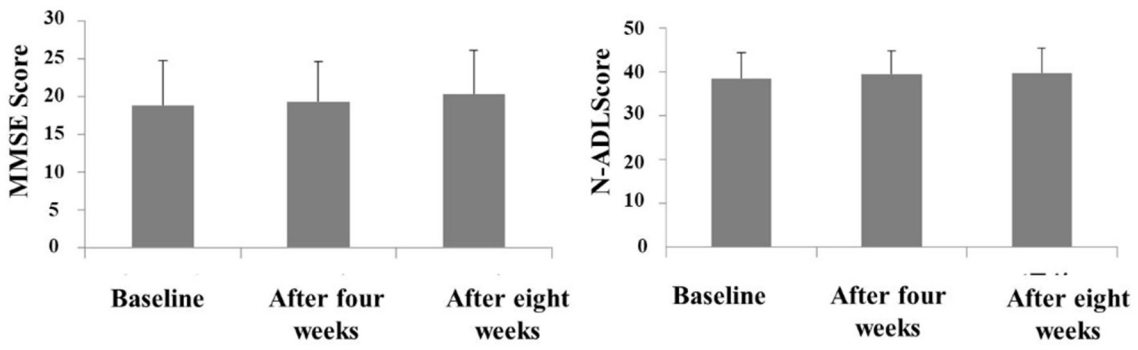

FIGURE 3 | The effects of YKSCH on MMSE and N-ADL. Data were MMSE scores and N-ADL scores (means ± SD and ranges) after YKSCH administration. MMSE results were on 20 cases (12 cases dropped because of missing data), and N-ADL results were on 21 cases (11 cases dropped because of missing data).

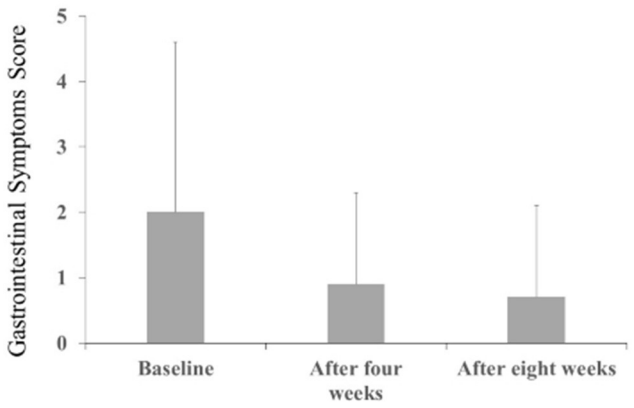

FIGURE 4 | The effects of YKSCH on Gastrointestinal Symptoms ($n = 28$). Data were gastrointestinal symptoms scores (means ± SD and ranges) after YKSCH administration. Four cases with missing data were dropped from the analysis. *Post hoc* Tukey's HSD tests were compared to baseline.

Because aggressiveness and circadian rhythm disorder are major contributors to caregivers' burden, alleviating both problems likely led to a reduction in the burden of care. The results suggest that YKSCH might be an effective treatment for patients who demonstrate significant aggressiveness and those who struggle with insomnia. There were no side effects of the administration of this medication observed throughout the study. Although this study has some limitations, such as the lack of a control group and the insufficient number of cases, YKSCH is considered a suitable treatment for AD sufferers.

CONCLUSIONS

This study clarifies the efficacy of YKSCH for BPSD. The results suggest that treatment with YKSCH, a Japanese herbal medicine, might benefit AD patients and their caregivers.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher on request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Osaka Association of Psychiatric Clinics. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TI designed the study plan and EK, TI, KK, MS, TT, YN, MN, TY, and YY registered the cases. EK conducted the analysis and prepared the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

This is an English language translation of Efficacy and safety of YKSCH on BPSD originally published in 2016 in *Igaku to yakugaku* 73(7) 846–853.

REFERENCES

1. The Japan Geriatrics Society, Nihon iryoukaihatukikou kenkyuuhi (Japan Agency for Medical Research and Development), Koureisya no yakubutsutiryou no anzensei ni kansuru kenkyuu kenkyuuhan (Research Group for Safety Pharmacotherapy Guidelines for the Elderly). *Koureisya no Anzen na Yakubutsuryouhou Gaidorain 2015 (Guideline for the Safe Pharmacotherapy for the Elderly 2015)*. Tokyo: The Japan Geriatrics Society (2015).
2. Matsuda Y, Kishi T, Shibayama H, Iwata N. Yokukansan in the treatment of behavioral and psychological symptoms of dementia a systematic review and meta-analysis of randomized controlled trials. *Hum Psychopharmacol Clin Exp*. (2013) 28:80–6. doi: 10.1002/hup.2286
3. Nakamura K. *Wakan kampou i jiten [Oriental Medicine Therapy Dictionary]*. Tokyo: Midori Shobo Co., Ltd. (2004). p. 624–5.
4. Ito A, Shin N, Tsuchida T, Okubo T, Norimoto H. Antianxiety-like effects of chimpi (dried citrus peels) in the elevated open-platform test. *Molecules*. (2013) 18:10014–23. doi: 10.3390/molecules180810014
5. Izumi Y. 14 cases of dementia: herb medicine improves clinical manifestations such as violence and wandering in fourteen dementia patients. *Kampo Newest Ther*. (2003) 12:352–6.
6. Miyazawa J. Study of clinical effects of Yokukansankachimpihange on Alzheimer's disease. *Psychiatry*. (2009) 14:535–42.
7. Magome A. Effects of Yokukansankachimpihange on dementia: including the point of view of oriental medicine. *Psychiatry*. (2011) 18:108–14.
8. Suzuki G. The effectiveness of using Yokukansankachimpihange on dementia patients as respite care from the standpoint of caregivers. *Jap. J. Med. Pharm. Sci*. (2013) 69:101–7.
9. Takahashi M. Yokukansankachimpihange ga yuukou na ninchisyou no rinsyoukei [The clinical pattern of dementia for which Yokukansankachimpihange is effective]. *Phil Kampo*. (2015) 53:20–1.
10. Takeda H, Cadakane C, Hattori T, Katsurada T, Ohkaware T, Nagai K, et al. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT_2 receptor antagonism. *Gastroenterology*. (2004) 134:2004–13. doi: 10.1053/j.gastro.2008.02.078
11. Igarashi Y. Yokukansan no sayoumekanizumu no kaimei [Revealing the action mechanism of Yokukansan]. *Geriat Med*. (2008) 46:255–61.
12. Egashira N, Nogami A, Iwasaki K, Ishibashi A, Uchida N, Takasaki K, et al. Yokukansan enhances pentobarbital-induced sleep in socially isolated mice: possible involvement of GABAA-benzodiazepine receptor complex. *J Pharmacol Sci*. (2011) 116:316–20. doi: 10.1254/jphs.11079SC

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

CASE REPORT
published: 27 January 2021
doi: 10.3389/fnut.2021.551373

Case Report: Nutritional Examination of Weight Loss Treatment Using Kampo

Yutaka Matsui and Ikuko Matsui*

Matsui Dietary & Dementia Clinic, Akashi, Japan

Loss of appetite is a common symptom in patients with dementia, and if weight loss or difficulty eating occurs without subjective symptoms, the patient can easily become malnourished. There is also a close relationship between dementia and physical frailty, such as weight loss and muscle weakness, and thus early intervention to address frailty in patients with dementia is important. In this study, 3 patients with dementia who complained of loss of appetite and weight loss showed increases in body weight and muscle mass after taking Ninjin'yoeito. Ninjin'yoeito was found to be a potentially effective treatment option for physical frailty in patients with dementia.

Keywords: muscle mass, weight loss, dementia, loss of appetite, Ninjin'yoeito

OPEN ACCESS

Edited by:
Masahiro Ohsawa,
Nagoya City University, Japan

Reviewed by:
Shin Takayama,
Tohoku University Hospital, Japan
Kanako Miyano,
National Cancer Center Research Institute, Japan

****Correspondence:***
Yutaka Matsui
yutakamatsui@matsui-network.com

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *13 April 2020*
Accepted: *05 January 2021*
Published: *27 January 2021*

Citation:
Matsui Y and Matsui I (2021) Case Report: Nutritional Examination of Weight Loss Treatment Using Kampo. Front. Nutr. 8:551373. doi: 10.3389/fnut.2021.551373

INTRODUCTION

Loss of appetite is a common symptom in patients with dementia, and when weight loss or difficulty eating occur in the absence of subjective symptoms, the patient can easily become malnourished. In such cases, the underlying causes should be carefully investigated. If the loss of appetite is caused by an organic abnormality of the digestive system or worsening of neurological function (e.g., dysphagia, constipation) associated with extrapyramidal symptoms, the patient should be treated for the dysfunction. If these possible causes are excluded, energy supplementation should be provided along with dietary nutritional guidance and prescriptions of oral and enteral nutritional supplements. Selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), or second-generation antipsychotics (atypical antipsychotics) are used to treat loss of appetite caused by apathy and depression in dementia. Patients who do not respond to treatment may experience repeated episodes of malnutrition and dehydration, leading to heart failure, infection, and potentially fatal outcomes. Therefore, loss of appetite in the elderly and patients with dementia has a significant impact on their life prognosis and should not be overlooked.

In addition, elderly individuals with loss of appetite and patients with dementia who remain malnourished are prone to fall into the frailty cycle, in which their basal metabolism and activity level are decreased due to reduced muscle mass and strength, leading to further loss of appetite and weight loss. Frailty is a condition in which physical and mental vitality, such as motor and cognitive functions, declines with age, leading to functional impairment of activities of daily living (ADLs), and a state that requires long-term care. There is a close relationship between dementia and frailty whereby physical frailty, such as weight loss and muscle weakness, causes a person to be at risk for cognitive decline or dementia, and conversely, cognitive impairment causes a person to be at risk for physical frailty. It has been reported that as cognitive decline progresses, the level of frailty increases (1, 2). Therefore, early intervention to address frailty, including weight loss and muscle weakness, is important in patients with dementia. Our hospital is conducting active nutritional

management, including a food frequency questionnaire, when loss of appetite or weight loss is a concern. However, in some patients nutritional status cannot be adequately controlled even with such guidance, in which case traditional Japanese herbal medicine (Kampo) may be introduced.

Ninjin'yoeito, Japanese herbal medicine (Kampo), is indicated for the treatment of loss of appetite and has been used since ancient times to improve nutritional status. In recent years, it has also been applied to treat frailty because of its multifaceted effects (3), and there have been numerous reports showing improvements in musculoskeletal strength and mass in addition to reversal of appetite loss, but none yet for muscle mass.

In this study, 3 cases of patients with dementia who experienced loss of appetite and weight loss, and who gained weight and muscle mass after receiving Ninjin'yoeito are reported. Informed written consent was obtained from the patients and their caregivers regarding this report.

CASE DESCRIPTIONS

Case 1

The patient was an 84-year-old female; 145.1 cm in height and 54.0 kg in weight at the time of her initial examination; a housewife; and described as sincere and calm in personality. Her chief complaints were loss of appetite and weight loss, and she had a medical history of angina with onset at the age of 75. With regard to the history of her present illness, she came to our hospital in January X−3 due to noticeable amnesia such as not being able to recognize the faces and names of relatives and grandchildren. She was diagnosed with Alzheimer's disease based on a 16-point score on the Mini-Mental State Examination (MMSE) and on functional imaging findings such as cerebral blood flow single-photon emission computed tomography (SPECT). She was treated with galantamine and outpatient care for lifestyle diseases such as hypertension, and her instrumental ADLs (IADLs) improved to the point where she was able to prepare meals, go shopping, and take the bus.

She was treated with galantamine. However, her functional ability to perform ADLs gradually declined; and in the summer of X−1, her functional impairment in ADLs progressed to the point where she required emergency treatment, including intravenous fluids, due to heat stroke. In December X−1, she fell on the stairs at a train station, fractured her right femur, and underwent an artificial femoral head replacement. Although she was discharged from the hospital in April X, she was unable to take her medication or do household tasks on her own that she was previously able to do. She was unable to prepare meals and often did not eat when her eldest daughter prepared meals for her. In October X, she had frequent hallucinations at home when someone was in the room; she started to eat much less food; and she weighed 50.4 kg, showing a decrease of 4 kg in 6 months. Although she was able to walk on her own, her IADLs declined as indicated by scores of 48 on the Dementia Assessment Sheet for Community-based Integrated Care System 21-items (DASC-21), 4 on the Lawton IADL scale, 3 on the level of long-term care required, 21 on the MMSE, and 14 on the Frontal Assessment Battery (FAB). Her muscle mass was 30.2 kg as measured by a body composition analyzer (InBody 270) using bioelectrical impedance analysis. The calculated weight after removing the body fluid weight (19.1 kg) from the total body weight (50.4 kg) was 31.3 kg. Since October X, she had been instructed to take 3.75 g of Ninjin'yoeito once before bedtime for her loss of appetite and weight loss.

In April X+1, improvement in her appetite was observed. Her weight was 52.2 kg, showing an increase of 1.8 kg. Her muscle mass was 31.1 kg, showing an increase of 0.9 kg. The weight after subtracting the body fluid weight was 52.2–19.7 kg = 32.5 kg, showing an increase of 1.2 kg. In July X+1, her weight dropped slightly by 0.8 to 51.4 kg, and her muscle mass also dropped by 0.3 to 30.8 kg. Eventually, there was an increase of 1.0 kg in body weight and 0.6 kg in muscle mass, and the weight after removing the body fluid weight was 51.4–19.5 kg = 31.9 kg, showing an increase of 0.6 kg. With regard to ADLs, a decrease in basic ADLs (BADLs) in addition to IADLs was observed, such as leaving a pot on the fire, requiring assistance to take medication, being unable to flush the toilet, and being unable to bathe and wash her hair, but her hallucinations and insomnia improved and she was no longer required to take sleeping medication and/or antipsychotic medication.

Case 2

The patient was a 74-year-old female; 152.9 cm in height and 56.0 kg in weight at the time of her initial examination; a housewife; and described as gentle in her personality. Her chief complaints were lack of motivation, irritability, loss of appetite, and weight loss. Her medical history included hypertension and dyslipidemia at the age of 68, and she had undergone a colectomy at the age of 73 in January X−1. She had no remarkable family medical history, but her husband had Lewy body dementia and was in need of long-term care. Regarding the history of here present illness, she came to our clinic in December X−1 for abnormal behavior based on amnesia and paranoia, such as buying too many vegetables that she could not use, rotting them in the refrigerator, being unable to clean her room, and repeatedly washing her clothes while saying "my husband is dirty." She had high neuropsychological test scores of 28 on the MMSE and 16 on the FAB; and her ADLs were not abnormally rated low, as indicated by scores of 33 on the DASC-21, 7 on the Lawton IADL scale, and 2 on the level of support required. Neurological examination showed no tendon reflex, left-right hyperactivity, or pathological reflex; and no atrophy was observed in the medial temporal lobe on magnetic resonance imaging (MRI) with coronal transection imaging. However, cerebral blood flow SPECT showed significant blood flow reduction in the anterior part of the bilateral precuneus, which led to the diagnosis of Alzheimer's disease.

She was attending local clinics for internal medicine and orthopedic surgery, but her conditions were poorly controlled, with polypharmacy and a diastolic blood pressure of over 100 mmHg. In our hospital, the patient was not treated with anti-dementia medication and adjustments to her medication regimen were initiated. She was also diagnosed with colorectal cancer and underwent a colectomy in January X. After discharge from the hospital, she weighed 50.6 kg, but her weight increased to 54.4 kg

in July X with repeated dietary and nutritional guidance aimed at improving the breakfast menu with a focus on increasing energy levels. However, in September X, depressive symptoms such as "feeling lightheaded and agitated" became noticeable, with frequent missed meals and a decrease in the amount of food intake, which decreased her weight by 1.1 to 53.3 kg in 2 months. Muscle mass measured by a body composition analyzer (InBody 270) using bioelectrical impedance analysis was 32.6 kg. The weight after removing the body fluid weight (24.5 kg) from the total body weight (53.3 kg) was 28.8 kg. Since September X, she started taking 3.75 g of Ninjin'yoeito once before bedtime for her loss of appetite and weight loss.

In November X+1, her appetite improved and she weighed 56.7 kg, with an increase of 3.4 kg. Her muscle mass was 33.0 kg with an increase of 0.4 kg. The weight after removing the body fluid weight was increased by 3.9 kg (56.7–24.0 kg = 32.7 kg).

Case 3

The patient was an 81-year-old male; 166.0 cm in height and 46.8 kg in weight at the time of his initial examination; running a restaurant business; and described as slovenly in his personality. His chief complaints were memory loss and weight loss, and he had a medical history of hypertension and reflux esophagitis. As to the history of the present illness, he had been suffering from memory loss since around November X−1. He had been treated at a local doctor's clinic for hypertension, but his forgetfulness worsened, as he was asking the same questions over and over again, unable to tell the date of the day, and unable to take his medication, and therefore he came to our clinic in September X. He was diagnosed with Alzheimer's disease, which was already several years advanced, with impairment in recent memory, semantic memory, executive function, and frontal lobe function shown by the scores of 11 on the Hasegawa's dementia scale, 18 on the MMSE, 10 on the FAB, and 10 on the clock-drawing test (CDT), as well as atrophy of the frontal and medial temporal lobes observed on MRI images. In September X, he complained that he was unable to stop drinking and was not eating, and he weighed 46.8 kg, with a decrease of 1.5 kg in 2 months. His muscle mass measured by a body composition analyzer (InBody 270) using bioelectrical impedance analysis was 34.3 kg. The weight after removing the body fluid weight (21.7 kg) from the total body weight (46.8 kg) was 25.1 kg. Since September X, he started taking Ninjin'yoeito 3.75 g once before bedtime.

In November X+1, improvement in his appetite was observed. His weight was 49.3 kg, showing an increase of 2.5 kg. His muscle mass was 36.7 kg, showing an increase of 2.4 kg. The weight after removing the body fluid weight was 49.3–23.2 kg = 26.1 kg, showing an increase of 1.0 kg.

DISCUSSION

In this study, 3 patients with dementia who complained of loss of appetite and had a weight loss of more than 1 kg in 2 months were treated with 3.75 g/day of Ninjin'yoeito, after which appetite, body weight, muscle mass, and body fluid loss were increased in all patients (**Figures 1**, **2**).

In the all 3 patients reported in this study, weight gain was achieved beginning around 3 months after initiating the treatment, suggesting that factors other than dementia, such as alcohol consumption, affected nutritional management and delayed the onset of effect.

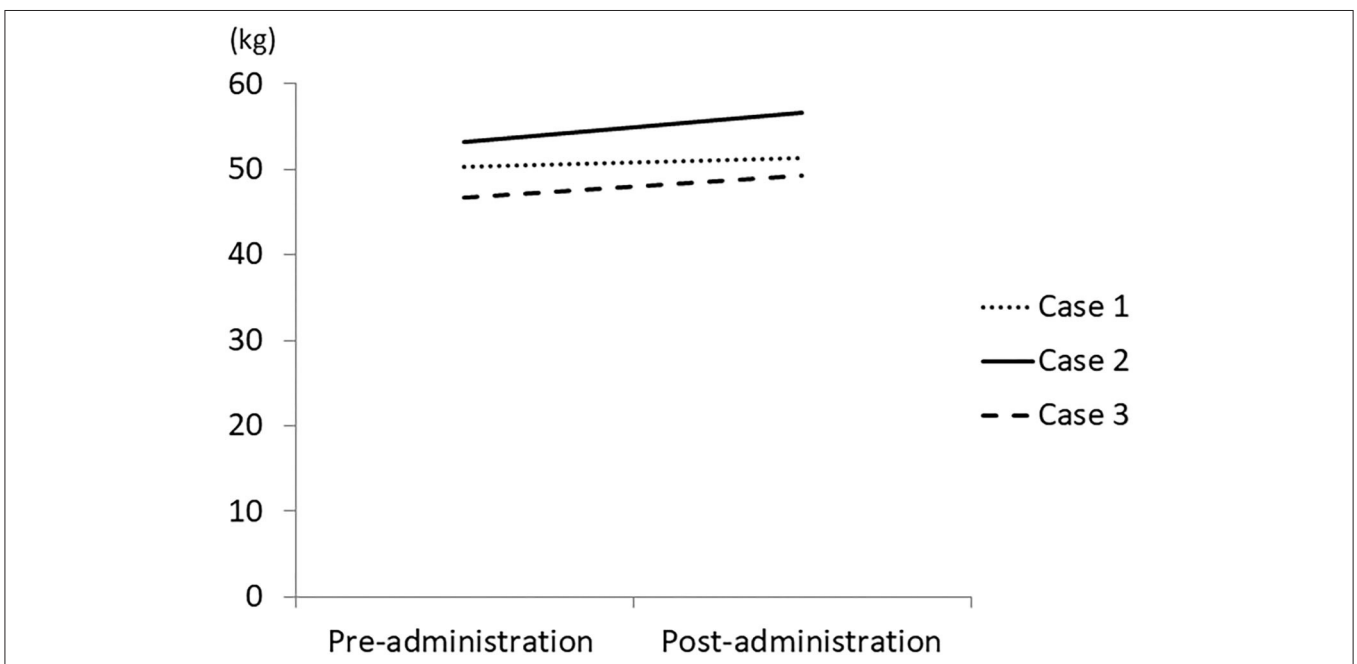

FIGURE 1 | Change in body weight by treatment with Ninjin'yoeito. Three patients who had weight loss of more than 1 kg in 2 months were treated with Ninjin'yoeito and their weight was measured using a body composition analyzer.

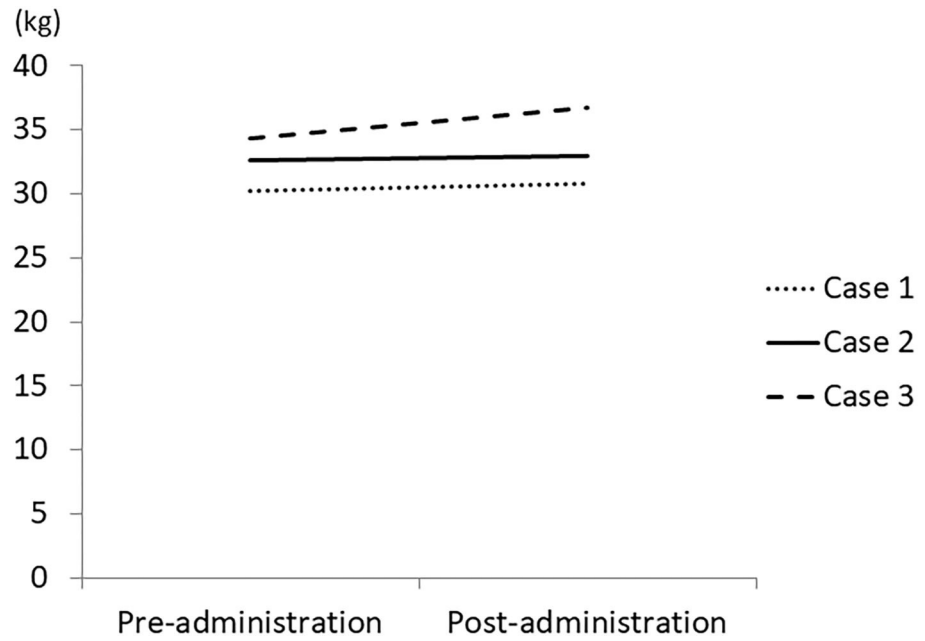

FIGURE 2 | Change in muscle mass by treatment with Ninjin'yoeito. Three patients who had weight loss of more than 1 kg in 2 months were treated with Ninjin'yoeito and their muscle mass was measured using a body composition analyzer.

The mechanism of loss of appetite involves not only organic and functional gastrointestinal lesions, but also central nervous system structures such as the frontal cortex, gustatory and olfactory centers, and the brainstem; and the involvement of the hormones ghrelin and orexin has received particular attention (4). In basic research, it has been reported that Ninjin'yoeito activates both ghrelin-responsive and non-responsive neuropeptide Y (NPY) neurons in the feeding center (5) and promotes ghrelin secretion by Citri Unshiu Pericarpium (6), which may have contributed to the improvement of appetite by Ninjin'yoeito in this study.

Although there was no significant increase in the amount of body fat in the patients reported in this study, I have had patients in the past who have gained weight due to an increase in body fat. However, ghrelin is said to maintain the homeostasis of the organism not only by stimulating feeding, but also as a result of fat accumulation and growth hormone (GH)-induced anabolic effects. Thus, the increase in fat induced by the administration of Ninjin'yoeito may represent a recovery from ghrelin-mediated impaired energy metabolism.

In this study, all 3 patients showed increases in muscle mass, but all had been taking the product for a relatively long period of time, between 9 and 13 months. In a previous report, it required 6 months to improve grip strength in elderly people over 65 years of age after administration of Ninjin'yoeito (7). On the other hand, in basic research, it has been confirmed that administration of Ninjin'yoeito suppresses food intake reduction and weight loss and reduces atrophy of the gastrocnemius and soleus muscles in klotho mice, which are a senescence-accelerated strain. The same study also reported that Ninjin'yoeito activated 4E-BP1, which is involved in protein synthesis, and inhibited the expression of atrogin-1 and LC3-II, which are involved in muscle proteolysis (8). The increase in muscle mass shown in this study may be attributed to the anorexia-reducing effect of Ninjin'yoeito, as well as the promotion of muscle protein synthesis. These findings suggest that Ninjin'yoeito may improve not only loss of appetite and weight loss, but also muscle weakness, which can significantly affect activity, when it is taken for some long periods of time.

A limitation of this study is that the possibility of measurement error due to sweating and salivation cannot be eliminated in the bioelectrical impedance analysis used to measure body composition. In order to confirm the findings of this study, it is necessary to conduct case-series studies with larger sample sizes and to establish measurement conditions.

CONCLUSION

There is a close relationship between dementia and physical frailty, such as weight loss and muscle weakness. Therefore, our hospital focuses not only on the administration of anti-dementia drugs in the treatment of dementia, but also on lifestyle-related diseases and nutritional management.

In this study, patients with dementia who complained of loss of appetite and weight loss were administered Ninjin'yoeito, Japanese herbal medicine (Kampo), which resulted in increases in body weight and muscle mass. Ninjin'yoeito was found to be a potentially effective treatment option for physical frailty in patients with dementia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the participant for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YM and IM conducted the study. YM wrote the manuscript.

REFERENCES

1. Kozaki K. Science and clinical findings in sarcopenia;2)Dementia and sarcopenia/frailty. *J Jpn Soc Int Med*. (2018) 107:1702–7.
2. Chong MS, Tay L, Lim WS, Ye R, Tan EK, Ding YY. Prospective longitudinal study of frailty transitions in a community-dwelling cohort of older adults with cognitive impairment. *BMC Geriatr*. (2015) 15:175–87. doi: 10.1186/s12877-015-0174-1
3. Takayama S, Arita R, Ohsawa M, Kikuchi A, Yasui H, Makino T et al. Perspectives on the use of ninjin'yoeito in modern medicine: a review of randomized controlled trials. *Evid Based Complement Alternat Med*. (2019) 2019:9590260. doi: 10.1155/2019/9590260
4. Iwasaki Y, Yada T. Mechanisms of regulation of feeding involving eating-related hormones transmitting information via the afferent vagus nerve. *Exp Med*. (2017) 35:917–25.
5. Goswami C, Dezaki K, Wang L, Inui A, Seino Y, Yada T. Ninjin-yoeito activates ghrelin-responsive and unresponsive NPY neurons in the arcuate nucleus and counteracts cisplatin-induced anorexia. *Neuropeptides*. (2019) 75:58–61. doi: 10.1016/j.npep.2019.03.001
6. Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K, et al. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. *Gastroenterology*. (2008) 134:2004–13. doi: 10.1053/j.gastro.2008.02.078
7. Sakisaka N, Mitani K, Sempuku S, Imai T, Takemoto Y, Shimomura H, et al. A clinical study of ninjin'yoeito with regard to frailty. *Front Nutr*. (2018) 5:73. doi: 10.3389/fnut.2018.00073
8. Takahashi R, Tiba S, Takemoto R, Michihara S, Han L, Fujita N. Effects of Ninjin'yoeito on survival times and senescent phenotype of mice. *Jpn J Psychosomat Intern Med*. (2018) 22:16–19.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 04 February 2021
doi: 10.3389/fnut.2021.525629

Ninjin'yoeito Alleviates Neuropathic Pain Induced by Chronic Constriction Injury in Rats

Risa Takemoto, Seiwa Michihara, Li-Kun Han, Nina Fujita and Ryuji Takahashi*

Kampo Research Laboratories, Kracie Pharma, Ltd., Toyama, Japan

Kampo medicines are frequently used empirically to treat pain in clinical practice. Ninjin'yoeito (NYT), which is associated with few adverse effects, is often used to treat the elderly, but has not yet been examined in detail. We herein investigated the effects of NYT, at 500 and 1,000 mg/kg p.o. (NYT500/NYT1000 group) in single and repeated administrations for 14 days, on pain in rats with peripheral neuropathy induced by loose ligation of the sciatic nerve (chronic constriction injury: CCI). Untreated CCI rats given distilled water were used as a control group. To assess induced pain, the pain threshold was measured using the von Frey test. To evaluate spontaneous pain, the ground-contact area of the paw with neuropathic pain was measured using the Dynamic Weight Bearing test. Serum samples were collected after the test to elucidate the mechanism of action of NYT, and brain-derived neurotrophic factor (BDNF) and corticosterone protein levels, which have been reported to change due to chronic pain, were analyzed. After single administration of NYT, the pain threshold rose in the NYT500 and NYT1000 groups. The pain threshold tended to rise on day 14 of repeated administration in the NYT500 group ($p = 0.08$) and it significantly rose at NYT1000 group ($p < 0.05$) compared to Control group. In addition, the foot contact area increased ($p = 0.09$). Therefore, CCI-induced pain was significantly remitted and spontaneous pain was remitted after repeated administration of NYT. Serum BDNF levels were higher in untreated CCI rats than in normal rats ($p = 0.05$), but decreased after the repeated administration of NYT (NYT1000, $p = 0.15$), while serum corticosterone levels were lower ($p = 0.12$) than those in normal rats and increased after the repeated administration of NYT (NYT1000, $p = 0.07$). The blood BDNF level has been suggested to influence pain intensity. The findings demonstrated NYT effectively treats neuropathic pain, suggesting that a NYT-induced decrease in blood BDNF contributed to the mechanism of pain relief. In addition, the variation of corticosterone was observed, suggesting that normalization of responsiveness to stress by NYT contributed to the pain relief.

Keywords: Ninjin'yoeito, CCI, neuropathic pain, corticosterone, BDNF, chronic pain

OPEN ACCESS

Edited by:
Masahiro Ohsawa,
Nagoya City University, Japan

Reviewed by:
Benoit Michot,
Harvard University, United States
Norikazu Kiguchi,
Wakayama Medical University, Japan

****Correspondence:***
Risa Takemoto
takemoto_risa@kracie.co.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *10 January 2020*
Accepted: *14 January 2021*
Published: *04 February 2021*

Citation:
Takemoto R, Michihara S, Han L-K, Fujita N and Takahashi R (2021) Ninjin'yoeito Alleviates Neuropathic Pain Induced by Chronic Constriction Injury in Rats. Front. Nutr. 8:525629. doi: 10.3389/fnut.2021.525629

INTRODUCTION

Chronic pain has been estimated to affect ∼25 million individuals in Japan (1), and has been divided into two categories: inflammatory and neuropathic pain (2). Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system. It reduces the quality of life of patients and is a frequently encountered condition that limits the doses and durations of treatments. A common treatment for neuropathic pain is antidepressant drugs and Ca^{2+} channel α2δ ligands; however, these are associated with adverse effects. In particular, these drugs must be administered with caution to mental disorder patients and the elderly.

Kampo medicines are frequently used empirically to treat pain in clinical practice. NYT is composed of Poria Sclerotium, Japanese Angelica Root, Rehmannia Root, Atractylodes Rhizome, Ginseng, Cinnamon Bark, Citrus Unshiu Peel, Polygala Root, Peony Root, Astragalus Root, Schisandra Fruit, and Glycyrrhiza (**Table 1**). NYT, which is associated with few adverse effects, is often used to treat the elderly. Mitsuhata reported the use of NYT and Yokkansan chimpi hange in pain treatments (3). Furthermore, NYT was shown to be effective for low back pain and lower abdominal pain accompanying anemia (4). In addition, it has recently been reported in a randomized controlled trial that NYT is useful to treat pain induced by an anticancer drug, oxaliplatin (5). In an animal study, both oxaliplatin-induced cold allodynia and mechanical hyperalgesia were markedly improved by NYT extract and a crude drug comprising NYT, Ginseng, suggesting that the active crude drug of NYT for pain is Ginseng. Furthermore, NYT extract, Ginseng, and Ginsenoside Rg3 inhibited oxaliplatin-induced suppression of neurite outgrowth of the primary dorsal root ganglion in a concentration-dependent manner in an *in vitro* system, identifying that ginsenoside Rg3 is one of the active ingredients (6). A rat model of chronic constriction injury (CCI) simulating symptoms of chronic nerve compression is a neuropathic pain model close to clinical cases (7, 8). It has been reported that liquiritin, an ingredient of licorice herb comprising NYT, ferulic acid, an ingredient of Angelica Sinensis Radix, and catalpol, an ingredient of Rehmanniae Radix, relieved neuropathic pain of CCI (9–11). However, the usefulness of NYT for neuropathic pain of CCI has not been investigated. Thus, in this study, we investigated whether NYT relieves CCI-induced neuropathic pain in rats.

TABLE 1 | Composition (daily dose*) of Kampo Formula Ninjin'yoeito (NYT).

Ingredient		Content (g)
English name	**Latin name**	
Poria sclerotium	*Poria*	4.0
Japanese angelica root	*Angelicae Radix*	4.0
Rehmannia root	*Rehmanniae Radix*	4.0
Atractylodes rhizome	*Atractylodis Rhizoma*	4.0
Ginseng	*Ginseng radix*	3.0
Cinnamon bark	*Cinnamomi cortex*	2.5
Citrus unshiu peel	*Aurantii Nobilis Pericarpium*	2.0
Polygala root	*Polygalae Radix*	2.0
Peony root	*Paeoniae Radix*	2.0
Astragalus root	*Astragali Radix*	1.5
Schisandra fruit	*Schisandrae Fructus*	1.0
Glycyrrhiza	*Glycyrrhizae Radix*	1.0

**Approximately 6,700 mg of dried water extract of NYT was prepared at the GMP-standardized factory of Kracie Pharma, Ltd. (Japan) based on the above described composition.*

MATERIALS AND METHODS

Animals

Male SD rats treated with CCI surgery in the left foot at 5 weeks of age were purchased at 6 weeks of age from Japan SLC (Shizuoka, Japan). The CCI model was prepared following the method created by Bennet et al. (12). Rats were anesthetized with isoflurane, the skin was incised along the gap of the left foot femoral muscle, and the sciatic nerve was exposed and loosely ligated with 4–0 silk at 4 sites at 1-mm intervals. Lepetan (buprenorphine) (Otsuka Pharmaceutical Co., Ltd., Japan) was subcutaneously administered twice as an analgesic after surgery and the following day. They were reared in an air-conditioned animal house facility (room temperature 23 ± 2°C; reversed 12-h light/dark cycle; relative humidity 55 ± 10%) at Kampo Research Laboratories in Kracie Pharma, Ltd. Rats were housed in a sterilized metal cage with a wire mesh floor and provided with laboratory pellet chow (CE-2; Clea Japan, Inc.) and water *ad libitum*. Before experimental procedures, they were acclimated to the room for 1 week. The experimental protocol was approved by the Experimental Animal Care Committee of Kracie Pharma, Ltd.

Drug Treatment

The dried extract powder of NYT (Lot No. 15112017) was used in the present study, and was manufactured by the GMP Pharmaceutical Factory of Kracie Pharma, Ltd. (Qingdao, China). The von Frey test was performed before NYT administration and animals with CCI which developed pain were selected (pain threshold: 6.0 g or lower). The selected CCI rats were divided into the following 3 groups: Control group, NYT 500 mg/kg treatment group (NYT500), and NYT 1,000 mg/kg treatment group (NYT1000), so as to make no significant difference in the pain threshold among the groups. Then, NYT was administered at 10 mL/kg B.W. once a day for 14 consecutive days starting on the 14th day post-surgery as follows: In the Control group, distilled water was orally administered. In the NYT500 and NYT1000 groups, NYT was suspended with distilled water before use and the specified dose was orally administered. The drug was administered by collaborators and efforts were made to ensure the experimenters were blinded in evaluation of the subsequent von Frey test and dynamic body weight bearing.

Von Frey Test for Mechanical Allodynia

Mechanical allodynia in rats was evaluated using the von Frey test. In the von Frey test, a series of calibrated von Frey

filaments (Touch-Test Sensory Evaluator, North Coast Medical, Inc., Morgan Hill, CA) with a bending force ranging between 1 and 15 g were applied to the midplantar skin of each hind paw at a rate of once per second. Specifically, the paw was stimulated 10 times at a speed of once per second in the order from the filament with the lowest strength and when escape behavior was noted once or more, the rat was judged as positive. Then, stimuli were added using the one-step weaker filament when the animal was positive and using the one-step stronger filament when the animal was negative. Stimulation was repeated until observing positive and negative responses to stimulation with 2 continuous filament types, respectively, and the filament strength to which a positive response was observed was recorded as the pain threshold. Fourteen days after the CCI surgery, the pain threshold of the left foot was measured before administration and on day 1 (single administration; 2 h after administration) and day 14 (repeated administration; the day following 13-day continuous administration) after initiation of administration. The pain threshold of the right foot without surgery before initiation of administration was recorded as the baseline.

Dynamic Weight Bearing for Spontaneous Pain

The dynamic weight bearing test (Bioseb, Pinellas Park, FL) was performed referring to the method reported by Quandros et al. (13). Briefly, the device was constituted with a small plexiglass chamber (22.0 × 22.0 × 30.0 cm) equipped with a floor sensor including a pressure transducer. In this system, software recording the mean weight loaded by each of the fore- and hind feet in grams without interference with an analyzer was used. To perform the test, the rat was placed in the chamber and allowed to freely move for 6 min. The device was used for acclimation for 1 min followed by recording for 5 min. To support data analysis, a camera was turned toward the side of the enclosure. All movements were photographed and investigated following the position of the rat on the device by the experimenters and the foot corresponding to the pixel set recognized by the sensor was identified as the right or left foot. DWB software provides data concerning the area (in mm^2) of the foot contacting the floor. The testis and tail were excluded from analysis. The results were presented as the area of the left foot. Fourteen days after the CCI surgery, measurement was performed before administration and on day 14 after initiation of administration (repeated administration) in each rat. The value measured in the right foot without surgery before initiation of administration was recorded as the baseline.

Serum Analyses

Blood samples were collected immediately following the sacrifice of each rat and clotted for 30 min at room temperature before centrifuging for 20 min at 2,000 rpm. The supernatant was

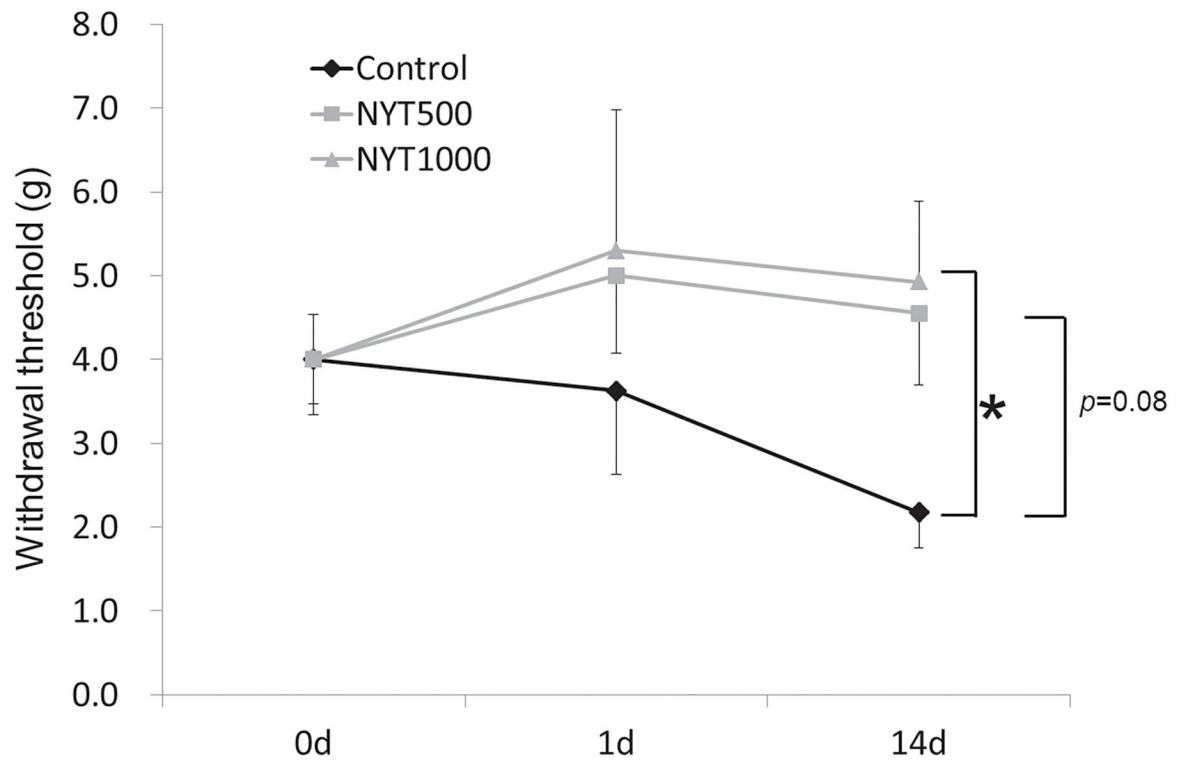

FIGURE 1 | Effects of NYT on CCI-induced mechanical allodynia. Effects of NYT on CCI-induced mechanical allodynia in rats. NYT at 500 and 1,000 mg/kg p.o. in single and repeated administrations for 14 days to rats. The black object group was administered water (Control); the gray object group was administered NYT (NYT500/NYT1000). Each column shows the mean ± S.E. of 8 mice.*$p < 0.05$ vs. the control group, evaluated using Dunnett's test.

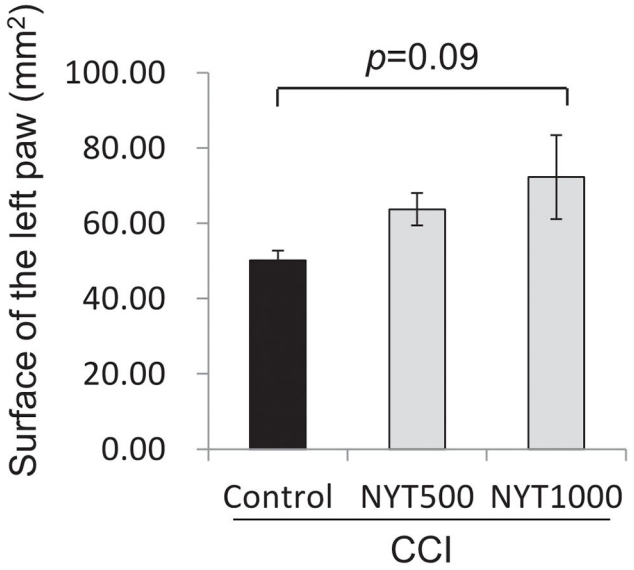

FIGURE 2 | Effects of NYT on CCI-induced spontaneous pain. Effects of NYT on CCI-induced spontaneous pain in rats. NYT at 500 and 1,000 mg/kg p.o. in repeated administrations for 14 days to rats. The black column group was administered water (Control); the gray column group was administered NYT (NYT500/NYT1000). Each column shows the mean ± S.E. of 4 mice. *p*-values evaluated using Dunnett's test.

then collected for an enzyme-linked immunosorbent assay (ELISA). Serum was stored at −40°C until assayed for brain-derived neurotrophic factor (BDNF) and corticosterone. Serum BDNF (BDNF/proBDNF Rapid ELISA Kit; BIOSENSYS) and serum corticosterone [corticosterone (Human, Rat, Mouse) ELISA (RE52211); IBL] were measured by ELISA following the manufacturer's instructions. Normal rats given distilled water were used as a Normal group.

Statistical Analysis

Data were expressed as the mean ± standard error of means. Significant differences were assessed by a one-way analysis of variance followed by Dunnett's test, or *post hoc* test followed by Steel test for multiple comparisons. Between normal group and control group were compared by Student's *t*-test. $P < 0.05$ were considered to be significant.

RESULTS

Effects of Single Administration of NYT on CCI-Induced Mechanical Allodynia

After single administration of NYT, the pain threshold rose in the NYT500 and NYT1000 groups (**Figure 1**) (Baseline: 9.1 g). Although the elevation was not significant, it was suggested that NYT exhibits an effect on pain, so administration was continued and the effect of NYT on pain by repeated administration was investigated.

Effects of Repeated Administration of NYT on CCI-Induced Mechanical Allodynia and Spontaneous Pain

The pain threshold tended to rise on day 14 of repeated administration in the NYT500 group ($p = 0.08$, by dunnet) and it significantly rose at NYT1000 ($p < 0.05$, Dunnett's test; **Figure 1**). In addition, the foot contact area increased ($p = 0.09$, Dunnett's test; **Figure 2**) (baseline: 78.52 mm2). Therefore, CCI-induced pain was significantly remitted and spontaneous pain was remitted after repeated administration of NYT.

Effects of NYT on BDNF Protein Expression in Serum

BDNF is an important marker and modulator of neural activity and N-methyl-D-aspartate receptor-dependent neuronal plasticity in ascending and descending pain transmission pathways (14). In animals, BDNF and its tropomyosin receptor kinase B receptor were shown to be increased in models of bladder inflammation and nerve injury (15, 16). Furthermore, when BDNF was neutralized by the anti-BDNF antibody or tropomyosin receptor kinase B receptor, mechanical allodynia (17) and thermal hyperalgesia were alleviated (18). Based on these findings, it was suggested that BDNF contributes to pain sensitivity and intensity. In a clinical study, the serum BDNF level was significantly higher in patients with pain of endometriosis than in patients without pain (19). Using serum BDNF, we investigated whether BDNF is involved in the action mechanism of this study. Serum BDNF levels were higher in untreated CCI rats than in normal rats ($p = 0.05$, by Student's *t*-test), but decreased after the repeated administration of NYT (1,000 mg/kg, $p = 0.15$, by Dunnett's test) (**Figure 3**).

Effects of NYT on Corticosterone Protein Expression in Serum

Glucocorticoid hormones are regarded as "stress hormones" because they typically increase in response to environmental challenges (20–23). Corticosterone, the primary glucocorticoid in some vertebrates, is released following the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Stress negatively affects hippocampal neurogenesis and plasticity through the activation of the HPA axis, resulting in the increased production of corticosterone and development of depressive-like symptoms (24). Since pain may activate the HPA axis (25, 26), we investigated whether our results were a consequence of the activation of the HPA axis in response to chronic pain as a stressful stimulus, and measured corticosterone serum levels in normal rats and CCI rats. In untreated CCI rats, serum corticosterone levels were lower ($p = 0.12$, by Student'*t*-test) than those in normal rats, and increased after the repeated administration of NYT (1,000 mg/kg, $p = 0.07$, by Steel test) (**Figure 4**).

DISCUSSION

We herein evaluated the efficacy of NYT to prevent neuropathic pain in CCI rats. Mechanical allodynia and spontaneous pain

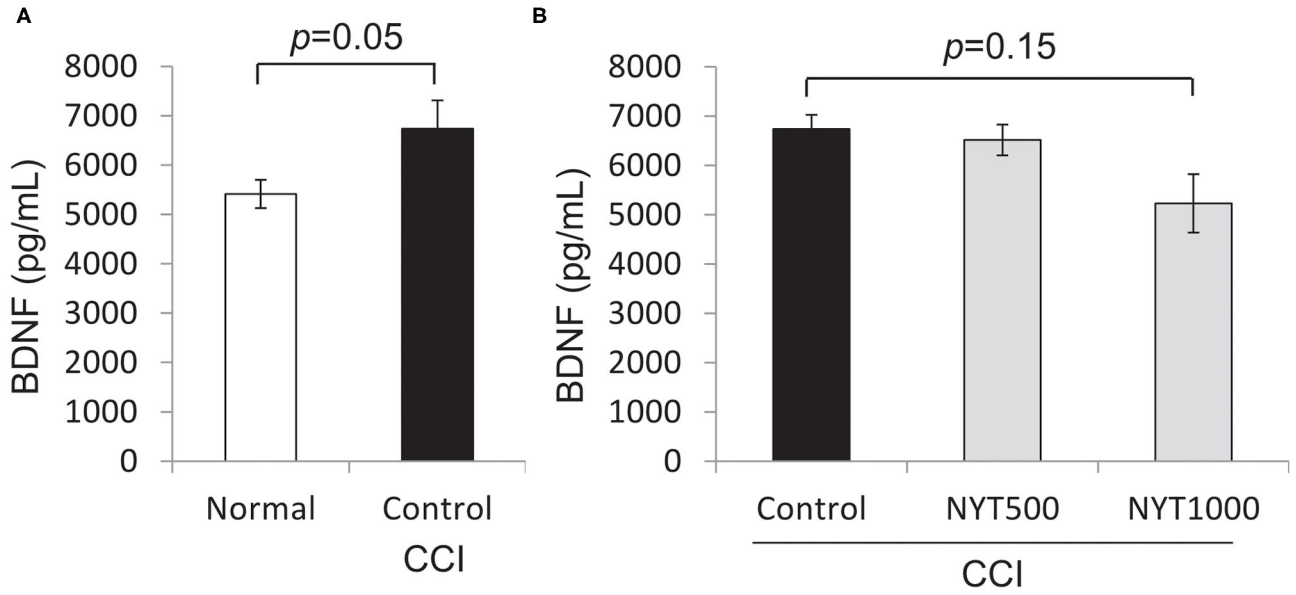

FIGURE 3 | Effects of NYT on BDNF protein expression in serum. BDNF protein levels in serum. The black column group was administered water (Control); the gray column group was administered NYT (NYT500/NYT1000). The white column group comprised normal rats (Normal). **(A)** Normal vs CCI. **(B)** CCI vs NYT. Each column shows the mean ± S.E. of 7–8 mice. *p*-values evaluated using *t*-test and Dunnett's test.

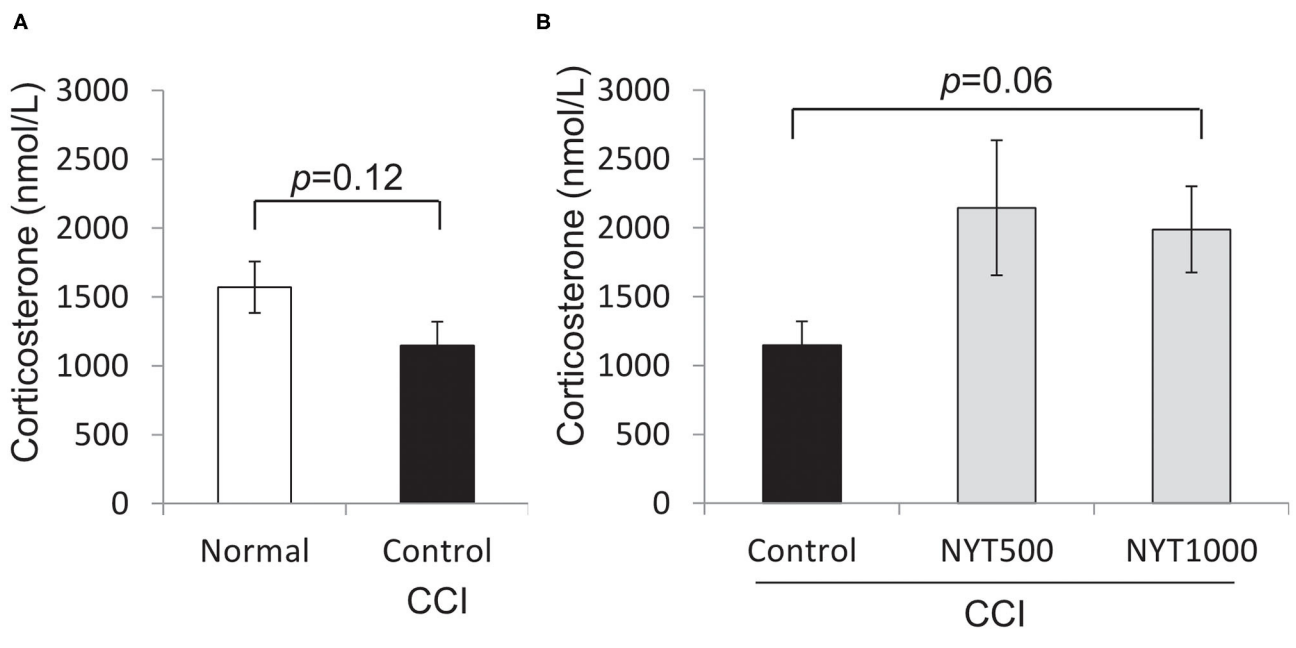

FIGURE 4 | Effects of NYT on corticosterone protein expression in serum. Corticosterone protein levels in serum. The black column group was administered water (Control); the gray column group was administered NYT (NYT500/NYT1000). The white column group comprised normal rats (Normal). **(A)** Normal vs CCI. **(B)** CCI vs NYT. Each column shows the mean ± S.E. of 8 mice. *p*-values evaluated using *t*-test and Steel test.

were significantly induced by CCI surgery and inhibited by NYT. These results are consistent with clinical findings. In addition, repeated administration of NYT decreased blood BDNF whereas blood corticosterone increased.

This study was focused on BDNF, which has been reported as a pain-related factor. A positive correlation between the blood and brain-tissue BDNF levels in rats has been reported (27), suggesting that the blood BDNF level reflects the brain-tissue BDNF level. In our study, the blood BDNF was measured expecting that the blood BDNF level reflects the brain-tissue BDNF level. In a preceding study, the BDNF level after CCI increased by 45% in the thalamus and by 27% in the midbrain (*p*

> 0.05) (28). In addition, it has been reported that the BDNF level in CCI markedly increased in the periaqueductal gray matter (PAG), which is the important pain control center on the spinal cord (29), and the thalamic nucleus is decisively involved in down regulation of harmful mechanical and heat-inducing reactions (30). These reports concerning an increase in cerebral BDNF in CCI and the increase in serum BDNF in CCI in this study were consistent, suggesting that an NYT-induced decrease in the BDNF level is a part of the mechanism of the analgesic effect. On the other hand, the mechanism in the CCI model is analyzed mainly in the spinal cord and BDNF expression in the spinal cord is very important. However, the correlation between spinal cord BDNF and serum BDNF is unclear and investigation of BDNF expression in the spinal cord and upstream factors is necessary to more closely analyze the mechanism.

When the serum corticosterone level was measured in rats influenced by chronic pain, it was lower than that in normal rats. It has been reported that in an SD rat continuous stress model, a significant increase was observed on day 1, the level gradually decreased from day 2, and no significant difference from the level in the control was noted on day 5 (31), suggesting that the corticosterone level rises in response to acute stress, but responsiveness to stress worsens through subsequently becoming chronic. Our study demonstrated that NYT elevated the corticosterone level. In a study on the levels of the ingredients of NYT, intraperitoneal administration of Ginseng saponin significantly increased plasma corticosterone in normal rats, but Ginsenoside Rg1 decreased an increased serum corticosterone level in a depression model in another study (useful for behavior of depression). Based on the ingredient levels, NYT may influence the blood corticosterone level in the normal body, but considering the results of the depression model described above and our model, it is also likely to return an increased/decreased serum corticosterone to the normal state. In addition, NYT and a component crude drug, onji (polygala root), has an antidepressant effect (32, 33). Chenpi (Citrus Unshiu Peel) has been demonstrated to exhibit an effect on serotonin in the nervous system (34). Our study suggested that normalization of responsiveness to stress leads to the analgesic effect. Stress responsiveness and the mechanism of pain relief are interesting and remain as issues in the future.

Multiple mechanisms resulting from sciatic nerve injury have demonstrated that demyelination, ectopic discharge, and macrophage infiltration are closely associated with the development of neuropathic pain behaviors (35, 36). Myelinated A fibers at the distal CCI stumps of the sciatic nerve undergoing nerve demyelination increase ectopic discharge, which is regarded as an injury-induced electrophysiological characteristic (37, 38). On the other hand, a crude drug composing NYT, Citrus Unshiu Peel, has been reported to influence demyelination in the mouse brain (39), suggesting that NYT relives pain only through myelination. In the future, We want to investigate the mechanisms of these.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by the Experimental Animal Care Committee of Kracie Pharma, Ltd. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

RiT, SM, L-KH, NF, and RyT performed the experiments and analyzed the data. SM, NF, and RyT initiated and supervised the study. RiT, SM, NF, and L-KH designed experiments. RiT and L-KH wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Yabuki S, Ushida T, Takesita K, Saura R, Ogawa F, Katsumata A, et al. A nationwide survey of chronic pain sufferers in Japan. *Clin Orthopaedic Surg.* (2012) 47:127–34. doi: 10.11477/mf.1408102249
2. St John Smith E. Advances in understanding nociception and neuropathic pain. *J Neurol.* (2018) 265:231–8. doi: 10.1007/s00415-017-8641-6
3. Mitsuhata H. Kampo treatment for pain in the elderly. In: Morimoto S, editor. *Jpn J Geriatr. 2016: Proceedings of the 58th Jpn J Geriatr, Supplement 53.* Kanazawa (2016). p. 73.
4. Ando N. Blood making effect of ninjin-yoei-to (Ren-shen-yang-rong-tang) as monotherapy in obstetric and gynecologic patients with anemia. *Jpn J Orient Med.* (1999) 50:461–70. doi: 10.3937/kampomed.50.461
5. Motoo Y, Tomita Y, Fujita H. Prophylactic efficacy of ninjin'yoeito for oxaliplatin-induced cumulative peripheral neuropathy in patients with colorectal cancer receiving postoperative adjuvant chemotherapy: a randomized, open-label, phase 2 trial (HOPE-2). *Int J Clin Oncol.* (2020) 25:1123–9. doi: 10.1007/s10147-020-01648-3
6. Suzuki T, Yamamoto A, Ohsawa M, Motoo Y, Mizukami H, Makino T. Effect of ninjin'yoeito and ginseng extracts on oxaliplatin-induced neuropathies in mice. *J Nat Med.* (2017) 71:757–64. doi: 10.1007/s11418-017-1113-6
7. Sumizono M, Sakakima H, Otsuka S, Terashi T, Nakanishi K, Ueda K, et al. The effect of exercise frequency on neuropathic pain and pain-related cellular reactions in the spinal cord and midbrain in a rat sciatic nerve injury model. *J Pain Res.* (2018) 11:281–91. doi: 10.2147/JPR.S156326
8. Da Silva JT, Evangelista BG, Venega RAG, Oliveira ME, Chacur M. Early and late behavioral changes in sciatic nerve injury may be modulated by nerve growth factor and substance P in rats: a chronic constriction injury long-term evaluation. *J Biol Regul Homeost Agents.* (2017) 31:309–19.
9. Zhang MT, Wang B, Jia YN, Liu N, Ma PS, Gong SS, et al. Neuroprotective effect of liquiritin against neuropathic pain induced by chronic constriction injury of the sciatic nerve in mice. *Biomed Pharmacother.* (2017) 35:186–98. doi: 10.1016/j.biopha.2017.07.167
10. Xu Y, Lin D, Yu X, Xie X, Wang L, Lian L, et al. The antinociceptive effects of ferulic acid on neuropathic pain: involvement of descending monoaminergic system and opioid receptors. *Oncotarget.* (2016) 7:20455–68. doi: 10.18632/oncotarget.7973
11. Wang Y, Zhang R, Xie J, Lu J, Yue Z. Analgesic activity of catalpol in rodent models of neuropathic pain, and its spinal mechanism. *Cell Biochem Biophys.* (2014) 70:1565–71. doi: 10.1007/s12013-014-0096-0

12. Bennet GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain.* (1988) 33:87–107. doi: 10.1016/0304-3959(88)90209-6
13. Quandros AU, Pinto LG, Fonseca MM, Kusuda R, Cunha FQ, Cunha TM. Dynamic weight bearing is an efficient and predictable method for evaluation of arthritic nociception and its pathophysiological mechanisms in mice. *Sci Rep.* (2015) 5:14689 doi: 10.1038/srep14648
14. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. *Neuron.* (2004) 44:5–21. doi: 10.1016/j.neuron.2004.09.012
15. Oddiah D, Anand P, McMahon SB, Rattray M. Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. *Neuroreport.* (1998) 9:1455–8. doi: 10.1097/00001756-199805110-00038
16. Suter MR, Wen YR, Decosterd I, Ji RR. Do glial cells control pain? *Neuron Glia Biol.* (2007) 3:255–68. doi: 10.1017/S1740925X08000100
17. Zhou XF, Deng YS, Xian CJ, Zhong LH. Neurotrophins from dorsal root ganglia trigger allodynia after spinal nerve injury in rats. *Eur J Neurosci.* (2000) 12:100–5. doi: 10.1046/j.1460-9568.2000.00884.x
18. Fukuoka T, Kondo E, Dai Y, Hashimoto N, Noguchi K. Brain-derived neurotrophic factor increases in the uninjured dorsal root ganglion neurons in selective spinal nerve ligation model. *J Neurosci.* (2001) 21:4891–900. doi: 10.1523/JNEUROSCI.21-13-04891.2001
19. Ding S, Zhu T, Tian Y, Xu P, Chen Z, Huang X, et al. Role of brain-derived neurotrophic factor in endometriosis pain. *Reprod Sci.* (2018) 25:1045–57. doi: 10.1177/1933719117732161
20. Bonier F, Martin PR, Moore IT, Wingfield JC. Do baseline glucocorticoids predict fitness? *Trends Ecol Evol.* (2009) 24:634–42. doi: 10.1016/j.tree.2009.04.013
21. Constantini D, Marasco V, Møller AP. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J Comp Physiol B.* (2011) 181:447–56. doi: 10.1007/s00360-011-0566-2
22. Pankhurst NW. The endocrinology of stress in fish: an environmental perspective. *Gen Comp Endocrinol.* (2011) 170:265–75. doi: 10.1016/j.ygcen.2010.07.017
23. Wingfield JC. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Funct Ecol.* (2013) 27:37–44. doi: 10.1111/1365-2435.12039
24. Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc Natl Acad Sci USA.* (1998) 95:3168–71. doi: 10.1073/pnas.95.6.3168
25. Aloisi AM, Albonetti ME, Muscettola M, Facchinetti F, Tanganelli C, Carli G. Effects of formalin-induced pain on ACTH, beta-endorphin, corticosterone and interleukin-6 plasma levels in rats. *Neuroendocrinology.* (1995) 62:13–8. doi: 10.1159/00012 6983
26. Harbuz MS, Lightman SL. Stress and the hypothalamopituitary-adrenal axis: acute, chronic and immunological activation. *J Endocrinol.* (1992) 134:327–39. doi: 10.1677/joe.0.1340327
27. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol.* (2010) 14:347–53. doi: 10.1017/S1461145710000738
28. Gardini AC, Dos Santos FM, da Silva JT, de Oliveira ME, Martins DO, Chacur M. Neural mobilization treatment decreases glial cells and brain-derived neurotrophic factor expression in the central nervous system in rats with neuropathic pain induced by CCI in rats. *Pain Res Manag.* (2017) 2017:7429761. doi: 10.1155/2017/7429761
29. Heinricher MM, Tavares I, Leith JL Lumb, BM. Descending control of nociception: specificity, recruitment and plasticity. *Brain Res Rev.* (2009) 60:214–25. doi: 10.1016/j.brainresrev.2008.12.009
30. Wilson AR, Maher L, Morgan MM. Repeated cannabinoid injections into the rat periaqueductal gray enhance subsequent morphine antinociception. *Neuropharmacology.* (2008) 55:1219–25. doi: 10.1016/j.neuropharm.2008.07.038
31. Ogawa T, Sei H, Konishi H, Shishioh-Ikejima N, Kiyama H. The absence of somatotroph proliferation during continuous stress is a result of the lack of extracellular signal-regulated kinase 1/2 activation. *J Neuroendocrinol.* (2012) 24:1335–45. doi: 10.1111/j.1365-2826.2012.02338.x
32. Murata K, Fujita N, Takahashi R, Inui, A. Ninjinyoeito improves behavioral abnormalities and hippocampal neurogenesis in the corticosterone model of depression. *Front Pharmacol.* (2018) 9:1216. doi: 10.3389/fphar.2018.01216
33. Hu Y, Liu M, Liu P, Guo DH, Wei RB, Rahman KY. Possible mechanism of the antidepressant effect of 3,6'-disinapoyl sucrose from polygala tenuifolia willd. *J Pharm Pharmacol.* (2011) 63:869–74. doi: 10.1111/j.2042-7158.2011.01281.x
34. Ito A, Shin N, Tsuchida T, Okubo T, Norimoto H. Antianxiety-like effects of chimpi (dried citrus peels) in the elevated open-platform test. *Molecules.* (2013) 18:10014–23. doi: 10.3390/molecules180810014
35. Gómez-Nicola D, Valle-Argos B, Suardíaz M, Taylor JS, Nieto-Sampedro M. Role of IL-15 in spinal cord and sciatic nerve after chronic constriction injury: regulation of macrophage and T-cell infiltration. *J Neurochem.* (2008) 107:1741–52. doi: 10.1111/j.1471-4159.2008.05746.x
36. Gould HJ III, Soignier RD, Cho SR, Hernandez C, Diamond I, Taylor BK, et al. Ranolazine attenuates mechanical allodynia associated with demyelination injury. *Pain Med.* (2014) 15:1771–80. doi: 10.1111/pme.12516
37. Gabay E, Tal M. Pain behavior and nerve electrophysiology in the CCI model of neuropathic pain. *Pain.* (2004) 110:354–60. doi: 10.1016/j.pain.2004.04.021
38. Wagner R, Heckman HM, Myers RR. Wallerian degeneration and hyperalgesia after peripheral nerve injury are glutathione-dependent. *Pain.* (1998) 77:173–9. doi: 10.1016/S0304-3959(98)00091-8
39. Sato N, Seiwa C, Uruse M, Yamamoto M, Tanaka K, Kawakita T, et al. Administration of chinpi, a component of the herbal medicine ninjin-youei-to, reverses age-induced demyelination. *Evid Based Complement Alternat Med.* (2011) 2011:617438. doi: 10.1093/ecam/neq001

Conflict of Interest: All authors are employees of Kracie Pharma, Ltd.

frontiers in Nutrition

CASE REPORT
published: 14 April 2021
doi: 10.3389/fnut.2021.547512

Case Report: Ninjin'yoeito May Improve Quality of Life After Hospitalization for Acute Illness in Patients With Frailty

*Masayuki Kashima**

Department of General Internal Medicine, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan

Introduction: Frail patients are susceptible to a large number of diseases, and frailty particularly is known to develop after acute illness. No conventional drugs are known to prevent such exacerbation after acute illness. However, traditional Japanese medicine, Kampo, is thought to confer efficacious energy and nutritional supplements and serve to improve malaise after acute illness. Ninjin'yoeito is a representative Kampo medicine for such situation.

Cases: We describe three frail patients hospitalized for acute illness who started taking ninjin'yoeito at the time of discharge.

Case 1: An 87-year-old man admitted with acute prostatitis complicated by hypertension and chronic obstructive pulmonary disease (COPD). His 36-Item Short Form Health Survey (SF-36) score, which is representative of total quality of life and comprises eight components, showed consistent improvements after 4 and 12 weeks of ninjin'yoeito administration, especially for body pain (BP; scores from 41 to 51 and 100, respectively), social function (SF; 50, 100, 100), and mental health (MH; 75, 75, 90).

Case 2: A 65-year-old man admitted with urinary tract infection complicated by primary sclerosing cholangitis and COPD. All SF-36 component scores showed improvement 12 weeks later: physical function (PF; 70–95), role physical (RP; 75–100), BP (72–84), general health (GH; 45–52), vitality (VT; 37.5–75), SF 75–100, role emotional (RE; 75–100), and MH (70–90).

Case 3: An 80-year-old man admitted for pneumonia complicated with hypertension. SF-36 score was improved 4 weeks later for RP (68.8–100), BP (52–61), GH (52–72), VT (43.8–62.5), SF (37.5–100), and RE (58.3–91.7).

Conclusion: Patients with frailty often have a worsened SF-36 score after discharge following acute illness, but the score may be improved by taking ninjin'yoeito.

Keywords: ninjin'yoeito, quality of life, acute illness, frailty, hospitalization

OPEN ACCESS

Edited by:
Nada Rotovnik Kozjek,
Institute of Oncology
Ljubljana, Slovenia

Reviewed by:
Keiko Mamiya,
Shinshu University, Japan
Akio Inui,
Kagoshima University, Japan

***Correspondence:**
Masayuki Kashima
huggi0727@gmail.com

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *28 April 2020*
Accepted: *26 February 2021*
Published: *14 April 2021*

Citation:
Kashima M (2021) Case Report: Ninjin'yoeito May Improve Quality of Life After Hospitalization for Acute Illness in Patients With Frailty. Front. Nutr. 8:547512. doi: 10.3389/fnut.2021.547512

INTRODUCTION

Frailty has been attracting increasing attention in today's aging society. It is associated with a need for nursing care, and preventing exacerbation of frailty is crucial. Frail patients are known to be susceptible to a large number of diseases, and frailty particularly is known to develop after acute illness (1, 2). No conventional drugs are known to prevent exacerbation of frailty after acute illness. Currently, only basic interventions such as nutrition therapy and rehabilitation are available to prevent exacerbation of frailty. Traditional Japanese medicine, known as Kampo, is thought to be efficacious as supplements for qi and the blood; qi can be considered to mean vital energy or life energy, and the blood the substance that supplies nutrition. Frailty is considered a state of deficiency of both qi and the blood. The experience of severe disease or trauma exhausts the qi and the blood, leading to frailty.

Drugs that have the effect of supplementing qi and the blood have been used traditionally to prevent or improve the medical condition known as frailty. Ninjin'yoeito, which consists of 12 types of crude drugs and whose basic composition was first described in the thirteenth century, is representative of Kampo medicines and is a supplement for qi and the blood. Some studies have shown that ninjin'yoeito can be effective in improving some frail conditions (3–5). Currently, the Japanese national medical insurance system has approved Kracie ninjin'yoeito extract granules (Kampo Research Laboratory, Kracie Pharma Ltd., Takaoka, Japan) for its efficacy in improving strength after illness. However, few scientific studies have demonstrated this efficacy.

Decreased strength is a part of frailty, yet it is difficult to measure. Attempts have been made to measure it using various indicators, such as quality of life (QOL), nutrition, and muscle strength. The 36-Item Short Form Health Survey (SF-36) is a 36-item, patient-reported survey of patient health. The SF-36 is a measure of health status, and an abbreviated variant of it and one of several established scoring systems for health-related QOL and its reliability and validity have been confirmed. The instrument consists of eight components: physical function (PF), role physical (RP), body pain (BP), general health (GH), vitality (VT), social function (SF), role emotion (RE), and mental health (MH), which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0–100 scale on the assumption that each question carries equal weight. The lower the score means the more disability. The higher the score means the less disability (6–8). Thigh circumference is related to whole body muscle mass and activities of daily living in the elderly (9). Grip strength is known to correlate with overall muscle strength and with mortality (10). Serum albumin level and the Prognostic Nutritional Index [PNI; serum albumin level (mg/dl $\times$ 10 + peripheral lymphocyte counts/mm^3) $\times$ 0.005] are known to correlate with nutritional status and prognosis (11–13).

This report describes three frail patients for whom supplementation with Kracie ninjin'yoeito improved frailty after acute illness, as evaluated using the SF-36, grip strength, serum albumin level, and PNI. The patients were provided with sufficient information and consent regarding the study in writing.

CASES

The three patients met the criteria for frailty of elderly age, low grip strength (dominant hand grip strength $<$26 kg in men and $<$18 kg in women), and low activity (no light exercise once a week) (14).

Case 1

An 87-year-old man was admitted to our hospital for acute prostatitis. He was treated with antibiotics and hospitalized for 8 days. His condition was complicated by comorbid hypertension, chronic obstructive pulmonary disease (COPD), and mild dementia.

His body length was 168 cm, and at the time of discharge, his body weight was 53.4 kg, body mass index (BMI) was 18.9, serum albumin level was 2.9 g/dl, and PNI was 38.34. He was started on Kracie ninjin'yoeito 7.5 g/day from the day of discharge. Grip strength, femoral circumference, SF-36 score, serum albumin level, and PNI were evaluated at discharge and 4 and 12 weeks later. Body weight increased to 51.8 kg at 4 weeks and 52.6 kg at 12 weeks after discharge. As shown in **Table 2**, grip strength in his dominant right hand increased from 24 kg at discharge to 24.5 kg at 4 weeks after discharge and 27 kg at 12 weeks after discharge. Right thigh circumference increased from 35 cm at discharge to 36 cm and then 38 cm at 4 and 12 weeks, respectively. SF-36 scores at discharge and after 4 and 12 weeks are shown in **Table 1**: PF 65.0, 75.0, and 50.0; RP 68.8, 68.8, and 56.3; BP 41.0, 51.0, and 100; GH 67.0, 77.0, and 62.0; VT 87.5, 87.5, and 81.3; SF 50.0, 100.0, and 100.0; RE 58.3, 66.7, and 58.3; and MH 75.0, 75.0, and 90.0. Serum albumin level was 4.0 g/dl at both 4 and 12 weeks, and PNI was 49.98 and 50.16, respectively.

Case 2

A 65-year-old man was admitted to our hospital for urinary tract infection. He was treated with antibiotics and hospitalized for 7 days. His condition was complicated by primary sclerosing cholangitis, COPD, and diabetes mellitus.

His body length was 173.4 cm, body weight was 47.8 kg, BMI was 15.9, serum albumin level was 3.0 g/dl, and PNI was 34.94 at discharge. He was started on Kracie ninjin'yoeito 7.5 g/day from the day of discharge. Grip strength, femoral circumference, SF-36 score, serum albumin level, and PNI were evaluated at discharge and 12 weeks later. Body weight increased to 50.0 kg at 12 weeks after discharge. As shown in **Table 2**, grip strength in his dominant right hand improved from 28 kg at discharge to 31 kg at 12 weeks. Right thigh circumference increased from 27 to 33 cm. SF-36 scores at discharge and 12 weeks are shown in **Table 1**: PF 70.0 and 95.0; RP 75.0 and 100.0, BP 72.0 and 84.0, GH 45.0 and 52.0, VT 37.5 and 75.0, SF 75.0 and 100.0, RE 75.0 and 100.0, and MH 70.0 and 90.0. Serum albumin level was 3.6 g/dl, and PNI was 40.94 at 12 weeks after discharge.

TABLE 1 | 36-item Short Form Health Survey component scores in 3 frail elderly patients who received ninjin'yoeito from the time of discharge after acute illness.

	Case	Discharge	4 weeks	12 weeks
PF	1	65	75	50
	2	70	–	95
	3	70	40	–
	Average	68.3	57.5	72.5
RP	1	68.8	68.8	56.3
	2	75	–	100
	3	68.8	100	–
	Average	70.9	84.4	78.2
BP	1	41	51	100
	2	72	–	84
	3	52	61	–
	Average	55	56	92
GH	1	67	77	62
	2	45	–	52
	3	52	72	–
	Average	54.7	74.5	57
VT	1	87.5	87.5	81.3
	2	37.5	–	75
	3	43.8	62.5	–
	Average	56.3	75	78.2
SF	1	50	100	100
	2	75	–	100
	3	37.5	100	–
	Average	54.2	100	100
RE	1	58.3	66.7	58.3
	2	75	–	100
	3	58.3	91.7	–
	Average	63.9	79.2	79.2
MH	1	75	75	100
	2	70	–	90
	3	95	85	–
	Average	80	80	95

PF, physical function; RP, role physical; BP, body pain; GH, general health; VT, vitality SF, social function; RE, role emotion; MH, mental health.

Case 3

An 80-year-old man was admitted to our hospital for community-acquired pneumonia. He was treated with antibiotics and hospitalized for 8 days. His condition was complicated by diabetes mellitus and hypertension.

His body length was 158 cm, body weight was 63.7 kg, BMI was 25.5, serum albumin level was 2.6 g/dl, and PNI was 32.8 at discharge. He was started on Kracie ninjin'yoeito 7.5 g/day from the day of discharge. His grip strength, femoral circumference, SF-36 score, serum albumin level, and PNI were evaluated at discharge and 4 weeks later. Body weight increased to 65 kg at 4 weeks after discharge. As shown in **Table 2**, grip strength in his dominant right hand increased from 17 kg at discharge to 20 kg at 4 weeks after discharge. Right thigh circumference increased from 44 cm at discharge to 45.7 cm at 4 weeks after discharge. Corresponding SF-36 scores are shown in **Table 1**: PF 70.0 and 40.0, RP 68.8 and 100.0, BP 52.0 and 61.0, GH 52.0 and 72.0, VT 43.8 and 62.5, SF 37.5 and 100.0, RE 58.3 and 91.7, and MH 95.0 and 85.0. Serum albumin level was 4.3 g/dl, and PNI was 54.95 at 4 weeks after discharge.

Overall, the average changes in the component SF-36 scores from discharge in these three cases were as follows: PF −10.00 at 4 weeks and +5.00 at 12 weeks after discharge, RP +15.60 and +6.25, BP +9.50 and +35.5, GH +15.00 and +1.00, VT +9.35 and +15.65, SF +56.25 and 37.50, RE +20.90 and +12.50, and MH −5.00 and +22.50 (**Figure 1**) (1, 2).

The average change in grip strength at discharge was +1.75 kg at 4 weeks and +3 kg at 12 weeks after discharge. The corresponding change in femoral circumference was +1.35 and 4.5 cm, in serum albumin level was +1.4 and +0.85 mg/dl, and in PNI was +16.89 and +8.88 (**Figure 2**) (1, 2).

DISCUSSION

This report has described our experience treating three frail patients with ninjin'yoeito after being discharged after acute illness and the beneficial effects it showed. We also have experience with five other frail patients (age, 66–90 years) who were discharged with acute illness who were not placed on ninjin'yoeito and were followed up for measurements of SF-36 score, grip strength, serum albumin, and PNI at discharge and for prognostic indices on an outpatient basis. The average change in SF-36 scores for these five patients from discharge to 4 and 12 weeks after discharge, respectively, were as follows: −13.75 and +5.00 for PF, −4.7 and +18.75 for RP, −29.25 and −8.75 for BP, −20.5 and −9.5 for GH, +4.67 and +14.05 for VT, −12.50 and −12.50 for SF, −41.70 and −8.32 for RE, and +1.25 and +10.00 for MH (**Figure 1**) (1, 2). The average grip strength was 13.30 kg at discharge, with the average change in grip strength +2.50 kg at 4 weeks and +2.16 kg at 12 weeks. The corresponding average change in femoral circumference was −0.70 and −0.43 cm, in serum albumin level from discharge was +0.70 and +0.82 mg/dl, and the average change in PNI was +9.07 and +12.51 (**Figure 2**) (1, 2). Comparing all three patients who received ninjin'yoeito treatment with the five patients who did not receive it showed that the SF-36 scores for GH, BP, SF, and RE seem to have improved markedly, and scores for RP and MH showed a slight improvement in the three patients who received ninjin'yoeito. Scores for PF and VT were almost the same in the two groups. MH, SF, and RE reflect psychosocial status. Pain is known to be related to psychological factors. Therefore, BP may have improved due to the psychological effect of ninjin'yoeito. In both groups, PH tended to decline after discharge (**Figure 1**) (1, 2). It is, however, possible that their scores at discharge were higher than their actual scores because baseline scores were measured during hospitalization, when exercise was restricted compared with daily life, and it was not possible to accurately define their level of activity. Also, activity may have decreased due to progression

TABLE 2 | PNI in our 3 elderly patients taking ninjin'yoeito after discharge.

		Case 1	Case 2	Case 3	Average	Percent average change
Body mass index		18.9	15.9	25.5	20.1	–
Grip strength (kg)	Discharge	24	28	17	23.0	–
	4 weeks	24.5	–	20	22.3	+9.86%
	12 weeks	27	31	–	29.0	+11.60%
Femoral circumference(cm)	Discharge	35	27	44		–
	4 weeks	36	–	45.7		+3.35%
	12 weeks	38	33	–		+15.39%
Serum albumin (mg/dL)	Discharge	2.9	3.0	2.6		–
	4 weeks	4.0	–	4.3		+51.65%
	12 weeks	4.0	3.6	–		+38.97%
PNI	Discharge	38.34	34.94	32.8		–
	4 weeks	49.98	–	54.95		+48.94%
	12 weeks	50.16	40.94	–		+23.99%

Grip strength: measured in dominant hand.
Femoral circumference: measured in the leg of the dominant hand side.
PNI: Prognostic Nutritional Index; serum albumin level (mg/dL) × 10+ peripheral lymphocyte counts (/mm^3) × 0.005.

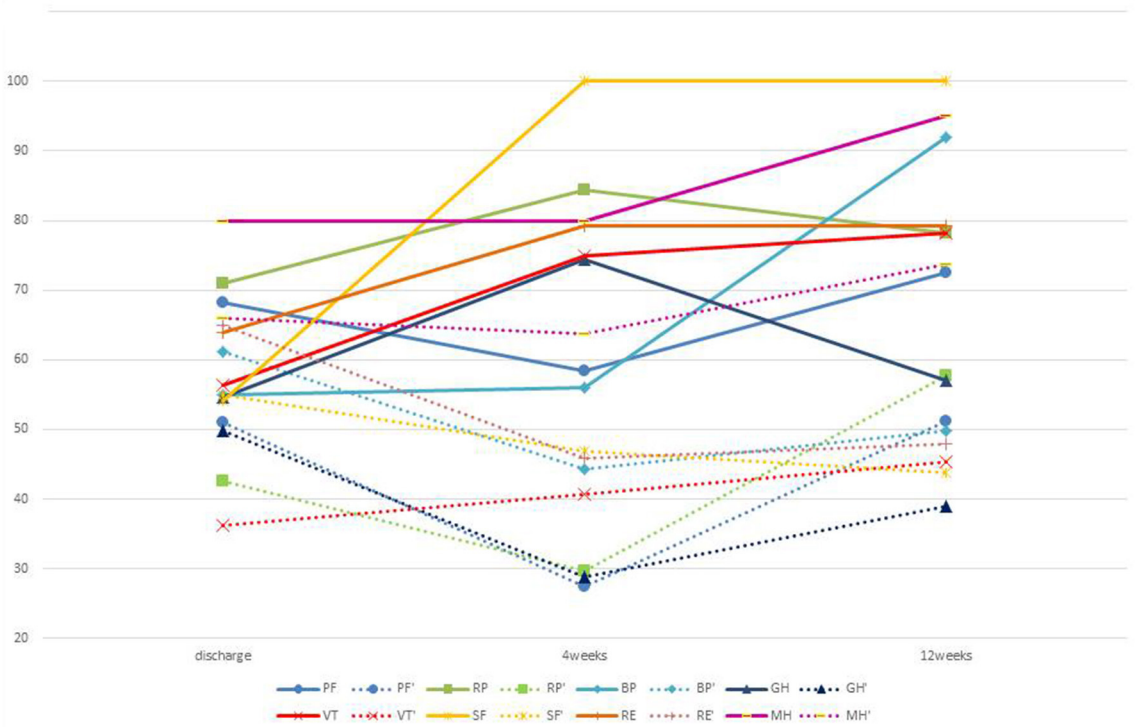

FIGURE 1 | Average transition of SF36 with and without ninjin'yoeito. PF, RP, BP, GH, VT, SF, RE, MH: average value of SF-36 of three people who received ninjin'yoeito. RF', RP', BP', GH', VT', SF', RE', and MH': average value of SF-36 of five people who did not receive ninjin'yoeito.

of underlying disease. Hochuekkito is a representative Kampo supplement for qi and is reported to improve SF-36 score (15). Ninjin'yoeito contains crude drugs such as Astragali radix, Ginseng, *Atractylodis* rhizome, and licorice, which are also components of hochuekkito and are thought to be qi supplements, so it is expected that SF-36 can be improved with ninjin'yoeito. Traditionally, ninjin'yoeito is considered to have an effect on the heart, which is the center of mental function that is addressed in Kampo medicine and is thought to be more likely to produce a mental effect.

Cases 1 and 2 have COPD. Hochuekkito is reported to improve total CAT (COPD assessment test) score and dyspnea, fatigue in COPD patients (16). As mentioned above, because ninjin'yoeito contains similar herbs to hochuekkito, ninjin'yoeito may improve dyspnea and fatigue as well. This may have contributed to the improvement of SF-36, especially VT in Cases

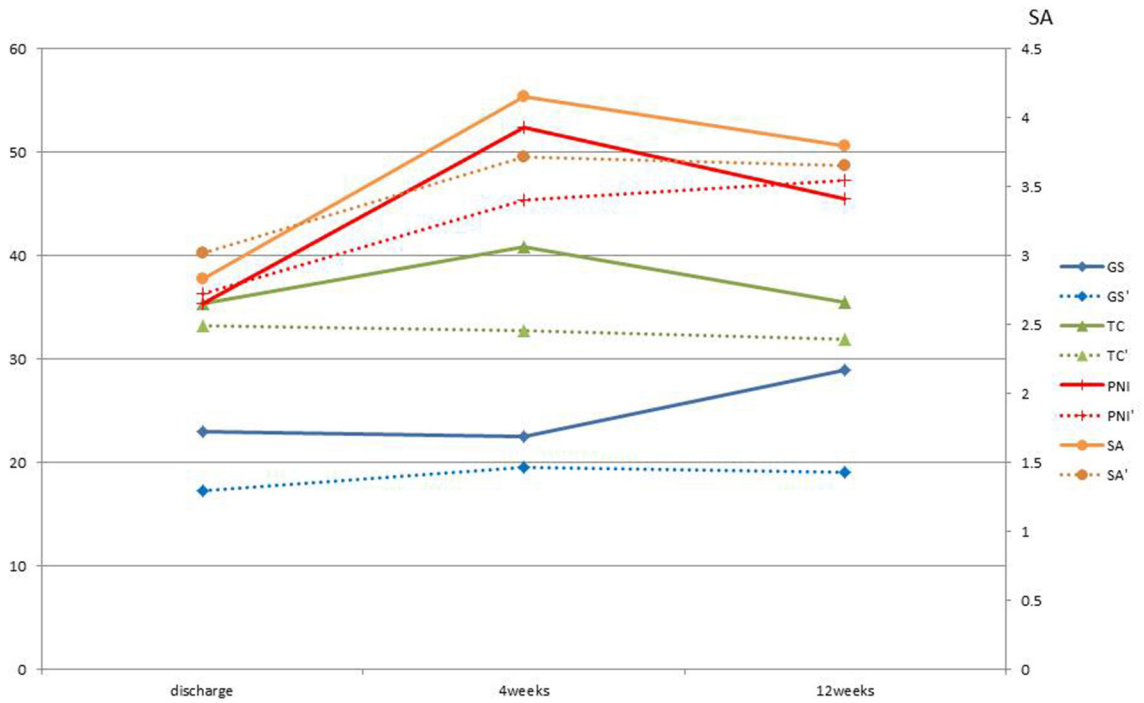

FIGURE 2 | Average change from discharge of each index with and without ninjin'yoeito. GS, Grip strength; kg; TC, Thigh circumference: cm; SA, Serum albumin; mg/dl; PNI, Prognostic Nutritional Index are the average change from discharge of the three patients who received ninjin'yoeito. GS', Grip strength; TC', Thigh circumference; SA', Serum albumin; PNI', Prognostic Nutritional Index are the average change from discharge of five patients who did not received ninjin'yoeito.

1 and 2. In addition, ninjin'yoeito is a Kampo supplement for not only qi but also the blood. Ninjin'yoeito may contribute to the improvement of nutritional status in COPD patients

In terms of grip strength, there was no significant change between the group treated with ninjin'yoeito and the group that was not treated with it. However, half of those not treated with ninjin'yoeito showed no change, whereas all the three subjects in the ninjin'yoeito-treated group showed improvement in grip strength. A previous randomized controlled trial reported an oral ninjin'yoeito administration group also showed improved grip strength (**Figure 2**) (1, 2, 5).

Regarding serum albumin and PNI, no significant difference was observed between the group that received ninjin'yoeito and the group that did not. However, Case 2 was complicated with primary sclerosing cholangitis. It might be possible that the poor improvement in serum albumin after 12 weeks was due to reduced albumin production capacity. PNI showed an improvement rate similar to that of albumin in both groups (**Figure 2**) (1, 2).

There are some limitations of this open-label pilot case report. First, the placebo effect was not taken into account especially in terms of the psychological effect because this was not a placebo-controlled trial. Second, this was not a randomized controlled trial, so the conditions of the two groups, in terms of treatment with ninjin'yoeito, were not the same. Thus, no test for significant difference could be applied for comparison between the two groups. Large randomized controlled trials are needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

Research Fund of Japanese Red Cross Kumamoto Hospital and Kracie Pharmaceutical Co., Ltd, Tokyo, Japan.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.547512/full#supplementary-material

REFERENCES

1. Rubenstein LZ. Specialized geriatric assessment units and their clinical implications. *West J Med.* (1981) 135:497–502.
2. Woodhouse KW, Wynne H, Bailtie S, James OF, Rawlins MD. Who are the frail elderly? *Q J Med*. (1988) 68:505–6.
3. Uto NS, Amitani H, Atobe Y, Sameshima Y, Sakaki M, Rokot N, et al. Herbal medicine ninjin'yoeito in the treatment of sarcopenia and frailty. *Front Nutr*. (2018) 5:126. doi: 10.3389/fnut.2018.00126
4. Ohsawa M. Tanaka Y, Ehara Y, Makita S, Onaka K. A possibility of simultaneous treatment with the multicomponent drug, Ninjin'yoeito, for anorexia, apathy, and cognitive dysfunction in frail Alzheimer's disease patients: an open-label pilot study. *J Alzheimers Dis Rep*. (2017) 1:229–35. doi: 10.3233/ADR-170026
5. Sakisaka N, Mitani K, Sempuku S, Imai T, Takemoto Y, Shimomura H, et al. A clinical study of Ninjin'yoeito with regard to frailty. *Front Nutr*. (2018) 5:73. doi: 10.3389/fnut.2018.00073
6. Fukuhara S, Bito S, Green J, Hsiao A, Kurokawa K. Translation, adaptation, and validation of the SF-36 Health Survey for use in Japan. *J Clin Epidemiol.* (1998) 51:1037–44.
7. Fukuhara S, Ware JE Jr, Kosinski M, Wada S, Gandek B. Psychometric and clinical tests of validity of the Japanese SF-36 Health Survey. *J Clin Epidemiol.* (1998) 51:1045–53.
8. Fukuhara S, Suzukamo Y. *Manual of SF-36v2 Japanese version*. Kyoto: iHope International Inc. (2004).
9. Nishiwaki T, Nakamura K, Ueno K, Fujino K, Yamamoto M. Health characteristics of elderly Japanese requiring care at home. *Tohoku J Exp Med.* (2005) 205:231–9. doi: 10.1620/tjem.205.231
10. Sasaki H, Kasagi F, Yamada M, Fujita S. Grip strength predicts cause-specific mortality in middle aged and elderly persons. *Am J Med*. (2007) 120:337–42. doi: 10.1016/j.amjmed.2006.04.018
11. Wu CY, Hu H-Y, Huang N, Chou Y-C, Li C-P, Chou Y-J. Albumin levels and cause-specific mortality in community-dwelling older adults. *Prev Med*. (2018) 112:145–51. doi: 10.1016/j.ypmed.2018.04.015
12. Hu Y, Shen J, Liu R, Feng ZM, Zhang CN, Ling L, et al. Prognostic value of pretreatment prognostic nutritional index in non-small cell lung cancer: a systematic review and meta-analysis. *Int J Biol Markers*. (2018) 33:372–8. doi: 10.1177/1724600818799876
13. Onodera, T, Goseki N, Kosaki G. Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients. *Nihon Geka Gakkai Zasshi.* (1984) 85:1001–5.
14. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* (2001) 56:M146–56. doi: 10.1093/gerona/56.3.m146
15. Satoh N. A randomized double blind placebo-controlled clinical trial of Hochuekkito, a traditional herbal medicine, in the treatment of elderly patients with weakness N of one and responder restricted design. *Phytomedicine*. (2005) 12:549–54. doi: 10.1016/j.phymed.2004.06.014
16. Hamada H, Sekikawa K, Murakami I, Aimoto K, Kagawa K, Sumigawa T, et al. Effects of Hochuekkito combined with pulmonary rehabilitation in patients with chronic obstructive pulmonary disease. *Exp Ther Med*. (2018) 16:5236–42. doi: 10.3892/etm.2018.6837

Conflict of Interest: The author declares that this study received funding from Kracie Pharmaceutical Co., Ltd. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

frontiers in Nutrition

CASE REPORT
published: 29 April 2021
doi: 10.3389/fnut.2021.548076

Case Report: Efficacy of Ninjin'yoeito Treatment for Idiopathic Pulmonary Fibrosis

Hisako Kushima[1]*, *Hiroshi Ishii*[2] and *Masaki Fujita*[1]

[1] Department of Respiratory Medicine, Fukuoka University Hospital, Fukuoka, Japan, [2] Department of Respiratory Medicine, Fukuoka University Chikushi Hospital, Chikushino, Japan

Ninjin'yoeito, a Kampo prescription, was administered to two patients with idiopathic pulmonary fibrosis (IPF) over a period of 12 weeks to improve loss of appetite and lassitude. In Case 1, improvements were observed in appetite, lassitude, and breathlessness, as well as increases or increasing tendencies in body weight, blood albumin level, and hemoglobin (Hb) level. Case 2 showed no changes in appetite but improvements in lassitude and no deterioration of breathlessness. His body weight and his blood albumin and Hb levels increased or showed increasing trends. In both cases, a trend for improvement of respiratory function was observed. In summary, ninjin'yoeito trended to improve the subjective symptoms and nutritional status of a patient with pulmonary fibrosis.

Keywords: idiopathic pulmonary fibrosis, loss of appetite, malaise, ninjin'yoeito, Kampo medicine

OPEN ACCESS

Edited by:
Akio Inui,
Kagoshima University, Japan

Reviewed by:
Tomoo Kishaba,
Okinawa Chubu Hospital, Japan
Keiko Ochiai Ogawa,
Kanazawa University, Japan

***Correspondence:**
Hisako Kushima
hkushi@fukuoka-u.ac.jp

Specialty section:
This article was submitted to Clinical Nutrition, a section of the journal Frontiers in Nutrition

Received: *01 April 2020*
Accepted: *29 March 2021*
Published: *29 April 2021*

Citation:
Kushima H, Ishii H and Fujita M (2021) Case Report: Efficacy of Ninjin'yoeito Treatment for Idiopathic Pulmonary Fibrosis. Front. Nutr. 8:548076. doi: 10.3389/fnut.2021.548076

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease in which advanced fibrogenesis results in irreversible honeycomb lungs. Antifibrotic agents can be used to treat IPF but the efficacy is limited. The mean lifespan after diagnosis of IPF is 3–5 years; and the mean lifespan after exacerbation of IPF is 2 months or less. IPF is associated with a very high mortality rate (1). Accompanying the progression of restrictive ventilatory defect, the IPF patient may complain of subjective symptoms, such as, exertional dyspnea, dry cough, weight loss, malaise, and/or fatigability. Since these symptoms significantly reduce patients' quality of life, we decided to administer ninjin'yoeito as an available medicine for malaise under universal health coverage in Japan. This report describes the administration of ninjin'yoeito to 2 IPF patients who had experienced loss of appetite and malaise, and a clear therapeutic response was observed.

CASE REPORT

Case 1 was a 59-year-old male with a history of rhabodomyolysis. The patient was referred to our hospital because of gradually progressive dyspnea on exertion. His height was 155.5 cm, the body weight was 51.8 kg and the percentage of oxygen saturation (SpO2) was 95%. His respiratory test results are shown in **Table 1**. Chest computed tomography (CT) demonstrated subpleural fibrotic opacities with traction bronchiectasis in the upper and lower lung fields. He was clinically diagnosed as IPF with pleuroparenchymal fibroelastosis-like lesions based on the guidelines (2) (**Figure 1**). He was treated with nintedanib ethanesulfonate and 7.5 g (divided into two doses) of ninjin'yoeito. The appetite score [Simplified Nutritional Appetite Questionnaire, SNAQ (3, 4)] showed improvement, increasing from 15 before administration to 16 after both 4 and 12 weeks of administration (SNAQ consists of four items related to appetite. All questions are scored from 1 to 5 points, with a total score ranging from 4 to 20 points. The lower the score, the poorer the appetite). Similarly, the

TABLE 1 | The results of pulmonary function tests and biomarkers before and after ninjin'yoeito treatment in Case 1.

	Before administration	After 12 weeks of administration
Vital capacity (VC) (L)	2.18	2.45
%Vital capacity (%VC)	63.2	72.5
Forced vital capacity (FVC) (L)	2.14	2.42
Forced expiratory volume 1.0 (FEV1) (L)	1.86	2.02
Forced expiratory volume 1.0% (FEV1%)	86.9	83.5
KL-6 (U/ml)	499	481
LDH (IU/L)	162	181

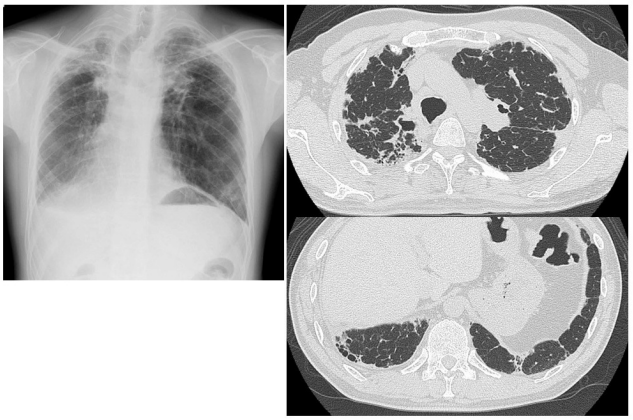

FIGURE 1 | Chest radiograph and CT scans of Case 1 showing elevation of bilateral hilar opacities and subpleural reticular opacities in the bilateral lung fields.

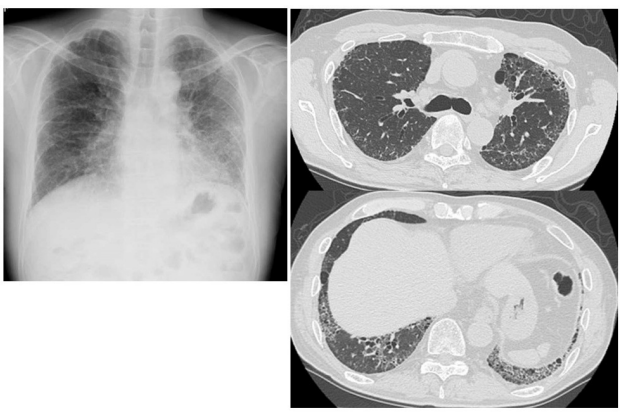

FIGURE 2 | Chest radiograph and CT scans of Case 2 showing reticular opacities, traction bronchiectasis, and some ground-glass attenuation in the bilateral lung fields.

lassitude score [Chalder Fatigue Scale, CFS (5, 6)] improved from six before administration to four after 4 and 12 weeks of administration (CFS consists of 11 items related to physical and mental fatigue. All questions are scored from 0 to 3 points, with a total score from 0 to 33 points. The higher the score, the stronger the fatigue). The breathlessness score [Modified Medical Research Council dyspnea scale, mMRC (7)] also improved from two before administration to one after both 4 and 12 weeks of administration (mMRC scale uses a 5-grade scale, with Grade 0 representing almost no breathlessness and Grade 4 representing severely impaired activities of daily living due to breathlessness. The higher the grade, the stronger the breathlessness). Body weight increased from 51.8 kg before administration to 54.5 and 55.2 kg after 4 and 12 weeks of administration, respectively. The blood albumin level increased from 3.6 g/dL before administration to 4.5 g/dL after both 4 and 12 weeks of administration, and the Hb level showed an increasing trend, changing from 14.9 g/dL before administration to 15.6 and 15.4 g/dL after 4 and 12 weeks of administration, respectively. The CONUT scores were zero before and 12 weeks after ninjin'yoeito administration. A trend for improvement was also observed with respect to respiratory function test results. Serum KL-6 decreased from 499 to 481 U/ml. LDH increased from 162 to 181 IU/L (**Table 1**). There was no occurrence of transaminitis during the treatment. He had an increase in walking distance from 383 to 440 m in a 6-min walk test (6MWT) 12 weeks after administration. The value of the lowest SpO2 at 6MWT remained unchanged at ∼90% for 12 weeks. The CT finding did not show significant changes before and after treatment with ninjin'yoeito.

Case 2, a 59-year-old male was referred to our hospital for respiratory failure and was clinically diagnosed as IPF based on the guidelines [Raghu et al. (2)] (**Figure 2**).

His height was 167.5 cm, the body weight was 50.2 kg and the SpO2 was 90%. His respiratory test results are shown in **Table 2**. He was treated with nintedanib ethanesulfonate and 7.5 g (divided into 2 daily doses) of ninjin'yoeito. CFS decreased from 27 before administration to 16 and 24 after 4 and 12 weeks of administration, respectively, showing improvement. The mMRC scale was three before administration and 2 and 3 after 4 and 12 weeks of administration, respectively, showing no deterioration. The SNAQ score was 14 before administration and after 4 and 12 weeks of administration, showing no change. Body weight increased from 50.2 kg before administration to 50.4 and 51.9 kg after 4 and 12 weeks of administration, respectively. The blood albumin level was 4.1 g/dL before administration to 4.1 and 4.3 g/dL after 4 and 12 weeks of administration, respectively, showing an increasing trend. The Hb level increased from 12.8 g/dL before administration to 13.7 and 14.3 g/dL after 4 and 12 weeks of administration, respectively. The CONUT score could not be measured because the serum cholesterol levels were not measured. The levels of albumin and lymphocyte counts were normal throughout the treatment period. A trend for improvement was also observed with respect to respiratory function. Serum KL-6 decreased slightly from 496 to 492 U/ml. LDH decreased from 201 to 196 IU/L (**Table 2**). There was no occurrence of transaminitis during the treatment. A 6MWT after

TABLE 2 | The results of pulmonary function tests and biomarkers before and after ninjin'yoeito treatment in Case 2.

	Before administration	After 12 weeks of administration
Vital capacity (VC) (L)	1.65	1.90
%Vital capacity (%VC)	42.3	48.7
Forced vital capacity (FVC) (L)	1.62	1.83
Forced expiratory volume 1.0 (FEV1) (L)	1.19	1.43
Forced expiratory volume 1.0% (FEV1%)	73.5	78.1
KL-6 (U/ml)	496	492
LDH (IU/L)	201	196

14 weeks of administration showed improvement in the lowest SpO2 (from 87 to 91%) while the walking distance decreased from 360 to 300 m. The CT finding did not show significant changes before and after treatment with ninjin'yoeito.

DISCUSSION

Ninjin'yoeito was administered to patients with IPF in this study. The treatment improved subjective symptoms such as, loss of appetite, malaise, and breathlessness, as well as scores related to nutritional status, including body weight and blood albumin and Hb levels. In a basic pharmacological study using a bleomycin-induced pulmonary fibrosis model, ninjin'yoeito was reported to have suppressed decreases in food intake and body weight, atrophy of the gastrocnemius, and expression of inflammatory cytokines in the lungs (8). Regarding the mechanism by which it improves loss of appetite, activation of ghrelin-responsive, and unresponsive neuropeptide Y neurons in the arcuate nucleus of the hypothalamus (9) and actions mediated by dopamine D_2 receptors (10) have been reported.

Ninjin'yoeito was administered to the two patients described herein to improve subjective symptoms, such as, loss of appetite and malaise. In addition to these improvements, trends for improvement were also observed with respect to respiratory function after 12 weeks of administration. Ninjin'yoeito is a Kampo prescription consisting of 12 crude drugs. Unlike hochuekkito and juzentaihoto, which are Kampo prescriptions that are similarly used to improve lassitude and malaise, ninjin'yoeito contains schisandra fruit, polygala root, and citrus unshiu peel, which all act on the respiratory system. A component of schisandra fruit, α-cubebenoate, has been reported to suppress accumulation of eosinophils, macrophages, and lymphocytes in the pulmonary alveoli in a bronchial asthma model and inhibited Th2 cytokine and TGF-β1 in pulmonary tissue (11). In a pneumonitis model, ninjin'yoeito was shown to suppress lung injury and death as well as production of nitric oxide in the serum (12). Pirfenidone is a therapeutic agent for IPF and has been shown to have an antifibrotic action in models of pulmonary fibrosis and hepatic cirrhosis (13, 14). Similarly, in a hepatic fibrogenesis model, ninjin'yoeito was found to suppress increases in the hepatic levels of hydroxyproline, which is an index of total collagen, as well as the production of cytokines that induce fibrogenesis (TGF-β1 and IL-13) (15). Investigation of additional cases will help to clarify the effect of ninjin'yoeito for the treatment of respiratory diseases. We believe that our study makes a significant contribution to the literature because the findings showed that NYT can improve fatigue and contribute to maintaining quality of life in IP patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Fukuoka University Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HK conducted the study and wrote the manuscript. HI and MF instructed the study. All authors contributed to the article and approved the submitted version.

REFERENCES

1. The Japanese Respiratory Society. *Guidelines for the Treatment of IPF 2017 (in Japanese)*. Tokyo: Nankodo (2017).
2. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med*. (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST
3. Nakatsu N, Sawa R, Misu S, Ueda Y, Ono R. Reliability and validity of the Japanese version of the simplified nutritional appetite questionnaire in community-dwelling older adults. *Geriatr Gerontol Int*. (2015) 15:1264–9. doi: 10.1111/ggi.12426
4. Rolland Y, Perrin A, Gardette V, Filhol N, Vellas B. Screening older people at risk of malnutrition or malnourished using the simplified nutritional appetite questionnaire (SNAQ): a comparison with the mini-nutritional assessment (MNA) tool. *J Am Med Dir Assoc*. (2012) 13:31–4. doi: 10.1016/j.jamda.2011.05.003
5. Tanaka M, Fukuda S, Mizuno K, Imai-Matsumura K, Jodoi T, Kawatani J, et al. Reliability and validity of the Japanese version of the Chalder Fatigue Scale among youth in Japan. *Psychol Rep*. (2008) 103:682–90. doi: 10.2466/pr0.103.3.682-690
6. Chalder T, Berelowitz G, Pawlikowska T, Watts L, Wessely S, Wright D, et al. Development of a fatigue scale. *J Psychosom Res*. (1993) 37:147–53. doi: 10.1016/0022-3999(93)90081-P

7. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. *Thorax*. (1999) 54:581–6. doi: 10.1136/thx.54.7.581
8. Shigekusa T, Tsubouchi H, Yanagi S, Nakazato M. Ninjin'yoeito improves cachexia in the respiratory system (in Japanese). *Annal Japanese Respir Soc*. (2019) 8:338.
9. Goswami C, Dezaki K, Wang L, Inui A, Seino Y, Yada T. Ninjin'yoeito activates ghrelin-responsive and unresponsive NPY neurons in the arcuate nucleus and counteracts cisplatin-induced anorexia. *Neuropeptides*. (2019) 75:58–61. doi: 10.1016/j.npep.2019.03.001
10. Yamada C, Mogami S, Kanno H, Nishi A, Shimobori C, Hattori T. Ninjin'yoeito ameliorates anorexia and decrease in the nest building *via* a dopamine D2 receptor-dependent manner in a novel murine apathy model (in Japanese). *Japanese Pharmacol Ther*. (2018) 46:207–15.
11. Shin JS, Ryu S, Cho YW, Kim HJ, Jang DS, Lee KT. Inhibitory effects of β-chamigrenal, isolated from the fruits of schisandrachinensis, on lipopolysaccharide-induced nitric oxide and prostaglandin E2 production in RAW 264.7 macrophages. *Planta Med*. (2014) 80:655–61. doi: 10.1055/s-0034-1368544
12. Tanaka K, Sawamura S. Therapeutic effect of a traditional Chinese medicine, ren-shen-yang-rong-tang (Japanese name: Ninjin'yoeito) on nitric oxide-mediated lung injury in a mouse infected with murine cytomegalovirus. *Int Immunopharmacol*. (2006) 6:678–85. doi: 10.1016/j.intimp.2005.10.011
13. Schelegle ES, Mansoor JK, Giri S. Pirfenidone attenuates bleomycin-induced changes in pulmonary functions in hamsters. *Proc Soc Exp Biol Med*. (1997) 216:392–7. doi: 10.3181/00379727-216-44187
14. Tada S, Nakamuta M, Enjoji M, Sugimoto R, Iwamoto H, Kato M, et al. Pirfenidone inhibits dimethylnitrosamine-induced hepatic fibrosis in rats. *Clin Exp Pharmacol Physiol*. (2001) 28:522–7. doi: 10.1046/j.1440-1681.2001.03481.x
15. Ochi T, Kawakita T, Nomoto K. Effects of Hochu-ekki-to and Ninjin'youeito, traditional Japanese medicines, on porcine serum-induced liver fibrosis in rats. *Immunopharmacol Immunotoxicol*. (2004) 26:285–98. doi: 10.1081/IPH-120037726

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership