Supplementary Material

1 Supplementary Data

Toxicity evaluation tests

1. Methods

1.1 Renal Function Test

Renal function tests were performed using commercial diagnostic kits to estimate serum urea and creatinine levels by following the method described in literature(1).

1.2 Liver Function Tests

The activity of liver enzymes, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT), was measured using King's technique and commercial reagent kits through a spectrophotometer (2).

2. Results

2.1 Toxicity evaluation

Liver and kidney functions were assessed to measure the effect of OA induction and the toxicity of GF and GL extracts in rats. Data exhibited that mean values of serum urea, creatinine, ALT, AST, and ALP varied significantly ($p < 0.01$) throughout the study with respective treatments. Significantly high ($p < .001$) serum urea, creatinine, ALT, AST, and ALP values were observed in the positive control (T_1) group (s.Table 1). Data unambiguously shows a significantly lower value in T_5 group.

3. Discussion

In this study, the toxicological evaluation of 50% of the guava fruit and leaf extract was also carried out by evaluating kidney and liver function parameters to evaluate the safety profile of guava fruit and leaf extract. Compared to the negative control group, slightly elevated serum urea and creatinine levels were observed in papain-induced osteoarthritic rats. This result is consistent with the outcome of Yamada et al. (2020), who observed elevated serum urea and creatinine levels in osteoarthritic rats (3). The current study's data evinced that high GF50% and GL50% doses potentially alleviated the elevated serum urea level in osteoarthritic rats. Various studies corroborate the nephroprotective properties of

guava fruit and leaf extract by curtailing serum urea and creatinine levels, eventually ameliorating renal function (4,5). ALT, AST, and ALP activity were assessed to evaluate the toxicity of high doses of guava fruit and leaf extracts on liver function. The outcomes of that study corroborated the hepatic protective activity of guava fruit and guava leaf extracts and indicated that 400 mg/kg GL50% significantly alleviated the liver enzymes. The result of the current study is aligned with previous studies showing that guava fruits and leaf extract have the potential to alleviate the elevated ALT level (6,7). This study endorsed the fact that these extracts, even at high doses, are non-toxic and safe for treating KOA. That research result accorded with the previous study (8). He concluded that 5 to 500 mg/kg of guava leaf extract for oral use in rats showed no toxicity signs or death, and it is safe even at 500 mg/kg.

References

1. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biokemistri*. 2003;15(2): 50–56.

2. King J. The transferases-alanine and aspartate transaminases. *Practical clinical enzymology*. 1965;

3. Yamada EF, Olin LC, Pontel CL, da Rosa HS, Folmer V, da Silva MD. Sida tuberculata reduces oxidative stress and pain caused by the knee osteoarthritis. *Journal of ethnopharmacology*. 2020;248: 112277.

4. Hamid M, Mohamed MM. Nephro-protective effects of grape and guava seeds extracts on gentamicin induced nephrotoxicity in rats. *Journal of Environmental Science. Ain Shams*. 2021;50(9): 333–357.

5. Wu T, Liu H, Lin S, Yu Y, Wei C. Extracts from guava fruit protect renal tubular endothelial cells against acetaminophen-induced cytotoxicity. *Molecular medicine reports*. 2018;17(4): 5544–5551.

6. Uboh FE, Okon IE, Ekong MB. Effect of aqueous extract of Psidium guajava leaves on liver enzymes, histological integrity and hematological indices in rats. *Gastroenterology research*. 2010;3(1): 32–38.

7. Nor NM, Yatim AM. Effects of pink guava (Psidium guajava) puree supplementation on antioxidant enzyme activities and organ function of spontaneous hypertensive rat. *Sains Malaysiana*. 2011;40(4): 369–372.

8. Babatola LJ, Oboh G, Ademiluyi AO. Toxicological evaluation of aqueous extract of different varieties of guava (Psidium guajava Linn) leaves. *Comparative Clinical Pathology*. 2019;28: 1689–1697.

2 Supplementary Figures and Tables

2.1 Supplementary Tables

sTable 1: Mean values (mean ± SD) for serum urea (mmol/L), creatinine (mmol/L), ALT (U/mL), AST (U/mL), ALP (U/mL) of papain induced osteoarthritic rats

Serum urea	**0 Day**	**Effect**	**15 Days**	**Effect**	**30 Days**
T0	57.99±0.854C	2.67^	59.53±0.843D	2.79 ∨	57.87±0.583E
T1	59.25±0.893Aa	2.10^	60.49±0.892Cc	3.20^	62.43±1.422Aa
T2	57.64±0.833Cc	7.89^	62.19±1.042Aa	1.76 ∨	61.09±0.733Bb
T3	56.93±0.853DdB	8.95^	62.03±1.954AA	1.51 ∨	61.09±0.754BbA
T4	57.86±0.583Cc	6.86^	61.83±0.742Bb	3.36 ∨	59.75±0.583Cc
T5	58.8±0.633BbA	5.07^	61.79±1.045BbB	5.71 ∨	58.26±0.943DdB
Creatinine					
T0	0.61±0.783C	2.17^	0.63±0.492E	0.00^	0.63±0.292D
T1	0.62±0.393Bb	13.98^	0.71±0.743Bb	5.19^	0.74±0.854Aa
T2	0.63±0.482Aa	16.49^	0.73±0.742Aa	0.91 ∨	0.72±0.872Cc
T3	0.60±0.872DdA	20.99^	0.73±0.853AaA	0.91 ∨	0.72±0.642CcA
T4	0.61±0.042Cc	10.38^	0.67±0.643CDc	7.92	0.73±0.482Bb
T5	0.61±0.643CcA	13.66^	0.69±1.322CdB	13.94 ∨	0.6±1.492EdB
ALT					
T0	66.48±1.302A	0.08^	66.53±0.492E	0.08 ∨	66.48±0.393E
T1	61.61±1.021Bb	30.10^	80.16±0.843Dd	36.72	109.6±0.482Aa
T2	66.49±0.643Aa	28.09^	85.17±0.733Aa	1.02 ∨	84.30±0.872Bb
T3	66.44±1.492AaA	26.97^	84.35±1.322BbA	1.21 ∨	83.33±0.042CcA
T4	66.71±1.032Aa	26.86^	84.63±0.854Bb	0.52 ∨	84.18±0.983Bb

T5	66.71±1.302AaA	24.10^	82.79±0.593CcB	16.82 ∨	68.87±1.032DdB
AST					
T0	117.82±0.854C	2.93^	121.27±0.733E	0.45^	121.81±1.492E
T1	117.53±0.893Cc	18.98^	139.84±0.432Cb	9.12^	152.59±0.733Aa
T2	117.54±0.833Cc	20.42^	141.54±0.482Aa	5.73 ∨	133.43±1.322Bb
T3	120.06±0.853AaA	17.26^	140.79±0.522ABaA	5.23 ∨	133.43±0.854BbA
T4	118.21±0.583Bb	17.59^	139±0.583Cb	4.41 ∨	132.87±0.593Cc
T5	117.39±0.633CcB	10.21^	129.37±1.422DcB	5.34 ∨	122.46±0.744DdB
ALP					
T0	78.29±0.783A	1.17^	79.20±0.633E	-0.57 ∨	78.75±1.103D
T1	75.86±0.393Dc	43.28^	108.69±0.873Aa	9.74^	119.28±0.933Aa
T2	71.78±0.482Fe	46.16^	104.91±0.783Bb	16.86 ∨	87.23±0.042Bb
T3	74.03±0.872EdB	39.99^	103.63±0.722CcA	15.82 ∨	87.23±0.983BbA
T4	77.25±0.042Ba	35.36^	104.56±1.001Bb	19.16 ∨	84.53±0.583Cc
T5	76.79±0.984CbA	26.26^	96.96±0.073DdB	24.91 ∨	72.80±0.393EdB

T_0: Control group, T_1: Positive group, T_2: 200 mg/kg GF50%, T_3: 400 mg/kg GF50%, T_4: 200 mg/kg GL50%, T5: 400 mg/kg GL50%

ALT: Alkaline phosphatase, AST: Aspartate transaminase, ALP: Alanine transaminase

Capital lettering show comparison among T_0 to T_5, Small lettering show comparison among T_1 to T_5, Colored lettering show comparison among T_3 & T_5

Effect D0-D15: (μ0 - μ15)/SD, Effect D15-30: (μ15 - μ30)/SD, ^: Effect size increase; ∨: Effect size decrease

means sharing the same letters in a column are not significantly different from each other at $p < 0.05$.


frontiers