Original Article

Graviola Attenuates Oxidative Stress and Histopathological Alterations in Diabetic Rats

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ABSTRACT

Background: Oxidative stress usually accompanies diabetes mellitus and contributes to liver lesions and pancreatic injury. *Annona muricata* (Graviola) has been reported to possess antioxidant and antihyperglycemic properties.

Objectives: This research aimed to assess the hepatoprotective and pancreatic protective potentials of *A. muricata* on alloxan–nicotinamide-induced diabetes in male rats.

Methods: The study was performed on 40 adult male rats randomly allocated into four groups: control, diabetic, diabetic + graviola (100 mg/kg), and graviola (100 mg/kg). Parameters assessed included serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), glutathione (GSH), and random blood sugar levels, and body weight. Liver and pancreatic histopathology were also examined.

Results: Diabetic rats exhibited significant increases in ALT, AST, MDA, and blood glucose levels ($P \leq 0.05$) compared with controls, whereas their GSH levels and body weight were significantly lower ($P \leq 0.05$). Graviola treatment significantly improved these changes by decreasing liver enzyme activities, oxidative stress, and hyperglycemia, while improving antioxidant status and body weight. Histopathological results showed pronounced hepatic and pancreatic damage in diabetic rats, while the graviola-treated group displayed qualitatively improved tissue architecture and reduced cellular injury.

Conclusion: These findings provide additional evidence that graviola may be a useful natural agent for treating diabetic complications. *A. muricata* extract has potent antioxidant, antihyperglycemic, hepatoprotective, and pancreatic protective effects on diabetes-induced tissue injury.

Keywords: *Annona muricata*, Experimental diabetes mellitus, Oxidative stress, Hepatic injury, Pancreatic histopathology

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by chronic hyperglycemia due to impaired insulin secretion and insulin resistance. In addition to its effects on systemic metabolism, diabetes is strongly associated with liver dysfunction and oxidative stress, which contribute to chronic hepatic hypoxia. Hyperglycemia promotes lipid peroxidation, mitochondrial dysfunction, and inflammatory reactions, leading to hepatocellular injury and diabetes-related complications, such as hepatic steatosis, inflammation, and fibrosis. Altered liver enzymes and related hepatic abnormalities have become major health concerns worldwide (Mohamed et al., 2016). Oxidative stress plays a major role in the progression of diabetes-induced liver injury and is generally associated with increased malondialdehyde (MDA) levels, decreased glutathione (GSH) levels, and increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels with therapeutic benefits for diabetes liver injury (Mohamed et al., 2016; Yazdi et al., 2019). Plant-derived bioactive compounds have increasingly been recognized as promising natural agents in the management of diabetes mellitus and its associated complications, owing to their antioxidant, anti-inflammatory, and metabolic-modulating properties (Mansourian et al., 2026). *Annona muricata* L. (Graviola), a medicinal plant belonging to the family Annonaceae, contains several biologically active phytochemicals, including flavonoids, alkaloids, acetogenins, tannins, and phenolic compounds. These constituents are known to exhibit antioxidant, anti-inflammatory, antihyperglycemic, and hepatoprotective properties (Son et al., 2021; Olas et al., 2023). Previous studies demonstrated that *A. muricata* leaf extracts reduce oxidative stress, improve glucose metabolism, and attenuate tissue injury in experimental diabetes models through the inhibition of lipid peroxidation and the enhancement of endogenous antioxidant defense systems (Olasehinde et al., 2022). Despite the growing evidence regarding the pharmacological importance of *A. muricata*, limited studies have comprehensively investigated its protective effect against diabetes-induced hepatotoxicity using combined biochemical, oxidative stress, physiological, and histopathological assessments over a prolonged experimental period. Therefore, the present study aimed to evaluate the hepatoprotective effect of *A. muricata* extract against alloxan-induced diabetic hepatic injury in rats through the assessment of liver enzymes, oxidative stress biomarkers, glycemic status, metabolic parameters, and detailed histopathological alterations. The novelty of this study lies in the integrated investigation of the relationship between oxidative stress modulation and hepatic tissue restoration following *A. muricata* administration in diabetic rats, using both biochemical and histopathological approaches during a continuous 60-day experimental period. This combined evaluation may provide further insight into the mechanistic role of *A. muricata* as a natural therapeutic agent against diabetes-associated liver injury.

Materials and Methods

Animals and experimental design

Forty adult male albino rats (200–250 g, 12 weeks old) were housed under standard laboratory conditions (22±2 ˚C, 12 h light/dark cycle) with free access to water and a standard pellet diet. Animals were randomly allocated into four experimental groups (n=10 per group):

Group I (control): This group received distilled water orally.

Group II (diabetic): Diabetes mellitus was induced by a single intraperitoneal injection of nicotinamide (150 mg/kg body weight), followed 15 min later by alloxan monohydrate (120 mg/kg body weight). The animals subsequently received distilled water orally (Vattam et al., 2016).

Group III (diabetic + graviola): Following diabetes induction, rats received *A. muricata* orally at a dose of 100 mg/kg/day for 60 consecutive days.

Group IV (graviola): Healthy rats received *A. muricata* orally at a dose of 100 mg/kg/day for 60 consecutive days.

This four-group experimental design was adopted in accordance with previously published studies on *A. muricata* in diabetic animal models (Alsenosy et al., 2019). No animals died or were excluded during the 60-day experimental period; all 40 animals completed the study and were included in the final statistical analysis.

Induction of type 2 diabetes mellitus

After 12 h of fasting, type 2 diabetes mellitus was induced by intraperitoneal administration of nicotinamide (150 mg/kg body weight), followed 15 min later by alloxan monohydrate (120 mg/kg body weight). To prevent acute hypoglycemia, the rats were supplied with 5% glucose solution for 24 h after injection. After 72 h, fasting blood glucose levels exceeding 200 mg/dL were considered indicative of diabetes mellitus (Vattam et al., 2016).

Extract preparation and gas chromatography–mass spectrometry (GC-MS) analysis

Commercially available *A. muricata* (graviola) fruit and leaf powder was used in this study. For oral administration, the powder was suspended directly in distilled water immediately before dosing to obtain the required dose of 100 mg/kg body weight. It should be noted that the commercial graviola powder was administered orally as an aqueous suspension, following previous studies that demonstrated the effectiveness of aqueous *A. muricata* preparations in experimental diabetic models (Ngueguim Tsofack et al., 2014). However, ethanol was used for GC–MS analysis because it is more efficient than water at extracting a wider range of phytochemicals (Sun et al., 2025). Therefore, the compounds identified by GC–MS represent the overall phytochemical profile of the graviola powder and may not exactly correspond to the composition of the aqueous suspension administered to the animals.

For GC–MS analysis, a separate portion of the same commercial powder was macerated in 70% ethanol at a ratio of 1:10 (w/v) for 72 h at room temperature. The extract was then filtered and concentrated under reduced pressure at 40 ˚C before being stored at 4 ˚C until use (Al Syaad et al., 2019). The ethanolic extract was analyzed using GC–MS (Shimadzu GC-MS-QP2010 SE) equipped with an HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.03 mL/min. The injector temperature was maintained at 250 ˚C, while the oven temperature was programmed from 60 ˚C to 280 ˚C at a rate of 10 °C/min. Electron ionization was performed at 70 eV using split injection mode (20:1, 1 µL). Compounds were identified using the NIST mass spectral library (Mishra et al., 2023).

Sample collection and biochemical assays

After eight weeks of treatment, overnight-fasted rats were anesthetized using chloroform. Blood samples were collected by cardiac puncture and centrifuged at 3000 rpm for 15 min to obtain serum samples. Serum ALT and AST levels were measured using commercial colorimetric kits (BIOLABO, France) at 340 nm according to the manufacturer's instructions (Burtis et al., 2012). Liver tissues were homogenized for the assessment of oxidative stress biomarkers. Reduced GSH levels were determined using ELISA kits (BT Lab, China), while MDA levels were estimated using the thiobarbituric acid reactive substances (TBARS) method at 532 nm (Burtis et al., 2012). Random blood glucose levels were monitored weekly using tail vein blood samples and a digital glucometer.

Histopathological examination

Liver and pancreatic tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological alterations were examined under a light microscope according to standard histological techniques (Suvarna et al., 2019).

Statistical analysis

Data were expressed as Mean±SD. Serum ALT, AST, GSH, and MDA levels were compared among groups using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test. Weekly random blood glucose and body weight measurements were similarly compared among groups at each time point using one-way ANOVA, followed by the LSD post hoc test, using SPSS software version 26. Differences were considered statistically significant at $P \leq 0.05$ (Kim, 2015). Although this time-point-wise approach does not account for within-subject correlation across repeated measurements over time, it was adopted to allow direct group comparisons at each interval; a repeated-measures or mixed-model analysis is recommended for future studies.

Results

Phytochemical profile (GC–MS analysis)

Figure 1 shows the chromatogram of the ethanolic extract of *A. muricata*. GC–MS analysis revealed 30 phytochemical constituents, identified by comparison with the NIST20 mass spectral library. The highest-matching compound was selected for each peak. The compounds are arranged in descending order by relative peak area (%) and are presented with their retention index (RI) and similarity index (SI%) in Table 1. Compounds with a relatively low SI (SI<70%) should be regarded as tentatively identified rather than confirmed.

Liver function (ALT and AST)

One-way ANOVA followed by LSD post-hoc test revealed highly significant differences ($P \leq 0.01$) among all four groups for both ALT and AST levels.

Regarding ALT, all four groups had distinct letters (a, b, c, and d for diabetes, diabetes + graviola, control, and graviola, respectively), indicating that ALT differed significantly among all groups. The diabetic group had the highest level (a), while the graviola-only group had the

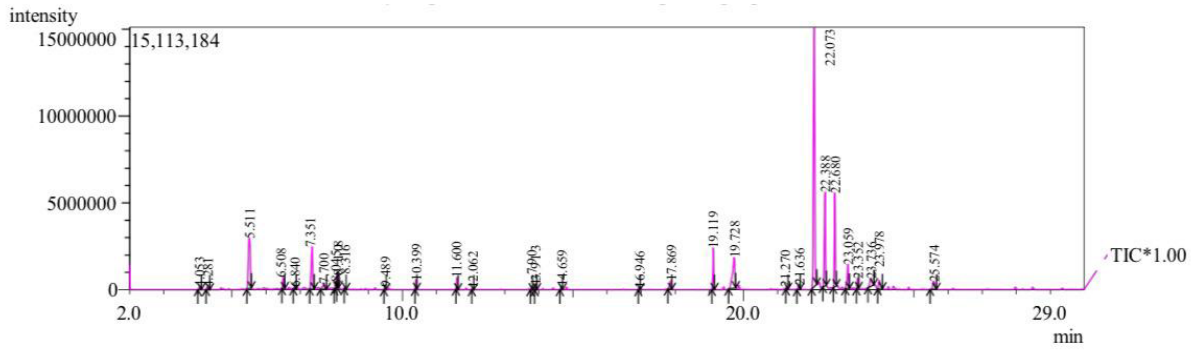


Figure 1. GC-MS chromatogram of the ethanolic extract of *A. muricata* (graviola)

lowest (d), which was significantly lower than the control group's level.

Regarding AST, the control and graviola-only groups shared the same letter (c), indicating no significant difference between them, despite the graviola-only group having a numerically lower mean (22.3 vs 24.2). The diabetes + graviola group (letter b) differed significantly from both, reflecting partial but significant recovery that did not reach control levels. The diabetic group recorded the highest value (a), which differed significantly from those of all the other groups, as presented in Table 2.

Oxidant/antioxidant status (GSH and MDA)

One-way ANOVA followed by LSD post-hoc test revealed highly significant differences (P≤0.01) among all four groups for both parameters.

Regarding GSH, the control and graviola-only groups shared the same letter (a), indicating no significant difference between them, the graviola-only group showing a numerically higher value (411.50 vs 398.90). The diabetes + graviola group (letter b) differed significantly from both, reflecting partial but significant recovery that did not reach control levels. The diabetes group recorded the lowest value (letter c), which differed significantly different from all the other groups.

Regarding MDA, All four groups had distinct letters (a, b, c, and d for diabetes, diabetes + graviola, control, and graviola, respectively), indicating that the differences among all four groups were statistically significant. The diabetes group showed the highest level (a), while the graviola-only group showed the lowest (d), which was significantly lower than the control group's level, as presented in Table 3.

Random blood sugar (RBS)

One-way ANOVA followed by LSD post-hoc test revealed highly significant differences (P≤0.01) among all four groups in overall mean RBS levels. No significant differences were detected among groups at baseline (W0, P=0.8872); however, significant differences among groups were observed at every time point from week 1 through week 8 (P≤0.0001). Group comparisons were performed independently at each time point using one-way ANOVA followed by LSD post-hoc test to assess treatment effects at each interval. This analysis does not account for within-animal correlation across repeated measurements, and no conclusions regarding group-by-time interaction are drawn. From week 1 through week 8, the diabetic group showed significantly higher RBS than all other groups at every time point (P≤0.0001), with values increasing progressively from 270.5±18 mg/dL to 334.3±25.1 mg/dL. The diabetes + graviola group had significantly lower RBS levels than the diabetic group at every time point (P≤0.0001), with RBS declining from 245.5±15.2 mg/dL at week 1 to 180.5±10.2 mg/dL at week 8, while remaining significantly higher than in the control and graviola-only groups throughout. The graviola-only group did not differ significantly from the control group at any time point, as in presented in Table 4.

Body weight

One-way ANOVA followed by LSD post-hoc test revealed highly significant differences (P≤0.01) among all four groups in overall mean body weight. No significant differences were detected among groups at W0 and W1 (P=0.7615 and P=0.3473, respectively); however, from week 2 onward, significant differences were observed among the groups at every time point (P≤0.05 to P≤0.0001). Group differences in body weight were similarly evaluated by one-way ANOVA at each time point; this analysis does not account for within-animal correla-

Table 1. GC-MS analysis of graviola

Peak No.	RT (min)	Compound Name	Molecular Formula	Molecular Weight	Area %	RI	SI%
1	5.511	Eucalyptol	$C_10H_{18}O$	154	10.19	1059	76
2	22.388	Cyproheptadine-type compound	$C_{21}H_{21}N$	287	9.65	2489	60
3	22.680	1-(4-Methyl-[1,1':4',1'']terphenyl-4''-yl) ethanone	$C_{21}H_{18}O$	286	9.22	2516	60
4	19.728	7-hexadecyn-1-ol	$C_{16}H_3OO$	238	7.25	1872	76
5	7.351	Camphor	$C_10H_{16}O$	152	6.00	1121	85
6	19.119	1-naphthalenepropanol	$C_20H_{34}O$	290	3.59	2016	79
7	23.059	Isocarnosol	$C_20H_{26}O_4$	330	3.05	2629	65
8	23.736	Beta-Sitosterol	$C_{29}H_5OO$	414	1.77	2731	51
9	25.574	9,19-cyclolanost-24-en-3-ol, acetate	$C_{32}H_{52}O_2$	468	1.68	2956	67
10	23.978	Demethylsalvicanol	$C_20H_3OO_3$	318	1.55	2642	56
11	17.869	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.22	1968	70
12	8.108	Carbonic acid, decyl nonyl ester	$C_20H_4OO_3$	328	1.19	2252	78
13	7.700	Isoborneol	$C_10H_{18}O$	154	1.10	1138	78
14	6.508	Dodecyl nonyl ether	$C_{21}H_{44}O$	312	1.05	2185	74
15	23.352	Cyclopentanecarboxylic acid, 4-methylene-2,2-diphenyl-, methyl ester	$C_20H_2OO_2$	292	1.01	2276	50
16	11.600	Isocaryophyllene	$C_{15}H_{24}$	204	0.98	1494	85
17	10.399	Alpha-terpinyl acetate	$C_{12}H_2OO_2$	196	0.74	1333	85
18	21.636	Isocarnosol	$C_20H_{26}O_4$	330	0.66	2629	56
19	8.045	Bicyclo[4.1.0]heptan-3-ol (4-Caranol)	$C_10H_{18}O$	154	0.63	1125	77
20	4.053	3-carene	C_10H_{16}	136	0.42	948	89
21	4.281	Camphene	C_10H_{16}	136	0.40	943	91
22	13.913	Tetramethyltricyclo[5.3.1.0(4,11)]undec-8-ene	$C_{15}H_{24}$	204	0.40	1403	82
23	13.790	9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{36}O_4$	352	0.31	2705	76
24	14.659	14-hydroxycaryophyllene	$C_{15}H_{24}O$	220	0.30	1737	84
25	6.840	trans-4a-methyl-decahydronaphthalene	$C_{11}H_20$	152	0.28	1175	78
26	9.489	Ethyl 1,7,7-trimethylbicyclo[2.2.1] heptan-2-yl carbonate	$C_{13}H_{22}O_3$	226	0.28	1453	86
27	21.270	Eudesma-4(14),11-diene	$C_{15}H_{24}$	204	0.28	1469	70
28	16.946	n-pentadecanol	$C_{15}H_{32}O$	228	0.18	1755	80
29	12.062	1,4,7-cycloundecatriene	$C_{15}H_{24}$	204	0.17	1579	88
30	8.316	Carbonic acid, decyl nonyl ester	$C_20H_4OO_3$	328	0.15	2252	78

Abbreviations: RT: Retention time; RI: Retention index; SI%: Similarity index.

Table 2. Comparison of serum ALT and AST levels among different groups

Group	Mean±SD	
	ALT (U/L)	AST (U/L)
Control	34.8±3.13^{c}	24.2±3.32^{c}
Diabetes	59.8±6.3^{a}	49.3±5.31^{a}
Diabetes and graviola	45±4.66^{b}	33.7±3.53^{b}
Graviola	30±1.15^{d}	22.3±0.94^{c}
LSD post hoc tes	3.864**	3.291**
P	0.0001	0.0001

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Note: Means with different letters in the same column differed significantly. **P≤0.01.

tion across repeated measurements, and no conclusions regarding group-by-time interaction are drawn. From W2 onward, the diabetic group showed significantly lower body weight than all the other groups at every time point (P≤0.05 to P≤0.0001), decreasing from 220±2.8 g at W2 to 202±3.7 g at W8. The diabetes + graviola group did not differ significantly from the control group at any time point from W2 onward. The graviola-only group showed the highest body weight values from W3 onward but this difference was not significantly different from the control or diabetes + graviola groups, as presented in Table 5.

Histopathological alteration in hepatic tissue

Light microscopy (H&E, 100×) revealed normal liver architecture in control rats (Figure 2A). Diabetic rats showed necrosis, hemorrhage, hepatocyte damage, and sinusoidal dilation/congestion (Figure 2B). The diabetic+ graviola group exhibited mononuclear cell aggregation, sinusoidal dilation, hypertrophic hepatocyte nuclei, and an apparent increase in Kupffer cell numbers (Figure 2C). Graviola treatment alone caused mild sinusoidal dilation, an apparent increase in Kupffer cell numbers, and mononuclear cell accumulation around the central vein (Figure 2D).

Histopathological alterations in pancreatic tissue

The histological section of the pancreas from the control group, which received distilled water orally for two months, showed normal pancreatic tissue architecture, including normal islets of Langerhans and acinar cells (Figure 3A). The predominant pancreatic lesion in the diabetic group was severe atrophy of the islets of Langerhans, associated with congestion of blood

Table 3. Comparison of GSH and MDA levels among different groups

Groups	Mean±SD	
	GSH (µmol/g Tissue)	MDA (µmol/g Tissue)
Control	398.9±14.53^{a}	58.8±7.13^{c}
Diabetes	101.2±13.3^{c}	315.8±10.47^{a}
Diabetes and graviola	298.2±31.51^{b}	158.8±28.49^{b}
Graviola	411.5±5.31^{a}	43.8±3.85^{d}
LSD post hoc test	16.983**	14.248**
P	0.0001	0.0001

**P≤0.01.

Note: Means with different letters in the same column differed significantly.

Table 4. Effect of groups on mean random RBS levels at different time points

Week	RBS (mg/dL)				LSD Test	P
	Control Group	Diabetes Group	Diabetes + Graviola Group	Graviola Group		
0	92.1±1.2	91.9±1.4	92.7±1.3	91.5±1.1	5.027 NS	0.8872
1	94±1.5^{b}	270.5±18^{a}	245.5±15.2^{a}	91±1.2^{b}	28.619**	0.0001
2	91.8±1.3^{c}	278.1±19.1^{a}	231.8±14.8^{b}	90.9±1.1^{c}	31.944**	0.0001
3	90±1.4^{c}	289.8±20.3^{a}	223.7±13.9^{b}	90.5±1^{c}	34.081**	0.0001
4	96.2±1.7^{c}	295.4±21.5^{a}	214.9±13.1^{b}	90±1.2^{c}	34.957**	0.0001
5	93.9±1.5^{c}	307.1±22.2^{a}	209.4±12.5^{b}	89.7±1.1^{c}	37.021**	0.0001
6	95.3±1.4^{c}	316.9±23^{a}	200.1±11.8^{b}	89.5±1^{c}	27.752**	0.0001
7	92.9±1.3^{c}	327.5±24.4^{a}	193.2±10.9^{b}	89.1±1.1^{c}	35.683**	0.0001
8	95.7±1.6^{c}	334.3±25.1^{a}	180.5±10.2^{b}	88.9±1^{c}	32.655**	0.0001
Mean	93.5±1.4^{c}	279±21.5^{a}	199±12.8^{b}	90.12±1.1^{c}	29.027**	0.0001

NS: Not significant.

**P≤0.01.

Note: Means with different letters in the same column differed significantly.

Table 5. Effect of group on mean body weight at different periods

Week	Body Weight (g)				LSD Test	P
	Control Group	Diabetes Group	Diabetes + Graviola Group	Graviola Group		
0	226±2.1	225±2.3	224±2	225±2.2	3.529 NS	0.7615
1	228±2.4	223±2.5	226±2.2	228±2.1	5.028 NS	0.3473
2	230±2.5^{a}	220±2.8^{b}	228±2.3^{a}	231±2.2^{a}	7.844*	0.0327
3	232±2.6^{a}	217±3^{b}	231±2.4^{a}	234±2.3^{a}	8.169**	0.0079
4	234±2.7^{a}	214±3.1^{b}	234±2.5^{a}	237±2.4^{a}	7.925**	0.0064
5	236±2.8^{a}	211±3.3^{b}	237±2.6^{a}	240±2.5^{a}	9.163**	0.0033
6	238±2.9^{a}	208±3.4^{b}	240±2.7^{a}	243±2.6^{a}	9.754**	0.0019
7	240±3^{a}	205±3.5^{b}	243±2.8^{a}	246±2.7^{a}	10.237**	0.0007
8	242±3.1^{a}	202±3.7^{b}	246±2.9^{a}	249±2.8^{a}	9.894**	0.0002
Mean	234±2.7^{a}	213.9±3.2^{b}	234.3±2.5^{a}	237±2.4^{a}		

NS: Not significant.

*P≤0.05, **P≤0.01.

Note: Means with different letters in the same column differed significantly.

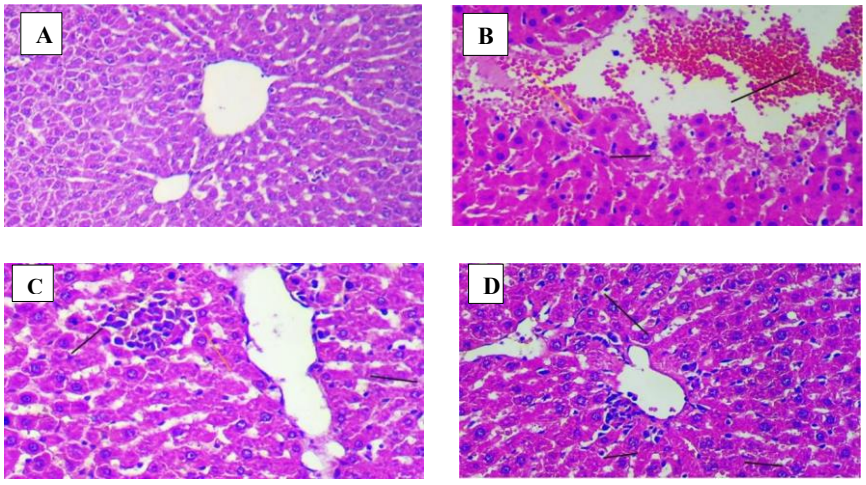


Figure 2. Histological sections of liver tissue stained with hematoxylin and eosin (H&E staining, ×100 magnification)

A) Control group, B) Diabetic group, C) Diabetic + graviola group, D) Graviola group

vessels (Figure 3B). In the diabetic + graviola group, the predominant pancreatic lesion was enlarged islets of Langerhans surrounded by normal acinar cells (Figure 3C). Microscopic examination of pancreatic tissue in the graviola group showed nearly normal islet architecture with mild congestion of blood vessels (Figure 3D). The histopathological evaluation in the present study was descriptive in nature. Retrospective semi-quantitative scoring of the original slides was not feasible at this stage; future investigations should incorporate a validated semi-quantitative grading system (e.g. a 0–3 severity scale) or morphometric quantification (e.g. islet area, cell counts per field) to strengthen histopathological interpretation.

Discussion

GC-MS analysis of graviola

GC-MS analysis of *A. muricata* extract revealed several bioactive compounds with antioxidant, anti-inflammatory, and antihyperglycemic activities, including eucalyptol, borneol, camphor, β-sitosterol, isocarnosol, and fatty acids. These compounds may contribute synergistically to the protective effects of graviola by reducing oxidative stress and inhibiting inflammatory pathways. Previous studies have reported that eucalyptol activates the Nrf2 signaling pathway, while borneol and camphor suppress

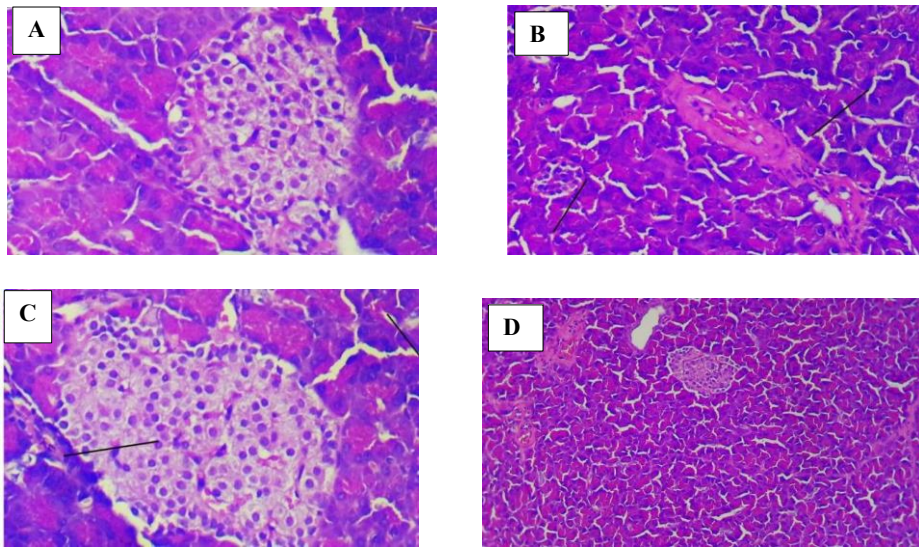


Figure 3. Histological sections of pancreatic tissue stained with hematoxylin and eosin (H&E staining, ×100 magnification)

A) Control group, B) Diabetic group, C) Diabetic + graviola group, D) Graviola group

inflammatory mediators through inhibition of NF-κB signaling (Juergens et al., 2020; Bansod et al., 2021; Li et al., 2023). β-Sitosterol may also contribute to increased insulin sensitivity and glucose uptake through the regulation of GLUT4 transporters (Sahebkar, 2020; Gupta et al., 2011). These proposed mechanisms may collectively contribute to the observed antioxidant and hepatoprotective effects of graviola in the present investigation. However, these mechanisms were not directly assessed in the present study and remain speculative in this context.

Liver function (ALT and AST)

As presented in Table 2, diabetes significantly elevated ALT and AST levels compared with those in the control group (59.8 vs 34.8 U/L and 49.3 vs 24.2 U/L, respectively), suggesting oxidative stress-mediated hepatocellular injury (Table 2). The diabetic group exhibited markedly increased levels of both enzymes; however, after administration of graviola extract, the levels were significantly reduced to 45.00 and 33.70 U/L, respectively. Notably, serum ALT levels in the graviola-treated group were significantly lower than that in the control group (30 U/L), while AST levels, although numerically lower than the control group's levels (22.3 vs 24.20 U/L), did not differ significantly. These findings suggest that graviola extract administration was safe for liver function. These results are consistent with previous studies (Adewole & Ojewole, 2009; Elhassaneen et al., 2023; Son et al., 2021). Therefore, graviola may exert a protective effect against diabetes-induced hepatic dysfunction.

Oxidant/antioxidant status (GSH and MDA)

As presented in Table 3, the diabetic group exhibited a marked depletion in GSH levels (101.2 vs 398.9 µmol/g tissue in the control group) and increased MDA concentrations (315.8 vs 58.8 µmol/g tissue in the control group), indicating hyperglycemia-induced oxidative stress. These findings are consistent with those reported by Yazdi et al. (2019). Administration of graviola extract significantly restored the oxidative balance by increasing GSH levels to 298.2 µmol/g tissue and reducing MDA levels to 158.8 µmol/g tissue. This effect may be attributed to graviola's rich content of bioactive compounds, including phenolic compounds, flavonoids, acetogenins, and vitamin C. These results agree with the findings of Juárez Rojop et al. (2012). Furthermore, the graviola-only group had significantly lower MDA levels than the control group (43.8 vs 58.8 µmol/g tissue), while GSH levels were numerically higher (411.5 vs 398.9 µmol/g tissue) but did not differ significantly from those in the control group. This finding suggests a potential enhancement of intrinsic antioxidant capacity, as previously reported by Pereira et al. (2020). Therefore, graviola may contribute to the restoration of redox homeostasis under conditions of diabetic oxidative stress. These antioxidant capabilities and the resulting mitigation of hepatic oxidative injury closely align with the mechanistic pathways described by Mustafa et al. (2025).

RBS

As presented in Table 4, no significant differences in random blood glucose levels were observed among the experimental groups at baseline (week 0), confirming the initial comparability of the animals before diabetes induction. Following induction, the diabetic group exhibited significantly higher blood glucose levels than the other groups from week 1 onward ($P<0.01$), reflecting persistent hyperglycemia throughout the experimental period. In contrast, treatment with *A. muricata* (graviola) significantly reduced blood glucose levels compared with the untreated diabetic group at all subsequent weekly measurements ($P<0.01$). However, blood glucose levels in the diabetic + graviola group remained significantly higher than those of the control group, indicating that graviola markedly improved, but did not completely normalize, glycemic status. Furthermore, no significant differences were observed between the control and graviola-only groups, suggesting that graviola did not induce hypoglycemia under normal physiological conditions. These findings are consistent with previous studies by Ngueguim Tsofack et al. (2014), which demonstrated that *A. muricata* exerts antihyperglycemic effects by preserving pancreatic β-cell function and enhancing glucose homeostasis. Similar improvements in glycemic control have also been reported by Brito et al. (2025). Collectively, the present findings indicate that chronic graviola administration effectively attenuates diabetes-induced hyperglycemia while maintaining normal blood glucose levels in healthy animals.

Body weight

As presented in Table 5, no significant differences in body weight were observed among the experimental groups at baseline (week 0) or during the first week of the experiment, confirming that initial body weights were comparable before the development of diabetes. From week 2 onward, the untreated diabetic group exhibited a significant and progressive reduction in body weight compared with the other experimental groups ($P<0.05$), reflecting the catabolic state associated with diabetes mellitus. This weight loss is primarily attributed to insulin deficiency, impaired glucose utilization, enhanced proteolysis, increased lipolysis, and the resulting negative energy balance.

In contrast, graviola treatment significantly attenuated diabetes-induced body weight loss. From week 2 onward, body weights in the diabetic + graviola group did not differ significantly from those in the control group, indicating substantial improvement in metabolic status. This beneficial effect may be attributed to improved glycemic control, preservation of pancreatic β-cell function, and reduced protein and lipid catabolism, as previously reported by Ngueguim Tsofack et al. (2014). Furthermore, the graviola-only group maintained body weights comparable to those of the control group throughout the experimental period, supporting the safety of graviola administration in healthy animals. These findings are consistent with previous reports by Brito et al. (2025) and Cintra et al. (2014), which demonstrated that improved glycemic control is associated with the attenuation of diabetes-induced body weight loss. Overall, the present findings suggest that chronic graviola administration effectively prevents diabetes-associated weight loss and contributes to the restoration of normal metabolic homeostasis.

Histopathological alteration in hepatic tissue

The diabetic group exhibited characteristic hepatic histopathological alterations, including necrosis, sinusoidal congestion, hemorrhage, and hepatocellular degeneration. These lesions may be attributed to oxidative stress and hyperglycemia-induced cellular injury associated with diabetes mellitus. Excessive production of reactive oxygen species (ROS) can induce lipid peroxidation and hepatocellular membrane damage, leading to hepatic dysfunction. Similar findings were reported by Lucchesi et al. (2015).

The graviola-only group exhibited mild sinusoidal dilatation, an apparent increase in Kupffer cell numbers, and mononuclear cell aggregation. These changes may partly reflect activation of hepatic defense mechanisms and enhanced phagocytic activity, or they may represent a mild inflammatory or adaptive hepatic response to graviola administration. Notably, no necrosis, hemorrhage, or marked hepatocellular degeneration was observed in this group, in contrast to the diabetic group. However, the safety of graviola at the administered dose should be interpreted with appropriate caution rather than considered conclusive, and further studies assessing dose-response relationships and the effects of chronic administration are warranted (Adewole & Ojewole, 2009).

In the diabetic + graviola group, hepatic architecture showed histological changes suggestive of qualitative improvement, with an apparent reduction in lesion severity compared with the untreated diabetic group, based on qualitative assessment. Sinusoidal dilatation was noted, and Kupffer cell numbers appeared slightly increased; however, hepatocellular architecture was improved, and overall tissue damage was less severe. These findings are consistent with those of Adewole & Ojewole (2009), who demonstrated the protective effect of graviola against diabetes-induced liver injury through reduced oxidative stress and enhanced antioxidant defense.

Histopathological alterations in pancreatic tissue

Marked pancreatic injury was induced by alloxan administration, as evidenced by severe atrophy of the islets of Langerhans and vascular congestion in the diabetic group. Alloxan selectively destroys pancreatic β-cells by inducing excessive reactive oxygen species generation, which causes oxidative stress, cellular degeneration, and decreased insulin secretion. These observations are in agreement with the finding of Al-Awar et al. (2021).

Histological findings in the diabetic + graviola group suggest a qualitative improvement in pancreatic tissue architecture, although no quantitative scoring was applied. These findings were thought to be due to phytochemicals in graviola, such as flavonoids and acetogenins, which protect pancreatic tissue against oxidative damage (Son et al., 2021). Diabetic rats administered graviola showed histological features suggestive of qualitative improvement in islet architecture, as evidenced by mild rather than severe vascular congestion; however, these observations remain descriptive and should be interpreted with caution in the absence of quantitative scoring. These findings could be due to the antioxidant activity of graviola and the protective effect of nicotinamide on residual β-cells.

In conclusion, these descriptive histopathological findings suggest that graviola administration was associated with reduced severity of pancreatic islet injury and apparently improved pancreatic tissue architecture in this experimental diabetes model, consistent with the findings of Adewole and Caxton-Martins (2006).

Conclusion

The present study demonstrates that *A. muricata* (graviola) exerts potent antioxidant, anti- hyperglycemic, hepatoprotective, and pancreatic-protective effects against alloxan-nicotinamide-induced diabetes in rats. Graviola administration significantly improved glycemic control, restored antioxidant status (GSH and MDA), reduced liver enzyme levels (ALT and AST), and attenuated

body weight loss compared with untreated diabetic animals. Histopathological findings were consistent with these biochemical improvements, with graviola-treated diabetic rats showing qualitatively reduced hepatic and pancreatic tissue injury relative to the untreated diabetic group. These protective effects may be attributed to the bioactive phytochemicals identified by GC-MS analysis, including eucalyptol, borneol, β-sitosterol, and phenolic compounds, which are known for their antioxidant and anti-inflammatory properties. Collectively, these findings provide additional evidence that graviola may serve as a useful natural agent for managing diabetic complications. Nevertheless, further studies incorporating a standard positive control, dose-response evaluation, and quantitative histopathological scoring are recommended to validate these findings and clarify the underlying mechanisms of action.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of AlQasim Green University, Al Qasim, Iraq (CODE: qgec/19/2025). All animal procedures followed the institutional and national ethical standards for research involving animals.

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Authors' contributions

Conceptualization, methodology, and supervision: Hassan K. Al-Awadi; Experiments: Zahraa Yousif Alsaady; Investigation and writing: Hawraa M. Murad.

Conflict of interest

The authors declared no conflict of interest.

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