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Assessment of Antioxidant Status, Hepatic Toxicity, and Liver Histopathology in Diabetic Rats Administered *Acacia polyacantha* Stem Bark Extract

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Abstract

Phytomedicine is increasingly incorporated into modern healthcare; however, safety concerns surrounding herbal remedies necessitate comprehensive toxicological evaluation. *Acacia polyacantha* is widely used in Nigerian ethnomedicine for diabetes management, yet data on its safety remain limited. This study evaluated the antioxidant capacity, hepatotoxicity, and histopathological effects of methanolic stem bark extract of *A. polyacantha* in alloxan-induced diabetic rats. Thirty rats were randomly assigned into six groups (n = 5): non-diabetic control, untreated diabetic control, metformin-treated diabetic rats (150 mg/kg), and diabetic rats treated with the extract at 100, 200, and 400 mg/kg body weight for 21 days. Untreated diabetic rats exhibited marked oxidative stress, characterized by reduced superoxide dismutase (46.08 ± 1.86 U/mL) and glutathione (612.40 ± 2.15 µg/mL), with elevated malondialdehyde levels (0.68 ± 0.04 µmol/L), compared with non-diabetic controls ($p \leq 0.05$). Diabetes induction also resulted in significant hepatic dysfunction, evidenced by elevated serum aspartate aminotransferase (AST; 60.60 ± 0.69 U/L) and alanine aminotransferase (ALT; 70.11 ± 1.08 U/L), reduced albumin (7.48 ± 1.78 g/dL) and total protein (9.63 ± 2.20 g/dL), increased total bilirubin (1.41 ± 0.21 mg/dL), and vascular congestion. Treatment with *A. polyacantha* extract improved liver function parameters, with the most pronounced effect observed at 200 mg/kg. The medium dose (200 mg/kg) significantly normalized AST (41.26 ± 0.89 U/L) and ALT (46.13 ± 1.02 U/L), restored albumin (6.02 ± 0.25 g/dL) and total protein (12.21 ± 0.96 g/dL), and reduced bilirubin levels (1.36 ± 0.08 mg/dL), comparable to metformin-treated and non-diabetic rats ($p \geq 0.05$). Histopathological findings corroborated the biochemical results, revealing near-normal hepatic architecture in extract-treated groups. These findings demonstrate that *A. polyacantha* stem bark extract exerts significant antioxidant and hepatoprotective effects, supporting its relative safety and therapeutic potential in diabetes management.

Keywords: *Acacia polyacantha*; albumin; diabetes mellitus; superoxide dismutase; vascular congestion.

INTRODUCTION

Phytomedicine is increasingly becoming an integral component of modern healthcare, bridging ancient traditional knowledge with contemporary scientific research and pharmaceutical innovation. This resurgence is driven by growing demand for natural therapies perceived to be safer, the urgent need for novel drugs to combat global health challenges such as antimicrobial resistance, and scientific advancements that enable the isolation, characterization, and optimization of plant-derived bioactive compounds. Additionally, the high cost and limited accessibility of synthetic drugs have encouraged many individuals, particularly in developing countries, to return to natural cures. Currently, more than 80% of the global population relies on medicinal plants for disease treatment and management (WHO, 2023). Furthermore, over 50% of clinically approved pharmaceutical drugs in use globally are derived from medicinal plants (Bareetseng, 2022).

The phytomedicine industry has experienced remarkable growth, with the global market valued at approximately USD 71 billion in 2023 and projected to exceed USD 328 billion by 2030 (Grand View Research, 2026). In Nigeria, phytomedicine is rapidly being integrated into mainstream healthcare, providing employment opportunities for thousands of people, including herbal practitioners, vendors, and researchers. In 2024, the Nigerian herbal medicine market was estimated at about USD 0.5 billion, with forecasts indicating growth to nearly USD 0.96 billion by 2033 (Reed Intelligence, 2026). Herbal medicines are widely used in Nigeria for managing various ailments, most notably malaria and typhoid fever. Other commonly

treated conditions include infections, gastrointestinal disorders, hypertension, diabetes, infertility, respiratory diseases, arthritis, rheumatism, and general body pain.

Despite their widespread use, concerns regarding the safety of herbal medicines persist. Several toxicological studies, including those by Anywar *et al*. (2021) and Yahaya *et al*. (2022), have demonstrated that medicinal plants are not invariably safe as traditionally believed. Hospitalizations following herbal medicine consumption have been documented in multiple studies and case reports (Amadi and Orisakwe, 2018; Tangnitipong *et al*., 2021). Fatalities associated with herbal medicine use often arise from inadequate regulation, intrinsic toxicity of certain plants, and contamination or adulteration with harmful substances (Ballotin *et al*., 2021). These factors may result in severe organ damage, cardiac complications, and adverse interactions with conventional medications. In Nigeria, several reports have highlighted fatalities linked to herbal medicine consumption due to plant misidentification, overdose, and adulteration, with children being especially vulnerable. Such incidents have contributed to hospital admissions and mortality, although exact figures vary across studies (Awodele *et al*., 2014; Akpan and Ekrikpo, 2015; Aina *et al*., 2024). Consequently, government efforts have intensified toward the standardization and regulation of herbal products to minimize unintended health consequences. This underscores the necessity for comprehensive toxicological evaluation of commonly consumed medicinal plants to provide scientific evidence that can inform herbal medicine policy and regulation in Nigeria.

Among the medicinal plants widely used in Nigeria, *Acacia polyacantha* (family Mimosaceae) is noteworthy (Hammad *et al*., 2024). *A. polyacantha* is a large, fast-growing, thorny tree widely distributed across tropical Africa and commonly referred to as white thorn or hookthorn due to its pale, often peeling bark and characteristic dark, hooked thorns (Ashu *et al*., 2020). In Nigerian traditional medicine, *A. polyacantha* is used in the management of diabetes, a property attributed to its rich phytochemical composition, including flavonoids, tannins, alkaloids, saponins, and triterpenoids (Tedi, 2023). However, scientific data on its toxicity and safe dosage remain limited, necessitating toxicological evaluation to support its potential integration into mainstream diabetes care.

Toxicological assessment of medicinal plants commonly involves the evaluation of antioxidant activity, hepatotoxicity, and histopathological effects using animal models. Antioxidant activity is frequently assessed using spectrophotometric methods due to their simplicity, cost-effectiveness, rapidity, and high sensitivity (Christodoulou *et al*., 2022). Hepatotoxicity is typically evaluated through liver function tests using automated biochemical analyzers, while histopathological examinations provide insight into tissue-level alterations. The liver is particularly targeted in toxicological studies because of its central roles in detoxification and excretion of xenobiotics. Therefore, this study evaluated the antioxidant capacity, hepatotoxic potential, and histological effects of methanolic stem bark extract of *Acacia polyacantha* in experimental rats.

MATERIALS AND METHODS

Plant sample collection and identification

Fresh stem bark of *Acacia polyacantha* was collected in May 2025 from Zuru, Kebbi State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Biological Sciences, Federal University Birnin Kebbi (FUBK), and assigned a voucher number (FUBK48). An authenticated specimen was subsequently deposited in the departmental herbarium.

Extract preparation

The stem bark was thoroughly washed to remove adhering debris, shade-dried at room temperature (25–30 °C) for 7 days, and pulverized using a mortar and pestle. Exactly 320 g of the powdered material was macerated in 70% ethanol for 72 h with intermittent shaking. The resulting mixture was filtered through a muslin cloth, and the filtrate was concentrated using a ZD 41K30RA-C rotary evaporator at 45 °C. The concentrated extract was transferred into Petri dishes and air-dried at room temperature, yielding approximately 6 g of crude ethanolic extract, which was subsequently stored in a desiccator until further use.

Source and management of animal samples

Thirty (30) healthy albino rats (*Rattus norvegicus*) of both sexes, aged 6–8 weeks and weighing 150–200 g, were obtained from the animal house of the Department of Biological Sciences, Federal University Birnin Kebbi (FUBK). The animals were acclimatized for one week in metal cages under standard laboratory conditions (28 ± 2 °C; 12-h light/dark cycle) and fed commercial rat pellets (Vital Feed Limited, Lagos, Nigeria) with clean water provided *ad libitum*.

Induction of diabetes in rats

Diabetes mellitus was induced using alloxan monohydrate following the method described by Kim (2024). Alloxan monohydrate was dissolved in 40 mL of 0.9% (w/v) normal saline and adjusted to pH 4.5. The solution was administered intraperitoneally at a dose of 150 mg/kg body weight. Seventy-two hours after alloxan administration, fasting blood glucose levels were measured using a glucometer (Fantastik-Accu Glucose Meter, IVD version 180705-1). Rats with blood glucose levels ranging from 200 to 450 mg/dL, accompanied by glucosuria, were considered to have developed moderate diabetes.

Experimental design

The thirty rats were randomly assigned into six (6) groups (n = 5 per group) as follows:

- Group 1: Non-diabetic rats (negative control)
- Group 2: Untreated diabetic rats (positive control)
- Group 3: Diabetic rats treated with metformin (150 mg/kg body weight; standard drug)
- Group 4: Diabetic rats treated with extract (100 mg/kg body weight; low dose)
- Group 5: Diabetic rats treated with extract (200 mg/kg body weight; medium dose)
- Group 6: Diabetic rats treated with extract (400 mg/kg body weight; high dose)

The extract and metformin were administered orally via gavage once daily for 21 days. Twenty-four hours after the last treatment, the rats were sacrificed by cervical dislocation. Blood samples were collected using heparinized capillary tubes and transferred into EDTA-containing tubes for liver function analysis. The livers were immediately excised, processed, and prepared for antioxidant, oxidative stress, and histopathological analyses.

Liver function tests

The biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were determined using ultraviolet, colorimetric, and spectrophotometric methods, respectively. Serum albumin and total protein concentrations were quantified using the Biuret method. All analyses were conducted as outlined by Yahaya *et al*. (2019).

Antioxidant status and lipid peroxidation evaluation

The antioxidant status of the rats was assessed by measuring the activities of superoxide dismutase (SOD) and reduced glutathione (GSH), while lipid peroxidation was evaluated by determining the level of malondialdehyde (MDA), a well-known biomarker of oxidative stress. All measurements were carried out in tissue homogenates using standard spectrophotometric methods, following the procedures described by Anyebe *et al*. (2021).

The excised tissues were rinsed in ice-cold normal saline, blotted dry, and homogenized in an appropriate phosphate buffer (pH 7.4). The homogenates were centrifuged at 3,000 rpm for 10 minutes at 4 °C, and the resulting supernatants were used for biochemical analyses. SOD activity was determined based on its ability to inhibit the auto-oxidation of epinephrine, while GSH levels were quantified using the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method. Lipid peroxidation was assessed by measuring MDA levels using the thiobarbituric acid reactive substances (TBARS) assay. All assays were conducted according to established protocols, and results were expressed per milligram of protein.

Histopathological examination

The liver tissues of the rats were prepared for histopathological examination following the method described by Yahaya *et al*. (2025). After sacrifice, the tissues were carefully excised and immediately fixed in 10% neutral buffered formalin for adequate preservation. The fixed tissues were dehydrated through ascending grades of ethanol (65, 80, and 100%), cleared in xylene, and embedded in paraffin wax. Sections of approximately 4–5 µm thickness were cut using a rotary microtome (model YR421), mounted on glass slides, and stained with hematoxylin and eosin (H&E) using standard protocols. Histopathological evaluation was carried out qualitatively under a light microscope, with emphasis on tissue morphology, architectural integrity, and cytological features.

Data analysis

Values were presented as mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22 for Windows. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Antioxidant and lipid peroxidation status in rats fed *Acacia polyacantha*

Table 1 shows the antioxidant and lipid peroxidation values in control and diabetic rats administered the stem bark extract of *Acacia polyacantha*. Untreated diabetic rats (Group 2) exhibited a marked decrease in superoxide dismutase (SOD) and reduced glutathione (GSH) levels, followed by a substantial elevation of malondialdehyde (MDA) concentration, compared with the non-diabetic control (Group 1), indicating enhanced oxidative stress due to diabetes. Treatment with metformin (Group 3) significantly improved antioxidant status, as evidenced by increased SOD and GSH levels and reduced MDA concentration relative to the untreated diabetic group. Similarly, rats treated with *A. polyacantha* extract (Groups 4–6) showed dose-dependent restoration of antioxidant defenses. The medium-dose extract (200 mg/kg; Group 5) produced the most pronounced effect among the extract-treated groups, with SOD and GSH values approaching those of the non-diabetic control and a marked reduction in MDA levels. Overall, administration of *A. polyacantha* stem bark extract ameliorated diabetes-induced oxidative stress by boosting endogenous antioxidant enzymes and suppressing lipid peroxidation, with efficacy comparable to the standard antidiabetic drug as expressed by the antioxidant values obtained.

Table 1. Antioxidant and lipid peroxidation values in control and diabetic rats administered stem bark extract of *Acacia polyacantha.*

	Concentration of antioxidants		
Groups	**SOD (U/mL) Mean**± SEM	**GSH (mg/ml) Mean**± SEM	**MDA (nmol/L) Mean**± SEM
1	66.15 ± 0.30	825.25 ± 0.67	0.22 ± 0.10
2	46.08 ± 1.86[a]	612.33 ± 1.67[a]	0.99 ± 0.03[a]
3	59.86 ± 4.67[b]	931.31 ± 0.96[b]	0.29 ± 0.06[b]
4	54.91 ± 1.79[b]	698.42 ± 0.96[b]	0.68 ± 0.09[b]
5	63.54 ± 1.16[b]	812.21 ± 0.55[b]	0.28 ± 0.14[b]
6	47.22 ± 1.29[b]	681.31 ± 1.60[b]	0.69 ± 0.06[b]

Note: values were expressed as mean ± SEM (n = 5 per group). **Group 1:** non-diabetic rats (negative control), **Group 2:** untreated diabetic rats (positive control), **Group 3:** diabetic rats treated with standard drug (150mg of metformin), **Group 4:** diabetic rats treated with low dose extract (100mg), **Group 5:** diabetic rats treated with medium dose extract (200mg), **Group 6:** diabetic rats treated with high dose extract (400mg). **SOD:** superoxide dismutase, **GSH:** reduced glutathione, **MDA:** malondialdehyde. Values within the same column bearing the same superscript letters (a, b, c, or d) are not significantly different at $p \geq 0.05$ (ANOVA), and values marked with an asterisk (*) are significantly different from the positive control at $p \leq 0.05$ (ANOVA).

Liver function parameters of rats fed *Acacia polyacantha*

Table 2 presents the effects of *A. polyacantha* stem bark extract on liver function indices in control and diabetic rats. Induction of diabetes caused marked hepatic dysfunction, as evidenced by significantly elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in untreated diabetic rats (Group 2) compared with the non-diabetic control (Group 1) ($p \leq 0.05$). Treatment with metformin (Group 3) and *A. polyacantha* extract (Groups 4 and 6) significantly reduced AST and ALT levels relative to the positive control, suggesting partial amelioration of diabetes-induced hepatocellular injury. However, the medium-dose extract (200 mg/kg, Group 5) markedly reduce AST and ALT activities compared with the diabetic control and showed the greatest hepatoprotective effect among the extract-treated groups.

Total protein and serum albumin levels were significantly altered in untreated diabetic rats, reflecting impaired hepatic synthetic function. Administration of *A. polyacantha* extract restored these parameters toward normal values, with the medium- and high-dose group showing no significant difference from the non-diabetic control ($p \geq 0.05$). Total bilirubin was significantly elevated in untreated diabetic rats compared with the negative control group, but was reduced following treatment with *A. polyacantha* extract, indicating a beneficial effect of the extract on bilirubin levels. Overall, administration of *A. polyacantha* stem bark extract improved liver function parameters in diabetic rats, with the 200 mg/kg dose producing the most pronounced hepatoprotective effect.

Table 2. Liver function parameters of control and rats fed *Acacia polyacantha.*

Groups	**AST (U/L) Mean**± SEM	**ALT (U/L) Mean**± SEM	**Albumin (g/dl) Mean**± SEM	**Total protein (g/dl) Mean**± SEM	**Total bilirubin (mg/dl) Mean**± SEM
1	13.88 ± 0.70	15.07 ± 0.25	4.03 ± 0.20	12.66 ± 0.16	1.06 ± 0.04
2	60.60 ± 0.69*	70.11 ± 1.08 *	7.48 ± 1.78 [a]	9.63 ± 2.20 [a]	1.41 ± 0.21[a]
3	18.71 ± 0.30[b]	59.88 ±0.24 [b]	6.27 ± 0.43 [b]	12.0 ± 0.13 [b]	1.23 ± 0.05 [b]
4	48.15 ± 1.23 [b]	56.81 ± 1.03 [b]	5.66 ± 0.20 [b]	9.80 ± 1.90 [b]	0.78 ± 0.12 [c]
5	23.96 ± 0.20*	29.77 ± 0.27*	4.95 ± 0.53 *	11.04 ± 1.48 *	1.18 ± 0.09*
6	39.80± 0.28 [b]	44.52 ± 0.89 [b]	6.19 ± 0.27 [b]	12.66 ± 1.63 [b]	1.32 ± 0.03 [b]

Note: values are expressed as mean ± SEM (n = 5 per group). **Group 1:** non-diabetic rats (negative control), **Group 2:** untreated diabetic rats (positive control), **Group 3:** diabetic rats treated with standard drug (150mg of metformin), **Group 4:** diabetic rats treated with low dose extract (100mg), **Group 5:** diabetic rats treated with medium dose extract (200mg), **Group 6:** diabetic rats treated with high dose extract (400mg). **AST:** Aspartate Aminotransferase, **ALT:** Alanine Aminotransferase. Values within the same column bearing the same superscript letters (a, b, c, or d) are not significantly different at $p \geq 0.05$ (ANOVA), and values marked with an asterisk (*) are significantly different from the positive control at $p \leq 0.05$ (ANOVA).

Histopathological effects of *Acacia polyacantha*

Figure 1(a–f) illustrates the hepatic histoarchitecture of control and diabetic rats treated with *A. polyacantha* stem bark extract. The non-diabetic control group (Figure 1a) showed normal liver architecture with well-preserved hepatocytes and intact vascular structures. In contrast, induction of diabetes resulted in marked vascular congestion and distortion of hepatic architecture in untreated diabetic rats (Figure 1b). Treatment with the standard antidiabetic drug markedly attenuated these pathological alterations, with near-normal hepatic morphology observed (Figure 1c). Similarly, administration of *A. polyacantha* extract produced a dose-dependent improvement in liver histology. Rats

treated with the low dose (100 mg/kg; Figure 1d) exhibited mild hepatocellular ballooning degeneration, indicating partial protection. However, rats administered the medium (200 mg/kg; Figure 1e) and high (400 mg/kg; Figure 1f) doses showed well-preserved hepatocytes and normal vascular architecture, comparable to those observed in the standard drug-treated and non-diabetic control groups. These findings suggest that *A. polyacantha* stem bark extract confers significant hepatoprotective effects against diabetes-induced hepatic damage.

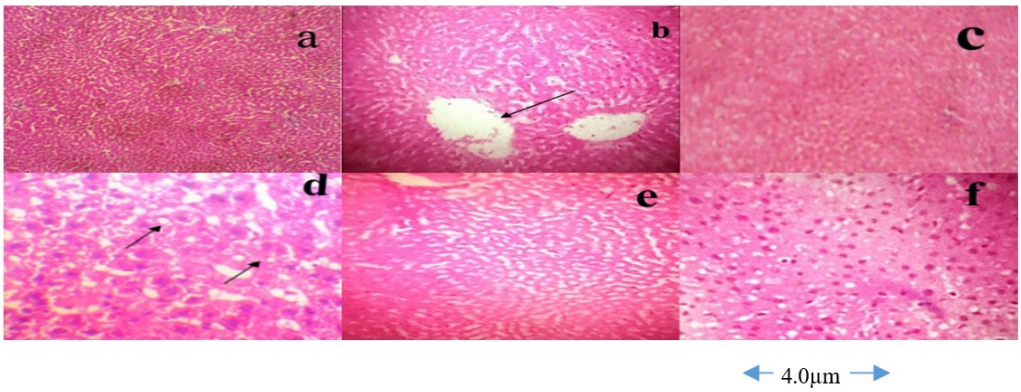

Figure 1. Photomicrographs of liver sections from rats treated with *Acacia polyacantha* stem bark extract (×100). (a) Liver of non-diabetic rats (Group 1) showing normal histoarchitecture; (b) liver of untreated diabetic rats (Group 2) showing marked vascular congestion; (c) liver of diabetic rats treated with metformin (Group 3) showing near-normal histoarchitecture; (d) liver of diabetic rats treated with 100 mg/kg extract (Group 4) showing hepatocellular ballooning degeneration; (e) liver of diabetic rats treated with 200 mg/kg extract (Group 5) showing normal histoarchitecture; and (f) liver of diabetic rats treated with 400 mg/kg extract (Group 6) showing normal histoarchitecture.

DISCUSSION

The present study demonstrates that the diabetes induction in rats is associated with marked oxidative stress, hepatic dysfunction, and structural liver damage, whereas treatment with *Acacia polyacantha* stem bark extract effectively attenuated these pathological alterations. The significant reductions in superoxide dismutase (SOD) and reduced glutathione (GSH) levels, coupled with elevated malondialdehyde (MDA) concentrations observed in untreated diabetic rats, confirm elevated production of reactive oxygen species and enhanced lipid peroxidation. These processes are well-established contributors to diabetes-related tissue injury and are consistent with the reports of Angie *et al*. (2024) and Qnais *et al*. (2025). The restoration of antioxidant enzyme activities and the suppression of MDA levels following metformin treatment validate the reliability of the experimental model and provide a benchmark for comparison, as previously demonstrated by Ahmed Mobasher *et al*. (2020) and Buczyńska *et al*. (2024). Notably, *A. polyacantha* improved antioxidant status, in line with the findings of Zaman *et al*. (2022) and Basira *et al*. (2025). The medium dose (200 mg/kg) exerted the most pronounced effect, suggesting an optimal concentration at which bioactive phytochemicals in the extract efficiently scavenge free radicals and enhance endogenous antioxidant defenses, as reported by Okpanachi *et al*. (2012). Although phytochemical analysis was not conducted in the present study, *A. polyacantha* has been widely reported to contain flavonoids, terpenoids, oleanolic acid derivatives, sterols, phenolic acids, and complex saponins, in addition to alkaloids, tannins, and steroids. These constituents are believed to underlie its traditional medicinal applications, particularly its notable antioxidant properties (Ashu *et al*., 2020; Batiha *et al*., 2022).

The increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities observed in untreated diabetic rats indicate compromised hepatocellular membrane integrity and leakage of intracellular enzymes into the circulation (Ouyang *et al*., 2024). The reduction of these enzymes following extract treatment reflects stabilization of hepatocyte membranes and improvement in liver function, consistent with earlier findings by Yahaya *et al*. (2022b). Similarly, the normalization of albumin and total protein levels suggests restoration of hepatic synthetic capacity, while decreased bilirubin concentrations indicate improved bilirubin metabolism and excretory function (Martínez Herreros *et al*., 2022).

Histopathological findings further corroborated the biochemical results. Severe vascular congestion and architectural distortion observed in untreated diabetic rats were markedly ameliorated by *A. polyacantha* extract, with medium- and high-dose treatments restoring near-normal hepatic morphology. These findings indicate that *A. polyacantha* stem bark extract possesses significant antioxidant and hepatoprotective effects in diabetic rats, with efficacy comparable to metformin, thereby highlighting its efficacy in the management of diabetes-associated oxidative stress and hepatic complications. Although reports on the hepatoprotective histoarchitectural effects of *A. polyacantha* are limited,

related species within the genus, such as *Acacia nilotica* (Omara *et al*., 2012) and *Acacia pennata* (Shao *et al*., 2022), have demonstrated comparable protective effects.

CONCLUSION

In conclusion, this study demonstrates that experimental diabetes induces significant oxidative stress, hepatocellular dysfunction, and structural liver damage in rats. Treatment with *Acacia polyacantha* stem bark extract effectively mitigated these alterations by restoring antioxidant defenses, reducing lipid peroxidation, and improving biochemical and histopathological indices of liver function. The medium dose (200 mg/kg) exhibited the most pronounced protective effect, suggesting an optimal therapeutic window for its bioactive constituents. The normalization of antioxidant enzymes, liver transaminases, serum proteins, and bilirubin levels, together with the marked improvement in hepatic architecture, underscores the potent antioxidant and hepatoprotective properties of the extract. Overall, the findings indicate that *A. polyacantha* stem bark extract is comparable to metformin in attenuating diabetes-associated oxidative stress and liver injury, highlighting its potential as a natural remedy for managing diabetic hepatic complications. Further studies, particularly phytochemical characterization and mechanistic investigations, are recommended to fully elucidate the active compounds and pathways responsible for these beneficial effects.

Completing Interest: The authors of the article stated that there is no competing interest.

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