Hepatoprotective Effects of Glibenclamide, Catechin, and Ethanolic Neem Leaf Extract in Alloxan-Induced Diabetic Rats

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ABSTRACT

Background*: Diabetes mellitus is a systemic disorder that causes hepatic damage, primarily through oxidative stress and inflammation. This study evaluated the hepatoprotective effects of glibenclamide, catechin, and ethanolic neem leaf extract in alloxan-induced diabetic male rats.*

Methods*: Fifty Sprague-Dawley rats were allocated into five groups: normal control, diabetic control, and three treatment groups receiving glibenclamide (5 mg/kg), catechin (40 mg/kg), or neem extract (250 mg/kg) orally for 14 days after diabetes induction (alloxan, 150 mg/kg i.p.). Diabetes was confirmed by a fasting blood glucose level ≥200 mg/dL after 72 h. Liver function biomarkers (AST, ALT, ALP, albumin, bilirubin, and total protein), oxidative stress markers (MDA, SOD, CAT, and GSH), lipid profiles, and liver histopathology were evaluated.*

Results*: Diabetic rats exhibited marked hyperglycaemia, elevated liver enzyme levels, lipid peroxidation, and hepatocellular damage. All treatments significantly improved glycaemic control, reduced hepatic enzyme levels and MDA, and enhanced antioxidant enzyme activities. Neem-treated rats demonstrated the most significant biochemical recovery and near-normal liver histology with preserved Kupffer cells.*

Conclusion*: These findings suggest that neem leaf extract exerts robust hepatoprotective effects, likely because of its high polyphenolic content and antioxidant properties, making it a promising candidate for mitigating liver injury in diabetes mellitus.*

Keywords*: Diabetes mellitus, liver, neem, catechin, glibenclamide*

ABSTRAK

Latar Belakang: *Diabetes melitus merupakan gangguan sistemik yang juga dapat menyebabkan kerusakan hati, terutama melalui stres oksidatif dan peradangan. Penelitian ini mengevaluasi efek hepatoprotektif glibenklamid, katekin, dan ekstrak etanol daun nimba pada tikus jantan Sprague-Dawley yang diinduksi diabetes dengan alloxan.*

Metode*: Lima puluh ekor tikus dibagi menjadi lima kelompok: kontrol normal, kontrol diabetes, dan tiga kelompok perlakuan yang menerima glibenklamid (5 mg/kg), katekin (40 mg/kg), atau ekstrak nimba (250 mg/kg) secara oral selama 14 hari setelah induksi diabetes (alloxan, 150 mg/kg i.p.). Diabetes dikonfirmasi dengan kadar glukosa darah puasa ≥200 mg/dL setelah 72 jam. Parameter fungsi hati (AST, ALT, ALP, albumin, bilirubin, protein total), penanda stres oksidatif (MDA, SOD, CAT, GSH), profil lipid, dan histopatologi hati dianalisis.*

***Hasil**: Tikus diabetes menunjukkan hiperglikemia signifikan, peningkatan enzim hati, peroksidasi lipid, dan kerusakan hepatoseluler. Semua perlakuan secara signifikan memperbaiki kontrol glikemik, menurunkan kadar enzim hati dan MDA, serta meningkatkan aktivitas enzim antioksidan. Tikus yang diberi ekstrak nimba menunjukkan pemulihan biokimia paling signifikan dan histologi hati yang hampir normal dengan sel Kupffer yang terpelihara.*

***Kesimpulan**: Temuan ini menunjukkan bahwa ekstrak daun nimba memiliki efek hepatoprotektif yang kuat, kemungkinan besar karena kandungan polifenolnya yang tinggi dan sifat antioksidannya, sehingga berpotensi sebagai kandidat untuk mengurangi cedera hati pada diabetes.*

***Kata Kunci:** Diabetes mellitus, hati, nimbi, katekin, glibenklamid*

INTRODUCTION

The hallmark of diabetes mellitus, a chronic metabolic disease, is persistent hyperglycaemia caused by deficiencies in insulin action, secretion, or both. Among its complications, liver dysfunction plays a significant role, as the liver is a key organ in glucose homeostasis and insulin metabolism[1]. In diabetic conditions, hepatic oxidative stress, inflammation, and fibrosis are common, often leading to non-alcoholic fatty liver disease and hepatocellular injury[2].

Alloxan-induced diabetes in experimental animals is a reliable model of type 1 diabetes mellitus. Alloxan selectively destroys pancreatic β-cells by generating reactive oxygen species (ROS), leading to insulin deficiency and hyperglycaemia, which in turn induces oxidative damage to the hepatic tissue[3]. The search for agents that mitigate diabetic complications, particularly liver damage, has intensified. Glibenclamide, a sulfonylurea class antidiabetic drug, possesses hypoglycaemic, modest antioxidant, and hepatoprotective properties[4]. However, long-term use may lead to adverse effects and limited organ protection.Phytochemicals, such as catechin (CTN), a natural flavonoid abundant in green tea and other plant sources, are known for their potent antioxidant, anti-inflammatory, and hepatoprotective properties. Studies have demonstrated the ability of catechin to scavenge free radicals and protect hepatic tissue from oxidative damage under diabetic conditions[5]. *Azadirachta indica* (neem) is a medicinal plant extensively used in traditional medicine. Its leaves contain various bioactive compounds with hypoglycaemic, antioxidant, and hepatoprotective properties. Neem leaf extract ameliorates hepatic dysfunction and oxidative stress in diabetes models by modulating inflammatory markers and improving liver histology[6,7].

A previous study from our group investigated the effects of glibenclamide, catechin, and neem leaf extract on pancreatic β-cell regeneration and pancreatic histology in alloxan-induced diabetic rats[8]. This study primarily focused on endocrine pancreatic recovery following diabetic injury. In contrast, diabetes mellitus induces significant hepatic metabolic disturbances, including oxidative stress and hepatocellular injury. Therefore, this study aimed to examine the hepatic effects of these therapeutic agents by evaluating liver function biomarkers, oxidative stress indices, lipid profile parameters, and liver histopathology. This approach provides complementary insights into the multi-organ protective effects of these treatments in diabetic conditions.

This study provides a direct comparative evaluation of neem leaf extract, a whole-plant phytotherapeutic preparation, against a standard antidiabetic drug (glibenclamide) and a purified bioactive flavonoid (catechin) in an alloxan-induced diabetic model. While previous studies have independently examined these agents, few have systematically compared conventional pharmacological therapy with both crude herbal extracts and isolated phytochemicals in the same experimental framework. By integrating biochemical, oxidative stress, lipid profile, and histopathological endpoints, this study offers novel insights into whether the therapeutic effects of neem leaf extract arise from synergistic phytoconstituents or mirror the activity of isolated antioxidant compounds. These findings contribute to the growing field of evidence-based phytomedicine and provide comparative data that may guide the development of complementary therapeutic strategies for diabetes-associated hepatic dysfunction.

METHODS

The study included 50 adult male Sprague-Dawley rats (n=10 per group) weighing between 150 and 200 g. The animals were kept in typical laboratory settings with a 12-hour light/dark cycle and were given unlimited access to water and a conventional pellet diet. Under the code OOUTH/HREC/010//026/

E120/2024AP, the Olabisi Onabanjo University Teaching Hospital Human Research Ethics Committee (OOUTH-HREC) authorised all experimental procedures, and animal handling complied with the guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Inclusion Criteria

This study involved healthy male Sprague-Dawley rats aged 8–10 weeks and weighing 150–200 g at the start of the experiment. All animals were screened to confirm normal baseline blood glucose and liver function before diabetes induction. Only rats that successfully developed diabetes, as confirmed by fasting blood glucose levels ≥200 mg/dL 72 h after alloxan injection, were included. Additionally, the animals were required to be free from any pre-existing illness, infection, or injury that could interfere with the experimental outcomes.

Exclusion Criteria

Rats with pre-existing hyperglycaemia (fasting blood glucose ≥200 mg/dL) before induction, or those that failed to develop diabetes after alloxan administration, were excluded. Animals with congenital anomalies, signs of liver disease, abnormal behaviour, or significant weight loss (>10% body weight) before the study were also excluded. Furthermore, any rat that developed severe illness, injury, or died during the induction phase was removed from the experiment to ensure reliable and consistent results.

Animal Grouping and Experimental Design

After the induction of diabetes mellitus, the rats were weighed and randomly assigned to groups **(Table 1).**

The animals used in this study were part of a broader experimental investigation examining the systemic effects of glibenclamide, catechin, and neem leaf extract in alloxan-induced diabetic rats. A previous publication using this dataset reported findings related to pancreatic β-cell regeneration and pancreatic histological changes[8]. The present study analyses distinct endpoints, focusing on hepatic biochemical markers, oxidative stress parameters, lipid profile indices, and liver histopathology, which were not included in the previous report.

Plant Material Collection, Preparation, and Extraction

Neem leaves (*Azadirachta indica*) were collected from Sagamu, Nigeria. The leaves were identified at the Department of Pharmacognosy, Olabisi Onabanjo University, Nigeria. The leaves were air-dried in the physiology laboratory for two weeks and crushed using a mechanical grinder.

Following Nazir et al.,[13] procedure, 100 g of the powdered leaf was dissolved in 500 ml of 99% ethanol and left in the refrigerator for three days while being shaken occasionally. Subsequently, muslin cloth and Whatman filter paper were used to filter the resultant combination. The filtrate was condensed into a semisolid mass at 40°C under reduced pressure using a rotary evaporator. The concentrate was reconstituted in distilled water to produce the dosage for the trials.

Induction of Diabetes

The procedure outlined by Yakubu et al.[9] was followed to induce diabetes. Alloxan monohydrate (0.9 g) was dissolved in 3 ml of distilled water, resulting in a stock solution with a concentration of 150 mg/0.5 ml. A single intraperitoneal injection of alloxan monohydrate (150 mg/kg b.w.) induced diabetes. Rats deemed diabetic for this study were those whose blood glucose levels were more than 200 mg/dl 72 hours after induction.

Preparation and Administration of Glibenclamide

A 10 mg glibenclamide tablet was crushed and dissolved in 5 ml of distilled water to obtain a concentration of 2 mg/ml. A 5 mg/kg bw dose of glibenclamide was administered daily from the stock solution, according to Shah & Khan,[10].

Table 1: Animal grouping and experimental design

Group		Treatment	References
A	Normal control	Distilled water	
B	Diabetes control	Alloxan (150 mg/kg bw) i.p.	Yakubu *et al.,*[9]
C	Glibenclamide treatment group	Alloxan (150 mg/kg bw) i.p. + glibenclamide (5 mg/kg BW) p.o.	Shah & Khan,[10]
D	Catechin treatment group	Alloxan (150 mg/kg bw) i.p. + CTN (40 mg/kg bw) p.o.	Nazir *et al.,*[11]
E	Neem leaf extract treatment group	Alloxan (150 mg/kg bw) i.p. + neem leaf extract (250 mg/kg bw) p.o	Dholi *et al.,*[12]

p.o = orally, i.p. = intraperitoneal, bw = body weight

Preparation and Administration of Catechin

Catechin (160 mg) was dissolved in 10 ml of distilled water to obtain a concentration of 8 mg/ml. A 40 mg/kg bw dose of catechin was administered daily from the stock solution, according to Nazir *et al.*[11]

Preparation and Administration of Neem Leaf Extract

Neem leaf extract (4.9 g) was dissolved in 49 ml of 70% ethanol to obtain a concentration of 50 mg/0.5 ml. A 250 mg/kg bw dose of neem leaf extract was administered daily from the stock solution, according to Dholi *et al.*[12].

Measurement of Fasting Blood Glucose

After a 14-hour overnight fast, fasting blood glucose was measured using a drop of blood from the rat tail and a glucometer (Accu-Check, Roche, Germany).

Procedure for Blood Collection

Blood was drawn into sterile sample tubes from the orbital venous sinus of each rat. To extract serum, the samples were centrifuged for 15 min at 3000 rpm after being allowed to clot for 30 min at room temperature. The serum was stored at -20°C until biochemical tests were performed.

Animal Sacrifice

After the study was completed, the animals were sacrificed by cervical dislocation. The liver, which was the organ of interest in this study, was removed after a midline abdominal incision.

Antioxidant Enzyme Activity Assays

Tissue samples (0.1 mg) were homogenised in 4 mL phosphate buffer solution (pH 7.2) and centrifuged at 3,000 rpm for 10 min. The supernatant was collected and stored at -80°C until further analysis. Antioxidant enzyme activity was determined using commercially available kits, including Glutathione Assay Kit (Arbor Assays, USA) for GSH, Superoxide Dismutase Assay Kit (Cayman Chemical, USA) for SOD, Catalase Assay Kit (Abcam, UK) for CAT, Total Antioxidant Capacity Assay Kit (Bio Vision, USA) for TAC, Bradford Protein Assay Kit (Bio-Rad, USA) for total protein, and Malondialdehyde Assay Kit (Bio Vision, USA) for MDA. All assays were performed according to the manufacturer's instructions using a microplate reader (BioTek Synergy HT, USA).

Biochemical Analysis of Liver Function Markers

Liver function parameters were assayed using standard enzymatic and colorimetric methods with commercial diagnostic kits (Randox Laboratories Ltd., UK) according to the manufacturer's instructions. All enzyme activities (AST, ALT, and ALP) were expressed as absolute enzyme activities in units per litre (U/L). Albumin and bilirubin concentrations were expressed as g/L and mg/dL, respectively. The reported values represent measured absolute concentrations and were not normalised to tissue weight or protein content.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method described by Reitman and Frankel,[14] which involves the formation of pyruvate or oxaloacetate measured through colorimetric detection with 2,4-dinitrophenylhydrazine.

Alkaline Phosphatase (ALP) activity was measured using a p-nitrophenyl phosphate (pNPP) substrate Tietz,[15] method wherein ALP catalyses the hydrolysis of pNPP to p-nitrophenol, measurable at 405 nm.

Serum albumin (ALB) concentration was measured using the bromocresol green (BCG) dye-binding method, as described by Doumas et al.[16] with absorbance measured at 630 nm.

Serum total protein content was assessed using the Biuret method according to Gornall,[17] which relies on the formation of a complex between copper ions and peptide bonds in an alkaline medium.

Total Bilirubin (TB) and Conjugated Bilirubin (CB) (mg/dL) levels were determined using the Jendrassik-Grof method[18]. Total bilirubin was measured after reaction with diazotised sulfanilic acid in the presence of caffeine, while conjugated bilirubin was measured without caffeine.

All absorbance readings were obtained using a UV-visible spectrophotometer, and the concentrations were calculated using calibration curves prepared with known standards.

Histological Procedure

For histological analysis, liver tissue samples were fixed in 10% neutral buffered formalin (NBF) for 24-48 hours, followed by dehydration in a series of ethanol solutions (70%, 80%, 90%, and 100%) and clearing in xylene. The tissues were then embedded in paraffin wax, sectioned into 5-µm-thick slices using a microtome, and deparaffinised in xylene. The sections were rehydrated in a series of ethanol solutions, stained with Harris' haematoxylin solution for 5-10 minutes,

and then stained with eosin Y solution for 1-2 minutes. After dehydration and clearing, the sections were mounted on glass slides using a mounting medium (DPX) and examined under a light microscope to observe the tissue morphology and architecture.

Statistical Analyses

All data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 8 for Windows. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using the Brown-Forsythe test. Differences among groups were analysed using one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc multiple comparison test. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of Glibenclamide, Catechin, and Neem Leaf Extract on Fasting Blood Glucose Level in Alloxan-Induced Diabetic Rats

The results presented in **Figure 1** demonstrate a significant increase in blood glucose levels in the diabetic group (Group B, 462 ± 0.64) compared to the control Group A (Group A, 82 ± 0.70). Treatment with glibenclamide (Group C), catechin (Group D), and ethanolic neem leaf extract (Group E) resulted in significant reductions in blood glucose levels (240 ± 0.12, 275 ± 0.73, and 199 ± 0.71, respectively) compared with the diabetic group. All values are expressed as mean ± standard error of the mean (SEM), with n = 10 animals per group. * Indicates a statistically significant difference at $P \leq 0.05$ between the groups

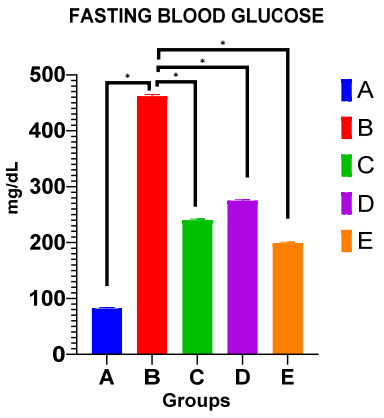

Figure 1: Effect of Glibenclamide, Catechin, and Neem Leaf Extract on Fasting Blood Glucose Level in Alloxan-Induced Diabetic Rats

Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Liver Oxidative Stress Markers in Alloxan - Induced Diabetic Rats

The results shown in **Figure 2** indicate a significant decrease in glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), and total protein (TP) levels, accompanied by a significant increase in malondialdehyde (MDA) levels in the diabetic group (Group B) compared to those in the control Group A. In contrast, treatment groups (Groups C, D, and E), which received glibenclamide, catechin, and neem leaf extract, respectively, showed significant increases in GSH, SOD, CAT, TAC, and TP levels and a significant decrease in MDA levels compared with Group B.

All values are expressed as mean ± standard error of the mean (SEM), with n = 10 animals per group. *Indicates a statistically significant difference at $P \leq 0.05$ between groups. GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; TAC, total antioxidant capacity; MDA, malondialdehyde

Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Liver Function Tests in Alloxan - Induced Diabetic Rats

The results presented in **Table 2** reveal a significant increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and conjugated bilirubin levels, accompanied by a reduction in albumin concentration in the diabetic group (Group B) compared with the control Group A. Treatment groups (Groups C, D, and E) demonstrated a significant decrease in AST, ALT, ALP, total bilirubin, and conjugated bilirubin levels, with a significant increase in albumin levels in Group E compared to Group B.

Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Lipid Profiles in Alloxan - Induced Diabetic Rats

The results illustrated in **Figure 3** show a significant increase in total cholesterol and low-density lipoprotein (LDL) levels, accompanied by a significant reduction in triglyceride and high-density lipoprotein (HDL) levels in the diabetic group (Group B) compared with the control group (Group A). The treatment groups (Groups C, D, and E) exhibited significant reductions in total cholesterol and LDL levels, along with significant increases in triglyceride and HDL levels compared with Group B.

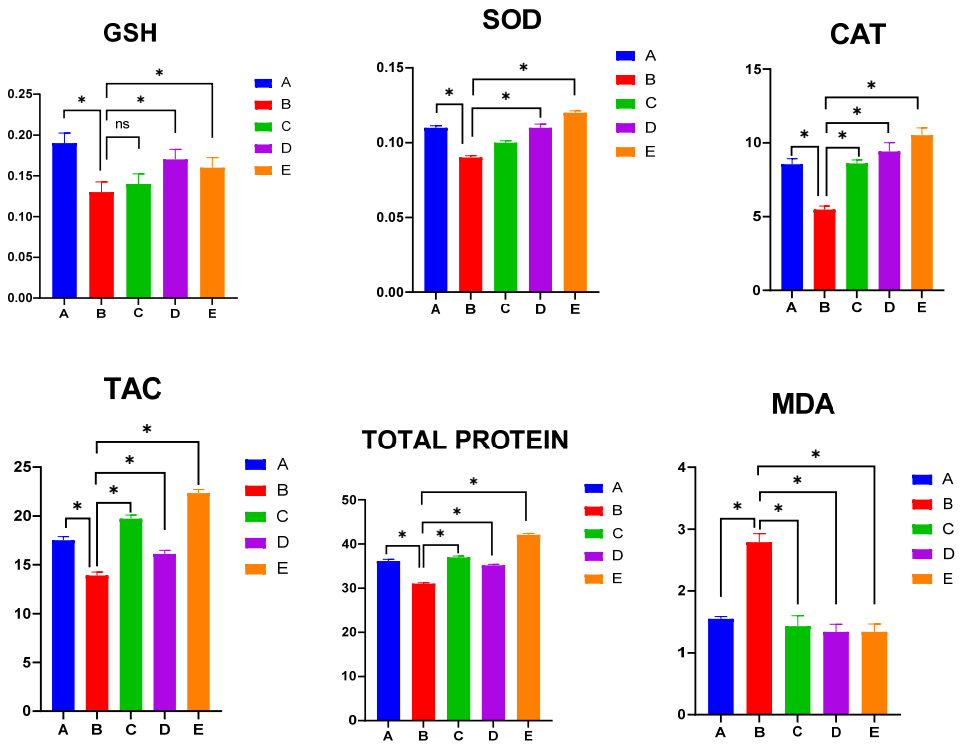

Figure 2: Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Liver Oxidative Stress Markers in Alloxan-Induced Diabetic Rats

Table 2: Effects of glibenclamide, catechin, and neem leaf extract on liver function tests in alloxan-induced diabetic rats

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)	ALB (g/L)	TB (mg/dL)	CB (mg/dL)
A	12±0.71	7±0.71	14±0.17	24.36±0.10	0.21±0.70	0.18±0.58
B	13±0.71*	10±0.71*	16±0.17*	20.5±0.18*	0.32±0.86*	0.3±0.71*
C	12±0.71**	8±0.71**	14±0.18**	20.7±0.13	0.28±0.86	0.2±0.70
D	13±0.71	6±0.71**	15±0.17**	22.7±0.13	0.3±0.71	0.19±0.43**
E	11±0.71**	7±0.11**	12±0.17**	25.26±0.92**	0.28±0.58	0.22±0.26

All values are expressed as mean ± standard error of the mean (SEM), with n = 10 animals per group. * = p<0.05 when compared with group A ** = p<0.05 when compared with group B. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ALB, albumin; TB, total bilirubin; CB, conjugated bilirubin.

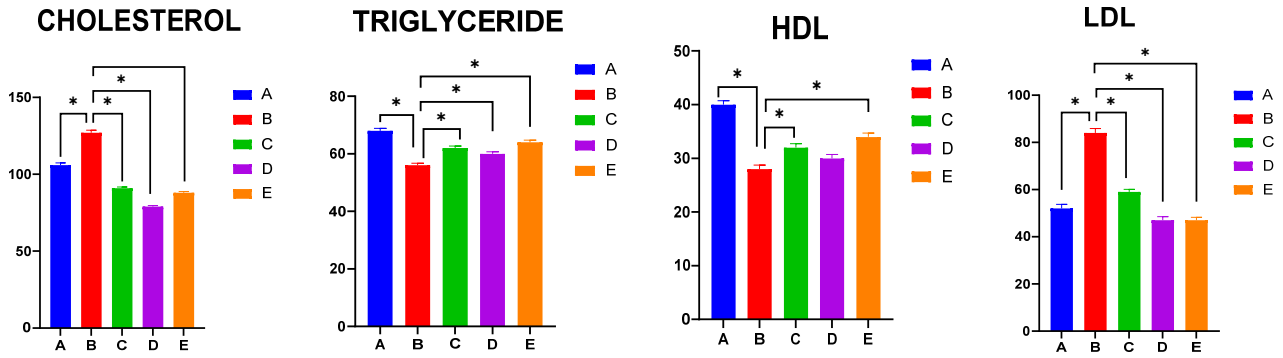

Figure 3: Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Lipid Profiles in Alloxan-Induced Diabetic Rats

All values are expressed as mean ± standard error of the mean (SEM), with n = 10 animals per group. * Indicates a statistically significant difference at P ≤ 0.05 between groups. HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Liver Histology in Alloxan - Induced Diabetic Rats

The results presented in **Figure 4** below show:

- The control group exhibited well-differentiated and organised hepatocytes (yellow circle), alongside the central vein (black thick arrow) and sinusoids (black thin arrow) containing Kupffer cells.
- The diabetes group showed dilated sinusoids (black thin arrow), a clogged central vein (black thick arrow), and uneven hepatocyte distribution (yellow circle).
- The group treated with glibenclamide showed a severely clogged central vein (black thick arrow), dilated sinusoids (black thin arrow), and a yellow circle representing the loss of hepatocytes and Kupffer cells.
- The group treated with catechin exhibited dilated sinusoids (black thin arrow), a congested central vein (black thick arrow), loss of Kupffer cells, and an uneven distribution of hepatocytes (yellow circle).
- The neem leaf extract-treated group exhibited sinusoids with Kupffer cells (black thin arrow), hepatocytes (yellow circle), and a clear central vein (black thick arrow). H/E X 400

DISCUSSION

Diabetes mellitus is a multifactorial metabolic disorder associated with impaired glucose metabolism and systemic complications that affect major organs, including the liver. The liver plays a central role in glucose and lipid homeostasis and is a major target of oxidative stress and inflammation in diabetes. While similar therapeutic agents were evaluated in our previous study on pancreatic β-cell regeneration[8], the present investigation focused on hepatic biochemical and histopathological outcomes, thereby providing additional insight into the systemic protective effects of these treatments in diabetes.

Alloxan induces diabetes by generating reactive oxygen species (ROS) in pancreatic β-cells, leading to selective cytotoxicity and insulin deficiency in the pancreas. This process resulted in elevated fasting blood glucose levels, as shown in **Figure 1**. Uncontrolled hyperglycaemia is a key driver of diabetic complications, including hepatic steatosis, oxidative stress, and inflammation[19]. Treatment with glibenclamide, catechin, and neem leaf extract significantly reduced hyperglycaemia. Glibenclamide stimulates insulin secretion by closing ATP-sensitive potassium channels on β-cells, whereas catechin and neem exert antidiabetic effects via antioxidant mechanisms, enhancement of insulin sensitivity,

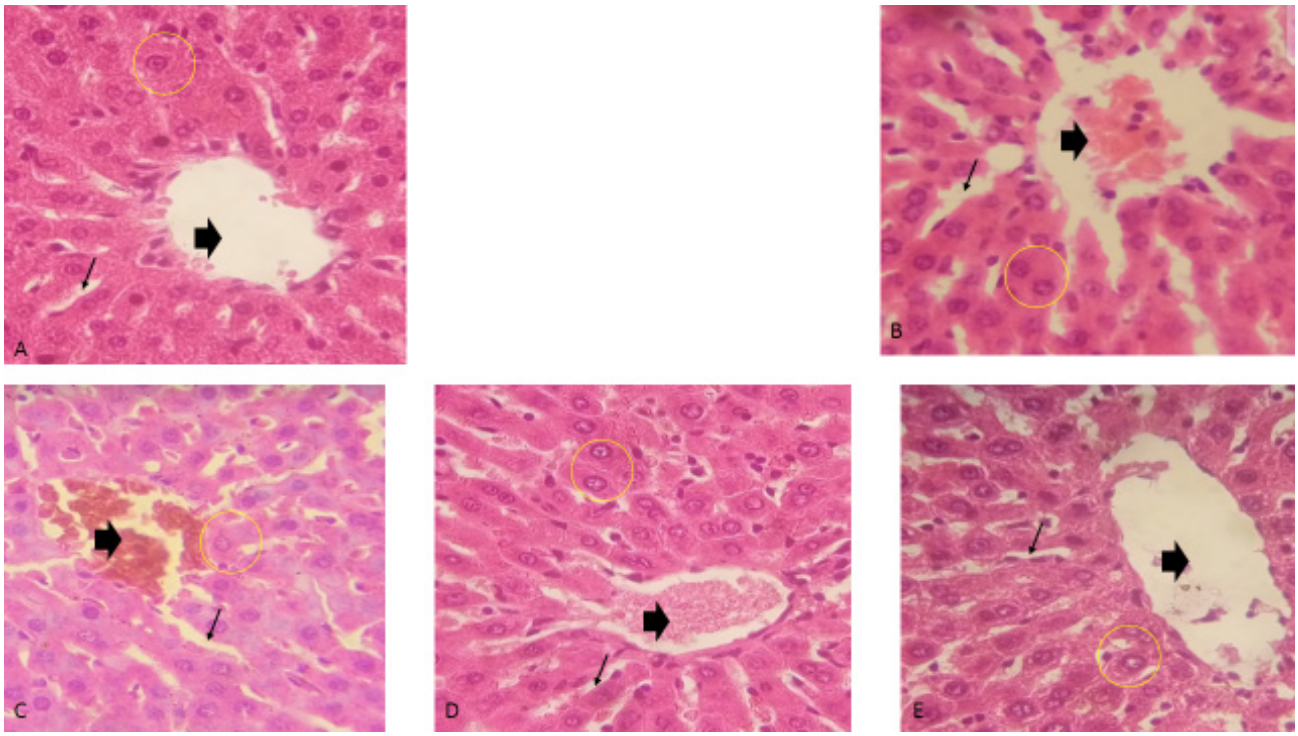

Figure 4: Effects of Glibenclamide, Catechin, and Neem Leaf Extract on Liver Histology in Alloxan-Induced Diabetic Rats

and modulation of glucose transporter expression, according to previous studies[20,21].

Oxidative stress is a major contributor to hepatic dysfunction in patients with diabetes. As shown in **Figure 2**, Group B (diabetic) exhibited significant reductions in antioxidant defences, including glutathione, superoxide dismutase, catalase, total antioxidant capacity, and total protein, accompanied by elevated malondialdehyde, a marker of lipid peroxidation. These findings are consistent with those of a previous study showing that diabetes causes severe oxidative damage within hepatic tissues, impairing mitochondrial function and promoting hepatocyte apoptosis[22]. Treatment with glibenclamide, catechin, and neem restored antioxidant enzyme activity and reduced MDA levels, with neem exhibiting the most pronounced effects. Neem's hepatoprotective efficacy is attributed to its polyphenolic content, especially nimbin and nimbolide, which can activate the Nrf2 pathway, thereby upregulating endogenous antioxidant genes and mitigating ROS-induced injury, as reported by Chattopadhyay[23]. The increase in TP levels may suggest improved liver synthetic function and reduced protein degradation due to the effects of oxidative stress.

Serum levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, albumin, which primarily reflects hepatic synthetic capacity, total bilirubin, and conjugated bilirubin serve as biochemical markers of hepatocellular integrity and biliary function. Elevated levels of AST, ALT, ALP, and bilirubin were observed **(Table 2)** in the diabetic group (Group B), which may indicate hepatocellular injury and impaired excretory function, common in diabetic hepatopathy, as supported by a study by Teshome[24]. Reduced serum albumin levels may suggest compromised hepatic protein synthesis rather than direct hepatocellular injury. Post-treatment reductions in AST, ALT, ALP, and bilirubin, as seen in groups C, D, and E, may demonstrate improved hepatocellular function and integrity, while improvement in albumin levels may indicate restoration of hepatic synthetic function. The superior performance of neem leaf extract highlights its dual role in protecting hepatocytes and restoring liver metabolism. This suggests that neem not only limits hepatocyte death but also promotes regenerative processes, likely through the modulation of inflammatory cytokines and oxidative pathways[25].

Dyslipidaemia is a hallmark of diabetes, often characterised by elevated total cholesterol and LDL levels, along with decreased HDL and triglyceride levels. These alterations **(Figure 3)** observed in the diabetic group contribute to fatty liver disease and atherosclerosis. These mechanisms include enhanced hepatic lipogenesis, impaired LDL clearance, and insulin resistance-mediated inhibition of lipoprotein lipase. Treatment with glibenclamide, catechin, and neem improved the lipid profiles. Neem leaf extract was particularly effective in lowering cholesterol and LDL levels while elevating HDL and triglyceride levels. This lipid-modulating effect may be due to improved insulin action, decreased hepatic lipogenesis, and enhanced lipid oxidation. Neem bioactives are known to influence lipid metabolism genes and possess antihyperlipidemic properties[23].

Liver histology provides morphological confirmation of biochemical findings. As seen in **Figure 4**, the diabetic group exhibited disrupted hepatic architecture: dilated sinusoids, congested central veins, and irregular hepatocyte arrangement, indicative of steatosis, inflammation, and vascular injury. Glibenclamide and catechin groups showed partial improvement but still had congestion and loss of Kupffer cells. The neem-treated group, however, demonstrated near-normal histoarchitecture, including intact hepatocytes, clear central veins, and preserved Kupffer cells. Kupffer cells play a critical role in clearing pathogens and maintaining hepatic immune homeostasis; their preservation reflects neem's immunomodulatory and cytoprotective properties[25].

CONCLUSION

This study demonstrates that diabetes-induced hepatic dysfunction is multifactorial, involving oxidative stress, enzymatic leakage, disrupted lipid metabolism, and structural damage. Glibenclamide partially mitigates these effects via glycaemic control but does not address oxidative damage directly. Catechin offers antioxidant benefits but is less effective than neem. Neem leaf extract stands out as a comprehensive hepatoprotective agent. Its benefits span glucose regulation, antioxidant restoration, enzymatic stabilisation, lipid modulation, and structural preservation. Physiologically, these effects may slow the progression of diabetic hepatopathy. The ability to restore both functional and morphological integrity of the liver underscores neem's potential as a natural therapeutic for diabetic hepatopathy.

LIMITATION

This study employed descriptive histological evaluation to assess hepatic architecture and cellular

integrity across experimental groups. While the qualitative observations provided valuable insight into structural alterations and treatment-associated improvements, the absence of a standardised histopathological scoring system may limit objectivity, reproducibility, and quantitative comparison between groups. Future studies incorporating validated liver injury scoring indices, such as semi-quantitative grading of necrosis, inflammation, and steatosis, would strengthen data interpretation and enhance comparability with existing literature.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study received no external funding.

AUTHOR CONTRIBUTIONS

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Supervision: Olusoji A. Oyesola
All authors read and approved the final manuscript.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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