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Effect of glibenclamide, catechin and ethanolic neem leaf extract on pacreatic beta cell regeneration in alloxan-induced diabetic rat

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ABSTRACT

Introduction. Type 1 diabetes mellitus is characterized by the destruction of pancreatic β-cells, leading to insulin deficiency and persistent hyperglycemia. This study investigates the regenerative potential of glibenclamide, catechin, and ethanolic neem leaf extract on β-cell function and architecture in alloxan-induced diabetic rats.

Material and methods. Thirty-five male Sprague-Dawley rats were divided into five groups: normal control (Group A), diabetic control (Group B), glibenclamide-treated (Group C), catechin-treated (Group D), and ethanolic neem leaf extract -treated (Group E). Diabetes was induced using alloxan monohydrate (150 mg/kg, i.p.), and treatments were administered orally for 14 days.

Results and conclusions. Biochemical analysis revealed marked hyperglycemia and hypoinsulinemia in diabetic controls, alongside elevated oxidative stress (↑MDA, ↓GSH, SOD, CAT, TAC, TP) and inflammatory markers (NF-κB, IL-6). Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly ameliorated these disturbances, with neem producing the most notable improvements. Ethanolic neem leaf extract -treated rats showed near-normal insulin levels, enhanced antioxidant status, and suppressed inflammatory responses. Furthermore, key regenerative markers (IGF-1, GLP-1, EGF, HGF, and betatrophin) were favorably modulated, particularly in the neem group, indicating stimulation of β-cell neogenesis and survival pathways. Histological examination supported the biochemical findings: ethanolic neem leaf extract-treated pancreases exhibited well-preserved islets and restored tissue architecture, contrasting with the degenerative features seen in diabetic controls. These findings suggest that ethanolic neem leaf extract, beyond its hypoglycemic and antioxidant effects, promotes β-cell regeneration through anti-inflammatory and growth factor-mediated mechanisms. This positions neem as a promising phytotherapeutic agent for diabetes management and β-cell restoration.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among its major forms, type 1 diabetes is marked by autoimmune-mediated destruction of pancreatic β-cells, while type 2 involves progressive β-cell dysfunction alongside insulin resistance [1]. A central pathological hallmark of both forms is the impairment or loss of pancreatic β-cell mass, which compromises insulin production and glucose homeostasis [2].

In type 1 diabetes, chronic oxidative stress and inflammation contribute significantly to pancreatic β-cell destruction, impairing their survival and regenerative capacity. Markers such as Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Total Antioxidant Capacity (TAC), and Total Protein (TP) reflect the oxidative balance, with elevated MDA and reduced antioxidant enzymes indicating oxidative damage [3,4]. Inflammatory cytokines like Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Interleukin-6 (IL-6) further exacerbate β-cell apoptosis and insulin dysfunction [5,6]. Additionally, regenerative and hormonal factors—Insulin-like Growth Factor 1 (IGF-1), Glucagon-like Peptide 1 (GLP-1), Epidermal Growth Factor (EGF), Hepatocyte Growth Factor (HGF), and betatrophin—are vital in promoting β-cell proliferation, differentiation, and function [7–9]. Evaluating these markers is crucial, as they directly influence β-cell loss and recovery.

Alloxan, a urea derivative, is widely employed in experimental models to induce type 1-like diabetes by selectively destroying insulin-producing β-cells via reactive oxygen species (ROS) generation and oxidative stress [10]. This oxidative mechanism mirrors key aspects of human diabetic pathology, making it suitable for evaluating β-cell regeneration and protective interventions.

Current antidiabetic drugs, such as glibenclamide—a sulfonylurea—stimulate insulin secretion by enhancing residual β-cell activity [11]. Phytochemicals with antioxidant, anti-inflammatory, and cytoprotective properties have attracted attention for their potential in β-cell preservation and regeneration. Catechin, a flavonoid found in green tea and cocoa, has demonstrated β-cell protective effects by mitigating oxidative damage and enhancing insulin secretion [12]. Similarly, ethanolic neem (*Azadirachta indica*) leaf extract, rich in bioactive compounds like nimbolide and nimbin—both classified as limonoids—exhibits antidiabetic, antioxidant, and pancreatic regenerative properties [13,14].

This study investigates and compares the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic β-cell regeneration in alloxan-induced diabetic rats. By integrating biochemical, histological, and functional assessments, this research aims to elucidate the regenerative potential of these agents, offering insight into novel therapeutic avenues for restoring pancreatic function in diabetes.

Material and methods

Thirty-five mature male Sprague-Dawley rats, seven weeks old were utilized for this study. The rats were given unrestricted access to food and water and were housed in plastic cages under typical laboratory conditions with a 12-hour day/12-hour night cycle. Ethical approval for the use of animal for research was obtained from Olabisi Onabanjo University Teaching Hospital Human Research Ethics Committee (OOUTH-HREC) with the number OOUTH/HREC/010//026/E120/2024AP. Strict adherence was made to all criteria regarding the use and care of laboratory animals. After the induction of diabetes, the animals were weighted and randomly assigned to groups followed by treatment for a period of 14 days as showed in the **Table 1**.

Plant material collection, preparation and extraction

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

The extraction was carried out according to the method of Nazir et al. [19] using 100 g of the leave powder dissolved in 500 ml of 99% ethanol for 3 days kept in the refrigerator with periodic shaking. The resulting mixture was then filtered using muslin cloth followed by whatman filter

Table 1. Animal grouping and experimental design.

	Group	No of rats	Treatment	References
A	Normal control	7	Distilled water only	Yakubu et al., [15]
B	Diabetes control	7	Alloxan (150 mg/kg bw) i.p	Yakubu et al., [15]
C	Glibenclamide treatment group	7	Alloxan (150 mg/kg bw) i.p + glibenclamide (5 mg/kg bw) p.o	Shan & Khan, [16]
D	Catechin treatment group	7	Alloxan (150 mg/kg bw) i.p + CTN (40 mg/kg bw) p.o	Nazir et al., [17]
E	Ethanolic neem leaf extract treatment group	7	Alloxan (150 mg/kg bw) i.p + neem leave extract (250 mg/kg bw) p.o	Dholi et al., [18]

p.o – orally; i.p – intraperitoneal; bw – body weight

paper. The filtrate was concentrated into a semi-solid mass at 40 °C under reduced pressure in the rotary evaporator. The concentrate was reconstituted in 70%ethanolto prepare the dose to be used for the study.

Induction of diabetes

The induction of diabetes was done according to the method described by Yakubu et al., [15]. Stock solution of alloxan monohydrate was prepare by dissolving alloxan monohydrate (0.9 g) in 3 ml of distilled water to give a stock concentration of approximately 150 mg/0.5 ml. Diabetes was induced by single i.p administration of alloxan monohydrate (150 mg/kg b.w.). The rats with blood glucose level greater than 200 mg/dl, 72 hours post-induction, was considered diabetic and used for this research work.

Preparation and administration of Glibenclamide

10 mg glibenclamide tablet was crushed and dissolved in 5 ml of distilled water to give a concentration of 2 mg/ml. 5 mg/kg bw dose of glibenclamide was administered from the stocked solution according to Shan & Khan [16].

Preparation and administration of Catechin

160 mg catechin (Central Drug House Ltd. India) was dissolved in 10 ml of distilled water to give a concentration of 8 mg/ 0.5 ml. 40 mg/kg bw dose of catechin was administered from the stocked solution according to Nazir et al. [17].

Preparation and administration of ethanolic neem leaf extract

4.9 g of ethanolic neem leaf extract was dissolved in 49 ml of 70% ethanol to give a concentration of 50 mg/ 0.5 ml. 250 mg/kg bw dose of ethanolic neem leaf extract was administered from the stocked solution according to Dholi et al. [18].

Measurement of fasting blood glucose

Fasting blood glucose was determined with a drop of blood from the rat tail using a glucometer (Accu-Check, Roche, Germany), after an overnight fast of 14 hours every 48 hours.

Procedure for blood collection

Blood was collected into sample bottle from the retro-orbital plexus using heparinized capillary tube according to the method of Diehl et al. [20].

Animal sacrifice and determination of organ weight

The animals were sacrificed by cervical dislocation after the expiration of research. The organs of study were harvested following midline abdominal incision, the organs weight was determined using a weighing scale.

Determination pancreatic tissue Antioxidant Enzymes Activity

Approximately 0.1 g of pancreatic tissue was excised and immediately washed with ice-cold saline to remove blood residues. The tissues were then homogenized in 4 mL of phosphate buffer solution (PBS; 0.1 M, pH 7.2). The homogenates were centrifuged at 3,000 rpm for 10 minutes at 4 °C using a refrigerated centrifuge (Thermo Scientific™ Heraeus™ Megafuge™ 16R, USA). The resulting supernatants were aliquoted and stored at -80 °C until further analysis.

GSH levels were determined using the Cayman Chemical Glutathione Assay Kit (Cat. No. 703002, USA), based on the reaction of GSH with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) forming a yellow-colored product measurable at 405

nm. Absorbance was read using a microplate reader (BioTek Epoch™, USA).

SOD activity was assayed using the Cayman Chemical Superoxide Dismutase Assay Kit (Cat. No. 706002). This method employs xanthine oxidase to generate superoxide radicals, which then react with a tetrazolium salt. SOD inhibits this reaction, and activity is inversely proportional to absorbance at 450 nm.

Catalase activity was measured with the Cayman Chemical Catalase Assay Kit (Cat. No. 707002). This kit uses the peroxidatic function of catalase to react with methanol in the presence of H_2O_2, forming formaldehyde, which is colorimetrically detected at 540 nm.

TAC was determined using the Abcam Total Antioxidant Capacity Assay Kit (AB65329, UK). This kit measures antioxidant capacity via the reduction of Cu^{2+} to Cu^{+} by antioxidants present in the sample, with absorbance measured at 570 nm.

Total protein concentration was quantified using the Bradford Protein Assay Kit (Bio-Rad, Cat. No. 5000006). The assay is based on the binding of Coomassie Brilliant Blue G-250 to proteins, forming a complex detectable at 595 nm.

MDA levels, an index of lipid peroxidation, were measured using the TBARS Assay Kit (Cayman Chemical, Cat. No. 10009055). This assay detects the MDA-TBA adduct, with absorbance read at 532 nm.

Quantification of pancreatic tissue Inflammatory Markers [Nuclear Factor kappa B (NF-κB), Interleukin-6 (IL-6)]

Levels of NF-κB in pancreatic tissue were quantified using a Rat NF-κB p65 ELISA Kit (Elabscience®, Cat. No. E-EL-R0676, USA). The assay is based on the sandwich ELISA principle using pre-coated 96-well plates with an NF-κB-specific capture antibody. After adding standards and samples, a biotinylated detection antibody was applied, followed by HRP-conjugated streptavidin and TMB substrate. Absorbance was measured at 450 nm using a microplate spectrophotometer (BioTek Epoch™, Agilent Technologies, USA). Concentrations were calculated against a standard curve.

Pancreatic IL-6 concentrations were determined using a Rat IL-6 ELISA Kit (Elabscience®, Cat. No. E-EL-R0015, USA). Following the manufacturer's protocol, standards and samples were incubated in wells pre-coated with a rat IL-6 monoclonal antibody. Detection was performed using biotin-conjugated antibodies and HRP-streptavidin, followed by TMB substrate development. The reaction was terminated with stop solution, and absorbance was recorded at 450 nm. Values were derived from a standard curve.

Determination of Betatrophin, IGF-1, GLP-1, EGF, and HGF in Pancreatic Tissue Homogenates Using ELISA

The quantification of pancreatic regeneration-associated proteins, including Betatrophin, Insulin-like Growth Factor-1 (IGF-1), Glucagon-like Peptide-1 (GLP-1), Epidermal Growth Factor (EGF), and Hepatocyte Growth Factor (HGF), was performed using enzyme-linked immunosorbent assay (ELISA) on pancreatic tissue homogenates.

Pancreatic tissues were excised promptly post-sacrifice, rinsed in ice-cold physiological saline to remove excess blood, and blotted gently on sterile filter paper. Approximately 0.1 mg of each tissue sample was homogenized in 4 mL of cold phosphate buffer solution (PBS, 0.1 M, pH 7.2) under ice-cold conditions to preserve protein integrity. The homogenates were then centrifuged at 3,000 rpm for 10 minutes at 4 °C using a refrigerated centrifuge (Thermo Scientific™ Heraeus™ Megafuge™ 16R). The resulting supernatants were carefully collected and stored at -80 °C until further analysis.

ELISA assays were carried out using rat-specific commercial kits according to the manufacturer's instructions. The following ELISA kits were used: Rat Betatrophin ELISA Kit (Elabscience®, Cat. No. E-EL-R2543), Rat IGF-1 ELISA Kit (Elabscience®, Cat. No. E-EL-R0022), Rat GLP-1 ELISA Kit (Cloud-Clone Corp., Cat. No. CEB887Ra), Rat EGF ELISA Kit (R&D Systems, Cat. No. RGE00), and Rat HGF ELISA Kit (Elabscience®, Cat. No. E-EL-R0713). These kits utilize the sandwich ELISA technique, wherein samples were loaded into 96-well microplates pre-coated with specific monoclonal antibodies against the target analytes.

After incubation with biotinylated detection antibodies and horseradish peroxidase (HRP)-conjugated streptavidin, tetramethylbenzidine

(TMB) substrate was added to induce a colorimetric reaction. The reaction was stopped with an acidic stop solution, and absorbance was read at 450 nm using a microplate reader (BioTek Epoch™, Agilent Technologies, USA). Concentrations of each analyte were calculated from standard curves generated using the supplied standards in each kit. All measurements were conducted in duplicates to ensure reproducibility and accuracy.

Histological Procedure for Pancreatic Tissue

For histological analysis, 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 deparaffinized in xylene. The sections were rehydrated in a series of ethanol solutions, stained with Harris' hematoxylin 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 tissue morphology and architecture.

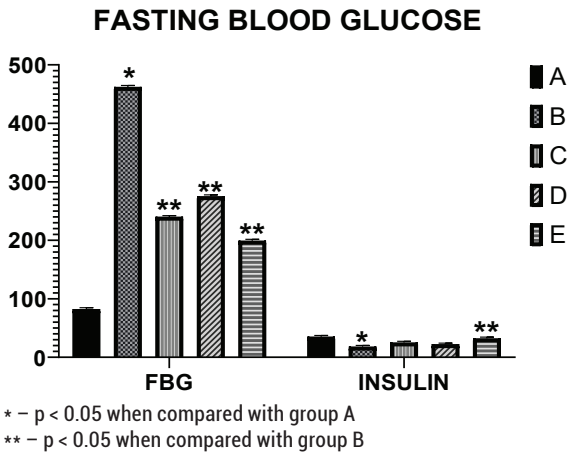
Statistical analysis

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using graph pad prism version 8 for windows. Differences between groups were analyzed by one-way anova followed by Bonferronipost-hoc test. Differences were considered significant when $p < 0.05$.

Results

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on glucose homeostasis in Alloxan-induced diabetic rat

Figure 1 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on glucose homeostasis in alloxan-induced diabetic rats. The results revealed a significant increase in blood glucose levels (462 ± 2.64) and a significant decrease in insulin level (18 ± 2.3) in the diabetic group B compared to the control group A (82 ± 2.70; 35 ± 2.2). Conversely, treatment with glibenclamide (group C), catechin (group D), and ethanolic neem leaf extract (group E) resulted in a significant decrease in blood glucose levels (240 ± 2.12, 275 ± 1.73, and 199 ± 2.71, respectively) compared to group B (462 ± 2.64) And a significant increase in insulin level in group E (32 ± 2.4) when compared with group B. All the values are expressed as mean ± standard error of mean (SEM).



* – $p < 0.05$ when compared with group A
** – $p < 0.05$ when compared with group B

Figure 1. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on glucose homeostasis in Alloxan-induced diabetic rat.

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Oxidative Stress Markers in Alloxan-induced diabetic rat

The results presented in **Table 2** demonstrate the effect of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic tissue oxidative stress markers in alloxan-induced diabetic rat. A significant decrease in GSH, SOD, and CAT activities, as well as TAC and TP levels, was observed in the diabetic group (Group B) compared to the control group (Group A). Conversely, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant increase in antioxidant enzyme activities (GSH, SOD, and CAT), TAC, and TP levels. Additionally, a significant decrease in MDA levels was noted in the treatment groups. All the values are expressed as mean ± standard error of mean (SEM).

Table 2. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Oxidative Stress Markers in Alloxan-induced diabetic rat.

Groups	GSH	SOD	CAT	TAC	MDA	TP
A	0.94 ± 0.11	0.86 ± 0.14	7.42 ± 0.88	8.89 ± 0.85	1.11 ± 0.57	36.14 ± 0.11
B	0.46 ± 0.71*	0.51 ± 0.79*	6.01 ± 0.91*	6.99 ± 0.65*	1.95 ± 0.82*	32.38 ± 0.58*
C	0.12 ± 0.78**	0.11 ± 0.13**	7.38 ± 0.88**	10.21 ± 0.96**	1.14 ± 0.64**	36.52 ± 0.37**
D	0.15 ± 0.71**	0.16 ± 0.14**	7.47 ± 0.87**	9.05 ± 0.78**	1.01 ± 0.63**	36.94 ± 0.51**
E	0.12 ± 0.60**	0.14 ± 0.94**	7.41 ± 0.91**	10.92 ± 0.93**	0.99 ± 0.58**	37.94 ± 0.51**

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM).

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Inflammatory Markers in Alloxan-induced diabetic rat

Table 3 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic tissue inflammatory markers, specifically NF-κB and IL-6 in Alloxan-induced diabetic rat. The results indicate a significant increase in NF-κB and IL-6 levels in the diabetic group (Group B) compared to the control group (Group A). In contrast, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant decrease in NF-κB and IL-6 levels compared to Group B.

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Beta Cell Regeneration Factors in Alloxan-induced diabetic rat

Table 4 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic beta cell regeneration factors in Alloxan-induced diabetic rat. The results indicate a significant increase in betatrophin and HGF levels, alongside a significant decrease in IGF-1, GLP-1, and EGF levels, in the diabetic group (Group B) compared to the control group (Group A). Conversely, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant decrease

Table 3. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Inflammatory Markers in Alloxan-induced diabetic rat.

Groups	NF-kB	IL-6
A	1.56 ± 0.71	144.9 ± 0.51
B	3.52 ± 0.37*	524.7 ± 0.75*
C	1.98 ± 0.93**	330.3 ± 0.51**
D	0.77 ± 0.51**	284.4 ± 0.20**
E	1.58 ± 0.51**	226.5 ± 0.32**

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM)

Table 4. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Beta Cell Regeneration Factors in Alloxan-induced diabetic rat.

Groups	Betatrophin	IGF-1	GLP-1	EGF	HGF
A	6.7 ± 0.3	12.4 ± 0.24	14.9 ± 0.46	0.92 ± 0.08	1.2 ± 0.26
B	12.2 ± 0.37*	5.2 ± 0.20*	7.11 ± 0.24*	0.22 ± 0.13*	2.8 ± 0.26*
C	10.7 ± 0.49	7.9 ± 0.46**	11.2 ± 0.37**	0.53 ± 0.24**	2.1 ± 0.24**
D	11.52 ± 0.33	6.42 ± 0.26	8.3 ± 0.30	0.81 ± 0.20**	2.2 ± 0.37**
E	8.5 ± 0.31**	8.21 ± 0.20**	10.2 ± 0.37**	0.72 ± 0.27**	1.7 ± 0.30

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM).

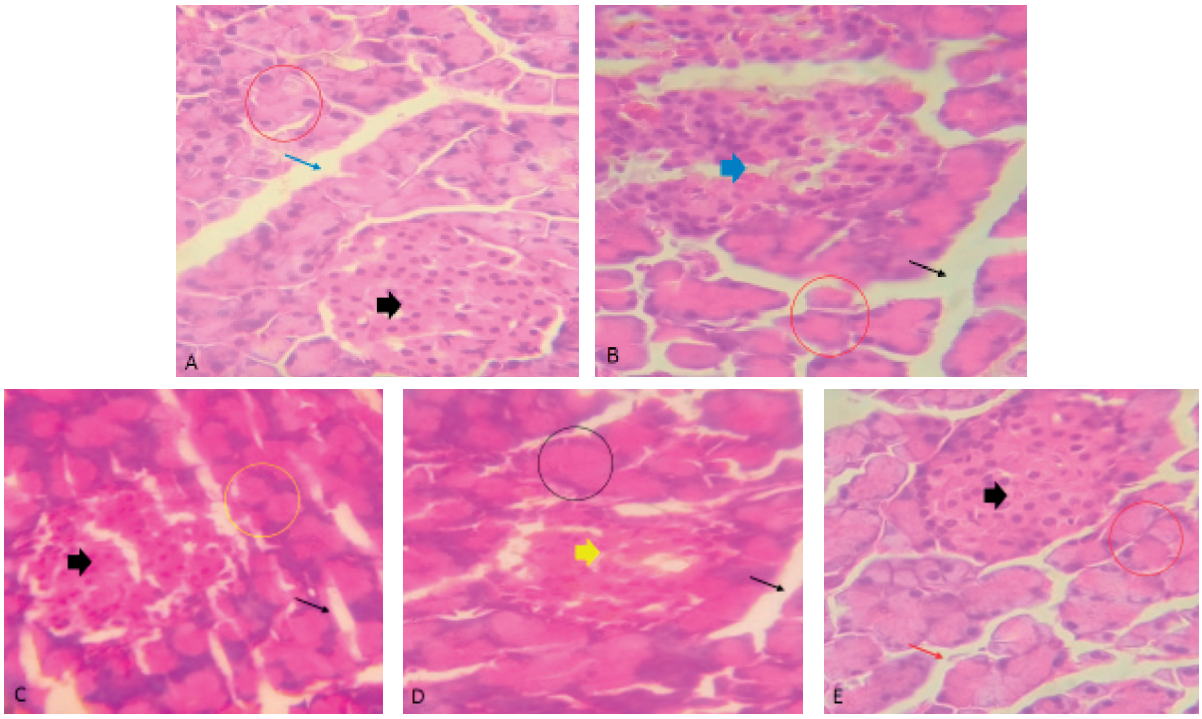


Figure 2. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic tissue histology Alloxan-induced diabetic rat. Photomicrograph of pancreatic histology showing: A – Control group showing a well differentiated and organized acinar cells (red circle), interlobular ducts (blue thin arrow) and islets of langerhans (black thick arrow); B – Diabetes shows degenerated islets of Langerhans (blues thick arrow), dilated interlobular ducts and irregular distributions of the acinar cells (red circle); C – Glibenclamide treated group shows congested interlobular ducts (black thin arrow), degenerated islets of Langerhans (black thick arrow) and acinar cells (yellow circle); D – Catechintreaed g roup shows congested and degenerated islets of Langerhans (yellow thick arrow), interlobular ducts (black thin arrow) and acinar cells (black circle); E – Ethanolic neem leaf extracttreated shows well differentiated islets of Langerhans (black thick arrow), acinar cells (red circle) and interlobular ducts(red thin arrow). H/E X 400.

in betatrophin and HGF levels, as well as a significant increase in IGF-1, GLP-1, and EGF levels, compared to Group B.

Discussion

Diabetes mellitus, particularly Type 1 diabetes, is marked by the selective destruction of pancreatic β-cells, resulting in insulin deficiency and persistent hyperglycemia. Alloxan, a toxic glucose analogue, is frequently used to induce experimental diabetes in animals due to its specific cytotoxicity to β-cells through oxidative stress mechanisms. This study examines the potential regenerative effects of glibenclamide, catechin, andethanolic neem (*Azadirachta indica*) leaf extract on β-cell function and integrity in alloxan-induced diabetic rats. Through a multifaceted physiological assessment—encompassing glucose-insulin homeostasis, oxidative stress, inflammatory markers, β-cell regenerative factors, and histological observations—the study offers deep insights into therapeutic prospects for restoring pancreatic function in diabetes.

Hyperglycemia and hypoinsulinemia are cardinal features of diabetes. In this study, Group B (diabetic rats) exhibited a significant elevation in blood glucose (462 ± 2.64 mg/dL) and a corresponding decrease in insulin levels (18 ± 2.3 µIU/mL), reflecting β-cell destruction and impaired insulin secretion. Alloxan'sdiabetogenicity stems from its selective accumulation in pancreatic β-cells via GLUT2 transporters, where it generates reactive oxygen species (ROS), leading to DNA fragmentation and apoptosis [10].

Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly lowered blood glucose and increased insulin levels, with neem (group E) showing insulin levels nearing the control (32 ± 2.4 µIU/mL). Glibenclamide, a sulfonylurea, enhances insulin release by inhibiting ATP-sensitive K^+ channels on β-cells [21] while catechin—a flavonoid—exerts antioxidant effects and

improves insulin sensitivity [22]. Neem's superior efficacy likely arises from its multifaceted action, including β-cell protection, enhancement of insulin secretion, and peripheral insulin sensitivity [23].

Oxidative stress plays a critical role in β-cell dysfunction due to their inherently low levels of antioxidant enzymes. In diabetic rats, significant reductions in GSH (glutathione), SOD (superoxide dismutase), CAT (catalase), total antioxidant capacity (TAC), and total protein (TP) were observed, alongside increased MDA (malondialdehyde), a marker of lipid peroxidation. These findings reflect profound oxidative damage in pancreatic tissues.

All three treatments reversed oxidative damage by elevating endogenous antioxidant enzyme activities and reducing MDA levels, indicating restored redox homeostasis. Catechin and ethanolic neem leaf extract are particularly potent in activating the Nrf2 pathway, a master regulator of antioxidant gene expression [24]. Neem's efficacy may also be attributed to its polyphenolic compounds, such as nimbolide and quercetin, which directly scavenge ROS and inhibit oxidative stress-induced β-cell apoptosis [25].

Chronic inflammation exacerbates β-cell damage in diabetes. The diabetic group displayed significant elevations in NF-κB (nuclear factor kappa B) and IL-6 (interleukin-6), two key inflammatory mediators involved in β-cell dysfunction and apoptosis. NF-κB, when activated by oxidative stress, promotes transcription of pro-inflammatory cytokines like IL-6, thereby perpetuating cellular injury.

Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly downregulated NF-κB and IL-6 levels. While glibenclamide shows modest anti-inflammatory action, catechin and neem offer more robust effects through direct inhibition of inflammatory signaling pathways. Neem's anti-inflammatory potency has been linked to inhibition of NF-κB activation, thereby reducing downstream cytokine production and preserving islet architecture [13,23].

A unique aspect of this study is its focus on β-cell regenerative markers: betatrophin, hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), glucagon-like peptide-1 (GLP-1), and epidermal growth factor (EGF). The diabetic group showed an increase in betatrophin and HGF, but a decrease in IGF-1, GLP-1, and EGF. Betatrophin and HGF upregulation may represent a compensatory but ineffective attempt at β-cell regeneration in response to injury [26].

Treatment groups showed normalized or reduced betatrophin and HGF levels, alongside increases in IGF-1, GLP-1, and EGF—markers associated with effective β-cell neogenesis, survival, and proliferation. IGF-1 promotes β-cell mass expansion through PI3K/Akt signaling, while GLP-1 enhances β-cell proliferation and reduces apoptosis [27]. EGF stimulates islet cell regeneration and has been shown to restore β-cell architecture in injured pancreases. Neem's ability to significantly boost these regenerative markers indicates its potential in restoring endocrine pancreatic function beyond mere symptom control.

Histopathological assessment of pancreatic tissue corroborated biochemical findings. Diabetic rats displayed disrupted islets of Langerhans, dilated ducts, and distorted acinar cells—consistent with alloxan-induced cellular degeneration. The glibenclamide and catechin groups showed partial restoration but retained signs of congestion and islet degeneration.

Strikingly, neem-treated rats exhibited near-normal pancreatic histology, with well-defined islets of Langerhans, intact acinar architecture, and preserved ductal structures. This morphological recovery aligns with the observed upregulation of regenerative and anti-inflammatory factors, indicating that neem not only protects but also actively restores β-cell architecture and function.

The physiological relevance of these findings lies in their demonstration that β-cell regeneration is a viable therapeutic target in diabetes management. The reversal of hyperglycemia, restoration of insulin secretion, normalization of redox and inflammatory states, and upregulation of pro-regenerative factors collectively suggest that ethanolic neem leaf extract offers a promising, multi-targeted approach to diabetes therapy. Unlike glibenclamide, which primarily enhances existing β-cell function, and catechin, which offers antioxidant support, neem exhibits both protective and regenerative capacities.

Such comprehensive β-cell restoration could translate into prolonged remission of diabetes symptoms, reduced dependence on exogenous insulin, and prevention of long-term complica-

tions. These findings underscore the potential of phytotherapeutics like neem as adjuncts or alternatives to conventional anti-diabetic drugs, especially in resource-limited settings.

Conclusion

This physiological study reveals the potential of glibenclamide, catechin, and ethanolic neem leaf extract in reversing pancreatic damage in alloxan-induced diabetic rats. While all treatments offer benefits, ethanolic neem leaf extract stands out for its ability to restore glucose-insulin balance, enhance antioxidant and anti-inflammatory responses, and stimulate β-cell regeneration both biochemically and histologically. These findings support neem's traditional use in diabetes and warrant further preclinical and clinical studies to explore its integration into mainstream diabetes management.

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Conflict of interest statement
The authors declare no conflict of interest.

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