Antidiabetic and antioxidant effects of methanol extract and fractions of *Glyphaea brevis* (Spreng.) Monachino in alloxan-induced diabetic rats

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ABSTRACT

Background: *Glyphaea brevis,* which has been reported to show considerable antibacterial, anti inflammatory, and antioxidant activities, was mentioned as a recipe for diabetes in our recent ethnomedicinal survey.

Objective: The present study investigated antidiabetic and antioxidant activities of methanol extract and fractions of Glyphaea brevis.

Methods: Antioxidant activity was evaluated by the total phenolic content (TPC), total flavonoid content (TFC) and DPPH assay. The antidiabetic effect of leaf extracts was evaluated on alloxan-induced diabetic rats. Solvent fractions were evaluated for in vitro α-amylase inhibitory activity using 3, 5-dinitrosalicylic acid assay.

Results: The TPC and TFC values of Glyphaea brevis leaf extract and its solvent fractions were comparable to that of gallic acid and quercetin included in the study as standard drugs. The ethyl acetate fraction had the highest DPPH radical-scavenging activity. The methanol leaf extract (1g/kg bw.) produced a significant antidiabetic activity ($p<0.05$) on day seven. Histopathological studies of the pancreas of the diabetic rats treated with G. brevis leaf extract showed comparable regeneration to glibenclamide-administered rats at of 1 mg/kg body weight. Solvent fractions effectively inhibited α-amylase.

Conclusion: Our study provides justification for the use of Glyphaea brevis leaf traditionally to manage diabetes. The polyphenolic constituents in the plant may be responsible for the antidiabetic and antioxidant effects.

Keywords: Diabetes, *Glyphaea brevis*, Phenolic content

Effets antidiabétiques et antioxydant de l'extrait de méthanol et les fractions de *Glyphaea brevis* (Spreng.) Monachino chez les rats diabétiques induits d'alloxane

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RESUME

Contexte: Le *Glyphaea brevis* qui, selon des études, a montré des activités antibactériennes, anti-inflammatoires et anti-oxydantes considérables, a été mentionnée comme une recette pour le diabète dans notre enquête ethno-médicale récente.

Objectif: La présente étude a examiné les activités antidiabétiques et anti-oxydantes de l'extrait de méthanol et des fractions de *Glyphaea brevis*.

Méthodes: L'activité anti-oxydante a été évaluée par la teneur totale en phénolique (TPC), la teneur totale en flavonoïdes (TFC) et le dosage de DPPH. L'effet antidiabétique des extraits de feuilles a été évalué sur des rats diabétiques induits d'alloxane. Les fractions de solvant ont été évaluées pour leur activité inhibitrice α-amylase in vitro en utilisant le dosage d'acide dinitrosalicylique 3, 5.

Résultats: Les valeurs PTC et TFC de l'extrait de la feuille de *Glyphaea brevis* et de ses fractions de solvant étaient comparables à celle de l'acide gallique et quercétine incluse dans l'étude en tant que médicaments standard. La fraction d'acétate d'éthyle a eu la plus forte activité anti-radicalaire DPPH. L'extrait de feuilles de méthanol (1g/kg de poids corporel.) a produit une activité antidiabétique significative ($p<0,05$) le jour sept. Des études histopathologiques du pancréas des rats diabétiques traités par extrait de feuilles *G. brevis* ont indiqué une régénération comparable à des rats auxquels on a administré la glibenclamide à 1 mg/kg de poids corporel. Les fractions de solvant ont inhibé efficacement le α-amylase.

Conclusion: Notre étude fournit une justification pour l'utilisation de la feuille de *Glyphaea brevis* traditionnellement pour gérer le diabète. Les constituants poly-phénoliques dans la plante peuvent être responsables des effets antidiabétiques et antioxydants.

Mots-clés: diabète, *Glyphaea brevis*, contenu phénolique

INTRODUCTION

Diabetes mellitus (DM) is an inherited and or acquired chronic metabolic disorder in which the pancreas produces insufficient amounts of insulin, or in which the body's cells fail to respond appropriately to insulin.[1] It is initially characterized by loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[2-3] Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.[4] The control of postprandial blood glucose level plays key roles in the treatment and decrease progression of diabetes mellitus.[1]

The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization[5] has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. Diabetes mellitus is known to affect 3% on average of adult Nigerians and the prevalence in northern Nigerian is put at 1.6%.[6] Presently the disorder has no known cure but could be controlled by agents that exhibit hypoglycaemic effect.[6] Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.[4] Medicinal plants contain phytochemicals with antioxidative potentials, which are responsible for their therapeutic effects.[7-9] Glucose oxidation, non-enzymatic glycation of proteins and oxidative degradation of glycated proteins generate free radicals, which cause damage to cellular organelles and enzymes, increase lipid peroxidation and lead to the development of insulin resistance.[10] In addition to antioxidative properties, these bioactive compounds have been shown to have other possible health benefits such as, anticarcinogenic, antihypertensive, antimutagenic, antidiabetic and antimicrobial activities.[11-12] To harness these benefits, nutrition recommendations for diabetes and related complications have been made based on scientific knowledge, clinical experience and experts' consensus. Increasing the awareness of clinicians and persons with diabetes about beneficial nutrition therapies can improve diabetes care.[13] Thus, it is possible to record better health outcomes as people learn to take foods as medicines. In the light of this, some of the medicinal plants of value in the treatment of diabetes can be recommended as food or food supplements for their beneficial health roles in alleviating diseases. *Glyphaea brevis* investigated in this study possesses antioxidant capacity that can tackle diseases. Natural antioxidants from plants have the ability to protect the body against oxidative damage[14], by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes.[15] Plants may exhibit hypoglycemic activity due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output, inhibiting the intestinal absorption of glucose or by facilitating metabolites in insulin dependent processes. Furthermore, the antidiabetic activity of plant polyphenols has been linked to the inhibition of α-amylase.[16] It is therefore imperative to seek for the incorporation of some of the edible medicinal plants in food constituents for their health benefits.

Glyphaea brevis, belonging to the family Tiliaceae, is a small tree when fully grown and found commonly in tropical regions. Various therapeutic uses, including the treatment of hepatitis, pulmonary troubles, oedema and gout paralysis, epilepsy, convulsions and spasms, dyspepsia, ulcers, and poisoning have been reported on *G. brevis*.[17] The root decoction is used as an aphrodisiac, appetizer, laxative, and as a remedy for chest pains, diarrhea, dysentery and sleeping sickness. *Glyphaea brevis* has been reported to show considerable antimicrobial[18], antiarthritis[19], antioxidant activities[20-21] and leaves when boiled with Vernonia *amygdalina leaves* help to reduce blood sugar level.[22] Glyphaeaside A1-A4, B1-B5, C, ten phenylalkyl-substituted iminosugars and cinnamic acid derived glucoside have been isolated from the root of *G. brevis*.[23] The HPLC analysis of *n-butanol* extract of the roots of G. brevis suggested the presence of phenolic compounds like protocatechuic acid (PCA).[19] Also, our recent ethnobotanical survey conducted from August to November, 2013 in Moniya, Akinyele Local Government Area, Ibadan, Oyo State on plants used for the treatment of diabetes indicated *G. brevis* as one of the plants frequently mentioned. This was therefore the basis of the present research. The present study was therefore designed to investigate antioxidant and antidiabetic activities of leaf extract and fractions of *Glyphaea brevis* on alloxan-induced diabetic rats.

METHODS

Plant collection and Extraction

Glyphaea brevis leaves were collected at Oluponna Community of Ayedire Local Government Area of Osun

State in June, 2014 and were authenticated by Mr. K. A. Adeniyi and Dr O. A. Ugbogu of Forest Herbarium, Ibadan (FHI) of Forestry Research Institute of Nigeria with a voucher specimen number, FHI: 110067. Pulverized leaf sample of *Glyphaea brevis* was macerated in methanol for 72 h at room temperature with occasional stirring. The extract was filtered through a defatted cotton plug followed by Whatman filter paper No. 1 and the filtrate was concentrated *in vacuo*. Percentage yield was calculated with reference to the initial weight of material macerated. The crude methanolic extract was partitioned with aliquots of *n-hexane*, chloroform and ethyl acetate successively and evaporated *in vacuo*.

Drugs and Chemicals

The 2, 2-diphenyl-l-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent and ferric chloride solution were purchased from Fisher Scientific (Springfield, NJ, USA). Other reagents were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

Animals

Eight weeks old Wistar male albino rat, with body weights ranging from 150-200 g were used for the study. The rats were fed on pelleted commercial growers mash and given water *ad libitum*. They were kept at room temperature. The animals were housed in plastic cages under condition of 12 h light/12 h dark cycle at room temperature. The weights of the animals were recorded prior to commencement and termination of the experiment using electronic weighing balance.

Phytochemical screening

Powdered leaf sample was screened for plant secondary metabolites such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides and anthraquinones using standard methods.[24-25]

Evaluation of Total Phenolic and Total Flavonoid Contents

The total phenolic content of the fractions was determined using the Folin-Ciocalteu method.[26] Briefly, to 0.1 mL of the extract was added 0.5 mL of Folin Ciocalteau reagent and 5.0 mL of sodium carbonate. The reaction mixture was allowed to stand for 30 min and the absorbance was measured at 765 nm using 752S spectrum lab UV/Visible spectrophotometer. Gallic acid was used as the standard. Extracts were analysed in triplicates. Total flavonoid content was determined using aluminium chloride method.[27] Briefly, 0.5 mL of methanolic extract was dispensed into test tube, followed by 1.5 mL of methanol, 0.1 mL of aluminum chloride (10%), 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The reaction mixture was shaken, allowed to stand at room temperature for 30 min before absorbance was read at 415 nm with a double beam 752s spectrum lab UV/Visible spectrophotometer. The TFC was expressed as quercetin equivalent (QE) in mg/g material.

Antioxidant activities evaluation

(DPPH radical scavenging assay)

The free-radical-scavenging ability of the methanolic extract against 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) free radical was estimated as described by Nabavi et al[26]with a slight modification. Briefly, appropriate dilutions of the extracts (0.024 to 0.096 mg/mL) totalling 1 mL was mixed with 3 mL of 60 µM freshly prepared DPPH solution (0.1 mM) in methanol; the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. All experiments were repeated three times independently. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

% inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100%. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), which was calculated from the linear regression curve.

Acute toxicity study

Acute toxicity study was conducted in two phases according to Lorke's method.[28] A total of sixteen male rats (105-200 g) were used. The first phase comprised nine male rats, randomly divided into three groups (n=3), and orally administered with 10,100, and 1000 mg/kg of the *G. brevis* leaf methanol extract (GBME) to possibly establish the range of doses producing any toxic effect . In addition, a fourth group of three rats was used as control group; animals in this group were not given the extract. The rats were observed for 24 h for death. In the second phase, further specific doses; 1600 mg/kg, 2,900 mg/kg and 5,000 mg/kg of the extracts were administered to a fresh batch of three animals at one animal per dose and the number of death in 24 h was recorded. The LD_{50} were calculated as the geometrical means of the maximum dose producing 0% (a) and minimum dose producing 100% mortality (b). All animals were observed frequently for signs of toxicity and possible deaths for 24 h, 72 h and 2 weeks.

Experimental design and Induction of diabetes mellitus

A total of 20 rats (5 normal; 15 alloxan-induced diabetic surviving rats) were used. The rats were divided into five groups of five rats each. The groups comprised: group I (non-diabetic rats), group II (diabetic control), group III (glibenclamide-treated rats), and group IV (*G. brevis* leaf extract-treated rats). The dosage of extract administered was within safe margin of 1000 mg/kg body weight while the standard drug, glibenclamide, was administered at a dose of 1 mg/kg. The extracts were dissolved in distilled water and administered once daily by oral route for seven days in group IV. Hyperglycemia was induced in 25 Wistar rats by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate 150 mg /kg, to overnight fasted rats. After 48 h, 20 animals with fasting blood glucose levels above 140 mg/dL were considered diabetic and included in this study. Animals that did not develop hyperglycaemia after 48 h of alloxan injection were excluded and new animals were used. After alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48h after alloxan injection. Fasting blood glucose estimation and body weight measurement ware done on day 0, 1, 4, and 7 of the study. The tail tip of the rat was cut swiftly with sterile scalpel and a drop of blood was squeezed onto the test area of strip inserted into Accu chek glucometer (Roche diagnostics, Germany)

On day 7, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar[28] was estimated. Serum was separated and analysed for serum cholesterol[29], serum triglycerides by enzymatic DHBS colorimetric method[31], serum HDL[32] and serum alkaline phosphatase hydrolyzed phenol amino antipyrine method[33] were estimated.

The whole pancreas from each animal was removed after euthanizing animals between 0900-1100 h to minimize diurnal variation and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections (5 µm thick) were cut and stained by haematoxylin and eosin (H & E) for histological examination.

Statistical analysis

The results were expressed as mean±S.E.M., the level of significance of various treatments was calculated by Student's t-test using SPSS 16.0, results were considered statistically significant at $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical analysis of pulverized *G. brevis* leaf revealed the presence alkaloids, flavonoids, saponons, tannins, cardiac glycosides and anthraquinones (Table 1).

Table 1: Phytochemical constituents identified in *G. brevis* leaf

Test(s)	Concentration
1. Alkaliods	
(a). Dragendroff	+++
(b). Mayer test	++
2. Anthraquinones glycosides	
(a). Borntrager test	++
3. Saponins	
(a). Foam test	+
(b). Emulssion test	+
4. Tanins	
(a). Ferric chloride test	+
5. Glycoside	
(a). Salkowski's test	++
(b). Keller-Kiliani test	++
6. Flavonoids	
(a). Lead acetate test	++

Keys: +++ = high concentration; ++ = moderate concentration; + = detected

Acute toxicity study

In this study, none of the rats treated with different dosages up to 5000 mg/kg dose of the extracts showed mortality or visible side effects after 14 days of observation.

Evaluation of total phenolic content, total flavonoid content and DPPH radical scavenging activity

The total phenolic contents of *G. brevis leaf* extract, its hexane, chloroform and ethyl acetate fractions were 9.24±2.34, 50.88±7.62, 63.88±9.24 and 43.89±2.37 mg gallic acid equivalent/g of extract, respectively while total flavonoid contents of *G. brevis* leaves extract, its hexane fraction, chloroform and ethyl acetate fractions were 21.89±0.07, 13.8±2.71, 10.05±0.77 and 20.69±1.59 mg quercetin equivalent / g of extract, respectively as presented in Table 2. The IC_{50} for DPPH radical-scavenging activity of *G. brevis leaf* extract, its hexane, chloroform and ethyl acetate fractions are 193.67±1.60, 217±1.10, 159.67±3.22 and 78.67±2.00 µg/mL, respectively (Table 2). The IC_{50} value of ascorbic acid was 18.44±1.20 µg/mL.

Table 2: Total phenolics, total flavonoid content and DPPH radical scavenging activity of *G. brevis* leaf

Samples	Total phenolics (mg Ascorbic acid equivalent/g dry weight)	Total flavonoid (mg quercetin equivalent/g dry weight)	IC50 values (µg/mL)
GBC	63.88±1.2	21.89±2.1	193.67±1.60
GBHF	50.88±1.5	10.05±1.0	217±1.10
GBCF	26.05±2.1	13.81±1.6	159.67±3.22
GBEF	48.87±3.2	20.69±3.0	78.67±2.00

GBC = G. brevis methanol extract, GBHF = *G. brevis n-hexane* fraction,
GBCF = G. brevis chloroform fraction and GBEF = *G. brevis ethylacetate* fraction.

Antidiabetic effects on alloxan-induced diabetic rats

Glyphaea brevis leaf extract at 1000 mg/kg body weight, reduced the fasting blood glucose level (FBGL) by 23.0%, while the standard drug, glibenclamide had percentage fasting blood glucose reduction of 31.9% as shown in Table 3. The weights of the animals depreciated after administration of alloxan. This parameter however, was significantly ameliorated after the administration of *G. brevis* methanol extract. At the end of the treatment there was increase in weight in group I while a decrease was observed in groups II, III and IV with percentage decreases of 7.2%, 10.6% and 10.1%, respectively after treatment for 7 days. This also showed consistency with FBGL of *G. brevis leaf* extract at 1000 mg/Kg (group IV) in which there was a moderate improvement in weight gain (Table 3).

Table 3: Effect of *G. brevis* leaf extract on blood glucose level (mg/dL) and body weight (g) of the experimental animals

Animal groups	Day 0		Day 1		Day 4		Day 7	
	BGL	Body weight	BGL	Body weight	BGL	Body weight	BGL	Body weight
Grp I (Control)	93.40 ±4.33^{a}	150.8 ±0.49^{a}	100.60 ±2.76 ^{a}	150.40 ±0.68^{ab}	92.60 ±2.44^{a}	155.4 ±3.07^{b}	94.80 ±4.19 ^{a}	159 ±4.11^{b}
Grp II (UDG)	367.67 ±15.00 ^{b}	163.20 ±1.53^{c}	372.80 ±25.00^{b}	159.60 ±1.75^{b}	378.20 ±30.05^{b}	154.60 ±1.60^{b}	382.04 ±10.04^{b}	148.80 ±1.98^{ab}
Grp III (glibenclamide, 1 mg/Kg)	373.17 ±72.74^{ab}	160.8 ±6.70^{bc}	298.60 ±73.85^{c}	145.00 ±11.01^{ab}	197.83 ±58.51^{c}	146.60 ±13.57^{ab}	254.20 ±58.11^{c}	143.20 ±15.93^{ab}
Grp IV (GBME, 1g/Kg)	543.67 ±36.33^{c}	149.80 ±0.20^{a}	463.33 ±61.46^{c}	140.60 ±1.50^{b}	477.00 ±29.50^{c}	139.20 ±2.31^{ab}	418.67 ±3.98^{c}	133.50 ±3.77^{ab}

Values expressed as mean±SEM; Means with the same superscript are not significantly different from each other ($p<0.05$). UDG = untreated diabetic group and GBME = G. brevis leaf methanol extract.

Assessment of biochemical parameters

Treatment with *G. brevis leaf* extract altered lipid profile in group IV (Figure 1). The abnormally high concentration of serum lipids was mainly due to increase in the mobilization of free fatty acids (FFA) from peripheral tissue due to activation of the hormone sensitive lipase during insulin insufficiency. There was an increase in ALT and significant decrease in ALP and AST in the plasma. Also, the activities of ALP slightly reduced and AST significantly ($p<0.05$) increased compared to the normal.

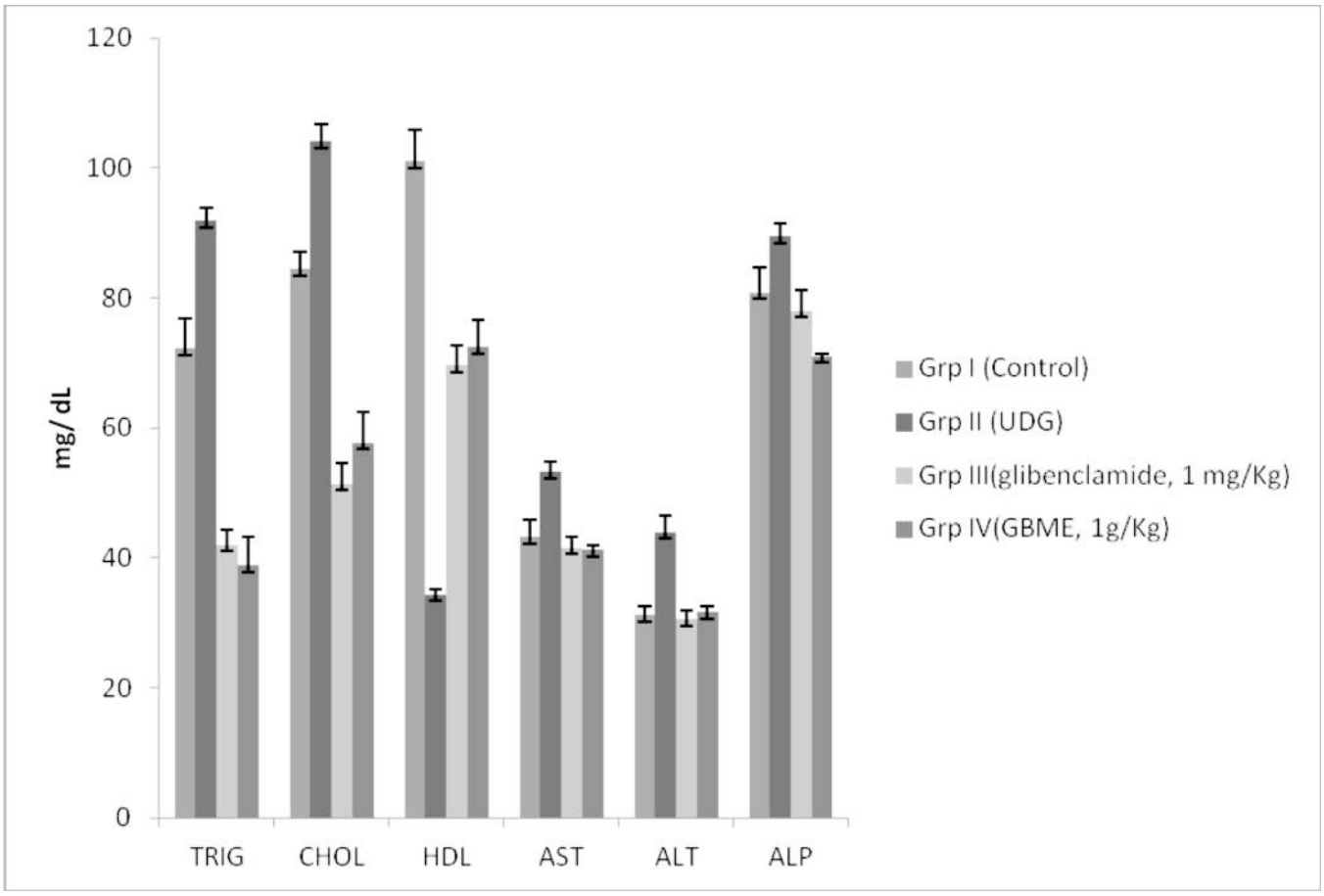

Figure 1: Biochemical parameters of the blood samples of experimental rats
Key: UDG=untreated diabetic group; GBME= Glyphaea brevis methanol extract

Histopathological study

Restoration of normal cellular population and size of islets with hyperplasia were seen in extract-treated groups as shown in Figure 2. Histopathological section of the pancreas of the normal control rat showed normal cellular population of islet of Langerhans (normal-sized pancreatic islets with regular outlines; the islet cells have normal nucleus and cytoplasm; normal exocrine acini and pancreatic ducts; no congestion of blood vessels; and no visible lesion). The pancreas of non-treated diabetic rats showed abnormalities such as decreased cellular density and changes in the islet of Langerhans with extravasated blood in exocrine pancreatic tissue. However, distinct clear, restoration of normal population was more profound in the rats treated with *G. brevis* methanol extract.

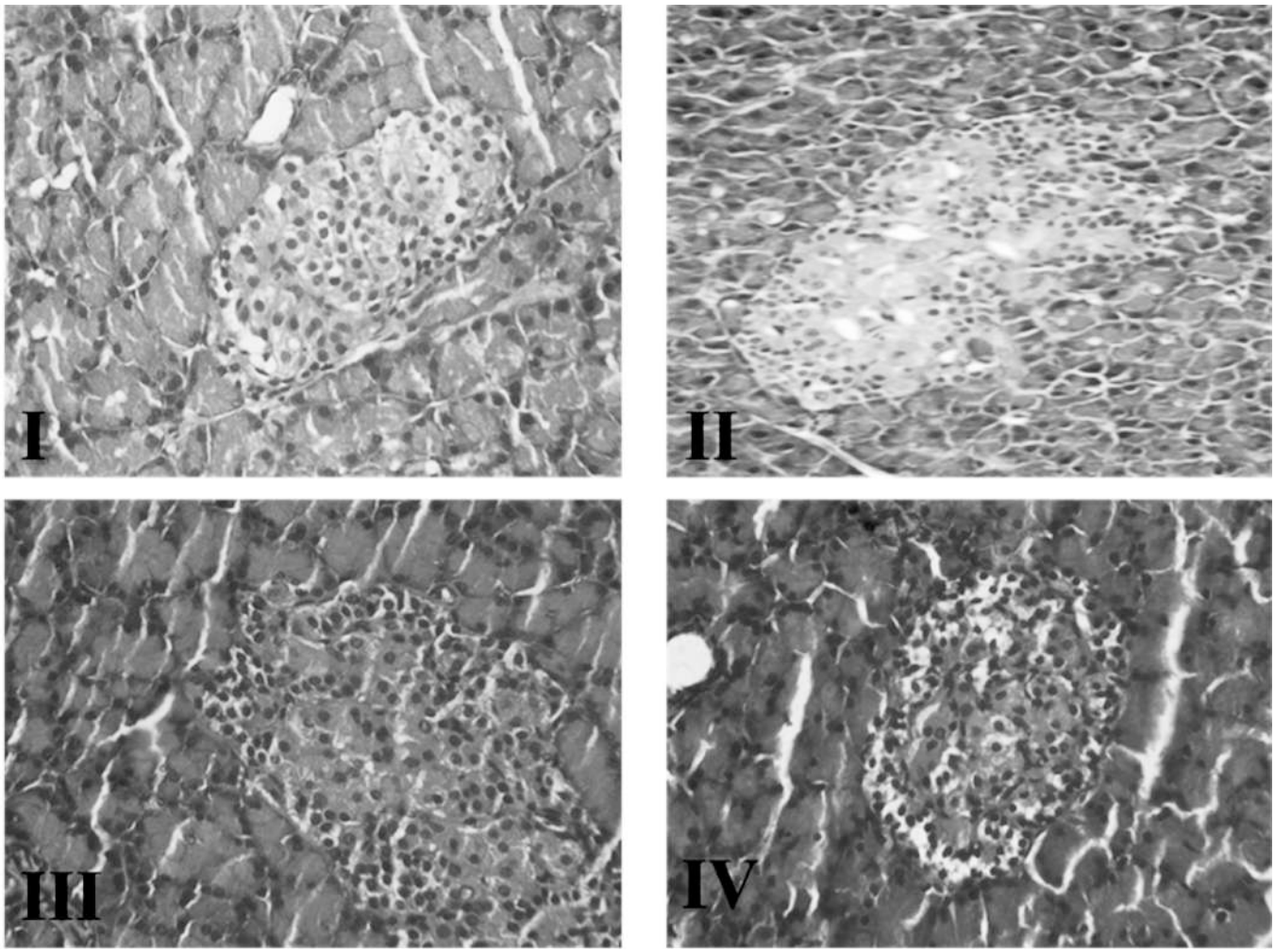

Figure 2: Histopathology of the pancreas of the experimental rats (X 400), I: Normal control, II: Untreated diabetic rats, III: Diabetic rats + glibenclamide, IV: Diabetic rats + GBME

Alpha amylase inhibitory activity

The *n-hexane*, chloroform and ethyl acetate fractions of *G. brevis leaf* extract were found to inhibit α-amylase activity with IC_{50} values of 253.41, 329.20, 277.06 µg /mL, respectively. The IC_{50} value of acarbose was 206.60 µg /mL.

DISCUSSION

The management of diabetes mellitus is still challenging globally in the face of many side effects of orthodox drugs. However, many medicinal plants have become promising in the treatment of chronic diseases in many countries of the world. *Glyphaea brevis* is one of such medicinal plants that have been reported to show wide medicinal application. The qualitative phytochemical screening result of this plant was in agreement with studies in literature.[34-35] In the present study, the classes of phytochemical compounds obtained in *G. brevis* leaves had been reported to show various important biological activities.[36-38] Important polyphenolic compounds, such as flavonoids, widely found in plants possess significant antioxidant activities that could decrease the incidence of certain human diseases .[39-42] In our study, flavonoids could be one of the important components of *G. brevis* upon which some of the pharmacological effects are based. Flavonoids from plants have been implicated in the reduction of lipids by inhibition of HMG-CoA reductase.[43-45] Also, saponins are known to elicit serum cholesterol lowering activity. Saponins have strong affinity for the aglycone moiety of membrane sterols, particularly cholesterol, with which they form insoluble complexes and this is believed to be the basis of both their hypocholesterolic and hemolytic effects.[46] It therefore follows that the underlying mechanism of lipid lowering effect of the *G. brevis leaf* extract could be by inhibition of lipid absorption due to the presence of saponins and tannins[47] or inhibition of cholesterol esterase, activation of fatty acid synthase, and production of triglyceride precursors such as

acetyl-CoA and glycerol phosphate.[48]

The effect of antioxidants on DPPH is due to their hydrogen donating ability.[49] Based on the DPPH radical scavenging activity obtained in the present study, it is possible that several compounds of different polarities might have contributed to the antioxidative properties of the extracts.

The results of acute toxicity study suggest that the extract has a low toxicity profile. [28] Hence, the experimental dose used (1000 mg/kg p. o.) was within safe margin. The crude extract of *G. brevis* showed significant antidiabetic activity by causing a decrease in the fasting blood glucose level of alloxan-induced diabetic rats. In diabetes, hyperglycemia is associated with dyslipidemia which is a risk factor for coronary heart diseases[50-51] resulting from insulin deficiency. In normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in a diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia.[52] Dyslipidemia is characterized by increase in TC, TG and fall in High Density Lipoprotein (HDL). The AST and ALT are released into the plasma when there is severe hepatocellular injury. [53] The increase in hepatic marker enzymes in alloxan treated group (group II) may be due to lipid peroxidation of bio-membranes, causing leakage of cellular components.[54] Additionally, the regeneration of β-cells may not be ignored as the probable mechanism by which *G. brevis leaf* extract produced a significant reduction in blood glucose in the treated rats. Alloxan induces extensive damage to the β cells of islets of Langerhans thereby inducing hyperglycaemia.[55-56] The partial restoration of normal cellular population was indicative of the antidiabetic potential of *G. brevis plant*, which may be due to its ability to prevent lipid peroxidation of pancreatic islet bio-membranes.[56]

Also, the control of human pancreatic α-amylase has been reported as an important aspect in treatment of type II diabetes.[57] Hence, retardation of starch digestion by inhibition of enzymes such as α-amylase plays a key role in the control of diabetes. Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels.[58] The findings in the present study also suggest that one of the mechanisms by which *G. brevis leaf* could be exhibiting hypoglycemic effect could be by inhibition of α-amylase activity leading to retardation of starch hydrolysis, eventually lowering postprandial hyperglycemia (PPHG). Kim et al.[59] demonstrated that the triterpenoid acids showed a significant inhibition effect on α -amylase. Moreover urosolic acid, pentacyclic triterpenoids and oleanolic acid derivatives exhibited the strongest α - amylase suppressing activity and are responsible for a major part of the activity of the total hexane extract of many antidiabetic plants.[60-61]

CONCLUSION

Methanolic extract of *G. brevis leaf* exhibited significant antihyperglycemic activities in alloxan-induced diabetic rats. This plant showed improvement in parameters like body weight, lipid profile and regeneration of β-cells of pancreas as well as inhibition of α-amylase and so might be of value in diabetes treatment. Further characterization of the bioactive compounds in the fractions may lead to the identification of lead compounds that may be of importance in antidiabetic drug discovery and development from *Glyphaea brevis leaf* extracts.

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