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Original Research Article

Biochemical and Hepatoprotective Effects of *Gangronema latifolium* Extract on Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disease associated with hyperglycemia, dyslipidemia, and hepatocellular damage. *Gongronema latifolium* has been widely explored in ethnomedicine for the treatment and management of various disorders. The biochemical, hepatoprotective, and histological effects of aqueous-ethanolic leaf extract of *G. latifolium* (Family: Asclepiadaceae) in alloxan-induced diabetic rats were investigated in this study. Thirty (30) juvenile male Wistar rats were placed into six groups, each with five rats: Normal rats made up Group 1, while diabetic rats in Groups 2–4 were given 200, 400, and 800 mg/kg body weight of aqueous-ethanolic leaf extract, respectively; diabetic rats in Group 5 were given a standard anti-diabetic drug (0.2 mg/kg glibenclamide), and diabetic rats in Group 6 were left untreated. When compared to control rats, alloxan-induced diabetic rats showed a significant ($p \leq 0.05$) rise in liver enzymes, low density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG), and fasting blood glucose (FBG), but a significant ($p \leq 0.05$) decrease in high density lipoprotein (HDL). The alterations in the following parameters were returned to normal levels when the diabetic rats were administered *G. latifolium* extract. At the end of the experimental period, the rats were sacrificed, and each experimental group's liver tissues were removed and used for histopathological examinations. When compared to the normal control and treatment groups, which had normal liver histological features, the diabetes group's histology showed cytoplasmic deterioration. The results indicate that *G. latifolium* may serve as a remedy for the management of diabetes.

Keywords: *Gongronema latifolium*, Diabetes, Wistar rats, Alloxan, Liver.

Introduction

Medicinal plants have provided valuable therapeutic agents for treating diseases and disorders, and they are widely employed in many regions of the world, particularly in rural areas where modern medical facilities are limited.[1] Recently, there has been an increase in plant-based therapeutic products in both developed and developing countries because they are mostly non-toxic, have fewer side effects, and are available at affordable prices.[2,3] One such medicinal plant is *Gongronema latifolium.* The plant belongs to the family of Asclepiadacae. It is an edible rainforest plant native to many parts of Nigeria and West Africa.[4] The plant is commonly used as a vegetable or spice and also widely been used in traditional medicine for treating diabetes and high blood pressure[5] and malaria.[6] *G. latifolium* is also used to treat various other ailments such as cough, loss of appetite, parasitic intestinal worms and stomach disorders. The leaf extract was also reported to exhibit antimicrobial properties[7] and anti-diabetic properties,[8-11] and in the United States, *G latifolium* leaves are processed into tea and marketed to diabetes mellitus patients.[12] Increasingly, diabetes management involves non-conventional drugs. It is estimated that 25 to 57% of people with diabetes have at one time or another resorted to complementary and alternative medicine, including medicinal plants.[13]

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Materials and Methods

Collection of plant material

Mr Felix Nwafor, a plant taxonomist at the Department of Pharmacognosy, University of Nigeria, verified fresh leaves of *G. latifolium* harvested in March 2021, from a farm in Nsukka, South-eastern Nigeria. For reference purposes, a voucher specimen with the specimen number PCG/UNN/0348 was deposited at the herbarium.

Extraction procedure

Extraction was carried out as previously described.[15] The leaves were rinsed with clean water within 2 hours of harvest, dried for 7 days in the shade, and then pulverized into a coarse powder. A 2 kg portion of powder was cold macerated in 8 L of 30% (v/v) ethanol (Sigma-Aldrich) at ambient temperature for 48 hours, and then filtered successively with a muslin cloth and Whatman No. 1 filter paper. The resulting filtrate was evaporated to a sticky dark brown paste under a constant stream of cool air for 48 h.

Phytochemical analysis of crude aqueous extracts

This was done using standard methods as previously described.[16] The phytochemicals tested for include; alkaloids, saponins, flavonoids, tannins, glycosides and essential oils.

Experimental animals

A total of 30 juvenile male Wistar rats weighing 160-190 g were obtained from the University of Nigeria, Nsukka's Faculty of Veterinary Medicine. Male rats were selected for this study because they are regarded to be more stable physiologically and less subject to hormonal changes, which could affect the results. The animals were housed in well-ventilated cages and allowed unrestricted access to feed and clean water. The animals were treated in accordance with the National Institutes of Health's (NIH) guidelines

for laboratory animal use and care.[17] The University of Nigeria Faculty of Veterinary Medicine Animal Care and Use Committee provided ethical approval for the study (approval number FVM-UNN-IACUC-2020-1059).

Induction of diabetes
Following a 12-hour fast, 25 rats were given an intraperitoneal injection of alloxan solution at the dose of 150 mg/kg. Rats with a fasting blood glucose level of greater than 140 mg/dl for 5 consecutive days were judged to have developed diabetes and were selected for the study.

Experimental groups and treatments
The experimental rats were randomly divided into 6 different experimental groups of 5 rats per group. The doses were fixed as previously described.[18]

Group 1: normal control (received 1ml distilled water/kg body)
Groups 2: diabetic rats (received 200mg/kg of *G. latifolium* extract).
Groups 3: diabetic rats (received 400mg/kg of *G. latifolium* extract).
Groups 4: diabetic rats (received 800mg/kg of *G. latifolium* extract).
Groups 5: diabetic rats (received 0.2 mg/kg of glibenclamide).
Group 6: diabetic rats (untreated)
The treatments were given orally twice a day, every 12 hours, for 21 days.

Measurement of blood glucose
The animals were fasted for 12 hours before blood samples were taken from the rats' tail veins and examined for fasting blood glucose levels using a glucometer (ACON Laboratories Inc, USA). Until the end of the treatment, measurements were taken every three days.

Biochemical parameters determination
The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG) and total Cholesterol (TC) were determined using assay kits from Randox Laboratories ltd (United Kingdom).

Acute toxicity study
This was carried out as previously described.[19] The extract was administered to six groups of five rats each at doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg. The rats were monitored for clinical signs of toxicity and mortality.

Histopathological examination
The histopathological examinations were performed according to the method previously used.[20] At the completion of the experiment, samples of the liver were taken from dissected rats and fixed in 10% neutral buffered formalin. After that, the fixed tissues were put through a series of alcohol and xylene modifications before being embedded in paraffin wax. The implanted tissues were sectioned at a thickness of 5 micron using a rotary microtone. The sections were chosen and floated in a water bath before being picked with a slide that had already been labeled. To expose overall tissue structure, the slides were dried on a hot plate, dewaxed with xylene, and hydrated with alcohol before staining with haematoxylin and eosin. Finally, the slides were viewed under a microscope (x40) and photomicrographs were taken using a Moticam 2.0 digital camera (Motic China).

Statistical analysis
Using IBM SPSS Statistics software version 23, the data was treated to one-way Analysis of Variance (ANOVA). Significance was accepted at $p \leq 0.05$

Results and Discussion

Diabetes mellitus is a health issue in many countries of the world. This necessitates the development of more effective and less hazardous therapeutic methods. Plants that have anti-diabetic properties could be a good source of novel hypoglycemic chemicals. Hyperglycemia and impaired glucose metabolism are the major indicators of diabetes, and following alloxan injection, the blood glucose of the experimental rats was significantly elevated compared with the normal control. Alloxan induce diabetes mellitus via stimulating selective pancreatic β cells destruction, and this damage trigger hyperglycemia. However, treatment with *G. latifolium* extract reversed the alloxan-induced hyperglycemia (Table 1). This observation is in consonant with the findings of earlier studies that reported the hypoglycemic effects of *G latifolium* extracts.[2,14] The hypoglycemic activity of *G. latifolium* leaves extract could be related to phytochemicals found in the leaves. Alkaloids, flavonoids, and saponin are among the phytochemicals present, and they are believed to have a hyperglycemic effect.[21,22] Also, it is known that these phytochemical constituents can stimulate insulin actions on the beta cells of the pancreas.[23] The preliminary phytochemical screening revealed the presence of glycosides, saponins, tannins, alkaloids, and flavonoids in the crude aqueous-ethanol leave extracts of *G. latifolium*.

Table 1: Blood sugar levels in rats before and after treatment with different doses of *Gongronema latifolium* extract

Stages	**Treatment groups**					
	Control (Normal rats)	**200 mg/kg bw**	**400 mg/kg**	**800 mg/kg**	**Standard control**	**Diabetic control**
Baseline	78.00 ± 7.65^{A1}	77.20 ± 6.53^{A1}	83.60 ± 7.50^{A1}	90.80 ± 8.04^{A1}	85.00 ± 17.03^{A1}	88.00 ± 2.00^{b}
After Induction	78.80 ± 9.01^{A1}	250.00 ± 70.21^{B45}	285.20 ± 61.88^{B3}	341.40 ± 86.71^{B3}	287.40 ± 71.02^{B3}	310.33 ± 43.89^{b}
Day 3	88.80 ± 5.40^{A1}	272.00 ± 74.93^{B5}	267.00 ± 28.20^{B3}	293.00 ± 54.89^{B3}	218.00 ± 33.37^{B2}	446.67 ± 46.15^{d}
Day 6	85.40 ± 7.92^{A1}	178.60 ± 25.53^{C34}	171.00 ± 33.71^{BC2}	183.80 ± 24.62^{C2}	110.20 ± 31.13^{AB1}	483.00 ± 73.82^{c}
Day 9	91.00 ± 8.19^{A1}	168.20 ± 20.47^{A234}	140.00 ± 34.15^{A}	143.20 ± 36.51^{A12}	91.60 ± 8.88^{A1}	412.67 ± 44.12^{b}
Day 12	87.80 ± 4.60^{A1}	143.40 ± 34.55^{A123}	124.40 ± 30.01^{A12}	123.20 ± 30.08^{A12}	83.20 ± 6.42^{A1}	514.00 ± 41.76^{b}
Day 15	85.80 ± 3.90^{A1}	112.00 ± 31.39^{A123}	102.20 ± 19.68^{A1}	101.80 ± 22.10^{A12}	83.00 ± 6.71^{A1}	442.33 ± 69.50^{b}
Day 18	79.60 ± 7.64^{A1}	$103.60 \pm 27.2\,5^{A123}$	87.40 ± 13.63^{A1}	83.60 ± 8.65^{A1}	78.20 ± 8.32^{A1}	378.33 ± 96.72^{b}
Day 21	86.40 ± 4.56^{A1}	84.20 ± 9.71^{A12}	76.80 ± 7.60^{A1}	83.60 ± 9.45^{A1}	78.20 ± 9.34^{A1}	552.00 ± 56.32^{b}

Values are represented as Mean (±) standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$

These bioactive compounds are present in varying concentrations in the plant extract, as shown in Table 6. In the present study, alloxan-induced diabetic rats developed dyslipidemia. The increased TG, TC, and LDL levels, and reduced HDL levels in alloxan-induced diabetic rats seen in this study are consistent with prior studies on changes in these markers in diabetic animals.[24,25] Diabetes-induced hyperlipidemia could be caused by increased mobilization of fat from adipose tissue due to glucose underutilization. However, treatment with *G. latifolium* extracts significantly ($P<0.05$) reduced the TC, TG and LDL when compared to the diabetic untreated rats. Similarly, the HDL which was reduced in the diabetic untreated rats was significantly increased ($P<0.05$) in the groups administered the *G. latifolium* extracts (Table 4 and 5), and this is consistent with previous studies that reported hypolipidaemic properties of *G. latifolium* extracts.[26]

The phytochemical constituents of *G. latifolium* could have contributed to the capacities of the plant extract to reverse diabetic dyslipidemia. Previous empirical investigations showed that phytochemicals such as saponins, flavonoids and tannins can ameliorate dyslipidemia.[27,28] The liver is severely damaged in patients with diabetes mellitus. These damages include abnormal liver enzymes levels, necrosis, inflammation, hepatocellular damage and acute liver failure. Liver enzymes are essential biomarkers in the body that are used to diagnose and measure whether the liver is functioning normally or not. Changes in liver enzyme levels are caused by major or subtle changes in the integrity of cellular membranes in liver tissues. Increased levels of ALP, AST, ALT, and reduced level of albumin as observed in the alloxan-induced diabetic rats are indicators of hepatocellular damage (Tables 2 and 3), and it is mainly due to exudation of these enzymes from the cytoplasm of liver cells into the blood stream.[29,30]

Table 2: Effect of oral administration of *Gongronema latifolium* leaf extract on aspartate transaminase (AST) and alkaline phosphatase (ALP) of alloxan-induced diabetic rats

Groups	Baseline	After Induction	7 Days	14 Days	21 Days	AST/ALP (IU/L)
Group 1	71.40 ± 4.45^{A1}	67.60 ± 2.61^{A1}	68.80 ± 1.64^{A1}	67.40 ± 3.65^{A1}	69.40 ± 1.14^{A1}	AST
	80.80 ± 2.59^{A1}	83.40 ± 4.98^{A1}	82.40 ± 5.03^{A1}	83.20 ± 4.97^{A1}	81.20 ± 3.11^{A1}	ALP
Group 2	74.20 ± 6.06^{A1}	101.20 ± 4.87^{B3}	91.40 ± 2.61^{B2}	81.20 ± 3.56^{C1}	75.00 ± 2.24^{A1}	AST
	82.60 ± 4.67^{A1}	133.60 ± 3.29^{C4}	114.20 ± 3.83^{B3}	97.40 ± 1.95^{C2}	84.60 ± 3.97^{A1}	ALP
Group 3	72.20 ± 8.96^{A1}	100.40 ± 4.04^{B3}	90.00 ± 4.47^{B2}	75.60 ± 2.41^{BC1}	70.80 ± 1.92^{A1}	AST
	78.60 ± 5.68^{A1}	128.60 ± 2.41^{BC4}	112.40 ± 2.88^{B3}	92.60 ± 2.41^{BC2}	79.40 ± 2.30^{A1}	ALP
Group 4	69.40 ± 6.54^{A1}	98.80 ± 7.05^{B3}	93.60 ± 2.41^{B3}	79.40 ± 1.52^{C2}	74.40 ± 2.70^{A12}	AST
	82.20 ± 4.32^{A1}	131.00 ± 5.48^{C4}	115.40 ± 5.03^{B3}	98.80 ± 3.03^{C2}	84.80 ± 4.44^{A1}	ALP
Group 5	70.80 ± 4.76^{A1}	100.00 ± 4.18^{B2}	89.20 ± 4.38^{B2}	73.60 ± 2.41^{B1}	69.20 ± 2.59^{A1}	AST
	81.20 ± 4.97^{A1}	127.40 ± 4.72^{BC2}	111.60 ± 2.07^{B2}	88.00 ± 4.18^{AB1}	81.20 ± 3.63^{A1}	ALP
Group 6	74.80 ± 5.45^{A1}	100.40 ± 5.77^{B2}	102.40 ± 5.46^{C2}	109.80 ± 3.56^{D23}	111.40 ± 5.68^{B3}	AST
	82.60 ± 3.85^{A1}	121.20 ± 3.11^{B2}	123.60 ± 4.16^{C2}	125.80 ± 4.15^{D2}	127.00 ± 3.54^{B2}	ALP

Values are represented as Mean (±) standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$)

Table 3: Effect of oral administration of *Gongronema latifolium* leaf extract on Alanine transaminase (ALT) and albumin of alloxan-induced diabetic rats

Groups	Baseline	After Induction	7 Days	14 Days	21 Days	ALT/ALBUMIN (mg/dl)
Group 1	4.90 ± 0.16^{A1}	4.88 ± 0.16^{A1}	4.88 ± 0.16^{C1}	4.86 ± 0.11^{AB1}	5.00 ± 0.16^{AB1}	ALBUMIN
	36.00 ± 2.92^{A1}	34.40 ± 3.29^{A1}	36.40 ± 2.70^{A1}	36.40 ± 2.70^{A1}	36.00 ± 2.65^{A1}	ALT
Group 2	4.88 ± 0.19^{A2}	4.50 ± 0.50^{A12}	4.36 ± 0.15^{AB1}	4.50 ± 0.10^{A12}	4.62 ± 0.22^{A12}	ALBUMIN
	40.00 ± 3.67^{A1}	71.00 ± 4.85^{B4}	59.80 ± 3.56^{B3}	48.20 ± 4.60^{C2}	39.40 ± 2.07^{A1}	ALT
Group 3	4.76 ± 0.30^{A23}	4.32 ± 0.19^{A1}	4.42 ± 0.13^{ABC12}	4.62 ± 0.26^{A123}	4.88 ± 0.19^{AB3}	ALBUMIN
	37.00 ± 2.24^{A1}	69.80 ± 4.97^{B4}	57.60 ± 2.70^{B3}	44.00 ± 2.00^{BC2}	36.40 ± 2.70^{A1}	ALT
Group 4	4.80 ± 0.42^{A1}	4.60 ± 0.56^{A1}	4.72 ± 0.41^{BC1}	5.20 ± 0.27^{B1}	5.28 ± 0.47^{B1}	ALBUMIN
	35.60 ± 3.05^{A1}	66.40 ± 4.04^{B4}	58.60 ± 2.30^{B3}	47.80 ± 2.86^{C2}	39.00 ± 2.24^{A1}	ALT
Group 5	4.82 ± 0.37^{A1}	4.54 ± 0.48^{A1}	4.46 ± 0.34^{ABC1}	4.58 ± 0.26^{A1}	4.88 ± 0.19^{AB1}	ALBUMIN
	37.00 ± 4.42^{A1}	71.40 ± 5.27^{B3}	54.60 ± 2.41^{B2}	39.20 ± 1.30^{AB1}	35.80 ± 1.92^{A1}	ALT
Group 6	4.76 ± 0.34^{A2}	4.22 ± 0.13^{A1}	4.18 ± 0.13^{A1}	4.58 ± 0.30^{A12}	4.48 ± 0.36^{A1}	ALBUMIN
	38.40 ± 6.11^{A1}	67.80 ± 2.86^{B2}	69.80 ± 0.34^{C2}	71.80 ± 3.83^{D2}	72.60 ± 2.70^{B2}	ALT

Values are represented as Mean (±) standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$)

Table 4: Effect of oral administration of *Gongronema latifolium* leaf extract on HDL and LDL of Alloxan-induced diabetic rats

Groups	Baseline	After Induction	7 Days	14 Days	21 Days	HDL/LDL (mg/dl)
Group 1	76.20 ± 5.35^{A1}	74.40 ± 3.64 ^{B1}	74.00 ± 2.73^{C1}	74.80 ± 2.77^{C1}	74.80 ± 1.92^{B1}	HDL
	15.40 ± 2.07^{A1}	17.20 ±1.92 ^{A1}	17.60 ± 1.14 ^{A1}	16.60 ± 1.94^{A1}	17.00 ± 1.58^{AB1}	LDL
Group 2	81.00 ± 6.78 ^{A4}	23.40 ± 3.84^{A2}	37.60 ± 1.81^{B2}	52.20 ± 4.65 ^{B3}	77.40 ± 5.07^{B4}	HDL
	17.20 ± 2.94^{A1}	86.20 ± 5.93 ^{B4}	55.00 ± 3.31 ^{B3}	33.20 ± 2.38 ^{BC2}	19.40 ± 1.81 ^{B1}	LDL
Group 3	74.80 ± 5.76^{A4}	26.60 ± 3.91 ^{A1}	38.60 ± 2.96 ^{B2}	59.60 ± 3.91 ^{B3}	76.40 ± 5.02 ^{B4}	HDL
	16.20 ± 1.92 ^{A1}	84.40 ± 4.82 ^{B4}	56.00 ± 3.53 ^{B3}	28.60 ± 2.07 ^{B2}	15.20 ± 1.30^{A1}	LDL
Group 4	77.40 ± 6.30 ^{A4}	27.20 ± 2.28^{A1}	36.00 ± 2.12 ^{B2}	52.60 ± 4.03 ^{B3}	71.40 ± 2.40 ^{B4}	HDL
	17.20± 3.56 ^{A1}	83.60 ± 5.77^{B4}	56.40 ± 2.60 ^{B3}	37.00 ± 2.54 ^{C2}	19.00 ± 2.23 ^{AB1}	LDL
Group 5	79.40 ± 5.17 ^{A4}	22.80 ± 2.38^{A1}	37.80 ± 3.03 ^{B2}	59.00 ± 3.53 ^{B3}	77.20 ± 6.30^{B4}	HDL
	17.60 ± 2.70^{A1}	85.20 ± 4.76 ^{B4}	55.20 ± 10.30^{B3}	31.40 ± 2.70 ^{B2}	18.60 ± 1.14 ^{AB1}	LDL
Group 6	74.20 ± 3.49 ^{A2}	21.40 ± 2.40 ^{A1}	20.60 ± 4.97^{A1}	20.20 ± 4.20 ^{A1}	20.20 ± 3.76 ^{A1}	HDL
	15.40 ±2.96^{A1}	84.60±5.36 ^{B2}	84.00 ±3.16 ^{C2}	83.60±3.57 ^{D2}	83.00 ±3.67^{C2}	LDL

Values are represented as Mean (±) standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$

Table 5: Effect of oral administration of *Gongronema latifolium* leaf extract on Triglycerides (TG) and total cholesterol (TC) of alloxan-induced diabetic rats

Groups	Baseline	After Induction	7 Days	14 Days	21 Days	TG/TC (mg/dl)
Group 1	84.00 ± 6.59^{A1}	83.60 ± 4.03 ^{A1}	85.00 ± 2.73 ^{A1}	85.80 ± 2.04 ^{A1}	86.00 ± 2.12 ^{A1}	TG
	112.60 ± 6.07^{A1}	112.80 ±1.92 ^{A1}	111.80 ±5.40 ^{A1}	111.20 ±6.22 ^{A1}	111.00 ±5.79 ^{A1}	TC
Group 2	85.40± 5.07^{A1}	129.00 ± 4.00^{B4}	109.40 ± 3.57 ^{B3}	99.80± 3.03 ^{B2}	87.20 ± 5.49 ^{A1}	TG
	111.80 ± 3.35 ^{A1}	142.20 ±5.40 ^{B3}	122.60 ±2.07 ^{B2}	115.60 ±1.82 ^{A1}	113.40 ±3.36 ^{A1}	TC
Group 3	84.00 ± 7.07 ^{A1}	125.4 ± 4.92 ^{B3}	108.4 ± 4.15 ^{B2}	100.6 ± 4.39 ^{B2}	81.2 ± 5.93 ^{A1}	TG
	108.40 ± 4.04 ^{A12}	138.00 ±4.18 ^{B4}	122.80 ±2.28 ^{B3}	114.40 ±1.14 ^{A2}	108.00 ±3.54 ^{A1}	TC
Group 4	83.80 ± 7.25 ^{A1}	125.00 ± 4.30 ^{B3}	107.80 ± 3.03 ^{B2}	103.00 ± 4.84^{B2}	87.20 ± 2.77^{A1}	TG
	111.40 ± 6.95 ^{A1}	137.60 ±6.73 ^{B3}	123.60 ±2.97 ^{B2}	117.00 ±2.92 ^{A12}	113.80 ±2.86 ^{A1}	TC
Group 5	87.80± 8.07465 ^{A1}	124.00 ± 3.53 ^{B3}	106.60 ± 3.50 ^{B2}	99.00 ± 4.63 ^{B2}	81.00 ± 2.91^{A1}	TG
	115.20 ± 7.46 ^{A1}	139.40 ±6.23 ^{B2}	120.20 ±5.26 ^{B1}	111.20 ±7.05 ^{A1}	110.20 ±3.77 ^{A1}	TC
Group 6	86.20 ± 6.72 ^{A1}	127.80± 2.77 ^{B2}	124.40 ± 4.82 ^{C2}	122.40 ± 7.09^{C2}	123.60 ± 4.77 ^{B2}	TG
	110.60 ± 8.17 ^{A1}	138.40 ±6.35 ^{B2}	134.80 ± 3.96 ^{C2}	133.40 ±2.41 ^{B2}	133.80 ±3.11 ^{B2}	TC

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$

Table 6: Results of Phytochemical Analysis

Phytochemicals	Amount
Alkaloids	++
Flavonoids	++
Tannins	+++
Saponins	++
Glycosides	++
Fats and Oil	+

Key: + slightly present, ++ moderately present, +++ Abundantly present

Since these enzymes are undeniably, markers of liver injury, the elevated levels of these enzymes in diabetes conditions were attributed to harm induced to the hepatocytes by alloxan, which now disrupts the normal activities of the liver.[31,32] Following treatment with the plant extract, the activities of these marker enzymes were significantly reduced in the *G. latifolium* extract treated diabetic rats (Tables 2 and 3), indicating the plant's hepatoprotective properties, which is consistent with some studies reporting the plant's hepatoprotective properties.[33,34] The ability of *G. latifolium* to exert a protective effect on the liver and lower the level of liver enzymes in the blood could be related to flavonoids' hepaprotective characteristics, which serve as membrane stabilizers to protect the liver cells from harm.[26]

In acute toxicity study, a scale proposed by Lorke (1984) roughly classifies substances administered via the oral route according to their LD_{50} as follows: Very toxic (LD_{50} < 1.0 mg/kg bw), toxic (LD_{50} up to 10.0 mg/kg bw), less toxic (LD_{50} up to 100.0 mg/kg bw) and only slightly toxic (up to 1000.0 mg/kg bw). Substances with LD_{50} values greater than 5,000 mg/kg bw are practically non-toxic. Acute toxicity evaluation revealed that *G latifolium* extract did not produce any mortality in the animals up to a dose of 5000 mg/kg. Hence LD_{50} > 5000 mg/kg. The high oral LD_{50} (> 5000 mg/kg) obtained suggested that the extract is practically non-toxic when administered via the oral route in ethnomedical use. The photomicrographs of the liver of experimental animals are shown in Figure 1. When compared to the normal control and diabetic rats treated with various doses of *G latifolium* extract, which showed normal hepatic histological features, the histology of the diabetic untreated rat revealed cytoplasmic

degeneration and hepatocytes distortion. The histological examination demonstrates that *G latifolium* extract has significant hepatoprotective effect against hyperglycemia-induced liver damage in rats.

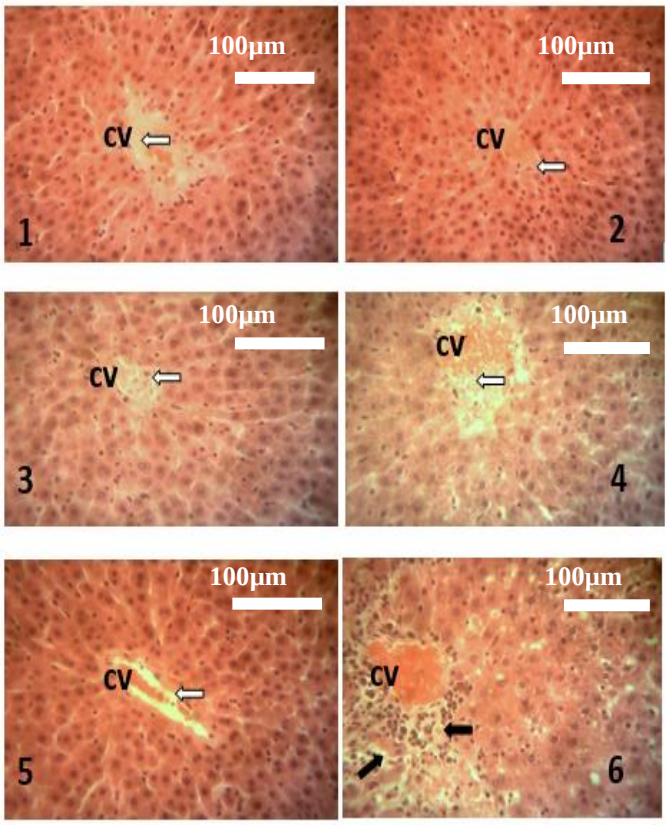

Figure 1: Photomicrograph of liver sections from the experimental group 1 (normal control) rats showing normal histological features of hepatocytes (white arrows) while groups 2 (diabetic and treated with 200mg/kg bwt extract), 3 (diabetic and treated with 400mg/kg bwt extract), 4 (diabetic and treated with 800mg/kg bwt extract), 5(diabetic and treated with standard drug) had no observable microscopic histological changes but group 6 (diabetic untreated) shows widespread necrosis of hepatocytes with severe mononuclear leucocytes infiltration of the centrilobular areas (black arrows).Note the central vein (cv). H and E x400.

Conclusion

This study revealed that *G latifolium* aqueous-ethanolic extracts have hypolipidemic, hypoglycemic and hepatoprotective effects in alloxan-induced diabetic rats. As a result, plant extracts may contribute beneficially in the treatment and management of diabetes mellitus and its associated complications. The plant extract was demonstrated to be non-toxic when taken orally in the acute toxicity study. However, more research on chronic toxicity is needed to determine the long-term effects of using this plant extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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