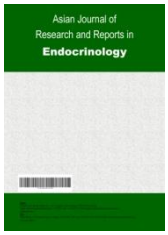

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Effect of Aqueous Leaf Extract of *Hamelia patens* jacq. on Some Biochemical Parameters in Alloxan-Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Medicinal plants is as old as man, they contain substances that can be used for therapeutic purposes with less toxicity. A number of these plants, leaf, herb and stem bark has been used in traditional medicine to treat diabetes for many years without sufficient scientific data.
Aim: Effect of aqueous leaf extract of *Hamelia patens*jacq. on some biochemical parameters in alloxan-induced diabetic albino rats was determined.
Method: The experimental animals (male Albino rats) were divided randomly into six groups of eight animals each. The test groups (2-6) were starved overnight followed by intraperitoneal administration of alloxan at 160mg/kg body weight. Treatment with the plant extract was for fourteen days. Group 1 was the normal control, group 2; diabetic untreated animals, group 3 received the standard drug gliclazide at 58mg/ml, groups 4, 5 and 6 were the extract treated groups at 400mg/kg body weight, 800mg/kg body weight and 1200mg/kg body weight respectively.
Results/Discussion: The mean lethal dose of the plant extract was above 5000mg/kg body weight. Total protein increased significantly ($P<0.05$) in the extract treated groups while, aspartate aminotransferase (AST), alanintransaminase (ALT), alkaline phosphatase (ALP) had significant decreases ($P<0.05$) when compared to group 2. Malondialdehyde (MDA), decreased significantly ($P<0.05$) in the group that received the standard drug alongside the extract treated groups when

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compared to group 2, while Glutathione (GSH) and Superoxide dismutase had a significant decrease in groups 2 and 3 at (P<0.05) and was non significant in the extract treatedgroups at (P>0.05). The C-peptidesincreased significantly (P<0.05) in groups 4 to 6 when compared to the group 2 that had a higher significant increase (P<0.05).
Conclusion: The results show that aqueous leaf extract of *Hamelia patens* may possess anti-diabetic,anti-inflammatory, antihepatotoxic, antianemic, antioxidant and antilipidemic properties at the tested doses.

Keywords: Blood glucose; antidiabetic; antioxidant; antilipidemic; antihepatotoxic; Alloxan.

1. INTRODUCTION

Plant products are shown to be of medicinal and therapeutic value to man. *Hamelia patens* Jacq. is a small tree, that grows up to1.4-3.0metres tall and sometimes could stretch up to 7metres. In the Atlantic tropical lowland of Costa Rica it could be seen as a growing tree. On eating the fruit birds are found dispersing the seed. The stems posess single and multiple plant twigs ranging from orange to purple with its leaves grouped in threes or fours, and finely hairy. There is presence of a berry edible fruit that is spherical to elliptical, and 7 to 10 mm long, turning red and then black on getting to maturity [1,2]. This plant is an ornamental plant that belongs to the *Rubiaceae*family, commonly known as Firebush. It could be used as diuretic and for the day to day treatment of inflammation, rheumatism, diabetes, wound healing, gastritis, stomach-ache, snake and scorpion bites, and high fever in traditional medicine [3].

Diabetes mellitus is known to be the most common disorder of the pancreas caused by an inability of the beta-islet of Langerhans to produce adequate insulin or a defect in the utilization of insulin. This disorder is highly characterized by polyuria (excessive urine production), polydypsia (excessive thirst for water) and polyphagia (excessive feeling for hunger); all for the inability of the body to metabolize glucose. It is also characterized by chronic hyperglycemia (high blood glucose) and glucosuria (presence of glucose in the urine), caused as a result of absolute or the relative lack of insulin. In this disease state a lot of disarrangement may occur ranging from, high presence of cholesterol, lack of weight gain, ketosis, arteriosclerosis, gangrene, some pathologic changes may occur in the eye, neuropathy, renal disease to coma [4].

There are different types of diabetes mellitus that can be classified under these two categories: The Type 1diabetes mellitus: that is known as the juvenile onset diabetes mellitus which is found to be insulin dependent, and Type 2 diabetes mellitus that is seen to be noninsulin-dependent; in this category β-cell mass is not completely lost. The present study is therefore designed to study the effect of aqueous leaf extract of *Hamelia patens* Jacq on some biochemical parameters in alloxan-induced diabetic albino rats.

Fig. 1. *Hamelia patens* leaf

2. MATERIALS AND METHODS

2.1 Plant Materials and Authentication

The leaves of *Hamelia patens* were harvested from a compound bush in Abia State University, Uturu, Abia State, Nigeria. The plant was authenticated at the department of Plant Science and Biotechnology, Abia State University Uturu by a taxonomist and voucher samples deposited in the Departmental herbarium. (Voucher number: ABSU/PSB/00043).

2.2 Preparation of Plant Extract

The extract was prepared using the method described by Daniel et al. [5] with slight

modifications. The leaves were air dried for four weeks into a constant weight. The dried leaves were milled into a fine powder and stored in a cellophane bag until use.

Hundred grams (100 g) of powdered leaves of *Hamelia patens* was soaked in 1000ml of water for 24hours with stirring for proper mixing and drained using a muslin cloth. It was filtered using Whatman no. 1 filter paper in order to get a clear filtrate. The concentration of the extract was determined to be 100 mg/ml.

2.3 Experimental Animals

A total of forty eight (48) apparently assumed healthy male Albino rats (9-10weeks old) weighing between (160 - 180 g) was procured from the animal house, Department of Biochemistry, Abia State University, Uturu. They had access to clean drinking water *ad libitum* and growers feed. All protocols for animal handling were strictly observed [6].

2.4 Induction of Diabetes

This was determined by the method described by Etuk E.U. [7].

Diabetes mellitus was induced by a single intraperitoneal injection of 160mg/kg body weight of Alloxan. Alloxan was dissolved in 0.9% normal saline as vehicle.

2.5 Measurement of Body Weight

Body weight was measuredon days 0, 7 and 14. Body weight noted was expressed as mean body weight (g).

2.6 Experimental Design

Randomized complete block experimental design recommended by Ogbeibu [8] was used in the study. The experimental animals were divided randomly into six (6) groups (Replicates) of eight (8) animals each:

***Group I*:** Normal control.
Group II: Diabetic group (negative control)
Group III: Diabetic group treated orally with 58mg/ml gliclazide: a standard anti-diabetic drug.
Group IV: Diabetic group treated orally with 400mg/kg body weight of aqueous leaf extract of *Hamelia patens.*
Group V: Diabetic group treated orally with 800mg/kg body weight of aqueous leaf extract of *Hamelia patens.*
Group VI: Diabetic group treated orally with 1200 mg/kg body weight of aqueous leaf extract of *Hamelia patens.*

2.7 Liver Function Test

2.7.1 Determination of Aspartate Aminotransferase (AST) and Alanine transaminase (ALT)

Activity was determined by the method of Reitman and Frankel [9].

2.7.2 Determination of alkaline phosphatase (ALP)

Activity was by the method of Julius et al. 1964 [10]. This method is based on the principle that serum alkaline phosphate hydrolyses a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH value turns into a pink colour that can be photometrically determined.

2.7.3 Determination of total protein was as described by Weichselbau [11]

Colorimetric determination of total protein based on the principle reaction (Copper salt in an alkaline medium). Protein in plasma or serum forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

2.8 Determination of Antioxidants Activities

2.8.1 Determination of superoxide dismutase activity (SOD)

The method of Sun and Sigma as described by Christine and Joseph [12], was adopted. The reaction mixture (3ml) contained 2.95ml sodium carbonate buffer (0.05M, ph. 10.2), 0.02ml of serum and 0.03ml of epinephrine in 0.005N HCL used to initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. An extinction coefficient for epinephrine at 480nm of 4020 $m^{-1}cm^{-1}$ was used in calculating activity.

2.8.2 Determination of catalase activity

The catalase activity was determined according to the method of Christine and Joseph [12],

2.8.3 Determination of Glutathione (GSH)

The GSH level was determined using the method described by Christine and Joseph [12], with slight modifications. Serum (0.5ml) was added to 2ml of 5% TCA and centrifuged at 3000 rpm for 10minutes. The supernatant (1ml) was added to 0.5ml of DTNB (10nm) in the presence of 3ml phosphate buffer (0.1M, pH 7.4). Absorbance was read at 420nm.

2.8.4 Determination of Malondialdehyde (MDA) activity

Lipid peroxidation was ascertained by formation of Malondialdehyde (MDA) and measured by thiobarbituric reactive (TBARS) method previously described by Onkawa et al. [13] Reaction mixture containing serum (0.5ml), TCA (0.5ml) and TBA (0.5ml) was incubated in boiling water for 15minutes. The pink color of chromogen formed was extracted in butanol solution (2.0ml). The mixture was centrifuged at 3000 rpm for 10minutes and the supernatant was read at 532nm.

2.8.5 Determination of C-peptides

The C-peptides values were determined using the approved guidelines of Clinical and Laboratory Standards Institute [14].

3. RESULTS

Table 1. Shows the acute toxicity test of aqueous leaf extract of *Hamelia patens.* At the highest dose of 5000mg/kg body weight there was no mortality. This shows that the extract is safe at 5000mg/kg body weight.

Table 2 shows the effect of *Hamelia patens* aqueous leaf extract on blood glucose concentration of alloxan-induced diabetic rats. Groups 2-6 showed marked significant increases and decreases at ($P<0.05$) as the treatment levels progressed. The standard drug treated group (Group 3) and extract treated groups (Groups 4,5, and 6) showed significant decreases in blood glucose levels post treatment.

Table 1. Acute (Oral) toxicity study of Albino rats after 24hours administration of *Hamelia patens* leaf extract

PHASE I	**Death/number of animals**		
Group	**Dose (mg/kg)**	**Mortality**	**Observation**
Control	0.00	0/3	
Hamelia patens leaf extract	10.00	0/3	-
	100.00	0/3	-
	1000.00	0/3	Calmness
PHASE II	**Death/number of animals**		
Group	**Dose (mg/kg)**	**Mortality**	**Observation**
Control	0.00	0/1	
Hamelia patens leaf extract	1600.00	0/1	Restlessness
	2900.00	0/1	Restlessness
	5000.00	0/1	Restlessness

Table 2. Effect of *Hameliapatens* aqueous leaf extract on Blood glucose concentration of alloxan-induced Diabetic Albino rats (mg/dL)

Treatment	**Pre-induction**	**Post-induction**	**Post-treatment**
Group 1 (Normal Control)	93.67 ± 6.66^{a}	90.67 ± 6.43^{a}	88.00 ± 7.55^{a}
Group 2 (Negative Control)	87.67 ± 2.08^{a}	354.00 ± 56.56^{b}	597.67 ± 2.08^{c}
Group 3 (Standard Drug)	88.00 ± 1.00^{a}	392.00 ± 95.06^{b}	225.33 ± 57.27^{c}
Group 4 (400mg/kg)	94.67 ± 3.21^{a}	381.67 ± 22.48^{b}	201.33 ± 6.66^{c}
Group 5 (800mg/kg)	96.00 ± 4.36^{a}	400.33 ± 70.06^{b}	260.00 ± 52.37^{c}
Group 6 (1200mg/kg)	86.00 ± 3.61^{a}	365.33 ± 67.09^{b}	186.33 ± 5.51^{c}

**Values are mean ± SD for N=8 (number of animals per group). Values across the samerow bearing the same letter of alphabets are not significantly different (P>0.05) while values with different subscript are significant at P<0.05.*

Key: *Negative control: (Diabetic group), Standard drug: (gliclazide 58mg/ml), Group 4-6: Hamelia patens aqueous leaf extract treated groups at different doses.*

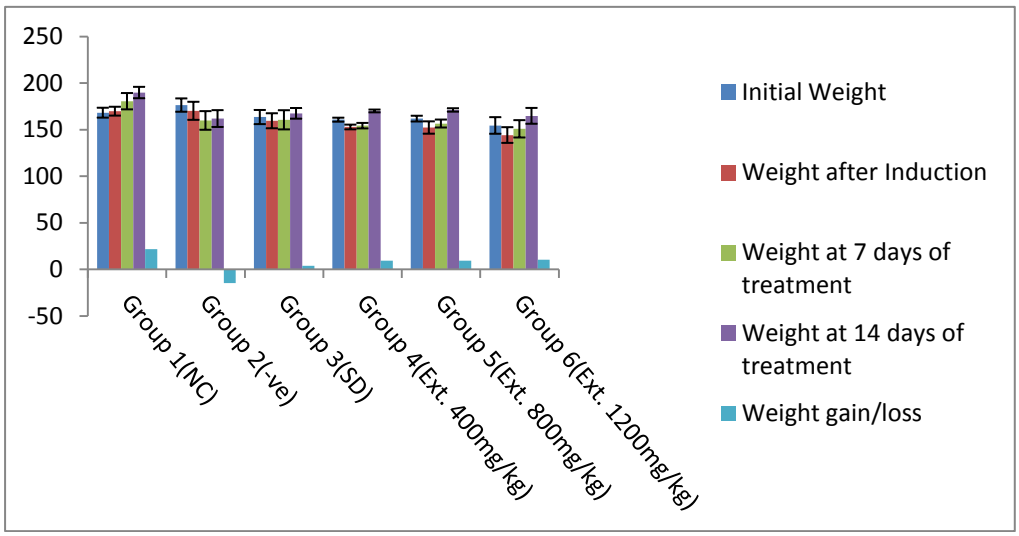

Fig. 2. Showing effect of *H. patens* aqueous leaf extract of alloxan-induced diabetic Albino rats on their body weights for 14 days of treatment

Table 3. Showing the effects of *Hamelia patens* aqueous leaf extract on liver function biomarkers of alloxan-induced diabetic Albino rats

Treatments	TP (g/dl)	AST (µ/l)	ALT (µ/l)	ALP (µ/l)
Group 1 (NC)	8.43±0.33^{a}	42.33±2.51^{a}	24.00±2.65^{a}	111.65±9.53^{a}
Group 2(-ve Control)	5.56±0.16^{b}	93.33±7.37^{b}	70.33±2.52^{b}	171.56±5.88^{b}
Group 3 (Strd Drug)	6.94±0.49^{ab}	57.33±6.68^{c}	43.00±4.36^{c}	133.37±4.24^{c}
Group 4 (400mg/kg b.w)	6.34±0.59^{c}	65.33±6.43^{d}	49.33±2.08^{d}	130.67±8.58^{d}
Group 5 (800mg/kg b.w)	6.49±0.09^{d}	58.00±3.00^{e}	51.67±6.35^{e}	135.71±0.90^{e}
Group 6 (1200mg/kg b.w)	6.55±0.58e	54.67±6.68f	48.00±7.00^{f}	128.50±2.48^{f}

**Values are mean ± SD for N=8 (number of animals per group). Values down the column bearing the same letter of alphabets are not significantly different (P>0.05) while values with different subscript are significant at P<0.05.*
***Key:** TP: Total protein, AST: Aspartateaminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, NC: Normal control, -ve control: Negative control, Strd drug: Standard drug (gliclazide 58mg/ml), B.W.: Body weight*

Table 4. Showing the effects of *Hamelia patens* aqueous leaf extract on Antioxidant biomarkers of alloxan-induced diabetic Albino rats

Treatments	GSH (µ/l)	SOD (µ/l)	CAT (µ/l)	MDA (mmol/l)
Group 1 (NC)	47.94±1.95^{a}	33.28±2.91^{a}	10.78±1.38^{a}	0.41±0.12^{a}
Group 2(-ve Control)	40.91±2.19^{b}	26.80±1.07^{b}	9.12±0.76^{a}	1.10±0.17^{b}
Group 3 (Strd Drug)	42.06±1.67^{c}	29.47±0.76^{c}	9.93±0.71^{a}	0.94±0.08^{c}
Group 4 (400mg/kg b.w)	45.13±2.08^{a}	31.03±1.65^{a}	11.51±1.10^{a}	0.81±0.05^{d}
Group 5 (800mg/kg b.w)	45.46±0.79^{a}	30.79±1.05^{a}	10.49±0.88^{a}	0.89±0.06^{e}
Group 6 (1200mg/kg b.w)	45.98±1.89^{a}	32.73±1.12^{a}	10.43±1.46^{a}	0.85±0.08^{f}

**Values are mean ± SD for N=8 (number of animals per group). Values down the column bearing the same letter of alphabets are not significantly different (P>0.05) while values with different subscript are significant at P<0.05.*
***Key:**GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, NC: Normal control, -ve control: Negative control, Strd drug: Standard drug, B.W.: Body weight*

The diabetic untreated group (Group 2) had the highest reduction in weight while group 1 (normal control) had the highest weight gain. There was improved weight gain in groups 3-4 as the treatment level progressed for 14 days.

Table 3 shows the effects of *Hamelia patens* aqueous leaf extract on liver function biomarkers of alloxan-induced diabetic Albino rats. TP, AST,ALT and ALP all had a significant increase on the diabetic group (P<0.05) and a significant

decrease on the extract treated groups. (P<0.05).

Table 4 shows the effects of *Hamelia patens* aqueous leaf extract on antioxidant biomarkers of alloxan-induced diabetic Albino rats. GSH and SOD had a significant decrease in groups 2 and 3 at P<0.05 and was non significant in the extract treatedgroups. (P>0.05). CAT had no significant increase in groups 3-6. (P>0.05). MDA had a marked significant increase in groups 3-6 when compared to the diabetic control group. (P<0.05)

Table 5. Showing the effects of *Hamelia patens* aqueous leaf extract on C-Peptides of alloxan-induced diabetic Albino rats

Treatments	C-Peptide (ng/ml)
Group 1 (NC)	1.61 ± 0.28^{a}
Group 2(-ve Control)	0.92 ± 0.05^{b}
Group 3 (Strd Drug)	0.10 ± 0.07^{c}
Group 4 (400mg/kg b.w)	0.95 ± 0.15^{d}
Group 5 (800mg/kg b.w)	1.10 ± 0.15^{e}
Group 6 (1200mg/kg b.w)	1.03 ± 0.07^{f}

**Values are mean ± SD for N=8 (number of animals per group). Values down the column bearing the same letter of alphabets are not significantly different (P>0.05) while values with different subscript are significant at P<0.05.*

***Key:** NC: Normal control, -ve control: Negative control (Diabetic group), Strd drug: Standard drug (gliclazide 58mg/ml), B.W.: Body weight, Group4-6 (Extract treated groups)*

Table 5 shows the effects of *Hamelia patens* aqueous leaf extract on C-Peptides of alloxan-induced Diabetic Albino rats. C-Peptides significantly increase in groups 3-6. (P<0.05) when compared to the diabetic control.

4. DISCUSSION

Most of the primary health care needs of the world are gotten from plant derived properties [15]. The mean lethal dose of a plant material is one of the ways to check for the short term poisoning effect of a particular plant material [16]. The oral acute toxicity LD_{50} figures of aqueous leaf extract of *Hamelia patens* was found to be above 5000mg/kg body weight with no mortality rate in any of the test groups after a 24 hour observation. This shows that the plant extract may have usefulphytoconstituents. The OECD guidelines which tests for acute toxicity shoes clearly that mortality is one of the ways to know how safe a particular plant extract could be for use [17].

Studies have it that an improvement in weight is due to an imbalance between dietary intake and the needed energy expenditure [18]. From this study we had observable increases and decreases in the body weight of all the groups (Group 1-6). However, these increases and decreases are not significant at 95% level of confidence (P<0.05). The mean body weight of the normal control (Group 1) increased for a period of 14 days although it was not significant (P>0.05) and had the highest weight gain while Diabetic control (Group 2) had the highest loss in weight. This could be as a result of lack of glucose metabolism and high level of tissue breakdown and that of protein as well [19,20].

The study revealed a significant (P<0.05) decrease in the glucose concentrationof the test animals treated with different doses of the extract (400-1200mg/kg body weight). This suggests the hypoglycemic effect of aqueous leaf extract of *Hamelia patens* in the alloxan-induced diabetic Albino rats. At 1200mg/kg body weight, *Hamelia patens* aqueous extract significantly (P<0.05) reduced the blood glucose level from 365.33±67.09 (mg/dL) to 186.33±5.51 mg/dL at 14 days of treatment. The blood glucose lowering effect of the extract at the highest dose was greater than that of the standard drug (gliclazide). Different research techniqueshad shown the inhibitory effect of gliclazide on hepatic gluconeogenesis and its sensitivity in NIDDM (Non-Insulin Depended Diabetes mellitus) [21]. The glucose lowering effect of the plant extract suggests the extract may possess antidiabetic potentials.

Increase in the concentrations of some liver biomarkers indicates some level of liver dysfunction [22]. Total Protein had a marked significant increase (P<0.05) from 5.56±0.16 of group 2 to 6.55±0.58 in group 6. This suggests protein synthesis effect of the plant extract in the test animals (translation). There was observable significant decrease (P<0.05) in AST, from 93.33±7.37 of group 2 to 54.67±6.68 of group 6, ALT from 70.33±2.52 of group 2 to 48.00±7.00 of group 6 and ALP 171.56±5.88 of group 2 to 128.50±2.48 of group 6. The increase in the liver enzyme following alloxan diabetic induction followed by a decrease at 14 days of treatment suggests that the plant has anti-inflammatory potentials [22]. When there is prolonged destruction of the hepatic cells it results to the release of more liver cells to exacerbate hepatic dysfunction with a decrease in the serum levels of some liver biomarkers [23]. This observation

suggests the plant extract may possess antihepatotoxic properties.

Oxidants and lipid peroxidation have serious debilitating impact thatcan be countered by an organized antioxidant defense mechanism for animals [24]. This mechanism deals with several enzymatic and non-enzymatic processes that protect living cells against oxidative damage [25]. Glutathione (GSH) and Superoxide dismutase (SOD) activities decreased significantly $P<0.05$ in group 2 and 3 and had a non significant decrease $P>0.05$ in the extract treated groups. This suggests that there could be an observable dismutation of superoxide radical into oxygen and hydrogen peroxide and could also play a significant role in hypertension reduction if evaluated [24], and reduction of free radicals in the experimental animals for GSH activity. Catalase (CAT) had a non significant decrease ($P>0.05$) in groups 2 and 3 and a non significant increase ($P>0.05$) in groups 4-6. This suggests that catalase has serious role in the ameriorative effects of hydrogen peroxide produced in several cellular processes such as photorespiration, oxidation, DNA synthesis [26]. This observation also suggests that the aqueous leave extract of the plant may possess antioxidant properties. Malondialdehyde decreased significantly ($P<0.05$) in the extract treated groups when compared to group 2 (diabetic group). In this study, the decreased levels of MDA (marker of lipid peroxidation) in diabetic rats clearly showed that diabetic rats were exposed to an increased oxidative stress via lipid peroxidation and had mild restoration in the extract treated groups [27]. This observation suggests the extract may also possess antilipidemic properties.

The C-peptide is a useful and widely used biomarker of insulin A and B made up of 31 amino acids peptide [28,29]. After cleavage of proinsulin, insulin and the 31-amino-acid peptide c-peptide are produced in equal amount. The c-peptide degradation rate in the body is slower than that of insulin (half-life of 20–30 min) [30], which affords a more suitable test response of fluctuating beta cells. Evaluation of C-peptide level is an important parameter to find out the amount of endogenous insulin secreted in the body [30]. There was observable significant differences $P<0.05$ in mean serum C-peptide levels between the control groups (Group 1, Normal Control) and Group 2, Negative Control). While there was observable increase that is statistically different in Group 2 (Diabetic untreated group) when compared to all the treatment groups (Group 3 – Group 8). These increases suggest thatthere was endogenous insulin secretion in the test diabetic rats treated with the leaf extract, which might be the cause of its blood glucose lowering effect in the animals [30].

5. CONCLUSION

From the study results show that aqueous leaf extract of *Hamelia patens*was able to give observable weight increase as the treatment progressed when compared to the untreated control. The study revealed significant ($P<0.05$) decrease in the blood glucose level of the animals treated with different doses of the extract (400-1200 mg/kg body weight). Which suggests that the plant has glucose lowering potentials [21]. On treatment we observed adequate improvement in some of the liver biomarkers, and lipid biomarkers in the animals. The c-peptides also showed significant improvement in the treatment groups when compared to the untreated control group as this biomarker is a measure of insulin A and B and its improvement in such a disease state [29]. Thus the plant extract may possess anti-diabetic, anti-inflammatory, antihepatotoxic, antianemic, antioxidant and antilipidemic properties at the tested doses.

ETHICAL APPROVAL

Ethical clearance was obtained from the Ethical Committee of Animal Care Use of the Faculty of Biological Sciences, Abia State University; Uturu, Nigeria.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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