Anti-Hyperglycemic Activity of Aqueous Extract of *Lawsonia Inermis* Leaf in Alloxan-Induced Diabetic Wistar Rats (Anti-Hyperglycemic Activity of Aelil)

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ABSTRACT

***Background:** Alterations in metabolism of fuel molecules in diabetes increase blood glucose. This causes long-term complications in many organs. Currently, available treatment options to maintain glycemic control are accompanied by various side effects. Therefore, there is need to develop newer anti-hyperglycemic agents of plant origin with no side effects.*

***Objective:** This study aimed to evaluate the anti-hyperglycemic effects of aqueous extract of Lawsonia inermis leaf in diabetic rats.*

***Methods:** Thirty albino rats of an average weight 203.21 ± 6.57 g were randomly divided into six groups. Diabetes was induced through intraperitoneal administration of 150 mg/kg body weight of alloxan in all animal groups except one. Group A, non-diabetic rats, and group B, a diabetic control group were treated with 0.5 mL of normal saline while group C was treated with 50 mg/kg body weight metformin. Groups D, E and F were administered daily dose of 200, 400, and 600 mg/kg body weight of aqueous extract of Lawsonia inermis leaf (AELIL) respectively for a period of 3 weeks. Parameters monitored are fasting blood glucose using glucometer, body weight, serum chemistry and liver enzyme markers.*

***Results:** Administration of AELIL showed a significant ($p < 0.05$) reduction in fasting blood glucose of diabetic rats in a dose dependent manner. Also, administration of AELIL has no negative effect on the body weight, serum chemistry and liver marker enzymes of diabetic rats. The AELIL showed no observable toxicity.*

***Conclusion:** AELIL possesses antidiabetic activity and safe for consumption.*

Keywords: Alloxan, diabetes mellitus, Lawsonia inermis

INTRODUCTION

Diabetes mellitus is a global disease and one of the leading causes of death worldwide [1]. It is a metabolic disorder characterized by hyperglycemia resulting in distortion of carbohydrate, fat and protein metabolism. The alterations in the metabolism of fuel molecules in the body could be due to defects in the secretion or action of insulin [2]. Insulin facilitates utilization of glucose by the body cells [3]. In diabetic condition, the levels of glucose build up in the blood and urine. This causes excessive urination, hunger, thirst and problems associated with the metabolism of fat and protein. Other symptoms associated with diabetes mellitus include weight loss, blurred vision, fatigue and delayed wound healing [4] [5]. In 2017, the

international diabetes federation (IDF), reported 451 million cases of diabetes mellitus in adults globally. This is projected to increase to about 693 million by 2045 if preventive measures are not adopted [6].

Plants and plant materials have been found useful from the time immemorial as they contribute to the maintenance of good health [7]. Many countries in Africa depend on herbs whose potential positive contribution in primary health care is acknowledged by the World Health Organization. More than 60% of the people of the world patronize medicinal plants for treating ailments and diseases [8]. The plants contain bioactive compounds called secondary metabolites with ability to prevent disease and promote health [9]. Many available plants within Nigeria, have been reported to have antidiabetic potential [10].

Lawsonia imermis, (Lythraceae) commonly known as henna is a perennial plant that is widely distributed across Africa. *L. inermis* is a multi-branched, glabrous shrub 2-6m tall with greyish-brown bark. The leaves are elliptical with depressed veins on the dorsal surface and grow opposite to each other on the stem. Henna fruits are small, brownish capsules usually 4-8mm in diameter having about 32-49 seeds per fruits. The leaves *of L. inermis* have been used traditionally in treating ailments like rheumatoid arthritis, ulcers, diarrheoa, leprosy, fever, diabetes and cardiac disease [11] [12]. *Lawsonia inermis* has also been reported to possess anti-inflammatory, antipyretic and analgesic properties [13].

Presently, there are different types of synthetic drugs used for the treatment of diabetes mellitus such as sulfonylureas, biguanides, thiazolidinediones, amongst others [14]. However, these drugs have been reported to show several side effects and are sometimes found to be ineffective in the treatment of chronic diabetic patients [15]. Metformin, for example is a biguanide which causes anorexia, diarrhea, nausea and lactic acidosis with renal impairment [16]. The treatment of diabetes mellitus and its complications using plant materials have been gaining interest. Therefore, the aim of this study was to evaluate anti-hyperglycemic effect of aqueous extract of *L.inermis* in alloxan-induced diabetic rats.

2. Materials and Methods

Collection and Identification of Plant Sample

Fresh leaves of *Lawsonia inermis* were collected from Suleja town, Suleja Local Government, Niger State, Nigeria. The plant was identified and authenticated by a botanist at the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, Niger state and a voucher specimen number IBB/BS/00007.

Preparation of Sample and Extraction

The leaves of the plant were air dried at room temperature for two weeks, pulverized with mortar and pestle, and then sieved. Aqueous extract of *L. inermis* was prepared by soaking 100g of the powdered leaves into 1000mL of distilled water for 72 hours. The resulting extract was filtered using muslin cloth followed by Whatman's filter paper (No 1). The filtrate was evaporated in a rotary evaporator at 40 °C under high pressure. The percentage yield of the extract is 8.7%. The resulting extract was freeze dried and stored in an air-tight container for further use. The extract was reconstituted in distilled water to give the doses used in this study.

Phytochemical analysis

The aqueous extract obtained was screened for the presence of Saponins, flavonoids, tannins, phlobatannins, alkaloids, steroids and Phenolics according to the methods described by [17] and [18].

Experimental animals

Thirty healthy albino rats with average body weight of 203.21 ± 6.57g were obtained from the Animal Holding Unit of the Department of Biochemistry, Kogi State University Ayingba. The rats were allowed to acclimatize in the Animal House of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger state. The animals were housed in clean metabolic cages (temperature range between 29 and 38 °C and relative humidity of between 51 and 67%). The rats were acclimatized for two weeks under conducive environmental conditions before the experiment and were allowed free access to standard animal feeds and distilled water during the period. Procedures of [19] guidelines on good laboratory practices were followed.

Diabetes Induction

The Induction of diabetes was done using method described by [7]. The albino rats were fasted overnight and induced into diabetes by the intraperitoneal administration of 150 mg/kg bw of alloxan dissolved in normal saline. The rats

were then given 10% glucose solution in water bottles for the next 24 hours to prevent hypoglycaemia and death. After 72 hours of intraperitoneal administration of alloxan solution, the fasting blood glucose levels were measured from the blood sample collected from the rats through their tails. The animals with blood glucose levels less than 250 mg/dL were not considered diabetic and rejected for the experiment.

Grouping of Animals

Thirty albino rats were divided into six groups with each group containing five rats. The animals were administered intra-peritoneal as follows:

Group A: Healthy rats administered with 0.5 mL of normal saline (Non-diabetic control)

Group B: Diabetic rats administered with 0.5mL of normal saline (Diabetic control)

Group C: Diabetic rats treated with standard drug metformin 50mg/kg bw

Group D: Diabetic rats treated with 200mg/kg bw of aqueous extract of *Lawsonia inermis* leaf.

Group E: Diabetic rats treated with 400mg/kg bw of aqueous extract of *Lawsonia inermis* leaf

Group F: Diabetic rats treated with 600mg/kg bw of aqueous extract of *Lawsonia inermis* leaf

The administration was done once daily for 21 days.

Determination of Fasting Blood Glucose Levels

Fasting glucose levels on days 3, 7, 14 and 21 were estimated using Accuchek glucometer. A 26 gauze sized needle was used to prick into the tail veins of the rats to obtain a drop of blood which was placed on the test area of the glucometer to determine the fasting blood glucose levels in each rats tested.

Acute Toxicity Study

The acute toxicity study was carried out according to [19] guidelines420. Twelve rats were divided into four different groups with each group consisting of three animals. The four groups were administered with single doses of 625mg/kg, 1250mg/kg, 2500 mg/kg and 5000mg/kg bw of the extract respectively. After dosing, the rats were observed continuously for signs of toxicity and mortality for 21 days.

Biochemical analysis

Biochemical analysis carried out for assessing liver function include; determination of serum total protein, total bilirubin and albumin, and the enzyme biomarkers; Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Alanine transaminase (ALT). Total protein was determined using Biuret method as modified by [20], albumin by the Bromocresol green method as described by [21] and total bilirubin by the colorimetric method described by [22]. The colorimetric method described by [23] based on the formation of 2,4-dinitrophenyl hydrazine with hydrazone was used for the determination of AST and ALT while ALP was estimated as described by [24].

Statistical analysis

Data obtained were analyzed using One-way analysis of Variance (ANOVA). The results were expressed as the mean ± SD (Standard deviation) of replicate analyses. Data were considered significantly different at $p < 0.05$.

3. Results

Phytochemical screening

The qualitative screening of secondary metabolites in the aqueous extract of *Lawsonia inermis* leaf is shown in Table 1. The extract contains; saponnins, flavonoids, tannins, terpenes, alkaloids, steroids and phenolics.

Acute toxicity

The effect of higher doses of AELIL on healthy rats is shown in Table 2. The three animals administered with different doses of the extract were observed for 21days and neither mortality nor toxic manifestation was recorded. Therefore, the LD_{50} for AELIL leaf as was observed in this study is greater than 5000mg/kg bw of the animal (LD_{50}>5000mg/kg).

Antihyperglycemia

Antihyperglycemic effect of aqueous extract of *Lawsonia inermis* leaf in alloxan-induced diabetic rats is shown in table 3. The results showed a significant ($p > 0.05$) reduction in the glucose level of the diabetic rats administered the extract in a dose dependent manner. The significant decrease observed during the administration of AELIL leaf also depend on the duration of administration. The glucose level of rats administered with 600 mg/kg bw was not significantly ($p < 0.05$) different from that of the non-diabetic animals after three weeks of administration of the extract. However, a significant ($p > 0.05$) difference was observed in the glucose levels of rats administered different doses of the extract.

Table 1. Phytochemical constituents of aqueous extract of *Lawsonia inermis*leaf.

Chemical constituents	Inference
Saponins	+
Flavonoids	+
Tannins	+
Phlobatanins	–
Terpenoids	+
Alkaloids	+
Steroids	+
Phenolics	+

Key: + = present, - = absent

Table 2. The LD_{50} of aqueous extract of *Lawsonia inermis* leaf using albino rats.

Dose (mg/kg body weight)	Sample size	Death	Response
625	3	nil	normal
1250	3	nil	normal
2500	3	nil	normal
5000	3	nil	normal

Animal weight

The weight of the diabetic rats before and during administration of aqueous extract of *Lawsonia inermis* leaf is represented in Table 4. The results showed that there is no significant ($p < 0.05$) difference in the weight of non-diabetic animals and the diabetic group of rats administered with 600 mg/kg b. wt. of the extract after 21 days of administration. Also, no significant ($p < 0.05$) difference was observed between the group of rats administered with the standard drug and the group administered with 600 mg/kg b. wt. of the extract after 21 days of administration. However, a significant ($p > 0.05$) reduction in weight was observed at lower doses (200 and 400 mg/kg bw) of the extract

Biochemical parameters

The effect of aqueous extract of *Lawsonia inermis* leaf on some biochemical parameters used in assessing the functionality of liver is represented in Table 5. The results obtained for the liver function test showed that there was no significant ($p < 0.05$) difference in the activities of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase in all groups except the diabetic non treated group. Also, no significant ($p < 0.05$) difference was observed in the albumin concentration in all groups of rats administered with the extract. However, there is a significant ($p > 0.05$) increase in the albumin concentration of the non-diabetic rats compared with the groups treated with both the extract and the standard drug. There is no significant ($p < 0.05$) difference in the serum total protein measured in non-diabetic group and the group of rats administered with the higher doses (200 and 400 mg/kg bw. Also, no significant ($p < 0.05$) difference was noticed in the serum total protein in the group administered with 200 mg/kg bw of

Table 3: Effects of aqueous extract of *Lawsonia inermis*leaf on glucose level of diabetic albino rats within three weeks of administration.

Treatments	Day 0	Day 3	Day 7	Day 14	Day 21
A	95 ± 2.31^{b}	94.7 ± 2.02^{a}	98.3 ± 1.45^{a}	105 ± 1.73^{a}	109 ± 1.86^{a}
B	89 ± 4.35^{ab}	345 ± 21.22^{b}	319 ± 11.4^{c}	347 ± 11.0^{e}	310 ± 10.5^{d}
C	84.3 ± 1.20^{a}	374 ± 16.5^{b}	336 ± 11.9^{c}	290 ± 2.60d^{e}	198 ± 0.58^{c}
D	85.7 ± 3.48^{a}	390 ± 18.02^{b}	337 ± 16.9^{c}	262 ± 18.68^{d}	191 ± 3.79^{c}
E	81.7 ± 1.20^{a}	373 ± 31.54^{b}	321 ± 12.0^{c}	224 ± 3.84^{c}	160 ± 15.1^{b}
F	89 ± 2.80^{ab}	395 ± 17.25^{b}	227 ± 13.5^{b}	145 ± 7.57^{b}	95.7 ± 3.48^{a}

Data are means ± SD of five determinations. Values with different superscripts were significantly ($p > 0.05$) different down the column and across the row.
A=Non-diabetic control, **B**=Diabetic control, **C**=Diabetic rats treated with 50mg/kg body wt. Metformin, **D**= Diabetic rats treated with 200mg/kg bw of AELIL, **E**=Diabetic rats treated with 400mg/kg bw of AELIL, **F**=Diabetic rats treated with 600mg/kg bw of AELIL

Table 4: Effects of aqueous extract of *Lawsonia inermis* leaf on body weight of diabetic albino rats.

Treatment	Day 0	Day 3	Day 7	Day 14	Day 21
A	190 ± 0.67^{a}	192 ± 0.33^{ab}	194 ± 0.82^{b}	196 ± 0.33^{c}	205 ±1.45^{d}
B	193 ± 2.51^{d}	185 ± 2.89^{c}	182 ± 1.73^{bc}	178 ± 1.45^{b}	171 ± 1.86^{a}
C	206 ± 4.0^{c}	198 ± 4.33^{b}	191 ± 2.08^{a}	195 ± 2.96^{ab}	203 ± 1.45^{c}
D	195 ± 1.67^{b}	185 ± 4.04^{a}	182 ± 1.76^{a}	180 ± 2.60^{a}	194 ± 2.19^{b}
E	193 ± 2.03^{b}	186 ± 2.65^{a}	184 ± 2.31^{a}	180 ± 0.82^{a}	183 ± 2.03^{a}
F	205 ± 2.89^{c}	197 ± 1.67^{b}	195 ± 0.88^{b}	190 ± 1.76^{a}	203 ± 1.73^{c}

Data are means ± SD of five determinations. Values with different superscripts were significantly ($p > 0.05$) different down the columnand across the row.
A=Non-diabetic control, **B**=Diabetic control, **C**=Diabetic rats treated with 50mg/kg body wt. Metformin, **D**= Diabetic rats treated with 200mg/kg bw of AELIL, **E**=Diabetic rats treated with 400mg/kg bw of AELIL, **F**=Diabetic rats treated with 600mg/kg bw of AELIL

the extract, standard drug, and the diabetic non treated group. For bilirubin, a significant ($p > 0.05$) difference was observed among the groups

4. Discussion

The use of plants or plant materials for the treatment of diabetes is a common practice all over the world. Many of the traditional plants

Table 5: Effect of aqueous extract of *Lawsonia inermis*leaf on some liver function parameters after 21 days of administration

Treatment	Alb (g/dL)	TP (g/dL)	Bilirubin (mg/dL)	ALP(U/L)	ALT (U/L)	AST (U/L)
A	3.86 ± 0.05^{b}	5.71 ± 0.26^{b}	1.47 ± 0.17^{b}	197.88 ± 6.06^{a}	14.46 ± 1.77^{a}	56.86 ± 1.68^{a}
B	3.62 ± 0.25^{ab}	4.81 ± 0.32^{a}	1.57 ± 0.16^{c}	223.16 ± 2.54^{b}	19.21 ± 0.66^{b}	65.77 ± 2.76^{b}
C	3.55 ± 0.11^{a}	4.73 ± 0.34^{a}	1.80 ± 0.38^{cd}	205.17 ± 4.65^{a}	15.09 ± 1.96^{a}	52.78 ± 0.69^{a}
D	3.28 ± 0.35^{a}	4.93 ± 0.43^{a}	1.99 ± 0.15^{c}	209.12 ± 2.76^{a}	14.74 ± 0.40^{a}	54.78 ± 2.57^{a}
E	3.33 ± 0.11^{a}	5.59 ± 0.69^{b}	2.19 ± 1.26^{e}	204.95 ± 3.47^{a}	13.91 ± 1.69^{a}	53.31 ± 4.27^{a}
F	3.36 ± 0.20^{a}	5.27 ± 0.78^{b}	1.33 ± 0.08^{a}	201.53 ± 5.12^{a}	13.65 ± 1.26^{a}	53.70 ± 3.76^{a}

Data are means ± SD of three determinations. Values with different superscripts were significantly ($p > 0.05$) different down the columnand across the row.
A=Non-diabetic control, **B**=Diabetic control, **C**=Diabetic rats treated with 50 mg/kg body wt. Metformin, **D**= Diabetic rats treated with 200mg/kg bw of AELIL, **E**=Diabetic rats treated with 400mg/kg bw of AELIL, **F**=Diabetic rats treated with 600mg/kg bw of AELIL
Alb-Albumin, **TP**-Total protein, **ALP**-Alkaline phosphatase, **ALT**-Alanine aminotransferase and **AST**-Aspartate aminotransferase.

used in the treatment or management of diabetes have undergone scientific investigation and have been recommended by the World Health Organisation (WHO) include but not limited to; *Momordica charantia, Azadirachta indica, Tinospora cordifolia, Enicostema littorae, Syzigium cumini, Gymnema sylvestre,* [25]. The aforementioned plants have been reported to be effective as Nigeria traditional plants usually employed in the treatment of diabetes [26] [27]. In this study, the aqueous extract of *Lawsonia inermis* showed the presence of saponins, flavonoids, steroids, tannins, alkaloids, terpenoids and phenols while phlobatannins was absent. This is in line with the findings of [28] who reported the presence of tannins, flavonoids, alkaloids, terpenoids saponins, phenols but absence of phlobatannins in aqueous extract of *L.inermis*.

The presence of secondary metabolites in the plant extracts have been reported to influence both physiological and biochemical processes in human and animals. The possible mechanism by which *L. inermis* leaves bring about its anti-hyperglycemic action may be through upregulation of insulin production and secretion from β-cell of islets of the pancreas or due to enhanced transport of blood glucose to peripheral tissue [29]. The presence of some phytochemicals, alkaloids, tannins, flavonoids etc, in plant have been reported to influence insulin secretion and glucose absorption [29].

The primary drawback in the use of traditional preparations for the treatment and management of various diseases is the lack of scientific data in support of the safety as well as the efficacy of the plant preparation. *Lawsonia inermis* has been identified as a potential hypoglycemic agent [29]. In this study, acute toxicity of the aqueous leaf extract of the plant show no toxic manifestations at an LD_{50} of 5000mg/kg body weight of the animal. This is in tandem with the work of [29] who reported that there are no symptoms of toxicity on administration of 3000 mg/kg bw and above of aqueous leaf extract of *Lawsonia inermis*. This study, according to [19] guidelines for the testing of chemicals indicates that the aqueous leaf extract of *Lawsonia inermis* at doses of 200mg/kg, 400mg/kg, and 600mg/kg may not be toxic/lethal to the animals. Based on the relatively high therapeutic index (TI) and the effective dose (ED_{50}) of this extract which are 24.61 bw and 203.67 mg/kg bw. respectively (Unpublished), the ED_{50}must be taken 25 times to get to the toxic dose which was found to be greater than 5000mg/kg bw of the animal. Therefore, aqueous extract of *Lawsonia inermis* leaf has a wide therapeutic range and also a wide

margin of safety.

The blood glucose level of all experimental groups, except the non-diabetic control group increased significantly after the intraperitoneal administration of alloxan. Within three days of intraperitoneal administration of alloxan, the average fasting blood glucose levels in the animals increased from 89± 4.35 to 347 ± 11.0. Administration of AELIL for three weeks consecutively produced remarkable fall in blood glucose levels of diabetic rats at all the doses. In contrast, the non-diabetic control rats remained normoglycemic throughout the experimental period of 21days (T**able 3).** Comparing blood glucose levels on day 3 after diabetic induction and day 21, AELIL at the dose 600 mg/kg produced a highly significant decrease ($p < 0.05$) in blood glucose levels as compared to lower doses; 200mg/kg and 400mg/kg of the extract and the positive control group administered with standard drug, metformin. Measurement of the body weights of rats in the experimental groups as represented in **Table 4** shows that the body weight increased normally in non-diabetic control rats, while alloxan induced diabetic rats (diabetic control) show a significant decrease in body weight as early as 3 days after the administration of alloxan. A progressive loss of body weight was noted 7 days after alloxan administration. The maximum decrease in body weight was observed at 21 days of alloxan administration in the diabetic non treated rats. The weight of animals in other groups was also decreased significantly till day 14 as compared to control group (Table 4). On day 21, the animals administered metformin (50 mg/kg bw), and those administered 200, 400, and 600 mg/kg bw of AELIL showed significant increase in their body weight as compared to diabetic non treated rats.

At doses used in this study, AELIL showed no toxic effect on the liver function of the experimental animals. Although, albumin, the main plasma protein that is responsible for the maintenance of osmotic pressure and distribution of body fluids, steroids and fatty acids was found to be significantly high in the non-diabetic control group compared to the groups of rats administered AELIL. The slight but significant reduction noted in albumin concentration of the rats administered AELIL may not be connected with liver damage, rather, it may be due to mobilization of proteins for the synthesis of some antioxidant enzymes required to mop up the free radicals generated as a results of alloxan assault on the organs of the rats. The fact that rats administered different doses of the extract did not show any significant difference in their albumin concentrations, indicates that albumin was not affected by the extract since there was no any dose dependent effect observed. Moreover, total protein, was found to be normal with no significant difference among all the doses tested.

Bilirubin which is the end product of heme metabolism is cleared off the blood by the liver, excess of which in the blood indicates cirrhosis, or blockade of the bile duct. Serum bilirubin in rats administered different doses of the extract was found to be within the normal bilirubin concentration.

Alkaline phosphatase (ALP) which is the enzyme of the cells lining the biliary duct of the liver is responsible for the hydrolysis of phosphate groups from many molecules (proteins). Blockade of the biliary duct elicit elevated plasma concentration of the enzyme. Tested doses of AELIL leaf show normal activity of ALP except at 200 mg/kg which is significantly ($p < 0.05$) higher than the non-diabetic control group. The elevated activity of ALP noted at 200 mg/kg of the extract may not be due to biliary duct blockage because the increase in the enzyme activity noted was not dose dependent when compared with other tested doses. Therefore, the elevated activity of the enzyme observed may not be as a result of the plant preparation.

ALT and AST are liver enzymes; plasma concentration of which indicates liver function. In the case of liver disease, ALT is usually higher than AST therefore ALT/AST ratio is high. This ratio is often used in the evaluation of liver function and disease. In this study, ALT and AST of the rats administered AELIL showed no significant difference to the non-diabetic control group. This indicates that the extract may not be injurious to the liver at doses administered in this study.

Conclusion

In conclusion, aqueous extract of *Lawsonia inermis* leaf at tested doses; 200 mg/kg, 400 mg/kg and 600 mg/kg bw showed significant anti-hyperglycaemic potential in diabetic rats after intra peritoneal administration. *Lawsonia inermis* has a high therapeutic index which makes it safer than some antidiabetic preparations. The results of the liver function tests suggest that the liver function is not impaired at the tested doses and therefore, can be concluded that, the aqueous leaf extract of the plant (L. *inermis*) is safe as an anti-diabetic preparation, most especially, at the therapeutic (effective) dose

which was far below the toxic dose. The plant can therefore be a good source for the discovery of safer and cheaper antidiabetic agent or drug.

Recommendation

Lawsonia inermis appears to be useful material for further studies, leading to possible drug development for diabetes as the extract had shown the potential for antidiabetic effect. Further investigations are required to elucidate the exact mechanism of action and also to ascertain the actual active agent(s) responsible for the plant extract anti-diabetic effect.

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Authors' contributions

This research work was carried out with the collaboration of all the authors. All the authors contributed to the writing of the final manuscript and approved that it should be sent for publication in Nigeria Journal of Nutritional Sciences

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Ethical approval

Verbal ethical approval for the use of animals in this study was sought and gotten from Ethical Review Committee of the Department of Biochemistry, IBB University, Lapai, Niger State, Nigeria during the proposal presentation on the research work.

Conflicts of interest

Authors declare no conflict of interest in this work and its publication

Consent for publication

Not applicable

Availability of data and materials

All data related to this manuscript are presented within the text..

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