

Full Length Research Paper

The effect of aqueous leaf extract of *Adansonia digitata* (baobab) on diabetes mellitus and the anterior pituitary of adult male wistar rats

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This study was carried out to evaluate the anti-diabetic properties of aqueous leaf extract of *Adansonia digitata* leaf (ALEAD) on blood glucose level. 36 of the rats were randomly distributed into 9. Group one served as the normal control and Group 2 rats were administered with alloxan (150 mg/kg) intraperitoneally. Groups 3, 4, and 5 were orally administered with alloxan (150 mg/kg) intraperitoneally and aqueous leaf extract of *A. digitata* (200, 400, and 600 mg/kg) once daily for 2 weeks. Group 6 were orally administered with metformin (150 mg/kg) once daily for 2 weeks. Groups 7, 8, and 9 were orally administered with aqueous leaf extract of *A. digitata* (200, 400, and 600 mg/kg) once daily for 2 weeks. The serum concentration of glucose of all the rats in each group was determined after the 8th and 15th dose of treatment. Groups 3, 4 and 5 showed a decrease after the first week of treatment but this decrease was not significant ($P>0.05$). The group treated with metformin (150 mg/kg) also showed a decrease which was also not significant ($P>0.05$). The result of the qualitative phytochemical analysis of aqueous leaf extract of *A. digitata* indicated the presence of glycosides, flavonoids, tannins, saponins, terpenoid and steroids. These results suggest that the aqueous leaf extract of *A. digitata* possess anti-diabetic effect on alloxan induced diabetic rats.

Key words: Diabetes mellitus, *Adansonia digitata*, anterior pituitary, wistar rats.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and defective metabolism of glucose and lipids (Muhammed and Hauwa, 2013). It has been shown that diabetes is a heterogeneous

syndrome characterized by an elevation of blood glucose level caused by relative or absolute deficiency of insulin (Mohammed and Hauwa, 2013).

Diabetes affects 177 million people worldwide in 2000

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and this figure is projected to increase to 300 million by 2025 (Chevenne and Fonfrede, 2007). It is a chronic condition which the body cannot properly convert food into energy and it is associated with long term complications that affect every part of the body (Bluestone et al., 2013). According to Williams and Randall (2015), diabetes mellitus alters the function of anterior pituitary gland either by hypo functioning or hyper functioning of the gland caused by lesion of the gland. The anterior pituitary gland secrete the following hormones adrenocorticotrophic hormone (ACTH), Thyroid stimulating hormone (TSH), Luteinizing hormone (LH) and follicle stimulating hormone (FSH), Prolactin and Growth hormone (GH). A study by Hisayo et al. (1996) shows that there is failure of secretion of TSH, ACTH on insulin dependent diabetes mellitus in the anterior pituitary of rats. The anterior pituitary growth hormone stimulates the release and oxidation of free fatty acids which leads to decreased glucose and protein oxidation and preservation, thereby enhancing glycogen stores into the liver (Arun et al., 2013). Amounts of growth hormone have been found to cause no increase in blood sugar in normal subjects but may decrease the sensitivity to injected insulin (Møller and Jørgensen, 2009). Alloxan have been found to selectively inhibit glucose-induced insulin secretion through its ability to inhibit the beta cell glucose sensor kinase (Sigurd, 2008). This permits the selective study to potential antidiabetic agents in rodents. Several studies have consistently used alloxan induced diabetes as an animal model of experimentally induced diabetes (Mohammed and Hauwa, 2013). Medicinal plants, also called medicinal herb have been and used in traditional medicine practices since pre-historic times, plants synthesize hundreds of chemical compounds for functions which include the cure of disease illness, defense against insects, fungi, and herbivores mammals (Sharangi, 2009).

The medicinal value of these plants lies in some chemical substance that produces a definite physiological action on the human body, this chemical substance has a potential or established biological activity that has been identified and they are known as phytochemicals (Sinija et al., 2008). The baobab plants are tropical trees native to Africa, Australia and Madagascar but dispersed widely by humans. The members of the genus are united by several derived characters that serve to distinguish them from other *Bombacacea* including a characteristic indehiscent fruit with deiform seeds and powdery pulp (David, 1995).

Adansonia digitata is commonly found in the thorn woodlands of African savannah, it is a very long-lived tree with multipurpose use. *A. digitata* is commonly found in thorn wood lands of African savannahs, which tend to be at low altitudes with 4-10 dry months per year. It tends to grow as solitary individuals though it can be found in small group depending on the soil type (Jitin et al., 2005). Baobab as a multipurpose tree offers protection and

serves as food, clothing and medicine as well as raw materials for many useful items (Chukwuma et al., 2017).

A. digitata plants parts are used to treat various ailments such as diarrhea, malaria and microbial infections. The plants and parts have interesting anti-oxidant and anti-inflammatory properties; hence, baobab is used extensively since ancient times in traditional medicines (David, 1995). *A. digitata* contain glycosides, saponins, steriods and flavonoids while alkaloids, tannins and resins were absent (Chukwuma et al., 2017). According to Muhammed and Hauwa (2013), baobab has been used in management of diabetes mellitus in Hausa land. According to WHO (1980) the number of people suffering from diabetes has risen from 108 million in 1980 to 422 million in 2014, furthermore in 2015 an estimated death of 1.6 million people were directly caused by diabetes. Being aware of all these, not excluding the side effects of most of the synthetic drugs used in the treatment of diabetes mellitus, arises the need to seek for an alternative in the herbal medicine. Although, studies have shown the antidiabetic effect of *A. digitata*, these studies only demonstrate the biochemical effect, and failure to access the histological effect the plant might have on the primary organ as well as the pituitary. *A. digitata* has been used in the management of diabetes and other metabolic activity. Traditionally *A. digitata* used to manage diabetes mellitus and a study by Muhammed and Hauwa (2013) have established the potential of these plant seed in the management of diabetes but no study have established on the effect of these extract on the pituitary hormones involved in the regulation of bodies carbohydrate metabolic activity. Hence this study is necessary to investigate the modulatory effect that the plant *A. digitata* might have on the pituitary histology and hormone secretion. The study investigated the effect of leaf extract of *A. digitata* (baobab) on alloxan-induced diabetes mellitus in adult male wistar rat.

MATERIALS AND METHODS

Collection and authentication of plant materials

Fresh leaves of *A. digitata* were procured from a local dealer in Kaduna state. It was identified at the Department of Agricultural Science, Enugu State University of Science and Technology, Agbani campus and a sample of it deposited at the herbarium unit.

Plant preparation and extraction

A sample of 5 g of each powdered plant materials was soaked in 100 ml of distilled water for 48 h. The solution was filtered using approximately 11 cm diameter whatman filter paper. The extract was subsequently collected after 24 h and immediately used for phytochemical analysis.

Phytochemical analysis

The aqueous extract of *A. digitata* was subjected to phytochemical

screening test to detect the presence or absence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloids, saponins, glycosides, sterols and triterpenes. Also proximate analysis to detect the presence or absence of moisture, protein, crude fibres, ash, fats and oil and carbohydrate at PRODA Emene Enugu state. Each of the tests was qualitatively screened; the presence or absence of the compound was expressed as positive or negative respectively.

QUALITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS

Test for alkaloids

The presence of alkaloid was determined as described by Zagga et al. (2018). A portion of the plant powder (5 g) was reacted with a few drops of hagers reagent (1.0 cm^3) and another 5 g portion was treated with Wengers reagent (1.0 cm^3) turbidity or precipitate with either of these reagents was taken as an evidence for the presence of alkaloids.

Test for tannins

A portion of the plant sample was diluted with distilled water in the ratio of 1:4 and a few drops of 10% ferric chloride was added to produce a blue black or green color. 5 g of dried powdered sample of the plant was boiled in 20 ml of distilled water in a test tube and then filtered using a hydrophilic filter (5.5 cm in size) and funnel (35 mm in size) placed in a conical flask. 0.1% FeCl_3 was added to the filtered samples and observed for brownish green or a blue-black coloration, which shows the presence of tannins (Trease and Evans, 1996).

Test for saponins

Five gram of powdered sample of the plant was boiled together with 20 ml of distilled water in a water bath and then filtered. 10 ml of the filtered sample was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing observed for the formation of emulsion which indicates presence of saponins (Odebiyi and Sofowora, 1978).

Test for flavonoids

A few drops of 1% NH_3 solution was added to the aqueous plant sample in a test tube, a yellow coloration was observed to indicate the presence of flavonoid (Sharma et al., 2013).

Test for terpenoid

Five gram of the plant sample was mixed with 2 ml of CHCl_3 in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to mixture to form a layer, an interface with a reddish-brown coloration is formed if terpenoid constituent is present (Sofowora, 1982).

Test for cardiac glycoside

Two milliliters of concentrated H_2SO_4 was prepared in a test tube. 5 g of plant sample was mixed with 2 ml of glacial acetic acid containing 1 drop of FeCl_3 . The mixture was carefully added to the 1 ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is present in the

sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent (Zagga et al., 2018).

Test for phenols

Two milliliters of the extracts was mixed with ferric chloride solution. A green or dirty green precipitate indicates the presence of phenolic compounds.

QUANTITATIVE ANALYSIS

Alkaloid determination

This was done using the method of Harborne (1973). 10 g of the test sample was weighed into 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4 h, then it was filtered and the extract was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to stand till its settlement (24 h). The precipitate was filtered out from the solution using filter paper and washed with dilute ammonium hydroxide. The residue was the alkaloid which was weighed after complete dryness and the percentage was calculated.

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of the sample}} \times 100$$

Saponin determination

Method of Obadoni and Ochuko (2001) was used for the determination of saponin. 10 g of test sample was put into 250 ml conical flask and 100 ml of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h, with constant stirring at about 55°C . The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C . The concentrate was transferred into 250 ml separator funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer (lower layer) was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added for washing two times. In both cases, the upper layer was collected while the lower layer discarded. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride, the remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

$$\% \text{ saponin} = \frac{\text{weight of the extract}}{\text{weight of the sample}} \times 100$$

Tannin determination

This was done using the method of Van-Burden and Robinson (1981). 500 mg of test sample in each case was taken in a plastic bottle and 50 ml of distilled water was added. Then it was shaken in a mechanical shaker for 1 h, and filtered in a 50 ml volumetric flask made up to mark. 5 ml of the filtrate was pipette out into the test tube and mixed with 2 ml of 0.1 M FeCl_3 , 0.1 ml N HCl and 0.008 M $\text{K}_4\text{Fe}(\text{CN})_6$ (potassium ferrocyanide). The absorbance was measured at 120 nm within 10 min. Absorbance was traced against concentration using tannic acid standard graph.

% Tannin = concentration × dilution × 100

Flavonoid determination

The method of Bohm and Kocipai-Abyazan (1994) was used. 10 g of test sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature using separating funnel. The whole solution was filtered through Whatman filter paper No. 42(125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity were calculated.

$$\% \text{ Flavoniod} = \frac{\text{Weight of extract}}{\text{weight of the sample}} \times 100$$

Proximate analysis

Ash, moisture, crude protein, crude fiber, fat and oil, and carbohydrate were determined according to the methods of Association of Official Analytical Chemist (1990).

Experimental animals

A total of 45 adult male wistar rats were purchased from the Animal House Unit of the College of Medicine, Enugu State University of Science and Technology Parklane GRA Enugu Nigeria. The animals were housed fly-proof metal cages and were provided with food (growers mesh) and water *ad libitum*. The animals were maintained under standard laboratory condition (24°C) with relative humidity of 60-70% under 12 h light/ dark cycles and were acclimatized for two weeks prior to the experiment.

Induction of diabetes using alloxan monohydrate

Stock solution of alloxan monohydrate (Sigma- Aldrich Canada) was prepared by dissolving alloxan monohydrate (0.9 g) in distilled water (6 cm³) and diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg). The volume of the solution containing 150 mg/kg given to each rat was determined by its weight. After a period of two days (48 h), the rats with blood glucose level greater than 200 mg/dl was considered diabetic and used for the research work. The method of Mohammed and Hauwa (2013) was adopted in the study with slight modification.

Experimental design

The animals were randomly divided into nine groups of five animals each. They were labeled group 1-9 of which Group 1 served as the control group.

Group 1: Normal control group and received 0.1 ml/kg normal saline as placebo

Group 2: Diabetic untreated group + feed and water *ad libitum*

Group 3: Diabetic + 200 mg/kg ALEAD

Group 4: Diabetic + 400 mg/kg ALEAD

Group 5: Diabetic + 600 mg/kg ALEAD

Group 6: Diabetic + 400 mg/kg metformin as standard drug

Group 7: Non- Diabetic + 200 mg/kg ALEAD

Group 8: Non- Diabetic + 400 mg/kg ALEAD

Group 9: Non- Diabetic + 600 mg/kg ALEAD

Determination of blood glucose level

Glucometer strips were inserted into the strip compartment of the

glucometer (Accu –Answer ZH–G01) and a sample of blood collected by tail snipping was used to touch the sensitive part of the strip and the values were displayed and recorded in mg/dl according to the method of Akpotu et al. 2018

Biochemical study

Hormonal assay

At the end of the experiment, blood samples were collected via cardiac puncture using 5 ml plane sample container. Sera were separated and stored at -20°C until ready for the analysis of the hormonal assay. Serum level of total growth hormone (GH) and thyroid stimulating hormone (TSH) were determined.

Method used for hormonal assay

TSH and GH ELISA (enzyme linked immune-solvent assay) by Uotila et al. (1981) procedure. The desired numbers of coated wells were secured in their holders' and 100 µl of standards, specimens and controls were dispensed into appropriate wells and thoroughly mixed for 30 s. They were later incubated at room temperature (18-25°C) for 60 min and the incubation mixtures were removed by flicking plate contents into a waste container. After, the micro titer wells were rinsed and flicked 5 times with distilled or deionizer water. The wells were sharply stroked onto absorbent paper or towels to remove all residual water droplets. Later 100 µl of TMB reagent was dispensed into each well and gently mixed for 10 s, the reaction was stopped by adding 100 µl of stop solution to each well and gently mixed for 30 s. Finally, absorbances were read at 450 nm with a micro titer well reader within 15 min.

Histopathology study

Tissue preparation

Twenty-four hours after the last treatment, all animals were sacrificed under anesthesia. The skull was opened and the brains of each rat were excised. The tissue was fixed in 10% neutral formal saline container with lids for 3 days to prevent autolysis, improve staining quality and aid optical differentiation of its cells. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70, 80 and 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5 µm thick with a rotary microtome, floated in water bath and incubated at 60°C for 30 min. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70%). The sections were then stained with hematoxylin for 15 min. Bluing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant;DPX

Slide examination

The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™5.0 megapixels microscope camera at x160 and x400 magnification

Statistical analysis

Data obtained were expressed as the mean ± standard deviation. They were fed into the computer using statistical package for social

sciences (SPSS, version 20; IBM SPSS, Chicago, Illinois, USA) software package. One-way analysis of variance (ANOVA) with Tukey post- hoc test was used to compare the statistically significant difference at $P < 0.05$.

RESULTS AND DISCUSSION

This study investigated the effect of aqueous leaf extract of *A. digitata* on alloxan induced wistar rats. The result of this study investigated the effects of *A. digitata* on the serum concentration of growth hormone and TSH. From the result there was an elevation in the growth hormone concentration in group 3 with the diabetic untreated group showing the highest increase when compared with the negative control. This elevation agreed with the research conducted by Mamza et al. (2013) hormone has an insulin antagonistic effect. During hypoglycemia, it is being secreted to restore blood glucose levels by stimulating glucose increase from the liver and inhibiting glucose uptake in peripheral tissues elevation (Mamza et al., 2013). There was a significant increase in growth hormone concentration in Group 7 and also in Group 8 which were given 200 and 400 mg/kg of extract only when compared to the negative control. This elevation suggests that it may be due to the presence of flavonoid in the extract, which has a glucose lowering property by inhibiting (Mohammed et al., 2013). Flavonoid inhibits glucose 6-phosphatase activity in the liver thereby suppressing gluconeogenesis and glycogenolysis and consequently reduces hyperglycemia (Chen, 1998). The increase in growth hormone concentration was more pronounced in Group 7 which suggest that it might be dose dependent. There was decrease in TSH level in the diabetes treated group when compared to Group 1. The serum TSH levels in Groups 1 and 2 which served as the negative control and diabetic untreated group respectively showed normal level of this hormone. This suggests that the diabetic states of the animals in Group 2 may have had no direct effect on TSH secretion on the anterior pituitary gland. This fact might be supported by the normal histology of the pituitary gland in Group 2 when compared with the histology of the Group 1 animals. The diabetes treated groups (Groups 3, 4, 5 and 6 respectively) however, showed fluctuations in the serum concentration of TSH whereas Groups 3, 5 and 6 showed decrease in the serum level of TSH while Group 4 showed an increase in the serum levels of TSH. Within these values, only the decrease in TSH levels brought about by the administration of Metformin (Group 6) was statistically significant ($P < 0.05$). This suggest that diabetes mellitus might have no direct stimulatory effect on TSH secretion but an anti-diabetic medications may pose an effect on the TSH level. However, the mechanism or process of this effect is still unknown and there is need for further research on the mechanism of action. Moreover, the administration of increased doses of the aqueous leaf extract of *A. digitata* had a decreasing effect

on TSH levels as seen in Group 7 and 8. However, the high doses of the aqueous leaf extract of *A. digitata* increased the secretion of TSH from the anterior pituitary gland. These values observed in these groups administered with the extract only were not significant ($P > 0.05$). The general administration of the plant extract suggests that the aqueous leaf extract of *A. digitata* has the potency of decreasing the serum levels of TSH. Though their mechanism of action is not known, it suggests that these effects were not directly on the anterior pituitary as the normal histology of this gland was noted in all the groups. There is need for further research on the mechanism of action. As revealed in Group 1 animals which served as the negative control group and received only normal saline, had a 19.2% increase in body weight at the end of the experiment. In contrast, Group 2 animals that served as the positive control (diabetic untreated) group showed a 10.7% decrease in body weight at the end of the experiment. This percentage decrease noticed in the group might be due to the adverse effects of diabetes on the body. This is in agreement with Lau et al. (2003) who stated that diabetes is often associated with a characteristic loss of body weight which is partially due to increased muscle wasting. Groups 7, 8 and 9 which served as the extract-treated groups were given low, medium and high doses of the aqueous leaf extract of *A. digitata*. Groups 7 and 8 had a 4.98 and 5.48% decrease in their body weights respectively, while group 9 had 7.4% increase in body weight at the end of the experiment. This suggest that high dose of the extract can cause weight gain as a result of increased blood glucose level (63.45%) at the end of the experiment (Tables 1 to 3, Figure 1).

However, comparing the percentage changes in body weight of animals in Groups 7, 8 and 9 to Group 1, it can be suggested that the aqueous leaf extract of *A. digitata* had no benefiting effect on the body weight of the animals and even the weight gain noticed with high dose administration were not up to the normal control group. Among the diabetic-treated Groups (3, 4, 5 and 6) which were given low, medium, high dose of the aqueous leaf extract of *A. digitata* and Metformin respectively, only Group 3 showed a percentage increase in body weight while Groups 4, 5 and 6 showed a decrease respectively in their body weights at the end of the experiment. Since the animals in this Groups 4, 5 and 6 also had increased blood glucose levels respectively as seen in Table 4, it may also be suggested that they also experienced the weight-decreasing effect of diabetes just as noticed in the Group 2.

The fasting blood glucose profile shows that *A. digitata* leaf extract was capable of lowering plasma glucose levels, as seen in the diabetic groups treated with low and medium doses of the extract (200 and 400 mg/kg respectively) after the first week of the experiment. This supports previous studies on other parts of *A. digitata* which were shown to possess antidiabetic potential

Table 1. Qualitative results of phytochemical screening of the leave of *A. digitata*.

Parameter	Ethanol	Water
Alkaloids	+	+
Tannin	-	-
Flavonoid	+	-
Glycoside	+	+
Saponin	+	+
Terpenoid	+	-
Phenol	-	-

Key: + =detected; - =not detected.

Table 2. Quantitative results of phytochemical screening of the leaf of *A. digitata*.

Parameter	Values (%)
Alkaloid	0.97
Flavonoid	3.63
Glycoside	0.21
Saponin	1.84
Terpenoid	1.02

Table 3. Results of proximate analysis of leaf of *A. digitata*.

Parameter	Values
Moisture	0.81
Protein	14.35
Crude fibre	7.22
Ash	1.43
Fats & oil	10.00
Carbohydrate	66.19

Values were analyzed as Mean $\pm$ SD using ONE WAY ANOVA with Tukey post hoc test. *P <0.05 compared with the control Group 1; β P <0.05 compared with the control Group 2; CDEFGHP <0.05 compared with the control Group 3, 4, 5, 6, 7, 8 and 9. Values without superscripts showed no significant difference among and between groups.

(Tanko et al., 2008; Saravanaraj et al., 2017). The blood glucose level of the groups treated with treated with a high dose of the extract (600 mg/kg), as well as the normal groups treated with different doses of the extract (Groups 7-9), on the other hand, were increased after administration. However, at the end of the second week of the experiment, the blood glucose levels increased in all the diabetic and non-diabetic groups treated with the extract. This increase in blood sugar might be as a result of the high carbohydrate content of the extract. As stated by Eizirik and Cnop (2010), carbohydrate consumption

increases demand on the β -cell for insulin secretion, which may lead to endoplasmic reticulum stress, as well as oxidative stress (Sung et al., 2012) both of which can result in β -cell damage after a long period of time. The postprandial fluctuations of glucose increase gradually with increased proportions of carbohydrates, as well as an increase in mean blood glucose which take longer times to decrease back to normal levels (Kang et al., 2013). It is therefore virtually impossible to match carbohydrates and insulin which leads to unpredictable blood glucose levels; but by reducing the doses of

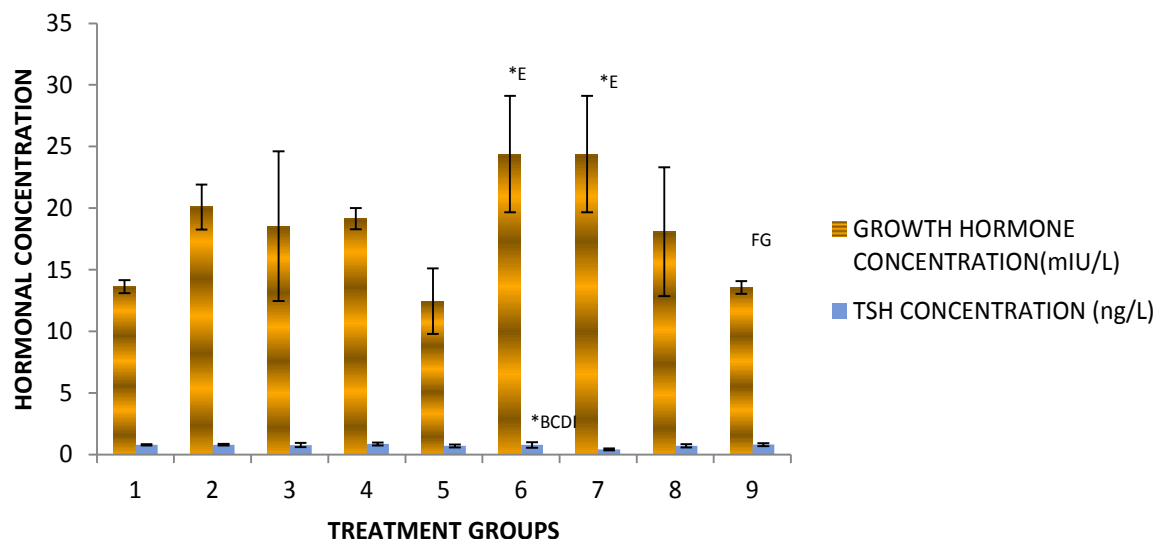


Figure 1. Result of the effect of the aqueous leaf extract of *A. digitata* on hormonal (GH and TSH) concentration. Values were analyzed as Mean ± SD using ONE WAY ANOVA with Tukey post hoc test. *P < 0.05 compared with the control Group 1; ^βP < 0.05 compared with the control Group 2; CDEFGHP < 0.05 compared with the control Groups 3, 4, 5, 6, 7, 8 and 9. Values without superscripts showed no significant difference among and between groups.

Table 4. Result of the effect of aqueous leaf extract of *A. digitata* on fasting blood sugar level in wistar rats.

Groups	Day 1	Day 8	Day 15	Changes (D15-D1)	% changes
1	85.5±3.7	95.2±6.8	110.0±4.3	24.5±0.6	28.7
2	422.7±135.9 [*]	439.2±13.21	466.5±110.6 [*]	43.8±25.3	10.4
3	181.8±71.3 ^β	153.8±92.1	223.5±68.4	41.8±2.9	22.9
4	191.3±134.1	120.33±69.2	217.3±142.6	26.0±8.0	13.6
5	149.8±64.3 ^β	199.0±200.9	254.5±117.9	104.8±53.6	69.9
6	389.5±185.2	198.8±77.5	309.8±111.0 [*]	63.8±74.2	17.1
7	76.3±4.3 ^{*β}	84.3±5.5	108.3±47.5 ^f	37.5±43.2	49.4
8	80.5±5.5 ^β	92.5±13.8	113.8±78.9 ^f	33.3±73.5	41.4
9	76.5±1.3 ^β	82.0±2.5	120.0±30.0 ^f	43.5±28.7	63.4

Values were analyzed as Mean ± SD using ONE WAY ANOVA with Tukey post hoc test. *P < 0.05 compared with the control Group 1; ^βP < 0.05 compared with the control group 2; ^{CDEFGHP} < 0.05 compared with the control Group 3, 4, 5, 6, 7 and 8.

carbohydrates and insulin, the size of the blood glucose fluctuations can be minimized (Bernstein, 1980). It is also possible that a high-carbohydrate/lower-fat diet such as contained in the extract, on a prolonged period of time could increase insulin sensitivity and lower fasting glucose levels, as reported by Gower et al. (2012). However, it was also observed that the blood glucose levels of the diabetic group treated with the extract were significantly lower than that of the untreated diabetic group as well as the group treated with the standard drug (metformin). This suggests that the extract has an antagonistic effect between its high carbohydrate content and its hypoglycemic property via unclear mechanisms (Figure 2).

Histological findings

Histology sections of the pituitary gland presented on all the slides (both in the control and treated groups) showed the normal histo-architecture of the rodent pituitary gland. The sections showed the bi-lobed pars distalis surrounding the pars intermedia and pars nervosa. The pars distalis is made up of 3 groups of cells; acidophilic chromophils, basophilic chromophils and chromophobes arranged in nests and cords interspersed within a rich fibro-vascular plexus. The acidophilic chromophils are characterized by small round to oval cells with a central nucleus with deeply eosinophilic cytoplasm while basophilic chromophils are more polyhedral with an

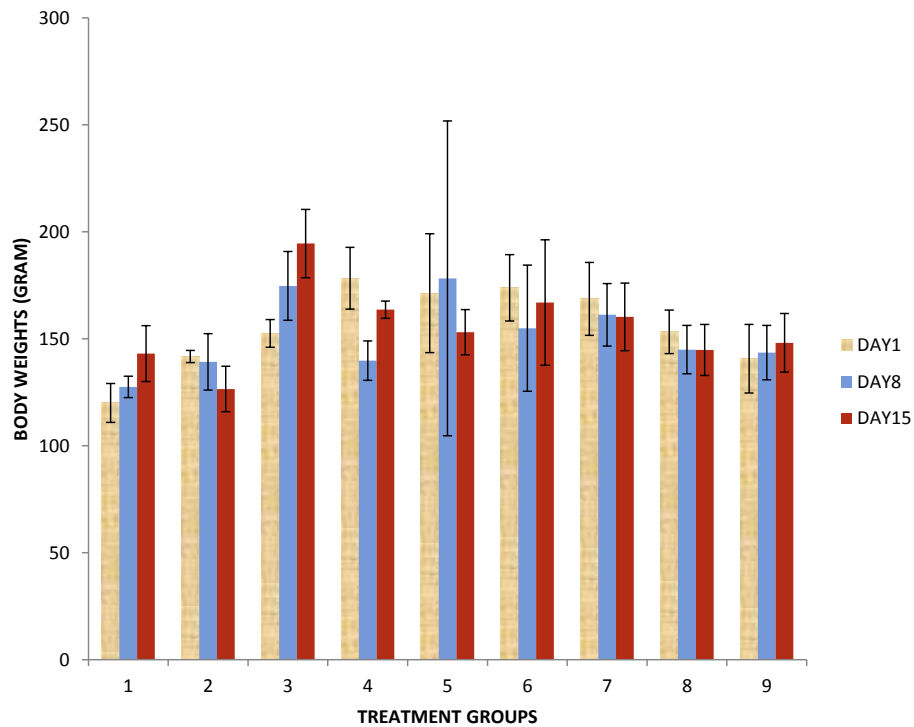


Figure 2. Result of the percentage changes in body weight of the experimental animals.

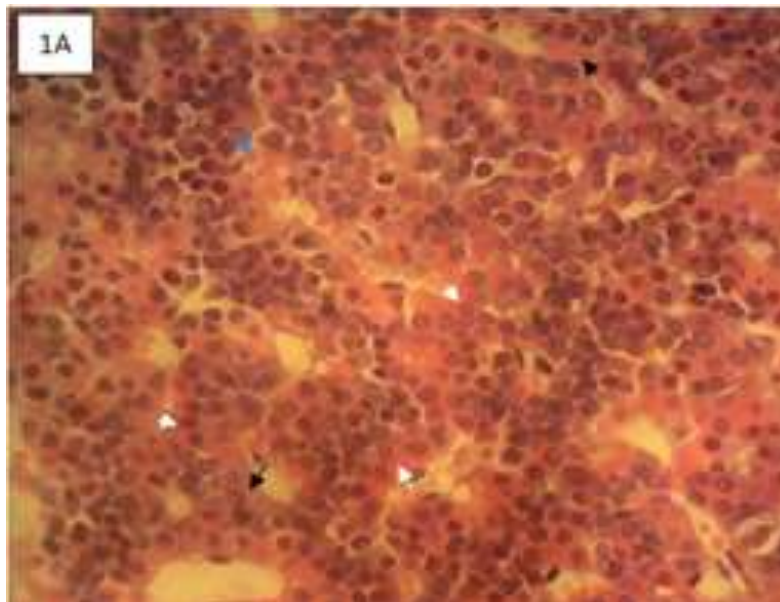


Plate 1. Photomicrograph of control animal group administered normal saline and given feed and water (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic chromophils (black arrow); acidophilic chromophils (white arrow); congested capillaries (blue arrow).

eccentric nucleus and pale basophilic cytoplasm. The chromophobic cells have a large nucleus with 1 or 2 nucleoli and abundant pale cytoplasm. The sections of

the pituitary glands examined in this study did not show any deviation from their respective normal histopathologies (Plates 1 to 9).

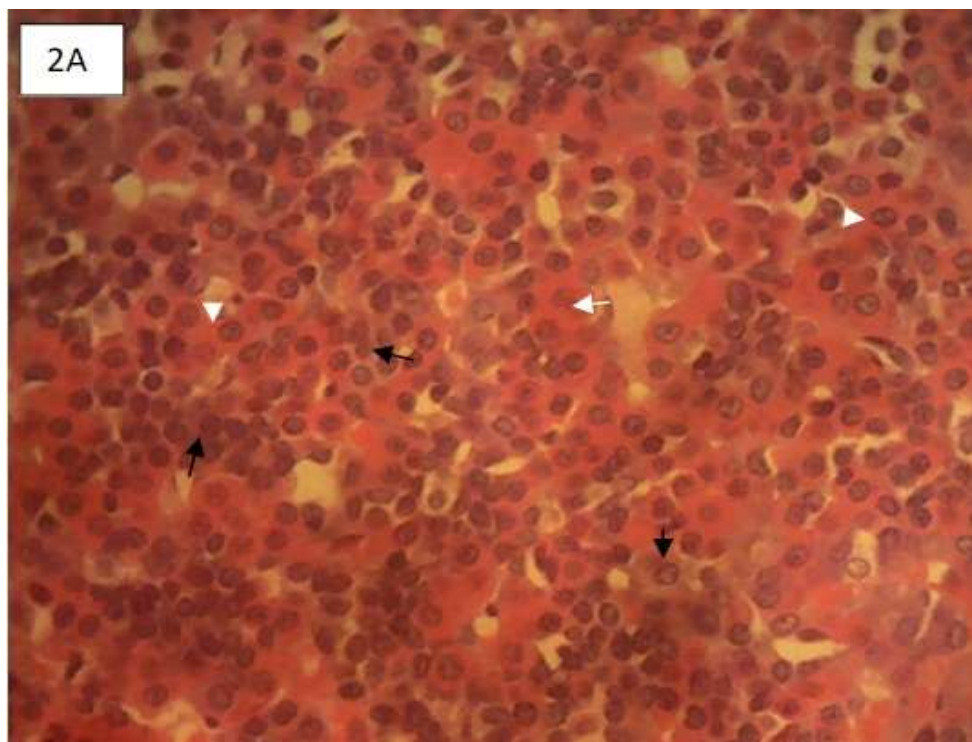


Plate 2. Photomicrograph of Group II given 150 mg/kg-bwt of Alloxan (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic chromophils (black arrow); acidophilic chromophils (white arrow); congested capillaries (blue arrow).

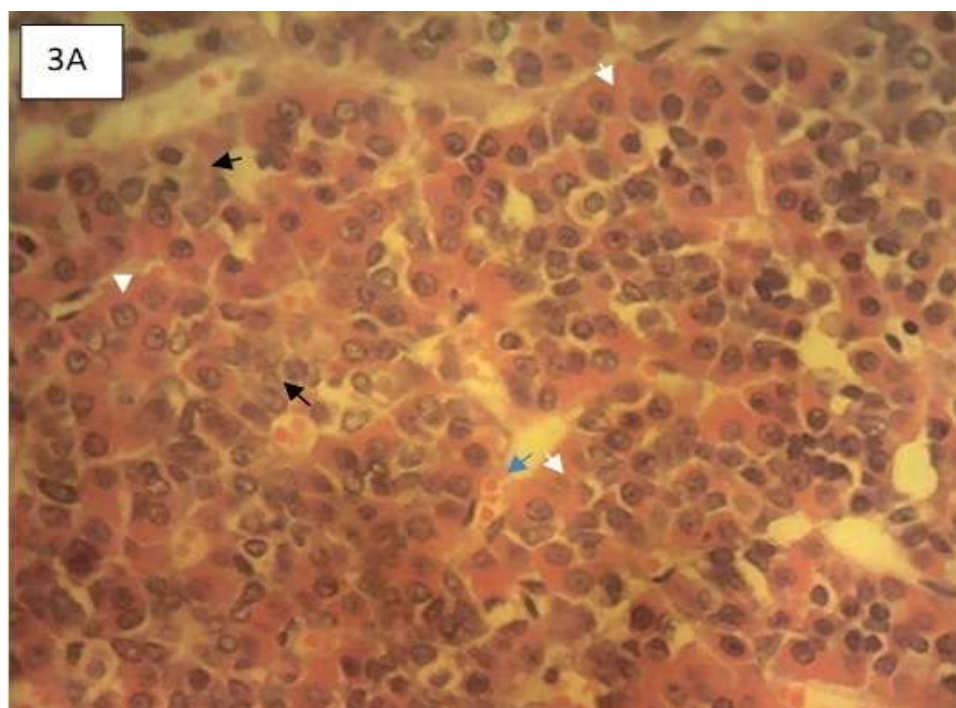


Plate 3. Photomicrograph of Group III given 150 mg/kgbwt of Alloxan and 200 mg/kg-bwt of extract (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic chromophils (black arrow); acidophilic chromophils (white arrow); congested capillaries (blue arrow).

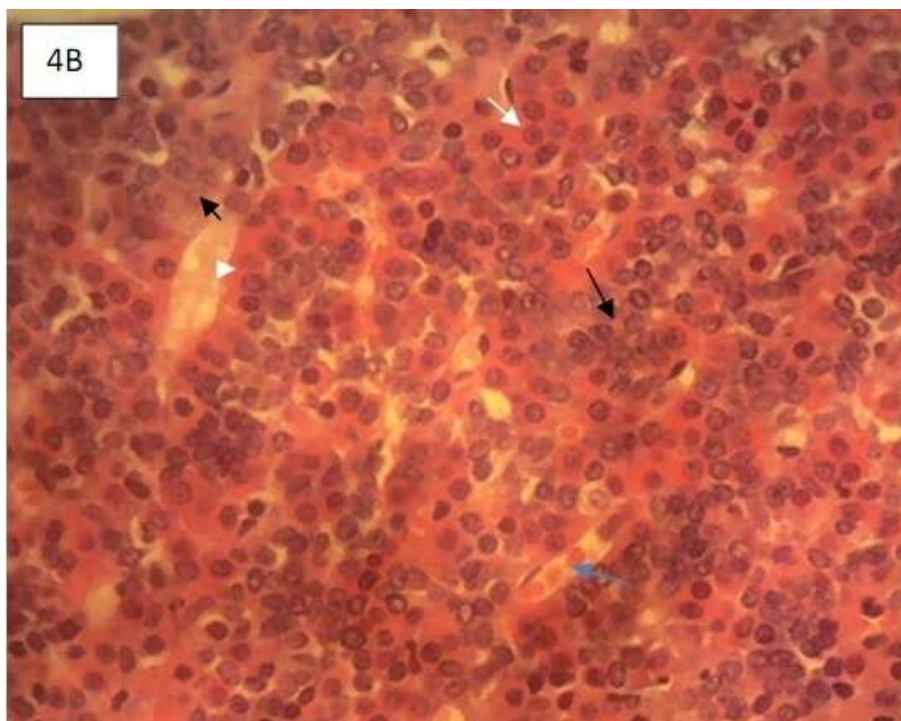


Plate 4. Photomicrograph of Group IV given 150 mg/kg -bwt of Alloxan and 400 mg/kg-bwt of extract (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic chromophobes (black arrow);acidophilic chromophils (white arrow);congested capillaries (blue arrow).

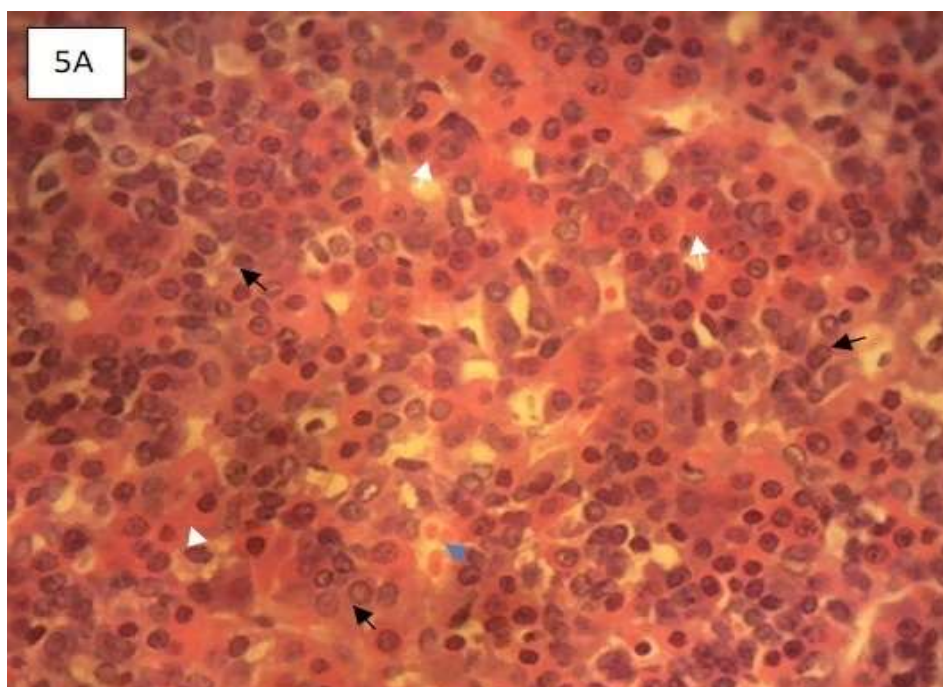


Plate 5. Photomicrograph of Group V given 150 mg/kg -bwt of Alloxan and 600mg/kg-bwt of extract (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic chromophils(black arrow);acidophilic chromophils(white arrow); congested capillaries (blue arrow).

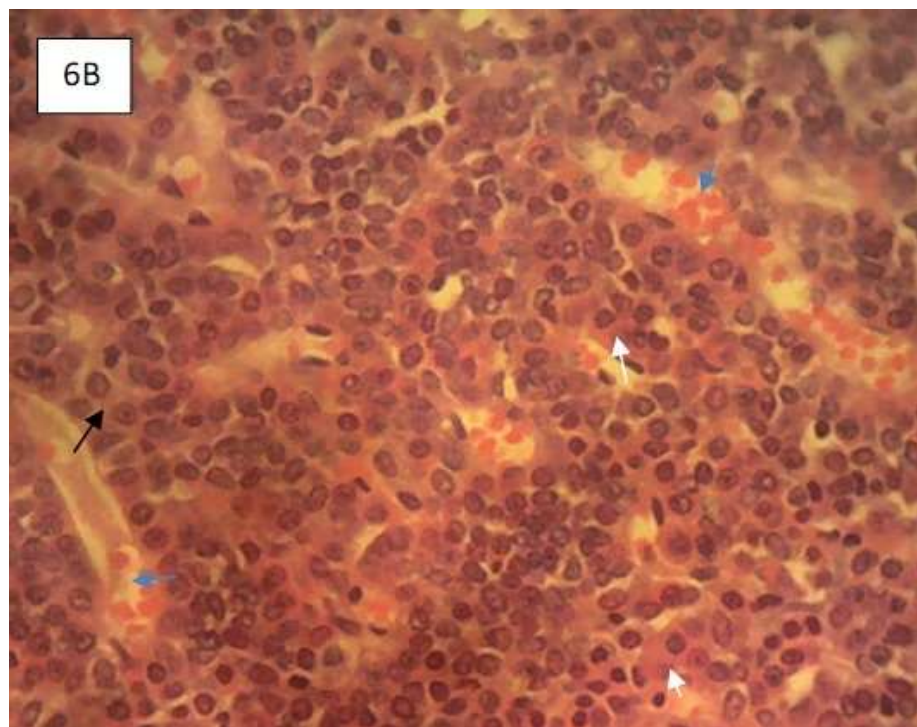


Plate 6. Photomicrograph of Group VI given 150 mg/kg -bwt of Alloxan and 150 mg/kg-bwt of metformin (H & E stain x 400) at high magnification showing the cells of the pars distalis; Basophilic Chromophils (black arrow); acidophilic congested capillaries; (blue arrow).

Conclusion

The results of this study provided evidence showing that ALEAD constitute viable phytochemical with anti-diabetic properties which accounts for its anti-diabetic potencies, which agrees with its anti-properties of its fruit pulp recorded in human and its use in traditional folk medicinal practices. This research work also showed that the ALEAD has the ability to increase growth hormone level and thyroid stimulating hormone level. Further studies should be carried out with longer time duration to confirm its mechanism of action as an anti-diabetic agent and its mechanism of action on its ability to decrease the level of TSH.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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