

The Effect of Methanolic Leaf Extract of *Adansoniadigitata* on the Histology of Wistar Rats Testes

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Abstract: This study investigated the effect of methanolic leaf extract of *Adansonia digitata* on the histology of the testes of adult albino wistar rats. A total of forty rats were divided into four groups (A, B, C and D) of ten rats each. Their body weights were measured on a weekly basis throughout the period of the experiment. In addition to standard animal feed and water, groups A, B and C were orally administered 40mg/kg, 80mg/kg and 200mg/kg of the extract per body weight respectively daily for twenty-one days after which were maintained on standard animal feed and water for a four week recovery period. Group D (control) received only standard animal feed and water for seven weeks. Two animals from each group were sacrificed at the end of first, second and third weeks of administration and also after a four week recovery period. The testes and blood were harvested for histopathologic and biochemical studies respectively. Result showed that the body weights of experimental animals were not significantly affected ($p=0.05$) by the extract when compared to control animals. Significant hormonal changes in the serum as well as histopathological changes in the testes were observed at a medium and high dose of the extract however only tissues that received low to medium dose of the extract recovered at the end of wash-out period. This result suggested that the toxic effect of the extract on the testes is dose-and time-dependent and is only reversible at a medium dose. In conclusion, high doses of methanolic leaf extract of *Adansonia digitata* had deleterious effect on the testes when consumed at regular and prolonged time.

Key words: Medicinal Plant • Toxicity • Hormones • Germ Cells • Degeneration

INTRODUCTION

Regular consumption of plant parts or herbs as food or for medicinal purposes has proved to be beneficial health-wise since it contains nutritional constituents and dietary antioxidants which reduce the risk and also protects the body against several diseases [1]. Baobab is one of such plant which possesses unique nutritional properties which have been of immense benefit to man. Baobab (*Adansonia digitata* L.) is a massive deciduous tree that is indigenous to Africa (African savannas) and belongs to the family Bombacaceae and genus *Adansonia* [2].

It is the largest succulent plant in the world with a diameter of 10-12m and a height of 23m or more [3, 4]. The tree was given the name *Adansonia* to commemorate the French Surgeon Michael Adanson (1726-1806) while the species *digitata* (meaning hand-like) was given referring to the shapes of the leaves. The genus

Adansonia comprises of eight species, six of which are found in the island of Madagascar, one in Australia while the remaining is widespread in Africa [5]. The African baobab is known by a large number of common names: Baobab, Cream of tartar tree, Monkey bread tree, Ethiopian Sour gourd, Senegal calabash, upside-down tree, lemonade tree and chemist tree (English); Kouka, kuka (Hausa); Boki, bokki (Fulani); Isimuhu, Umshimulu, Isimuku (Zulu); Kremetart, Kremetartboom; Mubugu, Muyu, Mbuyu, Mkulukumba, Mlambe (Afrikaans and others) [6].

The leaves serve as a staple food and a significant source of protein and minerals for many African populations [6-9]. Medicinally, the leaves are used to treat inflammation, diarrhea, dysentery, fever, malaria, fatigue, ophthalmia, toothache, gingivitis, kidney and bladder diseases and urinary tract diseases and for blood clearing [4,6,7&10-15]. The Aqueous leaf extract of *Adansonia digitata* has been used to ameliorate against carbon

tetrachloride-induced testicular toxicity, hence suggesting it may have a therapeutic role in free radical mediated diseases [16]. However, report on the toxicological profile of *Adansonia digitata* on organs are few and far in between. The aim of this study was to determine the effect of oral administration of methanolic leaf extract of *Adansonia digitata* on the body weight and histology of the testes.

MATERIALS AND METHODS

Ethical Considerations: Permission was granted by Research Ethics Committee, College of Graduate Studies, University of Port Harcourt, Nigeria to carry out this study for the required period of time.

Collection of Plant and Identification: The leaves of baobab (*Adansonia digitata*) was collected from Wondam in Mikang Local Government Area of Plateau State in the month of February 2015 and identified at the Department of Plant Science and Biotechnology of the University of Port-Harcourt, Rivers State, Nigeria.

Extraction of Plant Leaves: The plant leaves were washed with water, cut into smaller pieces and air-dried. The dried leaves were pulverized with a manual grinding machine or electric blender to a powder. From the powdered plant sample, methanolic extracts of the plant leaves was prepared using the Rotary Evaporation Method [17].

Procurement and Housing of Experimental Animals: Thirty five adult albino wistar rats weighing between 152 and 250g were purchased from the animal house of Faculty of Basic Medical Sciences, University of Port Harcourt and housed in a wire-netted wooden cage with wooden shavings as their beddings, under standard conditions within the animal house of Faculty of Basic Medical Sciences, University of Port Harcourt. They were fed with growers mash (Growers palletized feed) and water *ad-libitum* for a period of two weeks prior to the study to allow for acclimatization. The weights of the animals were estimated at procurement and during the period of acclimatization using an electronic analytical and precision balance.

Determination of Median Lethal Dose (LD50): The median lethal dose for *Adansonia digitata* methanolic leaves extract was conducted using the Organization for Economic Cooperation and Development [18]. Fifteen Swiss albino mice weighing 27-31 g were divided into five groups of three animals and each group

was administered with 2000mg/kg, 3000mg/kg, 4000mg/kg, 5000mg/kg and 6000mg/kg body weight of the extract respectively. The animals were observed for manifestation of physical signs of toxicity and the number of death within 24-72 hours.

Experimental Design: The body weights of experimental rats were measured on a weekly basis before and throughout the period of the experiment, using an electronic analytical and precision balance. Animals were randomly divided into four groups (A, B, C and D) with each group containing ten animals each (Table 1). In addition to standard animal feed and water, groups A, B and C were orally administered 40mg/kg, 80mg/kg and 200mg/kg of the extract per body weight respectively daily for twenty one day after which were maintained on standard animal feed and water for a four week recovery period. The four weeks recovery period was carried out to ascertain if the possible effect(s) seen during the first three weeks of extract administration could be reversed. Group D (control) received only standard animal feed and water for seven weeks. Two animals from each group (A, B, C and D) were weighed, anesthetized with chloroform and sacrificed on the 8th, 15th, 22nd and 50th days.

Collection of Organs and Blood Samples: Blood samples were obtained using a sterile syringe (5 mL) by cardiac puncture and carefully discharged into lithium heparin bottles prior to hormonal assay. The testes were exposed and excised through a ventral midline abdominal incision and stored in 10% formalin prior to tissue processing.

Hormonal Assays: The blood samples were taken to a medical diagnostic laboratory for hormonal (FSH and Testosterone) assay.

Histological Studies: Histological studies on the organs and tissues were done according to procedures described by Disbrey and Rack [19] and Drury and Wallington [20]. The sections were subjected to Hematoxylin and Eosin (H&E) then viewed under light microscopy. The permanent photomicrographs of each slide were recorded with a Kodak digital camera for subsequent histological analysis.

Statistical Analysis: All data were expressed as Mean \pm SEM and statistically analysed using One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test using statistical package for social sciences version 20 (SPSS 20.0). Values were considered significant at $p < 0.05$.

RESULTS

Acute Oral Toxicity Study: Mortality was observed after forty-six hours of administration in animals that received 6000mg/kg of the extract (group V) hence the LD50 was calculated to be 6000mg/kg body weight orally.

Weight of Rats: The effect of graded doses of methanolic extract of *Adansonia digitata* on body weights of male albino wistar rats at the end of the first, second and third weeks and also at the end of the reversal period is presented in table 1. The body weights of the animals increased proportionally in all four groups from the initial week of purchase to the end of the wash-out period hence the result showed no significant difference in the mean body weights of the experimental groups compared to control (Fig. 1). The extract didn't show any dose-dependent and/ or time-dependent effects on the body weights of the animals. The initial weights of the animals after purchase in control, groups A, B and C were 230.13 ± 15.96 , 198.50 ± 15.64 , 189.25 ± 11.06 and 182.88 ± 7.39 respectively. Although their mean weights differ, the difference was not significant ($p > 0.05$). The mean body weight during the acclimatization week prior to administration showed a non-significant decrease ($p > 0.05$) in groups A, B and C compared to control. At the end of the first week of administration of the extract, the mean body weight was reduced from group A to group C however this decrease was not significant ($p > 0.05$) compared to control. Control group had the highest mean body weight (240.38 ± 16.99) while the least body weight was recorded in group C animals which was received the highest amount of extract (200mg/kg per body weight). At the end of the second week of administration, groups A, B and C showed an insignificant decrease in their mean body weight compared to control. Similar trend of insignificant decrease in mean body weight of experimental animals compared with control was recorded at the end of week three and reversal period.

The histological sections of the testes in the control group as well as groups treated with 40mg/kg (group A) of extract appeared morphologically normal from the first week to the end of wash-out period (Figs. 2-5). A histological assessment of the testes in groups B and C animals showed normal architecture at the end of week one however at the end of the second to third weeks of administration there was a progressive histological disruption and cellular derangement such as degeneration of the germ cells in the seminiferous tubules and vacuolation of the seminiferous tubules (Figs. 2-4). At the end of the wash-out period the histopathological distortion in groups B (80mg/kg) reverted to normal whereas that in group C (200mg/kg) was irreversible (fig. 5).

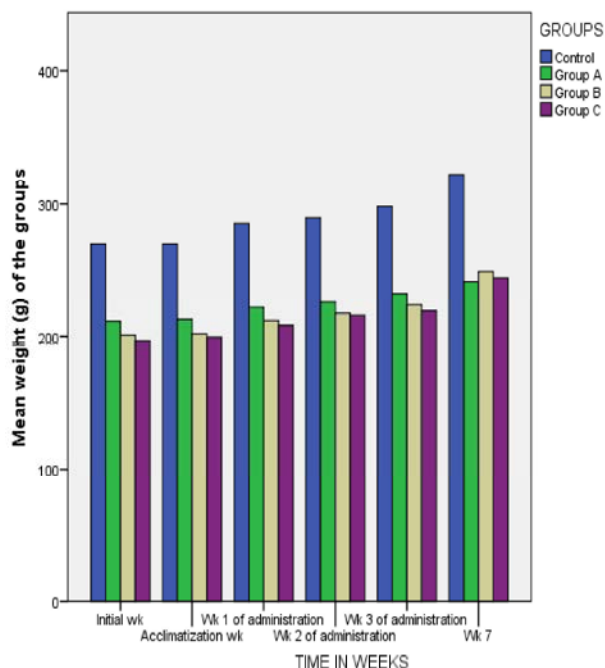


Fig. 1: Bar chart showing mean body weights of experimental and control groups of albino wistar rats during the period of the experiment.

Table 1: Effect of administration of different doses of methanolic leaf extract of *Adansonia digitata* on the weights of male albino wistar rats.

Time in weeks	Weight of Control (g)	Weight of Group A (g)	Weight of Group B (g)	Weight of Group C (g)
Initial week	230.13±15.96	198.50±15.64	189.25±11.06	182.88±7.39
Acclimatization week	230.75±15.98	198.75±16.10	188.63±10.28	182.13±7.06
Week 1	240.38±16.99	212.00±17.77	195.75±12.28	189.75±8.88
Week 2	251.33±22.93	218.67±25.18	197.17±18.29	197.17±15.33
Week 3	300.25±5.68	225.25±37.60	197.50±26.53	199.00±18.88
Week 7	322.50±2.50	241.50±63.50	249.00±71.00	244.00±52.00

Values are given as mean ± SEM for 2 rat in each group; experimental groups are compared with control. Values are statistically not significant.

Histological evaluation of the Testes

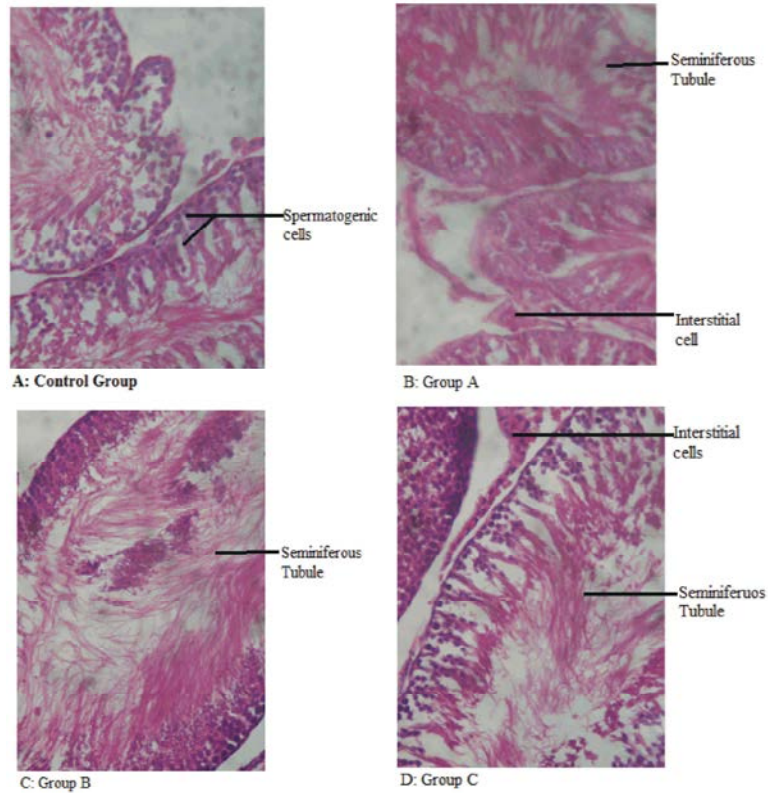


Fig. 2: Micrographs of albino Wistar rats' testis at the end of week one. (Mag. X 400; H&E stain)

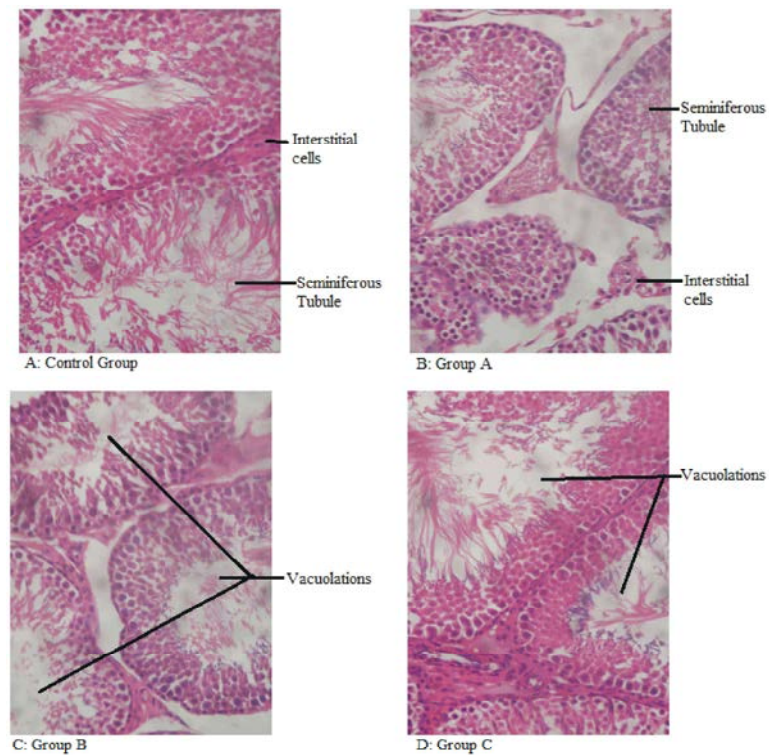


Fig. 3: Micrographs of Albino Wistar rats' testis at the end of week two. (Mag. X 400; H&E stain)

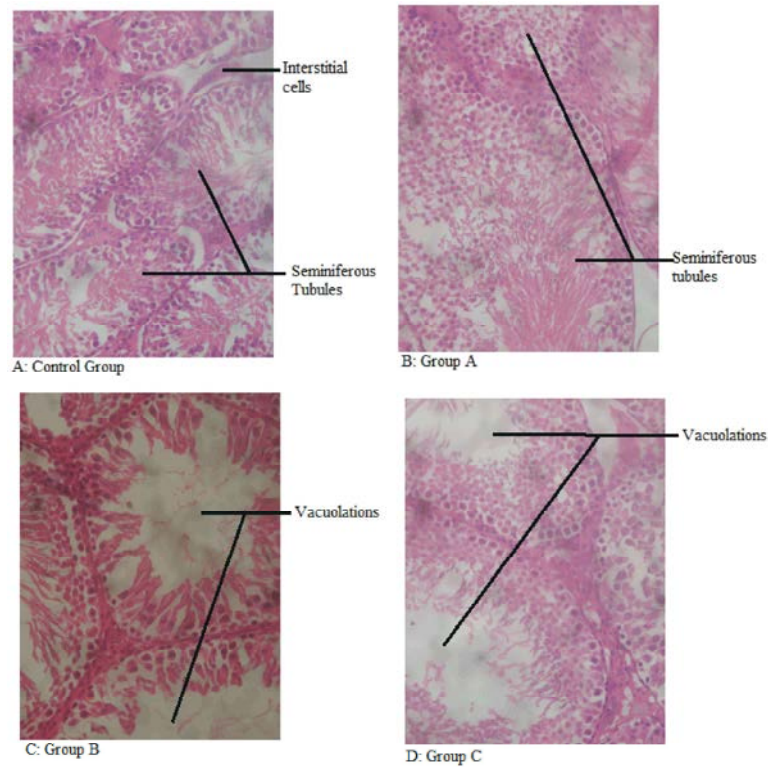


Fig. 4: Micrographs of Albino wistar rats' testis at the end of week three. (Mag. X 400; H&E stain)

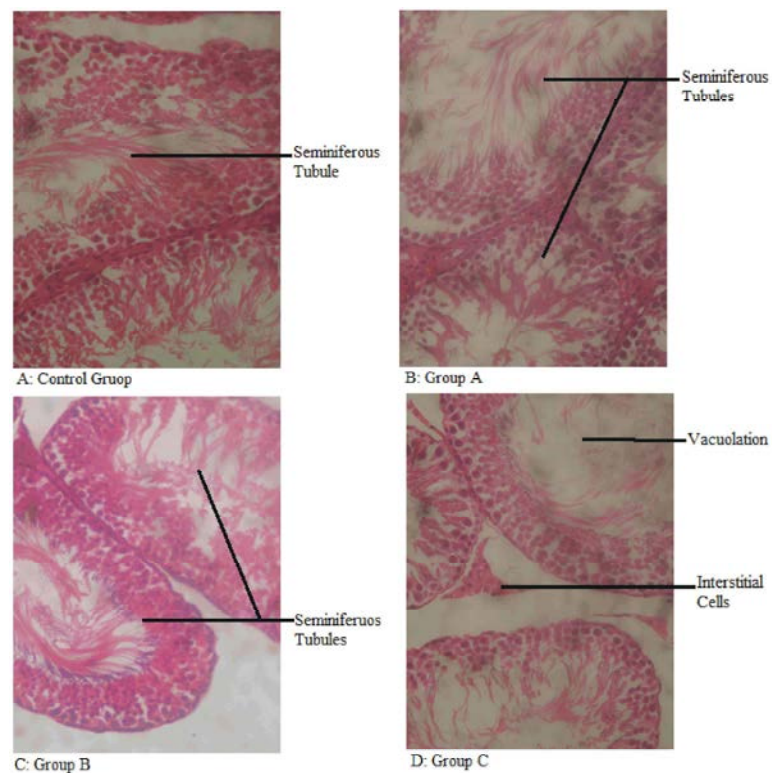


Fig. 5: Micrographs of Albino Wistar rats, testes at the end of the wash-out period. (Mag. X 400; H&E Stain)

Hormonal Assay: Tables 2-5 show the distribution of mean FSH and testosterone levels in the various groups at the end of the first, second, third weeks and the wash-out (reversal) period. Animals in groups A, B and C which were treated with 40mg/kg, 80mg/kg and 200mg/kg of the extract respectively, showed a progressive increase in mean FSH levels when compared to control at the end of the first and second weeks. Even though there was a progressive increase in mean FSH values across the experimental groups, only values in Groups B and C were statistically significant ($p > 0.05$). FSH values at the end of the third week decreased ($p > 0.05$) in Group A animals but increased significantly ($p < 0.05$) in groups B and C when compared with Control group. At the end of wash-out period, the mean FSH values was observed to decrease ($p > 0.05$) in group A and increase in Groups B and C when compared with the control group (Fig. 2). Although FSH values were elevated in groups B and C, only group C animals showed a significant increase ($p < 0.05$).

Results from biochemical analysis at the end of the first week of administration showed a progressive

increase in the mean testosterone values in all the experimental groups compared to that in the control group (Table 2-5 and Fig. 3) but only values in groups B (2.14 ± 0.01) and C (2.11 ± 0.01) were statistically significant ($p < 0.05$). At the end of the second week, mean testosterone values increased ($p > 0.05$) in group A (0.43 ± 0.01) animals and decreased ($p < 0.05$) in groups B and C as compared to animals in control group. The mean testosterone values as seen at the end of week two, further decreased ($p < 0.05$) in groups B and C and increased ($p < 0.05$) in group A at the end of third week of administration. When sex hormone values in groups B and C at the end of both week three and reversal period were compared, it was observed that mean testosterone values further decreased in groups C animals and improved in group B. At the end of wash-out period, the mean values of testosterone decreased in groups A, B and C animals as compared to the control group, however, only values in groups B and C animals significantly decreased ($p < 0.05$).

Table 2: The effect of different doses of methanolic leaf extract of *Adansonia digitata* on biochemical parameters at the end of first week of administration

Parameters	Control	Group A	Group B	Group C
FSH (ng/mL)	2.11 ± 0.01	2.17 ± 0.02	$2.24 \pm 0.01^*$	$2.27 \pm 0.03^*$
TESTOSTERONE (ng/mL)	0.43 ± 0.01	0.48 ± 0.01	$2.14 \pm 0.01^*$	$2.11 \pm 0.01^*$

Values are given as mean \pm SEM for 2 rats in each group; experimental groups are compared with control. Values are statistically significant at $*p < 0.05$.

P: statistical level of significance as determined by one-way ANOVA.

Table 3: The effect of different doses of methanolic leaf extract of *Adansonia digitata* on biochemical parameters at the end of second week of administration

Parameters	Control	Group A	Group B	Group C
FSH (ng/mL)	2.13 ± 0.01	2.18 ± 0.02	$4.06 \pm 0.01^*$	$5.25 \pm 0.03^*$
TESTOSTERONE (ng/mL)	0.42 ± 0.01	0.43 ± 0.01	$0.24 \pm 0.03^*$	$0.21 \pm 0.01^*$

Values are given as mean \pm SEM for 2 rats in each group; experimental groups are compared with control. Values are statistically significant at $*p < 0.05$.

P: statistical level of significance as determined by one-way ANOVA

Table 4: The effect of different doses of methanolic leaf extract of *Adansonia digitata* on biochemical parameters at the end of third week of administration.

Parameters	Control	Group A	Group B	Group C
FSH (ng/mL)	2.14 ± 0.01	2.12 ± 0.01	$3.21 \pm 0.01^*$	$3.35 \pm 0.01^*$
Testosterone (ng/mL)	0.44 ± 0.01	0.45 ± 0.01	$0.16 \pm 0.01^*$	$0.12 \pm 0.01^*$

Values are given as mean \pm SEM for 2 rats in each group; experimental groups are compared with control. Values are statistically significant at $*p < 0.05$.

P: statistical level of significance as determined by one-way ANOVA

Table 5: The effect of different doses of methanolic leaf extract of *Adansonia digitata* on biochemical parameters at the end of the recovery (wash-out) period.

Parameters	Control	Group A	Group B	Group C
FSH (ng/mL)	2.14 ± 0.01	2.13 ± 0.01	2.19 ± 0.01	$3.33 \pm 0.02^*$
TESTOSTERONE (ng/mL)	0.42 ± 0.01	0.41 ± 0.01	$0.28 \pm 0.01^*$	$0.10 \pm 0.01^*$

Values are given as mean \pm SEM for 2 rats in each group; experimental groups are compared with control. Values are statistically significant at $*p < 0.05$.

P: statistical level of significance as determined by one-way ANOVA

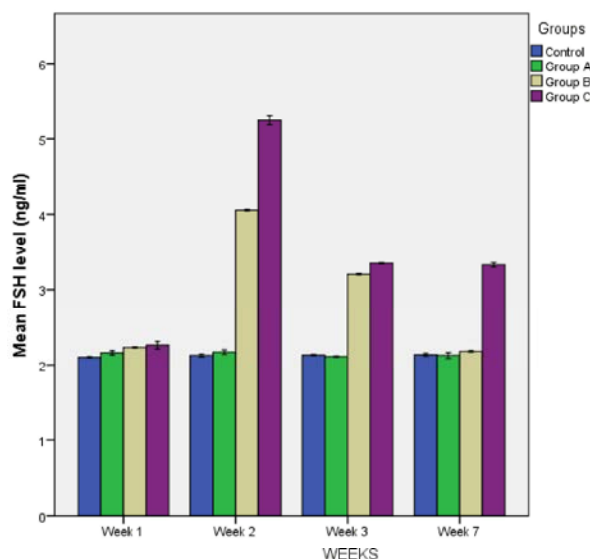


Fig. 6: Bar chart showing the mean FSH level of experimental and control groups of albino wistar rats during the period of the experiment

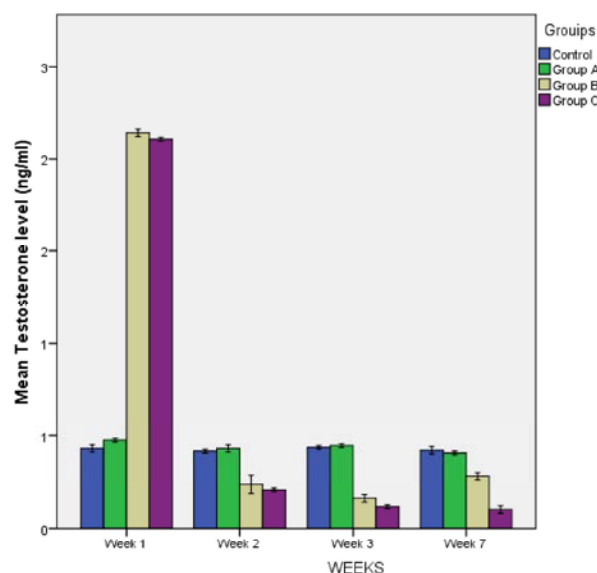


Fig. 7: Bar chart showing the mean testosterone level of experimental and control groups of albino wistar rats during the period of the experiment

DISCUSSION

Results from the body weight of the animals showed a progressive weight increase in both experimental and control animals from the initial weeks before administration to the end of the reversal (wash-out) period. Oral administration of the extract from week one to three did not show any significant increase in the mean

body weight of animals across the groups. These observations agree with the previous study of Oyetunji and colleagues [16], which reported that, the leaf extract of *Adansonia digitata* had no effect on body weight.

Histopathologic results from the study showed that the plant has adverse and severe effects on the histology of the testes. The study also showed a normal histoarchitecture of the testes in the control group from the end of the first week to the wash-out period. There was no change observed in the histoarchitecture of the testes in experimental groups A to C (40mg/kg, 80mg/kg and 200mg/kg respectively) at the end of the first week of administration. However, testicular damage in form of vacuolation was seen in groups B (80mg/kg) and C (200mg/kg) at the end of the second and third weeks of administration. It was also observed that the vacuolation persisted after washout period (four weeks). This suggests that the testiculotoxic effect of the extract is irreversible and is dose- and time-dependent. Hence the findings of this study disagree with the reports of [21,22] which stated that the leaves of *Adansonia digitata* possess antioxidant capacity. The testiculotoxic effect of the extract could be attributed to the presence of alkaloids [23] considering the phytochemicals of the plant [24-26]. It was also suggested that presence of tannins also known as an anti-oxidant could at high dose be pro-oxidant hence increasing lipid peroxidation [27,28].

The results also demonstrated a significant rise in both FSH and testosterone levels among the treated rats, at the end of the first week of extract administration. The increase in testosterone and FSH values indicates the spermatogenic property of the leaf extract as reported by Oyetunji *et al.* [16]. The increase could also be due to the antioxidant, as well as the bioactive constituents of the plant extract [16,21&22]. The effect of increased serum values of FSH and testosterone during this experimental period was clearly evidenced by the histological findings observed in this study in which there was a normal testicular architecture for this group. Results at the end of second and third weeks of administration, demonstrated an insignificant increase ($p > 0.05$) in plasma values of FSH and testosterone for animals treated with 40mg/kg per body weight (group A) of extract. Hormonal findings revealed that, consumption of 40mg/kg (low dose) of leaf extract for a prolonged period had no significant increase on both serum FSH and testosterone levels. However, for groups treated with 80mg/kg (group B) and 200mg/kg (group C) of the extract, an increased and decreased level

of FSH and testosterone values respectively were recorded at the end of both the second and third week. The concomitant increase and decrease in FSH and testosterone values respectively, may point to testicular impairment as evidenced from the histologic findings and may be due to the effect of a medium and high dose of the extract on serum testosterone. Evidence from researches conducted, have shown that, the decline in serum testosterone levels enhances FSH secretion through the hypothalamic-pituitary axis as a compensatory mechanism to elevate the testosterone level [29,30]. Conversely, FSH and testosterone levels decreased in animals treated with 200mg/kg body weight (group C) of the extract from the end of week three to the end of the reversal period. Despite the reduction in both hormonal values, for this period of time, animals in this group still maintained a high and low level of plasma FSH and testosterone respectively at the end of the wash-out period. The decreased testosterone level in these animals suggests testicular distortion as was confirmed by the histologic findings of this study. Conversely, serum testosterone values in groups treated with 80mg/kg (group B) body weight of the extract, improved at the end of the wash-out period when compared to that at the end of week three. Its FSH values however decreased for the aforementioned period of time. The lowered level of FSH could be as a result of suppression of FSH secretion by the high level of plasma testosterone. Elevation in mean testosterone levels could be attributed to regeneration of the distorted tissue as seen in Fig.5. This implies that the medium dose (80mg/kg) of the extract had a transient effect on sex hormones and hence the histology of the testes when administered for a prolonged time.

CONCLUSIONS

The methanolic leaf extract of *Adansonia digitata* was found to have LD₅₀ of 6000mg/kg. The extract was observed to have no significant effect on body weight of albino wistar rats during the experimental period, however, after a prolonged use, testicular distortions were observed at a medium and high dose. These testicular distortions were not reversible at a high dose, suggesting that the extract had a dose-and time-dependent effect on the testes. We therefore recommend that caution should be applied in the consumption of high doses of the extract as regular and prolonged consumption of methanolic leaf extract of *Adansonia digitata* have deleterious effect on the testes.

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