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# Effect of aqueous and methanolic extracts of *Prosopis africana* on some reproductive parameters of male albino rats

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## ABSTRACT

**Aim:** Purpose of the study was to investigate body weight, testicular weight, gonadosomatic index, testosterone assay and testicular histology of the male albino rats, Wistar strain, treated with graded concentrations of aqueous and methanol extracts of *Prosopis africana*.

**Methods and Materials:** Thirty-six (36) adult male albino rats of 12- 13 weeks old and weighing between 200 g and 250 g. Six (6) rats were used to determine the median lethal dose ($LD_{50}$) of the extracts while the remaining thirty rats were divided into five groups (A, B, C, D and E) with six rats in each group. Group A and B; D and E were given 500mg/kg and 1000mg/kg of aqueous and methanolic extract, respectively; group C was the control group and was given 10 mL of distilled water for 21 days. However, two (2) rats were randomly picked from each group and sacrificed on days 7, 14 and 21 to study the GSI, hormonal assay and histology using the blood and testicular samples.

**Results:** There was no significant difference *(p>0.05)* in the body weight, testicular weight, gonadosomatic index and testosterone levels within the experimental groups and when compared with the control group throughout the study. Histology of the testis for the experimental group and the control rats showed no visible pathologic lesions. However, the two extracts at both dosages, except the 1000mg/kg aqueous, increased testosterone titer in albino rats.

**Conclusions:** It was concluded that the extracts of *Prosopis Africana* seem to enhance testosterone titer in treated rats with cautious to the apparent inhibitory effect on GSI.

**Keywords:** Aqueous extract, Methanolic extracts, *Prosopis africana,* Reproductive parameters, Albino rats

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## Introduction

Plants from different botanical sources have been used by many Traditional Medical Practitioners (TMPs) in Nigeria for the treatment of locally endemic diseases (Asas *et al.*, 2005; Builder *et al.*, 2012). Numerous claims by the TMPs on the potency and use of many of these plants have been scientifically authenticated, thus establishing their efficacy, especially in the management of certain diseases (Riaz *et al*., 2023). *Prosopis africana* is a commercially important plant which has been used since ancient times, particularly for medicinal purposes (Santhaseelan *et al.*, 2017).



Traditionally, Paste, gum, and smoke from leaves and pods are applied for anticancer, antidiabetic, anti-inflammatory, and antimicrobial purposes (Santhaseelan *et al.*, 2017). It grows wildly in the Middle Belt and Northern parts of Nigeria (Barminas *et al.*, 1998; Aremu *et al*., 2006). *Prosopis Africana is* also known as African mesquite, *Prosopisoblonga*, Benth and *Prosopis lanceolata*, Benth, belong to the family *Mimosaceae* (*Leguminosae*) (Ogunshe *et al*., 2007). This is the only *Prosopis* native to inter-tropical Africa, occurring from Senegal to Ethiopia throughout the Sudan and Guinea ecozones, reaching the border of the Sahelian ecozones to the north. It is a small to large tree (4-20m), with very dark and scaly bark which is orange to red-brown and white streaks when slashed (Damola, 2004). The branches and twigs are thornless, leaves alternate with bipinnate

leaflets in 9 – 16 pairs, oblong-lanceolate (12 – 30 mm), and shortly pubescent. In different Nigerian languages, it is called *Kiriya* (Hausa), *Kohi* (Fulani), *Sanchi lati* (Nupe), *Kpaye* (Tiv), *Ayan* (Yoruba), *Ubwa* (Igbo) and *Ukpehie* (Igala). This plant has several medicinal applications in Nigeria, Mali, and other African countries (Ezike *et al.,* 2010; Kolapo *et al.,* 2009). It is very popular for its seeds, which, in fermented form, are used as a food condiment. The potential uses of its gum for gels, which is used in tablet formulation in pharmaceutical industries, have been reported (Adikwu *et al.,* 2001; Attama *et al.,* 2000). Because of its anti-tyrosine activity, the plant may also be useful in preventing skin whitening or as an anti-browning agent (Baurin *et al.,* 2002). It is listed among the plants used by local farmers to treat trypanosomiasis in northern Nigeria (Atawodi *et al.,* 2002). *Prosopis Africana* has been used singly for hardening teeth, and the leaves aremacerated for enhancing male sterility (Mann *et al.,* 2003).

The gonadosomatic index, which is either the testicular volume per body weight or testicular weight per body weight, is species-specific and varies with age and breeding season even within the same species (Pochron *et al,* 2002). Gomendio *et al.* (2006) reported that this index predicts the rates of sperm production as well as sperm function in a given species. Since the rate of spermatogenesis is determined by gonadal mass, males tend to grow relatively large testicles in species where the ejaculates of different males co-occur and compete for the fertilisation of a set of ova in the female reproductive tract at the time of ovulation (Purnima *et al.*, 2023). Evidence that sperm competition favoursthe evolution of larger testes relative to body mass (gonadosomatic index) comes from multiple comparative and experimental evolution studies across many taxa (Birkhead and Møller, 1998). According to Pizzari (2006), rodents, particularly murids, are an appropriate eutherian taxon for the study of sperm competition because the high interspecific variation in male gonadosomatic index values indicates different levels of sperm competition across species. Such variation has also been observed in the lemurs (Glander *et al,* 1992 and Pochron *et al.,* 2002).

Many of the claims about the plant's reproductive potential of the plant are based on folklore, and there has been virtually little or no scientific work to establish it. There is also a paucity of information on the effect of *Prosopis africana* on the reproductive parameters of male albino rats. Therefore, this study was designed to investigate thebody weight, testicular weight, gonadosomatic index, testosterone assay, and testicular histology of the male albino rats, Wistar strain, treated with graded concentrations of aqueous and methanol extracts of *Prosopis Africana.*

## Materials and Methods

The Study was carried out at the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University (A.B.U), Zaria. The University is located in the Northern Guinea Savannah Zone of Nigeria between latitudes 11°4′N and 0°N and longitudes 7°42′E and 0°E at an elevation of 650m above sea level. Its monthly temperature ranges from 13.8 °C to 36.7 °C (Sawa and Buhari 2011). The area has a tropical savannah climate withan annual rainfall of about 1,099 mm (Iloeje, 2004).

*Experimental animals and their management*

A total of thirty-six (36) male albino rats (Wistar strain) used for this study were procured from the animal house of the Department of Biochemistry, Federal University of Technology Minna, Niger state, Nigeria. The animals were kept in cages and allowed 14 days for acclimatisation before the commencement of the study. They were supplied with clean drinking water and fed with Grower's mash pellets of Vital Feeds Nigeria® Limited. Ad libitum. Six (6) rats out of thirty-six were used to determine the Median Lethal Dose ($LD_{50}$) of *Prosopis africana* Aqueous and Methanol extract, while thirty (30) were used to study the body weight, testicular weight, gonadosomatic index, and testosterone assay, and testicular histology of the rats. Ethical institutional guidelines for the management and use of animals were strictly followed.

*Plant collection and processing*

*Prosopis africana* leaves were collected from a forest near Emitete village along Bida-Doko Road in Lavun local Government area of Niger State, Nigeria as described by the traditional healers, and further authenticated at the Herbarium of University of Ibadan, Nigeria, with a Voucher number UIH-2285. The plant specimen was washed with tap water and then dried in the shade in a well-ventilated part of the laboratory at a room temperature of $25^0$C over 72 hours. The dried sample was cut into small pieces with a clean knife and then groundinto powder using a mortar and pestle.

*Preparation of Aqueous Extract*

Five hundred (500g) grams of powdered leaves were soaked in 4.0 litres of distilled water, shaken, and allowed to stand for 72 hours. The mixture was filtered using Whatman No.1 (115cm) filter paper. The filtrate was concentrated over a water bath at 50 °C. The dried extract was kept in sterile universal bottles and stored in a refrigerator for further use (Ogundiya *et al.,* 2006).

*Preparation of Methanol Extract*

Five hundred grams (500g) of powdered leaves were soaked separately in 70mls of methanol and 30mls of distilled water for four (4) days at 30 – 32°C. The extracts were filtered through Whatman No.1 (115cm) filter paper. The filtrate was concentrated over a water bath at 50 °C. The dried extract was kept in sterile universal bottles and stored in a refrigerator for further use (Ogundiya *et al.,* 2006).

*Determination of Median Lethal Dose (LD50)*

The Organisation for Economic Cooperation and Development (OECD) 2001 protocol was employed (Limit Test). Six (6) rats were randomly selected and grouped equally into two groups. Group 1 (n=3) was dosed with 2000mg/kg of Aqueous extract of *P. africana,* while Group 2 (n=3) was dosed with 2000mg/kg of methanol extract for five (5) consecutive days. The rats were observed for apparent signs of toxicity (abnormal gait, circling, teeth grinding, paralysis, seizure, gasping, etc.) and mortality throughout the administration (using an oral gavage needle).

*Experimental design*

The thirty (30) sexually mature rats were grouped into five groups (A, B, C, D, and E), with six rats per group. Group A and B were given 500mg/kg and 1000mg/kg of Aqueous extract respectively; group C was the control group and was given 10mls of distilled water while group D and E were given 500mg/kg and 1000mg/kg of the Methanol extract respectively on daily basis for 21days. However, two (2) rats were randomly picked from each group and sacrificed on days 7, 14, and 21 to study the body weight, testicular weight, gonadosomatic index, testosterone assay, and histology of the testis of the rats.

*Determination of body weight and testicular weight*

Body weight and testicular weight were measured in grams (g) using a sensitive digital electronic weighing machine (Lark® -LP502A). Body Weights were taken before the commencement of dosing (pre-treatment weight) of rats with extracts and before sacrifice (post-treatment weight). Following a standard surgical procedure, the testicles were exteriorised and weighed at days 7, 14 and 21using (Lark® -LP502A) machine.

*Extract administration*

Groups A, B, and D, E were the experimental groups of albino rats, dosed with graded concentrations of *Prosopis africana* Aqueous and Methanol extract of 500mg/kg and 1000mg/kg body weight, respectively. Group C served as the control group. Rats in groups A, B, and D, E were dosed with aqueous and methanol extract in the morning, between 7.00 am and 9.00 am using an oral gavage needle and tuberculin syringe for three weeks. The control group was given distilled water.

*Blood collection*

Blood samples (1ml each) were collected using heparinised capillary tubes through the orbital sinus route and dispensed into EDTA-free tubes from rats picked on days 7, 14, and 21 and prepared for hormonal assay. Samples were kept in coolers containing ice packs and immediately transported to the laboratory for analysis (Nasr *et al.,* 2017).

*Hormonal Assay*

Testosterone was assayed using an Enzyme-Linked Immunosorbent Assay (ELISA). Serum was harvested for Testosterone assay using commercial ELISA kits (Abcam Scientific, South Africa), and expressed as ng/ml (Nasr *et al*., 2017).

*Histological examinations*

This was carried out using the method employed by Ajonuma *et al.* (2005). The testes preserved in 10% formalin were processed into slides for histological examinations. Before embedding in paraffin wax, the tissue samples were dehydrated in graded ethanol. The embedded tissues were then processed using a KD-TS6A tissue processor. Sections (5 µm thick) were cut using a Shandon Finesse Manual Rotary Microtome, model 325, Thermo-scientific, and dried onto super frost microscope slides (Fisher Scientific, Pittsburgh, PA, USA). For hematoxylin and eosin (H&E) staining, slides were dewaxed in xylene and dehydrated in graded alcohol, and stained for light microscopy. Images were captured using a digital microscope attached to a computer.

*Statistical analysis*

Data obtained from the study were analysed using Repeated Measure ANOVA (RM-ANOVA) and Tukey Post Hoc testto compare the means for the treated groups and the control group for statistical difference using SPSS statistical software version 21. The values of $p< 0.05$ were considered significant at a 95% Confidence interval.

## Results and Discussion

The result of the median lethal dose ($LD_{50}$) of albino rats treated with Aqueous and Methanol extracts indicates that both aqueous and methanol extracts of *Prosopis africana we*re relatively safe at a 2000mg/kg dosage. The 500mg/kg of *Prosopis africana:* The aqueous extract caused 0.22% reduction compared to the methanol extract (Table 1), which caused about 0.25% reduction in GSI within D7 and D21. The 1000mg/kg of *Prosopis africana:* The Aqueous extract caused 0.17% reduction compared to the methanol extract, which caused about a 0.03% increase in GSI within D7 and D21. However, control rats showed a 0.16%reduction. The differences in the GSI between control and experimental (aqueous and methanol) rats were not significant ($p$>0.05). The 500mg/kg of *Prosopis africana:* The aqueous extract caused a 0.3ng/ml increase between D7 and D21 compared with the methanol extract, which caused a 0.7ng/ml increase in the testosterone assay (Table 2). The 1000mg/kg of *Prosopis africana:* The aqueous extract caused a 0.3ng/ml decrease between D7 and D21 compared with the methanol extract, which caused about 0.19ng/ml increase in TA between D7 and D21. Control rats showed a 0.1ng/ml increase in TA. The differences in testosterone assay between control and experimental (Aqueous and methanol) rats were not significant ($p$<0.05). Effects of aqueous and Methanol extracts of *Prosopis africana* were recorded non-significantly (Fig 1-5).

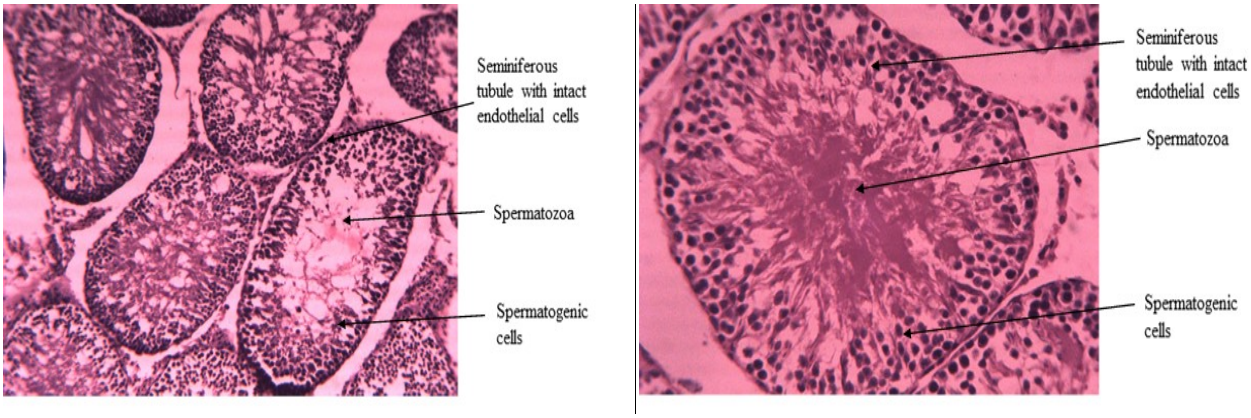


Fig 1: Photomicrograph of the seminiferous tubules (ST) from Group D rats (treated with 500mg/kg Methanol extract of *Prosopis Africana)* showing intact endothelial cells with numerous spermatogenic cells and scanty spermatozoa. There were no visible lesions. Numerous ciliated cuboidal linings were evidenced by decreased height of germinal epithelium and increased luminal width of the ST (x100, H&E). Fig **2:** Photomicrograph of the seminiferous tubules (ST) from Group E rats (treated with 1000mg/kg Methanol extract of *Prosopis africana*) showing intact endothelial cells with spermatogenic cells and containing a dense number of spermatozoa. There was a ciliated cuboidal lining evidenced by decreased height of germinal epithelium and increased luminal width of the ST (x 250, H&E).

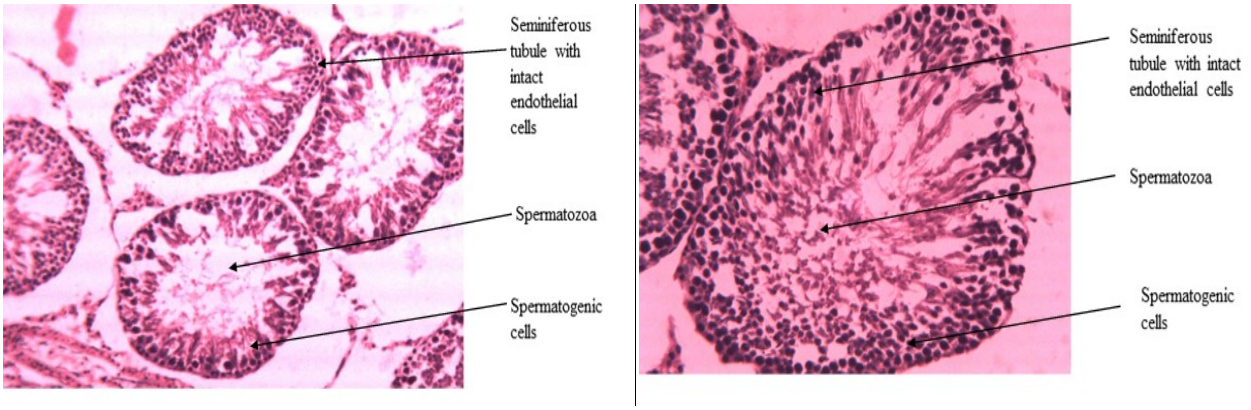


Fig 3. Photomicrograph of the seminiferous tubules (ST) from Group A rats (treated with500mg/kg aqueous extract of *Prosopis africana)* showing intact endothelial cells with numerous spermatogenic cells and scanty spermatozoa. There were no visible lesions (x100, H&E). Fig 4: Photomicrograph of the seminiferous tubules (ST) from Group B rats (treated with 1000mg/kg aqueous extract of *Prosopis africana*) showing intact endothelial cells with moderate spermatogenic cells and a slightly dense number of spermatozoa. Numerous tightly coiled tubules were evidenced by decreased height of germinal epithelium and increased luminal width of the ST (x250, H&E).

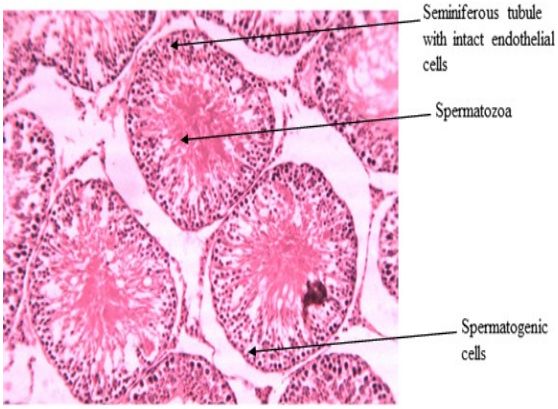


Fig 5. Photomicrograph of the seminiferous tubules (ST) from Group C rats (control) showing intact endothelial cells and spermatogenic cells. The lumen was lined with ciliated cuboidal cells containing a dense number of spermatozoa (x100, H&E).

The median lethal dose ($LD_{50}$) showd that no rat was lost during the six days of administration of 2000mg/kg of aqueous and methanol extracts of *Prosopis africana.* This observation indicates that both aqueous and methanol extracts of *Prosopis Africana* were relatively safe at a 2000mg/kg dosage (OECD, 2001; Builders *et al.*, 2012). The observations with the gonadosomatic index between D7 and D21 in the study appear to follow a similar trend (decrease) except for the rats administered 1000mg/kg methanolic extract of *P. Africana* (Table 1). Although the reason for this exception was unclear, it may not be faulty to opine that it may be connected with the quantity of *P. Africana* and the solvent of extraction. This position can be said to be fairly supported by the observation that a similar higher concentration of *P. Africana* (i.e. 1000mg/kg aqueous) produced an effect similar to other rat groups. The decreases observed with the gonadosomatic index in the study show a haphazard trend. This was not only because the reduction produced separately by a lower concentration of *P. Africana* (500mg/kg) in aqueous and methanolic solvents was dissimilar, but it was also higher than the 1000mg/kg aqueous with a higher concentration of *P. africana*. Clear inferences may not be easily made based on these findings, especially as the observations within experimental groups were not significant even after comparing with the control rats. This finding raised a lot of doubts about the clinical relevance of the extracts to male fertility. This was because careful considerations of the observations seemingly suggest that the extract possesses inhibitory properties on the gonadosomatic index. Although more studies were indicated, present observations strongly suggest careful use of aqueous and methanolic extracts of *P. Africana* on animals that fall within the reproductive ages. The observations with testosterone titre between D7 and D21 in the study appear to follow a similar trend (increase) except for the rats administered 1000mg/kg aqueous extract of *P. Africana.* Though the reason for this exception was unclear, it may not be faulty to opine that it may be connected with the quantity of *P. Africana* and the solvent of extraction. This position can be said to be fairly supported by the observation that a similar higher concentration of *P. africana* (i.e. 1000mg/kg methanolic) produced an effect similar to other rats' groups.

Table 1. Mean values of Body weights, testicular weights and gonadosomatic index for day7, 14 and 21.

| Days | Group | Pre-treatment body weight (g) | Post-treatment body weight (g) | Testicular weight (g) | Gonadosomatic index (%) |
|---|---|---|---|---|---|
| **7** | A | 210.54 ± 0.00$^{a}$ | 221.72 ± 15.20$^{b}$ | 3.39 ± 0.56$^{c}$ | 1.52 ± 0.25$^{d}$ |
| | B | 225.25 ± 0.00$^{a}$ | 229.24 ± 15.20$^{b}$ | 3.27 ± 0.55$^{c}$ | 1.42 ± 0.21$^{d}$ |
| | C | 205.50 ± 0.00$^{a}$ | 220.62 ± 6.00$^{b}$ | 2.20 ± 0.42$^{c}$ | 1.23 ± 0.21$^{d}$ |
| | D | 212.50 ± 0.01$^{a}$ | 220.00 ± 13.00$^{b}$ | 2.99 ± 1.25$^{c}$ | 1.35 ± 0.21$^{d}$ |
| | E | 232.50 ± 0.01 $^{a}$ | 240.00 ± 13.00 $^{b}$ | 2.84 ± 0.25 $^{c}$ | 1.18 ± 0.13 $^{d}$ |
| **14** | A | 218.75 ± 0.00$^{a}$ | 242.14 ± 6.00$^{b}$ | 3.09 ± 0.41$^{c}$ | 1.27 ± 0.10$^{d}$ |
| | B | 220.71 ± 0.00$^{a}$ | 222.52 ± 5.00$^{b}$ | 2.82 ± 0.30$^{c}$ | 1.26 ± 0.14$^{d}$ |
| | C | 214.75 ± 0.00$^{a}$ | 217.00 ± 0.00$^{b}$ | 2.29 ± 0.31$^{c}$ | 1.10 ± 0.15$^{d}$ |
| | D | 221.00 ± 0.01 $^{a}$ | 228.00 ± 6.25 $^{b}$ | 3.15 ± 1.20 $^{c}$ | 1.38 ± 0.14 $^{d}$ |
| | E | 215.00 ± 0.02 $^{a}$ | 230.00 ± 2.50 $^{b}$ | 2.67 ± 0.65 $^{c}$ | 1.16 ± 0.23 $^{d}$ |
| **21** | A | 241.50 ± 0.00$^{a}$ | 242.48 ± 0.00$^{b}$ | 2.86 ± 0.21$^{c}$ | 1.30 ± 0.21$^{d}$ |
| | B | 221.22 ± 0.00$^{a}$ | 225.52 ± 15.20$^{b}$ | 2.47 ± 0.31$^{c}$ | 1.25 ± 0.10 $^{d}$ |
| | C | 223.00 ± 0.00$^{a}$ | 231.50 ± 15.20$^{b}$ | 2.10 ± 0.51$^{c}$ | 1.07 ± 0.10 $^{d}$ |
| | D | 232.00 ± 0.00 $^{a}$ | 242.00 ± 13.20 $^{b}$ | 2.67 ± 1.36 $^{c}$ | 1.10 ± 0.23 $^{d}$ |
| | E | 228.00 ± 0.01 $^{a}$ | 232.00 ± 13.20 $^{b}$ | 2.83 ± 0.24 $^{c}$ | 1.21 ± 0.24 $^{d}$ |

Mean values with the same superscript in the same column are not significantly different ($p>0.05$)
Mean values with different superscripts in the same column are significantly different ($p<0.05$)

Table 2. Mean Testosterone assay of rats treated with aqueous and methanol extracts of *Prosopis africana*

| Days | Groups | Mean Testosterone level (ng/L) |
|---|---|---|
| **7** | A | 1.40 ± 0.01[a] |
| | B | 2.15 ± 0.10[a] |
| | C | 0.90 ± 0.05 [a] |
| | D | 1.10 ± 0.00[a] |
| | E | 1.26 ± 0.11[a] |
| **14** | A | 1.55 ± 0.19[b] |
| | B | 1.85 ± 0.00 [b] |
| | C | 1.50 ± 0.01 [b] |
| | D | 1.35 ± 0.10[b] |
| | E | 1.25 ± 0.00[b] |
| **21** | A | 1.70 ± 0.15[c] |
| | B | 1.85 ± 0.00[c] |
| | C | 1.00 ± 0.00 [c] |
| | D | 1.80 ± 0.20[c] |
| | E | 1.45 ± 0.00[c] |

Mean values with the same superscript in the same column are not significantly different ($p>0.05$)
Mean values with different superscripts in the same column are significantly different ($p<0.05$)

The increases produced with testosterone titre in the study showed separate dose-dependent increases in rats administered aqueous extract of *Prosopis* and also in rats administered methanolic extract. However, it was worthnoting that methanolic extract performed lower than both 500mg/kg and 1000mg/kg aqueous extracts of *Prosopis.* Based on these findings, it can be stated that Aqueous extract has a more stimulatory effect on testosterone titre than methanolic extract. However, the observations within experimental groups were not significant even after comparing with the control rats. This corroborates the work done by Joseph *et al.* (2019) when male Albino rats were dosed with methanol and oil extracts of *Ocimum gratissimum.* However, Oyedeji *et al.* (2013) reported that there was a significant difference in testosterone levels when male albino rats were dosed with the methanolic extract of *Vernonia amygdalina*. It was, however, worthnoting that a comparatively lower increment (~0.1ng/ml) in testosterone titre compared to other groups was obtained in the control rats. These findings established the clinical relevance, however of the extracts to male fertility (Libido). This was because careful considerations of the observations seemingly suggest that the extract possesses stimulatory properties on testosterone titre. Although more studies were indicated similar observations strongly suggest careful use of aqueous and methanolic extracts of *P. Africana* on animals that fall within the reproductive ages. From an extensive literature search, no work has been done to assay the effect of *P. africana* on reproductive hormones of albino rats, which can form a basis for comparison. Histological findings of the study in terms of testis showed that control rats containa dense number of spermatozoa, implying an increased spermatogenic activity within their testes. Similar observations were accounted for in both 1000mg/kg aqueous and methanol extracts. However, scanty spermatozoa were observed in both 500mg/kg aqueous and methanol extracts. However, no visible lesions were in the control as well as experimental rats. The observation were in agreement with increase seen in spermatozoa concentrations at 1000mg/kg (aqueous and methanol). This suggested that extract at high doses tends to enhance spermatogenic activity. This finding were in agreement with the work done by Joseph *et al.* (2019) when male Albino rats were dosed with methanol and oil extracts of *Ocimum gratissimum*. On the contrary, Oyedeji *et al.* (2013) reported that there was severe germinal erosion and necrosis in the seminiferous tubules of male albino rats dosed with methanolic extract of *Vernonia amygdalina* for 30 consecutive days compared to the control in the same study. From an extensive literature search, no work has been done to assay the effect of *P. africana* on the testicular architecture of albino rats, which can form a basis for comparison.

## Conclusion

It was concluded that extracts of *Prosopis africana* seem to enhance testosterone titer in treated rats, caution is advised due to apparent inhibitory effect on GSI. Further investigation should be carried out on *Prosopis Africana* since observations in the study were not significant; more studies should examine the actual effect and possible mechanisms of plant on reproductive parameters of male albino rats.

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