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Reproductive Effect of Different Doses of Ethanolic Stem-Bark Extract of *Prosopis Africana* on the Ovary of Wistar Rat

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ABSTRACT

The usefulness of *Prosopis africana* in many parts of Africa is diverse and its therapeutic uses are well known. Theaims of the study is to determine the effect of varying doses of ethanolic stem-bark extract of*Prosopis africana* on the ovary of wistar rats, with the objective on histology and hormone profiles.

Twenty-five adult female Wistar rats with an average weight of 100 – 200, were used. *Prosopis africana* extracts were administered for a period of two (3) weeks, with five (5) rats per group. Group 1 (control), received rat pellets and distilled water. Group 2 received 3000mg/kg. Group 3 received 2000mg/kg of *Prosopis africana* extract. Group 4 received 1000mg/kg of *Prosopis africana* extract. Group 5 received 500mg/kg of *Prosopis africana* extract orally. Twenty-four hoursafter the last administration, the animals were weighed, sedated with diethyl-ether and blood was collected. The blood was collected then ovaries were located, removed and weighed using an electronic sensitive analytical balance.The results were statistically significant $p>0.05$ and at high dose greater than 1000mg/kg the ovarian histology exhibited degenerative changes in the ovarian cortex; hemorrhage in between primary follicles was observed when compared with control group. While the histology for the lower doses especially that of 1000mg/kg showed better qualities. Conclusively, *Prosopis africana* ethanolic stem-bark extract improves fertility at a most preferable dose of 1000mg/kg.

Keywords: Degenerative, Hemorrhage, Multilaminar, *Prosopia-africana*

INTRODUCTION

The taxonomy of *Prosopia africana* describes that it belongs to kingdom of Plants, under the division of Magnoliophyta which are flowering plants, it belong to the class of Magnoliopsida or Dicotyledonous and is under the order of the Fabales family also known as the Pea family, its genus is *Prospis* and its species is *africana*[2].

There are various names given to *Prosopis africana*, from the common name to the local names given by different local areas in Nigeria. They include:Igbo (Okpei), Yoruba*(Ayan),* Idoma (*Okpeye),* Tiv (Kpaaye), Hausa (Kiriya or *Kiriaya)*, Arabic (Abu suru), English (Iron tree)[2].

For many centuries in the African continent, herbal plants have been used by natives for various purposes such as dietary adjunct and for the treatment of numerous diseases and wounds, aid for fertility and anti-fertility and many more[7]. It is said that herbal plants are continually of major importance in the health care systems in Africa since up to 80% of people rely on them for primary health care[4, 15]. As time passed, it has been observed that medicinal plants used in traditional medicine have mostly positive effects upon their usage but very minimal research has been done on these plants to know the way of their function, what they are composed of and how they may affect various organ of the body system.

Prosopis africana is a leguminous plant, commonly known as iron tree; it is one of the 44 species of the Prosopis family and is peculiar to Africa. In Nigeria, *Prosopis africana* is found in the middle belt of the nation between lat. 7° N and 10° N[8]. *Prosopis africana* is used locally to treat, pains in the body, anxiousness, headaches and to heal wounds. The unique properties of the plants are its hardwood, pale foliage drooping and dark brown pods, it has a height of about 40.6 feet when fully mature[10]. It is commonly known in Nigeria by its seeds which are used as food condiments when fermented[1]. The leaves, stem bark, twigs and root are for the treatment of tooth decay, dermatitis, bronchitis, dysentery, malaria and stomach pain in Mali. In Ghana, the roots are boiled and used as plaster for sore throat while the bark is used as dressing for wounds[10]. It has been studied for its different composition and it has been observed that all parts of *Prosopis africana* contain medicinal properties.

The diverse usage of *Prosopis africana* in various local communities makes it important for research to be carried out to discover more on its uses and how it can

contribute to the health care of humanity. Therefore, this research work reveals the effects the ethanolic extract of *Prosopis africana* stem bark has on the ovary. This is done in order to provide a precise prescription of the plant and to know the risk involved in its usage if there be any. Based on the research that states that *Prosopis africana* possess contraceptive properties and can also be used to treat menstrual cramps we find out being in the times were family planning is the order of the day with an increasing report of menstrual cramps, this plants could be of help to many people. For this reason, this study is aimed at investigating the effects of varying doses of ethanolic stem-bark extract *Prosopis africana* on the female reproductive organ.

MATERIALS AND METHODS

Chemicals and Reagents: All chemicals used in the course of the study were of pure analytical grade.The ELIZA kits for hormone profiles were bought from Nums Diagnostic Centre, Suleija. The histological staining was done in Anatomical-pathology Department, Obafemi Awolowo University, Ile-Ife, Osun State- Nigeria.

Preparation of Extracts: The Stem barks, fresh mature leaves and pods was obtained from selected natural environment was professionally identified. The plant leaves were washed with water, cut into pieces, dried in a cool environment. The dried plant leaves were pulverized into coarse powder in a grinding machine. The filtrate was concentrated using Rotary evaporator (Buchi) and further concentrated to dryness at 50oc in an electric oven (GallenKamp).After drying it was stored in the refrigerator at 40c until needed for use.

Acute Toxicity Test (Ld50): Fifteen mice were used to conduct the above test to determine the safe dosages and lethal dosage levels, according to Holford[3]. They were grouped into five (5), with three (3) mice per group. The acute toxicity of the Ethanol Extract of *Prosopis africana*extract was assessed by LD50 calculation, using a limit dose test at a limit dose of 3000mg/kg bw of the extract after oral administration in mice (three animals per group) (OECD-OCDE 425 Guide).Using the oral route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death. LD50 for *Prosopis africana*extract is 2000mg while the safe dose is 1000mg/Kg b.w.

Breeding of the Animals: Twenty five adult female Wistar rats with an average weight of 100-200g g. The rats, after procurement, were housed in cages (made from wood, wire gauze and net) under natural light and dark cycles at room temperature in the animal house of the Department of Biochemistry animal house, Federal University of Technology Akure (FUTA). The floor of the cages were made with wood to make it comfortable for the rats and it was covered with sawdust to provide a soft floor for the rats and to make cleaning of the cage convenient when littered. They were fed with rat pellets purchased from approved stores by Federal University of Technology Akure (FUTA) and water was given *ad libitum.* They were grouped and left to acclimatize for 2 weeks before the study commences.

Experimental Design: The total number of animals were twenty five (25) and were grouped into one (1) control and (4) experimental groups with consideration towards size variation. Using a feeding tube (size-6), distilled water and *Prosopis africana* extracts were administered to the control and treated animals respectively for a period of three (3) weeks.

- Group 1 (control): (n = 5): Given rat pellets and distilled water.
- Group 2: (n = 5): Given 3000mg/kg extract of *Prosopis africana* and rat pellets.
- Group 3: (n = 5): Given 2000mg/kg extract of *Prosopis africana* and along with the rat pellets in distilled water orally.
- Group 4: (n = 5): Given 1000mg/kg extract of *prosopis africana* along with the rat pellets in distilled water orally.
- Group 5: (n = 5): Given 500mgmg/kg extract of *Prosopis africana* along with the rat pellets in distilled water orally.

Animal Sacrifice and Sample Collection: The animals were weighed and sacrificed twenty four hours the last dosage, by use Diethyl ether as a sedative. When the animals were euthanized, the blood samples were collected using syringes through cardiac puncture through the midclavicular line and a midline surgical incision. The anterior abdomen wall was made to expose the internal organs of the thorax, abdomen and pelvis and the reproductive organs were removed.

Histology Analyses: Tissue specimens were taken from the Ovary of female wistar rats from each of the five groups and were fixed in 10% Paraformaldehyde for 24 hours. Then each specimen was sliced into small slabs (3-5mm thick) and further fixed in a change of the same fixative for another 15 hours. The fixed tissue specimens were trimmed and washed in tap water for 12 hours. An alcohol series (methyl, ethyl, and absolute) was used to dehydrate the tissue specimens. The tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned in 5-micron slices on a rotary microtome. The obtained tissue sections were collected on glass slides and stained with Hematoxylin and Eosin.

Luteinizing Assay Procedure: LH was quantitatively determined according to manufacturer instruction based on the method of Wennink *et al*[14]. Essentially, biotinylated monoclonal and enzyme labeled antibodies are directed against LH epitope— the immunologically active site. The reaction between LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well. Following the completion of the required incubation period, the enzyme-LH antibody bound conjugate is separated from

unbound enzyme-LH conjugated by separation and decantation. The activity in the antibody-bound fraction is directly proportional to the native antigen concentration. The activity of the enzyme present on the surface of the well was quantified by reaction with suitable substrate to produce color.

Twenty five microns (25 µl) of the standard, the specimens and controls will be dispensed into appropriate wells. Twenty five microns (25µl) of enzyme conjugate reagent will be pipetted into the wells and thoroughly mixed for 30seconds and incubate at a temperature of 36°C for 60minutes. The microtiter wells will be rinsed and flicked 5 times with three hundred micron (300µl) of washing solution. The wells will be struck sharply with absorbent paper to remove all residual all residual water. Hundred micron (100µl) of TMB substrate solution was added to each well and mixed and then incubate at room temperature for 15minutes. The reaction was stopped using hundred micron (100µl) of stopping solution. The samples will be gently mixed for 30 seconds until the blue colour changes to yellow. Absorbance will be read at 450nm with Rayto: RT-2100C, Microplate Reader within 15 minutes.

Follicule Stimulating Hormone Assay Procedure[13]*:* 25microns (µl) of the standard, the specimens and controls will be dispensed into appropriate wells. 50µl of enzyme conjugate reagent will be pipetted into the wells and thoroughly mixed for 30seconds and incubate at a temperature of (36°C) for 60minutes. The microtiter wells will be rinsed and flicked 5 times with three hundred micron (300µl) of washing solution. The wells will be struck sharply with absorbent paper to remove all residual water. Hundred micron (100µl) of TMB substrate solution will be added to each well and mixed and then incubate at room temperature for 15minutes. The reaction will be stopped using hundred micron (100µl) of stopping solution. The samples will be gently mixed for 30 seconds until the blue colour changed to yellow. Absorbance will be read at 450nm with a microtiter well reader within 15 minutes.

Serum Assay Estradiol Procedure: Before the procedure began, all reagents, serum references and controls were brought to room temperature (20 – 27°C). The microplates' wells were formatted for each serum reference, control and rat specimen that was to be assayed in duplicate. Any unused microwell strips were replaced back into the aluminium bag sealed and stored at 2 -8°C. 0.025 ml Pipette of appropriate serum reference, control to specimen assigned to the well. 0.05 ml of Estradiol Biotin Reagent was added to all wells. The microplate was swirled gently for 20 – 30 minutes at room temperature. 0.050 ml (50µl) of Estradiol Enzyme Reagent was added directly on top the reagents dispensed to all wells. The micro plate was swirled gently for 20 – 30 minutes to mix and after was covered and incubated for 90 minutes at room temperature. The contents of the microplate were then discarded by decantation and the plate was blotted to dry with absorbent paper. 350µl of wash buffer was added to properly decant the contents, this process was repeated 2 more times for a total of 3 washes with the use of a squeeze bottle. 0.100 ml (100µl) of substrate solution was added to all wells in the same order to minimize reaction time difference between the wells. After which incubation at room temperature was done for 20 minutes. 0.050ml (50µl) of stop solution was added in the same order to each well and was gently mixed for 15-20 seconds. The reading for the absorbance was taken in each well at 450nmusing a reference wavelength of 620-630nm. The results were read within 30 minutes of adding the stop solution.

Statistical Analysis: SPSS software was used for statistical analysis (version 22). Analysis of variance (ANOVA) and post HOC test were used to analyze the data. Turkey's multiple comparison will be represented to test for statistical significant difference between control and experimental group. Results will be represented as mean ± standard Error of mean (S.E.M). Result will be considered significant of the significant difference is taken as $p<0.05$.

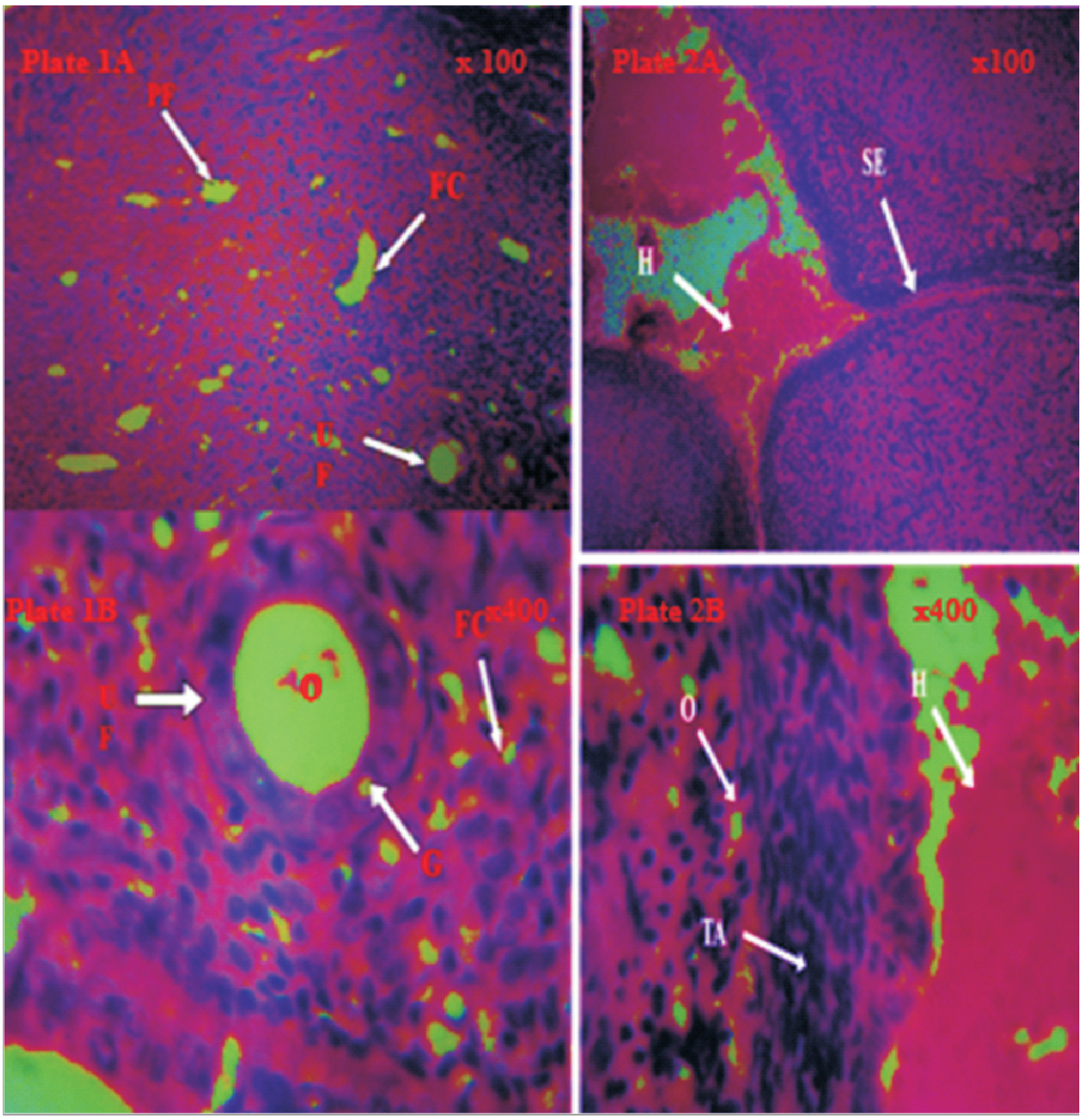
RESULTS

Histological Observations

- ✓ **Plate 1A & B**: A representative ovarian micrograph of wistar rats showing normal control structure. Several primary ovarian follicles (PF) are seen in the ovarian cortex which are still immature. Also a unilaminar primary follicle (UF) in which the granulose cells (G) form a single cuboidal layer around the large primary oocyte (O).Larger primary follicles were observed toward the cortex of the ovary. Stain is H & E and Magnification x 100 & 400.
- ✓ **Plate 2A& B:** Representative ovarian micrograph showing the cortical region of the ovary that is surrounded by surface epithelium **(SE).** The tunica albuginea **(TA),** an underlying layer to the surface epithelium.Hemorrhage **(H)** can be seen close to the ovarian cortex.The presence of hemorrhage is seen better at a bigger magnification which leads to degeneration**.** The stain is H&E magnification x100 & x400.
- ✓ **Plate 3A & B:**The representative of the photomicrograph of the ovary of wistar rat given 1000mg/kg/d of *Prosopis africana*. Showing maturing ovarian follicles having normal features of an ovarian follicle the antrum **(A)**, granulose cells **(G)**, primordial follicles **(PF)** and theca internal **(TI)**. Stain Hematoxylin magnification x100 & x400.
- ✓ **Plate 4A& B:** The micrograph shows the ovarian stroma around the collagen and containing some primary follicles. A larger

multilaminar primary follicle **(MF)** which has granulose cells **(G)**. It also shows the Theca interna **(TI)** around the follicule.This histological plate of the ovary shows a normal ovarian tissue. Stain is H & E and Magnification x 100 & x 400.

- ✓ **Plate 5A& B:** Representative ovarian micrograph of wistar rats given 500 mg of *Prosopis africana* showing the matured oocyte and the different cell layer: the antrum **(A)** granolosa cells **(G)**, the theca interna **(TI)**, and the theca externa **(TE)**. Stain Hematoxylin magnification x100 & x400.



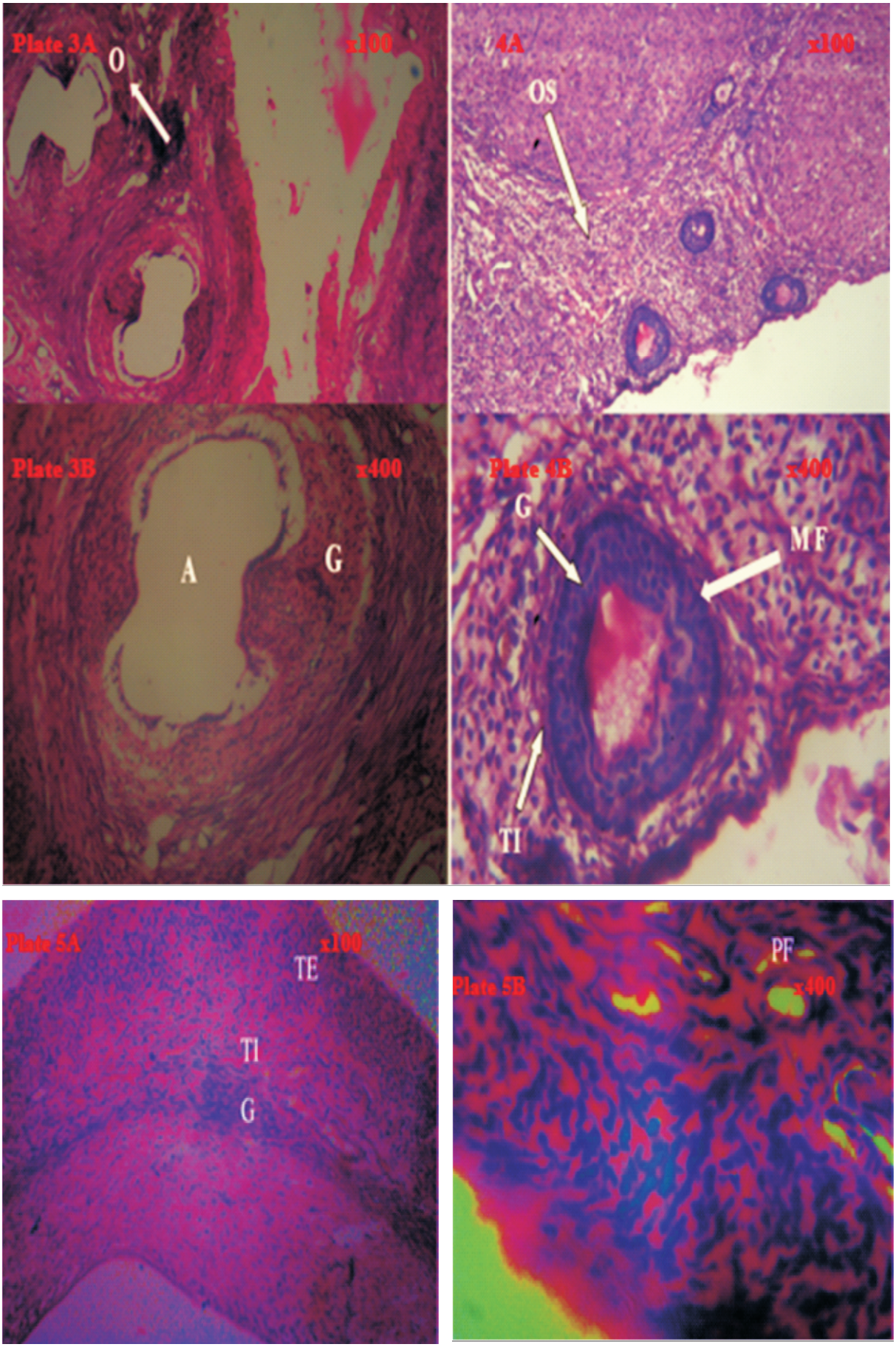


Plate 3A
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Weight and Hormone Profiles

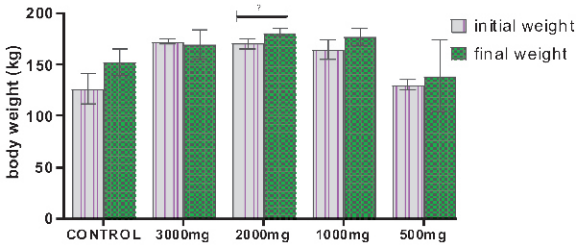
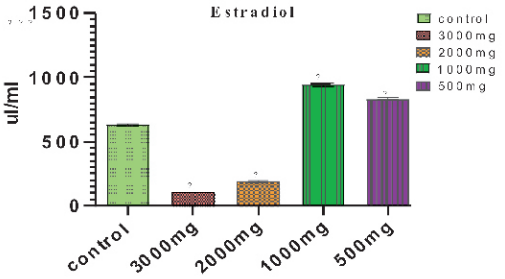


Figure 1: Variation in the weight of wistar rats
* Statistically significant to the control group p< 0.05.



Variation in the hormone profiles (Estradiol).
*Statistically significant to the control group at p>0.05.

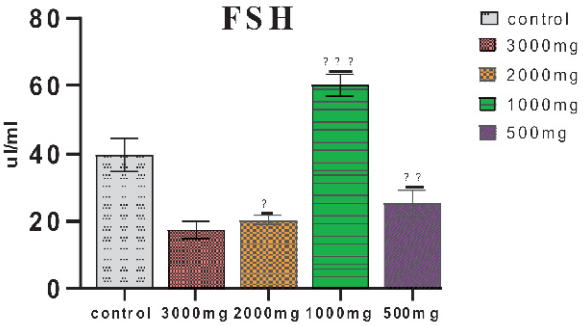


Figure 3: Variation in the hormone Profiles (FSH).
***Statistically significant to the control group p>0.05.
**Statistically significant to the 1000mg/kg and control groups p>0.05. *Statistically significant to the 1000mg/kg group and control group p>0.05.

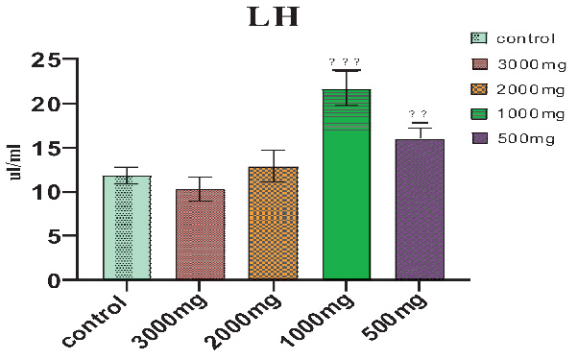


Figure 4: Variation in the hormone Profiles (FSH).
*** Statistically significant to the control group p>0.05.
**Statistically significant to the control group p>0.05.

DISCUSSION

Plant-base medicine has been wholly or partially a source of medical therapy for about 70% of the[12] with an estimated 80% of the world population currently seek therapeutic solution from herbal medicine as primary health care and this has gained recognition in several nations of the world as well as the World Health Organisation 'WHO'[9, 10, 16].

Based on this study, the general health condition of the control and experimental group was good; the water and food intake were normal for the experimental time. During the period used for the study, there was no visible mental or behavioral modification or illness in exception to the groups which were administered high doses (3000mg/kg and 2000mg/kg). Mortality was recorded in the groups with high doses, one from each group Plate 2, 3 A &B. In this study, this evaluation was undertaken and the result deduced can be used as diagnostic tool for the standardization of *Prosopis aficana* as a fertility drug.

The histological study showed the normal anatomical features of ovary in the control group (Plate 1A &B) having numerous primordial follicles in the ovarian cortex. In comparison with the 1000mg/kg group which also had normal feature including maturing ovarian follicles, the collagen and ovarian stroma showed no sign of degeneration or necrosis. The group of 500mg/kg also showed matured ovarian follicles which appeared to have just ovulated, while the 2000mg/kg group showed maturing oocyte a normal anatomical feature. In contrast to the 3000mg/kg group, hemorrhage was shown close to the ovarian stroma, some degenerative changes were also seen in ovarian cells. Mann[6], reported that*Prosopis africana* possess contraceptive properties and can also be used to treat menstrual cramps we find out being in the times were family planning is the order of the day with an increasing report of menstrual cramps, this plants could be of help to many people. The contraceptive property is dose dependent, at dose higher than 1000mg/kg it exhibited contraceptive property but at dose less the hundred it exhibited antioxidant property.

Jamal[5] reported that *Prosopis africana* contained a flavonoid compound 7,3',4'trihydroxy-3-methoxyflavanone was isolated from the ethylacetate extract. It is obviously exhibited that any compound that has flavonoid will exhibit antioxidant property. Hence, Plate 4 & 5 manifested the antioxidant properties. The ovarian tissue histology were normal in those group 4 & 5 and Plates 4 and 5 A &B. The micrographs show the ovarian stroma around the collagen and containing some primary follicles. A larger multilaminar primary

folliclewhich has granulose cells. It also shows the theca interna around the follicule. This histological plate of the ovary shows a normal ovarian tissue.
There were differences in the weights of the animals compare to the initial weight before *Prosopis africana* administration. Also, there were slight increase in the weight of the control groups; however the group taking 3000mg of *Prosopis africana* showed minor decrease in weight. The group given 1000mg/kg showed a larger increase in weight compared to the other two groups given 2000mg and 500mg/kg of *Prosopis africana*. The statistical significance $p>0.05$ (Fig. 1).

This investigation shows the effects that occur when different doses of *Prosopis africana* where used on wistar rats. The results from the hormonal analysis shows that *Prosopis africana* induced at a high dose of 3000mg and 2000mg had negative effects on the estradiol, LH and FSH. At a lower dose of 1000mg/kg and 500mg/kg the effects had more positive effect when compared to the control. The dose of 500mg/kg showed varying effect. It increased the hormonal levels of estradiol and FSH but not that of LH (Fig, 2, 3, 4). When compared with the control group, the 1000mg/kg dose increased the all levels of the hormones analyzed $p>0.05$.

In this study, the hormone profiles were all significant ($p<0.05$). The hormone profiles for follicle stimulating hormone, luteinizing hormone and estradiol all followed the same pattern that is 1000mg/Kg and 500mg/Kg body weight, relatively high as compared to the control group. These animals showed the highest level of hormones productions.

Marked differences exist between high dose and low dose of extract treatment. Based on our findings, the extract effects on hormone production is dose dependent. The use of *Prosopis africana* stem bark extract could have some negative effects on the ovary when the dose is higher than 1000 mg/Kg body weight.

Hormone levels were lowest in group 2 treated with 3000 mg/Kg body weight. This corresponds with the several pathologies observed in the histology of the group. The hormone profiles relative to the histology of ovaries indicates that the degree of consistency of histological sections with the control is dependent on the hormone levels. i.e, the higher the hormone levels the higher the consistency with control and the lesser the hormone levels the more pathologic features can be observed in the histological sections.

Moreover, the production of LH and FSH from the pituitary directly influence and is proportional to estradiol production. Animal groups with high LH and FSH production produce correspondingly high estradiol levels while groups with low FSH and LH production had correspondingly low estradiol level. This variation may be attributed to the differences in the basophilic cells population of the anterior pituitary and the granulosa, thecal cells of the ovaries.

CONCLUSION

The result of this study confirmed that therapeutic dose of *Prosopis africana* stem-bark has a positive influence on provides females fertility. The effect of *Prosopis africana* stem-bark is dose dependent and has a most preferable dose to bring out the best result. The study showed that at a dose higher than 1000mg/kg, there was a regressing effect on the ovary. However, at dose of 1000mg/kg there was drastic increase in the normal properties of the ovary. Therefore, it can be deduced that *Prosopis africana* ethanolic stem-bark extract improves fertility at a most preferable dose of 1000mg/kg.

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