



TOXICOLOGICAL EVALUATION OF METHANOL SEED EXTRACT OF HUNTERIA UMBELLATA ON REPRODUCTIVE FUNCTIONS OF TREATED WISTAR RATS

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ABSTRACT

ethnomedicinal survey have shown that the different parts of *Hunteria umbellata* plant are used in Western and Southern parts of Nigeria in managing various human diseases such as sexually transmitted infections and to induce or augment labor. This is however, without information on its effect on reproductive functions. This study was therefore designed to ascertain if there is any health benefit or risk in the ingestion of *Hunteria umbellata* on reproductive functions. In this study, qualitative phytochemistry, acute toxicity test and the sub-chronic toxicity effects of 250, 500, and 1000 mg/kg/day of *Hunteria umbellata* (HU) methanol seed extract on reproductive functions in male and female Wistar rats were investigated for 90 days. To achieve this, effect of repeated doses of methanolic extracts of *Hunteria umbellata* seed were investigated on selected reproductive parameters of the test animals. Eighty (80) (40 male and 40 female) Wistar rats, were randomly divided into 4 groups of 10 rats each. The first group received 10 ml/kg/day of distilled water and served as the control, while the second, third and fourth groups received 250, 500 and 1000 mg/kg/day of the extract for 90 days respectively. Blood samples were collected by retro-orbital puncture and delivered into plain tubes for hormonal assay, using ELISA hormone test kits (Biotec Laboratories Ltd, UK). After 90 days, the rats were anesthetized using chloroform. The histology of the testes and ovaries were also carried out. Result revealed that the prolonged treatment with methanol seed extracts of *Hunteria umbellata* for 90 days, caused minimal decreases ($p > 0.05$) in testosterone, estrogen, progesterone level in all the extract treated groups as compared with the control group. Also, no significant distortions were observed in the histology of testes and ovaries. The results suggests that the prolonged oral treatment with 250-1000 mg/kg/day of the methanol extract of the seed of *Hunteria umbellata* did not have any effect on the reproductive functions as well as the reproductive organs in males and females' rats treated, and could be safe following repeated use.

Keywords: *toxicological, methanol, seed, hunteria umbellata, reproductive, wistar rats*

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INTRODUCTION

Reproduction is the essential function of living organisms, especially mammals, that allows species continuity and improves the productivity and perpetuates animal species (Sorelle *et al.*, 2019). Today, reproduction, fertility, and population planning are considered critical issues especially in developing countries. In this regard, it is important to identify and use drugs with few side effects that are able to regulate fertility and help to reproduce through affecting the glands that secrete female hormones (Kooti *et al.*, 2014). At puberty and before pregnancy, sexual activity appears and large amounts of sex hormones, estrogen and progesterone, are produced. These hormones help to prepare uterus, vaginal, and oviducts. The perturbation or dysfunction of reproductive functions leads to negative consequences on the animal productivity (Sorelle *et al.*, 2019). Many factors can be responsible for this perturbation including stress (heat, feed, oxidative stresses), ageing, some drugs (Hafez and Hafez, 2005) and xenobiotics such as heavy metals. The infertility rate has increased tremendously in the past few decades (Oehninger, 2001; Venkatesh *et al.*, 2009).

The male reproductive system is a complex and intricate system that produces spermatozoa or sex cells to carry the genetic material of the male. The components of the male reproductive system include the hypothalamic–pituitary-gonadal (HPG) axis, and both the external and internal sexual organs. The male reproductive system forms during the early stages of embryonic development, becomes fertile during puberty and maintains the masculinity of the adult male. The external genitalia include the scrotum, testes, and penis whereas the internal genitalia include the epididymis, seminal ducts, spermatic cords, seminal vesicles, ejaculatory ducts, bulbourethral or Cowper’s glands, and the prostate gland. The testes produce the male gametes (spermatozoa). The excurrent duct system matures, stores, and transports the gametes to the penis for expulsion, and the accessory glands produce and modify the contents of the semen (Durairajanayagam *et al.*, 2016). Reproductive ability in the male contains the production of semen containing normal spermatozoa (quality) in the adequate number (quantity), together with the desire and ability to mate (Oyeyem *et al.*, 2008).

The female reproductive system functions to produce the female eggs, called the ova or oocytes necessary for production, and reproductive hormones. It also has the additional task of supporting the developing fetus and delivering it to the outside world (Effiong *et al.*, 2020). Unlike the male reproductive system, the female reproductive system is located primarily inside the pelvic cavity (Sorelle *et al.*, 2019). Reproduction begins with the development of ova in the ovaries, a single ovum is expelled from an ovarian follicle into the abdominal cavity in the middle of each monthly sexual cycle, this ovum then passes through one of the fallopian tubes into the uterus, and if it has been fertilized by a sperm, it implants in the uterus where it develops into a fetus, a placenta, and fetal membrane (Jain *et al.*, 2015). The female reproductive system is regulated by hormonal interaction between the hypothalamus, anterior pituitary gland, and ovaries (Marieb and Hoehn, 2015; Effiong *et al.*, 2020).

For thousands of years and even till today, medicinal plants also called or known as medicinal herbs are used in developed as well as developing countries for the management of various ailments or diseases, and has considerably contributed to the development of pharmaceuticals since about 25% of modern drugs are derived from plants (Effiong *et al.*, 2020). Medicinal plants constitute an effective source of both traditional and modern medicine. According to World Health Organization (WHO), medicinal plant is any plant which in one or more of its

organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs (Chukwuma *et al.*, 2015).

These plants have been shown to have genuine utility and, the World Health Organization (WHO) has estimated that about eighty percent of the world population relies on the use of medicinal plants for their psychological and physical health requirements (Mahomoodally, 2013), and in African countries this rate is said to be much higher (Akinyemi, 2000; Sofowora, 2013; Abd El-Ghani, 2016). This is so, since they cannot afford the products of Western pharmaceutical industries (Salie *et al.*, 1996), together with their side effects and lack of healthcare facilities (Griggs *et al.*, 2001). Recent reports indicate a wide use of medicinal herbs by pregnant women (Hepner *et al.*, 2002).

Hunteria umbellata (HU) *K. Kchum* (*Apocynaceae*) is a small tree of about 15–22 m in height with a dense evergreen crown (Oghenakogie *et al.*, 2014). It is found in rain forest zone of the southern part of Nigeria where it bears such local names as Osu (Edo), Erin (Yoruba) and Nkpokiri (Ibo) (Boone, 2006). It is also found in Ubongi-Shari in Ghana and the rain forest regions of Cameroon and Gabon. The leaves have been described as broad, abruptly acuminate and broadly lineate (Boone, 2006). The fruit is about 5–25 cm in diameter and consists of two separate globose mericaps 3–6 cm long, yellow, smooth. 8–25 seeds embedded in a gelatinous pulp (Oghenakogie *et al.*, 2014).

Hunteria umbellata (HU) have been widely employed in traditional herbal medicine in the treatment of peptic ulcers, piles yaws, dysmenorrhea, fever, infertility (Eluojoba, 1995), helminthic infection (Oluwemimo and Usifoh, 2001), bacterial infection (Anibijuwon *et al.*, 2011) and Diabetes (Igbe *et al.*, 2009). Recent research on the use of extracts of HU focuses on its use as a potent antidiabetic agent (Igbe *et al.*, 2009; Adeneye *et al.*, 2010).

JUSTIFICATION OF THE STUDY

Ethnomedicinal survey carried out showed that various parts of *Hunteria umbellata* (HU) plant, are used in managing myriad of ailments and the seeds are widely consumed within many localities in Nigeria. This is however, without information on its effect on reproductive functions. This study was therefore designed to provide information on the effect of methanol extract of *H. umbellata* seed on reproductive functions (testosterone, estrogen and progesterone). This is to ascertain if there is any health benefit or risk in the ingestion of *Hunteria umbellata* as medicine.

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The methanol seed extract of *Hunteria umbellata* was used for this study. The fruit were purchased from a local market in Benin City, Nigeria, and was identified by a taxonomist, in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. A voucher specimen with a reference number UBH-H557 was deposited in the herbarium of the Department for future reference.

PREPARATION OF EXTRACT

The seeds of *Hunteria umbellata* were first removed from the ripe fruits and the seeds were air-dried to a constant weight for three weeks. The dried material was then powdered using a mechanical grinder and kept in clean airtight amber bottle. The powdered material (800g) was cold macerated with 3L of methanol for 72 hours and the resulting solution was filtered using wire gauze and a sieve with pores. The filtrate was recovered and concentrated to dryness using water bath at 40°C and was preserved in a clean glass container in a refrigerator until use.

EXPERIMENTAL ANIMALS

The experiment was carried out using a total of 80 mature adult albino Wister rats of either sex (250-300g). The animals were purchased from the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka and transferred to the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, where they were used for the experiment. The rats were allowed two weeks acclimatization before they were randomly grouped into groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. All experimental animals were handled according to institutional and international guidelines for the use of experimental animals (Pub No. 85-23, revised 1985; Ozolua *et al.*, 2009). The Animal Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City, approved the experimental protocol. The approved ethical number is LS16074.

ACUTE TOXICOLOGICAL ASSESSMENT

The oral median lethal dose (LD₅₀) of the methanolic seed extract of *Hunteria umbellata* was evaluated by using modified Lorke method (Lorke, 1983). Nine mice were randomly assigned to three groups (n = 3 per group; both sexes represented) in the first phase. The groups were administered the extract at doses of 10, 100 and 1000 mg/kg body weight respectively. The animals were then monitored for 24 hours. Absence of death in the first phase led to the second phase. In the second phase using different rats, doses of 2000, 3000, 4000 and 5000 mg/kg of the extract was administered to one rat each. The control mice were given distilled water. After the administration of the extract, animals were observed for death and symptoms of toxicity within three days in the first instance and then for 30 min each day for another eleven days (Ozolua *et al.*, 2010). Gross toxicological symptoms were monitored and the LD₅₀ was calculated as follows;

$$LD_{50} = (D_0 + D_{100}).$$

Where: **D₀** = Highest dose that gave no mortality

D₁₀₀ = Lowest dose that produced mortality

PHYTOCHEMICAL EVALUATION

Preliminary phytochemical evaluation of the methanol extract of *Hunteria umbellata* was carried out using standard methods specified by Trease and Evans (1989) and Sofowora (1993). Ten percent (10%) preparation of the extract

in distilled water was considered as the test samples. Distilled water was used as a negative control throughout the phytochemical test.

EXPERIMENTAL DESIGN

From the outcome of the acute toxicity test, where no death was observed at 5000 mg/Kg, three doses of the extract 1/20th, 1/10th and 1/5 of 5000 mg/Kg (i.e 250, 500 and 1000 mg/Kg) were selected for the test (Prasanth *et al.*, 2015).

A total of eighty (80) rats, forty male rats and forty female rats were randomly divided into four groups of ten rats each as follows:

Group I - Control (received 1ml of distilled water)

Group II- Received 250mg/Kg of *H.umbellata* seed extract

Group III- Received 500mg/Kg of *H.umbellata* seed extract

Group IV- Received 1000mg/Kg of *H.umbellata* seed extract.

The extract was administered orally, once daily using an orogastric tube for ninety (90) days.

SAMPLE COLLECTION

Blood samples were collected by retro-orbital puncture on day 31st, 61st and 91st, and were delivered into plain tube. These were allowed to clot and were centrifuged at 3000 rpm for ten minutes using a table top centrifuge (90(1) Alpin Medical, England). The serum was carefully separated from the plasma using a micro-pipette into another set of plain tubes and was used for hormonal assay: progesterone, estrogen and testosterone using ELISA hormone test kits (Randox Lab Limited, UK).

HORMONAL ASSAY

The procedure described in the hormone assay kits was used according to the principle highlighted by Tietz, for estrogen and progesterone (Tietz, 1995), while serum testosterone concentrations were estimated using the Enzyme Immunology Assay (EIA) method as earlier described by Raji *et al.* (2003; 2006b).

DETERMINATION OF PROGESTERONE CONCENTRATION

The desired number of coated wells of micro-titer was secured in the holder. 25µl of specimens were dispensed into appropriate wells. 100µl of working progesterone – HRD conjugate reagent was dispensed into each well. 50µl of rabbit anti-progesterone reagent was dispensed into each well and was thoroughly mixed for 30 seconds. It was incubated at room temperature for 90 minutes. The micro-titer wells were rinsed and flicked 5 times with distilled water; 100µl of TMB reagent was dispensed into each well and was gently mixed for 10 seconds. It was again incubated at room temperature for 30 minutes, after which the reaction was stopped by adding 100µl of stop solution to each well. It was gently mixed for 30 seconds and was ensured that all blue colour changed to yellow completely. The absorbance was read at 450 nm with a micro-well reader within 15 minutes (MR – 9620A).

DETERMINATION OF ESTROGEN CONCENTRATION

The desired number of micro-titer coated wells was secured in the holder. 25µl of specimens were dispensed into the appropriate wells. 100µl of estrogen – HRP conjugate reagent was dispensed into each well. 50µl of rabbit anti-estrogen (E2) reagent was dispensed into each well and was thoroughly mixed for 30 seconds, and then incubated at room temperature for 30 seconds, and then incubated at room temperature for 90 minutes. The micro wells were rinsed and flicked 5 times with distilled water 100µl of TMB reagent was dispensed into each well and gently mixed for 10 seconds, then incubated at room temperature for 20 minutes. The reaction was stopped by adding 100µl of stop solution into each well and then mixed gently for 30 seconds to ensure that all blue colour changed to yellow completely. Absorbance was read at 450nm with a micro-titer well reader within 15 minutes using micro-plate Reader machine (MR – 9620A).

DETERMINATION OF TESTOSTERONE CONCENTRATION

The desired number of coated wells of micro-titer was secured in the holder. 10µl of the specimens were dispensed into appropriate wells. 100µl of testosterone HRP conjugate reagent was dispensed into each well. It was thoroughly mixed for 30 seconds and was incubated at 37°C for 90mins. The incubation mixture was removed by flicking plate contents into a waste container. The micro-titers wells were rinsed and flicked 5 times with distilled water. The wells were struck sharply onto absorbent paper to remove all residual water droplets. 100µl of TMB reagent was dispensed into each well and gently mixed for 5 seconds and was incubated at room temperature for 20 minutes. The reaction was stopped by adding 100µl of stop solution to each well, and then gently mixed for 30 seconds to ensure that all the blue colour changes to yellow completely. The absorbance was read at 450nm with a micro-titer well within 15 minutes using micro-plate reader machine (MR-9620A).

HISTOPATHOLOGICAL EXAMINATION

At the end of ninety (90) days, testes and ovaries were harvested and immediately fixed in 10% normal saline for histological processing (Drunny and Wallington, 1990). Following all standard safety operating procedures, tissues were dissected and placed in labeled tissues cassettes. The tissues did not exceed 3-5 mm thickness (Baker, 1962). Tissues were subjected to automatic tissue processing using Leica TP2010 automatic tissue processor for 18 hours passing them through the four stages of tissue processing namely: fixation (using 10% neutral buffered formalin) dehydration (using ascending grades of isopropyl alcohol) clearing or dealcoholisation (using xylene) and finally impregnation or infiltration (using molten paraffin wax) (Godwin, 2011). The tissues were then embedded in paraffin wax using the Leica automated tissue embedder and sectioned to get ultra-thin sections at five (5) microns, using the thermo scientific semi-automated rotary microtome. Tissues were floated out from the thermo scientific digital floating bath on frosted end pre labeled slides and dried on the thermo scientific digital slimline hot plate. Tissues were further dried in the hot air oven overnight and subjected to haematoxylin and eosin staining to demonstrate the general tissue structure. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope (Olympus, Japan) using X10 and X40 magnification (Kiernan, 2008). Photomicrographs were taken in bright field.

STATISTICAL ANALYSIS

Results were expressed as mean \pm standard error of mean (SEM) and data comparisons between treated and control groups were made using one-way analysis of variance (ANOVA) with Tukey post hoc test (SPSS version 20). $P < 0.05$ indicated statistically significant difference.

RESULTS

PHYTOCHEMICAL SCREENING

Results of phytochemical screening of the methanol seed extract of *Hunteria umbellata* showed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic and steroids.

ACUTE TOXICITY

The LD₅₀ could not be ascertained as no death was recorded after 24 hours of administration of the various doses of extract in the first phase (10, 100 and 1000 mg/Kg body weight), and in the second phase using different rats (2000, 3000, 4000 and 5000 mg/Kg body weight). This second phase lasted for 14 days. The LD₅₀ was therefore above 5000 mg/Kg.

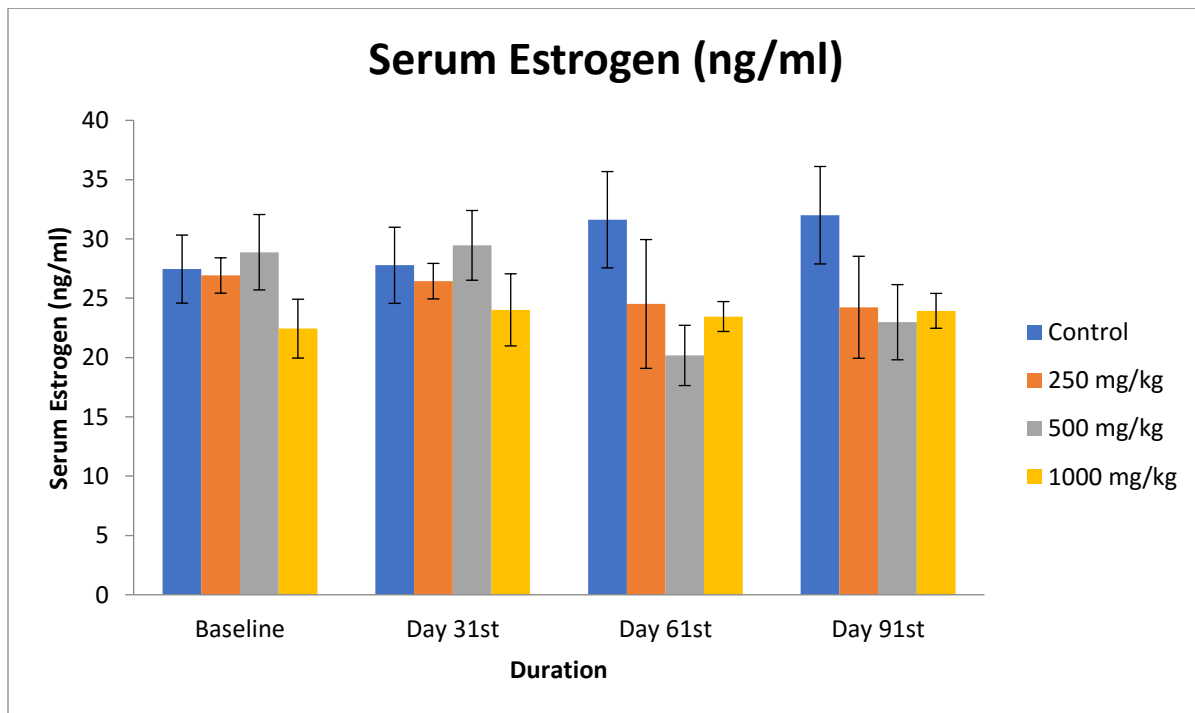


Figure 1. The effect of oral administration of methanol seed extract of *H. umbellata* on the estrogen level of female rats. Data are presented as mean \pm S.E.M, n = 5. $P > 0.05$: Not statistically significantly different from control group.

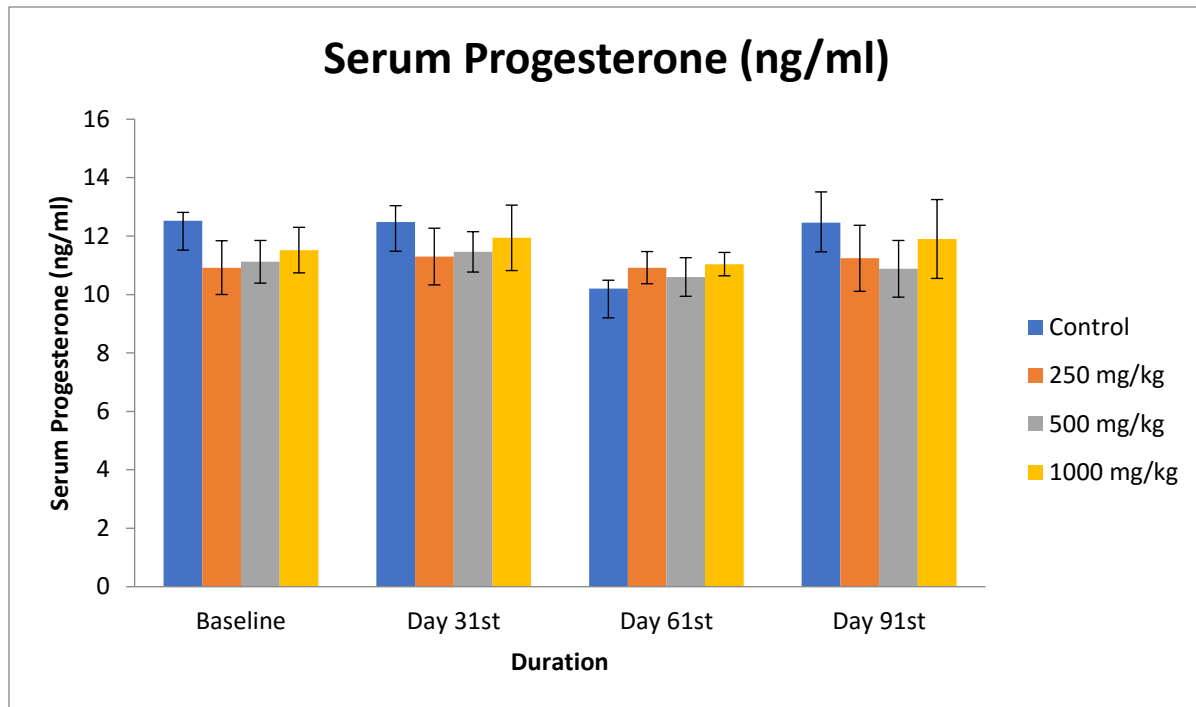


Figure 2. The effect of oral administration of methanol seed extract of *H. umbellata* on the progesterone level in female rats. Data are presented as mean \pm S.E.M, n = 5. P>0.05: Not statistically significantly different from control group.

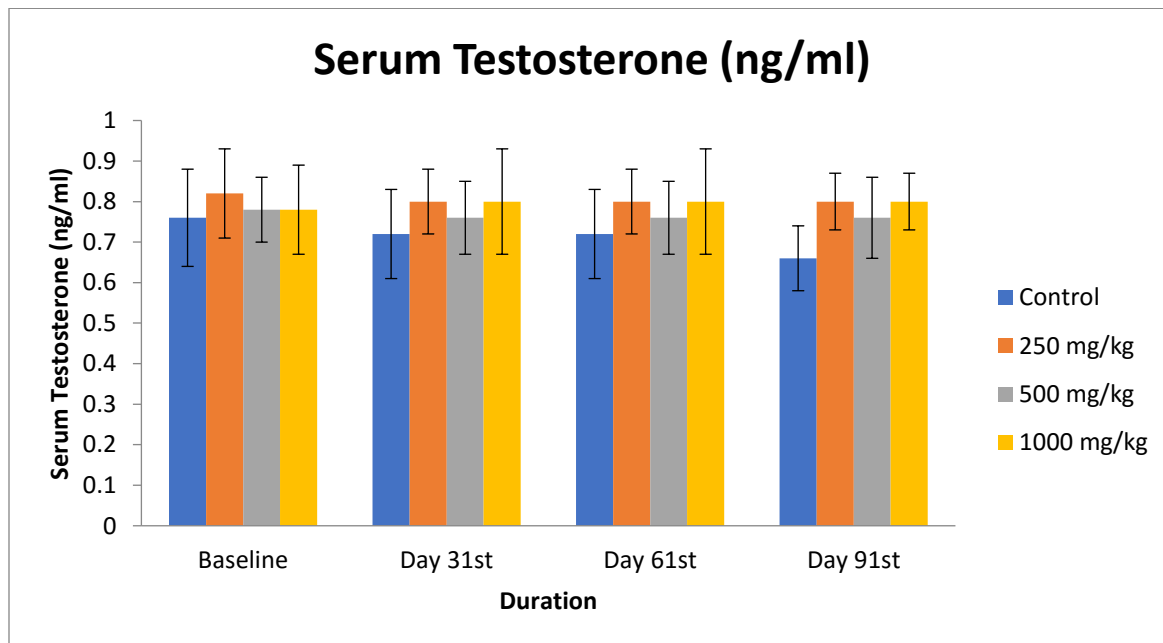
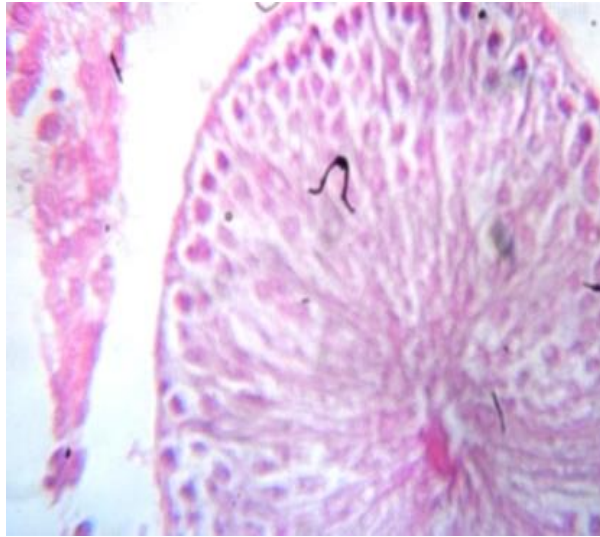
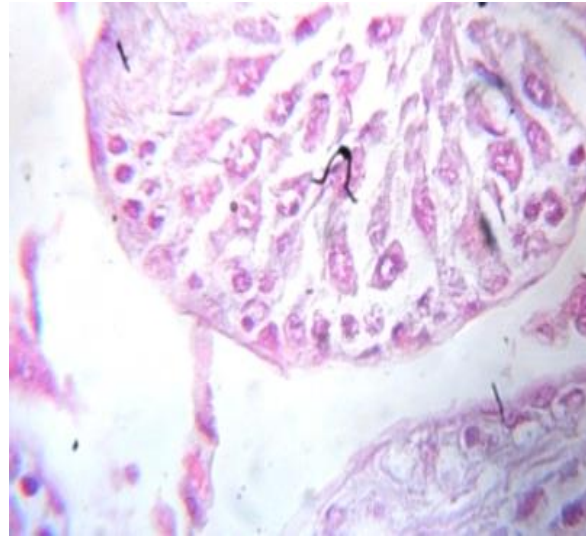


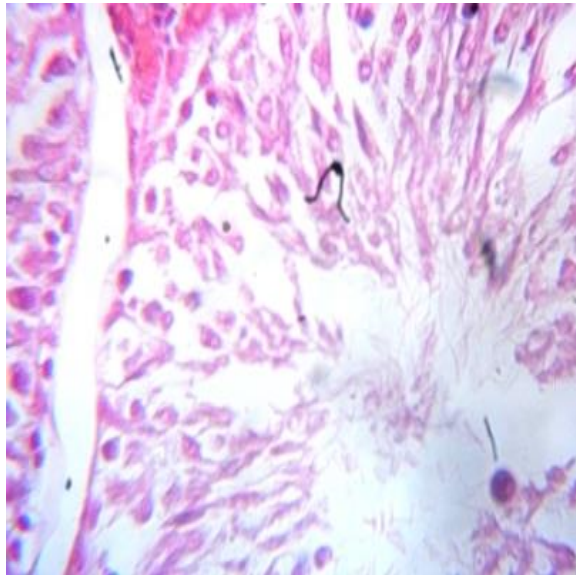
Figure 3. The effect of oral administration of methanol seed extract of *H. umbellata* on the testosterone level in male rats. Data are presented as mean \pm S.E.M, n = 5. P>0.05: Not statistically significantly different from control group.



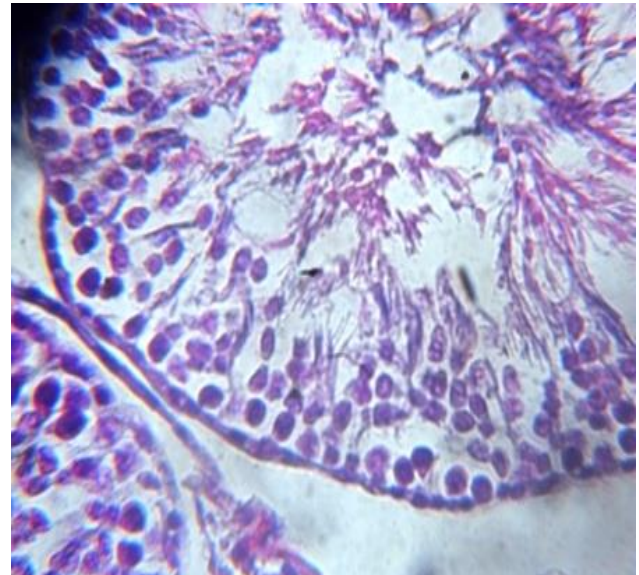
A



B



C



D

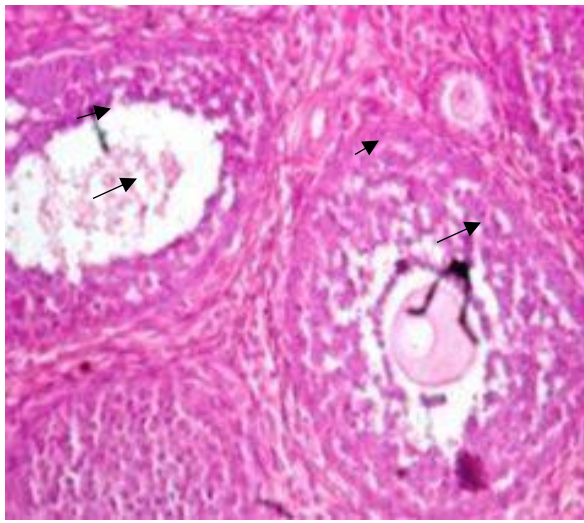
Figure 4. Photo micrograph of testis where A, B, C and D indicates, 250, 500, and 1000 mg/kg and normal control respectively after 90 days administration.

A. The testes show sections of the seminiferous tubules appearing moderately circular, transverse with normal epithelium showing sertoli cells of the spermatogenic series and spermatozoa within the lumen.

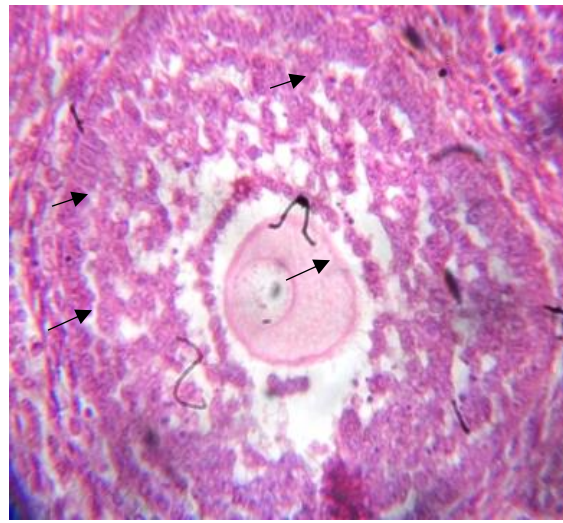
B. The testes reveal fairly circular seminiferous tubules with visible sertoli cells which appear vacuolated when compared to control. There are leydig cells seen at low power.

C. The testis reveals Hypo cellularity, reduction in cells of the spermatogenic series as a result of degeneration, sloughing and shortening of seminiferous epithelium. The seminiferous tubules show a thickened basal spermatogonia; widened empty lumen; widened interstitium due to tubular atrophy as a result of degeneration, areas of vascular haemorrhage.

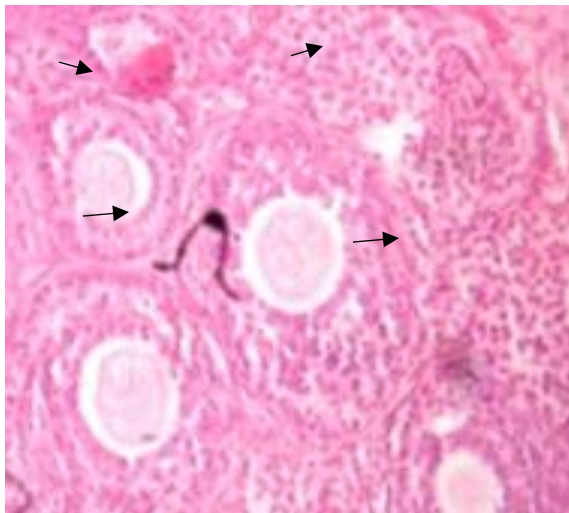
D. Testis shows sections of the seminiferous tubules are fairly circular in outline, with prominent Sertoli cells and other cells of spermatogenic series. There are prominent Leydig cells seen at low power.



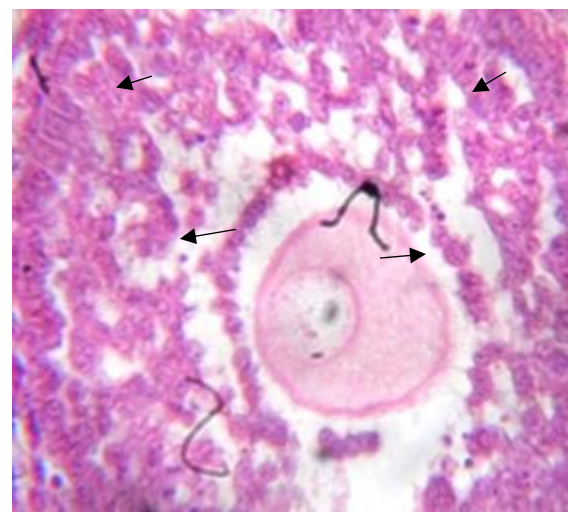
A



B



C



D

Figure 5. Photo micrograph of Ovaries where A, B, C and D indicates, 250, 500, and 1000 mg/kg and normal control respectively.

A: Section of the ovary reveal atretic tertiary follicle with ooplasm of oocyte surrounded by granulosa cells.

B: Section of the ovary reveal atretic tertiary follicle with ooplasm of oocyte surrounded by granulosa cells which appear slightly coarse.

C: Section of the ovary reveal atretic tertiary follicle with ooplasm of oocyte surrounded by granulosa cells however there is disruption in the theca folliculi which appears loosely bound and some visible congested capillaries.

D: Section of the ovary reveal atretic tertiary follicle with ooplasm of oocyte surrounded by granulosa cells.

DISCUSSION

The phytochemical analysis of the methanol seed extract of *Hunteria umbellata* indicated the presence of secondary metabolites such as alkaloids, tannins, steroids, flavanoids, glycosides, saponins, and phenol. Adeneye *et al.* (2012) and Kaur and Singh, (2016), have reported that secondary metabolites such as saponin, glycosides, steroid, tannins, phenols and copious amounts of alkaloids were present in the seeds of *Hunteria umbellata*. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds. It has been reported that several phytochemicals have been implicated in enhancing sexual functions in animal models. For example, saponins are responsible for aphrodisiac activity in *Fadogia agrestis* (Schweinf. Ex Hiern) and *Tribulus terrestris* (Linn.) so are alkaloids in *Pausinystalia yohimbe* (K. Schum) and *Microdesmis keayana* (J. Leonard) (Drewes *et al.*, 2003). Studies have shown that saponins enhance aphrodisiac properties due to its androgen increasing property (Gauthaman *et al.*, 2002; Alevtina and Zerihun, 2009) and also boost the level of testosterone in the body (Gauthaman and Adaikan, 2008). Also, saponins stimulate the leydig cells of the testes to directly increase testosterone production system (Ang *et al.*, 2004). Alkaloids were also present in the extracts and according to Dimitris *et al.*, (1997) they increase blood flow in the sexual organs due to vasodilatation thus sustaining male erection leading to enhanced sexual performance. Studies conducted by Ko *et al.*, (2004) indicate that flavonoids are phosphodiesterase (PDE) inhibitors. Phosphodiesterase enzyme breaks down cyclic AMP (cAMP) which activates synthesis of nitric oxide leading to vasodilatation thus increasing blood flow which sustains an erection (Rahimi *et al.*, 2009; Ko *et al.*, 2004).

These bioactive agents display aphrodisiac activity either by increasing androgen biosynthesis and secretion or by acting directly on the central nervous system in order to modulate the function of animal neurotransmitters and gonadal tissues (Bianchi, 2019). Specifically, saponins enhance androgen production (Graham, 2012) whereas alkaloids increase the dilation of blood vessels in the sexual organs, or increase nitric oxide that plays a key role in central erection and central sexual stimulation (Jian *et al.*, 2012). The presence of flavonoids and alkaloids in *Hunteria umbellata* (HU) could account for its protective effects on the testis and ovaries, since these phytochemicals have been documented to confer protection on organs, through prevention of tissue lipid peroxidation which explains their anti-oxidant and free-radical scavenging activities (Longe and Momoh, 2014). Studies have also shown that flavonoids bind to estrogen receptor sites on cell membranes in order to prevent over proliferation of these cells in response to estrogen (Azubuike *et al.*, 2018). Flavonoids block the enzymes that produce estrogen, and they do this by blocking estrogen synthetase (aromatase), a key enzyme used in the estrogen biosynthesis (Azubuike *et al.*, 2018). Aromatase is an enzyme required for conversion of androgens (testosterone and androstenedione) to estrogens (Estradiol and Estrone respectively). Interestingly, some of these phytochemicals such as saponins and steroids were also detected in the seed extract in this study and this could suggest the reason why the decreases in the sex hormones were not statistically significant.

The LD₅₀ gives a measure of the immediate or acute toxicity of a test substance (Oyediji *et al.*, 2013). The result of this study revealed that the acute toxicity (LD₅₀) test of the methanol seed extract of *Hunteria umbellata* did not show any toxicity on mice at the concentration below 5000 mg/kg body weight. This suggests that the methanol seed extract of *Hunteria umbellata* had no apparent toxic and lethal effects on the animals which probably indicate

that the extract has high safety index. Ihekwereme *et al.* (2018) reported that LD₅₀ values of test substances above 5000 mg/kg are considered safe. Also, Ahmed, (2015) stated that LD₅₀ values above 5000 mg/kg are classified as practically non-toxic. Toxicity studies are usually undertaken to define the toxicity and effect of extract, access the susceptible species, identify target organs, provide data for risk assessment in case of acute exposure to the chemical or drug, provide information for the design and selection of dose levels for prolonged studies (Wallace, 2001). The toxicity of plants is mostly dependent on the plant organs which may be as a result of some factors such as the storage form of the organ and seasonal variation considering the phytochemicals (Jaouad *et al.*, 2004).

There was no significant reduction ($p < 0.05$) in estrogen and progesterone level on day 31, 61 and 91st following the oral administration of the methanol seed extract of *H. umbellata* in female Wistar rats. This is in agreement with Osonuga *et al.* (2014) who reported that oral administration of leaf extract of *Momordica charantia* caused a non-significant reduction in estrogen levels of adult female Wistar rats. Studies have also shown that water based extract of combined *Lepidagathis longifolia* and *Phyllagathus rotundifolia* reduced progesterone and estrogen concentration in pseudopregnant and non-pregnant rats (Sulaiman *et al.*, 2001; Yakubu *et al.*, 2008). Alkaloids have been reported to inhibit the synthesis of cellular progesterone (Yakubu *et al.*, 2008). Therefore, the reduced level of progesterone by the extract could be due to the presence of alkaloid in the extract. Also a direct toxic effect on the corpus luteum may be a possible mechanism for decline in progesterone levels. The reduction in the levels of serum progesterone by *H. umbellata* seed extract may have consequential effect on conception in females; impede ovulation which may result in anovulation and sequelae. The attendant effect of this reduction in progesterone is spontaneous abortion and failure of implantation reported by other workers (Koneri *et al.* 2006; Sheeja *et al.* 2012).

In the same light, the reduction in the serum concentration of estrogen observed in this study may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis (Hsia *et al.*, 2007). Consequently, such decrease in estrogen levels may hamper ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of the pregnancy state (Hadley, 2000). Our findings contrast that of Ota *et al.* (1995) which demonstrated that herbal Shakuyaku (*Paeoniae radix*), Keihi (*Cinnamomi cortex*) and Botanpi (*Moutan cortex*) stimulated the aromatase activity in human granulosa cells and increased estrogen secretion *in vitro*. Kadohama *et al.* (1993) reported that several plant alkaloids inhibit aromatase activity, thus altering the potential for steroid production and reproductive performance.

These reductions in estrogen and progesterone may be caused by numerous chemical agents contained in plant extract. Benie *et al.* (2003) and Yakubu *et al.* (2005) reported that phytochemical screening has revealed many bioactive as well as toxic agents of plant extract that can affect the regulation of oestrous cycle, conception and reproduction. Alkaloids and flavonoids have been shown to reduce plasma concentrations of estradiol (Bianco *et al.* 2006). Therefore, the presence of these phytochemicals in *H. umbellata* may account for the alterations in the levels of the circulating hormone observed in this study as reported by Onyegeme-Okerenta *et al.* (2013). Therefore, the alkaloid in the extract may be responsible for the reduced level of estrogen probably by inhibiting aromatase activity.

Administration of the seed extract of *H. umbellata* to male Wistar rats did not produce any significant reduction in testosterone level of male Wistar rats on day 31, 61 and 91st (see Figure 3). Testosterone is a male

hormone that has significant impact on spermatogenesis (Lee *et al.*, 2001). It is secreted by the leydig cells of the testicles, the adrenals and the ovaries, and is the most important androgen secreted into the blood. Testosterone is responsible for the growth, maturation and transformation stages of spermatogenesis (Sembulingam and Sembulingam, 2010). A low sperm count may indicate a problem with testosterone levels. The reduction in testosterone level could suggest possible mechanisms of anti-spermatogenic action of the extract. Reduction in testosterone level might impair spermatogenesis and cause male infertility (Orisakwe *et al.*, 2004).

Another possible mechanism of the antispermatogenic actions of this extract could be the presence of steroids in its phytochemical constituents. Such steroids that are found in plants have been referred to as phytosteroids or phytoestrogens (Ejebe *et al.*, 2008). They have been reported to adversely affect the fertility in both male and female animals by acting as estrogen disruptors. Plants like soya beans which were initially widely promoted by nutritionist as rich sources of proteins (essential amino acids) have been found to be rich in these phytoestrogens and the antifertility effects of these agents have been confirmed in several disparate studies in animals and humans (Ejebe *et al.*, 2008). As a result of this possibility it is now recommended that phytoestrogens in plants be avoided unless contraception is a desired goal. Similar report had been reported by Das *et al.* (2009) and Lakshman and Changamma, (2012) that in rats treated with *Aegle mermelos* and *Carica papaya* extracts respectively, there was a reduction in the level of testosterone. It is well known that antioxidant compounds strengthen reproductive system and increase fertility in males. In male reproductive system, antioxidants reduce oxidative stress in testes, increase activity of Leydig cells and consequently increase testosterone secretion and improve spermatogenesis (Sheweita *et al.*, 2005; Glade and Smith, 2015). Presence of flavonoids was revealed in the seed extract of *H. umbellata*, and flavonoids have been shown to increase testosterone levels (Zohre *et al.*, 2015). Flavonoids inhibit 5-alpha reductase enzyme, which converts testosterone to dihydrotestosterone and increase testosterone levels (Azarneoshan *et al.*, 2009). Flavonoids also competitively bind to an enzyme called aromatase, decrease enzymatic expression, inhibit conversion of testosterone to estrogen and consequently increase testosterone levels (Van Meeuwen *et al.*, 2007).

Photomicrographs of rats' section of the ovary in all groups showed normal architecture, suggesting that the extract did not have any deleterious effect on normal ovarian function as substantiated by non-significant alteration in estrogen and progesterone levels. Photomicrograph of testes of extract treated rats revealed normal architecture, an indication of the absence of obvious toxicity. This is corroborated by non-significant alteration in testosterone levels.

CONCLUSION

Findings from this study have shown that the administration of the methanol seed extract of *Hunteria umbellata* did not produce any observable adverse effects or changes in the level of estrogen, progesterone, testosterone, and semen parameters investigated at doses of 250, 500 and 1000 mg/Kg/day for 90 days. Also, the histological architecture reveals the plant extract did not produce any obvious structural damage to the microanatomy of the testes and ovary. This suggests that the plant extract is not toxic on reproductive functions in the rats treated following repeated doses for 90 days.

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