



## Toxicological Evaluation of Methanol Extract of *Hunteria umbellata* Seed on Sperm and Haematological Parameters in Male Wistar Rats

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**ABSTRACT:** The effect of chronic administration of methanol seed extract of *Hunteria umbellata* (HU) on sperm and haematological parameters in male Wistar rats were examined for 90 days. Adult male Wistar rats were randomly divided into four groups of five rats each. Group 1 received 10 ml/Kg/day of distilled water and served as the control, while groups 2, 3 and 4 received 250 mg/kg/day, 500 mg/kg/day and 1000 mg/kg/day of the extract daily for 90 days by oral gavage. Sperm cells were collected from the vas deferens of the sacrificed male rats for the determination of sperm motility, sperm count and sperm morphology as well as blood samples via the abdominal aorta for haematological assays. Body weight was also recorded. Data were analyzed using One-way ANOVA and  $P < 0.05$  was recognized as significant. The result from this studies showed that the extract did not significantly alter ( $P > 0.05$ ) sperm count, motility, morphology as well as the haematological parameters except increase in platelet count as compared with the control group. In conclusion, 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 produce any sperm toxicity but may cause an increase in platelet activity.

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Infertility has become ominous problems, bedeviling so many marriages in Nigeria (Bodenner, 2016; Ukwubile *et al.*, 2018). Infertility is defined as the inability of becoming pregnant by the females or unable to induce pregnancy by the males, if the woman has no gynecological problems (Emokpae, 1999; Uadia and Emokpae, 2015), despite regular unprotected sexual intercourse for 1-year (Uadia and Emokpae, 2015). It also includes the inability to carry a pregnancy to the delivery of a live baby (WHO, 1992; Uadia and Emokpae, 2015). In males, the problem is as a result of defects in sperm in terms of a number of living cells, motility and testicular temperature, and this has created many problems in

some marriages (Manhal *et al.*, 2014). Haematology study has to do with the study of all the cellular elements of blood, both in health and disease condition (Ukwubile *et al.*, 2018). Blood is a tissue which consists of fluid plasma in which are suspended a number of formed elements (erythrocytes, leucocytes and thrombocytes), whose primary function is to provide a link between the various organs and cells of the body, and to maintain a constant cellular environment by circulating through every tissue, delivering nutrient to them and removing waste products (Bowman and Rand, 1980). The functions of blood are made possible by the individual and

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collective actions of its constituents; the biochemical and haematological components.

Medicinal plants have been used for thousands of years and even till today in developed as well as developing countries for the management of various ailments or diseases, and have considerably contributed to the development of pharmaceuticals since about 25% of modern drugs are derived from plants (Effiong *et al.*, 2020). This is so, since majority of the people 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). These plants have been shown to have genuine utility, and in 2001, the World Health Organization (WHO) 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). The medicinal value of medicinal plants is due to substances found in the plant tissues that produce a definite physiological action on the human body. The most important of these substances are the alkaloid, fixed oil, essential oil, tannins, resins, etc (Sharma *et al.* 2010), however, these active substances differ from plant to plant (Chevalier, 2000).

*Hunteria umbellata* (HU) is a small tree of about 15–22 m in height with a dense evergreen crown belonging to the family, Apocynaceae (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 or Abere (Yoruba) and Nkpokiri (Igbo) (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). 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 (Falodun *et al.*, 2006; Anibijuwon *et al.*, 2011).

It is important to evaluate the toxicity of medicinal plants in order to know their safety profile. Thus, the present study was designed to evaluate the safety of *Hunteria umbellata* seed extract on sperm and haematological parameters in male albino rats.

## MATERIALS AND METHODS

**Collection and Identification of Plant Material:** The ripe fruits of *Hunteria umbellata* were purchased from a local market in Benin City, Nigeria, in April 2016. The identification was done by Dr. H. A. Akinnibosun, 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 Plant Material:** The seeds of *Hunteria umbellata* were first removed from the ripe fruits and air-dried to a constant weight for three weeks. The dried material was then powdered using electronic grinder Lab. Mill (Model: Serial NO. 4745, Christy and Norris Ltd, England). The pulverized seeds were stored in air-tight plastic container for experimental analyses.

**Extraction of Plant Material:** The powdered material (800g) was transferred into a conical flask containing 3L of methanol and was allowed to soak for 72 hours. After 72 hours, the mixture was cold macerated using a sterile cotton wool. The resulting solution was filtered through a Whatman Filter Paper No. 1 using a glass funnel and the filtrate was recovered and concentrated to dryness using Water Bath at a temperature of 40°C and was preserved in a clean glass container in a refrigerator until use.

**Experimental Animals:** Forty (40) male Wister rats were used for this study. 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 shared 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 with ethical approval number of LS16074.

**Experimental Design:** From the outcome of the acute toxicity test, where no death was observed at 5000 mg/Kg, three doses of the extract 1/20<sup>th</sup>, 1/10<sup>th</sup> and 1/5

of 5000 mg/Kg (i.e. 250, 500 and 1000 mg/Kg) were selected for the test (Prasanth *et al.*, 2015).

The forty 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 oro-gastric tube for ninety (90) days.

*Samples collection, semen analyses and determination of haematological parameters:* Five rats from each group of both the experimental and control animals were sacrificed 24 hours after the last doses of the extract were administered to them by opening the abdominal cavity through a midline abdominal incision after anaesthesia by placing them in a closed jar containing cotton wool soaked with chloroform. The vas deferens of the sacrificed rats was located and sperm cells were collected from it.

*Semen Analysis:* The vas deferens was ligated with a minimum of 36mm length; both extremities of the vas deferens was ligated, cut and placed in a sterile petri dish. To the petri dish, 6 µl of normal saline already adjusted to 37±2°C was added. The Vas deferens was teased to allow the sperm cell diffuse out of it. A drop of the sperm cell from the petri dish was placed on a grease free clean slide and covered with a transparent cover slip. The slides were placed under the light microscope and viewed with x20 and x40 objective magnification lens for sperm parameters (sperm count, sperm motility and sperm pH) for both experimental and control animals and results were compared.

*Determination of Sperm Motility:* The sperm motility was evaluated with regards to three variables: progressive motile sperm (PMS), non-progressive motile sperm (NPMS) and non-motile sperm (NMS), distinguished by the movement of the sperm cells. It is usually expressed in percentile. A drop of the sperm cell was taken from the petri dish with a 1ml pipette and dispensed on cleaned slides and cover slips applied. The slides were placed on the microscope and viewed with the x20 and x40 objective magnification lens. The motility was scored in percentage according to their nature of motility as, Progressive, Non progressive and non-motile (immotile) sperm cells (Brazil *et al.*, 2004; WHO, 2010, and Ibeh *et al.*, 2018).

*Determination of Sperm Count:* The semen from each rat was diluted with a solution (1: 100) containing 5g

NaHCO<sub>3</sub>, 25 mg eosin and 1 ml 35% formalin in 100 ml distilled water. Each counting chamber of the improved Neubauer's haemocytometer was charged with 10 microlitres of diluted semen and allowed to stand for 5 minutes. The spermatozoa were counted under the light microscope at x 40 magnification. (Semen from each rat was counted twice). The count was expressed as million /ml of suspension.

*Determination of Semen pH:* The pH of semen was measured using a specially treated calibrated paper blot that changes color according to the pH of the semen that it is exposed to (WHO, 2010).

*Sperm Morphology:* The sperm cell morphology was examined by staining the slide with the Improved Eosin and Leishman stain (Ibeh *et al.*, 2017; 2018). A drop of the sperm cells was dispensed on a grease free clean slides and a smear was made. The slides were allowed to air dry and were flooded with the Improved Eosin and Leishman stain for 15 mins. The stain was rinsed and the back was blotted dry with cotton wool and left to air dry. The slides were placed in a microscope with the magnification lens at x100. The slide was viewed with at least 30 magnification fields, the normal and abnormal sperm cells were spotted and scored in percentage.

*Haematological Assays:* Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into EDTA bottles (BD Vacutainer®, BD-Plymouth, Plymouth, U.K), and the content thoroughly mixed by gentle rolling of the bottle for haematological assays. White blood cell (WBC), platelet count (PC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), monocytes (MON), lymphocytes (LYM), granulocyte (GRAN) were all analyzed by use of an automated blood analyzer (Automated sysmex KX-21 hematology analyzer, Sysmex Corporation, Kobe, Japan).

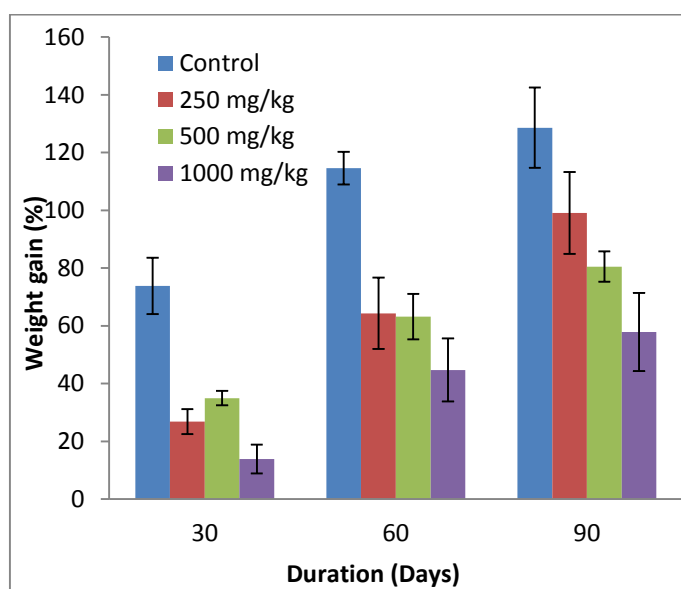
*Statistical Analysis:* Results were expressed as mean ± standard error of mean (SEM) and data comparisons between treated and control groups were made using one- way analysis of variance (ANOVA) with Tukey post hoc test (SPSS version 20). P < 0.05 indicated statistically significant difference.

## RESULTS AND DISCUSSION

Parameters such as sperm count, sperm motility and sperm morphology are key indices of male infertility (Zhou *et al.*, 2008). The quality of sperm is evaluated, by assessing the motility and morphology of sperms.

It is fundamental to evaluate the concentration and quality of sperm to assess the effects of medicinal plants on male reproductive functions. The sperm count, motility, pH and morphology were assessed in this study to evaluate the effect of prolonged administration of methanol seed extract of *H. umbellata* on sperm parameters using Wistar rats. The result revealed that there were no statistically significant differences ( $P>0.05$ ) in the total sperm count, motility, pH and morphology of rats in the test groups compared with those in the control group, following oral administration of *Hunteria umbellata* methanol seed extract (Figure 2). Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis

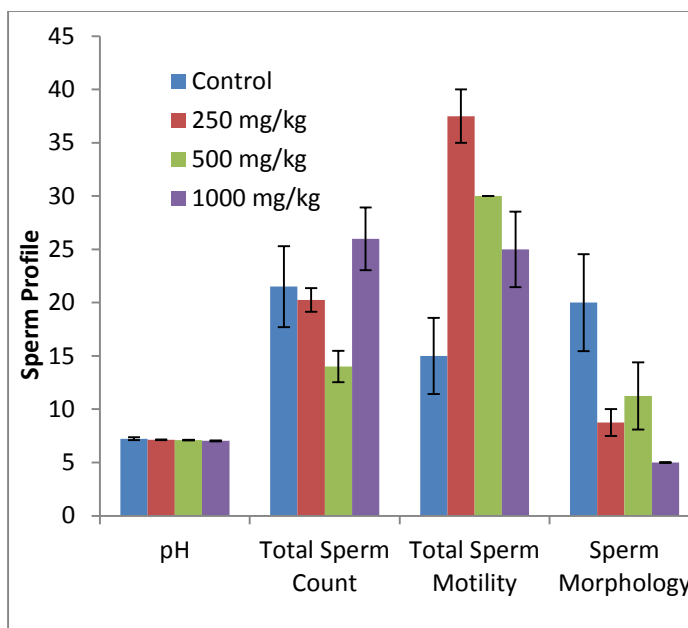
(Reddy *et al.*, 2006; Krishnamoorthy *et al.*, 2007). Sperm characteristics are important reproductive indices as they account for male fertility (Garner and Hafez, 1993). There was a reduction in the sperm count of rats treated with 500 mg/kg. However, the difference between sperm count values of both the control and experimental groups was not statistically significant. This suggests that the extracts did not affect the formation of spermatozoa. These findings were not in accordance with Mishra and Singh (2005), who reported a decrease in sperm count in male albino rats after treatment with aqueous leaf extract of *Azadirachta indica*. Similar report was also given by Krishnamoorthy *et al.* (2007) in *Terminalia chebula* extract treated rats.



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

In addition, the results showed that treatment of rats for 90 days with the extract produce non-significant increase in sperm motility. According to Bozkurt *et al.* (2006), sperm motility is one of the most important predictors of fertility. It is an important factor in the success of natural and experimental fertilization. In fertile individuals, sperm motility levels especially progressive sperms are directly related to the ability of fertilization (Zhou *et al.*, 2008). From the study, It was observed that there was increase in sperm motility by the extract at all the doses; 250, 500 and 1000 mg/kg ( $37.50\pm 2.50$ ,  $30.00\pm 0.00$  and  $25.00\pm 3.54$ ) when compared to the control ( $15.00\pm 3.56$ ), but this increase was not significant. A similar finding was reported by Heydari *et al.*, (2012) who reported that *F. parviflora* significantly increased the motility of spermatozoa in adult male rats. They administered *F.*

*parviflora* orally at doses of 750 and 1050 mg/kg for 3 days and 250 mg/kg for 5 days by gavage. Although there was no significant difference in the sperm motility of the treated groups with the control, there was an increase in this parameter in treated groups suggesting that the methanol extract of the seed of *Hunteria umbellata* could boost the metabolic activity in Sertoli cells. Sertoli cells are one of the major components of the seminiferous tubules and their number is greatly linked to total sperm production. Sertoli cells take care of germ cells during their maturity period, modulating the function of all hormone stimuli which regulate spermatogenesis process. The main energy source for motile spermatozoa is Adenosine triphosphate (ATP). This is released by the breakdown of glucose and fructose secreted by the seminal vesicles.



**Fig 2:** Effect of oral administration of methanol seed extract of *H. umbellata* on sperm profile on male albino rats for 90 days. Data are presented as mean ± S.E.M, n = 5. P>0.05: Not significantly different from control group.

**Table 1:** Mean values for Haematology test on Albino rats administered *Hunteria umbellata*

Parameters	Control Group	Group 1 (250mg/kg)	Group 2 (500mg/kg)	Group 3 (1000mg/kg)
WBC (10 <sup>3</sup> /µl)	6.76±1.40	4.02±0.64	6.66±1.39	4.72 ±0.14
LYM (10 <sup>3</sup> /µl)	69.02±11.32	80.72±4.94	82.06±2.54	81.82±10.58
MON (10 <sup>3</sup> /µl)	10.68±3.00	8.36±3.07	8.44±1.61	4.22±2.16
GRAN (10 <sup>3</sup> /µl)	20.30±8.39	10.94±1.93	9.50±1.23	14.76±9.25
HGB (g/dl)	14.02±1.19	14.82±1.02	13.06±0.42	17.08±0.52
HCT (%)	41.80±3.35	46.16±2.82	34.38±5.07	56.92±5.94
PLT (10 <sup>3</sup> /µl)	592.2±132.9	1100±146.5	644.8±92.76	882.6±92.72
MCV (µM3)	59.18±0.93	61.66±0.44	59.92±0.70	59.08±1.24
MCH (pg)	19.76±0.38	19.72±0.26	19.28±0.24	19.72±0.24
MCHC(g/dl)	33.42±0.37	32.02±0.45	32.32±0.36	33.48±0.53

Data are presented as mean ± S.E.M, n = 5. P>0.05: Not significantly different from control group. WBC, White blood Cell; LYM, Lymphocyte; MON, Monocyte; GRAN, Granulocyte; HGB, Heamoglobin; HCT, Heamatocrit; PLT, Platelet; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Heamoglobin; MCHC, Mean Corpuscular Heamoglobin Concentration.

Morphological alterations of spermatozoa along with reduction in total sperm count may affect male fertility and can cause significant reduction in fertility rate. But in this study, there was a statistically non-significant decrease in the percentage of morphologically sperm cells induced after treatment of rats for 90 days with the extract. In a similar study by Adeneye *et al.*, 2019, they reported an improved sperm morphology after repeated oral treatment with 100-400 mg/g/day of *Hunteria umbellata* for 60 days. This improvement could be due to improved steroidogenesis. The mean and standard error of mean (SEM) values of the effect of methanol extract of the seed of *Hunteria umbellata* on the haematological parameters of the male Wistar rats are given in Table 1. There was no significant difference (P>0.05) between the control group and the treatment groups for all the parameters evaluated; WBC, LYM, MON, GRAN, HGB, HCT, MCV, MCH and MCHC. However, platelets values for groups I

and II (control vs 250mg/Kg) were significantly different (P<0.05) from those of group III as well as those of group IV. These differences were such that the value for group II was higher than those of the treated groups and control group. Since there was absence of significant changes on the blood indices evaluated, it may therefore suggest that the extract is safe in the rats treated with no harmful effect on the haematological parameters. However, this observation is in contrast with the report of Longe and Momoh, (2014) who reported that rats treated for 14 days with methanolic seed extract of *Hunteria umbellata* at doses of 100-250mg/kg, produced significant elevations in RBC, PCV and Hb indicating the haematopoietic effect of *HU*. The absence of significant changes by the *Hunteria umbellata* extract, particularly on HGB values indicate that there is a balance between production and destruction of red blood cells. Hence, this will allow normal oxygen

availability for lung and tissue function, and consequently cell function. In the present study, platelet count increased at 250 mg/kg. Increase in the number of platelets is an indication of the clotting factor of the extract and this may be useful in the treatment of bleeding.

**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 haematological and semen parameters investigated at doses of 250, 500 and 1000 mg/Kg/day for 90 days. This suggests that the plant extract is not toxic on semen and haematological functions in the rats treated following repeated administration for 90 days.

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