EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 4
7-14
Year: 2024

# EFFECTS OF METHANOL STEM BARK EXTRACT OF *OCHNA SCHWEINFURTHIANA (EZEATA)* ON REPRODUCTIVE HORMONE AND SEMEN ANALYSIS IN MALE WISTAR RATS

**[1]Ifemenam K. E., [1]Akunneh Warriso C., [1]Ezeh A. K., [2]*Nwozor C. M., [1]Enwelum H. O., [3]Adinnu D. C., [4]Iwuamadi C. E.**

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Abia State University, Uturu, Abia State. Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. Anambra State. Nigeria.
[3]Department of Clinical Exercise Physiology, Faculty of Sport Health and Exercise Science, University of Hull, HU6 7RX, England, United Kingdom.
[4]Royal Bournemouth Hospital (University Hospital Dorset NHS Foundation Trust) Bournemouth, England, BH7 7DW United Kingdom.

***Corresponding Author: Dr. Nwozor C. M.**
Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. Anambra State. Nigeria.

**Article Received on 30/01/2024** **Article Revised on 20/02/2024** **Article Accepted on 12/03/2024**

## ABSTRACT
The effects of methanol extract of Ochna schweinfurthiana on reproductive hormone (testosterone), semen analysis in male wistar rats were studied. The phytochemical screening was evaluated and was found to contain glycosides, flavonoids, saponins, cardiac glycoside, steroids, trepenoids and the fractionated portion contained phytoestrogen. Twenty five (25) experimental animals were randomly grouped into five groups of 5 rats per group and were allowed to acclimatize for 2 weeks. Rats in group A served as the control and were fed with rat feed and water (normal saline), rats in group B severed as positive control received low dose Tamoxifen (20mg/kg) daily for 4 weeks, rat feed and water, rats in group C to E were fed with normal rat feed, water and were fed with different doses of Ochna schweinfurthiana extract at 200, 400 and 800mg/kg respectively for 4 weeks. The animals were sacrificed under anesthesia after the experiment and the male genitalia were surgically exposed and the cauda epididymis, left testicle were harvested and the weights were taken. Semen sample and blood were collected for semen analysis and testosterone level. The results showed that there was a significant decrease in the sperm motility of the test group $p< 0.05$, decrease in sperm count of the test group $p< 0.05$, decrease in the normal sperm morphology, and decrease in the testosterone level. Hence Ochna schweinfurthiana may be toxic to male reproductive organ.

**KEYWORDS:** Testosterone, semen analysis, ochna schweinfurthiana, male Wistar rats.

## INTRODUCTION
The director of World health Organization (WHO) on Traditional Medicine in 1993 reported that the use of plant based traditional medicine provided the primary healthcare need for 80% of human population.[1] In the present day medicine, the use of folk medicine and herbal medications is becoming popular both in the developed and developing world.[2]

World health Organization (WHO) defined infertility as a disease of the male or female reproductive system in which there is failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse.[3]

Infertility can be primary or secondary. Primary infertility is established when the couple or individual have never conceived despite cohabitation, adequate intercourse for 12 months. Secondary infertility is established when the woman has conceived previously but subsequently unable to conceive. The term subfertility which was recently introduced has been used by clinicians to define difficulty that is experienced by some couple, both of whom may have reduced fertility, jointly to conceive. This term seems more accurate and less stigmatizing for persons who experience fertility problems.[4] Infertility in male is the inability to produce sperm cells or enough sperm cell needed for fertilization of an ovum.[5]

The normal sperm count necessary to achieve fertilization is about 15 to 20 million count according to.[6] Male infertility factor may be induced by many factors which are but not limited to; diseases, environmental factor (environmental pollutant), adverse drug effect, plants or even herbal preparations. Male fertility is largely determined in spermatogenesis, the development of spermatozoa from spermatogonia in the testes which is by mitotic and meiotic divisions. This is followed by extensive morphological and biochemical differentiation, leading to a mature spermatozoon. Male infertility can be attributed to reduced sperm count, abnormal spermatozoa parameters such as total absence (azoospermia), oligospermia, teratozoospermia and asthenozoospermia. Infertility affects 15% of couples at reproductive age worldwide and those with male infertility accounts for up to half of the total cases.[7] About 20% of all infertility cases can be attributed to male factor, however, the true prevalence of male infertility is not clearly defined because of multiple factors which include: difference in the sources of data, differences in the definition of infertility and region/ population based studies.[8] Male infertility factor is usually diagnosed with abnormal semen analysis or by other sperm function defects. The age standardized prevalence of male infertility has been reported to increases by 0.3% annually.[9] Male infertility is mostly caused by problems that affect either sperm production or sperm transport through its tract. The causes of male infertility can range from: production problems which can result from undescended testis, infections (especially Mumps virus infection at younger age), torsion, varicocele, medicine and chemical, radiation and unknown causes to the blockage of sperm transport which can result from prostate related problems, vasectomy to hormone related problems which can result from pituitary tumors, congenital lack of Luteinizing hormone (LH) and Follicular Stimulating hormones (FSH). Although many couple can achieve pregnancy with Assisted Reproductive Technology (ART), investigation and evaluation of male factor is important for the most appropriate therapy to be given. Male fertility factor can be treated, medically, surgically, with the use of third party donor or even with adoption. Without the evaluation of male fertility factor, adequate management of the patient/ couple is not possible.[10]

The major challenge of infertility generally is the inability to conceive and in most developing countries especially Nigeria, the couple is often socially stigmatized.

**Ochna *schweinfurthiana***

Ochna *schweinfurthiana* (Os) belongs to the family of Ochnaceae which is a small tree that was named after Dr. Georg August Schweinfurth, a German botanical collector and taxonomist. It measures about 4 meters tall. It is commonly called Brick- red Ochna in English, 'Ezeata' by Nsukka people in Enugu located in the Eastern part of Nigeria, Jan-taru in Hausa, Hieke in Yoruba and Sa'aboule in Foufoulde.[11],[12] This plant for centuries has been used for Medicinal, agricultural, religious and social purposes.

It is an evergreen tree used in the treatment of different ailments. The leaves together with the roots of *O schweinfurthiana* have been prepared in several forms (powdered and decoctions). Chemical investigation carried out on different parts of the plant has been confined to phenolic compounds (Bioflavonoids, Glycosides, Steroids and Terpenes). Studies on the plant have shown it to be used as antimicrobial, analgesic, anti- inflammatory and anthelmintic agent.[11] In the Northern part of Cameroon, *O schweinfurthiana* is used in the treatment of many diseases such as rubella, burns, stomach ache and multiple sclerosis.[13] The root of the plant is used in the treatment of stomach ache, toothache, and headache while the leaves are used in the treatment of toothache.[12] The stem bark (pulverized) is used in the treatment of malaria, as anthelminthic, and used in wound dressing.[13] In the Northern part of Nigeria, *O schweinfurthiana* is used in the treatment of typhoid, measles, and fungal infections that are limited to the skin.[13]

The bark and flowers of O. schweinfurthiana are grown for ornamentation, dyes, stains, inks, tattoos, and mordant, among other uses in agro-horticulture. The timber is used to make equipment for forestry, farming, fishing, and hunting. The leaf has significance in society and religion, as well as in superstition and sorcery.[14]

No study has been carried out on the reproductive properties of the stem bark of Ochna *schweinfurthiana*, however, some people in the Eastern part of Nigeria have been using it as part of the ingredients in the preparation of their concoctions used in the treatment of infertility.

This study was undertaken to investigate the effects of stem bark of Ochna *schweinfurthiana* on the semen parameters and reproductive hormone (testosterone) of male Wistar rats.

## MATERIALS AND METHODS

### PLANT MATERIALS

Fresh stem bark of Ochna *schweinfurthiana* was collected from Obukpa, Nsukka Local Government Area, Enugu State Nigeria. It was authenticated by a taxonomist Mr. Ossai Ikenna Isaac at the Department of Botany, University of Nigeria Nsukka, Nigeria. A voucher number (UNH/04/0232) was deposited at the herbarium of the Department of Botany, University of Nigeria Nsukka, Nigeria. It was air dried under room temperature for 14 days and was pulverized with a mechanical grinding machine (GX160 Delmar 5.5HP).

### PHYTOCHEMICAL SCREENING

Phytochemical screening was done and reported by.[13],[15],[16]

**PREPARATION OF THE EXTRACT**

The methanol extract was done by measuring 500g of the ground Ochna Schweinfurthiana bark powder using a sensitive weighing balance (Bioevopeak, BA-T series). The measured powered sample was transferred into a 1000ml conical flask and 1000ml of 70% methanol was added to it and allowed to sit for 36 hours. The mixture was filtered using whatman filter paper number 4 and the filtrate was placed in a water bath at 100 centigrade for concentration, which was done until methanol was totally eliminated from the extract and paste was formed. The extract was stored in refrigerator between 0- 4 degree centigrade for further use.

**DRUG (TAMOXIFEN) AND TAMOXIFEN DOSE**

Tamoxifen (TAM) was purchased from Right Health Pharmacy, Awka, Anambra State, Nigeria and was used as the positive control based on its estrogen content and mechanism of action and several studies that have been done on its effect on the semen parameters. The drug was given as 5mg dissolved in 1ml of distilled water. Lower dose of TAM (20mg/kg) was used based on the study by.[17]

**PROCUREMENT OF EXPERIMENTAL ANIMALS**

Twenty-five Wistar rats were used for the study and were purchased from the Animal house of the Department of Botany Nnamdi Azikwe University, Awka, Anambra State. The animals were housed in the Animal house of Abia State University, Uturu under standard laboratory condition of 12 hours light, room temperature, 40- 60% relative humidity and they had constant access to feed (Growers feed) and water. Care and maintenance of the animals was carried out in accordance with EU directives 2010/63/EU for experimental animals. Guide for the care and use of Laboratory Animals, DHHS Publ. (NIH 86-123) was strictly followed. Ethical approval was obtained from the Animal Ethical Committee of Abia State University, Uturu. The animals were humanely handled in accordance with the Animal care and use Regulation Ethical Committee of Abia State University, Uturu, Nigeria.

**EXPERIMENTAL DESIGN**

The weights of the animals were taken at the beginning of the experiment and were between 90g to 110g. The experimental animals were randomly grouped into five groups of 5 rats per group and were allowed to acclimatize for 2 weeks. Rats in group A served as the control and were fed with rat feed and water, rats in group B severed as positive control and received low dose Tamoxifen (20mg/kg)[17] daily for 4 weeks, plus rat feed and water. Rats in group C to E were fed with normal rat feed, water and were fed with different doses of Ochna schweinfurthiana extract at 200, 400 and 800mg/kg respectively for 4 weeks.

**ACUTE TOXICITY STUDIES**

Acute toxicity analysis of the extract was carried out using Lorke's method as described by.[18] Intraperitoneal administration of the methanol leaf extract of the plant produced an LD50 774.6mg/kg, while the oral LD50 was about 5000mg/kg, hence they classified Ochna *schweinfurthiana* to be peritoneally toxic and orally safe.[16]

**SAMPLE COLLECTION AND ANALYSIS**

The rats were dosed for 4 weeks with the extracts and were humanely scarified under anesthesia (ketamine IM) at the end of the experiment and samples were collected.

**SEMEN ANALYSIS**

**Sperm count**

The male genitalia were surgically exposed and the cauda epididymis and left testicle were harvested and the weight of cauda epidymis was macerated in normal saline in the ratio of 1: 10 weight by volume and was diluted with normal saline and smeared on a glass slide for mortality and a portion was dipped into formal saline and Nauber counting chamber and a counting device was used for the sperm count and expressed in million/ml of suspension.[19]

**Sperm Morphology**

The stain on the slide was air dried and thereafter stained and viewed on the microscope for morphology. A portion of the sperm harvested from the cauda epididymis was stained with Eosin- Nigrosine on a glass slide and air- dried. The slides were examined through the aid of a digital microscope under the magnification of times 40 lens and the percentage of sperm cells was determined by the number of sperm cells that picked the stain. The percentage motility was taken and abnormalities were observed on each slide. The sperm motility, sperm concentration, viability and morphology were all determined.[20] Semen analysis remains the main method for the evaluation of male infertility although other advanced diagnostic tests have been developed to investigate sperm quality and function.[21] The semen qualities were assessed by examining sperm count, motility, morphology and vitality.

The sperm motility, sperm concentration, viability and morphology were all determined.[20]

**BLOOD SAMPLE**

Blood sample was collected by ocular puncture into a plain bottle and spanned in a centrifuge at 4000 RPM for 15 minutes and the serum was collected and sent to the lab for testosterone level.

**DETERMINATION OF SERUM TESTOSTERONE IN RAT**

Serum testosterone (Pg) was determined according to the method described by.[22]

**STATISTICAL ANALYSIS**

The data gathered from the study were analyzed using statistical package for social sciences (SPSS-24). Results were presented as mean ± Standard error of mean (SEM)

of sample replicates. P<0.05 was be considered to be statistically significant.

## RESULTS

### Phytochemical Analysis

The phytochemical analysis according to[13] contained flavonoids, steroid/ terpenes and saponin in the leaf extract of Ochna *schweinfurthiana.* A study done by[23] showed that the stem bark extract of Ochna *schweinfurthiana* contained: glycosides, flavonoids, saponins, cardiac glycoside, steroids, trepenoids and the fractionated portion contained phytoestrogen. This is represented in table 4.1:

**Table 1: Phytochemical analyses of extract of Ochna schweinfurthiana stem bark.**

| Phytochemical | Result |
|---|---|
| Alkaloids | - |
| Glycosides | + |
| Flavonoids | + |
| Saponins | + |
| Cardiac glycoside | + |
| Steroids | + |
| Trepenoids | + |
| Tannins | - |
| Key: Present + and Absent - | |

**Table 2: Effect of Ochna sweinfurthiana. on Testis and Body weight (grams).**

| Group(s) | Testis (g) | Body weight (g) |
|---|---|---|
| Group 1 | 2.00 | 151 |
| | 2.00 | 150 |
| | 1.90 | 149 |
| | 2.10 | 151 |
| | 2.00 | 150 |
| Group 2 (TAM) | 2.04 | 139 |
| | 2.51 | 130 |
| | 2.35 | 105 |
| | 2.45 | 136 |
| | 2.25 | 110 |
| Group 3 (Low Dose) | 2.43*** | 143 |
| | 2.31*** | 153 |
| | 2.40*** | 141 |
| | 2.32*** | 140 |
| | 2.39*** | 150 |
| Group 4 (Medium Dose) | 1.47 | 141 |
| | 1.93 | 147 |
| | 0.71 | 70 |
| | 1.50 | 145 |
| | 1.89 | 147 |
| Group 5 (High Dose) | 2.23 | 121 |
| | 1.79 | 124 |
| | 2.05 | 103 |
| | 2.21 | 120 |
| | 2.00 | 112 |

***Statistically significant, TAM (Tamoxifen)

Table 2 above shows the weight of testis. There was statistical difference between the control group and group 3 (low dose group) p is less than 0.05.

### SEMEN ANALYSIS

### PERCENTAGE MOTILITY

There was significant decrease in sperm motility of Group 2 to Group 5 when compared with the control group (P< 0.05). The mean for the control rat was 92 ± 1.02, the mean for the Group 2 (TAM fed rats) was 88 ± 1.05, the mean value for Group 3 (Low dose) was 72.2 ±2.28, the mean value for Group 4 (Medium Dose) was 65.4 ±2.04 and the mean value for Group 5 (High dose) was 78 ±0.95.

**Table 3: Effect of Ochna schweinfurthiana. on Actively Motile and Non Motile Sperm.**

| Group | Actively Motile Sperm (%) | Non Motile sperm (%) |
|---|---|---|
| Control Group | 90 | 10 |
| | 95 | 5 |
| | 92 | 8 |
| | 90 | 10 |
| | 94 | 6 |
| Group2 (TAM Fed Group)*** | 85 | 15 |
| | 90 | 10 |
| | 90 | 10 |
| | 86 | 14 |
| | 89 | 11 |
| Group 3 (Low Dose Group*** | 75 | 25 |
| | 70 | 30 |
| | 72 | 28 |
| | 74 | 26 |
| | 70 | 30 |
| Group 4 (Medium Dose)*** | 60 | 40 |
| | 70 | 30 |
| | 65 | 35 |
| | 62 | 38 |
| | 70 | 30 |
| Group (High Dose) 5*** | 80 | 20 |
| | 75 | 25 |
| | 80 | 20 |
| | 77 | 23 |
| | 78 | 22 |

***Statistically significant, TAM (Tamoxifen)

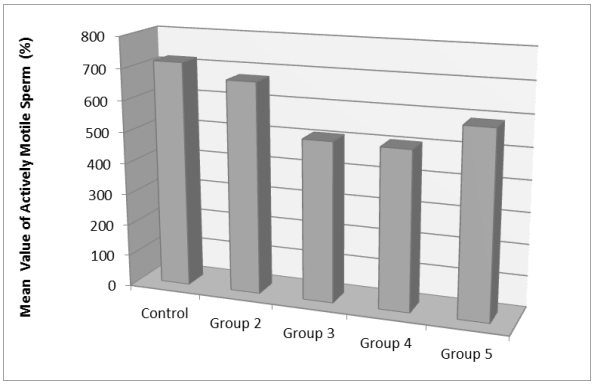


**Fig. 1: Actively Motile Sperm.**

**SPERM COUNT**

There was significant decrease in sperm count of Group 2 to Group 5 when compared with the control group P< 0.05. The mean for the control rat was 719 ± 3.32, the mean for the Group 2 (TAM fed rats) was 673.8 ± 5.14, the mean value for Group 3 (Low dose) was 510 ±1.81, the mean value for Group 4 (Medium Dose) was 506.2 ±4.42 and the mean Value for Group 5 (High dose) was 589.8 ±2.85.

**Table 4: Effect of Ochna schweinfurthiana. on Sperm count (106/ML).**

| Group | Sperm count ($10^6$/ML) |
|---|---|
| Control | 720 |
| | 730 |
| | 720 |
| | 710 |
| | 715 |
| Group 2 (Tamoxifen fed Group)*** | 690 |
| | 660 |
| | 670 |
| | 680 |
| | 669 |
| Group 3 (Low Dose Group) *** | 515 |
| | 506 |
| | 507 |
| | 514 |
| | 510 |
| Group 4 (Medium Dose Group)*** | 500 |
| | 518 |
| | 495 |
| | 515 |
| | 503 |
| Group 5 (High Dose Group)*** | 582 |
| | 590 |
| | 597 |
| | 585 |
| | 595 |

***Statistically significant, TAM (Tamoxifen)

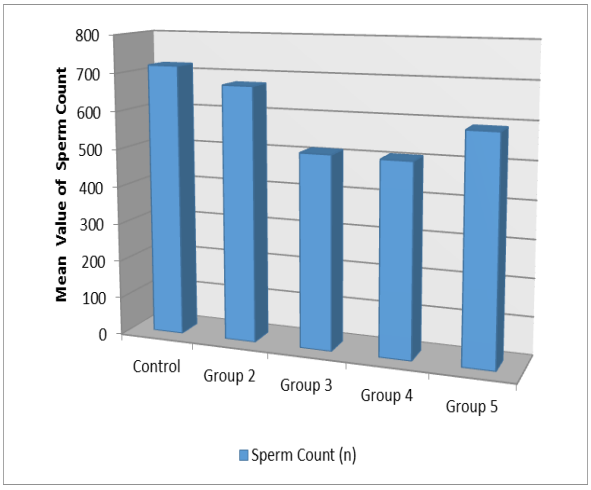


**Fig. 2: Sperm Count.**

**SPERM MORPHOLOGY**

The morphology of Group 3 (low dose) and Group 4 (medium Dose) were significantly decreased when compared with the control group P< 0.05. The mean of the control group was 83.20 ± 0.97, the mean for Group 2 (Tamoxifen Group) was 85.4± 4.06, the mean for Group 3 (low dose group) was 72.6± 0.93, the mean for Group 4 (Medium dose group) was 60.00± 2.02 and the mean for Group 5 (High Dose Group) was 73.00± 3.17.

**Table 5: Effect of Ochna sweinfurthiana. on Sperm Morphology.**

| Group | Normal Sperm cells (%) | Abnormal Sperm Cells (%) |
|---|---|---|
| Control | 85 | 15 |
| | 80 | 20 |
| | 82 | 18 |
| | 85 | 15 |
| | 84 | 16 |
| Group 2 (TAM Group) | 75 | 25 |
| | 92 | 08 |
| | 93 | 07 |
| | 76 | 24 |
| | 91 | 09 |
| Group 3 (Low Dose)*** | 70 | 30 |
| | 75 | 25 |
| | 71 | 29 |
| | 74 | 26 |
| | 73 | 27 |
| Group 4 (Medium Dose)*** | 55 | 45 |
| | 65 | 35 |
| | 60 | 40 |
| | 56 | 44 |
| | 64 | 36 |
| Group 5 (High Dose) | 80 | 20 |
| | 65 | 35 |
| | 75 | 25 |
| | 79 | 21 |
| | 66 | 34 |

***Statistically significant, TAM (Tamoxifen)

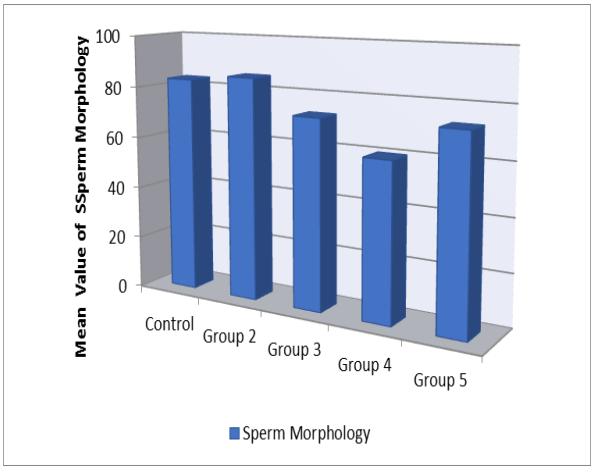


**Fig.3 sperm morphology.**

**TESTOSTERONE RESULT**

There was significant decrease in the testosterone level of groups fed with Tamoxifen and extract of Ochna S.( $P < 0.05$).

**Table 6: Effect of Ochna sweinfurthiana. on Testosterone Level Tg(ng/ml).**

| Group | Testosterone Tg(ng/ml) | Mean |
|---|---|---|
| Control (Group 1) | 12 | 10.92 |
| | 10 | |
| | 11 | |
| | 9.6 | |
| | 12 | |
| Group 2 (TAM) | 8.8 | 8.20 |
| | 7.8 | |
| | 7.9 | |
| | 7.9 | |
| | 8.6 | |
| Group 3 (Low Dose) | 2.1 | 2.4 |
| | 2.8 | |
| | 2.5 | |
| | 2.7 | |
| | 2.2 | |
| Group 4 (Medium Dose) | 4.8 | 5.22 |
| | 5.4 | |
| | 5.6 | |
| | 5.0 | |
| | 5.3 | |
| Group 5 (High Dose) | 2.1 | 2.54 |
| | 2.8 | |
| | 2.7 | |
| | 2.5 | |
| | 2.6 | |

***Statistically significant, TAM (Tamoxifen)

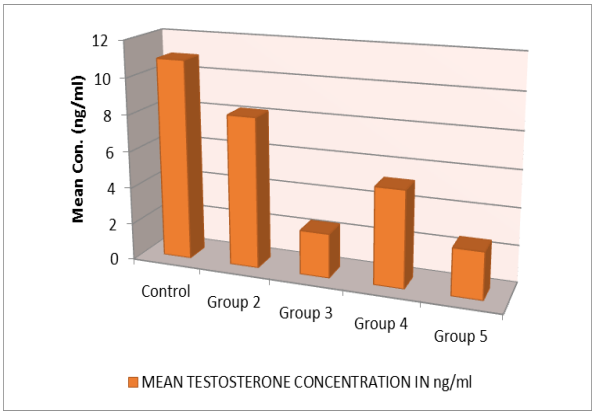


**Fig. 4: Testosterone Level.**

**DISCUSSION**

Stem bark extract of *Ochna. schweinfurthiana* caused a decrease in the reproductive parameters of male albino rats. Results obtained from the study revealed that there was significant decrease in the level of testosterone, and the organ weight of the testis (group 3 only) were significantly reduced in the groups fed with the extract of stem bark of Ochna *schweinfurthiana.* This effect may be attributed to some of the phytochemical constituents it contained, notably phytoestrogen. It has been revealed in the study done by.[23] That fractionated stem bark of Ochna *schweinfurthiana* contained phytoestrogen and the reduction in the testosterone level and semen analysis can be attributed to the estrogen content. This goes further to prove the study by.[17] That administration of TAM with increasing doses for a long period leads to weak sperm and also reduces sperm count and that this might lead to infertility.

The testis produces estradiol, through the enzyme aromatase, and acts locally through selectively expressed estradiol receptors (ER), (α and (β) in various testicular cells. It plays several important roles in male reproduction, such as differentiation of germ and somatic cells, spermiogensis, spermiation, maturation, transport and motility of spermatozoa, secretary activity of efferent tubules, scrotal testicular descent and long term fertility.[24]

Tamoxifen increases LH and FSH in the cases of lack of testosterone in the body and hence helps in spermatogenesis. LH stimulates Sertoli cells in the testicles to produce testosterone. Drugs with estrogenic effect work to improve the epididymis work on the maturity of sperm and not on the synthesis of sperm cells because it lacks effect inside the testicle and also works to inhibit the pituitary gland - adrenal gland axis (HPA axis) and hence increases LH and FSH to produce testosterone. However, increase in dose leads to a decrease in the production of LH due to estrogen receptors on the testicle mostly in the Leydig cells, which reduces spermatocytes count thus decreasing the chances and potential of fertility.[17][25][26] The administration of Ochna *schweinfurthiana* extract has been shown to decrease semen activity; the sperm motility and count were progressively decreasing. The tamoxifen group showed a slight decrease compared to the rats fed with extracts. This may suggest that the phytoestrogen content of Ochna *schweinfurthiana* might be the reason the semen activity was progressively declining with increase in dose of the Ochna *schweinfurthiana* extract.

*O schweinfurthiana* has been reported to be used in the induction of labor and as well to speed up prolonged labor and for evacuation of retained products of conception after a miscarriage[27] and most recently, a study by Agbason, 2023 revealed it was used in the treatment of post-menopausal osteoporosis however the doses of Ochna *schweinfurthiana* should be to the barest minimum as this study revealed a decline in testicular activity with increasing dose which can be attributed to its phytoestrogen content.

**CONCLUSION**

The findings from this research revealed the adverse effect of Ochna *schweinfurthiana* on the testosterone level and decrease in the sperm activity, motility and

production most likely due to its phytoestrogen content in Wistar rats. Considering the beneficial effects when used in the treatment of malaria, anthelminthic, and used in wound dressing and many others, (Abdullahi et al, 2010), it should be used with caution and hence the use of the stem bark of Ochna *schweinfurthiana* in the treatment of various ailment should be in moderation and based on this study, it should not be used on a long term basis in the treatment of other illness as it might pose a potential harm to the reproductive organ of the patient.

Further studies should be carried out to ascertain the dose which will not be deleterious to the testosterone and sperm quality when using it for treatment of other illness. Also further studies should be carried out on the effect on other organs like the liver, kidney etc.

**REFERENCES**


1. Elujoba, A. A., Odeleye, O. M. and Ogunyemi, C. M. Traditional medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 2005; 2(1): 46-61.
2. Haq, I., Safety of medicinal plants. *Pak J Med Res.*, 2004; 43(4): 203-10.
3. World health organization (WHO) (2018). International classification of Disease, 11th Revision (ICD- 11) Geneva: Classifying disease to map the way we live. *World Health Organization.* Who.int/news-room/spotlight/international-classification-of- diseases.
4. Baylis F. Infertility. *Encyclopedia of Applied Ethics.* Second Edition, 2012; 2: 712-720.
5. Gadea J, Sperm factor related to in vitro and in vivo porcine fertility. *Theriogenol*, 2005; 63(2): 431-444.
6. World Health Organization (WHO) (2015). World Health Statistics reports on global health goals for 194 countries. Https: //reliefweb.int/organization/who.
7. Agarwal A, Baskaran S, Parekh N, Cho C.L, Henkel R, Vij S, Arafa, m, Selvam M.K.P, Shah R. Male infertility. *Lancet*, 2021; 397: 319-333.
8. Barratt CLR, Björndahl L, De Jonge C.J., Lamb D.J, Osorio M.F, McLachlan R, Oates R.D., Van Der Poel .S., St John. B., Sigman. M., Sokol. R., Tournaye. H. The diagnosis of male infertility: An analysis of the evidence to support the development of global who guidance-challenges and future research opportunities. *Hum Reprod*, 2017; 23: 660.
9. Sun, H., Gong, T.-T, Jiang, Y.-T, Zhang, S, Zhao, Y.-H, and Wu, Q.-J. (2019) Global, regional and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990–2017: Results from a global burden of disease study, 2017; *Aging*. 11: 10952.
10. Schlegel P.N, Sigman M, Collura B, De Jonge C.J, Eisenberg M.L, Lamb D.J, Mulhall J.P, Niederberger C, Sandlow J.I, Sokol R.Z, Spandorfer S.D, Tanrikut C, Treadwell J.R, Oristaglio J.T, Zini A Diagnosis and Treatment of Infertility in men: AUA/ ASRM Guideline. *American society of reproductive medicine*, 2020; 1- 53.
11. Burkill MH. The useful plants of west Tropical Africa, Families J–L. *Royal Botanic Gardens Kew.*, 1985; 4: 275.
12. Messi A.N, Mbing J.N, Ndongo J.T, Nyegue M. A, Tchinda A.T, Yemeda M.F, Pegnyemb D.E. Phenolic compounds from the roots of Ochna *schweinfurthiana* and their antioxidant and antiplasmodial activities. *Phytochemistry Lett.*, 2016; 17: 119- 125.
13. Abdullahi M.I, Iliya I, Haruna A.K, Sule, M.I, Musa A.M, Abdullahi S.M. Preliminary phytochemical and antimicrobial investigations of leaf extracts of Ochna schweinfurthiana. *African J Pharm Pharmacol*, 2010; 4(2): 083-086.
14. Yusuf, A.J, Abdullahi, M.I and Muhammad, A.A. Review article on Ethnobotany, phytochemistry and pharmacology of Ochna *schweinfurthiana*: A review. *Universal journal of Pharmaceutical Research*, 2019; 4(6): 75-79.
15. Danmusa, U.M, Nasir, I.A., Abdullahi, M.I., Ahmad, A.A., Abdulkadir, I.S. Phytochemical analysis and antimicrobial activities of mMethanol stem extracts of *Ochna schweinfurthiana* F. Hoffm. *Journal of Pharmacy and Pharmacognosy*, 2015; 3(6): 171–182. Available online at http://jppres.com/jppres.
16. Ibrahim Z.Y.Y, Abdullahi, M.I, Musa A.M, Maje I.M, yusuf A.J, Aliyu I.M. Acute toxicity profile and Plasmodium berghei inhibitory activity of Ochna *schweinfurthiana* (F. Hoffm) Ochnaceae leaf extract in Laboratory animals. *Int j Pharma Sci res.*, 2015; 6(10): 1302-1306.
17. Amal K., Abbas, Aqeel H, and Qasim. Effect of tamoxifen drug on testes of albino male rats. Medico-legal updates://doi.org/10.37506/mlu.v20i4.1891, 2020; 20(4): 637-642.
18. Agyigra A.I., Ejiofor J.I., Magaji M.G., and Yakubu Y. Evaluation of methanol stem-bark extract of Ximenia Americana Linn (Olacaceae) for phytoconstituents and gastroprotection in rats. African Journal of Pharmacology and Therapeutics, 2017; 6(4): 161-165.
19. Ekaluo, U. B., Udokpoh, A. E., Ikpeme E. V. and Peter, E. U. Effect of chloroquine treatment on sperm count and weight of testes in male rats. *Global Journal of Pure and Applied Sciences*, 2008; 14: 175-177.
20. Lannuzel, A., Michel, P.P., Hoglinger, G.U., Champy, P., Jousset, A., Medja, Lombes A, Darios F, Gleye C, Laurens A, Hocquemiller R, Hirsch E.C, and Ruberg. M. The mitochondrial complex 1 inhibitor annonacin is toxic to mesencephalic dopaminergic neurons by impairment of energy metabolism. *Neurosci*, 2003; 121: 287–296.
21. Ashidi J.S, Owagboriaye O.F, Yaya B.F, Payne E.D, Lawal I. O, Owa O. S. Assessment of reproductive

function in male albino rats fed dietary meal supplemented with Mucuna Prurien seed powder. *Elsevier*, 2019; 5: e02716.

22. Lashansky, G. Saenger, P., Fishman, K., Gautier, T., Mayes, D., Berg G., Di Martino, j., Reiter, E. Normative data for adrenal steridogensis in a healthy pediatric population: age and sex related changes after adrenocorticotropin stimulation. *J clin Endocrinol Metab*, 1991; 73(3): 674-686.
23. Agbason. J. Macroscopic analysis and combination effects of Phytoestrogen and phenol- Rich fractions of Ochna Schweinfurthiana Stem Bark on animal model of Osteoporosis. *COOU library Igbariam Campus*, 2023; 15-40. Unpublished B.Sc. Thesis.
24. Carreau, S., & Hess, R. A. Oestrogens and spermatogenesis. Philosophical Transactions of the Royal Society B: *Biological Sciences*, 2010; 365(1546): 1517-1535.
25. Kotoulas, I. G., Cardamakis, E., Michopoulos, J., Mitropoulos, D., and Dounis, A. Tamoxifen treatment in male infertility. I. Effect on spermatozoa. *Fertility and sterility*, 1994; 61(5): 911-914.
26. Nussey, S., and Whitehead, S. (2001). Endocrinology: An integrated approach. *BIOS Scientific Publishers.* BIOS Scientific Publishers limited, Oxford. https://doi.org/10.4324/9780203450437
27. Bruschi P, Morganti M, Mancini M, Signorini M.A. Traditional healers and laypeople: A qualitative and quantitative approach to local Knowledge on medicinal plants in Muda (Mozambique). *J Ethnopharm*, 2011; 138: 543- 563.