

# Fertility Enhancing Effect of *Diopyros mespiliformis* (African Ebony) Stem Bark on Testes of Wistar Albino Rats: A hormonal study

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## Abstract

*Diospyros mespiliformis* (African ebony) is a plant that is highly recognized all over African and Asian countries, for its medicinal and curative properties. This study investigates the effects of *Diospyros mespiliformis* aqueous stem bark extract on the testis, epididymal weight and hormonal assay in Wistar albino rats at different doses. Thirty Adult Wistar rats were assigned into five groups of six rats each. With group I serving as control, while group (II, III, IV and V) were treated with 25mg/kg, 50mg/kg 100mg/kg and 100mg/kg (post treatment group) of the extract within the experimental period of twenty eight days. Twenty four hours after the last oral administration, the rats in groups (I- IV) were euthanized for tissue harvesting. The rats in group V were allowed to remain for fourteen days post-treatment and they were euthanized on day fifteen. Testes, epididymis tissues were harvested and weighed, blood samples were collected for hormonal analysis. Investigations revealed an increased in testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) serum level in groups (II, III and IV) when compared to groups (I and V). The testis and epididymis weight increased in groups (III and IV) when compared to the groups (I, II and V). The results of the study revealed that aqueous stem bark extract of *Diospyros mespiliformis* have fertility enhancing potentials on the testis, epididymis and hormonal serum level in Wistar albino rats.

**Keywords:** *Diospyros mespiliformis*, Epididymis, Fertility enhancers, Hormone, Weight.

## INTRODUCTION

*Diospyros mespiliformis* (African ebony) is a large deciduous tree found mainly in the Tropical and Sub-Saharan Africa (Shagal *et al.*, 2012). *Diospyros mespiliformis* belonging to the family Ebenacea in the order ebenales. It has a wide range of Ethno-medicinal uses which have not been scientifically evaluated (Belemtougri *et al.*, 2006). *Diospyros mespiliformis* has been used in traditional medical systems including Ayurveda, in China and African (Mallavadhni *et al.*, 1998). *Diopyros mespiliformis* has a large range of medicinal uses, it serves as a traditional food plant in Africa, it's fruit has potential to improve nutrition (Etkin, 1997). The leaves of *Diopyros mespiliformis* are used for treatment of skin infection, arthritis and headache. The

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leaves and fruits are chewed or applied as infusion for treating gingivitis, toothache, and for wound dressing to prevent infection (Burkill, 1995). Root infusion of *Diopyros mespiliformis* is used to treat stomach ache (Ruffo *et al.*, 2002). The bark and roots are used for treating infections such as malaria, pneumonia, syphilis, leprosy, dermatomycoses, as an anthelmintic and to facilitate child birth, also used as psycho-pharmacological drug and to treat tumor (Orwa *et al.*, 2009). The plant was also reported to have potent antibacterial and anti-trypanosomal activities (Freiburghaus *et al.*, 1998). Shagal *et al.*, (2012) reported the antimicrobial action of aqueous and ethanol extracts of leaves, stem bark and root of *Diopyros mespiliformis*, the function of extracts was tested against clinical evaluation of *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus spp*, *Shigella spp* and *Klebsiella pneumonia*. The LD50 of *Diopyros mespiliformis*, aqueous stem bark extract is above 5000mg/kg and doses ranging from 25mg/kg to 500mg/kg had been used in clinical trials (Orisakwe *et al.*, 2003). Herbals medication are among the methods used to treat male infertility, medicinal plant plays a major role in the treatment of erectile dysfunction, libido and sperm disorders (Nantia *et al.*, 2009). Variety of plants are claimed to be used as male fertility enhancers in traditional medicine, some of these plants have been evaluated in animal and human studies, some of these plants include: *Cissuspopulneas* (L.) *Tribulus terrestris* (L.) *Asparagus recemosus* Willd, *Gingko biloba* (L.) *Cochlospermum planchonii*, *Withaniasomnifera* (L.) *Dunal*, *Mucunaprureins* (L.) DC, *Lophiralanceolata van* and *Sesamum radiatum* (Moundjpa *et al.*, 2005; Belemtougri *et al.*, 2006). Hormone are chemical messengers that are secreted directly into the blood, which carries them to organs and tissue of the body to exert their functions. Physiologically, luteinizing hormone (LH) and follicles stimulating hormone (FSH) hormones are secreted in the anterior pituitary gland via gonadotropin releasing hormone (GnRH), secreted by the hypothalamus and circulate throughout the blood in the body to arrive and connect the specific receptors in Leydig cells and Sertoli in testicular tissue through increased production of secondary messenger, cyclic adenosine monophosphate (cAMP) stimulating cell activity (Gonzales *et al.*, 2001). It was reported that gonadotropins (LH, FSH) act on testis leading it to secrete testosterone. LH stimulates the Leydig cells to secrete testosterone, whereas FSH levels largely induce sertoli cells to regulate spermatogenesis. Testosterone is also important in the development of the male sexual characteristics (Al-sa'aidi *et al.*, 2009). The present study was designed to correlate recognized hormone and histological changes in the testis, following twenty eight days administration of *Diopyros mespiliformis* extract to Wistar albino rats.

## **MATERIALS AND METHODS**

### **Procurement of Plant.**

The stem bark of *Diopyros mespiliformis* was obtained from Madzi village in Michika Local Government Area of Adamawa State, Northern Nigeria. It was authenticated by a taxonomist in the Department of Biological Sciences University of Maiduguri, Nigeria.

### **Preparation of plant extract**

The stem bark of *Diopyros mespiliformis* was carefully removed, cleaned, air dried in shade for two weeks and then crushed into fine powder with a pestle and mortar. The powder sample of (800g) was macerated with 1 litre of distilled water for 24 hours with occasional mixing. The mixture was filtered using a filter paper and the filtrate was evaporated in an oven at 50°C.

### **Experimental Design**

Twenty-five adult wistar rats were allocated into five groups (I, II, III, IV and V) of five rats each, after which they were acclimatized for two weeks duration, they were fed with feed and water *ad libitum*. Group I served as the control group and was administered with feed and distilled water for 28 days.

Group II was given 25mg/kg of aqueous stem bark extract of *Diopyros mespiliformis* for 28 days.

Group III was given 50mg/kg of aqueous stem bark extract of *Diopyros mespiliformis* for 28 days.

Group IV was given 100mg/kg of aqueous stem bark extract of *Diopyros mespiliformis* for 28 days. The rats in groups (I, II, III and IV) was euthanized on the 29<sup>th</sup> day, after treatment. Testis and epididymis were harvested and weighed. Group V was also given 100mg/kg of aqueous stem bark extract of *Diopyros mespiliformis* for 28 days, and the extract was withdrawn, the rats was allowed to remain for 14 days, post treatment, and serve as the post treatment group. The rats in group V were euthanized on day 15<sup>th</sup> after treatment (Yiman *et al.*, 2018).

### **Testicular Measurement**

Testis length was measured vertically between the upper and the lower poles of the testis, using a digital vernier caliper, testis width was measured from the anterior to the posterior in the middle of the testis, using a digital vernier caliper, while the testis diameter, the maximum diameter of each testis was measured in the anterior to the posterior plane using a digital vernier caliper as method adopted by Thompson *et al.*, 2010.

### **Testis and Epididymis Weight**

Testis and epididymis were dissected out, blotted free of blood and weighed with a Sartorius digital balance.

### **Hormonal Analysis**

The rats was sedated using cotton wool soaked in chloroform, and blood sample was taken after sacrifice. The blood samples was centrifuged and the blood serum was taken to the Chemical Pathology Laboratory for hormonal assay. The Enzyme Linked Fluorescent Assay (ELFA) technique was used to measure LH, FSH and Testosterone levels in serum or plasma using VIDAS (BIOMERIEUX, France).

### **Assay Procedure (Technique)**

Step 1. The desired number of coated wells was secured in the holder.

Step 2. 100 µl of standards, specimens was dispensed and controls into appropriate wells.

Step 3. 100 µl of Enzymes Conjugate was dispensed into each well. Shake for 30 seconds. It is very important to shake the plate at this step.

Step 4. Incubate at 37 °C for 3 hours.

Step 5. The incubation mixture was removed by dumping plate contents into a waste container.

Step 6. The microtiter wells was rinsed five (5) times with diluted wash buffer.

Step 7. Paper towels was used to remove all residual water droplets.

Step 8. 100 µl of TMB solutions was dispensed into each well and gently mixed for 10 seconds.

Step 9. It was then incubated at room temperature for 20 minutes, in the dark.

Step 10. The reaction was stopped by adding 50 µl of 2 N HCl to each well.

Step 11. It was then mixed gently for 30 seconds to observe a colour change from blue to yellow.

Step 12. Optical density was read at 450 nm with a microtiter well reader (Mclachlan *et al.*, 1996).

**Statistical analysis**

The data was analyzed and compared by one way analysis of variance (ANOVA) by using Graph-Pad Prism Version 3.0 for Windows (Graph pad software, San Diego, California). Level of significance has been considered at the level of  $P < 0.05$ .

**RESULTS**

The results of the testis length, width and diameter were presented on (Table 1) analysis of testicular length showed there was significant ( $P < 0.05$ ) increase in the length of the testis in groups (III and IV) when compared to groups (I,II and V). Testis width showed there was significant ( $P < 0.05$ ) increase in the testis width in groups (III and IV) when compared to groups (I,II and V). Testis diameter showed there was significant ( $P < 0.05$ ) increase in the testis diameter in groups (II, III and IV) when compared to groups (I and V).

The results of the testis and epididymis weight were showed on (Table 2), the epididymis weight showed that, there was significant ( $p < 0.05$ ) increase in groups (II, III and IV) when compared to groups (I and V). Testis weight showed there was significant ( $p < 0.05$ ) increase in groups (II,III and IV) when compared to groups (I and V).

The results of hormonal assay was presented on (Table 3) showed that there was significant ( $P < 0.05$ ) increase in the testosterone serum level in groups (II, III and IV) when compared groups (I and V). Follicles stimulating hormone (FSH) showed that there was significant ( $p < 0.05$ ) increase in the FSH serum levels in groups (II, III and IV) when compared to groups (I and V). Luteinizing hormone (LH) showed that there was significant ( $p < 0.05$ ) increased in the LH serum level in group (II, III and IV) when compared to group (I and V).

Table 1: Showing the effect of *Diopyros mespiliformis* aqueous stem bark extract on Testis length, width and diameter.

Dose (mgkg <sup>-1</sup> )	Testis length (mm)	Testis width (mm)	Testis Diameter (mm)
0 (control)	1.83 ± 0.04	0.82 ± 0.03	1.22 ± 0.03
25	1.86 ± 0.05	0.84 ± 0.02	1.44 ± 0.06
50	1.99 ± 0.06*	0.91 ± 0.03*	1.51 ± 0.08*
100	2.08 ± 0.10*	0.93 ± 0.02*	1.93 ± 0.10*
100	1.87 ± 0.06	0.84 ± 0.02	1.94 ± 0.02

Table 2: Showing the effect of *Diopyros mespiliformis* aqueous stem bark extract on testis and

	0(control)	25mg/kg	50mg/kg	100mg/kg	100mg/kg
Weight of Epididymis (mm)	0.37±0.03	0.38±0.03*	0.42±0.03*	0.44±0.03*	0.40±0.02
Weight of testis (mm) epididymis weight.	1.34±0.17	1.39±0.11*	1.42±0.22*	1.51±0.17*	1.35±0.06

Table 3: Showing the effect of *Diopyros mespiliformis* aqueous stem bark extract on serum, Testosterone (TH), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone. (LH), levels.

	0 (Control)	25mg/kg	50mg/kg	100mg/kg	100mg/kg
TH (ng/ml)	5.36 ± 2.81	12.8± 3.47*	9.34 ± 3.60*	19.7± 21.8*	6.46±2.60
FSH (ng/ml)	0.08 ± 0.01	0.11 ± 0.01*	0.12 ± 0.01*	0.13 ± 0.01*	0.09±0.01
LH (ng/ml)	0.09 ± 0.01	0.11 ± 0.01*	0.11 ± 0.01*	0.13 ± 0.01*	0.10±0.01

Values presented as Mean ± SE, \* indicates significant difference with the control at P<0.05, SE= standard error of the mean, (n=5).

## DISCUSSION

Male infertility is a global health and social problems that causes stigma and marital disharmony (Okonofua *et al.*, 1996). Sequel to that, it has become of enormous importance to search for botanicals with proven track records of fertility enhancing potentials in order to explore their efficacy and levels of safety in order to counteract the menace, more than 50-80 millions couples are affected by infertility world-wide with 20-35 millions couples in Africa and 3-4 millions couples in Nigeria (WHO, 2010). The increase trend of male infertility and the need to search for affordable and available natural food or plant substances that enhances fertility by increasing hormonal serum level.

The study showed that the aqueous stem bark extract of *Diopyros mespiliformis* increased the testes length and width was significantly (P < 0.05) increased in groups III and IV when compared to groups I, II and V while the testicular diameter was significantly (P < 0.05) increased in size in groups II, III and IV when compared to groups I and V. Normal productions of thyroid hormones is said to directly increased testes weight and sperm production (Holberger, 2005).

The weight of the epididymis and testis were significantly (p < 0.05) increased in groups II, III and IV when compared to group I and V. It is a known fact that increased in epididymis weight is usually associated with increased in spermatogenesis (Martino- Andrade *et al.*, 2010). Several researchers have conducted studies on the administration of oral aqueous extract of *Garcinia kola*, and reported that this extract increases in testes weight of treated rats (Sharpe *et al.*, 2005).

The aqueous stem bark extract of *Diopyros mespiliformis* significantly increased serum gonadotropin hormone (LH, FSH) and testosterone serum in treated groups as compared to control groups. The testosterone levels showed a significant ( $p < 0.05$ ) increase in groups (II, III and IV) when compared to group (I and V). The Follicles stimulating hormone (FSH) showed significant ( $p < 0.05$ ) increase in the FSH level in groups (II, III and IV) when compared to groups (I and V). The Luteinizing hormone (LH) level shows there was significant ( $p < 0.05$ ) increased in groups (II, III and IV) when compared to groups (I and V). FSH has a stimulatory effect on the sertoli cells and is essential for normal sperm production. Thus, increased serum levels of LH may lead to increased in testosterone secretion of Leydig cells. Besides, increased serum levels of LH and FSH is suggested to be due to the plant extract directly inducing hypothalamic - pituitary - testicular axis, which in turn influences the gonads (Sharma *et al.*, 2013). Bendjeddou *et al.*, (2003) reported that the alkyl amide of *Anacyclus pyrethrum*, *Pellitorin* has positive effects on reproduction. Similarly, Zhenga *et al.*, (2000) reported that the extract of *lipidium meyenii* plant containing alkyl amide not only acts like testosterone, but also increases the weight of genital organs. Testosterone is a male hormone that has significant impact on spermatogenesis (Martino-Andrade *et al.*, 2010).

## CONCLUSION

The results of the study revealed that aqueous stem bark extract of *Diospyros mespiliformis* has compounds with fertility enhancing potentials on hormone, that could directly or indirectly promote male reproductive activity. Further work needed to be done to investigate the effect on the ultrastructural details of the testis. Also, analysis of sperm parameters would further authenticate findings from the work.

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