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Assessment of Sperm Quality in Wistar Rats Administered With Extracts of *Persea americana*

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Abstract
The present research study was aimed at investigating the effects of stem bark extract of Persea americana on some reproductive functions of male Wistar rats. In this study, male Wistar rats were randomly distributed into three (3) groups of 5 rats each. The control group (Group 1) received distilled water. Group 2 was administered 200mg/kg of the extract and group 3 was administered 400mg/kg of the extract daily for a period of 21 days after acclimatization. Blood sample was collected and some tissues harvested at sacrifice. The statistical analysis was done using Statistical Package for Social Sciences (SPSS). One-way analysis of variance (ANOVA) test was used to compare groups followed by post-hoc testing to determine whether there were significant differences between the control and treatment groups. P-value less than 0.05 was considered significant and result presented as Mean±SEM. The result indicated that the stem bark extract of Persea americana did not cause any significant alteration of anterior pituitary synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH), but may have caused changes in the gonads leading to decreased gonadal synthesis of testosterone. The sperm parameters including percentages of viable cells, cells with normal morphology, actively motile cells and sperm count of the rats were significantly reduced demonstrating that the stem bark extract of Persea americana possess anti-spermatogenic effects.

Keywords: *Reproductive functionss, Persea americana, sperm parameters, Wistar rats,*

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I. Introduction

The rate at which couples seek medical attention due to failure to reproduce and often necessitating several investigations to determine their reproductive wellbeing is becoming a source of concern to experts (Ericksen and Brunette, 1996). Infertility rate has become alarming at a global scale with an average of 8 to 12% of couples affected. In Africa, it is a common problem associated with reproductive health (Larsen, 2000). The use of the female's ability to conceive as a measure to differentiate between primary and secondary infertility is however problematic as it places responsibility for a couple's infertility at the doorsteps of the female partner. However, the inability of a couple to reproduce may be due to a problem of the male. Male factor infertility accounted for 25% and 26.8% of infertility cases in a study in south eastern Nigeria and south western Nigeria respectively. [Olatunji and Sule-Odu (2003) ; Nwajiaku *etal*, (2012)]. Abnormalities in sperm production including low sperm count and abnormal sperms has previously been reported as cause of male infertility (Nwajiaku *etal*, 2012) In addition, the mammalian testis is a sensitive organ which may react negatively to certain substances with the capacity to cause harm to it. Medicinal plants have very few harmful effects on humans, however some are toxic to both humans and animals, possessing the ability to harm certain organs in the body. *Persea americana* is a tropical or subtropical fruit bearing plant originating from South America. The fruit has been referred to as the most nutritious of all fruits. It is of high value not only for its unique texture, exquisite taste and aroma, and nutritional profile, but also for the numerous health benefits it possesses. Extracts of the plant has been reported to show antidiabetic effects [Alhassan, 2012], as well as analgesic and anti-inflammatory effects (Adeyemi etal, 2002).The bark extract of *Persea americana* exhibited much better antibacterial effect on test isolates compared to the leaf extract, however, it is difficult to find studies done to assess the effects of the bark extract on reproduction. This study was done with the objective to assess the effects of the stem bark extract of *Persea americana* on some male reproductive functions.

II. Materials And Methods

Animal Models

A total of fifteen (15) adult male Wistar rats used for this study were bred in the animal house of the Faculty of Basic Medical Sciences, Rivers State University, Nigeria. They were placed in standard cages and

allowed to acclimatize for 2 weeks while they were maintained in standard environmental conditions with proper ventilation and humidity. The animals were also given free access to food and water. Generally, the procedures conformed to the established principles and guidelines for the care and use of laboratory animals published by the United States Institute for Laboratory and Animal Research (1996). Appropriate institutional approval was obtained for this study.

Preparation of Plant Extract

A mature *Persea americana* (avocado) stem bark sample was collected from a tree within the premises of Rivers State University, Port Harcourt, Rivers State, Nigeria. The bark was removed from the stem, rinsed, and dried at 40°C in the oven. The plant was identified and authenticated by the taxonomist in the Department of Plant Science and Biotechnology, Rivers State University. A specimen (voucher number RSUPb041) was deposited in the herbarium. The ground stem bark of *Persea americana* was packed into a tiny bag weighing about 40g of each sample and placed into the thimble of the soxhlet apparatus using the soxhlet method of extraction. 250 mL of solvent (ethanol) was treated to minimal heat for 3 hours in a round-bottom flask using a heating mantle. The resulting solvent-oil mixture was put through a large condenser that was cooled by a constant flow of fresh water. The extract was then decanted into sample bottles after being separated using a rotary evaporator. The process was repeated until a sufficient amount of extract was recovered for analysis.

Experimental Design/Procedure

After a two-week acclimatization period, the 15 male Wistar rats were assigned into three groups. Group one represented the control and received distilled water, whereas groups 2 and 3 were experimental groups and received 200mg/kg and 400mg/kg of *Persea americana* ethanolic stem bark extract, respectively. Extract administration was carried out with an animal gavage tube. The animals remained on standard pelleted feeds and clean water while extract administration lasted for 21 days. At the end, animals were sacrificed under chlorofoam anaesthesia. Blood was collected and the serum was used for assay of some reproductive hormones including follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. An incision was made around the inguinal area to locate the testes and epidydimis from where semen was expressed for sperm analysis. Both testes and epidydimis were harvested and weighed. Sperm parameters including sperm viability, sperm morphology, sperm motility and sperm count was analysed in accordance with established methods (WHO, 2010), while hormonal assay followed standard procedures (Uotilo etal, 1981).

STATISTICAL ANALYSIS

The differences between the treatment and control groups were calculated using the SPSS (Statistical Package for Social Sciences) software program for Windows XP (version 21.0). The analysis of variance (ANOVA) test was used to compare groups. Least significant differences (LSD) and post hoc testing were used to determine whether there were significant differences between the control and treatment groups. P-values less than 0.05 were considered significant and result presented as mean±SEM.

III. Results

The results for the study are presented in tables 1-5

Table 1: Effect of extract of *Persea americana* on serum hormone levels.
Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Groups (mg/kg)	FSH (m/µ/ml)	LH (m/µ/ml)	Testosterone (ng/ml)
Control	0.59 ± 0.13	0.62 ± 0.12	3.07 ± 0.34
200	0.70 ± 0.18	0.66 ± 0.02	1.18 ± 0.20*
400	0.81 ± 0.16	0.44 ± 0.04	1.71 ± 0.19*

Table 2: Effect of extract of *Persea americana* on viable sperm cells.
Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Groups (mg/kg)	Viable sperm cells (%)	Relative change (%)	Level of significance
Control	74.00 ± 2.45	0	-
200	79.00 ± 4.00	6.76	0.25
400	63.00 ± 2.00*	- 14.86	0.02

Table 3:Effect of extract of *Persea americana* on sperm cells with normal morphology
Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Groups (mg/kg)	Normal morphology (%)	Relative change (%)	Level of significance
Control	73.00 ± 2.00	0	-
200	69.00 ± 3.32	- 5.48	0.26
400	60.00 ± 1.58*	-17.81	0.00

Table 4:Effect of extract of *Persea americana* on actively motile sperm cells
Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Groups (mg/kg)	Actively motile (%)	Relative change (%)	Level of significance
Control	69.00 ± 3.32	0	-
200	77.00 ± 3.00	11.59	0.07
400	55.00 ± 2.24*	- 20.29	0.01

Table 5:Effect of extract of *Persea americana* on sperm count
Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Groups (mg/kg)	Sperm count ($x10^6$/ml)	Relative change (%)	Level of significance
Control	370.00 ± 66.33	0	-
200	450.00 ± 65.19	21.62	0.32
400	196.00 ± 16.31*	- 47.03	0.04

IV. Discussion

This research study assessed changes in some reproductive parameters of male Wistar rats treated with ethanolic stem bark extract of *Persea americana.*. Following 21 days of oral administration of the extract to male rats, the result revealed that follicle stimulating hormone and luteinizing hormone were not significantly (P<0.05) altered, since the serum concentration of the hormones did not change significantly when test was compared to control rats. These hormones are gonadotrophins- glycoprotein hormones secreted by the anterior pituitary gland that act directly on the testes to stimulate somatic cell function in support of spermatogenesis. The somatic cells include, the interstitial steroidogenic cells of Leydig, whose function is primarily the production of testosterone; the myoid cells that surround the seminiferous tubules that provide physical support as well as contractile motion to the structures; and the Sertoli cells, which are in close contact with proliferating and differentiating germ cells in the seminiferous tubules, thus making them very essential as they provide physical and nutritional support for spermatogenesis [Maekawa etal, (1996), Mendis-Handagama (1997), Griswold, (1998)]

The serum concentration of testosterone was significantly (P<0.05) reduced in the group that received the higher dose (400mg/kg) of the extract suggesting that the extract may have caused changes at the level of the testis that led to reduction in the production of testosterone. Since testosterone is primarily secreted by the Leydig cells in males (Mendis-Handagama 1997); the extract of *Persea americana* may have exerted its action by inhibiting the interstitial steroidogenic cells of Leydig to release testosterone.

The extract of *Persea americana* significantly decreased percentages of viable spermatozoa and spermatozoa with normal morphology. The percentage of actively motile spermatozoa was also significantly decreased. These changes occurred with the higher dose (400mg/kg) of the extract. Also, the sperm count was significantly decreased in the group that took the higher dose of the extract. The decrease in sperm count may not be unrelated to the decrease in testosterone level. It has been reported that the amount of spermatozoa in the seminiferous tubules, which shows the degree of spermatogenesis is quantitatively maintained by testosterone and follicle stimulating hormone (Yakubu, 2012). Therefore, decrease in sperm count observed in this study could be attributed to the decreased testosterone level which followed the administration of *Persea americana* extract.

Male infertility may result from impaired sperm production. It has been suggested that pathological changes in seminiferous tubules and epididymis distorts testicular and epididymal functions giving rise to the reduction in quality and quantity of spermatozoa, including daily sperm output and caudal epididymal spermatozoa, percentage of motile sperm, live sperm and normal sperm morphology; all of which has the ability to cause infertility. There are several reports regarding infertility being related to reduction in quantity and quality of spermatozoa and testicular and epididymal damages. Infact, the sperm count estimation is an important test of spermatogenesis and is directly related to fertility (Nwoke etal.,2015).

In a study on male fertility, a decline in testosterone secretion was reported as the reason for an observed impairment of spermatogenesis (Abu et al.2013). The decrease in the density of spermatozoa has been

shown to be the possible mechanism in which natural substances used in the form of plant based contraceptive, inhibits male fertility (Sharma and Jocob., 2001). However, the reversibility of a depressed or inhibited fertility remains a cardinal feature of an ideal plant based male contraceptive.

V. Conclusion

The changes in serum testosterone level and the observed effect on testicular function and spermatogenesis showed that the stem bark extract of *Persea americana* inhibited spermatogenic activities in male Wistar rats. Furthermore, we recommend for further reversal study to determine the reversibility of the effects on these reproductive parameters of male Wistar rats.

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