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Original Research Article

Persea americana Mill (Lauraceae) Seed Extract Effect on Male Rat Fertility: Sperm Quality and Histomorphological Safety Assessment

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ABSTRACT

Poor sperm quality and morphological abnormalities have been cited as major causes of sperm functional incompetence and infertility. The current study focused on the effects of *Persea americana* Mill (Lauraceae) seed hydro-methanolic extract (PAS) on sperm quality and histomorphological changes in the reproductive and internal organs of male rats. The rats were assigned into 4 groups of 10 animals per group: control (no treatment and no intervention), and three extract treatment (20, 100 and 500 mg Kg^{-1}) groups. Treatment groups received a pre-assigned oral dose of PAS, once daily for 28 days. Five rats were randomly selected and sacrificed from each group on D_{27} for multi-parametric assessment, while the rest were used for reversibility analysis on day 14 post drug withdrawal. Sperm quality (count, motility, abnormality) and histomorphological safety of the internal organs (testes, brain, liver and kidney) were evaluated. The results showed a dose-dependent increase ($p<0.05$) in sperm count, motility, and sperm structural abnormalities. These abnormalities ranged from 1.1% to 3%, compared to the control group. The histopathology of the testes and internal organs showed no untoward effect; however, the liver showed some congestion at lower doses of PAS (20 and 100 mg Kg^{-1}) in the reversibility groups. Partial reversibility was observed in the measured parameters, including the testes weight in reversibility groups. The results revealed that PAS possibly caused a cumulative increase in spermatogenesis and other testicular functions. The extract could be further evaluated for holistic use in the management of male fertility, such as oligospermia and asthenospermia.

***Keywords*:** *Persea americana*, Male infertility, Testes, Sperm count, Sperm quality, Sperm motility

Introduction

In developing countries, such as Nigeria, the inability to have one's own biological child can be a major social life crisis.[1] In the past, women were often regarded and treated as the cause of couples' inability to conceive, and as a result, male partners sometimes sought other female partners for a possible test of their fertility and procreation. At times, it has a religious angle to it, where the couples are seen as being cursed, the gods being angry with them or as a punishment due to past events in their lives. This can be stressful and depressing to the couple and their household/relatives. Consequently, couples tend to seek help as soon as conception is delayed. The contribution of the male factor in infertility is increasingly being recognised as a major factor and is reported to be on the rise.[2-3]

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Male reproductive challenges as a contributing factor to infertility has been noted to be of the same ratio to that of the female, 30% to 40% (shared by both sexes), and 20% sole responsible for the state of infertility. [2] In Nigeria, male infertility is reported to be location-dependent and has a prevalence rate of 40% to 50%, challenging the traditional belief that infertility is solely the responsibility of women.[4]
The successful use of medicinal plants and herbal products to solve male-related infertility factors has been documented like, any other health challenge.[3] Many medicinal plants have also been screened for male fertility and contraceptive effects.
The cause of male infertility is multifactorial, ranging from dysfunctional erection, poor testicular development, lifestyle choices, reduced or low libido and low testosterone level, to unhealthy sperm.[5,3] An intact hypothalamic-pituitary testicular axis is needed to initiate and maintain qualitative and quantitative normal spermatogenesis and sexual function. The qualitative and quantitative health conditions of the sperm are important determinants of their functionalities and the ability of males to reproduce at will. Thus, any factor that can cause abnormalities and adverse effects in the production and the quality of produced sperm can cause male infertility.[3] Over 90% of infertility in male partners has been linked to poor quality and/or low sperm count.[3] Some conventional and recreational drugs have been documented to adversely affect the quality of sperm. Semen can be subjected to laboratory analysis to determine the quantity and quality of sperm and their possible effect on fertilization. Low-level and substandard quality of sperm will likely result in an inability to fertilize female eggs. Sperm morphology refers to the size and shape of sperm, and it is an important diagnostic aspect of male fertility analysis.[6] The percentage of sperm that appears "normal" under the microscope has a direct and indirect

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effect on the level of male fertility. Semen analysis also assesses the sperm volume and motility. The result of any semen analysis in relation to sperm count is compared to a known standard. It can be described as any of the following: hypospermia, oligozoospermia, and azoospermia, which means the absence of sperm in the semen.

Hypospermia is when the seminal volume is below the referenced limit. The normal average sperm count is within the range of 40-300 M/ml (https://www.medicalnewstoday.com/articles/324821).

Oligozoospermia is when the total sperm count is below reference figures. Complete absence of sperm in semen is known as azoospermia. The absence of semen is technically known as aspermia. A 2022 meta-analysis showed a gradual decline in human sperm counts by observed by fall of 1.16% per year from 1973 to 2000 and higher (2.64%) post 2000.[2,7]

An estimated 80% of the population in developing countries relies on traditional herbal medicine for healthcare. Medicinal plant remedies in the form of herbal products are easily accessible, affordable and culturally accepted in developing countries and even in developed countries (as phytomedicine products).[8] The high patronage of traditional healers and herbal medicine use by infertile couples is documented and, in some cases, it started before clinical evaluation. [9,10,11] The first set of herbal medications are likely herbs recommended by family and friends. Often the seeking for traditional medicine treatment options is based on cultural beliefs/acceptability, religion, low cost, availability etc.[11] The efficacy and safety claims of most of these home herbal remedies are yet to be scientifically evaluated and documented.[12] However, a good number are traditionally used, non-conventional proof of successful conception exists, without scientific backup.[12] The discovery and development of many conventional drugs from the phytoconstituents of medicinal plants have encouraged more search for newer drugs from plants.

Internet scientific literatures and review search showed that several medicinal plant extracts have been reported to contain bioactive phytoconstituents that either enhances or inhibits hormonal, testicular, and spermatogenic functions (male reproductive health).[3,13] For example, *Khaya grandifoliola* stem bark extract has been reported to increase testosterone concentration and sexual behaviour in normal male rats.[14] Some other plants that elicit male fertility activity by various mechanism of activities such as spermatogenesis-inducing, androgenic effect, antioxidant, anti-inflammatory, etc. due to the bioactive principle contained. Some examples include *Fagara tessmannii*, *Mucuna pruriens*, *Nigella sativa*, *Panax ginseng*, *Morinda officinalis*, *Lepidium meyenii*, *Anacyclus pyrethrum*, *Astragalus membranaceus*, etc.[15] The administration of the ethanolic extract of the roots of *Martynia annua* to male rats caused Leydig cell atrophy. Medicinal plants can improve male fertility by regulating estrogen, FSH (follicle-stimulating hormone), LH (luteinizing hormone), and progesterone levels, which are all crucial for male reproductive health.[16] *Persea americana* Mill (Lauraceae), commonly known as avocado pear, contains a single seed which is normally disposed of after the use of the pulp. In traditional medicine in Nigeria and some Asia countries, this seed is used as female contraception,[8] in the management of hypertension,[17] in the treatment of diabetes and inflammation.[8,17] It also exhibits local anaesthetic effects on the muscles and teeth, and exhibits hypoglycemic and anticancer effects.[8,17]

This study is an ongoing investigation by this research team, seeking answers concerning the potential effects of *Persea americana* Mill (Lauraceae) seed hydro-methanol crude extract (PAS) on male reproductive indices, with a focus on sperm production, quality, and histomorphological changes in the reproductive organs. The study was conducted with only adult male albino rats. The result of the study will be additional information on the potential or possible role of *Persea americana* seed as an integrative approach in male fertility management.

Materials and Methods

Plant collection and identification

The fruits of *Persea americana* were bought from Mushin Market **(**6.5215° N, 3.3548° E), Mushin local government area, Lagos state, Nigeria in June 2021. The fruit was identified and authenticated by Mr. Nwafor, F. of Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State. The herbarium specimen was prepared and deposited with voucher number PCG/UNN/0012.

Preparation and extraction of plant extract

The pulp of the fruits was removed to isolate the seed. The seeds were extracted from the fruits and washed to remove the remaining pulp. The seeds were sliced into small pieces and air dried. The dried seed slices were blended, and800 g weighed out for extraction. Extraction was done using cold maceration method. The cold maceration was done using 4200 mL of 80% methanol. It was soaked for 3 days and filtered using muslin cloth. This was repeated 3 more times, and all the filtrates were pooled together, and the solvent removed using Buchi Rotavapor rotary evaporator and water bath (at 40ºC). The dried crude extract was weighed (94.8 g) and stored at 4 °C in an air-tight container.

Experimental animals

The number of animals used was 40, all male albino rats and weighing between 110 - 122 g (within the weight range used in our previous study of the potential effect of the plant on female hormonal profile)[10]. The animals were obtained from Animal Laboratory Centre, College of Medicine University of Lagos and housed in the animal house. The animals were housed in cages, under standard animal housing conditions. The housing conditions in the Animal House, College of Medicine University of Lagos include a 12 h dark and 12 h light cycle, access to standard pelleted diet and water *ad libitum* and room temperature. The environment of the animals was kept clean. All animal experiment procedures were approved by the Health Research Ethics committee of College of Medicine of the University of Lagos (ethical approval number: CMUL/HREC/11/19/677) and according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 407, OECD 2008.[18] The animals for the study were acclimatized to the laboratory conditions for 7 days post collection from source.

Ethical approval

This study was approved by the Health Research Ethics Committee of College of Medicine of the University of Lagos with the CMULHREC number: CMUL/HREC/11/19/677.

Administration of extract and test specimen collection

The male rats were weighed to record their baseline weight after being acclimatized for 7 days and maintained on animal feed. The range for the baseline weights recorded was 118 - 135 g. The weighed rats were divided into 4 groups:

- Negative control (no treatment, no intervention); and

Three extract treatment groups, namely,

- T2 that received 20 Kg^{-1} of PAS
- T3 that received 100 Kg^{-1} of PAS
- T4 that received 500 Kg^{-1} of PAS

Each group consisted of 10 animals (as described and reported in Orabueze *et al.*, 2021)[8]. The negative control group received no treatment, and no intervention. The different extract treatments with PAS were administered orally at the various designated dose levels of 20, 100 and 500 mg Kg^{-1}.

The administration of the test drug (PAS extract) at various doses was done once daily and at approximately the same time every day for a period of 28 days (D_0 – D_{27}). The animals were weighed every 7 days using automatic Electronic Balance, to monitor their weight gain or loss and to adjust the dose to the weight. On the last day of the study, day 28 (D_{27}), the treatments were stopped (ceased) across all groups. Approximately 24 h after the last dose on D_{27}, five animals were randomly selected from each group, weighed and sacrificed by cervical dislocation for collection of experimental specimens. The remaining 5 animals in each group were not given any extract again. They had access to rat pellet and water *ad libitum* for another 14 days post dosage stoppage, designated as recovery or reversibility period, thus making a total of 42 study days (D_1 - D_{41}).[19] The reversibility designated animals were then sacrificed 24 h after their 14 days recovery period elapsed.

Reversibility sub-study groups below were created from the treatment

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groups:

- RT1= Reversibility for Negative Control
- RT2 = Reversibility for 20 mg Kg^{-1}
- RT3 = Reversibility for 100 mg Kg^{-1}
- RT4= Reversibility for 500 mg Kg^{-1}

The internal organs associated with the reproductive functions such as the testes, and systemic organs associated with general functioning of the body such as brain, liver and kidney were harvested, weighed, and prepared for analytical use. The harvested organs were trimmed of fat and rinsed in normal saline, then kept in 10% buffered formalin until needed. The testes were kept in the Bouin's solution for preservation until needed. Semen was extracted from the testes for sperm quality and motility analysis.

The average weight of the rats was within the range of 170-180 g.

Preparation of specimen for sperm quality parameter evaluations

The cauda epididymis from each side of the testis of each animal were removed using scissors and cut into pieces inside a petri dish. The sperm was washed off using warm normal saline (0.9 % NaCl), forming a saline sperm suspension. This was labelled as stock and samples collected from this stock ware then used for the sperm quality parameter assays (sperm motility, count, and abnormalities). [19]

a) Sperm motility

An aliquot of the suspended sperm was dropped on a microscope slide and covered immediately with a cover slip. The slide was mounted on the microscope stage, and the area covered with the coverslip was focused using the ×40 objective lens. Sperm motility here is defined as any movement made by the sperm flagellum within an observation duration of 30s and expressed as a percentage compared to actively motile sperm in total sperm. The percentage motility of the sperm cells was recorded as the average of three observed microscopic fields.[19-21]

b) Sperm count

The total count of the sperm was determined by first diluting the collected semen with sodium bicarbonate (1 M; 20 ml). A drop of the semen dilution was taken and dropped on charged Neubauer's counting chamber and mounted on a microscope. The chamber was then viewed using the x10 objective lens and the number of sperm cells counted in three fields. The counting was repeated 3 times and averaged to minimize human counting error. The obtained result was divided by three to get the mean. Calculation was done using the standard formula:[19,21]

Formula for total count = Mean No of sperm counted x dilution factor /volume x 1000

c) Sperm morphology: Abnormality Assay of the sperm head

A drop of semen was collected into a petri dish and three drops of Eosin-Nigrosin stain were added into the petri dish and stirred for even distribution as described by El-Sherbiny (1987)[22] with some modifications. Smears of the semen sample were made on slides and labeled accordingly. The sperm slide samples were air dried and then observed under a microscope for morphological normal and abnormal spermatozoa. The percentage for each abnormality was calculated based on the number of spermatozoa present in the field of view with the aid of light microscope (x400). Normal spermatozoa are expected to have an oval-shaped head, a short middle piece, and a long thin tail.[21] According to the 5th and 6th editions of the World Health Organization (WHO) guidelines on human semen analysis, a sample can be considered to have normal morphology if at least 4% of the sperm exhibit normal shape and appearance, although this may be associated with slightly reduced fertility. [6,21]

Weights of the organs and the body

The body weight of the animals was taken at weekly intervals. The initial and the final weights were also documented. The internal organs: liver, brain and kidney were removed along with the testes. They were trimmed of fat, weighed separately, and documented.

Histopathology

The organs were processed in the histopathology laboratory of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria for histopathological studies according to the methods already described in a similar study done by this research team.[8] The microscopical view was done using oil immersion lens with x100 magnification. Microscopical views of the organs from the treatment groups were compared with those from the healthy animals - the negative control animals that received no treatment.

Statistical analysis

The result values were expressed as Mean ± S.E.M and analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett´s multiple comparison test. Pearson correlation was used to determine the association between hormonal levels and sperm count. The significance level was set at $p < 0.05$.

Results and Discussion

Evaluation of sperm quality parameters is an easy, reliable, and effective way of using reproductive biomarkers in determining fertility profile of male partners. Studies and case reports have shown that consumption of some medicinal plants enhances fertility profile resulting in pregnancy.[3,23] Male fertility enhancing medicinal plants act through various mechanisms such as improving the hormonal profile, increasing sperm quality (health, viability, and motility of sperm), and by increasing the numerical strength of the testicular vessels and survival of the sperm .[3,24,25]

The selected doses administered to the treatment groups were determined as described by Orabueze *et al.* (2021)[8], which reported that the LD_{50} of the extract is greater than 4000 mg kg^{-1}. The treatment doses used in this study were all below 4000 mg kg^{-1} and were therefore considered safe for the animals.

This study evaluates the possible effects and nature of the influence of PAS on male health, particularly in relation to sperm quality, testicular toxicity, and the impact on other indirectly affected internal organs such as the brain, kidneys, and liver.

Table 1 shows that the animals gained weight across the groups. There was significant increase ($p < 0.05$) in the body weight of the male rats between weeks 1 to 4 of administration of hydro-methanolic extract of PAS. The negative control group (T1) exhibited the highest percentage increase in body weight (34.7%, Table 1), followed by group T2, the lowest treatment group. Upon withdrawal of the test drug-extract, the reversibility groups (RT1 - RT4) exhibited continued weight gain, week 5 - 6. The percentage increase in body weight was not dose-dependent and not statistically significant ($p < 0.05$) across the treatment groups or between the negative control and the treatment groups. This could be a normal growth pattern of healthy animals eating well but not at the same capacity or due to individual body metabolic differences. The continuous weight gain observed during the reversibility period and the absence of significant differences among the groups suggest that the general metabolic rate of the animals was not affected by the presence of PAS in the body.

The relative organ weight of the brain, liver, testes, and the kidney of the treatment group was not significantly different ($p < 0.05$) from the negative control group that received no treatment. However, a non-significant ($p < 0.05$) decrease in the liver was observed at 100 and 500 mg Kg^{-1} doses compared to the negative control group, T1. This decrease in the weight of the liver at the high doses, though not significant compared to the control, may require further investigation. Histopathology can help confirm or give more insight into the existence of any silent or hidden toxicity concern. Experimentally, any significant change in organ weight indicates potential toxicity or harmful effects of a chemical or drug.[26]

The highest increase in the weight of the testes occurred in the group that received 500 mgKg^{-1} and it was not significant at ($p < 0.05$). The increase in the testes was observed to be dose-dependent in the treatment groups. The weight-organ analysis of the testes showed a slight insignificant drop in weight with the lowest dose, 20 mg Kg^{-1} and then gradual increase as the dose increased (100 and 500 mg Kg^{-1}) in Table 2 and 3. The same pattern occurred in the reversibility groups (Table 3), first drop in the testes weight in the low dose of 20 mg Kg^{-1} and then gradual increase with increased doses. This could mean that a dose higher than 20 mg Kg^{-1} is needed to initiate pharmacological effect as noticed in the higher doses.

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Table 1: Effect of *Persea americana* seeds (PAS) on the body weight of male rats after administration of the extract at various doses

	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	% gain (wk 1 to 4)	Week 5 (g)	Week 6 (g)	% gain (wk 5 to 6)
T1	121.7±2.5	137.0±14.4	150.0±16.5*	163.0±18.2*	34.7			
T2	119.0±2.0	132.7±7.4	143.3±5.9*	155.7±11*	30.8			
T3	122.7±4.7	135.7±3.2	147.0±2.6	151.0±2.8*	23.8			
T4	123.0±4.4	135.0±5.3	147.0±3.6	159.7±6.4*	29.8			
RT1	127.0±1.0	137.3±1.5	150.7±3.8*	161.0±6.2*	26.8	168.7±4.7	177.7±4.7	5.3
RT2	120.3±0.6	126.3±3.8	137.3±3.5	150.0±5.6*	25.0	160.7±2.3	171.0±2	6.4
RT3	122.3±1.5	134.2±2.8	144.0±3.6	153.0±3.6*	25.4	162.0±3.6	172.3±4.7	6.4
RT4	121.7±2.1	131.7±3.1	146.3±5.9	153.3±4.5*	25.9	166.3±4	175.7±3.2	5.7

n=5. Values are mean ± SEM; statistically significant at $p < 0.05$; Data were analyzed by one-way ANOVA;
Values marked with superscript [a] in the same column are significantly different from the negative control for $P < 0.05$
Values marked with superscript * in the same row are significantly different from mean weight of week 1 for $P < 0.05$

KEY:
T1= Negative Control (No treatment and No intervention), T2= 20 mg Kg^{-}, T3= 100 mg Kg^{-1}, T4= 500 mg Kg^{-1}
Reversibility sub-study groups were created from the treatment groups
RT1= Reversibility for Negative Control
RT2 = Reversibility for 20 mg Kg^{-1}
RT3 = Reversibility for 100 mg Kg^{-1}
RT4= Reversibility for 500 mg Kg^{-1}

The observed increase in the weight of the testes in Table 2 is partially irreversible compared to the negative control (T1) and is presented in Table 3.

Table 2: Effect of Hydro-methanolic extract of *Persea americana* seed on the relative organ weight (g) of vital organs of rats after 28 days administration.

Organ	T1 (g)	T2 (g)	T3 (g)	T4 (g)
Brain	1.54±0.1	1.59±0.2	1.47±0.16	1.72±0.05
Liver	5.51±1.72	5.75±1.03	4.48±0.54	5.04±0.53*
Testes	2.13±0.38	1.95±0.27	2.05±0.12	2.36±0.04
Kidney	0.92±0.11	0.94±0.03	0.89±0.11	1.03±0.16

n= 5; Values are expressed as mean ± SEM; statistically significant at $p < 0.05$ compared to the negative control; Data were analyzed by one-way ANOVA; Values marked with superscript * in the same row are significantly different from the negative control for $p<0.05$

T1= Negative Control (No treatment and No intervention)
T2= 20 mg Kg^{-1}
T3= 100 mg Kg^{-1}
T4= 500 mg Kg^{-1}

Table 3: Effect of Hydro-methanolic extract of *Persea americana* Seeds on the relative organ weight (g) of vital organs of rats after 28 days administration and 14-day reversibility.

	RT1 (g)	RT2 (g)	RT3 (g)	RT4 (g)
Brain	1.62±0.03	1.61±0.04	1.62±0.04	1.64±0.03
Liver	8.14±0.68	7.57±0.1	8.36±0.87	9.09±0.4*
Testes	2.14±0.15	1.88±0.17	2.02±0.29	2.26±0.07
Kidney	1.04±0.11	1.03±0.02	1.03±0.06	1.04±0.02

n= 5; Values are expressed as mean ± SEM; statistically significant at $p < 0.05$ compared to the negative control; Data were analyzed by one-way ANOVA; Values marked with superscript * in the same row are significantly different from the negative control for $p<0.05$

Reversibility sub-study groups were created from the treatment groups
RT1= Reversibility for Negative Control
RT2 = Reversibility for 20 mg Kg^{-1}
RT3 = Reversibility for 100 mg Kg^{-1}
RT4= Reversibility for 500 mg Kg^{-1}

The irreversible weight of the testes after 14 days of withdrawal of the extract may be regarded as an exhibition of prolonged acting effect or permanent change caused by the phytoconstituents contained in the herbal extract. Changes in the reproductive organ weights are significant indicators to possible changes in the functionality of the organs or toxicological concern.[26-27] Increase in the weight of the testicular tissue as recorded in this study is a possible indication of changes in seminiferous tubules since no toxicity effect has been observed and the dose range used is low compared to the Orabueze, *et al.*, record of "LD_{50} being greater than 4000 mgKg^{-1}. Thus, the observed effect can be attributed to the increased sperm production (spermatogenesis) as recorded by the dose dependent increase in sperm count (Table 4).[28-29] The weight and size of the testes and other closely related organs are regulated by the androgen hormones such as the testosterone, resulting in possible increase in sperm production and libido. Increase in some female reproductive hormones after oral administration of PAS has been reported in a previous study by this research team and such investigation in the male counterpart may help to understand the increase observed in testes and in the sperm count.[8] Key functions of the testes include spermatogenesis and steroidogenesis which are processes needed for reproduction.[29] PAS possibly supports the process associated with spermatogenesis, and steroidogenic enzymatic activity. Decrease in testicular weights (testicular atrophy) and its accompanied reduction in spermatogenic cells in the testes is an indication of contraceptive effect.[30] Men with infertility challenges, especially azoospermia have increased risk of testicular cancer compared to males with normal sperm counts.[2,31-33] It is documented that 5 to 8% of patients with testicular cancer have been diagnosed with azoospermia.[2,34] The results of this study warrant further in-depth study of the relationship between the control intake of PAS and prevention or management of male reproductive cancers.

Table 4 shows the effect of hydro-methanolic extract of PAS on sperm motility, sperm count and sperm morphology. A dose-dependent significant ($p < 0.05$) increase was observed in the motility analysis, where T4 (500 mg Kg^{-1}) showed the highest sperm motility of 85.3 ±1.53% among the treatment groups. The spermatozoa maintained some form of movement for more than 4 hours, indicative of viability and motility. The reversibility groups that were administered with the extract for 28 days and allowed to recover (RT2 -RT4) for 14 days exhibited dose-dependent and continued increase in motility. Thus, indicating that little or no reversibility occurred after the withdrawal of

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the PAS extract. The continued increasing rate of motility observed after the 14-day recovery period may be an indication permanent change or prolonged/accumulative effect of the PAS, however, a longer reversibility or recovery observation period may be required before it can conclusively be reported. A dose-dependent increase in sperm count was also observed across all treatment groups. The difference between the sperm count for the negative control and treatment groups were all significant ($p<0.05$). The reversibility groups all showed little or no reversibility for sperm count.

Table 4: Effect of hydro-methanolic extract of PAS on sperm motility, sperm count and sperm morphology

GROUP	MOTILITY (%)	COUNT (M/mL)	MORPHOLOGY Deformed (%)
T1	70.0 ± 2.00	92.50 x 10^6	0.33 ± 0.57
T2	75.0 ± 3.00	101.67 x 10^6	1.33±0.57*
T3	80.0 ± 0.00*	111.25 x 10^{6*}	2.0 ± 1.41*
T4	85.3 ±1.53*	119.17 x 10^{6*}	3.0 ± 1.00*
RT1	70.0 ± 5.00	90.00 x 10^6	0.32 ± 0.57
RT2	75.0 ±5.00	100.42 x 10^{6**}	0.67 ± 0.57
RT3	80.0 ± 5.00**	107.50 x 10^{6**}	1.0 ±1.00**
RT4	88.33 ± 7.64**	109.17 x 10^{6**}	1.0± 0.00**

n=10 (5 for treatment + 5 for reversibility) per group; *, ** Represents significant difference at $p<0.05$ when compared to T1 negative control and RT1 Reversibility negative control respectively

KEY

T1= Negative control — RT1= Reversibility for negative control

T2= 20 mg Kg^{-1} — RT2= Reversibility for 20 mg Kg^{-1}

T3= 100 mg Kg^{-1} — RT3= Reversibility for100 mg Kg^{-1}

T4= 500 mg Kg^{-1} — RT4= Reversibility for 500 mg Kg^{-1}

The percentage of abnormal sperm increased as the dose increased. The highest treatment dose group, 500 mg Kg^{-1} showed a deformity rate of deformity of 3% indicating good morphology of over 90 -95%. Incomplete reversibility was obtained on drug withdrawal across the treatment groups, and indication of permanent damage.

Healthy and quality sperm are needed for result oriented fertilization. Abnormal, sluggish and damaged sperms may not be able to swim through the female reproductive path, oviduct, for fertilization. The results of this study suggest that PAS exhibits effects comparable to those of *Panax ginseng* roots, including enhanced spermatogenesis, a significant reduction in sperm abnormalities, and increases in sperm count, viability, and motility in the evaluation of male fertility.[35-36] There is evidence of plants possessing androgenic activity, positive effects on spermatogenesis and improvement of the quality of sperms produced.[36]

Sperm count and motility are semen parameters that are generally correlated with the testes weight and concentration of testosterone. In the present investigation, the administration of PAS significantly increased sperm count (number) and motility (swimming). An increased testicular sperm count may be considered a marker or indicator of enhanced spermatogenesis.[37] The study extract PAS may contained phyto-constituents that help to mitigate oxidative stress and any other mechanism that improve production of healthy and mobile sperms.[3] Co-administration of ethanol with *Tetracarpidium conophorum* aqueous leaf extract was able to mitigate the ethanol effect of decrease in testicular sperm count and other male reproductive parameters.[38] Likewise, garlic and *Garcinia cola* have documented data about their ability to improve the spermatogenesis and hormonal profile of the pituitary-testicular axis of male Wistar rats respectively.[39-40] Powder of *Mucuna puriens* seeds has also been reported to improve sperm count, motility and sexual activity.[41-42] Literature search has shown that avocado fruit pulp has been reported to have significant positive effect on the male reproductive health while the extract of the avocado leaves was reported to affect the male reproductive profile negatively.[43-44] Plants that exhibit beneficial effects on male reproductive health system may be useful in solving every day male fertility challenges.

An increase or decrease in testicular spermatogenesis or sperm flagellum function can cause increased or slowed sperm motility respectively.[45] Decrease in sperm motility can be associated with testicular or epididymal toxicity, or changes in any other sperm parameters. The motilities observed in this study were within the total motility scores of 3 and 4, in the range of 85%–96%.[46-47] A review of the literature revealed that reported values vary among authors; however, there is a consensus that higher sperm motility grades increase the likelihood of sperm reaching and fertilizing the ovum. According to the WHO laboratory manual, a minimum of 40% progressive motility is considered sufficient to achieve pregnancy.[21] Sperm motility is a strong marker and predictive parameter to sperm fertility potential.[5,45-46] Good motility is needed to achieve fertility in both natural and assisted conception. Increased male fertility index is directly linked to active and effective progressive motile movement of sperm through the female reproductive tract.[5,48]

One of the after-effects of disturbances in testicular spermatogenesis is the increase in the number of abnormal sperm, according to Skoracka *et al.* 2020.[47,49,50] Abnormal sperm morphology of less than 10% as observed in the study is good and adequate for fertility.[21,46] T4 (500 mg Kg^{-1}) gave the highest percentage of malformed sperm. Increased number of malformed spermatozoa is one of the leading causes of disturbed fertility or infertility in animals.[51] High mobile sperm but with fertility limiting abnormality, such as abnormal heads can cause a reduction in fertility profile.[5,48] The recommendable dose for any extract in the management of male infertility should be aimed at reaching a balance between the parameters that affect fertility such as increased motility, increased sperm count and decreased percentage of abnormal cells. Hence, in relation to the PAS, an effective dose would be 100 mg Kg^{-1}.

Figure 1 shows the photomicrographs of the internal organs of male rats that were administered hydro-methanolic variable doses of PAS (20, 100 and 500 mg Kg^{-1}). Figure 1 also includes the photomicrographs of the internal organs of male rats for Reversibility groups, 20, 100 and 500mg Kg^{-1}.

From the histological results, all treatments groups showed normal internal organs. PAS had no negative effect on the internal organs that were harvested and subjected to histopathological evaluation. No abnormalities were observed irrespective of dose. The photomicrographs of T2, T3 and T4 of the testes showed numerous luminal spermatozoa. Hence, the hydro-methanolic seed extract of *Persea americana* can be said to enhance the function of male reproductive organs, particularly the testes, thereby improving male hormonal activity. However, for the reversibility groups, vascular congestion was observed at RT2 (20 mg Kg^{-1}) and RT3 (100 mg Kg^{-1}) in the liver. The vascular congestions observed, though noticed post withdrawal of the extract, may be an indicator that prolonged exposure to the extract should be done with caution even at low dose. The organ-weight increase in the liver mentioned earlier and the observed result for histology of the liver may need to be investigated further since this is an extract that is used daily for various health reasons in traditional and supplementary medicines. The reversibility study showed total recovery in the organ-weight for the liver, but vascular congestion was still seen in the reversed groups 20 and 200 mg Kg^{-1}. Thus, a longer-term study needs to be done for holistic understanding of the toxicity level on male rats.

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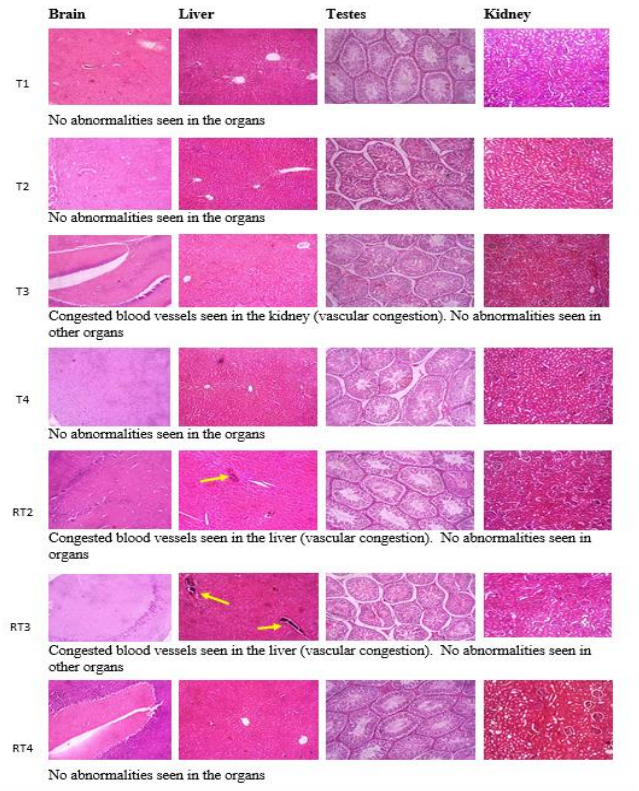

Figure 1: The photomicrographs of the internal organs (brain, liver, testes and the kidney) of male rats treated with PAS for 28 days and the reversibility groups.

KEY
T1= Negative control
T2= 20 mg Kg^{-1} RT2= Reversibility for 20 mg Kg^{-1}
T3= 100 mg Kg^{-1}, RT3= Reversibility for100 mg Kg^{-1}
T4= 500 mg Kg^{-1}, RT4= Reversibility for 500 mg Kg^{-1}
RT1= Reversibility for Control

The histopathological investigation done in this study showed no untoward effect on the brain or any other organ. The toxicity studies reported that the seed extract of PAS is relatively safe. However, there is need for further investigation on the long-term safety of the seed use. The result of this study confirms that the hydro-methanolic seed extract of *Persea americana* Mill (Lauraceae) investigated has positive effect on male reproductive functions such as increased spermatogenic indices, increased semen quality, increased testicles-body weight and does not change the histological architecture and structure of the testes in male rats.

Conclusion

The result of this study confirmed that the hydro-methanolic seed extract of *Persea americana* Mill (Lauraceae) investigated has positive effect on spermatogenesis and improved parameters that measures the sperm abnormalities index (quality of sperms) and their characteristics in male rats. The extract could be further evaluated for holistic use in management of male infertility related with poor sperm count (oligospermia) and motility (asthenospermia). Furthermore, the potential use of PAS as a supplement for the prevention or management of male reproductive cancers may be explored, given the reported association between testicular cancer and azoospermia.
Further investigations are needed to determine if there is any accumulative effect on prolonged use in the liver.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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