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Evaluation of potentials of aqueous ethanolic extract of *Psidium guajava* on reproductive functions of male Albino rats

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Abstract

The therapeutic utilization of plants for managing several diseases by people of all continents, especially Africa, is as old as tradition. The objective of this study was to evaluate the potentials of aqueous ethanolic leaf extract of *Psidium guajava* on male reproductive parameters. Twenty-four male Albino rats were randomly assigned into four groups of six rats per group. Rats in the control group were administered Tween 20®. Rats in groups 2, 3, and 4 were administered *Psidium guajava* aqueous ethanolic leaf extract orally at the doses of 100, 200, and 400 mg/kg body weight, respectively, once daily for 60 days. The body weights of the rats were determined at the beginning and end of the experiment. Sperm parameters and some reproductive organs weight of each rat was also determined, some organs were collected for histopathology. Assay for follicle-stimulating hormone, luteinizing hormone, and testosterone was done using Enzyme-Linked Immunosorbent methods. Some reproductive organs were collected for histopathological analyses. The results showed a dose-dependent increase ($p<0.05$) in the weight of male reproductive organs, sperm parameters, and hormones. The extract significantly increased ($P < 0.05$) serum testosterone in the group treated 400 mg/kg body weight when treated groups were compared with control. Furthermore, the results of FSH and LH revealed a significant ($p < 0.05$) increase when the treated groups were compared with the control. The histopathological analysis did not reveal any form of damage to the architectural integrity of the testis. However, there was degenerating germinal epithelium in the group administered 400 mg/kg of the aqueous ethanolic leaf extract of *P. guajava*. Conclusion: aqueous ethanolic extract of *P. guajava* has positive effects on male reproductive parameters.

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Introduction

Natural products constitute a potential source of alternative medicine in the developing world to treat and manage diseases (Kumar *et al.*, 2013). In developing countries like Ghana, Mali, Nigeria and

Zambia, 80% of the rural dwellers rely largely on herbal medicine as first line of treatment of illnesses as most synthetic and orthodox drugs are not affordable (WHO, 1998). Similarly, in developed countries, despite advancements in allopathic drugs, over 25% of prescribed medicines are derived directly or indirectly from plants (Newman *et al.*, 2000).

Some plant extracts are known to have chemo preventive and chemotherapeutic as well as stimulatory activity on the reproductive function. For example, study by Chaturapanich *et al.* (2008) showed that many plant extracts can enhance fertility.

Psidium guajava (common name-guava) is a well-known tropical tree that is abundantly grown for fruit. It belongs to phylum *Magnoliophyta*, class *Magnoliopsida*, and *Myrtaceae* family with all its parts having a history of therapeutic value (Dakappa *et al.*, 2013).

In folk medicine, especially in some traditional African localities, decoctions from *Psidium guajava* leaves are normally used to treat reproductive disorders. *Psidium guajava* leaf extracts are particularly believed to improve erection and impotency in males. All parts (roots, fruits, and leaves) are used in traditional medicine for the treatment of diseases (Levy & Carley, 2012). Anti-inflammatory, hepatoprotective, and antioxidant properties of *P. guajava* leaf extract has been reported Roy *et al.* (2006); Ryu *et al.* (2012). Although many have reported on the therapeutic efficacy of *P. guajava* leaves, there is dearth of scientific information on its effects on reproductive functions.

Thus, this work evaluates the potentials of aqueous ethanolic extract of *P. guajava* leaves on the reproductive parameters of male Albino rats.

Materials and Methods

Chemicals and reagents

Testosterone (INTERCO UK), FSH (Cat. No. KT-59859) and LH (Cat. No. KT – 21064) Kamiya Biomedical Company (USA), Nigrosine (Burgoyne Urbioges and Co. India, Mumbai), Eosin (Lab Tech Chemicals), Sodium citrate (JHD®), (Guangdong Guanghua Sci.Tech Co. Limited China), "Tween® 20" (kernel, China), Giemsa Stain, pentobarbitone sodium (Simagchem Corporation China).

Collection and identification of plant materials

Fresh leaves of *P. guajava* were collected within Makurdi metropolis and identified by a botanist. A voucher specimen number (FUAMH 1027) has been deposited in the Herbarium College of Forestry, Federal University of Agriculture, Makurdi.

Extraction of plant materials

The guava leaves were washed under tap water and air-dried for three (3) weeks at room temperature. They were pulverized with an electric grinding machine and stored in an airtight container. One hundred grams (100 g) of the leaf powder was placed in a conical flask containing a mixture of extracting solvent (absolute ethanol and distilled water) in a ratio of 4:1. The mixture was intermittently stirred throughout the 48 hours extraction period, using a glass rod stirrer. Thereafter, the content of the flask was filtered with a Whatman filter paper No.1 into a measuring cylinder, concentrated at 37℃ in a water bath, and stored in a refrigerator at 4℃ until required for use.

Experimental animals

Twelve (12) weeks old male Albino rats weighing 160 ± 30gm were used for the study. The rats were housed in plastic cages in the Animal House of the Department of Veterinary Physiology, University of Agriculture, Makurdi, under a photoperiod (12L, 12D) and were fed growers mash® (Grand Cereals and Oil mills Limited Bukuru, Jos). The rats were acclimatized for 14 days and were provided water daily. The experimental protocol was under the guidelines on the care and wellbeing of research animals (National Institute of Health U.S, 1985) and was approved by the Departmental Ethics Committee.

Experimental procedure: Twenty-four (24) male Albino rats were randomly assigned into four groups of six rats each:

- Group A (control) were treated with "Tween 20®" which represent the vehicle used
- Group B, treated with aqueous ethanolic extract of *P. guajava* at 100 mg/kg body weight
- Group C, treated with aqueous ethanolic extract of *P. guajava* at 200 mg/kg body weight
- Group D, treated with aqueous ethanolic extract of *P. guajava* at 400 mg/kg body weight once daily for 60 days.

The body weights of the rats at the end of the experimental period were recorded

Collection of blood samples: The procedure of Parasuraman *et al.* (2017) was used for the collection of blood samples. The rats were fasted on day 60 and were anesthetized on day 61 with pentobarbitone sodium (40 mg/kg). Five (5) ml of blood was collected via cardiac puncture into an EDTA and plain sample bottles, where the latter were used for the hormonal

assay, the former were centrifuged at 3000 rpm for 5minutes to obtain serum and stored at -4℃ until used for biochemical analysis.

Measurement of the body and organs weights: The bodyweight of the rats at the end of the experimental period was recorded, and at autopsy, a mid-ventral abdominal incision was made on each of the male rats. Testes, epididymides, vas deferens, seminal vesicles, and ventral prostate gland were exteriorized, blotted free of blood, connective tissues removed and weighed using a Metler balance (C282001, China).

Collection of the organs for histopathology: Animals from each group were sacrificed 24 hours after the last treatment following an ethical procedure. For histopathological examination, a mid-ventral incision of the scrotal sac. The testes and epididymides were exteriorized, blotted free of blood, trimmed of adjoining tissues, and stored in a 10% formalin solution. Samples embedded in paraffin wax were used for serial sections at 5 µm and stained with haematoxylin-eosin, and mounted on a glass slide for microscopic evaluation (Mahmood, 2014).

Semen analysis

Semen motility: Determination of cauda epididymal sperm motility was done using the method described by WHO (1999) and Brar *et al.* (2011). The individual motility was determined by the formula;

$$\text{Motility (Individual) (\%)} = \frac{\text{Number of motile sperm}}{\text{Total no. of sperm (motile + immotile)}} \times 100$$

Sperm concentration: Sperm count was determined using an improved Neubauer haemocytometer by the method described by WHO (1999); Oyeyemi & Ajani (2015). The cauda epididymal tissues were minced and then placed in a sterile universal specimen bottle containing 1 ml of normal saline to allow motile sperm to swim up from the epididymides. Five µl of epididymal fluid was delivered onto a glass slide covered with a 22×22 mm coverslip and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed.

Sperm viability test: The viability (percentage of live spermatozoa was determined using eosin nigrosin stain as described by WHO (1999); Gupta (2014).

$$\text{Motility (Individual) (\%)} = \frac{\text{Number of viable sperm}}{\text{Total no. of sperm (viable + non-viable)}} \times 100$$

Sperm morphologies: Sperm morphology was determined by examining air-dried slides under oil immersion as described by WHO (1999). The abnormal spermatozoa were counted and the percentage calculated.

Hormone assay: Serum testosterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) were assayed using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Results were expressed as mean ± standard deviation (Mean ± SD). The data were analyzed by one-way analysis of variance (ANOVA). The mean was considered to be significant at $P < 0.05$ and was separated using Duncan Multiple Range Test. All statistical analysis was carried out using the Graph Pad Prism version 6.01 for windows.

Results

Body and organ weights of male Albino rats

There was no significant ($p > 0.05$) difference in mean body weight of epididymis, vas deferens, seminal vesicle, and prostate of rats in groups B and C as compared with the control. However, there was a significant increase ($P < 0.05$) in the mean weight of testes, seminal vesicle, and prostate gland of the group D treated with 400 mg/kg body weight as compared with the control (Table 1).

Sperm characteristics of male Albino rats

Administration of the aqueous ethanolic leaf extract of *P. guajava* significantly increased ($P<0.05$) motility, viability, concentration, and normal morphology of sperm cells in a dose-dependent manner. However, dead sperm cells and abnormal morphology of sperm cells were significantly decreased ($p <0.05$) as compared with the control group (Table 2).

Hormone assay

The hormone assay results of male Albino rats following oral administration with aqueous ethanolic extract of P. guava leaves after 60 days of treatment are presented in Figures 1, 2 and 3 for testosterone, FSH and LH, respectively. The extract significantly increased ($P<0.05$) serum testosterone in the treated groups were compared with control. Furthermore, the results of FSH and LH revealed a significant ($p<0.05$) increase when the treated groups were

Table 1: Effects of aqueous ethanolic extract of *Psidium guajava* leaves on the body and relative organ weights of Albino rats

Weight (grams)	Groups			
	Group A	Group B	Group C	Group D
Body weight gain	49.13 ± 1.00	49.5 ± 1.00	47.47 ± 1.00	42.17 ± 1.00
Testis	1.28 ± 0.13[a]	1.37 ± 0.26[a]	1.58 ± 0.11[b]	1.68 ± 0.22[b]
Epididymis	0.49 ± 0.07	0.50 ± 0.07	0.51 ± 0.12	0.52 ± 0.20
Vas deferens	0.06 ± 0.01	0.07 ± 0.03	0.08 ± 0.04	0.28 ± 0.43
Seminal Vesicle	0.52 ± 0.20[a]	0.59 ± 0.39	0.75 ± 0.20	0.86 ± 0.10[b]
Prostate gland	0.18 ± 0.05[a]	0.25 ± 0.12	0.26 ± 0.07	0.34 ± 0.09[b]

Values are mean ± SD. n=6. Values with different alphabet superscripts in the same row are significant at p<0.05. Group A=Control, Group B=100 mg/kg, Group C=200 mg/kg, Group D=400 mg/kg

Table 2: Effects of aqueous ethanol extract of *P. guajava* leaves on sperm motility, viability, concentration and morphology of Albino rats

Dose (mg/kg)	Sperm motility (%)	Viability (%)		Sperm concentration ($\times 10^6$/ml)	Morphology (%)	
		Life Sperm	Dead Sperm		Normal	Abnormal
Control	78.73 ± 7.63[a]	81.00 ± 2.37[a]	19.00 ± 2.37[a]	71.83 ± 4.26[a]	72.83 ± 2.04[a]	27.17 ± 2.04[a]
100	86.32 ± 2.65[b]	85.00 ± 2.00[b]	15.00 ± 2.00[b]	79.67 ± 3.88[b]	75.50 ± 1.87 [a]	24.17 ± 1.72[ab]
200	87.99 ± 1.15[b]	87.17 ± 1.47[b]	12.83 ± 1.47[b]	83.83 ± 5.12[b]	78.33 ± 2.34[b]	21.67 ± 2.34[bc]
400	89.94 ± 1.11[b]	90.83 ± 2.14[c]	9.17 ± 2.14[c]	90.17 ± 4.07[c]	81.67 ± 2.94 [c]	20.00 ± 4.47[c]

Values are mean ± SD n=6. Values with different alphabet superscripts in the same column are significant at p<0.05.

Control = Group A, 100 mg/kg=Group B, 200 mg/kg=Group C, 400 mg/kg=Group D

compared to the control (Figures 2 and 3, respectively).

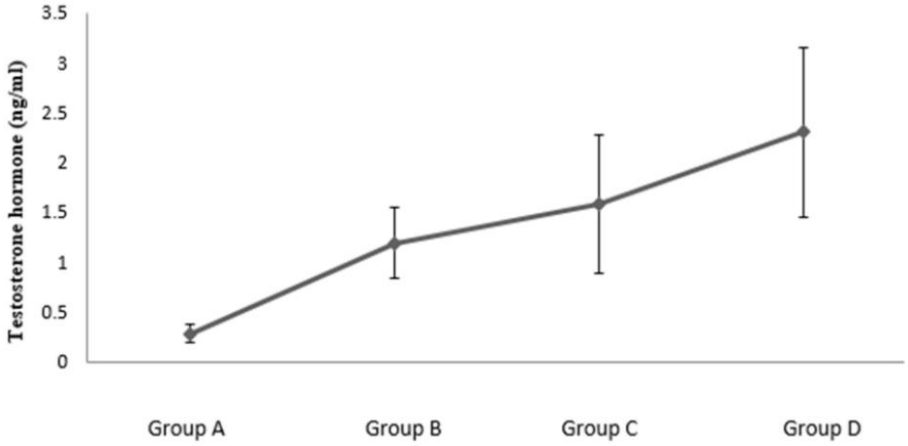

Figure 1: Concentration of testosterone (ng/ml) against graded doses of aqueous ethanolic extract of *Psidium guajava* leaves in male Albino rats (n = 6)

The histopathological analysis does not reveal any lesion to the testis of the rats in the control group (plate 1A), the testis of the rats treated with 100 mg/kg aqueous ethanolic extract of *Psidium guajava* leaf showed normal testicular appearance with a clear seminiferous tubular outline and primary and secondary spermatocytes (plate 1B) and the testicular architecture of the rats treated with aqueous ethanolic extract of *Psidium guajava* leaf at the dose of 200 mg/kg between also showed normal seminiferous tubules with clearly defined basal and luminal compartment and supporting Sertoli cells (plate 1C, however, for the group that was administered 400mg/kg between of the aqueous ethanolic extract of *Psidium guajava*, it revealed degenerating germinative epithelium (G) in the seminiferous tubules (plate 1D).

Discussion

Administration of aqueous ethanolic extract of *P. guajava* to male rats induced a significant increase in the body weights and relative weights of reproductive organs of the rats in the treatment group but especially to the group administered 400 mg/kg body weight. This result corresponds to that of other investigators, Ekaluo *et al.* (2013) and Akinola *et al.* (2007).

The increase in the weight of the reproductive organs in the *P. guajava* treated groups (B, C, and D) may be due to the effect of the extract on the testicular tissues resulting in increased tubular sizes, and this can enhance steroid biosynthesis of the Leydig cells. The increase in the weight of these reproductive organs can equally enhance testicular, epididymal and seminal vesicle functions, and this is closely associated with the androgen regulatory effect, which has been reported to play a fundamental role in the development, growth and normal functioning of the testis and the male accessory reproductive organs (Prins *et al.*, 1991).

The administration of aqueous ethanolic extract of *Psidium guajava* leaves orally for 60 days improved the sperm parameters evaluated (Table 2). This suggests that the extract enhances sperm motility, count, viability, concentration and decreases spermabnormality. The improved sperm qualities observed in the present study agree with the findings of Akinola *et al.* (2007). Oyeyemi *et al.* (2008) in rats treated with *Telfaira occidentalis* leaf extract. Farombi *et al.* (2013), had similarly reported an improvement in sperm quality of rats administered *Garcinia kola* seed extract, which,

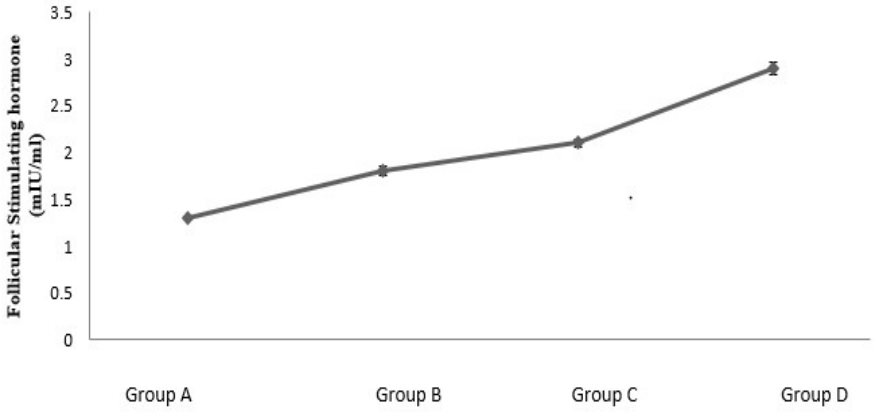

Figure 2: Concentration of follicle-stimulating hormone (ng/ml) against graded doses of aqueous ethanolic extract of *Psidium guajava* leaves in male Albino rats (n = 6)

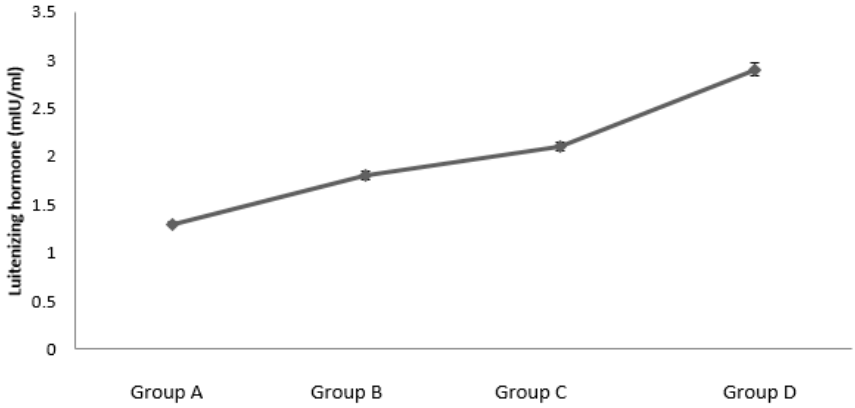

Figure 3: Concentration of luteinizing hormone (ng/ml) against graded doses of aqueous ethanolic extract of *Psidium guajava* leaves in male albino ats (n = 6)

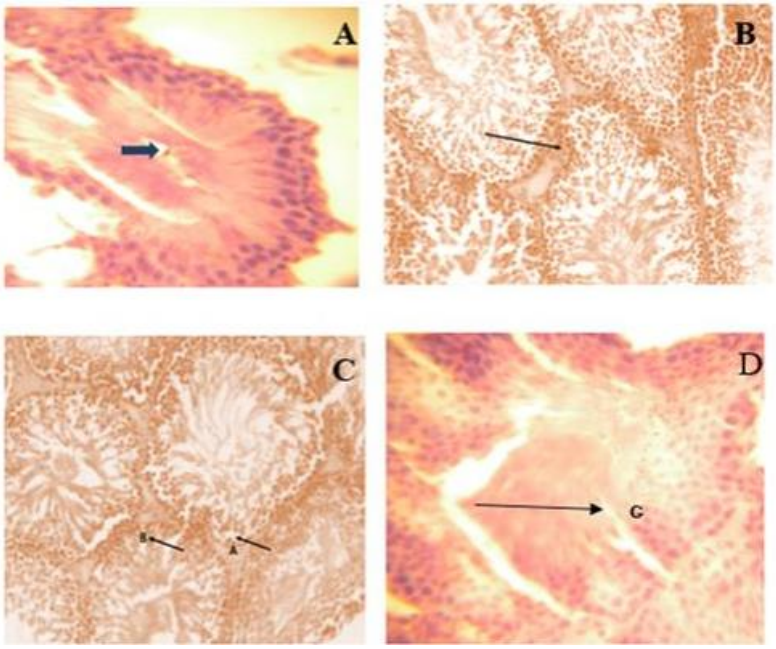

Plate 1: Photomicrographs showing the effect of *Psidium guajava* leaf on the testes of male Albino rats

however, is in contrast with the findings of Abu *et al.* (2013), who observe marked reductions in concentration, motility, viability and morphology of spermatozoa in rats treated with *Garcinia kola*. It has been documented those motile spermatozoa in high concentration and void of abnormalities are greatly connected with fecundity (Aitken *et al.*, 1984) as immotile spermatozoa are similarly correlated with infertility (Abu & Uchendu, 2010). Additionally, it has been stated that the rise in both sperm concentration and the bulkiness of sexual organs is a sign of improvement in male fertility (Woode *et al.*, 2011). This increase in spermatogenesis can be attributed to the rich antioxidant properties of the aqueous ethanolic extract of *Psidium guajava* leaves. It is known that the sperm membrane is endowed with polyunsaturated fatty acids, making them exclusively susceptible to reactive oxygen species (ROS) damage derived from oxygen metabolism. In addition to the action on membrane phospholipid bilayer, the ROS may also damage macromolecules like DNA and proteins (Grignard, 2005). These ROS can cause lipid peroxidation of the sperm plasma membrane leading to damage to the structure of the axoneme, problems in the course of capacitation or the acrosome reaction, and loss of motility, ensuing infertility (Tramer *et al.*, 1998). Akinola *et al.* (2007) reported that ethanolic leaf extract of *P. guajava* demonstrated a valuable effect on gossypol-induced sperm toxicity, and hence it may enhance male fertility due to the rich natural antioxidants it contains. It is conceivable that guava leaf extract also has spermatogenic potentials as the dose-dependent increase in sperm parameters evaluated could be attributed to the antioxidant present in the guava leaves.

In the present study, there was a significant increase in serum testosterone production in the *Psidium guajava* treated groups as compared with the control and this is consistent with the findings of Uboh *et al.* (2010b), who reported a similar increase in this hormone when aqueous extract of *Psidium guajava* leaves was administered to Albino rats. The increase in serum testosterone observed in this study might suggest that the extract contains agents that enhance the maturation of sperm cells in the epididymis and increase the weight of the gonads and accessory sex glands (Bhasin *et al.*, 1988).

This present study also revealed a significant increase in serum FSH and LH following oral administration of guava leaves extract in male Albino rats. This finding is in agreement with Uboh *et al.* (2010a), who recorded an increase in serum testosterone when Albino rats were orally dosed with guava leaves extract. However, Uboh & co-investigators did not observe a significant increase in FSH and LH concentrations. Several investigators who evaluated different medicinal plants reported a similar increase in FSH and LH (Krishnamoorthy *et al.*, 2013; Ofem *et al.*, 2014; Onyegeme-Okerenta & Essien, 2015). It has been observed that as testosterone levels decrease, levels of FSH and LH are expected to increase to stimulate the production of more testosterone (Emanuele & Emanuele, 2001). In this study, high testosterone levels in animals treated with *Psidium guajava* extract were accompanied by corresponding high levels of LH and FSH. This increase in serum gonadotropins and testosterone concentration may be attributed to the bioactive content of the aqueous ethanolic extract of *P. guajava* (Uboh *et al.*, 2010b). However, the specific chemical agent(s) responsible for this increase and the precise mechanism(s) of action is subject to further investigation. From our investigation, the administration of aqueous ethanolic extract of *Psidium guajava* leaves ensued an increase in male sex hormone and size and weight of reproductive organs in a dose-dependent manner. This increase may act directly or indirectly on the pituitary gland secretory function, causing an increase in the androgen. However, the specific chemical agent(s) accountable for the enhanced effect of *Psidium guajava* leaf extract and the mechanism(s) of gonadal hormones stimulating effect was not determined.

The histopathology of the testis of the group treated with 100 mg/kg and 200 mg/kg aqueous ethanolic extract of *Psidium guajava* leaf showed a normal testicular appearance. However, the group administered 400mg/kg aqueous ethanolic extract of *Psidium guajava* revealed degenerating germinative epithelium (G) in the seminiferous tubules. Though there was degeneration of the germinal epithelium of the seminiferous, the sperm concentration, motility and viable sperm cells were higher in this group as compared to the other groups, and this looks contradictory, but it could be that the epithelial degeneration was mild and at the onset stage. This perhaps informs that a very high dose of *P. guajava* leaves extract may be deleterious to the male reproductive organ and invariably spermatogenesis.

In conclusion, the aqueous ethanolic leaf extract of *Psidium guajava* stimulated the production of testosterone and gonadotropins (FSH and LH). It also improved sperm parameters and male reproductive organs weights in a concentration-related manner.

The extract thus has the potential of enhancing male reproductive functions.

Conflict of Interest

The authors declare that there is no conflict of interest.

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