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Assessment of *polyscias fruticosa* (L.) Harm (Araliaceae) leaf extract on male fertility in rats

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ABSTRACT

Background: *Polyscias fruticosa* is used widely as food, remedy for diseases, and as an ornamental across Afro-Asian countries. For instance, *P. fruticosa* is used traditionally as an anti-asthma, anti-tussive, and a muco-suppressant herbal remedy for asthmatics in Ghana. Although many studies have investigated the pharmacological basis of the ethnobotanical uses of *P. fruticosa*, however, its effect on the reproductive system remains completely unknown.

Aim of study: This study assessed effects of *P. fruticosa* leaf extract (PFE) on male fertility and toxicity in adult male Wistar rats.

Materials and methods: After crude preparation of PFE, it was subjected to qualitative phytochemical, thin layer chromatography and gas chromatography mass spectrometry analyses. The effect of PFE was assessed on male fertility and toxicity by using healthy adult male Wistar rats. Rats were randomly assigned to normal saline (5 ml/kg *po*, *n* = 5), Clomiphene Citrate (CL; 50 mg/kg *po*; *n* = 5), and PFE (100, 200, and 500 mg/kg *po*; *n* = 5, respectively) groups and treated for 21 days. On day 22, rats were sacrificed and male fertility parameters (left testis weight, relative testis weight, caudal epididymal weight, caudal epididymal sperm count, sperm motility, sperm morphology, and assessment of male sex hormones and testicular histology) were assessed.

Results: There were no significant changes in bodyweight, weight of left testis, weights of right and left caudal epididymides between treatment groups (PFE and CL) and control. Caudal epididymal sperm count increased in PFE (100 and 500 mg/kg)-treated rats relative to control. Sperm motility relatively increased in PFE-treated rats compared to control. Sperm abnormality decreased in PFE-treated rats, especially in PFE (100 mg/kg) group compared to control. Serum testosterone levels decreased inversely with serum luteinizing hormone levels in PFE-treated rats compared to control. There were minimal-to-no-alterations in histological sections of testis, except vacuolations at primary spermatocyte stage. Glycosides, saponins, cyanogenic glycosides, sterols, and alkaloids were detected in PFE.

Conclusion: PFE improved caudal epididymal sperm count and may be useful as male fertility enhancer but exhaustive safety studies on key male sex organs needs to be established.

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Luteinizing hormone; Male fertility; *Polyscias Fruticosa*; Testicular toxicity; Testosterone

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Introduction

Herbal medicine is not only popular but also its use has increased among populations entrapped in economically challenged tropical regions of the world [1,2] and recently among populations in developed countries. In the USA, it is reported that one out of every four adult Americans admitted to using herbal products in one way or the other in the past years for medical reasons [3,4]. Increase in herbal product usage is not paralleled by the requisite scientific ascertainment of safety, efficacy, and other pharmacological properties relevant for informed and evidence-based herbal therapy. For instance, many herbal medicines have not been scientifically screened for their safety and efficacy for specific indications, yet they are still in common use including those with known or unknown effects on reproduction [2,5-7]. Pharmacologically, many herbs have been confirmed for their activity against specific indications but lack scientific information on their effects on the reproductive system, despite their systemic exposure. One of such herbs with extensive ethnobotanical use but having no scientific ascertainment of its effects on the reproductive system is *Polyscias fruticosa* (L.) Harms. [syn. *Panax fruticosum* L., *Notho panax fruticosum* Miq.] (Family: Araliaceae).

Ecologically, *P. fruticosa* is distributed across Eastern Asia, tropical islands of the pacific region [8] as well as in Africa, particularly in Ghana and Togo [9,10]. Traditionally, crude forms of *P. fruticosa* are used in Asia as tonic, anti-inflammatory, anti-toxin, anti-microbial as well as a spice and a digestive agent [8]. A crude root extract of *P. fruticosa* is documented as a diuretic, febrifuge, treatment for dysentery, neuralgia and rheumatic aches [11]. In Ghana, the extracts of the aerial parts of *P. fruticosa* were shown to be effective against asthma and other symptoms of upper respiratory disease [9,10,12]. Aside from its use as food and disease remedy, it is also used as an ornamental herb [8]. Phytochemical analysis has shown that *P. fruticosa* contains oleanoic acid saponins and polyacetylenes isolated from leaves [13,14] and roots [8,15]. Previously, we demonstrated anti-inflammatory, anti-tussive, muco-suppressant, and anti-asthmatic effects of *P. fruticosa* in guinea pigs and rats [9,10,12]. Quite recently, we demonstrated reproductive toxicity of *P. fruticosa* in pregnant rats (manuscript under review). However, it remains to be established the effect of *P. fruticosa* on reproduction, specifically male fertility in view of the fact that it is orally administered and therefore systemically exposed. This study investigated effects of *P. fruticosa* leaf extract (PFE) on male fertility in rats by specifically assessing the following: sperm count, sperm motility, sperm morphology, testicular male sex hormones [testosterone and luteinizing hormone (LH)], and testicular histology. This has become necessary in view of the utility of *P. fruticosa* as an herbal medicine and also as food among many populations across Afro-Asian regions of the world.

Materials and Methods

Chemicals and drugs

The chemicals and drugs secured for the study were absolute ethanol (PS-Park Scientific Limited, Northampton, UK), clomiphene citrate (CL; DOPPEL FARMACEUTICI S.p.a, Via delle Ande, 15 – 00144-ROME, Italy), dihydrated aluminium potassium sulphate (E. Merck, Darmstadt. Mol.-Gew.474,39), normal saline (Amanta Healthcare Ltd., Gujarat, India), silica gel (VWR International bvba/spr, Haasrode, Belgium, Batch: 09B200018), phosphate buffered saline, DPX, chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), 10% neutral buffered formalin, 1% Eosin W/V (BDH Chemicals LTD, England), and sodium hydrogen carbonate (PROLABO^{R}, EC-EMB 45053).

Collection, identification and authentication of P. fruticosa

P. fruticosa leaves were sourced from the botanical gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Identification and authentication of *P. fruticosa* leaves were done at the Herbal Medicine Department by a pharmacognosist. A voucher specimen (KNUST/HM/13/W010) was deposited at KNUST herbarium as previously reported [9,10].

Preparation of PFE

PFE was prepared by following a previously described method [9,10,12] with some modifications. Briefly, fresh *P. fruticosa* leaves (2.6 kg) were washed, shade-dried completely, and pulverised into powder (1.8 kg). The powder was soaked in absolute ethanol (4.8 L) for 72 hours, and then filtered. A rotary evaporator (B'U'CHI Olibath B – 485) was used to retrieve the ethanol from a dark-green filtrate at 50°C. The residue was completely dried in a desiccator containing activated silica gel. The crude extract yielded 63.0 g, given

a percentage yield of 3.4%. The whole extraction procedure was repeated until enough extract was obtained for the study. The final crude extract obtained was named PFE. PFE was stored in a refrigerator at 4°C until use.

Qualitative phytochemical screening of PFE

Phytochemical composition of PFE was ascertained by using previously described standard qualitative methods [16,17]. Subsequently, thin layer chromatography (TLC) and gas chromatography mass spectrometry (GC-MS) analysis were conducted on PFE as reported earlier (manuscript under review).

Dose selection

PFE (100, 200 and 500 mg/kg *po*) was selected based on our previous studies [9,12,18]. Dose of CL was based on clinical doses (50 mg/kg *po*) [19]. Bodyweight of rats were measured daily and doses were accordingly adjusted to reflect bodyweight changes.

Acquisition of experimental animals and husbandry

Eight weeks old healthy male Wistar rats weighing 120–200 g were obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana. The rats were housed in aluminum cages (40 × 35 × 15 cm) with a base dressed with saw dust as bedding. Rats were maintained under 12 hours light/dark cycle, ambient temperature and normal humidity. Rats were fed standard pellet diet (Essaar grower mash, Essaar Agro-West Africa Ltd, Ghana) and had access to water *ad libitum*. Rats were allowed to acclimatize with laboratory conditions for two weeks before the start of experimentation. Rats were handled and treated humanely in strict adherence to standard guidelines as enshrined in the "Principles of laboratory animal care" (NIH publication No.85–23, revised 1985) as well as specific national and institutional requirements regarding the use of animals in scientific studies.

Measurement of body and organ weights

Bodyweights of rats were measured daily. Subsequently, bodyweights were measured prior to induction of anesthesia in rats using pentobarbital (60 mg/kg ip) on day 22. Testis and epididymis were surgically removed, trimmed of connective tissues and weighed (Sartorius LP 1200) as previously described [20] with some modifications. Relative organ weight (ROW) for testis and epididymis were determined as previously described [18] with some modifications. The formula used in calculating ROW is shown below:

ROW=Absolute organ weight (g)Body weight gon day 22

Measurement of epididymal sperm count

Epididymal sperm count was carried out according to previous methods [21,22] with some modifications. Briefly, the left and right caudal epididymides from a rat were removed into two sterile petri dishes. They were minced with sharp scissors and the ripped epididymides were transferred to two test tubes labeled as L (left) and R (right) containing 4 ml phosphate buffered saline (PBS) at 37°C. The sperms were then allowed enough time (5–10 min) to disperse. Approximately 10 µl of the diluted sperm suspension was transferred to each counting chamber of an improved Neubauer counting chamber (Haemocytometer) using a fine bore Pasteur pipette to charge the chamber and was allowed to stand for 4 minutes. The chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was calculated by multiplying the number of counted spermatozoa by five and expressed as shown below:

Sperm count = 5 x 106ml,

where (*X*) is the number of spermatozoa counted in five 16-celled squares.

Assessment of sperm motility

Epididymal sperm motility was carried out as described previously [4,23,24]. Briefly, a cut was made at the cauda of the contralateral epididymis. Resulting fluid from the cut was diluted in PBS. A drop of diluted sperm suspension was dropped on a pre-warmed slide, covered with a cover slip and observed under a microscope. Sperm motility was estimated in six separate randomly selected fields through a light microscope at a magnification of 40×. The mean of the six (6) estimations was used as the final motility score. Sperm motility was estimated in three (3) categories as previously described [25]. These categories were defined as progressive (where the spermatozoa are exhibiting a linear motion), nonprogressive (where the spermatozoa are exhibiting a local motion but not to the front or backward) or immotile (where the spermatozoa are not in motion at all). These three parameters

were recorded and expressed in percentages (%) as shown below:

% *P* or *N* or *I* motility

=[*P* or *N* or *I*][Sum of the three Categories for one field] 100; *P*: Progressive motility, *N*: Non progressive, *I*: Immotile

Assessment of sperm morphology

Sperm morphology was evaluated according to a previous method [26] with some modifications. Briefly, a 1 ml sperm suspension from rats in each group was pipetted into an appropriately labeled test tube. Subsequently, 5 drops of 1% Eosin yellow was added and gently mixed manually. The resultant suspension was incubated for 45 minutes at room temperature to facilitate staining. By using a pipette, the suspension was gently agitated. A drop of each suspension was transferred to a clean slide. Smears were prepared by simply spreading the drop in a circular pattern until its size was just big enough (1.8–2.0 cm^2) to facilitate early air-drying. Dried smear was preserved by mounting with cover slips using DPX mountant. Well over 100 sperms per rat from each experimental group were examined as previously described [23].

Measurement of serum levels of testosterone and luteinizing hormone

Blood samples were collected by cardiac puncture using a needle (23 g) and a syringe (3 ml). Collected blood was emptied into blood labeled tubes. Blood samples were centrifuged at 3,500 rpm for 20 minutes few minutes after collection. The serum obtained was separated from the cells into Eppendorf tubes and stored at −20°C until use. Serum levels of LH and testosterone were determined by using an ELISA assay kit (Human Gesellschaft fur Biochemica und Diagnostica mbH Max-Planck-Ring 21 65205, Wiesbaden, Germany) by strictly following manufacturer's instructions. The sensitivity of hormone detection was 0.005 ng/ml. To prevent inter-assay errors all samples were analyzed in a single assay.

Histological assessment of testis

Left testis of each rat in each group was isolated, fixed, sectioned, and stained with hematoxylin and eosin (H&E) according to a previous method [27] with some modifications. Briefly, isolated left testis was fixed in 10% formalin, embedded in paraffin, cut at 3 μm sections, de-waxed, rehydrated, and stained with H&E. Sections were first stained with hematoxylin, washed in running water until appearance of blue color. Subsequently, sections were stained with eosin and viewed under light microscope at a magnification of 40× by three independent persons. Representative photomicrographs were generated for rats in each group.

Statistical analysis

Data are expressed as mean ± SD. Normality analysis was conducted to determine the nature of data distribution before selection of statistical test. Mean comparisons between groups was done by One Way ANOVA using GraphPad Prism for Windows Version 6.01 (GraphPad Software, San Diego, CA, USA) followed by Tukey's *post hoc* multiple comparison. $P < 0.05$ was considered statistically significant in all analysis.

Results

Phytochemical analyses of PFE

Phytochemical screening showed the presence of glycosides, cyanogenic glycosides, saponins, alkaloids, and sterols in PFE as observed earlier (manuscript under review). Subsequently, a TLC analysis showed four (4) spots. Subsequently, a GC-MS profiling of PFE showed twelve (12) peaks out which eight (8) marched library compounds.

Body weight and relative organ weight assessments

Although bodyweights of treatment groups compared to control on day 22 was not significantly ($P \geq 0.05$) different from that of control. PFE (500 mg/kg) was lower compared to control and all other groups. Weight of left testis was comparable among all groups even though it was lower in the case of PFE (500 mg/kg) group. Except PFE (100 mg/kg), the weight of left caudal epididymis was comparable among treatment and control groups. Mean weight of left caudal epididymis of PFE (500 mg/kg) and CL groups was significantly different compared to PFE (100 mg/kg). Except CL group, weight of right caudal epididymis was comparable among all groups (Table 1). There was a dose-dependent increase in mean caudal epididymal weight and relative caudal epididymal ratio in PFE (100, 200, and 500 mg/kg) groups compared to control and CL groups (Figs. 1 and 2).

Epididymal sperm count, sperm motility and morphology

Except PFE (200 mg/kg) and CL groups, the mean left and right caudal epididymal sperm counts of PFE

Table 1. Assessment of body and testicular weights in male Wistar rats after PFE and CL treatments.

Groups	Wt of rats (g)	Wt of left testis (g)	Wt of Left testis/ Wt of Rat	Wt of left caudal epididymis (g)	Wt of Right caudal epididymis (g)
NS	202.52 ± 8.50	1.32 ± 0.15	0.0065 ± 0.001	0.20 ± 0.01	0.20 ± 0.01
LD	189.55 ± 33.04	1.20 ± 0.06	0.0064 ± 0.001	0.14 ± 0.02*	0.15 ± 0.02
MD	198.70 ± 8.68	1.33 ± 0.03	0.0067 ± 0.000	0.17 ± 0.02	0.17 ± 0.02
HD	191.29 ± 13.82	1.06 ± 0.37	0.0055 ± 0.001	0.21 ± 0.04#	0.22 ± 0.06
CL	196.98 ± 7.24	1.26 ± 0.12	0.0064 ± 0.000	0.23 ± 0.05#	0.28 ± 0.08#†
F-value	0.289	1.0028	0.629	3.375	3.717
P-value	0.878	0.439	0.653	**0.041**	**0.042**
N	3	3	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS: Normal saline group, †significantly different in comparison with MD: Medium dose (200 mg/kg) of PFE, CL: Clomiphene citrate (50 mg/kg); Wt: weight; PFE: *Polyscias fruticosa* leaf extract.

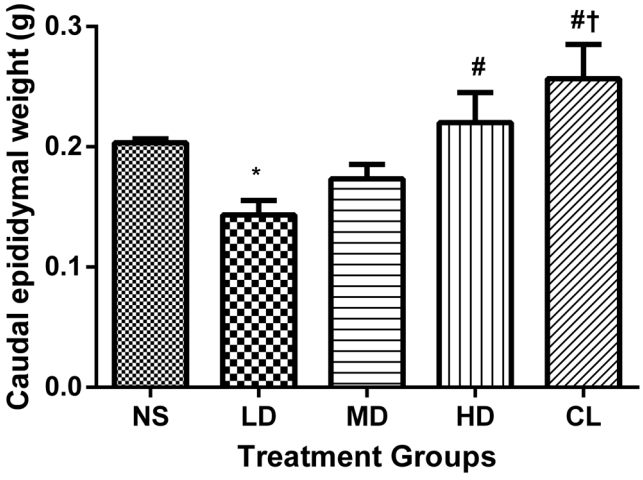

Figure 1. Average changes in weight of caudal epididymis in male rats after 21-day oral treatments. *Significantly different in comparison with NS: Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group; CL: clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

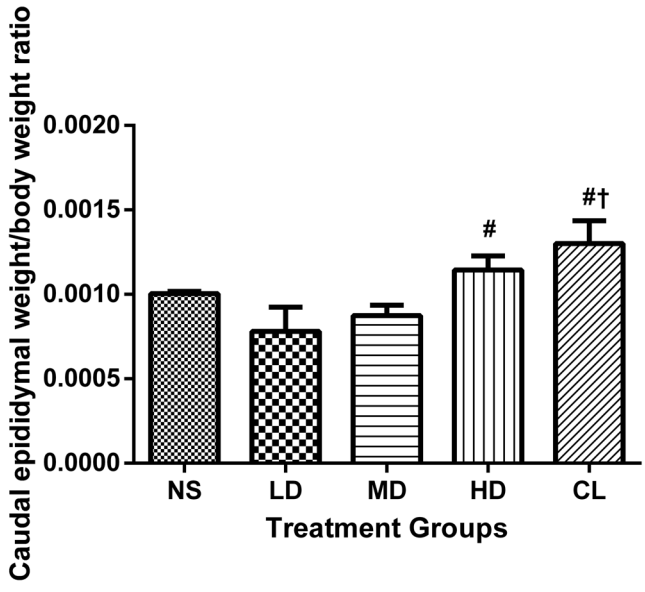

Figure 2. Changes in relative caudal epididymal weight to body ratio of male rats after 21-day oral treatments. *Significantly different in comparison with NS: Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group; CL: clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

(100 and 500 mg/kg) increased significantly compared to control (Fig. 3 and Table 2). There were no significant differences between mean relative left and right caudal epididymal weights between treatments and control except in the case of PFE (500 mg/kg) and CL groups (Table 2). Average caudal epididymal sperm count significantly ($P \leq 0.05$) increased in PFE (100 and 500 mg/kg) groups compared to control (Fig. 3 and 4). Sperm motility with respect to progressive and immotile was comparable among treatment groups (PFE and CL) and control (Table 3). Sperm motility with respect to non-progressive was significantly reduced in treatment groups (PFE and CL) compared to control (Table 3). Except PFE (100 mg/kg) group which showed low sperm abnormality, the remaining treatment groups and the control had comparable sperm abnormality and morphology (Fig. 5 and Table 4).

Evaluation of serum testosterone and luteinizing hormones

Mean serum testosterone levels decreased in PFE (100, 200, and 500 mg/kg) and CL groups inversely with serum LH levels compared to control (Table 5) but the mean difference was statistically insignificant ($P \leq 0.05$). Mean serum LH levels decreased in PFE (100 and 500 mg/kg) and CL groups compared to control, while that of PFE (200 mg/kg) was comparable to that of control (Table 5).

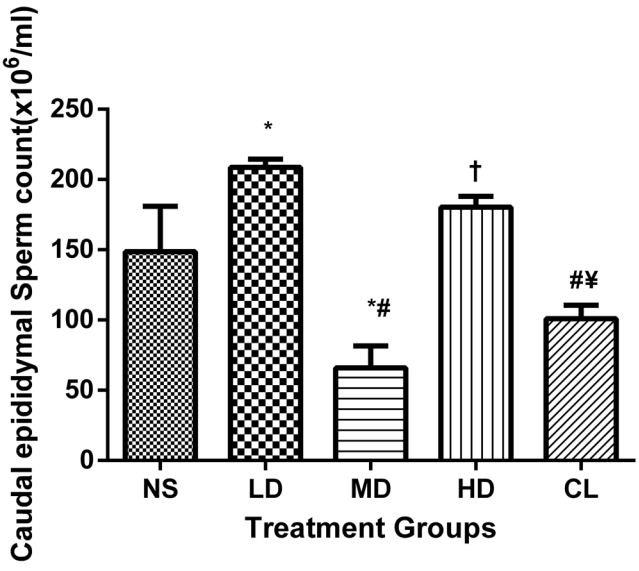

Figure 3. Spermatozoa spread from caudal epididymal spermatozoa suspensions of male rats after 21-day treatment with drugs observed under a microscope. (A) NS: Normal saline group, (B) LD: Low dose (100 mg/kg) group, (C) MD: Medium dose (200 mg/kg) group, (D) HD: High dose (500 mg/kg) group, and (E) CL: clomiphene citrate (50 mg/kg).

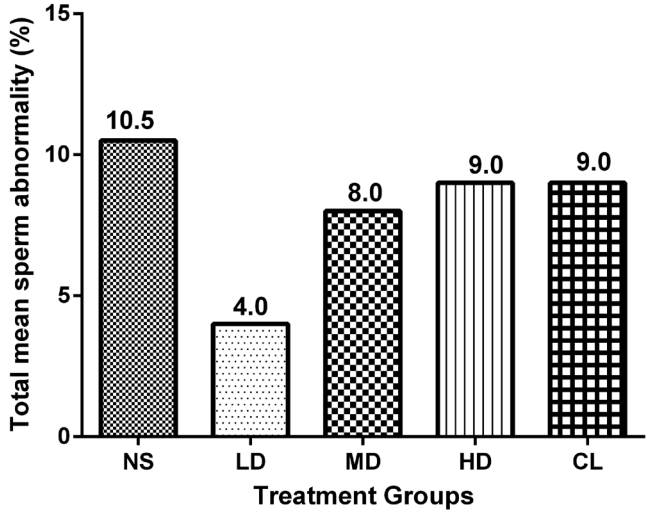

Figure 4. Average caudal epididymal sperm count of male rats after 21 day oral treatments. *Significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group, ¥significantly different in comparison with HD: High dose (500 mg/kg) group and CL: Clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

Table 2. Assessment of caudal epididymal sperm count in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Groups	Left caudal epididymal sperm count (×10⁶/ml)	Right caudal epididymal sperm count(×10⁶/ml)	Left Caudal epididymal wt/body wt ratio	Right Caudal epididymal wt/body wt ratio
NS	151.67 ± 50.08	145.00 ± 62.65	0.001 ± 0.000	0.001 ± 0.000
LD	240.00 ± 5.00*	176.67 ± 23.63	0.001 ± 0.000	0.001 ± 0.000
MD	66.67 ± 44.81*#	65.00 ± 10.00*#	0.001 ± 0.000	0.001 ± 0.000
HD	191.67 ± 17.56†	168.33 ± 30.14†	0.001 ± 0.000#	0.001 ± 0.000
CL	70.00 ± 26.46*#¥	101.67 ± 10.41#¥	0.001 ± 0.000#	0.001 ± 0.000#†
F-value	3.717	15.543	2.766	3.170
P-value	**0.042**	**<0.0001**	0.087	0.063
N	3	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD - Low dose (100 mg/kg) of PFE, †significantly different in comparison with MD - Medium dose (200 mg/kg) of PFE, ¥significantly different in comparison with HD - High dose (500 mg/kg) of PFE; CL-Clomiphene citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract.

Table 3. Assessment of sperm motility parameters after 21-day PFE and Clomiphene Citrate treatments in male Wistar rats.

Groups	Progressive (%)	Nonprogressive (%)	Immotile (%)
NS	41.50 ± 10.83	41.27 ± 11.11	31.10 ± 12.88
LD	53.13 ± 4.60	20.83 ± 4.35*	24.67 ± 7.85
MD	49.97 ± 3.57	15.07 ± 7.35*	29.70 ± 12.14
HD	44.97 ± 11.01	26.90 ± 5.28*	28.10 ± 11.42
CL	40.87 ± 8.33	15.97 ± 2.36*	39.60 ± 8.11
F-value	1.253	7.515	0.811
P-value	0.350	0.005	**0.546**
N	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD - Low dose (100 mg/kg) of PFE, †significantly different in comparison with MD - Medium dose (200 mg/kg) of PFE, ¥significantly different in comparison with HD - High dose (500 mg/kg) of PFE; CL - Clomiphene Citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract. Progressive (Many spermatozoa exhibit linear motion); Non-progressive (Many spermatozoa exhibits local motion but not to the front or backward); Immotile (Many spermatozoa were not in motion at all).

Table 4. Assessment of sperm morphology in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Group	Pinhead (%)	Flattened head (%)	Tailless head (%)	Headless sperm (%)	Bent neck (%)	Broken tail (%)	Coiled tail (%)	Bent tail (%)
NS	0	1	3.5	2	2.5	0	0.5	1
LD	0	0	1	1	1	0	1	0
MD	0	0.5	1.5	1.5	1.5	0	1.5	1.5
HD	0	1	2	1.5	2	0	1	1.5
CL	0	0	2.5	2	1.5	0	1	2

NS – normal saline (5 ml/kg); LD – Low dose (100 mg/kg); MD – Medium dose (200 mg/kg); HD - High dose (500 mg/kg); CL – Clomiphene citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract

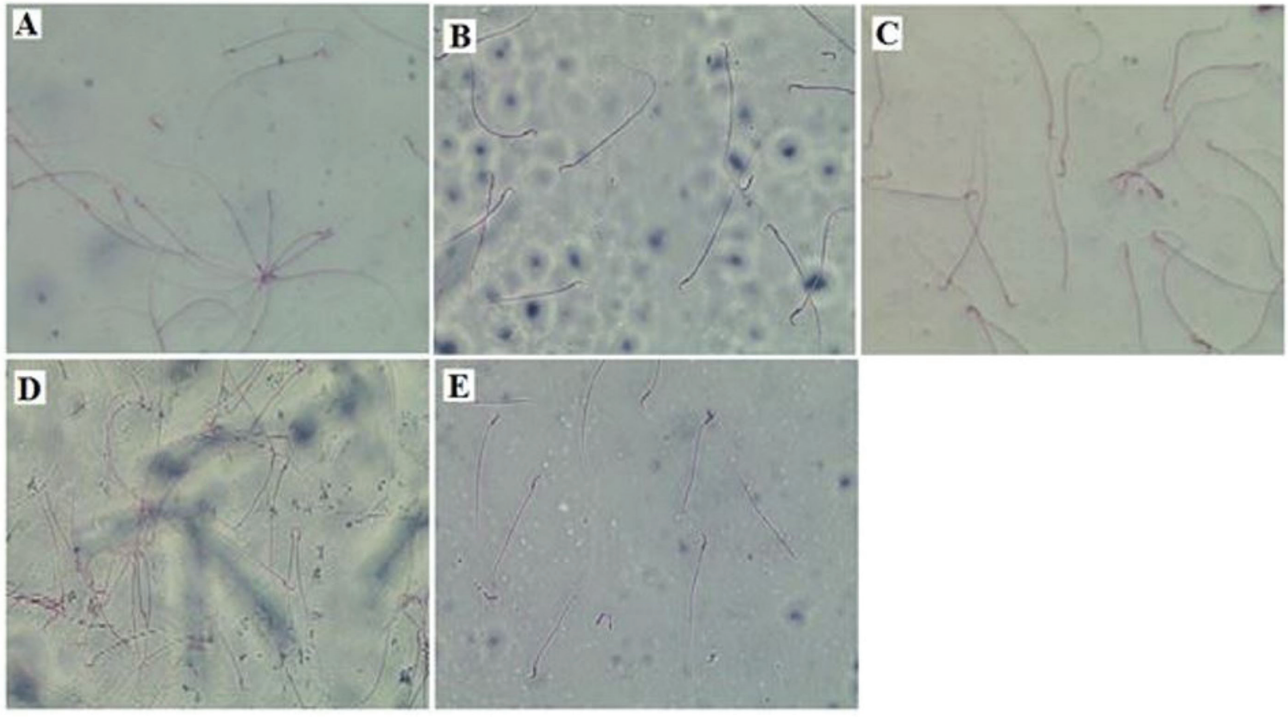

Figure 5. Total mean sperm abnormality of male rats after 21 day oral treatments. NS: normal saline (5 ml/kg), LD: low dose (100 mg/kg), MD: medium dose (200 mg/kg), HD: high dose (500 mg/kg), CL: clomiphene citrate (50 mg/kg).

Table 5. Assessment of serum levels of male sex hormones in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Groups	Testosterone (ng/ml)	LH (IU/L)	Testosterone /LH ratio	LH/Testosterone ratio
NS	3.11 ± 2.42	0.39 ± 0.09	8.57 ± 6.62	0.53 ± 0.35
LD	2.38 ± 1.73	0.11 ± 0.13	58.74 ± 31.36	0.22 ± 0.21
MD	0.30 ± 0.27	0.40 ± 0.59	8.77 ± 7.59	4.78 ± 4.55
HD	0.59 ± 0.35	0.18 ± 0.04	3.86 ± 2.53	3.54 ± 3.31
CL	0.12 ± 0.11	0.07 ± 0.05	82.51 ± 81.74	1.19 ± 0.81
F-value	1.307	0.92	0.828	0.619
P-value	0.332	0.489	0.537	0.659
N	3	3	3	3

ANOVA column comparison at $P < 0.005$, NS – normal saline (5 ml/kg), LD – Low dose (100 mg/kg) of PFE, MD – medium dose (200 mg/kg) of PFE, HD – high dose (500 mg/kg) of PFE, CL – clomiphene citrate (50 mg/kg), IU – Internal unit and LH – Luteinizing hormone, PFE – *Polyscias fruticosa* leaf extract.

Evaluation of testicular histology

The histological sections of left testis of PFE (200 mg/kg and 500 mg/kg)-treated rats compared to control rats were fairly of normal architecture with oval outline, normal seminiferous epithelium, and numerous spermatozoa within the lumen as well as intact interstitium (Fig. 6). However, vacuolation was common across all groups.

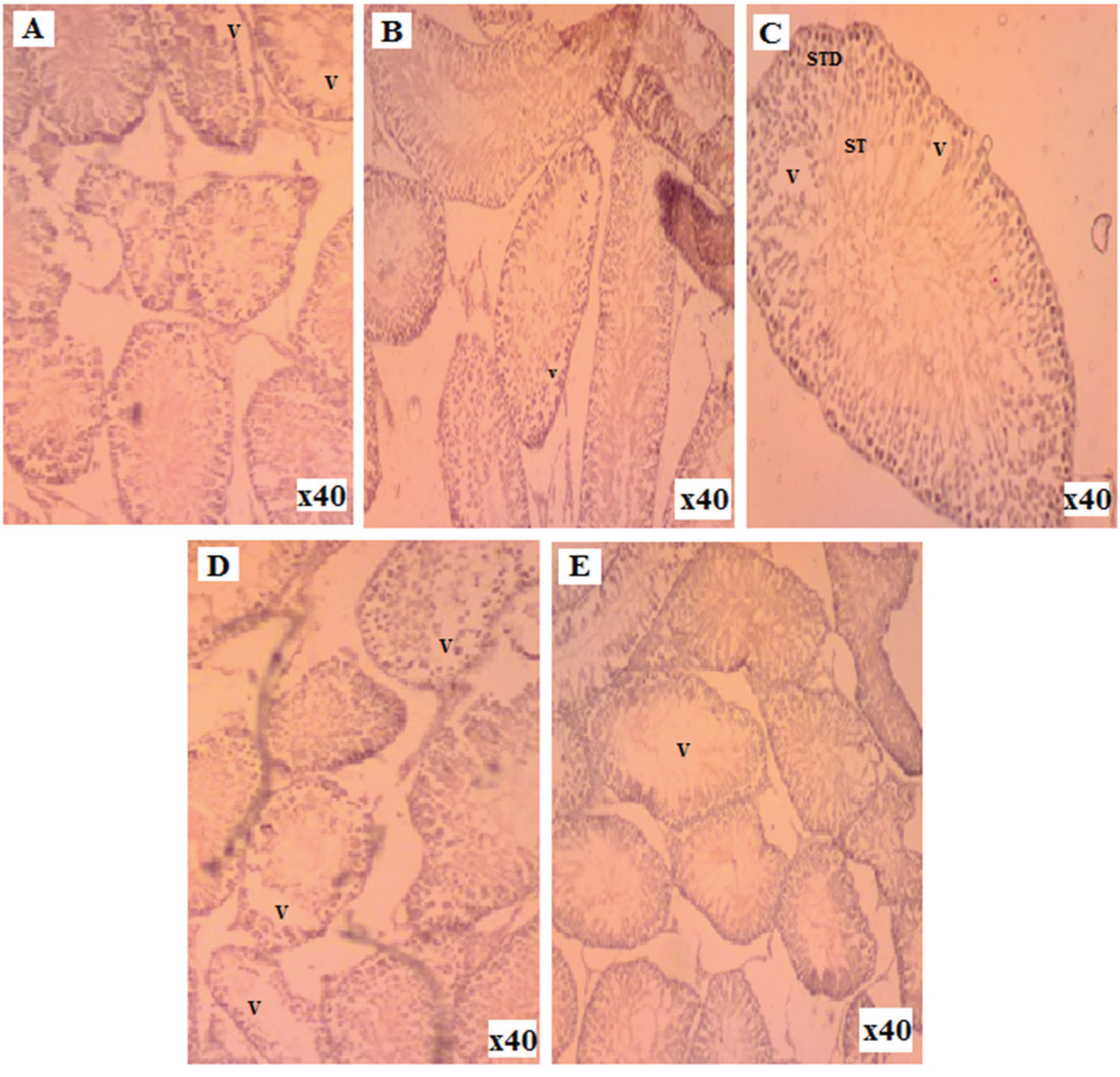

Figure 6. Histomicrographs showing effects of 21-day drug treatments on left testicular microstructure of control (A), LD (B), MD (C), HD (D), and CL (E). NS: normal saline (5 ml/kg), LD: Low dose (100 mg/kg), MD: medium dose (200 mg/kg), HD: high dose (500 mg/kg), CL: CL (50 mg/kg), ST: Sperm tail, STD: Spermatids and V: Vacuolation.

Discussion

Most herbal medicines are orally administered for the treatment of specific diseases and more often their pharmacological assessment is limited to efficacy with respect to the claimed ethnobotanical and folk uses. In view of the systemic exposure of herbal medicines, they certainly produce unknown physiological and pharmacological effects on non-target organ systems of the body including the reproductive system. Previously, folk uses of *P. fruticosa* in Ghana such as anti-inflammatory, anti-tussive, muco-suppressant, and anti-asthma effects were studied [9,10,12] but how *P. fruticosa* affects male fertility and any possible toxic effects on male reproductive organs in view of its systemic exposure to both men and women remain unknown. Presently, we demonstrated that 21-day oral administration of PFE to male rats improved epididymal sperm count (Fig. 3), sperm motility (Table 3), caudal epididymal weight, decreased sperm abnormality (Fig. 4 and Table 4), especially in PFE (100 mg/kg) group and decreased serum levels of testosterone inversely with LH without producing significant changes in bodyweight, weight of male reproductive structures

(testis, left and right caudal epididymides) and testicular structure.

Improvement in weight and size of reproductive structures such as testis and seminiferous tubules are directly related to normal male sexual development [28]. In effect, increase in weight of reproductive organs secondary to drug treatments indicates that perhaps such agents may improve reproductive processes including spermatogenesis. In this study, although there were changes in bodyweight, left testis and caudal epididymal weights between treatment (Low dose, medium dose, high dose and CL) and control groups, these changes were not statistically significant, indicating that PFE may promote normal development of male reproductive organs since it did not decrease the weight of the tested male reproductive structures. Of note reproductive organs are hormone-sensitive, especially to sex hormones [29,30]. As a result, normal development of reproductive organs can be improved with natural products having hormone-like effects mediated through the hypothalamo-pituitary-gonadal axis which plays a crucial role in sexual reproduction [31]. The hypothalamus via a system of releasing hormones regulates the actions of the pituitary gland which secretes many tropic hormones including LH; LH in turn stimulates Leydig cells to secrete testosterone. Free circulating testosterone promotes spermatogenesis through its stimulatory effects on Sertoli cells in the seminiferous tubules [28,32,33]. In effect, increase in circulating levels of testosterone is shown to be correlated with increased epididymal sperm count while LH only promotes testicular development [34]. Also, male reproductive structures such as seminal vesicles and epididymis are highly androgen-sensitive [30,35,36]; therefore, improvement in sperm output and viability is a direct manifestation of the actions of androgens, specifically testosterone. Interestingly from the present study, it was observed that epididymal sperm count and sperm motility in PFE-treated male rats particularly those in PFE (100 and 500 mg/kg) group increased relative to control while their corresponding serum testosterone levels decreased inversely with LH even though increased testosterone level is crucial for spermatogenesis which normally manifest as increased sperm count and sperm motility [6,34]. The inverse serum levels of testosterone and LH as observed in this study was expected in view of the inhibitory effect of testosterone on LH [29] which serves as part of a negative feedback mechanism to ensure homeostatic regulation. Although this observation is the first to be observed in male rats treated with *P. fruticosa* leaf extract, it however mirrors a previous observation in adult men treated with Maca (*Lepidium meyenii*), where the sperm count and sperm motility improved quiet independently of testosterone, LH and follicle stimulating hormone [37] indicating the possibility of herbal medicines such as PFE having an unknown mechanism of improving sperm count and sperm motility in an androgen-independent manner. Contrastingly, the present observation is at variance with that of other herbs which demonstrated in male rodents a direct relationship between testosterone levels and sperm count as in *Cichorium intybus* [38], *Taraxacum officinale* [6], and *Achillea millefolium* [39], in which it was demonstrated that decreased caudal epididymal sperm count correlated with decreased androgen levels particularly testosterone levels. Comparing the present observation with others [6, 38, 39] with respect to the relationship between testosterone levels and sperm count, it is possible that herbal medicines such as PFE may have multiple mechanisms of improving sperm quality. Although the present result is insufficient to completely explain, it is possible that PFE may have improved epididymal sperm count, sperm motility and decreased sperm abnormality in a testosterone-independent manner through an unknown mechanism.

Although PFE treatment improved important male fertility parameters including sperm count, sperm motility, sperm abnormality, left testicular weight, and caudal epididymal weight, it produced minimal-to-no alteration in histological sections of left testis, mostly dominated by vacuolation (Fig. 6) which is commonly mistaken to be a sign of testicular toxicity [40].

Biological and pharmacological activities of herbs are attributable not only to their diverse phytochemical composition but also the complex phytochemical-to-phytochemical interactions [1,41]. In this study, saponins, cyanogenic glycosides, glycosides, sterols, and alkaloids were identified in PFE confirming our previous report [9,10,12]. Although this study did not assay individual phytochemicals in PFE, but given that all the identified phytochemicals were in PFE and could have interacted chemically in unknown ways, it is not unreasonable to attribute the observed spermatogenic effects of PFE to its phytochemical constituents. Saponins from *Albizia lebbeck* and *Cissus populnea* were shown to have no spermatogenic effects in men after 72 hours exposure and also decreased testis, epididymis,

and seminal vesicle weights [42,43]. Similarly sterols have been shown to decrease sperm quality [44,45] just as some alkaloids extracted from herbs [46,47], which deductively indicate that the spermatogenic effect of PFE may not be related to individual phytochemicals but could be attributed to a possible synergistic interactions between all the constituent phytochemicals. This study could have benefited from determining caput and corpus epididymal sperm counts as well as the spermatogenic mechanism of PFE particularly how it may modulate androgen receptors. Nonetheless, the present results provides not only preliminary evidence for possible utility of *P. fruticosa* leaf as a male fertility enhancer but also provides a compelling reason for further studies on *P. fruticosa*, particularly its safety on key male sex organs.

Conclusion

P. fruticosa leaf extract has increased caudal epididymal sperm count in 8 weeks old male Wistar rats quiet independently of testosterone. PFE may be relevant for improving male fertility parameters but its safety on key male sex organs needs to be established.

Author Contributions

A Boye conceived the idea, designed the experiments, wrote the draft manuscript, and critically revised the final manuscript for an important intellectual content. **VYA Barku** carried out phytochemical screening and TLC analysis. **GA Koffuor** read and edited the final manuscript for important intellectual content together with **A Boye**. **AKO Owusu** and **EA Asiamah** performed experiments, data analysis, and literature searches.

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Conflict of Interest

Authors declare no conflict of interest.

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