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Research Article

THE LEAF-AQUEOUS EXTRACT OF *Pseudopanax arboreus*(Pa)(ARALIACEAE) (L.F.PHILLIPSON)REPAIRS SOME REPRODUCTIVE INDICES IMPAIRED BY AMITRIPTYLINE IN MALE RATS

***[1]Egbe B. Besong, [2]Nchegan Benjamin, [3]Ateufack Gilbert and [3]Albert Kamanyi**

[1] Zoology Laboratory, Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon.
[2] Department of Fisheries and Aquatic Ecosystems Mnagement ; Institute of Fisheries and Aquatic Science at Yabassi ; University of Douala ; Cameroon.
[3] Animal Physiology and Phytopharmacology Laboratory, Department of Animal Biology, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.

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ABSTRACT

Antidepressants have been associated with toxicity of the male reproductive system, altering testicular architecture, affecting hormonal profile and sperm characteristics, especially when administered for a long duration. Earlier, we reported the sex-enhancing potentials of *Pseudpanax arboreus* in normal male rats and its potentials to correct sexual deficiencies induced by amitriptyline in male rats. We undertook the present investigation to determine the curative properties of the leaf-aqueous extract of *P. arboreus* (Pa) on some indices of amitriptyline-induced male sexual impairments in rats. Sexually mature Albino male rats pre-exposed to10 mg/kg body weight of amitriptyline hydrochloride suspension for 56 days then allowed to self-recover for 11.5 days, were divided into 5 groups of 8 rats each and treated as follow: Group 1, pre-treated with amitriptyline, was allowed time for self-healing; Group 2 received 10ml/kg distilled water and served as negative control; Group 3 received 6mg/kg sildenafil citrate™ (Viagra) (positive control); while Groups 4 and 5 were administered 75 and 150mg/kg of the aqueous extract of *P. arboreus*, respectively. All extract-treated animals showed significant increase in relative weight of some sex and accessory organs, increased sperm motility and concentration as well as increased plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone compared to the negative control animals. Also, the extract induced a significant improvement in the histo-architecture of the testes in sexually impaired animals, compared to the control group. In conclusion, the leaf-aqueous extract of *Pseudopanax arboreus* possesses curative properties on some male reproductive indices induced by amitriptyline.

Keywords: Amitriptyline, Antidepressant, Male Reproductive System, *Pseudopanax arboreus*, Sexorgans, Sperm.

INTRODUCTION

Reproduction is the biological process by which new individual organisms called offspring are produced from their parents. Reproduction remains a fundamental feature of all known life since each individual organism exists as the result of reproduction. Unfortunately, like every other physiological process, reproduction can be impaired by a number of factors which may result in sexual dysfunction.

Male sexual dysfunction (MSD) can result from various lifestyle and medical factors such as antidepressants. These are drugs used to correct psychological disorders including stress, mood disorders, personality disorder, affective psychosis, schizophrenia and whose prolonged administration has been linked to various forms of male sexual dysfunction. There are 3 major groups of antidepressants: (1) monoamine oxidase inhibitors (MAOIs), blamed for about 40% of MSD cases and

*Corresponding Author: Egbe B. Besong, Zoology Laboratory, Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon Email: egbe.ben@ubuea.cm.

involved in decreased libido, erectile dysfunction, delayed orgasm, and impaired ejaculation; (2) tricyclic antidepressants (TCAs) linked with about 30% of MSD and having similar actions with the MAOIs; and (3) selective serotonin reuptake inhibitors (SSRIs), blamed for between 58% and 73% of MSD cases and whose effects are similar to those of the first two groups (Conaglen and Conaglen, 2010). All categories of antidepressants provoke MSD by altering the availability and actions of neurotransmitters or hormones that intervene in the male sexual response cycle either at the central or the peripheral level (Stein and Hollander, 1994; Balon 1997; Nurnberg *et al*., 2003). The use of these drugs remains rampant in most Cameroonian rural settings today where a large part of the population relies on self-medication with poor or no knowledge on the dosage or possible side effects. Some have been extensively used and can be considered "over-the-counter" drugs. These patients are therefore likely to suffer from AIMSD. There is about 24.9% global prevalence of antidepressant-induced MSD (AIMSD), though statistics on its prevalence in Africa and Cameroon still remain scanty. Male sexual impairment impacts a heavy psychological toll and loss of self-esteem on the individual and remains one of the major causes of male infertility, accounting for about 25% of infertility among couples (Nwajiaku *et al*., 2012). As stated by (Yakubu *et al*., 2008; Yakubu *et al.*, 2011; Ajiboye *et al*., 2013), male sexual deficiencies require attention since everyone needs to procreate (reproduce) to ensure the continuity of his/her lineage, whether depressed or not. An example of antidepressant is amitriptyline which has been reported to weaken sperm parameters via increased production of ROS and toxicity (Ladan *et al*., 2018). Also, Lauren and Mary (2020) have reported that Fluoxetine has gonadotoxic effects that can result in decreased sperm concentration and motility, increased deoxyribonucleic acid fragmentation as well as decreased reproductive organ weights. Studies by Solek *et al*. (2021) have clarified the patho mechanisms of antidepressants action and their associated toxicity towards the reproductive system, emerging issues linked with animal or human reproductive health, and treatment of mood disorders. In a previous study, we demonstrated that prolonged consumption of amitriptyline results in male sexual dysfunction (Egbe *et al*., 2018). Antidepressants can therefore be blamed for male reproductive deficiencies ranging from lack of sexual interest (libido), through inability to achieve and maintain an erection, to lack of the capacity to produce motile and viable sperm capable of fertilizing an ovum. Since time immemorial, man has used different substances, methods or approaches to address potential issues like improving on sexual performance optimize fertility or resolve other reproductive deficiencies. Many techniques and pharmacologic substances exist especially in developed countries which are used to correct these deficiencies. However, in the developing countries, the high cost of these treatments makes them inaccessible and unaffordable for the majority of the populations. In addition, these substances are non-tolerable to most sufferers and the secondary effects they induce are not easily endurable; which motivate these populations to prefer the traditional medicine using local plants or plant parts (Archana *et al*., 2005).The use of medicinal plants (Nelisiwe *et al*., 2020) has gained much notoriety since they are considered cheaper and tolerable by some users, readily available and accessible in some areas and most importantly lack side or undesired effects. The World Health Organization (WHO) encourages the use of herbal medicine as alternative medicine for the prevention and treatment of diseases and according to this organ, more than 80% of the population in Africa uses medicinal plants and other natural products to meet their healthcare needs (Etame *et al*., 2017). Earlier, we reported the sex-enhancing potentials of *Pseudpanax arboreus* ("Five fingers") in normal male rats (Egbe *et al*., 2017) and its potentials to reverse sexual deficiencies induced by amitriptyline in male rats (Egbe *et al*., 2018). We undertook the present investigation to determine the curative properties of the leaf-aqueous extract of *P. arboreus* (Pa) on some indices of amitriptyline-induced male sexual impairments in rats.

MATERIALS AMD METHODS

Preparation of plant extracts and their doses

Fresh leaves of *P. arboreus* (commonly called "Five fingers" in Manyu Division) were collected from Ntenako village, Manyu Division, South-West Region of Cameroon in the month of February 2022 and as described in our previous study (Egbe *et al*., 2017). A botanical sample comprising a full branch and an attached flower of the plant was carefully preserved and taken to the National Herbarium in Yaounde for authentication, where a voucher of the specimen is found at the number 2734/SRFK (YA). Part of the collected leaves were air-dried for about a month and ground into a powdered form using an electric grinder. Preparation of the aqueous extract was based on its traditional usage and following the instructions and guidelines of the traditional healers. Traditionally, the plant is taken in the form of maceration of 20 leaves in 250ml of water. The extraction protocol used by Egbe *et al*., (2017) was repeated here with little modification. Part of the residue was handed to the Plant and Organic Chemistry Unit of the Department of Chemistry, University of Buea for a qualitative phytochemical screening which revealed the presence of alkaloids, flavonoids, phenols, saponins, sterols, tannins and triterpenoids as obtained in the previous study (Egbe *et al*., 2017).

The administrative doses of each extract were prepared with respect to the information obtained from folk users of this plant and the traditional healers. According to them, the leaf-maceration is used to improve male sexual performance or treat male sex-related ailments such as erectile dysfunction (ED), ejaculatory dysfunction, disorders of sexual desire and low sperm count by administering a total daily volume of about 250 ml of maceration of 20 leaves to an adult male. Based on these and other screening tests, the therapeutic dose used in improving on their sexual performance or resolving their

sexual difficulties was determined. This stood at 12.10 mg/kg from which the animal equivalent dose (AED) following the method of Nair and Jacob (2016) was calculated and stood at 75 mg/kg. Consequently, to obtain a stock solution of 7.5 mg/ml concentration, a total of 750 mg of the extract were weighed using a micro-scale (NVT 1601/1, OHAUS Corporation, USA) and dissolved in 100 ml of distilled water (vehicle). From this solution, 1ml was administered to 100g body weight of each male rat, which was equivalent to 75 mg/kg or 7.5 mg/100g body weight dose.

In order to investigate the effects of the aqueous extract at higher doses, a second dose of 150 mg/kg (i.e. therapeutic dose x2) body weight was introduced. Here, to obtain a stock solution of 15 mg/ml concentration, a total of 1500mg of the aqueous extract were weighed and dissolved in 100 ml of distilled water (vehicle). From this solution, 1ml was administered to 100g body weight of each male rat, which was equivalent to 150 mg/kg or 15 mg/100g body weight dose.

Reagents and chemicals

Products used in this study were of analytical quality and included amitriptyline hydrochloride 25 mg (Qualitest Pharmaceuticals Inc, Malvern, PA, USA), sildenafil citrate (Viagra)(Pfizer Inc., USA); Bioassay kits for ffollicle stimulating hormone (FSH)(DRG Diagnostics, Germany), luteinizing hormone (LH)(DRG Diagnostics, Germany), Testosterone (Omega Diagnostics LTD, Scotland, UK), ethyl-ether (Mark and Baker LTD, Dagenham, England). All were purchased and stored under recommended conditions until used.

Raising of animals

Male albino rats were raised in the Animal facility of the Department of Animal Biology and Conservation, Faculty of Science, University of Buea under standard conditions as reported in our previous studies (Egbe *et al*., 2017, 2018, 2019, 2022).

Treatment with amitriptyline hydrochloride

Mature and healthy males with fully developped testes obtained from our bred were divided into two groups. Group A served as control and was given vehicle only (distilled water) while group B served as the drug-treated group and was administered an oral dose of 10 mg/kg body weight of amitriptyline hydrochloride suspension (suspension prepared daily in distilled water). The 10mg/kg dose of Amitriptyline hydrochloride (amit. HCl) was selected considering the 400 mg maximum human daily dose (HED) from which the animal equivalent dose (AED) was obtained by extrapolating it in animals using the methods described by Nair and Jacob (2016) and reported in our previous study (Egbe *et al*., 2018). Treatment was given once a day and at the end of each week, a few rats were randomly selected from either group and used in assessing the relative weight of sex and assessor organs, sperm characteristics and plasma levels of FSH, LH and testosterone following procedures described earlier(Egbe *et al*., 2018).

To this effect, each animal selected was deprived of food and water for 12 hours and then anesthesized using ethyl-ether. The thoracic region was rapidly dissected, the heart identified, blood collected through cardiac puncture (Njoku-Orji *et al*. 2015) using a 5ml syringe and immediately transferred into heparinized test-tubes. The animal was then terminated through cervical dislocation. Blood was kept for 24 hours after which the supernatant was collected and put into test tubes. It was then centrifuged for 15 minutes at 2500rpm in the Eppendorf Centrifuge. At the end of this, the supernatant was again collected. The plasma FSH, LH and testosterone concentrations of each animal were determined using the procedure outlined in the manufacturer's instruction manual. This was based on the principle of competitive binding between the hormone in the plasma and hormone-HRP conjugate for a constant amount of rat anti-hormone (Tietz, 1995; Egbe *et al*., 2018). In each case, a blank solution was prepared to help calibrate or standardise the Eliza reader.

The relative weight of sex and accessory organs were determined following termination of animals. To this effect, upon sacrifice of each animal, the abdominal cavity was dissected and the following organs were identified, then isolated: testes, epididymis, vas deferens, prostate, seminal vesicles and the penis. Each was rinsed thoroughly and wiped with clean absorbent paper, carefully freed from all connective tissue and then weighed using an electronic balance (NVT 1601/1, OHAUS Corporation, USA). Their individual weights were then expressed as a percentage of the total body weight (Egbe *et al*., 2018) as shown in the formula below:

$$\textbf{Relative mass } (\%) = \left[\frac{OM}{AM}\right] x\ 100$$

Where *OM* = mass of organ (g); *AM* = mass of animal (g).

Sperm characteristics were also evaluated following sacrifice of animals. Sperm motility and concentration analyses were done according to the procedures described by Ralebona *et al*. (2012). Following sacrifice of animals, the cauda epididymis was carefully separated, weighed and placed in a Petri dish with 10 ml of normal saline, pH 7.2. It was minced using a pair of scissors and the Petri dish placed in a water-bath at 37°C to allow sperm to swim into the warmed normal saline to form a suspension. From this suspension, a volume of 0.10 ml was collected and added to 19.99 ml of normal saline for dilution, from which 15 µl were loaded into the Neubauer improved cell counting chamber (Deep 1/10 mm, Labart, Germany). Motility was done immediately after dilution, whereas, concentration was determined 24 hours after dilution. Five (5) squares were counted in triplicate per sample. Sperm motility was

reported as a percentage (%) while concentration was expressed as number of sperm X 10^6 (number of sperm/µl).

The testes were also examined macroscopically and microscopically for possible pathological alterations induced by exposure of male rats to amitriptyline treatment. In effect, following their removal from the sacrificed animals, each testis was freed from all connective tissue, weighed, fixed in 10% buffered formalin, dehydrated through ascending grades of ethanol (75%, 80% and 96%), cleaned in xylene and embedded in paraffin wax. Consecutive sections were cut at a thickness of 5µm, stained with haematoxylin and eosin (H&E). A minimum of three fields of each tissue slide were examined under the light microscope (UNICO 380, USA) at X100 for interstitial edema, seminiferous tubule degeneration, hypertrophy or aplasia of cells, congestion, necrosis of seminiferous tubules (Kalid, 2009; Branimir *et al.*, 2012; Ndukui *et al.*, 2014).

Treatment with the aqueous extract of *P. arboreus*

At the end of the 8th week (on day 57) from commencement of treatment with amitriptyline, 40 amitriptyline-treated animals showed significant reduction in relative weight of sex and accessory organs, sperm characteristics and hormonal profile following procedures explained earlier. However, no animal mortality and signs of morbidity were recorded throughout the induction of sexual impairment. They were randomly divided into 5 groups of 8 rats each. Group 1, pre-treated with amitriptyline, was allowed time for self-healing; Group 2 received 10ml/kg distilled water and served as negative control; while rats of group 3 were given 6mg/kg Sildenafil Citrate™ (Viagra) (positive control). Groups 4 and 5 were administered 75 and 150mg/kg of the aqueous extract of *P.a*, respectively. All substances were administered orally once a day using the metal oropharyngeal cannula and treatment lasted 58 days to complete one cycle of spermatogenesis. On day 59 after treatment, all animals were sacrificed and the relative weight of sex and accessory organs, sperm characteristics and plasma hormonal profile were evaluated following procedures described earlier. Also, histological sections were prepared as described in the preceding section.

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee (Yaounde, Cameroon) for animal handling and experimental procedure (Reg No. FW-IRB00001954). Efforts were made to reduce animal suffering and the number of animals used. Ethical clearance was obtained from the Faculty of Science, University of Buea Institutional Animal Care and Use Committee (UB-IACUC) Approval Number UB-IACUC N° 07/2023.

Statistical analyses

Values were expressed as Mean ± SEM. Mean values were calculated for each animal and quantitative comparisons between groups established from those means. Analysis of Variance (ANOVA) followed by Duncan test was used in the SPSS for windows version 20.0 software. Significant levels were tested at $p<0.05$

RESULTS AND DISCUSSION

Compared to the control animals, chronic treatment of sexually mature male rats with 10 mg/kg amit. HCl provoked a significant ($p<0.05$) drop in the relative weight of their sex and accessory organs (Table 1) while the 11.5 days withdrawal period did not produce any significant effect (Tables 1 and 2). Subsequent treatment of these rats with either dose of the leaf-aqueous extract of Pa registered a significant ($p<0.05$) increase in testicular, epididymal, vas deferens and penile weights; while its effects on seminal vesicles and prostatic weights were not significant (Table 3). Meanwhile, the Viagra-treated animals registered a non-significant ($p<0.05$) increase in relative weight of the seminal vesicles and prostate.

Table 1. Data obtained on the relative weight of sex and accessory organs of sexually drilledmale rats treated with 10 mg/kg amitriptyline hydrochloride for 56 days.

	Organ					
Treatment	Testes	Epid.	V. def	S. ves.	Prost.	Penis
DW (10 ml/kg)	1.64 ± 0.03^a	0.55 ± 0.03^a	0.46 ± 0.01^a	0.27 ± 0.05^a	0.37 ± 0.05^a	0.17 ± 0.03^a
Amit. HCl (10 mg/kg)	1.36 ± 0.14^b	0.34 ± 0.06^b	0.35 ± 0.04^b	0.19 ± 0.05^b	0.28 ± 0.07^b	0.11 ± 0.04^b

Values presented as Mean ± SEM; DW: distilled water; Amit. HCl: Amitriptyline hydrochloride; Epid.: epididymis; V. def: vas deferens; S. ves.: seminal vesicles; Prost.: prostate; within the same column, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different;($p<0.05$).

Table 2. Data obtained on relative weight of sex and accessory organs of sexually drilled male rats following 56 days treatment with and 11.5 days withdrawal from 10 mg/kg amitriptyline hydrochloride.

	Organ					
Treatment	Testes	Epid.	V. def	S. ves.	Prost.	Penis
DW (10 ml/kg)	1.63 ± 0.13^a	0.55 ± 0.07^a	0.47 ± 0.06^a	0.27 ± 0.08^a	0.37 ± 0.05^a	1.47 ± 0.04^a
Amit. HCl (10 mg/kg)	1.37 ± 0.12^b	0.35 ± 0.06^b	0.36 ± 0.05^b	0.20 ± 0.07^a	0.29 ± 0.10^a	1.23 ± 0.05^b

Values presented as Mean ± SEM; DW: distilled water; Amit. HCl: Amitriptyline hydrochloride; Epid: epididymis; V. def: vas deferens; S. ves.: seminal vesicles; Prost.: prostate; within the same column, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; ($p<0.05$).

Table 3. Table 3. Effects of the aqueous leaf-extract of *P. arboreus* on relative weight of sex and accessory organs of amitriptyline-induced sexually impaired male rats.

Organs	Treatment				
	Amit. Only	Amit.+DW	Amit,+Viagra	Amit.+Pa75	Amit.+Pa150
Testes	1.39 ± 0.05^a	1.56 ± 0.03^b	1.58 ± 0.14^b	1.84 ± 0.11^c	1.92 ± 0.06^c
Epididymis	0.52 ± 0.03^a	0.56 ± 0.03^a	0.66 ± 0.06^b	0.69 ± 0.05^b	0.72 ± 0.04^b
Vas deferens	0.34 ± 0.01^a	0.37 ± 0.01^a	0.48 ± 0.44^b	0.55 ± 0.03^c	0.61 ± 0.01^c
Sem. Vesicles	0.22 ± 0.02^a	0.25 ± 0.05^a	0.28 ± 0.05^a	0.29 ± 0.03^a	0.29 ± 0.06^a
Prostate	0.29 ± 0.04^a	0.34 ± 0.05^a	0.35 ± 0.10^a	0.32 ± 0.10^a	0.31 ± 0.05^a
Penis	1.27 ± 0.11^a	1.58 ± 0.03^b	1.66 ± 0.14^b	1.80 ± 0.11^c	1.83 ± 0.06^c

Values presented as Mean ± SEM; Amit. only: Animals treated with Amitriptyline only; Amit.+DW: Amitrityline -Treated + distilled water; Amit.+Viagra: Amitriptyline-Treated + Viagra (6 mg/kg); Amit.+Pa75: Amitriptyline -Treated + *Pseudopanax arboreus* 75 mg/kg; Amit.+Pa150: Amitriptyline-Treated + *Pseudopanax arboreus* 150 mg/kg; $p<0.05$; Epid.: epididymis; V. def: vas deferens; S. ves : seminal vesicles; Prost : prostate; within the same row, values accompanied by different letters are significantly different; ($p<0.05$).

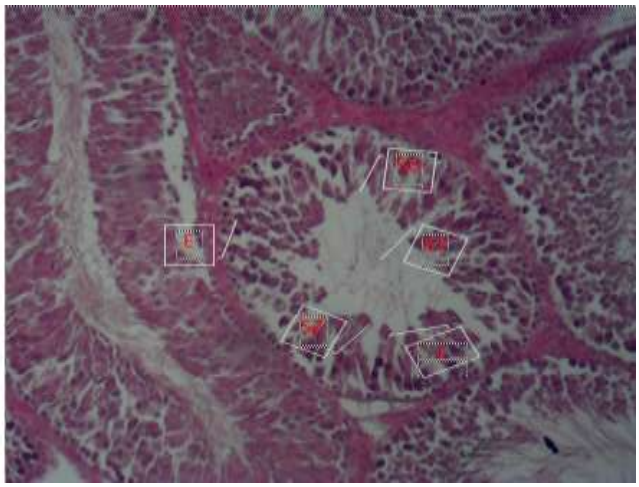

Figure 1. Histo-architecture of the testis of sexually trained male rats treated with 10mg/kg amit. HCl for 56 days (H/E; X400).

No signs of necrosis, interstitial edema, seminiferous tubular degeneration nor congestion are noticed; but poorly developed seminiferous tubular epithelium, spermatogonia and spermatozoa; large seminiferous tubular lumen indicating normal (low) spermatogenesis; E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

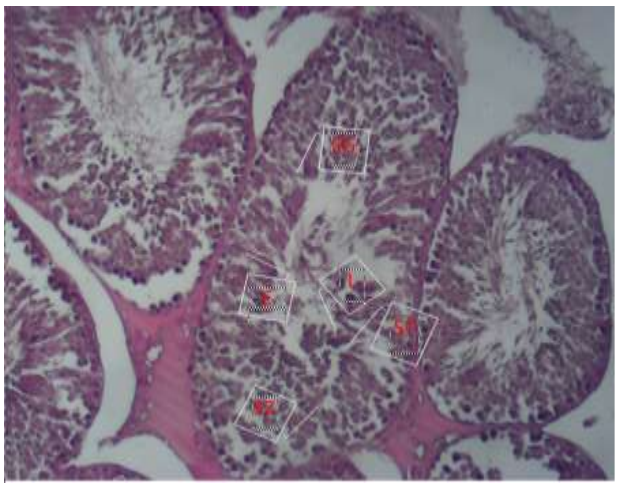

Figure 2. Histo-architecture of the testis of sexually trained male rats treated with 10 mg/kg amit. HCl for 56 days and following 11.5 days.

No signs of necrosis, interstitial edema, seminiferous tubular degeneration nor congestion are noticed; but poorly developed seminiferous tubular epithelium, spermatogonia and spermatozoa; large seminiferous tubular lumen indicating normal (low) spermatogenesis; E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

The effects of chronic administration of 10 mg/kg amit. HCL and its withdrawal on the sperm motility and concentration of sexually mature male rats were significant ($p<0.05$) compared to the control group (Table 4). Table 5 illustrates the data obtained from sperm analyses of these rats following their subsequent treatment with the leaf-aqueous extract of *P. arboreus*(Pa). According to the data, administration of the leaf-aqueous extract of Pa to the amitriptyline-treated rats at either dose resulted in a significant ($p<0.05$) increase in sperm motility and concentration in animals treated with both doses, compared to their Viagra and distilled water-treated counterparts. Meanwhile, there was no significant difference in sperm characteristics between the 2 control groups.

Table 4. Data obtained from a 56 days treatment and 11.5 days withdrawal of 10 mg/kg Amit HCl on the sperm characteristics of sexually drilled male rats.

Treatment	Parameter Sperm characteristics (After 56 days of treatment)		Sperm characteristics (Post withdrawal period)	
	Motility (%)	Concentration ($X10^6$)	Motility (%)	Concentration ($X10^6$)
DW (10 ml/kg)	53.59 ± 11.20^{ac}	2.08^{ad}	53.80 ± 9.55^{ac}	2.10^{ad}
Amit. HCl (10 mg/kg)	41.83 ± 11.55^{be}	1.06^{bf}	43.20 ± 10.16^{be}	1.05^{bf}

Values presented as Mean ± SEM; DW: distilled water; Amit. HCl: Amitriptyline hydrochloride; within the same column, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; within the same row, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; $p<0.05$.

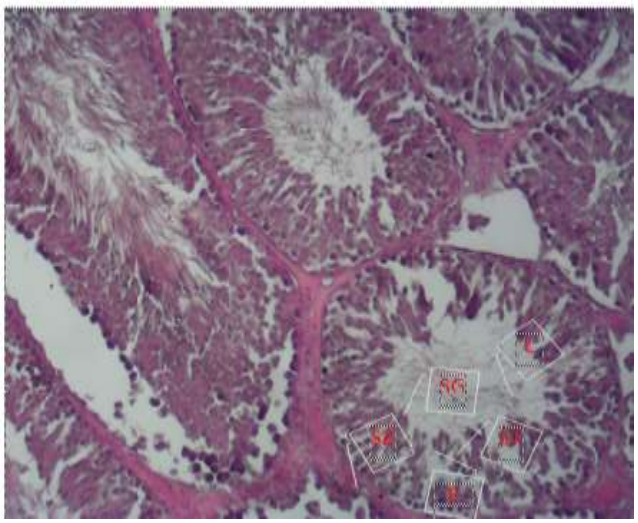

Figure 3. Histological section of the testes of sexually trained male rats treated with 10 ml/kg distilled water for 56 days (H/E; X400).

No signs of necrosis, interstitial edema, seminiferous tubular degeneration or congestion are noticed. Note: poorly developed (scanty) seminiferous tubular epithelium, spermatogonia and spermatozoa; large seminiferous tubular lumen indicating normal spermatogenesis; E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

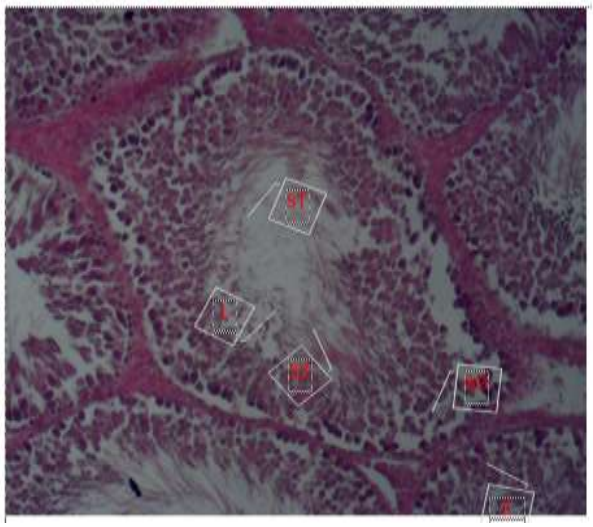

Figure 4. Histological section of the testes of sexually trained male rats treated with 10ml/kg distilled water for 56 days and following 11.5 days post-treatment (withdrawal) period (H/E; X400).

Note: poorly developed (scanty) seminiferous tubular epithelium, spermatogonia and spermatozoa; large seminiferous tubular lumen indicating normal spermatogenesis; E: Epithelium; L: Lumen; ST: spermatids; SG: spermatogonia; SZ: spermatozoa.

Table 5. Effects of the aqueous leaf-extract of *P. arboreus* (Pa) on sperm characteristics of Amitriptyline-treated male rats.

Sperm characteristics	Treatment Amit. Only	Amit.+DW	Amit,+Viagra	Amit.+Pa75	Amit.+Pa150
Motility (%)	42.57 ± 11.02^{a}	43.22 ± 11.43^{a}	67.25 ± 13.78^{b}	78.64 ± 10.36^{b}	76.71 ± 13.31^{b}
Concentration ($x10^6$/ml)	1.11^{a}	1.21^{a}	1.19^{a}	2.38^{b}	2.58^{b}

Values presented as Mean ± SEM; Amit. Only: Animals treated with Amitriptyline only; Amit.+ DW: Amitrityline-Treated + distilled water; Amit.+Viagra: Amitriptyline-Treated + Viagra (6 mg/kg); Amit.+Pa75: Amitriptyline-Treated + *Pseudopanax arboreus* 75 mg/kg; Amit.+Pa150: Amitriptyline-Treated + *Pseudopanax arboreus* 150 mg/kg; within the same row, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; $p<0.05$.

Following treatment of sexually mature male rats with 10 mg/kg of amit. HCl for 56 days and an11.5day withdrawal period from the drug, there was no significant ($p<0.05$) difference in their plasma concentrations of FSH, LH and testosterone compared to the control group (Table 6). Their subsequent treatment with either dose (Amit.+Pa75 or Amit.+Pa150) of the leaf-aqueous extract of *P. arboreus* produced a significant ($p<0.05$) difference in the plasma levels of these hormones, compared to the control animals. The effects caused by Viagra were not significantly different compared to those of the distilled water-treated animals (Table 7).

Table 6.Data obtained from 56 days treatment with and 11.5days withdrawal from 10 mg/kg Amit HCl on the plasma hormonal profile of sexually mature male rats.

Treatment	Parameter					
	Hormonal profile (After 56 days treatment period)			Hormonal profile (Post withdrawal period)		
	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)
DW (10 ml/kg)	10.34±2.38[a]	9.67±2.27[a]	10.89±1.65[a]	10.48±2.40[a]	10.13±2.88[a]	10.37±1.80[a]
Amit. HCl (10 mg/kg)	8.26±2.54[a]	7.92±2.59[a]	7.64±2.14[a]	8.12±2.90[a]	7.97±2.59[a]	7.69±2.55[a]

Values presented as Mean ± SEM; DW: distilled water; Amit. HCl: Amitriptyline hydrochloride; within the same row, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; and within the same column, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; $p<0.05$.

Table 7.Effects of the aqueous and methanol leaf-extracts of *P. arboreus* on the plasma levels of FSH, LH and testosterone of Amitriptyline-induced sexually impaired male rats.

Organs	Treatment				
	Amit. Only	Amit.+DW	Amit,+Viagra	Amit.+Pa75	Amit.+Pa150
FSH (mIU/ml)	10.37±3.71[a]	11.35±2.46[a]	11.18±3.11[a]	14.38±1.89[b]	14.87±1.55[b]
LH (mIU/ml)	9.22±2.93[a]	9.52±1.39[a]	11.35±1.56[a]	13.47±2.61[a]	13.82±2.41[a]
Testosterone (ng/ml)	8.26±1,33[a]	8.69±2.33[a]	8.74±3.37[a]	13.81±2.43[b]	13.44±1.72[b]

Values presented as Mean ± SEM; Amit. only: Animals treated with Amitriptyline only; Amit.+DW: Amitrityline-Treated + distilled water; Amit.+Viagra: Amitriptyline-Treated + Viagra (6 mg/kg); Amit.+Pa75: Amitriptyline-Treated + *Pseudopanax arboreus* 75 mg/kg; Amit.+Pa150: Amitriptyline-Treated + *Pseudopanax arboreus* 150 mg/kg; within the same row, values accompanied by different letters are significantly different, while values accompanied by the same letter are not significantly different; $p<0.05$. Exposure of sexually trained male rats to prolonged treatment with 10 mg/kg amit. HCl did not result in any alteration of the testicular architecture. No signs of necrosis, interstitial edema, seminiferous tubular degeneration nor congestion were noticed (Figures 1 and 2), compared to the distilled water-treated animals (Figures 3 and 4, respectively).

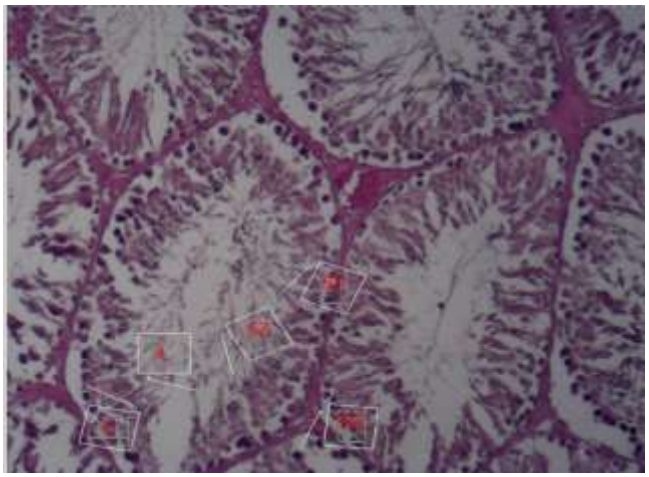

Figure 5. Histo-architecture of the testis of Amitriptyline-induced sexually impaired male rats treated with distilled

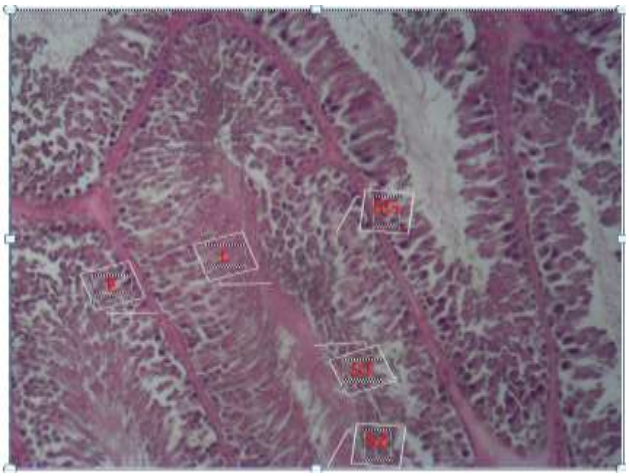

Figure 6. Histo-architecture of the testis of Amitriptyline-induced sexually impaired male rats treated with 6 mg/kg

water (AMIT.-DW) (control)(H/E; X400).

Notice a poorly developed seminiferous tubular epithelium with few spermatogonia and spermatozoa indicating low spermatogenesis, compared to the extract-treated groups (Figures 8 and 9); E: Epithelium; L: Lumen; SG: spermatogonia; ST: Spermatids; SZ: spermatozoa.

In this study, we determined the weights of testis, epididymis, vas deferens, seminal vesicles, prostate glands and the penis in amitriptyline pre-treated animals and noticed that chronic exposure of sexually mature male rats to amit. HCl caused significant effect on the relative weight of these organs, findings which are in corroboration with those of (Lauren and Mary, 2020). Their subsequent treatment with the leaf-aqueous extract of *P. a*. resulted in a significant rise in their values. The weight of the testes is a useful index in evaluating the efficiency of steroidogenesis. Like in the Viagra-treated animals, extract-treated rats recorded increase in the relative weights of the sex and accessory organs compared to control group rats, thus exhibiting androgenic effect. Androgens, including testosterone, have been reported to be useful for the histo-morphometric development and maintenance of the testes and ultimately the biochemical process of sperm production

Viagra (AMIT.-Viagra) (H/E; X400).

Notice Hyperplasia of seminiferous tubular epithelium, many spermatogonia and spermatozoa, all indicating intense spermatogenesis compared to Figure 6; E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

(Adimoedja, 2000; Walker, 2010); low serum levels may have adverse effect on fertility. The enhancements in the weights of sex and accessory organs of male rats are usually associated with androgenic activity and anabolic function. Androgens can stimulate the growth of accessory sexual organs like testis, seminal vesicles and prostate and increase their weights (Chauhan *et al*., 2009). Drugs or natural compounds that increase the weights of sex and accessory organs are considered to possess androgenic properties (Luo *et al*., 2006). This increase in the relative weight of sex and accessory organs recorded in extract-treated rats could be a reflection of the increased plasma levels of testosterone. Meanwhile, its non-significant effect on the weights of the seminal vesicles and the prostate could be attributed to the actions of phenols present in the extract (Alireza *et al*., 2003).

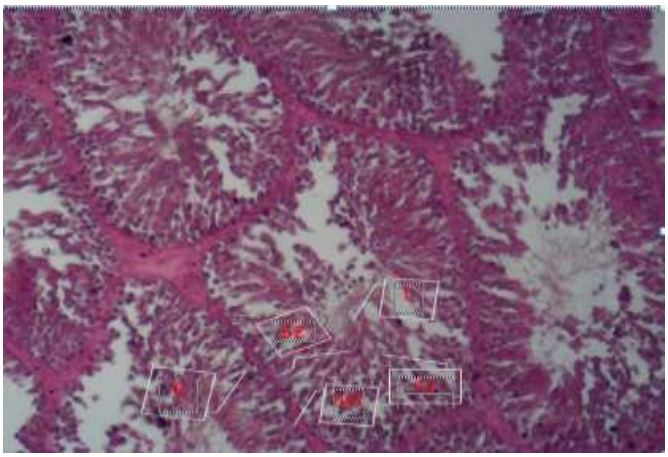

Figure 7. Histo-architecture of the testis of Amitriptyline-induced sexually impaired male rats treated with the 75 mg/kg (AMIT.Pa75) dose of the aqueous extract of *P. arboreus*(H/E; X400).

Noticed here ishyperplasia of seminiferous tubular epithelium, spermatogonia and spermatozoa, all indicating intense spermatogenesis; All stages of spermatogenesis are clearly observed and distinct here compared to Figure 6 including: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa; as well as a well-developed basement membrane and Leydig's cells. E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

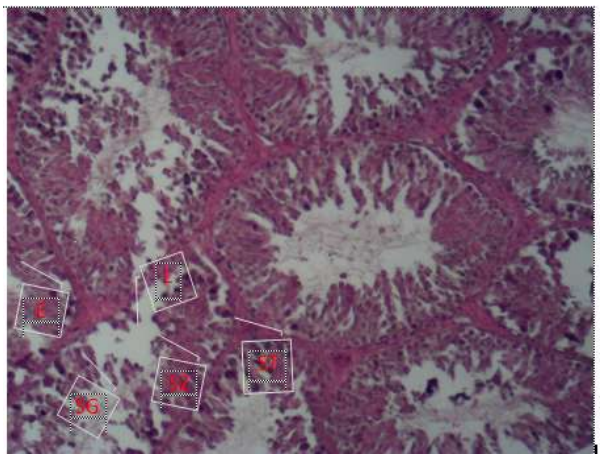

Figure 8. Histo-architecture of the testis of Amitriptyline-induced sexually impaired male rats treated with the 150 mg/kg (AMIT.-AE2) dose of the leaf-aqueous extract of *P. arboreus* (H/E; X400).

Like in Figure 6, here, there is hyperplasia of seminiferous tubular epithelium, several spermatogonia and spermatozoa; also notice large seminiferous tubular lumen indicating intense spermatogenesis. All stages of spermatogenesis are clearly observed and distinct here compared to Figure 6 including: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa; as well as a well-developed basement membrane and Leydig's cells. E: Epithelium; L: Lumen; SG: spermatogonia; ST: spermatids; SZ: spermatozoa.

Furthermore, amit. HCl-treated males registered significantly low sperm motility and concentration, compared to the distilled water animals. These results are similar to those of Ladan *et al*. (2018) who reported that amitriptyline consumption can weaken sperm parameters as a result of increased production of ROS and to those of Lauren and Mary (2020). Still in 2020, Ehab *et al*. Reported that amitriptyline induces testicular toxicity through oxidative stress by inactivating thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR) and glutathione peroxidase (GPx). In 2021, Solek *et al*. provided evidence that antidepressant treatment may contribute to spindle apparatus assembly defects and organelle distribution during cell division *in vitro*, a study which shed new light on the pathomechanisms of antidepressants action and their associated toxicity towards the reproductive system. Treatment of the amitriptyline pre-exposed rats with the leaf-aqueous extract witnessed a significant increase in sperm motility and concentration, compared to those treated with distilled water. Tanins, a class of phytoconstituents found in our extract have been reported to enhance spermatogenesis. According to Mohamed *et al*. (2022), tannins are known to have antioxidant and other health-promoting effects and may serve as binders/acceptors to reduce the deleterious effects of excessive ROS and as a consequence help improve on sperm motility and viability. Wurlina *et al*.(2020) have also reported spermatogenc potentials of tanins at low doses. Another class of phytocompounds found in our extract were the saponins which are effective in the recovery of the male reproductive organ and can induce an increase in the number and viability of germ cells (Minyoung *et al*., 2007).The effects of Viagra noticed in our experiment are similar to the findings of ken *et al*. (2002) who observed that Sildenafil does not have an adverse effect on sperm function or ejaculate quality.

Also in our experiment, there was a significant effect on the hormonal profile of the animals. These results are in line with those of Ehab *et al*.(2020) who observed that Amitriptyline repressed reproductive hormonal activity which is evidenced by histopathological lesions, DNA damage and p53 protein expression. However, an increase in plasma testosterone levels was noticed in these animals when treated with the aqueous extract of P. a. Testosterone is synthesized and secreted by the Leydig cells of the testis under the influence of LH (Luteinizing hormone), a gonadotrophin. Unfortunately, plasma levels of both LH and FSH were less significant; which means some phytoconstituents of the leaf-extract must have mimicked the role of LH to stimulate the Leydig cells to synthesise testosterone. Viagra enhanced a significant increase in plasma concentration of the reproductive hormones including LH, FSH and testosterone. Viagra is known to increase serum testosterone levels through a decline in the levels of serum LH (luteinizing hormone) and the inverse relationship between LH and testosterone has been explained by Spitzer *et al*. (2013) and Hackett *et al*.(2016) Exposure of sexually mature male rats to prolonged treatment with 10mg/kg amit. HCl did not result in any alteration of the testicular architecture, even though Fikret *et al*. (2014) have shown the damaging effects of some antidepressants on the histology of the testis. No signs of necrosis, interstitial edema, seminiferous tubular degeneration nor congestion were noticed. However, upon treatment of these amitriptyline pre-treated male rats with the leaf-aqueous extract of *P. arboreus*, there was a general improvement on the histo-architecture of the testes of these animals like that of the Viagra-treated animals. The testes of the animals witnessed hyperplasia of seminiferous tubular epithelium, spermatogonia and spermatozoa, all indicating intense spermatogenesis. Aphrodisiacs, in addition to enhancing copulatory behavior in laboratory animals, have the potentials to promote testicular development, steroidal synthesis and spermatogenesis (Yakubu *et al*., 2008b). These improvements in testicular architecture and spermatogenesis correlate with an increase in testicular weight and could be as a result of the increase in plasma testosterone recorded. Testosterone, an androgen, isuseful for the development and maintenance of the architecture and morphology of the testes and consequently spermatogenesis (Adimoedja, 2000; Walker, 2010). These actions of the plant on the testicular architecture could have been induced by the androgenic effect of the extract explained earlier. Our standard drug Viagra stimulated an improvement in the histo-architecture of amitriptyline pre-treated rats, findings that are in corroboration with those of El-Sayed *et al*. (2022).

CONCLUSION

In conclusion, the leaf-aqueous extract of *Pseudopanax arboreus* possesses curative properties on some male reproductive indices induced by amitriptyline and could be a potential solution to antidepressant-induced male sexual dysfunction.

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