Available online on 15.07.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Androgenic effects of the aqueous extract of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae) wood in hemicastrated male Wistar rats

Yaya Younous Charfadine [1,2], Rodolphe Koularambaye [1,3], Sara Nathalie Edjenguèlè Béboy [1*], Joseph Rodrigue Fifen Ngapout[2], Yannick Sani Jignoua[1], Désiré Paul Djomeni Dzeufiet[2], Paul Fewou Moundipa[1]

[1] *Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon.*

[2] *Laboratory of Animal Physiology and Medicinal Plant Studies, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon.*

[3] *Laboratory of Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364 Yaoundé, Cameroon.*

Article Info:

Article History:

Received 19 April 2024
Reviewed 30 May 2024
Accepted 24 June 2024
Published 15 July 2024

Cite this article as:

Charfadine YY, Koularambaye R, Béboy SNE, Ngapout JRF, Jignoua YS, Dzeufiet DPD, Moundipa PF, Androgenic effects of the aqueous extract of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae) wood in hemicastrated male Wistar rats, Journal of Drug Delivery and Therapeutics. 2024; 14(7):10-17

DOI: http://dx.doi.org/10.22270/jddt.v14i7.6683

*Address for Correspondence:

Sara N. E. Béboy, Department of Biochemistry, University of Yaoundé I, PO Box 812 Yaoundé, Cameroon

Abstract

Background: Androgen deficiency is the most common disorder of reproductive function and can lead to male sexual disorders.

Objective: This study was designed to evaluate the androgenic effects of *Pycnanthus angolensis* (Welw.) Warb in hemicastrated rats.

Materials and methods: Forty-two male rats were divided into 6 groups of 7 rats each, including a group of uncastrated rats that received distilled water (10 ml/kg); a group of castrated rats that received 10 ml/kg of distilled water; a group of castrated rats that received testosterone enanthate (3 mg/kg BW) per week intramuscularly; and 3 groups of castrated rats that received 43, 86 and 172 mg/kg of the aqueous extract of *Pycnanthus angolensis*, respectively. After 14 days of oral treatment, the rats were killed by decapitation. The blood was collected and the androgen-dependent organs were collected for histological sectioning, and biochemical analysis. The tail of the epididymis was used to assess sperm quality.

Results: Treatment with the aqueous extract at doses of 43 and 86 mg/kg, significantly improved the sexual behavior of castrated rats, with increases of 25.92% and 22.74% intromissions frequency, and 67.06% and 56.46% mount frequency, compared to those in the castrated rats which did not receive any treatment. The extract also enhanced sperm quality in castrated rats. Both doses also significantly increased serum testosterone levels with rates of 45.07% and 49.00%, respectively; compared to those in the negative control group.

Conclusion: In view of the aforesaid results, *Pycnanthus angolensis* (Welw.) Warb could be considered as a promising natural agent in hypogonadism management.

Keywords: Androgen, hypogonadism, castrated male rats, unilateral castration, *Pycnanthus angolensis* (Welw.) Warb.

INTRODUCTION

Male reproduction is closely related to the production of androgenic hormones that are essential for the development and maintenance of male sexual characteristics [1]. Hypogonadism, characterized by the inability of the testes to produce adequate levels of testosterone, can have a significant impact on male health and well-being [2]. However, the exact prevalence of hypogonadism is unclear and remains a major concern in medicine, particularly in older men [3,4]. Conventional medicinal treatments, such as testosterone supplementation [5], have been shown to be effective but, are limited due to undesirable side effects and constraints such as disruption of treatment and high cost [6]. Consequently, there is a growing need for alternative solutions such as plant-based treatments to regulate reproductive function. Several medicinal plants like waterlily *Nymphaea lotus* (Nymphaeaceae) and *Allanblackia floribunda* (Clusiaceae) have been studied for their potential to improve reproductive function, particularly because of their androgenic activities [7,8]. Among these numerous plants, *Pycnanthus angolensis* (*P. angolensis*) is a tropical plant from the Myristicaceae family whose trunk bark is used to treat inflammation, microbial infections, anaemia and pain [9,10]. The wood of this plant is used in the southern Cameroon region by '*Baka*' traditional healers to treat erectile dysfunction. *In vitro* and *in vivo* studies have shown its androgenic and aphrodisiac activities [8,11]. However, despite these promising results, no *in vivo* studies have been carried out to assess the effect of *P. angolensis* on male reproductive function under hypogonadal conditions. The present study aimed to evaluate the effect of the aqueous extract of *P. angolensis* wood in hemicastrated male rats.

MATERIALS AND METHODS

Plant collection and preparation of extracts

Fresh wood of *P. angolensis* was collected in the Centre region of Cameroon in November 2022 and identified at the National

Herbarium of Cameroon under the voucher number HNC31369. The wood was ground with an electric grinder. Three hundred grams of the dried powder of wood of *P. angolensis* was used to prepare the 10% (m/v) aqueous extract by decoction for 30 using distilled water. The obtained extract was evaporated to dryness, weighted and kept at 4°C. The extraction yield was 6.5 %.The doses used in this study were prepared by dissolving in distilled water the evaporated extracts in order to obtain the three required doses: 43, 86 and 172 mg/kg of body weight (BW).

Animal handling

One-month old male albino Wistar rats weighing between 40-45 g were used for this study. The animals were acclimatized for 2 weeks and given food and water *ad libitum*. On the 46th day after birth, the rats were subjected to surgery and the left testicle was removed. Fifteen one-month old female rats, weighing between 40-45 g, were brought to the estrus by subcutaneous injection of 100 µg/kg estradiol benzoate and an intramuscular injection of progesterone (5 mg/Kg) at 48 and 6 hours respectively prior to the pairing, according to the modified method of Kameni *et al.* [7].

Castration

Castration was performed according to the method described by Sulaiman *et al.*, [12]. After anaesthetizing the rats by intraperitoneal injections of ketamine (50 mg/kg) and valium (10 mg/kg), the scrotum was lightly incised aseptically with scissors to remove the left testis which was gently separated from the epididymis. Normal rats underwent a blank surgery consisting of opening the scrotum and closing it without removing the testis. Then, a Betadine solution and Neomycin (Baneocin®) were applied to the wound until complete healing was achieved to avoid infection.

Experimental design

One week after castration, the rats were randomly divided into 6 groups of 7 rats each, as follows:

- The normal group G1 consisted of non-castrated rats that received orally distilled water (10 mL/kg, BW) daily;
- The negative control group G2 consisted of castrated rats that received orally distilled water (10 mL/kg, BW);
- The positive control group G3 consisted of castrated rats that received intramuscular testosterone enanthate (3 mg/kg, BW) per week;
- Three test groups G4, G5 and G6, consisted of castrated rats that orally received the aqueous extract at doses of 43, 86 and 172 mg/kg, BW, respectively.

After 14 days of treatment, the rats fasted for 12 hours. The following day, the animals were killed by decapitation under ether anesthesia. The blood was collected in dry tubes and centrifuged for 15 min at 1000 rpm. The supernatant was transferred to Eppendorf tubes and stored at -20°C for subsequent biochemical analysis. Organs such as the right testis, epididymis, prostate, seminal vesicles, and penis were carefully removed, weighed, and then subdivided for histological, biochemical, and sperm analyses.

Sexual behavior study

To measure the effects the aqueous extract of *P. angolensis* on the sexual behavior of rats; on the last day of treatment, the animals were individually introduced into observation cages under dim light in a quiet room. After 10 minutes of acclimatization, each male rat was paired with a receptive female for 30 minutes. Then, the following sexual behavior parameters were measured:

- penile licking (PL) which is the number of times the rat bent to lick the penis, indicating the frequency of erections;
- mount frequency (MF) which is the number of mounts preceding ejaculation, with or without intromission;
- ejaculation frequency (EF) which is the number of observed ejaculations;
- intromission frequency (IF) which is the number of intromissions preceding ejaculation;
- mount latency (ML) which is the time interval in seconds between the introduction of the receptive female in the cage and the first mount;
- intromission latency (IL) which is the time interval in seconds between the introduction of the receptive female in the cage and the first intromission;
- ejaculation latency (EL) which is the time interval in seconds between the first intromission and the first ejaculation.

Homogenate preparation

The epididymis, prostate, seminal vesicle, penis, and right testis tissues were removed after laparotomy, washed, weighed, and homogenized separately in ice cold appropriate buffers using a mortar and pestle. Then a 20% (w/v) homogenate of testes and seminal vesicles were prepared separately in sodium phosphate buffer (0.1 M, pH 7.3) and distilled water, respectively. The tissues of the prostate, penis, and epididymis were used to prepare a 10% (w/v) homogenate of each organ in phosphate buffer (0.1 M, pH 6.5), Krebs solution, and potassium phosphate buffer (0.1 M, pH 6.8), respectively. These homogenates were centrifuged at 1000 rpm for 10 min at 4°C and kept at -20°C until biochemical analysis.

Biochemical analysis

Serum testosterone levels were determined by the Enzyme-Linked Immunosorbent Assay (ELISA) using a *MONCENT* kit (Ref: EL1-1263). Acid phosphatase activity in prostate homogenate was determined by the Hillmann method using a *LABKIT* kit (Ref: LKBEDTT 06) while the activity of α-glucosidase activity in the epididymal homogenate was assessed using a *CHRONOLAB* kit (Ref: 101-0311). The level of fructose in seminal vesicles were determined as described by Gonzales and Villena [13], and the total proteins were determined according to the method described by Gornall *et al.* [14].

Sperm analysis

The tail of the epididymis was sampled and cut in physiological fluid previously maintained at 37°C in a water bath to allow visualization of the spermatozoa. Two parameters were observed: viability, mobility and count. These parameters were calculated according to the following formulas [15]:

$$\%\ Viability = \frac{Live\ spermatozoa}{Total\ spermatozoa} \times 100$$

$$Mobility = \frac{Mobile\ spermatozoa}{Total\ sperm\ count} \times 100$$

$$Number\ of\ spermatozoa = \frac{X \times df \times 10^6}{4}$$

X = number of spermatozoa in 4 Malassez cell quadrants;

df = dilution factor (10)

Histological analysis

All organs removed (testes, penis, seminal vesicles, prostate, epididymis) were kept in 10% formalin. The histological analysis technique used was described by Suvarna *et al.* [16].

Ethical considerations

The experiment was carried out with the approval of the Institutional review committee. The research was carried out after obtaining an ethical clearance (reference number BTC-JIRB2022-053).

Statistical analysis

Data are expressed as mean ± standard deviation (n=7) and were analyzed using R software (version 4.2.3, Lyon). Results were considered significant at P<0.05 and compared using the Kruskal-Wallis test followed by Dunn's post hoc test.

RESULTS

Effect of administration of the aqueous extract of *P. angolensis* on the relative weight of reproductive organs in rats

After 14 days of treatment, no significant effect was observed on the relative weights of epididymis, prostate, and seminal vesicle in the different groups compared to those of the normal control group (G1) (Table 1). However, significant increases (P < 0.05) in the relative weight of the penis and testes in the treated groups at doses of 43 and 86 mg/kg, when compared to the normal control and negative control groups were observed.

Table 1: Effect of the aqueous extract of *P. angolensis* on relative weights of reproductive organs

	Groups					
Organs (g)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
Right testis	0.70 ± 0.05	0.72 ± 0.06	0.69 ± 0.06	0.82 ± 0.07*,§	0.86± 0.07*,**,§	0.81 ± 0.07
Epididymis	0.41 ± 0.03	0.30 ± 0.03	0.32 ± 0.04	0.32 ± 0.07	0.34 ± 0.06	0.30 ± 0.07
Penis	0.15 ± 0.00	0.16 ± 0.02	0.18 ± 0.02*	0.16 ± 0.02	0.17 ± 0.02*	0.15 ± 0.01
Prostate	0.16 ± 0.03	0.14 ± 0.03	0.22 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.15 ± 0.03
Seminal vesicle	0.40 ± 0.10	0.40 ± 0.10	0.48 ± 0.05	0.42 ± 0.10	0.48 ± 0.09	0.35 ± 0.10

Results are expressed as mean ± SD (n=7). $^{*}P < 0.05$ versus the normal control group (G1); $^{**}P < 0.05$ versus the negative control group (G2; $^{§}P < 0.05$ versus the group G3. G1: uncastrated rats; G2: castrated rats; castrated rats treated with testosterone enanthate: G3 (3 mg/kg); with aqueous extract at a dose of 43 mg/kg: G4; 86 mg/kg G5 and 172 mg/kg: G6.

Effects of *P. angolensis* on sexual behavior parameters at the end of the treatment

As summarized in Table 2, when compared to the normal control group, the ejaculation, intromission and mount frequencies as well as the penile licking were significantly decreased (P < 0.05) in the negative control group by 40.80 %; 65.16 % and 27.27 %; respectively. Treatment of castrated rats with the aqueous extract at the doses of 43 and 86 mg/kg resulted in significant increases (P < 0.05) in ejaculation, intromission, and mount frequencies as well as the penile licking and the ejaculation latency, in comparison to those of the negative group. At the dose of 43 mg/kg, the extract treatment significantly decreased (P < 0.05) the latency times of ejaculation, intromission and mount when compared either to the negative control group or the positive control group.

Table 2: Effect of the aqueous extract of *P. angolensis* on sexual behavior parameters

	Groups					
Copulatory parameters	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
PL	294.71±7.23	76.14±15.33*	207.57±12.26*	274.07±13.50**	283.42± 6.36**	129.60±3.16*,††
MF	209.34±5.34	152.80±3.34*	207.34±6.64	255.28 ±16.53**	239.07±12.70**	83.00±0.74
EF	1.74±0.56	1.03±0.04	1.69±0.09	1.82±0.51**	1.72±0.02	1.03±0.01
IF	201.64±14.67	70.25±5.33*	197.32±3.63	252.35±7.32**,§	230.00±10.36**	74.34±0.50†,††
ML(s)	14.29±1.53	29.43±7.43	12.00±0.33	3.67±0.76*,**,§	6.00±0.36**	5.80±0.00**
IL (s)	14.29±1.53	29.43±7.43	12.00±0.33	3.67±0.76*,**,§	6.00±0.36**	5.80±0.00**
EL (s)	227.58±81.16	291.25±34.31	206.43±68.84**	205.67±59.64**	206.72± 95.33**	248.50±94.73§

Results are expressed as the mean ± SD (n=7). $^{*}P < 0.05$ versus the normal control group (G1); $^{**}P < 0.05$ versus the negative control group (G2; $^{§}P < 0.05$ versus the group G3; † $P < 0.05$ versus the group G4; †† $P < 0.05$ versus the group G5. G1: uncastrated rats; G2: castrated rats; castrated rats treated with testosterone enanthate: G3 (3 mg/kg); with aqueous extract at a dose of 43 mg/kg: G4; 86 mg/kg G5 and 172 mg/kg: G6. EF: ejaculation frequency; IF: intromission frequency; MF: mount frequency; EL: ejaculation latency; IL: intromission latency; ML: mount latency.

Effect of *P. angolensis* on several biochemical parameters

As shown in Table 3, the rats treated with all doses of the plant extract or the reference product (G3) recorded significant increases (P < 0.05) in testicular and total cholesterol level, compared to those in the normal and negative control groups. Results indicate that the different doses of the plant extract significantly increased testosterone levels (P < 0.05) when compared to those in the normal control and negative control groups. The highest serum testosterone level was obtained in the group of rats treated with the plant extract at the dose of 86

mg/kg, with an increase of 183.85% compared to the normal control group. Treatments with the plant extract and the reference product resulted in significant increases (P < 0.05) in the activity of both α-glucosidase and acid phosphatase compared to those in the negative control group. These increases were 23.41% and 79.48% in the castrated group treated with the plant extract at a dose of 43 mg/kg, respectively for the α-glucosidase and acid phosphatase activities.

Table 3: Effect of the aqueous extract of *P. angolensis* on several biochemical parameters of androgen-dependent organs

	Groups					
Parameters	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
Testosterone (ng/mL)	1.61 ± 0.07	1.45 ± 0.86	2.09 ± 0.52	2.56 ± 0.03*	4.57 ± 0.08*,**,§	2.49 ± 0.50*,**,§
Testicular cholesterol (mmol/L)	0.48 ± 0.00	0.18 ± 0.00	0.71 ± 0.00*,§	0.75 ± 0.00*,§	0.46 ± 0.00§,†	0.15 ± 0.00§,†
Total cholesterol (mmol/L)	1.34 ± 0.04	1.21± 0.00	1.64 ± 0.04*	1.64 ± 0.02*,**	1.36 ±0.00**	1.50 ± 0.04
α-Glucosidase (U/L)	5.64 ± 0.24	1.70 ± 0.51*	5.04 ± 0.63**	5.68 ± 0.34**	3.73 ± 0.53†	3.02 ± 0.07
Acid phosphatase (U/L)	6.01 ± 0.33	3.51 ± 0.02*	5.01 ± 0.01*	6.3 0± 0.07**	6.03 ± 0.77†	2.74 ± 0.76†
Fructose (mmol/L)	0.89 ± 0.02	0.42 ± 0.00	0.92 ± 0.00**	0.94 ± 0.00**	0.87 ± 0.04**	0.47 ± 0.00§,†,††

*Results are expressed as the mean ± SD (n=7). *P < 0.05 versus the normal control group (G1); **P < 0.05 versus the negative control group (G2); §P < 0.05 versus the group G3; † P < 0.05 versus the group G4; †† P < 0.05 versus the group G5. G1: uncastrated rats; G2: castrated rats; castrated rats treated with testosterone enanthate: G3 (3 mg/kg); with aqueous extract at a dose of 43 mg/kg: G4; 86 mg/kg G5 and 172 mg/kg: G6.*

Effect of *P. angolensis* on protein levels

Figure 1 illustrates the effect of *P. angolensis* on the serum, prostatic, and epididymal total protein levels. The results show that castration led to a significant reduction in protein levels in the prostate and epididymis. Treatment with testosterone enanthate significantly increased protein levels (P <0.05) in the serum and the prostate, when compared to those in the negative control group. Treatment of castrated rats with the aqueous extract at the doses of 43 and 86 mg/kg induced significant increases (P <0.05) in the protein levels in the prostate and epididymis compared to those in the negative control group.

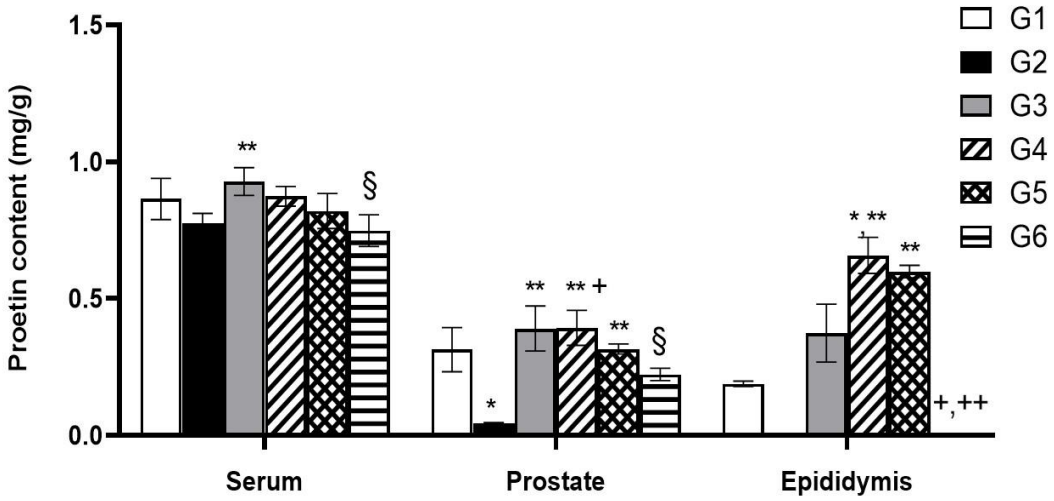

Figure 1 Effect of *P. angolensis* on the protein levels in the epididymis, prostate and serum

*Results are expressed as the mean ± SD (n=7). *P < 0.05 versus the normal control group (G1); **P < 0.05 versus the negative control group (G2); §P < 0.05 versus the group G3; † P < 0.05 versus the group G4; †† P < 0.05 versus the group G5. G1: uncastrated rats; G2: castrated rats; castrated rats treated with testosterone enanthate: G3 (3 mg/kg); with aqueous extract at a dose of 43 mg/kg: G4; 86 mg/kg G5and 172 mg/kg: G6.*

Effect of *P. angolensis* on sperm quality

The effects of the aqueous extract of *P. angolensis* on sperm quality are summarized in table 4. No significant change was observed in the nonprogressive motility of spermatozoa (P >0.05). The table shows that the castration led to a significant decrease (P <0.05) in the viability of spermatozoa compared to those in the normal group. The percentage of immobile spermatozoa in the castrated group was 2-fold the percentage of immobile spermatozoa in the normal control group. After the different treatments, the viability of spermatozoa significantly increased (P <0.05), compared to that in the negative control group. The aqueous extract at the dose of 43 mg/kg significantly decreased P <0.05) the percentage of immobile spermatozoa, compared to that in the negative control group.

Table 4: Effect of the aqueous extract of *P. angolensis* on sperm quality

	Groups					
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
Viability (%)	86.59±9.20	62.95±14.10*	87.11±13.80**	88.58±10.50**	88.32 ± 9.60**	67.59 ± 14.27
NPM (%)	39.72±19.32	27.43±13.16	28.00±13.51	19.57±6.51	27.15 ± 11.39	18.72 ± 5.05
PM (%)	399.72 ±36.62	228.72±56.33	253.29±28.21*	399.00±38.17**, §	407.15 ± 12.80**, §	242.58 ± 92.27
Immobility (%)	16.29±5.76	32.58±2.79*	26.00±4.85*	23.00±9.05**	26.00 ± 4.50*	18.86 ± 3.02

*Results are expressed as the mean ± SD (n=7). *P < 0.05 versus the normal control group (G1); **P < 0.05 versus the negative control group (G2); §P < 0.05 versus the group G3; † P < 0.05 versus the group G4; †† P < 0.05 versus the group G5. G1: uncastrated rats; G2: castrated rats; castrated rats treated with testosterone enanthate: G3 (3 mg/kg); with aqueous extract at a dose of 43 mg/kg: G4; 86 mg/kg G5and 172 mg/kg: G6.*

Effect of *P. angolensis* on reproductive organ histology

The microarchitectures of several androgen-dependent organs are illustrated in Figures 2 and 3. The different doses of the extract did not show adverse effects on the organs. The testes sections in the normal control group showed normal seminiferous tubules with a normal distribution of sperm cells. However, compared to the normal control group, several testicular histopathological changes such as the reduced presence of spermatozoa and loss of the connective tissue in the negative control group. Figure 2 shows that the rats in the normal control group have a normal and pseudostratified epididymis with sperm cells until maturation. Several histopathological changes were observed in the negative control group, including disrupted spermatogenesis, decreased epididymal sperm density and agglutination, decreased secretion of prostatic amylaceous bodies, wall thinning, decreased eosinophilic secretions of seminal vesicles, and loss of penile tissue integrity (Figure 3). The administration of the plant extract at the different doses or the reference substance induced a reorganization of these organs similar to those in the normal control group.

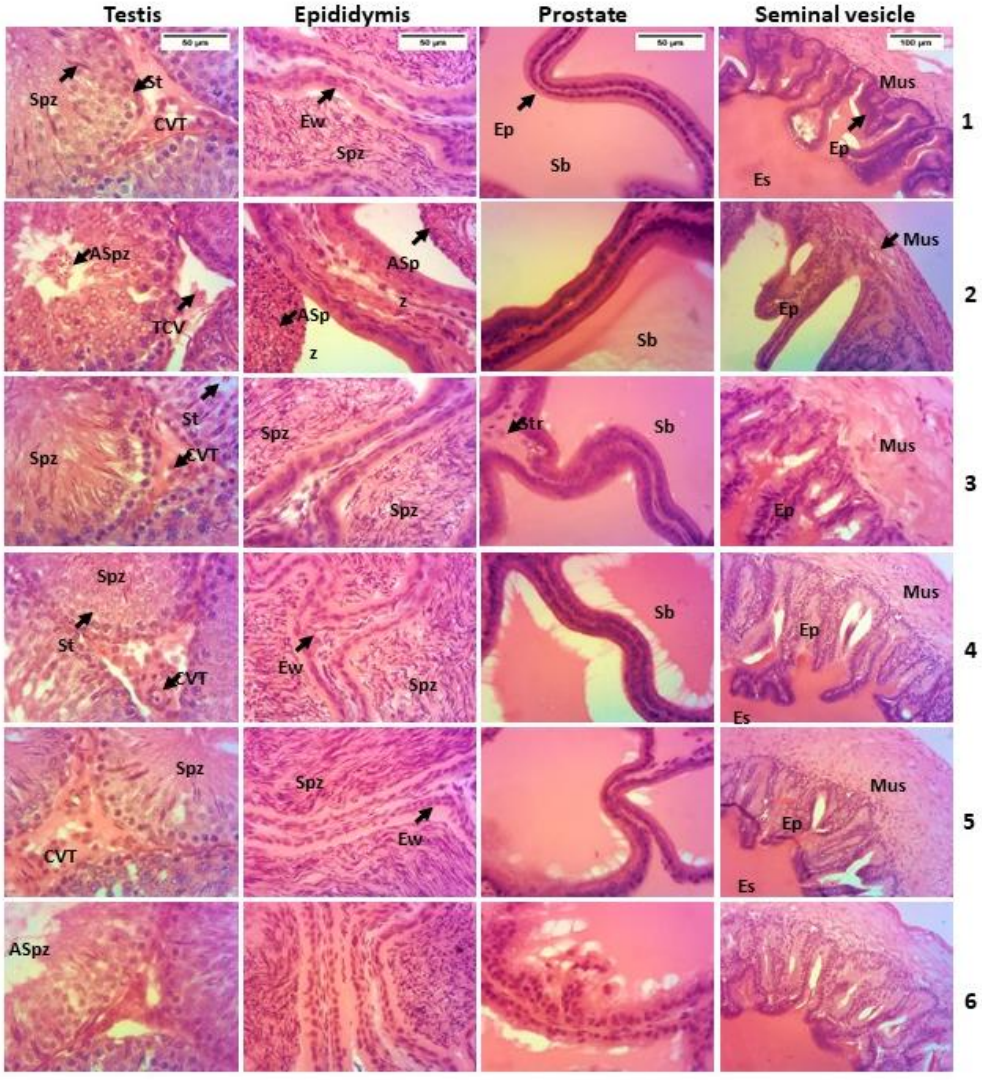

Figure 2 Microphotographs of testis (X250), epididymis (X250), prostate (X250) and seminal vesicle (X40); Hematoxylin-eosin staining,

ASpz: Altered spermatozoa; CVT: Connective vascular tissue; Ep: Epithelium; Es: Eosinophilic secretion; Ew: Epididymal wall; Mus: Muscularis; Sb: Starchy bodies; Spz: Spermatozoa; St: Seminiferous tube; Str: Stroma. 1: uncastrated rats; 2: castrated rats; 3: castrated rats treated with testosterone enanthate (3 mg/kg); 4: with aqueous extract at a dose of 43 mg/kg; 5: with aqueous extract at a dose of 86 mg/kg; 6: with aqueous extract at a dose of 172 mg/kg.

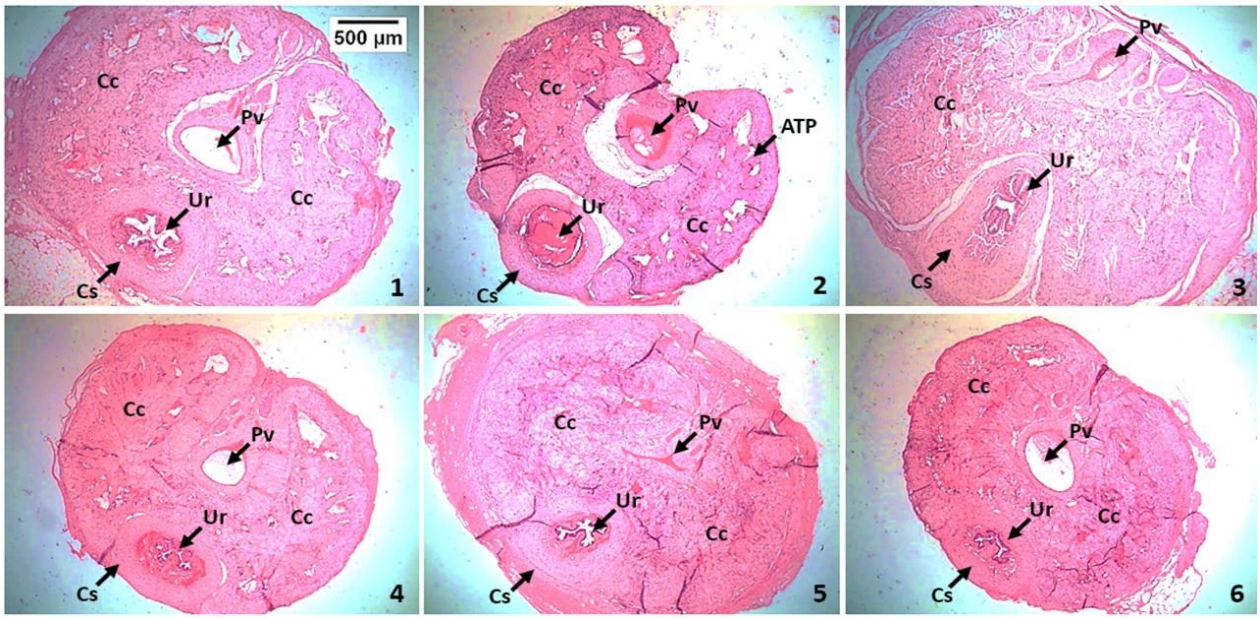

Figure 3 Microphotographs of the penis (X40); stained with Hematoxylin and eosin.

Cc = Corpus cavernosum; Cs = Corpus spongiosum; Pv = Penile vein; Ur = Urethra. 1: uncastrated rats; 2: castrated rats; 3: castrated rats treated with testosterone enanthate (3 mg/kg); 4: with aqueous extract at a dose of 43 mg/kg; 5: with aqueous extract at a dose of 86 mg/kg; 6: with aqueous extract at a dose of 172 mg/kg.

DISCUSSION

In this study, we investigated the effects of the aqueous extract of *P. angolensis* on hemicastrated male rats. Surgical castration is commonly used to induce hypogonadism in the laboratory [17]. Castration leads to a significant decrease in protein levels in reproductive organs and a reduction in enzyme activity, as well as testosterone levels [18,19]. The reproductive organs play a crucial role in sperm production, storage, and ejaculation, as well as in the modulation of sex hormones [20–22]. The increase in protein levels in these organs after the administration of the aqueous extract of *P. angolensis* suggest an improvement in their function and development that may be due to androgens. Testosterone is the key androgen that regulates sperm production [23,24], libido, and secondary sexual characteristics [25,26]. Therefore, increasing testosterone levels can improve sperm quality as well as overall male sexual and reproductive function. In our study, the groups treated with 43 and 86 mg/kg had higher testosterone levels compared to those in the negative control group. Indeed, the phytochemical screening of the aqueous extract of *P. angolensis* revealed the presence of alkaloids and saponins [8] which can be responsible for the ability of the plant extract to stimulate testosterone production and protein synthesis [27–29]. Acid phosphatase and α-glucosidase are main enzymes involved in sperm maturation, motility, and capacitation [30,31]. As the plant extract led to increases in their activity these results could suggest that the plant extract exhibits androgenic properties and can improve the sperm quality. This result was supported by the histological sections of the testes and epididymis that showed normal organ structures even after castration followed by the plant treatment.

Orchidectomy or castration is surgical removal of one or both testes to prevent or treat prostate cancer. In our study, the hemicastration of rats resulted in a significant decrease in the ejaculation, intromission and mount frequencies. Thus, castration reduces sexual performance [32] as well as libido because the latency times of ejaculation, intromission, and mount were significantly increased in the castrated rats. Nevertheless, the plant treatment significantly restored the sexual performance of the castrated rats after 14 days of treatment; and appeared to be more effective than the treatment with the reference product. Results also showed that castrated rats treated with doses of the aqueous extract or the reference product recorded significant decreases in the ejaculation, intromission, and mount latencies, which are markers of sexual motivation or desire. These results suggest that the aqueous extract of *P. angolensis* could be an alternative treatment for the management of male sexual dysfunctions, specially caused by hypogonadism.

Administration of the plant extract increased fructose levels in male rats. Fructose is an important component of sperm as it provides energy to the sperm cells, affecting their motility. Consequently, these observed results suggest an androgenic effect of the plant extract, particularly an improvement in the biochemical processes required for sperm function, which could improve male fertility. The beneficial effects of *P. angolensis* can be attributed to the secondary metabolites contained in its aqueous extract. Previous phytochemical screening studies of *P. angolensis* also revealed the presence of saponins, flavonoids, steroids, alkaloids, terpenoids, and glycosides [8,33]. These compounds could act on the central nervous system by stimulating the androgen synthesis pathway or behave as structural analogues of androgens to improve male reproductive function. To assess the impact of post-castration treatment on androgen-dependent organ structures, histological sections were performed. The results showed that the administration of the extract at doses of 43 and 86 mg/kg restored the architecture of the reproductive organs. However, the administration of the plant extract at a dose of 172 mg/kg resulted in the destruction of epididymal spermatozoa and and prostatic cells. Therefore, our results suggest that the aqueous extract of *P. angolensis* at the doses of 43 and 86 mg/kg increased testosterone levels, enzyme activities, sperm quality, and androgen-dependent organ structure in hemicastrated male rats. These effects could be attributed to the phytochemical composition of the extract, mainly saponins and alkaloids. Therefore, further studies with anti-androgenic compounds are needed to deepen our understanding of the mechanisms of action of this plant and assess its therapeutic potential in androgen-related disorders.

CONCLUSION

The results of this study demonstrated that the aqueous extract of *P. angolensis* has significant effects on androgen levels in hemicastrated male rats. Administration of this extract at the doses of 43 and 86 mg/kg improved reproductive function, particularly by stimulating the production of testosterone and other markers of androgenic activity, such as α-glucosidase and prostatic acid phosphatase. These results suggest that *P. angolensis* extract may have therapeutic potential in the treatment of hormonal disorders and dysfunctions linked to androgenicity. However, precautions must be taken because some biomolecules present in the extract may be toxic at high doses. Further studies are needed to determine the optimal safety and efficacy of this extract, as well as to better understand its underlying mechanisms of action.

Acknowledgements

The authors are grateful to the 'Baka' Pygmies who kindly provided the medicinal plants. The authors also thank M. Keumedjio Teko Paulin for the collection of the medicinal plant.

Conflicts of interest

The authors have no conflicts of interest regarding this investigation.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Sanda KA, Fotsing D, Mahamat O, Natesan V, Rajendran J, Nantia AE, *et al.* "Effect of Allanblackia floribunda stem bark aqueous extract in male albino wistar rats : androgenic activities and penile nitric oxide (NO) production". Asian Journal of Pharmacy and Pharmacology, 2021; 7: 63-73. http://dx.doi.org/10.31024/ajpp.2021.7.2.5 .

2. Kerlan V & Young J, "Les hypogonadismes masculins". MCED, 2017; 86: 40-49.

3. Marcelli M & Mediwala SN, "Male hypogonadism : a review". Journal of Investigative Medicine, 2020; 68: 335-356. http://dx.doi.org/10.1136/jim-2019-001233 .

4. Grossmann M & Matsumoto AMA, "Perspective on Middle-Aged and Older Men With Functional Hypogonadism: Focus on Holistic Management". Journal of Clinical Endocrinology and Metabolism, 2017*:* 102:1067-1075. http://dx.doi.org/10.1210/jc.2016-3580 .

5. Galbiati FF, Goldman AL, Gattu A, Guzelce EC & Bhasin S, "Benefits and risks of testosterone treatment of older men with hypogonadism". Urologic Clinics of North America, 2022; 49: 593-602. http://dx.doi.org/10.1016/j.ucl.2022.07.011.

6. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, *et al.* "Testosterone Therapy in Men With Hypogonadism : An Endocrine Society * Clinical Practice Guideline". Journal of Clinical Endocrinology and Metabolism, 2018; 103:1715-1744. http://dx.doi.org/10.1210/jc.2018-00229

7. Kameni PM, Djomeni DPD, Bilanda DC, Mballa MF, Yolande, Mengue NYS, *et al.* "*Nymphaea lotus* Linn. (Nymphaeaceae) Alleviates Sexual Disability in L-NAME Hypertensive Male Rats". Evidence-Based Complementary and Alternative Medicine, 2019; 9 pages. http://dx.doi.org/10.1155/2019/8619283 .

8. Keumedjio TP, Béboy ESN & Moundipa FP, "*In vitro* screening of the potential of six Cameroonian medicinal plants on male reproductive biomarkers". Investigational Medicinal Chemistry and Pharmacology, 2023; 6: 72-82.

9. Sofidiya MO & Awolesi AO, "Antinociceptive and antiulcer activities of *Pycnanthus angolensis"*. Revista Brasileira de Farmacognosi, 2015; 25: 252-257. http://dx.doi.org/10.1016/j.bjp.2015.05.004 .

10. Achel DG, Alcaraz M, Kingsford-Adaboh R, Nyarko AK & Gomda Y, "A review of the medicinal properties and applications of *Pycnanthus angolensis* (WELW) warb". Pharmacologyonline, 2012; 2:1-22. http://dx.doi.org/10.30574/wjarr.2023.18.2.077 7.

11. Béboy ESN, Mbog LMC, Jignoua SY, Keumedjio TP, Feune MPK, Moundipa FP. "Effects of the aqueous extract of Pycnanthus angolensis (Myristicaceae) on male rat sexual behavior and nitric oxide release". World Journal of Advanced Reearch. Reviews, 18: 329-337 (2023).

12. Sulaiman I, Mabrouk M, Adamu L & Dissi G, "Sexual behavior and fertility of male rats following subacute hemi-orchidectomy". International Journal of Biosciences, 2015; 6:1-8. http://dx.doi.org/10.12692/ijb/6.8.1-8 .

13. Gonzales GF & Villena A, "True corrected seminal fructose level: A better marker of the function of seminal vesicles in infertile men". International Journal of Andrology, 2001; 24: 255-260.

14. Gornall AG, Bardawill CJ & David MM, "Determination of serum proteins by means of the biuret reaction". Journal of Biological Chemistry, 1949; 177: 751-766.

15. Ngoula F, Guemdjo Tekam M, Kenfack A, Tadondjou Tchingo CDA, Nouboudem S, Ngoumtsop H, *et al.*, "Effects of heat stress on some reproductive parameters of male cavie (Cavia porcellus) and mitigation strategies using guava (*Psidium guajava*) leaves essential oil". Journal of Thermal Biology, 2017; 64, 67-72. http://dx.doi.org/10.1016/j.jtherbio.2017.01.001 .

16. Suvarna SK, Layton C & Bancroft JD. Bancroft's Theory and practice of histological techniques. 8th ed health sciences, 2019.

17. Nieschlag E & Nieschlag S, "The history of testosterone and the testes: From Antiquity to Modern times". Testosterone, 2017; 19 pp. http://dx.doi.org/10.1007/978-3-319-46086-4

18. Han X, Zhou Y, Zeng Y, Sui F, Liu Y, Tan Y, *et al.*, "Effects of active immunization against GnRH versus surgical castration on hypothalamic-pituitary function in boars". Theriogenology, 2017. http://dx.doi.org/10.1016/j.theriogenology.2017.04.038

19. Palmer C, Pedersen HG & Sandøe P, "Beyond Castration and Culling: Should we use Non-Surgical , Pharmacological methods to control the sexual behavior and reproduction of animals ?" Journal of Agricultural and Environmental Ethics, 2018; 31: 197-218. http://dx.doi.org/10.1007/s10806-018-9718-7 .

20. Gadella BM, "Reproductive tract modifications of the boar sperm surface". Molecular Reproduction and Development, 2017; 822-831. http://dx.doi.org/10.1002/mrd.22821

21. Chemek M, Venditti M, Boughamoura S, Mimouna SB, Messaoudi I, Minucci S, "Involvement of testicular DAAM1 expression in zinc protection against cadmium-induced male rat reproductive toxicity". *J. Cell. Physiol.* 2017; 9999: 1-11. http://dx.doi.org/10.1002/jcp.25923 .

22. Seven I, Seven PT, Baykalir BG, Ak TP, Kaya SO, Yaman M,. "Bee glue (propolis) improves reproductive organs , sperm quality and histological changes and antioxidant parameters of testis tissues in rats exposed to excess copper". *Andrologia,* 2020; 1-11. http://dx.doi.org/10.1111/and.13540

23. Azubuike NC, Okwuosa CN, Onwukwe OS, Onyemelukwe AO, Ikele I, Achukwu PU, "Effects of *Phyllanthus amarus* on epididymal sperm characteristics , testosterone levels and histology of reproductive organs of male rats". Pharmacologyonline, 2018; 3: 57-67.

24. Dutta S, Sengupta P, Muhamad S. Male reproductive hormones and semen quality. Asian Pacific Journal of Reproduction, 2019; 8:5. http://dx.doi.org/10.4103/2305-0500.268132 .

25. Karabasoglu C & Erbas O, "Rat sexual behaviours". *Journal of Experimental and Basic Medical Sciences* , 2021; 2: 139-146. http://dx.doi.org/10.5606/jebms.2021.75650 .

26. Dogani M, Askari N, Kalantari-hesari A, Hosseini F. "The effects of P. atlantica as a libido booster and sexual enhancer on the reproductive system of male rats". Journal of Traditional and Complementary Medicine, 2022*;* 12: 345-353.

27. Watcho P, Watio HM, Wankeu-Nya M, Ngadjui E, Defo PD, Nkeng-efouet PA, *et al.* "Androgenic effects of aqueous and methanolic extracts of *Ficus asperifolia* in male Wistar rats". BMC Complemenary and Alternative Medicine, 2017; 1-9. http://dx.doi.org/10.1186/s12906-016-1547-5

28. Salgado RM, Carvalho CF, Andreoli C V, Mathias AC, "The Conundrum of the Efficacy of Tribulus terrestris Extract as Supplement for Muscle Mass Gain. Journal of Pharmacognosy and Natural Products, 2018, 4: 4-7. http://dx.doi.org/10.4172/2472-0992.1000145 .

29. Rusmiati, Sari, S. & Budyarti, C. Potential of Leaves of Water Mimosa (Neptunia plena) in Increasing Spermatogenesis Activity of Male Rat. Earth Environmental Sciences, 2020; 499-. http://dx.doi.org/10.1088/1755-1315/499/1/012020

30. Liu Y, Zhang C, Wang S, Hu Y, Jing J, Ye L, *et al.*, "Dependence of sperm structural and functional integrity on testicular calcineurin isoform PPP3R2 expression". Journal of Molecular Cell Biology, 2020; 12, 515-529. http://dx.doi.org/10.1093/jmcb/mjz115 .

31. Vashisht A & Gahlay GK, "Understanding seminal plasma in male infertility : emerging markers and their implications". Andrology, 2023; 1-20. http://dx.doi.org/10.1111/andr.13563

32. Roberts SL, Powell JG, Hughes HD & Richeson JT, "Effect of castration method and analgesia on inflammation , behavior , growth performance , and carcass traits in feedlot cattle". Journal of Animal Science, 2018, 96; 66-75. http://dx.doi.org/10.1093/jas/skx022 .

33. Nono ECN, Mkounga P, Kuete V, Marat K, Hultin PG, Nkengfack AE, "Pycnanthulignenes A-D, antimicrobial cyclolignene derivatives from the roots of *Pycnanthus angolensis*". Journal Nat. Prod. 2010; 73: 213-216. http://dx.doi.org/10.1021/np9007393 .