World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Effects of the aqueous extract of *Pycnanthus angolensis* (Myristicaceae) on male rat sexual behavior and nitric oxide release

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World Journal of Advanced Research and Reviews, 2023, 18(02), 329–337

Publication history: Received on 19 March 2023; revised on 03 May 2023; accepted on 06 May 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.2.0777

Abstract

Erectile dysfunction is a major public health problem leading to harmful consequences for couples. Hence the need to find adequate treatments using medicinal plants such as *Pycnanthus angolensis*, a Cameroonian plant used to treat erectile dysfunction. The effects of aqueous extract of *Pycnanthus angolensis* wood were evaluated on male rat sexual behavior and nitric oxide release. Adult male *Wistar* rats were divided into 4 groups of 5 each that included a group receiving orally: distilled water (10 ml/Kg BW), plant extract treatment (43 or 86 or 172 mg/kg BW). Sexual behaviors were monitored on days 1, 4, 7, and 14 by pairing male rats to receptive females and nitric oxide levels were assessed in penile homogenate. At the end of the 14 days of treatment, rats were killed and sexual organs weighed. No significant effect was observed on sexual organs weights. When administered at a dose of 43 mg/Kg, body weight; the plant extract significantly increased ($p<0.05$) the sexual performance as well as the libido of male rats. Nitric oxide release was significantly increased ($p<0.05$) in that group of extract-treated rats, while compared to the control group. The study demonstrates that *Pycnanthus angolensis* is a medicinal plant which can enhance the sexual behaviors of male rat. Hence, it can be used for the management of erectile dysfunction, mainly at a dose of 43 mg/Kg, body weight.

Keywords: *Pycnanthus angolensis*; Aqueous extract; Sexual behavior; Nitric oxide release

1. Introduction

Sexuality disorders are disturbances that affect the sexuality of an individual or a couple. These disorders, which mainly include a decrease in libido, ejaculatory disorders and erectile dysfunction (ED) [1,2], constitute an important health problem with harmful consequences for man. In Cameroon, 52.7% of men with sexual problems suffer from erectile dysfunction [3] , which is defined as the inability for a man to achieve or maintain an erection sufficient for satisfactory sexual activity [4]. ED is widespread and affects men of all ages; primarily those between the ages of 40 and 70. The World Health Organization (WHO) estimates that 15% of the world's male population is affected each year. In Cameroon, the prevalence is 17.3% [3]. ED can be due to multifactorial causes such as vascular and neurological problems, hormonal disorders, sedentary lifestyles and drugs [5]. Normal penile erection is a neurovascular occurrence which involves the relaxation of cavernous smooth muscle of the penis [6]. This relaxation depends on the production of cyclic guanosine monophosphate which is triggered by an increase of the release of nitric oxide. The relaxation results in the decrease of intracellular calcium levels and the increase of the blood flow leading to the penis erection.

In order to manage erectile dysfunction, conventional medicine has developed several treatments over the years such as psychosexual therapy, penile prostheses, trans-urethral and oral therapy as well as revascularization and intra-cavernosal injections [7]. However, despite the beneficial effect of these treatments on ED, there are several

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shortcomings in their use such as penile insensitivity after operations, occurrence of cancers, infectious risks, irreversibility of the therapy, control of sometimes complicated medical techniques and effectiveness at only 40% of treatments [8]. Therefore, there is a great need to improve the quality of life of men with this condition as well as their sexual partner(s) through adequate treatment.

The medicinal plants used in the world and mainly in Africa, constitute a real therapeutic source thanks to their components that are secondary metabolites. Among these numerous plants, some have aphrodisiac effects [9]; such as *Pycnanthus angolensis* (Pa) which is a plant of the Myristicaceae family. It is found in tropical Africa [10] where the trunk bark is used for the treatment of inflammation, microbial infections, anemia and pain [11]. In Cameroon, specifically in the South region, *Baka* traditional practitioners use the aqueous decoction of *Pycnanthus angolensis* wood for the treatment of erectile dysfunction [12].

However, little has been done with regards to the aphrodisiac use of *Pycnanthus angolensis* and its effects on parameters of male reproduction. The aim of the present work was to evaluate the effects of the aqueous extract of *Pycnanthus angolensis* wood on male rat sexual behavior and nitric oxide release.

2. Material and methods

2.1. Chemicals and apparatus

The chemical and reagents used were of analytical grade. Phosphoric acid, N-naphthylethylene diamine, sodium nitrate, tris-base, sodium chloride, calcium chloride, anhydrous sulfanilamide were purchased from Sigma (Steinheim, Germany). All chemicals and reagents were of high quality grade. Absorbances were read using a Spectrolab spectrophotometer and a Wi-Fi Panorama Camera was used to record sexual behaviors.

2.2. Preparation of plant extract

Pycnanthus angolensis (Myristicaceae) extract was prepared from fresh wood collected in Yaoundé in Cameroon on November 2020 and identified at the Cameroonian National Herbarium, as specimen number HNC31369. The wood of P. angolensis was washed, dried under mild sunlight and grinded into powder. The aqueous extract 10% (m/v) was prepared by decoction during 30 min in distilled water regarding the recommendation of the traditional healer. The aqueous extract obtained was filtered, evaporated and then kept in a sealed glass container until use. The freeze-dried extract as reconstituted in distilled water in order to obtain the required doses 43, 86 and 172 mg/Kg of body weight.

2.3. Animals

2.3.1. Experimental design

Twenty male *Wistar* albino rats of thirteen weeks, weighing between 160 g and 180 g were obtained from the Animal house of the Department of Biochemistry, University of Yaoundé I. Animals were housed under standard conditions in cages and were allowed to *ad libitum* free access to feed and water. After acclimatization, animals were randomly divided into 4 groups as follow:

- Control group (G1): normal rats received distilled water (10 ml/kg, bw);
- EA1 (G2): rats received 43 mg/kg, bw of *P. angolensis* aqueous extract;
- EA 2 (G3): rats received 86 mg/kg, bw of *P. angolensis* aqueous extract;
- EA 3 (G4): rats received 172 mg/kg, bw of *P. angolensis* aqueous extract.

Animals received daily an oral administration of distilled water at a dose of 10 ml/kg of body weight and the plant extract at different doses during 14 days.

2.3.2. Sexual behavioral test

To assess the effects of *P. angolensis* aqueous extract on sexual behavior, rats were monitored for sexual behavior after 1, 4, 7 and 14 days. Thirty minutes following the administration, sexual behavior of male rats was studied by pairing them with female rats in their receptive phase of the estrus cycle which was induced by a subcutaneous administration of 50 µg/Kg estradiol benzoate [13] . Sexual behaviors parameters includes sexual arousal parameters (mount latency, intromission latency, and ejaculatory latency) and performance parameters (mount frequency, intromission frequency, and ejaculation frequency) were assessed. Ejaculation frequency is the number of observed ejaculations. Mount frequency is the number of observed mounts without intromission. Intromission frequency is the number of observed intromissions from the time of introduction of the female. Ejaculatory latency is the time interval in seconds between

the introduction of the receptive female and the first ejaculation. Intromission latency is the time interval in seconds between the introduction of the receptive female and the first intromission. Mount latency is the time interval in seconds between the introduction of the receptive female and the first mount.

2.4. Preparation of penile homogenate

After the sacrificing of rats, the penis of rats were carefully isolated and weighed to prepare a 10% (w/v) homogenate which was mixed with 0.1 M Tris-Hcl buffer (pH 8.0) containing 1 mM $CaCl_2$ and 50 mM Nacl. The homogenate was centrifuged at 1620×g for 25 min at 4 °C. Then, supernatant was kept and used to assess the nitric oxide (NO) levels.

2.5. Effects of plant extract on NO release

NO as measured in penile homogenate according the method of Fermor *et al.* [14] which is a diazotation reaction. The diluted sample in distilled water (1:4, v/v) was added to 500 µL of Griess reagent/ the same volume of Griess reagent was added to the blank tube made up of 500 µL of distilled water. After homogenization, tubes were incubated at room temperature for 10 minutes. Then, absorbance was measured at 546 nm and a standard curve was obtained using a serial known concentrations of $NaNO_2$ allowing to calculate the levels of NO which was expressed as µmol/g of organ BW.

2.6. Data analysis

Data were analyzed using R software (version 4.2.3, Lyon). Test for normality was performed using Shapiro-Wilk test. One-way analysis of variance (ANOVA) and Dunnet's post-hoc test were used for normally distributed data. Non parametric analysis between the groups were done using Kruskal-Wallis test followed by a post-hoc Dunn's test. Data were expressed as mean(SD) or median(IQR). Results were regarded as median(IQR) for the comparison between groups and considered statistically significant at p values < 0.05.

3. Results

3.1. Effects of P. angolensis treatment on relative weights of reproductive organs

After 14 days of treatment with the plant extracts, no significant increase neither nor decrease was observed in the reproductive organs' weight of control group and *P. angolensis* treated rats. Nevertheless, at the dose 86 mg/Kg of the plant extract (Table 1), the weight of the penis muscle significantly ($p<0.05$) decreased compared to the control group.

Table 1 Relative organ weights after 14 days of treatment with aqueous extract of P. angolensis

		Doses (mg/Kg)		
Organs	**Control**	**43**	**86**	**172**
Epididymis				
Mean (SD)	0.274(0.070)	0.230(0.053)	0.201(0.011)	0.248(0.021)
Median(IQR)	0.254(0.068)	0.219(0.052)	0.197(0.011)	0.241(0.020)
Penis				
Mean(SD)	0.140(0.033)	0.140(0.019)	0.150(0.009)	0.146(0.009)
Median(IQR)	0.122(0.029)	0.133(0.017)	0.152(0.009)	0.149(0.008)
Penis muscle				
Mean(SD)	0.684(0.175)	0.448(0.079)	0.299(0.011)	0.342(0.045)
Median(IQR)	0.734 (0.170)	0.426 (0.077)	0.303 (0.011)*	0.319 (0.040)
Testes				
Mean(SD)	0.608(0.064)	0.651(0.050)	0.616(0.051)	0.631(0.028)
Median(IQR)	0.638(0.058)	0.650(0.050)	0.622(0.051)	0.622(0.027)

Values are expressed as mean(SD) or median(IQR), n=5. *p < 0.05 versus control.

3.2. Effects of P. angolensis treatment on sexual behaviour of male rats

3.2.1. Effects of P. angolensis extracts on the performance of male rats

No significant results were observed for the ejaculation frequency (Table 2). Table 3 disclosed a significant increase ($p<0.05$) of the intromission frequency of extract-treated rats at the dose 43 mg/Kg, compared to rats of the control group. The same significant results were obtained for the mount frequency (Table 4).

Table 2 Effects of *P. angolensis extracts* on the ejaculation frequency of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	0.667(0.577)	0.667(0.577)	0.333(0.577)	1.333(1.155)
Median(IQR)	1.000(0.500)	1.000(0.500)	0.000(0.500)	2.000(1.000)
D4				
Mean (SD)	1.667(0.577)	1.333(0.577)	1.000(1.000)	0.667(1.155)
Median(IQR)	2.000(0.500)	1.000(0.500)	1.000(1.000)	0.000(1.000)
D7				
Mean (SD)	1.667(0.577)	3.333(1.155)	0.667(0.577)	1.333(1.528)
Median(IQR)	2.000(0.500)	4.000(1.000)	1.000(0.500)	1.000(1.500)
D14				
Mean (SD)	1.667(0.577)	3.333(0.577)	1.000(1.000)	2.000(1.000)
Median(IQR)	2.000(0.500)	3.000(0.500)	1.000(1.000)	2.000(1.000)

Values are expressed as mean(SD) or median(IQR), n=5. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14.

Table 3 Effects of *P. angolensis extracts* on the intromission frequency of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	8.333(0.577)	18.000(7.000)	3.333(2.887)	3.000(2.646)
Median(IQR)	8.000(0.500)	15.000(6.500)	5.000(2.500)[a]	5.000(2.500)[a]
D4				
Mean (SD)	14.667(2.082)	30.140(10.504)	11.333(10.203)	10.000(2.646)
Median(IQR)	14.333(2.000)	30.000(10.500)*	14.000(10.000)	11.000(2.000)
D7				
Mean (SD)	10.667(1.155)	26.667(6.506)	14.000(11.533)	15.333(14.742)
Median(IQR)	10.000(1.000)	27.000(6.500)*	18.000(11.000)	10.000(14.000)
D14				
Mean (SD)	15.333(3.055)	36.667(5.686)	16.333(16.010)	22.667(21.079)
Median(IQR)	16.000(3.000)	35.000(5.500)*	17.000(16.000)	17.000(20.500)

Values are expressed as mean(SD) or median(IQR), n=5. *$p < 0.05$ versus control. [a]$p < 0.05$ versus EA1. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14.

Table 4 Effects of *P. angolensis extracts* on the mount frequency of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	14.000(2.646)	13.667(4.041)	12.333(5.859)	7.333(4.509)
Median(IQR)	15.000(2.500)	13.000(4.000)	10.000(5.500)	7.000(4.500)
D4				
Mean (SD)	15.000(3.000)	34.000(12.000)	29.000(10.000)	13.667(2.517)
Median(IQR)	15.000(3.000)	34.000(12.000)*	29.000(10.000)	14.000(2.500)
D7				
Mean (SD)	15.333(3.512)	32.000(2.000)	29.333(7.506)	21.333(11.372)
Median(IQR)	15.000(3.500)	32.000(2.000)*	29.000(7.500)	18.000(11.000)
D14				
Mean (SD)	18.667(3.786)	43.333(8.021)	22.667(18.824)	40.667(9.866)
Median(IQR)	17.000(3.500)	44.000(8.000)*	32.000(17.000)	36.000(9.000)

Values are expressed as mean(SD) or median(IQR), n=5. *p < 0.05 versus control. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14.

3.2.2. Effects of P. angolensis extracts on sexual arousal of male rats

Table 5 Effects of *P. angolensis extracts* on the ejaculatory latency time (seconds) of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	828.000(842.385)	285.000(16.093)	1098.667(608.113)	1196.333(523.585)
Median(IQR)	374.000(745.000)	290.000(15.500)	778.000(541.000)	917.000(464.500)
D4				
Mean (SD)	166.000(11.269)	153.667(23.180)	1013.000(681.584)	832.333(839.369)
Median(IQR)	172.000(10.000)	157.000(23.000)	625.000(593.000)	396.000(749.500)
D7				
Mean (SD)	187.000(12.288)	107.333(22.502)	940.667(744.376)	675.667(888.855)
Median(IQR)	182.000(11.500)	98.000(21.000)	527.000(652.500)[a]	169.000(773.000)
D14				
Mean (SD)	204.333(12.503)	52.000(5.568)	686.000(964.754)	181.667(15.567)
Median(IQR)	210.000(11.500)	53.000(5.500)	131.000(836.500)	180.000(15.500)

Values are expressed as mean(SD) or median(IQR), n=5. [a]p < 0.05 versus EA1. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14

Ejaculatory, intromission and mount latencies are indicative of sexual arousal. Tables 5, 6 and 7 show the effects of the aqueous extract of *P. angolensis* on ejaculatory, intromission and mount latencies times respectively. After 7 days of treatment, a significant increase ($p<0.05$) of the ejaculatory latency time of extract-treated rats at the dose of 86 mg/Kg compared to those treated with the plant extract at the dose of 43 mg/Kg (Table 5). Table 6 depicted a significant

decrease ($p<0.05$) of the intromission latency time of the extract-treated rats at the dose of 43 mg/Kg on during all the treatment. Table 7 indicated on day 4, a significant increase of the mount latency time of extract-treated rats at dose of 172 mg/Kg compared to those treated with the plant extract at the dose of 43 mg/Kg. A significant decrease ($p<0.05$) of the mount latency time was also observed among the extract-treated rats at dose of 43 mg/Kg, whereas the values obtained by the other dose group were not significantly different from the control group.

Table 6 Effects of *P. angolensis extracts* on the intromission latency time (seconds) of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	208.667 (37.166)	288.333(10.017)	973.333(717.145)	1123.333(551.696)
Median(IQR)	198.000(36.000)	289.000(10.000)[b]	602.000(641.000)*	811.000(481.000)*
D4				
Mean (SD)	102.333(0.570)	148.667(8.083)	618.000(911.072)	526.667(479.205)
Median(IQR)	102.000(0.500)	150.000(8.000)*	97.000(791.500)	252.000(416.000)
D7				
Mean (SD)	81.667(9.452)	95.000(3.606)	92.667(8.142)	656.333(852.233)
Median(IQR)	85.000(9.000)	94.000(3.500)	89.000(7.500)	189.000(750.000)*
D14				
Mean (SD)	41.333(6.506)	25.333(2.517)	41.000(14.107)	44.000(22.113)
Median(IQR)	41.000(6.500)	25.000(2.500)*,[b]	39.000(14.000)	36.000(21.000)

Values are expressed as mean(SD) or median(IQR), n=5. *$p < 0.05$ versus control (ED); [b]$p < 0.05$ versus D1 and D14 of group EA. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14.

Table 7 Effects of *P. angolensis extracts* on the mount latency time (seconds) of male rats

	Groups			
Day of observation	**ED**	**EA1**	**EA2**	**EA3**
D1				
Mean (SD)	190.000(55.678)	263.000(22.605)	880.333(796.511)	1083.000(545.249)
Median(IQR)	180.000(55.00)	252.000(20.500) [b]	430.000(694.500)*	819.000(495.000)*
D4				
Mean (SD)	74.000(3.606)	37.333(2.082)	612.333(905.605)	413.000(444.294)
Median(IQR)	75.000(3.500)	38.000(2.000)	97.000(788.000)	161.000(387.000)*,[a]
D7				
Mean (SD)	67.667(7.024)	69.000(4.583)	59.667(17.039)	563.667(724.287)
Median(IQR)	67.000(7.000)	68.000(4.500)	61.000(17.000)	147.000(628.000)
D14				
Mean (SD)	37.000(2.646)	14.000(3.606)	20.667(5.686)	22.333(5.132)
Median(IQR)	38.000(2.500)	13.000(3.500) *, [b]	19.000(5.500)	21.000(5.000)

Values are expressed as mean(SD) or median(IQR), n=5. [a]$p < 0.05$ versus EA1; [b]$p < 0.05$ versus D1 and D14 of group EA1. ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW; D1: day 1; D4: day 4; D7: day 7; D14: day 14.

3.3. Effects of *P. angolensis* extracts on in vitro NO release

As shown on figure 1, NO release was significantly increased ($p<0.05$) in the group of extract-treated rats at the dose of 43 mg/kg while comparing with the control group.

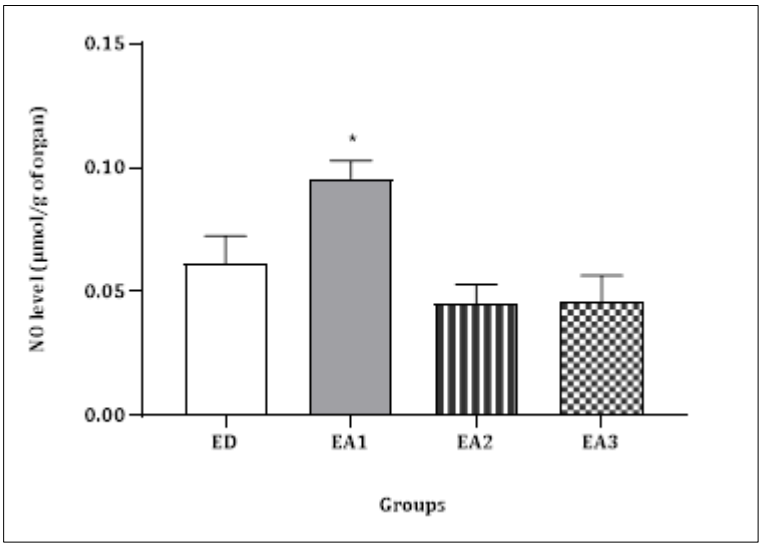

Figure 1 Effects of *P. angolensis* extracts on the level of nitric oxide of male rats Values are expressed as mean ± SD, n=5. *p < 0.05 versus control (ED). ED: distilled water; EA1: aqueous extract 43 mg/kg; EA2: aqueous extract 86 mg/kg; EA3: aqueous extract 172 mg/kg, BW

4. Discussion

Despite the traditional use of the wood of *Pycnanthus angolensis* by healers of the Southern region of Cameroon to treat erectile dysfunction, to the best of our knowledge no attempt has been made to scientifically investigate its aphrodisiac properties. Therefore, the present study was undertaken in order to study the effects of aqueous extract of *P. angolensis* wood were evaluated on male rat sexual behavior and nitric oxide release. Administration of the plant extract showed no significant effect of the plant extract on the relative weight of androgenic organs of male rats, even on the ejaculation frequency. Nevertheless, after 4 days of treatment with plant extract at the dose of 43 mg/kg, significant increases of intromission and mount frequencies while compared to the control group. These results suggest that the plant extract at dose of 43 mg/kg, could enhance the performance of male rats. Mount, intromission and ejaculation frequencies are indicators of sexual performance while mount, intromission and ejaculatory latencies are indicative of sexual motivation or desire, while [15,16]. Results also exhibited significant decreases of mount and intromission latencies in rats treated with the plant extract at dose of 43 mg/kg. These results are improved than those obtained by Kenjale *et al.* [17] with the aqueous extract of the roots of *Chlorophytum borvilinanum* at the dose of 125 mg/kg and 250 mg/kg, BW. Similar results were also obtained with the roots of *Arctium lappa L.* [15] and the stem of *Massularia acuminata* [18] but with higher doses than those used in the present study. The reductions of mount and intromission latencies described a reduction of hesitation of male rats to sniff and approach the female rats [19]. Hence, the aqueous extract of *P. angolensis* significantly enhanced the libido as well as the sexual arousal. These pro-sexual effects of the plan could be due to the presence of some secondary metabolites. Indeed, previous phytochemical studies revealed that the aqueous extract of *P. angolensis* revealed the presence of second metabolites such as alkaloids, phenols, flavonoids, saponins, terpenoids and steroids [20,21]. Several studies showed that metabolites such as alkaloids, phenols, flavonoids and saponins can enhance sexual behavior of male rats [22,23] probably by enhancing the androgen biosynthesis and secretion. Moreover, it is well known that alkaloids enhance vascular relaxation by stimulating NO production [22,23].

Present results also showed that NO release was significantly increased ($p<0.05$) in the group of extract-treated rats at the dose of 43 mg/kg while comparing with the control group. This results indicates that the plant extract can stimulate the production of NO which is involved in erection.

Nitric oxide, which is the main mediator of penile erection, plays a crucial role in the relaxation of smooth muscle that leads to erection. It is an essential neurotransmitter for erection. These stimulating effects on NO production are thought to be due to the saponins and alkaloids contained in the plant extract [24], which have the ability to stimulate NOS activity in penile tissue and consequently endothelial and neuronal NO synthesis

5. Conclusion

This study showed that the orally administration of the aqueous extract of *P. angolensis* at the dose of 43 mg/Kg, BW could be used for the management of erectile dysfunction. However, further investigations need to be undertaken in order to support the use of this plant as an aphrodisiac agent.

Compliance with ethical standards

Acknowledgments

The authors are grateful the 'Baka' Pygmies who kindly provided the medicinal plants. They also thank Pr Pefura Yone and *the Institut de Formation Professionnelle pour l'Education et la Recherche Appliquée* (IFPERA) for the proofreading of this paper.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All experiments in this study were conducted under the principles and procedures of the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Cameroon Institutional National Ethics Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954).

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