Aqueous extract of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae) alleviates paroxetine-induced erectile dysfunction in male rats

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**ABSTRACT**

**Background:** Erectile dysfunction is the inability to achieve and maintain an adequate erection for sexual performance. *Pycnanthus angolensis* (Welw.) Warb. is a medicinal plant used by the traditional healers of the Southern region of Cameroon to manage male infertility and especially erectile dysfunction. **Aim and objectives:** This study aimed at investigating the effects of aqueous extract of *Pycnanthus angolensis* wood were investigated on paroxetine-induced erectile dysfunction in male rats. **Material and methods:** Thirty *Wistar* albino male rats (160-180 g) were randomly distributed into six groups of five animals each. Erectile dysfunction was induced in 5 groups for 21 days by oral administration of paroxetine (10 mg/kg), once a day. After induction, rats were orally treated during 14 days with three doses of plant extract (43, 86 and 172 mg/kg) except 2 groups which were given distilled water and sildenafil (5 mg/kg), respectively. Sexual behaviors were monitored on days 1, 4, 7, and 14 by pairing male rats to receptive females. After 14 days-treatment, the rats were killed by decapitation under ether anesthesia. The reproductive organs were collected for histological and biochemical analysis. **Results:** Paroxetine induced erectile dysfunction characterized by a significant decrease (p<0.05) in sexual arousal and performance of male rats. Nevertheless, this erectile dysfunction was improved by administration of the aqueous extract of *Pycnanthus angolensis* at the different doses. The administration of paroxetine significantly (p<0.05) reduced the nitric oxide level by 66.67% when compared to the distilled water-group. After 14 days-treatment, the extract induced significant increases (p<0.05) in the sexual performance parameters (mounts, intromissions and ejaculations frequency) as well as in the sexual arousal of the male rats. Results were markedly pronounced with the plant extract at the doses of 86 and 172 mg/kg. The nitric oxide levels in the erectile dysfunction-induced rats with paroxetine were restored after treatment with the plant extract. The degenerated seminiferous tubules and the low density of spermatozoa observed in the histological sections of the erectile dysfunction-induced rats with paroxetine, were restored after treatments with sildenafil and the aqueous extract at either doses (43, 86 and 172 mg/kg). **Conclusions:** Aqueous extract of *Pycnanthus angolensis* (Welw.) Warb alleviated the paroxetine-related erectile dysfunction by enhancing sexual behavior and the production of nitric oxide. In view of the abovementioned results, *Pycnanthus angolensis* (Welw.) Warb could be considered as an alternative treatment in the erection dysfunction management. **Keywords:** Male impotence, Paroxetine, *Pycnanthus angolensis*, Aqueous extract, Sexual behavior.

**INTRODUCTION**

Infertility is a reproductive health problem that affects many couples around the world. Many factors can lead to this health condition. Among the multiple cases of male infertility, sexual dysfunction is responsible of about 30 % of adult men worldwide [1], and of 17.3% of men in Cameroon [2]. The worldwide prevalence of sexual dysfunction will increase from 152 million to 365 million in 2025 [3,4]. Sexual dysfunction can occur in men at any age but it is more common in the elderly [5]. Types of sexual dysfunction leading to male infertility are erectile dysfunction and premature ejaculatory disorders C. Besides this, erectile dysfunction appears to be a function of severity of semen quality impairment which is higher in men with azoospermia than in fertile men [6,7].

Erectile dysfunction, also called male impotence, is the inability to achieve and sustain sufficient penile erection to allow a satisfactory sexual intercourse or the inability to ejaculate or both [1,3]. Normal penile erection is a neurovascular occurrence which involves the relaxation of cavernous smooth muscle of the penis [8].
This relaxation depends on the production of cyclic guanosine monophosphate (cGMP) which is triggered by an increase of the production of nitric oxide (NO). The relaxation results in the decrease of intracellular calcium levels and the increase of the blood flow leading to the penis erection. Erectile dysfunction is associated with a reduced quality of sexual life characterized by anorgasmia, premature ejaculation and/or delayed ejaculation [10]. Then, erectile dysfunction can be considered as a pathology which could be due to multifactorial causes such as vascular and neurological problems, hormonal disorders, sedentary lifestyles and drugs such as antidepressants like paroxetine. In fact, paroxetine is a drug from the family of selective serotonin reuptake inhibitors (SSRIs) used for the treatment of anxiety, insomnia, and depression with several associated side effects including sexual dysfunction, anorgasmia and sleeping challenge [11]. Erectile dysfunction can be managed by conventional therapies including psychosexual therapy; pelvic surgery [12]; penile implants and drugs such as phosphodiesterase type 5 inhibitors like Sildenafil, Tadalafil or Vardenafil, Tadalafil or Vardenafil. These therapies are effective but not easily affordable, hence the necessity to look for cheaper and reduced side effects with medicinal plants [15,16].

**Pycnanthus angolensis** (Welw.) Warb. (**P. angolensis**) is a medicinal plant used by traditional healers of the Southern region of Cameroon to treat male infertility and especially erectile dysfunction. Biological activities of **P. angolensis** reported in literature include antihermimetic, anticancer, antihyperglycemic, antimicrobial [17], antioxidant activities [18-20], anti-inflammatory, antimituganet [21]. The plant has also been screened for the stimulation of testosterone and nitric oxide production [22,23]. The purpose of this study was to assess the effects of the aqueous extract of **Pycnanthus angolensis** on paroxetine-induced erectile dysfunction in male rats.

**MATERIAL AND METHODS**

**Plant collection and extraction**

**P. angolensis** extract was prepared from fresh wood collected in Yaoundé in Cameroon in December 2021 and identified at the Cameroon National Herbarium, as specimen number HNC31369. Dried wood of **P. angolensis** was ground using a blender. Fifty grams of the powder were dissolved in 500 mL of distilled water and boiled during 30 min in order to get the aqueous extract which was filtered, dried in the oven at 40°C and kept in the freezer until use. The extraction yield was 13.33%. The three doses administered were estimated based on posology used by traditional healers. The amount of powder administered to a man weighing 70 kg for the management of erectile dysfunction is equivalent to 86 mg/kg. Thus, the obtained extract was dissolved in distilled water to obtain the required doses 43, 86 and 172 mg/kg of body weight (bw), half and two-folds of the required dose.

**Animal care and experimental design**

Thirteen weeks male *Wistar* albino rats, weighing between 160-180 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 food and water.

**Ethical considerations**

The study was ethically approved by the Institutional review committee (Reg. number FWA-IRD 0001954).

**Induction of erectile dysfunction**

After 7 days of acclimatization, male *Wistar* albino rats were randomly divided into 6 groups (G1 to G6) with 5 animals per group. In order to induce erectile dysfunction, 25 rats received an oral administration of 10 mg/kg of paroxetine, daily for 21 days. The choice of the dose of paroxetine was in accordance to Ademosun et al. [11].

**Plant extract administration and assessment of mating behavior parameters**

At the end of the 21 days of induction, the erectile dysfunction-induced rats were then orally treated for 14 days with the plant extract at 3 different doses except 2 groups which were given distilled water and sildenafil, respectively. Rats were randomly divided as follow:

- **Normal control group (G1):** normal rats that received distilled water (10 ml/kg, bw);
- **Negative control group (G2):** rats that received paroxetine (10 mg/kg, bw) and distilled water (10 ml/kg);
- **Positive control group (G3):** rats that received paroxetine and treated with sildenafil citrate (5 mg/kg, bw);
- **Test group (G4):** rats that received paroxetine and treated with the aqueous extract of *P. angolensis* at the dose of 43 mg/kg, bw;
- **Test group (G5):** rats that received paroxetine and treated with the aqueous extract of *P. angolensis* at the dose of 86 mg/kg, bw;
- **Test group (G6):** rats that received paroxetine and treated with the aqueous extract of *P. angolensis* at the dose of 172 mg/kg, bw.

Rats were monitored for sexual behavior at day 1, 4, 7 and 14. Their behavior was observed for 30 min in presence of 30 female rats brought to estrus by subcutaneous administration of 50 µg/Kg estradiol benzoate [24]. Then, sexual behaviors parameters such as sexual arousal parameters (mount latency, intromission latency, and ejaculation latency) and desire parameters (mount frequency, intromission frequency, ejaculation frequency) were assessed.

The following sexual parameters were recorded during the recordings: mount frequency (MF) or number of mounts recorded preceding ejaculation; ejaculation frequency (EF) which is the number of ejaculations recorded during the observation; intromission frequency (IF) or number of intromissions recorded preceding ejaculation; mount latency (ML) or time elapsed from the introduction of the receptive female in the cage to the first mount; intromission latency (IL) or time elapsed from the introduction of the female to the first intromission; ejaculation latency (EL) which is the time elapsed from the first intromission of the first ejaculation.

Then, relative sexual parameters (RP) were calculated from those parameters comparing with the negative group, using the following formula:

\[
\text{RP} = \frac{\text{Considered parameter (Gx on Dx)}}{\text{Considered parameter (Gref on Dx)}}
\]

With Gx= each group  
Dx= day 1 or 4 or 7 or 14  
Gref= Reference negative group

**Measurements of reproductive organ relative weights**

At the end of the 14-days treatment period, animals were killed under ether anesthesia, and the reproductive organs (testes, epididymis and penis) were isolated, rinsed, wiped, clean, and weighted. Their relative weights were expressed as a percentage of the total body weight of the rat. The reproductive organs were also kept in Bouin solution for histological analysis.

**Determination of Nitric oxide (NO) levels**

NO levels were assessed according to the method of Fermor et al. [25] in a 10% (w/v) penile homogenate, prepared in 0.1 M Tris-HCl buffer (pH 8.0) containing 1 mM CaCl2 and 50 mM NaCl.
Briefly, 100 µL of diluted sample in distilled water (1:4, v/v) or blank were added to 500 µL of Griess reagent followed by an incubation at room temperature for 10 minutes. Then, absorbances were read at 546 nm and the levels of NO were calculated from the standard curve of sodium nitrite. NO levels of were expressed as µmol/g of organ, bw.

**Effects of paroxetine and plant extract on the histology of reproductive organs in rats**

For microscopic examination, the testes, epididymis and penis of each animal were fixed in Bouin’s solution for a fortnight. Then, they were dehydrated with ascending grades of alcohol (50°, 70°, 95° and 100°), cleaned in xylene, and embedded in Paraffin wax. Thin Paraffin slices of 5 µm were prepared with a microtome, and stained with haematoxylin-eosin for histological examination under a light microscope [20].

**Data analysis**

Statistical analysis were performed using R software (version 4.1.3, Lyon). Data were expressed as mean ± standard error of the mean (SEM) of three replicate. Data analysis between the groups were done using One-way analysis of variance (ANOVA) test followed by a Tukey’s post-hoc test. Significant differences were accepted at *p* < 0.05.

**RESULTS**

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

Administration of paroxetine induced significant decreases (*p* < 0.05) in the relative weights of the testes and penis, compared to those in the normal control group (Table 1). Oral treatments of paroxetine-induced sexually impaired male rats with either the aqueous extract of *P. angolensis* or Sildenafil citrate, had significant effects on the relative weights (*p* < 0.05) of the testes and penis, compared to those of the negative control. However, no significant effect was observed of the treatments was observed on the relative weights of the epididymis.

**Evaluation of copulatory parameters after treatment with paroxetine, Sildenafil citrate and the plant extract**

Effects of the aqueous extract of *P. angolensis* on the sexual performance parameters

Results obtained from the study of the effects of the plant extract on the sexual performance of male rats with impaired dysfunction displayed that the plant extract administration reversed the erectile dysfunction due to administration of paroxetine (10 mg/kg) in male rats. It can be seen that oral administration of paroxetine significantly reduced the mount (Figure 1), intromission (Figure 2) and ejaculation (Figure 3) frequencies when compared to those in the normal control group. At day 14, compared to the normal group, the administration of paroxetine significantly lowered (*p* < 0.05) the frequencies of mount by 14.20%, the frequencies of intromission by 39.40%, respectively compared to the normal control group. After 14-days of continuous oral administration, the aqueous extract at the dose of 172 mg/kg resulted in a mount frequency 3-fold higher than those in the normal control and negative groups. This value was also greater than that in the positive control group (G3), though not significant (*p* > 0.05). Compared to the normal (G1) and negative (G2) control groups, the erectile dysfunction-induced rats and treated with aqueous extract at a dose of 43 mg/kg (G4) recorded higher ejaculation frequencies (*p* < 0.05). During treatment, values increased with a rate of 14% and 30%, compared to the normal and the negative control groups, respectively. The administration of the aqueous extract at the dose of 172 mg/kg resulted in the highest values in relative intromission (25.85) on day 7 and in the relative mount (24.23) on day 14.

**Effects of the aqueous extract of *P. angolensis* on the arousal parameters**

Paroxetine administration in male rats also altered sexual arousal by significantly (*p* < 0.05) prolonging the time lag of mount (Figure 4), intromission (Figure 5) and ejaculation (Figure 6), compared to those in the normal control group. After 14-days treatment, oral administration of paroxetine significantly raised (*p* < 0.05) the latencies of mount, intromission, and ejaculation by 1.22; 19.30; and 1.77-fold respectively, when compared to the normal control group. The plant extract administration significantly reversed (*p* < 0.05) the paroxetine administration related changes in the relative mount, intromission and ejaculation latencies by shortening the respective time lags.

**Nitric oxide level**

As shown in Figure 7, administration of paroxetine significantly (*p* < 0.05) reduced the NO level by 66.67% when compared to the normal control group. After 14-days treatment with Sildenafil citrate and the aqueous extract at the dose of 86 mg/kg, the NO levels were 4.45 and 2.86-fold, respectively higher (*p* < 0.05) than that in rats with paroxetine-induced erectile dysfunction. The highest increase in the NO level in rats was obtained with the Sildenafil citrate treatment (0.09 µmol/g).

**Histological examination of the testis, epididymis and penis**

Figure 8 illustrates the effects of the aqueous extract of *P. angolensis* on the microarchitecture of some androgen-dependent organs. Results showed that normal rats that received distilled water presented normal testicular parenchyma with seminiferous tubules showing male sperm cells at different stages of development from the periphery (spermatogonia) to the lumen (spermatozoa). Their epididymis showed a high density of spermatozoa, and the penis with a cavernous body, and well-differentiated dorsal nerves. On the other hand, in the negative control group, paroxetine led to a clarification of the lumen of the seminiferous tubes, indicating an alteration in spermatogenesis and a reduction in sperm secretion and density in the epididymis. The paroxetine-induced erectile dysfunction male rats also showed a disorganization of the corpora cavernosa of the penis and a reduction in the surface area of the dorsal vein. The Treatments with sildenafil citrate and the aqueous extract at either doses repaired the tissue damages.

**DISCUSSION**

The present study explored the effect of aqueous extract of *P. angolensis* wood on paroxetine-induced erectile dysfunction in male rats. Oral administration of paroxetine significantly induced erectile dysfunction in the male rats after 21 days. This was illustrated by the deterioration of sexual motivation and performance in male rats. Paroxetine significantly increased (*p* < 0.05) sexual motivation parameters such as the latencies of mounts, intromissions and ejaculations while parameters of sexual performance like frequencies of mounts, intromissions and ejaculations were significantly lowered (*p* < 0.05) in paroxetine induced group. These results are similar to (Ademosun *et al.* [11]; Ogunro and Yakubu [27] and Rahman *et al.* [28] findings. They revealed that paroxetine was able to induce erectile dysfunction in male rats. Paroxetine is a drug used for the management of depression and anxiety. Paroxetine treatment can cause side effects which alter the sexual behavior of rats and also decrease the nitric oxide production leading to erectile dysfunction [29].

Aqueous extract of *Pyrananthus angolensis* wood is frequently used by the Pygmies Baka of the Southern region of Cameroon to treat impaired sexual functions and to arouse libido. Results obtained from the treatment of paroxetine-induced erectile dysfunction male rats with the aqueous extract of *P. angolensis* exhibited its aphrodisiac potential by significantly increasing (*p* < 0.05) the performance and...
sexual arousal parameters likewise the positive control, sildenafil citrate. The plant extract significantly increased the frequency of sexual behavior parameters and decreased the time lag in the paroxetine-induced group. Then, the traditional use of that plant was clearly justified by the increase of sexual arousal and performance of the paroxetine-induced erectile dysfunction male rats as a result of the oral administration of the plant extract. These results suggest that the plant extract could enhance erectile function by stimulating the biosynthesis of testosterone which stimulates the mechanism including lido and NO production by the NO/cGMP signal pathway \[30\].

Moreover, results depicted that the plant extract treatment better reversed the effect ($p < 0.05$) of paroxetine than the sildenafil citrate treatment by significant increases of the frequencies of mount, intromission and ejaculation. Additionally, results were more pronounced with the aqueous extract at the doses of 86 and 172 mg/kg. This suggests that *P. angolensis* could be a more potent inhibitor of phosphodiesterase type 5 than sildenafil citrate.

Previous studies have demonstrated that paroxetine can induce erectile dysfunction by inhibiting the activity of nitric oxide synthase which catalyzes the production of NO from arginine \[31,32\]. Nitric oxide, the main mediator of penile erection, plays a crucial role in the smooth muscle relaxation which eventually leads to erection. It is an essential neurotransmitter for erection. The production of nitric oxide in the penile homogenate showed a significant decrease ($p < 0.05$) in the negative control group which received only paroxetine, compared to the normal control group and the treated groups especially with sildenafil citrate (G3) and the aqueous extract of plant at the dose of 86 mg/kg (G5) ($p < 0.05$). Then, the stimulation of NO production by the plant extract could explained the responses obtained from the study of sexual behavior parameters as NO is known to be involved in penile erection by initiating smooth muscle relaxation after a sexual stimulation \[31\]. The results showed that the aqueous extract at the doses of 86 and 172 mg/kg administered once daily for 14 days increased the NO level (figure 7). The mechanism involved could be the stimulation of NO production through the NO/cGMP pathway.

The microscopic examination displayed degenerative changes in the seminiferous tubules of the rats showing reduced spermatogenesis in the lumen in the paroxetine-induced erectile dysfunction male rats. Those results indicate that the paroxetine administration altered the testis and epididymis but the plant extract treatments reversed the changes related to the paroxetine administration.

It is well-known that paroxetine inhibits the serotonin reuptake into the presynaptic nerve through the serotonin transporters \[31,32\] leading to delayed ejaculation and inhibition of NO synthase resulting in male sexual disorders. Then, our findings showed that the damages related to the oral administration of paroxetine in male rats were all reversed by the plant extract treatment which could contain substances capable to act as agonists of serotonin receptors.

Otherwise, the phytochemical analysis of the aqueous extract of *Pycnanthus angolensis* revealed many active metabolites such as saponins, flavonoids, alkaloids and polyphenols \[19,33\]. Then therapeutic effects of the plant extract could be due to the secondary metabolites such as flavonoids which are known to improve testosterone synthesis regarding the similar structure of flavonoids with cholesterol \[34\].

Flavonoids have also been reported to increase the level of nitric oxide in vascular tissue, enhancing the sexual behavior of male rodents \[16,35\]. Likewise, the HLPC-MS/MS of the aqueous extract of *P. angolensis* revealed the presence of flavonoids and phenolic compounds such as catechin, genistein, prunetin \[21,36\] which could be responsible of the effects of the plant extract. As the mechanism underlying the effects of the aqueous extract of *P. angolensis*, remains unknown; further studies on the assessment of testosterone production, sperm quality or the activities of key enzymes involved in erection mechanism will be worthy to be conducted.

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

<table>
<thead>
<tr>
<th>Organs (% )</th>
<th>Groups</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td></td>
<td>0.646 ± 0.007</td>
<td>0.586 ± 0.010</td>
<td>0.641 ± 0.015</td>
<td>0.627 ± 0.007</td>
<td>0.632 ± 0.009</td>
<td>0.681 ± 0.020</td>
</tr>
<tr>
<td>Epididymis</td>
<td></td>
<td>0.235 ± 0.019</td>
<td>0.251 ± 0.005</td>
<td>0.254 ± 0.011</td>
<td>0.224 ± 0.001</td>
<td>0.234 ± 0.007</td>
<td>0.231 ± 0.008</td>
</tr>
<tr>
<td>Penis</td>
<td></td>
<td>0.121 ± 0.001</td>
<td>0.181 ± 0.011</td>
<td>0.140 ± 0.016</td>
<td>0.154 ± 0.015</td>
<td>0.149 ± 0.005</td>
<td>0.148 ± 0.003</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). $p < 0.05$ versus the normal control group (G1); $p < 0.05$ versus the negative control group (G2); G1: normal rats that received distilled water; G2: erectile dysfunction-induced rats with paroxetine (10 mg/kg); G3: erectile dysfunction-induced rats with paroxetine and treated with sildenafil citrate (5 mg/kg); G4: erectile dysfunction-induced rats with paroxetine and treated with aqueous extract at a dose of 43 mg/kg; G5: erectile dysfunction-induced rats with paroxetine and treated with aqueous extract at a dose of 86 mg/kg; G6: erectile dysfunction-induced rats with paroxetine and treated with aqueous extract at a dose of 172 mg/kg.

Results are expressed as mean ± SEM (n=5). $p < 0.05$; $p < 0.01$; $p < 0.001$ compared to the normal control group; $p < 0.05$; $p < 0.01$; $p < 0.001$ compared to negative control group. Normal rats that received distilled water: G1 or normal control group; erectile dysfunction-induced rats with paroxetine (10 mg/kg): G2 or negative control group; erectile dysfunction-induced rats and treated with sildenafil citrate (5 mg/kg); G3 or negative control group; with aqueous extract at a dose of 43 mg/kg: G4; with aqueous extract at a dose of 86 mg/kg: G5; with aqueous extract at a dose of 172 mg/kg: G6.

**Figure 1**: Relative mount evolution during 14-days treatment with the aqueous extract of *P. angolensis*
Results are expressed as mean ± SEM (n=5). *p < 0.05; **p < 0.01; ***p < 0.001 compared to the normal control group; #p < 0.05; ##p < 0.01; ###p < 0.001 compared to negative control group. Normal rats that received distilled water: G1 or normal control group; erectile dysfunction-induced rats with paroxetine (10 mg/kg): G2 or negative control group; erectile dysfunction-induced rats and treated with sildenafil citrate (5 mg/kg): G3 or negative control group; with aqueous extract at a dose of 43 mg/kg: G4; with aqueous extract at a dose of 86 mg/kg: G5; with aqueous extract at a dose of 172 mg/kg: G6

**Figure 2:** Relative intromission evolution during 14-days treatment with the aqueous extract of *P. angolensis*

**Figure 3:** Relative ejaculation evolution during 14-days treatment with the aqueous extract of *P. angolensis*

**Figure 4:** Relative mount latency evolution during 14-days treatment with the aqueous extract of *P. angolensis*
CONCLUSION

The oral administration of paroxetine altered the indices of the sexual behavior of male rats and decreased the production of nitric oxide. The plant extract (43, 86 and 172 mg/kg) significantly alleviated the paroxetine-induced erectile dysfunction in male rats by raising the frequencies of mount, intromission and ejaculation and shortening their time lag. The results of the present study support that the aqueous extract of *Pycnanthus angolensis* wood can be used as a natural complementary and alternative medicine to manage paroxetine-induced sexual dysfunction.

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Conflict of interest

The authors declared no conflict of interest.

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