Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Original Article

Evaluation of the Androgenic Properties of the Aqueous Extract of the Trunk Bark of *Spathodea Campanulata P. Beauv*. (Bignoniaceae) in Male Rats

Evaluation des propriétés androgéniques de l'extrait aqueux de l'écorce du tronc de Spathodea Campanulata P. Beauv. (Bignoniaceae) chez les rats mâles

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Key Words: Spathodea campanulata, androgenic, aqueous extract, male rat.

Mots clés : Spathodea campanulata, androgénique, extrait aqueux, rat male.

ABSTRACT

Introduction. Several causes among which the deficit of androgens are at the origin of the erectile dysfunction. In the West region of Cameroon, more particularly in the locality of Dschang, traditional healers use the bark of *Spathodea campanulata* P. Beauv. (*S.campanulata*), belonging to the family Bignoniaceae, for the treatment of erectile dysfunction. The purpose of this study was to evaluate the androgenic properties of the aqueous extract of *S. campanulata* trunk bark on the androgenic parameters of adult male rats. **Methods**. To do this, 25 male rats were used and divided into 5 groups that were treated: group I received the reference substance (testosterone enanthate) at 5 mg / kg weekly and constituted the positive control. Group II was the negative control and received distilled water at a dose of 5 ml / kg daily. The others were the experimental groups and received extract at doses of 200 mg / kg (group III) and 400 mg / kg (group IV) and 800 mg / kg (group V) daily. Administration was single-dose at the same time each day and treatment lasted 28 days. On the 29th day, the animals were weighed and sacrificed and the organs were removed. The following parameters were then determined: The relative weight of the organs, the serum and testicular cholesterol levels, the serum, testicular and epididymal protein levels, the rate of penile nitrogen monoxide and vesicular fructose, as well as the histology of the testis and epididymis. **Results**. The results of this study show that the treatment had an impact on some androgenic parameters. In fact, at the dose of 200 mg / kg the extract allowed a significant increase ($P < 0.05$) compared to the negative control of the rate of penile nitrogen monoxide, in the order of 136.36% and the vesicular fructose of 19.58%. In addition, there was an increase in the weight of certain androgen-dependent organs: the testes, of 11.20% and penis, of 31.81% and the prostate, of 7.70%. These results reflect an activity of androgens whose incidence is felt on these parameters. The analysis of the testicular and epididymal histological sections of the animals treated with the extract for 28 days suggests that the active ingredient of *S. campanulata* has an effect, although minimal on testicular and epididymal morphology of experimental animals compared to negative controls. **Conclusion**. These results indicate an androgenic potential of *S. campanulata* in male rats and support the empirical use of *S. campanulata* trunk bark in the treatment of erectile dysfunction in West Cameroon.

RÉSUMÉ

Introduction. Plusieurs causes parmi lesquelles le déficit en androgènes sont à l'origine des dysfonctions sexuelles. A l'Ouest-Cameroun, plus particulièrement dans la localité de Dschang, les tradipraticiens utilisent les écorces de tronc de *Spathodeacampanulata* P. Beauv. (*S. campanulata*), appartenant à la famille des Bignoniacées, pour le traitement des dysfonctions sexuelles chez les hommes. Cette étude avait pour but l'effet de l'extrait aqueux des écorces de tronc de *S. campanulata*sur les paramètres androgéniques des rats mâles adultes. **Méthodologie**. Pour ce faire, 25 rats mâles ont été utilisés et répartis en 5 groupes qui étaient traités quotidiennement : le groupe I recevait la substance de référence (l'énanthate de testostérone) à 5 mg/kg et constituait le témoin positif. Le groupe II était le témoin négatif et recevait quant à lui de l'eau distillée à la dose de 5 ml/kg. Les autres constituaient les groupes expérimentaux et étaient traités à l'extrait aux doses 200 mg/kg (groupe III) et 400 mg/kg (groupe IV) et 800 mg/kg (groupe V). L'administration se faisait en dose unique à la même heure chaque jour et le traitement a duré 28 jours. Le 29^{e} jour, les animaux étaient pesés puis sacrifiés et les organes prélevés. Les paramètres suivant étaient alors déterminés : Le poids relatif des organes, le taux de

cholestérol sérique et testiculaire, le taux de protéines sériques, testiculaires et épididymaires le taux de monoxyde d'azote pénien et de fructose vésiculaireétaient déterminés. Enfin, nous avons réaliséles coupes histologiques du testicule et de l'épididyme. **Résultats**. Les résultats de cette étude montrent que le traitement avait un impact sur certains paramètres androgéniques. L'effet de l'extrait à la dose de 200 mg/kg a permis une augmentation significative ($P < 0,05$) du taux de monoxyde d'azote pénien par rapport au témoin négatif, de l'ordre de 136,36 % et du fructose vésiculaire de 19,58 %. En outre, on a noté une augmentation du poids de certains organes androgéno-dépendants, notamment les testicules, de 11,20 %, le pénis, de 31,81 % et la prostate, de 7,70 %. **Conclusion**. Ces résultats traduisent une activité des androgènes dont l'incidence se fait ressentir sur ces paramètres. L'analyse des coupes histologiques testiculaires et épididymaires des animaux traités à l'extrait pendant 28 jours, laissent penser que le principe actif de *S. campanulata* a un effet quoique minime sur la morphologie testiculaire et épididymaire des animaux expérimentaux par rapport aux témoins négatifs. Ces résultats indiquent un potentiel androgène de *S. campanulata* chez les rats mâles et soutiennent l'utilisation empirique de l'écorce du tronc de *S. campanulata* dans le traitement de la dysfonction érectile dans l'ouest du Cameroun.

INTRODUCTION

In many emerging countries, traditional medicine is widely used in the treatment of several diseases on the basis of empirical evidence [1]. Indeed, many medicinal plants are used to solve the problems related to erectile dysfunction with multiple causes. Several causes among which, toxic effects of some substances on the testes and related organs and the disorder of androgenic parameters are among the factors responsible for this sexual impotence [2]. Thus, to try to overcome these dysfunctions, several plants like *Litsea chinensis* and *Ochis maculata* are used for their aphrodisiac properties [2]. The leaves of *Hibiscus macranthus* and *Basella alba* are used for their androgenic activity [3]. In addition, the barks of *Spathodea campanulata* are used as aphrodisiacs. Previous studies have shown that the aqueous extract of the trunk bark of *Spathodea campanulata* stimulates the sexual behavior of adult rats. This by reducing the latency of monte and intromission, by increasing the erection number and the riding frequency, as well as the frequency of intromission[4]. *Spathodea campanulata* has effects on penile erection and copulatory parameters that are controlled by androgens and several other factors. Indeed, the more in-depth study of the mechanisms leading to erection has made it possible to reduce all pathologies to a common denominator, the secretion of nitric oxide (NO), necessary to activate penis relaxation and erection [5]. Therefore, in order to try to understand the mechanism by which *S. campanulata* would act, it is advisable to conduct a study aimed at "the evaluation of the androgenic properties of the aqueous extract of the trunk barks of *Spathodea campanulata* P. Beauv. (Bignoniaceae) in the male rat" such, the secretory activity of the testes and certain ancillary organs whose operation is controlled by androgens.

MATERIALS AND METHODS

Chemicals

Chemicals Testosterone enanthate (Androtardyl®) was purchased from a pharmacy in Yaoundé.

Preparations of aqueous extract of *Spathodea campanulata* stem barks

Fresh Stem barks of *Spathodea campanulata* were collected in Dschang, Cameroon. Then, botanical identification was done in comparison with the specimen N°8647/HNC at the Cameroon National Herbarium (HNC) in Yaoundé. The Stem barks were cut into small pieces, air-dried and powdered using an electric grinder. The dried powder (150g) was macerated in 2 liters of distiled water for 48 hours. The resultant extract was filtered and the filtrate was evaporated at 45°C.

Repartition and treatment of animals for the fertility test

The Faculty of Medecine and Biomedical Sciences of the University of Yaounde I Committee approved the animal protocol for animal experimentation and the experiments were performed according to the OCDE Principles of Laboratory Animal Care (National Institute of Health guideline; publication no. 86-23, revised 1984). A total of 25 adult male Wistar rats (160-200 g) were obtained from our colony, raised at room temperature (23-25°C) with a natural light-dark cycle (12/12 h) and maintained at standard laboratory rat diet and tap water given *ad libitum*. The male rats were randomly assigned into five equal groups (n = 5 per group) and orally administered in the following manner: testosterone enanthate (5 mg/Kg) according to previous studies Moundipa et al (1999), distilled water was administered at 10 ml/Kg and aqueous extract of *S. campanulata* extract was administered at three different doses of 200, 400 and 800 mg/Kg by gavage. These doses were chosen based (on our previous studies) and on indications of the traditional practitioner. Testosterone was injected intramuscularly once weekly whereas distilled water and aqueous extract of the plant were given once daily for 28 days. This was to evaluate their effects on male reproductive function. After the treatment periods, animals were killed, their blood collected, the testes and some annex glands removed for histological and biochemical analysis[3],[6]. The following reproductive parameters were then computed according to the method ofYakubu et *al*[7].

Relative weight and biochemical analysis of androgens dependent organs

The animals were sacrificed after 28 days of treatment, by cervical decapitation and testis, seminal vesicles, epididymis, muscle levator ani and prostate glands were removed carefully and each organ was weighted with a sensitive electronic balance.

Biochemical analysis

Cholesterol was determined in serum and testis by colorimetric method [8]. The fructose levels were determined in seminal vesicle according to protocols described in a WHO manual [9]. Total proteins in serum and sexual organs (testis and epididymis) were determined using colorimetric methods described by [10] and [11], respectively.

Histological analysis

Both testes were weighted and after that, to determine histological changes in the testes following treatment with distilled water, testosterone enanthate or aqueous extract of *S. campanulata*, a routine paraffin fixation for the testicular and epididymis tissues was performed. First, the tissues were fixed in Bouin liquid for two weeks. This was followed by a dehydration procedure using a series of graded alcohol mixtures. Then, the dehydrated tissue was immersed in xylene for two hours and 30 minutes. Tissues were then embedded in paraffin and were cut at a thickness of 5µm. The tissues were mounted on slides and stained by immersing them in Mayer hematoxylin solution. To remove excess hematoxylin the slides were rinsed under running tap water. The slides were dipped in alcohol, eosin solution and then dehydrated through a series of graded alcohols. Finally, the tissues were mounted under a synthetic resin. Microscopic evaluation of the slides was undertaken and variations in histoarchitecture were recorded [12].

Statistical analysis

One-way analysis of variance (ANOVA) followed by post-hoc Student-Newman-Keuls multiple comparison test was performed using GraphPad Instat software version 3.10. A probability of $P < 0.05$ was significant.

RESULTS

The yield of the extraction was 12.66 % (w/w in term of dried material).

Effects of *S. campanulata* on body weight

Figure 1 shows the effects of *S. campanulata* on the body weight of the experimental animals.

The analysis of this evolution curve shows no significant variation ($P> 0.05$) over the duration from the 1st day to the 14th day of treatment in the positive and negative control groups. However, the evolution of the curve of the animals treated with the extract (200 mg / kg, 400 mg / kg and 800 mg / kg) compared to the negative controls shows a significant decrease ($P <0.001$). In addition, there was a significant increase ($P <0.001$) in the change in the weight of the animals in the treated batches between day 14 and day 28. This variation isin the order of 33.30% (20.81 ± 2.42 Vs 27.74 ± 1.04) of the relative change in body weight; for the batch at 200mg / kg; 38.74% (13.55 ± 0.57 Vs 18.80 ± 1.66) of the relative change in body weight for the batch at 400mg / kg and 26.79% (14.82 ± 0.69 Vs 18, 79 ± 0.83) of the relative change in body weight for the batch at 800mg / kg.

Effects of *S. campanulata* on the relative weight of organs

The effects of *S. campanulata* extract on the relative weight of androgen-dependent organs are summarized in Table I. Testosterone enanthate resulted in a significant decrease in testicular weight ($P <0.001$) in the order of 65.17% (1.12 ± 0.06 Vs 0.39 ± 0.03) of the epididymis ($P <0.05$) in the order of 40.62% (0.32 ± 0.02 Vs 0.19 ± 0.03), of the seminal vesicle in the order of 28.57% (0, 14 ± 0.01 Vs 0.10 ± 0.01) and prostate in the order of 7.70% (0.13 ± 0.01 Vs 0.12 ± 0.00), compared to the negative control . The administration of the extract at 200 mg / kg increased the size of the testes by 11.20% (1.12 ± 0.06 Vs 1.22 ± 0.05), 7.70% prostate (0.13 ± 0.01 Vs 0.32 ± 0.02) and penis enhancer muscle in the order of 31.81% (0.22 ± 0.00 Vs 0.29 ± 0.04) compared to the negative control. In addition, the increase in penis size in the order of 22.22% (0.09 ± 0.01 Vs 0.11 ± 0.01) for the positive control batches, 33.33% (0, 09 ± 0.01 Vs 0.12 ± 0.01) for the batch treated at 200 mg / kg extract, 11.11% (0.09 ± 0.01 Vs 0.10 ± 0.01) for the batch treated at 400mg / kg and 800mg / kg of extract, was observed after 28 days of treatment.

Effects of *S. campanulata* extract on biochemical parameters

Effects of S. campanulata extract on serum, epididymal and testicular protein levels

Figure 2 shows serum, epididymal and testicular protein levels. There was a significant increase in total epididymal protein levels in the animals treated with the extract at the doses of 200 mg / kg ($P <0.01$) and 800 mg / kg ($P <0.05$) in the order of 112% (0.25 ± 0.02 Vs 0.53 ± 0.05 mg / dL) and in the order of 136% (0.25 ± 0.02 Vs 0.59 ± 0.08 mg / dL) respectively. In addition, there was an increase, albeit not significant, in the testicular proteins at the doses of 200 mg / kg of the order of 20.24% (0.65 ± 0.10 Vs 0.88 ± 0.06 mg). / dL) compared to those of the negative control group. In addition, the comparison with the positive control group showed a non-significant increase of 4.8% (0.84 ± 0.08 Vs 0.88 ± 0.06 mg / dL) in the level of epididymal proteins in rats treated at the dose of 200 mg / kg.

Effects of S. campanulata extract on serum and testicular total cholesterol

Figure 3 shows the serum and testicular cholesterol levels after 28 days of experimental animal treatment. Treatment of the animals with the extract at doses of 200 mg / kg and 800 mg / kg resulted in a non-significant increase ($P > 0.05$) in serum cholesterol levels compared to the negative control in the range of 27, 43% (12.03 ± 0.82 Vs 15.33 ± 1.57 mg / dL) and 28.60% (12.03 ± 0.82 Vs 15.47 ± 1.40 mg / dL) respectively.

Table I: Effects of the aqueous extract of S. campanulata on the relative weight of androgen-dependent organs (expressed as a percentage of body weight).

Relative weight of adrogenic organs Dependent	Distilled water (10 ml/kg)	Testosterone Enanthate (5 mg/kg)		*Spathodea campanulata* (200 mg/kg)		*Spathodea campanulata* (400 mg/kg)		*Spathodea campanulata* (800 mg/kg)	
Testis	1,12 ± 0,06	0,39 ± 0,03	↓65,17%	1,22 ± 0,05	↑11,20 %	1,17± 0,02	↑4,46 %	1,11± 0,07	↓0,89 %
Epididymis	0,32 ± 0,02	0,19± 0,03	↓40,62	0,32± 0,02	0,00	0,28 ± 0,02	↓12,5 %	0,31 ± 0,02	↑3,12 %
Prostatis	0,13 ±0,01	0,12 ± 0,00	↓7,70 %	0,14 ± 0,01	↑7,70 %	0,09 ± 0,00	↓30,77 %	0,11 ± 0,01	↓15,38 %
Elevateur Muscle	0,22 ± 0,00	0,22 ± 0,01	0,00 %	0,29 ± 0,04	↑31,81 %	0,23 ± 0,02	↑4,54 %	0,26 ± 0,02	↑18,18 %
Seminal Vesicle	0,14 ± 0,01	0,10 ± 0,01	↓28,57 %	0,14 ± 0,01	0,00 %	0,10 ± 0,01	↓28,57 %	0,11 ± 0,01	↓21,43 %
Penis	0,09 ± 0,01	0,11 ± 0,01	↑22,22%	0,12 ± 0,01	↑33,33 %	0,10 ± 0,01	↑11,11 %	0,10 ± 0,00	↑11,11 %

↑: increase; ↓: Decrease; 3 = P <0.001 compared to the negative control group; % V: Percentage change from controls

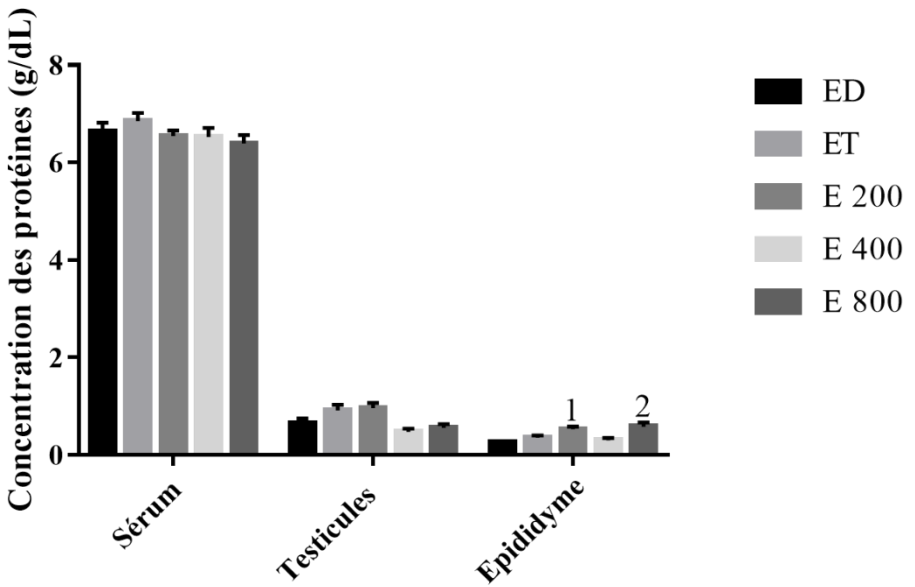

Figure 2: Effects of the aqueous extract of S. campanulata on the rate of total serum, testicular and epididymal proteins; Each column representsthe mean ± ESM, n = 5.1: P <0.01; 2: P <0.05; 3: P <0.001 compared to the negative control group

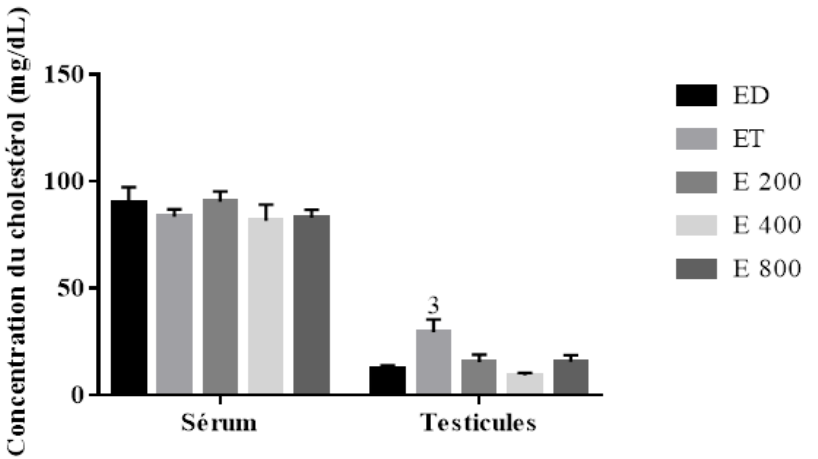

Figure 3: Serum and testicular cholesterol levels after 28 days of treatment of experimental animals. 1: P <0.01; 2: P <0.05; 3: P <0.001 compared to the negative control group

However, there was a non-significant decrease ($P > 0.05$) in testicular cholesterol levels in the animals treated with the extract at doses of 200 mg / kg, 400 mg / kg and 800 mg / kg compared to rats with received testosterone enanthate. This decrease is respectively of the order of 91.12% (29.30 ± 2.66 Vs 15.33 ± 1.57 mg / dL), of 221.27% (29.30 ± 2.66 Vs 9, 12 ± 0.53 mg / dL), 89.40% (29.30 ± 2.66 Vs 15.47 ± 1.40 mg / dL)

Effects of S. campanulata extract on vesicular fructose

Figure 4 shows the rate of vesicular fructose after 28 days of treatment of experimental animals. It should be noted that the animals which received the extract at the dose of 200 mg / kg showed a significant ($P < 0.05$) increase in the rate of vesicular fructose compared to the negative control group. This increase is of the order of 19.58% (14.66 ± 0.23 Vs 17.53 ± 0.91 µmol / g). Compared to the positive control, the batches that received the extract showed a non-significant increase of about 18.05% (14.85 ± 0.35 Vs 17.53 ± 0.91 µmol / g) compared to positive control.

Effects of S. campanulata extract on nitric oxide rate

Figure 5 shows the rate of penile nitric oxide after 28 days of treatment of experimental animals. It is noted that the animals which received the extract at a dose of 200 mg / kg showed a significant increase ($P < 0.05$) in the penile nitric oxide level relative to the negative control group. This increase is in the order of 136.36% (0.11 ± 0.02 Vs 0.26 ± 0.01 µmol / g). Compared to the positive control, the batches that received the extract showed a non-significant increase of the order of 18.05% (0.13 ± 0.02 Vs 0.26 ± 0.01 µmol / g).

Effects of *S. campanulata* extract on histological parameters

Effects of S. campanulata extract on the testes

Figure 6 shows the cross-section of the testis in the groups treated with distilled water, testosterone enanthate and the extract at doses of 200, 400 and 800 mg / kg for a period of 28 days; they indicate that there were slight changes in testicular architecture in the positive control group during the experimental period. In fact, it is observed in testosterone-positive control batch, a densification of the basement membrane between the seminiferous tubes and an increase in the number of spermatozoa in the lumen of the seminiferous tubes separated by an interstitial tissue. Each seminiferous tube shows the wall to light, a basement membrane and male sex cells at different stages of development. The spermatozoa are well identifiable in the tubular lumen, with their flagellum.

Effects of *S. campanulata* extract on the epididymis

The histological sections of the epididymis in different batches have a normal structure (Figure7). The epididymal ducts separated from each other by a connective tissue, show a basement membrane and spermatozoa in the epididymal lumen.

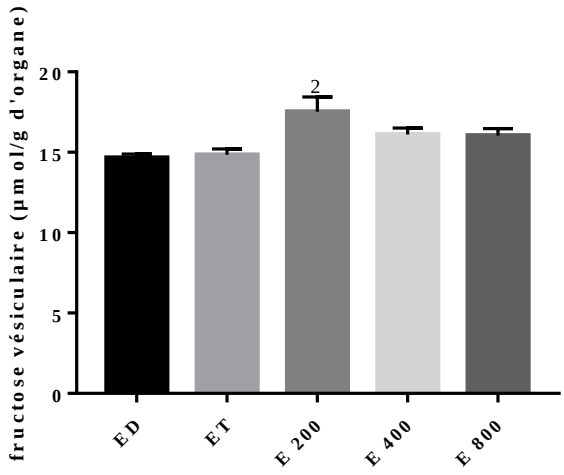

Figure 4: Effects of the aqueous extract of *S. campanulata* on the rate of fructose vesicular (Each column represents the mean ± SEM, n = 5. 2: $P < 0.05$; 3: $P < 0.001$ compared to the negative control group)

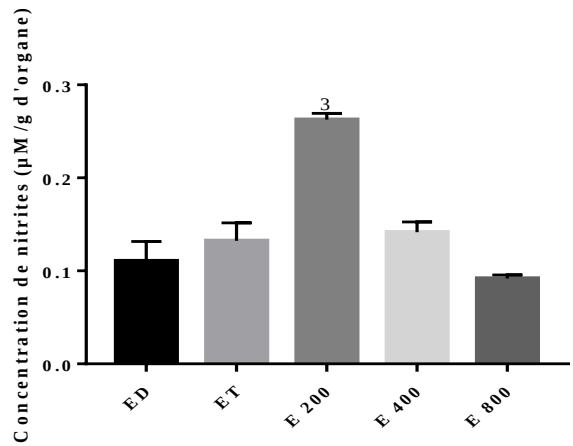

Figure 5: Effects of the aqueous extract of *S. campanulata* on the rate of vesicular fructose (Each column represents the mean ± SEM, n = 5. 1: $P < 0.01$; 2: $P < 0.05$; 3: $P < 0.001$ compared to the negative control group)

DISCUSSION

The empiric claims of *Spathodea campanulata* stem barks as an aphrodisiac has encouraged investigation into its potential as an androgenic agent. Considering the fact that male sexual behavior is influenced by the circulating levels of testosterone in the blood, testosterone enanthate was used as standard referent to compare the androgenic properties. Distilled water, which did not affect the testosterone levels, and equaly aphrodisiac or sexual activity in adult male rats was used as negative control also for comparison purpose. The evaluation of biochemical parameters of organ/body weight ratio, concentrations of testicular secretory constituents like total protein and cholesterol, fructose and nitric oxide in penile can give useful information on the androgenic potential of chemical compounds and plant extracts.

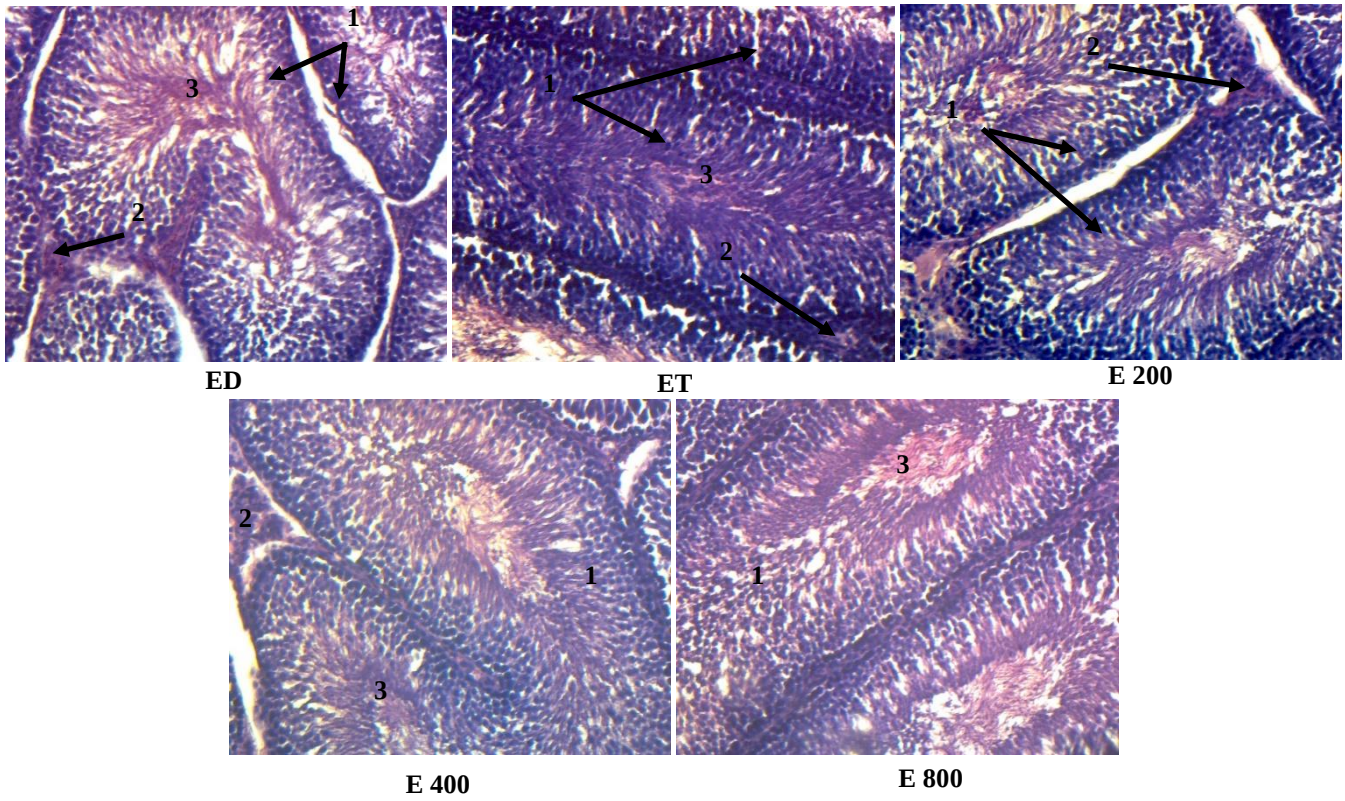

Figure 6: Photomicrograph of the testes. (Hematoxylin-Eosin X40).
ED = Normal control batch receiving distilled water; ET = Positive control batch receiving testosterone enanthate at the dose of 5 mg/kg; E 200, E 400, E 800 = Batch tests receiving the aqueous extract of5 mg/kg at the respective doses of 200, 400 and 800 mg / kg; 1 = seminiferous tubules; 2 = interstitial tissue; 3 = Spermatozoa.

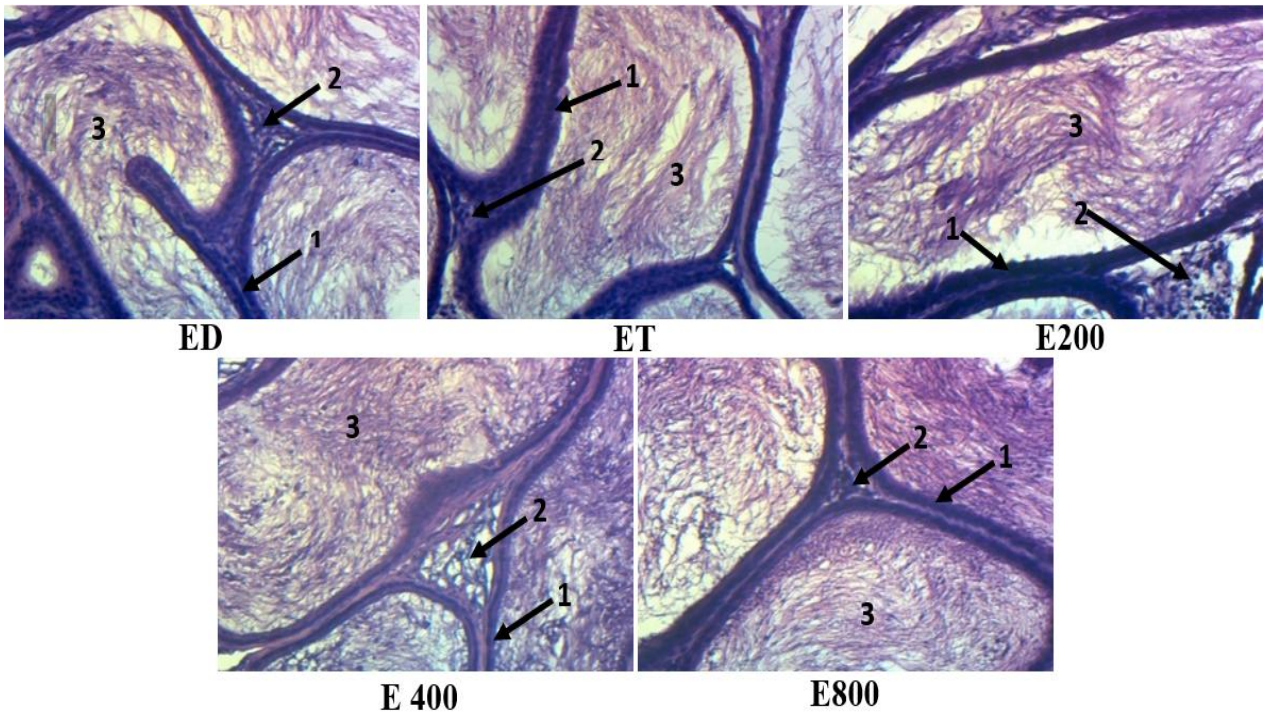

Figure 7: Photomicrograph of the epididymis. (Hematoxylin-Eosin X40).
ED = Normal control batch receiving distilled water; ET = Positive control batch receiving testosterone enanthate at a dose of 5 mg / kg; E 200, E 400, E 800 = Batch tests receiving the aqueous extract of *S. campanulata* at the respective doses of 200, 400 and 800 mg / kg; 1 = seminiferous tubules; 2 = interstitial tissue; 3 = Spermatozoid.

These parameters can also be used to evaluate normal functioning capacity of the testes [13,14]. Treatmentwith

the aqueous extract of *Spathodea campanulata* P.Beauv. Stem barks influenced the parameters of the treated animals in a dose-dependent manner. Meanwhile we observed at the end of treatment, a non-significant increase in certain sexual organ relative weight such as penis, testis) and ventral prostate This increase may either indicate an inflammation or an increase in the secretory ability of the organ while a reduction in the parameter can imply cellular constriction. However, no evidence of inflammation in any of the tested groups has been reportedhistomorphological analysis. Therefore, the increase in the relative organs weight observed following the administration of the plant extract might be attributed to increased secretory activity of the testes [15]. In fact, aconversion of cholesterol to testosterone may be reflected by a decrease in the concentration of cholesterol in testes [16]. Thus, the decreasing cholesterol rate observed in the testes of extract-treated male rats at the dose of 200 mg/kg compared to control clearly indicated an increased conversion of cholesterol into testosterone. The synthesis of steroid hormones requires a constant supply of cholesterol [17] and this requirement for normal testicular activity has been well established[14]. In the same way, it has been reported that, increased protein concentrations enhance sperm maturation which is an important component of androgenicity [18]. Testicular proteins are among the constituents that ensure the maturation of spermatozoa [19]. Increased weight and high protein concentration of the testes observed in animals treated with the plant extract at the dose of 200 mg/kg indicates enhancement of testicular growth and androgenic activities. As fructose is consumed during fructolysis to provide energy to immotile spermatozoa seminal fructose content is an important parameter for evaluating the normal sexual functioning in male [20]. Treatment with the aqueous extract at both doses decreased seminal fructose content. Some authors reported that after ejaculation, the spermatozoa in a process named fructolysis consume fructose. At higher sperm counts, the process will be stronger resulting in a low [21], suggesting that high fructose concentrations observed in animals treated with *S. campanulata*, have been released to supply motile sperm in energy after ejaculation. These effects are considerably restrained in the testosterone treated group compared to *S. campanulata* treated groups. On the other hand, the improvement of relative weight of the androgen dependent organs and all others fertility parameters observed after 28 days of treatment with aqueous extract of *S. campanulata* confirm aphrodisiac, reproductive and androgenic-like effects of our plant extract.

ACKNOWLEDGEMENTS

Bafoussam medical and surgical center
FMSB multidisciplinary galenic laboratory.
Organic chemistry laboratory of the Faculty of Sciences of the University of Yaounde I.

SOURCE OF FUNDING

This research has not received any funding from any international organization, public or private.

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