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RESEARCH ARTICLE

APHRODISIAC ACTIVITY OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF THE STEM BARK OF *STRYCHNOS CAMPTONEURA* (LONGANIACEAE) IN WISTAR RAT

[1,*]Cyr Jonas Morabandza, [1]Radard Ondele, [1]Romaric De Garde Elion Itou, [1]Arnaud Wilfrid Etou Ossibi, [1]Céty Imbiella, [1]Etienne Mokondjimobe, [2]Pascal Robin Ongoka and [1]Ange Antoine Abena

[1]Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien Ngouabi University, P.O. Box 69, Brazzaville-Congo

[2]Master departments of Exact Sciences, E.N.S, Marien Ngouabi University, P.O. Box 69, Brazzaville-Congo

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ABSTRACT

This study was initiatedto evaluate aphrodisiac activity of aqueous and hydro-ethanolic extracts of the stem bark of *Strychnos camptoneura* in male rat. Aqueous and hydroethanolic extracts of *S. camptoneura* were prepared and, orally administered at the doses of 100 and 250 mg/kg for sexual parameters (sexual mounting; erection number; ejaculationnumber and latency time) evaluation. Distilled water (0,5ml/100g) and yohimbine (10mg/kg) being respectively negative and positive control. Aphrodisiac effect mechanisms were studied in the presence of L-NAME, Haloperidol and Atropin. Obtained results revealed that aqueous and hydroethanolic extracts of *S. camptoneura* at the doses of 100 and 250 mg/kg act favorably and significantly on sexual parameters (sexual mounting; erectionnumber; ejaculation number and latency time) in male rat. The two extracts would act by dopaminergic and cholinergic way. The preliminary results thus obtained explain the traditional use of the stem bark of *S. camptoneura* in sexual purposes and could constitute a good remedy in the treatment of erectile dysfunction.

INTRODUCTION

Medicinal plants have since long time subject of use by the man in various purposes. *Strychnos camptoneura* (Loganiaceae) commonly called yindza is a plant of traditional pharmacopeia in north of Congo-Brazzaville, commonly used in the treatment of malaria, ulcers, rheumatisms, diabetes, fever, microbial infections, hernia and parasitizes (Bouquet, 1979). It would be often used by the populations against erectile dysfunction, absence of libido and many other pathologies, which affect people sexual life. However, in spite of several scientific investigations on the pharmacological properties and the nontoxic character of the extracts of this species (Morabandza *et al*., 2016; Morabandza *et al*., 2016; Morabandza *et al*., 2017), no scientific study nowadays approached the aphrodisiacs potentialities claimed by rural populations.

Corresponding author: **Cyr Jonas Morabandza,**
Biochemical and Pharmacological Laboratory, Health Sciences Faculty, Marien Ngouabi University, P.O. Box 69, Brazzaville-Congo.

Moreover, the chemical study of the stems and barks of this plant revealed the abundance of flavonoids and sterols, compounds which are known for their aphrodisiac properties (Morabandza *et al*., 2016). The present study, undertaken comparatively day and night, aims to evaluate the aphrodisiac activity of aqueous and hydroethanolic extracts of *S. camptoneura* in Wistar rat.

MATERIALS AND METHODS

Vegetal materials: The stems barks collected at M'voula village (Itoumbi, Cuvette west around 765 km from Brazzaville-Congo) constituted material of this study. The plant specimen was identified in the Institute of Research of Exact and Natural Sciences (I.R.E.N.S.) of Congo and recorded under the N° 2271.

Animals: Male and female's Wistar rats of 4 months, weighing between 150-200g of The Faculty of Health Sciences of Marien Ngouabi University (Brazzaville-Congo), were used. They were high under standards conditions (12 H of light/12 H darkness) with free access food.

Preparation of extracts: The collected stem bark was air dried at 25 ± 1°C during 14 days in the laboratory and grounded into powder, using a wood mortar. 50 g of powder was subjected to maceration under magnetic agitation in 500 ml of each solvent (distilled water; distilled water/ethanolV/V) during 48 hours. The maceratewere filtered and concentrated at 55°C using steem room and the concentrate was preciously kept at 4°C.

Evaluation of*S. camptoneura* extracts activity on sexual parameters

Sexual activity in rat was evaluated by using classical method (Davidson, 1982; Carro-juarez *et al*., 2004). 6 groups of 5 male rats each one were treated as follow: group 1(negative control), treated with 0.5 ml/100g of distilled water; group 2 (positive control) treated with 10 mg/kg of yohimbine; groups 3 and 4 treated with aqueous extract; groups 5 and 6 with hydroethanolic at 100 and 250 mg/kg respectively. After 4 days of treatment of male rats, 30 female rats received under cutaneous way 600 µg of œstradiol (Oromon®) by animal during 3 days to make them receptive to the males (Watcho *et al*., 2007; Ondélé *et al*., 2011). Six (6) hours after the last administration (7 days for male rats and 3 days for females), animals are placed by couple in cages. Sexual parameters observation were made at day and night during 1 hour. The following parameters were evaluated: number of sexual mounting, erection, intromission andlatency time (interval time which separates two sexual mounting consecutive).

Evaluation of *S. camptoneura* extracts on sexual activity in the pretreated rat with

Haloperidol, Atropine and L-NAME

This experience was realized in order to determine the mechanism by which *S.camptoneura* extracts act by using Haloperidol, Atropine and L-NAME respectively nonspecific inhibiting of the dopaminergic receivers, muscarinic and NO synthesis.5 groups of 5 animals each one were treated as follow: group 1 (negative control) treated with physiologic solution (NaCl 0.9 %); group 2 with 100 mg/kg of hydroethanolic extract; groups 3, 4 and 5 received respectively 10 mg/kg Haloperidol (i.p), Atropin(i.p) and L-NAME (i.m) follow by 100 mg/kg of hydroethanolic extract. One hour after products administration, animals were placed by couple in the cages. Females rats used for this experiment were treated each one with 600 µg of œstradiol during 3 days as in the preceding experiment. The number of sexual mounting, erection, intromission and latency time were determinate during 1 hour.

Statistical Analyze

Statistical analysis of results was carried out by using variances analysis (ANOVA), "T" test of Student and Mann-Whitney to compare the experimental groups with the control groups; the significance level was set at p≤0.05.

RESULTS AND DISCUSSION

The objective of this study was to evaluate aphrodisiac activity of aqueous and hydroethanolic extracts of *S. camptoneura* on sexual parameters (sexual mounting, erection, ejaculation and latency time) and to identify the probable mechanism of action of those extracts.

Effect of aqueous and hydroethanolic extracts on sexual mounting

Figure 1 indicates the effect of aqueous and hydroethanolic extract of *S. camptoneura* on sexual mounting in rat at day and night during 1hour. At day, only hydroethanolic extract (100 mg/kg) and yohimbine (10 mg/kg) present a significant increase ($p<0.05$) number of sexual mounting compared to the negative control (distilled water):66.97 ± 0.57 and 64.2 ± 4.27 respectively against 34.4 ± 14.09 for negative control. At night, the same tendency was observed: 61.40 ± 3.46 for hydroethanolic extract and 51.73 ± 2.11 for yohimbine against 28.8 ± 9.22 for negative control. The aqueous extracts (100 and 250 mg/kg) and hydroethanolic (250 mg/kg) do not cause however significant increases of sexual mounting at day as night.

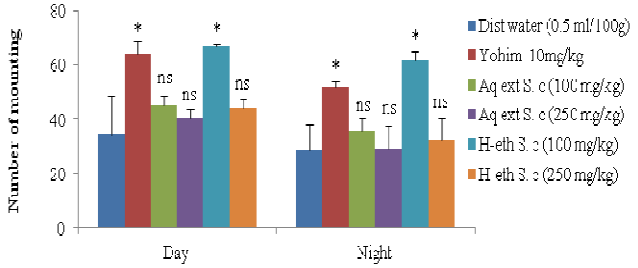

Fig. 1. Effect aqueous and hydroethanolic extracts of *S. camptoneura* on number of sexual mounting in rat.The values are Means ± ESM, n = 5, *p< 0,05 significative difference, ns= non significative difference compare to the control

Effect of aqueous and hydroethanolic extracts of *S. camptoneura* on number of erections

Figure 2 presents effects of aqueous and hydroethanolic extracton erections number in the rat during 1 hour, day and night.

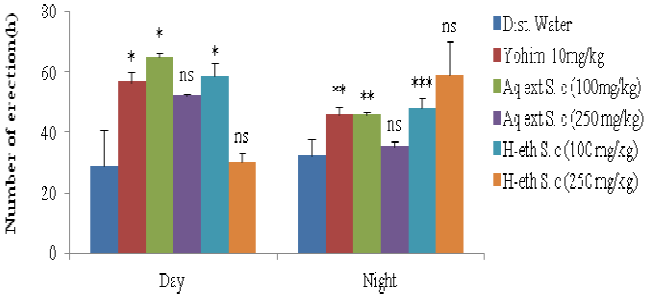

Fig. 2. Effect aqueous and hydroethanolic extracts of *S. camptoneura* on number of erection in male rat. The values are means ± ESM, n = 5, *p< 0,05 or **p<0,01, significative difference, ***p<0,001, very significative difference compare to the control

At day, aqueous, hydroethanolic extracts (100 and 250 mg/kg) and yohimbine (10 mg/kg) induces significant increases ($p<0.05$;$p<0.01$) of erection number compared to negative control (distilled water):64.6 ± 1.63 and 52.21 ± 0.70; 58.50 ± 4.30 and 30.2 ± 3.27 and 56.80 ± 3.10respectively against 29.2 ± 11.56 for control group. At night, the same tendencies are observed with 100 mg/kg, with significant increases ($p<0.01$;$p<0.001$): 46.01 ± 0.70; 48.11 ± 3.39 and 46.10 ± 2.66 against 32.21 ± 5.56 for the control respectively for the aqueous extracts, hydroethanolic and the yohimbine.

Effect of aqueous and hydroethanolic extracts of *S. camptoneura* numberof ejaculation

The effects of aqueous and hydroethanolic extracts number of ejaculations are presented in figure 3.At day, aqueous and hydroethanolic extracts (100 mg/kg) do not cause a significant increase of number of ejaculation whereas this number is significant ($p<0.05$) with yohimbine (10 mg/kg) compared to the negative control (distilled water). Number of ejaculation is 1.36 ± 0.22;1.8 ± 0.2 and 2.18 ± 0.20 respectively for the aqueous, hydroethanolic extract and yohimbine against 0.81 ± 0.48 for negative control.Atnight, however, aqueous and hydroethanolic extracts (100mg/kg) as yohimbine provoke a significant increase ($p<0.05$) number of ejaculation: 2.60 ± 0.41;2.41 ± 0.24 and 2.32 ± 0.17 respectively against 1.01 ± 0.54 for negative control.

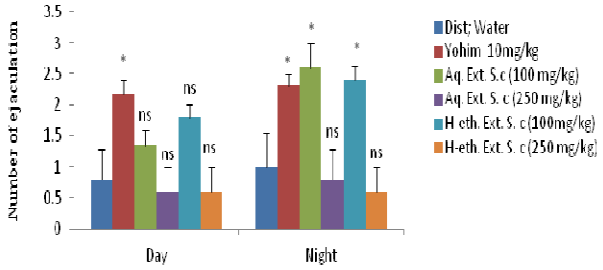

Fig. 3. Effect of aqueous and hydroethanolic extracts of *S. camptoneura* on number of ejaculationin male rat The values are means ± ESM, n = 5, *p< 0,05 significative difference, ns = no significative difference compare to the control

Effect of aqueous and hydroethanolic extracts of *S. camptoneura* on the latency time

Figure 4 presents the effects of extracts on latency time.At day, the two extracts1 (00 and 250 mg/kg), have not induce significant reduction of latency time compared to the negative

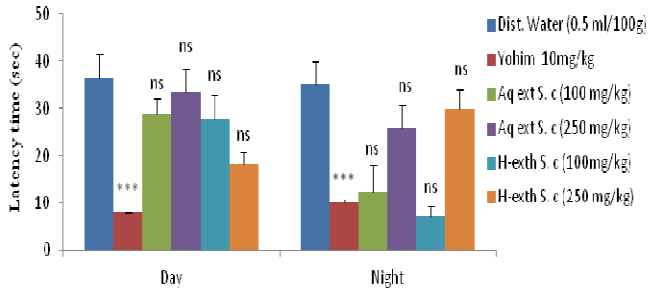

Fig. 4. Effect aqueous and hydroethanolic extracts of *S. camptoneura* on latency time in male rat. The values are means ± ESM, n = 5, ***p<0,001 significative difference, ns = no significative difference compare to the control

control, contrary to yohimbine (10 mg/kg) that reduce significantly (***$p<0.001$) latency time. At studied doses, aqueous extract, presents a latency time of 28.99 ± 3.09 and 33.42 ± 0.50 seconds and, hydroethanolic extract 27.8 ± 5.07 and 18.4 ± 2.45 seconds respectively. Compared to yohimbine (10 mg/kg), this reduction is significant (***$p<0.001$) with a time of 7.76 ± 0.19 seconds against 36.55 ± 0.51 seconds for negative control. The same tendencies are observed at night, with the two extracts (100 and 250 mg/kg) compared to the negative control: 12.55 ± 5.51 and 26.12 ± 12.50 seconds for the aqueous extract;7.45 ± 2.12 and 30.10 ± 0.21 seconds forhydroethanolic extract against 35.01 ± 5.12 seconds for negative control. On the other hand, yohimbine induces a significant reduction (***$p<0.001$) of latency time: 10.10 ± 0.71 seconds.

Effect of hydroethanolic extract of *S. camptoneura* on sexual parameters in the pretreated rat with Atropine, Haloperidol and L-NAME

Sexual mounting

Figure 5 presents the effect of hydroethanolic extract (100 mg/kg) number of sexual mounting in the pretreated rat with Atropine, Haloperidol and L-NAME.The results show that hydroethanolic extract (100 mg/kg) only increases (***$p<0.001$) number sexual mounting: 56.20 ± 5.68 against 53.2 ± 6.34 for control group (NaCl 0.9 %). L-NAME pretreated rats the extract decreases significantly (***$p>0.001$) number of sexual mounting compared to the control: 43.01 ± 5.14 against 56.20 ± 5.68 in rats received hydroethanolic extract only and 53.20 ± 6.34 for the treated rats with physiologic solution (NaCl 0.9%);correspondent toan inhibition of 19.17 % of L-NAME. In the pretreated animals with Atropine and haloperidol however, no sexual mounting was observed, which corresponds to a total inhibition of 100 % compared to the control and those treated only with hydroethanolic extract.

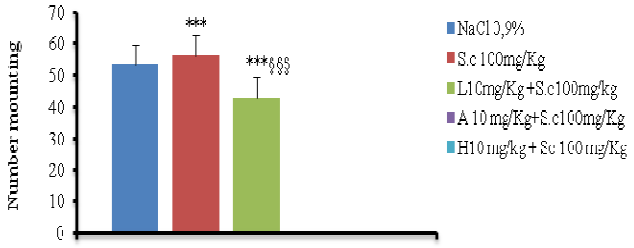

Fig. 5. Effect of Hydroethanolic extract of *S. camptoneura* on sexual mounting in pretreated male rat with L- NAME, haloperidol and atropine. The values are means ± ESM, n = 5, ***p< 0.001 very significative difference compare to the control, §§§p<0.001 very significative difference, compare to treated with extract only. L = L-Nitro Arginine Methyl Ester; A = Atropine; H = haloperidol; S.c = *Strychnos camptoneura*

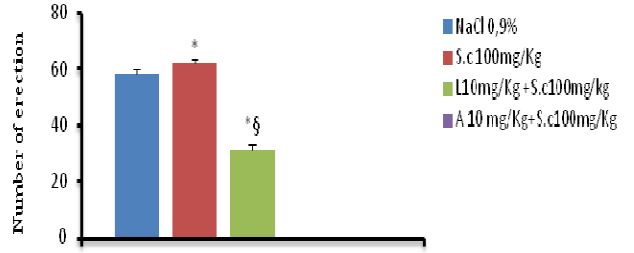

Fig. 6. Effect of hydroethanolic extract of *S. camptoneura* on number of erection in pretreated rat with L-NAME,haloperidol and atropine. The values are means ± ESM, n = 5, *p< 0.05 significative difference, compareto the control. §p<0.001 significative difference, compare to the treated rats with extract only.ns = no significative difference compare to the control. L = L-Nitro Arginine Methyl Ester; A = Atropin; H = haloperidol; S.c = *Strychnos camptoneura.*

Number oferection

Figure 6 shows the effect of hydroethanolic extract (100 mg/kg) number of erection in the pretreated rat with Atropine,

Haloperidol and L-NAME. The extract (100 mg/kg) significantly increases (*p<0.05) number erection compared to the control group (NaCl 0.9 %) : 62.41 ± 6.04 against 58.22 ± 1.71. But, administration ofhydroethanolic extract (100 mg/kg) in the pretreated rats with L-NAME significantly decreases (p>0.05) number of erection : 31.23 ± 8.88 against 62.4 ± 6.04 for hydroethanolic extract only and of 58.20 ± 1.71 for the rats treated with the physiologic solution (NaCl 0.9 %) correspondent to an inhibition of 43.39 % induce by L-NAME. In the pretreated animals with the Atropine and Haloperidol, no erection is observed for all experiment period.

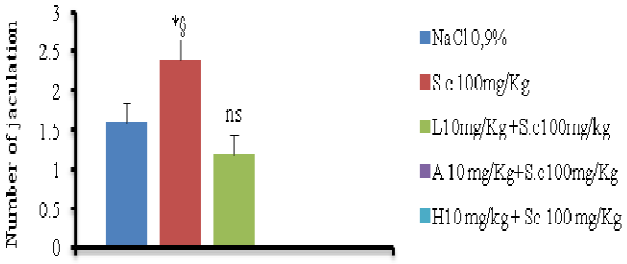

Fig. 7. Effect of hydroethanolic extract of S. *camptoneura* on ejaculations number in pretreated with L-NAME,halopéridol and atropine. The values are means ± ESM, n = 5, *p< 0.05 significative difference, compareto the control. §p<0.001 significative difference, compare to the treated rats with extract only. ns = nosignificative difference compare to the control. L = L-Nitro Arginine MethylEster; A = Atropin; H = haloperidol; S.c = *Strychnos camptoneura*

Number of ejaculation

Figure 7 shows the effect of hydroethanolic extract (100 mg/kg) number of ejaculation in the pretreated rat with the Atropine, Haloperidol and L-NAME. The extract (100 mg/kg) only significantly increases number of ejaculation compared to the control (NaCl 0.9%): 2.41 ± 0.24 (p<0.05) against 1.60 ± 0.24 for control group. In the presence of L-NAME, this extract had not significant increase number of ejaculations compared to the extract only and to the control (NaCl 0.9%) with : 1.22 ± 0.37 against 2.41 ± 0.24 for extract only and 1.60 ± 0.24 for physiologic solution (NaCl 0.9 %). However, in the presence of atropine and of haloperidol no ejaculation was observed. The obtained results of ours investigations revealed that aqueous and hydroethanolic extracts of *S. camptoneura* (100 and 250 mg/kg) significantly increase the number of sexual mounting in male rats. The same results were obtained with aqueous extract of *Monsonia angustifolia* (Fouche *et al*., 2015) that led to the hypothesis that *S. camptoneura* extracts could act on sexual desire. At the studies doses (100 and 250mg/kg), results shows a significant increase (**p<0.01; ***p<0.001)of number of erection at day and night. Erection being a physiological phenomenon due to the clogging of the cavernous and spongy bodies of the penis by blood (Andersonet *al*., 1995); an increaseof erection number after extracts administration, suggests that the stem barks extracts of *S.camptoneura* would act favorably on blood flow in the cavernous and spongy bodies of the penis. These results join those of others studies carried out with *Rauvolfia* obscura; *Buchholzia coriacea* and *Ferula asafoetida* (Ondélé *et al*., 2015; Ondélé *et al*., 2015; Bargheri *et al*., 2015). Physiologically, when there is erection, it follows by intromisation and probably an ejaculation. The results show a significant increase (*p<0.05) of number of ejaculation marked by many consecutive lickings with the withdrawal of the penis after an intromission especially at night. It suggests that plant extracts, could induce sexual pleasure thus, confirming the first results. One study proved that the plants suppose as aphrodisiacs had a remarkable action on the pleasure in animal species (Zanolari, 2003). These results are also confirmed by those of latency time which represents the time between two sexual consecutive mounting. Indeed, ours two extracts seem to decrease the latency time (time between two sexual consecutive mounting).

A critical analysis of this work on sexual parameters induced by the two extracts of *S. camptoneura* reveals a similar effect to that induces by yohimbine (10mg/kg), used as reference drug in this study. Yohimbine is a famous aphrodisiac and powerful vasodilator, acting favorably on the increase in the flow of necessary blood towards the penis to the release and the maintenance of erection (Sabna *et al*., 2013). Ours results shows that the effect of hydroethanolic extract would be more important on certain parameters at 100 mg/kg than aqueous extract. This would be probably explained by the fact that the ethanol in combination with water would allow a good extraction of the polyphenols (Mulicacci*et al*., 2004). Indeed, a preceding study had revealed the presence of the flavonoïdes and of sterols in the stem and the bark of *S. camptoneura* (Morabandza *et al*., 2016).

Another study had already shown that the flavonoids are able to release the cavernous bodies, thus facilitating blood flow towards these bodies erectiles what would support erections (Drewes *et al*., 2003). These results justify certainly the use of the barks of this specie in the palm wine by the rural populations to treat sexual failures. In this study, it was also, question to identify the influence of *S. camptoneura* on certain neurotransmitters implied in erectile function. The effect of hydroethanolic extractonly (the most active extract) was thus evaluated in the presence of the antagonists: L-NAME, Haloperidol and Atropine. The obtained results revealed a persistence of the sexual activity in the pretreated animals with L-NAME; but a total absence of sexual activity in those pretreated with Atropine and Haloperidol. It's let think that the extract would not act by nitronergic way but by dopaminergic and cholinergic way. Dopamine and acetylcholin being neurotransmitters playing a role in the male sexual behavior (Velasco *et al*., 1998). Our results join those obtained by others studies which showed respectively by the same mechanism as the aqueous extracts of *B. ferruginea* and *B coriacea* acted by dopaminergic and cholinergic way (Ondélé *et al*., 2015). The comparative analysis of the results, shows that the effect *S. camptoneura* on sexual parameters would be more remarkable in night than in day light although nonsignificant. Another study had already proven that in many animal species, the light would be limiting factor for sexuality (Téry, 1990).

Conclusion

The present study showed that aqueous and hydroethanolic extracts of stems barks of *S. camptoneura* have an interesting aphrodisiac activity approaching yohimbine. The two extract would probably act by dopaminergic and cholinergic way. This activity can explain the abondant traditional use of this species for this purpose and, could constitute an alternative in the care of the sexual failures.

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