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Effect of Aqueous and Ethanolic Extracts of *Caesalpinia bonduc* Root on Sexual Behaviour of Male Wistar Rats

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Abstract

The empiric use of the aphrodisiac plants such as Caesalpinia bonduc is growing up. The practice of self- medication with natural aphrodisiacs increases the risks of adverse reactions. It is then necessary to undertake studies in sight of reasoned use of these substances. The objective of the current study was a characterization of the sexual behavior of male wistar rats treated or not with 100 mg/kg and 300 mg/kg of aqueous or ethanolic extract of C. bonduc roots, comparatively with Viagra. The survey of the sexual behavior was carried in two sets of observation, each during 15 minutes: the first, 15 minutes after treatment and the second, 105 minutes after treatment. The result of phytochemical screening of C. bonduc roots powder revealed the presence of various compounds including tannins, flavonoids, anthocyans, leucoanthocyans, mucillag, saponosids, anthracemic, heterosids, alkaloids and quinons. At the first observation, no significant difference (P>0.05) was noticed for the mean frequencies of sniff and the mean frequencies of goes up while the Neperian logarithm of the latent time of goes up varied significantly (P<0.05) among treatments. At the second observation, no significant difference (P>0.05) was noticed for the mean frequencies of sniff and the Neperian logarithm of the latent time of goes up contrary to the mean frequencies of goes up, which varied significantly among treatments (P<0.05). It had been concluded that the chemical groups of C. bonduc roots could potentially induce aphrodisiac activities. However, these results need further investigations.

Keywords: *Aqueous extract, ethanolic extract, Caesalpinia bonduc, sexual behavior, male wistar rats*

Introduction

Men and women try by all means to develop, preserve or regain their own sexual capacities and to stimulate their partner's desire. The repeated inability of the male to perform sexual function, at least effectively, or a disorder that interfere with his full sexual response cycle could come from various origins including personal life styles (chronic alcohol abuse, cigarette smoking), androgenic deficiency, ageing, psychological disorders, side effects of some psychiatric medications, antidepressants and chronic medical conditions like diabetes and pulmonary cancer [1]. This men sexual disturbance could take different forms, such as disorders of desire and orgasm, erectile dysfunction, disorders of ejaculation or recurrent ejaculation with minimum sexual stimulation that occur before, during, or shortly after the penetration [2]. Several synthesis aphrodisiac substances, such as, Viagra®, Cialis®, Levitra® are extensively used for palliate these disorders. In the developing countries, particularly in Africa, the high cost of these treatments makes them inaccessible for the majority of the populations [3]. In addition, the secondary effects induced by these treatments are not easily endurable. Therefore, these populations prefer the traditional medicine using local plants [4]. Plant-derived chemicals that have sex-enhancing potentials for animals have therefore received more attention and become worldwide known as a treatment [5]. These phytochemicals increase libido (sexual desire and arousal), sexual potentiality (effectiveness of erection) and sexual pleasure [6]. Nowadays, the empiric use of the medicinal plants is spilled in the treatment of various affections bound to the sexuality. Nevertheless, the growing practice of the self-medication with natural aphrodisiacs increases the risks of induced diseases [7]. The effects on man could be, ache in the penis, burning of urethra, infections, pains and bleeding [1]. It is, therefore, necessary to undertake investigations on the use of the natural aphrodisiacs. This study is in interested on the case of *Caesalpinia bonduc* which tree is widely available in Benin and whom roots are used as aphrodisiac material by peoples. The

pharmacological properties of different parts of *C. bonduc* have been already reported [3] including the activities of anti-microbial, anti-tumor, anti-inflammatory and anti-diabetic. This study aimed to investigate the preliminary data on the biological properties of *C. bonduc* roots bound to the sexual behavior of the male Wistar rats.

Material and methods

Plant material

Fresh and healthy *Caesalpinia bonduc* roots were purchased from herb sellers at the markets of the Cotonou city (Bénin) and authenticated at the Department of Plant Biology, University of Abomey-Calavi (Bénin). The roots were rinsed with tap water to remove dust and other foreign particles, carefully cleaned and dried for fourteen days in dark place at temperatures between 26 °C and 30 °C. After drying, the roots were thoroughly pulverized using an electric grinder type Flour Mills, El. Motor No.1827. The ground material obtained was preserved at room temperature for various analyzes.

Animal material

Animal material was Wistar strain rats of both sexes 10 to 14 weeks old and weighing between 150 and 200 g. The rats were acclimated in the animal laboratory for sufficient ventilation with constant temperature (24 ± 2 °C) and a light which varies in a circadian rythm 12 h light: 12 h dark. Animals have *ad libitum* access to water and a diet balanced in proteins, carbohydrates, fats, vitamins and minerals in the form of croquettes. The survey has been achieved with 18 male and 18 females. The male have been distributed at random in six groups of 3 rats each. These experimental groups of rats have received, respectively, 100 mg/kg of distilled water [Control (-)], 50 mg/kg of citrate of Sildenafil (Viagra) [Control (+)], 100 mg/kg (E 100) and 300 mg/kg (E 300) of ethanolic extract and 100 mg/kg (A 100) and 300 mg/kg (A 300) of aqueous extract. The extracts of *C. bonduc* roots as well as the citrate of Sildenafil (product of reference) and distilled water have been managed orally with the help of a œsophageal probe. The treatment has been done between 9 PM and 6 AM in order to use female rats always in the phase of œstrus [8].

Phytochemical screening

The phytochemical profiling of the *C. bonduc* roots to determine the major constituents was performed according to the classic procedure [9].

Preparation of aqueous and ethanolic extracts

The extraction was performed with the common method [10] currently used in our laboratory. Indeed, 50 g of *C. bonduc* powder were macerated in 500 mL of solvent (distilled water and ethanol-distilled water: 1/1) for 72 hours. The obtained extract was filtered three times using Whatman filter paper. The filtrate was evaporated by dryness at 40°C using a rotavapor coupled to water cooling. The gotten dry residual is then weighed for the determination of the yield in mass.

Test on the sexual behavior of the male Wistar rats

The assessment of the effects of the extracts on the sexual behavior of the rats has been carried out according to the methods used by Suresh Kumar *et al.* (2000) [11] and Tajuddin *et al.* (2005) [12]. Therefore, the measured parameters are: Frequency of Sniff (FS: the number of sniff of the male rat in the presence of the female rat during 15 minutes), Frequency of goes up (FG: the number of bring up the female by the male during the same time of 15 minutes) and Latent time of goes up (LTG: the time (expressed in minutes) that separates the moment of the introduction of the female in the cage of the male and the first goes up). Two sets of observations have been carried out. The first observation has been done 15 minutes after the administration of the substances; the female rat was introduced into the cage of the male during 15 minutes for that first observation. Then, the male and the female were separated during 105 minutes and brought together again during 15 minutes for the second observation.

Statistical analyses

The mean values of FS, FG and LTG of the first and the second observations have been compared separately and in combined way, for the different treatments by one way analysis of variance (ANOVA) after verifying data normality and homogeneity of variance. The LTG data have undergone a Neperian logarithmic transformation (Ln) before fulfill normality condition. All statistic tests were performed using the statistical software R.3.0.3. The differences were considered significant at the level of 5% ($P<0.05$).

Results

Phytochemical screening

The result of phytochemical screening of the *Caesalpinia bonduc* roots powder revealed the presence of various chemical compounds such as tannins catechic, gallic tannins, flavonoids, anthocyans, leucoanthocyans, mucillag, saponosids, free anthracemic, combined anthracemic, O-hétérosids, C-heterosids, cardiotonic heterosids , alkaloids, derivative quinons. They are therefore rich in secondary metabolits.

Influences of the extracts on the sexual behavior of the male wistar rats

At the first observation, (Table 1), no significant difference was noticed among the treatments, for FS and

Table 1: Comparison of the mean frequencies of sniff (FS), the mean frequencies of goes up (FG) and the mean of Neperian logarithm (Ln) of latent time of goes up (LTG) among the treatments at the first observation

Treatments	FS		FG		Ln (LTG)	
	Mean	CV	Mean	CV	Mean	CV
A 100	6.67[a]	31.23	5.33[a]	103.27	3.54[ba]	49.56
A 300	5.67[a]	26.96	4.67[a]	44.61	3.73[ba]	17.01
E 100	8.67[a]	43.68	1.00[a]	173.21	5.70[a]	-
E 300	5.00[a]	40.00	8.33[a]	88.45	1.35[b]	26.68
Control (-)	9.00[a]	40.06	8.00[a]	78.06	4.44[ba]	11.04
Control (+)	3.67[a]	78.73	6.67[a]	160.39	4.44[ba]	11.04

The means followed by the same letter in a same column are not significantly different (p>0.05).
CV = Coefficient of variation

Table 2: Comparison of the mean frequencies of sniff (FS), the mean frequencies of goes up (FG) and the mean of Neperian logarithm (Ln) of latent time of goes up (LTG) among the treatments at the second observation

Treatments	FS		FG		Ln (LTG)	
	Mean	CV	Mean	CV	Mean	CV
A 100	2.33[a]	24.74	0.67[b]	173.21	1.79[a]	-
A 300	2.00[a]	50.00	2.00[b]	100.00	4.09[a]	0.00
E 100	2.33[a]	24.74	0.00[b]	-	-	-
E 300	2.00[a]	50.00	11.33[ba]	25.47	3.79[a]	59.79
Control (-)	3.33[a]	96.44	16.00[a]	43.75	4.02[a]	71.85
Control (+)	2.33[a]	24.74	6.67[ba]	173.21	2.30[a]	-

The means followed by the same letter in a same column are not significantly different (p>0.05).
CV = Coefficient of variation

Table 3: Comparison of the mean frequencies of sniff (FS), the mean frequencies of goes up (FG) and the mean of Neperian logarithm (Ln) of the latent time of goes up (LTG) among the treatments for the two sets of observation

Treatments	FS		FG		Ln (LTG)	
	Mean	CV	Mean	CV	Mean	CV
A 100-1	6.67[a]	31.23	5.33[ba]	103.27	3.55[a]	49.56
A 100-2	2.33[a]	24.74	0.67[b]	173.21	1.79[a]	-
A 300-1	5.67[a]	26.96	4.67[ba]	44.61	3.73[a]	17.01
A 300-2	2.00[a]	50.00	2.00[b]	100.00	4.09[a]	0.00
E 100-1	8.67[a]	43.68	1.00[b]	173.21	5.70[a]	-
E 100-2	2.33[a]	24.74	0.00[b]	-	-	-
E 300-1	5.00[a]	56.57	7.00[ba]	141.42	1.35[a]	26.68
E 300-2	2.75[a]	62.10	11.25[ba]	21.00	3.79[a]	59.79
Control-1 (-)	9.00[a]	40.06	8.00[ba]	78.06	4.44[a]	11.04
Control-2 (-)	3.33[a]	96.44	16.00[a]	43.75	4.02[a]	71.84
Control-1 (+)	3.67[a]	78.73	6.67[ba]	160.39	4.41[a]	11.04
Control-2 (+)	2.33[a]	24.74	6.67[ba]	173.21	2.30[a]	-

The means followed by the same letter in a same column are not significantly different (p>0.05).
CV = Coefficient of variation

FG means (P>0.05). However, the mean value of FS varied from 3.67 (positive control) to 9 (negative control) and the mean of FG varied from 1 (E 100) to 8.33 (E 300). On the other hand, a significant difference was exhibited for the mean of Ln (LTG) (P<0.05). The lowest Ln (LTG) value (1.35) was noticed for E 300 and the highest (5.70) for E 100.

For the second observation (Table 2), no significant difference was noticed for the mean of FS and Ln (LTG) (P>0.05) but the mean of FG varied significantly among treatments (P<0.05) from 0 (E 100) to 11.33 (E 300).

The results of the combination of the two sets of observation (Table 3) revealed that only the mean of FG varied significantly (P<0.05) among the treatments. The action of the treatment E 100 nearly remained constant during the two sets of observation (0 to 1). The action of the treatment A 100 was significantly better at the first observation (5.33) than during the second observation (0.67).

Discussion

The search for aphrodisiacs substances of various origin dates back to ancient civilizations. *Caesalpinia bonduc* is extensively used as aphrodisiac plant in many countries in Africa particularly in Benin [13]. These findings

corroborate those of others reports about the use of *Caesalpinia benthamiana* (plant belonging to the family of *C. bonduc*, caesalpiniacea) [14]. To highlight the reasons behind these claims, we investigated in this study the effects of aqueous extract and ethanolic extract of *C. bonduc* roots on the sexual behaviour of male Wistar rats. The phytochemical composition discovered for *C. bonduc* roots was closed to the one reported for *C. benthamiana* (sterols, polyterpens, flavonoids, saponin, tannins, reducing sugars, coumarins and proteins). Besides, the results of the phytochemical screening revealed the presence of flavonoids, and tannins also discovered in the seed and leaves of *C. bonduc* [15, 16] and saponin present in the leaves [16]. The results of our phytochemical sifting agree with those found in aqueous extract of *Massularia acuminata* stem that entailed an accelerator effect on the sexual behavior of the male Wistar rats [2].

The results of the observation on the sexual behavior of the male rats upon high dose of aqueous or ethanolic extract showed trends to reduction of the latent time of goes up (LTG) comparatively to the free treatment control animals. These results are similar to those previously reported [2]. It is usually admitted that among the normal and very driven rat, the latent time of goes up constitutes a parameter inversely proportional to the sexual incentive, whereas the frequency of go up is considered like an indicator of sexual performance or libido [7]. Nevertheless, it has been noted during our investigations that the treatment with ethanolic extract at the highest dose (E 300) induced a latent time of goes up lower than that of rats treated with Viagra, even if the deference was not statistically significant. These results are contrary to those observed with the aqueous extract of *Uapaca guinensis* a plant also used for its aphrodisiac properties [9]. On the other hand, the observations showed that the highest dose of ethanolic extract (E 300) induced among the rats an increase of the frequency of goes up compare to the trend exhibited by the rats of positive control. This result is contrary to the findings reported with the ethanolic extract of the roots of *Mimosa pudica*, also a leguminous aphrodisiac plant like *C. bonduc* [17]. The frequency of goes up noticed with the two doses of aqueous extract is similar to the results obtained with *Syzygium aromaticum* extract that entailed a reduction of frequency of goes up one hour and three hours after treatment of male rat [18]. These reports also corroborated those obtained with ethanolic extract of *Vanda tessellate* [11].

Conclusions

The results of this study revealed the presence, in the extracts of *C. bonduc* roots, of several chemical groups commonly present in the parts of the aphrodisiac plant. This proves, the effects induced on the sexual behavior of the male Wistar rat treated by its acqueous and ethanolic extracts. The ethanolic extract exhibited an accelerator effect by decreasing the latent time of goes up while the aqueous extract increased the frequency of goes up. These results justify the use of *C. bonduc* roots by several peoples for aphrodisiac properties. However, these findings need further deepened investigations.

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