Ethanol extract of *Caesalpinia bonduc* (L.) Roxb root improves the sexual performance of male Wistar rats

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ABSTRACT

Background: *Caesalpinia bonduc* (CB) is an African tropical plant whose roots are used in traditional medicine as ethanol maceration for many purposes, especially for erection impairment. **Objective:** This study aims to examine the aphrodisiac activity of the ethanol extract from the root of CB in male Wistar rats. **Materials and Methods:** Eighteen male rats were divided into three groups (n = 6): The Group 1, control received dimethyl sulfoxide (vehicle), Group 2 received Viagra[®] (*Sildenafil citrate*) at 25 mg/kg body weight, and Group 3 received ethanol extract of CB root at 500 mg/kg body weight. Each treatment was administered once daily by gavages for 28 days, with the exception of Viagra[®] which was administered 1 h before each mating. On days 1, 14, and 28, the male rats were mated with artificially estrus female rats by hormonal treatment with benzoate estradiol and progesterone. The sexual behavior parameters as intromission frequency (IF), intromission latency (IL), mount frequency (MF), and mount latency (ML) were evaluated. The effect of the extract on serum testosterone level and histoarchitecture of testis was also assessed. **Results:** CB root extract increased significantly the IF (P < 0.001) and MF (P < 0.01) when compared with the control group. Significantly decreased (P < 0.05) of ML and IL of rats were observed. Significant increases (P < 0.01) in testosterone levels of extract-treated group were observed. This is supported by cross sections of the testis that showed an increase in the diameter of the seminiferous tubes compared to the control group and those with Viagra[®] group. **Conclusion:** Findings in this study revealed that CB root enhanced sexual behavior in male rats and may play an aphrodisiac role that justifying its use in alternative medicine.

KEY WORDS: Caesalpinia bonduc; Wistar Rat; Aphrodisiac; Testosterone; Histology

INTRODUCTION

Male sexual behavior comprises a complex pattern of genital and somatomotor responses, elicited, directed, and

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maintained by external and internal signals.^[1] This male sexual behavior may be facing some difficulty, such as disorders of desire and orgasm, erectile dysfunction, disorders of ejaculation, or recurrent ejaculation with minimum sexual stimulation that occurs before, during, or shortly after the penetration.^[2-21] Sexual disorders and erectile dysfunction are among the most abundant.^[10] Several factors would be involved in erectile dysfunction including psychological and physiological factors. These factors induce among others, the production of a large number of free radicals at the origin of the oxidative stress, resulting in a decrease in sexual

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performance.^[22] To face this problematic, main alternatives can be envisaged: It is, on the one hand, the use of various synthetic substances such as dopamine, *Amyl nitrite*, and *Sildenafil citrate*, the use of aphrodisiac plant extracts such as *Caesalpinia bonduc* (CB).^[2-18,22-29] An aphrodisiac being defined as any agent capable of arousing the sexual instinct, inducing venereal desire and increasing pleasure and performance.^[12] Aphrodisiac substances are often used to enhance sexual performance and treat sexual dysfunction. Aphrodisiacs are commonly classified into two categories: Psycho-physiological stimuli preparations (olfactory, visual, tactile, and aural) and internal agents, mainly obtained from plants (food, alcoholic drinks, and love potion).^[19]

CB is an important plant that belongs to the family Caesalpiniaceae, a popular traditional medicinal plant which is widely distributed throughout the tropical and subtropical regions of Southeast Asia.^[9] This ethnomedicinal plant grows up to a height of 1 m, includes of yellow hooked prickles all over the stem, leaf rachis. Leaf, root, bark, and seeds of this plant are highly medicinal.^[26] All parts of the plant have medicinal properties, so it is a very valuable medicinal plant which is utilized in traditional system of medicine. The plant has been reported to possess, antidiarrheal, antidiabetic, adaptogenic, anthelmintic, anti-inflammatory, antimalarial, antimicrobial, analgesic, antibacterial, antispasmodic, antioxidant, antiproliferative, antipsoriatic, antitumor, larvicidal, muscle contractile, and hepatoprotective.^[29] The root-bark is good for tumors and for removing the placenta, it possesses number of properties such as febrifuge and anthelmintic. The roots are used in intermittent fever and diabetes, taken in cases of hernia and aphrodisiac.^[2,29] It has been studied that plant root extract possesses antiinflammatory and antimicrobial activities, whereas root bark shows antifertility effects.^[24] The leaves and young twigs of CB are used as an antimalarial decoction.^[13]

Our previous work validated a dose-dependent aphrodisiac claim of CB root 100 and 300 mg/kg BW ethanol extract in the male Wistar rats.^[2] It is therefore logical to investigate the recognized aphrodisiac potential of the plant root in a complete randomized design at a dose greater than those previously used to validate the well-known use of CB as an aphrodisiac plant. It is in this context that this research project that fully meets the objective three of sustainable development (ensuring a healthy life and promoting wellbeing for all at all ages)^[14] was undertaken on the properties of CB (L.) Roxb root ethanol extract to improve the sexual performance of male Wistar rats.

MATERIALS AND METHODS

Sample Collection

CB with herbarium number AA 6743/HNB was collected from the area in Sèhouè (6° 55'54" N-2° 16'27" E),

Atlantique Department of southern Benin. Species were identified by a botanist at the National Herbarium of Benin, University of Abomey-Calavi where the voucher sample was deposited. Root was gently washed twice under running tap water and then washed again in distilled water to remove any dirt and dried at room temperature for 21 days and crushed into coarse powder with electric grinder (Flour mills Nigeria, El Motor N°1827). The powder obtained was used for the preparation of the ethanol extract.

Extraction of Plant Material

Two hundred and fifty grams powder of CB root were extracted with 500 ml of ethanol (95%) in enclosed glass jars using cold maceration with continuous shaking for 72 h under orbital shaker (VWR® Advanced Orbital Shaker, Model 5000) at 180 rpm at room temperature. The process was repeated 3 times for the collection of maximum contents. The homogenate was filtered on cotton and Whatman N°1 filter paper (Whatman International Ltd: Maidstone, England), then concentrated using a rotary evaporator (VWR® IKA, RV8) at 40° C under reduced pressure. The dried extract obtained after oven-dried at 40° C was stored in light-resistant air-tight container, labeled and was stored at 4°C until the pharmacological assays after determining the percentage yield according to the formula: % Yield of extraction = [Weight of dry crude extract obtained (g)/Weight of plant material powder before extraction (g)] \times 100.^[3] The extract was reconstituted in dimethyl sulfoxide (DMSO) for use during the experiment.

Qualitative Phytochemical Analysis

The phytochemical screening of CB root powder was assessed to identify the major natural chemical groups. The groups include phenolic compounds (anthocyanins, tannins, flavonoids, and coumarins); terpenoids (triterpenoids, and sterols); heterosides (saponins, glycosides, heterosides, cardiac glycosides, derived cyanogenic, derived quinines, and free antracenics); alkaloids; reduced sugars; and mucilages.^[5-17] The phytochemical analysis of the ethanol extract for the presence of phytochemical constituents was performed using standardized methods.^[23]

Animals

A total of 36 Wistar albino rats (*Rattus norvegicus*) of both sex made up of equal number of male $(220 \pm 20 \text{ g})$ and female $(200 \pm 20 \text{ g})$ of 3 months old were obtained from the laboratory animal room of the Teaching and Research Unit in Human Biology of the Faculty of Health Sciences (FSS) of Cotonou, University of Abomey-Calavi, Benin. The rats were kept in a controlled environment at ambient temperature with 12 h/12 h light natural and dark cycles. They were fed with standard rat pellet feed (Complete Food, Group Veto Services S. A., Benin) and drinking water *ad libitum* during the experiment. The pellet diet consisted of 18% crude protein, 1.16% calcium, 0.80% phosphorus, 0.82% lysin, 14.4% crude fiber, and 7.12% crude fat. Paddy husk was used as the bedding material that was cleaned 3 times/week. The experiments were carried out to minimize animal suffering in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes.^[28]

Induction of Estrus in Female Rats

Female rats were brought in estrus phase artificially by sequential administration of Estradiol benzoate[®] (Sigma-Aldrich) (100 μ g/ml BW) and Progesterone[®] (Sigma-Aldrich) (5 mg/ml BW), 52 h and 4 h, respectively, before mating.^[30] The drugs were dissolved in olive oil (60°C during 1 h and then thoroughly shaken) before injected subcutaneously in a volume of 0.2 ml/rat.^[30] They were further mated with male rats and only those exhibiting good sexual receptivity (displayed a high degree of lordosis in response to male's stimulation) and no rejection behavior were selected for the heterosexual copulatory tests.^[19]

Sexual Training and Selection of Male Rats

Each male rat was mated with estrus female rats, and the sexual behavior-related parameters were recorded. Single male rat was placed in test cage and an adaptation period (5–10 min) was allowed. An estrus female rat was introduced in the cage, and the copulatory behavior was permitted for 15 min.^[19] Male rats exhibiting active sexual behavior were considered as sexually experienced and used for testing aphrodisiac activity of the extract.

Experimental Design

Male Wistar rats (18) were divided into three groups (n = 6 in each group). Group 1, control was administered of DMSO (vehicle) while Group 2 received 25 mg/kg BW of Viagra[®] (reference drug). Group 3 was administered of ethanol extract at 500 mg/kg BW of CB root. Each treatment was administered once daily by oral gavages for 28 days, with the exception of Viagra[®] which was administered 1 h before started each mating.^[31] During the period of the experiment, food and water were given *ad labium*.

Sexual Behavioral Protocol

Copulation parameters to characterize the effects of CB root on the sexual behavior of male rats were recorded following the standard procedure of Agmo repeated by Yakubu *et al.* and several other authors.^[2-19,22-33] The sexual behavior of the animals was noted on the 1st, 14th, and 28th, days of the study after extract administration. Male rats were placed individually in the mating cages, after 10 min of acclimation; sexually receptive female rats were introduced into the cages

in a 1:1 ratio. The tests lasted for 30 min for each rat pair and the sexual behavior parameters were recorded: Mount frequency (MF): The number of mounts without intromission from the time of introduction of the female until ejaculation; intromission frequency (IF): The number of intromissions from the time of introduction of the female until ejaculation; mount latency (ML): The time interval between the introduction of the female and the first mount by the male; and intromission latency (IL): The time interval from the time of introduction of the female to the first intromission by the male. During observation period, any jerking movement of the mating area was avoided to enable the rats to chase each other; and cleaning of the mating area was performed after each trial since the urine trails left by one rat might alter the sexual behavior of the next rat as reported by Che-Musa *et al.*^[25]

Testosterone Estimation

After administration of the extract, Viagra® and DMSO at the 1st, 14th, and 28th days of treatment, animals were anesthetized in a saturation closed jar containing cotton wool soaked in diethyl ether. When rats became unconscious, blood samples were collected from the retro-orbital plexus using hematocrit microtube in appropriately labeled sample tubes. The blood samples were left at room temperature for 1 h and then centrifuged at 3000 revolutions/min (rpm) for 15 min after coagulation.^[2-7,25-29] Sera was pipetted by micropipette and transferred into new Eppendorf tubes then stored to freeze at -20°C before being used in the measurement of testosterone of the experimental animals using radioimmunoassay commercial kits.^[16] The assay was done according to the protocol of the radioimmunoassay service of the Faculty of Health Sciences of the University of Abomey-Calavi.

Histopathological Studies

Sexual organ isolated, testis, was fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated with subsequent 50%, 70%, 90%, and 100% ethanol, and then cleared in xylene.^[2-6,22-29,33] After incubation of paraffin in a 60°C incubator, they were embedded and blocked in paraffin at same temperature. Fine serial sections (5 μ m thick) obtained by cutting the embedded tissue with microtome, then mounted on gelatinous water coated slides and dried for 24 h at room temperature. The sections on the slides were deparaffinized with xylene, rehydrated in a descending series of alcohol and water before stained with hematoxylin and eosin dyes, dried and mounted on a light microscope for microscopic examination and histopathological analysis.^[2-6,22-29,33]

Statistical Analysis

The data were expressed as mean (\pm SEM). The averages were analyzed using analysis of one-way analysis of variance

and supplemented by Student's *t*-test. Post-test analysis was performed using Dunnett's multiple comparison tests to determine significant differences in all parameters. Values were considered significantly different when P < 0.05. The analysis and construction of the graphics were done using the GraphPad Prism software version 6.00 (GraphPad Prism Software, Inc., San Diego, California).

RESULTS

Plant Extract and Qualitative Phytochemical

CB root after complete drying yielded about 6.95% of the extract brown color and oily appearance. Qualitative phytochemical screening revealed the presence of some secondary metabolites in the roots of CB. The results [Table 1] show that there is the presence of alkaloids, tannins, flavonoids, saponosides, triterpernoids, coumarins, and free anthracenics as well in the powder as in the ethanol extract of CB root.

Effect of CB Root Extract on Sexual Behavior of Male Rats

The results of the sexual behavior study are presented in Figures 1-4. A significant decrease in ML (P < 0.05) at day 28 was observed in the rats treated with ethanol extract [Figure 1]. The IL [Figure 2] of the rats administered with ethanol extract significant decrease with day of administration from 14th to 28th days. Ethanol extract increases significantly (P < 0.01), the MF of rats at day 28 [Figure 3] as well as IF (P < 0.001) of rats [Figure 4]. Throughout the experiment sexual behavior was notable among the treated rats. The results confirmed the potential of ethanol root extract of CB as a sexual stimulant, however, the reference drug; Viagra was more effective than the extract.

Table 1: Phytocl	hemical	screening	of root of
Cae	esalpinia	bonduc	

Phytochemical	Tests performed	Inference	
		Powder	Ethanol extract
Alkaloids	Mayer	+	+
	Dragendorff	+	+
Tannins	Ferric chloride	+	+
Flavonoids	Shinoda	-	+
	Ferric chloride	+	+
Saponins	Foam	+	+
Triterpenoids	Liebermann-Burchard	+	+
Coumarins	Ammonia	+	+
Free anthracenics	Ammonia	+	+
Quinoid derivatives	Bornträger	-	+
Mucilages	Precipitation	+	—
Steroids	Liebermann-Burchard	-	+

+: Presence of the compounds, -: Absence of the compounds

Effect of Ethanol Extract of CB Root on Serum Testosterone Level

There was no significant difference in the serum levels of testosterone in rats at day 1 when compared with the control group. The serum testosterone concentration was found to be significative (P < 0.001) in the Viagra group at day 14 while it was no significative (P > 0.05) in treated group. However, the

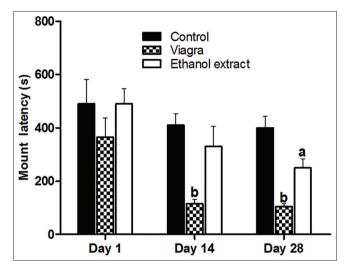


Figure 1: Effect of *Caesalpinia bonduc* root extract on mount latency of control and treated male Wistar rats at days 1, 14, and 28. The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. Values are means of six replicates \pm SEM; the bars carrying the letter "a» and «b" are significantly different from the control at P < 0.05 and P < 0.01 for the same day

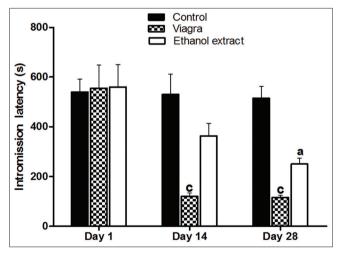


Figure 2: Effect of *Caesalpinia bonduc* root extract on intromission latency of control and treated male Wistar rats at days 1, 14, and 28. The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. Values are means of six replicates \pm SEM; the bars carrying the letter "a» and «c" are significantly different from the control at P < 0.05 and P < 0.001for the same day

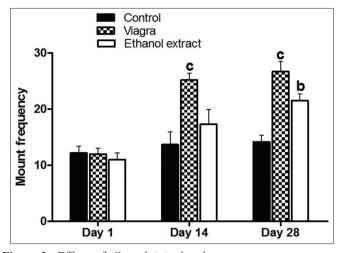


Figure 3: Effect of *Caesalpinia bonduc* root extract on mount frequency of control and treated male Wistar rats at days 1, 14, and 28. The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. Values are means of six replicates \pm SEM; the bars carrying the letter "b» and «c" are significantly different from the control at P < 0.01 and P < 0.001 for the same day

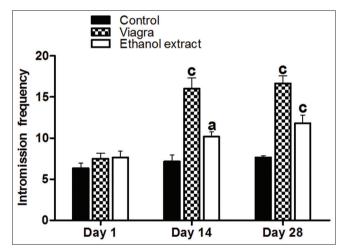


Figure 4: Effect of *Caesalpinia bonduc* root extract on intromission frequency of control and treated male Wistar rats at days 1, 14, and 28. The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. Values are means of six replicates \pm SEM; the bars carrying the letter "a» and «c" are significantly different from the control at P < 0.05 and P < 0.001 for the same day

rats treated with CB root extract at the dose of 500 mg/kg BW showed significant increase (P < 0.01) in serum testosterone level when compared with that of control rats on day 28 [Figure 5].

Effect of Ethanol Extract of CB Root on Histoarchitecture of Testis of Male Rats

The testicular section of the control group compared to those of the treated groups presented with observable differences

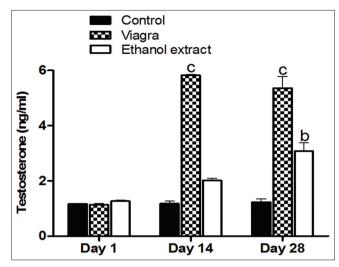


Figure 5: Effect of *Caesalpinia bonduc* root extract on testosterone level of control and treated male Wistar rats at days 1, 14, and 28. The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. Values are means \pm SEM; the bars carrying the letter "b» and «c" are significantly different from the control at *P* < 0.01 and *P* < 0.001 for the same day

in spermatogenic stages [Figure 6]. The cross section of the testis of control rats reveals the presence of germ cells at various stages of development with a normal density as spermatogonia, spermatocytes, and spermatozoa. Cross sections of the testicles of the animals treated with Viagra[®] once every 2 weeks showed a normal histoarchitecture, Leydig cells were observed, the seminiferous tubes (ST) were in normal numbers. The cross section of the testes of rats treated with ethanol extract reveals an increase in the diameter of the ST compared to the control rats and those treated with Viagra[®] once every 2 weeks. A proliferation of the different cells of these ST with a good presentation and uniform arrangement was observed.

DISCUSSION

Herbal medicines as well as their derivatives have been used as an alternative to allopathic medicines in many countries for the treatment of various diseases including sexual dysfunctions.^[27] Some plants with aphrodisiac properties have been suggested as a viable alternative to relief different sexual dysfunctions, and their usefulness has been demonstrated by means of animal models.^[2-18,27-29] Hence, to evaluate the action of ethanol extract of CB root on sexual behavior, 18 female and 18 male Wistar rats were used. Animals were grouped, and sexual behavior was evaluated as per Agmo^[30] and Yakubu et al. protocol and some sexual behavior parameters (mounting number, mounting latency, intromission number, and IL) were monitored. Blood sample was collected at the end of the experiment for evaluation of testosterone level. Results revealed that administration of CB root extract increased the IF and MF when compared with

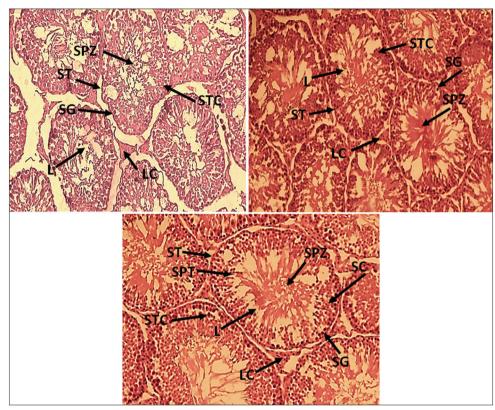


Figure 6: Effect of *Caesalpinia bonduc* root extract on testis c of control and treated male Wistar rats (×100, hematoxylin and eosin). The control group received DMSO daily over 28 days, Viagra group received 25 mg/kg BW of Viagra[®] 1 h before the start of each mating, and the ethanol extract group received 500 mg/kg BW ethanol extract of CB root daily for 28 days. The sertoli cells (SC) recognizable by their nucleus; spermatogonia (SG) that proliferates and differentiates from the base to the light (L) filled with spermatozoa (SPZ); spermatocytes (STC) occupying a median position in the tube; spermatids (SPT) grouped in colonies are observed on the liminal side of the seminiferous tube (ST); Leydig cells (LC) grouped in colony between the seminiferous tubules are observed

animals in the control group. In addition, it was found that the mount latency and the intromission latency of the rats treated with ethanol extract of CB root were significantly decreased. The results indicated also significant increases in testosterone synthesis in rats when they were treated with ethanol extract of CB root, and this is supported by cross sections of the testicle that has a well-organized histological structure without alteration.

Phytochemicals are produced by plants as secondary metabolism. They generally have biological activities and play a role in plant growth or defense against predators, pathogens, or competitors.^[32] They are commonly found in fruits, vegetables, nuts, legumes, and grains. Some phytochemicals possess incredible health benefits while others are toxic.^[32] The phytochemical test helps in determining the class of chemical compounds present in the extracts which may lead to their quantitative estimation and also identifying the source of pharmacologically active phytoconstituents.^[3] In the present work, major groups of chemical compounds have been characterized such as alkaloids, tannins, saponin, coumarins, flavonoids, free anthracenics, triterpene, quinoid derivatives, and steroids. The alkaloids present in the root of CB are chemical compounds belonging to organic substances of vegetable origins and they are alkaline nitrogen

metabolites which generally precipitate with iodobismuthate reagents (Dragendorff reagent).^[23] The sterols, triterpenes, and saponins also present in the roots of CB belong to the class of terpenes. Phenolic compound is present in the CB root in the form of flavonoids. These phenolic compounds also exist in the CB root as tannins and coumarins. Flavonoids alter androgen levels, which play an important role in sexual stimulation. They also enhance the activities of antioxidants, thereby imparting an indirect potentiating effect on sexual behavior parameters.^[8] Similarly, alkaloids enhance vascular relaxation by promoting nitric oxide production and by protecting the synthesized nitric oxide against ROS.^[8] Saponins exhibit a sex stimulating activity by enhancing androgen levels through binding to hormone receptors to provoke conformational changes or through binding to enzymes that are affected by the synthesis of such hormones.^[8] In addition, phenolic compounds stimulate the secretion of FSH and testosterone.[8] These results are consistent with those found by Gbankoto et al. and partially agree with those of Wadkar and Sayyad, who detected in addition to our compounds glycosides and sugars in the ethanol extract.^[19] Note that these researchers worked on the bark of the root of CB. Pingale et al. who worked on CB leave identified carbohydrates and sugars.^[15] The results of these authors show a similarity with our compound; this

resemblance is certainly due to a specific property of the plant. Other chemical compound differs from one researcher to another. This difference can be explained by the screening method adopted, the geographical location of the species, the type of organ studied, the country of collection, the harvest season, the stage of leaf maturation, and the physicochemical characteristics of the species soils.

The significant increase in the mount and IF observed in this study as well as the significant decrease in mount and intromission latencies are indicators of the aphrodisiac potential.^[19] The marked effects on the sexual behavior parameters, compared with the control, are indications of stimulation of sexuality. Such an increase in the frequency of mount and intromission suggests that libido, sexual vigor, and sexual performance were unimpaired.^[20] The decrease recorded in mount and IL can be explained by stimulation of sexual motivation and arousal and may also be an indication of improved sexual behavior in treated male animals, which further supports the effect of the administered extract on sexual enhancement.^[10] Similar findings have been reported by Gbankoto et al. and Zamble et al. who were worked with Caesalpinia benthamiana roots; Akassa et al.who were worked on Pausinystalia Yohimbe.[2-29,31,34] In the current study, the improvement of sexual behavior was also observed in rats treated with Sildenafil citrate, the most common drug used to treat erectile dysfunction. This observation was also made by Haripriya et al. with a dose of 5 mg/kg BW of Viagra.^[4]

Apart from the desire that is essential for initiation of sex, penile tumescence, and rigidity as well as the accessory muscles that help in providing additional penile rigidity and ejaculation are dependent on testosterone for normal sexual activity.^[20] An increase in testicular and serum-free testosterone concentration will confirm aphrodisiac potential inherent in the plant extract.^[25] Commonly, high level of testosterone in the male reproductive system is closely related to the increase of luteinizing hormone (LH) concentration and the presence of secondary phytochemical, which capable to mimic the function of LH to stimulate interstitial cells in testosterone production.^[25] Abedi et al. have worked on Phoenix dactylifera, also reported an increase in testosterone levels in treated rats compared to the control, as well as Lee et al. who studied the aphrodisiac properties of the aqueous extract of Cynanchum wilfordii. Our results are in contradiction with those of Che-Musa et al. who did not observe a significant increase in testosterone levels in rats treated with the aqueous extract of the leaves of Aquilaria malaccensis.

The testes of rats treated with the ethanol extract of the CB root showed a distinct histological change compared to the control group characterized by an improvement in the histological appearance of the testicular tissue. The histological structure seemed better than the picture of animals treated with Viagra. There was an increase in the number of spermatogenesis cells with an increase in the diameter of the ST. This same observation was made.^[11] These researchers have shown through the results of their work on the aphrodisiac effects of *Smilax kraussiana* root methanol extract in the experimental rat that the extract was nontoxic to the reproductive organs in the male rat and it promoted the proliferation of ST cells responsible for spermatogenesis.

CONCLUSION

Results from this study provide important information and data on aphrodisiac properties of CB root, which is traditionally used by local healers. Phytochemical screening has shown its wealth of secondary metabolites whose biological functions are numerous and varied, particularly their involvement in the improvement of sexual behavior. These results clearly demonstrate that the ethanol extract of CB root at a dose of 500 mg/kg body weight significantly enhances sexual behavior parameters, thus confirming the aphrodisiac activity of the plant.

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