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Effect of methanolic extract of *Alchornea cordifolia* leaves on the sexual behavior of senescent and sexually inexperienced rats

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ABSTRACT

Alchornea cordifolia, a shrub found in tropical Africa, is used to treat several physiological disorders, including male infertility and impotency. The main objective of this study was to determine the effect of the methanolic extract of *Alchornea cordifolia* leaves on the sexual behavior of senescent and sexually inexperienced rats. In order to evaluate aphrodisiac properties and reproductive performance, rats were divided into groups: group I receiving distilled water, group II receiving testosterone, group III receiving sildenafil citrate, group IV and V receiving the methanolic extract of *Alchornea cordifolia* leaves at 200 mg / kg and 400 mg / kg, respectively. The treatment was done by gavage for groups I, III, IV and V and subcutaneous injection of testosterone every three days for group II for 14 days. On days 1, 7 and 14, the rats were mated to the receptive females. At the end of this last treatment, the rats were mated with gonad-intact females. 14 days after of treatment, the extract leads to an increase in libido and sexual performance parameters (mounts, intromissions and ejaculations frequency) and an increase in sexual desire (decrease of mount and intromission latency). The extract also prolonged the duration of coitus by increasing the ejaculation latency and the average interval of copulation. An increase in the percentage of female's rats impregnated as well as the numbers of implantations were recorded in females' rats mated with the males treated with our extract. These results reveal the potential of the methanolic extract of *Alchornea cordifolia* leaves to improve male reproductive performance justifying its use in traditional medicine.

Keywords: *Alchornea cordifolia*, rats, sexual behavior, mount, intromission, ejaculation, reproductive performance.

INTRODUCTION

Reproductive disorders are steadily increasing worldwide due to etiological factors and aging [1, 2]. Male sexual behavior comprises a complex pattern of genital and somatomotor responses, elicited, directed, and maintained by external and internal signals [3]. This male sexual behavior 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 [4]. Sexual disorders and erectile dysfunction are among the most abundant.

Research on masculine sexual dysfunction have mainly focused on understanding erectile and ejaculatory disorders, which represent the most prevalent sexual problems [5]. Erectile dysfunction (ED) or male impotence is the preferred clinical term describing the persistent or recurrent disability to achieve and maintain a penile erection rigid enough to allow satisfactory sexual activity for at least 3 months [6]. Erectile function decreases with age and the prevalence of ED increases with age. It goes from 5% for men aged 20-39 to over 70% for men aged over 70 [7]. Steinarch, 1994 [8] reported that inexperienced male rat's exhibit mating behavior post castration, for two weeks.

Erectile dysfunction is an important public health problem that deserves increased support. Enhanced sexual behavior may provide increased relationship satisfaction and self-esteem in humans [9]. Treatment of ED with several medicines includes drugs having aphrodisiac properties [10]. Aphrodisiacs can be defined as substances that are ingested, applied topically, smoked / sniffed or delivered to the body to induce sexual arousal, increase sexual experience, and improve sexual performance [11]. An aphrodisiac also includes any food or drug that arouses the sexual instinct, induces venereal desire and increases pleasure and performance [12]. Methods commonly used in aphrodisiac study can be assigned into

physical methods including male sexual behavior (Mount Frequency, Mount Latency, Intromission frequency, Intromission latency, Ejaculation frequency, etc.) [13]. In developed countries, the therapy involves the implantation of penile prostheses, intracavernous injections, and the use of certain pharmaceutical products (phosphodiestérase type V inhibitors) [14]. Sildenafil citrate is known as one of the first therapies for male sexual dysfunction, particularly erectile dysfunction and ejaculatory dysfunction with excellent overall efficacy and satisfactory side effect profiles [15].

Despite this, because of its fewer side effects, there is a hunt for natural supplement from medicinal plants being intensified mainly. The use of plants extract for treatment of sexual disorders is gaining ground every day. Many of the effective herbal preparation are accessible and have slight or no side effects [16] such as *Alchornea cordifolia*.

Alchornea cordifolia is found in Senegal, eastern Kenya, and southern Tanzania and throughout Central Africa. It is generally found in tropical Africa in secondary forests, usually located near water, wetlands or swampy areas. It grows at a considerable height but is always a shrub or muddy habit. The plant develops well in acidic soil [17]. The leaves are normally used in infusion for the treatment of respiratory problems such as sore throat, cough and bronchitis, and to solve intestinal problems such as gastric ulcers, diarrhea, amoebic dysentery and worms [18]. The poultice of the leaves is used for the treatment of wounds. The leaves and root bark of *Alchornea cordifolia* are applied externally to treat leprosy and also serve as an antidote to snake venom [19]. Literature have also reported the phytochemicals constituents present in *Alchornea cordifolia* such as terpenoids, steroid glycosides, flavonoids, tannins, saponins, carbohydrate, imidazopyrimidine, alkaloids, alchorneine, alchornidine and several guanidine alkaloids [20]. Therefore, this work is a preliminary study aimed at evaluating the effect of oral administration of methanolic extract of *Alchornea cordifolia* leaves on the sexual behavior of senescent and sexually inexperienced rats

METHODOLOGY

Preparation of plant extracts

Fresh leaves of *Alchornea cordifolia* harvested in central region of Cameroon, was compared with the sample No. 33548 / CST at the National Herbarium of Cameroon. The leaves was Air dried and then ground into powder. The powder of leaves was macerated with methanol at the ratio 1:10 (g: L) at room temperature for 3 days with constant shaking. The solvent were filtered and freeze-dry to obtain the methanolic extract of leaves of *Alchornea cordifolia*.

Animals

Thirty mature males *Wistar* rats (15 –16 weeks) was use for this study. The rat were housed in polypropylene 2 per cages under 12:12 hours of light/dark cycles at a temperature of 23 ± 20C under hygienic conditions maintained in the animal house University of Douala. Water and food in the form of standard pellets were given *ad libitum* to the rats. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC).

Experimental design

The rats were randomly divided into five treatment of 6 rats each as follow:

Treatment I: Control group - received 0.7 ml of distilled water orally per day for 14 days.

Treatment II: Experimental rat - received 10 mg/kg body weight testosterone by injected subcutaneously every 3 days for 14 days.

Treatment III: Experimental rat - received 5 mg/kg BW of sildenafil citrate (Viagra) orally per day for 14 days.

Treatment IV: Experimental rat - received 200 mg/kg body weight of methanolic extract of leaves of *Alchornea cordifolia* orally per day for 14 days.

Treatment V: Experimental rat - received 400 mg/kg body weight of methanolic extract of leaves of *Alchornea cordifolia* orally per day for 14 days.

Ovariectomized females induced artificially in oestrus were also used in the study. Females were made receptive by successive administration subcutaneous of estradiol benzoate (15 µg/kg), followed by progesterone (60 µg/kg) after 48 hr. and 6hr respectively prior mating.

Mating Behavioral study

Observation of sexual behavior was made on the 1st, 7th and 14th day of the treatment

The receptive female rats were introduced to the male rats, 30 min after administration of treatment of male rats, in a locally manufactured wooden cage. Treated male and estrus female rats were paired in the ratio 1:1 (1 female to 1 male). Any female animal that do not show receptivity was replaced by another receptive female.

Observation was carefully made for 30 min and the parameters of male sexual behavior were recorded. These parameters includes: "Mount (MF) and Intromission frequency (IF) - the number of mounts and intromissions from the time of introduction of the female until ejaculation), Mount (ML) and Intromission latency (IL) - the time interval between the introduction of the female and the first mount or intromission by the male, Ejaculation latency (EL) - the time interval between the first intromission and ejaculation", ejaculatory frequency (EF) (number of ejaculatory recorded and average interval of copulation (AIC) (times between the first intromission of a series and ejaculation marking the end of these series).

RESULTS

Effects of the methanolic extract of the leaves of *Alchornea cordifolia* on mounts frequency

Figure 1 shows the effect of treatment with the methanolic extract of *Alchornea cordifolia* leaves on mounts frequency during days 1, 7 and 14 of the treatment.

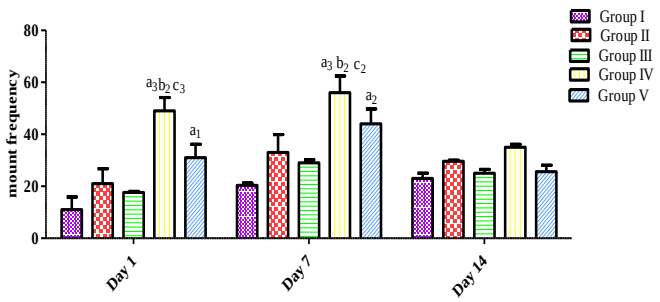


Figure 1: Effect of methanolic extract of *Alchornea cordifolia* leaves on mount frequency

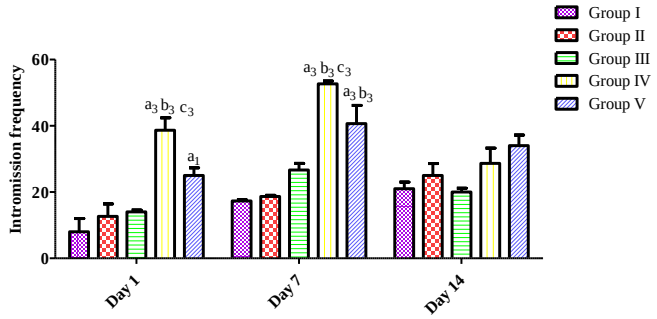
Each diagram represents the mean ± ESM. Number of animals per

group = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a^1P <0.05 with respect to group 1; a^2P <0.01 with respect to group 1; b^2P <0.01 compared to group 2; b^3P <0.001 relative to group B; c^2P <0.01 compared to group 3; c^3P <0.001 compared to group 3.

On the first day, rats treated at dose of 200 mg / kg showed a significant increase in mount frequency when compared to control and those receiving sildenafil citrate group (p <0.001) and rats receiving testosterone (p <0. 01). Rats treated at dose of 400 mg / kg also showed a significant increase in mount frequency (p <0.05) when compared to group I. On the 7th day, there was an increase in this value individually when compared to the first day. Group IV showed a significant increase (p <0.001) in mount frequency when compared to group I, II and III while group V showed a significant increase in mount frequency when compared to group I (p <0.001), II and III (P<0.05) with a rate of 32.26% and 40.03% respectively. At the 14th day, the same trend was observed with no statistical difference.

Effects of the methanolic extract of leaves of *Alchornea cordifolia* on intromission frequency

The effects of the methanolic extract of *Alchornea cordifolia* leaves on the intromission frequency during days 1, 7 and 14 are shown in Figure 2.



Each bar represents the mean ± ESM. Number of animals per group = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a^1P <0.05 with respect to group 1; a^3P <0.001 compared to group 1; b^3P <0.001 compared to group 2.

On day 1 and 7, administration of 200 mg / kg (Group IV) resulted in a significant (p <0.001) increase in the number of intromissions when compared to group I, II and III while on day 1, at dose of 400 mg / kg this parameter significantly increased (p <0.05) when compared to group I. On day 7, the dose of 400 mg / kg resulted in a significant (p <0.01) increase in intromission frequency when compared to group I and II, with an increase of 34.43% when compared to group III. On day 14 no significant difference was noted between treated groups and control one.

Effects of the methanolic extract of leaves of *Alchornea cordifolia* on the ejaculation frequency

The number of ejaculations observed after administration of the extract is shown in figure 3.

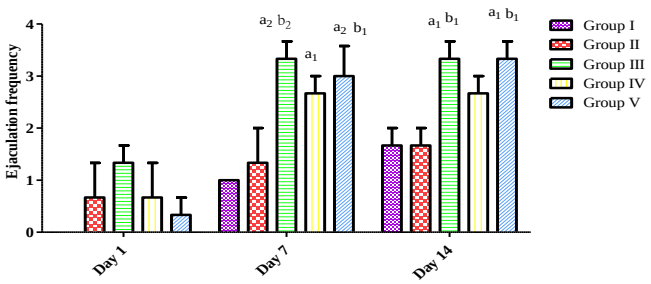


Figure 3: Effects of the methanolic extract of leaves of *Alchornea cordifolia* on the ejaculation frequency

Each bar represents the mean ± ESM. Number of animals per group = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a^1P <0.05 with respect to group 1; a^2P <0.01 compared to group 1.

On day 1, no significant difference (p> 0.05) was noted between all groups and no ejaculation was noted during the observation period concerning group I (distilled water). In Group III (sildenafil citrate), the greatest number of ejaculations were recorded, although this increase was not significant (p> 0.05). At day 7, there was an increase in values in all groups when compared to the values recorded on the first day. It was also noted that group III shows a significant increase (p <0.01) when compared to groups I and II. Group IV showed a significant increase (p <0.05) when compared to group I, while group V showed a significant increase when compared to group I (p <0.01) and group II (p <0.05). At day 14, groups III and V showed a significant increase (p <0.05) when compared to groups I and II. Group IV did not show any change when compared to groups I and II.

Effects of the methanolic extract of leaves of *Alchornea cordifolia* on the mounts latency

The variations in the mount latency after 14 days of treatment are shown in Figure 4.

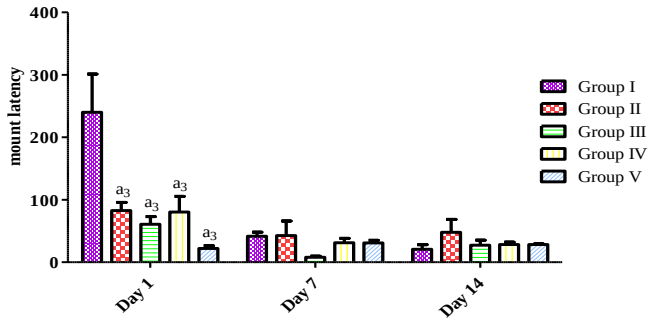


Figure 4: Effects of the methanolic extract of leaves of *Alchornea cordifolia* on the mounts latency

Each bar represents the mean ± ESM. n (number of animals per group) = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a3P <0.001 compared to group I.

On Day 1, when compared to control (Group I), there was a significant reduction (p <0.001) in the mount latency at doses of 200 and 400 mg / kg while on day 7 and 14, no significant difference was observed between treated groups and controls one.

Effects of methanolic extract of *Alchornea cordifolia* leaves on intromission latency

Figure 5 shows the variations in intromission latency after extract administration for 14 days.

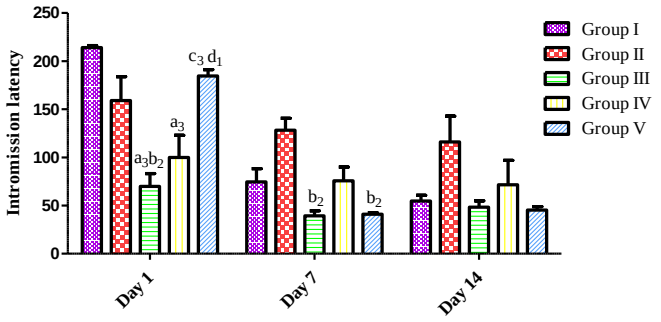


Figure 5: Effects of methanolic extract of leaves of *Alchornea cordifolia* on intromission latency.

Each bar represents the mean ± ESM. n (number of animals per group) = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves, b2 P <0.01 compared to group 2, c3P <0.001 compared to group 3.d1P <0.0001 compared to group 4.

Between all the three periods of treatment, it is only on day 7 that we noticed the significant decrease in intromission latency of group IV and V when compared to group II.

Effects of methanolic extract of leaves of *Alchornea cordifolia* on ejaculation latency

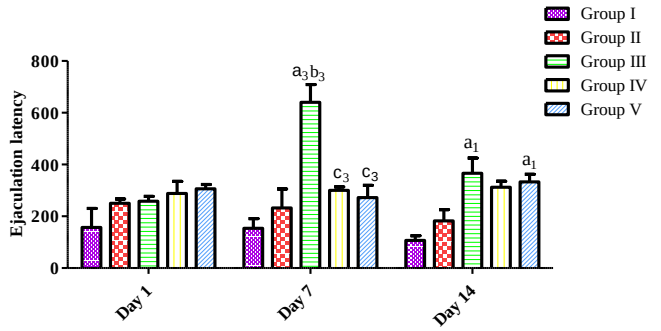


Figure 6: Effects of methanolic extract of leaves of *Alchornea cordifolia* on ejaculation latency

Each bar represents the mean ± ESM. n (number of animals per group) = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a^1P <0.05 with respect to group 1; c^3P <0.001 compared to group 3.

It appears that: On day 1, there was no significant increase (p> 0.05) in ejaculation latency in all groups when compared to Group I. Nevertheless, on day 7, this parameter significantly decreased (p <0.001) in Group IV and V when compared to Group III. On day 14, the ejaculation latency significantly (p <0.05) increased in Group V when compared to Group I.

Effect of methanolic extract of *Alchornea cordifolia* leaves on average interval of copulation

Values of average interval of copulation de copulation are represented on figure 7.

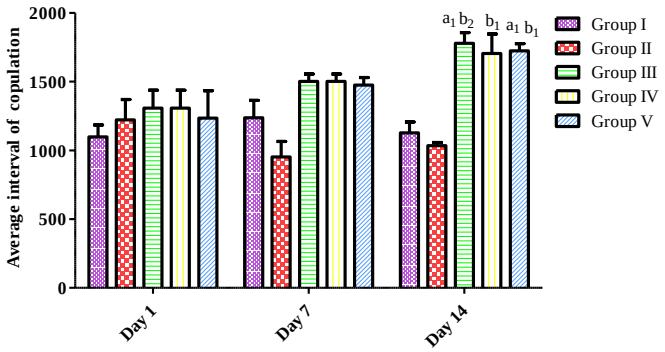


Figure 7: effect methanolic extract of *Alchornea cordifolia* leaves on average interval of copulation

Each bar represents the mean ± ESM. n (number of animals per group) = 6. Group I = Rat receiving 0.5 ml of distilled water. Group II = Rat receiving testosterone (10mg / kg) by subcutaneous injection. Group III = Rat receiving 5 mg / kg of citrate sildenafil. Group IV = Rat receiving 200 mg / kg of the methanolic extract of *A. cordifolia* leaves. Group V = Rat receiving 400 mg / kg of the methanolic extract of *A. cordifolia* leaves. a^1P <0.05 relative to group 1; b^1P <0.05 compared to group 2; b^2P <0.01 compared to group 2.

On day 1 and 7, no significant change was observed concerning the average interval of copulation, however on day 14, this parameter significantly increased (p <0.05) in group IV and V when compared to group II and I.

DISCUSSION

Sexual behavior is one of the fundamental principles of reproduction including sexual desire, mating, erectile function and ejaculation. They can express their effects through mechanisms such as vasodilatation, nitrite oxide and gonatropine production, and also elevation of androgens [10]. The methanolic extract of *A. cordifolia* leaves has shown interesting properties in sexual behavior.

This study evaluated the sexual behavior of aged and sexually inexperienced rats coupled with susceptible females. The administration of the extract induced a significant increase in the mount and intromission frequencies compared to the different controls. This is also pronounced at the small dose level and is different from the one obtain the first day of observation. On the other hand, it has been observed an opposite trend in the mount and intromission latency, which are significantly reduced. The mount latency is considered as a reflection of sexual motivation and is said to be inversely proportional to motivation or sexual desire. In addition, alteration of mount frequency is considered an indication of libido, strength, potency, sexual performance, sexual motivation and vigor [21, 4]. Mount and intromission frequencies are known as useful indices of vigor, libido, and power [22], so increased of mount frequency is an indicator of increased sexual motivation while increasing of intromission frequency of is an indicator of the efficacy of erection, penile orientation and increased activation of ejaculatory reflex by the administered extract.

In addition, mount and intromission latency represent indicators of sexual motivation and are inversely proportional to sexual motivation.

This allows to say that, after the administration of the methanolic extract of the leaves of *A. cordifolia*, male rats were highly excited, and this was expressed by increased of performance, motivation and vigor. The decrease recorded in mount and intromission latency 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 [23]. The results of this study show that the administration of the low dose (200 mg / kg) of extract to sexually unexperienced rats leads to good performance at both mount and intromission level (increases frequencies and reduces latencies) when compared with effect observed after treatment at high dose (400 mg / kg). Similar results were obtained by Kada *et al.*, (2012) [24] who suggested that this may be due to saturation of receptors for bioactive compounds present in the extract or to a sedative effect of the extract. One can conclude that, the methanolic extract of *A.cordifolia* leaves contains metabolites that could exert an aphrodisiac effect on different levels (central nervous system, testosterone production, relaxation of blood vessels) and also synergistically.

Increase of intromission frequency induced by our extract shows the activation of the mechanism of erection. This could be due to the action of the alkaloids present in the extract that have estrogenic properties. It is well known that, estrogens can induce vasodilatation of the blood vessels of the penis which would result in an erection of penis. In addition, saponins present in the extract have erogenous properties in the vasodilatation of blood vessels and consequently can initiate erection [23]. Since intromission cannot be achieved without adequate erection and coordinated activity of the penile muscle, the increased frequency of intromission after administration of the extract in this study suggests that the mechanism of erectile dysfunction penis has been activated. Therefore, the extract can increase sexual potency by allowing or supporting erection [22], possibly by facilitating the flow of blood into the penis [10]. In the other hand, several lines indicated that, in erectile function, androgens stimulate the expression of the neuronal isoform of nitric oxide synthase (nNOS) and modulate the activity of phosphodiesterase type 5. Then, flavonoids and sterols present in the extract could act by inducing changes in the level of neurotransmitters involved in erectile function, and by this way, modulate the action of these neurotransmitters at their target cells or raising the androgen level [21, 25]. Flavonoids could also induce a complex mechanism of action including an increase in testosterone levels by inhibiting the cytochrome P450, enzyme aromatase responsible for converting testosterone to estrogen [26], and, at the central nervous system, an increase in dopaminergic transmission (neurotransmitters involved in sexual behavior) in the brain [27, 28].

Another important factor in this study is the elevation of ejaculation latency, which is considered as an indicator of sexual pleasure and performance, and ejaculation frequency which represents strength, vigor and endurance [29]. Administration of the methanolic extract of *A. cordifolia* leaves led to an increase in ejaculation latency in the rats treated at doses of 200 and 400 mg / kg when compared to negative control group and rats treated with sildenafil citrate on the first day of treatment. This increase becomes more significant ($p < 0.5$) from 7 to 14 days when compared to negative controls. The prolongation of ejaculatory latency after administration of the extract may be considered as a strong indication that the sexual function of male rats has been improved by prolonged coitus and suggesting aphrodisiac activity. According to Malviya *et al*. (2011) [12] and Shamloul (2010) [30], substances that act on male sexual reflexes such as ejaculation are considered as aphrodisiacs. These observations are similar to those reported by Abedi *et al*. (2012) [3] who studied the effect of the aqueous extract of pollen grains of Phoenix dactylifera on the sexual behavior of rats. The extract also caused an increase in the frequency of ejaculation that may explain the increase of its aphrodisiac effect. This observation is in correlation with the report of Fouche *et al*., (2015) [23] during the study of the effect of the aqueous extract of *Monsonia angustifolia* on the sexual behavior of rats.

One of the mechanism involved in the physiological mechanisms for evaluating male sexual behavior is sexual arousal and sexual performance. Then this pro-sexual effects of aphrodisiacs can be perceived in terms of performance (potency) [31]. We have also noticed in this study that, the methanolic extract of *Alchornea cordifolia* leaves has supported an increase in the average interval of copulation (indicator of sexual vigor) which increased from the 1st to the 14th day. This would have been the consequence of a lasting erection due to the presence in the extract of the metabolites that maintain the erection and increase the sexual motivation. Moreover, this increase in overtime could also be explained by the fact that the animals would have acquired a sexual experience. This improvement in libido could be the consequence in a possible elevation in the concentration of testosterone, since saponins could improve androgen production [32], mainly testosterone which is hormone involved in the improvement of sexual desire, motivation and sexual performance in this study

CONCLUSION

In view of the above, our results have revealed that, the methanolic extract of *Alchornea cordifolia* leaves could be used as a stimulator of sexual behavior of aging and unexperienced male rats. This effect could be attributed to the secondary metabolites present in the extract. Therefore, the result encourages the use of the plant to solve sexual problems of the third age people. Nevertheless, further investigations are needed to characterize the bioactive agents of these components of *Alchornea cordifolia* leaves.

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