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THE BEHAVIORAL EFFECTS OF *CLEOME ARABICA* AQUEOUS EXTRACT IN WISTAR RAT

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Abstract: Medicinal plants are used all over the world and have rapidly gained economic importance. The study was conducted to investigate the effects of the aqueous extract of *C. arabica* leaves on the sexual behavior of Wistar rats. *C. arabica* is a medicinal plant with a foul odor, toxic, and has hallucinogenic effects. The experimental study was carried out on white rats (male and female) of the Wistar strain from the Pasteur Institute of Algiers (Kouba, Algeria) weighing between 150 and 200g sexually naive. The animals are raised in polyethylene cages, the rats were divided into 4 groups (n=10 rats/group) which received a saline solution (male and female control group), 0.17 µg/ml of the aqueous extract of *C. arabica* leaves for 7 days orally (male and female treated group). The sexual behavior test was carried out according to four crosses types. The results of the treated groups showed a significant decrease in the mating frequency compared to the control group. Overall, the results showed that *C. arabica* significantly altered the sexual behavioral parameters (mounts frequency, mating time, licking time, first contact time, contacts number, intromission, latency time, and frequency). The aqueous extract of the plant caused an inconsistent decrease in the parameters of sexual behavior and orientation activity that were recorded in the treated animals. The *C. arabica* leaves aqueous extract caused an inconsistent decrease in the sexual behavioral parameters and orientation activity that were recorded in the treated animals.

Keywords: *Rattus rattus*, sedative effect, sexual behavioral parameters, *C.arabica.*

INTRODUCTION

The plants use for medicinal and mythological purposes and to solve problems related to ill health have been practiced in African and other societies for many years (Mohammed et al. 2014). Some of the factors that contribute to the increased use of medicinal plants include their availability, cultural significance, history of known efficacy, and, most importantly, their ease of use (Thomford *et al*., 2015). Approximately 40-50% of all marketed drugs are either obtained from herbs or their use is agitated by medicinal plants (Ekor, 2014). The World Health Organization estimates that 80% of the African population depends on traditional medicines for their primary health care needs (WHO, 2005).

Management of degenerative diseases such as mental illness, sexual dysfunction, and microbial infections is one area in which a lot of people in developing countries depend on herbal medicine (Adimoelja, 2000; Ajali, 2002; Fang and Schinkle, 2007).

Plant materials are central to tradiomedical practices and have remained useful sources of new drugs (O'Brien, 2004). Although orthodox medical practices are generally acceptable, alternative health care is still relied on all over the world (O'Brien, 2004; Leckridge, 2004). In the world developing countries, traditional herbal medicine is often used side by side the Western medicine, with herbal medicine taking the upper hand when the cost of Western medicine is beyond reach (Busia, 2005).

The need for plants with sex-enhancing potential or aphrodisiacs with little or no side effects is of great concern. Various substances of animal and plant origin have been used in the folk medicine of different cultures as an aphrodisiac, some of which have been identified pharmacologically to exert their effects on the hypothalamic-pituitary-testicular axis (Mills *et al*., 1996; Yakubu *et al*., 2011).

The Algerian Sahara has exceptional floristic biodiversity, consisting of more than 500 species, of which there are 162 endemic species in the northern Sahara alone and to which is added a tradition of traditional pharmacopeia (Ozenda,1991). Several species are known for their remarkable therapeutic properties (Quezel, 1978).

Spontaneous plants in arid areas are considered as phytogenetic resources of agronomic, economic, ecological, and strategic interest (UNESCO, 1960). Plant extracts have been used as insecticides by humans before Romans time, this practice continues to exist with many plant species known for their insecticidal properties (Balandrin *et al*., 1985; Isman, 2002). Plant products can be degraded more rapidly in the environment than synthetic compounds, and some may have increased specificity that may favor beneficial insects for the plant **(**Desneux *et al*., 2007). The need for plants with sex enhancement potential or aphrodisiacs with little or no side effects is of great

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concern. Various substances of animal and plant origin have been used in traditional medicine from different cultures as aphrodisiacs, some of which have been pharmacologically identified to exert their effects on the hypothalamic-pituitary-testicular axis (Mills *et al*., 1996; Yakubu, 2011). Aphrodisiacs are substances that stimulate or increase sexual desire and sexual performance. Sexual desire is controlled and regulated by the central nervous system which integrates tactile, olfactory, auditory, and mental stimuli. Sexual performance, which is quite different and not always dependent on sexual desire, is also called erectile performance or capacity. But erectile dysfunction can occur even if sexual desire remains strong, in which case sexual performance depends on a neurovascular event via the hemodynamic mechanisms of the erect penis (Anderson, 2001; Anderson and Wagner, 1995; Chandler, 1988).

The reproductive system is extremely sensitive to several factors such as lifestyle, radiation, medications, and toxic substances (Fucic *et al*., 2012). Exposure to these and other factors could lead to birth defects in infants and even functional alterations in adults (Knapp *et al*. 2012). Any interference that alters the normal functioning of the reproductive organs affects the animal's ability to reproduce.

In this work, we are trying to test the aqueous extract effect of an Algerian Saharian plant *Cleome arabica* (*Capparidaceae*) on Wistar rat. The *C. arabica* plant is a green, briefly hairy, glandular, viscous, and annual herb, 30-50 cm high, with erect and branched stems, trifoliolate leaves, elongated silica fruit opening through 2 valves, seeds covered with hairs as long as the seed diameter (Ozenda, 1991). This study aims to evaluate the aqueous extract effect of this plant on the sexual behavioral parameters in Wistar rats.

MATERIEL AND METHODS

Animal

We used the adult rats "*Rattus rattus*" of the Wistar strain, from the Pasteur Institute of Algiers (Algeria), to carry out the various experiments. The rats were raised in plastic cages lined with sawdust and fitted with steel lids and water-filled bottles. The rats were fed with sticks of corn, barley, milk, and vitamin supplements. These animals have been acclimatized to the laboratory conditions (temperature 25±2°C and humidity 70-80% and photoperiod 12:12h. The sexually inexperienced (naive) Wistar rats were used in this study. These animals had free access to food and water.

Cleome arabica (Capparidaceae)

It is a plant with a foul odor, toxic and hallucinogenic effects **(**Gubb, 1913; Ozenda, 1991). The plant is used in traditional medicine as a diuretic and against rheumatism; it is also a therapeutic and anti-bacterial plant (Ladhari *et al.*, 2013). These species effects have also been proven against different orders of insects **(**Ozenda, 1991; Doumandji-Mitiche and Doumandji, 1993; N'Guessan *et al*., 2009; Koïta et al., 2012, Habbachi *et al*., 2019, Habbachi, 2020). The plant studied was collected in the Bousaada region (M'sila, Algeria) (33°48′24″ north latitude, 2°52′56″ east longitude).

Preparation of *C. arabica* aqueous extract

For the extract, we put 170g of powdered leaves in 1 liter of distilled water and boil for 30 minutes on a hot plate at 180°C. The resulting mixture is filtered through filter paper and 750 ml of the filtrate (1 g/l stock solution) is recovered. For the present study, the leaves of *C. arabica* were harvested in Hodna (M'sila, central Algeria), in the month of February 2019. The plant was identified by Pr Rebbas Khellaf, Department of Biology, Faculty of Science, and University of M'sila.

Treatments

Forty rats were separated into two groups, a control group (20 individuals: 10 males and 10 females) and a treatment group (20 individuals: 10 males and 10 females) who will undergo the intoxication, by gavage, of 1 ml of *C. arabica (*0.17µg/ml) for 7 successive days.

Sexual behavior

In mammals, male sexual behavior includes a pre-copulatory phase (motivational or appetitive) and a copulatory (or consummatory) phase leading ultimately to ejaculation (Raskin et al., 2011). During the pre-copulatory phase, male rodents sniff the genitals of females, emit ultrasonic vocalizations, mark their territory with urine and show a clear preference for estrus (or receptive) females (Raskin *et al*., 2011). During the consumptive phase, the male performs climbs followed by intromissions during which he performs back and forth movements corresponding to rhythmic pelvic thrusts, culminating in ejaculation (Raskin *et al*., 2011). Thus, in a normal rat, copulatory parameters take place according to the following chronology (Bekker, 1996):

Mounting: the male rat climbs on the receptive female in heat from the rear flank, and makes pushing movements with his pelvis (Bekker, 1996). The latency is generally 3 to 6 seconds (Bekker, 1996).

The intromission: it takes place in a similar way to the mating, but immediately followed by a deep pelvic thrust when the penis enters the vagina (Bekker, 1996). This penetration phase lasts about 200 to 400 ms in well-trained rats (Bekker, 1996).

Measurement of Sexual Behavior

After the last administration, the animals were placed in a mating cage (one couple per day) in a quiet room at room temperature (24±2°C) with a light/dark cycle of 12:12h and a humidity of 60 to 65%. The animals had free access to water and food.

Before each test the male rat was placed in the observation cage (42cmx 10cm x 21cm), he was allowed to get used to the test chamber for 5 min. Afterward, a female was introduced into the cage and the mating behavior observation started immediately after the introduction of the female, and the parameters were recorded as the test progressed.

The copulatory activity (rise latency, intromission latency, rise frequency, and intromission frequency) of each male was evaluated in the presence of a female, in a quiet room as described by Watcho *et al* (2017).

Orientation activities

Male rat orientation activities towards females (riding, licking, and sniffing), towards oneself (genital grooming, non-genital grooming) were observed during the period of the copulatory behavior test, according to the method described by Malminas (1973) and evaluated according to Hull's method (1984). No male was exposed to the same female more than once during the experiment.

All trials were videotaped, and event tracking software was used to evaluate the experiment parameters. The following sexual performance parameters were recorded using standard methods. The camera was placed in a horizontal view that facilitates the observation of the mating cage. The standard elements of sexual behavior are normally recorded using surveillance DVR.

These tests are carried out according to four types of crosses: control male X control female, treated male X treated female, control male X treated female, and treated male X control female. Tests are normally completed immediately after the first post-ejaculatory intromission. In this test, female rats were introduced into the cages of the male animal with a ratio of one female to one male (Yakubu *et al*., 2007).

Sexual Behavior Parameters

The following parameters of sexual behavior were measured as described by Dewsbury et al (1970); Szechtman *et al* (1981), Hart *et al* (1983), Ageel *et al* (1994), Agmo (1997), Amin et al (1996), Gauthaman et *al* (2002), Carro-Juarez *et al* (2004), Yakubu *et al* (2007), Guohua *et al* (2009); Palaniyappan et al (2009) ,Mokhtari *et al* (2011), Fouche *et al* (2015), Zain *et al* (2018), Nur Hidayat *et al* (2019):

-***Mating latency time (ML):*** is the time between the introduction of a female into the cage and the first breeding.

-***The latency time of the intromission (IL):*** is the time that separates the introduction of the female and the first intromission.

-***The frequency of mounts (MF):*** are the mounts numbers, with or without intromissions preceding ejaculation.

-***The frequency of intromissions (IF)****:* corresponds to the number of intromissions preceding ejaculation.

-***Penile licking (PE):*** is the number of times the rat bent over to lick the penis.

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Data Analysis

The various study data were analyzed by descriptive and comparative methods (variances analysis) on XLStat 2009 software.

RESULTS

The results show that the aqueous extract of the *C. arabica* leaves administered at a dose of 0.17µg/ml for 7 consecutive days has an effect on certain parameters of performance and sexual motivation in rats. When observing the rat nuptial courtship, we recorded that the sequences are done in the following order: contacts, intromission, mounting, mating, and licking.

It was noted that ***the first contact' time*** is about 2.00±0.40 seconds on average, while in treated couples (treated male X treated female) is more important and can reach 11.25±1.31 seconds (Tab. 1). So that treated couples take much longer to establish first contact with their partners compared to control couples; these times are significantly different (*p*: 0.02) **(**Tab.1).

For ***the intromission latency' time (IL):*** the *C. arabica* aqueous extract disturbed this time, we recorded a null value in the couples (treated male X treated female) so there was no intromission compared to the control couples which (IL) is 190.25±58.98 seconds (Tab. 1). Data analysis shows that the intromission latency' times are highly significantly different (p: 0.0001) (Tab .1).

Table 1.

Effect of the aqueous extract of *C. arabica* on the parameters of sexual behavior in the Wistar rat: Time of 1st contact, the latency time of the intromission, the frequency of intromission, and mating latency time

	1st Contact time (s)	Contacts number	Intromission latency time (s)	Intromissions frequency	Mounting latency time (s)
♂**C X**♀**C**	2.00±0.40	106.00±19.90	190.25±58.98	105.75±65.64	105.75±65.64
♂***C.a*** **X**♀***C.a***	11.25±1.31	17.75±2.49	0.00±0.00	0.00±0.00	0.00±0.00
♂**C** **X**♀***C.a***	4.00±0.70	65.25±2.17	142.00±63.27	68.75±13.46	68.75±13.46
♂***C.a*** **X**♀**C**	5.75±0.85	27.75±5.36	382.50±158.39	4.25±1.43	4.25±1.43
F_{obs}	***4.33***	**5.32**	***24.18***	***7.57***	***7.57***
P	***0.02****	**0.01***	***<0.0001*****	***0.004****	***0.004****

[**Mean:** *Mean*; **SEM:** *Standard deviation of the mean*; ♂**C:** *Control male*; ♂***C.a*****:** *Male treated with C. arabica*; ♀ **C:** *Control female*; ♀***C.a*****:** *Female treated with C. arabica*]

The *C. arabica* aqueous extract decreased in a highly significant way ***the intromission frequency (FM)*** in the pairs (treated male X treated female) compared to the control pairs which is 105.75±65.64 seconds (Tab. 1).

Similarly, we recorded that ***the mounting latency time (ML)*** is null in the treated male X treated female

pairs compared to the control pairs which is 105.75±65.64 seconds (Tab. 1). We, also, recorded the highly significant difference (p: 0.004) (Tab .1).

For the ***mounts frequency (FM)***: is null in the couples "treated male X treated female" compared to the control couples which is 105.75±65.64 seconds (Tab. 2). We found significant differences between mounts frequency (*p*: 0.004) (Tab. 2).

The same result is observed for ***the mounting latency time*** and intromission during the observation period; *C. arabica* affects significantly the mounts frequency (0.004) (Tab. 2).

Table 2.

Effect of the aqueous extract of *C. arabica* on the parameters of sexual behavior in the Wistar rat: The frequency of mounts, mating time, time of licking, number of licking

	Mounts frequency	**Mating time (s)**	**Licking time (s)**	**Lickings number**
♂C X♀C	105.75±65.64	78.00±15.23	534.50±107.23	44.00±11.05
♂*C.a*X♀*C.a*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
♂C X♀*C.a*	68.75±13.46	137.50±26.92	490.75±112.46	22.25±7.36
♂*C.a* X♀C	4.25±1.43	8.50±2.87	1.50±0.95	6.25±5.60
F_{obs}	***7.57***	***15.88***	***4.11***	***2.47***
p	***0.004*****	***0.000******	***0.03****	***0.11***

[**Mean:** *Mean*; **SEM:** *Standard deviation of the mean*; ♂**C:** *Control male*; ♂***C.a*:** *Male treated with C. arabica*; ♀ **C:** *Control female*; ♀***C.a*:** *Female treated with C. arabica*]

DISCUSSION

In the present study the parameters of sexual behavior observed were mating latency time (ML), the latency time of the intromission (IL), the frequency of mounts (MF), the frequency of intromissions (IF), penile licking (PE). Despite the fact that the frequency of ejaculation was not performed in this study, the frequencies of mounts and intromission were sufficient, which are valuable indices of vigor, libido, and power (Dasofunjo *et al*.,2013).), it was extremely difficult, even for an experienced observer, to distinguish between intromission and ejaculation when observing sexual behavior (Wawata et al., 2010). The effects of the *C. arabica* aqueous extract on sexual behavior are summarised in this study.

The administration of the aqueous extract from the leaves of *C. arabica* caused a sedative effect in terms of reduction of sexual behavior and activity of orientation towards the female and towards oneself. In addition, animals treated with the aqueous extract of *C. arabica* did not indicate an attraction to females either, but rather appeared tired or drowsy and were not ready to move towards females. This was consistent with the findings of Yakubu *et al,* (2009) and Yukubu *et al*, (2011) who observed sexual behavioral parameters with 100 mg/kg body weight of *Bulbine natalensis* stem and 200 mg/kg body weight of *Massularia acuminata* root respectively in male rats. This condition also resulted in a reduction of MF and IF in treated animals and this sedative effect was evident after 30 minutes of observation.

However, the latencies of mounts and intromission for the treatment groups showed significant differences from the control group.

The aqueous extract of *C. arabica* has a highly significant influence on the mating time (Fobs= 15.88; *p*: 0.000) (Tab. 2).

In the end, we show that males lick their penises; we noted that C. arabica aqueous extract acts this sequence and we recorded the high significant differences between lickings times (*p*: 0.03) (Tab. 2).

Moreover, during the observation of the experimental period, we revealed that sexual function in treated male X female couples was not improved, especially in some parameters of sexual behavior such as mounting frequencies (MF) and intromission frequencies (IF) which had no value (Tab.1) and (Tab.2). A similar finding was reported on *Garcinia kola* seeds in male Wistar rats (Yakubu *et al*.2011). The higher values of mounting and intromission latencies observed in pairs treated male X control female with the aqueous extract are one of the main indicators of an increase in the hesitation time of male rats towards female mice (Yakubu and Afolayan, 2009; Fouche *et al*.,2015**)**. This was demonstrated when the vast majority of treated rats were not attracted to their opposite sex. In addition, the dissimilarity of MF and IF values were also recorded in treated animals throughout the experimental period. This situation was supported by the previous study, which suggested that each number of frames recorded in treated animals was not determined by the success of intromission (Fouche *et al.*, 2015).

The results obtained revealed a decrease in the time of self-care licking (genital grooming) in males treated with the aqueous extract of *C. arabica* compared to control, while males of male couples treated with X treated female showed a zero value of genital grooming compared to temois couples.

Contrary to the control couples, during the observation period the male rats, as soon as they were introduced, reacted with immediate advances towards the females and showed a sexual interest towards the female rats, such as chasing, sniffing, and licking of the genitals, which finally ended with mounting and intromission (Yakubu and Quadri, 2012).

The mounting frequency (MF) and the intromission frequency (IF) are useful indices of vigor, libido, and power. While mounts number of (MF) reflects sexual

motivation, the increase in the intromissions number (IF) shows the erection effectiveness, the penis orientation, and the ease with which ejaculatory reflexes are activated (Yakubu *et al*., 2009; Yakubu *et al*., 2010). Mounting latency (ML) and intromission latency (IL) are indicators of sexual motivation (Ratnasooriya and Dharmasiri, 2000; Yakubu *et al.*, 2009).

The higher mounting latency observed in rats receiving the lowest dose of the extract is an indication of the hesitation time of male rats towards receptive females (Yakubu *et al.*,2009). This was consistent with the findings of Yakubu *et al.* 2010, the root of *Massularia acuminata* in male Wistar rats at concentrations of 25, 50, and 100 mg/kg body weight.

Most of the sexual behavior sequences are affected by the plant extract. In this study, we recorded a perturbation of the behavioral sequences (frequencies of intromissions, mounts, mount latency time, and intromissions) in the Wistar rat that lead to mating with the *C. arabica* aqueous extract, this perturbation is remarkable when both partners are treated and when the male is treated and the female is control. The same results were recorded in *Drosophila melanogaster* (research model) if it's treated by *C. arabica* aqueous extract or ethanolic extract (Habbachi *et al.,* 2019; Habbachi, 2020)

Several studies have highlighted the different effects of aromatic plants in animals sexual behavior such as *Peganum harmla, Daphne gnidium, Citrullus colocynthis* (L.), *Mentha rotundifolia, Drimia maritima* (Merabti *et al.,* 2015; Benhissen, 2016; El-Bah, 2016; Merabti, 2016; Masna, 2016, Bekhakhache *et al.,* 2018, Kheroubi *et al*.,2020; Saadane *et al.*,2021).

CONCLUSION

This study results show that male rats treated with a leaves aqueous extract of *C. arabica* plant in the presence of a female did not increase their sexual performance during the observation period. The administration caused an inconsistent decrease in the sexual behavior parameters and orientation activity (towards the female and towards oneself) that were recorded in the treated animals. This study thus revealed that the *C. arabica* leaves aqueous extract has a lack of aphrodisiac properties with respect to rats.

The study shows that this plant extract acts on the key sequences (mount latency time ML, and intromission latency time IL) leading to mating.

AUTHORS CONTRIBUTION

All authors equally contributed to this study. Nour El Iméne BOUBLATA, Fatma Zohra SAADANE, Sarra HABBACHI, Abir BOUZAR, Wafa HABBACHI, Saliha BENHISSEN, Khellaf REBBAS, Abedkrim TAHRAOUI, designed and carried out the experimental study and writed the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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