

# Effects of ethanolic extract of the *Cleome arabica* on sexual behavior in Wistar rats



Nour El Iméne Boublata<sup>a\*</sup>  | Sarra Habbachi<sup>b</sup>  | Fatma Zohra Saadane<sup>b</sup>  |

Abir Bouzar<sup>b</sup>  | Wafa Habbachi<sup>b</sup> 

<sup>a</sup>Laboratory of Environmental Biosurveillance, Department of Biology, Faculty of Sciences, University Badji Mokhtar Annaba 23000, Algeria.

<sup>b</sup>Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of Sciences, University Badji Mokhtar Annaba 23000, Algeria.

\*Corresponding author: [imeneboulata@gmail.com](mailto:imeneboulata@gmail.com)

**Abstract** Traditional medicinal plants are widely used as immunomodulatory medicines that help improve health. A total of 50 plants used for the treatment of toxicity were screened for their protective effects. Traditional medicinal are globally used and have rapidly grown in economic importance. Intrinsically active compounds are well-known for their antioxidant, anti-tumor, anti-viral, and anti-inflammatory activities. The study was conducted to investigate the effects of the ethanolic extract of *C. arabica* leaves on sexual behavior in Wistar rats. *C. arabica*, 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 were raised in polyethylene cages and divided into two groups (n = 10 rats/group), which received a saline solution (male and female control group), 0.20µg/ml of the ethanolic extract of *C. arabica* leaves for seven days orally (male and female treated group). The sexual behavior test was performed according to three types of crossing. The results of the treated groups showed a significant increase in mating frequency compared to the control group. Overall, the results showed that *C. arabica* significantly affects sexual behavior. The ethanolic extract of *C. arabica* increased sexual behavior and orientation activity performance recorded in the treated animals. Thus, this study found that *C. arabica* has a significant effect on the rats' sexual behavior.

**Keywords** aphrodisiac effect, *Rattus rattus*, spontaneous plant, sexual behavioral parameters

## 1. Introduction

Medicinal plants serve as critical therapeutic agents and valuable raw materials for manufacturing numerous traditional and modern medicines. In many developing countries, traditional medicine is still the mainstay of health care, and most of the drugs and cures come from natural sources, such as a plant (Motaleb et al 2011).

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 contributing 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 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).

During the last years, the demand and utilization of medicinal plants have increased globally. There is now a consensus regarding the importance of medicinal plants and traditional health systems in solving medicinal plants' health

care problems, efficacy, and safety in curing various diseases (Motaleb et al 2011).

Plants produce a wide range of secondary metabolites such as phenolic compounds, alkaloids, vitamins, terpenoids, and other secondary metabolites with proven antioxidant activity (Kaur and Kapoor 2002; Wojdylo et al 2007).

The *Cleome* genus contains 250 species, of which only seven are used in traditional medicine (Wollenweber and Dorr 1992). Many species of the *Cleome* genus have been studied for their medicinal properties, and some have been evaluated for their activating (Selloum et al 1995; Nagaya et al 1997; Fushiya et al 1999; Bouriche et al 2003; Simoes et al 2006; Sharma et al 2010) and analgesic (Singh and West 1991; Paraimaladevi et al 2003; Bose et al 2007) properties. *Cleome* species have a long history of medicinal uses such as rubefacients, scabies treatment, inflammation, and rheumatic fever (Boulos 2000). They are also used in various disorders, such as fever, diarrhea, inflammation, bronchitis, liver disease, malaria, and skin diseases (Nishant and Vinod 2009; Jane and Patil 2012). The leaves and stems were decocted for the diabetes treatment by the Bedouins of South Sinai (El-Askary 2005) and were famous as an antihyperglycemic agent (Abedel-kawy et al 2008; AbdelMotaal et al 2011; Boublata et al 2020; Boublata et al 2021).

It is a northern species growing in the Maghreb and Saharan regions, common in the Hodna (M'sila) and some regions of the Algerian Sahara: *Cleome arabica* (Ozenda 1991; Beniston 1984). It is locally called "Netten" and "Netteina" (Baba Aissa 2000) and, like other species, it's rich in flavonoids (Touil et al 1998; Bouriche et al 2003; Wollenweber and Dorr 1992). *C. arabica* L. [Capparidaceae] is common species well acclimatized in the desert areas. The leaves are described as having hallucinogenic effects and sedatives for abdominal and rheumatic pain (Boulos 1983; Ahmad et al 1990; Tschritzis et al 1993; Baba Aissa 2011).

The present study of *C. arabica* focuses on evaluating the ethanolic extract effects on sexual behavior and its sequences leading to the mating of Wistar rats couples.

## 2. Materials and Methods

### 2.2. Animals

We used adult rats (*Rattus rattus*) - Wistar strain from the Pasteur Institute (Algiers, Algeria) for all experiments. Rats were raised in sawdust-lined plastic cages with a steel lid and baby bottles filled with water. Rat's food is made in the sticks form consisting of corn, barley, milk, and vitamin supplement. These animals were acclimatized to laboratory conditions (temperature  $25\pm 2$  °C and humidity 70-80% and photoperiod 12:12h).

### 2.2. *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). The effect of this species has also been proven against different orders of insects (Doumandji-Mitiche and Doumandji 1993; Ozenda 1991; Aouinty et al 2006; Kemassi and Oued El-hadj 2008; N'Guessan et al 2009; Lebouz 2010; Kemassi et al 2012; Koita et al 2012; Habbachi et al 2013; Merabti et al 2015; Benhissen 2016; Merabti 2016, Masna 2016; Korichi-Almi et al 2016; Almi et al 2016; Benhissen et al 2018; Bekhakheche et al 2018; Seglab et al 2019; Habbachi et al 2020; Boublata et al 2020; Saadane et al 2021; Boublata et al 2021). For the present study, the plant was collected in the Bousaada region (M'sila, Algeria) ( $33^{\circ}48'24''$  N latitude,  $2^{\circ}52'56''$  E longitude).

### 2.3. Preparation of the *C. arabica* ethanolic extract

Ethanolic maceration follows the protocol of Bouharb et al 2014). This one consists of macerating 400g of plant powder for 24 hours in one liter of ethanol solvent at (99.8%) at room temperature and in the shade. After filtration, the solution obtained was evaporated in the shade. With the help of a magnetic stirrer to drive out the solvent (Keita et al 1998) using a hot plate at 50 °C until a paste was obtained, kept at 4 °C until its use. On each bottle are noted the preparation date, extraction type, and concentration. The plant was identified by Pr. Rebbas Khellaf, Department of Biology, Faculty of Science, and University of M'sila (Algeria).

### 2.4. 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), which were undergo the intoxication, by gavage, of 1ml of *C. arabica* (0.20µg/ml) for seven successive days.

### 2.5. Sexual behavior

In mammals, male sexual behavior includes a precopulatory phase (motivational or appetitive) and a copulatory (or consummatory) phase leading ultimately to ejaculation (Raskin et al 2011). During the precopulatory 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 rats, copulatory parameters take place according to the following chronology (Bekker 1996): (1) In the *mounting*, the male rat climbs on the receptive female in heat from the rear flank and makes pushing movements with his pelvis. The latency is generally 3 to 6 seconds; (2) The *intromission* occurs similarly to the mating but is immediately followed by a deep pelvic thrust when the penis enters the vagina; this penetration phase lasts about 200 to 400 ms in well-trained rats.

### 2.6. Behavioral measurements

After the last administration, the animals were placed in a mating cage (one couple per day) in a quiet room at room temperature ( $24\pm 2$  °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).

#### 2.6.1. 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 classic elements of sexual behavior usually are recorded using surveillance DVR.

These tests are carried out according to three types of crosses: control male × control female treated male × treated female and treated male × control female. Examinations usually are completed immediately after the first post-jaculatory 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).

### 2.6.2. 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 (2019): *Mating latency time (ML)* is the time between introducing 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)* is 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.



**Figure 1** Sexual behavior test.

### 2.7. Data Analysis

All parameters measured in this study were statistically analyzed by XLStat 2009 software, using descriptive metric methods giving the mean, the standard deviation of the mean. The results were, also, analyzed by a

variances comparison (ANOVA, whose significance level  $\alpha = 0.05$ ).

### 3. Results

The results show that the ethanolic extract of the *C. arabica* leaves administered at a dose of 0.20 $\mu$ g/ml for seven consecutive days affects specific performance parameters 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.

We noted that the first contact time is 25.60 $\pm$ 5.30 seconds in the control couples, while in the treated couples is 9.20 $\pm$ 0.80 seconds. The treated couples take less time to establish the first contact with their partner than the control couples; these times are significantly different ( $P = 0.04$ ; Table 1).

For the intromission latency time (IL), the ethanolic extract of *C. arabica* acts on this time, and we recorded an average of 6693.60 $\pm$ 3374.76 seconds (Table 1) in the treated couples (treated male × treated female) compared to the control couples, which IL is 12316.20 $\pm$ 11444.51 seconds (Table 1). Ethanolic extract very-highly significant increase the intromission frequency (IF) in treated pairs compared to control pairs, which was 3.80 $\pm$ 1.39 intromissions (Table 1).

Similarly, we recorded that the mounting latency (ML) is 4.20 $\pm$ 1.20 seconds in the treated pairs compared to the control pairs, which was 14.60 $\pm$ 6.12 seconds (Table 1). We also recorded a very-highly significant difference ( $P = 0.000$ ) (Table 1).

The mounting frequency (FM) is 19.40 $\pm$ 8.90 mounts in the couples (treated male × treated female) compared to the control couples, which is 3.80 $\pm$ 1.39 mounts (Table 2). We found highly significant differences between the mounts frequencies ( $P = 0.000$ ; Table 2).

For the rise latency time, *C. arabica* affects very-highly significant the rise latency time ( $P = 0.000$ ; Table 2). The *C. arabica* ethanolic extract has a very-highly significant influence on the mating time ( $P = 0.000$ ; Table 2). In the end, we show that the males lick their penises; we noted that the *C. arabica* ethanolic extract does not act on this sequence, and we recorded no significant effect between the licking times ( $P = 0.67$ ; Table 2).

### 4. Discussion

In the present study, the parameters of sexual behavior observed were mating latency time (ML), the intromission latency, the frequency of mounts, the frequency of intromissions, penile licking. Although 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). Even for an experienced observer, it was challenging to distinguish between intromission and ejaculation when observing sexual behavior (Wawata et al 2010). The effects of

the *C. arabica* ethanolic extract on sexual behavior were summarised in this study.

Several studies reported that aphrodisiac plants are good alternatives for improving sexual behavior (Carro-Juarez et al 2004; Preedapirom et al 2018), probably due to their efficacy and availability. This sexual behavior may also be due to androgenic and gonadotropic activities of *C. arabica* ethanolic extract; these results were observed for *M. acuminata* stem in male rats (Yakubu et al 2008; Yakubu and Akanji 2011).

Treat animals with the ethanolic extract of *C. arabica* shows that the treated males go directly towards their treated females in the first 30 seconds of the test, contrary to the control couples, which take a little more time to join their females. This rapid orientation step towards the conspecific indicates the extract's stimulating effect, which probably

includes aphrodisiac compounds. Our results are consistent with those of Yakubu and Akanji (2011), which suggest that male rats, upon introduction to the test cage, responded with immediate advances toward females and showed precopulatory behaviors such as chasing anogenital sniffing that eventually resulted in mounting.

ML and IL are considered indicators of sexual motivation (Yukubu and Afolayan 2009). The significative reduction in these parameters observed in rats treated with ethanolic extract of *C. arabica* could imply an improvement in sexual motivation and sexual appetite, further justify the folk use of this plant as a sexual stimulant. Besides, increased MF is an indicator of vigor, libido, and potency. Increased MF indicates sexual motivation (Cicero et al 2001). The pro-sexual effect of *C. arabica* was also established by increasing the MF after treatment.

**Table 1** Effect of the ethanolic extract of *C. arabica* on 1<sup>st</sup> contact, the latency time of the intromission, the frequency of intromission, and mating latency time of Winstar rats.

	1st Contact time (s)	Contacts number	Intromission latency time (s)	Intromissions frequency	Mounting latency time (s)
♂C × ♀C	25.60± 5.30	111.20± 36.51	12316.20±11444.51	3.80±1.39	14.60±6.12
♂C.a × ♀C.a	9.20±0.80	168.40±15.68	6693.60±3374.76	19,40±8,90	4.20±1.20
♂C.a × ♀C	19.40±8.17	120.40± 25.33	12107.60±9877.75	15.60±8.80	5.00±1.41
F <sub>obs</sub>	0.08	2.96	1.50	16.53	16.83
P-value	0.04*	0.10	0.60	0.000***	0.000***

[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*]

**Table 2** Effect of the ethanolic extract of *C. arabica* on the frequency of mounts, mating time, time of licking, number of licking number of Winstar rats.

	Mounts frequency	Mating time (s)	Licking time (s)	Lickings number
♂C × ♀C	3.80±1.39	7.60±2.78	165.20±104.30	12.40±6.54
♂C.a × ♀C.a	19.40±8.90	38,80±17,81	214.40±144.91	23.00±7.42
♂C.a × ♀C	15.60±8.80	31.20±17.60	167.00± 79.87	18.00±5.16
F <sub>obs</sub>	16.53	16.53	0.41	0.63
P-value	0.000***	0.000***	0.67	0.54

[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*]

When tested with a female, the copulatory behavior of the male rat consists of a repeated series of mounts and intromissions culminating in ejaculation (Mbongue et al 2005). The administration of ethanolic extract of *C. arabica* caused a stimulatory effect, increasing sexual performance and orientation activity towards the female. These results are similar to those of Whatcho et al (2007); Watcho et al (2014); Watcho et al (2017), and Watcho et al (2019) Long-term administration of the aqueous and methanolic extracts of *Bersama engleriana* leaves significantly increased the sexual behavior of the animals during the first observation period.

These sexual behaviors, for example, ear wagging, jumping, and lordosis of the receptive female rats in this study implied intense proceptivity. At the same time, the precopulatory behavior of the extract-treated male rats also indicated that the animals were generally aroused. The pursuit of female animals (males running behind female animals in close contact) suggested impending copulation (Yakubu and Akanji 2011).

Several proceptive behaviors of females and precopulatory behavior parameters of males were observed on the cage side when extract-treated male rats were presented to receptive female rats. The proceptive behavior

displayed by the female rats included ear wagging, characterized by rapid anteroposterior vibration of the ears, a short run in which the female rats suddenly stop and present their posteriors to the male rats (darting), and a short jump with stiff legs followed by immobility and presentation (hopping). Receptive female rats also showed lordosis before, at the beginning of, and during the mounts. There was genital grooming after each mounting that resulted in intromission. The effect of ethanolic extract of *C. arabica* in rats was similar to that of aqueous extract of the plant *Massularia acuminata* (Yakubu and Akanji 2011).

The present study results demonstrate the ability of *C. arabica* to stimulate the copulatory activity of sexually naïve rats. The pro-sexual effect of *C. arabica* could be attributed to the existence of flavonoids and sterols revealed by phytochemical tests (Drewes et al 2003).

## 5. Conclusions

Our findings highlight the sexually stimulating effects of the ethanolic extract of *C. arabica* in Wistar rats and thus give value to its aphrodisiac reputation. It appears from this work that this extract has a sexually stimulating activity which would confirm the interest of its traditional use as a sexual stimulant.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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