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ORIGINAL ARTICLE

Dracaena arborea extracts delay the pro-ejaculatory effect of dopamine and oxytocin in spinal male rats

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Dracaena arborea is a medicinal plant with ethnopharmacological aphrodisiac reputation. In the present study, the effect of an intravenous administration of aqueous and ethanolic extracts from the dried roots of this plant on the ejaculatory pattern of spinal cord-transected and urethane-anaesthetized rats was investigated. In addition, the effects of these extracts were also determined on dopamine and oxytocin-induced ejaculation. Systemic administration of aqueous and ethanolic extracts (5, 20, 60, 100 mg kg^{-1}) of *D. arborea* did not activate fictive ejaculation, whereas dopamine (0.1 μM kg^{-1}) and oxytocin (0.5 UI kg^{-1}) provoked ejaculation evidenced by the rhythmic contractions of the bulbospongiosus muscles accompanied with penile erection and sometimes with expulsion of the seminal plugs. Pretreatment of spinal rats with *D. arborea* extracts dose-dependently blocked the pro-ejaculatory activity of dopamine and oxytocin. In conclusion, the present study shows that the bioactive substances present in the extracts of *D. arborea* inhibit the activity of the bulbospongiosus muscles through the blockade of dopaminergic and oxytocinergic receptors in rats.

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INTRODUCTION

Dracaena arborea (Wild) Link (Dracaenaceae) is a tall tree native to West Africa where it is used against gonorrhoea, small pox, malaria and leishmaniasis.[1] In the western part of Cameroon, the mixture of 'palm wine' with the roots of this plant, identified by its local name as Keubgouh, has been used over the years as an aphrodisiac.[2] In a pilot study, we observed that the aqueous and ethanolic extracts from the dried roots of *D. arborea* in normal and castrated adult sexually experienced rats promotes male sexual behaviour, with main effect centred in a delay in the ejaculation function. Further, pretreatment of rats with haloperidol, a centrally acting dopamine antagonist, blocked the pro-sexual effects of *D. arborea*.[3] This blocking action of haloperidol prompted us to investigate the effects of *D. arborea* on the ejaculation as the integrity of the dopaminergic system is necessary for the occurrence of this sexual response.[4,5] Because oxytocinergic receptors can be activated by dopamine during the induction of male sexual behaviour,[6,7] the effects of *D. arborea* extracts on oxytocin-induced ejaculation were also investigated. In the present study, we used the fictive ejaculation model which permits the recording and visualization of the rhythmic contractions of the bulbospongiosus muscles that accompany ejaculation and that can be induced by pharmacological means.[8,9]

METHODS

Collection of plant material

The plant material was harvested in Bagnoun, West Region of Cameroon and authenticated by Dr Pinta Jonas of the Botany Department, Faculty of Science, University of Dschang, Cameroon. The harvested fresh roots were cut into small pieces, dried at room temperature and ground into a fine powder.

Preparation of aqueous and ethanolic extracts

Six hundred grams of the powdered roots were dissolved in 5 l of distilled water and kept for 72 h at 4 °C and occasionally stirred. After filtration, the filtrate was concentrated in an oven (55 °C) to give 35.10 g of brownish residue corresponding to an extraction yield of 5.85%. The aqueous extract used in the study was prepared at a final concentration of 100 mg ml^{-1} in distilled water. For ethanolic extracts, ground roots (1 kg) of *D. arborea* were macerated with ethanol (95%) (5 l, 2 ×) for 72 h to yield, after solvent evaporation under reduced pressure, 30 g of a brownish extract corresponding to an extraction yield of 3%. The ethanolic extract used in the study was prepared by dissolving 1 g of the residue in a known volume of distilled water and the final volume adjusted to 10 ml (100 mg ml^{-1}).

Phytochemical screening

The test of Shinoda was used to determine the presence of flavonoids,[10] Libermann Buchard's test revealed the existence of sterols[11] while saponins were revealed as described by Hostettmann *et al.*[12]

Animals and groups

Adult male Wistar rats (250–300 g bw) were obtained from the animal house of the 'Laboratorio de Comportamiento Reproductivo of the 'Escuela de Medicina Veterinaria' of the 'Universidad Autónoma de Tlaxcala, México'. They were housed in groups (four rats per cage), under an inverted LD cycle 12:12 h, at 22 °C and with free access to food and water. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals 'NOM-062-ZOO-1999'. Male rats were trained for sexual experience as previously described[3] and only those exhibiting good copulatory behaviour were selected for the study. Animals were divided into 21 groups of 5 rats each. Group 1 received saline solution (1 ml kg^{-1}) and served as control. Groups 2–21 were used to analyse the effect of the following treatments: aqueous extract of *D. arborea* (5, 20, 60, 100 mg kg^{-1}), ethanolic extract of *D. arborea* (5, 20, 60, 100 mg kg^{-1}), oxytocin (0.5 UI kg^{-1}), dopamine (0.1 μM kg^{-1}), the sequential treatments with aqueous extract (100 mg kg^{-1}) plus oxytocin

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(0.5 UI kg^{-1}) or aqueous extract (5, 20, 60, 100 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}), ethanolic extract (100 mg kg^{-1}) plus oxytocin (0.5 UI kg^{-1}) or ethanolic extract (5, 20, 60, 100 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}). In all sequential treatments (Groups 7, 8, 10), the second drug application was performed 3 min after the first one. The doses of plant extracts were chosen on the basis of our pilot studies (unpublished), and the infusion time was 5 s.

Surgical preparation

Animals were urethane-anaesthetized (1.5 g kg^{-1} intraperitoneally), and by performing a surgical incision on the perineum, the bulbospongiosus genital muscles were identified. Two platinum wires (Grass, Grass Medical Instruments, Quincy, MA, USA) were inserted into the muscles to record electromyographic activity, which was registered on a polygraph (Grass M6). For a better visualization of the motor genital activity associated with ejaculation, an additional surgery was performed to expose the bulbar portion of the penis and its anatomical connections with the striated bulbospongiosus muscles. At the end of the surgical approach, the spinal cord was blunt transected at T6 level and prepared for recording.[13] Treatments were administered by infusing the selected extracts and compounds into the jugular vein.

Table 1. Qualitative phytochemical analysis on aqueous and ethanolic extracts of *Dracaena arborea* dried roots

Components	Saponins	Phenols	Flavonoids	Sterols	Alkaloids
Observation	Present	Present	Present	Present	Absent

Drugs

Urethane (Sigma Chemicals, St Louis, MO, USA), oxytocin (Sigma Chemicals) and dopamine (Tecnofarma, México D.F., México) were used in the study.

Activation and recording of the rhythmic genital motor pattern of ejaculation

This was done following the technique previously described.[8] In brief, in all animals immediately after spinal cord transection, ejaculatory motor patterns

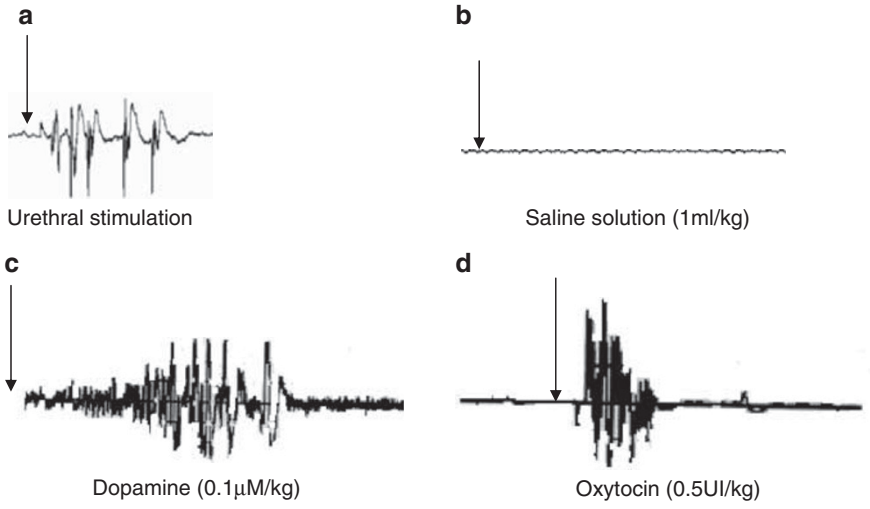

Figure 1. Original electromyographic tracings showing the effects of urethral stimulation (**a**), saline solution (**b**), dopamine (**c**) and oxytocin (**d**) on the rhythmic contractions of the bulbospongiosus muscles in sexually experienced male spinal rats. Arrows indicate the time of injection. Calibration bar 100 mv, 60 s.

Table 2. Effect of urethral stimulation, drugs and *Dracaena arborea* extracts administration on the number and frequency of discharges of the bulbospongiosus muscles in spinal rats

Treatment	*Number of discharges* (n)	*Frequency of discharges* (n s^{-1})
Urethral stimulation ($n=42$)[a]	06.22 ± 0.50***	0.46 ± 0.03***
Saline solution (0.9%) (0.1 ml kg^{-1})	0	0
Dopamine (0.1 μM kg^{-1}) ($n=5$)	09.00 ± 0.58***	0.44 ± 0.02***
Aqueous extract of DA (5, 20, 60, 100 mg kg^{-1}) ($n=5$)	0	0
Aqueous extract (5 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	04.00 ± 0.00$^{\alpha}$	0.55 ± 0.08
Aqueous extract (20 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	03.33 ± 0.88$^{\alpha}$	0.29 ± 0.07$^{\alpha}$
Aqueous extract (60 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	01.33 ± 0.67$^{\beta}$	0.11 ± 0.06$^{\beta}$
Aqueous extract (100 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	0	0
Ethanolic extract of DA (5, 20, 60, 100 mg kg^{-1}) ($n=5$)	0	0
Ethanolic extract (5 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	02.67 ± 0.33$^{\beta}$	0.44 ± 0.03
Ethanolic extract (20 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	03.33 ± 0.88$^{\beta}$	0.42 ± 0.01
Ethanolic extract (60 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	02.67 ± 1.33$^{\beta}$	0.29 ± 0.15$^{\alpha}$
Ethanolic extract (100 mg kg^{-1}) plus dopamine (0.1 μM kg^{-1}) ($n=5$)	0	0
Oxytocin (0.5 UI kg^{-1}) ($n=5$)	07.33 ± 1.45***	0.51 ± 0.09***
Aqueous extract (100 mg kg^{-1}) plus oxytocin (0.5 UI kg^{-1}) ($n=5$)	0	0
Ethanolic extract (100 mg kg^{-1}) plus oxytocin (0.5 UI kg^{-1}) ($n=5$)	0	0

Abbreviation: DA, *Dracaena arborea*. All values are expressed as mean ± s.e.m. n = number of rat per group. $^{***}P<0.001$ significantly different compared with saline solution. $^{\alpha}P<0.01$, $^{\beta}P<0.001$ significantly different compared with dopamine. For each rat, the frequency of contractions was calculated by dividing the number of contractions (n) by its duration (s). [a]Represents the mean value of all urethral stimulations carried out in this study (three stimulations per rat).

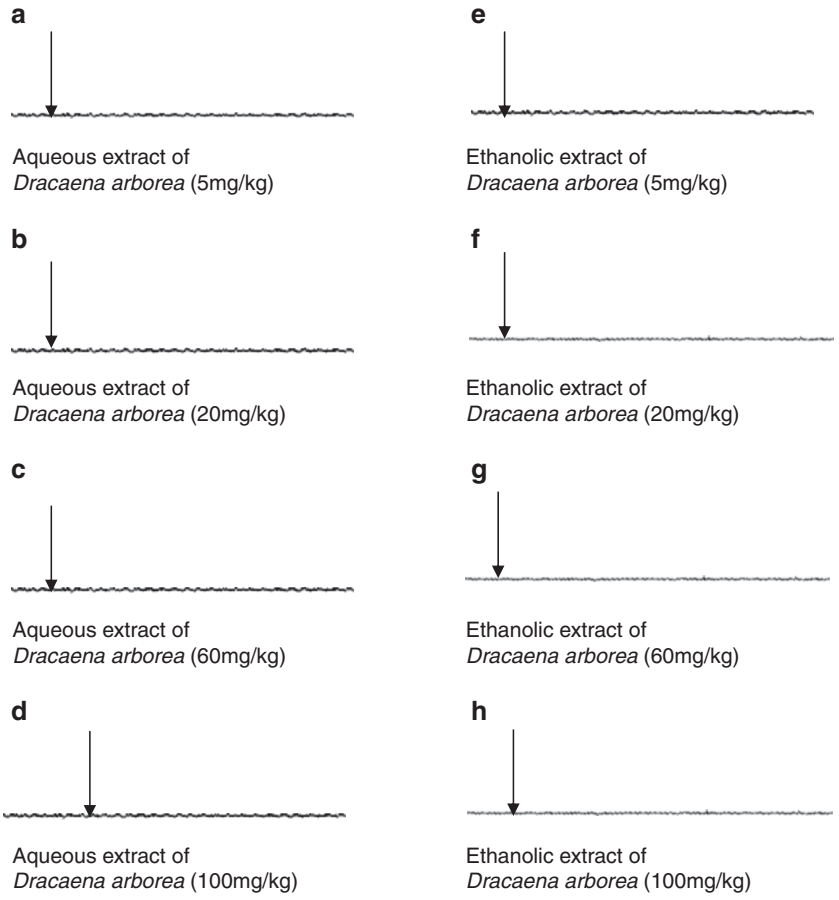

Figure 2. Original electromyographic tracings showing the effects of intravenous injection of aqueous (**a**–**d**) and ethanolic (**e**–**h**) extracts from the roots of *Dracaena arborea* on the rhythmic contractions of the bulbospongiosus muscles in sexually experienced male spinal rats. Arrows indicate the time of injection. Calibration bar 100 mv, 60 s.

(EMP) could be reflexively expressed and recorded in the genital muscles. To establish the capacity of the spinal apparatus to produce the genital rhythmic pattern after signalization, two consecutive EMP were recorded at 3-min intervals. Reflexively induced EMP were activated by mechanical stimulation of the urethra.[8] In brief, after sipinalization, EMP were repeatedly evoked at 3-min intervals by the injection of saline solution (200 μl min^{-1}) through a PE-50 catheter (Harvard Instruments, Holliston, MA, USA) (0.965 mm optical density) inserted into the pelvic urethra via a bladder incision. Thereafter, one of the selected treatments was applied, and the response obtained under their influence was recorded. The parameters recorded for each motor train were the number and frequency of electromyographic bursts. The number of motor contractions included all motor contractions expressed in the motor ejaculatory train evoked by the sensorial or pharmacological stimuli. The frequency of contractions of the bulbospongiosus muscles was calculated by dividing the number of contractions by its duration.

Statistical analysis

Values were expressed as mean ± s.e.m. Mean values were calculated for each animal, and quantitative comparisons between groups were established from those means. One-way analysis of variance followed by Tukey's Honestly Significant Difference test was performed using the SPSS for Windows version 10.0 (New York, NY, USA). Comparisons with *P*-values < 0.05 were considered to be statistically significant.

RESULTS

Phytochemical screening

Qualitative phytochemical screening of the aqueous and ethanolic extracts of *D. arborea* showed the presence of saponins, phenols, flavonoids and sterols (Table 1).

Effects of urethral stimulation on fictive ejaculation

In all the spinal cord-transected and urethane-anesthetized rats, injection of saline solution (200μl min^{-1}) into the pelvic urethra (urethral stimulation) before each drug administration provoked rapid rhythmic contractions of the striated bulbospongiosus muscles (Figure 1). The number and the frequency of discharges of all sensory-induced motor patterns averaged 5.75 ± 0.87 ($P < 0.001$) and 0.86 ± 0.16 Hz ($P < 0.001$), respectively.

Effects of dopamine and oxytocin on fictive ejaculation

The pro-ejaculatory effects of dopamine and oxytocin in spinal rats are outlined in Figure 1 and Table 2. At the doses used, dopamine (0.1 μM kg^{-1}) and oxytocin (0.5 UI kg^{-1}) provoked rhythmic contractions of the bulbospongiosus muscles. Dopamine was the most efficient with a frequency of discharges of 0.82 ± 0.19 ($P < 0.001$) against 0.51 ± 0.09 ($P < 0.001$) for oxytocin. These rapid contractions were accompanied by sustained erection of the penis and sometimes with expulsion of the urethral contents.

Effects of aqueous and ethanolic extracts of *D. arborea* on fictive ejaculation

Intravenous administration of either the aqueous or ethanolic extract (5, 20, 60, 100 mg kg^{-1}) of *D. arborea* in spinal rats did not produce any contraction of the bulbospongiosus muscle. Injection of saline solution (1 ml kg^{-1}) produced similar results (Figure 2, Table 2).

Effects of *D. arborea* on the expression of dopamine and oxytocin-induced fictive ejaculation

Pretreatment of spinal rats with *D. arborea* extracts dose-dependently prevented the pro-ejaculatory effects of dopamine (0.1 $\mu M\,kg^{-1}$; Figure 3 and Table 2). At the dose of 100 mg kg^{-1}, *D. arborea* completely blocked the effects of oxytocin (0.5 UI kg^{-1}; Figure 4 and Table 2).

DISCUSSION AND CONCLUSION

In behaving male rats, ejaculation is accompanied by the rhythmic contractions of the bulbospongiosus and ischiocavernosus muscles at the base of the penis and of anal sphincter and skeletal muscles.[14] These contractions are mediated by a spinal control centre referred to as spinal pacemaker,[15] spinal pattern generator,[16] spinal ejaculation generator[17] or central pattern

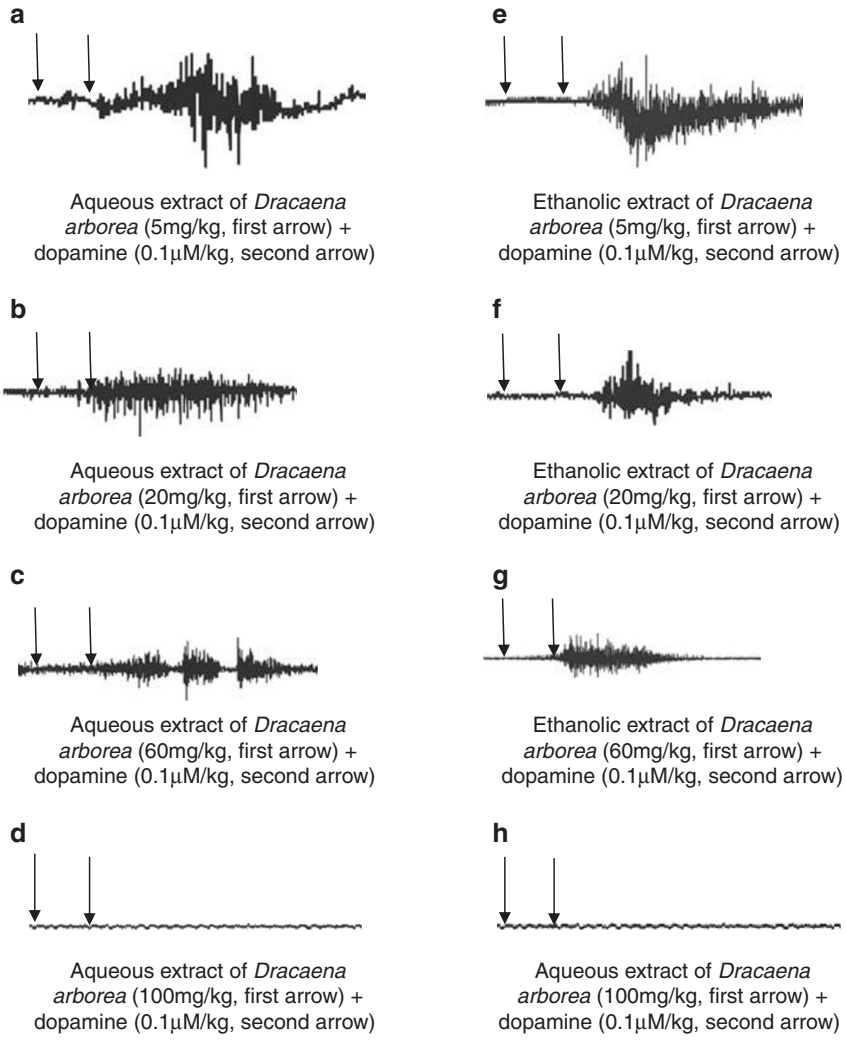

Figure 3. Original electromyographic tracings showing the dose-dependent preventive effects of *Dracaena arborea* extracts on the pro-ejaculatory activities of dopamine in sexually experienced spinal male rats. Notice that both the aqueous as well as the ethanolic *Dracaena arborea* extracts prevented the pro-ejaculatory potential of dopamine in a similar dose-dependent manner (**a**–**h**). Arrows indicate the time of injection of drugs. Calibration bar 100 mv, 60 s.

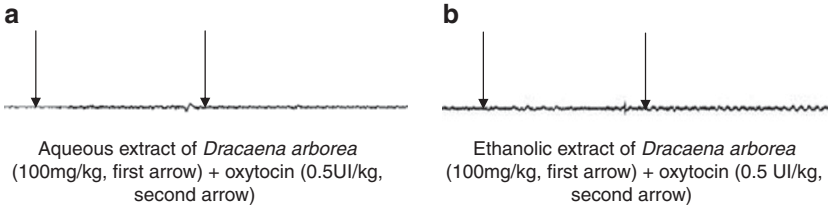

Figure 4. Original electromyographic tracings showing the preventive effects of *Dracaena arborea* extracts on the pro-ejaculatory activities of oxytocin in sexually experienced spinal male rats. Traces in panels **a** and **b** show that effective doses of both *Dracaena arborea* extracts completely blocked the expression of ejaculatory motor pattern induced by oxytocin. Arrows indicate the time of injection of drugs. Calibration bar 100 mv, 60 s.

generator.[8] In the present study, the effects of *D. arborea* extracts on the rhythmic contractions of the bulbospongiosus muscles were evaluated. In order to establish the capacity of the spinal cord to produce the genital muscular rhythmic contractions and to eliminate the reciprocal connections with supraspinal structures in the brain, rats used in this work were urethane-anaesthetized and spinal cord-transected at T6 level.[8,18] Under this condition, the ability of a urethral stimulation (peripheral stimulation) to induce rhythmic discharges of the striated bulbospongiosus muscles implies that the spinal cord, specifically the lombosacral spinal cord, contains a control centre for ejaculation that receives somatosensory inputs deriving only from external stimulation. Thus, under urethral stimulation, these sensory nerves are stimulated and inputs are generated and sent to the lumbosacral spinal cord, which interprets the message and induces the rhythmic discharges of the striated bulbospongiosus muscles (ejaculation). Intravenous administration of the aqueous and ethanolic extracts of *D. arborea* did not produce any discharge of the bulbospongiosus muscle during the period of observation (5 min). This result suggests a non-activation effect of these extracts on the spinal circuit controlling ejaculation. Basically, ejaculation is divided in two successive phases: emission corresponding to the secretion of seminal fluid by sexual accessory glands and the transport of spermatozoa into the prostatic urethra, and expulsion, corresponding to the forceful expulsion of semen via the urethral meatus.[19] During the expulsion phase, the bulbospongiosus muscles have a primary role by contracting in a rhythmic manner.[17,20] It could then be thought that, following intravenous administration, the bioactive constituents found in the extracts of *D. arborea* may interact directly with the outputs of the spinal ejaculation generator leading to a preventive effect upon the activity of the bulbospongiosus muscles, the main muscles involved in the expulsion phase.

In order to determine the possible mechanism(s) of action of *D. arborea* in the preventive effect of the ejaculation expression, the pretreatment with the extracts of this plant followed by systemic dopamine and oxytocin were evaluated, and the ejaculatory response, if present, was described. Dopamine is one of the most studied neurotransmitters that activates oxytocinergic neurons and facilitates penile erection and sexual activity.[7] Dopamine originates from the paraventricular nucleus of the hypothalamus (as well as oxytocin) and acts mainly on the dopamine receptors of D_2 type to increase Ca^{2+} influx in the cell bodies of oxytocinergic neurons; this causes the release of nitric oxide, which in turn activates oxytocinergic neurons to release oxytocin in higher brain areas and also in the spinal cord to induce penile erection and sexual activity.[7] Systemic administration of dopamine and oxytocin to spinal rats induced the activation of the ejaculatory motor pattern, confirming previous observations in animals with the spinal cord transected in which ejaculatory reflexes remain intact and can be activated pharmacologically or electrically.[8,17,21] Pretreatment with either the aqueous or ethanolic extract of *D. arborea* before the intravenous injection of dopamine or oxytocin of spinal rats resulted in the blockade of the EMP induced by dopamine and oxytocin. Thus, the ability of *D. arborea* to completely prevent the pro-ejaculatory properties of dopamine and oxytocin indicates the potential involvement of both dopaminergic and oxytocinergic receptors in the ejaculation-delaying effect of this plant extract.

In conclusion, the systemic administration of *D. arborea* extracts prevents the contractions of the bulbospongiosus muscles through the blockade of dopaminergic and oxytocinergic receptors. Present data support traditional use of this plant as an aphrodisiac agent that could be used to prevent rapid ejaculation in men.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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