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Original article

Aphrodisiac activity of *Phlegmariurus saururus* in copulating and noncopulating male rats

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ABSTRACT

Background: *Phlegmariurus saururus* is popularly known in Argentina as aphrodisiac. For this reason, it was previously investigated and determined that the decoction of this plant elicits pro-ejaculatory activity and increases the ejaculatory potency in the Fictive Ejaculation Model.
Hypothesis/Purpose: the decoction of *P. saururus* facilitates sexual behavior in sexually experienced male rat and induces copulatory behavior in non-copulating male rats.
Methods: The extraction method (decoction) was validated through Selectivity, Accuracy and Precision, by identification of the majority alkaloids, expressed as sauroxine. Male (sexually experienced and non-copulating) and female (receptive) Wistar rats were used to determine sexual behavior. Sildenafil was used as positive control. The following variables were evaluated: Mount Latency, Intromission Latency, Ejaculation Latency, Post Ejaculatory Interval, as well as the Mounts and Intromissions Number.
Results: In sexually experienced male rats, *P. saururus* decoction stimulates sexual arousal and facilitates sexual execution. In noncopulating male rats, this decoction induces copulation with behavioral characteristics similar to sexually experienced animals.
Conclusion: *P. saururus* possesses aphrodisiac activity in copulating and noncopulating male rats.

Introduction

Despite the increasing availability of effective conventional medical treatments for male sexual complaints, to stimulate sexual desire and to enhance performance and enjoyment, plant-derived and herbal remedies continue as a widespread alternative of the population. Medicinal plants with pro-sexual activity are considered as aphrodisiac plants (Birri et al., 2014; Carro-Juárez et al., 2004). Aphrodisiacs can be classified according to their effects when consumed or administered. They can stimulate psychological effects, thereby increasing sexual desire and pleasure aside other hallucinogenic or mood stimulating properties (Sandroni, 2001). Aphrodisiacs can also impact physiologically, for instance by increasing blood flow and by inducing enhancing erection after hormonal changes (Sandroni, 2001).

A variety of medicinal plants with aphrodisiac potential have been used in traditional medicine of different countries to promote, vitalize and improve sexual function (Carro-Juárez et al., 2004; Watcho et al., 2009, 2014; Sharma et al., 2012, 2014; Estrada-Reyes et al., 2013; Munglue et al., 2014; Chauhan et al., 2014) but out of these, very few others have been scientifically analyzed.

One of the traditionally used plant species in Argentina reputed as an aphrodisiac (Hieronymus, 1882; Toursarkissian, 1980) and also as a memory improver agent (Martínez Crovetto, 1981) is *Phlegmarirurus saururus* (Lam.) B. Øllg. (ex *Huperzia saururus* (Lam.) Trevis.; *Urostachys saururus* (Lam.) Herter; *Lycopodium saururus* Lam.) (Lycopodiaceae). Component of Andean-Pampas vegetation (Ponce, 1996), *P. saururus* is known as “cola de quirquincho” (Amorín, 1974; de la Sota, 1977; Ratera and Ratera, 1980), or “armadillo tail” because aerial parts of the species resemble to the

Abbreviations: CF, Chloroform fraction; GLC-MS, Gas liquid chromatography-mass spectrometry; NC, noncopulating animals; DER, drug extract ratio means the ratio between the quantity of herbal substance used in the manufacture of a herbal preparation and the quantity of herbal preparation obtained; SE, sexually experienced male rats; C_{SE}, sexually experienced male rats control; C_{NC}, noncopulating male rats control; S_{SE}, sexually experienced sildenafil; ML, time from the introduction of the female in the arena until the first mount with pelvic thrusting; IL, Intromission Latency time from introduction of the female until the first mount with pelvic thrusting and vaginal penetration; EL, Ejaculation Latency time from the first intromission until ejaculation: PEI, Post Ejaculatory Interval time from ejaculation until the next intromission in the following copulatory series.

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animal tail. The presence of alkaloids characterizes the species. These are Lycopodium alkaloids and hitherto, ten of them have been isolated and identified (Ortega et al., 2004ab; Vallejo et al., 2013). From them, three are the major constituents, sauroine, sauroxine, and 6-hydroxylycopodine. All the remaining alkaloids occur in a very small concentration, including several of them at trace level (Ortega et al., 2007; Vallejo et al., 2013).

Previous studies in our labs in anaesthetized and spinalized male rats revealed that the administration of a decoction of *P. saururus* elicits pro-ejaculatory activity and showed aphrodisiac activity at the level of the sexual potency (Birri et al., 2014). The decoction of *P. saururus* provokes these pro-ejaculatory effects by targeting at least partially the nitrergic, cholinergic, adrenergic and oxitocinergic spinal systems (Birri et al., 2014).

Since the aphrodisiacs can be categorized according to their mode of action into 3 groups; those that increase libido (i.e. sexual desire, arousal); those that increase sexual potency (i.e. effectiveness of erection) and those that increase sexual pleasure (Sandroni, 2001), the aim of the present research was to investigate if *P. saururus* possesses aphrodisiac activity in copulating and noncopulating male rats. We hypothesized that the decoction of *P. saururus* facilitates sexual behavior in sexually experienced male rat and induces copulatory behavior in noncopulating male rats. The evaluation of the sexual performance of sexually active males allows the assessment of the sexual behavior components, i.e., motivation and performance, which are modified – facilitated or blocked – by a given treatment (Beach, 1956; Hull and Rodríguez-Manzo, 2009) and the use of noncopulating (sexually inactive) male rats is a model commonly used to specifically evaluate sexual arousal (Canseco-Alba and Rodríguez-Manzo, 2013).

As the aphrodisiac activity of this plant was probed by using a decoction and facing that it could potentially be used with medicinal purposes, the validation of analytical method was also an objective in this investigation.

Materials and methods

Plant material

Plant material (consistent in aerial parts of the Lycophyta *P. saururus*) was collected in the Pampa de Achala, San Alberto Department, Province of Córdoba, Argentina, in October 2012 (Spring, in Argentina). The plant was identified by Dr. Gloria Barboza (Instituto Multidisciplinario de Biología Vegetal (IMBIV), Universidad Nacional de Córdoba). A voucher specimen is deposited at the herbarium of the Museo Botánico de Córdoba (CORD) as CORD 684.

Chemicals

Sodium hydroxide and Chloroform, both purchased from Biopack (Buenos Aires, Argentina); ethanol Porta (Córdoba, Argentina). Chloroform and ethanol were additionally distillated; cyclohexanone BDH (Poole, England); All chemicals were of analytical grade. Estradiol benzoate and progesterone purchased from Sigma Chemical Co. (St. Lois, USA); sildenafil citrate (Pfizer Inc., Mission – KS, USA)

Preparation of P. saururus extract

Aerial parts of *P. saururus* were dried in the shade and ground before use. In agreement to the manner that this species is consumed in popular medicine, the method selected for extract preparation was a decoction. This was prepared according to Farmacopea Argentina (Farmacopea Argentina VII, 2014). Briefly, to 5 g of the dried and ground sample 100 mL of water were added allowing the system boils for 20 min. Decoction was filtered, cooled, and posteriorly, lyophilized. The lyophilized decoction was prepared as a solution by using saline at the time of use, for animal experimentation.

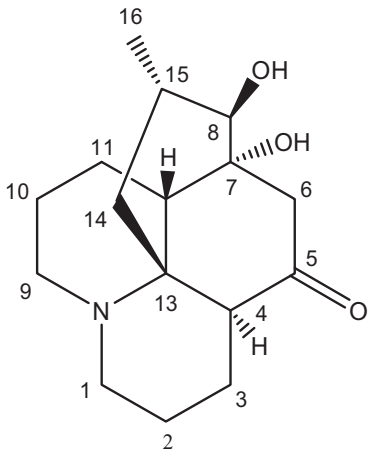

Fig. 1. 7α,8-endo-dihydroxylycopodine, sauroine.

Another decoction was prepared for validation purpose. DER was calculated as indicated by European Medicines Agency (EMA, 2010). After preparation, this decoction was alkalinized by using NaOH 0.1 N to pH 12, then, partitioned with Cl_3CH three times (100 mL each) in a decantation funnel. The chloroform fractions (CF) obtained were jointed, concentrated to dryness and retaken with ethanol for Gas–Liquid Chromatography (GLC) analysis at a known concentration. As for decoction, DER for CF was obtained.

Analytical method validation

Validation was developed through different parameters, following the European Medicines Agency (EMEA, 1995; EMA, 2010).

A chromatographic methodology was selected for identification and quantitation of the CF alkaloid constituents. The GLC–MS apparatus was a Clarus 600 from Perkin Elmer (Shelton, Connecticut, USA). Turbo Mass software was used to control and acquire data from the GLC–MS. All the separations were conducted through a Perkin Elmer fused silica DB 5 MS capillary column (60 m, 0.25 mm ID, 0.25 mm film thickness) using helium as a carrier gas (49.6 psi). The split injection mode was selected. Ionization was carried out in the mass spectrometer under vacuum by electron impact with a −70 eV ionization energy.

For the most abundant (majority) alkaloids separation the following program was used: 60 °C (1 min), 60–230 °C at a 40 °C/min, 230 °C (1 min), 230–250 °C at 20 °C/min, 250 °C (2 min), 250–275 °C at 2 °C/min, 275 °C (1 min). Injector and detector temperature were 280 °C, injection volume 0.1 µL, using EtOH as a solvent.

To carry out a quantitation with the minor possible error, a Calibration Curve was created with five stock solutions of sauroine, each one in triplicate and in a concentration range of 1.5–10.00 mg/mL. Internal standard method was employed by using cyclohexanone. Sauroine (7α, 8-*endo*-dihydroxylycopodine, Fig. 1) (Ortega et al., 2004b) was used as analyte in view that it is one of the major alkaloids in *P. saururus* (Ortega et al., 2007). Linearity was determined by calculating the regression plots by the linear regression method and expressed as determination coefficient (r^2).

Three CF of the decoction were analyzed (each in triplicate) during the same day to know the major alkaloids content and expressed as sauroine. Media of these samples was considered the 100%. After that, CF at 80% and 120% were analyzed, both in triplicate, to know the alkaloid proportion in each case. This way, the smallest value of precision will be achieved because the results

are obtained by the same operator, with the same equipment and within short intervals of time.

Animals

Sexually vigorous and sexually inactive male Wistar rats (50–75 days and 250–350 g body weight) were used. Female Wistar rats of 50–75 days and 140–170 g body weight were used. The female receptivity was guaranteed at the experimental time as it can be seen in the protocol. Animals were housed in groups (7 rats per cage) under an inverted 12:12 h LD cycle at 22 °C with food and water *ad-libitum*.

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. In turn, procedures were approved by the Ethics Committee of Experimental Protocols on the animals used for scientific projects by Res. Decanal 752, Facultad de Ciencias Químicas – Universidad Nacional de Córdoba, Córdoba, Argentina, both, in accordance with internationally accepted principles for laboratory animal use and care.

Groups

Before experimental testing all animals received at least five sexual behavior tests; sexually active males, those showing ejaculation latency equal or shorter than 20 min in at least the last three consecutive sessions, were selected and considered sexually experienced (SE). Sexually inactive male rats that failed to show sexual activity in the five consecutive experimental tests were classified as noncopulating (NC) male rats.

Sexually experienced rats

SE rats were divided into 6 groups of 6–7 rats each. Group 1 received saline solution (C_{SE}, Control), Group 2 (S_{SE}) was administered with doses of 10 mg/kg of sildenafil and used as a positive control. Groups 3–6 (G3–G6) received *P. saururus* decoction at 1, 3, 10, and 30 mg/kg, respectively. Animals of all the groups were administered orally and the behavioral analysis started 30 min after treatment.

Noncopulating rats

NC rats were divided into 3 groups of 6 animals each. The first group (G7) received saline solution, the second one (S_{NC}) was considered as positive control and treated with sildenafil 10 mg/kg, and the third group (G9) received 3 mg/kg of *P. saururus* decoction. As SE, NC male rats were orally administered and behavioral tests were carried out 30 min after the administration.

Sexual behavior testing protocol

Male rats were introduced into a cylindrical observation cage and a 5 min adaptation period was allowed for each case. Thereafter, a stimulus-receptive female was introduced and sexual behavior was recorded along 20 min. Female receptivity was induced by the subcutaneous injection of estradiol benzoate (4 µg/rat) followed 44 h later by progesterone (2 mg/animal). Behavioral observations were conducted 4 h after progesterone administration. The sexual behavior parameters analyzed were Mount Latency (ML, time from the introduction of the female in the arena until the first mount with pelvic thrusting); Intromission Latency (IL, time from introduction of the female until the first mount with pelvic thrusting and vaginal penetration, this means intromission); Ejaculation Latency (EL, time from the first intromission until ejaculation); and the Post Ejaculatory Interval (PEI, time from ejaculation until the next intromission in the following copulatory series). The number of mounts and intromissions displayed in an ejaculatory series was also recorded.

Male rats belonging to the NC group were assayed 15 days after they received treatments and their sexual behavior was observed. The entire copulatory assay was repeated under the same conditions with the exception that males did not receive any (further) treatment.

Data analysis

For validation purposes, statistical differences of the means were assumed to be significant when $p < 0.05$ by one way analysis of variance (ANOVA), followed by the Bonferroni Multiple Comparisons Test or *t*-test, as appropriate. Data were expressed as the media ± SD. To determine statistical significant differences among treatments, the sexual behavior data were analyzed by Kruskal–Wallis One Way Analysis of Variance on Rank and Mann-Whitney Test, in view that the variables being analyzed do not follow a normal distribution.

Data was expressed as median numbers (95% confidence interval). Mount and Intromission Latencies were expressed in seconds while EL and PEI were expressed in minutes.

The proportion of copulating male rats was analyzed using the Fisher *F*-test and expressed in percentages.

The Sigma Stat program, version 2.03 (Systat Sofware Inc., San José, California, USA) and GraphPad InStat (GraphPad Software Inc., San Diego, California, USA) were employed for all statistical analyses.

Results and discussion

Extracts of P. saururus

As a result of the decoction preparation a yield of 12.00–13.14% was obtained. After an alkaline extraction of the decoction by using CH_3Cl, a range of 0.63–0.88% of this chloroformic fraction (CF) was obtained. According to the European Medicines Agency (2010), DER is defined as the ratio between the quantity of herbal substance used in the manufacture of an herbal preparation and the quantity of herbal preparation obtained. The number (given as the actual range) written before the colon is the relative quantity of the herbal substance; the number written after the colon is the relative quantity of the herbal preparation obtained. As a consequence, Decoction DER was 7.6–8.3:1 and for CF DER was 111.6–158.7:1.

Validation

By using the above explained methodology, three major alkaloids were identified. Sauroine, 6-hydroxylycopodine and sauroxine identification was achieved by means of the mass spectra provided by the GLC-MS equipment obtained at the run time in base on their characteristic breakdown patterns in comparison to our previous studies (Ortega et al., 2004a, b, 2007; Vallejo et al., 2013).

A comprehensive validation of the present method was conducted. The results indicated that a good linearity with a correlation coefficient of $r^2 = 0.998$ ($n = 5$) was achieved for sauroine, with linearity range = 1.5–10 mg/mL (Fig. 2).

The concentration of the majority constituents of the quantitation of CF, expressed as sauroine, did not show significant differences among the three CF studied ($p = 0.321$). As a result, a concentration of 32.76 ± 7.09 mg/mL was obtained. This means that CF is constituted by a percentage that ranks between 44.97% and 59.82% of alkaloids, expressed as sauroine.

Results obtained comparing the media concentration of the major alkaloids between the expected theoretical values and the real values obtained for the 80% and 120% CF, allow affirm that there is

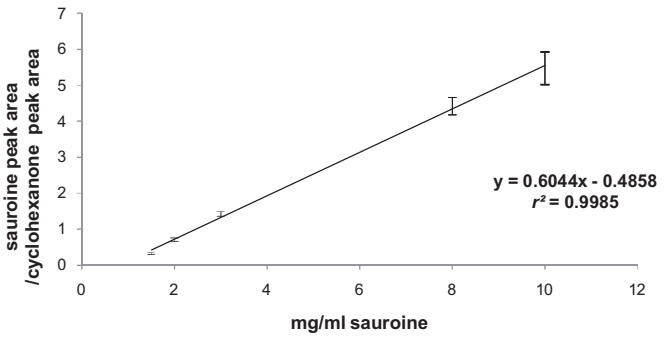

Fig. 2. Calibration curve of sauroine. Five concentrations in a range between 1.5 and 10.00 mg/ml were prepared. Cyclohexanone was used as internal standard. Data were expressed as the media ± SD.

not significant differences between both ($p = 0.33$ and $p = 0.80$, respectively). Selectivity, accuracy and precision conditions are guaranteed according to all the above exposed.

Groups

Sexually experienced rats

Analysis of the different parameters of male rat sexual behavior revealed that for NM (Fig. 3B), IL (Fig. 3C) and PEI (Fig. 3F) no significant differences among treatment groups (G3–G6) and C_{SE} were promoted by the decoction ($p > 0.05$). In relation to NI and EL, the decoction promoted a statistically significant diminution in these parameters in comparison to C_{SE} ($p < 0.05$). Decoction also importantly influenced the NI. Thus, compared to control animals, intromissions were statistically significantly decreased after the administration of all decoction concentrations, as it is shown in Fig. 3D. This suggests that the decoction of *P. saururus* promotes

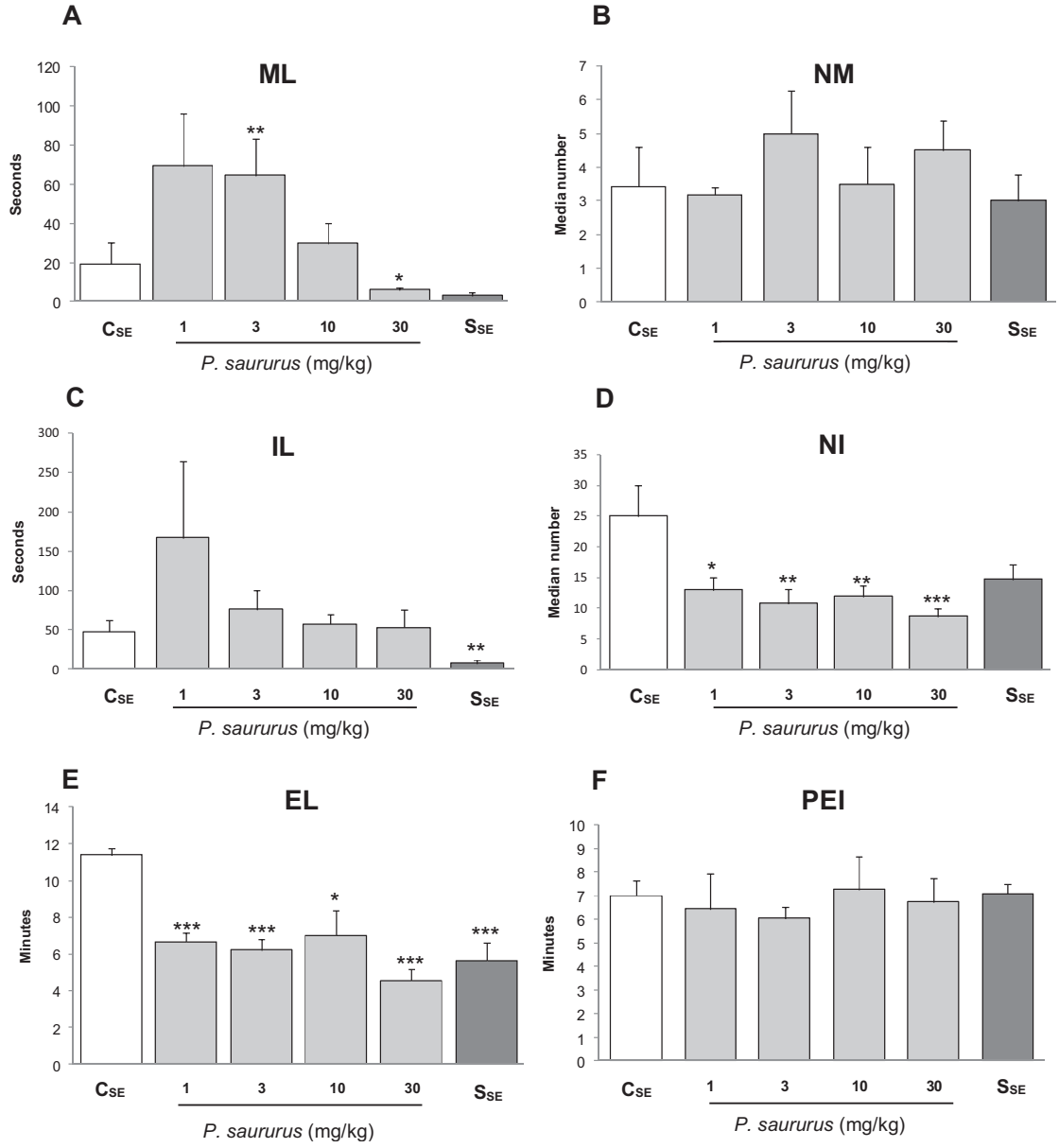

Fig. 3. Specific sexual behavior parameters of sexually active male rats treated with *P. saururus* decoction, sildenafil citrate and vehicle (control). (A) ML = mount latency, B) NM = number of mounts, C) IL = intromission latency, D) NI = number of intromissions, E) EL = ejaculation latency, F) PEI = post ejaculatory interval. The results were analyzed by Mann–Whitney U Test and were expressed as mean ± standard error of mean (SEM) and significantly different when compared treatments to controls, (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

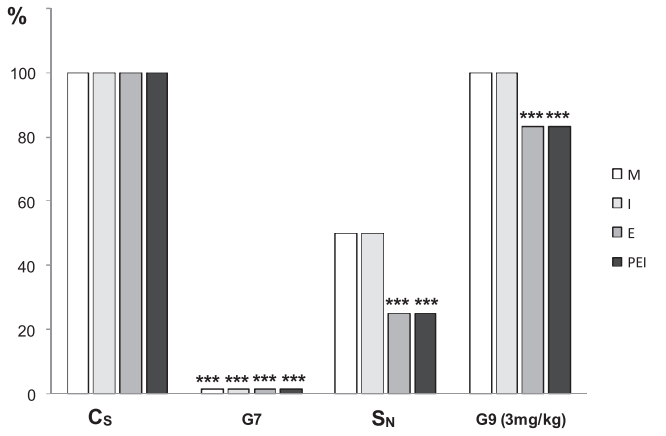

Fig. 4. Percentage of noncopulating male rats treated with 3 mg/kg of *P. saururus* decoction (G9) that completed the copulatory series in comparison to Sexually Experienced Control (C_{SE}), Noncopulating Control (G7) and the Positive Control (S_{NC}). The proportion of copulating male rats was analyzed using the Fisher F-test, *** $p < 0.001$. M = mount; I = intromission; E = ejaculation; PEI = post ejaculatory period.

better sexual performance since even with a number of significantly less intromissions than controls, the treated animals could ejaculate faster (facilitator effect). Besides, in Fig. 3D it can be seen that sildenafil does not promoted similar facilitatory results since similar results between S_{SE} and C_{SE} were obtained. Thus, the decoction of *P. saururus* seems to produce a better performance than sildenafil in copulating rats.

Fig. 3E shows the dose response curve for EL parameter. It is important to take into account that this parameter is related to the male rat sexual performance. Particularly, the statistical analysis revealed that *P. saururus* decoction produces a statistically significant diminution in the ejaculation latency without concentration dependency. Besides, *P. saururus* decoction exhibited a comparable effect to sildenafil (S_{SE}). However, it is important to stress this result knowing that *P. saururus* is a mixture of several compounds administered in a similar concentration to that of sildenafil, which is a sole pure compound.

Noncopulating animals

In view that in sexually experienced male rats doses of 1, 3, 10 and 30 mg/kg were effective to reduce the EL -the most important parameter of male rat sexual behavior- the concentration of 3 mg/kg was selected to assay NC animals. At this concentration, all NC rats exhibited mounts and intromissions, and 83.3% displayed the complete copulatory series in the treatment group (G9), as it can be seen in Fig. 4. In relation to the positive control, we observed that 50% of the sildenafil treated male rats executed mounts and intromissions and only 25% completed ejaculatory behavior and reassumed copulation. In both cases G9 showed better results (Fig. 4).

When the analysis of the parameters related to the copulatory series (Mount, Latency and Number; Intromission, Latency and Number; Ejaculation latency, and Post Ejaculatory Interval) was proceeded, it was seen that the NC males treated with 3 mg/kg *P. saururus* decoction (G9) presented a statistically similar execution to that observed for the Control group among the SE male rats ($p > 0.05$) with the exception of IN, which showed a statistically significant diminution in comparison to C_{SE} ($p < 0.05$) (Table 1). On the contrary, when comparing G9 to the Control of NC male rats (G7), statistically significant differences for the parameters analyzed were found ($p < 0.001$) (Table 1). These observations show that NC males under the influence of *P. saururus* decoction, display a sexual behavior similar to copulating animals.

Table 1.
Sexual behavior parameters for decoction treated noncopulating males (G9) in comparison to sexually experienced control (C_{SE}), noncopulating control (C_{NC}), and sildenafil (S_{NC}).

	C_{SE}	C_{NC} (G7)	S_{NC}	Decoction (G9)
ML (sec)	5.25–17.25	–	8–97.5	9–90
MN	1–6.5	$0 \pm 0^{***}$	10–24	$2–5^{\#\#}$
IL (sec)	20.5–74	–	24–572	$8–29^{\#}$
IN	15–36.5	$0 \pm 0^{***}$	$1.5–10.5^{**}$	$10–16^{*}$
EL (min)	11.13–12.19	–	$18.37–23.28^{*}$	7.76–17.14
PEI (min)	5.39–7.80	–	$13.78–28.74^{*}$	$5.92–9.97^{\#}$

The parameters are expressed as the 95% confidence interval. Mount (ML) and intromission (IL) Latency are expressed in seconds; Ejaculation Latency (EL), and Post Ejaculatory Interval (PEI) are expressed in minutes. Number of mounts (NM), and number of intromissions (NI) are expressed in frequency.
The results were analyzed by Mann-Whitney U Test being significantly different when compared to C_{SE} (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and G9 in comparison to S_{NC} ($^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$).

In relation to the positive control, we observed that these animals displayed less intromissions that C_{SE}, and at the same time their EL and PEI were longer to that exhibited by C_{SE}.

When comparing G9 and $S_{NC,}$ we observed that G9 showed a decrease in the MN ($p < 0.01$) and IL ($p < 0.05$). Thus, G9 displayed better performance and motivation since males of this group could ejaculate at the same time than those for C_{SE} group. Therefore, it is very important to emphasize that 3 mg/kg of *P. saururus* decoction made that noncopulating rats behave as copulating ones. Additionally their performance was better in the presence of the decoction. The PEI which was shorter for G9 than that for S_{NC} ($p < 0.05$), again showed the G9 animals motivation. Thus, the decoction improves sexual performance in view that the parameters related to motivation (PEI) and performance (LE) respectively, were better for G9 than those for sildenafil, and equal than those for C_{SE} doing that G9 males were more sensitive to the decoction than those of S_{NC}. Thus, all these findings support the notion that the decoction of *P. saururus* not only induces the expression of male sexual behavior in non-copulating rats, but also that the decoction promotes a better execution.

After the outstanding results obtained for NC males under the influence of the *P. saururus* decoction, a question raised, were the NC male rats converted into copulating ones when received the decoction? To answer this question, NC rats were subjected to a resting period along 15 days with water and food *ad-libitum* and without additional copulatory tests provided. On the 16th day, G9 was again assayed without any treatment to induce sexual behavior. As a result, 100% of male rats displayed mounts and intromissions. Even more, 50% of NC rats studied completed the ejaculatory series confirming that *P. saururus* is capable to transform NC rats into copulating ones. This datum is indicative that even when 50% of NC rats did not ejaculate in the 20 min of the copulatory test, all of them were motivated to copulate (Fig. 5).

Finally, analysis of data shows that there are no statistically significant differences when the diverse parameters of the copulatory behavior for the decoction treated NC male rats are evaluated on day 1 and 15 days after (Table 2, LM, $p = 0.1486$; NM, $p = 0.4362$; LI, $p = 0.5000$; NI, $p = 0.3153$; LE, $p = 0.0714$ and PEI, $p = 0.1964$). These remarkable results show the effectiveness of the treatment as a copulating promoter. As it was said, decoction of *P. saururus* induces noncopulating animals behave as copulating ones. A final interesting datum is that when comparing the EL exhibited by rats between day 1 and day 16, we observed ELs statistically significant decreases on the 16th day in relation to the C_{SE}, suggesting that this decoction permanently converted NC rats into copulating ones. Noncopulating male rats can be transformed into copulating ones after receiving naloxone or anandamide

Table 2
Sexual behavior parameters of male rats treated with *P. saururus* decoction (G9) at days 1st and 16th without treatment and in comparison to sexually experienced control (C_{SC}) and sildenafil (S_{NC}).

	C_{SE}	S_{NC}		G9 (3 mg/kg)	
		Day 1	Day 16	Day 1	Day 16
ML (sec)	5.25–17.25	8–97.5	17.75–255.5	9–90	7–51
MN	1–6.5	10–24**	2.5–11.5	2 - 5	1 - 8
IL (sec)	20.5–74	24–572	67.75–290.5	8 - 29	9 - 16
IN	15–36.5	1.5–10.5**	6–10.5**	10–16*	7–20
EL (min)	11.13–12.19	18.37–23.28*	7.89–11.45	7.76–17.14	3.30–8.29**
PEI (min)	5.39–7.80	13.78–28.74	4.74–7.58	5.92–9.97	3.78–6.55

The parameters are expressed as the 95% confidence interval. The mounts (ML) and intromissions (IL) Latency are expressed in seconds; Ejaculation Latency (EL), and Post Ejaculatory Interval (PEI) are expressed in minutes. Number of mounts (NM), and number of intromissions (NI) are expressed in frequency.
The results were analyzed by Mann-Whitney U Test being significantly different when compared to C_{SE} (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

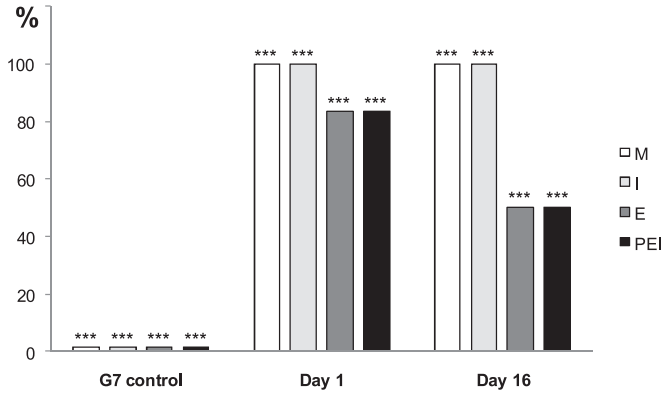

Fig. 5. Percentage of noncopulating male rats treated with 3 mg/kg of *P. saururus* decoction (G9) that completed the copulatory series on days 1 and 16. The proportion of copulating male rats was analyzed using the Fisher F-test, *** $p < 0.001$. M = mount; I = intromission; E = ejaculation; PEI = post ejaculatory interval.

(Gessa et al., 1979; Canseco-Alba and Rodríguez-Manzo, 2013). Several lines of evidence suggest the involvement of endogenous opioids in this sexual inhibitory state. Endogenous opioids and endocannabinoids are neuromodulators of neurotransmitter release, although through different mechanisms (Gessa et al., 1979; Canseco-Alba and Rodríguez-Manzo, 2013). It is probable that the opioidergic and endocannabinoid systems could be at least partially targeted by the compounds contained in the decoction here analyzed. Further experimental approaches to elucidate the central mechanisms targeted by the decoction to permanently convert noncopulating into copulating ones are required. Related to positive control, we observed that for G9 there were no significant changes in the male rat sexual parameters between day 1 and day 16 (Table 2).

Conclusions

All in all, data of the present study show that *P. saururus* decoction possesses aphrodisiac activity. This notion is supported since the decoction of *P. saururus* a) stimulates sexual arousal and facilitates the sexual performance of sexually experienced male rats; and b) since induces copulatory behavior in noncopulating male rats and converts non-copulating male rats into copulating ones. Thus, more research is necessary to complete the pre-clinical studies of this promissory natural drug in the field of sexual function.

Conflict of interest

The authors declare no conflict of interest.

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