Journal of Ethnopharmacology 157 (2014) 38–44

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

# *Huperzia saururus* Lam. Trevis. (Lycopodiaceae) facilitates ejaculation in spinal cord transected male rats

M.A Birri [a], M.A Franco [b], M.G Vallejo [a], M. Carro-Juárez [b], A.M Agnese [a,*]

[a] IMBIV, CONICET y Farmacognosia, Departamento de Farmacia, Fac. de Cs. Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[b] Laboratorio de Comportamiento Reproductivo, Escuela de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tlaxcala, C.P. 90000, Col. Centro, Tlaxcala, Mexico

ARTICLE INFO

*Article history:*
Received 9 May 2014
Received in revised form
30 August 2014
Accepted 10 September 2014
Available online 22 September 2014

*Chemical compounds studied in this article:*
Atosiban (PubChem CID: 5311010)
Hexamethonium (PubChem CID: 5938)
Prazosin (PubChem CID: 4893)
L-NAME (PubChem CID: 39836)

*Keywords:*
*Huperzia saururus*
Aphrodisiac
Spinal rat
Ejaculation potency
Spinal mechanisms

ABSTRACT

*Ethnopharmacological relevance:* *Huperzia saururus* (Lam.) Trevis. has an extensive ethnopharmacological use, mainly because of its aphrodisiac properties. The species is consumed as decoctions or infusions in traditional medicine. The purpose of the present research was to determine if *Huperzia saururus* is able to increase sexual potency by evaluating the ejaculatory response, in the presence of a decoction in spinal cord transected male rats.

*Materials and methods:* The fictive ejaculation model to record the rhythmic contractions of the bulbospongiosus muscles that accompany ejaculation as an indicator of ejaculation occurrence was used. Sexually experienced male Wistar rats were used. The activation of the fictive ejaculation by the i.v. administration of a decoction was tested, as well as the effects of the oxytocinergic, cholinergic, adrenergic and nitrergic antagonism upon the pro-ejaculatory activity of *Huperzia saururus*.

*Results:* Decoction (3 μg/animal) was able to activate the fictive ejaculation in spinal male rats, producing a statistically significant diminution on the latency of discharge parameter and a statistically significant augment for the number of discharges. Moreover, when sequential treatments using antagonists plus decoction were administered, the effects produced showed that prazosin prevent the pro-ejaculatory effect of the decoction and that the four antagonists assayed blocked the facilitatory effect of *Huperzia saururus* since the facilitation in the latency of response was prevented, and the number of discharges was reduced. Together these findings support the notion that the decoction exerts an aphrodisiac effect influencing the ejaculatory potency which is partially mediated by oxytocinergic, cholinergic, adrenergic and nitrergic spinal mechanisms.

*Conclusion:* In agreement to the ethnopharmacological uses, *Huperzia saururus* decoction has aphrodisiac properties by influence on the ejaculatory potency.



## 1. Introduction

The plants are a valuable resource in the Health System of developing countries. Although there are no accurate data to assess the extent of global use of medicinal plants, the World Health Organization (WHO) has estimated that over 80% of the world's population uses traditional medicine routinely to meet their primary health care (Ortega et al., 2006; Vallejo et al., 2007), and that much of the traditional treatments involve the use of plant extracts or their active principles (Akerele, 1985).

One of the traditionally used species in Argentina is *Huperzia saururus* (Lam.) Trevis. (ex *Urostachys saururus* (Lam.) Herter.; *Lycopodium saururus* Lam.) (Lycopodiaceae). Component of Andean-Pampas vegetation, *Huperzia saururus* is known as "cola de quirquincho" and is used in the folk medicine because it is reputed as an aphrodisiac agent and also has been reported as a memory improver agent (Martínez and Crovetto, 1981). The consumption of this plant has been reported early in the XVIII century (Hieronymus, 1882) and posteriorly, by Toursarkissian (1980). In addition, *Huperzia saururus* is used to solve mood and sentimental disorders (Amorín, 1977). Recent studies have corroborated the reputation of *Huperzia saururus* as an aphrodisiac and it has been shown in guinea pigs that dichloromethane and methanolic extracts of this plant exert relaxation of the smooth muscle of the penis, thus promoting penile erection (Hnatyszyn et al., 2003). In agreement to Sandroni (2001) aphrodisiacs can be classified by their mode of action into three types: those that increase libido (i.e. sexual arousal), potency (i.e. sexual reflexes)

* Corresponding author. Tel.: +54 351 5353865.
*E-mail address:* magnese@fcq.unc.edu.ar (A. Agnese).

http://dx.doi.org/10.1016/j.jep.2014.09.016
0378-8741/

or sexual pleasure (a criterion only applied to humans). It is well known that aphrodisiac compounds can act at different levels of the central nervous system to promote changes in neurotransmitters concentrations among other mechanisms (Sandroni, 2001). We wonder if *Huperzia saururus* possess aphrodisiac properties to increase sexual potency largely targeting ejaculation. Thus, the aphrodisiac effects of *Huperzia saururus* would be modulated by neural mechanisms controlling ejaculation. In the present study we carried out an experimental study to provide evidence about the ability of *Huperzia saururus* to increase sexual potency by evaluating the ejaculatory response, in the presence of an aqueous extract (a decoction) of *Huperzia saururus* in spinal cord transected male rats. For this purpose, we used the fictive ejaculation (FE) model to record the rhythmic contractions of the bulbospongiosus muscles that accompany ejaculation as an indicator of ejaculation occurrence. In this model in spinally-transected and anaesthetized male rats, the ejaculatory response can be induced by physiological-like stimulation and by pharmacological means and permits the quantitative evaluation of the genital motor pattern of ejaculation and the visualization of penile events accompanying expulsion of seminal material (Carro-Juárez et al., 2003) as a response of the activation of the spinal generator for ejaculation. Thus, this animal model has been useful for the study of the neural control of the ejaculatory response by the spinal pattern generator of ejaculation (Carro-Juárez et al., 2003; Carro-Juárez and Rodríguez-Manzo, 2008; Watcho et al., 2014).

## 2. Materials and methods

### 2.1. Plant material

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

### 2.2. Preparation of Huperzia saururus extract

Aerial parts of *Huperzia saururus* were dried in the shade and ground before use. In agreement to the way 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 VI Edición, 1976) using a proportion of 1:20 W/V. After filtered, the decoction was lyophilized and weighed to calculate the yield. The solution was prepared by dissolution of the lyophilized decoction in saline at the time of use (LDS).

### 2.3. Phytochemical screening

An aqueous extract (a decoction) prepared as already explained was analyzed in relation to the different kind of compounds that could be present. Tests for phenolic compounds were developed including tannins (Wagner et al., 1984; Trease and Evans, 2002), flavonoid glycosides (Shinoda, 1928; Markham, 1982; Trease and Evans, 2002), sulphated flavonoids (Markham, 1982), anthocyanines (Harborne, 1973). Other secondary metabolites were also tested like saponins (Harborne, 1973), alkaloids (Harborne, 1973; Wagner et al., 1984), coumarins (Wagner et al., 1984), anthraquinones (Wagner et al., 1984), steroids (Reich and Schibli, 2006). Primary metabolites were investigated as well, including reducing sugars (Trease and Evans, 2002) and amino acids (Bradford, 1976; Trease and Evans, 2002).

### 2.4. Animals

Sexually experienced male Wistar rats (300–350 g body weight) were used. Animals 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 (Harlan®, México, S.A. de C.V.) 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".

Ten groups (G) of four animals each selected at random were used to develop the experiments, named G1 (control), G2 (*Huperzia saururus* LDS, 3 μg/animal), G3 (atosiban, 1 mg/kg), G4 (hexamethonium, 30 mg/kg), G5 (prazosin, 10 μg/kg), G6 (L-NAME, 30 μg/kg), G7 (atosiban, 1 mg/kg plus *Huperzia saururus* LDS, 3 μg/animal), G8 (hexamethonium, 30 mg/kg plus *Huperzia saururus* LDS, 3 μg/animal), G9 (prazosin, 10 μg/kg plus *Huperzia saururus* LDS, 3 μg/animal), and G10 (L-NAME, 30 μg/kg plus *Huperzia saururus* LDS 3 μg/animal). All the drugs were i.v. administered.

### 2.5. General surgical procedures

All animals were anesthetized with urethane (0.7 g/kg, i.p.) and the adequacy of anesthesia was assessed by the absence of a withdrawal reflex after noxious paw pinch. The bulbospongiosus genital muscles were identified after a surgical incision on the perineum and two platinum wires (Grass) were inserted into the muscles to record electromyographic (EMG) activity, which was registered on a polygraph (Grass M7). An additional surgery was performed to expose the bulbar portion of the penis and its anatomical connections with the striated bulbospongiosus muscles, for a better visualization of the genital rhythmic motor pattern of ejaculation and its associated genital events. The right femoral vein was cannulated for the LDS and drugs administration. At the end of the surgery the spinal cord was blunt transected at T6 spinal cord level, a cord level that clearly permit the expression of sexual reflexes without disrupting significantly thoracic, lumbosacral and coccygeal reflexive responses.

### 2.6. Activation and recording of the fictive ejaculation response

The FE response consists in the expression of the genital motor pattern of ejaculation that accompanies expulsion of intraurethral secretions and the expression of penile reflexes by the spinal cord, in anesthetized and spinal cord transected male rats. Thus, after the spinal cord transection, the FE is immediately and spontaneously expressed with a mean latency of 1–3 min. In order to assess the capacity of the spinal cord to produce the FE response, after spinalisation, one spontaneous genital motor pattern of ejaculation (GMPE) was allowed to be expressed and then recorded in the bulbospongiosus muscles. This muscle was selected as a monitor of the genital muscles activity since it discharges during ejaculation in synchrony with all genital muscles and given its superficial position on the perineum. Immediately after the expression of a spontaneous GMPE, we evoked the ejaculatory response by sensory stimulation of the urethra, which consisted in its distension produced by the injection of saline solution with a syringe pump (200 μL/min) during 10 s, through a PE-50 catheter (0.965 mm OD) inserted into the pelvic urethra via a bladder incision, while occluding the penis meatus to achieve an intraurethral stimulation by increasing its pressure that ranged from 20 to 30 mm Hg. Once the GMPE was obtained by physiological-like (by urethral mechanical stimulation) means, the

LDS was administered (3 μg/animal) and the responses under its influence were analyzed. Finally, the GMPE was repeatedly activated at 3m in intervals by urethral stimulation under the presence of circulating LDS until its inhibition. The criterion used to consider the inhibition of this response was the absence of the GMPE expression upon two consecutive stimulation periods following its repeated elicitation. At this moment, the stimulation protocol was finished.

#### 2.6.1. Activation of FE by the i.v. administration of the LDS of Huperzia saururus in spinal cord transected male rats

In order to establish the aphrodisiac properties of *Huperzia saururus* upon ejaculation in spinal male rats, in animals from G2, one GMPE was elicited after spinalisation by urethral simulation to establish the capacity of the spinal cord to produce the ejaculatory motor response. The ejaculations obtained occurred within a 3-min period. Immediately after the expression of the first urethrally-induced ejaculatory pattern, the *Huperzia saururus* LDS was i.v. injected and the GMPE responses observed under its influence were registered and graphed. After the LDS injection, to verify the expression of ejaculation by the spinal cord, additional urethral stimulations were applied and its resulting genital responses, if present, were observed. If no response was obtained, the experiment was ended. If expressed, the ejaculation responses were evoked by mechanical stimulation of the urethra at 3 min intervals, as below described, until the inhibition of the ejaculatory motor pattern. Data were compared to those obtained in control (G1) animals.

#### 2.6.2. Effects of the oxytocinergic, cholinergic, adrenergic and nitrergic antagonism upon the pro-ejaculatory activity of Huperzia saururus

This experiment was designed to uncover possible roles of oxytocinergic, cholinergic, adrenergic or nitrergic mechanisms in the establishment of the proejaculatory effects of the LDS of *Huperzia saururus* in spinal male rats. To this purpose, in all animals of G3–G6 immediately after spinal cord transection, urethral stimulation was applied and once a FE response was expressed, the intravenous injection of antagonistic doses of atosiban (1 mg/kg; G3), hexamethonium (30 mg/kg; G4), prazosin (10 μg/kg; G5), and L-NAME (30 μg/kg; G6), respectively, were administered and its effects upon the FE response were depicted. Besides, all of G7–G10 animals were treated as previously described but the sequence of administration was the antagonist first and 3 min later, the *Huperzia saururus* LDS in a concentration of 3 μg/animal, upon the expression of FE. After the administration of individual treatments, the animals received repeated mechanical stimulation of the urethra at 3 min intervals, as below described, until the inhibition of the ejaculatory motor pattern. Data were compared to those obtained in control (G1) and *Huperzia saururus* (G2) animals.

### 2.7. Quantitation of GMPE variables

In order to analyze the pro-sexual properties of *Huperzia saururus*, we used electromyographic techniques to register the rhythmic activity of the bulbospongiosus muscles. In the present study we defined a GMPE as the expression of the first motor train of a GMPE (which could be accompanied or not by an after-discharge component) obtained in response to mechanical stimulation of the urethra or after the systemic administration of the LDS. The parameters considered for the analysis of the GMPEs registered were the latency to the expression of the GMPE after mechanical stimulation of the urethra and after the administration of the LDS, the number of discharges in the GMPEs and its frequency, as well as the number of GMPEs expressed prior to its inhibition. The after-discharge component of the GMPE is only observed in urethrally-evoked responses. Since this type of sensorial stimulation exerts an almost immediate cumulative inhibitory effect on the expression of the after-discharge component, we determined to exclude the after-discharge component from the quantitative analysis of both the physiological-like- and drug-induced GMPEs.

### 2.8. Drugs

Urethane, atosiban, hexamethonium, prazosin and L-NAME were all purchased from Sigma Chemical Co. St. Louis, USA. Urethane was dissolved in saline solution and administered at 20%. Doses of the selected antagonists were decided based on previous studies (Carro-Juárez et al., 2003, 2006; Carro-Juárez and Rodríguez-Manzo, 2006; Estrada-Reyes et al., 2013).

### 2.9. Data analysis

Bulbospongiosus electromyographic activity was recorded differentially, amplified and filtered (1000×, 0.1–1 kHz bandpass) (Poliview Data Acquisition System; Grass Astro-Med Inc. USA, 2003). Quantitative comparisons among groups were calculated from means of GMPEs and statistically analyzed by using a one-way ANOVA followed by the *Tukey* test. The variables analyzed from the GMPE included the latency of response, the number and frequency of discharges and the total number of evoked GMPE. A $p < 0.05$ was considered to be statistically significant. The GraphPad InStat program was used for all statistical analyses.

## 3. Results

### 3.1. Decoction yield

According to the way that *Huperzia saururus* is mainly consumed in Argentina, a decoction of the aerial parts was prepared. After lyophlization this extract gave a yield of 7.19%. The solution was prepared by dissolution of the lyophilized decoction in saline at a concentration of 7.5 μg/mL (W/V) at the time of use. In relation to the phytochemical screening tannins and reducing sugars were positive. It is interesting to point out that besides alkaloids (Vallejo et al., 2009; 2013a) and amino acids (Vallejo et al., 2013b), whose presence was already detected in our lab, another kind of secondary metabolites were detected. The presence of saponins deserves to be investigated because these chemical compounds are well known in relation to their biological activity.

### 3.2. General observations on the activation of the fictive ejaculation by the LDS of Huperzia saururus

As previously reported, in all studied control animals the GMPEs elicited by urethral stimulation were registered as highly rhythmic motor patterns of bulbospongiosus muscle activity and included a first ejaculatory motor train and an after-discharge component. Rhythmic contractions elicited by urethral stimulation were always accompanied by the expulsion of seminal secretions and always coincided with phasic penile erections including penile movements such as flaring, flips and cups. Consecutive stimulation of the urethra induced an inhibitory effect on the GMPE, which was gradually evidenced in the parameters of the first ejaculatory motor trains and in its after-discharge component and a progressive reduction in the number of motor discharges and its frequency. In these animals the first sensory elicited ejaculatory phase was the most potent and the last one previous to its

inhibition, the weakest. The ejaculatory capacity of control animals consisted on the expression of a mean number of seven GMPEs. Once the ejaculatory capacity maximum level was accomplished, no further GMPEs including its associated penile movements or expulsion of seminal contents occurred. At this moment, the ejaculatory ability was considered as inhibited.

*Huperzia saururus* LDS was able to activate the GMPE in spinal male rats. Thus, systemic administration of the LDS elicited GMPEs that always consisted on the expression of highly rhythmic motor patterns registered in the bulbospongiosus muscles very similar to those registered in control animals (Fig. 1). LDS of *Huperzia saururus* elicited ejaculatory motor responses always accompanied by penile movements including flaring, flips and cups, but without the after-discharge component. General visual observations of penile erections and movements elicited by the LDS here analyzed, permitted us to notice that the expression of these sexual responses was significantly more potent as compared to that elicited by urethral stimulation.

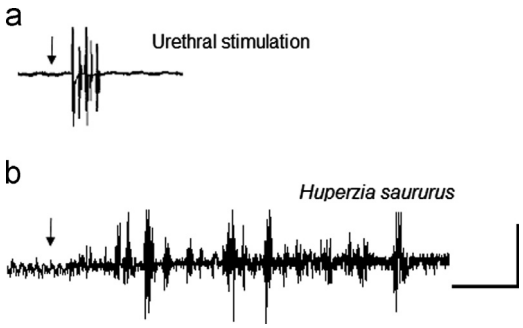


**Fig. 1.** Activation of ejaculatory rhythmic motor patterns in sexually experienced spinal male rats by urethral stimulation (A), and by the intravenous injection of the *Huperzia saururus* decoction (3 μg/animal) (B). Downward arrows indicate the *Huperzia saururus* administration. Calibration bar 50 mV, 10 s.

### 3.3. Effects of Huperzia saururus LDS upon the GMPE parameters

The latency of discharge for *Huperzia saururus* LDS 3 μg/animal (G2) was statistically significantly diminished (Fig. 2a), showing an improvement of this parameter. On the other hand, administration of the *Huperzia saururus* LDS provoked a statistically significant augment for the number of discharges (Fig. 2b). In relation to the frequency of discharge another situation was evidenced, since no statistically significant difference when compared to control animals after *Huperzia saururus* administration was found. The number of GMPE was not significantly modified after *Huperzia saururus* administration. The modified parameters revealed an improvement in the ejaculatory potency elicited by the *Huperzia saururus* LDS.

### 3.4. Effects of the oxytocinergic, cholinergic, adrenergic and nitrergic antagonism upon the pro-ejaculatory activity of Huperzia saururus

#### 3.4.1. Antagonists effect on FE parameters

With respect to the use of the antagonists above mentioned upon the expression of ejaculation in spinal cord transected male rats, it was found that with the exception of L-NAME, atosiban, hexamethonium, and prazosin, all them did not provoke any ejaculatory responses (Table 1). When L-NAME was administered we observed in the latency of discharge a statistically significant increase with respect to control animals, the number of discharges was significantly diminished in comparison to control and no statistically significant variations were observed for the frequency of discharge and the number of genital motor patterns showed a decrease in relation to control animals (Table 1).

#### 3.4.2. Antagonists effect upon the pro-ejaculatory activity of Huperzia saururus

When sequential treatments using the antagonists plus LDS were administered, the effects produced on the four analyzed parameters were as follows: the oxytocinergic antagonist atosiban significantly reduced the number of discharges, but not its frequency. Otherwise, latency of discharge and the ejaculatory capacity was not changed as evidenced by a non-significant number of ejaculatory motor patterns (Table 2).

With regard to the administration of the cholinergic antagonist hexamethonium followed by the LDS, we observed that hexamethonium partially blocked the pro-ejaculatory effect of the LDS since the number of discharges was significantly reduced. On the other hand, the frequency of discharge, the latency of response and the number of GMPE were not significantly modified (Table 2).

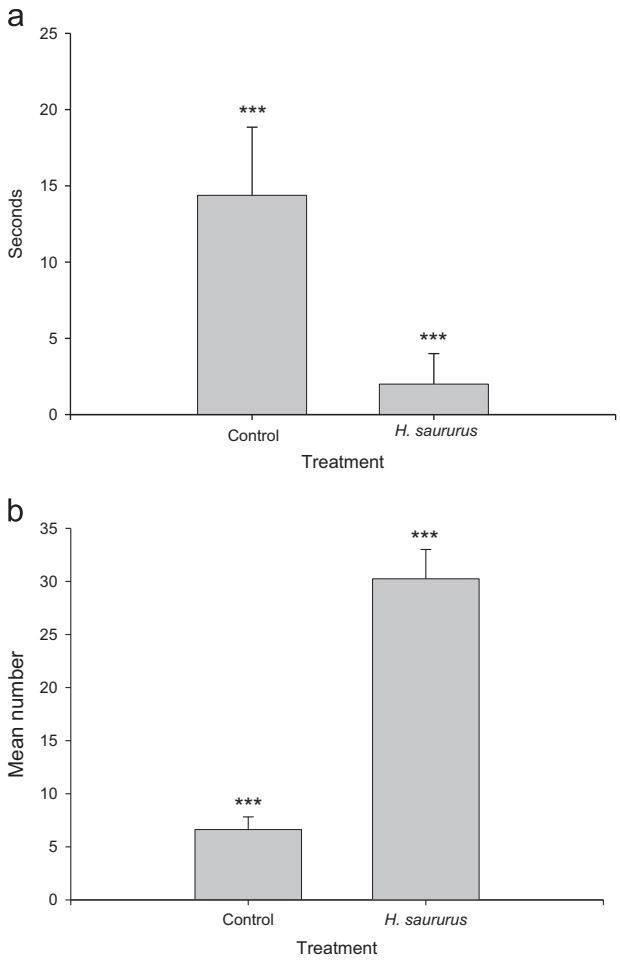


**Fig. 2.** Specific parameters of the ejaculatory motor patterns obtained in response to *Huperzia saururus* decoction (G2, 3 μg/animal). Values are expressed as mean ± - standard error of the mean. (a) Latency of discharge was significantly diminished ($T_{LD}$ (3)=5.185; $p$= 0.0002); (b) statistically significant augment for the number of discharges ($T_{ND}$ (10)=21.359; $p < 0.0001$). *T*- test, *** $p <$ 0.001.

Animals pre-treated with the adrenergic antagonist prazosin blocked the facilitatory effect of *Huperzia saururus* since the facilitation in the latency of response was prevented, the number of discharges was reduced, but without significant effects on the frequency of discharge and on the expression of the GMPE (Table 2).

Finally, for animals pre-treated with L-NAME only the number of discharges was influenced and a statistically significant reduction was registered (Table 2). Together, these data indicate that the

**Table 1**
Comparison among the parameters of the genital motor patterns of ejaculation registered in response to urethral stimulation, after the injection of *Huperzia saururus* decoction and after the administration of the antagonists used in this study.

| | Control | Decoction | Atosiban | Hexamethonium | Prazosin | L-NAME |
|---|---|---|---|---|---|---|
| **LD** | 14.38 ± 1.58**a | 2.00 ± 1.00*b,***c | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 23.0 ± 4.30***c |
| **ND** | 6.63 ± 0.42**a,***c | 30.23 ± 1.38*b,***c | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 1.75 ± 0,48***c |
| **FD** | 1.16 ± 0.07**a | 0.95 ± 0.03*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 0.98 ± 0,05 |
| **GMPEs** | 7.63 ± 0.71**a,*c | 8.50 ± 2.50*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 0.00 ± 0.00**a,*b | 1.75 ± 0,85*b,*c |

*T*- test, LD= latency to discharge; ND= number of discharges; FD= frequency of discharge; GMPEs= genital motor patterns of ejaculation.
**a control group versus the antagonist groups for each parameter.
*b decoction group versus the antagonist groups for each parameter.
***c L-NAME group compared to the decoction or control groups for LD and ND.
*c control group versus the antagonist L-NAME.

**Table 2**
Comparison among the parameters of the genital motor patterns of ejaculation registered in response to the *Huperzia saururus* decoction (3 μg/mL), and after the pre-treatment with the selective antagonists followed by *Huperzia saururus* decoction.

| | Decoction | Atosiban + Decoction | Hexamethonium + Decoction | Prazosin + Decoction | L-NAME + Decoction |
|---|---|---|---|---|---|
| **LD** | 2.00 ± 1.00* | 30.5 ± 26.6 | 10.5 ± 9.84 | 43.0 ± 17.0* | 6.00 ± 4.70 |
| **ND** | 30.23 ± 1.38***a,b,c,d | 9.50 ± 2.50***a | 4.25 ± 2.53***b | 7.75 ± 1.84***c | 9.00 ± 3.08***d |
| **FD** | 0.95 ± 0.03 | 0.99 ± 0.01 | 0.50 ± 0.29 | 0.95 ± 0.05 | 0.64 ± 0.24 |
| **GMPE** | 8.50 ± 2.50 | 3.75 ± 1.03 | 1.75 ± 1.43 | 5.25 ± 1.49 | 4.25 ± 1.75 |

*T*-test, LD= latency to discharge; ND= number of discharges; FD= frequency of discharge; GMPEs= genital motor patterns of ejaculation.
***a decoction group versus atosiban plus decoction group.
***b decoction group hexamethonium plus decoction group.
***c decoction group versus prazosin plus decoction group.
***d decoction group versus L-NAME plus decoction group.

compounds contained in *Huperzia saururus* LDS that promote pro-ejaculatory actions target at least partially the oxitocinergic, cholinergic, adrenergic and nitrergic spinal system to exert its effects.

## 4. Discussion and conclusions

Previous studies on the medicinal properties of *Huperzia saururus* showed that it facilitates penile erection and thus, an aphrodisiac component on sexual potency was evidenced (Hnatyszyn et al., 2003). Here, evaluation of the LDS pro-ejaculatory activities was focused on the analysis of the latency of response, the number and frequency of discharges of the ejaculatory motor pattern and the number of motor patterns of ejaculation evoked.

In the animal model here employed, it has been shown that the latency to express ejaculation after the application of physiological-like stimulation (urethral stimulation) or after the administration of standard drugs or plant extracts reflects the responsiveness of the spinal cord to the sexual stimuli and mirrors the threshold of ejaculation that could be seen in conscious male rats (Carro-Juárez and Rodríguez-Manzo, 2009). Present findings show that LDS, when acutely administered, significantly reduces the latency to induce ejaculatory activity (expression of the genital motor patterns of ejaculation, penile erections and movements and the expulsion of urethral contents) implying that LDS possesses aphrodisiac activity which, could be acting on the spinal generator of ejaculation. As a result, chemical compounds contained in LDS could provoke a substantial modification of the ejaculatory threshold, after modifications in the intraspinal inhibitory tone, which control the rhythmic expression of the ejaculatory response. If the aphrodisiac capacity of LDS on the ejaculatory threshold here obtained is enough to affect male copulatory behavior is a matter that deserves future studies.

The discharges registered in the motor responses obtained after *Huperzia saururus* administration were also significantly augmented. It has been showed that the augmented number of ejaculatory discharges reflects the robustness of the rhythmic motor pattern and this parameter is strongly associated to the ejaculatory potency (Carro-Juárez et al., 2014). This datum is in line with a previous study of Hnatyszyn et al. (2003). Together, these results suggest that *Huperzia saururus* improve sexual potency promoting the ejaculatory potency.

The frequency of discharge obtained after LDS was not significantly modified in the fictive ejaculatory responses. The frequency of discharge of the genital motor pattern contributes to the evaluation of the ejaculatory potency. Thus, if a reduced frequency of discharge is obtained when physiological-like or pharmacological stimuli are applied, a diminished potency could be expected and on the contrary, when an augmented frequency of discharges is obtained, improvements in the potency of the ejaculatory trains are expected (Carro-Juárez and Rodríguez-Manzo, 2009; Carro-Juárez et al., 2014). Besides, when pharmacological and physiological stimuli provoke the facilitation of ejaculatory motor patterns, augmented numbers and frequencies of discharges in the ejaculatory trains would be expected. In the present study, we observed that LDS augmented number of discharges but not elicited significant modifications in its frequency. We have not an explanation for these unexpected results, but we can offer probable explanations. Thus, it has been proposed that at least two modules or neural nucleus mainly comprise the spinal generator for ejaculation (SGE) (Carro-Juárez and Rodríguez-Manzo, 2008). These SGE modules could be separately controlling the expression of the motor discharges and its frequency of discharge and if this were the case, it is tempting to suggest that molecules contained in LDS act on the different modules of the ejaculation generator, on one side targeting those neurons in charge of the expression of the motor train discharges and consequently, promoting the force to expel

the seminal secretions and, on the other side, targeting those neurons in charge of the quality or refinement of the movement, those involved in the control of the frequency of discharges. Further electrophysiological studies are necessary to test this proposal.

In the fictive ejaculation model the number of genital motor patterns of ejaculation evoked by urethral or pharmacological stimulation is related to the ejaculatory capacity of male rats (Carro-Juárez et al., 2009, 2014), with sustained expression of ejaculatory motor patterns previous to its inhibition by repeated urethral stimulation (Carro-Juárez and Rodríguez-Manzo, 2000; Carro-Juárez et al., 2003). We observed no significant changes in the ejaculatory capacity of male rats after the administration of LDS. This is a very interesting result since it seems that the compounds contained in LDS acts only in the neural elements conforming the modules of the spinal pattern generator in charge of the expression of the ejaculatory train but not in those neural elements in charge of the pacemaker activity, in which the intraspinal ejaculatory rhythm arises. In all probability the LDS acts targeting synaptically interacting excitatory interneurons that synchronize the ejaculation generator spiking activities (Brocard et al., 2010). Further physiological studies are necessary to test this hypothesis.

With respect to the potential actions of the compounds contained in LDS upon spinal neural circuits in charge of ejaculation, it is important to point out that the main biologically active constituents of *Huperzia saururus* are alkaloids (Lycopodium alkaloids). For this reason, many studies have been developed with the alkaloid extract (AE). Thus, AE has demonstrated to possess a marked capacity to inhibit the acethylcholinesterase enzyme (AChE) *in vitro* and *ex vivo* studies (Ortega et al., 2004a; Vallejo et al., 2007). However, when isolated alkaloids were assayed, our findings showed that alkaloids have not the same behavior, i.e., sauroine does not inhibit AChE (Ortega et al., 2004b) and on contrary, sauroxine (Puiatti et al., 2013) and *N*-de-methylsauroxine (Vallejo et al., 2013a) are active. In addition, we have demonstrated in an electrophysiological *ex vivo* model of rat hippocampal slices that AE induce and maintain the long-term potentiation (LTP) (Ortega et al., 2006). Finally, in electrophysiological experiments (on hippocampus slices) and behavioral tests (step down test), sauroine have shown significative effects on memory retention by significantly increasing hippocampal plasticity (Vallejo et al., 2009). All these data could suggest that alkaloids present in LDS act by different mechanisms and at different modules of the spinal generator for ejaculation. Specific studies using individual alkaloids from LDS upon the functioning of the ejaculation generator are necessary to test suggestions.

The exact mechanism (s) through the aqueous crude extract of *Huperzia saururus* exerts its pro-ejaculatory actions is at present unknown. A role for the oxytocinergic (Carro-Juárez et al., 2004, 2006), dopaminergic (Kada Sanda et al., 2013), cholinergic (Lee et al., 2011), adrenergic (Mao et al., 2014) and nitrergic (Singh and Singh, 2012; Estrada-Reyes et al., 2013) mechanisms in the mediation of the pro-sexual actions promoted by aphrodisiac plants have been described. In the present study we evaluated the antagonism of atosiban, hexamethonium, prazosin and L-NAME on the facilitation of the ejaculatory potency induced by LDS. Data revealed that all antagonists here employed prevent the pro-ejaculatory effect seen after LDS administration, suggesting that the oxytocinergic, cholinergic, adrenergic and nitrergic spinal mechanisms are partially turned on by the aphrodisiac activity of LDS, interestingly centered on the ejaculatory potency, but not in the ejaculatory capacity. Thus, present data support the notion that in spinal cord transected male rats, LDS is promoting its aphrodisiac actions on ejaculation after the improvement of the ejaculatory potency with the participation of all the four spinal mechanisms studied.

All in all, present study shows that LDS exert important aphrodisiac actions acting on the spinal circuits that control the male rat ejaculatory potency. It seems that pro-ejaculatory effects of LDS are mediated by differential activity of the compounds contained in it upon different modules of the ejaculation generator, with the participation of the oxytocinergic, cholinergic, adrenergic and nitrergic mechanisms.

## Acknowledgments

To Dr. Gloria Barboza for the plant material identification. This Project was supported by SeCyT-Universidad Nacional de Córdoba 05/C548, PICT 1576 ANPCyT (FONCyT), PIP 00607 CONICET, Argentina and PID No 0113/2011 Agencia Córdoba Ciencia.

## References

Akerele, O., 1985. The WHO traditional medicine program: policy and implementation. International Traditional Health Newsletter 1, 1–3.
Amorín, J.L., 1977. Cola de quirquincho *Urostachis saururus* (Lam.) Heter (Lycopodiaceas). Una peligrosa planta usada en medicina popular Argentina. Farmacobotánica 16, 3–6.
Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.
Brocard, F., Tazerat, S., Vinay, L., 2010. Do pacemakers drive the central pattern generator for locomotion in mammals? The Neuroscientist 16, 139–155.
Carro-Juárez, M., Alcazar, C., Ballesteros-Polvo, E., Villalobos-Peñalosa, P., 2009. Increase of ejaculatory capacity by systemic administration of the oquichpatli (*Senecio cardiophyllus*) aqueous crude extract in male rats. Journal of Ethnopharmacology 126, 506–511.
Carro-Juárez, M., Cervantes, E., Cervantes-Méndez, M., Rodríguez-Manzo, G., 2004. Aphrodisiac properties of *Montanoa tomentosa* aqueous crude extract in male rats. Pharmacology Biochemistry and Behavior 78, 129–134.
Carro-Juárez, M., Cruz, S.L., Rodríguez-Manzo, G., 2003. Evidence for the involvement of a spinal pattern generator in the control of the genital motor pattern of ejaculation. Behavioural Brain Research 975, 222–228.
Carro-Juárez, M., Franco, M.A., de Lourdes Rodríguez-Peña, M., 2014. Increase of the ejaculatory potency by the systemic administration of aqueous crude extracts of cihuapatli (Montanoa genus) plants in spinal male rats. Journal of Evidence-Based Complementary & Alternative Medicine 19 (1), 43–50.
Carro-Juárez, M., Lobaton, I., Benitez, O., Espiritu, A., 2006. Pro-ejaculatory effect of the aqueous crude extract of cihuapatli (*Montanoa tomentosa*) in spinal male rats. Journal of Ethnopharmacology 106, 111–116.
Carro-Juárez, M., Rodríguez-Manzo, G., 2000. Sensory and motor aspects of the coital reflex in the spinal male rat. Behavioural Brain Research 108, 97–103.
Carro-Juárez, M., Rodríguez-Manzo, G., 2006. α-adrenergic agents modulate the activity of the spinal pattern generator for ejaculation. International Journal of Impotence Research 18, 32–38.
Carro-Juárez, M., Rodríguez-Manzo, G., 2008. The spinal pattern generator for ejaculation. Behavioural Brain Research 58, 106–120.
Carro-Juárez, M., Rodríguez-Manzo, G., 2009. Participation of endogenous opioids in the inhibition of the spinal generator for ejaculation in rats. The Journal of Sexual Medicine 6, 3045–3055.
Estrada-Reyes, R., Carro-Juárez, M., Martínez-Mota, L., 2013. Pro-sexual effects of *Turnera diffusa* Wild (Turneraceae) in male rats involves the nitric oxide pathway. Journal of Ethnopharmacology 146, 164–172.
Farmacopea Argentina VI Edición, 1976. Ministerio de Salud de la Nación Argentina.
Harborne, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman A. & Hall, London. Great Britain.
Hieronymus, G., 1882. Plantae Diaphoricae Florae Argentinae: ó Revista sistemática de las plantas medicinales, alimenticias ó de alguna otra utilidad y de las venenosas, que son indígenas de la República Argentina ó que, originarias de otros países se cultivan o se crian espontáneamente en ella, Guillermo Kraft; Buenos Aires. República Argentina. p. 329, http://www.biodiversitylibrary.org/item/34544#page/7/mode/1up.
Hnatyszyn, O., Moscatelli, V., Garcia, J., Rondina, R., Costa, M., Arranz, C., Balaszczuk, A., Ferraro, G., Coussio, J.D., 2003. Argentinian plant extracts with relaxant effect on the smooth muscle of the corpus cavernosum of guinea pig. Phytomedicine 10, 669–674.
Kada Sanda, A., Miegueu, P., Bilanda, D.C., Faleu Nya Ngassam, M., Watcho, P., Djomeni, P.D., Kamtchouing, P., 2013. Ejaculatory activities of *Allanblackia floribunda* stem bark in spinal male rats. Pharmaceutical Biology 51 (8), 1014–1020.
Lee, M.R., Yun, B.S., In, O.H., Sung, C.K., 2011. Comparative study of Korean white, red, and black ginseng extract on cholinesterase inhibitory activity and cholinergic function. Journal of Ginseng Research 35 (4), 421–428.
Mao, H., Wang, H., Ma, S., Xu, Y., Zhang, H., Wang, Y., Niu, Z., Fan, G., Zhu, Y., Gao, X. M., 2014. Bidirectional regulation of bakuchiol, an estrogenic-like compound,

on catecholamine secretion. Toxicology and Applied Pharmacology 274 (1), 180–189.
Markham, K.R., 1982. Thechniques of Flavonoid Identification. Academic Press Inc., London. Great Britain.
Martínez Crovetto, R., 1981. (Miscelanea). Las plantas utilizadas en medicina popular en el Noroeste de Corrientes (República Argentina), 69. Fundación Miguel Lillo, Argentina p. 15.
Ortega, M.G., Agnese, A.M., Cabrera, J.L., 2004a. Sauroine-a novel Lycopodium alkaloid from *Huperzia saururus*. Tetrahedron Letters 45 (38), 7003–7005.
Ortega, M.G., Agnese, A.M., Cabrera, J.L., 2004b. Anticholinesterase activity in an alkaloid extract of *Huperzia saururus*. Phytomedicine 11 (6), 539–543.
Ortega, M.G., Vallejo, M.G., Cabrera, J.L., Pérez, M.F., Almirón, R.S., Ramírez, O.A., Agnese, A.M., 2006. *Huperzia saururus*, activity on synaptic transmission in the hippocampus. Journal of Ethnopharmacology 104, 374–378.
Puiatti, M., Borioni, J.L., Vallejo, M., Cabrera, J.L., Agnese, M., Ortega, M.G., Pierini, A. B., 2013. Study of the interaction of *Huperzia saururus* Lycopodium alkaloids with the acetylcholinesterase enzyme. Journal of Molecular Graphics and Modelling 44, 136–144.
Reich, E., Schibli, A., 2006. High Performance for the Analysis of Medicinal Plants. Thieme, New York, Stuttgart, USA.
Sandroni, P., 2001. Aphrodisiacs past and present: a historical review. Clinical Autonomic Research 11, 303–307.
Shinoda, J., 1928. A new biologically active flavone glycoside from the roots of *Cassia fistula* Linn. Journal of the Pharmaceutical Society of Japan 48, 214–220.
Singh, A.P., Singh, R., 2012. Potent natural aphrodisiacs for the management of erectile dysfunction and male sexual debilities. Frontiers in Bioscience 1 (4), 167–180.
Toursarkissian, M., 1980. Plantas medicinales de la Argentina: Sus nombres botánicos, vulgares, usos y distribución geográfica. Hemisferio Sur, Buenos Aires.
Trease, G., Evans, W., 2002. Pharmacognosy, 15th Ed. Saunders Publishers, London. Great Britain.
Vallejo, M.G., Ortega, M.G., Cabrera, J.L., Carlini, V.P., Rubiales de Barioglio, S., Agnese, A.M., 2007. *Huperzia saururus* increases memory retention in rats. Journal of Ethnopharmacology 111, 685–687.
Vallejo, M.G., Ortega, M.G., Cabrera, J.L., Carlini, V.P., Rubiales de Barioglio, S., Almirón, R.S., Ramírez, O.A., Agnese, A.M., 2009. Sauroine, an alkaloid from *Huperzia saururus* with activity in Wistar rats in electrophysiological and behavioral assays related to memory retention. Journal of Natural Products 72, 156–158.
Vallejo, M., Ortega, M., Cabrera, J.L., Agnese, M., 2013a. N-demethyl-sauroxine, a novel Lycodine group alkaloid from *Huperzia saururus*. Tetrahedron Letters 54, 5197–5200.
Vallejo, M., Dimmer, J., Ortega, M., Cabrera, J., Agnese, M., 2013b. Amino acids content and acetylcholinesterase inhibition of *Huperzia saururus* infusion and decoction. Pharmaceutical Biology 51 (10), 1341–1345.
Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer-Verlag, Berlin, Heidelberg, Germany.
Watcho, P., Modeste, W.N., Albert, K., Carro-Juárez, M., 2014. *Dracaena arborea* extracts delay the pro-ejaculatory effect of dopamine and oxytocin in spinal male rats, International Journal of Impotence Research, 2014 May 1. http://dx.doi.org/10.1038/ijir.2014.13.