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Pro-ejaculatory effect of the aqueous crude extract of cihuapatli (*Montanoa tomentosa*) in spinal male rats

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Abstract

In the present study, the pro-sexual effect of the cihuapatli (*Montanoa tomentosa*) and its possible pro-ejaculatory properties in spinal male rats were examined. Systemic administration of the aqueous crude extracts of *Montanoa tomentosa* exerted a pro-ejaculatory effect and produced an increase in the number of discharges in the ejaculatory motor patterns in the spinal rats. The cihuapatli-induced ejaculatory responses included the expression of penile erections and penile movements and the potent expulsion of urethral contents and in some cases the expulsion of seminal plugs. The cihuapatli-induced ejaculatory motor patterns were similar to that obtained after systemic oxytocin. Cihuapatli- and oxytocin-induced ejaculatory motor responses and the penile erections and movements were abolished by the pre-treatment with hexamethonium, a selective oxytocin antagonist. Present data show that the cihuapatli extract acts directly at the spinal system in charge of the expression of the ejaculatory motor patterns and suggest that the aqueous crude extract exerts its aphrodisiacs properties by increasing sexual potency acting as an oxytocic agent.

Keywords: Cihuapatli; *Montanoa tomentosa*; Ejaculation; Aphrodisiac; Oxytocin; Sexual potency; Male rat

1. Introduction

Cihuapatli, the Mexican zoapatle (*Montanoa tomentosa*) is a medicinal plant with an extensive use as a traditional remedy for reproductive impairments (Levine et al., 1981; Gallegos, 1985). Mexican traditional medicine mentions that the aqueous crude extract of this plant possesses contraceptive activity in women during the early stages of pregnancy (Hahn et al., 1981; Levine et al., 1981; Gallegos, 1983; Ponce-Monter et al., 1983). The experimental studies with cihuapatli aqueous crude extract or its purified fractions confirm popular observations and it have been shown that the contraceptive effects of *Montanoa tomentosa* aqueous crude extract are provoked by inhibition of implantation, cervical dilation and uterine bleeding (Hahn et al., 1981) without influence the endocrine status. Administration of cihuapatli tea did not produce any cardiovascular changes, and it did not influence the hematological status, liver, kidney, and thyroid function, blood lipids, proteins, and electrolyte status (Hahn et al., 1981). Thus, early experimental studies on the cihuapatli aqueous crude extract support a unique contraceptive, oxytocic-like effect on the female reproductive tract.

In a recent study, the aphrodisiac properties of cihuapatli in the male rat were demonstrated. By evaluating the pro-sexual properties of *Montanoa tomentosa* we described that in sexually active male rats acute administration of the extract of this plant facilitates both motivation and performance. Thus, in genitally anaesthetized animals, the administration of *Montanoa tomentosa* extracts significantly increases mounting behaviour and improves the expression of sexual behaviour in non-copulating males, revealing a specific effect of this extract on sexual motivation (Carro-Juárez et al., 2004). Interestingly, behavioural studies in sexually naive and in non-copulating male rats revealed that *Montanoa tomentosa* induces a shortage in ejaculation latency (Carro-Juárez et al., 2004). Given the aphrodisiacs effects of cihuapatli, it could be thought that the compounds contained in this plant act at the neural circuits of ejaculation, though the exact mechanism(s) or potential site(s) through the aqueous crude extract of *Montanoa tomentosa* exerts its pro-ejaculatory actions are not known.

Aphrodisiacs are substances able to excite libido or arouse sexual instinct (Sandroni, 2001). These substances can be cat-

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egorised according to their mode of action into three groups: by increasing libido (i.e. sexual desire), by increasing potency (i.e. effectiveness of erection) and by increasing sexual pleasure (Sandroni, 2001). The aphrodisiacs act at the level of the central nervous system by altering specific neurotransmitters or specific sex hormone concentrations. Some of them can be effective in both sexes, though most act through an increase in testosterone concentration and are therefore male-specific (Sandroni, 2001). In the present study the possibility that the aqueous crude extract of *Montanoa tomentosa* exerts an increase in sexual potency by acting directly upon the ejaculatory response was tested. To this aim, we analysed the effect of intravenous administration of cihuapatli aqueous crude extract upon the expression of the rhythmic genital motor pattern, a response considered the main component of ejaculation in the male rat. We employed a model for the study of ejaculation in spinal and urethane-anaesthetised male rats: the fictive ejaculation model (Carro-Juárez et al., 2003). The fictive ejaculation model permits the recording and visualization of the rhythmic motor pattern of ejaculation accompanied by complex pelvic activity that includes phasic and strong penile erections, as well as penile movements followed by the potent expulsion of urethral contents (Carro-Juárez et al., 2003). The rhythmic motor pattern of ejaculation registered in this experimental model is elicited by mechanical stimulation of the urethra and can be induced by the systemic administration of several drugs (Carro-Juárez and Rodríguez-Manzo, 2003, 2005).

It has been established on one side, that the aqueous crude extract of cihuapatli is an oxytocic-like agent (Levine et al., 1981; see for review, Perusquía et al., 1985), and on the other side, that the fictive ejaculation response can be induced by the systemic administration of oxytocin (Carro-Juárez and Rodríguez-Manzo, 2005). Thus, we wonder if a physiological mechanism involving the oxytocinergic system could be involved in the sexual potency increase promoted by the cihuapatli extract. To evaluate this possibility the present study analysed the potential involvement of the spinal oxytocinergic system in the mediation of such response. To this purpose the specificity of the cihuapatli-induced and the oxytocin-induced ejaculatory motor patterns was evaluated by using the selective oxytocin antagonist, hexamethonium, previous to the administration of the cihuapatli extract and oxytocin.

2. Materials and methods

2.1. 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 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".

2.2. General surgical procedures

All animals were urethane-anaesthetised (0.7 g/kg i.p.), and by performing a surgical incision on the perineum, the bulbospongiosus genital muscles were identified. Two platinum wires (Grass) were inserted into the muscles to record electromyographic (EMG) activity, which was registered on a polygraph (Grass M7). For a better visualisation of the motor genital activity associated to the ejaculation, an additional surgery was performed to expose the bulbar portion of the penis and its anatomical connections with the striated bulbospongiosus muscles. Treatments were administered by infusing the selected extracts and compounds into the femoral vein. At the end of the surgical approach the spinal cord was blunt transected at T6 level and prepared for recording.

2.3. Groups

Animals were divided into six groups. The first group ($n = 3$) served as control group and was used to record sensory-elicited ejaculatory motor patterns and to evaluate the effect of i.v. administration of saline solution. Groups 2–6 ($n = 3$, each) were employed to analyse the effect of the following treatments: aqueous crude extract of cihuapatli (25 mg/animal), oxytocin (0.5 IU/kg), the selective oxytocin antagonist, hexamethonium (25 mg/animal) and the sequential treatments with hexamethonium plus aqueous crude extract of cihuapatli (25, 25 mg/kg, respectively) and hexamethonium plus oxytocin (25 mg/kg, 0.5 IU/kg, respectively), on the expression of the ejaculatory motor pattern.

2.4. Activation of the rhythmic genital motor pattern of ejaculation

In all animals, immediately after spinal cord transection, ejaculatory motor patterns could be reflexively expressed and recorded in the genital muscles. Animals in all groups were used to register the induced ejaculatory motor patterns. To establish the capacity of the spinal apparatus to produce the genital muscular rhythmic pattern after spinalisation, two to three consecutive ejaculatory motor patterns were recorded during at least a 10 min-interval. Reflexively induced ejaculatory motor patterns were activated by mechanical stimulation of the urethra as previously described (Carro-Juárez et al., 2003). In brief, after spinalisation, ejaculatory motor patterns were repeatedly evoked at 3 min intervals by the injection of saline solution (200 μl/min) through a PE-50 catheter (0.965 mm o.d.) inserted into the pelvic urethra via a bladder incision. Thereafter, one of the selected treatments was i.v. injected immediately after the expression of a genital motor pattern and the response obtained under their influence, registered. After the injection of the selected treatment, two additional motor patterns, if present, were permitted to express before the next injection. Otherwise, when no response was obtained a 1 min recording period was allowed between injections. Three consecutive i.v. injections were provided and their respective responses recorded. A similar protocol was considered for animals in group 1.