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Investigation of Local Anesthetic Effect and Tocixity of *Ottonia Propinqua* (Piperaceae)

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SUMMARY. The local anesthetic effect of the ethanolic extract of *Ottonia propinqua* roots was tested *in vivo* with twich response of guinea-pig skin. The extract trends to produce reversible and no dose dependent anesthesia. The extract displayed signs of local irritation and its LD_{50} in mice was 33 mg/kg, i.p.

RESUMEN. "Investigación del Efecto Anestésico Local y Toxicidad de *Ottonia Propinqua* (Piperaceae)". El efecto anestésico local *in vivo* del extracto etanólico de las raíces de *Ottonia propinqua* fue estudiado utilizando el método de la anestesia intradérmica comparada con conejillos de las Indias. Los resultados demostraron una actividad anestésica que fue independiente de la dosis. Se observan también muestras de irritación local. La dosis letal mediana (DL_{50}) en ratones fue 33 mg/kg, i.p.

INTRODUCTION

The Piperaceae family comprises 12 genera and about 1400 species of mainly pantropical distribution. *Piper* and *Peperomia* are the best represented genera in the brazilian flora, with about 170 and 150 species, respectively [1].

The majority of phytochemical research in Piperaceae was performed with the genus *Piper*. The most characteristic chemical constituents are amides, lignans and essential oils [2,3]. The genus Ottonia has 23 described species, of which 21 are from Brazil [4]. To the best of our knowledge only amides as piperovatine and piperlongumine are reported for this genus [5-7].

Several species display pungent taste probably due to amides. Many of them provide intense salivation and are known as "jaborandi". This popular name is derived from indian's language "Tupi-Guarani" and means "that makes you slobber". Besides Piperaceae "jaborandi" is employed to name many plants from diferents families which have the same property (e.g *Ptlocarpus sp.*, Rutaceae). Therefore, some species of *Piper* and *Ottonia* have been used in homemade medicine as anesthetic for toothache [8].

KEY WORDS: Ottonia sp., *Ottonia propinqua*, Piperaceae, Anesthetic, Amides.
PALABRAS CLAVE: Ottonia sp., *Ottonia propinqua*, Piperaceae, Anestesia, Amidas.

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In this work we evaluated the anesthetic effect and acute toxicity of *Ottonia propinqua* popularly known as "jaborandi". Its main folk use is as local anesthetic in toothache. According to the ethnobotanical data from South Brazil the roots are chewed or used as hand made alcoholic preparations. The people that use this plant report mouth anesthesia and intense salivation.

MATERIALS AND METHODS

Plant material

Roots of *Ottonia propinqua* were colected in January 1994 in Rio Grande do Sul, South Brazil. The plant was identified by M. Sobral (Graduate Course of Pharmaceutical Sciences - Universidade Federal do Rio Grande do Sul).

Extraction procedure

The air-dried roots were powdered and extracted three times by maceration with ethanol. The combined extracts were evaporated under reduced pressure to dryness. The yield of dry extract was 6,7% (w/w), relative to the dried starting material. The dilutions of the extract were made fresh in saline + polisorbate 4% on the day of experiments.

Detection of amides

The ethanolic pungent extract was applied for thin layer chromatography using toluene: ethyl acetate (70:30 v/v) as mobile phase and silica gel $60GF_{254}$ as stationary phase. After spraying with vanilin-sulphuric acid and heating for 10 min at 100 °C, three majoritary lemmon-yellow bands, probably due to amides, were detected [9].

Twitch response of guinea-pig skin

Adult guinea pigs (300-400 g) were prepared one day before the experiment by first clipping and than shaving the hairs on the lower back. It was done 24 h in advance to obtain the disappearance of any irritation produced by shaving. To correct the variation in sensivity of different parts of the shaved skin and the variation between animals, the doses of the test drugs (extract 1%, 0.5%, 0.25%, 0.125%, cocaine hydrochloride 0.2% and saline + polisorbate 4%) were given in different areas in a number of random combinations. Equal volumes (0.25 ml) of the drugs were injected intradermally and the weals raised by the injected volumes were outlined with a marking pen. Five minutes after the injection, the sensivity of the outlined area was tested by pricking with a needle. Six light pricks were made on the skin in the area bordering the site of injection. The pricks were repeated in 5 min intervals for 30 min. The total score (twitch) for each weal was added up and expressed as the total number of positive responses out of 36 possible changes. Six animals were used for each test dose.

Toxicity

Male swiss mice (20 - 25 g) were injected , i.p., with the alcoholic root extract at the 100, 80, 60, 40 and 20 mg/kg with injection volume of 10 ml/kg. The control group received an equivalent volume of vehicle (saline + 4 % polisorbate

80). The test and the control groups (10 animals each) were observed for 24 h under normal conditions with free access to food and water. The median lethal dose (LD_{50}) was determined taking into account deaths in the first 45 minutes of observation after administration.

Statistical analyses

The results of twitch guinea pig responses were analysed by one way analyses of variance -ANOVA- with repeated measures and *post hoc* comparation was performed by Bonferroni's test. The LD_{50} was calculated using Probit method.

RESULTS AND DISCUSSION

The ethanolic extract produced a response comparable to both, saline and cocaine, taking into account total positive responses (Table 1), except for the dose 0,50 %, which had not anesthetic effect at all ($F_{5,30} = 4,47$; $p<0,01$). The tendency of the extract to induce anesthesia was transient and no dose-dependent (Fig.1). We have no explanation for that. Probably this effect is erratic and no specific, but more studies are necessary because the rapid onset of actions with short duration would justify the emergencial and primary care by traditional communities. Nevertheless, the extract showed high acute toxicity. The LD_{50} in mice (i.p.) was 33 mg/Kg and all animals showed intense signs of central stimulation. The deaths were precocious and preceded of tonic-clonic convulsion. Furthermore, we observed signs of local irritation at the injection site. Thus, this plant not seems to be appropriate for homemade medicine or to manufacture phytotherapic products. Further investigations are necessary in order to clarify the chemical composition of this plant and to identify its active compounds. They could be amides, commonly found in the genus *Ottonia* and detected in this study. Isobutilamides as piperovatine and piperlongumine were isolated from *Ottonia martiniana* [6], that has the same use. These compounds have a general chemical feature analogue to capsaicin, isolated from genus *Capsicum* (Solanaceae). This compound has been employed in neurological research, especially in chronic pain study [10] and to relieve pain that follows *Herpes zoster* infection [11]. Its utilization for a long time can make peripheral sensory nervs insensitive to a painful stimulus, probably by substance P depletion [11]. In this way, this group of natural products could be relevant for the development of prototype drugs for interfering with nociceptive processing.

Treatment	Total response
Extract 0,125 %	19,7 3,24
Extract 0,25 %	20,5 2,46
Extract 0,5 %	24,8 1,42 **
Extract 1,0 %	19,7 2,11
Cocaine hidrochloride 0,2 %	10,2 3,91*
Control (Saline Polisorbate 80)	29,0 3,88
Values are means SEM (n=6) ; p < 0,01	

* Significant difference in relation to control.

** Significant difference in relation to cocaine.

Table 1. Effect of alcoholic extract of *Ottonia propinqua* on total twitch response of guinea-pig skin.

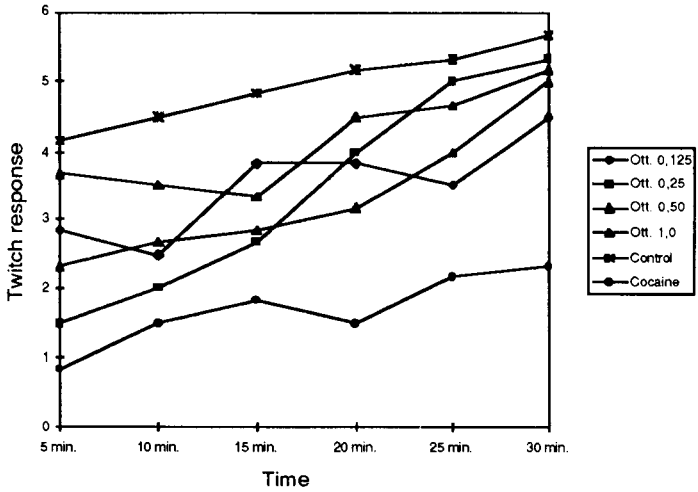

Figure 1. Effect of alcoholic extract of *Ottonia propinqua* on twitch response of guinea-pig skin through the time.

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