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Cocaine-Like Actions of *Erythroxylum argentinum* Schulz *(Erythroxylaceae)*

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SUMMARY. The crude alkaloid fraction (200 mg/kg, i.p.) from the leaves of *Erythroxylum argentinum* Schulz *(Erythroxylaceae)* was able to reverse the reserpine induced ptosis in mice and showed a local anesthetic effect (1%) in the twitch response in the guinea-pig skin test. The acute toxicity of the crude alcoholic extract was not excessive up to 1250 mg/kg in mice.

RESUMEN. "Acciones Símil-cocaína producidas por *Erythroxylum argentinum* Schulz *(Erythroxylaceae)*". El extracto alcaloidal crudo (200 mg/kg, i.p.) de hojas de *Erythroxylum argentinum* Schulz *(Erythroxylaceae)* demostró capacidad de reversión de la ptosis inducida por reserpina en ratones y una actividad anestésica (1%) por el método de la anestesia comparada con conejillos de las Indias. El efecto tóxico de extractos etanólicos crudos no fue significativo (1250 mg/kg) en ratones.

INTRODUCTION

The genus *Erythroxylum* is the exclusive natural source of cocaine, being represented in the state of Rio Grande do Sul (Brazil) by nine species. Several species have been used as purgative, astringent and central stimulant [1]. *Erythroxylum argentinum* Schulz *(Erythroxylaceae)* is a shrub or a small tree widespread in Argentina and the south of Brazil used in folk medicine as a stomachic and as a treatment for sinusitis or flu[1] . From the leaves of this species tropane alkaloids as tropacocaine, hygrine, cuscohygrine [2], methylecgonidine, 4-hydroxyhigrinic acid and 3-benzoyloxynortropane [3] were isolated. Analgesic and anti-inflammatory effects of the crude extracts were reported using animal models [4]. Novák *et al.* [5] demonstrated *in vitro* a cocaine-like activity of tropacocaine, the main alkaloid isolated from this species [2,3]. Therefore, the aim of this study was to investigate a possible pharmacological cocaine-like profile for *Erythroxylum argentinum*, as well as to assess its acute toxicity.

MATERIAL AND METHODS

Plant Material

The leaves of *Eythroxylum argentinum* were collected in Porto Alegre, Brazil, and identified by M. Sobral; a voucher specimen has been deposited at the Herbarium of the Departamento de Botânica do Instituto de Biociências - UFRGS, Porto Alegre, Brazil (ICN 87535).

Preparation of the crude alcoholic extract

Dry and powdered leaves (1000 g) were exhaustively extracted with 70° ethanol in a Soxhlet apparatus and the extracts were evaporated under reduced pressure at temperature lower than 60 °C (100 g of dry leaves affords 17,8 g of crude extract).

Preparation of the crude alkaloidal fraction

The ground, air-dried, leaves (200 g) were soaked with 25% aqueous NH_4OH and then exhaustively extracted with CH_2Cl_2 in a Soxhlet apparatus. This concentrated extract was parti-

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PALABRAS CLAVE: Erythroxylon argentinum , Alcaloides tropánicos, Efecto anestésico, Acción símil-cocaína.

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tioned with diluted HCl (0.1 N). The aqueous layer was alkalized with concentrated NH_4OH (pH 9,0), partitioned with CH_2Cl_2 and concentrated under vacuum to give a crude alkaloidal fraction (yield: 100 g of dry leaves provides 180 mg of crude alkaloidal fraction).

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 sensitivity of different parts of the shaved skin and the variation between animals, the doses of the test drugs (crude alkaloidal fraction 1%, cocaine hydrochloride 0.2% and saline + polysorbate 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 sensitivity 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 to eight animals were used for each test.

Reserpine-induced ptosis

Prevention

Groups of adult male Swiss mice (n = 8) were treated with crude alkaloidal fraction (200 mg/kg, i.p.), cocaine hydrochloride (40 mg/kg; i.p.) or saline + polysorbate 80 (i.p.) followed by reserpine (2mg/kg, i.p.) 30 min. later. Observation was taken 3 h after reserpine injection. All animals were touched before observation to obtain accurate scores of palpebral ptosis and recorded by two independent observers using the Rubin's ptosis scores [6].

Reversal

Groups of adult male Swiss mice (n = 8) were treated with reserpine (2 mg/kg, i.p.) and 3 h later received crude alkaloidal fraction (200 mg/kg, i.p.), cocaine hidrochloride (40 mg/kg, i.p.) or saline + polysorbate 80 (i.p.). Observation was taken 30 min after injection as described for prevention assay.

Toxicity assessment

Excessive toxicity

Male Swiss mice (25-40 g) were i.p. injected with the crude alcoholic extract (200 mg/kg; injection volume: 10 ml/kg). The control group received an equivalent volume of vehicle (Tris buffer 0.02 M). The test (n = 8) and the control (n = 7) groups were observed for 48 h under normal conditions with free access to food and water. The same procedure was done with 0.5% and 1.0% crude alkaloidal fraction (8 animals each group injected i.p. with 0.5 ml). The control group received an equal volume of vehicle (saline + 4 % polysorbate 80).

Median lethal dose (LD_{50})

For preliminary estimation of the median lethal dose (LD_{50}) male swiss mice (20-30 g) were i p. injected with the crude alcoholic extract at 250, 500, 750, 1000 and 1250 mg/kg (injection volume: 10 ml/kg). The control group received an equivalent volume of vehicle (saline + 4% polysorbate 80). The test and the control groups (2 animals each) were maintained under normal laboratory conditions with free access to food and water and observed 30 min, 45 min, 24 h, and 48 h after injection.

Statistical analysis

The data of twitch guinea pig responses were analyzed by one way analyses of variance -ANOVA- with repeated measures and post hoc comparison was performed by Student Newman Keull's test. The results of ptosis experiments were analyzed by Kruskall-Wallis followed by Dunn's test.

RESULTS

Twitch response of guinea-pig skin

The crude alkaloidal fraction reduced significantly the total twitch response of guinea-pig skin ($F_{2,19} = 4.76$; $p < 0.05$, Figure 1). This anesthetic effect was comparable to cocaine effect at tested doses in the first 10 minutes, when it was maximal (Figure 2).

Reserpine-induced ptosis

The crude alkaloidal fraction was abble to prevent reserpine-induced ptosis (H2 = 10,2; p < 0,01) (Table 1) but did not induce significant reversion of it (Table 2). Despite of this, it is possible observe in the Figure 3 that the extract showed a tendency to reverse reserpine-induced ptosis.

Toxicity assessment

Mice treated i.p. with the crude alcoholic extract at 200 mg/kg or with 5 mg of crude alka-

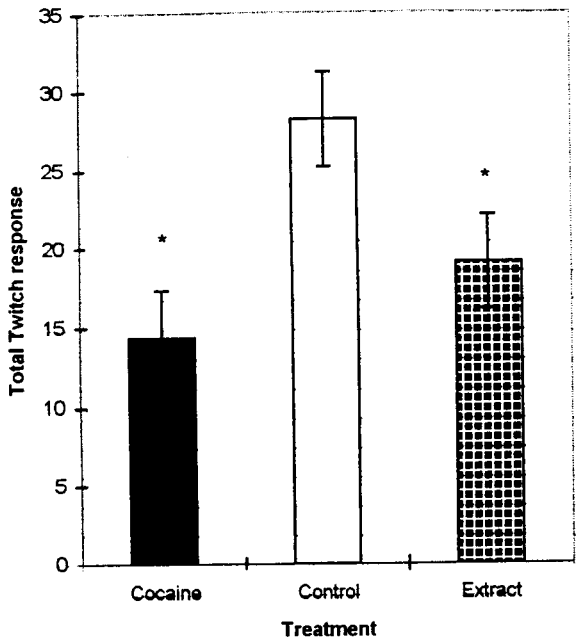

Figure 1. Effect of crude alkaloidal fraction of *E. argentinum* leaves (1%; s.c.) and cocaine hydrochloride (0.2%; s.c.) on total twitch response of guinea-pig skin * $F_{2.19} = 4.76$; $p < 0.05$; one way ANOVA with repeated measures and *post hoc* comparation by Student Newman Keull's test. The values are expressed as means ± standard deviation.

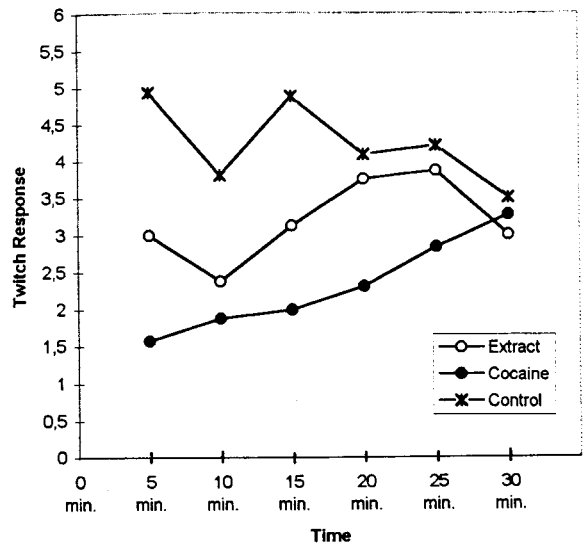

Figure 2. Effect of crude alkaloidal fraction of *E. argentinum* leaves (1%; s.c.) and cocaine hydrochloride (0.2%; s.c.) on twitch response of guinea-pig skin through the time.

Group	Median	25%	75%
Cocaine	3.50	3.00	4.00
Control	4.00	3.50	4.00
Extract*	2.00	2.00	2.75

* $H_2 = 10.2$; $p < 0.01$. Kruskall-Wallis followed by Dunn's test.

Table 1. Effect of crude alkaloidal fraction of *E. argentinum* leaves (200 mg/kg; i.p.) and cocaine hydrochloride (40 mg/kg; i.p.) on prevention of reserpine (2 mg/Kg; i.p.) induced ptosis.

Group	Median	25%	75%
Cocaine*	1.00	0.250	1.75
Control	3.75	2.5	4.00
Extract	1.75	1.00	3.00

* $H_2 = 7.86$; $p < 0.05$. Kruskall-Wallis followed by Dunn's test.

Table 2. Effect of crude alkaloidal fraction of *E. argentinum* leaves (200 mg/kg; i.p.) and cocaine hydrochloride (40 mg/kg; i.p.) on reversal of reserpine (2 mg/Kg; i.p.) induced ptosis.

loidal fraction (absolute value) observed for 48 h did not differ from controls, suggesting that toxicity of the extracts is not excessive.

The i.p. administration of crude alcoholic extract at 250, 500, 750, 1000, and 1250 mg/kg did not cause any deaths during the all observation period This is in agreement with low toxicity of *E. argentinum*.

DISCUSSION

The crude alkaloidal fraction of *E. argentinum* leaves reduced significantly the total twitch response in the guinea-pig skin. This anesthetic effect was transient, with maximal effect at the 10 min. These results are in agreement with aforetime reports about anesthetic cocaine-like activity of tropacocaine [5], which is the main alkaloid isolated from *E. argentinum* [3].

The crude alkaloidal fraction promoted the prevention of reserpine-induced ptosis but did not cause significant reversion of it although it was possible to observe a strong tendency to achieve that response. This effect could be attributed, at least in part, to the tropacocaine, since this alkaloid displayed sympathomimetic activity [7] and induced inhibition of dopamine

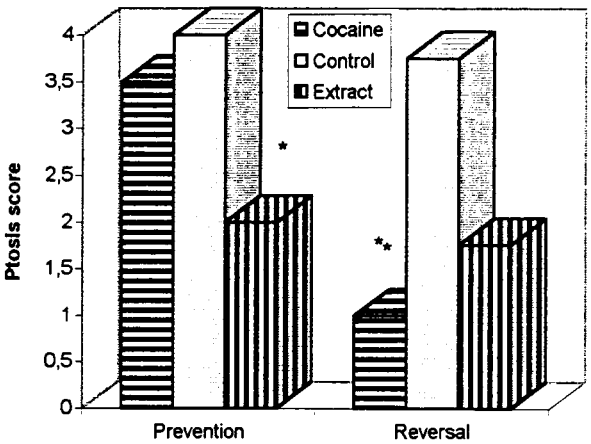

Figure 3. Effect of crude alkaloidal fraction of *E. argentinum* leaves (200 mg/kg; i.p.) and cocaine hydrochloride (40 mg/kg; i.p.) on prevention and reversal of reserpine (2 mg/Kg; i.p.) induced ptosis. * H_2=10.2; p<0.01. ** H_2 = 7.86; p<0.05. Kruskall-Wallis followed by Dunn's test. The values are expressed as median.

and norepinephrine neuronal reuptake [5]. The reserpine antagonism was the earliest animal model for detection of antidepressant drugs. However, in view of the diversity of protocols employed, it is difficult to draw general conclusion about the reserpine syndrome. The ptosis is considered mainly a peripheral symptom and psychostimulants, anticholinergics, analgesics and peripherally acting sympathomimetics can antagonize it [8].

In conclusion, the results obtained indicate that the leaves of *E. argentinum* possesses compounds with a pharmacological profile similar to cocaine in relation to the peripheral actions, however with low toxicity, since no signs of acute toxicity were observed for the alkaloidal and crude alcoholic extracts at the assayed doses. Considering our results and previous reports, the pharmacological data for *E. argentinum* match with the popular use as stimulant and for the sinusitis and flu.

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