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.

**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 tropine alkaloids as tropacocaine, hygrine, cuscohygrine 2, methylcgonidine, 4-hydroxyhygrinic acid and 3-benzoyloxyntropane 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 *Erythroxylum 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 87555).

**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 NH₄OH and then exhaustively extracted with CH₂Cl₂ in a Soxhlet apparatus. This concentrated extract was parti-
tioned with diluted HCl (0.1 N). The aqueous layer was alka
dized with concentrated NH₄OH
(pH 9.0), partitioned with CH₂Cl₂ and concen-
trated 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 then shaving the hairs on the lower back. It
was done 24 h in advance to obtain the disap-
pearance of any irritation produced by shaving.
To correct the variation in sensitivity of different
parts of the shaved skin and the variation be-
tween animals, the doses of the test drugs
(crude alkaloidal fraction 1%, cocaine hydro-
chloride 0.2% and saline + polysorbate 4%)were
given in different areas in a number of random
combimations. Equal volumes (0.25 ml)
of the drugs were injected intradermally and
the weals raised by the injected volumes were out-
lined 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 ex-
pressed as the total number of positive respons-
es out of 36 possible changes. Six to eight ani-
mals 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 (2 mg/kg, i.p.) 30 min. later. Obser-
vation was taken 3 h after reserpine injec-
tion. All animals were touched before observa-
tion to obtain accurate scores of palpebral ptosis
and recorded by two independent observers us-
ing 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 hydrochloride (40 mg/kg,
i.p.) or saline + polysorbate 80 (i.p.). Obser-
vation 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; in-
jection 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 con-
trol group received an equal volume of vehicle
(saline + 4 % polysorbate 80).

**Median lethal dose (LD₅₀)**

For preliminary estimation of the median lethal
dose (LD₅₀) male swiss mice (20-30 g) were
i.p. injected with the crude alcoholic ex-
tract at 250, 500, 750, 1000 and 1250 mg/kg (in-
jection 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 signifi-
cantly the total twitch response of guinea-pig
skin (F₂,₁₉ = 4.76; p < 0.05, Figure 1). This anes-
thetic 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 able to
prevent reserpine-induced ptosis (H² = 10.2; p <
0.01) (Table 1) but did not induce significant re-
version of it (Table 2). Despite of this, it is pos-
sible 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 ex-
tract 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 comparison by Student Newman Keull’s test. The values are expressed as means ± standard deviation.

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* *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.

The alkaloidal 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 aforesaid reports about anesthetic cocaine-like activity of tropacocaine $^4$, 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 \textit{E. argentimum} 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. 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.

In conclusion, the results obtained indicate that the leaves of \textit{E. argentimum} 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 \textit{E. argentimum} match with the popular use as stimulant and for the sinuses and flu.

REFERENCES