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CNS pharmacological effects of the hydroalcoholic extract of *Sida cordifolia* L. leaves

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Abstract

Sida cordifolia L. (Malvaceae), known as "malva branca", is a plant used in the popular medicine for the treatment stomatits, of asthma and nasal congestion. This work researched the acute toxicity of *Sida cordifolia* and its action on the central nervous system (CNS) because no data in the literature have been found about of pharmacological activity of this plant in the CNS. The hydroalcoholic extract of *Sida cordifolia* leaves (HESc) was used and the psychopharmacology approach began with the determination of LD_{50}, where a low toxicity was observed in mice. Depressive activity on CNS was demonstrated by several alterations in mice's behavior in the pharmacological screening. In the motility test, the HESc showed significant reduction of spontaneous activity at a dose of 1000 mg/kg (i.p.) at 30 and 60 min. The same form the HESc also decreased the ambulation and rearing in open-field test at 30, 60 and 120 min at a dose of 1000 mg/kg (i.p.).

Keywords: *Sida cordifolia*; Malvaceae; Medicinal plants; CNS depressant activity

1. Introduction

In Brazil *Sida cordifolia* is popularly known as "malva branca" or "malva branca sedosa" and found throughout the country with considerable distribution in the northeast region. It is used in the popular medicine for the treatment stomatits, of asthma and nasal congestion (Balbach, 1978).

This plant contains mainly alkaloids, oils, steroids, resin acids, mucin and potassium nitrate (Diwan and Kanth, 1999). Studies showed that the roots possess diuretic and tonic properties and administered for nervous disorders such as hemiplegia and facial paralysis (Rastogi and Malhotra, 1985).

Pharmacological investigation carried out with an aqueous extract of this plant's leaves demonstrated an anti-inflammatory and analgesic activity (Antoniolli et al., 2000). Although preliminary pharmacological studies with *Sida cordifolia* have been undertaken, there are no data about the pharmacological effects of this species on behavior and CNS. The aim of this study was to carry out a pharmacological behavioral screening, determine the acute toxicity and to evaluate the effects of HESc in psychopharmacological animal's models.

2. Material and methods

2.1. Plant material and preparation of extract

Sida cordifolia was colleted in the botanical garden of Universidade Federal de Sergipe (UFS) (Brazil) in January 1999. The plant was identified by Dr. C. Dias Silva Jr. and a voucher specimen (no. 30171) is deposited in the Herbarium of the Biology Department at the same institution. *Sida cordifolia*

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leaves were dried at 40 ± 1 °C and ground into a granulated powder. The extract was obtained using 494 g of this powder with EtOH 70% at 50 °C for 72 h in Soxhlet followed by filtration. The filtrate was concentrated in rotaevaporator at 50 ± 5 °C for 48 h, lyophilized for 8 h and stored at 5 °C, yielding 88 g of lyophilized active material. The extract was freshly prepared with 0.9% saline and cremophor (vehicle) for pharmacological experiments.

2.2. Animals

Male Swiss mice (weighing 25–35 g, 90 days old) were obtained from our research animal house and were maintained at controlled room temperature (21 ± 2 °C) on a 12 h light/dark cycle (lights on at 06:00–18:00 a.m.) with free access to food and water. All experiments were conducted between 8:00 and 13:00 h. Procedures were approved by the Laboratório de Tecnologia Farmacêutica Animal Care and Use Committee.

2.3. Drugs

Sodium pentobarbital and cremophor were purchased from Sigma (USA). All drugs and the HESc were immediately prepared before each assay and administered in a volume of 0.1 ml/10 g body weight (mice). At the time use, the extract was suspended in vehicle (saline 0.9% and one drop of cremophor) at the desired concentrations and the sodium pentobarbital was diluted in a saline 0.9% solution.

2.4. Acute toxicity

Different doses of HESc were administered intraperitonally (i.p.) (500–3000 mg/kg) and orally (p.o.) (500–5000 mg/kg), while the control group received only the vehicle. The groups were observed for 48 h and at the end of this period mortality was recorded for each group (Dietrich, 1983).

2.5. Pharmacological behavioral screening

Groups of 10 mice were treated with HESc at the dose of 1000 mg/kg, i.p. or p.o. (experimental) or vehicle (control) while behavioral effects were observed and quantified as described by Almeida et al. (1999). After the treatment, the mice were observed from 30 min up to 4 h for studying behavioral changes.

2.6. Spontaneous locomotion test

This experimental model was described by Carlini (1973) to evaluate the interference of a substance in the motor activity of the animals. Groups of 10 mice were treated with HESc of dose of 1000 mg/kg (i.p. or p.o.) or vehicle. The animals were placed in the activity cage (with a square area of 48 cm, 30 cm in height and demarcation squares of 12 cm × 12 cm). After 30, 60 and 120 min of treatment, the number of squares invaded within a period of 3 min were counted (De Lima et al., 1993; Almeida et al., 2001). The invasion criterion adopted was the presence of all paws of the animal within the square (Vásquez-Freire et al., 1994).

2.7. Open-field

This method is used to evaluate exploratory activity and emotionality of animals (Carlini et al., 1986). The open-field consisted of a white painted arena measuring 55 cm in diameter with a 100 W lamp. The floor of the arena was divided into several units by black painted lines. Groups of 10 mice were treated with HESc at dose of 1000 mg/kg (i.p. or p.o.) or vehicle. After 30, 60 and 120 min of administration, each mouse was placed in the center of the arena and defecation, ambulation, rearing and grooming were recorded for 5 min (Arletti et al., 2000).

2.8. Rotarod test

This method was described by Dunham and Miya (1957). Mice were placed on a rotating rod (2.5 cm diameter, rotating at 7 rpm) for a pre-selection and those able to remain on the rod for 3 or more minutes in two successive trials were selected for testing (Morais et al., 1998). After 24 h of pre-selection, groups of ten mice were treated with HESc at dose of 1000 mg/kg (i.p. or p.o.) or vehicle. After 30, 60 and 120 min of treatments the animals were placed on a rotative bar of the rotarod apparatus for 5 min and the time spent by each animal on the rotarod was recorded (Carlini and Burgos, 1979; Morais et al., 1998).

2.9. Pentobarbital-induced sleep time

This methodology evaluate the depressive action of a given drug in CNS that possess sedative activity and characteristics of a hypnotic drug (Carlini et al., 1986). Groups of 10 mice were treated with HESc at a dose of 1000 mg/kg (i.p. or p.o.) or vehicle. After 60 min of the pre-treatment the animals were treated with sodium pentobarbital (50 mg/kg, i.p.) (Pal et al., 1996; Perez et al., 1998; Morais et al., 1998). The time between loss and recovery of the righting reflex, taken as sleeping time, was recorded for the saline and the drug pre-treated animals (Speroni and Minghetti, 1988).

2.10. Statistical analysis

Calculation of the LD_{50} values with 95% confidence limits and comparisons of the results were performed using computerized linear regression analysis, in GraphPad Prism, version 3.02, a registered trademark of GraphPad Software Inc. The statistical analysis of data was made by analysis of variance (ANOVA) followed by Bonfferoni test. In all cases differences were considered significant if $p < 0.05$.

3. Results

3.1. Acute toxicity of HESc

The hydroalcoholic extract of *S. cordifolia* was toxic at high doses administered (i.p.). The LD_{50} values were 2639 mg/kg with 95% confidence limits of 2068–3367 mg/kg for i.p. administration. Deaths were not observed among orally treated animals.

3.2. Pharmacological behavioral screen of HESc

The HESc at a dose of 1000 mg/kg (i.p. and p.o.) produced sedation, decrease of the ambulation, reduction of answer to the touch, analgesia and decrease of urination. The effects of HESc were more pronounced in animals treated intraperitonally.

3.3. Effect of HESc on spontaneous motor activity in mice

The mice treated with HESc at a dose of 1000 mg/kg (i.p.) caused significant reduction ($p < 0.001$) of the spontaneous locomotor activity in comparison with the control group at 30 and 60 min. The animals treated p.o. showed a decrease ($p < 0.05$) of ambulation at 60 min (Fig. 1).

3.4. Effect of HESc in open-field

The HESc at a dose of 1000 mg/kg (i.p.) significantly ($p < 0.05$) reduced the ambulation of mice at 30, 60 and 120 min. On the other hand, the animals treated p.o. presented a decrease ($p < 0.05$) of ambulation at 60 and 120 min. Another important data observed was the reduction of rearing in animals treated at 30, 60 and 120 min, both i.p. or p.o. (Table 1).

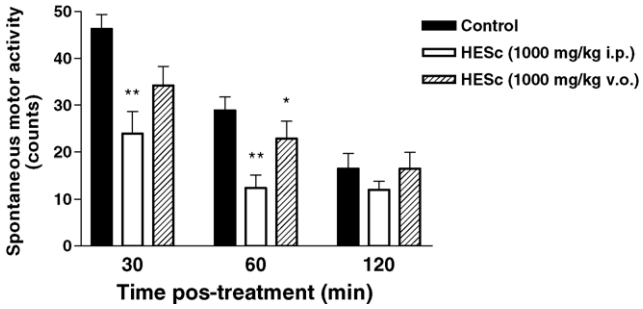

Fig. 1. Effect of HESc on spontaneous locomotion in mice. The values represent mean ± S.E.M. ($n = 10$); * $p < 0.05$, ** $p < 0.001$ significantly different from control.

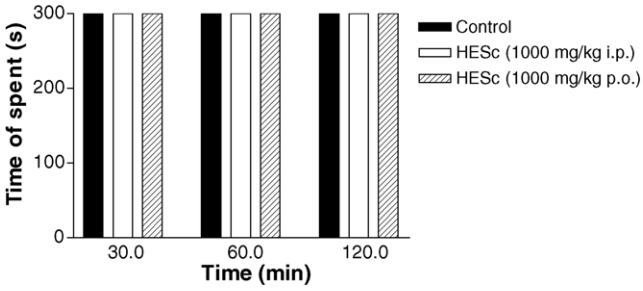

Fig. 2. Effect of HESc on the motor coordination. The values represent mean ± S.E.M. ($n = 10$).

3.5. Effect of HESc on rotarod test

The HESc at a dose of 1000 mg/kg (i.p. or p.o.) did not cause a significant difference in the motor coordination of the treated animals in comparison with the control group (Fig. 2).

3.6. Effect of HESc on pentobarbital-induced sleep time

The HESc at a dose of 1000 mg/kg (i.p. or p.o.) did not produce a significant alteration of the latency and the time of sleep of the treated animals in comparison with those from the control group (Fig. 3a and b).

Table 1
Effect of HESc on the open-field in mice

Time (min)	Groups	Dose/route (mg/kg)	Ambulation	Rearing	Grooming	Defecation
30	Control	–	54.2 ± 2.8	23.4 ± 3.9	1.0 ± 0.2	0.2 ± 0.2
	HESc	1000/i.p.	31.7 ± 7.5*	5.5 ± 2.8**	1.0 ± 0.3	0.1 ± 0.1
	HESc	1000/p.o.	54.9 ± 4.9	8.6 ± 2.8**	0.6 ± 0.2	0.3 ± 0.1
60	Control	–	41.6 ± 2.4	18.0 ± 2.7	1.8 ± 0.4	0.1 ± 0.1
	HESc	1000/i.p.	28.4 ± 4.4*	1.8 ± 0.8***	1.7 ± 0.5	0.1 ± 0.1
	HESc	1000/p.o.	29.4 ± 5.2*	6.5 ± 2.6**	1.7 ± 0.5	0.1 ± 0.1
120	Control	–	33.6 ± 3.8	10.1 ± 1.7	1.2 ± 0.4	0.1 ± 0.1
	HESc	1000/i.p.	12.9 ± 3.9*	1.5 ± 1.0**	1.5 ± 0.4	0.0 ± 0.0
	HESc	1000/p.o.	14.6 ± 3.7*	3.8 ± 2.1*	1.2 ± 0.4	0.2 ± 0.2

$n = 10$; values represent mean ± S.E.M.
* $p < 0.05$ significantly different from control.
** $p < 0.01$ significantly different from control.
*** $p < 0.001$ significantly different from control.

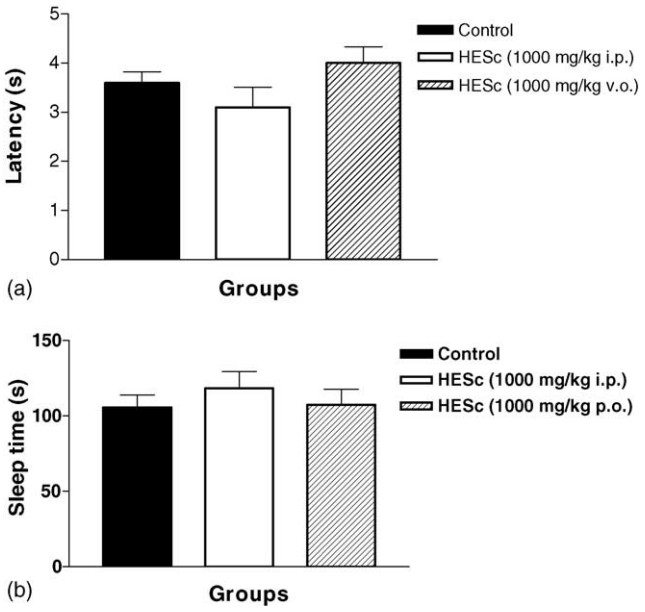

Fig. 3. Effect of HESc on: (a) the latency of pentobarbital-induced sleep and (b) the time of sleep induced pentobarbital. The values represent mean ± S.E.M. ($n = 10$).

4. Discussion and conclusion

In this work, the effects of the hydroalcoholic extract of *Sida cordifolia* leaves was studied in several behavioral animal models for the evaluation of central activity: pharmacological behavioral screening, spontaneous locomotion, open-field, pentobarbital-induced sleeping time and rotarod test. These are classical animal models of preliminary pharmacological tests of activities on CNS, which provide information about action upon psychomotor performance, motor behavior and neurotoxicity. Increasing doses of the hydroalcoholic extract of *Sida cordifolia* up to 5 g/kg administered to mice p.o. were not lethal, this is an indication of the low toxicity of the extract (Dietrich, 1983).

In pharmacological behavioral screening, the animals treated with HESc showed decrease of response to the touch and reduction of motor activity. These data are indicative of depressive activity of the CNS according to Almeida et al. (1999). It is important to note that the i.p. dose chosen make with basis in preliminary tests that showed more effective.

The general depressive activity was confirmed in the spontaneous locomotion test where the HESc significantly reduced spontaneous motor activity. The decrease in motor activity gives an indication of the level of excitability of the CNS (Masur et al., 1971) and this decrease may be related to sedation resulting from depression of CNS (Ozturk et al., 1996). This behavioral effect is similar to those obtained in previous studies of depressive drugs of CNS in agreement with Moore and Kenyon (1994, pp. 114–123), cited in Almeida et al. (2001); Radhakrishnan et al. (2001) affirmed that the reduction of motor activity can be due to the inhibitory effects of the extract in SNC or the muscular relaxing activity.

The HESc decreased the ambulation and the rearing in the open-field, test confirming the depressive property of the extract. According to Masur et al. (1971) the rearing is a response to the levels of excitability of CNS. It was also observed that mice treated with HESc did not present significant alteration in the time of spent in the rotative bar. Vale et al. (1999) affirmed that the absence of interference with the motor coordination in the rotative bar discards the possibility of a muscular relaxing effect.

Another methodology carried out to assess the depressant effect of HESc in CNS was the pentobarbital-induced sleep test. The results showed that the animals treated with HESc did not present any alteration in latency and sleep time. According to Lovell (1986), the animals' response in this model can be affected by environmental (diet, temperature and bedding material) and genetic factors. However, the depressive activity of HESc in mice was better evidenced by the decrease of ambulation in spontaneous locomotion and open-field tests. Therefore the absence of effects in motor coordination performance and in pentobarbital-induced sleep time suggests a possible absence of neurotoxicity.

On the basis of the present study, we may suggest that the HESc has depressant effect on CNS without interfering with motor coordination with a low toxicity, thus justifying its extensive use by the northeast Brazilian population. It is necessary to determine the major evidence indicating depressant activity as well as the possible action mechanisms.

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