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Acute toxicity and general pharmacological effect on central nervous system of the crude rhizome extract of *Kyllinga brevifolia* Rottb.

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Abstract

Acute toxicity and general pharmacological activities of the crude hydro-alcoholic rhizome extract of *Kyllinga brevifolia* Rottb., a popular medicine used in Paraguay, were investigated on mice. The intraperitoneal LD_{50} was found to be 575 mg/kg. Oral administration of doses up to 3.0 g/kg did not provoke any toxic symptoms. Oral administration of 100 mg/kg of the extract induced a significant increase in gastrointestinal transit. In open field studies, a decrease of spontaneous locomotor activity, piloerection, passivity, palpebral ptosis, catatonia and a stereotyped behaviour was produced by the extract when administered orally (1, 10 and 100 mg/kg). A significant decrease in respiration rate was observed (1, 10 and 100 mg/kg, p.o.) using a continuous flow respiration system. Lastly, doses of 1, 10 and 100 mg/kg, p.o. of the extract produced a significant increase in the hypnotic effect induced by pentobarbital in a dose-dependent manner. The latest effects could probably explain its rational use in traditional medicine to alleviate stress or as a sedative agent.

Keywords: *Kyllinga brevifolia* Rottb. rhizomes; Sedative activity; Central nervous system activity; Acute toxicity; Respiration

1. Introduction

The rhizomes of *Kyllinga brevifolia* Rottb. (Cyperaceae) (*Cyperus brevifolius* (Rottb.) Hassk) (Kapi-i katí) are used in Paraguayan traditional medicine as refreshing beverage and are claimed to possess diuretic, sedative and antispasmodic properties (Gonzalez Torres, 1992; Basualdo et al., 1995). However, we have found no scientific references on any experimental evaluation either about central nervous system activity, which traditional medicine ascribes to this plant, or about the toxicity of this plant.

Mental ailments are apparently a member of heterogeneous diseases and, as such, will probably always require a selected arsenal of antidepressive,

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antipsychotic, or anxiolytic agents with different modes of action for full treatment of the various manifestations of problems among the affected population (Baldessarini, 1990). Various cultures frequently maintain within their collection of traditional medicine substances valued as tonic or stimulant. Utilized by indigenous peoples for thousands of years to revitalize the body and mind, such substances may represent a potentially valuable, untapped source of active psychoactive drugs for treating mental diseases such as depression (Elisabetsky et al., 1992). The present study was undertaken to determine, on one hand, the acute toxicity (LD_{50}), and to evaluate the influence of the plant on respiration, sleeping time induced by pentobarbital and the general activity on central nervous system, by administration of the crude hydro-alcoholic extract of *K. brevifolia* Rottb. in mice.

2. Materials and methods

2.1. Plant material and preparation of extract

The rhizomes of *K. brevifolia* Rottb. (Cyperaceae) were collected in Paraguarí Department, Paraguay, in February 1991 and were identified at the herbarium of the Faculty of Chemical Sciences, where a voucher herbarium specimen has been deposited (Basualdo 2.900). The guaraní name of this plant is Kapi-i katí (meaning smelling grass). Fresh rhizome samples were air-dried and ground, yielding 459.5 g of powder. The powder was extracted with a mixture of ethanol:water (70:30) by a conventional reflux method for 1 h. The extraction was repeated three times and the filtered hydro-ethanolic extracts were mixed and evaporated under reduced pressure. The concentrated extract was frozen and finally freeze-dried to yield 31 g of lyophilized extract. Thus, 1 mg of lyophilized extract was obtained from 14.8 mg of dry rhizome powder.

2.2. Animals

The acute intraperitoneal and oral LD_{50}, the effect on gastrointestinal transit (propulsion) and the general behaviour activity of the crude extract were evaluated in Swiss adult albino mice of either sex and weighing between 20 and 30 g. A 12-h dark-light cycle, 23–25°C temperature and 50–60% humidity were maintained inside the animal room. The animals received standard food and before the experiments they were fasted overnight with water ad libitum.

2.3. Drugs

Sodium chloride was obtained from Sigma (St. Louis, MO, USA), neostigmine methyl sulphate from Shionogi Pharmaceutical (Japan), atropine sulphate from Fuso Pharmaceutical (Japan), and pentobarbital (Nembutal) from Abbott (Japan); ethanol, propylenglycol and charcoal for pharmaceutical use were purchased locally.

2.4. Acute toxicity

The extract was suspended in saline containing 1% propylenglycol and administered intraperitoneally to five groups of ten mice, and orally to another five groups of ten mice. The mice were kept under observation for 48 h.

2.5. Effect on general behaviour

The behavioural profile of albino mice under the influence of the extract was studied by placing the animals individually into the observation cage. The modifications or not in mood, awareness, spontaneous motor activity and autonomic activity by direct and simple observation were scored (0 to 4 +). Groups of five female mice (20–30 g) were administered orally with saline as a control (0.1 ml/10 g body weight), 1, 10 and 100 mg/kg of extract; likewise the other four groups were treated intraperitoneally with saline, 1, 10 and 100 mg/kg of plant extract. Observations were made during 1 h after extract administration.

2.6. Effect on locomotion (open field)

Plastic cages ($50 \times 40 \times 20$ cm) divided by permanent red marker into squares of 10 cm^2 at the bottom (on the external surface) were used. The

open field method, as described by Carlini (1973), was employed. First, four groups of five female mice (20–30 g) were intraperitoneally administered (in an individual sequence) with a volume of vehicle (0.1 ml saline/10 g body weight), 1, 10 and 100 mg/kg of extract, respectively. Immediately after injection, each mouse was introduced into a cage and the number of squares invaded during 2 min was counted. The observation was performed during 2 h.

2.7. Effect on respiration

The continuous flow measurements of respiration were performed by using five units of Respiration Monitor RM-80-1 (Columbus Instruments). All the animals received (both orally and intraperitoneally) for 1 week, a volume of 0.1 ml/10 g of body weight of saline, as a training for adaptation to the experimental conditions. After this training period, groups of five female mice (20–30 g) were administered (orally and intraperitoneally) a volume of vehicle (0.1 ml saline/10 g body weight), 1, 10 and 100 mg/kg of extract. The respiration rate 20 min before drug administration (considered as 100%) and during 3 h after both vehicle and extract administration were electronically recorded.

2.8. Pentobarbital sleeping time

Adult albino mice of either sex (20–30 g) were divided into groups of seven animals per cage. The extract, at doses of 1, 10, 100 mg/kg, and normal saline (0.1 ml/10 g body weight) respectively, were orally administered to each group, and 30 min after, each animal was injected with sodium pentobarbital (30 mg/kg, i.p.). The time since the injection to loss of the righting reflex (induction time) and the time from the loss of righting reflex to awakening (sleeping time in min) were registered for each animal (Carlini, 1973).

2.9. Effect on gastrointestinal transit in mice

Groups of 12 mice were treated with: (a) 0.3 ml of distilled water, (b) 10 μg/kg s.c. of neostigmine methyl sulphate, (c) 1 mg/kg, i.p. of atropine sulphate, and (d), (e) and (f) with 1, 10 and 100 mg/kg, p.o., respectively, with crude root extract suspended in saline solution. Both chemicals and extract were administered in a volume of 0.1 ml/10 g body weight. Then 30 min later, 0.3 ml of 10% aqueous charcoal suspension were administered orally to all the animals. After 40 min, the mice were sacrificed by ether anaesthesia and cervical dislocation, the small intestine was rapidly and carefully removed and aligned parallel to a ruler. The length traversed by the charcoal was calculated as a percentage of the total intestinal length (from pyloric sphincter to the ileo-coecal junction).

2.10. Statistical analysis

The results are expressed as mean $\pm$ S.D. and the statistical analysis of the data was performed by Student's t-test. Probability level of 0.05 was considered as statistically significant.

3. Results

3.1. Acute toxicity (LD_{50}) and effect on general behaviour

The intraperitoneal LD_{50} was found to be 575 mg/kg in 48 h of observation (95% C.L. 476–667 mg/kg). Oral administration of doses up to 3.0 g/kg did not show any toxic symptom in mice. Administration of 1, 10 and 100 mg/kg, p.o. of the extract and doses of 1 and 10 mg/kg, i.p. did not provoke any significant change in their general behaviour. However, a dose of 100 mg/kg, i.p. of the extract induced a slight decrease in locomotor activity, piloerection, passivity, palpebral ptosis, catatonia and a stereotyped behaviour.

3.2. Effect on mice locomotor activity and respiration

In the open field studies, a decrease of spontaneous locomotor activity was observed with 10 and 100 mg/kg, i.p., in a dose-dependent fashion (Fig. 1). Using the continuous flow respiration

system, the oral administration of 1, 10 and 100 mg/kg of the extract produced a significant decrease in respiration rate (Fig. 2). The intraperitoneal administration of 10 and 100 mg/kg of the extract provoked a decrease in respiration rate in a significant manner, whereas a dose of 1 mg/kg induced a significant increase in respiration rate (Fig. 3).

3.3. Effect on sleeping time induced by pentobarbital

Oral administration of 1, 10 and 100 mg/kg of the extract produced a significant increase in the hypnotic effect induced by pentobarbital in a dose-dependent fashion (Fig. 4).

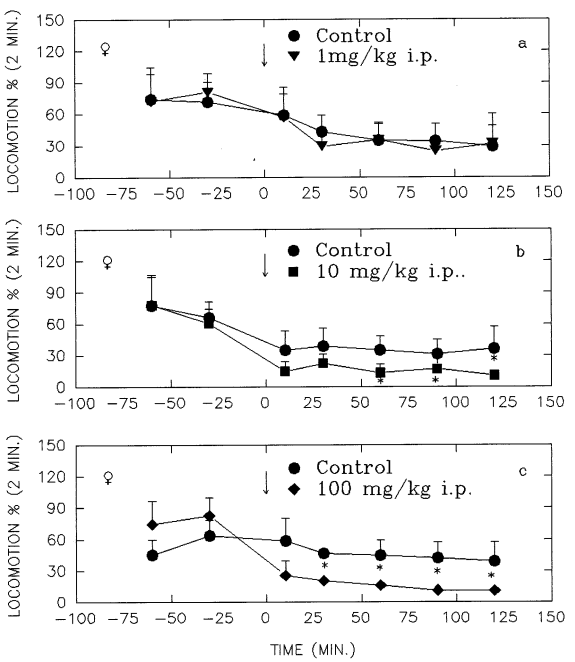

Fig. 1. Locomotion variation in mice (open field) after intraperitoneal administration of *K. brevifolia* extract. (a) Control: vehicle (●--●), extract 1.0 mg/kg (▼--▼). (b) Control: vehicle (●--●), extract 10.0 mg/kg (■--■). (c) Control: vehicle (●--●), extract 100.0 mg/kg (◆--◆). Locomotion before extract administration was considered to be 100%. The x-axis represents time course (min) for each dose. Each point represents the mean ± S.D. ($n = 5$). $^*P < 0.05$ was considered as significant.

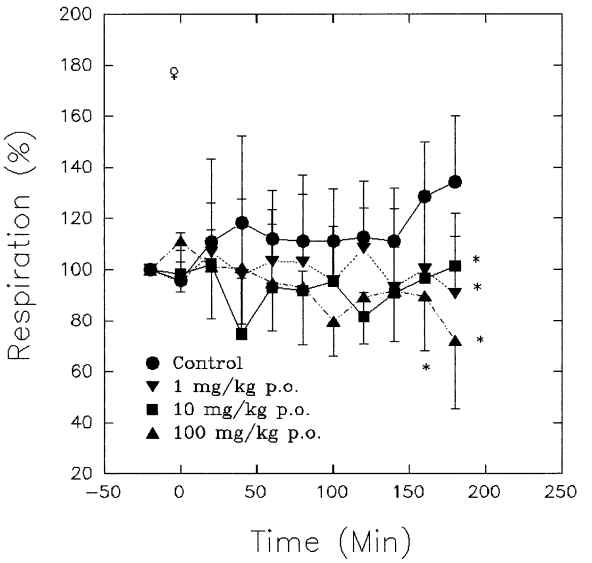

Fig. 2. Respiration rate variation in mice (continuous flow measurement) after oral administration of *K. brevifolia* extract. The respiration rates 20 min before extract administration were considered to be 100%. The x-axis represent time course (min) for each dose. Each point represents the mean ± S.D. ($n = 5$). $^*P < 0.05$ was considered as significant.

3.4. Effect on gastrointestinal transit

Oral administration of 100 mg/kg of the extract induced a significant increase in gastrointestinal transit in mice (Fig. 5).

4. Discussion and conclusions

The rhizome of *K. brevifolia* Rottb. is one of the resources of popular medicine in Paraguay for which activity on the central nervous system is claimed, although no scientific record exists about this effect. However, other members of Cyperaceae family, such as the genus *Rhynchospora*, have been previously studied (*R. corniculata*), showing antimicrobial activity in vitro against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Trichophyton mentagrophytes*, *Mycobacterium smegmatis*, *Candida albicans* and *Saccharomyces cerevisiae* (Mc Chensey and Adams, 1985). Concerning the chemical composition of this genus, studies per-

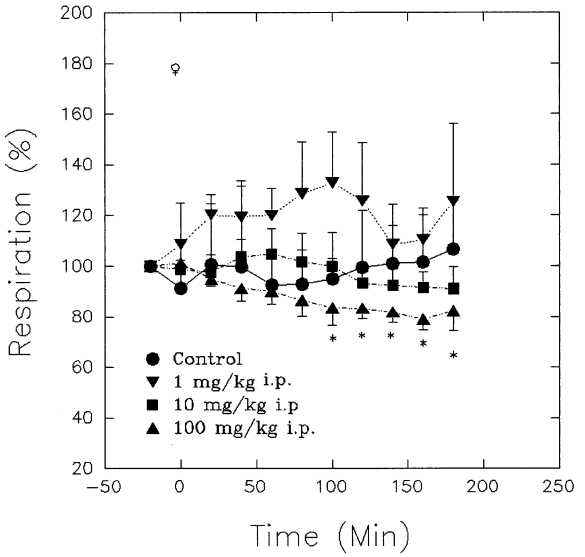

Fig. 3. Respiration rate variation in mice (continuous flow measurement) after intraperitoneal administration of *K. brevifolia* extract. The respiration rates 20 min before extract administration were considered to be 100%. The *x*-axis represent time course (min) for each dose. Each point represents the mean ± S.D. ($n = 5$). *$P < 0.05$ was considered as significant.

formed on leaves and inflorescence of *Rhynchospora brownii*, *Rhynchospora eximia*, *Rhynchospora gracillima*, *Rhynchospora heterochaeta*,

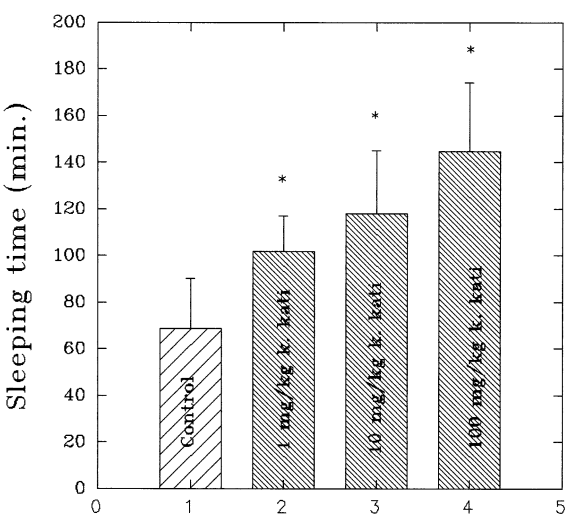

Fig. 4. Effects of *K. brevifolia* on sleeping time induced by pentobarbital ($n = 12$ per each dose). Each bar represents the mean ± S.D. *$P < 0.05$ was considered as significant.

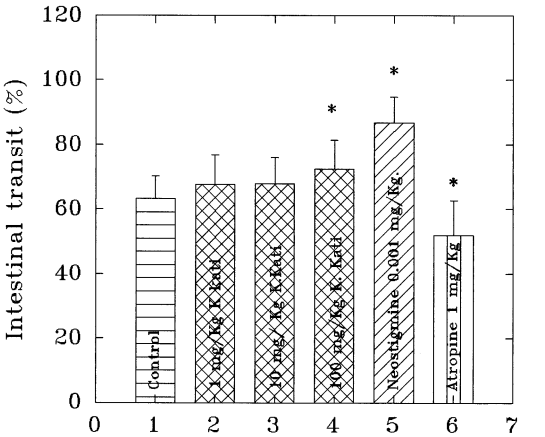

Fig. 5. Effects of *K. brevifolia* on mice gastrointestinal transit ($n = 12$ per each dose). Each bar represents the mean ± S.D. *$P < 0.05$ was considered as significant.

Rhynchospora leae, *Rhynchospora rubra* and *Rhynchospora triflora* showed the presence of flavonoids (Williams and Harborne, 1977; Harborne et al., 1985). Concerning the popular use of plants of this genus, only *Rhynchospora corymbosa* seeds are mentioned in the literature for the treatment of toothache in Puerto Rico (Stimson, 1971).

The results of the present study show that extract of *K. brevifolia* Rottb. exhibits very low toxicity, which is reflected by high LD_{50} values for both intraperitoneal and oral administrations and the absence of toxic symptoms. This property of the extract could be related to the popular use of the plant as a refreshing beverage (Gonzalez Torres, 1992). Oral administration of the extract induced a significant increase in gastrointestinal transit in mice. These effects suggest that the extract probably has no antispasmodic activity, as claimed by the Paraguayan folk medicine. Depression of general activity, decrease in spontaneous locomotor activity and decrease in respiration rate were observed by intraperitoneal administration of the extract in mice. These effects are the first parameters suggesting the sedative activity of the extract.

Concerning the effect on the central nervous system, doses of the crude hydro-alcoholic rhizome extract of *K. brevifolia* Rottb. (1, 10 and 100

mg/kg, p.o.) produced a significant increase in the hypnotic effect induced by pentobarbital, in a dose-dependent manner, thus suggesting a probable sedative activity. It should be emphasized that the method employed for this assay is considered as a very sensitive way to denote agents with depressor activity on the central nervous system (Carlini, 1973). The sedative effect recorded here may be related to an interaction with benzodiazepine receptors, since many benzodiazepines and related compounds that bind to receptors in the central nervous system have already been identified in certain plant extracts (Medina, 1990; Viola et al., 1993; Medina and Merder, 1996). Moreover, ligand for benzodiazepine receptors with anticonvulsant activity (Wolfman et al., 1993) and another with antagonistic activity on the receptor site (that may function as a memory enhancer) have been reported (Da Cunha, 1993). It would be important to analyse the potentially antianxiety, antidepressant, antipsychotic or anticonvulsant activities of the crude extract and purified fractions of *K. brevifolia* Rottb. in different animal models. Studies are underway to isolate the active principle(s) of the plant, and to determine the specific activity on the central nervous system.

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