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PHARMACOLOGICAL AND TOXICOLOGICAL EVALUATION OF *ALPINIA SPECIOSA*

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Alpinia speciosa *Schum or* A. nutans *is a plant of the Zingiberaceae family, known popularly as "colony" (colônia) and used as a diuretic and to control hypertension.*

We have determinated the concentration of Na^+ and K^+ found in the alcoholic extract and in the tea concoction. They contained 51.0 mEq Na^+, and 132 mEq K^+ in the extract, and 0,0 mEq of Na^+ and 26 mEq K^+ in the tea.

Phytochemical analysis of the leaves demonstrated the presence of catecquic tanins, phenols and alkaloids, and also some essential oils.

When injected intra-peritoneally the hydroalcoholic extract, in a dose range of 100 to 1400 mg/kg, (or 2500-18000 mg/kg orally) produced in mice: writhing, psychomotor excitation, hypokinesis and pruritus. The LD_{50} by ip was 0.760 ± 0.126 g/kg and 10.0 ± 2.5 g/kg by oral administration for the hydroalcoholic extract.

Subacute toxicity made by injecting daily for 30 days the LD_{10} in rats caused an increase in transaminases and lactate dehydrogenase, whereas other parameters such as blood glucose, urea and creatinine were normal. A histopathological analysis of liver, spleen, gut, lung and heart showed no alterations.

The drug also produced a prolongation of the sleeping time.

The hydroalcoholic extract induced in the rat and in the dog a dose-dependent fall in blood pressure in doses of 10 to 30 mg/kg. In isolated atria the extract induced a reduction of the frequency and in the inotropic responses.

Neither the extract nor the tea had an effect on the diuresis of the rat.

Key words: *Alpina speciosa* – Zingiberaceae – diuresis – toxicity – mice

Alpinia speciosa Schum or *A. nutans* is a plant of the Zingiberaceae family (Fig. 1), known popularly as "colony", originated from tropical Asia (Baily, 1953) and adapted in tropical America (Braga, 1976). It is often used in northeast Brazil as a tea concoction as a diuretic and a hypotensive agent (Matos, 1987, 1988).

In view of these properties we have decided to probe its pharmacological effects on experimental models using mainly its hydroalcoholic extract obtained from the leaves of the plant and to study its toxicity in acute and subacute models.

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MATERIALS AND METHODS

The hydroalcoholic extract from *A. speciosa* was obtained by trituration of 10 g of fresh leaves in a hot (previously boiled for 5 min) 50% hydroalcoholic solution.

After allowing the mixture to settle, the extract was filtered and the ethanol evaporated in a water bath at 80 °C. The final concentration of the extract was 20 mg/ml of dried material. The extract was stored at 2 °C, until its use.

Fig. 1: *Alpinia speciosa* Schum – (photo obtained in the medicinal botanical garden of the Federal University of Ceará).

To prepare the tea, 3 g of fresh leaves were added to make up to 200 ml of infusion in boiling water. This preparation was used only in subacute toxicological studies.

Na^+ and K^+ were determined by flame photometry (Moura, 1982).

The acute toxicity tests were made in mice by using the method of Miller & Tainter (1944). In some animals this was done by oral route, while in others by intraperitoneal (IP) injection of the test material. We have also determined the sub-acute toxicity in Wistar rats, by treating the animals for 30 days with extract, tea or water. For this we have administered the LDIO, of the drug daily. At the end of these experiments we have made a histopathological analysis of the liver, kidney, spleen, lungs and heart.

Several biochemical parameters were also evaluated such as urea, creatinine, glucose, transaminases, lactate dehydrogenase and alkaline phosphatase.

The effects of the drug on sleeping time was made by the method of Dandiya & Collumbine (1959).

Blood pressure was measured in rats and dogs by cannulation of the carotid and iliac arteries respectively and recording it with a Narco-Biosystems transducer and polygraph. For the injection of drugs the jugular or the femoral vein were catheterized.

The diuretic effects of the drugs were evaluated in rats according to the method of Timmermann et al. (1964) and Aston (1959) with some modifications. A positive control with hydrochlorathiazide (10 mg/kg) was also administered in a group of rats. The control animals received the same volume of vehicle. The urine was collected every hour for 5 h (Martz et al., 1962). Besides the urine volume we also measured the excreted sodium and potassium.

The general pharmacological properties of the extract was examined by experiments *in vivo* such as: the isolated rat atrium by the cumulative method of Van Rossum (1963); the isolated toad heart according to the method of Bulbring (1930); the guinea pig ileum preparation according to Magnus (1904); the isolated rectus abdominalis muscle of the toad by the method of De Jalon (1947); and the

isolated uterus by the method of Holton (1948).

RESULTS

The electrolyte concentration found in extract was 51.0 mEq for Na^+ and 132 mEq for K^+. The tea contained 26.0 mEq/L of K^+ and 0.00 for Na^+. Phytochemical analysis of the leaves demonstrated the presence of catechic tanins, free phenols, alkaloids and essential oils.

The drug promoted CNS excitation which was followed by depression and hypokinesia. A neural effect is also suggested by the finding that the extract promoted a prolongation of the sleeping time when 500-1000 mg/kg were administered orally, raising it respectively from 133 ± 7.0 min for the controls (pentobarbital) to 180 min ± 14.6 and 188.8 ± 15 min respectively. This demonstrates that the extract of Alpinia prolongs the sleeping time by acting either on the central nervous system or by prolonging the drug metabolism in the liver.

TABLE I

Acute toxicity of the hydroalcoholic extract of *Alpinia speciosa* by intraperitoneal injection of mice

Group[a]	Dose (mg/kg)	Number of dead animals	% of mortality	% of corrected mortality	Probit.	LD_{50} S. E. M. mg/kg
I	100	0/10	0	2.5	3.04	
II	400	2/10	20	20.5	4.15	
III	700	5/10	50	50,0	5,00	760 ± 1,26
IV	1000	7/10	70	70,0	5,52	
V	1400	10/10	100	97,5	6,96	

a: 10 animals per group.

TABLE II

Acute toxicity of the hydroalcoholic extract of *Alpinia speciosa* by oral route administration of mice

Group[a]	Dose (mg/kg)	Number of dead animals	% of mortality	% of corrected mortality	Probit.	LD_{50} S. E. M. mg/kg
I	2500	0/10	0	2.5	3.04	
II	5000	1/10	10	10,5	3,71	
III	8000	3/10	30	30,0	4,47	10000 ± 2500
IV	12000	6/10	60	60,0	5,25	
V	18000	10/10	100	97,5	6,96	

a: 10 animals per group.

TABLE III

Sub acute toxicity of the tea and extract of *Alpinia speciosa* or *nutans* in rats

Transaminase (U/ML x 0,482)				Lactate dehydrogenase (U/ML x 0.482)				
TGO (A)	TGO (B)	TGO (C)	TGP (A)	TGP (B)	TGP (C)	LDH (A)	LDH (B)	LDH (C)
11,90 ± 3,95*	77,40 ± 27*	64,0 ± 11,3	63,40 ± 8,76^{NS}	64,22 ± 11,09*	47,10 ± 2,64	1000,27 ± 82,75*	866,2 ± 108,6*	272,6 ± 53,9

n: 10 animals per group.
Groups of rats: (A): treated with tea (15 ml/kg) of weight – 1,5%; (B): treated with hydroalcoholic extract (5 g/kg of body weight); (C): control group – Rats treated with distilled water (1,5 ml/kg of body weight).
NS: (non significant); * – $P < 0,01$.

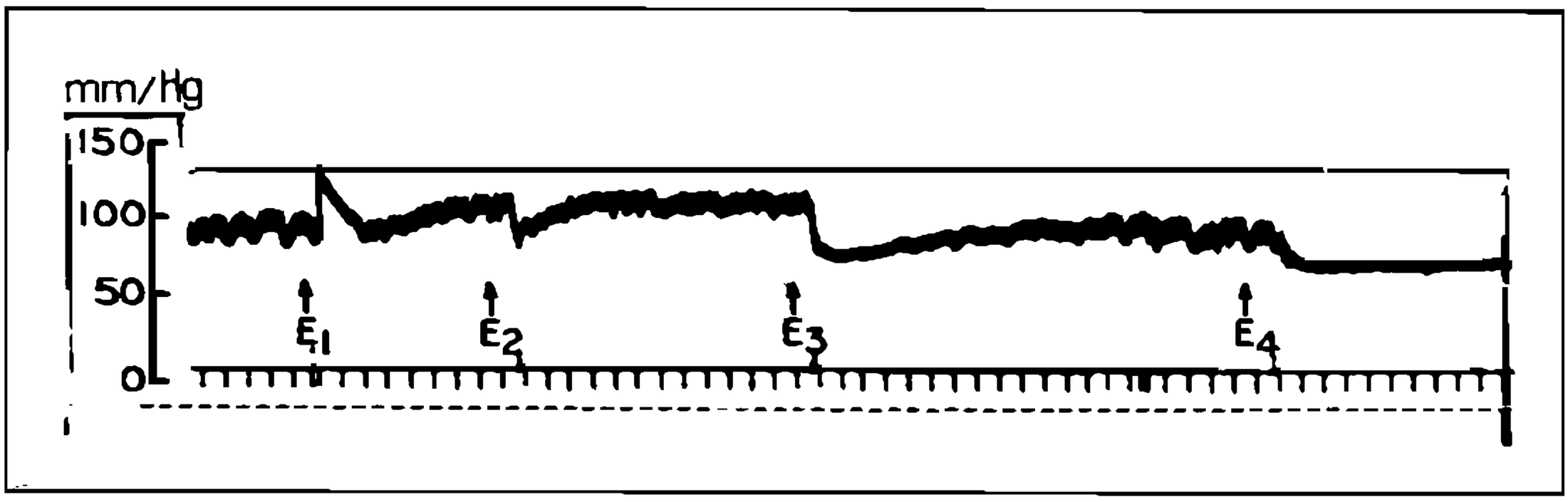

Fig. 2: effects of the hydroalcoholic extract of *Alpinia speciosa* on the rat blood pressure – E_1: Noradrenaline ($5{,}9 \times 10^{-5}$ M); E_2: extract (10 mg/kg); E_3: extract (20 mg/kg); E_4: extract (30 mg/kg).

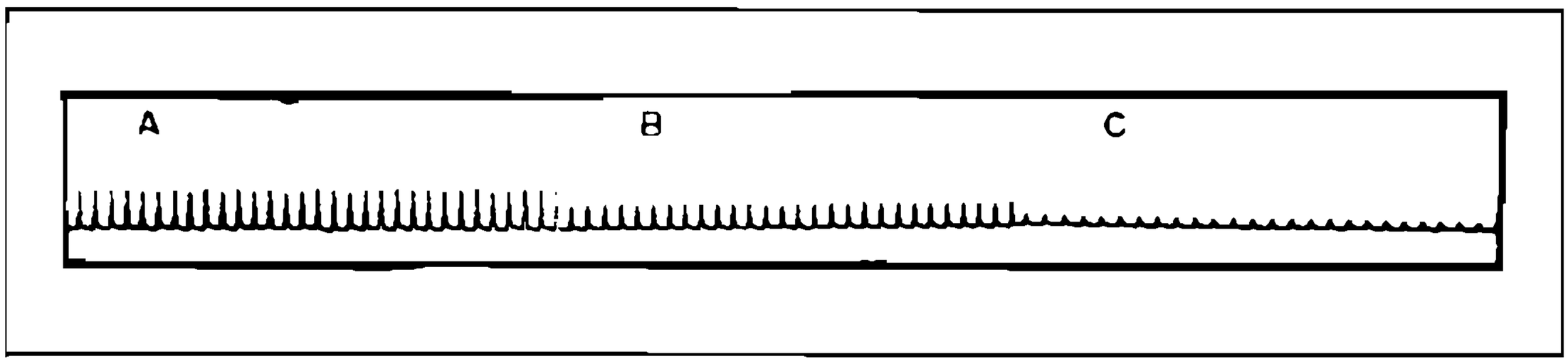

Fig. 3: effects of the hydroalcoholic extract of *Alpinia speciosa* in the isolated rat atria – A: control; B: 800 μg/ml (*Alpinia*); C: 1000 μg/ml (*Alpinia*).

The results obtained with acute and subacute toxicity are summarized in Tables I, II and III. Urea, creatinine, glucose and alkaline phosphatase values were all normals. The histopathologic analysis of the liver, spleen, kidney, lung and heart showed no signs of toxicity. The LD_{50} by oral route was 10 g/kg. During the subacute treatment by the drug in the rat we observed elevation of TGP, TGO and LDH. The hemogram made with peripheral blood demonstrated only elevated lymphocyte counts. The aqueous extract of leaves was practically atoxic in our experiments.

The results observed with the effects of the drug on the rat blood pressure are shown on Fig. 2. As in the dog, there is a remarkable fall in blood pressure which is maintained and can be seen after the first few minutes of injection. This is not caused by the high amount of K^+ present in the extract, because we know that the effect of these ions are transient.

The hypotensive effects induced by the extract were not blocked by atropine (1 mg/kg), propranolol (2 mg/kg) or hexamethonium (1 mg/kg).

As for the study of the diuresis, animals treated with either the tea or the extract, showed no increase of this parameter.

Our experiments *in vitro* demonstrated a nonespecific blockade of the extract for the action of Ach (10^{-6} M) and bradykinin (BK) (10^{-8} M) on guineapig ileum. As for histamine, we observed a potentiation which was followed by blockade. The extract inhibited also the action of Ach (10^{-6} M), oxytocin (10^{-5} M) and barium chloride (10^{-3} M) on the rat uterus. In the rectus abdominalis muscle, the effect of Ach was potentiated by the use of the drug. The extract promoted also a decrease in heart rate and inotropic response in isolated atria and in the perfused toad heart (Fig. 3).

DISCUSSION

Most of the studies done with *A. speciosa* are related to the rhyzomes, where several substances have been identified (Itokawa et al., 1980). The results presented here with the leaves have no parallel in the literature and only in rare occasions a screening of the leaves and flowers were done by Matos et al. (1966) and Carline et al. (1972).

Carline's group observed that the flower extracts were very toxic, results which are contrary to the generally observed data in our experiments. The plant is very well tolerated as seen in Tables I and II, by intraperitoneal and oral route respectively.

The hypotensive effects observed herein correspond with those of Vanderlinde et al. (1988).

The phytochemical analysis of the leaves demonstrated the presence of alkaloids, a finding that differs from earlier work of Mattos et al. (1966) and Haggag & Shamy (1977) which reported only saponins in leaves and rhizomes.

So, it is possible that the hypotensive effect verified in our experiments could be due to the alkaloids present in the leaves. The drug promotes also a prolongation of the sleeping time in rat what favors a central effect mediating the fall in blood pressure. Importantly, this effect is not blocked by the known autonomic antagonists. Though the extract promoted also a decrease in heart rate and in the inotropic response properties linked to an action at peripheral sites.

In conclusion, we observed in alcoholic extract of *A. speciosa* a very powerful effect in lowering the blood pressure in rats and dogs. The tea is less effective and the drug is devoided of significant toxicity, except for the increase in LDH, TGO and TGP which was observed during subacute treatment of rats. This of course, could be a species related observation. The results confirm ethnopharmacology claims for the effects of the plant.

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