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The Central Nervous System stimulant effects of the ethanolic extract from the toxic brazilian plant *Pseudocalymma elegans**

O extrato etanólico da planta tóxicca brasileira, *Psedocalymma elegans* apresenta efeitos estimulantes sobre o Sistema Nervoso Central

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SUMMARY

The effects of the ethanolic extract from the Brazilian toxic plant *Pseudocalymma elegans* (Vell.) Kuhlm. upon the behavior of mice were studied. Mice that received 1.6 to 3 g/kg of body weight extract presented seizure-like signs dying with a mean latency of 8 min. The LD_{50} in this situation was estimated in 1.8 g/kg. Mice that received extract 1 g/kg i.p. were observed in an open field 30 min later, presented a decrease in the number of rearings and an increase in the freezing time, without significant differences in the number of crossings, grooming time or number of fecal boluses compared to the control group. Mice submitted to the same treatment and tested on the elevated plus-maze presented a reduction in the percentage of entries and in the time spent in the open arms of the maze. These animals reduced the locomotor activity measured automatically and presented no difference in the muscular tonus, measured by the time of permanence hanging from a wire. These data suggest that the plant extract has compounds with stimulant effects upon the central nervous system: In a lower dose (1 g/kg) we observed behavioral effects that suggest an anxiogenic action of the extract without affecting the muscular tonus, and higher doses resulted in convulsions and death.

UNITERMS: Pseudocalymma elegans; Poisonous plants; Convulsions; Central nervous system; Mice.

INTRODUCTION

Pseudocalymma elegans (Vell.) Kuhlm. (Bignoniaceae) is a toxic plant that occurs in Brazil in the State of Rio de Janeiro (Mello[21], 1941; Tokarnia *et al.*[34], 1969). The plant is toxic to cattle (Tokarnia *et al.*[34], 1969), rabbits, guinea-pig (Tavares *et al.*[33], 1979) and goats (Tokarnia *et al.*[36], 1993). Signs of intoxication in cattle were generalized tremors, instability and death. In rodents intoxications, excitation, thachipnea, muscular tremors, pedling movements and death were observed. The signs of intoxication in goats were reluctance to walk, sternal decubitus, dispnea, frequent bleating, muscular tremors, lateral decubitus and death. Authors also reported that exercise aggravates the signs. Postmortem examinations and histopathological changes indicated no important lesions: The common signs observed in many animal species were slight alterations of the liver. In rodent's; cases of hemorrhages in the lungs were related (Tavares *et al.*[33], 1974) and in cattle and goats (Tokarnia *et al.*[34,36] 1969, 1993), alterations in the epithelial cells of the proximal convoluted tubules of the kidneys and necrosis of heart fibers of the myocardium.

Krebs[16,17] (1987, 1991) isolated three compounds from the plant: the iridoid glucosides lamiide and durantoside II and the flavonoid compound sorbarin. There were not found any publications about the pharmacology of these compounds in the last 10 years.

The pharmacological properties of the extract are relatively unknown, since no other publications were found in the last fifteen years. Since many medicaments and other biological active compounds have been found in plants, we decided to study the effects of the administration of the ethanolic extract of the plant to mice. This work describes the first results about the toxicity of the plant and some effects it produces on the central nervous system (CNS) of those animals. These results may be the starting point to knowing better the toxicity of the plant, and they may suggest pharmacological treatments in cases of intoxication. Results also suggest mechanisms of action of the active compounds from the plant. The biological tests used in this work may also be appropriate to guide a future work of purification of the active principles of the plant. Last, the compounds purified from this plant may represent promising drugs to the therapeutical arsenal, or may help us understand the functioning of the mammalian CNS.

MATERIAL AND METHOD

The Plant Extract

The growing purple stems and leaves of *P. elegans* were collected near Itaguaí city, Seropédica district, Rio de Janeiro state, Brazil, from August to October 1993, by Dr. Jürgen Döbereiner and Dr. Carlos H. Tokarnia, who gently gave us this material. They obtained the correct classification of the plant from the Botanic Museum Garden, of Rio de Janeiro. A picture of the plant is presented on Fig. 1. The samples were dried under ventilation and crushed in a mechanical mill. An amount of 50 g of the powder was suspended in 400 ml of ethanol 50% (v/v) and left in a boiling water bath for 15 min. Two hundred ml more of ethanol were added, and the suspension was maintained under agitation overnight. The extract was filtered twice in filter paper and concentrated in a rota-vapor in order to evaporate the ethanol. The remaining extract was re-extracted 5 times with 200 ml of petroleum ether and 3 other times with ethyl ether. The extracts were evaporated and dissolved in KOH 1 M. In the case of the ethanolic extract, dimethy ll-sulphoxide 20% (v/v) was added and the pH was corrected to 7 with HCL. Other extracts were dissolved in KOH 1 M and the pH was adjusted to 7 with HCL. Both the plant dried powder and the extracts were stored at -20°C until used. The flow card of the purification steps are presented in Fig. 2.

Figure 1
Pseudocalymma elegans, in natura, Itajaí city, Seropédia district, Rio de Janeiro state, Brazil

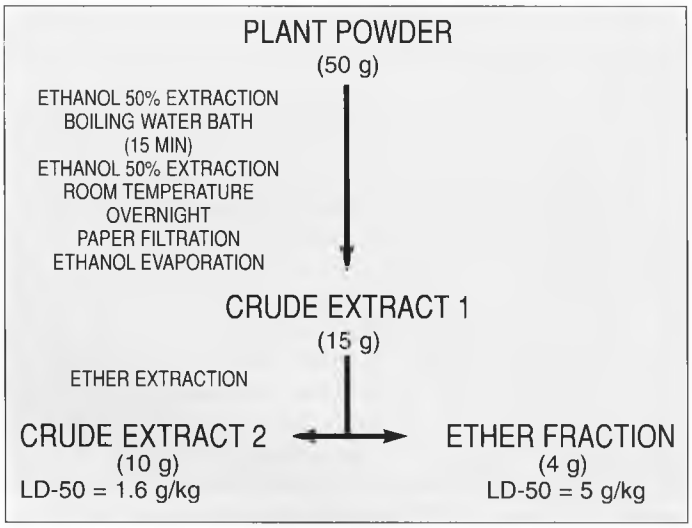

Figure 2
Flow chart showing the main steps in the preparation of the crude extract of *Pseudocalymma elegans* leaves. For more details see the description in Material and Method

Animals

Adult male mice (older than 2,5 months, 27-35 g) from our own breeding stock were used. Mice were housed after arrival in groups of 15 in 60 x 25 x 25 cm plexi-glass boxes in controlled temperature (25°C) and maintained on a 12-h light dark period (lights on 7:00h), with food and water available ad libitum. Tests were always performed from 12:00h p.m. to 06:00 p.m.

Tests of Toxicity

The initial experiments indicated that the main part of the toxic compound(s) from the plant was present in the ethanolic fraction (Fig. 2). For this reason, all the experiments were done with i.p. administration of the ethanolic extract. Parallel experiments showed that the plant extract is toxic to mice when administered i.p. or orally. In the oral administration, 14 animals were left without food for 12 hr before receiving the extract (3.5-9.0 g/kg of body weight). In experiments with i.p. administration, groups of 10 to 14 mice received the plant extract (0.1-3.0 g/kg; 0.1 ml/10 g of body weight; i.p.). A control group received the same volume of vehicle (dimethyl- sulphoxide 20% (v/v)) in NaCl 0,09% (p/v), i.p. The animals were observed for 48 hr. General effects, occurrence and latencies of seizure-like signs and deaths were registered.

Behavioral Tests

Two groups of 13 animals received ethanolic extract (1 g/kg, 0.1 ml/10 g of body weight) or a solution of NaCl 0,09% (p/v), dimethyl-sulphoxide 20% (v/v). After 30 min the animals were submitted to the following tests, successively:

1) open-field test; 2) elevated plus-maze test; 3) free locomotor activity test; 4) wire test. The battery of tests took about 18 min.

In the open field test each animal was allowed to explore freely an open field for 5 min. The open field consists of a round arena (100 cm diameter) painted in white and divided in 19 quadrants separated by marks of two concentric circles and lines dividing the spaces between the circles. The edges of the arena are limited by a 40 cm-high white wall. All the apparatus is covered with a fine curtain and illuminated with four 60 watts lamps, and during the tests a 60 dB white noise was turned on. Two observers registered the number of crossings by the marks of the field, the number of rearings, time spent with grooming, time the animal stays immobile (freezing) and, at the end of the session, the number of fecal boluses left by the animal. This procedure was described in detail by Masur[23] (1972) and the parameters measured are said to express the "emotionality" of the animal (Masur[23], 1972; Bike; Archer[1], 1974).

The elevated plus maze consists of four perpendicularly disposed wooden arms (5 x 30 cm, two had 15 cm-high wooden walls, and two were open), linked by a central 5 x 5 cm square. The maze was suspended 50 cm from the room floor. The animals were placed on the central part of the maze facing a closed arm. The time spent and the number of entries in the open and closed arms were counted during 5 min. This test was proposed first to rats (Pellow *et al.*[27], 1985) and has proved to be a good measure of the anxiety state of the animals. Lister [22] adapted the method for mice.

The free locomotor activity of the animals was tested in an authomatized box (20 x 50 x 20 cm) with three light arrows pointed to photocells. Whenever the animal crosses a light arrow, the apparatus counts a crossing. In this test the number of crossings was counted during 5 min.

The wire test consists in counting how long the animal hangs on to a tightly stretched wire. In this test we wait for the mouse to fall from the wire up to a top of 120 s. This test is sensitive to drugs with muscle relaxant properties.

Statistical Analysis

Differences in the latencies of death were evaluated by one-way ANOVA analysis of variance. LD_{50} was calculated from a linear regression of probit plot by the least squares method. Differences in the open field, elevated plus maze and free locomotor activity scores among groups were evaluated by Student's t-test. Differences in the latency of permanence in the wire-test were evaluated by Mann-Whitney U-test.

RESULTS

Toxic Effects of the plant

After i.p. administration of the ethanolic extract, animals ran quickly around their home box, sometimes stretched the posterior paws and walked with the back dorsum upraised. Few minutes after receiving doses higher than 1.6 g/kg, some animals presented convulsion-like signs and died. "Convulsions" consisted in oral and facial movements followed by stretching of all the body, and, sometimes, clonic-like convulsion movements. Duration of the "convulsion" is no more than 2 min and in almost all cases is followed by death. In most cases, death occurred around 8 min after the extract administration, and this latency is independent of the dose (Fig. 3). These results suggest that there may be a causal correlation between the occurrence of the convulsion-like signs and death (fatigue of diaphragm muscles?). Only in 3 cases-against 25 others-death occurred 1 to 2 days after extract administration. Here, the cause of lethality was probably different.

Parallel experiments were done with 14 animals that received from 3.5 to 9 g/kg of the extract orally. In this case the same signs of intoxication were observed, but they occurred around 10 to 60 min later, and the lethal dose was 3 times larger compared with the i.p. administration.

Fig. 4 shows extract dose curve versus lethality. The LD_{50} was 1.82 g/kg of body weight (linear regression from the probit plot; r = 0.962). As it must be observed, there is a small difference between the LD_{50} and the lowest dose causing incidence of death (0.7 vs 1.6 g/kg).

Behavioral Effects of the Plant Extract

Mice that received 1 g/kg of body weight of the plant extract, and were observed in an open field 30 min later, showed a reduction of 81% in the number of rearings ($t = 7.105$; $p < 0.001$) and a 50-time improving of the freezing time ($t = 2.238$; $p < 0.05$) compared to the control group. On the other hand, the number of crossings, time of grooming and number of fecal boluses presented no significant

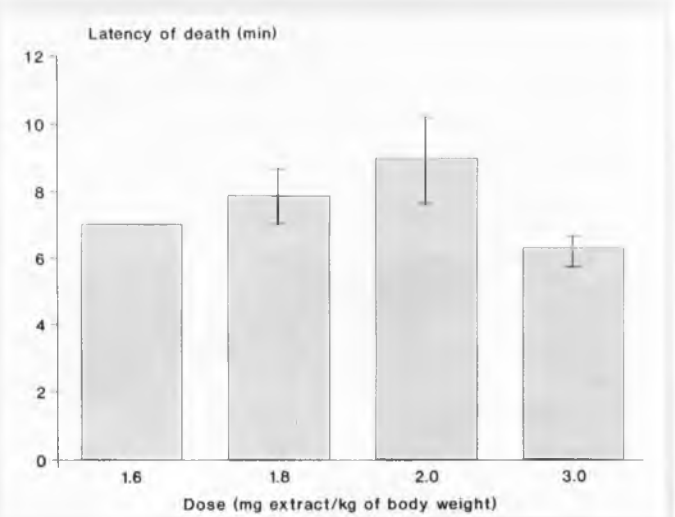

FIGURE 3
Mean latencies of death after the administration of the ethanolic extract from *Pseudocalymma elegans*. There is no significant difference among the groups (One-way ANOVA F(3,21) = 0,436. For calculations there were considered the animals that received the extract and died, that is: 1,6 g/Kg N = 1; 1,8 g/Kg N = 6; 2,0 g/Kg N = 10; 3,0 g/Kg N = 8.

differences from the control group (Tab. 1). The group that received the plant extract showed a preference for the closed arms of the elevated plus maze compared to the control group, as it can be seen on Tab. 2: The group that received the plant extract spent 21% more time in the closed arms ($t = 2.763$; $p < 0.02$), spent 17% less time in the open arms ($t = 3.013$; $p < 0.01$) and entered 18% less times in the open arms of the maze ($t = 4.076$; $p < 0.001$). Animals that received the plant extract also showed a reduction in the total number of entries in the arms of the maze (closed + open) ($t = 2,977$; $p < 0,01$) Locomotor activity of the mice was depressed by the plant extract administration ($t = 2,323$; $p < 0,05$), as it can be seen on Tab.3. This reduction was not caused by an alteration in the muscular tonus, since the time animals stayed hanging from a tightly stretched wire was not significantly different from the control group ($U = 81.5$; $p > 0.2$), as it can be seen on Tab. 4.

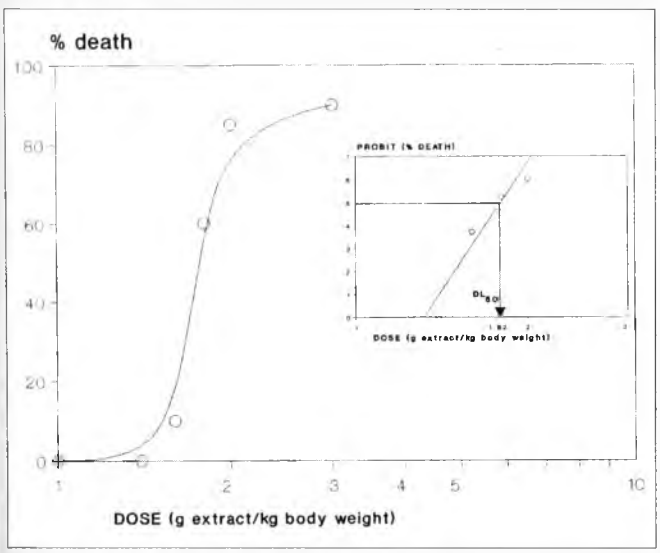

Figure 4
Lethality curve of the ethanolic extract from *Pseudocalymma elegans*. The data from groups of 10 to 14 animals were considered. Semi-log and problit-log plot. LD_{50} = 1.82 g of plant extract per Kg of body weight.

DISCUSSION

The general observations of intoxication signs with the ethanolic extract of *P. elegans* are similar to the previous descriptions of intoxication with the plant in other animal species. The observation that the animals ran quickly may be related to the "instability" described for cattle (Tokarnia *et al.*[34], 1969) or "excitation" in rabbits and guinea-pig (Tavares *et al.*[33], 1974). The convulsion-like signs resemble those of "generalized tremors" in cattle (Tokarnia *et al.*[34], 1969), "muscular tremors" and "pedling movements" in rabbits (Tavares *et al.*[33], 1974) and "muscular tremors" in a goats (Tokarnia *et al.*[36], 1993). Also, the reluctance of mice to walk are similar to those described by Tokarnia *et al.*[36], 1993 in goats. The lethal doses (upper then 1.6 g/kg) are similar to those observed for other animal species: 1 g of leaves/kg of body weight for cattle (Tokarnia *et al.*[34], 1969); 0.8 g of leaves/kg body weight for rabbits and guinea-pigs and 0.5 g/kg of leaves/kg of body weight for goats (Tokarnia *et al.*[36], 1993). The latency of onset of the signs varied from some minutes to few days in the studies mentioned above, while in the present study it always occurred in few minutes. The difference may be due to the fact that plant extract was administered i.p., while other authors used the intra-gastric via. In our hand, when it was administered orally, the latency for signs of intoxication to appear and the time of death accurred later. Other authors also used the plant homogenized instead of extracts. In this case, the coincidence of signs indicated that the ethanolic extract contains the toxic principles of the plant and that they are absorbed both when adminstered i.p. or orally.

P. elegans belongs to a class of Brazilian plants causing sudden death in cattle: *Palicourea*, *Mascagnia*, *Arrabidaea* and *Pseudocalymma* (Tokarnia *et al.*[35], 1990). The signs described above correlate in some ways with the signs reported to *P. marcgravii* in rodents. Peixoto *et al.*[26] (1987) described that in rabbits it causes violent uncontrolled movements followed by difficult and intermittent breathing and death. Gorniak *et al.*[12] (1989) reported that a crude extract of this plant caused tonic-clonic convulsions following administration in rats and rabbits, but caused depression in mice or even in rats when the extract is given i.v. or s.c.,

Table 1
Effect of the administration of the ethanolic extract from *Pseudocalymma elegans* upon the behavior of mice in an open field. Curitiba - PR, 15 a 20/09/93.

	N	Number of Crossings	Numbers os Rearings		Time of Freezing (s)		Time of Grooming (s)	Fecal Boluses
Vehicle	13	118.9 ± 4.9	37.4 ± 3.3		0.8 ± 0.5		8.5 ± 1.9	0.62 ± 0.29
Extract	13	106.8 ± 17.9	6.8 ± 2.8	(#)	40.5 ± 17.7	(*)	7.5 ± 4.4	0.08 ± 0.08

The animals received 1 g/kg of body weight of the plant extract i.p. and after 30 min they were observed in an open field during 5 min. The results are expressed as means ± standard error.
* $p < 0.05$; # $p < 0.001$, Student's t-test.

Table 2
Effect of the administration of the ethanolic extract from *Pseudocalymma elegans* upon the behavior of mice in an open field. Curitiba - PR, 15 a 20/09/93.

	N	total number entries		% entries open arms		% times spent open arms (s)		% time spent closed arms	
Vehicle	13	23.5 ± 1.7		57.2 ± 2.2		36.6 ± 3.7		34.7 ± 4.0	
Extract	13	16.1 ± 1.9	(#)	39.8 ± 3.7	(+)	19.9 ± 4.1	(#)	55.8 ± 6.5	(*)

The animals received 1 g/kg of body weight of the plant extract i.p. and after 35 min they were submitted to a 5 min session on an elevated plus maze. The results are expressed as means ± standard error.
* $p < 0.02$; # $p < 0.01$; $p < 0.001$, Student's t-test.

Table 3
Effect of the administration of the ethanolic extract from *Pseudocalymma elegans* upon the free locomotor activity of mice. Curitiba - PR, 15 a 20/09/93.

	N	Number of Crossings
Vehicle	13	67.00 ± 8.35
Extract	13	40.46 ± 7.80 (*)

The animals received 1 g/kg of body weight of the plant extract i.p. and after 40 min they were submitted to a 5 min session in an automated free locomotor apparatus. The results are expressed as means ± standard error.
* $p < 0.05$, Student's t-test.

Table 4
Effect of the administration of the ethanolic extract from *Pseudocalymma elegans* upon the muscular tonus of mice. Curitiba - PR, 15 a 20/09/93.

	N	time (s)
Vehicle	13	40 (13.7 / 120)
Extract	13	46 (16 / 99)

The animals received 1 g/kg of body weight of the plant extract i.p. and after 45 min were left hanging from a tightly stretched wire. The results are expressed the medians (interquartile range) of the time of permanence hanging from the wire. The Mann-Whitney U-test detected no differences between the group that received vehicle or the one that received the plant extract.

respectively. The same author also described occurrence of salivation and movement difficulties after intoxication. The toxic effect of *P. marcgravii* and *A. bilabiata* is supposed to be caused by the presence of the toxin monofluoracetic acid (MFA) in the leaves of these plants (Echkschmidt *et al.*[9], 1989; Krebs *et al.*[18], 1994). Echkschmidt *et al.*[9] (1989) showed that some general neurotoxic signs observed in rats intoxicated with *P. marcgravii*, such as tonic-clonic convulsions, are similar to the signs observed after intoxication with MFA. This compound inhibits the aconitase enzyme from the tricarboxylic acid cycle and blocks the synthesis of glutamine (Echkschmidt *et al.*[9], 1989). If MFA is one of the toxic compounds of *P. elegans* it could decrease the energy supply to the neurons, modifying the level of all neurotransmitters. In this case, its action would be inespecific and not addressed to a particular neurotransmitter or neurorreceptor. In the case of *P. elegans*, although there is no notice of purification of MFA from this plant, the possibility that the neurotoxic signs observed were caused by this compound cannot be excluded.

Previous studies suggested that the animals intoxicated with the plant presented slight anatomopathological alterations (Tokarnia *et al.*[34, 36] 1969, 1993; Tavares *et al.*[33], 1974). In the present study, acute effects of the plant extract were observed so it is not probable that these effects are due to the anatomopathological changes described previously.

The seizures elicited by the plant extract parallel the effects of many CNS stimulant drugs. In particular, antagonists of the complex receptor benzodiazepine/ GABA/ cloride channel, like picrotoxin, bicuculin and anxiogenic beta-carbolines (BCCB, DMCM), may elicit seizures in mice (Amabeoku[1], 1992). As it was expected, GABA receptor agonists such as muscimol, and baclofen or benzodiazepine receptor agonists such as diazepam, or barbiturates such as phenobarbitone, antagonize chloroquine elicited seizures (Amabeoku[1],1992). Caffeine also induces tonic convulsive seizures when applied intravenously in mice (Inano[15], 1992).

The plant extract administration reduced free locomotion activity of mice, an effect opposite to that observed after ethanol administration (Lister[7], 1988; Durcan *et al.*[8], 1989; Paeivaerinta; Korpi[25], 1993), benzodiazepines (Lister[20], 1988) and adenonise receptor antagonists (Griebel *et al.*[13], 1991).

The elevated plus maze test is particularly sensitive to anxiolytic drugs such as benzodiazepines and GABA receptor agonists (File; Aranko[11], 1988; Pellow *et al.*[27], 1985), and ethanol (Lister[22], 1988). In general these drugs improve the preference of mice for the open arms of the maze, an anxiolytic action (Lister[21, 22], 1987, 1988). The behavior of mice and rats in the elevated plus maze is also affected by antagonists of the glutamate receptor type N-methyl l-D-aspartate (NMDA) (Sharma; Kulkarni[30], 1991; Guimarães *et al.*[14], 1991), by drugs that alter serotonergic function (Audi *et al.*[2], 1991; Benjamin *et al.*[3], 1990; Briley *et al.*[5], 1990), by adrenergic receptor agonists (Lapin[19], 1993), by cocaine (Rogerio; Takahashi[28, 29], 1992) and by adenonise receptor antagonists (Zangrossi *et al.*[39], 1992).

The plant extract administration caused a decrease in the number of rearings and an increase in the time of freezing in the open field test. This test is also sensitive to the "fear/anxiety state" of the animal. Treatments that generate anxiety such as inescapable footshock developed a decrease of behavioral activity and an increase of defecation in an open field (Lemoine *et al.*[20], 1990; Vandijken *et al.*[37], 1992; Weyers *et al.*[38], 1990). The responses observed in the open field correlate to the MAO (monoamino-oxidase) activity (Lemoine *et al.*[20], 1990) and higher plasma catecholamine levels (Weyers *et al.*[38], 1990), an indicative of increased autonomic activity. Anxiolytic drugs like ethanol (Lister[22], 1988) that act in the 5-HT receptors (Stefanski *et al.*[32], 1992), increase the exploration of mice in the open field. Dopaminergic DA_1/DA_2 agonists (SHF 38393/bromocriptine) increase while DA_2/DA_3 antagonists (SCH 23390/zetidoline) decrease the exploration behavior in this test (Bruhwyler *et al.*[6], 1991).

The results discussed above indicate the presence of CNS stimulant compounds with "anxiogenic" actions in the plant extract. Although some CNS depressants, such as the benzodiazepine receptor agonists (diazepam, midazolam, tetrazepam) cause muscle relaxant effects (Farkas *et al.*[10], 1989; Simiand *et al.*[31], 1989), the administration of the plant extract did not alter the muscular tonus of mice.

In conclusion, signs of mice intoxication with the plant extract are similar to the signs previously described to cattle, rabbits, guinea-pig and goats. These signs, in particular the seizure-like one, and, at lower doses, the anxiogenic-like effects of the extract in bahavioral tests suggest that the plant contains CNS stimulant compounds. These compounds may be similar to, or perhaps the proper MFA purified from the toxic plant *P. marcgravii*, but alternatively, they may be new compound(s). These compounds may interact with many CNS neurotransmitter systems to produce the intoxication signs reported in this work.

Results presented in this work contribute to the establishment of a model to study the signs of the plant intoxication in mice, animals appropriate to large scale tests, in a future purification step. The results may also contribute to the design of new experiments, in a future project to find pharmacological treatments in cases of intoxication with this plant.

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RESUMO

Os efeitos do extrato etanólico da planta tóxica *Pseudocalymma elegans* (Vell.) Kuhlm. sobre o comportamento de camundongos foi estudado. Camundongos que receberam injeções intraperitoneais (i.p.), nas doses de 1.6 a 3 g/kg de peso corporal, apresentaram convulsões e morreram com uma latência média de 8 min. A LD_{50} foi estimada em 1.8 g/kg. Os camundongos que receberam 1 g/kg (i.p.) do extrato apresentaram um maior número de "rearings" e um maior tempo de "freezing" do que o grupo controle, quando observados em um campo aberto 30 min após a injeção. Durante o tempo em que esses animais foram observados no campo aberto não ocorreram alterações significativas no número de cruzamentos, tempo de "grooming" e número de bolos fecais. Quando esses animais foram colocados em um labirinto em cruz elevado exploraram menos os braços abertos do labirinto que os animais controle: apresentaram uma menor porcentagem de entradas e uma menor porcentagem de tempo de permanência nos braços abertos do labirinto. Esses animais apresentaram também uma menor atividade locomotora medida de forma automatizada e nenhuma alteração no tônus muscular, avaliado pelo tempo de permanência em um arame esticado. Os três primeiros testes sugerem que a administração de doses moderadas do extrato desencadeia um efeito "ansiogênico" contrário ao observado com a administração de ansiolíticos depressores do sistema nervoso central (SNC). Doses maiores do extrato provocam uma super-estimulação do SNC com convulsões que, eventualmente, podem contribuir para a letalidade do extrato.

UNITERMOS: *Pseudocalymma elegans*; Plantas venenosas; Convulsões; Sistema nervoso central; Camundongos.

REFERENCES

1-AMABEOKU, G. Involvement of GABAergic mechanisms in chloroquine-induced seizures in mice. **General Pharmacology**, v.23, p.225-9, 1992.

2-AUDI, E.A.; OLIVEIRA, R.M.W.; GRAEFF, F.G. Microinjection of propranolol into the dorsal periaqueductal gray causes an anxiolytic effect in the elevated plus-maze antagonized by ritanserin. **Psychopharmacology**, v.105, p.553-7, 1991.

3-BENJAMIN, D.; LAL, H.; MEYERSON, L.R. The effects of 5-HT sub (1B) characterizing agents in the mouse elevated plus-maze. **Life Science**, v.47, p.195-203, 1990.

4-BIKE, L.I.A.; ARCHER, J. Open-field behaviour of oestrous and dioestrous rats: Evidence against an "emotionality" interpretation. **Animal Behaviour**, v.23, p.509-12, 1975.

5-BRILEY, M.; CHOPIN, P.; MORET, C. Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. **Psychopharmacology**, v.101, p.187-9, 1990.

6-BRUHWYLER, J.; CHLEIDE, E.; LIEGEOIS, J.F.; DELARGE, J.; MERCIER, M. Effects of specific dopaminergic agonists and antagonists in the open-field test. **Pharmacology Biochimestry Behavior**, v.39, p.367-71, 1991.

7-DURCAN, M.J.; LISTER, R.G. Time course of ethanol's effects on locomotor activity and anxiety in mice. **Psychopharmacology**, v.96, p.67-72, 1988.

8-DURCAN, M.J.; LISTER, R.G.; LINNOILA, M. Behavioral effects of alpha sub(2) adrenoceptor antagonists and their interactions with ethanol in tests of locomotion, exploration and anxiety in mice. **Psychopharmacology**, v.97, p.189-93, 1989.

9-ECHKSCHMIDT, M.; BRIZOLA, M.; TARRAGA; PALERMO NETO, J. Is monofluoracetic acid the active neurotoxic principle in *Palicourea marcgravii* (St Hill) leaves? **Brazilian Journal of Medical and Biological Research** v.22, p.975-7, 1989.

10-FARKAS, S.; TARNAWA, I.; BERZSENYI, P. Effects of some centrally acting muscle relaxants on spinal root potentials: A comparative study. **Neuropharmacology**, v.28, p.161-73, 1989.

11-FILE, S.E.; ARANKO, K. Sodium valproate and chlordiazepoxide in the elevated plus-maze test of anxiety in the rat. **Neuropsychobiology**, v.20, p.82-6, 1988.

12-GORNIAK, S.L.; PALERMO NETO, J.; SPINOSA, H.J.; SPINOSA, H.J. Effects of *Palicourea marcgravii* on laboratory rodents. **Journal of Ethnopharmacology**, v.25, p.221-6, 1989.

13-GRIEBEL, G.; SAFFROY-SPITTLER, M.; MISSLIN, R.; REMMY, D.; VOGEL, E.; BOURGUIGNON, J.J. Comparison of the behavioral effects of an adenosine A sub(1)/A sub(2)-receptor antagonist, CGS15943A, and A sub(1)-selective antagonist, DPCPX. **Psychopharmacology**, v.103, p.541-4, 1991.

14-GUIMARÃES, F.S.; CAROBREZ, A.P.; AGUIAR, J.C.; GRAEFF, F.G. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey: **Psychopharmacology**, v.103, p.91-4, 1991.

15-INANO, S. Effects of agonists and antagonists of benzodiazepine, GABA and NMDA receptors, on caffeine-induced seizures in mice. **Japanese Journal Psychopharmacology**, v.12. p.199-205, 1992.

16-KREBS, H.C. Ingredients from *Pseudocalymma elegans* (Vell.) Kuhl.: I Isolation of allantoin, lamiide and sorbarin. **Zeitschrift für Naturforschurig Bio Chemie Sciences**, v.42, p.1361-4, 1987.

17-KREBS, H.C. Ingredients from *Pseudocalymma elegans* (Vell.) kuhlm.: I Isolation of a new iridoid glucoside and of durantoside. **Zeitschrift für Naturforschurig Bio Chemie Sciences**, v.46, p.1258-60, 1991.

18-KREBS, H.C.; KEMMORLING, W.; HABERMEHL, G. Qualitative and quantitative determination of fluoracetic acid in *Arabidea bilabiata* and *Palicourea marcgravii*. **Toxicon**, v.32, p.909-13, 1994.

19-LAPIN, I.P. Anxiogenic effect of phenylethylamine and amphetamine in the elevated plus-maze in mice and its attenuation by ethanol. **Pharmacology, Biochemistry and Behavior**, v.44, p.241-3, 1993.

20-LEMOINE, A.P.; ARMANDO, I.; BRUN, J.C.; SEGURA, E.T.; BARONTINI, M. Footshock effects heart and brain MAO and MAO inhibitory activity and open field behavior in rats. **Pharmacology, Biochemistry and Behavior**, v.36, p.85-8, 1990.

21-LISTER, R.G. The use of plus-maze to measure anxiety in the mouse. **Psychopharmacology**, v.92, p.180-5, 1987.

22-LISTER, R.G. Interactions of ethanol with benzodiazepine receptor ligands in tests of exploration, locomotion and anxiety. **Pharmacology, Biochemistry and Behavior**, v.31, p.761-6, 1988.

23-MASUR, J. Sex differences in "emotionality" and behavior of rats in the open-field. **Behavioral Biology**, v.7, p.749-54, 1972.

24-MELLO, E.M.M.; FERNANDES, J.S. **Contribuição ao estudo de plantas tóxicas brasileiras**. Serviço de Informação Agrícola, Min. Agricultura, Rio de Janeiro, 1941.

25-PAEIVAERINTA, P.; KORPI, E.R. Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. **Pharmacology, Biochemistry and Behavior**, v.44, p.127-32, 1993.

26-PEIXOTO, P.V.; TOKARNIA, C.H.; DOBEREINER, J.; PEIXOTO, C.S. Intoxicação experimental por *Palicourea marcgravii* (Rubiaceae) em coelhos. **Pesquisa Veterinária Brasileira**, v.7, p.117-29, 1987.

27-PELLOW, S.; CHOPIN, P.; FILES, S.; GRILEY, M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. **Journal of the Neuroscience Methods**, v.14, p.149-67, 1985.

28-ROGERIO, R.; TAKAHASHI, R.N. Anxiogenic action of acute but not repeated cocaine administration in handling-habituated mice in the plus-maze test. **Brazilian Journal of Biological Research**, v.25, p.713-6, 1992.

29-ROGERIO, R.; TAKAHASHI, R.N. Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze. **Pharmacology, Biochemistry and Behavior**, v.43, p.631-3, 1992.

30-SHARMA, A.C.; KULKARNI, S.K. MK-801 produces antianxiety effect in elevated plus-maze in mice. **Drug Development Research**, v.22, p.251-8, 1991.

31-SIMIAND, J.; KEANE, P.E.; BIZIERE, K.; SOUBRIE, P. Comparative study in mice of tetrazepam and other centrally active skeletal muscle relaxants. **Archives Internationales de Pharmacodynamie et de Therapie**, v.297, p.272-85, 1989.

32-STEFANSKI, R.; PALEJKO, W.; KOSTOWSKI, W.; PLAZNIK, A. The comparison of benzodiazepine derivatives and serotonergic agonists and antagonists in two animal models of anxiety. **Neuropharmacology**, v.31, p.1251-8, 1992.

33-TAVARES, M.I.; RESENDE, A.M.L.; DÖBEREINER, J. Intoxicação experimental por *Pseudocalymma elegans* em coelhos e cobaias. **Pesquisa Agropecuária Brasileira**, v.9, p.91-4, 1974.

34- TOKARNIA, C.H.; DöBEREINER, J.; CANELLA, C.F.C.; GUIMARÃES, D.J. Intoxicação experimental por *Pseudocalymma elegans* (Vell.) Kuhlm. em bovinos. **Pesquisa Agropecuária Brasileira**, v.4, p.195-204, 1969.

35-TOKARNIA, C.H.; PEIXOTO, P.V.; DÖBEREINER, J. Poisonous plants affecting heart function of cattle in Brazil. **Pesquisa Veterinária Brasileira**, v.10, p.1-10, 1990.

36-TOKARNIA, C.H.; PEIXOTO, V.; DÖBEREINER, J. Intoxicação experimental por *Pseudocalymma elegans* (Bignoniaceae) em caprinos. **Pesquisa Agropecuária Brasileira**, v.4, p.195-204, 1993.

37-VAN-DIJKEN, H.H.; MOS, J., VAN-DER-HEYDEN, J.A.M.; TILDERS, F.J.H. Characterization of stress-induced long-term behavioral changes in rats: Evidence in favor of anxiety. **Physiology and Behavior**, v.52, p.945-51, 1992.

38-WEYERS, P.; BOWER, D.B.; VOGEL, W.H. Relationships of plasma catecholamines to open-field behavior after inescapable shock. **Neuropsychobiology**, v.22, p.108-16, 1990.

39-ZANGROSSI JUNIOR, H.; LEITE, J.R.; GRAEFF, F.G. Anxiolytic effect of carbamazepine in the elevated plus-maze: Possible role of adenosine. **Psychopharmacology**, v.106, p.85-9, 1992.

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