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Pharmacological Evaluation of Ricinine, a Central Nervous System Stimulant Isolated from *Ricinus communis*

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FERRAZ, A. C., M. E. M. ANGELUCCI, M. L. DA COSTA, I. R. BATISTA, B. H. DE OLIVEIRA AND C. DA CUNHA. *Pharmacological evaluation of ricinine, a central nervous system stimulant isolated from Ricinus communis*. PHARMACOL BIOCHEM BEHAV **63**(3) 367–375, 1999.—The extract of the pericarp of castor bean (*Ricinus communis*) showed some typical central nervous system stimulant effects when administered to mice. The animals became exophthalmic, presented tremors and clonic seizures and died a few minutes after receiving larger doses of the extract. At lower doses the extract improved memory consolidation and showed some neuroleptic-like properties, such as a decrease in exploratory behavior and catalepsy. The memory-improving effect and the seizure-eliciting properties of the extract were also observed with the administration of ricinine, a neutral alkaloid isolated from the extract. However, the neuroleptic-like properties of the extract were not observed with ricinine. As the therapeutic index of ricinine is of the order of 200, the compound may be considered as a promising cognition-enhancing drug that may be used for the treatment of human amnesias. © 1999 Elsevier Science Inc.

Ricinus communis Ricinine Seizures Memory Mice

THE toxic plant castor bean (*Ricinus communis*, L., Euphorbiaceae) is found in all regions of Brazil, where it is known by the names of "mamona" or "carrapateira" (49). The intoxication of cattle (12,48) or rabbits (27) by the leaves or the fruit pericarps causes lack of equilibrium and the animals are unable to walk short stretches. They also show muscular tremors, sialorrhea, and erucation. Sudden recovery or death follows these signs. These effects are different from those observed with the intoxication by the seeds of the plant. In this case, the animals exhibit anorexia, diarrhea, weakness, apathy, and eventually death (49). Some of these signs may be due to the toxin ricin (11).

The toxins isolated from this plant so far are ricin, the *R. communis* agglutinin-60 (RCA), obtained from the seeds (31), and ricinine, obtained from the leaves (1,26,49). Ricin is a proteic toxin formed by an A and a B chain. The A chain inactivates ribosomes, impairs protein synthesis, and causes cell death (1). RCA, initially isolated as a contaminant of ricin preparations, is a toxic lectin with hemagglutinin properties (31). RCA has also the property of being taken up and transported retrogradely by axons of peripheral neurons, causing their death (29). Ricinine is a neutral alkaloid supposed to inhibit the respiratory chain (15).

There are no reports on the study of the fruit pericarp toxins of *R. communis*, although Tokarnia (49) has shown that this part of the plant is four times more toxic than the leaves. The signs of intoxication with pericarp suggest that they have central nervous system (CNS) stimulant compounds. These compounds may be useful as tools to study CNS physiology, as putative drugs and also as models of intoxication that show similarities with current pathologies, such as epilepsy. Because many CNS stimulants also exhibit cognition enhancing properties, we decided to study the pericarp of the fruits of *R. communis* and to isolate the active compound.

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METHOD

Preparation of the Plant Extract and Purification of Ricinine

The fruits of *Ricinus communis* were collected in the vicinity of Curitiba (Brazil). A voucher specimen of the plant was deposited at the UPCB Herbarium, Department of Botany, UFPR, and Professor Olavo Guimarães identified it. The fruit pericarp was dried and milled, and the powder obtained was extracted with 50% ethanol. The solvent was evaporated and the aqueous solution was washed with petroleum ether and ethyl ether to yield the crude extract. A portion of the extract was redissolved in hot ethanol and left to cool. The crystals thus obtained were recrystallized three times, dried at 40°C, and stored at room temperature. The identity of ricinine was determined by spectroscopic methods (IR and ^{1}H-NMR).

Animals

The experiments were performed on adult male mice (2.5 months, 20–30 g) from our own breeding stock. The mice were housed in groups of 15 in Plexiglas boxes (60 × 25 × 25 cm) and maintained at 22 ± 2°C on a 12 L:12D-period (lights on 0700 h), with food and water available ad lib.

Drugs and Injection Procedure

The tests were always carried out between 0700 and 1200 h, except for Irwin's test, which was carried out between 0700 and 1800 h. All drugs and plant extracts were administered at doses of 0.01 ml/kg. The extract was prepared in 20% (v/v) dimethylsulphoxide. The range of doses is described below for each set of experiments. Ricinine was prepared in a mixture of corn oil:water (1:1), with a drop of Tween 80 for every 1 ml of water, and was administered subcutaneously (SC). Haloperidol and apomorphine (Sigma Chem. Co., St. Louis, MO) were dissolved in saline (0.9% NaCl) and administered at doses of 4 mg/kg (IP) and 1 mg/kg (SC), respectively. Control groups received the same volume of saline or vehicle IP or intragastrically (IG).

Behavioral Tests, Apparatus, and Procedures

A battery of behavioral tests was performed with animals that received the plant extract. The tests in which a significant and robust effect was observed were also performed with ricinine, isolated from the extract.

Experiment 1. General and toxic effects. Forty-four mice were divided into five groups of 12 animals that received IP 0.2, 0.3, 0.35, or 0.7 g/kg extract or vehicle, respectively, and were observed for 48 h. During this period general effects, occurrence of seizure-like signs and death were recorded. Fifty additional mice were divided into five groups of 10 animals that received IG 1.0, 2.0, 4.0, 5.6, or 7.8 g/kg extract or vehicle, and were submitted to the same observation procedure.

The general effects of ricinine were observed with the protocol of the Irwin's Hippocratic Test (21). Sixty mice were divided into six groups of 10 animals that received saline, vehicle or 10, 20, 30, or 40 mg/kg of ricinine. These animals were submitted to a battery of 49 observation tests in which the occurrence of an event or a score was attributed according to Irwin's protocol. All the 49 tests were sequentially repeated every 30 min from the time of drug administration to 210 min. Three independent observers performed the observations in a double-blind schedule. These animals were maintained in the vivarium for 14 additional days for the observation of death occurrences.

Experiment 2. Effect of the extract and ricinine on tests for anxiolytic and neuroleptic drugs. Sixty-one mice were divided into four groups that received respectively: IP 0.1 g/kg extract (n = 15); IP vehicle (n = 14); IG 1.0 g/kg extract (n = 15); or IG vehicle (N = 17). After 30 min, each animal was successively submitted to the following tests: open filed, elevated plus-maze, and muscular tonus test. The total duration of the test battery was 20 min. The effect of ricinine on the elevated plus-maze test was observed in another 75 mice divided into five groups of 15 animals that received IP 0.1, 1.0, 5, and 10 mg/kg ricinine or vehicle, respectively. In the open-field test, each animal was allowed to freely explore for 5 min a round arena (1 m in diameter) illuminated by four 60-watts lamp 40 cm above the floor. During the session, a 60-dB white noise was turned on. The experimental parameters were scored according to Masur (32), and are said to express the "emotionality" of the animals (32,40). The elevated plus-maze test was performed according to Lister (30), who adapted to mice the test originally designed for rats (39). This test is widely used to assay anxiolytic and anxiogenic drugs. The muscular tonus test consisted of counting how long the animal hangs by its paws from a tightly stretched wire. A ceiling of 300 s was imposed for this test (45).

Catalepsy (41) and locomotor activity (51) were tested in another 98 mice divided into six groups of 19, 19, 15, 15, 15, or 15 animals that received IP vehicle, 4 mg/kg haloperidol, or 0.25, 2.5, 50, or 100 mg/kg extract, respectively. The animals were submitted successively to the two tests 30 min after treatment. Two other groups of 15 or 17 animals received vehicle or 1 g/kg extract IG. Ninety additional mice were divided into four groups of 25, 22, 20, and 23 animals, which received SC vehicle, or 15, 20, or 25 mg/kg ricinine, and were submitted to the same tests. Free locomotor activity of the animals was tested in a box (20 × 50 × 20 cm) with three infrared light beams pointing to photocells. During 5 min of free exploration, the apparatus counts the number of times the animals crosses a light beam (51). The catalepsy test (41) consisted of placing the animal's forepaws on a bar elevated 4.5 cm from the floor. The time the animal stayed still at this position was computed until a maximum of 30 s. The procedure was repeated three times, and the highest result was reported as time of catalepsy for that animal.

Twenty other animals were divided into two groups of 10, which received IP 250 mg/kg of extract or vehicle, respectively. After 30 min these animals received SC 1 mg/kg of apomorphine. Over the next 2 h the animals were observed every 10 min, and stereotyped behavior was scored according to Setler (46).

Experiment 3. Effect of the extract and ricinine on the passive avoidance test. The step-through passive avoidance test (22) was performed under the following conditions: footshock = 0.16 mA, training ceiling latency = 30 s, test ceiling latency = 300 s, training-test interval = 24 h, drug administration = immediately after the training session. Ninety mice were equally divided into six groups that received IP vehicle, or 0.05, 0.5, 2.5, 50, or 200 mg/kg, respectively. Seventy-five additional mice were divided equally into five groups that received SC vehicle, or 0.08, 0.10, 0.12, or 0.15 mg/kg ricinine, and were submitted to the passive-avoidance test.

Statistical Analysis

Most of the behavioral data were analyzed by Kruskall–Wallis ANOVA, followed by Mann–Whitney U-test. In the case of censured data (when a ceiling was imposed) Kaplan–

Meier survival analysis was employed, and the survival curves were compared by the nonparametric test of Log rank (28). Elevated plus-maze data were analyzed by one-way ANOVA. When the locomotion of the animals in the maze differed between groups, the percentage of time and frequency of entries into the closed and open arms of the maze were analyzed by two-way ANOVA, with locomotion as a covariate. Frequency data were analyzed by the Fisher test. LD_{50} were estimated by linear regression (mean error squares method) of the log (dose) vs. probit (death occurrences) plot. The smoothing curves of passive avoidance results were fitted by the least-squares smoothing procedure. Dose–effect curves were fitted by nonlinear regression (least-squares method) to the Boltzmann equation, or according to the distance-weighted least squares. All the statistical and graphic procedures were carried out using the softwares Statistica 5.1 for Windows (StatSoft Inc., 1996) or GraphPad Prisma 2.0 (1995).

RESULTS

Spectroscopic Identification of Ricinine

The identity of ricinine (Fig. 1) was deduced from spectroscopic data. The IR spectrum showed an intense band at 2222 cm^{-1} attributed to the nitrile group; the band relative to the carbonyl group was at 1659 cm^{-1}. The ^{1}H-NMR spectrum showed the two doublets of the olefinic protons at 8.00 and 6.37 ppm; the singlets of the methoxyl and methyl groups were at 4.04 and 3.50 ppm, respectively.

Experiment 1: General and Toxic Effects

The LD_{50} was estimated at 0.34 g/kg for IP administration of the extract (linear regression from a log-logit plot; $r = 0.96$) and at 3.0 g/kg ($r = 0.99$) for IG administration. No effect was observed after vehicle administration. Before death the animals became exophthalmic, presented muscular tremors, and sometimes walked dragging the hind paws. A few minutes after receiving doses above 0.3 g/kg (IP) or 2.0 g/kg (IG) the animals presented signs of clonic seizures and died 1 or 2 min later. Only two animals died 1 h to 14 days after extract administration. In all cases of sudden death, after the animals stopped breathing their hearts continued beating for several minutes.

Ricinine presented the following effects on Irwin's test as seen in Table 1: 1) a prominent exophthalmic effect was observed 30 min after the administration of 20 mg/kg ricinine (Fisher test, $p \leq 0.05$). 2) A mydriatic effect was observed 30 and 60 min after ricinine administration —20 mg/kg, 30 min: Kruskall–Wallis ANOVA, $H(5, 41) = 16.25$, $p \leq 0.01$; Mann–Whitney U-test = 8.5, $p \leq 0.01$; 30 mg/kg, 60 min: Kruskall–Wallis ANOVA, $H(5, 39) = 12.72$, $p \leq 0.05$; Mann–Whitney U-test = 0.00, $p \leq 0.05$. This effect was not observed when ricinine was administered topically to the rabbit conjunctiva (results not shown). 3) The sensorimotor response of the pinna was increased 90 min after the administration of 20 mg/kg ricinine [Kruskall–Wallis ANOVA, $H(5, 41) = 13.23$, $p \leq 0.05$, Mann–Whitney U-test = 15, $p \leq 0.05$ and 120 min [Kruskall–Wallis ANOVA, $H(5,41) = 15.59$, $p \leq 0.01$; Mann–Whitney U-test = 10.5, $p \leq 0.05$]. 4) The grip strength reaction increased in animals treated with 20 mg/kg ricinine 120 min after administration [Kruskall–Wallis ANOVA, $H(5, 41) = 11.89$, $p \leq 0.05$; Mann–Whitney U-test = 12.5, $p \leq 0.05$]. 5) All animals that died after ricinine administration presented clonic convulsions a few minutes before death. The effect was significant at doses of 30 mg/kg ($p \leq 0.005$, Fisher test) and 40 mg/kg ($p \leq 0.005$, Fisher test). 6) LD_{50} was estimated at 25 mg/kg (linear regression from a log-logit plot; $r = 0.95$). As observed for the extract-treated animals, after the animals stopped breathing their hearts continued beating for several minutes. No deaths were observed among the animals that survived 1 h to 14 days after drug administration.

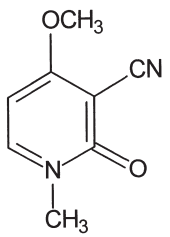

FIG. 1. Ricinine.

Most of the effects listed above seemed to be more significant at doses of 20 mg/kg, because higher doses led to higher mortality and the number of remaining animals was too small to allow an appropriate statistical analysis.

The other scores or Irwin's test listed above did not show any significant change with ricinine treatment compared to vehicle (Kruskall–Wallis ANOVA, $p \leq 0.05$): 1) the behavioral observations made were: sleep, locomotor activity, bizarre behavior, alley progression, transfer arousal, touch–escape, position struggle, grasp–irritability, provoked biting, provoked freezing, finger withdrawal, finger approach, position passivity, vocalization, urination–defecation, visual placing, tail pinch, toe pinch, corneal, startle, pelvic elevation, tail elevation, limb rotation. 2) The neurologic observations were: body tone, abdominal tone, limb tone, wire maneuver, righting reflex, ataxic gait, hypotonic gait, total incapacity, tremors, twitches. 3) The autonomic observations were: pupil reaction to light, palpebral closure, salivation, lacrimination, diarrhea, hypothermia, piloerection, skin color, and respiratory rate.

Experiment 2: Effect of the Extract and Ricinine Upon Tests for Anxiolytics and Neuroleptic Drugs

1. In the open-field test, extract-treated animals (0.1 g/kg, IP) spent more time in the "freezing" position [median (Q25/75): vehicle = 54 (4 of 90); extract = 0 (0 of 0); $U = 20.0$, $p \leq 0.005$, Mann–Whitney U-test], decreased the number of crossings [vehicle = 100 (47 of 134); extract = 1 (0 of 17); $U = 21.0$, $p \leq 0.005$], the number of rearings [vehicle = 8 (1 of 23); extract = 0 (0 of 0); $U = 21.0$, $p \leq 0.005$] and the time of grooming [vehicle = 1 (0 of 78); extract = 0 (0 of 78); $U = 49$, $p \leq 0.005$] compared to the control group. The number of fecal boluses was not different from the control group ($U = 102.0$, $p > 0.2$). The animals that received IG 1 g/kg extract spent more time in the "freezing" position [vehicle = 0.0 (0.0 of 0.7), extract = 3 (0 of 21.2); $U = 63.0$, $p \leq 0.05$], and decreased the number of rearings [vehicle = 46 (27 of 59)]; extract = 17 (3 of 32); $U = 41.5$, $p \leq 0.005$]. This treatment did not affect the number of crossings ($U = 95.0$, $p \geq 0.2$), time of grooming ($U = 91.0$, $p \geq 0.2$), or defecation ($U = 92.0$, $p \geq 0.2$).
2. In the elevated plus maze, extract-treated animals (0.1 g/kg, IP) reduced the total number of entries into the open + closed arms of the maze [mean ± SEM: vehicle = 8.8 ± 0.9; extract = 3.1 ± 0.8; $FG(1, 27) = 22.5$, $p \leq 0.001$, one-way ANOVA]. ANOVA with the total number of entries

TABLE 1

EFFECT OF RICININE ON SOME SCORES OF THE IRWIN HIPPOCRATIC TEST

		Saline							Vehicle							Ricine 10 mg/kg					
Time Post drug	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death
0 min	10	0	4	4	6	0	0	10	0	4	3	6	0	0	10	0	4	6	6	0	0
30 min	10	0	4	1	6	0	0	10	0	4	4	6	0	0	10	2	4	6	6	0	0
60 min	10	0	4	1	6	0	0	10	0	4	1	6	0	0	10	0	4	6	6	0	0
90 min	10	0	4	0	6	0	0	10	0	4	0	6	0	0	10	0	4	6	6	0	0
120 min	10	0	4	0	6	0	0	10	0	4	0	5	0	0	10	0	4	6	4	0	0
150 min	10	0	4	0	6	0	0	10	0	4	0	5	0	0	10	0	4	4	4	0	0
180 min	10	0	3	0	6	0	0	10	0	3	0	6	0	0	10	0	4	4	6	0	0
210 min	10	0	3	0	5	0	0	10	0	3	0	6	0	0	10	0	4	4	6	0	0
		Ricinine 20 mg/kg							Ricinine 30 mg/kg							Ricinine 40 mg/kg					
Time Post drug	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death	*n*	Exophtalmos	Pupil Size	Pinna	Grip Strength	Convulsions	Acute Death
0 min	10	0	4	6	6	0	0	10	0	4	3	6	0	0	10	0	4	6	6	0	0
30 min	9	4*	6*	6	6	1	1	5	2	3	0	6	5*	5*	2	2	4	6	6	8*	8*
60 min	8	0	4	6	6	1	1	3	0	6*	5	6	2	2	2	0	4	0	6	0	0
90 min	8	0	4	6*	6	0	0	3	0	4	6	6	0	0	2	0	4	0	6	0	0
120 min	8	0	4	6*	6*	0	0	3	0	2	5	6	0	0	2	0	4	0	6	0	0
150 min	8	0	4	6	6	0	0	3	0	3	1	4	0	0	2	0	4	6	6	0	0
180 min	8	0	4	6	6	0	0	3	0	4	5	6	0	0	2	0	4	6	6	0	0
210 min	8	0	3	6	6	0	0	3	0	4	6	6	0	0	2	0	4	4	6	0	0

Groups of 10 animals received 0.9% saline, vehicle (water:corn oil, 1:12% Tween 80) or ricinine 10–40 mg/kg and were submitted to a battery of 50 standard observations repeated every 30 min for 210 min. The observations encompass behavioral, neurologic, autonomic, and toxic parameters. Results presented above show only observations in which the drug treatment produced a significant alteration ($*p \leq 0.05$) compared to the vehicle group. *n* represents the number of animals still alive at the time of observation. The table shows the median values of exophatalmus, pupil size, and grip strength scored according to Irwin's scale. Convulsions and acute death are represented as number of occurrences. Data scores were analyzed by Kruskall–Wallis ANOVA followed by the Mann–Whitney *U*-test. Frequencies were analyzed by the Fisher test.

as covariate showed that, discounting the influence of these parameter, there was no significant alteration into the percentage of entries in the open arms $F(1, 26) = 0.14$, $p \geq 0.2$, or percentage of time spent in the closed arms, $F(1, 26) = 0.40$, $p \geq 0.2$. IG administration of 1.0 g/kg extract also decreased the total number of entries in the closed + open arms of the maze [vehicle = 15.9 ± 1.8; extract = 10.8 ± 1.1; $F(1, 28) = 5.28$, $p \leq 0.05$]. The same analysis performed for the results of IG extract administration showed that, regardless of the decrease in the total number of entries, there was a small but significant decrease in the percentage of time spent in the open arms [vehicle = 29.4 ± 4.6; extract = 23.7 ± 4.7; $F(1, 27) = 9.79$, $p \leq 0.01$] and an increase in the percentage of time spent in the closed arms [vehicle = 52.7 ± 3.9; extract = 57.5 ± 5.5, $F(1, 27) = 28.29$, $p \leq 0.001$], without affecting the percentage of entries into the open arms, $F(1, 27) = 0.0005$, $p \geq 0.2$. The IP administration of ricinine (0.1 to 10 mg/kg) did not affect the total number of entries, $F(4, 70) = 1.26$, $p \geq 0.2$, the percentage of time spent in the open arms, $F(4, 70) = 0.66$, $p \geq 0.2$, the percentage of time spent in the closed arms, $F(4, 70) = 0.41$, $p \geq 0.2$, and the percentage of entries into the open arms, $F(4, 70) = 0.45$, $p \geq 0.2$.

3. IP or IG administration of the extract did not affect the results of the muscular tonus test ($p \geq 0.2$, Kaplan–Meier survival analysis, Log rank test).
4. As can be seen in Fig. 2A, IP administration of the extract-induced catalepsy at doses of 0.25 to 100 mg/kg ($H = 46.25$, $p \leq 0.001$, one-way Kruskal–Wallis ANOVA, followed by Kaplan–Meier survival analysis). A nonlinear regression (Boltzmann sigmoidal equation, GraphPad Prism Software) showed a dose–effect S-shaped curve with an $r^2 = 0.99$ (maximal effect = 102 s; $EC_{50} = 0.9$ mg/kg) for IP doses. This effect is common to many neuroleptics such as haloperidol, which in this study caused a more prominent effect (mean ± SEM = 231 ± 22, $p \leq 0.005$, Kaplan–Meier survival analysis followed by the log rank test). As can be seen in Fig. 2B, the extract also decreased the free locomotor activity at all doses administered either IP [one-way Kruskal–Wallis ANOVA, $H = 46.25$, $p \leq 0.001$; two-tailed Mann–Whitney U-test, $p \leq 0.05$] or IG [1.0 g/kg extract, $n = 15$ animals per group; vehicle median = 35.0 (29 of 37); extract = 24.0 (17 of 31.5)]; $U = 47.50$, two-tailed Mann–Whitney test, $p \leq 0.005$. A nonlinear regression (Boltzmann sigmoidal equation) showed a dose–effect S-inverted curve with an $r^2 = 0.96$ for IP doses. Haloperidol treatment completely abolished locomotor activity (mean ± SEM = 0.42 ± 0.11, $p \leq 0.005$, Kaplan–Meier survival analysis followed by the log rank test).
   Ricinine did not alter the scores of catalepsy [Kruskal–Wallis ANOVA, $H(3, 90) = 5.74$, $p = 0.12$] or of locomotor activity of the animals [Kruskal–Wallis ANOVA, $H(3, 89) = 5.34$, $p = 0.15$].
5. The plant extract (250 mg/kg; IP) did not impair the stereotyped behavior induced by SC 1 mg/kg apomorphine [vehicle median = 22.5 (17 of 28.5); extract = 26.5 (23 of 28.5)]; $U = 33.5$, $p \geq 0.2$, two-tailed Mann–Whitney U-test.

Experiment 3: Effect of the Extract and Ricinine on the Passive-Avoidance Test

The results of the passive-avoidance test are presented in Fig. 3. In training session scores no difference was detected between the vehicle and extract-treated groups [mean = 13.5 ± 0.6, one-way ANOVA, $F(5, 84) = 3.74$, $p \geq 0.2$]. Similarly, no difference was detected between the vehicle and ricinine-treated groups in training session scores [mean = 10.1 ± 0.7, one-way ANOVA, $F(4, 70) = 0.12$, $p \geq 0.2$]. The animals learned to avoid the dark compartment because the latency to enter into the dark compartment was greater in the test session compared to the training session (extract-vehicle group: $U = 0{,}0$ $p \leq 0.001$; ricinine–vehicle group: $U = 2.0$, $p \leq 0.001$, Mann–Whitney). As can be seen in Fig. 3A extract administration improved retention of the passive-avoidance task at doses of 0.5 to 50 mg/kg, [H (5, 90) = 15.96, Kruskal–Wallis ANOVA, $p \leq 0.001$—see figure legend for individual differences between groups]. Pilot experiments were done with doses ranging from 0.01 to 10 mg/kg to find the range of doses at which ricinine affects memory retention (data not shown). As can be seen in Fig. 3B, ricinine improved retention at doses of 0.10 to 0.12 mg/kg [$H(4, 75) = 12.70$, Kruskal–Wallis ANOVA, $p \leq 0.05$—see figure legend for individual differences between groups].

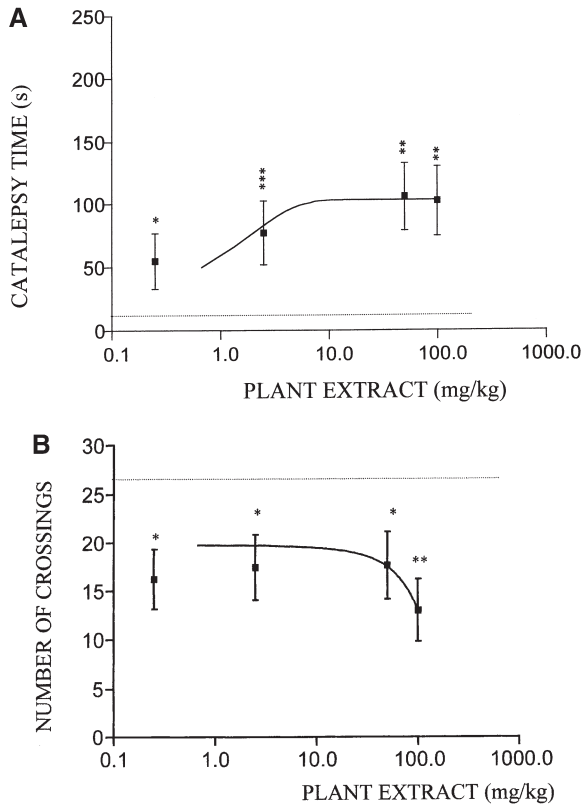

FIG. 2. Effect of IP administration of the extract from *Ricinus communis* on catalepsy time (A) or on free locomotor activity (B). The animals received the vehicle or the plant extract 30 min before being tested. The results are expressed as means ± SEM. The dotted line represents the values for the control group (vehicle). *$p \leq 0.05$; **$p \leq 0.005$; ***$p \leq 0.001$, log-rank test for catalepsy or Mann–Whitney U-test for free locomotor activity.

DISCUSSION

The present results suggest that *R. communis* extract has typical CNS stimulant and neuroleptic effects. The stimulant effects, such as exophthamus, hyperreactivity (evidenced by tremors or by the pinna and grip-strength reaction), memory

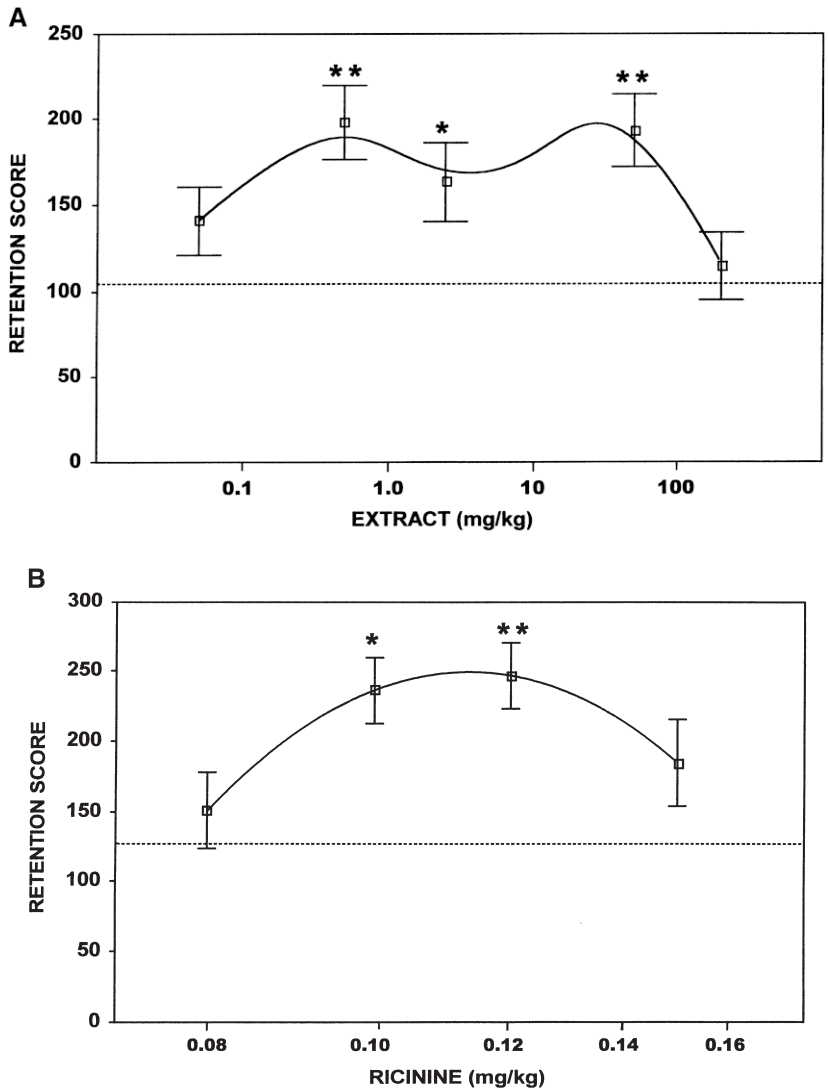

FIG. 3. Effect of the administration of the extract from *Ricinus communis* (A) or ricinine (B) on the retention of the passive avoidance task in mice. The animals received the vehicle or plant extract (IP) or ricinine (SC) immediately after the training session. The dotted line represents the values for the control group (vehicle). The results are expressed as means ± SE. $*p \leq 0.05$; $**p \leq 0.005$ compared to the vehicle group (Kaplan–Meier analysis of survival followed by the log-rank test). The curves are fitted by the least-squares smoothing procedure.

improvement, and clonic seizures, seem to be due to the presence of the alkaloid ricinine. The main toxic compound of the extract also seems to be ricinine, because animals who died after administration of extract or ricinine showed similar signs: they all died after the occurrence of clonic seizures followed by an apparent breathing arrest. On the other hand, compounds other than ricinine may be responsible for the neuroleptic-like effects of the extract, because ricinine did not cause reduction of locomotor activity or catalepsy in the mice.

The *R. communis* extract and ricinine improved retention of the passive-avoidance task, with a dose–effect curve with an inverted *U*-shape, typical of many other CNS stimulants that act as cognition enhancers (34). The range of doses at which ricinine is effective is narrower than that observed for the extract. The latter also presents a bimodal curve, possibly indicating that it contains more than one active compound. The effect of the extract or ricinine on passive avoidance can not be explained by an alteration in the behavior of the animals in the training session, because both ricinine and the plant extract were injected after training. Therefore, this effect is more likely to be explained by an improvement of memory consolidation.

The results observed with ricinine place it among other compounds known to have memory-improving effects. The literature presents a large list of cognition enhancers effective in animal models of learning and memory (19,23,42,43,54). However, few of them proved to be effective and nontoxic for the treatment of the cognitive impairments of human dementias (42,54). Most of the CNS stimulants, like GABA/BZ receptor antagonists or excitatory amino acid receptor agonists, improve memory retention (24). The main problem in the use

of these drugs for the treatment of amnesias is their low therapeutic index (LD_{50}/ED_{50}) and their anxiogenic and proconvulsant properties. Ricinine proved to be a convulsant, but while the $GABA_A$-Cl^- channel blocker, picrotoxin, showed a therapeutic index of the order of 3×10^0 (3,18), ricinine has a therapeutic index in the order of 2×10^2. The same ratio is observed when comparing the EC_{50} of ricinine for the convulsant effect with the EC_{50} for the memory-improving effect. Most of the memory-enhancing β-carbolines, inverse agonists of the central benzodiazepine receptor, like *n*-butyl β-carboline-3-carboxylate (β-CCE) (36), methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), and FG7142 (25) are highly anxiogenic. Even the theoretically harmless central benzodiazepine receptor-antagonist flumazenil, which has memory-enhancing properties in rats (8), caused signs of anxiety when administered into the rat amygdala (9) or when administered to humans (37). Ricinine, on the other hand, did not show an anxiogenic effect. In view of these results, ricinine-like compounds may represent a new class of cognition-enhancing drugs useful for the treatment of amnesic symptoms related to Alzheimer's and other neurodegenerative diseases.

Most of the known memory improving compounds, as pointed out above, are convulsants at large doses. The signs of intoxication caused by convulsants, like picrotoxin, pentilenetetrazole, strychnine, and others, have been used as chemical models of epilepsy in the screening of anticonvulsant drugs (10). Pilocarpine, a cholinergic muscarinic receptor agonist, improves memory at lower doses (42,43,47) and, when administered at a larger dose to rats, produces one of the most extensively studied animal model of epilepsy (5,50). Likewise, mouse intoxication with ricinine may be a useful model of convulsive seizure.

Some of our results suggest that *R. communis* contains neuroleptic compounds. The extract decreased free locomotor activity and exploratory behavior and caused catalepsy, common effects of neuroleptic drugs (4,40). These effects were not due to an impairment of muscular tonus, because the extract did not alter the time the animals stayed hanging from a tightly stretched wire. It is also not probable that this effect is related to preliminary reactions to seizures because it was observed with doses that did not induce seizures (0.25–100 mg/kg). Seizures were observed with doses above 200 mg/kg extract. The other point suggesting that the decrease in free locomotor activity was not a preseizure sign is that the animals that received ricinine, the seizure-eliciting compound, did not show these neuroleptic-like effects. Although presenting the neuroleptic-like effect described above, the plant extract did not alter the stereotyped behavior induced by apomorphine, another test sensitive to D_2 dopamine receptor antagonists (46). However, the effect of the extract suggesting a D_2 receptor-antagonist action may not be related to the nigrostriatal pathway where apomorphine acts to produce stereotyped behavior (7), but rather may be related to the mesolimbic pathway.

Anxiogenic drugs and large doses of anxiolytics that may cause sedation (40) also induce reduction of exploratory behavior. Anxiogenic-like properties are observed in many other CNS stimulants, like benzodiazepine/$GABA_A$ antagonists (6,9) and glycine site agonists at the NMDA receptor (33,44). Another test more specific for anxiolytic and anxiogenic drugs, the elevated plus-maze test, showed that the plant extract has none of those effects when administered IP. A minor anxiogenic effect was observed with IG administration of the plant extract. Ricinine is not anxiogenic, because it did not reduce exploratory behavior and did not alter the elevated plus-maze scores.

Ricinine has been isolated previously by other groups [see (14)]. Farah (15) suggested that it might inhibit the mitochondrial respiratory chain. Other compounds with this mechanism of action, like monofluoracetate (13), also have seizure-eliciting properties. In a related study (16), we further discussed why we believe that Farah's hypothesis seems not to be applicable to ricinine intoxication.

Other compounds isolated from plants present some pharmacological and toxic effects similar to those observed with *R. communis* extract and ricinine. Seizures and other CNS stimulant-like effects were observed in animals intoxicated with monofluoracetate (13), a compound isolated from *Palicourea marcgravii*. Other plants from the Euphorbiaceae family, like *Glicine max* (52), *Croton zehntneri* (2), and securinine, isolated from *Philanthus amarus* (17), also elicit seizures. When animals intoxicated with ricinine or *R. communis* extract died, they stopped breathing before their hearts stopped beating. Other Euphorbiaceae plants present similar effects: *G. max* contains a soytoxin that causes dyspnea (52), and *Phyllanthus urinaria* causes trachea contraction in guinea pigs (38). Like *R. communis*, *Croton zehntneri* extract also decreases exploratory behavior in an open field (2). Some phorbol esters (35) and diterpenes (20) have been isolated from other Euphorbiaceae plants. Phorbol esters bind to protein kinase C (PKC), which plays an important role in synaptic plasticity, including learning and memory processes. they also improve dopamine neurotransmission, which may itself modulate memory storage (53). Some diterpenes, as well as α-tocopherol, inhibit phospholipase A2, an enzyme that participates in the formation of platelet-activating factor (PAF), which is supposed to participate in long-term potentiation (LTP), a proposed cellular model of learning and memory (22).

The present results suggest that ricinine is a putative memory-enhancing drug. At larger doses this compound has major CNS-stimulating properties, eliciting seizures. Other compounds of *R. communis* have neuroleptic-like properties. Future studies of these compounds may lead to a better understanding of the plant toxicology and to the development of new neuroactive drugs.

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