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Full Length Research Paper

Alterations in behavior and memory induced by the essential oil of *Zingiber officinale* Roscoe (ginger) in mice are cholinergic-dependent

Cícero Francisco Bezerra Felipe[1]*, Kamyla Sales Fonsêca[1], André Luiz dos Reis Barbosa[1], José Noberto Sousa Bezerra[2], Manoel Andrade Neto[2], Marta Maria de França Fonteles[1] and Glauce Socorro de Barros Viana[1]

[1]Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, Brazil.
[2]Department of Organic and Inorganic Chemistry, Federal University of Ceará, Fortaleza, Brazil.

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The objective of the work was to investigate the effects of the essential oil of *Zingiber officinale* Roscoe (EOG - 25, 50 and 100 mg/kg, i.p) on the behavior and cognition of male Swiss mice (25 - 30 g), treated daily for 7 days. In the 7th day, animals were submitted to the elevated plus maze, open field and rota rod tests to evaluate anxiolytic, sedative and motor coordination effects, respectively. Memory tasks and cholinergic system effects of EOG were also assessed by the T-maze, passive avoidance, oxotremorine and pilocarpine-induced convulsions tests. EOG did not show anxiolytic effects in the elevated plus maze test, while it showed a sedative effect in the open field test at the doses of 50 and 100 mg/kg. EOG did not alter motor coordination of the animals, while it produced a cognitive impairment, in the passive avoidance test, in animals treated with the dose of 100 mg/kg. In the T-maze test, cognitive impairment was also evident and occurred in a dose-dependent manner. In addition, EOG (50 and 100 mg/kg, i.p.) blocked the oxotremorine-induced tremors and significantly increased the latency of pilocarpine-induced seizures, as well as animals survival. Altogether, our results suggest that EOG effects are, at least in part, dependent upon an antagonist action on the central muscarinic cholinergic system.

Key words: *Zingiber officinale* Roscoe, memory, scopolamine, oxotremorine, pilocarpine.

INTRODUCTION

There is a long history on the use of ginger (the rhizomes of *Zingiber officinale* Roscoe) in Chinese and Ayurvedic medicines where it is used as an antiemetic, anti-inflammatory, antipyretic, analgesic, for toothache, insomnia, baldness, urinary tract infections, as well as a therapy for various gastrointestinal disorders (Balch, 1996). Numerous chemical investigations of this plant material have led to the isolation and identification of a large number of biologically active compounds (Yu et al., 1998). Ginger is valued both for its aromatic, volatile compounds and by its spicy, pungent constituents. The latter principles are non-volatile phenols with various side chains, the so-called gingerols, shogaols and paradols.

Extracts prepared from rhizomes of *Z. officinale* are known to present several pharmacological activities, such as analgesic, anti-inflammatory, hypoglycemic, hypolipemic and antioxidant ones (Bhandari et al., 2005; Grzanna et al., 2005; Lantz et al., 2007; Ojewole, 2006). Among those, the most studied one is the anti-inflammatory action that distinguishes ginger from non-steroidal anti-inflammatory drugs. In this sense, ginger behaves as a dual inhibitor of cyclooxygenase and 5-lipoxygenase and, thus, may have a better therapeutic profile and fewer side effects than non-steroidal anti-inflammatory drugs (Grzanna et al., 2005). A recent work (Lantz et al., 2007) demonstrated that compounds found in ginger are capable of inhibiting PGE_2 production. Some works indicated that the ethanolic extract of *Z. officinale* can protect tissues from lipid peroxidation, also exhibiting significant lipid lowering activity in diabetic rats (Bhandari et al., 2005). A recent work (Vishwakarma et al., 2002)

*Corresponding author. E-mail: cicerof@hotmail.com

reported a benzene fraction from ginger to possess anti-convulsant, anxiolytic and antiemetic activities. Furthermore, combined extracts of *Z. officinale* and *Gingko biloba* showed an important antioxidant activity on aged rats (Topic et al., 2002a) which is implicated in the improvement of memory in animals submitted to cognitive tasks (Topic et al., 2002b).

Dried ginger consists of 40 - 60% starch, 10% proteins, 10% fats, 5% fibers, 6% inorganic material, 10% residual moisture and 1 - 4% essential oil. Essential oils are known to present antimicrobial, antifungal and insecticidal properties against wide spectra of organisms such as fungi, bacteria and insects (Singh et al., 2005). The major constituents of the essential oil of *Z. officinale* are the sesquiterpenes zingiberene (35%) and farnesene (10%). Monoterpenes (1,8-cineol, linalol, borneal, neral and geraniol) are also found, however, in lower concentrations (Andrews et al., 1995; Sakamura et al., 1986). Although the volatile chemical constituents of the essential oil from *Z. officinale* rhizomes are well known, there are only a few studies on its possible pharmaco-logical properties. A recent work (Lim et al., 2005) showed that the inhalation by mice of the ginger essential oil resulted in the reduction of immobility time in the for-ced swimming test, indicating an antidepressant effect.

In view of the limited literature on the effects of volatile constituents of ginger, the aim of the present work was to study the effects of sub-acute intraperitoneal (i.p.) ad0-ministration of the essential oil of ginger (EOG) on several parameters of mice behavior, attempting to clarify its mechanism of action.

METHODS

Plant material

Ginger (*Z. officinale*) was collected at the city of Baturité, state of Ceará, Brazil, and the exsiccatae deposited at the Prisco Bezerra Herbarium, of the Federal University of Ceará, under the number 32,420. To extract the essential oil (EOG), the rhizomes of ginger were washed out with water and then dried and grinded. The material was hydro-distilled for 3 h, and the essential oil sample was collected and dried over anhydrous sodium sulfate. The analysis of the chemical constituents of EOG was accomplished at the Federal University of Ceará. The oil sample was analyzed in a Hewlet-Parkard GC / MS model 5971A, under the following conditions: column - dimethylpolysiloxane DB-1 fused silica capillary column 30 m x 0.25 mm, film thickness 0.1 μm; carrier gas – helium (1 ml/min); injector temperature - 250ºC; detector temperature: 200ºC; column temperature 35 – 180ºC at 4 ºC/min, then 180 – 250ºC at 10ºC/min; MS - electronic impact 70 eV. The identification of the constituents was achieved with the aid of the respective Kovats Indices and comparing the mass spectra with those of the library.

Animals

Male Swiss mice (25 - 30 g) were obtained from the animal house of the Federal University of Ceará, and maintained in a room with a controlled temperature of 22 ± 2ºC, and a 12 h light/dark cycle. They had free access to food and water. Fifteen animals per group were used, and all the experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the U.S. Department of Health and Human Services, Washington, DC (1985).

Drugs

Atropine sulfate, scopolamine hydrochloride, oxotremorine and pilocarpine sulfate were purchased from Sigma Chemical Co., USA, and diazepam (20 mg/2 ml) was purchased from União Química, Brasil. All other drugs were from analytical grade.

Experimental procedure

EOG was emulsified in distilled water with Tween 80 (0.5%) and this vehicle was used in the control group. Three solutions were prepared to be used with the following doses: 25 mg/kg (EOG 25), 50 mg/kg (EOG 50) and 100 mg/kg (EOG 100). These doses were selected according to previous studies of toxicity developed in our laboratory, and used in this work as they showed to be safe and pharmacologically active. Except for sedation, no toxicity signs were observed with doses up to 400 mg/kg, i.p. (data not shown). Animals were treated daily for 7 days with EOG or vehicle (1 ml/kg, i.p.) and, in the last day, the mice were subjected to behavioral tests (elevated plus maze, open field, rota rod, passive avoidance, T-maze, oxotremorine-induced tremor, and pilocarpine-induced seizures tests) in order to evaluate the central actions of EOG. Each test was performed with different groups of animals, and all experiments were conducted in a quiet room at constant temperature (23 ± 1°C) and poorly illuminated with a 15-V red light, to avoid animal stress. With the exception of EOG, all other drugs were dissolved in distilled water before use.

Elevated plus maze (EPM)

Mice were divided into five different groups: control, diazepam, EOG 25, EOG 50 and EOG 100. Animals from the diazepam group received vehicle for 6 days; only on the 7th day, this group received diazepam. Ampoules containing diazepam were diluted in distilled water up to the desired concentration, immediately before use. This drug, at the dose of 1 mg/kg, i.p., was administered once, 30 min before the test, as a positive control. All other groups were treated for 7 days with vehicle (1 ml/kg, i.p.) and OEG (25, 50 and 100 mg/kg, i.p.). In the last day of treatment, each animal was subjected to the EPM test.

The plus maze for mice (Lister, 1987) consisted of two perpendicular open arms (30 x 5 cm) and two closed arms (30 x 5 x 25 cm) also in perpendicular position. The open and closed arms were connected by a central platform (5 x 5 cm). The platform and floor were made from black acrylic, and the lateral walls of the closed arms were made of transparent acrylic. The maze was 45 cm above the floor. After treatment (30 min), each animal was placed at the center of the plus maze with its nose facing one of the closed arms, and was observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time spent in the open and closed arms (after the animal entered the maze with the four paws). Anxiolytic compounds reduce the natural animal's aversion to the open arms, and promote the exploration thereof. On the other hand, the animal's forced or voluntary passages into the open arms are associated with hormonal and behavioral changes, indicative of increased anxiety.

Open field test

The mice were divided into four different groups: control, EOG 25, EOG 50 and EOG 100. Each group was treated for 7 days with

vehicle (1 mL/kg, i.p.) and OEG (25, 50 and 100 mg/kg, i.p.), respectively. In the 7th day, 30 min after drug treatment, each animal was subjected to the open field test.

The open field area was made of acrylic (30 x 30 x 15 cm) with transparent walls and a black floor, divided into nine squares of equal areas. The open field was used to evaluate the exploratory activity of the animal for 5 min (Archer, 1973). The observed parameters were: number of squares crossed (with the four paws) and numbers of grooming (stereotyped behavior) and rearing (vertical exploratory activity).

Rota rod test

The mice were divided into four different groups: control, EOG 25, EOG 50 and EOG 100. Each group was treated for 7 days with vehicle (1 mL/kg, i.p.) and OEG (25, 50 and 100 mg/kg, i.p.) respectively.

In the 7th day, 30 min after drug treatment, each animal was placed with the four paws on a 2.5 cm diameter bar, 25 cm above the floor, which was rotating at 12 rpm. For each animal, the number of falls and the time spent on the bar (without falling off) were registered. In case of falling off from the bar (up to three falls), the mouse was replaced, and the test proceeded until reaching the test cut off time (60 s) (Dunham and Miya, 1957).

Passive avoidance test

The mice were divided into 8 different groups: control, scopolamine, EOG 25, EOG 50, EOG 100, scopolamine + EOG 25, scopolamine + EOG 50 and scopolamine + EOG 100. Animals from the scopolamine and scopolamine + EOG (25, 50 and 100) groups received, respectively, vehicle (1 mL/kg, i.p.) and vehicle (1 mL/kg, i.p.) + EOG (25, 50 and 100 mg/kg, i.p.) for 6 days; only on the seventh day, these groups received scopolamine (0.5 mg/kg, i.p.), a positive control for cognitive impairment. All other groups were treated for 7 days with vehicle (1 mL/kg, i.p.) and OEG (25, 50 and 100 mg/kg, i.p.). In the last day of treatment, each animal was subjected to the passive avoidance test.

A two-compartment apparatus (48 x 22 x 22 cm) from Ugo Basile, Italy, was used. The starting compartment was illuminated while the shock compartment was dark. A stainless steel bar floor was used for delivery of a scrambled constant current. Each experiment was started with one pre-training trial (30 min after the drug injection), in which the mouse was placed into the illuminated compartment for 60 s for habituation and, then, removed from the apparatus. After 30 s in the acquisition trial (training trial), each mouse was placed individually in the light compartment. When the animal entered the dark compartment, a foot shock of 1.0 mA (1 s) was delivered through the grid floor. The latency time to enter the dark compartment was measured up to a cut off time of 300 s (baseline). The animal was removed from the apparatus, and the trial repeated 15 min later (short-term memory), even with those mice that reached the cut off time. The retrieval trial was performed in the same manner, 24 h later (long-term memory). When replaced into the apparatus for short-term and long-term memory measurements, each animal entering the dark compartment received a foot shock again, and the latency time was registered (Cunha et al., 2000).

T-maze

The mice were divided into five different groups: control, scopolamine, EOG 25, EOG 50 and EOG 100. Animals from the scopolamine group received vehicle for 6 days; only on the 7th day, this group received scopolamine (10 mg/kg, i.p.), a positive control for cognitive impairment. All other groups were treated for 7 days with vehicle (1 ml/kg, i.p.) and OEG (25, 50 and 100 mg/kg, i.p.). In the last day of treatment, each animal was subjected to the T-maze test.

The T-maze for mice consisted of two perpendicular open arms (30 x 5 cm) and one closed arm (30 x 5 x 25 cm) also in a perpendicular position. The floor was made from black acrylic and the lateral walls of the closed arms were made of transparent acrylic. The maze was 45 cm above the floor. Each mouse was placed in the closed arm and the time it spent to reach the open arms was registered. This trial procedure was accomplished two times, 48 h before the final test, which consisted in placing again the animal in the closed arm to register the latency to reach the open arms. The cut off time was 300 s (Viana et al., 1994).

Oxotremorine-induced tremors

EOG (25, 50 and 100 mg/kg, i.p.), vehicle, and atropine (10 mg/kg, i.p.) were administered to different groups of mice, 30 min before the oxotremorine (0.5 mg/kg, i.p.) administration. The tremor was scored visually in individual animals, at 10, 20, and 30 min after oxotremorine administration, using a rating scale from 0 to 3, as described by Coward et al. (1977): 0 (no tremor); 1(occasional isolated twitches); 2 (moderate or intermittent tremor associated with short periods of quiescence); 3 (pronounced continuous tremor).

Pilocarpine-induced seizures

In the last day of treatment and 30 min after the drug administration, all animals received a single injection of pilocarpine (300 mg/kg, i.p.). The latency for the first convulsion and the percentage of deaths at 24 h after pilocarpine administrations were the studied parameters.

Statistical analysis

Results were expressed as mean ± S.E.M. and analyzed with analysis of variance (ANOVA) and the Student-Newman-Keuls as a *post hoc* test. Results were considered significant at $p < 0.05$.

RESULTS

In the elevated plus maze test, no significant effect was observed in the time spent in the open (TSOA) or closed arms (TSCA), as compared to controls, after the administration of EOG to mice, daily for 7 days (25, 50 and 100 mg/kg, i.p.). As expected, diazepam (1 mg/kg, i.p.) significantly increased by 35% and decreased by 27% TSOA and TSCA, respectively. Diazepam also increased by 71% the number of entrances in the open arms (NEOA), with no effect on the number of entrances in the closed arms (NECA). Except for a 22% increase in the NECA, observed only with the dose of 100 mg/kg, i.p., EOG did not change NEOA nor NECA, as compared to controls (Table 1).

In the open field test (Figure 1), the intraperitoneal administration of EOG, 50 and 100 mg/kg, decreased dose-dependently the number of crossings of the animals in approximately 31 and 38%, when compared to controls. Furthermore, EOG (50 and 100 mg/kg, i.p.) reduced

Table 1. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the elevated plus maze test, in mice.

Group	TSOA	TSCA	NEOA	NECA
Control	121.0 ± 9.1 (26)	127.9 ± 8.4 (27)	6.4 ± 0.4 (25)	7.0 ± 0.5 (26)
Diazepam	163.3 ± 10.9 (28) [a]	94.0 ± 8.7 (28) [a]	11.0 ± 0.8 (26) [a]	7.3 ± 0.6 (28)
EOG 25	94.5 ± 6.3 (15) [b]	159.0 ± 8.1 (16) [b]	6.5 ± 0.2 (16) [b]	8.4 ± 0.3 (16)
EOG 50	84.3 ± 7.5 (13) [b]	151.9 ± 7.0 (13) [b]	6.0 ± 0.3 (13) [b]	8.5 ± 0.9 (13)
EOG 100	129.8 ± 11.4 (16) [b]	135.6 ± 12.3 (17) [b]	5.8 ± 0.4 (16) [b]	5.4 ± 0.4 (17) [a,b]

Values are means ± SEM of the number of mice in parentheses. a and b. against controls and diazepam, respectively, at p <0.05 (ANOVA and Student-Newman-Keuls as the *post hoc* test). TSOA (time spent in the open arms), TSCA (time spent in the closed arms), NEOA (number of entries in the open arms) and NECA (number of entries in the closed arms) were the parameters studied.

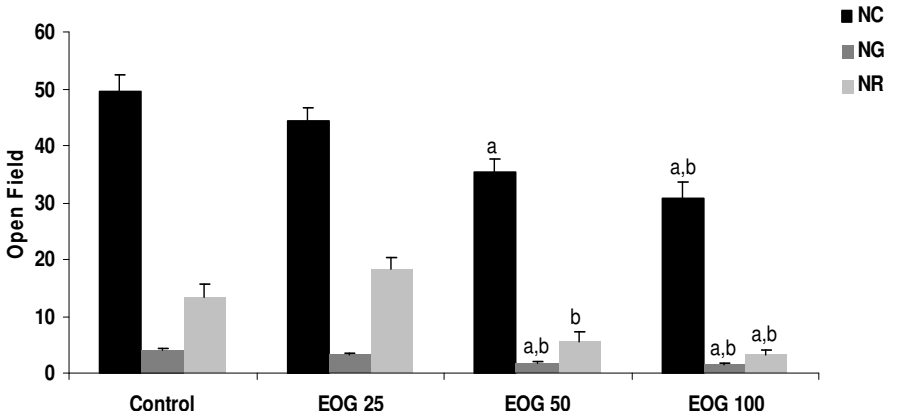

Figure 1. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the open field test, in mice.

Values are means ± SEM. The number of mice per group is presented in parentheses; a and b against controls and OEG 25, respectively, at p < 0.05 (ANOVA and Student-Newman-Keuls as the *post hoc* test). NC (number of crossings), NG (number of grooming), and NR (number of rearing) were the parameters studied.

significantly the number of grooming, and the maximum effect seemed to be reached at the dose of 100 mg/kg (63% reduction). The intraperitoneal administration of EOG also decreased the number of rearing (76% in the EOG 100 group and 59% in the EOG 50 group). However, there was no statistical difference between the doses, indicating that the maximum effect (decrease in the number of rearings) was reached with a dose around 100 mg/kg, i.p. The EOG dose of 25 mg/kg did not alter any observed parameter. Conversely, EOG (25, 50 and 100 mg/kg, i.p.) daily treated animals did not show any alteration in motor coordination, when tested on the rota rod apparatus (Table 2), as compared to controls.

Scopolamine alone or associated with EOG (25, 50 and 100 mg/kg, i.p.) caused a deficit in the short-term memory of mice, as assessed by the passive avoidance test. Scopolamine (S) reduced the values of latency in 83%, when compared to controls. The values of the latency of the groups S, EOG 25 + S, EOG 50 + S, and EOG 100 + S did not show any difference among them, indicating that EOG when associated with scopolamine does not reverse the amnesic effects of this standard drug. Also, EOG 100 mg/kg alone decreased the latency time in approximately 35%. After 24 h, the animals treated with scopolamine alone or associated with EOG showed a recovery of their cognitive functions. The cognitive impairment was however maintained in the EOG 100 group, and this was more evident after its association with scopolamine (EOG 100 + S group). Then, the association of S with EOG seems to potentiate the amnesic effects of scopolamine (Table 3).

A cognitive impairment caused by EOG was also seen in the T-maze test (Figure 2). EOG, 50 and 100 mg/kg i.p., decreased dose-dependently the latency of the animals to reach the open arms of the apparatus, in approximately 53 and 84%, respectively, when compared to controls. Scopolamine (10 mg/kg i.p.) decreased the latency in approximately 73%, as compared to controls. In the test of oxotremorine-induced tremors, EOG (25, 50 and 100 mg/kg, i.p.) reduced the intensity of tremors

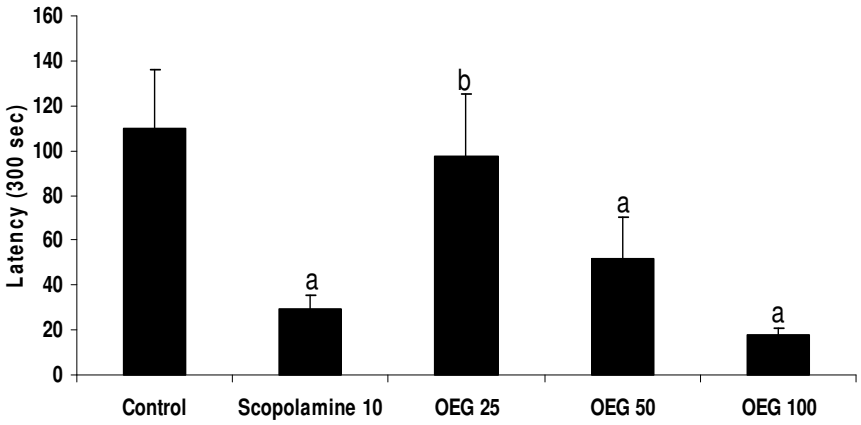

Figure 2. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the T-maze test, in mice.

Values are means ± SEM. The number of mice per group is presented in parentheses; a and b against controls and Scopolamine 10, respectively, at $p < 0.05$ (ANOVA and Student-Newman-Keuls as the *post hoc* test).

Table 2. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the rota rod test, in mice.

Group	TS on the bar (s)	NF
Control	44.1 ± 2.9 (25)	2.3 ± 0.1 (27)
EOG 25	52.4 ± 2.9 (14)	2.2 ± 0.2 (14)
EOG 50	52.1 ± 2.7 (13)	2.0 ± 0.3 (15)
EOG 100	44.9 ± 3.9 (17)	2.1 ± 0.2 (17)

Results are expressed as means ± SEM. The number of mice per group is presented in parentheses. EOG was administered intraperitoneally, at the doses of 25, 50 and 100 mg/kg. The parameters studied were the time spent on the bar (TS) and the number of falls (NF).

in approximately 34, 15 and 54%, respectively, as compared to controls. On the other hand, the pre-treatment with atropine completely abolished the oxotremorine-induced tremors (Figure 3).

In the test of pilocarpine-induced convulsions, EOG significantly increased the latency time for the 1st convulsion, at the doses of 50 and 100 mg/kg (148 and 128%, respectively), accompanied by a significant increase in the percentage of survival, as compared to controls administered with pilocarpine alone (Table 4).

DISCUSSION

Rhizomes from *Z. officinale* (ginger) have been used for medicinal purposes since antiquity, around the world, especially in Asia. Several species from the genus *Zingiber*, including *Z. officinale*, are reported to have anti-inflammatory, antiulcer, antioxidant and antimicrobial properties among others (Somchit and Nurshukriyah, 2003). Recently (Haghighi et al., 2005), the ginger extract was demonstrated to be very effective in the symptomatic treatment of osteoarthritis. Another work (Kalejaiye et al., 2002) showed that the aqueous extract of *Z. officinale* rhizomes exhibited a hypoglycemic activity, in streptozotocin and glucose-induced diabetic rats. Although ginger presents, in its chemical composition, non-volatile as well as volatile constituents, most of the work in the literature was carried out with ginger extracts and, thus, with the non-volatile components of rhizomes. As far as we know, the present work is the first report on the central effects of the essential oil of ginger.

Essential oils represent the volatile components of plants and their antimicrobial, antifungal and insecticidal properties are well established against wide spectra of organisms (Singh and Maurya, 2005). For instance, the essential oil from rhizomes of ginger was shown to exhibit a high antifungal activity (Jantan et al., 2003). Furthermore, a report on a six-month clinical experience demonstrated that a 5% solution of the essential oil of ginger was very effective in preventing post-operative nausea and vomiting, when administered naso-cutaneously before surgery (Geiger, 2005).

The elevated plus maze is a good model for studying compounds acting on the GABAergic neurotransmitter system, and is very sensitive in evaluating the influence of the complex GABA receptor/benzodiazepine drug, in anxiety. In the present study, we showed that the daily administration of EOG did not alter the animals' behavior in the plus maze task, when compared to diazepam and controls. This result suggests that EOG is possibly devoid of any benzodiazepine-type pharmacological activity. This effect differs from other studies (Hasenöhrl et al., 1996) showing that the combined extracts of *Z. officinale*

Table 3. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the passive avoidance test, in mice.

Group	Latency (15 min)	Latency (24 h)
Control	257.6 ± 13.7 (31)	295.4 ± 3.7 (34)
Scopolamine	44.5 ± 5.2 (26)[a]	102.4 ± 15.9 (24)[a]
EOG 25	270.8 ± 22.4 (9)	293.0 ± 6.9 (10)
EOG 50	253.4 ± 23.2 (7)	300.0 ± 0.0 (7)
EOG 100	167.2 ± 43.2 (9)[a]	256.0 ± 37.9 (6)
EOG 25 + Scopolamine	52.9 ± 9.8 (14)[a]	177.7 ± 30.8 (13)[a]
EOG 50 + Scopolamine	51.4 ± 6.4 (12)[a]	187.3 ± 34.8 (12)[a]
EOG 100 + Scopolamine	72.1 ± 21.1 (14)[a]	65.1 ± 17.0 (14)[a]

Values are means ± SEM. The number of animals per group is presented in parentheses; a against control, at $p < 0.05$ (ANOVA and Student-Newman-Keuls as the *post hoc* test).

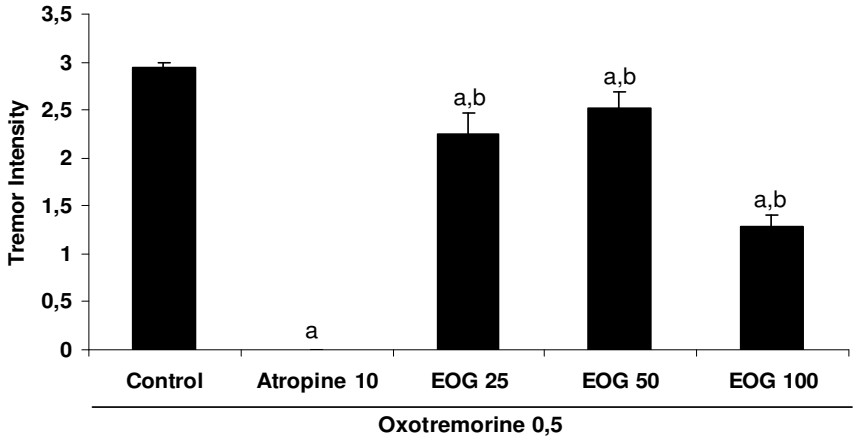

Figure 3. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the oxotremorine-induced tremor test, in mice**.**

Values are means ± SEM of the number of mice in parentheses; a and b against control and Atropine 10, respectively, at $p<0.05$ (ANOVA and Students Newmans-Keuls as the *post hoc* test).

Table 4. Effects of the daily intraperitoneal administration for 7 days of the essential oil of *Z. officinale* (EOG) on the pilocarpine-induced seizures, in mice.

Group	Pilocarpine- induced convulsions test	
	Latency (s)	Deaths (%)
Control	515.0 ± 54.0 (8)	100.0
EOG 25	492.7 ± 45.1 (8)	87.5
EOG 50	1279.0 ± 109.5 (7) [a]	75.0
EOG 100	1174.1 ± 86.7(6) [a]	57.2

Values are means ± SEM. The number of animals per group is presented in parentheses. EOG was administered intraperitoneally at the doses of 25, 50 and 100 mg/kg, 30 min before the pilocarpine injection (300 mg/kg, i.p.); a against controls at $p < 0.05$ (ANOVA and Student-Newman-Keuls as the *post hoc* test).

and *G. biloba* presented anxiolytic-like effects, on the elevated plus maze test. Such differences are possibly due to different chemical compositions of the essential oil and the combined extracts. Compounds that increase the number of entries in the open arms, as benzodiazepines, were reported (Pellow et al., 1985) to decrease the animals' exploratory behavior and motor activity. However, compounds that do not show any effect on anxiety, as haloperidol and tricyclic antidepressants, also decrease these parameters. This agrees with the results observed with EOG in the open field test, since its daily administration decreased the exploratory behavior, without having any significant effect, on the elevated plus maze test.

Furthermore, the daily administration of EOG promoted a decrease in the horizontal exploratory activity, vertical exploratory activity (rearing) and grooming. Thus, our results reveal an interesting CNS depressant (sedative)

effect of EOG, which was dose-dependent and reached its maximum at the dose of 100 mg/kg. An earlier work (Kawasaki et al., 1979) showed that the aqueous extract from *Z. mioga* produced an intense reduction in locomotor activity of mice, in the open field test. The extract also lowered significantly the rectal temperature, and prolonged the thiopental-induced sleeping time, de-noting a central depressant effect, as we similarly show-ed. The open field test is one of the most popular models used in the field of psychopharmacology. The increase of the locomotor activity can be considered as a stimulant effect, while the decrease in vertical exploratory (rearing) and horizontal (spontaneous) activities are more related to sedation. Grooming is a type of behavior common to almost all animal species. Although several neurotransmitters modulate this behavior expression, dopamine (DA) seems to be particularly involved in the process. The daily administration of EOG provoked a decrease in the number of grooming, in the open field test, indicating an effect upon the dopaminergic system. Decreases in locomotor activity and exploratory behavior, in the groups treated with EOG, reveal pharmacological effects similar to those observed with antipsychotic drugs.

The rota rod test is largely used to evaluate neurologic deficits in rodents. The daily treatment with EOG did not produce any motor coordination. This indicates that EOG safety is in the range of doses used in the present study. According to Massaquoi and Hallett (1998), the loss of motor coordination is characteristic of many neurological disorders, being a pharmacological effect easily detected in cases of intoxication.

Clinical evidences give support to the hypothesis that the brain cholinergic system is involved in mnemonic processses (Decker and Mcgaugh, 1991; Fibiger, 1991; Gallagher and Colombo, 1995). Earlier works (Hasselmo, 1995; Hasselmo and Bower, 1993) studied the cholinergic modulatory influence on learning and memory, in the hippocampus and cortex of rats. Acetylcholine (ACh) is a neurotransmitter responsible for the transference of impulses from cholinergic neurons in cholinoceptive nervous cells to innervated tissue (Tucek and Dolezal, 1993). Physiological studies indicate that the ACh neuromodulator effect at the cell level is variable, causing synaptic facilitation and suppression as well as direct hyperpolarization and depolarization, at the same cortical area.

Surprisingly, EOG administration induced a deficit in memory of mice submitted to the T-maze test. In this test, EOG 50 and 100 caused cognitive impairments (like scopolamine 10 mg/kg, i.p.), as it decreased the latency of the animals to reach the open arms of the apparatus. In the passive avoidance test, EOG also induced an important cognitive deficit. This effect was more evident with the dose 100 mg/kg of EOG alone. When associated with scopolamine, EOG seemed to potentiate the deficit produced by scopolamine in cognitive functions. In the passive avoidance model, EOG seemed to have an anticholinergic effect. This evidence agrees with the findings of Prado-Alcala et al. (1993), who say that, under several conditions, central or systemic administration of anticholinergic drugs or cholinergic system lesions produce memory deficits, while drugs that increase the cholinergic activity show an opposite effect. Our results disagree from those (Hanumanthachar and Parle, 2006) that show *Z. officinale* extract to significantly improve learning and memory in young mice, and also to reverse amnesia induced by diazepam and scopolamine. However, their extract had a different chemical composition, presenting mostly non-volatile components.

Oxotremorine is a muscarinic receptor agonist, and its convulsant activity seems to be mediated through the central cholinergic stimulation (Bebbington et al., 1966). The blockade of oxotremorine tremors by EOG indicates that it exerts an antagonist effect on the cholinergic system, and justifies the cognitive deficit observed with EOG and the potentiation of the scopolamine effect in the passive avoidance model. Similarly to oxotremorine, pilocarpine is also a muscarinic receptor agonist known to induce convulsions at high doses (> 250 mg/kg). The increase in the latency to the 1st pilocarpine seizure, associated with the increase in the animals' survival shown by EOG, confirms the involvement of the cholinergic system with the central actions of this essential oil.

Our work showed that the daily administration of the essential oil obtained from ginger rhizomes (EOG) presented a sedative effect, since it decreases the number of crossings, grooming and rearing, in the open field test. EOG, however, did not show any anxiolytic effect on the elevated plus maze test, nor impaired the motor coordination of mice. In the passive avoidance test, EOG (100 mg/kg) alone or associated with scopolamine impaired both mice short-term and long-term memories, indicating that the essential oil presents an anticholinergic effect on the CNS. This was observed in the oxotremorine-induced tremor, as well as in pilocarpine-induced seizures, since EOG in both models reversed the effects of these cholinergic muscarinic agonists. Further-more, EOG also blocked ACh-induced contractions, in the isolated rat uterus preparation (data not shown). However, surprisingly, the acetylcholinesterase activity was not signifycantly changed in brain homogenates of animals treated with EOG (100 mg/kg, i.p., for 7 days) (data not shown). This result suggests that the effect of EOG on the cholinergic system may be due to its interaction with brain muscarinic receptors. Altogether, our results show that the effects of EOG are, at least in part, dependent upon the muscarinic cholinergic system.

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REFERENCES

Andrews LS, Cadwalader KR, Gronder RM, Chung HY (1995). Chemical and microbial quality of irradiated ground ginger. J. Food. Sci.pp 29-32.Archer J (1973). Tests for emotionality in rats and mice: A review.

Animal Behav, 21: 205-235.

Balch JF, Balch PA (1996). Prescription for nutritional healing: A practical A-Z reference to drug-free remedies using vitamins, minerals, herbs & food supplements. New York: Avery Publishing Group.

Bebbington A, Brimbelcombe R W, Shakeshaft D (1966). The central and peripheral activity of acetylenic amines related to oxotremorine. Brit. J. Pharmacol. 26: 56–67.

Bhandari U, Kanojia R, Pillai KK (2005). Effect of ethanolic extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. J Ethnopharmacol. 97(2): 227-230.

Coward DM, Doggett NS. Sayers AC (1977). The pharmacology of N-carbamoyl-2-(2,6-dichlorophenyl) acetamidine hydrochloride (LON-954) a new tremorogenic agent. Arzneimittleforschung/Drug Research. 27: 2326-2332.

Cunha GM, Bezerra PJ, Saldanha MD, Cavalcante MC, Bruin VM, Viana GS (2000). Pentoxifylline improves learning and memory in glutamate-lesioned rats. Pharmacol Biochem. Behav. 66: 687-694.

Decker MW, Mcgaugh JL (1991). The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. Synapse, 7: 151–168.

Dunham NW, Miya TS (1957). A note on a simple apparatus for detecting neurological deficits in rats and mice. J. Am. Pharmac Assoc. 46: 208-212.

Fibiger HC (1991). Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. Trends Neurosci. 14: 220–223.

Gallagher M, Colombo PJ (1995): Aging and the cholinergic hypothesis of cognitive decline. Current Opinion in Neurobiology. 5: 161–168.

Geiger JL (2005). The essential oil of ginger, *Zingiber officinale*, and anaesthesia. Int. J. Aromatherap. 15(1): 7-14.

Grzanna R, Lindmark L, Frondoza CG (2005). Ginger- an herbal medicinal product with broad anti-inflammatory actions. J. Med. Food. 8(2): 125-132.

Haghighi M, Khalvat A, Toliat T, Jallaei S (2005). Comparing the effects of ginger (*Zingiber officinale*) extract and ibuprofen on patients with osteoarthritis. Arch. Iranian. Med. 8(4): 267-271.

Hanumanthachar J, Parle M (2006). *Zingiber officinale*: evaluation of its nootropic effect in mice. African J Trad Compl Alternative Med, 3(1): 64-74.

Hasenöhrl RU, Nichau CH, Frisch CH, De Souza Silva MA, Huston JP, Mattern CM, Häcker R (1996). Anxiolytic-like effect of combined extracts of *Zingiber officinale* and *Ginkgo biloba* in the elevated plus-maze. Pharmacol Biochem Behav; 53: 271–275.

Hasselmo ME (1995): Neuromodulation and cortical function: Modeling the physiological basis of behavior. Behav. Brain. Res. 67: 1-26.

Hasselmo ME, Bower JM (1993). Acetylcholine and memory. Trends in Neurosci. 16: 218–222.

Jantan IB, Yassin MSM, Chin CB, Chen LL, Sim NL (2003). Antifungal activity of the essential oils of nine *Zingiberaceae* species. Pharmac. Biol. 41(5): 392-397.

Kalejaiye OF, Iwalewa EO, Omobuwajo OR, Oyedapo OO (2002). Hypoglycaemic effects of Nigerian *Zingiber officinale* rhizome on experimental diabetic rats. Nigerian. J. Nat. Prod. Med. 6: 33-35.

Kawasaki H, Gomita Y, Fukamachi K, Moriyama M, Ichimaru Y, Uchikado A, Nonaka G, Nishioka I (1979). Behavioral and EEG effects of *Zingiber mioga* Roscoe (water soluble fraction). Nippon Yakurigaku Zasshi, 75(6): 601-615.

Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine 14 (2-3): 123 – 128.

Lim WC, Seo JM, Lee CI, Pyo HB, Lee BC (2005). Stimulative and sedative effects of essential oils upon inhalation in mice. Arch. Pharm. Res. 28(7): 770-774.

Lister RG (1987). The use of a plus-maze to measure anxiety in the mouse. Psycopharmacol. 92: 180-185.

Massaquoi SG, Hallett M (1998). Ataxia and other cerebellar syndromes. In: Parkinson's disease and movement disorders. Baltimore: Williams & Wilkins.

Ojewole JAO (2006). Analgesic, anti-inflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (*Zingiberaceae*) in mice and rats. Phytother. Res. 20(9): 764 – 772.

Pellow S, Chopin P, File SE, Briley M (1985). Validation of open, closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14: 149-167.

Prado-Alcala RA, Fernandez-Ruiz J, Quirarte GL (1993). Cholinergic neurons and memory, in: T.W. Stone (Editor), Synaptic Transmission 2, London: Taylor and Francis Ltd. pp. 57–69.

Sakamura F, Ogiharat K, Suga T, Taniguchi K, Tanaka R (1986). Volatile constituents of *Zingiber officinale* rhizomes produced by in vitro shoot tip culture. Phytochemist. 25: 1333.

Singh G, Maurya S (2005). Antimicrobial, antifungal and insecticidal investigations on essential oils: An overview. Natural Product Radiance. 4: 179-192.

Somchit MN, Nurshukriyah MH (2003). Antiinflammatory property of ethanol and water extracts of *Zinziber zerumbet*. Indian. J. Pharmacol. 35: 181-182.

Topic B, Hasenohrl RU, Hacker R, Huston JP (2002b). Enhanced conditioned inhibitory avoidance by a combined extracts of *Zingiber officinale* and *Gingko biloba*. Phytother. Res. 16(4): 312-315.

Topic B, Tani E, Tsiakitziz K, Kouronakis PN, Dere E, Hasenohrl RU, Hacker R, Mattern CM, Huston JP (2002a). Enhanced maze performance and reduced oxidative stress by combined extracts of *Zingiber officinale* and *Gingko biloba* in the aged rat. Neurobiol. Aging. 23(1):135-43.

Tucek S, Dolezal V (1993). The non-quantal release of acetylcholine from motor nerve terminals: comment on its likely size. Prog. Brain. Res. 98: 209-212.

Vishwakarma SL, Pal SC, Kasture VS, Kasture SB (2002). Anxiolytic and antiemetic activity of *Zingiber officinale*. Phytother. Res, 16 (70): 621-626.

Yu Z, Wu H, Ding J (1998). The volatile chemical components of fresh *Zingiber officinale*. Acta Botanica Yunnanica. 20: 113-118.