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Research Article

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Anxiolytic Properties of Garcinia Lucida Vasque (Clusiaceae) in Mice and Its Possible Action

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Abstract Anxiolytic effects of *Garcinia lucida (G. lucida)* barks aqueous extract and its possible action mechanisms were assessed in mice. Stress induced hyperthermia (SIH), Elevated plus maze (EPM), Open field (OF), and hole-board tests were used. Mice received distilled water as negative control, Phenobarbital (20 mg/kg) and diazepam (3 mg/kg) as positive control or gradual doses of *G. lucida* (200, 400 and 800 mg/kg) as test groups. After 24 hours acclimatization period, animals received appropriate treatment and 1 hour later individually subjected to behavioural paradigms and parameters recorded for 5 minutes. Involvement of $GABA_A$ receptor complex in *G. lucida* extract activity was inferred from experiments with known $GABA_A$-targeting agents. Administration of *G. lucida* extract induced a significant ($p<0.05$) decrease of SIH from 1.17°C to 1.03°C compared to negative control. In EPM test, open arms entries increased from 5.16±0.74($p<0.01$) to 8±0.57($p<0.001$) at 200 and 800 mg/kg respectively. In OF test, crossing number significantly ($p<0.001$) increased from 74±11.66 to 92±8.53 respectively at 400 and 800 mg/kg. *G. lucida* significantly decreased rearing number from 22.16±1.044 ($p<0.05$) at 200 mg/kg to 14.66±0.71 ($p<0.001$) at dose 800 mg/kg, suggesting anxiolytic-like effects as observed with diazepam. These anxiolytic effects of *G. lucida* were significantly inhibited by anxiogenic activities of bicucilline (5 mg/kg), flumazenil (5 mg/kg) and N-methyl-β-carboline-3-carboxamide (10 mg/kg). Thus, *G. lucida* has anxiolytic properties that might be due to interactions with benzodiazepine-binding sites of $GABA_A$ receptor complex. These observed effects may in part explain the use of *G. lucida* in traditional medicine.

Keywords Mice, *G. lucida*, anxiety, anxiolyic, $GABA_A$ receptor complex

1. Introduction

In today's life of stress and strain, there is a need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory function of the brain [1]. Stress involves complex biochemical, neural and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes

mellitus, male impotency and cognitive dysfunctions to cardiovascular disease, hypertension, peptic ulcers, migraine, allergies, asthma, carcinoma, premature aging, rheumatic diseases and ulcerative colitis [2]. Importantly, stress is also known to interfere with cognitive functions, tending to retard the memory engram rather than the acquisition of learning [3]. During the last two decades, pharmacotherapy with psychoactive drugs has been increasingly recognized as most effective in the management of anxiety, stress and psychosomatic disorders. However, the prolonged use of tranquilizers and psychotropic drugs leads to a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. Moreover, such agents primarily relieve the symptoms and offer a palliative relief of a temporary nature [4,5]. Approximately 10% of the world population is suffering from one or many forms of anxiety disorders, for example, panic attacks, social phobias, or generalized anxiety disorders [6].

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. Only 10% of plants have been studied for their pharmacological properties [7]. *G. lucida*, is one of the medicinal plants used in Africa. The stem bark, the leaves, the roots, and the fruit of this plant are used to treat various types of diseases. In Cameroon, *G. lucida* is used in the treatment of gastro-intestinal, lungs and gynaecological affections, and sexually transmitted diseases. According to Cameroonian traditional healers, the plant is also used as poison and venin antidote, and to fight against devils [8]. It's also used as palm or raphia wine ferment [9]. Previous experiments have shown that *G. lucida* has components which also exhibit anti-inflammatory, anti-acid and antibacterial activities [10]. Our study was intended to explore the anxiolytic properties of aqueous extract of bark of this medicinal plant in mice, using hyperthermia-induced stress (SIH), elevated plus maze (EPM), open field (OF), and hole-board tests. Its potential action mechanisms were also investigated in regard of receptor systems involved in the anxiolytic-like effects. Part of these results has been published in abstract form.

2. Material and methods

2.1. Plant material

The barks of *G. lucida* used were collected at Mfou, the vicinity of Yaoundé (Cameroon), during the dry season (November 2016). A voucher specimen of the plant (N^{o} 43677/SRF/Cam) was authenticated at the National Herbarium of Cameroon in Yaoundé.

2.2. Animals

Swiss albinos naïve mice of either sex aged about 2 to 3 months, weighing approximately 18-25 g were used for experimental purpose. Animals provided from the animal house of the laboratory of Animal Physiology of the Higher Teacher's Training College of Yaoundé. They were housed in standard cages with the temperature maintained at 25°C, and 12 h alternating light and dark cycle. They were supplied with food and water ad libitum. The study was conducted in accordance with the Cameroon National Ethics Committee (Reg. No. FWA-IRB00001954), and experiments were designed to minimize the number of animals used and to minimize suffering.

2.3. Chemicals

All the chemicals used in this study were as analytic. These chemicals included: Bicuculline, Flumazenil (RO151788), N-methyl-β-carboline-3-carboxamide (FG7142) from sigma (USA), Phenobarbital and Diazepam from Roche (France).

2.4. Methods

2.4.1. Preparation of the aqueous extract

800 g powder of bark of *G. lucida* were weighted and boiled in 4 liters of distilled water during 20 minutes. After cooling, the decoction was collected and filtered using Wattman filter paper N^{o} 1. The filtrate was then evaporated to dryness using an oven at 60°C giving aqueous extract with 11.32% yield.

2.4.2. Pharmacological tests

2.4.2.1. Stress-induced hyperthermia test

Animals were marked and housed 10 per cage. Mice were removed from the cage one after the other in a precise order and treated with distilled water (negative control group), phenobarbital 20 mg/kg ip (positive control group), or one of three doses of the decoction of *G. lucida* (test groups) per os. All animals within a given cage were consecutively treated at 1-minute intervals. After 60 minutes, mice were again consecutively removed from the cage (1 minute intervals) and their body (rectal) temperature was recorded. This experiment is based on the fact that among animals in the same cage, mice removed later have a higher body temperature than those removed earlier [11,12]. SIH was defined as the difference between the mean temperature of the first three mice and the mean temperature of the last three mice.

2.4.2.2. Elevated plus maze test

The apparatus comprised two open arms (15 x 5 x 10 cm) and two closed arms (15 x 5 x 10 cm) that extended from a common central platform (5 x 5 cm). The entire maze was elevated to a height of 50 cm above floor level. The negative control group received distilled water, the positive control group received diazepam (3 mg/kg), and the three test groups received three different doses of *G. lucida*. One hour after treatment, the mice were individually placed on the EPM center platform facing an open arm and observed for 5 minutes [13]. The number of entries by each animal into open or closed arms and the time spent by each animal in either open or closed arms (conventional parameters) were recorded with stopwatches by two trained experimenters. Time on the center platform and ethological parameters such as rearing and head dipping were also recorded.

2.4.2.3. Open field test

One hour after appropriate treatment, naive mice were placed in the center of the OF. The OF used was a wooden square box 40 x 40 x 45 cm; the floor was divided into 16 smaller squares of equal dimensions (10 x 10 cm) [13]. Animals placed one by one in the center of the box could explore the box for 5 minutes. Mice were observed for 5 minutes to evaluate the effects of the plant on both exploratory activity and anxiety [14-16]. Hand-operated counters and stopwatches were used to score crossing (number of square floor units entered), rearing (The time the animal stood on its hind legs), grooming, and defecation. The positive control group received diazepam 0.3 mg/kg.

2.4.2.4. The hole-board test

The mice hole-board was a wooden box (40 cm x 40 cm 2, 2 cm) with 16 equidistant holes 3 cm in diameter in the floor. The center of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 25 cm above the ground and divided into squares of 10 cm x 10 cm [17]. After treatment administration, each animal was placed in the center of the apparatus and allowed to freely explore the floor for 5 minutes. The behavioural parameters recorded was the latent period of first head dipping appearance, the number and duration of head dipping, and the number of rearing. The positive control group received diazepam 3 mg/kg.

2.4.2.5. Study of the involvement of GABA site of GABA-A receptors complex on the anxiolytic properties of *G. lucida* by using bicuculline

The involvement of GABA site of GABA-A receptor complex for anxiolytic properties by *G. lucida* was evaluated. The bicuculline, a competitive antagonist of GABA site of GABA-A receptor complex were used in this study. Mice were treated with bicuculline (5 mg/kg; i.p) 30 minutes before administration of *G. lucida* decoction (400 mg/kg). One hour after administration of the test substance, the mice were placed one after the other in the center of the elevated plus maze. Conventional and ethological parameters were observed and recorded for 5 minutes period.

2.4.2.6. Study of benzodiazepines site of GABA-A receptors complex on the anxiolytic properties of *G. lucida* by using β-carboline and flumazenil

For the evaluation of the involvement of benzodiazepines site of the GABA-A receptor complex in the anxiolytic properties of *G. lucida*, groups of mice were treated with flumazenil (5 mg/kg, i.p), a GABA-A benzodiazepine

receptor antagonist or β-carboline (10 mg/kg, i.p) an inverse agonist of the receptor site of the GABA-A receptor complex of benzodiazepines, 30 minutes before the administration of *G. lucida* extract (400 mg/kg, p.o). The dose flumazenil and β-carboline administered has been found to block the GABA receptors [18]. The anxiety evaluation using elevated plus maze test was carried out one hour after the administration of *G. lucida*. Between each trial, the maze was wiped with 10% ethanol to prevent olfactory cue from animals. Classic and ethological parameters were observed and recorded for a period of 5 minutes.

2.5. Data analysis

Values were expressed as mean ± SEM (Standard Error of the Mean). All data were analysed by one way analysis of variance (ANOVA). Post hoc tests were then performed using Tukey HSD test, with the level of significance set at $p \leq 0.05$.

3. Results

3.1. Effects of aqueous extract of *G. lucida* on the mean rectal temperature

G. lucida, like Phenobarbital (20 mg/kg) had a significant and dose dependant decrease of mean rectal temperature of mice compared to the negative control group treated with distilled water (figure 1). The mean rectal temperature in that group from 33.97 ± 0.19°C decreased to 33.2 ± 0.2 °C, 32.79 ± 0.16 °C and 30.87 ± 0.18°C ($p<0.001$) in mice treated with *G. lucida* respectively at the doses of 200, 400 and 800 mg/kg. In mice treated with phenobarbital, the mean rectal temperature was 28.24 ± 0.04 °C.

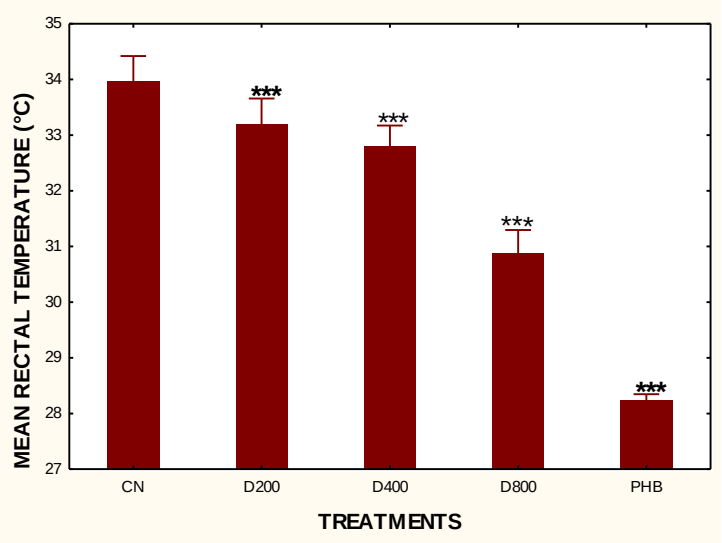


Figure 1: Effects of aqueous extract of G. lucida on mean rectal temperature

Each bar represents the mean rectal temperature + SEM, n=10. P values for groups were obtained by one way ANOVA followed by Tukey HSD post-hoc test. ***$p<0.001$ significantly different with respect to distilled water treated group. CN: negative control group treated with distilled water. PHB: positive control group treated with Phenobarbital (20 mg/kg). D200, D400 and D800: *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg.

3.2. Effects of aqueous extract of *G. lucida* on Stress-Induced Hyperthermia

As shown in figure 2, the aqueous extract of *G. lucida* decreased significantly ($p<0.05$) and dose dependant the SIH. The negative control group had a SIH of 1.26°C. The mice which received *G. lucida* (800 mg/kg) and phenobarbital (20 mg/kg) expressed respectively 1.03°C and 0.13°C values of SIH.

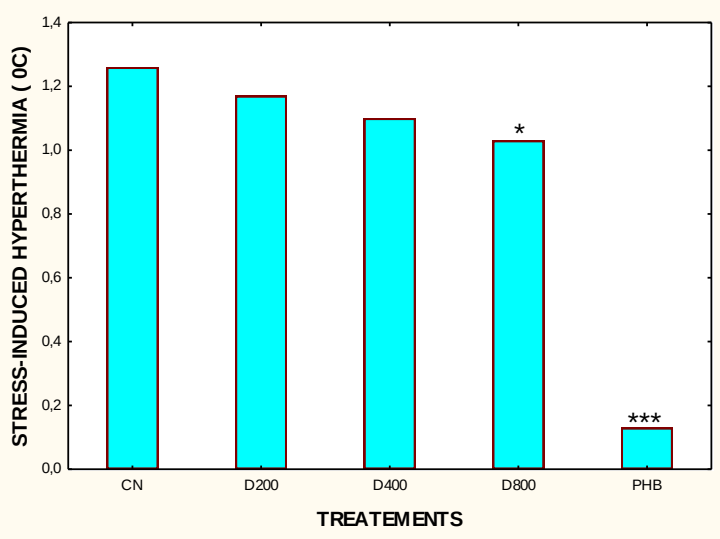
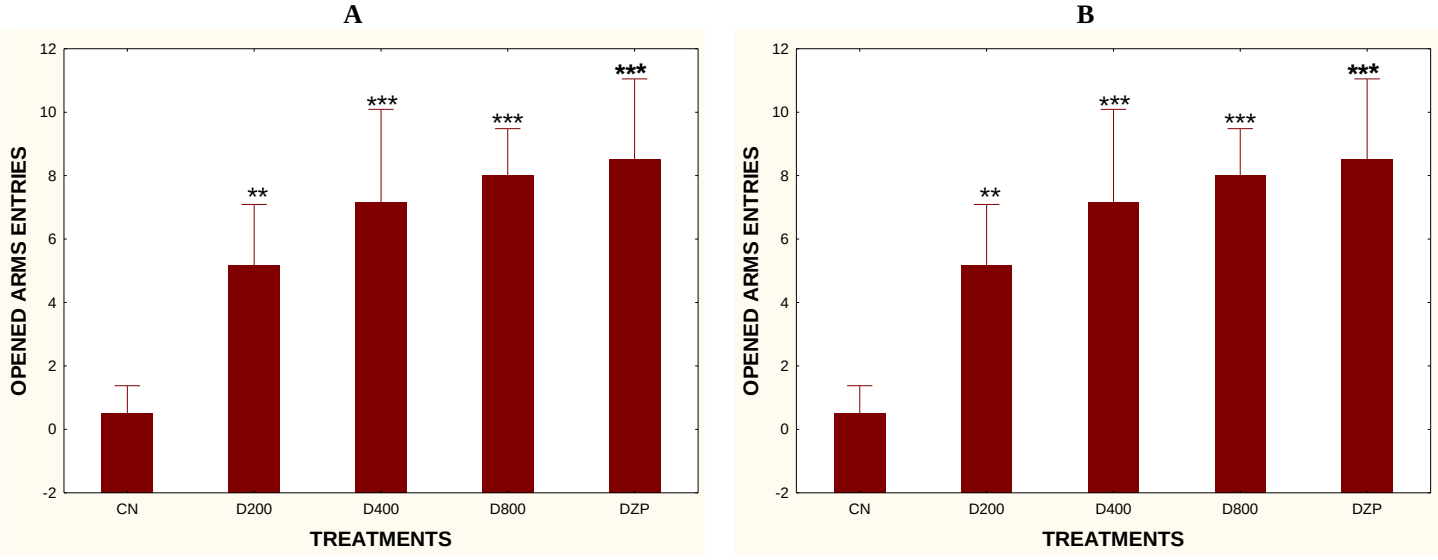


Figure 2: Effects of aqueous extract of G. lucida on stress-induced hyperthermia

Each bar represents the SIH, n=10. P values for groups were obtained by one way ANOVA followed by Tukey HSD post-hoc test. *p<0.05, ***p<0.001 significantly different with respect to distilled water treated group. CN: negative control group treated with distilled water. PHB: positive control group treated with Phenobarbital (20 mg/kg). D200, D400 and D800: *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg.

3.3. Effects of single administration of aqueous extract of *G. lucida* on Elevated Plus Maze parameters

According to figures 3 (A, B, and C), a single administration of *G. lucida* aqueous extract in mice at the doses 200, 400 and 800 mg/kg increased significantly and dose dependant open arms entries (5.16 ± 0.74; p<0.01; 7.16 ± 1.13; p<0.001; and 8 ± 0.57; p<0.001 respectively), percentage of open arms entries (60.77%; p<0.001; 72.91%; p<0.001; and 80%; p<0.001 respectively), and percentage of time spent in open arms (18.93%; p<0.05; 52.8%; p<0.01 and 77.89%; p<0.001 respectively) of the EPM compared to the negative control group treated with distilled water (0.5 ± 0.34, 8.33% and 1.82% respectively). As expected for a positive control group, a single administration of diazepam (3 mg/kg; i.p) increased significantly (p<0.001) open arms entries, the percentage of open arms entries and time spent in the open arms of the EPM.



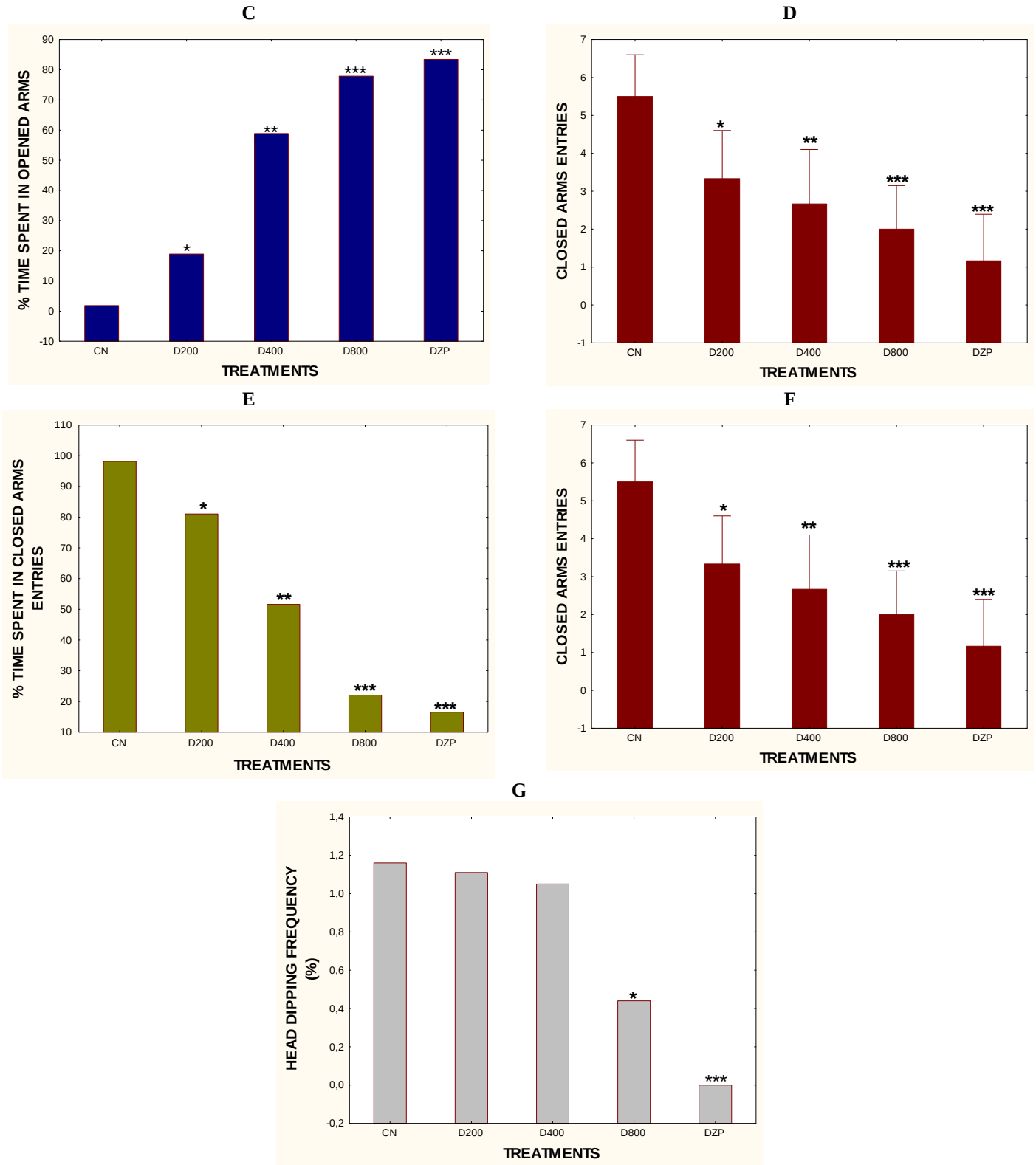


Figure 3: Effects of a single administration of the aqueous extract of G. lucida on EPM parameters. (A): opened arms entries; (B): percentage of open arms entries; (C): percentage of time spent in opened arms; (D): closed arms entries; (E): percentage of closed arms entries; (F): rearing frequency; (G): head dipping frequency

As shown in figures 3 (D, and E), *G. lucida* extract at the doses 200, 400 and 800 mg/kg) decreased significantly and dose dependant closed arms entries (3.33 ± 0.5; $p<0.05$; 2.66 ± 0.4; $p<0.01$ and 2 ± 0.4; $p<0.001$ respectively) and percentage of time spent in closed arms (81.06%; $p<0.05$; 47.2; $p<0.01$ and 22.10; $p<0.001$ respectively) compared to the negative control group.

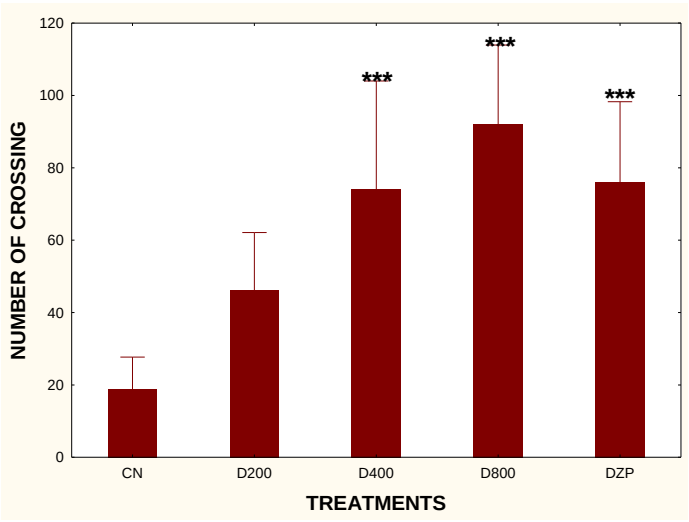
Figures 3 (F and G) indicated that the plant extract (200, 400 and 800 mg/kg) decreased the frequency of rearing (significantly at the dose 800 mg/kg; $p<0.05$) and head dipping (significantly at the doses 400 mg/kg; $p<0.05$ and 800 mg/kg; $p<0.01$) in treated mice compared to the negative control group. The same trend was observed in the positive control group treated with diazepam.

Each bar represents the mean + SEM, n=6. P values for groups were obtained by one way ANOVA followed by Tukey HSD post-hoc test. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significantly different with respect to distilled water treated group. CN: negative control group treated with distilled water. DZP: positive control group treated with diazepam (3 mg/kg). D200, D400 and D800: *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg.

3.4. Effects of single administration of aqueous extract of *G. lucida* on Open Field parameters

Figure 4 expresses the evolution of open field (OF) parameters in mice treated with graded doses of extract aqueous of *G. lucida.* As shown in figure 4A, the number of crossing significantly increased from 18.83 ± 3.45 in the negative control group to 74 ± 11.66 ($p<0.001$) and 92 ± 8.53 ($p<0.001$) at the doses of 400 and 800 mg/kg respectively in mice treated with the plant extract. This number was 76 ± 8.66 ($p<0.001$) in the positive control group received diazepam (0.3 mg/kg). *G. lucida* aqueous extract also significantly ($p<0.05$; $p<0.01$ and $p<0.001$) increased the frequency of crossing and grooming and the time spent in center of OF compared to the negative control group (figure 4 (B, C, and D). Time spent in periphery was 212 ± 4.32 s in the negative control group. A single administration of our plant extract decreased significantly this time to 190.83 ± 2.31 s ($p<0.05$), 157.5 ± 6 s ($p<0.01$) and 144.5 ± 5.75 s ($p<0.01$) respectively at the doses of 200, 400 and 800 mg/kg. Diazepam (0.3 mg/kg) in the positive control group also significantly ($p<0.001$) decreased the time spent in periphery compared to the negative control group (figure 4E). The frequency of rearing significantly decreased in mice treated with *G. lucida* aqueous extract with a minimum at the dose 800 mg/kg ($p<0.01$) with a value of 0.011 compared to 0.034 for negative control group (figure 4F). The mass of fecal boli from 0.4 ± 0.094 g in the negative control group significantly ($p<0.001$) decreased to zero at the dose of 800 mg/kg of *G. lucida* aqueous extract (figure 4G) as in the positive control group treated with diazepam (0.3 mg/kg).

A



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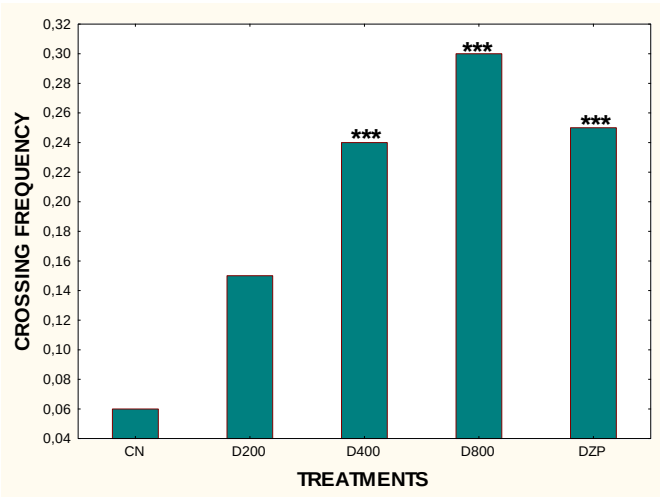

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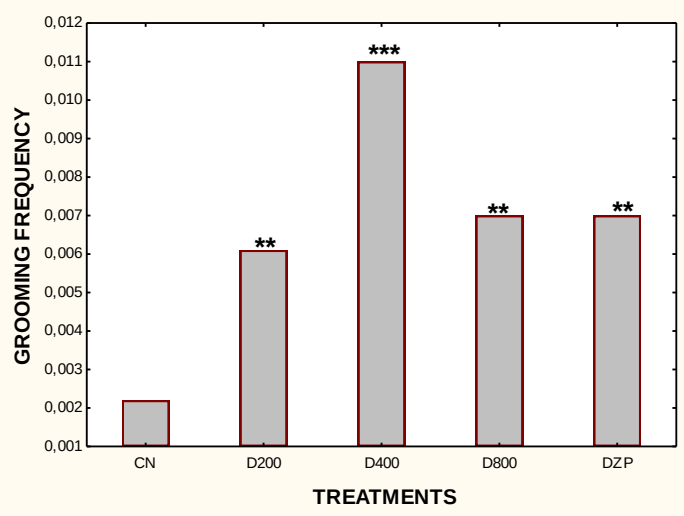
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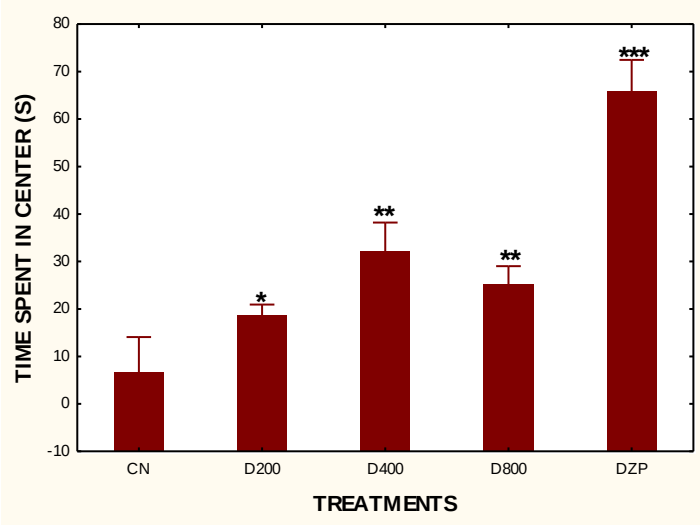
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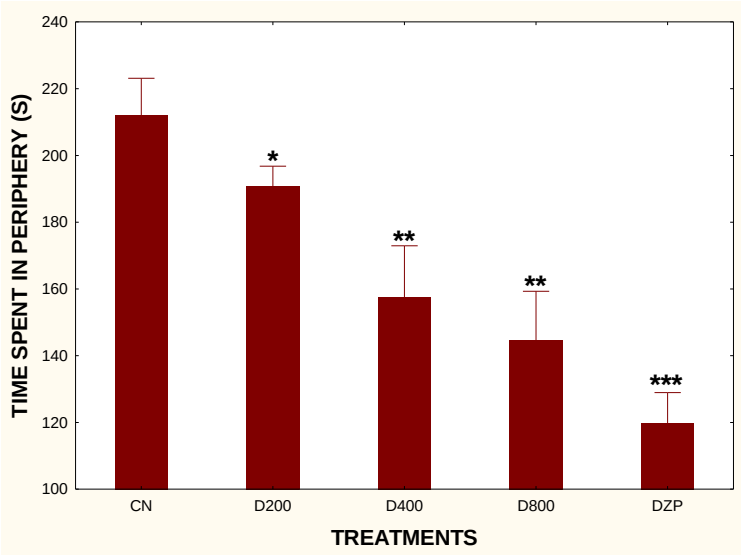
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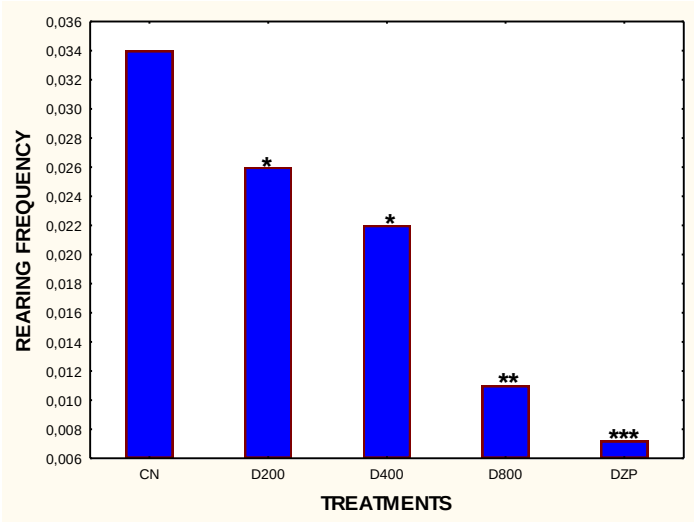
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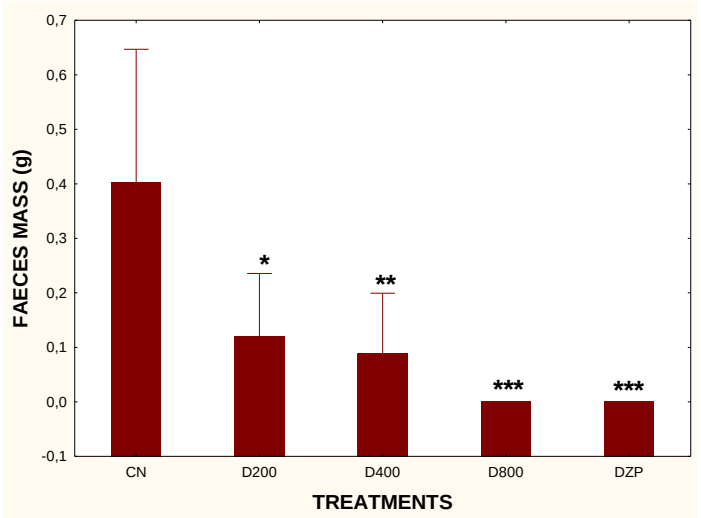
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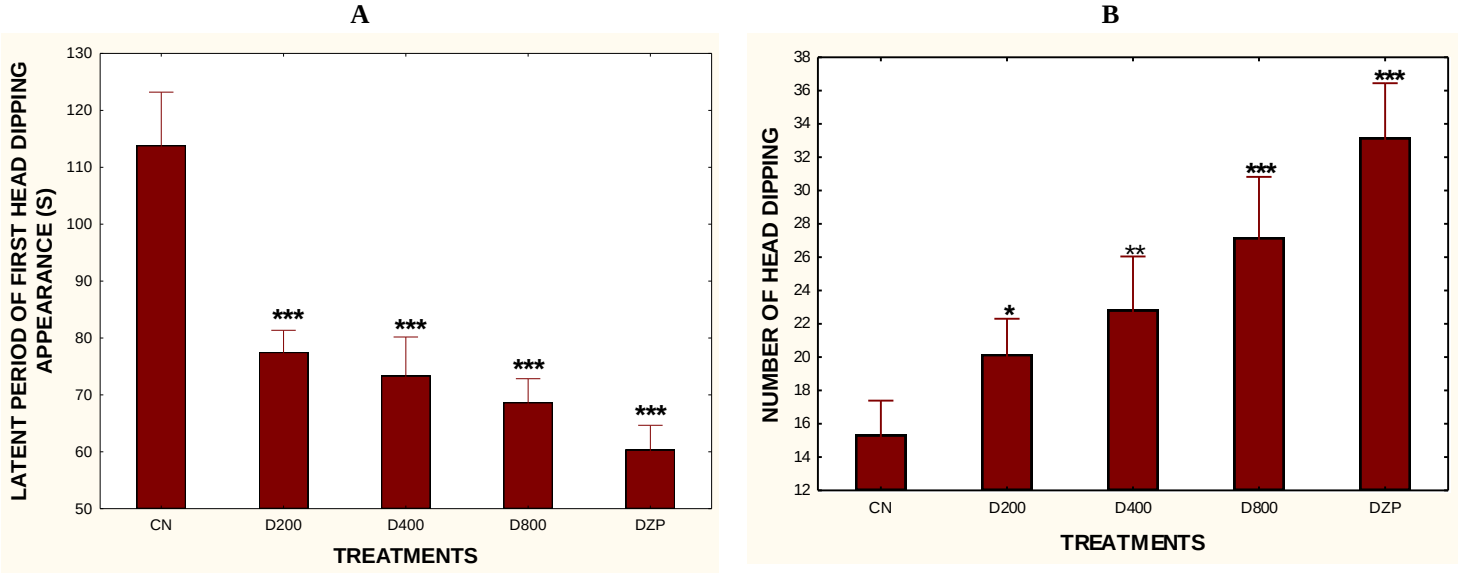
TREATMENTS

Figure 4: Effects of a single administration of the aqueous extract of G. lucida on open field parameters. (A): number of crossing; (B): crossing frequency; (C): grooming frequency; (D): time spent in center; (E): time spent in periphery; (F): rearing frequency; (G): faeces mass

Each bar represents the mean + SEM, n=6. P values for groups were obtained by one way ANOVA followed by Tukey HSD post-hoc test. *p<0.05, **p<0.01, ***p<0.001 significantly different with respect to distilled water treated group. CN: negative control group treated with distilled water. DZP: positive control group treated with diazepam (0.3 mg/kg). D200, D400 and D800: *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg.

3.5. Effects of single administration of aqueous extract of *G. lucida* on Hole-Board parameters

G. lucida aqueous extract significantly (p<0.001) and dose dependant decreased the latent period of first head dipping appearance with values being 77.5 ± 1.5 s, 73.33 ± 2.66 s and 68.66 ± 1.62 s respectively at the doses 200, 400 and 800 mg/kg, compared to negative control value which was 113.83 ± 3.46 s. This latent period also has been significantly (p<0.001) decreased by diazepam (3 mg/kg) in the positive control group (figure 5A). From 15.33 ± 0.8 in the negative control group, the number of head dipping was significantly increased by *G. lucida* aqueous extract with a maxima value of 27.16 ± 1.42 (p<0.001) at the dose of 800 mg/kg. Like the plant extract, diazepam (3 mg/kg) also significantly (p<0.001) increased this number at 33.16 ± 1.27 (figure 5B). As shown in figure 5C, single administration of *G. lucida* aqueous extract significantly (p<0.01) increased the head dipping duration at doses of 400 and 800 mg/kg with respective values of 11.33 ± 0.76 s à 14.5 ± 1.05 s compared to negative control group where this duration was about 5.33 ± 0.76 s. The number of rearing in the negative control group was 27.33 ± 1.02 (figure 5D). *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg significantly decreased this parameter with values of 22.16 ± 1.044 (p<0.05), 18.33 ± 0.88 (p<0.01) and 14.66 ± 0.71 (p<0.001) respectively. The same trend was observed with the positive control group with a value of 12.66 ± 0.55 (p<0.001). The mass of fecal boli from 0.32 ± 0.1 g in the negative control group significantly (p<0.05) decreased to 0.05 ± 0.05 g at the dose of 800 mg/kg of *G. lucida* aqueous extract (figure 5E) and 0.016 ± 0.01 g in the positive control group treated with diazepam (3 mg/kg).



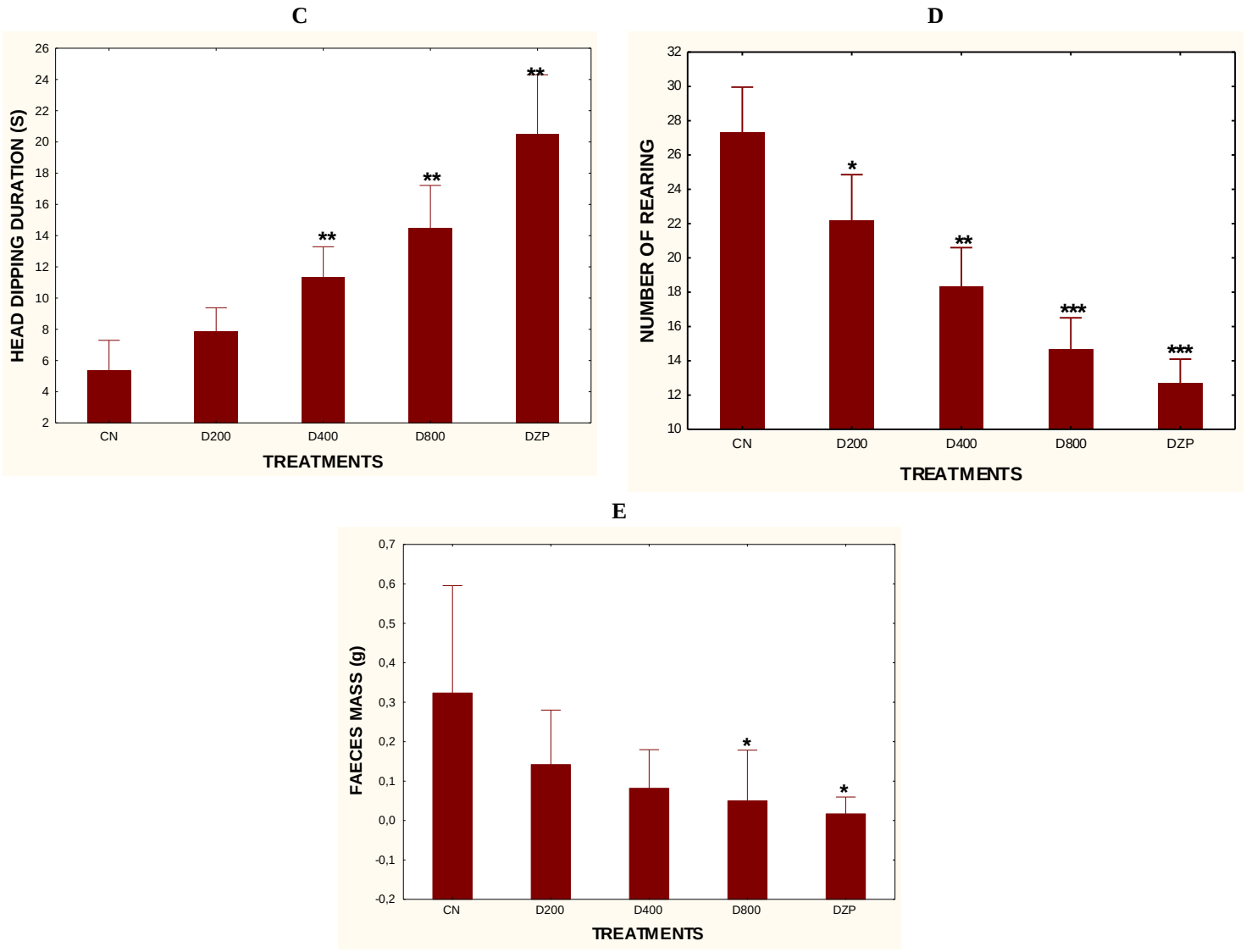


Figure 5: Effects of a single administration of the aqueous extract of G. lucida on hole-board parameters. (A): latent period of first head dipping appearance; (B): number of head dipping; (C): head dipping duration; (D): number of rearing; (E):): faeces mass.

Each bar represents the mean + SEM, n=6. P values for groups were obtained by one way ANOVA followed by Tukey HSD post-hoc test. *p<0.05, **p<0.01, ***p<0.001 significantly different with respect to distilled water treated group. CN: negative control group treated with distilled water. DZP: positive control group treated with diazepam (3 mg/kg). D200, D400 and D800: *G. lucida* aqueous extract at doses of 200, 400 and 800 mg/kg.

3.6. Involvement of GABA site of GABA-A receptor complex on the anxiolytic properties of *G. lucida* in mice using bicuculline

Based on the dose-activity data in EPM from 3 doses (200,400 and 800 mg/kg) of *G. lucida* aqueous extract, we used the medium dose (400 mg/kg) to investigate antagonist study. The administration of distilled water (DW), distilled water followed by bicuculline 5 mg/kg (DW+BIC) and dose 400 mg/kg of *G. lucida* aqueous extract followed by bicuculline 5 mg/kg (D400+BIC) resulted in significant (p<0.001) decrease of open arms entries and time spent in open arms, compared to effect observed in mice treated with *G. lucida* aqueous extract (400 mg/kg).

However, closed arms entries, time spent in closed arms, number of rearing and number of head dipping significantly increased (table 1).

Table 1: Effects of *G. lucida* aqueous extract in the presence of bicuculline on EPM parameters

Parameters	DW	DW+BIC	D400	D400+BIC
Opened arms entries	0.5±0.34***	0.16±0.16***	7.16±1.13	0.83±0.3***
Time spent in opened arms	5.33±3.71***	4.16±2.66***	44±6.75	4.83±0.7***
Closed arms entries	5.5±0.42**	6.66±0.49***	2.66±0.55	5.5±0.42**
Time spent in closed arms	287.16±2.72***	293.66±0.98***	39.33±10.55	267.66±1.76***
Number of rearing	13.33±0.55**	19.66±0.66***	7.5±1.64	16.33±0.66***
Number of head dipping	3.50±0.56	7.00±0.96**	3.16±0.60	6.16±0.70*

Values are expressed as mean ± SEM, n=6. *p<0.05, **p<0.01,***p<0.001, significant difference compared to dose 400 mg/kg of aqueous extract of *G. lucida* (D400) by one way ANOVA followed by TUKEY HSD post-hoc test. DW: Distilled water. DW+BIC: Distilled water and bicuculline (5mg). D400: Dose 400 mg/kg of *G. lucida* aqueous extract. D400+BIC: Dose 400 mg/kg of *G. lucida* aqueous extract associated to bicuculline (5mg).

3.7. Involvement of benzodiazepine site of GABA-A receptor complex on the anxiolytic properties of *G. lucida* in mice using FG 7142 and RO 151788

Action mechanisms of anxiolytic properties of *G. lucida* aqueous extract was evaluated, using flumazenil (RO 151788), a GABA-A benzodiazepine receptor antagonist and N- methyl-β-carboline-3-carboxamide (FG 7142) an inverse agonist of the receptor site of the GABA-A receptor complex of benzodiazepines, in mice placed in EPM. Results shown that a single administration of RO 151788 (5 mg/kg) or FG 7142 (10 mg/kg) followed by 400 mg/kg of *G. lucida* aqueous extract single administration, significantly decreased open arms entries ($p<0.001$) and time spent in open arms ($p<0.05$), compared to effect observed in mice treated with *G. lucida* aqueous extract (400 mg/kg). Meanwhile close arms entries ($p<0.001$), time spent in close arms ($p<0.001$), number of rearing ($p<0.001$) and number of head dipping ($p<0.001$) significantly increased. Similar effects also occurred when mice received a single administration of RO 151788 (5 mg/kg) (table 2) or FG 7142 (10 mg/kg) (table 3) followed by 3 mg/kg of diazepam.

Table 2: Effects of *G. lucida* aqueous extract in the presence of flumazenil on EPM parameters

Parameters	DW	D400	D400+FLU	DZP	DZP+FLU
Opened arms entries	0.5±0.34***	7.16±1.13	1±0.25***	8.5±0.99	1.5±0.42***
Opened arms time	5.33±3.71*	44±6.75	6±1.39*	71.5±15.9	6.5±1.4*
Closed arms entries	5.5±0.42*	2.66±0.55	6.16±0.79**	1.16±0.47	6.5±0.56***
Closed arms time	287.16±2.72***	39.33±10.55	279.5±1.65***	14.16±6.4	281.16±1.6***
Number of rearing	13.33±0.55**	7.5±1.64	15.50±0.67***	1.66±0.66**	20.33±0.71***
Number of head dipping	3.5±0.56	3.16±0.6	8.16±0.79***	0.0**	10.50±0.76***

Values are expressed as mean ± SEM, n=6. *p<0.05, **p<0.01,***p<0.001, significant difference compared to dose 400 mg/kg of aqueous extract of *G. lucida* (D400) by one way ANOVA followed by TUKEY HSD post-hoc test. DW: Distilled water. D400: Dose 400 mg/kg of *G. lucida* aqueous extract. D400+FLU: Dose 400 mg/kg of *G. lucida* aqueous extract associated to flumazenil (5mg/kg). DZP: Diazepam (3 mg/kg). DZP+FLU: Diazepam (3 mg/kg) associated to flumazenil (5mg/kg).

Table 3: Effects of *G. lucida* aqueous extract in the presence of N- methyl-β-carboline-3-carboxamide on EPM parameters

Parameters	DW	D400	D400+βC	DZP	DZP+ βC
Opened arms entries	0.5±0.34***	7.16±1,13	0.66±0.21***	8.5±0.99	0.83±0.3***
Opened arms Time	5.33±3.71*	44±6.75	6,5±0.42*	71.5±15.9	8±0.51*
Closed arms entries	5.5±0.42**	2.66±0.55	6.66±0.66***	1.16±0.47	6.33±0.42***
Closed arms Time	287.16±2.72***	39.33±10.55	281.16±1.6***	14.16±6.44	279±1.65*
Number of rearing	13.33±0.55**	7.5±1.64	16.83±0.60***	1.66±0.66**	19.83±0.60***
Number of head dipping	3.50±0.56	3.16±0.60	6.83±0.60***	0.0**	8.16±0.60***

Values are expressed as mean ± SEM, n=6. *p<0.05, **p<0.01,***p<0.001, significant difference compared to dose 400 mg/kg of aqueous extract of *G. lucida* (D400) by one way ANOVA followed by TUKEY HSD test. DW: Distilled water. D400: Dose 400 mg/kg of *G. lucida* aqueous extract. D400+βC: Dose 400 mg/kg of *G. lucida* aqueous extract associated to N- methyl-β-carboline-3-carboxamide (10mg/kg). DZP: Diazepam (3 mg/kg). DZP+ βC: Diazepam (3 mg/kg) associated to N- methyl-β-carboline-3-carboxamide (10mg/kg).

4. Discussion

The aim of the present study was to evaluate the anxiolyic like effects of *G. lucida* barks aqueous extract and the potential involvement of GABA-A receptors complex. In the current investigation, we examined for the first time the anxiolytic effects of *G. lucida* aqueous extract using stress-induced hyperthermia test (SIH), elevated plus maze test (EPM), open field test (OP), and hole-board test. SIH test is often used to identify benzodiazepines like anxiolytic agents well known for their Phenobarbital sleep potentiating effects [19]. Therefore, the observed effects of *G. lucida* aqueous extract in the doses finding experiments could as well be due to their modulating effects on the benzodiazepine site of GABA receptors. *G. lucida* extract would thus act on the barbiturates receptor sites by extending the opening of chloride voltage operating channel to produce anxiolytic effects [20,21]. Previous studies showed an important relationship between anxiolytic drugs and the reduction of body temperature [21,22]. On the EPM paradigm, *G. lucida* aqueous extract displayed a pronounced anxiolytic activity. *G. lucida* aqueous extract exhibited an increase of open arms entries, percentage of open arms entries and percentage of time spent in open arms. A drug which increases the number of entries into open arms is anxiolytic [23,24]. Furthermore, an increase in the percentage of time spent in the open arm and in the percentage of open arms entries indicates an anxiolytic effect [25]. The plant extract shown a decrease of closed arms entries and percentage of time spent in closed arms. A substance which reduces the number of entries into closed arms and percentage of time spent in closed arms also reduces anxiety [26]. In another hand, *G. lucida* also decreased rearing and head dipping frequency in mice. The results are agree with previous studies where it's established that a decrease of rearing and head dipping frequency in mice in EPM is a sign of anxiety decrease [27,28]. In the open field test, *G. lucida* aqueous extract increased the number and frequency of crossing, time spent in the center and the frequency of grooming. An increase of locomotive activity or exploration level in rodents by a pharmacological drug in open field test expresses anxiety decrease [29]. In contrast, a low exploratory or locomotive activity indicates anxiety increase [30]. An increase of time spent in the center or a decrease of time spent in periphery in rodents in open field test suggests a reduction of anxiety [22, 31]. An increase of grooming frequency is a sign of anxiety decrease [17]. *G. lucida* like diazepam (0.3 mg/kg) also decreased the frequency of rearing and faeces mass in open field test. A drug able to reduce the frequency of rearing or faeces mass has anxiolytic activity [17]. In the hole-board test, an anxiolytic-like state may be reflected by an increase in head dipping behaviours [17, 32]. *G. lucida* increased the number and duration of head dipping in mice, and also decreased the number of rearing and faeces mass corroborating the anxiolytic-like effect previously shown in elevated plus maze and open field tests. Taken together, all these observations suggest that *G. lucida* barks aqueous extract has anxiolytic properties which could be modulating by GABA neurotransmission in cerebral cortex and hippocampus [33]. Previous studies on the chemical components of *G.lucida* revealed the presence of certains compounds such as flavonoids, saponins, anthocianes, tannins, triterpens, carbohydrates, alkaloids, phenols and polyphenols which activate barbiturates, benzodiazines and GABA receptors in GABA-A receptor complex [34-36] and therefore generate anxiety decrease [37,38]. Flavonoids have been recently implicated for various pharmacological activities and they have been identified as a new type of ligand with in vivo anxiolytic properties. Some natural and synthetic flavonoids have been found to bind specifically and competitively to benzodiazepine receptors and to possess anxiolytis effects [39-41]. The GABA-A receptor is known to be a mediator of unconditioned anxiety. In this study, the involvement GABA receptor in the anxiolytic activity of *G. lucida* was assessed using bicuculline as antagonist. Bicuculline-sensitive GABA receptors are part of the super family of cys-loop pentameric ligand-gated ion channel receptors that include nicotinic, acetylcholine, glycine and $5HT_3$ receptors [42]. Bicuculline acts as competitive antagonist on GABA-A receptors in the fact that it competitively inhibits GABA binding to the receptors [43]. Inhibition of GABAergic neurotransmission way is anxiogenic, while the

stimulation leads to anxiolytic effects [21,22]. Anxiolytic effects of *G. lucida* aqueous extract in EPM test were inhibited by bicuculine. These results suggest that the plant extract could act on bicuculline GABA receptors to induce anxiolytic effects on mice. Involvement of benzodiazepine site of GABA-A receptor complex was also assessed on the anxiolytic properties of *G. lucida* in mice using β-carboline (FG 7142) an inverse agonist of the receptor site of the GABA-A receptor complex of benzodiazepines and flumazenil (RO 151788), a competitive antagonist of the receptor site of benzodiazepines of GABA-A receptor complex. Like with bicuculline, anxiolytic effects of *G. lucida* in mice were reversed by β-carboline and flumazenil in EPM test. The inhibition of anxiolytic activity of our plant by these drugs suggest a possible interaction between GABAergic system and anxiolytic properties of secondary metabolites of *G. lucida* aqueous extract [18].

5. Conclusion

In summary, our investigations show that aqueous extract of *G. lucida* induces anxiolytic effects in mice in stress-induced hyperthermia, elevated plus maze, open field and hole-board tests. These anxiolytic effects are inhibited by GABA-A receptor complex antagonists. Taken together, our findings suggest that *G. lucida* has anxiolytic-like effects mediated by action on GABA-A receptor complex.

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