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Dual Effect Of The Ethanolic Extract Of Hibiscus Acetosella Leaves In The Mice Central Nervous System

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Abstract:
Introduction: *Hibiscus acetosella (Welw.) or "vinegar-purple" possesses bioactive compounds in its leaves, flowers and roots of recognized anti-inflammatory and antioxidant activities.*
Objective: *This study aimed to investigate the effect of the ethanolic extract from Hibiscus acetosella (EEHa) leaves on behavioral and neurochemical parameters in mice.*
Methods: *The animals (Swiss mice) received EEHa (10, 50, 100 mg/kg) per oral by acute or sub-chronic daily treatment. Diazepam or imipramine were used as reference drugs. Behavioral tests were performed at 60 minutes or 14 days after EEHa treatment to evaluate the parameters: exploratory activity (open field); anxiety (hole board, elevated plus maze, social interaction); depression (tail suspension) and sleep time. The prefrontal cortex, hippocampus and striatum of euthanatized animals were collected for quantification of the oxidative stress markers malondialdehyde (MDA) and reduced glutathione (GSH).*
Results: *EEHa (50-100 mg/kg) increased the n° of crossings and rearing but reduced the n° of grooming (open field), the n° of dives (hole board), the permanence time (TPBA) and the n° of entries (NEBA) in the open arms (elevated plus maze), the interaction time (social interaction), and reduced the immobilization time (tail suspension) and sleep time of the animals. However, at 10 mg/kg presented opposite effect on these parameters. In addition, EEHa (10, 50, 100 mg/kg) presented antioxidant effect via reduction of MDA (41, 57, 55%) and increase of GSH (2.1, 2.3, 2.6-fold).*
Conclusion: *EEHa per se presents anxiolytic and antidepressant effects in mice associated to the antioxidant capacity.*
Key Word*: Hibiscus acetosella. Anxiety. Depression. Oxidative stress*

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I. Introduction

Neurobehavioral disorders affect individuals of all age groups in the world, corresponding to almost 1 billion people[1]. In the brazilian population, 63% present anxiety disorders, and 59% depression. These diseases show behavioral changes such as negative feelings and exacerbated fear, that may occur simultaneously[3]. Pathophysiological manifestations of neurological diseases are often related to impaired cellular redox homeostasis and, subsequently, oxidative and nitrosative stress[4].

The currently pharmacological treatment of these disorders may fail or cause adverse effects, including extrapyramidal (tremors, aggressive behavior), increased anxiety, depression, hypnosis and sedation[5,6]. In this context, biomolecules from plants of recognized actions on the central nervous system (CNS) can be considered as alternative therapy.

It has been reported for Hibiscus species the presence of a variety of biochemical compounds, such as polyphenols, flavonoids and anthocyanins, along with neuroprotective and antioxidante effects in rodents[7,8]. *Hibiscus acetosella Welw* (Malvaceae), "vinagreira roxa", is widely distributed in Brazil, being its leaves, flowers and fruits used in typical dishes preparation, and as appetite suppressant, antipyretic and anti-anemic[9]. Phytochemical analysis of different extract preparation of *H. acetosella* leaves revealed the presence of phenolic compounds, such as tannins and flavonoids, recognized for their antibacterial, anti-inflammatory and antioxidant activities[10,11].

The objective of this study was to investigate the effect of the ethanolic extract from *Hibiscus acetosella* leaves on behavioral parameters and neurochemical markers of anxiety, depression, hypnosis, and exploratory activity.

II. Material And Methods

Leaves collection, ethanolic extract preparation and phytochemical prospection

Leaves of *Hibiscus acetosella* were collected in São Luis-Maranhão (Brazil) and a voucher specimen (n° 4683) was deposited in the Herbarium Prisco Bezerra of Federal University of Ceará, Brazil.

The leaves were dried at 50 °C, ground into powder, and the extraction process performed in 70% ethanol for 15-day (1:10 w/v). The resulting material, named ethanolic extract of *Hibiscus acetosella* leaves (EEHa), was filtered, concentrated and lyophilized[12]. The identification of the active constituents of EEHa was performed by qualitative phytochemical analysis of the following: saponins, organic acids, reducing sugars, polysaccharides, proteins and amino acids, phenols, tannins, flavonoids, alkaloids, purines, cardiac glycosides, catechins, steroids and triterpenoids, lactones, depsides, coumarin derivatives, anthraquinones[13].

Animals

Male Swiss mice (25-30 g; n=8/group) were maintained at 22-25 °C (12h light/dark cycle), receiving water and food *ad libitum*, and allowed to adapt to the laboratory for at least 1 hour before experiments. The experimental protocols were conducted in accordance with NIH guidelines (publication nº 85-23, revised 2011) and approved by the Animal Care and Use Committee of University State of Ceara (CEUA/UECE n° 3281238/2016).

Experimental design

Animals received EEHa (10, 50, 100 mg/kg) *per oral* by acute (1h) or sub-chronic treatment (daily for 14-day) and were evaluated in behavioral tests (open field, hole board, elevated plus maze, social interaction, tail suspension and sleep time). Diazepam or imipramine (Sigma-Aldrich; St. Louis, MO, USA), used as reference drugs, were solubilized in sterile saline (0.9% NaCl).

After euthanasia, the brain areas prefrontal cortex, hippocampus and striatum were dissected to quantify oxidative stress markers (malondialdehyde-MDA, reduced glutathione-GSH).

Open field

Animals were individually placed in the open-field apparatus consisting of an acrylic box (30 x 30 x 15 cm) with floor divided into 9 squares. The number of squares crossed with all paws (crossing), elevations (rearing) and self-cleaning (grooming) were counted for 5 minutes[14].

Hole Board

Animals were placed in the equipment (20 x 20 cm) containing 16 equidistant holes. Both frequency and duration of the spontaneous behavior of head dips were measured for 5 minutes[15].

Elevated plus maze

Mice were placed in the center of the elevated plus maze consisting of two opposing open (30 × 5 × 25 cm) and closed arms (30 × 5 × 25 cm). The number of entries and the time spent on both arms were registered during 5 minutes[16,17].

Social interaction

Animals were placed in pairs on acrylic apparatus (30 x 30 x 15 cm) to evaluate the individual behaviors. One hour before the test, they were adapted to the environment. The frequency and the time spent in different types of interaction were measured during 10 minutes and classified into two categories: aggressive behavior (grabbing, boxing, kicking, biting) and non-aggressive behavior (smelling, self-cleaning)[18].

Tail suspension

Animals were suspended 50 cm from the ground by a tape fixed 1 cm from the tip of the tail and the immobility time was recorded for 5 minutes[19].

Sleep time

In the acute protocol, animals received intraperitoneal administration of pentobarbital (40 mg/kg) 60 minutes after EEHa or diazepam (1 mg/kg) and were placed in dorsal position. The time in which the animal loses the postural reflex was recorded as sleep latency, and the time between loss and voluntary recovery of the postural reflex was recorded as sleep time. The loss of righting reflex was considered for the animal inability to return to the prone position when placed in supine position for 3 consecutive times[20].

Brain oxidative stress

Tissues of the prefrontal cortex, hippocampus and striatum were homogenized in phosphate buffer sodium (pH = 7.4) to quantify the concentrations of MDA by the TBARS method[21] at 535 nm and GSH by DTNB method[22] at 412 nm.

Statistical analysis

Data were expressed as Mean ± SEM (n=8) and analyzed by One-Way ANOVA followed by Bonferroni test. Values of $p<0.05$ were considered significant.

III. Result

EEHa phytochemical prospection

The qualitative analysis of EEHa revealed the presence of saponins, phenols, tannins, flavonoids, alkaloids, cardiac glycosides, steroids and triterpenoids, coumarin derivatives and anthraquinones.

EEHa effect on locomotor and exploratory activities in the open field test

The number of crossings was reduced by EEHa at 10 mg/kg (14-day: 5.25 ± 0.70) and increased at 50 (1h: 54.86 ± 1.19; 14-day: 30.63 ± 3.81) and at 100 mg/kg (14-day: 30.25 ± 2.53) compared to control (1h: 41.71 ± 3.86; 14-day: 30.25 ± 2.53) (Figure 1A and D). The number of rearing was reduced by EEHa at 10 mg/kg (14-day: 5.14 ± 1.29) and increased at 50 (1h: 30.86 ± 1.20; 14-day: 28.00 ± 2.14) and at 100 mg/kg (1h: 29.0 ± 1.13; 14-day: 25.57 ± 3.56) compared to control (1h: 21.71 ± 2.80; 14-day: 14.00 ± 1.86) (Figure 1B and E). The number of grooming was reduced by EEHa at 10 mg/kg (14-day: 2.25 ± 0.55), 50 (1h: 1.00 ± 0.21; 14-day: 1.37 ± 0.46) and 100 mg/kg (1h: 0.85 ± 0.14; 14-day: 1.12 ± 0.22) compared to the control (1h: 3.14 ± 0.50; 14-day: 4.50 ± 0.59) (Figure 1C and F). Diazepam (2 mg/kg) reduced the number of crossing (1h: 15.8 ± 1.59; 14-day: 5.75 ± 0.79), rearing (1h: 4.10 ± 0.76; 14-day: 5.62 ± 1.36) and grooming (1h: 0.60 ± 0.16; 14-day: 1.12 ± 0.44) compared to control (Figure 1A-F).

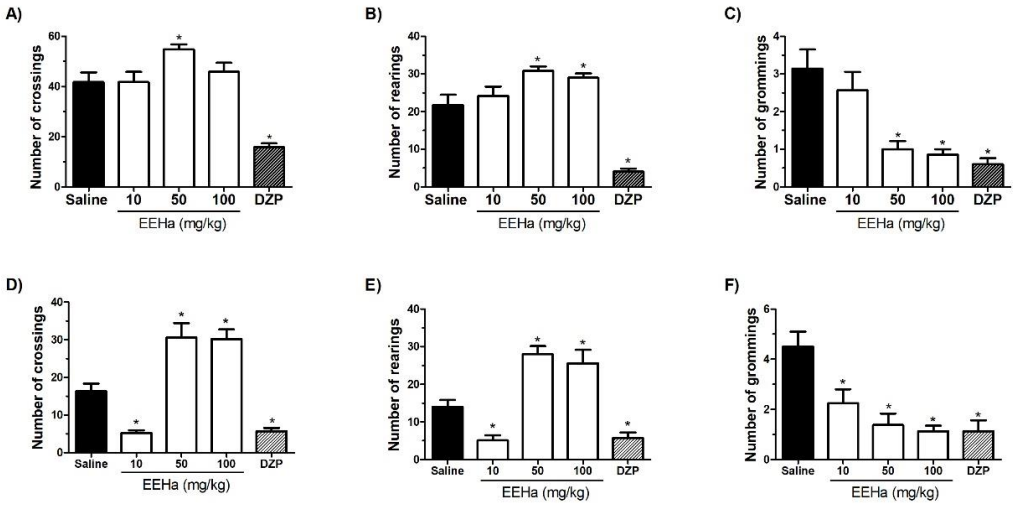

Figure 1. Dual effect of EEHa on locomotor and exploratory activities after 60 minutes and 14-day in the open field test. (**A-C**) 60min or (**D-F**) 14-day after. The n° of (**A/D**) crossing; (**B/E**) rearing; and (**C/F**) grooming. ANOVA and Bonferroni test. *p<0.05 *vs.* saline. DZP: diazepam (2 mg/kg).

EEHa effect on anxiolytic behavior in the hole board and elevated plus maze tests

In the hole board test, the number of head dips was decreased by EEHa at 10 mg/kg (1h: 18.57 ± 2.12; 14-day: 15.63 ± 1.87), and increased at 50 (1h: 47.86 ± 4.14; 14-day: 36.25 ± 2.54) and 100 mg/kg (1h: 56.00 ± 5.30; 14-day: 35.88 ± 1.61) compared to control (29.14 ± 0.85). The anxiolytic dose of diazepam (1 mg/kg) increased the number of head dips (1h: 58.86 ± 6.89; 14-day: 41.13 ± 3.06) (Figure 2A and C). In addition, the time of permanence in the holes was decreased by EEHa at 10 mg/kg (1h: 12.29 ± 1.40s; 14-day: 8.28 ± 1.20s), and increased at 50 (14-day: 30.43 ± 3.11s) and at 100 mg/kg (1h: 32.43 ± 3.57s; 14-days: 30.86 ± 3.51s) compared to control (1h: 22.29 ± 1.16s; 14-day: 19.29 ± 1.82s). Diazepam (1 mg/kg) increased this time of permanence (1h: 34.86 ± 3.46s; 14-day: 35.71 ± 3.85s) (Figure 2B and D).

In the elevated plus maze test, EEHa after 1 hour increased the number of entries in the open arms at 50 (6.14 ± 0.63) and 100 mg/kg (5.85 ± 0.70) compared to control (2.57 ± 0.81). However, these doses also increased the number of entries in the closed arms: 50 (8.71 ± 0.71) and 100 mg/kg (8.71 ± 0.28) compared to control (6.14

± 0.88). Diazepam (1 mg/kg) increased the number of entries in the open arms (9.00 ± 0.32) and decreased the number of entries in the closed arms (2.50 ± 0.52) (Figure 2E). EEHa at all doses increased the permanence time in the open arms (10 mg/kg: 68.29 ± 16.52; 50 mg/kg: 67.71 ± 9.80 s; 100 mg/kg: 70.14 ± 3.95s *vs*. control: 20.29 ± 8.94 s) and reduced the permancence in the close arms (50 mg/kg: 182.0 ± 11.24; 100 mg/kg: 173.1 ± 5.21s *vs*. control: 244, 3 ± 17.27s). Similarly, diazepam (1 mg/kg) increased the permanence time in the open arms (116.7 ± 11.75s) and reduced in the close arms (88.75 ± 14.32s) (Figure 2F). After 14 days, EEHa reduced the number of entries in the open arms at the dose of 10 mg/kg (1.50 ± 0.46), but increased this number at 50 (11.25 ± 1.20) and 100 mg/kg (11.50 ± 1.53) compared to the control (6.00 ± 0.92). In the closed arms, EEHa increased this parameter at 10 mg/kg (18.14 ± 1.45) and decreased at 50 (6.5 ± 0.50) and 100 mg/kg (5.75 ± 0.64) compared to control (11.13 ± 0.47). Diazepam (1 mg/kg) increased the number of entries (16.00 ± 1.11) and reduced this parameter in the close arms (2.0 ± 0.56) (Figure 2G). In addition, EEHa decreased the permanence time in the open arms at 10 mg/kg (9.12 ± 1.77s) and increased at 50 (95.13 ± 7.35s) and 100 mg/kg (89.25 ± 5.80s) compared to control (34.63 ± 1.53s). In the closed arms, this parameter was increased at 10 mg/kg (244.8 ± 7.86s), and decreased at 50 (126.1 ± 5.85s) and 100 mg/kg (126.6 ± 5.35s) compared to control (172.5 ± 5.18s). Diazepam (1 mg/kg) increased the permanence time in the open arms (109.0 ± 17.67s) and reduced this parameter in the close arms (108.30 ± 5.61s) (Figure 2H).

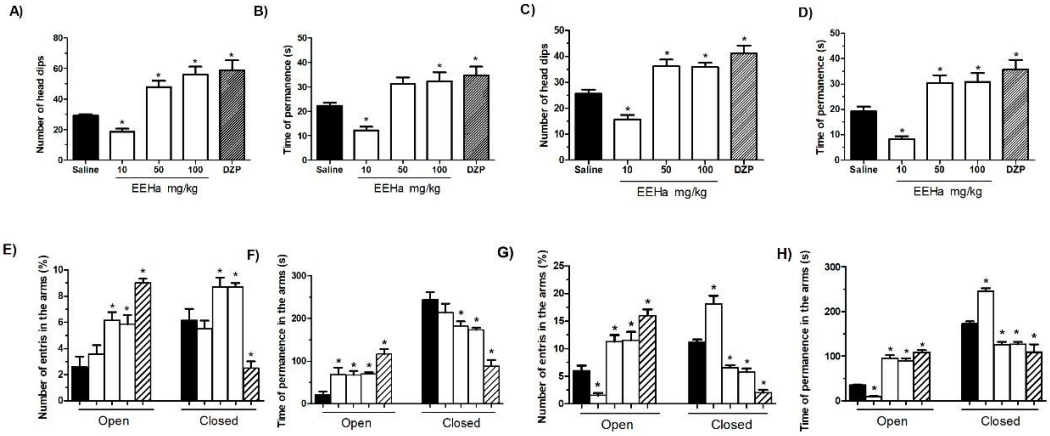

Figure 2. Dual effect of EEHa on anxiolytic behavior in the hole board and elevated plus maze test after 60 minutes and 14 days. (A-D): Hole board. (**A**) N° of head dips after 60min; (**B**) permanence time after 60min; (**C**) n° of head dips after 14-day; (**D**) permanence time after 14-day. (**E-H**): Elevated plus maze. (**E**) N° of entries in the open and closed arms in % after 60min; (**F**) permanence time in the open and closed arms after 60 min; (**G**) n° of entries in the open and closed arms in % after 14-day; (**H**) permanence time in the open and closed arms after 14-day. ANOVA and Bonferroni test. *p<0.05 *vs*. saline. DZP: diazepam (1 mg/kg).

EEHa effect on the social interaction test

After 1 hour, EEHa (50 mg/kg) increased: grab (11.57 ± 1.86 *vs*. control: 1.85 ± 0.45), boxing (10.71 ± 1.75 *vs*. control: 1.85 ± 0.73) and sniffing (12.00 ± 1.71 *vs*. control: 2.42 ± 0.36) (Figure 3A). The interaction time at this dose increased (400.1 ± 31.35s) compared to control (324.4 ± 15.14s) (Figure 3B).

After 14 days, EEHa at 10 mg/kg reduced: grab (0.42 ± 0.20 *vs*. control: 13.43 ± 1.60), boxing (0.57 ± 0.20 *vs*. control: 11.57 ± 2.72), sniffing (4.00 ± 0.72 *vs*. control: 14.00 ± 1.15), self-cleaning (3.71 ± 0.68 *vs*. control: 12.57 ± 0.94) and locomotor activity (47.57 ± 4.59 *vs*. control: 46.14 ± 2.85); and at 50 mg/kg increased: grabbing (25.86 ± 1.73 *vs*. control: 13.43 ± 1.60), boxing (22.57 ± 2.61 *vs*. control: 11.57 ± 2.72) and sniffing (28.14 ± 2.77 *vs*. control: 14.00 ± 1.15) (Figure 3C). The interaction time reduced at 10 mg/kg (245.0 ± 16.02s) and increased at 50 mg/kg (465.1 ± 18.02) compared to control (350.4 ± 16.39s) (Figure 3D).

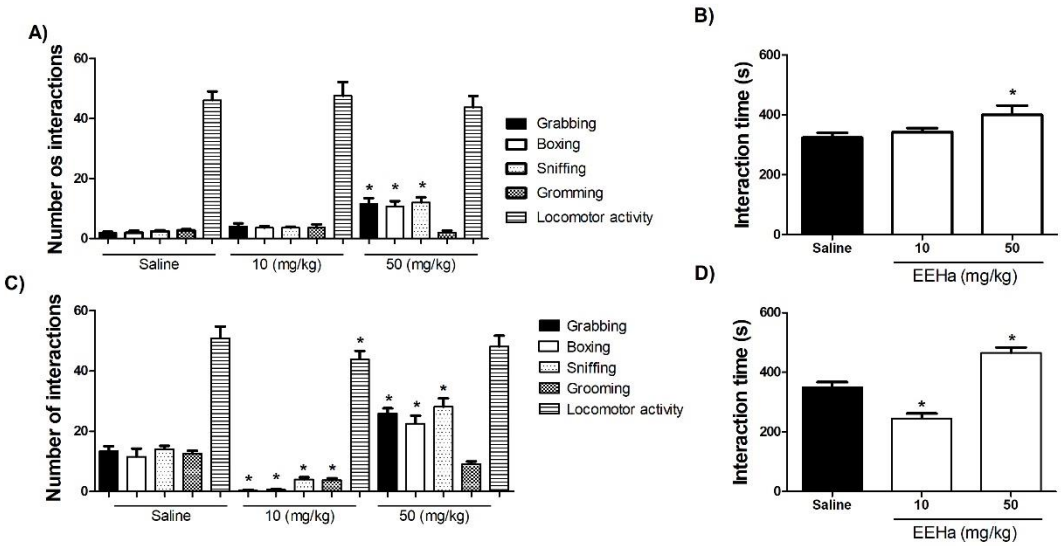

Figure 3. Dual effect of EEHa on the social interaction after 60 minutes and 14 days. (A) N° of interactions after 60min; (B) interaction time after 60min; (C) n° of interactions after 14-day; (D) interaction time after 14-day. ANOVA and Bonferroni test. *p<0.05 *vs*. saline.

EEHa effect on depressor behavior in the tail suspension test

After 1 hour, the immobility time was increased by EEHa (10 mg/kg: 148.9 ± 4.22s) and reduced by imipramine (10 mg/kg: 22.29 ± 7.27s) compared to control (125.5 ± 5.61 s) (Figure 4A). After 14 days, this time was increased by EEHa at 10 mg/kg (164.6 ± 9.48s), and reduced at 50 (72.43 ± 7.13s) and 100 mg/kg (62.14 ± 8.07s) compared to control (110.6 ± 5.87s). Imipramine (10 mg/kg) reduced the immobility time (22.63 ± 6.51s).

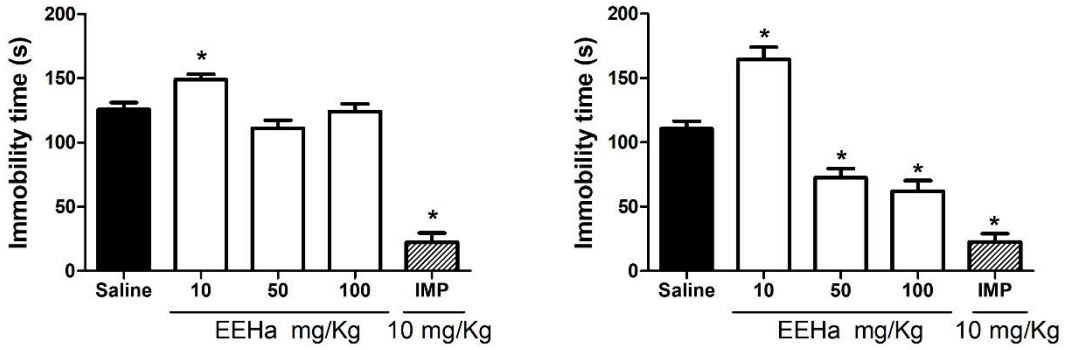

Figure 4. Dual effect of EEHa on the immobility time after 60 minutes and 14 days in the tail suspension test. (A) Immobility time after 60min; (B) immobility time after 14-day. ANOVA and Bonferroni test. *p<0.05 *vs*. saline. IMP: imipramine (10 mg/kg).

EEHa effect of sleep latency in the sleep induced by sodium pentobarbital

The latency time was reduced by EEHa at 10 mg/kg (27%) and increased at 50 mg/kg (43%) compared to control (240.0 ± 15.83s). Diazepam (1 mg/kg) decreased this behavior (57%) as expected (Table 1). The sleep time was increased by EEHa at 10 mg/kg (61%) and reduced at 50 mg/kg (50%) and at 100 mg/kg (60%) compared to control (3086 ± 300.6s). Diazepam (1 mg/kg) increased the sleep time (2.1 fold) (Table 1).

Table 1. EEHa effect in the latency time and sleep time induced by sodium pentobarbital

Group	Latency time (s)	Sleep time (s)
Control (Saline)	240.0 ± 15.83	3086 ± 300.6

EEHa 10 mg/kg	174.8 ± 8.74*	4982.0 ±279.0*
EEHa 50 mg/kg	343.5 ± 20.14*	1543.0 ± 173.3*
EEHa 100mg/kg	300.5 ± 22.84	1875.0 ± 316.2*
Diazepam 1 mg/kg	104.5 ± 8.13*	6481.0 ± 249.8*

ANOVA and Bonferroni test. *p<0.05 *vs.* saline.

Brain oxidative stress

After 14 days, EEHa reduced MDA levels at 100 mg/kg in the prefrontal cortex by 78.5% (8.34 ± 2.23 *vs*. control: 38.57 ± 5.14 µmol/g tissue) and in the hippocampus by 81% (8.82 ± 1.43 *vs.* control: 45.52 ± 6.84 µmol/g tissue). In the striatum, EEHa at all doses reduced MDA levels by 41, 57 and 55%, respectively (10 mg/kg: 64.20 ± 13.02, 50 mg/kg: 47.29 ± 6.61; 100 mg/kg: 49.60 ± 6.58 µmol/g tissue) compared to control (109.5 ± 4.12 µmol/g tissue) (Figure 5A). EEHa increased GSH levels in the prefrontal cortex at 100 mg/kg by 2.2-fold (1341 ± 225.9 *vs*. control: 588.4 ± 71.58 µmol/g tissue); in the hippocampus at 50 mg/kg by 2.1 (1049 ± 116.3) and 100 mg/kg and by 2.2-fold (1063 ± 169.4) compared to control (479.3 ± 88.14 µmol/g tissue); and in the striatum at all doses by 2.1, 2.3 and 2.6-fold, respectively (10 mg/kg: 1069 ± 107.1; 50 mg/kg: 1191 ± 91.81; 100 mg/kg: 1311 ± 118.6 µmol/g tissue) compared to control (498.6 ± 78.95 µmol/g tissue) (Figure 5B).

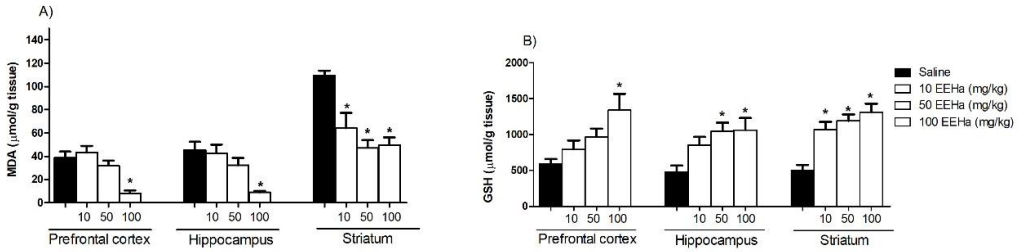

Figure 5. EEHa reduces MDA and increases GSH levels in the brain tissues of mice after 14 days. (A) malondialdehyde (MDA); (B) reduced glutathione (GSH). ANOVA and Bonferroni test. *p<0.05 *vs*. saline.

IV Discussion

The present study demonstrated a protective effect of the ethanolic extract of *Hibiscus acetosella* leaves (EEHa) on behavioral parameters of anxiety and depression, and also on markers of oxidative stress. The dual effect stimulatory/anxiolytic and inhibitory/anxiogenic of EEHa on CNS was demonstrated on the behavioral tests.

The open field test is a classic model that evaluates the exploratory/locomotor activity, as well as the anxiolytic or anxiogenic effects of compounds on the animal behavior in a new environment[14]. In this test, the rodent behavior to explore the environment, expressed by the movement of raising the front paws (rearing) and crossing (locomotor activity), had been associated to dopaminergic hyperactivity[23]. In respect to the self-cleaning behavior (grooming), it is associated with the modulation of several neurotransmitters[24], including dopamine[25]. It is well known that the modulator activity of dopamine on D1 receptors increases locomotion[26], whereas on D2 receptors induces stereotyped behaviors[27].

EEHa at the highest doses increased the number of crossing and rearing in the acute and sub-chronic treatment protocols, suggesting excitatory action on the CNS. However, EEHa at the lowest dose in the sub-chronic treatment decreased the number of crossing and rearing, which suggests an inhibitory effect. Some substances elicit opposite effects depending on the dose[28]. Diazepam, a drug from the benzazepine class and agonist of GABA-A receptor, at 2 mg/kg a sedative and anticonvulsant dose[29], also decreased the locomotor activity, demonstrating sedative effect. In addition, EEHa reduced the number of grooming (self-cleaning) in the open field at all doses, suggesting anxiolytic effect, similar to diazepam.

The elevated plus maze and the hole board (diving behavior) tests are models used to evaluate anxiolytic activity of drugs[16]. In the plus maze test, EEHa at the highest dose (in both protocols – acute and sub-chronic) increased the number of entries and permanency time of the mice in the open arms, indicating exploratory activity in unprotected and potentially dangerous areas for the animal. However, the lowest dose administered in the sub-chronic model stimulated the increase in the number of entries and permanence time of animals in closed arms, indicating anxiogenic effect. The dual effect of substances on anxiety behaviors have been described on rodent models[30].

The results obtained in the hole board test with EEHa corroborate those presented in the plus maze, providing evidence of a dual effect in the sub-chronic treatment on anxiety. In addition, EEHa does not present difference in effects between both protocols (acute and sub-chronic).

The increase in the social interaction indicates anxiolytic effect, weather the decrease indicates anxiogenic effect. Besides, a variety of neuropsychiatric disorders are characterized by disruptions in social behavior and cognition, including depression and autism spectrum disorders[31]. EEHa increased social interaction and anxiolytic effect in both acute and sub-chronic administration, in addition to other behavioral alterations not associated with locomotor activity. In this test, the treatment with EEHa was not performed at 100 mg/kg due to the observation of aggressive behavior appeared at 50 mg/kg. Substances that alter anxiety levels without modifying locomotor activity have been associated to serotonin modulation via 5-HT2 receptor[32]. EEHa at the lowest dose administered sub-chronically decreased the interaction time, corroborating most of previous tests performed.

The tail suspension test is applied to screening for biological substances with antidepressant effect[19]. The results showed that EEHa at the highest doses by sub-chronic treatment reduced the immobility time, suggesting antidepressant effect. The action onset of the most antidepressants drugs is between 7-14 days at clinical doses, and about 4-8 weeks to reach the full therapeutic effect[33]. However, the EEHa lowest dose, acute or sub-chronically, increased immobility time, suggesting inhibitory effect on CNS and depressant activity. Imipramine, a tricyclic antidepressant which acts via neuronal reuptake of noradrenaline and serotonine[34], decreased the immobility time.

Previous phytochemicals studies using leaves and flowers of the Hibiscus genus revealed the presence of the phenolic compounds quercetin and cyanidin[35,36]. Quercetin and isoquercitrin have been described to present antidepressant effect evidenced in the forced swimming test[37].

In the present study it was clear that EEHa has anxiolytic effect at the higher doses used in the two protocols (acute and sub-chronic) and antidepressant effect at the higher doses in the sub-chronic protocol. However, the dose of 10 mg/kg administered sub-chronically proved to be a CNS depressant. To clarify this effect, it was observed that EEHa at the lower sub-chronic dose presented sedative/hypnotic effect in the barbiturate-induced sleep time test, decreasing latency and increasing the animals' sleep time. This effect explains that, especially when given cumulatively over 14 days, EEHa 10mg/kg appears to be a sedative agent.

Phenolic compounds possess antioxidant properties shown by their effective scavenging activity on reactive oxygen species (ROS) and free radicals. The phenolic compounds found in Hibiscus plants belogs to the group of flavonoids and anthocyanins[11]. In *H. acetosella* the presence of caffeic acid, a phenolic acid, was associated to the antioxidant and antibacterial properties[11]. In our study, EEHa reduced MDA levels and increased that of GSH in homogenized of prefrontal cortex, hippocampus and striatum, indicating reduction in oxidative stress and increase in the antioxidant system. MDA is a lipid peroxidation final product, toxic to proteins and DNA[38]. GSH is an antioxidant molecule that participates in the conversion of H_2O_2 into H_2O[39]. A phenolic compound isolated from *Hibiscus vitifolius* named gossypin in a similar manner reduced MDA levels, increase GSH levels, and the activity of GPx and catalase in the brain tissue of mice[40].

V Conclusion

EEHa, in both acute and sub-chronic protocols, presents a dual effect on the CNS, corresponding to excitatory, but no hypnotic-sedative activity at high-doses, and inhibitory and hypnotic-sedative activities at low-doses. Besides, EEHa possesses antioxidant activity in brain tissues of mice subjected to stress generated by the behavioral tests.

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