3. Pharmacological studies of extracts from *Bellis perennis* L. (Asteraceae) on central nervous system

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Abstract. The main objective of this study was to evaluate pharmacological properties of organic extract of *Bellis perennis* L. on central nervous system in mice. *In vitro* studies to evaluate the antioxidant activity of ethanolic extract of flowers (EEF) of *B. perennis* showed there was a significant reduction in the production of acid reactive compound (tiobarbituric acid) and increase in the scavenger activity against hydroxyl and nitrite radicals. In the studies using as the model of seizure being induced by pilocarpine, the group treated with EEF at doses of 50, 100, and 150 mg/kg (i.p.) presented a significant reduction in rates of tremors, seizures, status epilepticus and mortality rate. In histopathological examinations of...
the pretreated animals with EEF (50 mg/kg) and then after they received stimulus of seizures, it showed a reduction in the number of brain damage and degree of involvement in the striatum and hippocampus. In the other hand, the groups treated with vehicles or three evaluated doses with EEF and the animals treated with EEF (100 and 150mg/kg) and then pilocarpine (400 mg/kg, i.p.) didn´t present any histopathology changes in their striatum and hippocampus regions. Studies were conducted to verify the effects of EEF on enzymatic activity of acetylcholinesterase, and our results showed a significant stimulatory effect of EEF on the activity of this enzyme in the presence of seizure by pilocarpine stimulation and, moreover, inhibition of enzyme activity when absent seizure stimulation by the cholinergic drug. In turn, the animals treated with the same doses have showed a decrease in the number of rearing and crossings in open field tests. In the forced swim test, the results have showed that EEF presents a significant decrease in immobility time. This way, our results suggests that the EEF has antioxidant properties in vitro and also possess an anticonvulsant, antidepressant, anxiolytic and neuroprotective effects in mice, as well as stimulates and inhibits the acetylcholinesterase.

1. Introduction

The knowledge about medicinal plants symbolizes sometimes the only therapeutic resource in many communities and ethnic groups. The use of plants for the treatment and cure of diseases is as old as mankind. Even today in the poorest regions of the country and even in large cities, medicinal plants are sold in fairs, markets and found in popular residential backyards. Thus, users of medicinal plants worldwide, maintains the practice of herbal medicine consumption, result information therapies that have been accumulated for centuries. In an indirect way, this type of medical culture aroused the interest of researchers in studies involving multidisciplinary areas such as botany, pharmacology and phytochemistry, which aim to enrich the knowledge of medicinal plants (MACIEL et al., 2002). Until the nineteenth century, medicines were available almost exclusively formulated herbal remedies. According to Oliveira and Akisue (1998), a medicinal plant is any plant that contains in one or more of their organs, substances that can be used for therapeutic substances or precursors for such purpose. Initially, the discovery of the therapeutic properties of plants was merely intuitive or, sometimes, by observing the animals, seeking relief from the herbs in their affections (ALVES et al., 2000). In recent years, medicinal plants began to be studied in a systematic and rigorous, scientific point of view, in order to identify its chemical components and demonstrate
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their pharmacological actions, as well as be aware of the side effects. In this sense, Brazil with its enormous biodiversity, presents itself as a great potential for research and exploration in the area of medicinal plants (ALVES et al., 2000) and may contribute to the development of new drugs. Despite the wealth of flora, with about 500,000 species, there is consensus on the lack of scientific studies on the subject (FERREIRA, 2002), and previous data show that only 15% of remaining plants of tropical forests were evaluated by phytochemical studies (VERPOORTE, 2000).

Among various botanical families, we highlight several plants with biological potential, such as Asteraceae, which comprises approximately 1500 genera and 2300 species (JUDD et al., 2009), including Bellis perennis, a species popularly known as “daisy” (Figure 1).

Previous works reported the use of B. perennis due its expectorant properties, healing, anti-inflammatory, anti-hemorrhagic, anesthetic, antiparasitic, antifungal, antimicrobial and antioxidant activities (AVATO; TAVA, 1995; OBERBAUM et al., 2005; MORIKAWA et al., 2008; KAVALCIOĞLU et al., 2010).

The chemical composition of B. perennis is well determined and it produces a range of compounds including volatiles, sterols and alkaloids. However saponins found in flowers such as perennesosides and others oleanane type of triterpene saponins are chemical markers and call attention due the anti-hyperlipidemic activities (MORIKAWA et al, 2008). Flavonoids and glucoside derivatives from apigenin and isorhamnetin, specially the glucuronopyranosides are also considered compounds of chemotaxonomic and biological importance (YOSHIKAWA et al, 2008).

In the present work "Pharmacological study of properties from B. perennis L. (Asteraceae) on central nervous system", we analyzed brain areas (striatum and hippocampus) of the animals using pilocarpine model (400 mg/kg body weight). Behavioral, pharmacological and neurochemical studies of these animals were performed in order to contribute to research into new formulations to treatment of neurological disorders.
2. Materials and methods

Plant material and extract preparation

The species was collected in October 2010, in Pacoti, state of Ceará, Brazil. The voucher specimen was identified and deposited at the Graziela Barroso Herbarium at the Federal University of Piauí (Number 27276).

The flowers of *B. perennis* were dried at 25 °C under shade and powdered mechanically. Crush yielded of flowers (310 g) was extracted with hexane, followed by ethanol (EtOH) in a Soxhlet apparatus (8 h for each solvent) and after the solution was concentrated in a vacuum evaporator yielded 3.2% (w/w) of extract. The dried extract was kept at 4 °C in refrigerator in the air tight bottles until use.

**Phytochemical studies**

The ethanolic extract was submitted to a phytochemical screening for detection of presence of unsaturated sterols, triterpenes, alkaloids, flavonoids, lactones/esters, and protein/amino acids and carbohydrates and/or glycosides (STAHL, 1969).

**Animals and experimental procedures**

Male albino mice (*Mus musculus*), Swiss variety (25-30 g, two months-old), were used. The animals were randomly housed in appropriate cages at 23 ± 2°C under 12 h light/dark cycle (lights on 06:00 am - 18:00 pm) with access to food (Purina®) and water *ad libitum*. All experiments were carried out between 08:00 am and 18:00 pm in a quiet room. Experimental protocols and procedures were approved by the Ethics Committee on Animal Experiments at the Piauí Federal University (CEEA/UFPI nº 077/2010). All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**Behavioral screening**

Behavioral screening was performed following parameters described by Almeida and coworkers (1999) and animals were observed at 14 days consecutive after oral route (o.r.) administration of ethanolic extract (EEF) from *B. perennis* (50, 100 and 150 mg/kg, o.r.). During 14 consecutive days, the animals were observed to detect general signs of toxicity: piloerection, prostration, writhing, increased evacuation, grooming, discrete groups, dyspnea, sedation, analgesia and palpebral ptosis. The spontaneous locomotor
activity of the animals was assessed in a cage activity (50 cm × 50 cm × 50 cm) after 30 min of the last dose of treatment (ASAKURA et al., 1993).

**Acute toxicity (LD$_{50}$) and effect on general behavior**

The acute intraperitoneal LD$_{50}$ and the general behaviour activity of EEF were evaluated in *Swiss* adult albino mice of both sex and weighing between 25 and 30 g. A 12-h dark-light cycle, 25 ± 2 °C temperature and 50–60% humidity were provided. The animals received standard food and before the experiments they were fasted overnight with water *ad libitum*. The EEF was suspended in 0.05% Tween 80, dissolved in 0.9% saline and administered intraperitoneally in growing doses (1000, 2000, 3000 and 4000 mg/kg). Mice were kept under observation for 14 days.

**Evaluation of biochemical and hematological parameters in the blood of mice**

Hematological and biochemical analyzes were performed in the laboratory of the University Hospital at the Piauí Federal University. Animals were anesthetized with sodium pentobarbital (40 mg/100 g body weight, i.p.) and blood collection was performed by puncture of the retro-orbital plexus, using needles and syringes and microhematocrit tubes. Blood was packed into two kinds of tube: one with anticoagulant HB (Laborlab®) for determining hematological parameters, and the other without anticoagulant to obtain serum for assessment of biochemical parameters.

Complete blood counts (hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and platelets were determined in a fully automated analyzer (Siemens Diagnostics, Tarrytown, NY). Smears of blood stained with May-Grunwald-Giemsa were used for differential leukocyte counts (neutrophils, eosinophils, lymphocytes and monocytes).

Glucose, uric acid, urea, creatinine, alkaline phosphatase, triglycerides, total protein, total bilirubin, direct bilirubin, total cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined enzymatically by standard methods using specific kitsLabtest® and measurement the optical density at a wavelength corresponding to a Labmax 240.

**Determination of the content of acid reactive substances to thiobarbituric acid (TBARS) in vitro**

The TBARS assay was used to quantify lipid peroxidation (LOPES; SCHULMAN; HERMES-LIMA, 1999) and adapted according to the
previously described method (ESTERBAUER; CHEESEMAN, 1990). Lipid peroxidation was induced by adding 0.1 mL of 2,2'-azobis-2-aminopropane (AAPH) 0.12 M. The absorbance in concentrations of 10, 25, 50, 100 and 150 mg/ml of EEF was measured using a Biospectro SP 220 spectrophotometer at 532 nm. The results were expressed as percentage of TBARS formed in comparison to that produced by AAPH (reaction medium).

**Determination of the antioxidant activity against the formation of hydroxyl radical in vitro**

The formation of hydroxyl radical (OH-) was quantified using the oxidative degradation of 2-deoxyribose (LOPES; SCHULMAN; HERMES-LIMA, 1999). The principle of antioxidant activity of EEF was quantified by the degradation of 2-deoxyribose. It was determined the level of malondialdehyde (MDA) produced by their condensation with 2-thiobarbituric acid (TBA).

Briefly, the reactions were initiated by the addition of Fe²⁺ + 6 mM to solutions containing 2-deoxyribose 5 mM, H₂O₂ 100 mM and phosphate buffer 20 mM (pH 7.2) to determine the antioxidant activity of the EEF in concentrations of 10, 25, 50, 100 and 150 mg/ml against the formation of hydroxyl radical. The absorbance was measured at 532 nm using a spectrophotometer Biospectro SP 220 and the results were expressed as percentage in relation to the reaction medium.

**Determination of the antioxidant activity in removing the contents of nitric oxide (NO) in vitro**

Nitric oxide was generated from the decomposition of sodium nitroprusside (SNP) in phosphate buffer 20 mM (pH 7.4) by Griess reaction (BASU; HAZRA, 2006). The absorbances of EEF concentrations (10, 25, 50, 100 and 150 mg/ml) were measured using a Biospectro SP 220 spectrophotometer at 540 nm. The results were expressed as percentage of nitrite formed compared to NPS (reaction medium).

**Forced swimming test (FST)**

This test is the most widely used and recognized pharmacological model (PORSOLT et al., 1977a) for assessing antidepressant activity (PORSOLT et al., 1977b, 1978). The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice had a swimming-stress session for 15 min (pre-test), 24 h before being individually placed into glass cylinders
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(height: 25 cm; diameter: 10 cm; containing 10 cm of water a 25 ± 2 °C) for 5 min (test). A mouse was judged to be immobile when it ceased struggling and remained floating motionless on the water, making only small movements necessary to keep its head above water.

Open field test

The open-field arena was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm), divided into nine squares of equal areas. The open-field was used to evaluate the exploratory activity of the animal (ARCHER, 1973). The mouse was placed individually into the center of the arena, and allowed to explore it freely. The observed parameters were: ambulations (the number of squares crossed with all four paws), numbers for grooming and rearing, recorded for the 5 min testing period.

Experimental protocols

Animals were treated with EEF and submitted to the open-field test 30 (i.p.) min later. Another group of animals was submitted to the forced swimming test. Controls were administered with vehicle (0.05% Tween 80 dissolved in 0.9% saline; vehicle group). In order to elucidate the possible mechanism involved in the anxyolitic effects from EEF, we used diazepam and flumazenil administered alone or associated with EEF in open-field test.

Similarly, in order to clarify the antidepressant effect of the EEF, imipramine and paroxetine alone or associated with EEF were used in the forced swimming test. Imipramine was chosen because this drug is a classical antidepressant that acts through noradrenergic and serotonergic pathways. Paroxetine, another antidepressant drug, is more selective, and acts predominantly through serotonergic pathways. Both antidepressants were then used, alone as positive controls or in combination with higher dose of EEF to investigate alteration/interference of imipramine or paroxetine on the antidepressant effect of EEF. Reserpine, a drug known to cause depletion of biogenic amines (noradrenaline, dopamine and serotonin) from storage granules, was also used to evaluate the participation of those amines in the EEF antidepressant effect. In a combination protocol, imipramine, paroxetine or reserpine was administered 10 min before EEF, and the test was performed 30 min later.

Evaluation of anticonvulsant activity in mice (pilocarpine-induced seizures test)

Adult male Swiss mice were divided into eight groups. The control group was treated with 0.9% saline (i.p., n=7). The P400 group was treated with pilocarpine (400 mg/kg, i.p., n=7). EEF 50, EEF 100 and EEF 150 groups
were treated with EEF at doses of 50, 100 and 150 mg/kg (i.p.), respectively. The groups EEF 50 + P400, EEF 100 + P400 e EEF 150 + P400 were pre-treated with the same doses of EEF of the groups treated only with the extract of *B. perennis*, and 30 min after this treatment, received pilocarpine (400 mg/kg, i.p.).

**Behavioral studies in mice in pilocarpine-induced seizures**

The treated animals and controls were separated into cages containing a maximum of 6 animals and placed in reserved environment, being made direct observation. All experimental groups were observed after the treatment, according to experimental protocols, making a total of 24 h of observation.

The following parameters were observed: the presence of peripheral cholinergic signs, miosis, piloerection, chromodacryorrhea, salivation, diarrhea and diuresis), tremors, stereotyped movements [increased activity of biting, scratching, chewing and wet-dog shakes], motor seizures (including clonic movements of the upper extremities occurring at approximately 30 minutes after administration of pilocarpine), progressing to status epilepticus (characterized by motor limbic seizures as defined for a continuous occur when period greater than 30 minutes) and still determined the number of dead animals in each experimental group.

**Histopathological studies in mice in the pilocarpine-induced seizures**

Animals of all experimental groups that survived after the observation period were euthanized (as described above) and decapitated; their brains were removed and fixed in 10% formalin for 72 h for submission to histopathological analysis. Sagittal slices were made at intervals of 1 mm from an initial cut close to the mammillary bodies. For microscopic study was made of 10 μm sections stained with hematoxylin-eosin, and analyzed with the aid of an optical microscope.

The brain areas were observed and classified according to the data of the Paxinos and Watson atlas (1986). For the analysis of brain lesions were observed the following parameters: normal aspects similar to controls or the presence of cells tumescent, atrophied, pyknotic nuclei, neuronal death, gliosis and vacuolization. The degree of injury was expressed by a percentage scale from 0 (none) to 100 (total) for each frame examined (CLIFFORD et al., 1987; BUREAU; PEREDERY; PERSINGER, 1994). The animals were defined as having brain damage when at least 50% change in one or more structures as described previously by De Bruin et al (1999).
Dissection of the brain area (hippocampus) to achieve the neurochemical studies

After 24 hours of observation all animals which survived this observation period were euthanized by administering sodium pentobarbital intraperitoneally at a dose 40 mg/100 g in weight. The animals were decapitated with a guillotine (Harvard, USA) and then the brains quickly removed and placed on aluminum foil in a petri dish on ice. After completion of the dissection, each area was placed in foil, labeled, weighed and stored at -80 °C for later use.

Protein content for determination of the activity of acetylcholinesterase (AChE)

The test dose of protein was performed by the method described by Lowry et al (1951) in hippocampal homogenates of tissues to 10% of treated mice, and the results were used for the determinations of AChE activity.

Testing acetylcholinesterase activity (AChE)

Acetylcholinesterase activity was determined according to the method described by Ellman and coworkers (1961), having as basic principle the measurement of the speed of production of the thiocholine ratio as acetylthiocholine (ATC) used as substrate was hydrolyzed.

This was accompanied by continuous reaction of the thiol with the 5:5 ion-dithiobis-2-nitrobenzoate (I) to produce yellow anion of 5-thio-2-nitrobenzoic acid (II), whose absorbance was measured at 412 nm in a Biospectro SP 220 spectrophotometer, enabling automatic readings in digital system and provided increased sensitivity.

The enzymatic activity was measured by reading the change in absorbance per minute for 3 minutes, and the reaction linear for at least 10 minutes. The specific activity was expressed as nanomoles of ATC hydrolyzed per milligram of protein per minute (nmol/mg protein/min).

The hippocampal tissues were homogenized in phosphate buffer (pH 8.0, 0.1 M) and 10% homogenate (10 μl) was added to a cuvette containing 500 μL of buffer 890 μl of distilled water and 50 μl ditiobisnitrobenzoico acid (DTNB) 0.01 M and absorbance zeroed. After being allowed to zero absorbance, the cuvette was removed and to it was added acetyltihiocholine iodide 0.075 M. Absorbance was recorded for 3 min at 412 nM. Enzyme activity was calculated as the change in absorbance minute 3 to minute 0 on the protein content contained in the homogenate.
The complete procedure was done using a Biospectro SP 220 spectrophotometer adjusted to a wavelength of 412 nM.

**Statistical analyses**

The data obtained were evaluated by one-way analysis of variance (ANOVA) followed by *t*-Student-Neuman-Keuls as *post hoc* test. Differences were considered to be statistically significant when p<0.05 to p<0.001.

**3. Results**

The phytochemical screening of EEF revealed the presence of alkaloids, phenolic compounds, flavonoids, carbohydrates, steroids and proteins. So, the chemical screening results are in accordance with the chemical composition described for this plant.

**Table 1.** Effects of acute treatment with EEF for determining the LD$_{50}$ for 14 days of observation.

<table>
<thead>
<tr>
<th>Doses (g/kg)</th>
<th>Number of Animals/group</th>
<th>Number of Death/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>00</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>00</td>
</tr>
<tr>
<td>1500</td>
<td>10</td>
<td>02</td>
</tr>
<tr>
<td>2000</td>
<td>10</td>
<td>06</td>
</tr>
<tr>
<td>3000</td>
<td>10</td>
<td>05</td>
</tr>
<tr>
<td>4000</td>
<td>10</td>
<td>08</td>
</tr>
</tbody>
</table>

The EEF at doses of 50, 100 and 150 mg/kg (o.r.) showed no behavioral changes in animals during 14 days of treatment. The intraperitoneal LD$_{50}$ value was 2310 mg/kg of body weight, according Table 1. Oral administration of doses up to 3.0 g/kg did not show any toxic symptom in mice. Administration of 50 and 100 mg/kg (i.p.) of the EEF did not cause alterations in their general behaviour. However, a dose of 150 mg/kg (i.p.) of the EEF induced a slight decrease in locomotor activity, piloerection, passivity, palpebral ptosis and a stereotyped behavior.

The absence of significant clinical effects following acute administration of EEF in hematological parameters was observed in Table 2.

In biochemical parameters in the groups treated with EEF or vehicle, after 24 h of intraperitoneal administration, the EEF caused no clinical significant changes in blood glucose, uric acid, urea, creatinine, alkaline phosphatase, triglycerides, total protein, total bilirubin, direct bilirubin, total cholesterol and transaminases (TGO/TGP) (Table 3). All parameters remained within the physiological range throughout the experimental period of 24 hours.
Table 2. Effect of acute intraperitoneal administration of EEF in hematological parameters in mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle</th>
<th>EEF 50</th>
<th>EEF 100</th>
<th>EEF 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (mm$^3$)</td>
<td>8.63 ± 0.03</td>
<td>8.36 ± 0.13</td>
<td>8.46 ± 0.09</td>
<td>8.57 ± 0.10</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.17 ± 0.17</td>
<td>13.14 ± 0.34</td>
<td>13.61 ± 0.42</td>
<td>13.69 ± 0.53</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.90 ± 0.48</td>
<td>41.56 ± 1.41</td>
<td>42.10 ± 1.28</td>
<td>41.57 ± 1.82</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>49.71 ± 0.04</td>
<td>49.71 ± 1.50</td>
<td>49.76 ± 1.79</td>
<td>48.51 ± 1.20</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.42 ± 0.03</td>
<td>15.71 ± 1.21</td>
<td>16.09 ± 1.04</td>
<td>15.97 ± 0.94</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.03 ± 0.38</td>
<td>31.62 ± 0.67</td>
<td>32.33 ± 0.80</td>
<td>32.93 ± 0.85</td>
</tr>
<tr>
<td>Platelets (mm$^3$)</td>
<td>292.4 ± 0.84</td>
<td>301.6±33.36</td>
<td>287.10±15.08</td>
<td>291.90±20.19</td>
</tr>
<tr>
<td>Leukocytes (mm$^3$)</td>
<td>8.53 ± 0.77</td>
<td>8.31 ± 0.31</td>
<td>8.43 ± 0.41</td>
<td>8.38 ± 0.89</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>17.00 ± 0.79</td>
<td>16.38 ± 1.03</td>
<td>16.25 ± 0.88</td>
<td>16.88 ± 0.87</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.35 ± 0.02</td>
<td>0.36 ± 0.07</td>
<td>0.37 ± 0.09</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>78.12 ± 0.31</td>
<td>79.50 ± 3.36</td>
<td>76.75 ± 4.76</td>
<td>78.33 ± 4.87</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.70 ± 0.21</td>
<td>2.62 ± 0.26</td>
<td>2.62 ± 0.37</td>
<td>2.75 ± 0.37</td>
</tr>
</tbody>
</table>

EEF was administered in a single dose intraperitoneally to groups of mice (n = 10 per group) with the following doses: 50 (EEF 50), 100 (EEF100), and 150 mg/kg (EEF 150) for 24h. In a separate group, vehicle were administered (Tween 80 0.05%). Data are expressed as mean ± S.E.M.

Table 3. Effect of acute intraperitoneal administration of EEF in biochemical parameters in mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle</th>
<th>EEF 50</th>
<th>EEF 100</th>
<th>EEF 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>88.73 ± 0.11</td>
<td>87.00 ± 1.59</td>
<td>89.89 ± 1.86</td>
<td>89.63 ± 2.58</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>54.67 ± 0.30</td>
<td>25.43 ± 1.81$^a$</td>
<td>25.00±0.24$^a$</td>
<td>18.00 ± 2.25$^a$</td>
</tr>
<tr>
<td>Creatinim (mg/dl)</td>
<td>0.43 ± 0.04</td>
<td>0.62 ± 0.01$^a$</td>
<td>0.61 ± 0.05$^a$</td>
<td>0.58 ± 0.05$^a$</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.61 ± 0.04</td>
<td>2.43 ± 0.52$^a$</td>
<td>2.62 ± 0.26</td>
<td>2.71 ± 0.04</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>106.7 ± 0.05</td>
<td>92.9 ± 0.91$^a$</td>
<td>98.56 ± 0.41$^a$</td>
<td>97.67 ± 0.01$^a$</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>86.07 ± 0.57</td>
<td>85.63 ± 9.53</td>
<td>85.00 ± 0.72</td>
<td>86.67 ± 1.65</td>
</tr>
<tr>
<td>AST (U/ml)</td>
<td>91.40 ± 1.81</td>
<td>36.86 ± 3.48$^a$</td>
<td>32.00 ± 1.93$^a$</td>
<td>33.57 ± 0.55$^a$</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>57.83 ± 0.81</td>
<td>32.86 ± 3.92$^a$</td>
<td>40.14 ± 6.70$^a$</td>
<td>24.71 ± 2.75$^a$</td>
</tr>
</tbody>
</table>

EEF was administered in a single dose intraperitoneally to groups of mice (n = 10 per group) with the following doses: 50 (EEF 50), 100 (EEF100) and 150 mg/kg (EEF 150) for 24h. In a separate group, vehicle were administered (Tween 80 0.05%). Data are expressed as mean ± S.E.M. *p <0.001 versus vehicle group (ANOVA and t-Student-Newman-Keuls as post hoc test).

Antioxidant activity of EEF was investigated (MARQUES; MELO; FREITAS, 2012). The EEF (10, 25, 50, 100 and 150 mg/ml) produced a significant decrease in TBARS production at all concentrations tested, while Trolox (0.45 mg/ml) produced a decrease of 42.64% in the production of TBARS (Table 4). It also produced hydroxyl radical removal at all concentrations tested when compared to the system group (Table 4). The concentration of 150 mg/ml of the EEF produced the largest increasing in removal of hydroxyl radical when compared to other concentrations. Trolox
Table 4. Antioxidant activity in vitro of EEF for removing nitric oxide, hydroxyl radical and the production of TBARS.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/ml)</th>
<th>Antioxidant activity of EEF in vitro (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>System</td>
<td>00</td>
<td>99.90 ± 1.35</td>
</tr>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>65.30 ± 2.87*</td>
</tr>
<tr>
<td>EEF</td>
<td>25</td>
<td>59.01 ± 3.68*</td>
</tr>
<tr>
<td>EEF</td>
<td>50</td>
<td>53.66 ± 3.93*</td>
</tr>
<tr>
<td>EEF</td>
<td>100</td>
<td>52.59 ± 3.84*</td>
</tr>
<tr>
<td>EEF</td>
<td>150</td>
<td>51.91 ± 2.38*</td>
</tr>
<tr>
<td>Trolox</td>
<td>0.45</td>
<td>42.54 ± 2.82*</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M., n = 10 experiments in duplicate. \*p <0.001 versus System; (ANOVA and t-Student-Neuman-Keuls as post hoc test).

already produced hydroxyl radical removal (71.06%). In the nitrite production, the EEF at the same doses was able to reduce nitrite production at all concentrations tested (Table 4).

The Trolox, a synthetic analogue of α-tocopherol used as standard antioxidant, decreased by 57.46% the production of nitrite.

The EEF possible antidepressant effect in the forced swimming test (MARQUES; MELO; FREITAS, 2012) was studied (Table 5). Under this condition, EEF was used at doses (50, 100 and 150 mg/kg, i.p.), since at these doses the sedative and anxiolytic effects of EEF do not mask the antidepressant activity. Significant decreases of 24 and 28% in the immobility time were also observed after EEF administration (150 mg/kg) when compared to the EEF 50 and EEF 100 groups, respectively. The results showed that EEF presents a significant antidepressant effect at 50, 100 and 150 mg/kg, suggested by the respective decrease in 58, 56 and 68% of the time of immobility.

The association of EEF 150 mg/kg with imipramine (IMI) showed a greater decrease of the immobility time in 67 (p<0.01) and 75% (p<0.01), respectively, in comparison with the groups treated with EEF alone (150 mg/kg) or IMI (50 mg/kg) alone. However, the association of EEF with paroxetine did not alter the effect observed with EEF or paroxetine alone (p>0.05), suggesting that the serotonergic system is not involved in the EEF antidepressant effect. On the contrary, the EEF activity was totally blocked by the previous administration of reserpine. These data suggest that the noradrenergic system participates in the EEF antidepressant action.

EEF (50, 100 and 150 mg/kg) showed no sedative effects (MARQUES; MELO; FREITAS, 2012) as assessed by the open-field test (Figure 2). Significant effects were detected with at doses tested that produced similar
Table 5. Antidepressant effect of EEF in the forced swimming test in mice and the possible involvement of the noradrenergic system and/or serotonin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Immobility time (s)</th>
<th>Values of Immobility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (24)</td>
<td>195.00 ± 2.60</td>
<td>-</td>
</tr>
<tr>
<td>EEF 50 (12)</td>
<td>81.90 ± 9.70*</td>
<td>↓58*</td>
</tr>
<tr>
<td>EEF 100 (12)</td>
<td>85.70 ± 9.50*</td>
<td>↓56a</td>
</tr>
<tr>
<td>EEF 150 (12)</td>
<td>62.40 ± 13.40*</td>
<td>↓68a ↓24b ↓28c</td>
</tr>
<tr>
<td>IMI 50 (12)</td>
<td>82.20 ± 1.80*</td>
<td>↓58*</td>
</tr>
<tr>
<td>IMI 50 + EEF 150 (12)</td>
<td>20.67 ± 1.76**,**</td>
<td>↓67* ↓75**</td>
</tr>
<tr>
<td>PAROX 20 (12)</td>
<td>72.20 ± 1.80*</td>
<td>↓58*</td>
</tr>
<tr>
<td>PAROX 20 + EEF 150 (12)</td>
<td>66.15 ± 17.10</td>
<td>NS</td>
</tr>
<tr>
<td>RESERP 0.25 (12)</td>
<td>192.20 ± 1.80</td>
<td>NS</td>
</tr>
<tr>
<td>RESERP 0.25 + EEF 150 (12)</td>
<td>189.60 ± 15.00</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of immobility time. ( ) = number of animals per group. Vehicle = Tween 80 (0.05%) = EEF 50 (EEF 50 mg/kg); EEF 100 = (EEF 100 mg/kg); EEF 150 = (150 mg/kg); IMI = imipramine (50 mg/kg); PAROX = paroxetine (20 mg/kg); RESERP = reserpine (0.25 mg/kg). Drugs were administered 10 minutes before EEF and the test performed 30 min later. *p<0.05 and **p<0.01 when compared to controls (ANOVA followed by t-Student-Newman-Keuls as post hoc test). *p<0.05 when compared to the vehicle group (Chi-square) \(^p<0.05\) when purchased at the EEF 50 group (Chi-square). *p<0.05 when purchased at the EEF 100 group (Chi-square). ↓ = decreases; ↑ = increase. NS = not significant.

percentages of decrease (57, 58 and 55%, respectively) in the number of crossings. The number of rearing was reduced of 59, 55 and 56% with EEF 50, EE 100 and EE 150 mg/kg, respectively, whose results were similar to those observed with diazepam, used as a positive control. In the number of grooming, no changes in behavior were detected. In addition, the number of grooming was increased of 166, 181 and 216% with EEF 50, 100 and 150 mg/kg respectively, when compared to the diazepam group. Flumazenil was used for evaluating the mechanism of action about the EEF sedative effect. So, 15 min after the flumazenil injection, EEF (150 mg/kg) was administered and under these conditions, the animals’ behavior no was similar to the controls, indicating that EEF do not have a benzodiazepine-type of sedative effect.

The behavioral studies (MARQUES et al., 2011) were performed as described previously (Figure 3). All adult animals treated with pilocarpine (400 mg/kg, i.p., n=7) for 24 h of observation, showed peripheral cholinergic signs, stereotyped movements, wet-dog shakes and tremors. Seizures and status epilepticus occurred in 100% (n=7) of the animals, and all died in this group.

Pretreatment with EEF at a dose of 150 mg/kg, 30 min before administration of pilocarpine, produced behavioral changes (MARQUES et al., 2011). No change in rates of peripheral cholinergic signs and stereotyped movements, observed during studies in behavioral P400 group, was verified.
Figure 2. Effects of EEF (i.p.) in the open field test in mice. Control=Tween 80 0.05% dissolved in saline 0.9%.; EEF 50=EEF 50 mg/kg, i.p.; EEF 100=EEF 100 mg/kg, i.p.; EEF 150=EEF 150 mg/kg, i.p.; DZP=diazepam 1 mg/kg, i.p.; FLU=flumazenil, 2.5 mg/kg, i.p. Drugs were administered 10 minutes before EEF and the test was performed 30 min later. a<p<0.05 when compared to vehicle group. b<p<0.05 when compared to DZP (ANOVA followed by t-Student-Newman-Keuls as post hoc test).

by pre-treatment with the extract. However, in this group (EEF150 + P400) was observed a significant reduction of 50% over the tremors, the rates of 72% and 72% of seizures and status epilepticus, but also there were no deaths of animals in comparison to P400 group. None of the animals treated only with saline 0.9% showed abnormal behavioral characteristics of seizures induced by pilocarpine.

The seizure model induced by pilocarpine was used to investigate the pharmacological actions of EEF in behavioral parameters in adult mice during the acute phase of the convulsive process (MARQUES et al., 2011). Immediately after pilocarpine administration, the mice showed persistent behavioral changes, including piloerection, akinesia, ataxia, tremor, myoclonus and masticatory automatisms of facial muscles, and movements like wet dog (wet-dog shakes), which persist of 10-15 minutes. These behavioral changes were continued until installation of the motor limbic seizures, including clonic movements of the upper extremities which occurred in 100% of animals. In the same groups, convulsions progressed to the development of status epilepticus in 100%. There of the same group was observed a mortality rate of 100%.
**Figure 3.** Effect of EEF in adult mice during seizures induced by pilocarpine. P400 group was treated with pilocarpine (400 mg/kg, i.p.). EEF 50, EEF 100 and EEF150 groups were treated with EEF doses respectively of 50, 100 and 150 mg/kg (i.p.). EEF 50 + P400, EEF 100 + P400 and EEF 150 + P400 groups were pre-treated with EEF doses respectively of 50, 100 and 150 mg/kg (i.p.) and 30 min after treatment received P400 (i.p.). After treatment, the animals were observed for 24 h. P400 = pilocarpine 400 mg/kg; EEF 50 = EEF 50 mg/kg; EEF 100 = EEF 100 mg/kg; EEF 150 = EEF 150 mg/kg. a p<0.05, when compared to P400 group. b p<0.05, when compared to the EEF 50 + P400 group. c p<0.05, compared to EEF 100 + P400 group. Chi-square test.

The latencies for the installation of first seizures and status epilepticus (MARQUES et al., 2011) were analyzed (Figure 4).

There was an increase in time to first seizure and status epilepticus in the groups EEF 50 + P400, EEF 100 + P400 and EEF 150 + P400 compared to the group. In control, EEF 50, EEF 100 and EEF 150 groups, seizures or status epilepticus were not observed.
Effect of EEF in adult mice during seizures induced by pilocarpine in latencies for installations of first epileptic seizure and status epilepticus. P400 group was treated with pilocarpine (400 mg/kg, i.p.). EEF groups were treated with EEF doses respectively of 50, 100 and 150 mg/kg (i.p.). EEF 50 + P400, EEF 100 + P400 and EEF 150 + P400 groups were pre-treated with EEF doses respectively of 50, 100 and 150 mg/kg (i.p.) and 30 min after treatment received P400. After treatment, the animals were observed for 24 h. P400 = pilocarpine 400 mg/kg; EEF 50 = EEF 50 mg/kg; EEF 100 = EEF 100 mg/kg; EEF 150 = EEF 150 mg/kg. \(^a\)p < 0.05 when compared to P400 group. \(^b\)p < 0.05 when compared to the EEF 50 + P400 group. \(^c\)p < 0.05 compared to EEF 100 + P400 group. (ANOVA followed by t-Student-Newman-Keuls as post hoc test).

Effect of EEF in striatal histopathological changes in adult mice after pilocarpine-induced seizures (MARQUES et al., 2011) was observed (Figure 5 and Table 6).

On the other hand, only the animals that were pretreated with EEF 50, 30 minutes before administration of pilocarpine, was a 58.34% reduction in the number of animals with brain damage, and those that showed convulsion and status epilepticus was detect histopathologic change in the striatum in only 2 animals (16.66%). Also detected was a reduction of 42.58% in the degree of involvement of the striatum in the EEF 50 + P400 group compared to P400 group. The groups treated only with Tween 80 0.05% solution (control group) or EEF groups showed no histopathologic alteration in the striatum.

Effect of EEF in hippocampus histopathological changes in adult mice after pilocarpine-induced seizures (MARQUES et al., 2011) was observed (Figure 6 and Table 7). Animals pretreated with EEF 50, 30 min before administration of pilocarpine, showed a 58.33% reduction in the number of animals with brain damage, and those that showed convulsion and status epilepticus was detect histopathologic change in the hippocampus in three animals (25%). Also detected was a reduction of 49.76% in the degree of impairment of the hippocampus in EEF 50 + P400 group compared to P400 group.
Figure 5. Effect of EEF in striatal histopathological changes observed in adult mice after pilocarpine-induced seizures. A - No histopathological changes in the striatum of adult mice observed for 24 h after administration of the vehicle (i.p.); B - Neuronal loss, gliosis, atrophy and degeneration of mice striatum that had convulsions, status epilepticus 24 h after administration of P400 (400 mg/kg, i.p.); C - No histopathological changes in the mice striatum pretreated with EEF (50 mg/kg, i.p.) and after 30 min received pilocarpine (400 mg/kg, i.p., EEF 50 + P400). D - No histopathological changes in the striatum of adult mice pretreated with EEF (100 mg/kg, ip) and after 30 min received pilocarpine (400 mg/kg, i.p., EEF 100 + P400). E - Absence of histopathological changes in the striatum of adult mice pretreated with EEF (150 mg/kg, i.p.) and after 30 min received pilocarpine (400 mg/kg, i.p., EEF 150 + P400). F - No histopathological changes in mice striatum pretreated with EEF (50 mg/kg, i.p., EEF 50). G - No histopathological changes in mice striatum pretreated with EEF (100 mg/kg, i.p., EEF 100). H - No histopathological changes in mice striatum pretreated with EEF (150 mg/kg, i.p., EEF 150). N=8/group; Hematoxylin - Eosin (HE) - X40.
Table 6. Effect of EEF in striatal histopathological changes observed in adult mice after pilocarpine-induced seizures.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lesion (%)</th>
<th>Severity of lesion (%)</th>
<th>n</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>P400</td>
<td>75</td>
<td>57.49 ± 0.92</td>
<td>06</td>
<td>08</td>
</tr>
<tr>
<td>EEF 50 + P400</td>
<td>25</td>
<td>10.50 ± 0.72(^{b})</td>
<td>02</td>
<td>08</td>
</tr>
<tr>
<td>EEF 100 + P400</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 150 + P400</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 50</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 100</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 150</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
</tbody>
</table>

After treatment all groups were observed for 24 h. The degree of impairment striatal (severity of lesion) was expressed as the mean ± S.E.M. scores of brain damage in the number of animals with lesions in the striatum. The animals were defined as having brain damage when there was at least 50% of impairment in the striatum. n = number of animals with lesions/group; N = total number of animals/group. P400 = pilocarpine 400 mg/kg; EEF 50 = EEF 50 mg/kg; EEF 100 = 100 mg/kg; EEF 150 = EEF 150 mg/kg. \(^{a}\)p<0.05 when compared to the P400 group (Chi-square). \(^{b}\)p<0.05 when compared to the P400 group (ANOVA and t-Student-Newman-Kewls as post hoc test).

The effects of EEF in the activity of acetylcholinesterase (AChE) in homogenates of hippocampus of mice (MARQUES et al., 2011) were determined (Figure 7). EEF, at doses of 50, 100 and 150 mg/kg was able to inhibit AChE activity by 81, 83.5 and 82.2%, respectively, compared to vehicle group. When the results of AChE activity in the groups pretreated with EEF was verified a reduction of 80.9, 83.5 and 82.1% in EEF 50, EEF 100 and EEF 150 groups, respectively, compared to P400 group.

We also found that the groups treated with the combination of EEF and 30 min later with pilocarpine in three different doses (EEF 50 + P400, EEF 100 + P400 and EEF 150 + P400) produced a significant increase in AChE activity of 177, 114.1 and 96.3%, respectively, compared to vehicle. Moreover, these pre-treatment followed by treatment with P400 also induced a significant increase of 178.4, 215.2 and 97.2% in AChE activity, respectively, compared to the P400 group.

4. Discussion

The phytochemical screening of EEF revealed the presence of alkaloids, phenolic compounds, flavonoids, carbohydrates, steroids and proteins. Traditional medicine plants, also known as herbal medicine, herbal medicine or phytomedicine refers to products for medical plant roots, stems, leaves, bark, seeds, flowers and fruits that can be used to promote general health and
Figure 6. Effect of EEF in hippocampus histopathological changes observed in adult mice after pilocarpine-induced seizures. A - Absence of histological changes in the hippocampus of adult mice observed for 24 h after administration of the vehicle (i.p.);
B - Neuronal loss, gliosis, atrophy and degeneration in mice hippocampus that had convulsions, status epilepticus 24 h after administration of P400 (400 mg/kg, i.p.);
C - No histopathological changes in mice hippocampus pretreated with EEF (50 mg/kg, i.p.) and after 30 min received pilocarpine (400 mg/kg, i.p., EEF 50 + P400).
D - No histopathological changes in mice hippocampus pretreated with EEF (100 mg/kg, i.p.) and after 30 min received pilocarpine (400 mg/kg, i.p., EEF 100 + P400).
E - No histopathological changes in mice hippocampus pretreated with EEF (150 mg/kg, i.p., EEF 150 + P400).
F - No histopathological changes in mice hippocampus pretreated with EEF (100 mg/kg, i.p., EEF 50).
G - No histopathological changes in mice hippocampus pretreated with EEF (150 mg/kg, i.p., EEF 100).
H - No histopathological changes in mice hippocampus pretreated with EEF (150 mg/kg, i.p., EEF 150). N=8/group; Hematoxylin - Eosin (HE) - X40.
Table 7. Effect of EEF in hippocampus histopathological changes observed in adult mice after pilocarpine-induced seizures.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lesion (%)</th>
<th>Severity of lesion (%)</th>
<th>n</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>P400</td>
<td>87.50</td>
<td>61.92 ± 0.73</td>
<td>07</td>
<td>08</td>
</tr>
<tr>
<td>EEF 50 + P400</td>
<td>37.5</td>
<td>16.75 ± 0.72</td>
<td>03</td>
<td>08</td>
</tr>
<tr>
<td>EEF 100 + P400</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 150 + P400</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>EEF 50</td>
<td>00</td>
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<td>08</td>
</tr>
<tr>
<td>EEF 100</td>
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<td>00</td>
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<td>08</td>
</tr>
<tr>
<td>EEF 150</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>08</td>
</tr>
</tbody>
</table>

After treatment all groups were observed for 24 h. The degree of impairment of hippocampus (severity of lesion) was expressed as the mean ± S.E.M. scores of brain damage in the number of animals with lesions in hippocampus. Animals were defined as having brain damage when there was at least 50% of impairment in striatum. n = number of animals with lesions/group. N = total number of animals/group. P400 = pilocarpine 400 mg/kg. EEF 50 = EEF 50 mg/kg. EEF 100 = 100 mg/kg. EEF 150 = EEF 150 mg/kg. a p<0.05 when compared to the P400 (Chi-square). b p<0.05 when compared to the P400 (ANOVA and t-Student-Newman-Kewls as post hoc test).

Figure 7. Effect of EEF in mice hippocampus on acetylcholinesterase (AChE) activity. Values represent the mean ± S.E.M. of number of animals used in the experiments. The AChE activity was determined in 10 μl of homogenate. Statistical analysis used: ANOVA and t-Student-Neuman-Keuls as post-hoc test. a when compared to vehicle (p<0.05). b when compared to P400 group (p<0.05). c when compared to EEF 50 + P400 group (p<0.05).

Treat diseases. These different plant products can be used directly on a prescribed formula or processed into different products ready for use. Traditional medicine has been used to maintain wellness and to treat or prevent diseases (CHENG, 2000; WANG; REN, 2000; CHAN, 1995).

Toxicological and pharmacological studies are very important for the safety of clinical use in the discovery of new pharmacological agents. For example, the volatile oil from Artemisia afra Jacq. Ex Willd as isolated by
Van der Lingen (WATT; BREYER-BRANDWIJK, 1962) is said to be as toxic as Sabina oil and produces hemorrhagic nephritis, hepatic degenerative changes and pulmonary edema when administered orally to rabbits, and sometimes abortion in rabbits and guinea pigs (WATT; BREYER-BRANDWIJK, 1962). The volatile oil also produces hallucinogenic effects that can be attributed to thujone, so overdoses or continuous uses for long periods are potentially harmful (VAN WYK; VAN OUDTSHOORN; GERICKE, 2000).

Previous studies have determined the toxicity biochemistry, hematology and histopathology of plant extracts administrations after acute, sub-acute and/or chronic in animals, in order to obtain information on the safety of the plant and provide guidance for the selection of a safe dose for use in traditional medicine (THANABHORN, 2006; MUKINDA; SYCE, 2007; FERRERO et al., 2007).

In the acute toxicity study intraperitoneally, mice given doses up to 150 mg/kg did not show any signs of adverse effect. Higher doses induced mortality and pronounced adverse effects.

There are several levels of toxicity in the technical literature are intended safety for human health, including the table of relative toxicity of Crowl. 1995. The LD$_{50}$ for intraperitoneal administration of the EEF in mice was estimated by linear regression (2.31 g/kg). Thus, intraperitoneal administration of EEF can be considered harmful.

Toxicological findings demonstrate that acute and subacute LD$_{50}$ selected for the hydroalcoholic extract from Wedelia paludosa DC Asteraceae is greater than 4 g/kg, and has low toxicity when administered orally in mice. demonstrating that the extract is virtually devoid of toxicity (BURGER et al., 2005). The ethanolic extract of Eremanthus erythropappus (DC) McLeisch (Asteraceae) was not toxic to animals treated, with LD$_{50}$ above 3 g/kg (SILVÉRIO et al., 2008).

From the toxicological point of view, any substance can be considered a toxic agent, depending on exposure conditions: or absorbed dose, duration and frequency of exposure and routes of administration (BARROS; DAVINO, 1996). Our studies suggest that the use of EEF from B. perennis is safe since it did not produce acute toxicity in animals. However, further studies to evaluate the subchronic and chronic toxicity of this extract are needed.

Analysis of blood parameters is relevant to the risk assessment of the hematological changes in humans (OLSON et al, 2000). The tests are intended to demonstrate that the use of extracts for medicinal purposes will not harm the overall health of living organisms, such as hepatic and renal systems, by clinical examination.
As to hematology study of animals treated with EEF of *B. perennis*, no changes were detected in the following parameters: erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, leukocytes, neutrophils, eosinophils, lymphocytes and monocytes. The analyzed values remained within reference values (BRITO, 1994).

In general, the biochemical profile of animals remained within reference values (HARKNESS; WAGNER, 1993). Thus, it can be suggested that the EEF of *B. perennis* at the doses tested did not produce acute renal failure or liver toxicity in animals (JAHN; GÜNZEL, 1997). There was a significant decrease in triglycerides and transaminases in the three tested doses of EEF compared to the control group, suggesting a possible hypolipidemic and hepatoprotective effect, which can be further explored in the future.

However, more studies should be conducted for a long period of treatment so that we can ensure the safety of the use of this species in humans.

It was established that exposure of organisms to exogenous and endogenous factors generates various free radicals such as superoxide anion radical (O$_2^-$) and hydroxyl (OH$^-$) and other ROS, such as H$_2$O$_2$ and single oxygen (O$_2$), which induce changes in cells, cytotoxicity and/or genotoxicity indirectly, favoring the acceleration of aging and cancer, cardiovascular disease and degenerative diseases (DASGUPTA; DE, 2007; TRIPATHI; MOHAN; KAMAT, 2007).

A biological antioxidant is defined as "any substance that, when present at low concentrations compared to oxidizable substrate, it can significantly reduce or prevent oxidation of the substrate" (BENZIE; STRAIN, 1996). Thus, free radicals and ROS are usually removed or inactivated *in vivo* by endogenous antioxidant enzymes as superoxide dismutase, peroxidase and low molecular weight compounds, such as tocopherol, ascorbic acid, polyphenols, thereby reducing the damage induced by oxidative stress. However, the ROS become harmful when produced in excess, under certain abnormal conditions, such as ischemia, inflammation and in the presence of catalytic ions (Fe$^{2+}$) (ADELMAN, 2005).

With respect to the neurochemical studies, a study with ubiquinone (SANTOS et al., 2010) show no significant changes in superoxide dismutase activity in the striatum of adult rats after convulsions. However, pretreatment with ubiquinone produced a significant rise in superoxide dismutase activity by 24% when compared to pilocarpine. The results indicate that seizures induced by pilocarpine produce behavioral changes characteristic of epileptic activity and there may be changes in superoxide dismutase activity during the acute phase of seizures. However, one may suggest that the anticonvulsant effect of ubiquinone may be due to this enzyme neuromodulation. Behavioral
changes and superoxide dismutase activity observed suggest an extensive involvement of the striatum in epileptogenesis, and ubiquinone was able to prevent these changes and presents itself as a potential anticonvulsant in this model of epilepsy.

We tried to demonstrate in vitro antioxidant potential of EEF. Therefore, the antioxidant activity was analyzed by TBARS assay, which is a method used to quantify lipid peroxidation which corresponds to a cell membrane damage caused by oxidative stress. The AAPH, a water-soluble azo compound is used as free radical generator. Their decomposition produces molecular nitrogen and carbonyl radicals, which, in turn, react with thiobarbituric acid, resulting in the formation of TBARS (ZIN; ABDUL-HAMID; OSMAN, 2002; FITÓ; DE LA TORRE; COVAS, 2007; MOON; SHIBAMOTO, 2009; SERAFINI et al., 2011).

During the oxidation process, the peroxides are gradually broken down into lower molecular weight compounds. One of the compounds is malonaldehyde, which is measured by TBA method (ZIN; ABDUL-HAMID; OSMAN, 2002).

The antioxidant activity of some essential oils is primarily attributable to one or more compounds with hydroxyl groups (OH−) on the aromatic ring or unsaturation. Some studies have shown that oxygenated monoterpenes constitute one of the major classes of antioxidant compounds in large part due to its functional group (hydroxyl).

Lipid peroxidation involves a series of chain reactions wherein an initiator radical extracts a hydrogen atom from a lipid and oxygen molecules react with lipid radicals, although this process to form lipid peroxyl and peroxides, which in turn draws atoms hydrogen from other lipids (HALLIWELL; GUTTERIDGE, 2007). Thus, it is possible that EEF interact more strongly with specific types of lipids, and in a system rich in lipids, such as the TBARS assay, lipids with lower affinity with EEF and/or hydrophilic portions of amphipathic lipids are more susceptible to radical attack allowing the beginning of the chain reaction of lipid peroxidation.

The EEF, at all concentrations tested was capable of preventing lipid peroxidation inhibiting amount of TBARS formed. A similar result was obtained with trolox, a synthetic analogue of α-tocopherol used as antioxidant standard. This result suggests that EEF can exert antioxidant effect which protects the lipid biomolecules.

Other studies investigating the use of plant parts as antioxidants have also used the TBARS method and obtained similar results (LIMA et al., 2006; BROINIZI et al., 2007).

Another methodology employed was the test of elimination of NO. This method is based on NO production from the decomposition of sodium nitroprusside in aqueous solution, NO, in turn, interacts with oxygen to
produce nitrite ions, which can be measured by Griess reaction (BASU; HAZRA, 2006). These nitrite ions have a strong oxidizing power, reacting with various biological molecules, leading to cell damage (HALLIWELL; GUTTERIDGE, 2007; GUIMARÃES et al., 2010). Substances with action elimination of NO compete with oxygen, leading to reduced production of nitrite, featuring the antioxidant activity (AHMADI-ASHTIANI et al., 2011; SERAFINI et al., 2011). In this regard, it has been demonstrated that high concentrations of free radicals can be highly toxic, and that the nitric oxide metabolites produced via the oxidative stress, such as nitrite and nitrate may contribute to this toxicity (FREITAS et al., 2005).

Many studies suggest that neuronal damage can be induced by excitotoxic stimulation of receptors that induce the production of free radicals, among them, nitrite and nitrate (BONDY; LEE, 1993; BONFOCO et al., 1995; BRUCE; BAUDRY, 1995; SHULZ et al., 1995; UEDA et al., 1997; VANHATALO; RIIKONEN, 2001). Oxidative stress induced by cerebral seizures can be blocked or at least reduced significantly by blocking the inhibition of the enzyme nitric oxide synthase (NOS), therefore a possibility for treating the effects of oxidative stress in epileptic patients could be done through the use of drugs that can modulate the enzymatic activity of NOS (RAJASEKARAN, 2005).

In the inhibition assay of NO, EEF reduced nitrite production, indicating a potential role as an agent for inhibiting NO. Reactions between certain ROS (including superoxide) and NO are generally faster and can result in inhibition of NO in some cells with consequent inhibition of some of its biological effects (SALVEMINI; DOYLE; CUZZOCREA, 2006). However, the combination of multiple ROS and NO invariably results in formation of peroxynitrite, which is a strong oxidizing agent which reacts with various biomolecules under physiological pH, leading to severe cellular damage (HALLIWELL; GUTTERIDGE, 2007). Thus, EEF may prevent the formation of peroxynitrite by their ROS scavenging capacity and NO. Together, these results suggest that EEF is able to act as an antioxidant and protects general biomolecules, such as membrane lipids against the damage induced by free radicals.

Nitric oxide plays a role in several types of inflammatory processes. It is produced by macrophages in the course of the inflammatory response. NO is mutagenic and can interfere with the processes of DNA repair (OKOKO, 2009).

In this study, EEF significantly decreased the production of nitrite, once again demonstrating its antioxidant property against damage caused by free radicals. Other studies with plant extracts with antioxidant activity can also decreased nitric oxide (KUMAR et al., 2005; MAIA et al., 2010).
Methodology quite employed to evaluate the antioxidant activity of a substance based on inhibition ability of free radicals formed in less reactive species (HOELZL et al., 2005). The ability of a substance to eliminate the hydroxyl radical is directly related to its antioxidant activity. The hydroxyl radical is a highly reactive species that can damage DNA, proteins and lipids (HALLIWELL; GUTTERIDGE, 2007; HUANG; OU; PRIOR, 2005; SHUKLA et al., 2009; SERAFINI et al., 2011). In this method, the hydroxyl radical is generated by Fenton reaction. In the presence of the hydroxyl radical, the deoxyribose is degraded to malonaldehyde, being quantified (PAYA; HALLIWELL; HOLT, 1992; HUAN; OU; PRIOR, 2005; SERAFINI et al.. 2011). However, this method has a drawback because many antioxidants also have the property of chelating metals. Thus, it is impossible to distinguish whether the action of the substance was indeed removal or simply chelating OH⁻ (HUANG; ORU; PRIOR, 2005).

The hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging pathology. This radical has the ability to join nucleotides in DNA and cause strand breakage, which contributes to mutagenesis, carcinogenesis and cytotoxicity (HOCHSTEIN; ATALLAH, 1998; MANIAN et al., 2008; SHUKLA et al., 2009). The ability of an extract to remove the hydroxyl radical is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins (SHUKLA et al., 2009). It was demonstrated that EEF produced removing the hydroxyl radical, exhibiting a significant antioxidant activity which may be capable of inhibiting cell damage caused by this radical. The trolox (default) also significantly reduced the amount of this radical.

In the trial of hydroxyl radical, EEF significantly decreased the production of hydroxyl, reinforcing its antioxidant activity. Previous studies with other plant extracts obtained similar results to this study (KUMAR et al., 2005; DUARTE-ALMEIDA et al., 2006).

The effect of *B. perennis* in spontaneous activity was studied by analyzing the number of crossings, rearings and groomings in open field test. In this test, it is the assessment of self-cleaning (groomings) by inhibition occurs following exposure to stress. This behavior is related to the paraventricular nucleus of the hypothalamus due to high activation of the hypothalamic-pituitary-adrenal (HPA) (DO-REGO et al., 2005). In the experiments, EEF in the three tested doses produced no significant effect on grooming behavior during acute treatment of animals, suggesting that it cannot exert influence on the HPA axis.

In turn, the increased number of crossings (horizontal movement) is indicative of CNS stimulant properties. Likewise, the number of rearings
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(vertical movement) is indicative of an effect on locomotor activity (VOGEL, 2002). It is known that treatment with diazepam, an anxiolytic drug, used as a positive standard pharmacological studies decreases significantly when these two parameters assessed in the open field test. The experiments showed that the treatment with EEF at doses of 50, 100 and 150 mg/kg, intraperitoneally significantly decreased behaviors rearings and the number of crossings in the open field test. The literature suggests that drugs that decrease these parameters have potential anxiolytic, as the plant *Ocimum gratissimum* L. (OKOLI et al., 2010).

Aiming to clarify the probable mechanism of action of EEF, we evaluated the possible involvement of the benzodiazepine site of the GABA<sub>A</sub> receptor in the anxiolytic effects of EEF suggested for *B. perennis*. Thus, animals were pretreated with flumazenil at a dose of 2.5 mg/kg (i.p.), which is a selective antagonist of the benzodiazepine site of GABA<sub>A</sub> receptors. and 15 minutes later these animals were treated with same vehicle. EEF different doses or diazepam (DZP) at a dose of 2 mg/kg (positive control). Exactly after 30 minutes of these treatments, the animals were evaluated again in the open field test and found that flumazenil was only able to reverse the effects of diazepam, suggesting that the anxiolytic effects demonstrated by EEF cannot be due to direct interaction with GABAergic receptors.

Several neurotransmitters are involved in the physiology of anxiety, depression and seizures, including serotonin, norepinephrine and dopamine (DAILY et al., 2004; MOLTZEN; BANG-ANDERSEN, 2006). The monoamine hypothesis is based on the assumption that depression is due to a deficiency of either of these neurotransmitters (RANG et al., 2007), although many others may be involved, including the hypothalamic-pituitary-adrenal (HINDMARCH, 2002).

Mood disorders, anxiety and sleep are prevalent in many psychiatric comorbidity (KESSLER et al., 2005) who were treated with botanical medicines since antiquity. Data from a representative sample of more than two thousand people interviewed in Brazil during 1997-1998 revealed that 57% of people who suffer from anxiety attack, and 54% of people with major depression reported using herbal therapies and complementary and alternative medicine during the 12 months prior to the treatment of their disease (KESSLER et al., 2001). Furthermore, depression which is thought to result from biochemical changes in the brain is a common disease in adult life (RICHELSON, 1991; GREENBERG; STIGLIN; FINKELSTEIN, 1993; JUDD, 1995; BAKER; COUTTS; GREENSHAW, 2000).

Compounds antidepressants are known to act by different mechanisms in different level receivers, probably by stimulating similar paths in sub-cellular
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level (YILDIZ; GONUL; TAMAM, 2002). EEF (50, 100 and 150 mg/kg) was also able to decrease the immobility time of mice in the forced swimming test. At higher doses (150 mg/kg), the antidepressant effect was not masked by actions sedative and hypnotic drugs. The effect of the EEF was increased with imipramine, a tricyclic antidepressant that blocks the reuptake of serotonin and norepinephrine. However, there were no changes after EEF association with paroxetine, a known selective inhibitor of serotonin reuptake. Moreover, EEF effects were totally blocked by pretreatment with reserpine, a known inhibitor of vesicular carrier of catecholamines (which facilitates storage vesicle). A similar process occurs in storage locations of 5-HT, which may finally lead to a depletion of biogenic amines. Moreover, this finding suggests that the antidepressant effect of EEF is probably related, at least in part, to increased noradrenergic activity in the CNS.

This study shows that oral administration of EEF decreases the immobility time in the forced swimming test in mice, an animal model of depression (PORSOLT et al., 1978) compared with the control group. EEF 50, EEF 100 and EEF 150 and antidepressant drugs decreased immobility time in the forced swimming test in animals tested, compared to vehicle group, and the effect of higher doses of extract (150 mg/kg) was comparable to the effect observed for the IMI 20 group, which was given a classical tricyclic antidepressant. IMI 20 + EEF 150 significantly decreased the immobility time in the forced swimming test when compared to IMI 20 and EEF 150 groups. These results suggest a possible antidepressant effect of the extract.

Among the diseases that can benefit from such studies, temporal lobe epilepsy can interfere with numerous social and psychological aspects of the patient. It is currently considered one of the most common neurological disorders. Shows a prevalence rate of 5%, and approximately 30 to 50% of epileptic patients are symptomatic. There are estimates that 50 million people worldwide are epileptic and only 25 to 45% are completely free of seizures after treatment. The absence of an appropriate drug treatment for epilepsy and seizures may aggravate render the patient more susceptible to epileptic other neuropathologies (POST, 2004).

Epilepsy is a serious neurological condition highly prevalent in the world and that can induce difficulties in the execution of professional epileptic patient (GILLIAM et al., 2004). The incidence varies with geographic location. It occurs more frequently in developing countries, where there is a higher rate of malnutrition, infectious diseases, disabilities and medical needs in the pharmacological treatment (DE LORENZO et al., 2001). This disease is more common in childhood due to increased vulnerability of CNS
infections, accidents and head injuries and complications from other diseases which can cause seizures. It can also be manifested during the aging process whose physiological complications increase susceptibility to neuronal hyperexcitation culminated in the development of epilepsy.

The model of epilepsy induced by pilocarpine in mice may be useful for investigating the development and neuropathology of temporal lobe epilepsy. The model is characterized by an initial precipitating stimulus such as status epilepticus prolonged (12-18 h) that induces neuronal loss in the hippocampus and may still result in the development of spontaneous recurrent seizures (MELLO et al., 1993; WALZ et al., 1999; BONAN et al., 2000).

Previous studies have shown the involvement of several neurotransmitter systems and oxidative stress in the pathophysiology of pilocarpine-induced convulsive process. Through studies on neurochemical levels in brain regions involved in these seizures epileptogenesis (ERAKOVIC et al., 2000; BARROS et al., 2007; XAVIER et al., 2007; FREITAS et al., 2009).

The model of epilepsy with high-dose pilocarpine convulsive allows to investigate the process through behavioral and electroencephalogramic changes and the presence of brain damage (CLIFFORD et al., 1987; PERSINGER et al., 1993; MARINE et al., 1997), which are similar to temporal lobe epilepsy in humans, also allowing the study of changes in level receivers (MARINE et al., 1998), and/or the levels of monoamines and their metabolites in the status epilepticus (EL-ETRI et al., 1993; KHAN et al., 2000).

Making use of this model of seizures induced by pilocarpine, it was possible to evaluate the pharmacological effect of the EEF in behavioral parameters of adult mice during the acute phase of the convulsive process. After acute administration of pilocarpine, all mice showed the following behavioral changes: peripheral cholinergic signs (miosis, piloerection, salivation, diarrhea and diuresis), tremors and stereotyped movements (increased activity of biting, scratching, chewing and wet-dog shakes, which is the act of bobbing like a wet dog). These behavioral changes were continued until installation of the motor limbic seizures, including clonic movements of the upper extremities occurred in all animals. In the same group, convulsions progressed to the development of status epilepticus in all animals and the survival of unverified none of the animals in this group.

The P400 group showed seizures, status epilepticus and high mortality. The group pretreated with EEF before induction of seizures by P400 showed similar behavior to P400 group: presence of peripheral cholinergic signs, tremors and stereotyped movements. However, there was a significant decrease in installation time of the first seizure and status epilepticus as well
as a significant decrease in the mortality rate, suggesting the anticonvulsant action of EEF.

Our results (MARQUES et al., 2011) regarding behavioral changes during seizures are consistent with the observations described previously by other studies (SANTOS et al., 2008; FREITAS et al., 2009). We demonstrated that systemic administration of EEF 50 reduces the number of animals that have seizures induced by pilocarpine and status epilepticus, and also increases the survival rate of the animals. This was evidenced by the observed behavioral changes in animals receiving the convulsant stimulus. The results suggest that EEF 100 and EEF 150 groups can present a significant anticonvulsant action in seizure model induced by pilocarpine. It is suggested that this plant could serve, after conducting further studies to clarify fully the mechanism of action, as a potential therapeutic agent for the treatment of epilepsy in the future.

Thus, the results suggest that EEF can present a significant anticonvulsant action in seizure model induced by pilocarpine, probably due to potentiation or increase inhibitory neurotransmission.

Associated with behavioral studies and evaluation of the activity of AchE, we investigated the effects of EEF from B. perennis in mice on histopathological changes observed in seizure model induced by pilocarpine. In these histopathological studies, we observed a high number of animals with brain injury and a significant impairment of the areas investigated in the P400 group. Furthermore, in the striatum and hippocampus of animals pretreated with three doses of EEF, 30 minutes before administration of pilocarpine, was detected a significant reduction in the number of animals with brain injury and degree of impairment of the area analyzed in animals that had seizures and status epilepticus.

In the model of epilepsy induced by pilocarpine, all animals treated with EEF 100 and 150 mg/kg showed no changes in the hippocampus and striatum during acute seizures. However, hippocampus and striatum of animals of the group treated with the lower dose during this phase had a lower involvement area.

Neuronal death observed in the hippocampus and striatum of animals after pilocarpine-induced seizures can be attributed to excitotoxicity produced by glutamatergic system (CAVALHEIRO et al., 1994). However, in histopathological studies, animals pretreated with EEF showed complete blockage of neuronal damage in the hippocampus and striatum at doses of 100 and 150 mg/kg and a decrease of 67 and 57.14% with the lowest dose in these two brain areas, respectively. Literature data suggest that oxidative stress may be involved in the installation of pilocarpine-induced seizures, probably due to increased production of free radicals and there is a decreased
activity of enzymatic antioxidant defenses (SHULTS; HAAS, 2005; FREITAS et al., 2005; CHATUVIERDI; BEAL, 2008).

The data demonstrate a possible neuroprotective effect of EEF during these seizures, although further studies should be conducted to try to clarify the mechanism of action of this extract. It is also possible to suggest that the effects are due to the removal of free radicals produced during seizures or by modulation of neurotransmitter systems, but the data show a possible anticonvulsant and neuroprotective effect of the EEF in pilocarpine-induced seizures model in adult mice.

Aiming to corroborate previous studies was performed to evaluate the effect of EEF of *B. perennis* in the absence and presence of convulsive stimulation (pilocarpine) on the activity of the enzyme acetylcholinesterase (AChE) in hippocampus homogenate of mice.

The results suggest that EEF of *B. perennis* has potential for clinical use in the treatment of Alzheimer's disease, since it reduced significantly the AChE activity *in vivo*. When the effects of EEF of *B. perennis* were evaluated in the AChE activity in seizure model induced by pilocarpine, it was observed that EEF was able to inhibit the enzyme activity of AChE in all three dose levels in the hippocampus of animals showing its future use in the treatment of neurodegenerative diseases. In the groups treated with three different doses of EEF and then pilocarpine (400 mg/kg), there was significant increase in acetylcholinesterase activity compared to the control and P400 groups, suggesting a possible regulatory mechanism of the enzymatic activity of AChE to reduce or convulsions triggered by modulating cholinergic.

The cholinergic transmission is completed mainly by the hydrolysis of acetylcholine (ACh) by the enzyme AChE (SILVER, 1974; 1999; MASSOULIÉ et al., 2006). This enzyme contributes substantially to synaptic transmission during convulsions, so it is important to describe the effects of EEF of *B. perennis* in this enzymatic activity. AChE have a crucial role in cholinergic neurotransmission by hydrolyzing the neurotransmitter acetylcholine to terminate nerve impulses mediated by this brain system (LOCKRIDGE et al., 2003), and can also influence the pathophysiology of neurodegenerative diseases and addiction (GRUSLIN; DESCOMBES; PSARROPOULOU, 1999; MACÊDO et al., 2006).

On the other hand, in the groups that were pretreated with EEF of *B. perennis* and then received P400, a significant increase in the activity of the enzyme AChE was observed. It is suggests the neuroprotective effect of EEF during seizures, trying to decrease the action of acetylcholine in the synaptic cleft and blocking the seizure. Previous studies describing plant
Bellis perennis L. on central nervous system extracts capable of enhancing the activity of AchE, such as Syzygium cumini L. (MAZZANTI et al., 2004).

A substance isolated from EEF of B. perennis, isorhamnetina 3-O-β-D-(6''-acetyl)-galactopyranoside, showed anticholinesterase activity in vitro (MARQUES et al., 2013). It is suggested that the flavonoid can more effectively enhance the cholinergic stimulation and thus produce an insecticidal effect more potent than other insecticides such as carbamates and organophosphates for use in agriculture or in domestic environments. In vivo studies, there was also a decrease of enzymatic activity of acetylcholinesterase when administered EEF or flavonoid in animals, suggesting potential use in the treatment of neurodegenerative diseases such as Alzheimer's Disease. The isolated substance may be used, after further research, in product formulation(s) pharmaceutical(s) for the prevention and/or treatment of Alzheimer's pathology associated with reduced cerebral neurotransmitters, especially acetylcholine.

This work led to a patent "Pharmaceutical applications of the extract of Bellis perennis and/or substance isorhamnetina 3-O-β-D-(6''-acetyl)-galactopyranoside isolated from this extract as an anticonvulsant agent in the treatment of epilepsy" (MARQUES et al., 2012) and patent application "Bellis perennis L. extract, isolated substances of the same agents and their applications in insecticides by inhibiting the enzyme acetylcholinesterase" (FORTES et al., 2012).

5. Conclusion

This study, using approaches in vivo and in vitro, suggests through acute toxicity studies conducted that EEF has high LD$_{50}$ value and is moderately toxic. The haematological and biochemical profiles of animals remained within reference standards, and suggest a possible hypolipidemic and hepatoprotective effect of EEF. However, more studies during a subchronic and chronic treatment are needed to confirm this finding. EEF produced a decrease in the levels of lipid peroxidation in vitro, and was able to exert activity in the removal of nitrite and hydroxyl radicals at different concentrations. The antioxidant activity in vitro suggests that this medicinal plant can be used in the future, after conducting further studies in the treatment of neurodegenerative diseases which are characterized by the occurrence of cellular damage produced by free radicals. EEF reduced the time of immobility in the forced swimming test and the number of crossings and rearings in the open field test, suggesting possible anxiolytic and antidepressant effects for the species studied, but new pharmacological studies should be conducted to define its possible mechanism of action. EEF
demonstrate anticonvulsant activity by decreasing the number of animals displaying seizures and status epilepticus induced by pilocarpine, and was able to increase survival rate of animals in this model. The seizures induced by pilocarpine exhibit behavioral changes and histopathological characteristics of epileptic activity in adult mice. EEF showed a significant anticonvulsant effect (by reducing the number of animals that had seizures and the mortality rate as well as increase the latency to first seizure and installation of status epilepticus) and neuroprotective (for reducing injuries in regions hippocampus and striatum caused by process changes associated with seizure). EEF reduced episodes of pilocarpine-induced seizures in mice, probably by increasing the activity of the enzyme AChE in mice hippocampus. There was also an increase of enzymatic activity when administered to animals with EEF, suggesting its possible use in the treatment of neurodegenerative diseases such as Alzheimer's Disease. Somehow, the results presented here are relevant because they validate the use of B. perennis, an important medicinal plant used in South America.

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