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**Antioxidant and cicatrizing activity of the species Abarema cochliacarpos (Gomes) Barneby & J. W. Grimes**

Saskya Araújo Fonseca¹,²*, J. R. M. Costa¹, N. R. S. Gomes⁴, Amanda Lima Cunha³, João Gomes da Costa¹, Thiago José Matos-Rocha¹,⁴, Karwhory Wallas Lins da Silva³, Paulo Henrique Barcellos França², Francisco Feliciano da Silva Júnior¹, Antônio Euzébio Goulart Sant’Ana² and Aldenir Feitosa dos Santos¹,³

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In equine clinics, skin injuries are some of the most frequently treated injuries, this research evaluated the healing potential and antioxidant activity of *Abarema cochliacarpos* in order to develop a pharmaceutical formulation for treating skin lesions in horses. Firstly, the antioxidant activity of several fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical method and the thiobarbituric acid test. The determination of phenolic compounds was done by means of the Folin-Ciocalteau test. To assess the wound healing activity the dry powdered stem bark and the aqueous, ethanolic and propylene glycol extracts from the same plant, material were incorporated in a gel formulation and evaluated in equines with induced wounds. Results show that all obtained extracts and their fractions have antioxidant action. Regarding the healing evaluation in equines, the extracts tested and the dry powdered stem bark showed statistically significant activity in the contraction of wounds. Histological analysis revealed the powdered stem bark as the best treatment to induce healing of cutaneous lesions in horses. Experimental studies in animals with different dosages and formulations, besides the isolation of its chemical components, as a tool in the discovery of new healing agents or optimization of the existing ones is recommended.

**Key words:** *Abarema cochliacarpos*, antioxidant activity, wound healing activity.

**INTRODUCTION**

*Abarema cochliacarpos* (Fabaceae) is a native tree from Brazil (popularly called in this country by “barbatimão”) found mainly in the Atlantic forest and Caatinga. Many traditional communities in northeast Brazil use its bark in folk medicine (Agra et al., 2008; Pereia et al., 2013). An ethnopharmacological survey in a Brazilian traditional
community has documented popular uses of this species to treat inflammatory disorders, including ulcerations, and for wound healing purposes (Tenório et al., 2016).

The stem bark used in the preparation of herbal medicines, comprise chemical compounds that include alkaloids, flavonoids, terpenes, stilbenes, steroids, protease inhibitors, and tannins. The latter class of secondary metabolites comprises the majority of components of A. cochliacarpos and have been regarded as the main responsible compounds for its antioxidant activity, as they can scavenge free radicals (Shimizu et al., 2009).

Plant extracts thus represent an important alternative to traditional medicine for the treatment of wounds, because they can act on the surface of the lesion, and through anti-inflammatory activity, create a microenvironment that facilitates fibroplasia. The lesions may heal faster because the wound is decontaminated by the extract's antimicrobial effects, or through changes of pH in the wound, which create an unfavorable environment for the growth of microorganisms.

In equine clinics, skin injuries are some of the most frequently treated injuries, affecting mainly the locomotor limbs. They occur due to the animals' active behavior and rapid reactions, especially in horses involved in sporting activities (Paganela et al., 2009). Horses have sudden reactions that can lead to limb trauma. In addition, inadequate facilities and grazing are also crucial factors for the occurrence of these lesions. Incorrect treatments and the tendency to chronicity of the lesions also hamper the cicatricial process (Viana et al., 2014). Given the interest in development of herbal medicines for the treatment of wounds, and the high incidence of equine skin lesions, the research aims to assess the phytochemical profile, antioxidant activity, and effects of powdered stem bark, natrosol gel formulation obtained from aqueous, ethanolic and propylene glycol extracts of the stem barks of A. cochliacarpos, on healing of skin wounds in horses.

MATERIALS AND METHODS

Study type and setting

The experiment involved both in vitro and in vivo laboratory evaluation. The research was conducted in the multidisciplinary research laboratory of the Centro Universitário Cesmac, located in Maceió, Alagoas; Brazil, and in the Clinical Veterinary School of the same institution, which is located in the city of Marechal Deodoro, Alagoas, Brazil.

Ethical aspects

This study was approved by the Ethics Committee for the use of animals (CEUA) of the Federal University of Alagoas with opinion substantiated n° 43/2012.

Collection and identification of botanical material

The specimen was collected in the municipality of Marechal Deodoro, Alagoas, Brazil. Three samples were collected for botanical identification the specimen was identified at the local of collection by a botanist and samples from stem and leaves were stored in the Herbarium of the Environment Institute, in Alagoas, under MAC registration number: 25.370. The stem bark was dried in the shade at room temperature and powdered afterwards by means of a mill that was used in the preparation of the extract. The stem bark powder was then stored in a tightly sealed dark container. Aliquots of this material were used for incorporation into the gel of natrosol, production of extracts, and phytochemical screening.

Preparation of extracts

Aqueous extract

Extracts from the bark of the stems of A. cochliacarpos were obtained by decoction over 30 min, in a proportion of 100 g of dry powdered bark for each 1000 ml of distilled water. The aqueous extract was then stored at room temperature until it was incorporated into the natrosol gel via the method of Verza (2006).

Propylene glycol extract

To obtain this extract, the powdered material was macerated with an 80% mixture of propylene glycol in distilled water for 24 h. After this period, the extract was filtered in a filter paper (Whatman No 1). The propylene glycol extract was further stored at room temperature until its incorporation into the natrosol gel via the method of Verza (2006).

Ethanolic extract

The ethanolic extract from the barks of the stem of A. cochliacarpos was prepared by a steeping method, in which the plant material was first crushed, then placed in a percolator tube containing absolute ethanol for 72 hours. After this period, the extract was filtered in (Whatman No 1 filter paper). This procedure was repeated until the plant material was exhausted. A portion of the liquid sample was then concentrated in a rotary evaporator under reduced pressure until a crude ethanolic extract (BSE) was obtained (Weber, 2013). The other part of the liquid sample was stored at room temperature until it was incorporated in the natrosol gel.

Preparation of natrosol gel and obtaining gel from A. cochliacarpos

The natrosol gel was prepared according to previously described methodology (Wettasinghe and Shahidi, 1999). After the

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preparation of the natrosol gel, each extract was incorporated into the natrosol gel in a 10% v/w concentration for a seven-day stability test.

**Chemical study of the ethanolic extract**

The Crude Ethanolic Extract (CEE) of the A. cochliacarpos stem bark was fractioned through a liquid-liquid partition method. The extract was dissolved in mixture of methanol and water at a ratio of 6:4, respectively. From the methanol/water phase, the process of partition was initiated using the solvents hexane, chloroform and ethyl acetate, resulting in Hexane Fraction (HF), Chloroform Fraction (CF) and Ethyl Acetate Fraction (EAF), respectively. The fractions were then subjected to HPLC-DAD (High Performance Liquid Chromatography-Diode Array Detector), TLC (thin layer chromatography), phytochemical prospecting, evaluation of antioxidant activity, and determination of phenolic compounds.

**Phytochemical screening**

Phytochemical screening was performed for searching the following metabolites: pyrogallic tannins, phlebotonic tannins, phenols, anthocyanins, anthocyanidins, flavonoids, xanthones, flavonones chalcones, aurones, flavononols, leucoanthocyanidins, catechin, flavonones, flavanones, xanthones, steroids, triterpenoids, and saponins (Matos, 1997).

**Analysis of antioxidant activity by radical 2,2-diphenyl-1-picrilidrazila**

In the analysis of antioxidant activity by radical 2,2-diphenyl-1-picrilidrazila (DPPH), vegetable samples, HF, CF and AEF were dissolved in 95% ethanol, applied in chromatoplates (Gel silicic F254, disabled) and eluted in solvent system containing ethyl acetate/formic acid (8:2). After elution, chromatoplates were immersed in ethanolic solution of DPPH 0.3 μg/mL for 10 seconds. The appearance of yellow spots underneath the purple coloration is indicative of antioxidant activity.

Absorbance values were converted to antioxidant activity percentages (AAO%) using the formula: AAO% = 100 - ([ABSA - ABSB] X 100)/ABSC, where ABSA represents the absorbance of the sample, ABSB is the absorbance of the blank, and ABSC is the absorbance of the control sample. Quantitative assessment of antioxidant activity was done by monitoring the free radical DPPH consumption and by measuring the decrease in absorbance of solutions at different concentrations (Eurides et al., 2007). Samples from BSE, HF, CF and AEF were diluted in triplicate in ethanol with final concentrations of 250, 125, 50, 10 and 5 μg/mL. Reactions were carried out at room temperature for 30 minutes. Readings of the absorbances were subsequently made at 518 nm.

**Evaluation of antioxidant activity by the Thiobarbituric acid method**

For evaluation of the antioxidant activity by the Thiobarbituric acid method (TBA), homogenized egg yolk solution was used as a rich medium in lipids. The procedure was performed triplicate, in accordance with the methodology described by Ferreira (2010). Five test tubes were set up containing 0.5 mL of egg yolk (10% w/v) and 0.1 mL of the plant samples was dissolved in the least amount of methanol and the volume diluted to 1 mL with water. Three concentrations were tested for each BSE solution: 100, 500 and 1000 μg/mL.

Next, 2.2 chloride 1-azo-bis (2-amidinopropano)-ABAP (0.07 mol/L) was added to each tube to induce lipid peroxidation, followed by a 20% solution of acetic acid (pH 3.5) and 1.5 mL of TBA (0.8% w/v) solution in sodium dodecyl sulfate-SDS (1.1% w/v). To allow observation of the complete lipid peroxidation, the antioxidants 3.5-di-tert-butyl-4-α-tocopherol and butylated hydroxytoluene BHT were used as positive standards in the same conditions to which plant extracts were subjected. The vials were placed in a water bath at 95°C under stirring over 60 min. After cooling, 5 mL of 1-butanol were added to each tube, and the tubes were centrifuged at 3000 rpm for 10 min.

To allow observation of the complete lipid peroxidation, the antioxidants 3.5-di-tert-butyl-4-α-tocopherol and butylated hydroxytoluene BHT were used as positive standards in the same conditions to which plant extracts were subjected, allowing the observation of the complete lipid peroxidation. Absorbance of organic supernatant layer was measured in a spectrophotometer at a wavelength of 532 nm. The values obtained were applied in the following formula to determine the antioxidant content of plant samples in percentage (IA%): IA% = (1 - A/C) x 100, where C is the absorbance of the control and the fully oxidized, the arithmetic mean of the sample tested absorbances.

**Determination of phenolic compounds**

The vegetable samples CEE, HF, CF, and EAF were evaluated at a concentration of 2.0 mg/mL. 0.5 mL of a 2N Folin-Ciocalteau reagent solution was added to 0.5 mL of each sample, followed by 1.0 mL of water (in triplicate). After stirring for 2 min, 0.5 mL of 10% (w/v) aqueous sodium carbonate (Na₂CO₃) was added to the tube. Then the samples were incubated for 2 h at room temperature (while protected from light). Absorbance values were obtained by readings at 750 nm, using the Folin-Ciocalteau reagent in methanol as blank sample. In order to build a calibration curve, gallic acid solutions were used at concentrations of 0.15, 0.1, 0.05, 0.025, 0.01, and 0.005 μg/mL. Total phenol values were expressed as gallic acid equivalents (Junqueira, 1999).

**EC calculation**

Results of in vitro tests were expressed in EC₅₀ values (Nascimento et al., 2014), which is the concentration required to produce half (50%) of maximum effect estimated at 100% for the plant extract.

**In vivo assay**

**Animal selection**

Two male and three female horses were selected from the farm of the CESMAC Veterinary Medicine School. All animals were of mixed race, aged between 2.5 and 3 years, weighing from 270 to 320 kg, and were clinically healthy and well-nourished. The animals were fed with a balanced diet (corn + wheat + soy) at a rate of 2.5 kg/day, bulky (Tifton Grass) 28 kg/animal/day and water ad libitum. The animals were accommodated in individual stalls covered with sand.
Surgical procedure

After anesthesia, administered intravenously using 10% xylazine (0.5 mg/kg/EV), the animals were trichotomized, and a skin antisepsis was made with 1% povidone-iodine. Then, five circular and symmetrical wounds were made aseptically in the thoracic region, at the lateral to dorsal midline of each animal with the help of scalpel blade No. 23, after local anesthesia with lidocaine hydrochloride 2%, according to the method of Andriguetto and Perly (2002). The five wounds were made with the help of a punch of 3 cm, measuring approximately 3.0 × 3.0 cm, with a distance of 5 cm between them. The damage included the epidermis, dermis, and subcutis. After the surgical procedure, the wounds were measured with Vernier Calipers 200 × 0.05 mm/8 × 1/128, France.

Clinical evaluation of wounds

Clinical evaluations were performed visually by the same individual every 72 h, observing the presence of hyperemia, edema, pain, discharge, itching, crust, contraction, granulation tissue, and scar tissue in accordance with Ardisson et al. (2002).

Morphometric analysis of wounds

For the acquisition of the area of wounds, measurements of diameters at the time of biopsy to 0, 3, 7 and 14 days post-op were carried out. All wounds were measured with the aid of calipers (Andriguetto and Perly, 2002) and from these elements, the following equation was formulated: where A represents the area (cm²), and r the radius. The degree of contraction, expressed in percentage, was measured by the equation proposed by Ramsey et al. (1995) where Wo = initial wound area and Wi = wound area on the day of the biopsy: 100 x (Wo-Wi)/Wo = % contraction.

Treatment for the wounds

Wounds were treated daily at 24 h intervals, using powdered stem barks as well as aqueous, ethanolic and propylene glycol extracts of A. cochliacarpos in a gel formulation. Four wounds were treated with A. cochliacarpos and the remaining wound was treated with Dakin (0.5% NaOCl). Treatments were applied on wounds of different positions for each animal. Throughout the experiment, bandages were not applied to the wounds.

Histological procedures

The animals underwent incisional biopsy of the skin for bilateral microscopic analysis of the healing process. Microscopic assessments were carried out at the end of the trial period. Material collection was performed for histopathological analysis, with tissue removal foursquare measuring about 2 x 2 cm, in order to include the entire diameter of the wound, as well as tissue adjacent to injury. The collected material was fixed in 10% buffered formalin for 24 h, being subjected to routine histological processing, included in paraffin and then cut into 6 cm. set microtome. Then, the cuts were stained by hematoxylin and eosin and then examined using a light microscope (Barroso et al., 2010). Microscopic analysis of the healing of each of the wounds was descriptive, qualitative evaluating the morphology of the inflammatory process, granulation tissue, cellularity, neovascularization and fibroplasia, as well as the evolution of the healing process throughout the ages (Barroso et al., 2010).

Statistical analysis

The results of the area and contraction of the wounds had been expressed on average ± shunting line standard, submitted to analyses of variance, and also to Tukey’s test, considering significant comparative values to the level of 5% of significance. Statistical analysis was performed using SPSS to determine the linear regression and the coefficient of determination (R²), which best explain the antioxidant potential of plant samples.

RESULTS

Preparation and fractionation of the ethanolic extract

From the crude extract (598 g) of stem bark, approximately 35 grams (5.85% yield) of the CF, 15 grams of EAF (2.50% yield) and 2 g of HF (0.33% yield) were obtained through primary fractionation. Fractionation of the crude extract with solvents of increasing polarity allows inferring the classes of substances extracted in the different fractions, in accordance with the known solubilities and polarities presented by various substances (Müller, 2006).

Phytochemical prospection

The phytochemical screening revealed different classes of secondary metabolites including phlebotonic tannins, flavonols, xanthones, flavones, catechins, steroids, and saponins, as shown in Table 1.

Quantitative assessment by DPPH assay

A significant radical scavenging activity was found for the crude extract at low concentrations as shown in Figure 1. Furthermore, fractionation of the crude extract showed that the activity was retained by the EAF (Figure 2) since higher scavenging of radicals could be attained at lower concentrations. In contrast, similar scavenging activity for CF (Figure 3) and HF fractions (Figure 4) were achieved with higher concentrations and indicates that the compounds responsible for the activity in the crude extract had greater affinity by ethyl acetate solvent.

Determination of phenolic compounds

Total phenolic content found in the screening was of 181.8 mg Gallic acid equivalents/g of the sample with regard to CEE extract, and 50% of this content was found in AEF.

Antioxidant content percentage

The most common technique used to measure lipid peroxidation is the test of Thiobarbituric Acid (TBA), a
Table 1. Prospection of the chemical constituents of stem bark of *A. cochliacarpos*.

<table>
<thead>
<tr>
<th>Secondary metabolite</th>
<th>CEE</th>
<th>EAF</th>
<th>CF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogalic tannins</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Phlebotonic tannins</td>
<td>N</td>
<td>P</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Phenols</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Anthocyanins and anthocyanidins</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Flavonols, xanthones and flavonones</td>
<td>N</td>
<td>P</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Chalcones and aurones</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Flavononols</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Leucoanthocyanidins</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Catechin</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Flavonones</td>
<td>P</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Flavanonols and xanthones</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Steroids</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Saponins</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>N</td>
</tr>
</tbody>
</table>

(P) indicate the presence of compounds; (N) indicate the absence of compounds. CEE, Crude Ethanolic Extract; EAF, Ethyl Acetate Fraction; CF, Chloroform Fraction; HF, Hexanic Fraction.

Figure 1. Antioxidant activity percentage of CEE from stem bark of *A. cochliacarpos*.

The spectrophotometric method that measures the concentration peroxidation products. The final product measured is the malondialdehyde or reactive substances formed from barbituric acid. In TBA test, the CEE extract presented the highest antioxidant capacity when compared to BHT at concentrations of 1000, 500 and 100 μg/ml, as shown in Table 2.

**Evolution of the wound areas**

The wound areas gradually decreased with the evolution of time over the course of 14 days of treatment (Figure 5). After 24 h from the beginning of the treatment, hyperemic areas were observed in the wounds, with zones of intense vascularization. The edges were well defined geometrically; however, the presence of edema and crusting areas were sometimes displayed at the outer regions of the wounds. Throughout the course of the experiment, those wounds treated with *A. cochliacarpos* presented irregular thick crusts and dryness.

The reduction of the wound area occurred due to the mechanism of contraction and the centripetal movement of the wound border towards the center, in order to reduce the area to be covered by the proliferating...
epithelium. The diameter and contraction data as a function of the different treatments are shown in Table 3. Based on results shown in Table 3, it was attested that the dry powdered stem bark as well as the ethanolic and

**Figure 2.** Antioxidant activity percentage of EAF from stem bark of *A. cochliacarpos*.

**Figure 3.** Antioxidant activity percentage of CF from stem bark of *A. cochliacarpos*.

**Figure 4.** Antioxidant activity percentage of the HF from stem bark of *A. cochliacarpos*.
Table 2. Antioxidant activity of CEE extract from stem bark of *A. cochliacarpos*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentrations of the samples/AI Values%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 μg/ml</td>
</tr>
<tr>
<td>CEE</td>
<td>30.00</td>
</tr>
<tr>
<td>Control BHT</td>
<td>28.66</td>
</tr>
</tbody>
</table>

Figure 5. Wound areas of different groups following 14 days treatment.

Table 3. Mean and standard deviation of the diameter and contractions of the lesions according to the application of the different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Area</th>
<th>Diameter</th>
<th>Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic</td>
<td>692 ±78</td>
<td>30 ± 2a</td>
<td>22 ± 9a</td>
</tr>
<tr>
<td>Aqueous</td>
<td>729 ±103a</td>
<td>30 ± 2a</td>
<td>12 ± 6b</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>697±72c</td>
<td>29 ± 2b</td>
<td>19 ±9c</td>
</tr>
<tr>
<td>Powdered bark</td>
<td>683±85c</td>
<td>30 ± 2b</td>
<td>22 ±10a</td>
</tr>
<tr>
<td>Dakin’s solution</td>
<td>679±88c</td>
<td>29 ± 2b</td>
<td>25 ± 7a</td>
</tr>
<tr>
<td>Saline</td>
<td>710 ±57b</td>
<td>30 ± 1.23b</td>
<td>16 ± 7b</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column do not differ statistically at the 5% probability level by the Duncan or Scott-Knott test.

propylene glycol extracts had the best performances in relation to reducing diameters and areas of induced wounds, with a wound healing activity comparable to Dakin’s solution (p<0.05).

**Histological analysis**

Histological evaluation of wounds on the 14th day revealed significant differences regarding the inflammatory response and deposition of granulation tissue between the aqueous, ethanolic, propylene glycol extracts and powdered bark of *A. cochliacarpos*. The best healing occurred using the bark powder, followed by propylene glycol and ethanolic extracts. Because the treated animals exhibited less ulceration associated with neutrophilic inflammation and a greater organization of connective tissue, as illustrated by Figure 6A, B and C. The water extract and the Dakin fluid were less effective treatments. Wounds treated with these...
Figure 6. (A): Histopathological appearance of the lesion treated with: A. powdered stem bark. Re-epithelialization process. Presence of granulation tissue HE organized. 10 x objective; (B) Propylene glycol extract. Edema and fibrin. Bacterial colony. Ulceration with edema and fibrin. 40 x objective; (C) Propylene glycol extract. Injury with ulceration and inflammatory neutrophilic infiltrate. Irregular re-epithelialization. 40 x lens; (D). Propylene glycol extract. Ulceration associated with neutrophilic infiltration. Eosinophilic inflammation. Granulation tissue. 10x lens; (E). Dakin fluid. Ulcer associated with neutrophilic infiltrate. Granulation tissue with infiltrated mononuclear cells; (F). Powdered stem bark (Granulation tissue). Eosinophilic inflammation. 40 x lens.

substances presented greater inflammatory response, sharp ulceration associated with moderate neutrophilic inflammation, and disordered of connective tissue. These changes are not expected with that intensity on the 14th day. Histological features of the healing provided by these extracts are shown in Figure 6D and E. The presence of mononuclear infiltrates (macrophages and lymphocytes) and granulomas was observed as expected. The inflammatory phase was characterized by the presence of inflammatory cells in scar tissue, especially polymorphonuclear leukocytes (PMN) and macrophages. The wound treated with powdered stem bark accelerated re-epithelialization by the end of 14th day, which indicates the end of the healing process and suggesting greater efficacy of the product. All lesions presented a moderate to severe eosinophilic inflammatory reaction Figure 6F. Probably due to the response to induced tissue injury in the dermis of horses.

DISCUSSION

The DPPH assay is a widely used antioxidant procedure that asserts the ability of a compound to scavenge free radicals (Bendini et al., 2007). Several advantages have contributed to its dissemination as a first choice method of evaluation for antioxidant potential of foods (Floegel et al., 2011), plant extracts (Mensor et al., 2001) or isolated compounds (Villeño et al., 2007) which include its low cost, ease of handling and valid accuracy (Kedare and Singh, 2011).

The extracts obtained herein were able to scavenge the DPPH radical and act as potential antioxidants. This property correlates to the presence of some classes of secondary metabolites in the stem barks of *A. cochliacarpos*. As we have shown by the preliminary phytochemical screening, tannins and flavonoids are present in the stem bark of this species, and may be regarded as responsible for the antioxidant properties of the extracts since they are widely acknowledged as potent radical scavengers (Hatano, 1995; Nanjo et al., 1996; Cai et al., 2006).

Polyphenolic compounds comprise a large class of secondary metabolites featuring more than one phenolic ring in their core structure, which is derived from shikimate or polyketide metabolic pathway (Quideau et al., 2011). Their antioxidant mechanisms have been known for some time and relate to the presence of the phenolic ring, which provides the ability to scavenge free radicals and form itself stable free radicals, by hydrogen-
atom transfer, or cation radicals, via single-electron transfer (Leopodini et al., 2011; Aquino et al., 2017). The stability of the radical formed is explained by the delocalization of the unpaired electron through the framework of phenolic rings existent in the molecular structure of these secondary metabolites (Quideau et al., 2011; Leopodini et al., 2010).

Wound healing is a complex and stepwise process that comprises different but complementary biochemical and cellular events in order to provide anatomical reconstruction and physiological regeneration of damaged tissue in most cases (Broughton et al., 2006). We believe in the viewpoint that describes the wound healing as a four-stage process: coagulation, inflammation, proliferation and remodeling. The first stage, coagulation, takes place immediately after injury and involves the formation of blood clot to avoid excessively bleeding. Secondly, recruitment of inflammatory cells ensues due to the release of chemoattractant substances so that bacterial dissemination is prevented and removal of damaged tissue is carried out. At the same time, proliferation occurs and includes re-epithelialization and angiogenesis which altogether leads to wound contraction. Finally, this is followed by remodelling of the extracellular matrix and deposition of collagen to restore tensile strength of the newly formed tissue (Velnar et al., 2009).

Wound contraction involves a reduction in the size of the wound from the day of operation until complete epithelialization occurs. The qualitative histological criteria analysed in order to assay the degree of wound healing comprise the presence of inflammatory cells and necrotic tissue, the extent of epithelialization and angiogenesis, and the organization of connective tissue (Thakur et al., 2011). The results in Table 3 confirm increased rate of wound contraction. These observations are further supported by histopathological evidences that confirmed enhanced epithelialization by ethanolic and propylene glycol extracts, as well as the powdered stem bark, when compared to the control.

The inflammatory stage features the infiltration of mononuclear and polymonuclear leukocytes at the skin continuity solution in order to provide defense against micro-organisms and to promote debridement of necrotic tissue (Koh et al., 2011). Even though inflammation is indispensable to wound healing, the migration of neutrophils to the wound site has marked influence in increasing the levels of Reactive Oxygen Species (ROS) locally. This can induce tissue damage to healthy cells by destroying their membranes through lipid peroxidation or by causing damage to essential macromolecules such as proteins and nucleic acids. This ultimately might delay or impair wound healing (Schäfer and Werner, 2008).

There are reports of the use of *A. cochliacarpos* to alleviate inflammatory diseases, through studies using experimental models in vitro and in vivo (Sánchez-Fidalgo et al., 2013). Furthermore, the antiulcer effect of the aqueous extract of stem bark of *A. cochliacarpos* was tested in experimental alcohol gastric ulcers and had efficacy in promoting the healing of colonic lesions and reducing neutrophil infiltration, which may be related to the decrease of proinflammatory cytokines and down-regulation of inflammatory COX-2 (cyclooxygenase-2) and iNOS proteins (inducible Nitric Oxide Synthase), as well as JNK activation (c-Jun N-terminal Kinase) (Da Silva et al., 2010).

In addition, *A. cochliacarpos* has pharmacological potential against Gram-positive bacteria, mainly of the genus *Staphylococcus*, and can be exploited in the future to obtain bioactive compounds with antibacterial action (Tenório et al., 2016). The hydroethanolic extract from stem barks of *A. cochliacarpos* for the treatment of rats with induced burns and infected with strains of *S. aureus* reported that the animals showed complete healing in 21 days (Soares et al., 2013).

The relevance of the antioxidant activity in wound healing is well described (Süntar et al., 2012). Since phenolic compounds such as flavonoids and tannins are present in *A. cochliacarpos* extracts, and as these agents influence one or more phases of healing process, the wound healing activity might be related to these compounds. Several plant species from the Brazilian Caatinga ecoregion are known for their high total phenolic content, and hence are used for medicinal purposes by traditional communities to treat inflammatory conditions and for healing wounds (Oliveira et al., 2013). The phytochemistry of *A. cochliacarpos* was investigated in more detail by nuclear magnetic resonance studies that pointed to the presence of proanthocyanidins, mainly catechins (Da Silva et al., 2010).

Polyphenols are able to prevent lipid peroxidation directly by scavenging free radicals or by delaying the onset of cell necrosis and improving vascularity, which contributes to increase collagen viability, reduce cell damage and promote DNA synthesis (Getie et al., 2002). Moreover, tannins are known for promoting capillary vasoconstriction, which decrease vascular permeability and cause a local anti-inflammatory effect in addition to their action as scavengers of reactive species, which greatly contributes to their antioxidant potential and hence favours the healing (Lopes et al., 2005). As radicals can damage cell structures including membrane lipids, proteins, enzymes, and nucleic acids, the antioxidant role of tannins might be one of the most important components of wound healing (Edwin et al., 2008).

The solubility of polyphenols is determined by the chemical nature of the plant sample, as well as the polarity of the solvents used for extraction procedure. In addition, other factors such as the ratio between solid and solvent, and the particle size of the sample may influence the content of polyphenols (Dai and Mumper,
Ethanol extracts of barks usually present a higher content of polyphenols in their composition when compared to aqueous extracts of the same plant parts, as shown by several investigations (Igbinosa et al., 2011; Olajuyigbe and Afolayan, 2011; Iloki-Assanga et al., 2015; Pawar and Dasgupta, 2016). Since the wound healing activity of A. cochliacarpos might be connected to the antioxidant activity of its chemical constituents, this may partially explain the lower efficacy of the aqueous extract.

**Conclusion**

The crude ethanolic extract and the ethyl acetate fractions of the A. cochliacarpos showed antioxidant action in quantitative and qualitative DPPH and TBA assays. After partition, the ethyl acetate fraction was particularly distinctive, with 95.45% of antioxidant activity occurring in a concentration of 50 µg/ml for DPPH. These results are likely due to the presence of flavonoids, as well as hydrolysable and condensed tannins, indicated by the chromatographic profile and phytochemical screening. The ethyl acetate fraction also presented the highest content of phenolic compounds: 0.010 mg of Gallic acid/g of the sample.

This study confirmed microscopically and macroscopically that the healing process in horses was mediated by the use of A. cochliacarpos ethanolic and propylene glycol extracts, as well as the powdered stem bark, being the latter the best in terms of decrease in diameter of wounds and wound contraction. Thus the enhanced ability of wound healing in equines with the extracts could be explained on the basis of antioxidant effects of the chemical constituents of A. cochliacarpos and the in vivo model in equines showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced re-epithelialization.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


Kedare SB, Singh RP (2011). Genesis and development of DPPH...


Acute toxicity evaluation of ethanolic extract of the air parts of *Sida rhombifolia* L., in wistar rats

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*Sida rhombifolia* L., popularly known in Brazil as “SIDA” or “mata-pasto”, is considered a weed; a plant of the American continent and widely distributed in North Africa, belongs to the Malvaceae family. In Brazil, *S. rhombifolia* L. is scattered throughout the national territory, infesting agricultural crops. Certain species of the genus Sida, including *S. rhombifolia*, are widely used in Indian, Chinese, African and American medicine. The present study was carried out with the objective of evaluating the non-clinical acute toxicity of crude ethanolic extract (CEE) obtained from *S. rhombifolia* L. In treated males, there was a statistically significant reduction in water and feed intake. Biochemical analyzes showed statistically significant changes in the parameters of aspartate aminotransferase, alanine aminotransferase and creatinine; hematological parameters showed altered erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin and eosinophil parameters; observed only in treated male animals. The animals' organs showed no significant changes. The results suggest that the ethanolic extract obtained from *S. rhombifolia* L. presents low acute dose toxicity. However, chronic toxicological studies should be performed to demonstrate the safety of long-term use of the drug.

**Key words:** *Sida rhombifolia* L., acute non-clinical toxicity, hematological parameters, biochemical analyses, histopathological parameters.

INTRODUCTION

From antiquity, medicinal plants have been the most important and best known therapeutic resource; their usage represents a characteristic link with the human species (Almeida et al., 2008). At present, due to the
immense biological diversity of flora on the planet and the apparent shortage of new drugs proceeding from this same diversity, there is a growing interest in natural product research, which could uncover new treatments for various diseases. Thus, phytotherapy in popular medicine, the seeking of new products with therapeutic properties based on ethno-pharmacological studies has grown (Elisabetksy, 2001; Maciel et al., 2002; Butler, 2004; Milição et al., 2012).

According to the World Health Organization (2011), from 70 to 95% of underdeveloped country populations depend on medicinal plants as their only form of disease treatment. This is because of the high cost of synthetic drugs. Most natural products come from popular culture, in the form of infusions, decoctions, tinctures and alcoholic solutions obtained from artisanal techniques, without having proven pharmacological properties, at least through non-clinical studies. This confirms the need to carry out toxicological and pharmacological studies aiming to transform such natural products into safe, effective and quality drugs (Veiga and Pinto, 2005; Franca et al., 2008; WHO, 2011).

*Sida rhombifolia* is a botanical genus inserted in the *Malvaceae* family, belonging to the order Malvales which contains 243 genera and 4225 species (Stevens, 2003), which present as sub-shrubs, shrubs and rarely as trees (Baracho, 1998). Species of this family are greatly distributed around the world, being found predominantly in tropical regions, and especially in South America (Heywood, 1993). In Brazil, it is scattered throughout the national territory, infesting agricultural crops. According to Fleck et al. (2003) *S. rhombifolia* L. is the most widespread species of *Sida* in the country. *S. rhombifolia* L. is popularly known in Brazil as “matapasto”, “guanxuma”, and “relógio”. Certain species of the genus *Sida*, including *S. rhombifolia* are widely used in Indian, Chinese, African and American medicines. Differing types of extracts and components isolated from these plants have demonstrated antimicrobial, anti-inflammatory, analgesic, anti-ulcerogenic, hypotensive, antioxidant and anti-diabetic activities, confirming the folk lore and beliefs about the species (Ajithabai et al., 2012; Pradhan et al., 2013; Galal et al., 2015).

Studies have reported isolated and identified phytochemicals from aerial parts of *S. rhombifolia* L. using chromatographic and spectroscopic methods. The study led to the isolation of the scopoletin, escoporone, ethoxy-ferulate, kaempferol, kaempferol-3-O-D-glycosyl-6-O-D-rhamnose, quindolinone, 11-methoxy- quindoline, quindoline and the salt of cryptolepine.

In addition, quindolinone and the salt of cryptolepin induced vasorelaxation dependent on the vascular endothelium, justifying the use of the species in folk medicine in India (Chaves., et al 2017). Based on the search for new pharmacologically active and safe agents, having several phytochemical constituents isolated from the aerial parts of *S. rhombifolia* L. (Chaves., Et al. 2017), this study evaluated the toxicity of the crude extract of *S. rhombifolia* which used non-clinical tests following the recommendations of the National Agency of Sanitary Surveillance (ANVISA).

**MATERIALS AND METHODS**

**Plant collection**

Aerial parts of *S. rhombifolia* L. (*Malvaceae*) were collected in the municipality of Santa Rita-Paraiba and botanical identification was performed by Dr Maria de Fátima Agra of Federal University of Paraiba. The exsiccate material is filed at the Prof. Lauro Pires Xavier Herbarium of Federal University of Paraiba under No. Agra 7045.

**Preparation of *S. rhombifolia* L. crude ethanolic extract**

The crude ethanolic extract was prepared by the staff at the Phytochemical Laboratory of Professor Dr. Maria de Fátima Vanderlei. Aerial parts of *S. rhombifolia* L. were dehydrated in an oven with circulating air at an average temperature of 40°C for 96 h. They were then ground in a mechanical mill, obtaining approximately 5.5 kg of powder. It was macerated in 95% ethanol (EtOH) for 72 h for extraction of the organic constituents. The extractive solution was concentrated in an evaporator at 40°C, providing approximately 570.0 g of crude ethanolic extract (CEE).

**Experimental animals**

24 *Wistar* rats were used, albinos, adults, male and female (nulliparous and non-pregnant), weighing between 180 and 220 g, as provided by the Prof. Thomas George bioterium of Research Institute for Drugs and Medicines of Federal University of Paraiba (IPeFarM/UFPB). The experimental protocol was approved by the Ethics in Animal Experimentation (CEUA) of Federal University of Paraiba (UFPB) , (process No. 029/2015). All were grouped in polyethylene cages, containing six animals each, and maintained under controlled conditions at a temperature of 21 ± 2°C, without any medications, and having free access to food (pellets) and water.

**Acute toxicological testing**

The parameters evaluated for acute toxicological tests were based on ANVISA Resolution RE 90/2004 (Brazil, 2004), using Wistar rats of both sexes. The rats were divided into two groups: control and treated. Each group consisted of 12 animals, 6 males and 6 females. The control group was distributed in two boxes that

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separated the animals by sex and the animals received water by gavage. The treated groups were equally distributed and received the dose of crude ethanolic extract (EEC) at 2000 mg / kg body weight (bW). After the administration of EEC, the observation of behavioral parameters with pharmacological screening was performed at intervals of: 30, 60, 120, 180 and 240 min, according to the experimental protocol developed, as previously described (Almeida et al., 1999). After 14 days of experimentation, the animals were by sacrificed administration of excess anesthetic (anesthesia of 80 mg / kg of xylazine and 5 mg / kg of ketamine), following the recommendations of the scientific community. Blood was withdrawn for laboratory analysis of hematological and biochemical parameters.

**Laboratory analysis of the blood**

Collection of the samples was carried out by bleeding the brachial plexus. The blood was collected in tubes with the anticoagulant ethylenediamine tetraacetic acid (EDTA) for determination of hematological parameters, and in tubes with separator gel - Microtainer®BectonDickson® - which were centrifuged for 10 min at 2026 g of force, to obtain serum for determination of biochemical parameters. The hematological analyses consisted in the study of the red cell series (erythrogram), white cell (WBC), and the platelet count. The erythrogram included the erythrocyte count, hematocrit, mean corpuscular volume (MCV), hemoglobin, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The WBC included a global leukocytes and cell differentiation counts. The biochemical analyses were performed for the serum samples. The total cholesterol, urea, glucose, triglycerides, alkaline phosphatase (ALP), albumin, globulin, and transaminases; Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT), uric acid, creatinine, total protein, calcium ions and magnesium were analyzed in an automated biochemical apparatus ChemWell-T®.

**Anatomy-pathological examination**

The organs of the animals (livers and kidneys) were sectioned and immersed in a fixative solution. After 12 h of fixation, samples for histopathological processing were obtained by inclusion in paraffin and stained with hematoxylin and eosin.

**Statistical analysis**

For statistical analysis of the results, we used the Mann-Whitney and test "t" un-paired, using the software GraphPadPrism® 6.0. The results were considered significant for p values < 0.05.

**RESULTS**

**Behavioral evaluation and lethality**

In the evaluation of behavioral changes after administration of the oral dose of the crude ethanolic extract (S. rhombifolia L.) at the dose of 2000 mg/kg body weight (bW), no motor and / or sensorial deficiencies were observed, nor did the dose tested cause no deaths in the animals within 14 days.

**Weight evolution**

Compared to their respective control groups, there was no statistically significant change in weight evolution of the male or female rats treated with S. rhombifolia L. of crude ethanolic extract (CEE) at an oral dose of 2000 mg/kg body weight (bW). The results are seen in Table 1.

**Water and food consumption**

The ingestion of water and feed was measured daily during the acute treatment with the substance. In the treated males, a statistically significant decrease in the consumption of water and ration was observed. On the other hand, the females did not show any changes in either parameter. The results are presented in Table 2.

**Biochemical parameters**

Biochemical findings were obtained from animal serum analyzes after the 14-day experimental period (Table 3). The animals treated (male) presented higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine.

**Hematological parameters**

The hematological alterations obtained from the plasma analyses of the animals after the 14-day experimental period are described in Table 4. For the males treated with S. rhombifolia L. of crude ethanolic extract CEE at an oral dose of 2000 mg/kg body weight (bW), there were significant differences between the control and treated groups for erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and eosinophils at 2000 mg/kg bw.

**Anatomy pathological study**

Macroscopically, the organs did not present significant anatomical changes (Figure 1).

**DISCUSSION**

During the study, and after the administration of S. rhombifolia L. CEE at an oral dose of 2000 mg/kg body weight (bW), no sign of severe toxicity or death of animals was detected during the 14 days of evaluation, which corroborates previous studies conducted by Sireretawong et al. (2008). In the behavioral screening assessment, the first four hours after administration of the
Table 1. Weight evolution of Wistar rats, male and female, after 14 days of administration of *S. rhombifolia*.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control</th>
<th>Treated (2000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males 1º</td>
<td>43.92 ± 29.09</td>
<td>22.38 ± 6.80</td>
</tr>
<tr>
<td>Males 2º</td>
<td>22.45 ± 11.23</td>
<td>11.42 ± 3.10</td>
</tr>
<tr>
<td>Females 1º</td>
<td>6.28 ± 6.70</td>
<td>4.27 ± 6.19</td>
</tr>
<tr>
<td>Females 2º</td>
<td>2.30 ± 4.10</td>
<td>5.25 ± 2.43</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney.*p< 0.05.

Table 2. Water consumption and ration of male and female Wistar rats after administration of *S. rhombifolia* L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Treated (2000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water consumption (ml)</td>
<td>259.8±15.10</td>
<td>221.0±27.68***</td>
</tr>
<tr>
<td>Ration consumption (g)</td>
<td>151.3±3.80</td>
<td>131.5±13.05***</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water consumption (ml)</td>
<td>192.8±27.94</td>
<td>184.9±23.08</td>
</tr>
<tr>
<td>Ration consumption (g)</td>
<td>100.7±10.43</td>
<td>107.8±7.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney.*p<0.05, **p< 0.01, ****p<0.001.

Table 3. Biochemical parameters obtained from the serum of rats treated with *S. rhombifolia* L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Male Treated (2000 mg/kg)</th>
<th>Control</th>
<th>Female Treated (2000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/l)</td>
<td>6.20±0.83</td>
<td>6.83±1.25</td>
<td>6.49±0.68</td>
<td>7.60±0.72</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>59.40±14.19</td>
<td>124.70±50.64*</td>
<td>65.80±23.04</td>
<td>63.50±13.43</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>162.80±62.15</td>
<td>337.7±192.80*</td>
<td>185.60±84.50</td>
<td>178.30±42.04</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>339.2±114.1</td>
<td>346.0±19.30</td>
<td>176.0±58.15</td>
<td>189.20±31.45</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.47±0.91</td>
<td>4.10±1.34</td>
<td>3.48±0.76</td>
<td>4.22±0.41</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>66.00±8.28</td>
<td>74.83±15.69</td>
<td>66.40±9.55</td>
<td>80.50±15.54</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>125.125.00±33.59</td>
<td>100.30±36.15</td>
<td>98.25±26.83</td>
<td>155.30±49.83</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>12.89±2.91</td>
<td>11.89±1.38</td>
<td>10.15±1.14</td>
<td>11.14±1.78</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>0.99±0.33</td>
<td>0.97±0.10</td>
<td>1.19±0.48</td>
<td>1.24±0.27</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>60.00±1.87</td>
<td>72.00±13.37</td>
<td>59.33±8.08</td>
<td>70.00±9.50</td>
</tr>
<tr>
<td>Serum-creatinine (μmol/L)</td>
<td>0.30±0.06</td>
<td>0.43±0.04*</td>
<td>0.42±0.09</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>2.73±0.28</td>
<td>2.74±0.15</td>
<td>3.04±0.17</td>
<td>3.37±0.36</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>102.6±12.48</td>
<td>112.5±15.68</td>
<td>89.20±18.74</td>
<td>107.8±12.91</td>
</tr>
</tbody>
</table>

ALT, Alanine Amino Transferase; AST: Aspartate Amino Transferase; ALP: Alkaline phosphatase. Values are expressed as mean ± S.D. (n=6).*"t" test Mann-Whitney.*p<0.05.

CEE no changes at the level of the Central Nervous System (CNS) or (Autonomic Nervous System (ANS) were detected, indicating that the plant has no activity on these systems.

Analyzing any possible toxic effects, the body weight gains of the animals and consumption of water and ration were observed and are shown in Tables 1 and 2, respectively. A significant decrease in the consumption of water and rations of treated males was detected, which may be associated with general discomfort, leading to a decrease in feeding of the treated rats, as suggested previously by Adeneye and Agbaje (2008), or it may be
Table 4. Hematological parameters obtained from the plasma of rats treated with *S. rhombifolia* L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (2000 mg/kg)</td>
<td>Control (2000 mg/kg)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>16.32 ± 0.62</td>
<td>16.33 ± 0.71</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.58 ± 1.17</td>
<td>38.76 ± 1.28</td>
</tr>
<tr>
<td>MCV (μm³)</td>
<td>51.04 ± 3.17</td>
<td>43.28 ± 0.71**</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.06 ± 1.38</td>
<td>17.65 ± 0.34**</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>41.24 ± 0.72</td>
<td>40.78 ± 0.44</td>
</tr>
<tr>
<td>Leukocytes (10³/mm³)</td>
<td>8.62 ± 3.43</td>
<td>5.15 ± 2.17</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>26.40 ± 4.40</td>
<td>21.20 ± 4.55</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.00 ± 0.00</td>
<td>0.00 ± 0.00**</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>66.80 ± 7.80</td>
<td>76.17 ± 8.28</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>4.40 ± 3.21</td>
<td>4.83 ± 2.56</td>
</tr>
<tr>
<td>Platelets (10³/mm³)</td>
<td>900.80 ± 143.10</td>
<td>671.50 ± 173.96</td>
</tr>
</tbody>
</table>

MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration. Values are expressed as mean ± S.D. (n=6). "t" test Mann-Whitney *p < 0.05 **p < 0.01.

Figure 1. Histopathology of liver (A) and kidney (B) organs of male and female rats treated with the crude ethanolic extract of *S. rhombifolia* L. at an oral dose of 2000 mg / kg. None of the animal organs presented histological peculiarities (liver and kidneys). Hepatic tissue and space-door (black arrow) without particularities (Female liver - A). Liver tissue and space-door (black arrow) without particularities (Male liver - B). Renal tubules and glomeruli (black arrow) without particularities (female kidneys - C). Renal tubules and glomerulus (black arrow) without particularities (male kidney - D). H & E 200x.
that *S. rhombifolia* L., interferes directly in the lipid metabolism of treated animals, which leads to a decrease in the body weight of these animals. However, the decrease was not statistically significant, indicating that CEE has low toxicity; since in general changes behavior and weight gain are critical parameters for assessment of effects of a compound on animals; such changes are often the first signs of toxicity and indicative of adverse drug effects (Auletta, 1995; Teo et al., 2002; El-Sanusi and El-Adam, 2007).

During biochemical parameter analyses we observed an increase in the levels of ALT and AST for the treated males, whereas in females, there were no statistically significant changes. The liver is one of the most important organs in the body, being responsible for the metabolism and detoxification of all toxins that enter the body. Liver function may be evaluated through blood tests to provide information about the status of the liver and cellular integrity. Certain enzymes and proteins can be used as indicators of liver problems, such as ALT, AST, gamma-glutamyl transferase and bilirubin (Brandt et al., 2009). Certain drugs and medications are known to induce lipid peroxidation, causing swelling and necrosis of the liver cells, which results in the release of cytosolic enzymes, such as ALT, AST and ALP (Agbor et al., 2005). Thus, increases of ALT and AST in plasma may be indicative of hepatic lesions.

ALT is considered the most sensitive parameter for the liver, in cases of liver damage this enzyme leaks into the bloodstream. As an example of drugs that have high hepatotoxicity and cause changes in the levels of ALT and AST, stanozolol and acetaminophen are highlighted, yet they are routinely used (Basu et al., 2009; Mosallanejad et al., 2011). The increase in AST and ALT caused by the administration of CEE for *S. rhombifolia* L. indicates that the plant presents some signs of hepatotoxicity; as support of Ouédraogo et al. (2013), with similar results. When we observed the values obtained, there was a significant increase in creatinine levels for treated rats compared to the control group. However, this result has no clinical significance, since it is within the reference values (Giknis and Clifford, 2006; Castello Branco et al., 2011). Regarding the values obtained from the treated females, we did not obtain significant alterations of this group.

Blood parameter analysis is important for risk assessments of certain substances when administered to humans; the hematological system has great value to predict the first signs of toxicity. The hematopoietic system is very susceptible to toxic substances; an important system for analyzing physical health, and to evidence pathology in humans and animals (Olson et al., 2000; Li et al., 2010). Few statistically significant differences were found among the majority of hematological parameters between the control and treated groups. However, a significant decrease between the controls and the treated groups for the parameters of erythrocytes, MCV, MCH, and eosinophils for the males treated was observed. In the females, no parameter suffered statistically significant alteration, indicating that *S. rhombifolia* L. CEE presented low toxicity relative to the hematological system (Konaté et al., 2012).

The increase in the erythrocyte values of the male rats may be related to sex, because the erythrocyte number varied and males obtain higher values than those of women. Another factor that controls the emission of erythrocytes in the blood is the level of oxygenation of the tissues, in conditions of low oxygen pressure, during oxygen depletion erythropoiesis stimulation occurs (Lorenzi, 2006). The mean corpuscular volume (MCV) is considered one of the main criteria for the classification of anemic disorders, however, the decline in the MCV of the males cannot be considered as indicative of anemia, since the value was still close to reference and the other parameters that may indicate an anemia were not significantly altered (Bessman et al., 1983). The changes in the values of MCH and for eosinophils, despite being statistically significant do not have clinical relevance, since the values were close to reference. Such differences can be explained by biological variability among rats (Lewis et al., 2002; Giknis and Clifford, 2006; Castello Branco et al., 2011).

Qualitative macroscopic analyzes revealed that the dose tested did not produce changes in the vital organs of the treated animals and no changes suggestive of toxic effects were observed in the histopathological analyzes. These results are in agreement with the data obtained in the biochemical analyzes.

**Conclusion**

After acute treatment at oral dose of 2000 mg / kg body weight (bW) in male and female rats with crude ethanolic extract of *S. rhombifolia* L. (CEE), it was observed that the plant had no activity at the levels of the CNS or ANS, and has little influence on animal feeding, leading to only small weight losses. Regarding the toxicity, evaluated by biochemical and hematological parameters, it was observed that CEE has low toxicity. Few parameters showed significant changes. This justifies the extensive popular use found in the Brazilian northeast and allows a more comprehensive evaluation being necessary to evaluate the potential toxicity of this plant species when used chronically.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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