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Full Length Research Paper

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

Luciana da Silva Nunes Ramalho[1*], Gabriela Tafaela Dias[2], Edla Julinda Ribeiro Coutinho Espinola Guedes[1], Micaelly da Silva Oliveira[1], Andressa Brito Lira[2], Kardilândia Mendes de Oliveira[1], Otemberg Souza Chaves[2], Josué do Amaral Ramalho[1], Alexandre Rolim da Paz [3], Janine Agra Padilha[2], Hilzeth de Luna Freire Pessoa[4], Maria de Fátima Vanderlei de Souza[6], Caliandra Maria Bezerra Luna Lima[5] and Margareth de Fátima Formiga Melo Diniz[6]

[1]PostGraduate Program in Technology Development and Innovation in Medicines, Health Sciences Center, Federal University of Paraiba, 58051-900 Paraiba, Brazil.
[2]PostGraduate Program in Natural and Synthetic Bioactive Products, Health Sciences Center, Federal University of Paraiba, 58051-900 Paraiba, Brazil.
[3]Department of Medical Sciences, Health Sciences Center, University of Paraiba, 58051-900 Paraiba, Brazil.
[4]Department of Biological Sciences, Health Sciences Center, University of Paraiba, 58051-900 Paraiba, Brazil.
[5]Department of Physiology and Pathology, Health Sciences Center, University of Paraiba, 58051-900 Paraiba, Brazil.
[6]Department of Pharmaceutical Sciences, Health Sciences Center, University of Paraiba, 58051-900 Paraiba, Brazil.

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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 (Elisabetsky, 2001; Maciel et al., 2002; Butler, 2004; Militão 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-60-α 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* L. 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

*Corresponding author. E-mail: luciananramalho@yahoo.com; Tel. +55083987758018.

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 - MicrotainerBectonDickson® - 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 weigt (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 Sireeratawong 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*.

Weeks	Control	Treated (2000 mg/kg)
Males 1º Week	43.92± 29.09	22.38± 6.80
Males 2º Week	22.45± 11.23	11.42± 3.10
Females 1º Week	6.28±6.70	4.27±6.19
Females 2º Week	2.30± 4.10	5.25 ± 2.43

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.

Variable	Control	Treated (2000 mg/kg)
Males		
Water consumption (ml)	259.8±15.10	221.0±27.68***
Ration consumption (g)	151.3± 3.80	131.5±13.05***
Females		
Water consumption (ml)	192.8±27.94	184.9± 23.08
Ration consumption (g)	100.7± 10.43	107.8± 7.01

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.

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

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.

Variable	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg)
Hemoglobin (g/dl)	16.32 ± 0.62	16.33 ± 0.71	16.20 ± 0.25	16.30 ± 0.54
Hematocrit (%)	39.58 ± 1.17	40.05 ± 1.63	38.76 ± 1.28	39.38 ± 1.43
MCV (μm^3)	51.04 ± 3.17	43.28 ± 0.71**	50.80 ± 2.02	50.75 ± 2.45
MCH (pg)	21.06 ± 1.38	17.65 ± 0.34**	21.24 ± 0.78	21.03 ± 1.084
MCHC (g/dl)	41.24 ± 0.72	40.78 ± 0.44	41.82 ± 0.83	41.40 ± 0.90
Leukocytes (10^3 /mm^3)	8.62 ± 3.43	5.15 ± 2.17	5.40 ± 2.07	6.08 ± 1.69
Neutrophils (%)	26.40 ± 4.40	21.20 ± 4.55	24.20 ± 5.54	28.33± 3.77
Eosinophils (%)	1.00 ± 0.00	0.00 ± 0.00**	2.00 ± 2.34	1.50 ± 0.84
Lymphocytes (%)	66.80 ± 7.80	76.17 ± 8.28	69.00 ± 6.28	67.67± 4.08
Monocytes (%)	4.40 ± 3.21	4.83 ± 2.56	4.00± 0.70	2.50 ± 1.22
Platelets (10^3/mm^3)	900.80±143.10	671.50 ± 173.96	674.60 ± 193.46	769.83± 217.62

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.

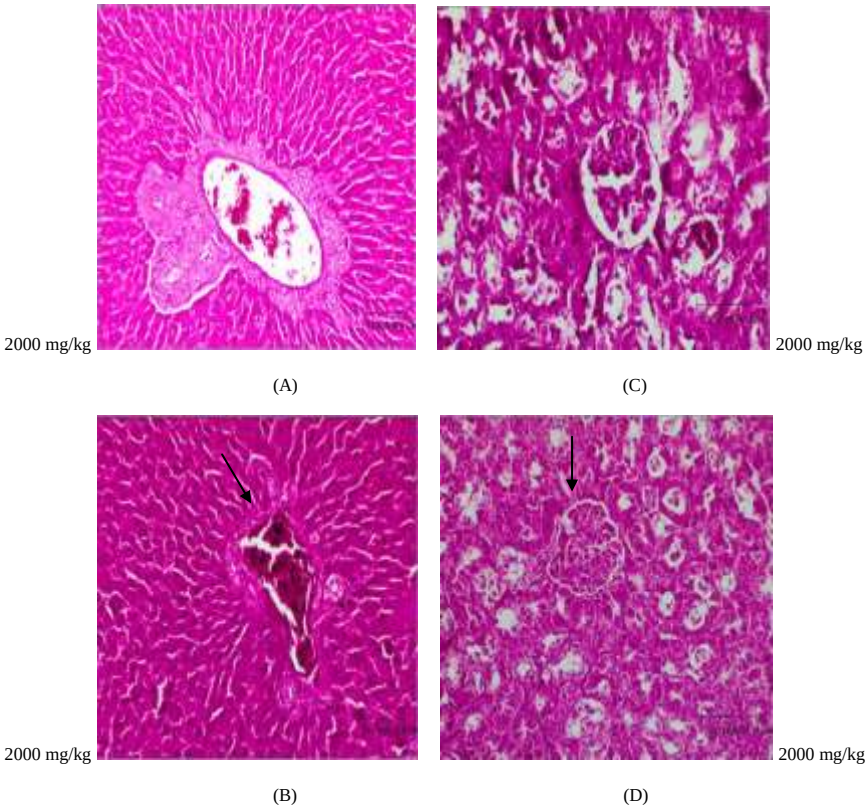

Figure 1. Histopathology of liver (A) and kidney (B) organs of male and female rats treated with the crude ethanolic extract of *S. rhombifolia* L. at an oral dose of 2000 mg / kg . None of the animal organs presented histological peculiarities (liver and kidneys). Hepatic tissue and space-door (black 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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REFERENCES

Adeneye AA, Agbaje EO (2008). Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves ethanol extract of Morinda Lucida Benth. in normal and alloxan-induced diabetic rats. African Journal of Biomedical Research 11(1).

Agbor GA, Oben JE, Nkegoum B, Takala JP, Nigogang JY (2005). Hepatoprotective activity of Hibiscus cannabinus (Linn.) against carbon tetrachloride and paracetamol induced liver damage in rats. Pakistan Journal of Biological Sciences 8:1397-1401.

Ajithabai MD, Rani S, Jayakumar G (2012). Review on the species of Sida used for the preparation of nayopayam kashayam. International Journal of Pharmaceutical Sciences and Research 2:173-195.

Almeida RN, Falcão ACGM, Diniz RST, Quintans-Júnior LJ, Polari RM, Barbosa-Filho, JM, Agra MF, Duarte JC, Ferreira CD, Antoniolli AR, Araújo CC (1999) Metodologia para avaliação de plantas com atividade no Sistema Nervoso Central e alguns dados experimentais. Revista Brasileira de Farmácia 80:72-76.

Almeida RBA, Carretto CFP, Santana RS, Furlan MR, Junqueira JC Jorge AOC (2008). Antimicrobial activity of Cymbopogon citratus (DC.) Staf on Candida spp. Journal of Dentistry, UNESP 37:147-153.

Auletta CS (1995). Acute, subchronic and chronic toxicology. CRC Press: London.

Baracho GS (1998). de. Taxonomy of the genus Sida L. section cordifolia (DC.) Fryxell (Malvaceae) in Brazil. Dissertation (Master in Botany), Recife-PE.

Basu SK, Rupeshkumar M, Kavitha K (2009) .Hepatoprotective and antioxidant effect of Andrographis echioides N. against acetaminophen induced hepatotoxicity in rats. Journal of Biological Sciences 9:351-356.

Bessman JD, Gilmer PR, Gardner FH (1983). Improved classification of anemias by MCV and RDW. American Journal of Clinical Pathology 80:322-326.

Brandt AP , Oliveira LFS, Fernandes FB, Alba J (2009) . Evaluation of prospective hypocholesterolemic effect and preliminar toxicology of crude extract and decoction from Vitex megapotamica (Spreng) Moldenke (V. Montevidensis Cham.) in vivo. Revista Brasileira de Farmacognosia 19(2):388-393.

Brasil Resolução-RE nº90, de 16 de março de (2004). Guia para a realização de estudos de toxicidade pré-clinica.

Butler MS (2004). The role of natural product chemistry in drug discovery. The role of natural product chemistry in drug discovery. Journal of Natural Products 67(12):2141-2153.

Castello BACS, Diniz MFFM , Almeida RN ,Santos HB , Oliveira KM , Ramalho JA, Dantas JD (2011). Biochemical and hematological parameters of Wistar rats and Swiss mice of the Vivarium Professor Thomas George. Brazilian Journal of Health Sciences 15:209-214.

Chaves OS, Teles YCF, Monteiro MMO, Junior LGM , Agra MF, Braga VA ,Silva TMS, Souza MFV (2017). Alkaloids and Phenolic Compounds from *Sida rhombifolia* L. (Malvaceae) and Vasorelaxant Activity of Two Indoquinoline Alkaloids. Molecules 22:94.

Elisabetsky E (2001).Ethnopharmacology how to do research on active substances. In: SIMÕES CMO, Schenkel EP, Gosman G, Mello JC, MENTZ LA, Petrovick PR (eds). Farmacognosia: da planta ao medicamento. 3.ed. Porto Alegre: UFSC. p. 91-104.

El-sanusi NI, El-adam S (2007). The effect of low levels of dietary Ruta graveolens and Solenostemma argel or their mixture on bovans chicks. Asian Journal of Animal and Veterinary Advances 2:27-31.

Fleck NG, Rizzardi MA, Agostinetto D, Vidal RA (2003). Seed production by black sting and guanxuma as a function of weed density and soybean sowing time. Plant Weed 21:191-202.

França ISX, Souza JA, Baptista RSB, Britto VRS (2008) .Popular medicine: benefits and harms of medicinal plants. Brazilian Journal of Nursing, pp. 201-208.

Galal A, Raman V, Khan IA (2015). Sida cordifolia, a Traditional Herb in Modern Perspective–A Review. Current Traditional Medicine 1:5-17.

Giknis MLA, Clifford CB (2016). Clinical laboratory parameters for Crl:CD (SD) rats. Charles River Laboratories, pp. 1-18.

Heywood VH (1993). Flowering Plants on the World, Ed. B. T. Batsford Ltda., London.

Konaté K, Bassolé IHN, Hilou A, Aworet-Samseny RRR, Souza A, Barro N, Dicko MH, Jacques Y, Datté BM (2012). Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of *Sida acuta Burn* f. and *Sida cordifolia* L. (Malvaceae), medicinal plants of Burkina Faso. BMC Complementary and Alternative Medicine 12:1-11.

Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F (2002). Recognition of adverse and nonadverse effects in toxicity studies. Toxicologic Pathology 30:66-74.

Li X , Luo Y, Wang L, Li Y, Shi Y, Cui Y, Xue M (2010). Acute and subacute toxicity of ethanol extracts from *Salvia przewalskii* Maxim in rodents. Journal of Ethnopharmacology 131:110-115.

Lorenzi TF (2006). Manual of hematology: propaedeutic and clinical. Guanabara Koogan 711 p.

Maciel MAM, Pinto AC, Veiga VF, Grynberg NF, Echevarria A (2002). Plantas medicinais: a necessidade de estudos multidisciplinares. Química Nova 25(3):429-438.

Militão GCG, Dantas INF, Ferreira PMP, Alves APNN, Chaves DC, Monte FJQ. (2012). *In vitro* and *in vivo* anticancer properties of cucurbitacin isolated from Cayaponia racemosa. Pharmaceutical Biology 50:1479-1487.

Mosallanejad B, Avizeh R, Varzi H (2011). Najafzadeh. Successful treatment of stanozolol induced-hepatotoxicity with silymarin in a bitch. Asian Journal of Animal Sciences 5:213-218.

Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B, Heller A (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regulatory Toxicology and Pharmacology 32:56-67.

Ouédraogo M, Zerbo P, Konaté K, Barro N, Sawadogo LL (2013). Effect of Long-term use of *Sida rhombifolia* L. Extract on Haemato-biochemical Parameters of Experimental Animals. British Journal of Pharmacology and Toxicology 4:18-24.

Pradhan DK, Kumar PA, Kanta BR, Shivesh J, Ranjan MM, Ashutosh M, Sanjay C (2013). Ethnomedicinal and therapeutic potential of Sida acuta Burm. f. International Research Journal of Pharmacy 4:88-92.

Sireeratawong S, Nirush L, Umarat S, Amornat T, Anongnad N, Nadthaganya S, Jaijoy K Acute and subchronic toxicity study of the water extract from root of *Sida rhombifolia* Linn. in rats. Songklanakarin Journal of Science and Technology 30:729-737.

Stevens F (2003). Angiosperm Phylogenic, website.Version 4, Mayo 2003. http://www.mobot.org/MOBOT/research/APweb/

Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani VA (2002). A 90-day oral gavage toxicity study of d-methylphenidate and d, l-methylphenidate in Sprague–Dawley rats. Toxicology 179:183-96.

Veiga VFJ, Pinto AC (2005). Maciel, M.A.M. Medicinal plants: safe cure. New Chemistry 28:519-528.

World Health Organization (WHO) (2011). The World Medicines Situation 2011-Traditional Medicines: Global Situation, Issues and Challenges. World Health Organization: Geneva, Switzerland.