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Vol. 10(4), pp. 53-58, 29 January, 2016
DOI: 10.5897/AJPP2015.4476
Article Number: D58E73A56862
ISSN 1996-0816
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http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Preclinical assessment of the acute toxicity and anti-inflammatory activity of *Wissadula periplocifolia* (L.) C. Presl ("jangadeira")

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Received 6 November, 2015; Accepted 10 December, 2015

***Wissadula periplocifolia* (L.) C. Presl is a plant of the Malvaceae family that is popularly known as white velvet leaf ("jangadeira" and "malva-malva" in Brazil). Species of this family are used in folk medicine as diuretic, anti-inflammatory and anti-rheumatic agents. This study evaluated the acute toxicity and anti-inflammatory effects of a crude ethanolic extract of *W. periplocifolia*. Acute toxicity testing revealed reduced food intake and decreased serum creatinine and alkaline phosphatase levels in males, as compared to the control group, suggesting a loss of muscle mass. Assessment of anti-inflammatory activity using a rat paw carrageenan-induced edema model showed that 25 mg/kg of this ethanolic extract significantly reduced edema, although this was not observed at higher doses. These findings indicated that this *W. periplocifolia* extract was non-toxic and had some anti-inflammatory effects. However, a more comprehensive assessment is necessary to evaluate other possible indicators of toxicity, particularly during chronic administration.**

Key words: *Wissadula periplocifolia*, acute toxicity, toxicological test, ethanolic extract, anti-inflammatory activity.

INTRODUCTION

The toxicity of medicinal plants places a burden on public health, because there is no strict control of the marketing of medicinal plants to the general population in Brazil (Veiga-junior et al., 2005). According to the National

System of Toxic-pharmacological Information (SINITOX), 1,185 cases of poisoning caused by plant ingestion were reported in 2012; 796 of these had a confirmed cure and 1 case was fatal.

According to Fryxell (1997), the *Wissadula* genus is composed of 26 species distributed from Texas (in the south of the United States) to Argentina, and also found in other parts of the world. The genus is characterized mainly by constricted carpidia, often with two seeds in the top constriction and one in the bottom constriction. The species chosen for this study, *Wissadula periplocifolia,* is popularly known as “jangadeira” and “malva-malva” in Brazil. In selecting *W. periplocifolia* for this study, the study took into account the fact that various species of the Malvacea family are popularly used in the treatment of various ailments, as emollients, diuretics, and anti-febrile, anti-inflammatory, and anti-bacterial agents (Schultz, 1968).

Two mixtures of steroids were identified in the aerial parts of *W. periplocifolia*: sitosterol/stigmasterol and sitosterol 3-O-β-D-glucopyranoside/stigmasterol 3-O-β-D-glucopyranoside; four derivatives of chlorophyll; and four triterpenes. The presence of steroids led the study to assess possible anti-inflammatory properties, since these are found in other species of the Malvaceae family that present this activity (Teles et al., 2014). These include *Malva sylvestris* (Billerter et al., 1991) widely used in European folk medicine for the treatment of coughs and inflammatory diseases of the mucous membranes and an herb found in Indian swamps, *Sida rhomboidea* Roxb, whose roots and leaves are used as a tonic to cure fevers, heart disease, and all types of inflammation. Pharmacological studies using extracts of this plant indicated their antinociceptive and anti-inflammatory activities (Venkatesh et al., 1999).

Based on the need to find new pharmacologically active and safe agents, and the phytochemical constituents of the aerial parts of this plant (Teles et al., 2014), the present study was conducted to evaluate the toxicity of a crude extract of *W. periplocifolia* using pre-clinical tests, in accordance with the recommendations of the National Health Surveillance Agency (ANVISA), and to assess its anti-inflammatory potential.

MATERIALS AND METHODS

Plant material

W. periplocifolia was collected at Pedra da Boca, municipality of Ararúna - PB, in August 2011. The botanical identification was performed by Prof. Maria de Fatima Agra at the Research Nucleus in Natural Products, Federal University of Paraiba (UFPB). A dried specimen of the material is archived in the Herbarium Prof. Lauro Pires Xavier (UFPB) under number 6498.

Plant material-leaves

The leaves of *W. periplocifolia* was collected at Pedra da Boca, municipality of Ararúna, state of Paraíba, Brazil, in August 2011. The botanical identification was performed by Prof. Maria de Fatima Agra at the Research Nucleus in Natural Products, Federal University of Paraiba (UFPB). A dried specimen of the material is archived in the Herbariumof Prof. Lauro Pires Xavier (UFPB) under number 6498.

Preparation of the extract

Fresh plant material was dried in an oven at 40°C for 72 h. Thereafter, it was ground in a mechanical mill, yielding 8.9 kg of powder; the powder was then subjected to maceration with ethanol for three days. This process was repeated in order to maximize the extraction. The ethanolic extract was concentrated using a rotary evaporator at 50°C, resulting in 705 g of crude ethanol extract (CEE).

Animals

Adult male and female (nulliparous and non-pregnant) Wistar rats (*Rattus novegicus)* weighing between 200 and 300 g, were provided by Professor Thomas George of the Institute for Research on Drugs and Medications (IPeFarM-UFPB). The animals were grouped in polyethylene cages and maintained under controlled temperature conditions of 21 ± 2°C, with a 12 h light-dark cycle, without the use of any medication. The rats had free access to food (ration pellets) and drinking water was available in graduated polyethylene bottles with glass nozzles. The animals were placed in the study environment for at least 30 min prior to the execution of each experiment. The use of animals was approved by the Ethics Committee for Animal Research (CEUA-UFPB) under number 169/2015.

Preclinical acute toxicity testing

The acute toxicity tests were conducted according to the Resolution RE 90/2004 ANVISA (Brasil, 2004), using *Wistar* rats of both sexes. The rats were divided into two groups: a control group and a treated group, with 12 animals in each group. The control animals were administered the extract vehicle (distilled water). The animals in the treated group received a single dose of 2000 mg/kg conjugated equine estrogens (CEE) by oral gavage on day zero of the study. After the administration of CEE, behavioral parameters were observed for signs of pharmacological effects on the central nervous system at 30, 60, 90, 120, 180, and 240 min, as earlier described (Almeida et al., 1999). The intake of water and ration was evaluated daily. After 14 days, the animals were sacrificed by administration of excess anesthetic (80 mg/kg xylazine and 5 mg/kg ketamine), as recommended by the scientific community. Blood was

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Table 1. Water and ration intake by Wistar rats treated with 2000 mg/kg *W. periplocifolia* ethanolic extract.

Groups	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg)
Water intake (mL)	287.1±359.0	268.9±25.9	170.4±19.3	116.4±3.3
Ration feed intake (g)	185.8±15.4	161.6±10.1***	116.4±12.4	114.8±10.7

The values are expressed as mean ± standard deviation (N = 6); *p<0.05 unpaired Student's t test; ***p<0.001 Mann-Whitney test.

removed for laboratory analysis of hematological and biochemical parameters.

Biochemical parameters

Biochemical analyses were carried out using a Cobas Mira Plus® automated biochemical analyzer (Roche) to determine the serum levels of glucose, urea, creatinine, cholesterol, triglycerides, uric acid, gamma-glutamyl transferase (GGT), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, total protein, albumin, globulin, amylase, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and very high-density lipoproteins (VLDL). Calcium and magnesium were assessed using a selective ion Electrolyte Analyzer (AVLAB).

Hematological parameters

The hematological analyses included assessment of the red series (erythrogram), white series (leukogram) and platelet count. For the erythrogram, the erythrocytes were counted, and the hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), the concentration of corpuscular hemoglobin (MCHC) and red cell distribution width (RDW) were determined. The leukogram included a total leukocyte count and a determination of cell differentiation.

Paw edema induced by carrageenan

Anti-inflammatory activity was analyzed according to the methodology described by Winter et al. (1962). Wistar rats were subjected to the following oral treatments: vehicle (0.9% saline solution), the non-steroidal anti-inflammatory standard (indomethacin), and 3 different doses of *W. periplocifolia* CEE (25, 50, and 100 mg/kg, respectively). The volume of the rat paw was immediately measured (corresponding to 1 h). After 60 min, an inflammatory process was induced by the administration of 0.1 µL of 1% carrageenan in the subplantar region of the right hind paw of each animal. The volume of the paw was measured immediately after the carrageenan injection (corresponding to time zero) and at 60 min intervals for 4 h, with the aid of a plethysmometer. The effects of the substances tested were expressed in terms of percentage inhibition. This was calculated according to the equation: Anti-inflammatory activity (%) = [(mean edema volume in the control group - mean edema volume in the test group)/mean edema volume in the control group] × 100, for each time-point.

Statistical analysis

The results of the acute toxicity study were analyzed by GraphPad Prism 6.0 software using Student's t test and the Mann Whitney test for unpaired samples. Differences were considered to be statistically significant when they exhibited p values < 0.05. The carrageenan inflammation data were evaluated by one-way analysis of variance (ANOVA) followed by Turkey's test.

RESULTS

Acute toxicity

Behavioral screen

No motor and/or sensory impairments were observed following one oral dose of 2000 mg/kg CEE, and this dose did not cause the death of any animal within 14 days (data not shown).

Water and food intake

There was a statistically significant decrease in ration food intake by treated males, as compared to the control group and the females (Table 1).

Biochemical parameters

The biochemical analyses showed a statistically significant decrease in serum creatinine and alkaline phosphatase in treated males, as compared to the control group (Table 2).

Hematological parameters

The acute toxicity test of 2000 mg/kg CEE did not reveal any significant changes in hematological parameters (Table 3).

Anti-inflammatory activity

The results presented in Table 4 (as percentage inhibition), indicate that 25 mg/kg CEE produced anti-inflammatory activity that was comparable to that of

Table 2. Biochemical parameters in Wistar rats treated with 2000 mg/kg *W. periplocifolia* ethanolic extract.

Variables	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg)
Glucose (mg/dL)	147.7±10.9	125.5±18.0	128.2±10.4	137.6±19.7
Urea (mg/dL)	40.0±2.9	36.67±4.4	39.6±1.7	41.6±3.4
Creatinine (mg/dL)	0.46±0.09	0.33±0.06*	0.45±0.43	0.49±0.49
Cholesterol (mg/dL)	51.00±10.4	49.80±13.50	58.20±11.50	56.80±9.58
HDL (mg/dL)	21.30±3.10	18.70±3.30	21.60±4.28	22.20±2.50
LDL (mg/dL)	13.06±8.46	31.10±26.14	22.20±8.29	25.40±7.77
VLDL (mg/dL)	16.71±2.98	11.62±5.43	14.40±6.42	9.40±3.36
Triglycerides	83.3±14.24	59.0±27.30	72.00±31.88	46.0±17.35
Uric acid (mg/dL)	0.47±0.15	0.73±0.73	0.58±0.19	0.62±0.23
AST (U/l)	125.9±34.00	135.30±72.00	96.80±26.00	127.20±37.10
ALT (U/l)	52.30±11.54	55.33±10.17	42.60±8.09	47.20±7.76
GGT	14.71±3.00	10.00±4.30	11.40±7.83	12.60±4.10
Alkaline phosphatase (U/l)	381.00±63.90	257.80±84.60*	206.40±146.20	248.60±96.42
Total protein (g/dL)	6.21±0.39	6.08±0.23	6.50±0.10	6.54±0.31
Albumin (g/dL)	2.53±0.14	2.37±0.15	2.69±0.04	2.62±0.26
Globulin (g/dL)	3.68±0.27	3.71±0.21	3.82±0.08	3.92±0.29
Calcium (g/dL)	10.36±0.34	10.37±0.34	10.10±0.21	10.24±0.47
Amylase (g/dL)	1165±81.01	1015±143.7	673±74.60	782.60±102
Magnesium (mg/dL)	2.04±0.12	2.25±0.22	1.92±0.08	2.02±0.13
LDH (mmol/L)	1876±351.00	1731±604.50	1307±605.50	1723±854

The values are expressed as mean ±standard deviation (N = 6); *p<0.05 unpaired student's t test Mann-Whitney.

Table 3. Hematological parameters in Wistar rats treated with 2000 mg/kg *W. periplocifolia* ethanolic extract.

Variable	Male		Female	
	Control	Treated (2000 mg/kg)	Control	Treated (2000 mg/kg)
RBC (10^6/mm^3)	7.88±0.31	8.44±0.99	8.05±0.57	7.18±0.32
Hemoglobin (g/dL)	15.04±0.42	14.69±0.52	14.39±0.87	13.00±0.27
Hematocrit (%)	42.80±1.49	42.5±1.63	41.50±2.20	37.5±0.65
MCV (μ^3)	54.32±1.51	50.85±5.47	51.63±0.87	52.3±1.96
MCH (pg)	19.06±0.38	17.63±2.35	17.93±0.29	18.15±0.61
MCHC (%)	35.12±0.42	34.55±1.03	34.67±0.40	34.70±0.45
RDW	10.27±0.58	10.09±0.23	10.6±0.10	10.63±0.19
Leukocytes (10^3/mm^3)	7.19±0.83	6.05±1.12	3.56±0.62	4.77±2.04
Neutrophils (%)	22.00±1.87	29.50±7.05	30.33±4.51	31.25±4.99
Eosinophils (%)	0.80±0.44	0.00±0.00	0.33±0.57	0.00±0.00
Lymphocytes (%)	72.00±2.35	66.75±6.70	66.67±3.51	65.71±5.31
Monocytes (%)	5.20±2.77	3.75±0.95	2.67±1.15	3.00±1.15
Platelets (10^3/mm^3)	750000±7336	749500±5432	596300±3597	628300±2430

The values are expressed as mean ±standard deviation (N = 6); *p<0.05 unpaired Student's t test Mann-Whitney.

indomethacin, a cyclooxygenase inhibitor, as it reduced edema significantly ($p < 0.05$) throughout the study period.

These findings indicated that CEE contained one or more compounds with anti-inflammatory activity.

DISCUSSION

The present study indicated that the *W. periplocifolia* CEE showed no relevant toxicity in the pharmacological screening at a dose of 2000 mg/kg, did not influence

Table 4. Effect of the oral administration of doses of 25, 50 and 100 mg / kg W. BSE periplocifolia, the formation of paw edema induced by carrageenan in rats.

Time (hours)	Volume paw edema (ml)				
	Control	CEE of *W. periplocifolia* 25 mg/kg	CEE of *W. periplocifolia* 50 mg/kg	CEE of *W. periplocifolia* 100 mg/kg	Indometacin 25 mg/kg
1	1.08±0.17	0.83 ± 0.83	0.79 ± 0.10*	0.93 ± 0.37	0.78±0.48**
2	1.18±0.19	0.87 ± 0.06*	0.95 ± 0.15	0.96 ± 0.27	0.96±0.06**
3	1.25±0.18	0.89 ± 0.07*	0.95 ± 0.12*	0.98 ± 0.05	0.80±0.09**
4	1.16±1.14	0.83 ±0.05**	1.08 ± 0.10	0.94 ± 0.09	0.77±0.09**

The values are expressed in mean One Way ANOVA, followed by the test of Tukey, with *p < 0.05, **p < 0.01, ***p < 0.001.

motor coordination or reflexes, and did not change central or autonomic nervous system activities. No deaths were observed during the 14 days of the study. According to the criteria for the classification of toxicity of the European Community and the FAO/WHO Expert Committee on Food Additives, the non-occurrence of death after oral administration of 2000 mg/kg of a plant product indicates a probable lack of toxicity for the recipient species (Barros and Davino, 2003; Husna et al., 2013). Although this assessment indicated a low toxicity, more detailed and longer term studies are required to evaluate the potential toxicity of this extract.

The administration of CEE only led to a statistically significant decrease in food intake in treated males; this may or may not be due to changes in the physiology of the animal, such as increased stress. The ingestion of water and nutrients are important parameters in safety studies of potential therapeutic products, as they are essential to the proper functioning of the animal's physiological system and, consequently, for an adequate response to the drug tested, since inadequate nutrition may compromise the response (Stevens and Mylecraine, 1994; Husna et al., 2013).

Creatinine is formed as a result of the non-enzymatic degradation of muscle creatine and therefore has a direct relationship with muscle mass. Conditions that reduce creatinine include age, muscle loss, and a diet that is poor in meat (Ravel, 1997; Henry, 2008). Therefore, the reduction in chow consumption by males and the reduced creatinine level observed in this group indicated that loss of muscle mass occurred. Serum alkaline phosphatase activity derives from a range of isoenzymes originating in different organs, with a predominance of renal, bone, and liver fractions. This enzyme is present in virtually all tissues of the body, especially in the membranes of cells in the renal tubules, bone (osteoblasts), placenta, and intestinal tract (Henry, 2008; Sadeghi et al., 2011). Although this activity was reduced in male rats treated with *W. periplocifolia* CEE, the study did not find any specific reason for this unique finding, which occurred in the absence of any significant changes in other liver enzymes. More extensive studies will be required to verify this finding.

There were no changes in the hematological parameters of rats treated with the *W. periplocifolia* CEE. The erythrocyte series was maintained in terms of number and morphology, with no observable anemia, polycythemia, or hemolysis. The leukocyte series was also unchanged in this 14-day study when compared with literatura data (Castello Branco et al., 2011).

The study used the rat paw carrageenan-induced edema model to evaluate the anti-inflammatory activity of *W. periplocifolia* CEE. This provides a well-characterized model of acute inflammation, involving the participation of several inflammatory mediators, and has been widely used in the evaluation of anti-inflammatory activity, including that of other species belonging to the Malvaceae family (Sadeghi et al., 2011).

Other species of the Malvaceae family have chemical constituents that have shown anti-inflammatory activity, for example *Gossypium hirsutum* L. and *Abutilon indicum* (L.) Sweet, which contain terpenoids (Sharma and Ahmad, 1989; Williams et al., 1995; Silva et al., 2006), *Sida galheirensis* (Ulbr.) and *Herissantia tiubae* (K. Schum.) Brizicky, which contain steroids, phenolic acids, flavonoids, and flavonoid glycosides (Nakatani et al., 1986; Silva et al., 2006; Silva et al., 2009) and *Gossypium arboreum* L., which contains flavonoid glycosides (Bakoma et al., 2013)

The results of the present study demonstrated that 25 mg/kg of *W. periplocifolia* CEE produced anti-inflammatory activity comparable to that of indomethacin. However, this effect was not CEE dose-dependent. No significant effects were observed in the other CEE-treated groups, as compared with the control group. This acute preclinical toxicological evaluation of a *W. periplocifolia* CEE did not reveal any toxic effects and indicated some anti-inflammatory activity. The acute toxicological tests conducted at a dose of 2000 mg/kg CEE showed no toxic effects, and no animal death. Although this indicates a probable lack of toxicity in rats (Barros and Davino, 2003; Husna et al., 2013), a more

comprehensive assessment is required to evaluate the potential toxicity of this plant species when used chronically, and to determine its anti-inflammatory potential.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Brazilian agencies: CAPES and CNPq for their financial support.

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