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Hippocratic screening and subchronic oral toxicity assessments of the methanol extract of *Vatairea macrocarpa* heartwood in rodents

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Abstract: *Vatairea macrocarpa* (Benth.) Ducke, Fabaceae, is popularly known as 'angelim'. Its heartwood macerate is used to treat inflammation, gastric ulcer, diabetes and infections. The oral acute and subchronic toxicity of the methanol extract of *V. macrocarpa* heartwood (ME*Vm*) was evaluated. In the Hippocratic screening, a single administration of MEVm was given orally to mice at doses ranging from 100 to 5000 mg/kg. In the subchronic study, ME*Vm* was given orally as a daily administration for thirty days to Wistar rats at doses of 20, 100 and 500 mg/kg. In Hippocrtaic screening, doses of ME*Vm* up to 5000 mg/kg did not cause any relevant behavioral changes or deaths thus making it impossible to establish the LD50. In subchronic assay, body weight gains and food intake were significantly reduced at the last week of treatment with 20 and 500 mg/kg dose. Serum triacylglycerides, total proteins and γ-glutamyltransferase activity were significantly reduced, while alkaline phosphatase activity was elevated. In hematological parameters, ME*Vm* increased the percentage of segmented neutrophils cells at the highest dose. All alterations observed were minor in nature and were not accompanied by any relevant clinical signs or any histopathological changes. In conclusion, the results demonstrate relative safety profile of ME*Vm* in the experimental animals.

Introduction

Medicinal plants have been essential in the treatment of several diseases and preservation of human health, being many times the only therapeutical resource in many communities and ethnic groups.

The uses of medicinal plant-derived medicines have been on the increase in the past few decades in many countries, which mainly stem from the perception that their "natural" condition makes them beneficial with no health risks (García-Cortés et al., 2008). In the developing countries, medicinal plants are extensively utilized for food, economic, and medicinal purposes. Unfortunately, very little information is available on the toxicology of these plants. However, evidence on the toxicity risks associated with a wide variety of such remedies has emerged in the last few years, and these herbal remedies are now the most common cause of hepatotoxicity in Asian countries (Norris et al., 2008). The ability of these substances to cause organ damage often results from the interaction of a series of complex cellular processes that are involved in their pharmacological activities (Udem & Ezeasor, 2010).

In Brazil, medicinal plants and herbal drugs are freely sold on the street and in popular markets, besides being cultivated in many homes and are of easy reach in the rural areas. The uses of these natural products are most time based on anecdotal proof of efficacy and safety (Tagliati et al., 2008).

Vatairea macrocarpa (Benth) Ducke, Fabaceae, popularly known as 'angelim', 'gengelim', 'savanna angelim', 'faveiro' and 'bitter', is a tree which may grows up to 5 to 10 m high. It is commonly found in the Central, Central-West, Northeastern and Southeastern Brazil (Pott & Pott, 1994; Lorenzi, 2002). In folk medicine, its heartwood is used for the treatments of infections (Matos et al., 1988), inflammations (Jesus et al., 2009), gastric ulcers (Silva, 2007; Jesus et al., 2009), rheumatism (Moreira & Guarim-Neto, 2009) and diabetes (Oliveira et al., 2008). Among its main pharmacological properties are antibacterial activity of the heartwood extract (Matos et al., 1988) and antidiabetic activity of its stem-bark ethanol extract (Oliveira et al., 2008). Baviloni et al. (2010) showed that the hypoglycemic effect of this species is due to the stimulation of insulin signaling pathway in peripheral tissues, particularly in the liver and adipose tissue. Lectins

identified in the seeds of *V. macrocarpa* (Calvete et al., 1998; Cavada et al., 1998), presented bacterial anti-adhesion property (Mysore et al., 1999; Teixeira et al., 2006) and pro-inflammatory effect (Alencar et al., 2003; 2004). Crysophanic acid, an anthraquinone, was isolated from the heartwood of this plant by Matos et al. (1988). Derivatives of anthraquinones have been implicated in hepatocellular damage induced by *Cassia angustifolia* (Matos & Martins, 2005).

Despite widespread use of *V. macrocarpa* in popular medicine, studies addressing the potential toxicity of this plant are hard to come by. Acute and sub-chronic toxicological studies were carried out to evaluate the safety profile of this plant in experimental animals.

Materials and Methods

Animals

Male *Rattus norvegicus* rats, Wistar strain (180-200 g) and *Mus musculus*, a variety of Swiss Webster mice (25-30 g) used in this study were obtained from the Central Animal House of Federal University of Mato Grosso (UFMT), Brazil. They were maintained in polypropylene cages at 22±2 ºC, with controlled 12 h light/dark cycles, with free access to Purina® (Labina, Goiás, Brazil) standard feed and water *ad libitum*. This study protocol was approved by the Ethics Committee of Animal Experimentation of UFMT (CEPA UFMT 23108.002841/08-4), in accordance with the Federal Government Legislation on Animal Care.

Plant material

The plant was collected at Nossa Senhora do Livramento, located 24 km from Capão Grande District, Várzea Grande, Mato Grosso. Voucher specimen of the plant was dried and deposited in the Central Herbarium of UFMT and was identified under the register number 36375. Authenticated samples of the plant with flowers were sent to the Plantarum Institute for Floral Studies, in Nova Odessa, São Paulo, Brazil, for taxonomic ratification.

Preparation of the plant's extract

The heartwood of *Vatairea macrocarpa* (Benth) Ducke, Fabaceae, was cleaned and dried in the oven at a temperature of 40 ºC for seven days and then ground in a knife mill (model TE-625 Tecnal, São Paulo, Brazil). The dried powder (2 kg) was macerated with methanol (1:3 w/v) for 7 days at room temperature. The macerate was filtered and concentrated under reduced pressure in a rotaevaporator (model 801-Fisatom, Sao Paulo, Brazil) at 40 ºC, resulting in the methanolic extract of *V. macrocarpa* (ME*Vm*). At the time of use, ME*V*m was dissolved in distilled water.

Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out according to the methodology proposed by Matos (1998).

Hippocratic screening (Malone & Robichaud, 1962)

Male mice received MEVm (*p.o.*) at doses of 100, 250, 500, 1000, 3000 and 5000 mg/kg bw. Three animals were used for each dose of the extract and one control animal received the vehicle (distilled water, 10 mL/kg). Animals were observed individually in open field at 5, 10, 15, 30, 60, 120 and 240 min and once a day, for a period of fourteen days, noting any clinical signs or mortality if any.

Subchronic toxicity

Subchronic toxicity was evaluated as proposed by Chan et al. (1982). Animals (n=6/group) were treated daily via oral with the vehicle (1 mL/100 g) and ME*Vm* (20, 100 and 500 mg/kg bw) for thirty days. Animals were kept in metabolic cages, the body weight gain and food consumption were determined at every six days. Signs and symptoms of behavioral alterations were recorded, including skin, eyes, gastrointestinal, respiratory, central nervous system and peripheral alterations, including any other general changes. At the end of the treatments, animals were anesthetized with ethyl ether and blood collected via vena cava for biochemical and hematological analyses. Animals were then sacrificed by cervical dislocation, the organs (liver, heart, lung, kidneys, stomach and spleen) were removed, and relative weights were calculated (weight of the organ/body weight x 100) and were subjected to a complete necropsy, including external body macroscopic examinations. The organs were fixed in 10% formol solution for histological analysis.

Histological analysis

Organ samples from each animal were further processed for histopathology. Samples were routinely processed and sectioned at 5 µm, stained by hematoxylin and eosin technique (Prophet et al., 1992) and analyzed under light optical microscope.

Vital organs such as liver, kidneys and lungs were preserved for histopathological analysis. The organs were fixed in 10% formalin, dehydrated in ethanol and clarified in xylene. After processing, the

tissues were embedded in paraffin and then sectioned to a thickness of 5 µm using a Hyrax M60 (Carl Zeiss MicroImaging GmbH, Germany).The sections were stained with hematoxylin and eosin. The tissues were examined under a microscope in a random order and without the knowledge of animal or group. The renal injury was based on degeneration of Bowman space and glomeruli, degeneration of proximal and distal tubules, vascular congestion and interstitial edema. The criteria for liver injury were vacuolization of hepatocytes and pyknotic hepatocyte nuclei, number of Kupffer cells and enlargement of sinusoids. Moreover, the histopathological change of lungs was based on congestion, edema, inflammation and haemorrhage.

Measurement of hematological and biochemical parameters in rats

Whole blood cell (WBC), segmented, lymphocytes, monocytes, eosinophils, red blood cell (RBC), platelet (PLT), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were determined with hematology analyzer ABX Pentra 60 (HORIBA ABX Ltda, Brazil).

For biochemical analysis, blood without additive was centrifuged at 3000 × *g* at 4 °C for 10 min. Serum was separated and stored at -20 °C until determination of alanine amino transferase (ALT), aspartate amino transferase (AST), uric acid (UA), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), total protein (TP), glucose (GLU), albumin (Alb), creatinine (Cre), total cholesterol (CHO) and triacylglycerides (TG) using an automatic biochemistry analyzer Wiener lab. BT 3000/CB 350i (Wiener lab group, Argentina).

Data analysis

Results of parametric tests were expressed in terms of mean±SEM. In the assays involving comparison of more than two means, one-way ANOVA was used, followed by the Tukey-Kramer test when statistical difference was detected among the groups. Values of $p<0.05$ were considered significant.

Results

Preliminary phytochemical analysis

Phytochemical analysis of ME*Vm* revealed the presence of phenolic compounds, pyrogallic tannins, flavonols, flavonones, free steroids, quinones and resins.

Hippocratic screening

ME*Vm* did not cause any behavioral alterations in mice for doses up to 5000 mg/kg *p.o*. No other signs and symptoms or occurrence of deaths were observed in any of the treated groups. As a result of the data obtained at this stage, it was not possible to determine LD50.

Table 1. Effect of the methanolic extract of *Vatairea macrocarpa* (MEVm, mg/kg *p.o.*) on the body weight gain in rats after thirty days of treatment.

Group	Body weight gain (g)				
	M1	M2	M3	M4	M5
Vehicle	14.33±1.83	36.16±1.64	14.83±2.10	17.16±3.66	12.66±2.88
ME*Vm* 20	17.00±3.53	30.83±3.93	10.00±2.50	21.66±2.45	4.16±2.34*
ME*Vm* 100	14.00±3.22	31.83±6.10	17.60±2.97	20.00±2.30	8.16±1.01
ME*Vm* 500	17.80±4.86	29.4±3.10	12.00±3.03	18.80±3.80	1.20±0.96**

M1-M5: Mean of body weight gain each six days. The results were expressed in mean±SEM using. One way ANOVA followed by Tukey-Kramer test. *$p<0.05$ and **$p<0.01$ represent significant difference from the control group.

Table 2. Effect of the methanolic extract of *Vatairea macrocarpa* (ME*Vm*, mg/kg *p.o.*) on the food intake of rats after 30 days of treatment.

Group	Food intake (g)				
	M1	M2	M3	M4	M5
Vehicle	88.83±2.73	109.50±2.06	103.50±1.76	123.16±3.1	130.00±1.04
ME*Vm* 20	86.5±3.18	108.33±1.45	109.83±2.0	126.83±1.5	130.00±2.01
ME*Vm* 100	88.66±1.14	106.33±1.14	111.16±2.9	128.16±1.3	117.50±3.57**
ME*Vm* 500	78.83±2.19*	100.66±2.29*	113.00±4.15	117.40±2.75	118.00±2.07*

M1-M5: Mean of food intake for each six day. The results were expressed in mean±SEM using. One way ANOVA followed by Tukey-Kramer test. *$p<0.05$ and **$p<0.01$ represent significant difference from the control group.

Subchronic toxicity

Body weight and feed consumption

There was gain in body weights in all treated groups including the control. However, the body weight gain was significantly suppressed, in a non-dose dependent manner, in the last week of treatments for the 20 ($p<0.05$) and 500 ($p<0.01$) mg/kg groups when compared with the control, as shown in Table 1. Modest but significant decreases in the food consumption were observed for the last six days (days 25-30, M5) in the 100 (9.6% $p<0.01$) and for the first 12 (M1, M2) and 25th to 30th (M5) days in the 500 mg/kg groups (11.3%, $p<0.1$, 8%, $p<0.05$ and 9.2%, $p<0.05$, respectively), as shown in Table 2.

Relative organ-body weight and histological analysis

Values of relative organs-weights of the treated groups as depicted in Table 3 did not differ significantly from the control. No apparent histological alterations were found in the comparative analysis of tissues in the different groups.

Hematological and biochemical analysis

As depicted in Table 4, significant increases in the relative values of segmented neutrophils were observed for the dose of 500 mg/kg, without any alteration in other parameters analyzed in the erythrogram and leukogram.

Significant increases by 39% ($p<0.05$), 68% ($p<0.01$), 75% ($p<0.001$) in the alkaline phosphatase enzyme activity in a dose-dependent manner were observed in the 20, 100 and 500 mg/kg groups respectively. Total plasma protein was increased as well in the 100, and 500 mg/kg treated groups. GGT activity significantly decreased only in the 100 mg/kg group by about 80% ($p<0.05$). In all the treated groups, the levels of triacylglycerides decreased in a dose-dependent manner by 58%, 64%, 69% ($p<0.001$) for 20, 100 and 500 mg/kg treated groups, respectively.

Discussion

Preclinical evaluation of drugs and plant products safety is currently performed in animals. A good correlation has been reported between toxicological effects in rats and humans while a weaker correlation is observed between humans and mice (Olson et al., 2000). Thus numerous studies investigate the acute effects of high doses in mice and the chronic effects of lower doses in rats including the doses potentially usable in humans (Mu et al., 2011). ME*Vm* presented no signs and symptoms of acute toxicity.

Changes in body weight have been used as an indicator of side-effects of drugs and chemical substances (Santos et al., 2009). In the subchronic toxicity study, changes in the body weight gain and feed consumption of rats orally treated with ME*Vm* were present only in higher doses, which were not persistent and also non-dose dependent. These signs were accompanied by no changes observed in the macroscopic analysis of animal organs and histopathological analysis, indicating that these alterations may not be of toxicological importance (Mu et. al., 2011). According to Quideau et al. (2011), plant with polyphenols present poor oral bioavailability, in this way, observed differences in patterns of inhibition of feed intake and body weight gain observed in the study, may be due to irregularities in the absorption of the active components of the extract.

Alterations in some serum biochemical parameters were observed, such as increase in ALP and decrease in GGT activity. ALP enzyme, a phosphohydrolase, is found in several tissues with higher concentrations in the liver, bones, kidneys and placenta (Henry, 1995). The increased activity of ALP may be due to induction of the enzyme or of the

Table 3. Relative weight of organs of rats treated with methanolic extract of *Vatairea macrocarpa* (ME*Vm*) after thirty days of treatment.

Organ	Control (g)	ME*Vm* (mg/kg *p.o.*)		
		20	100	500
Left kidney	0.37±0.01	0.39±0.01	0.38±0.01	0.38±0.01
Right kidney	0.38±0.01	0.38±0.01	0.43±0.02	0.38±0.01
Stomach	0.46±0.03	0.49±0.02	0.45±0.03	0.48±0.01
Liver	4.20±0.13	4.30±0.18	3.90±0.20	3.80±0.10
Lung	0.56±0.04	0.51±0.01	0.62±0.05	0.63±0.01
Spleen	0.22±0.01	0.21±0.01	0.21±0.02	0.22±0.01
Heart	0.31±0.01	0.29±0.01	0.30±0.01	0.31±0.01

The results were expressed in mean±SEM using one way ANOVA.

Table 4. Effect of the methanolic extract of *Vatairea macrocarpa* (ME*Vm*) on the hematological and biochemical parameters in rats after 30 days of treatment.

Parameter	Control	ME*Vm* (mg/kg *p.o.*)			Reference range[1]
		20	100	500	
Hematological parameters					
RBC (x10^6/mm^3)	7.7±0.18	7.5±0.06	7.8±0.2	7.7±0.1	7.27-9.65
HGB (g/dL)	15.6±0.2	15.7±0.1	16.6±0.4	15.9±0.4	13.7-17.6
HCT (%)	38.7±0.8	38.6±0.4	40.7±0.9	39.5±1.1	39.6-52.5
MCV (fL)	50.2±0.5	51.2±0.3	51.8±0.9	50.8±0.7	48.9-57.9
MCH (pg)	20.2±0.3	20.9±0.1	21.0±0.3	20.5±0.1	17.1-20.4
WBC (x10^3/mm^3)	7.0±0.7	5.4±0.5	5.0±0.8	7.7±0.9	1.96-8.25
Seg. (%)	19±2.0	25±2.9	23±1.9	32±3.3*	6.2-26.7
Lymphocytes (%)	77±2.5	71±2.9	74±2.0	66±3.3	66.6-90.3
Monocytes (%)	2.3±0.6	1.3±0.3	1.3±0.3	1.4±0.4	0.8-3.8
Eosinophils (%)	2.0±0.6	1.5±0.6	1.0±0.2	0.6±0.2	0.2-3.5
PLT (x10^3/mm^3)	894±43	855±34	906±59	812±42	638-1177
Biochemical parameters					
GLU (mg/dL)	190±16	209±19	258±52	337±61	70-208
Cre (mg/dL)	0.91±0.01	0.96±0.03	0.95±0.03	0.94±0.04	0.2-0.5
UA (mg/dL)	4.2±0.3	4.2±0.5	4.7±0.5	3.7±0.6	-
AST (UI)	131±16	113±3	110±2	117±4	74-143
ALT (UI)	50±5	50±2	50±2	51±2	18-45
ALP (UI)	460±80	641±13*	777±24**	805±52***	62-230
GGT (UI)	6.8±2.2	1.5±0.7	1.3±0.3*	3.4±1.2	-
TP (g/dL)	7.8±0.05	7.9±0.06	8.3±0.1**	8.2±0.1±*	5.2-7.1
Alb (g/dL)	3.1±0.06	3.2±0.09	3.1±0.2	3.0±0.3	3.4-4.8
TG (mg/dL)	247±13	103±28***	88±8***	75±7***	20-114
CHO (mg/dL)	101±8.7	110±8.3	104±10.7	98±3.3	37-85

The results were expressed in mean±SEM. One way ANOVA followed by Tukey-Kramer test. *p<0.05, **p<0.01 and ***p<0.001 represent significant difference from the control group. WBC: whole blood cells, RBC: red blood cell, PLT: platelets, HCT: hematocrit, HGB: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, Glu: glucose, ALT: alanine amino transferase, AST: aspartate amino transferase, ALP: alkaline phosphatase, GGT: γ-glutamyltransferase, Alb: albumin, TP: total protein, TG: triacylglycerides, CHO: total cholesterol, UA: uric acid, Cre: creatinine. [1]Giknis & Clifford (2008).

microsomal enzymes by the components of the extract, rather than due to hepatobiliary injury (Smith et al., 2002). In corroboration to this assumption, there were no accompanying alterations of the aminotransferases.

GGT is a hepatic microsomal enzyme, which is usually determined with regard to hepatobiliary disease (Henry, 1995). GGT has about 90% sensitivity and specificity to confirm hepatic origin of increased phosphatase serum activity (McIntyre, 2004). However, in this present study, GGT activity was rather reduced as opposed to elevated activity of the ALP enzyme. Perhaps, this reduction may be related to the presence of an unknown antioxidant phytochemicals/metabolites in the plant extract, as it has been shown in some epidemiological studies that concentrations of serum antioxidant related inversely to serum GGT level in dose-response manners (Lee et al, 2004). Therefore, when taken together, the dose-dependent decreases in GGT are not of toxicological importance (Mu et al., 2011). Significant but slight increase (about 6.4%) in the concentration of total protein was noted, but this was not accompanied by a corresponding increase in albumin concentration. This slight increase is of doubtful toxicological importance.

No significant alterations were observed for AST and ALT, suggesting no inflammatory or necrotic damage of liver due to ME*Vm* (Table 4). Histological observation of sections of liver of MEVm treated rats attests to the fact that, there is no hepatic damage, as shown by the normal looking integrity of the hepatocytes.

Vatairea macrocarpa, Fabaceae, is popularly used as an anti-diabetic agent. Its hypoglycemic activity has been experimentally substantiated as noted earlier. As

observed in this study, the levels of triacylglycerides in animals treated with the extract decreased significantly in a dose-dependent manner and are thus treatment related. However, these reductions fall within the reference limits by Boehm et al. (2007), and therefore of no toxicological significance. The dose-dependent reductions in triacylglycerides levels might be due to the presence of hypolipidemic agent in the extract. This finding is similar to what is obtained of fibrates, a class of amphipathic carboxylic acids used for a range of metabolic disorders, mainly hypercholesterolemia (high cholesterol) (Bertolami, 2005). It is thus possible that the hypolipidemic mechanism of action of ME*Vm* may in a way, be similar to that of the fibrates. It will be interesting therefore to carry out studies on the mechanism of action of ME*Vm* in order to get a better understanding of its hypolipidemic effects.

Kidney functions were evaluated by means of serum uric acid and creatinine. Increase blood creatinine is a good indicator of negative impact in kidney functions (Atsamo et al., 2011). In the present study, blood creatinine and uric acid were not affected by ME*Vm* treatment.

Analysis of blood parameters is relevant to risk evaluation as the changes in the haematological system have a higher predictive value for human toxicity, when the data are translated from animal studies (Olson et al., 2000). The hematological profile of treated rats showed no significant difference compared with control group, except for the segmented neutrophils which increased in the group treated with ME*V*m at the dose of 500 mg/kg. The increase in the neutrophils at the highest dose may suggest the presence of immune stimulating agent in the extract. However, since this increase is sporadic and is also non-dose dependent, it is of no toxicological value (Mu et al., 2011). Furthermore, histological analysis revealed no apparent histological alterations in the comparative analysis of tissues of the different groups. This further reinforces the fact that *V. macrocarpa* is relatively safe at the doses tested in rats for the period of thirty days.

Conclusion

In summary, based on the toxicological parameters evaluated in the studies performed, it is concluded that ME*Vm* is not acutely toxic and is well tolerated in rats upon repeat dosing to at least 500 mg/kg bw/day.

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References

Alencar NM, Assreuy AM, Alencar VB, Melo SC, Ramos MV, Cavada BS, Cunha FQ, Ribeiro RA 2003. The galactose-binding lectin from *Vatairea macrocarpa* seeds induces in vivo neutrophil migration by indirect mechanism. *Int J Biochem Cell B 35*: 1674-1681.

Alencar NM, Assreuy AM, Criddle DN, Souza EP, Soares PM, Havt A, Aragão KS, Bezerra DP, Ribeiro RA, Cavada BS 2004. *Vatairea macrocarp*a lectin induces paw edema with leukocyte infiltration. *Protein Peptide Lett 11*: 195-200.

Atsamo AD, Nguelefack TB, Datté JY, Kamanyi A 2011. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina senegalensis* DC (Fabaceae) in rodents. *J Ethnopharmacol 134*: 697-702.

Baviloni PD, Santos MP, Aiko GM, Reis SR, Latorraca MQ, Silva VC, Dall'Oglio EL, Sousa-Jr PT, Lopes CF, Baviera AM, Kawashita NH 2010. Mechanism of anti-hyperglicemic action of *Vatairea macrocarpa* (Leguminosae): Investigation in peripheral tissues. *J Ethnopharmacol 131*: 135-139.

Bertolami MC 2005. Mecanismos de hepatotoxicidade. *Arq Bras Cardiol 85*: 25-27.

Boehm O, Zur B, Koch A, Tran N, Freyenhagen R, Hartmann M, Zacharowski K 2007. Clinical chemistry reference database for Wistar rats and C57/BL6 mice. *Biol Chem 388*: 547-554.

Calvete JJ, Santos CF, Mann K, Grangeiro TB, Nimtz M, Urbanke C, Sousa-Cavada B 1998. Amino acid sequence, glycan structure, and proteolytic processing of the lectin of *Vatairea macrocarpa* seeds. *Febs Lett 425*: 286-92.

Cavada BS, Santos CF, Grangeiro TB, Nunes EP, Sales PV, Ramos RL, De Sousa FA, Crisostomo CV, Calvete JJ 1998. Purification and characterization of a lectin from seeds of *Vatairea macrocarpa* Duke. *Phytochemistry 49*: 675-80.

Chan PK, O'Hara GP, Hayes AW 1982. *Priciples and methods for acute and subchronic toxicity.* In: Hayes AW. Principles and Methods of Toxicology. Raven press, New York, p. 1-51.

García-Cortés M, Borraz Y, Lucena MI, Peláez G, Salmerón J, Diago M, Martínez-Sierra MC, Navarro JM, Planas R, Soria MJ, Bruguera M, Andrade RJ 2008. Liver injuries induced by "natural remedies": an analysis of cases submitted to the Spanish Liver Toxicity Registry. *Rev Esp Enferm Dig 100*: 688-695.

Giknis MLA, Clifford CB 2008. *Clinical Laboratory Parameters For Crl:WI (Han) Rats* http://www.criver.com. Assessed 24 Jan 2012.

Henry JB 1995. Diagnósticos clínicos e tratamento por métodos laboratoriais. São Paulo. Manole.

Jesus NZT, Lima JCS, Silva RM, Espinosa MM, Martins DTO 2009. Levantamento etnobotânico de

plantas popularmente utilizadas como antiúlceras e antiinflamatórias pela comunidade de Pirizal, Nossa Senhora do Livramento-MT, Brasil. *Rev Bras Farmacogn 19*: 130-139.

Lee DH, Blomhoff R, Jacobs DR 2004. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radical Res 38*: 535-9.

Lorenzi H 2002. Árvores brasileiras - Manual de identificação e cultivo de plantas arbóreas do Brasil. Nova Odessa, SP.

Malone MH, Robichaud RC 1962. A Hippocratic screen for pure or crude drug materials. *Lloyd 25*: 320-332.

Matos FJA 1998. Introdução à fitoquímica experimental. Fortaleza, CE. Imprensa Universitária da Universidade Federal do ceará.

Matos FJA, Aguiar LMBA, Silva MGV 1988. Constituintes químicos e atividade antimicrobiana de *Vatairea macrocarpa* Duke. *Acta Amaz 18*: 351-352.

Matos LC, Martins B 2005. Hepatites tóxicas: revisão da literatura. *Med Intern 12*: 239-258.

McIntyre N 2004. *Clinical biochemistry of the liver*. In: Bittar EE (ed.) The Liver in Biology and Disease, Principles of Medical Biology 15: 291-316.

Moreira DL, Guarim-Neto G 2009. Usos múltiplos de plantas do cerrado: um estudo etnobotânico na comunidade Sítio Pindura, Rosário Oeste, Mato Grosso, Brasil. *Polibotánica 27*: 159-190.

Mu LH, Huang ZX, Liu P, Hu Y, Gao Y 2011. Acute and subchronic oral toxicity assessment of the herbal formula Kai-Xin-San. *J Ethnopharmacol 138*: 351-357.

Mysore JV, Wigginton T, Simon PM, Zopf D, Heman-Ackah LM, Dubois A 1999. Treatment of *Helicobacter pylori* infection in rhesus monkeys using a novel antiadhesion compound. *Gastroenterology 117*: 1316-1325.

Norris W, Paredes AH, Lewis JH 2008. Drug-induced liver injury in 2007. *Curr Opin Gastroen 24*: 287-297.

Oliveira HC, dos Santos MP, Grigulo R, Lima LL, Martins. DTO, Lima JC, Stoppiglia LF, Lopes CF, Kawashita NH 2008. Antidiabetic activity of *Vatairea macrocarpa* extract in rats. *J Ethnopharmacol 115*: 515-519.

Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharm 32*: 56-67.

Pott VJ, Pott A 1994. Plantas do Pantanal. Corumbá-MS. EMBRAPA-CPAP.

Prophet EB, Mills B, Arrington JB, Sobin LH 1992. Laboratory methods in histotechnology. American Registry of Pathology.

Quideau S, Deffieux D, Douat-Casassus C, Poységu L. 2011. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew Chem Ind 50*: 586-621.

Santos SR, Rangel ET, Lima JCS, Silva RM, Lopes L, Noldin VF, Cechinel Filho V, Delle Monache, Martins DTO 2009. Toxicological and phytochemical studies of *Aspidosperma subincanum* Mart. Stem bark (Guatambu). *Pharmazie 64*: 1-4.

Silva IHLGP 2007. Educação, cultura e tradição: Tessituras de uma comunidade tradicional. Cuiabá, MT. 184p. Dissertação de mestrado. Universidade Federal de Mato Grosso.

Smith GS, Hall RL, Walker RM 2002. Applied clinical toxicology in preclinical toxicology testing. In: Haschek WM, Rousseaux CG, Wallig M (eds), *Handbook of Toxicologic Pathology 1*: 123-155.

Tagliati CA, Silva RP, Féres CAO, Jorge RM, Rocha OA, Braga FC 2008. Acute and chronic toxicological studies of the Brazilian phytopharmaceutical product Ierobina®. *Rev Bras Farmacogn 18*: 676-682.

Teixeira EH, Napimoga MH, Carneiro VA, de Oliveira TM, Cunha RM, Havt A, Martins JL, Pinto VP, Gonçalves RB, Cavada BS 2006. *In vitro* inhibition of Streptococci binding to enamel acquired pellicle by plant lectins. *J Appl Microbiol 101*: 111-116.

Udem SC, Ezeasor CK 2010. The acute and subchronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* ker (Zingiberaceae) in mice. *Comp Clin Pathol 19*: 75-80.

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