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Full Length Research Paper

Subchronic toxicity evaluation of the hydroethanolic extract from *Endopleura uchi* (Huber) Cuatrec in Wistar rats

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***Endopleura uchi* (Huber) Cuatrec. (Humiriaceae) is a species from Brazilian Amazon rainforest, popularly used against menstrual disorders and uterine inflammation. This study aimed to evaluate the subchronic toxicity of hydroethanolic extract from *E. uchi* (EHEEu) in Wistar rats based on biochemical and hematological parameters. Rats were treated with daily doses of EHEEu (500 mg/kg - gavage), and then hematological and biochemical parameters were observed. The results shows that the treatment performed produced no signs of toxicity or death, as well as no changes in weight gain or daily intake of water and food. Biochemical and hematological parameters were not modified by EHEEu administration, with the exception of erythrocyte index of rats (males) in the treated group, however, it was not assigned clinical relevance once it remained within the reference range for the species. Thus, subchronic administration of EHEEu produced no toxic effects in Wistar rats.**

Key words: *Endopleura uchi*, subchronic treatment, hematology, biochemistry.

INTRODUCTION

The traditional use of medicinal plants based on popular knowledge, along with the belief that being natural does not cause adverse reactions, made only a few medicinal plants were evaluated through pre-clinical and clinical studies to prove its effectiveness and security (Turolla and Nascimento, 2006). However, over time it was realized that certain plants have potentially dangerous substances and therefore should be used with care,

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respecting their toxicological risks (Veiga-Junior and Pinto, 2005).*Endopleura uchi* (Huber) Cuatrec. (Humiriaceae) is a botanical species typical from the Brazilian Amazon rain-forest This plant is found in not too flooded ground forest, which is widely spread in the Amazon Basin.It is popularly known in the region as uchi, uxi, axuá, uchi-pucu, uxi-amarelo, uxi-liso e uxi-ordinário (Cuatrecasas, 1961; Schultes, 1979). The stem bark of *E. uchi* is used as tea for menstrual disorders and uterine inflammation (Revilla, 2001). Other indications are also known and cited as antimicrobial activity (Politi, 2009), high anti-oxidant activity and absence of cytotoxicity (Politi et al., 2011). These results justify the increasingly growing interest in the study of this plant.

According to Politi (2009), the bark of *E. uchi* consists mainly of three types of secondary metabolites: tannins, coumarins and saponins. Another phytochemical study from crude ethanol extract of bark led to the isolation of bergenin isocoumarins and 8, 10 dimetoxibergenina, pentacyclic triterpenoids, maslinic acid and its methyl ester maslininate (Luna et al., 2000).

The bergenin extracted from the bark of *E. uchi* have been identified as mainly responsible against the biological activities such as anti-inflammatory (Nunomura et al., 2009), anti-microbial (Silva et al., 2009), neuroprotective (Takahashi et al., 2003) and antinociceptive (Oliveira et al., 2011). Until this moment, toxicological studies do not support the safe use of *E. uchi*. However, Politi et al. (2010) evaluated the acute oral toxicity in male rats with an extract of powdered bark of *E. uchi*. Thus, the present study aimed to evaluate the subchronic toxicity of crude hydroethanolic extract of the stem bark of *E. uchi* in Wistar rats of both sexes.

MATERIALS AND METHODS

Collection and authentication

The stem barks of *E. uchi* were collected in April, 2005 in the city of Ananideua, Pará State, Brazil. The voucher specimen of the plant (number 180611) was deposited in the Herbarium of the Brazilian Agricultural Research Corporation - EMBRAPA, Belém, Pará, Brazil.

Extract preparation

The stem bark of *E. uchi* were dried in an air circulating oven at 40°C for 72 h and after drying, it was triturated in a knives mill, obtaining the powder of the plant (464 g). Subsequently, this material was macerated in a 75% hydroalcoholic solution in a ratio of 1:5 at room temperature for 7 days, under stirring. Then, the mash was filtered and concentrated on a rotaevaporator (Quimis Model Q 218.2) at 45°C until complete evaporation of the solvent. The concentrated filtrate was subjected to lyophilization providing 10.65% yield.

Analysis of the content of total polyphenols and total tannins

The method of Brazilian Pharmacopeia, 5th edition (2010) described for Rhatany species (*Ratanhiae radix*) was adapted and subjected to linear regression curve prepared with pyrogallic acid, all analyses were performed in triplicate. The polyphenols contents were calculated from the equation of the line obtained by standard curve of acid pyrogallic in concentrations from 0.01 to 0.05 mg/ml submitted to reaction with phosphomolybdic tungstic acid in alkaline medium following the method described by Carvalho et al. (2013).

Animals

Rats (*Rattus norvegicus albinus*), Wistar strain (males and females), weighing around 170 to 215 g from the vivarium of the Center for Reproductive Biology, Federal University of Juiz de Fora were used. These went through a period of adjustment, kept under controlled lighting conditions (cycle 12 h light/dark), temperature (23 ± 2°C) and received water and food *ad libitum*. The experimental protocol was approved by the Ethics Committee of the Federal University of Amapá (Case No. 001A/2012).

Treatment of animals and evaluation of biochemical and hematological parameters

Twenty rats corresponding to two groups n = 10/group (5 males and 5 females) were randomized into five subgroups and treated for 22 consecutive days orally with EHEEu at a dose of 500 mg/kg (treated group) and distilled water (control group). Parameters indicative of toxicity and blood-biochemical analyzes were performed according to the method described by Silva et al. (2005), with some adaptations. Parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, HDL cholesterol, triglycerides, alkaline phosphatase (ALP), albumin, glucose, creatinine, total and differential blood count were determined using the multiparametric equipment for biochemical determination (Alizé) from Biomérieux and automatic hematologic cells analyzer HumaCount Plus.

Statistical analysis

The results obtained in different analyzes were expressed as average ± standard error of the average (average ± SEA) for each experimental group. For biochemical and hematological analysis, we used Mann - Whitney, and to compare the data of weight gain, water and food intake the student "t" test (unpaired) was used. Test was performed using GraphPad Prism software® (version 5.03). Results with $p < 0.05$ were considered statistically significant.

RESULTS

The lyophilized extract showed total polyphenols equal to 7.01% corresponding to an average of 0.021 ± 0.008 mg/ml (n = 3). The percentage of total tannins was 1.5% with 0.0045 ± 0.0011 mg/ml (n = 3) representing 21.4% of all polyphenols in the extract (Figure 1). The oral administration for 22 days with EHEEu at a dose of 500 mg/kg in rats, males and females, did not affect the weight gain

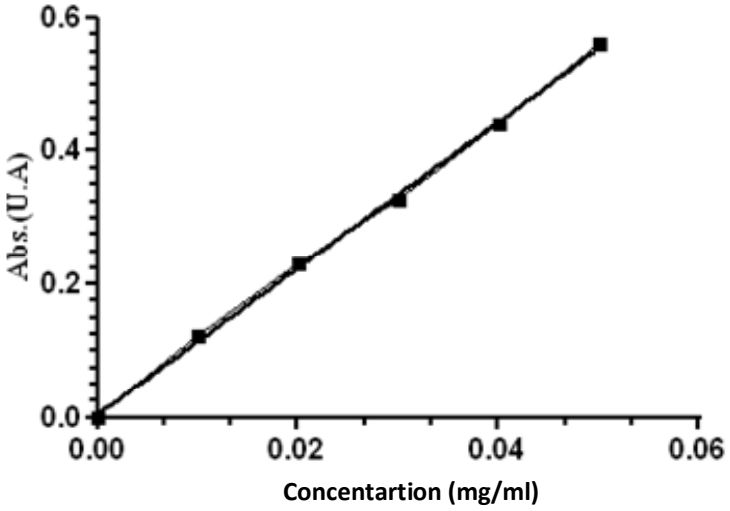

Figure 1. Standard curve of pyrogallic acid for spectrophotometrically (λ = 760 nm), concentrations from 0.01 to 0.05 mg/mL subjected to reaction with phosphomolybdictungstic acid in alkaline medium with read-after 2 minutes of reaction.
Linear regression of the results obtained r^2 = 0.9987 correlation coefficient with the equation of the line y = 10.450x 0.0118.

Table 1. Effect of the treatment (po) of EHEEu (500 mg/kg) and distilled water (control) for 22 consecutive days, on the biochemical parameters of Wistar rats (males and females).

Parameter	Female		Male	
	Control	EHEEu	Control	EHEEu
AST (U/L)	241.6 ± 50.0	245.0 ± 39.9	193.2 ± 27.6	228.0 ± 37.1
ALT (U/L)	83.6 ± 22.9	79.0 ± 17.8	53.0 ± 4.8	54.5 ± 5.8
Total cholesterol (mg/dl)	66.5 ± 1.8	73.8 ± 4.6	61.6 ± 3.3	65.6 ± 4.2
HDL cholesterol (mg/dl)	31.6 ± 1.2	30.7 ± 1.4	31.2 ± 1.1	30.4 ± 0.8
Triglycerides (mg/dl)	82.0 ± 4.5	76.8 ± 6.3	55.5± 2.3	74.4 ± 1.6
ALP (U/L)	23.6 ± 2.4	26.2 ± 6.2	90.8 ± 8.9	90.0 ± 17.9
Albumin (g/dl)	3.5 ± 0,0	3.6 ± 0,1	3.7 ± 0.0	3.6 ± 0.0
Glucose (mg/dl)	111.7 ± 20.9	123.3 ±17.3	154.5 ± 6.75	146.4 ± 13.5
Creatinine (mg/dl)	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.0

The values represent mean ± S.E.M. (n = 5/group). *p < 0.05 compared to the control group (Mann-Whitney test). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; FA: Alkaline phosphatase.

gain and daily food and water intake of these animals, since significant changes were not observed compared to the control group (Figures 2, 3 and 4). The level of serum AST, both in males and females (control and treated) and ALT in males (control and treated) were above the reference values (Table 1). The treatment of animals with EHEEu did not alter the levels of triglycerides and total cholesterol, however, the levels of HDL cholesterol in males and females (control and treated group) animals remained below the reference values (Table 1). Oral treatment of animals with EHEEu did not interfere in hematologic rates, except for the values of mean corpuscular hemoglobin concentration (MCHC) of animals (males), which showed a significant increase compared to the control group. The differential count of lymphocytes, monocytes, neutrophils and eosinophils showed similar values, with no statistically significant differences between the control group and treated with EHEEu (Table 2).

DISCUSSION

Polyphenols vary from simple molecules to complex

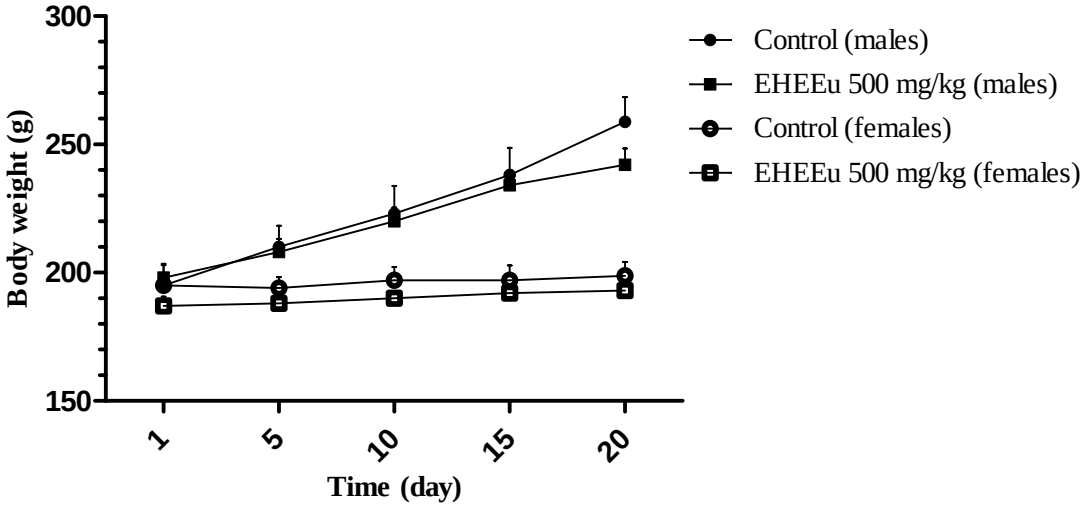

Figure 2. Effects of the administration (po) of EHEEu (500 mg/kg) and distilled water (control) on the body weight of Wistar rats (males and females), by 22 consecutive days. The values represent mean ± SEM. (n = 5/group). *p < 0.05 compared to the control group.

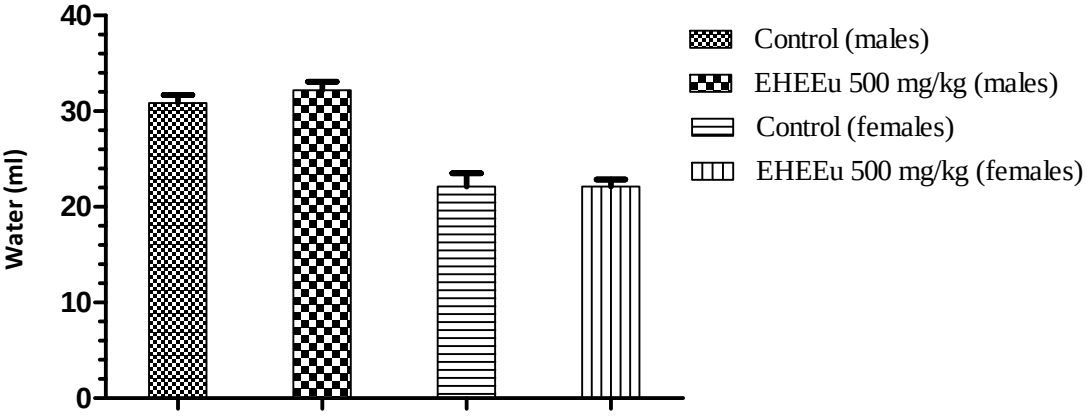

Figure 3. Effects of the administration (po) of EHEEu (500 mg/kg) and distilled water (control) on the daily water consumption of Wistar rats (males and females), for 22 consecutive days. The values represent mean ± S.E.M. (n = 5/group). *p < 0.05 compared to the control group.

molecules with a high degree of polymerization and are classified into several classes of secondary metabolites such as flavonoids, phenolic acids, simple phenols, coumarins, tannins, lignins, and tocopherols among others and are easily oxidizable by metals light, heat and enzymatic processes and are associated the antioxidant actions (Shahidi and Naczk 1995; Simões et al., 2004). In this study, the EHEEu showed concentration significant for tannins.

One of the indicators of adverse effects of drugs and chemicals is the change in animal body weight (Tofovic and Jackson, 1999; Raza et al., 2002; Teo et al., 2002). Systemic toxicity can also be diagnosed by decreased water intake, diet, behavioral changes such as apathy and prostration, and by the appearance of rough hair coat (Melo, 2001). In this study, it was observed that the gain in body weight in rats (males) from control and EHEEu treated groups was higher than those rats (females) from both groups. However, it was found that oral administration for 22 days with EHEEu in rats, males and females did not affect the weight gain and daily food and water intake of these animals (Figures 2, 3 and 4).

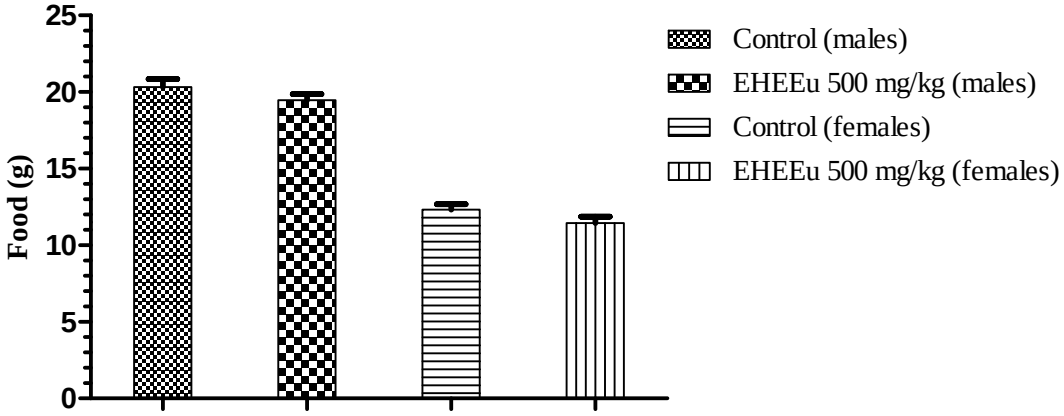

Figure 4. Effects of the administration (po) of EHEEu (500 mg/kg) and distilled water (control) on the daily feed intake of Wistar rats (males and females), for 22 consecutive days. The values represent mean ± S.E.M. (n = 5/group). *p < 0.05 compared to the control group.

Table 2. Effect of the treatment (po) of EHEEu (500 mg/kg) and distilled water (control) for 22 consecutive days, on hematological parameters of Wistar rats (males and females).

Parameter	Females		Males	
	Control	EHEEu	Control	EHEEu
RBC (x10^6/mm^3)	7.9 ± 0.1	7.9 ± 0.3	9.2 ± 0.0	9.1 ± 0.2
HGB (g/dl)	14.8 ± 0.1	15.1 ± 0.5	15.7 ± 0.1	15.9 ± 0.2
HCT (%)	41.3 ± 0.4	40.8 ± 1.7	48.0 ± 0.3	47.0 ± 1.7
MCV (fl)	52.2 ± 0.6	51.5 ± 0.6	52.0 ± 0.3	51.2 ± 0.5
MCH (pg)	18.6 ± 0.1	19.1 ± 0.4	17.0 ± 0.1	17.4 ± 0.2
MCCH (g/dl)	35.7 ± 0.3	37.1 ± 0.5	32.7 ± 0.1	34.7 ± 0.4*
WBC (×10^3/mm^3)	3.7 ± 0.6	3.4 ± 0.4	3.6 ± 0.6	3.2 ± 0.7
Lymphocytes (%)	56.2 ± 3.8	52.2 ± 3.6	63.8 ± 2.1	61.8 ± 1.3
Monocytes (%)	2.4 ± 0.6	3.0 ± 0.3	1.6 ± 0.4	2.0 ± 0.5
Segmented (%)	40.0 ± 3.5	43.0 ± 3.6	32.6 ± 2.2	34.6 ± 1.2
Eosinophils (%)	1.4 ± 0.2	1.8 ± 0.3	2.0 ± 0.8	1.6 ± 0.5
Platelets (×10^3/mm^3)	1069.0 ± 35.5	996.25 ± 13.9	1026.3 ± 159.0	1007± 63.1

The values represent mean ± S.E.M. (n = 5/group). *p < 0,05 compared to the control group (Mann-Whitney test). RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MVC: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCCH: Mean corpuscular hemoglobin concentration.

Although the food intake of the treated group was lower than in the control group (Figure 4), the fact is not associated with EHEEu toxic effects because there was no interference with the body mass gain of these animals during treatment. During the experimental period, the animals of different groups showed no clinical signs of toxicity and no death was registered. The decrease in glomerular filtration, in general, leads to increased plasma creatinine concentrations. In rats, plasma levels of creatinine change can be a reliable indicator for the presence of renal injury, because its serum level is not influenced by diet, age or sex (Alves, 2007). The administration EHEEu does not change serum creatinine levels in animals, which indicates that renal function was not affected (Table 1).

According to Alves (2007), some enzymes can be used as indicators of hepatic injury, such as ALT, AST and ALP. ALT is found mainly in the cytoplasm of hepatocytes, while 80% of AST is present in mitochondria. In light hepatocellular damage the serum is predominantly cytoplasmic, while in severe injuries the mitochondrial enzyme is released (Motta, 2003). The ALP is present

mainly at bone tissue, at the hepatobiliary system and the gastrointestinal mucosa, it is indicative of cholestasis, which may lead to increased serum levels up to 10 times (Scheffer and Gonzalez, 2003). In the present study, these enzymes were not altered after administration of EHEEu, however the level of serum AST, both in males and females (control and treated) and ALT in males (control and treated) were above the reference values (Table 1) (Clifford and Giknis, 2008).

The importance of lipid levels is fundamental because elevated levels of total cholesterol are closely related to risk of ischemic coronary disease (Araújo et al., 2011). Treatment of animals with EHEEu did not alter the levels of triglycerides and total cholesterol, probably indicating that there was no change in the lipid metabolism of these animals, however, the levels of HDL cholesterol in males and females (control and treated group) animals remained below the reference values (Table 1) (Dantas et al., 2006).

Despite the existence of own mechanisms to control the physiological parameters values, it is known that in certain groups of animals, such as rats and mice, these parameters can vary, mainly related to gender, strain, genotype and may be influenced by age, diet, handling, environment, among other factors (Pinheiro et al., 2003). Although some biochemical parameters in animals treated with EHEEu are below or above the reference values, cannot be assigned clinical importance because the same results were found in the control group, with no statistically significant difference between them (Table 1). However, it is necessary for the histopathological study of the liver and kidneys of these animals.

According to Silva et al. (2012), hematological parameters are important for the toxicity study, due to the hematopoietic system that is highly sensitive to the toxic agents' activities, such as those with cytotoxic or mutagenic potential. These toxicants may result in many changes such as qualitative or quantitative, transient or permanent and may also limit the use of drugs. In oral treatment of animals with EHEEu, in hematologic rates, only the values of mean corpuscular hemoglobin concentration (MCHC) of animals (males) was changed, which showed a significant increase compared to the control group (Table 2). According to Alves (2007), analysis of red blood cell (RBC) indices are important indicators in determining the morphological type of anemia. The increased erythrocyte MCHC index of male animals had no clinical relevance because they have values within the range recommended by the study of Charles River (Giknis and Clifford, 2008).

Conclusion

With the obtained results, we can suggest that oral treatment with EHEEu at a dose of 500 mg/kg for 22 days produced no signs of systemic toxicity in Wistar rats males and females when compared to the control group. So the EHEEu showed high degree of safety at dose and period in which the animals were exposed.

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Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

Alves NM (2007). Estudo farmacognóstico e da toxicidade experimental (aguda e subaguda) do guatambu (*Aspidosperma subincanum* Mart.). Dissertação de Mestrado, Universidade de Brasília, Brasil.

Araújo TOP, Goes LEJ, Rocha AG, Costa MP, Santos LHS, Bertato AC, Gileno MC (2011). Benefícios da cafeína sobre os níveis séricos de colesterol e triglicerídeos. Rev. Uniara 14(1):118-126.

Brazilian Pharmacopeia (2010). Métodos aplicados a medicamentos e físicos e químicos. Farmacopeia Brasileira, Brasília (DF), 5th edition 2:59-93.

Carvalho HO, Medeiros BJL, Sá BM, Araújo JTC, Kawakami MYM, Favacho HAS, Carvalho JCT (2013). Study of dissolution profiles and desintegration of capsules containing the dried hydroethanolic extract of *Calophyllum brasiliense*. Braz. J. Pharmacogn. 23(1):194-199.

Clifford CB, Giknis MLA (2008). Clinical Laboratory Parameter for Crl: WI(Han). Charles River Laboratories.

Cuatrecasas JA (1961). A taxonomic revision of Humiriaceae. Contributions from the United States National Herbarium. Bull. US Nat. Mus. 35(2):25-214.

Dantas JA, Ambiel CR, Cuman RKN, Baroni S, Bersani-Amado CA (2006). Valores de referência de alguns parâmetros fisiológicos de ratos do Biotério Central da Universidade Estadual de Maringá, Estado do Paraná. Acta Sci. Health Sci. 28(2):165-170.

Luna JS, Silva TM, Bento ES, Sant´ana AEG (2000). Isolamento e Identificação estrutural dos constituintes químicos de *Endopleura uchi* (Humiriaceae). In: Reunião Anual da Sociedade Brasileira de Química, Minas Gerais, Brasil.

Mello FB (2001). Estudo dos efeitos de *Lantana camara* (Verbenaceae) sobre a fertilidade e reprodução de ratos**.** Dissertação de Mestrado, Universidade Federal do Rio Grande do Sul, Brasil.

Motta VT (2003). Bioquímica Clínica para o Laboratório: princípios e interpretações. Ed. Robe, São Paulo, Brasil.

Nunomura RCS, Oliveira VG, DA Silva SL, Nunomura SM (2009). Characterization of bergenin in *Endopleura uchi* bark and its anti-inflammatory activity. J. Braz. Chem. Soc. 20(6):1060-1064.

Oliveira CM, Nonato FR, Lima FO, Couto RD, David JP, David JM, Soares MBP, Villarreal CF (2011). Antinociceptive properties of bergenin. J. Nat. Prod. 74(10):2062-2068.

Pinheiro DCSN, Favali CBF, Filho AAS, Silva ACM, Filgueiras TM, Lima MGS (2003). Parâmetros hematológicos de camundongos e ratos do biotério central da Universidade Federal do Ceará. Bol. Inf. COBEA (3):6-9.

Politi FAS, Mello JCP, Migliato KF, Nepomuceno ALA, Moreira, RRD, Pietro RCLR **(**2011). Antimicrobial, Cytotoxic and Antioxidant

Activities and Determination of the Total Tannin Content of Bark Extracts *Endopleura uchi*. Int. J. Mol. Sci. 12:2757-2768.
Politi FAS, Moreira RRD, Salgado HRN, Pietro RCLR (2010). Testes preliminares de motilidade intestinal e toxicidade oral aguda com extrato de cascas pulverizadas de *Endopleura uchi* (Huber) Cuatrec. (Humiriaceae) em camundongos. Rev Pan-Amaz Saude 1(1):187-189.
Politi FAZ (2009). Estudos farmacognósticos e avaliação de atividades biológicas de extratos obtidos das cascas pulverizadas de *Endopleura uchi* (Huber) Cuatrec. (Humiriaceae). Dissertação de Mestrado, Universidade Estadual Paulista Júlio de Mesquita Filho, Brasil.
Raza M, AL-Shabanah OA, EL-Hadiyah TM, AL-Majed AA (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci. Pharmac. 70:135-145.
Revilla J (2001). Plantas da Amazônia: oportunidades econômicas e sustentáveis. Ed. Sebrae/Inpa, Manaus, Brasil.
Scheffer MC (2002). Fisiologia pós-colheita de espécies medicinais, condimentares e aromáticas. In: Wachowicz, CM, Carvalho, RIN. Fisiologia vegetal: Produção e pós-colheita. Curitiba: Champagnat, Pp.383-404.
Scheffer JF, González FHD (2003). Enzimologia clínica em medicina veterinária. Available at: http://www6.ufrgs.br/favet/lacvet/restrito/pdf/rev_jfss.pdf [Accessed April 2012.
Schultes RE(1979). De plantis toxicariis e mundo novo tropicale commentationes XXI. Interesting native uses of the Humiriaceae in the northwest Amazon. J. Ethnopharmacol. 1(1):89-94.
Shahidi F, Naczk M (1995). Food Phenolics: Sources,Chemistry,Effects and Applications. Technomic Publishing Company Inc., Lancaster (Pennsylvania). Pp. 281-319.
Silva EJR, Aguiar FJS, Gonçalves ES, Souza IMV, Dimech GS, Fraga MCCA, Coelho MCOC, Wanderley AG (2005). Avaliação do tratamento subcrônico com o estrato hidroalcólico de *Calendula officinalis* L. sobre os parâmetros bioquímicos e hematológicos em ratas Wistar. Ver. Bras. Farmacogn. 15(2):88-93.
Silva SL, Oliveira VG, Yano T, Nunomura RCS (2009). Antimicrobial activity of bergenin from *Endopleura uchi* (Huber) Cuatrec. Acta Amaz. 39(1):187-191.
Silva SN, Abreu IC, Silva GFC, Ribeiro RM, Lopes AS, Cartagenes MSS, Freire SMF, Borges ACR, Borges MOR (2012). The toxicity evaluation of *Syzygium cumini* leaves in rodents. Rev. Bras. Farmacogn. 22(1):102-108.
Simões CMO, Schenkel EP, Gosmann, G, Mello JCP, Mentz LA, Petrovick PR (Org.) (2004). Farmacognosia: da planta ao medicamento. 5 ed. Porto Alegre/ Florianópolis: Editora UFRGS/Editora UFSC.
Takahashi H, Kosaka M, Watanabe Y, Nakade K, Fukuyama Y (2003). Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. Bioorg. Med. Chem. 11(1):1781-1788.
Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V (2002). A 90 day oral gavage toxicity study of D- methylphenidate and D, L-methylphenidate in Sprague Dawley rats. Toxicology 179:183-196.
Tofovic SP, Jackson EK (1999). Effects of long-term caffeine consumption on renal function in spontaneously hypertensive heart failure prone rats. J. Cardiovasc. Pharmacol. 33(3):360-366.
Turolla MSR, Nascimento ES (2006). Informações toxicológicas de alguns fitoterápicos utilizados no Brasil. Rev. Bras Ciên. Farm. 42(2):289-306.
Veiga-Júnior VF, Pinto AC (2005). Plantas medicinais: cura segura? Quim. Nov 28(3):519-528.