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TOXICOLOGICAL ACTIVITY EVALUATION OF *Cocos nucifera* L. IN EXPERIMENTAL MODELS

(Avaliação da atividade toxicológica de *Cocos nucifera* L. em modelos experimentais)

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ABSTRACT

The objective of this study was to evaluate the acute, subchronic and chronic toxicity of the liquid of green coconut husk fiber (LGCHF) and butanol extract obtained from LGCHF in mice and rats. In acute toxicity by oral route non-lethal effects were observed in mice at a 3.000 mg/kg dose in both extracts. But intraperitoneally, LGCHF and butanol extract were responsible for the deaths of all animals at doses of 500 and 700 mg/kg, respectively. In subchronic toxicity test, the rats treated with LGCHF showed white blood cell, neutrophils, red blood cell and platelets count in addition hematocrit significantly higher. The group treated with LGCHF, in the chronic toxicity test, showed higher values for white blood cell, neutrophils, basophils and platelets count ($P<0.05$). However, in subchronic and chronic toxicity any hematological parameters varied in the group treated with butanol extract ($P>0.05$). The biochemical analysis, only triglycerides were higher ($P<0.05$) in the group treated with LGCHF, during the chronic toxicity test. Rats treated with both extracts had no changes in histopathological analysis related to toxicity. Weight gain did not differ between treated and control groups ($P>0.05$). In conclusion, both extracts showed, for those parameters, low toxicity.
KEY WORDS: mice, rats, toxicity, *Cocos nucifera*.

RESUMO

O objetivo desse estudo foi avaliar a toxicidade aguda, subcrônica e crônica do líquido da casca do coco verde (LCCV) e do extrato butanólico do LCCV em camundongos e ratos. Na toxicidade aguda por via oral foram observados efeitos não-letais em camundongos na dose de 3.000 mg/kg de ambos os extratos. Porém, intraperitonealmente, o LCCV e o extrato butanólico foram responsáveis pela mortalidade de todos os animais nas doses de 500 e 700 mg/kg, respectivamente. No teste de toxicidade subcrônica, os ratos tratados com LCCV apresentaram contagem total de leucócitos, neutrófilos, hemácias e plaquetas, além de hematócrito significativamente maiores. O grupo tratado com LCCV, no teste de toxicidade crônica, apresentou valores maiores para contagem total de leucócitos, neutrófilos, basófilos e plaquetas ($p<0,05$). Entretanto, na toxicidade subcrônica e crônica,

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nenhum parâmetro hematológico variou no grupo tratado com o extrato butanólico (p>0,05). Na análise bioquímica, somente os triglicerídeos apresentaram valores maiores (p<0,05) no grupo tratado com LCCV, durante a toxicidade crônica. Os ratos tratados com ambos os extratos não apresentaram alterações histopatológicas relacionadas com toxicidade. O ganho de peso não diferiu entre os grupos tratados e controle (p>0,05). Em conclusão, ambos os extratos apresentaram, dentro dos parâmetros analisados, baixa toxicidade.
PALAVRAS-CHAVE: camundongos, toxicidade, ratos, *Cocos nucifera*.

INTRODUCTION

Cocos nucifera L. is a plant commonly found along the northeastern coast of Brazil. Most commonly, it is sought for the flavor and nutritional qualities of its water obtained from fruit (Senhoras, 2003). Medicinal uses for aqueous extracts of coconut fiber as tea have been reported (Esquenazi et al., 2002; Mendonça-Filho et al., 2004). The decoction coconut husk fiber is used in northeastern Brazil as a traditional medicine to treat diarrhea, arthritis, bleeding and hemorrhages (Duke, 1992). Popular medicine also uses the flesh of the green coconut to treat teniasis, schistosomiasis, and ancylostomiasis (Blini & Lira, 2005).

In recent years, it has been shown that aqueous extract of coconut husk fiber have antibacterial, antiviral (Esquenazi et al., 2002) and antiprotozoal activities (Calzada et al., 2007). In our laboratory, *in vitro* tests on *Haemonchus contortus* showed that liquid of green coconut husk fiber (LGCHF) and butanol extract obtained from LGCHF had maximum ovicidal effectiveness at concentrations of 2.5 and 10 mg/mL, respectively, and high larvicidal efficacy at concentrations of 65 and 80 mg/mL, respectively (personal communication). But there are no published studies in scientific literature on the toxicological profile of *C. nucifera* extracts.

Therefore, the purpose of this study was to evaluate the acute, subchronic and chronic toxicity of the LGCHF and butanol extract obtained from LGCHF in mice and rats.

MATERIAL AND METHODS

Plant material and extract preparation

The LGCHF provided by EMBRAPA/ Agroindústria Tropical was obtained from coconut collected in the fruit industry in the city of Fortaleza, Ceará, Brazil. The green coconut husk fiber was crushed, pressed and filtered to obtain the LGCHF. To obtain the butanol extract, the LGCHF was subjected to three washes with solvents in 10:1 proportion using a decantation funnel. Initially, ethyl acetate was used and then butyl alcohol (butanol). The butanol solvent was eliminated in a rotary evaporator to produce the LGCHF butanol extract. To perform the tests, the LGCHF and butanol extract were diluted in distilled water and 3% dimethylsulfoxide (DMSO), respectively.

Animals

Swiss albino mice, female, weighing 25-30g were used in acute toxicity tests. The subchronic and chronic toxicity tests were performed in rats, female, weighing 150-200g. Animals were housed in polyethylene cage with sterile wooden scraps. Mice and rats were kept at 25C° and fed standard pellets (Purina®) and water *ad libitum*. All procedures were approved by the Ethical Committee of Ceará State University (Process Number: 07227499-9).

Acute Toxicity

To evaluate acute toxicity of LGCHF were used 110 Swiss albino mice. The animals

were divided into 11 groups (n = 10): G1 - received distilled water by oral administration; G2 to G5 - 500, 1000, 2.000 and 3.000 mg/kg LGCHF orally; G6 - distilled water by intraperitoneal administration; G7 to G11 - 100, 200, 300, 400 and 500 mg/kg LGCHF intraperitoneally.

The acute toxicity evaluation of butanol extract used 110 Swiss albino mice divided into 11 groups (n = 10): G12 - received 3% DMSO by the oral route; G13 to G16 - 500, 1.000, 2.000 and 3.000 mg/kg extract butanol orally; G17 - 3% DMSO by the intraperitoneal route; G18 to G22 - 300, 400, 500, 600 and 700 mg/kg extract butanol intraperitoneally.

After each administration, the animals were observed for a period of 6 hours and the effects observed were recorded in the appropriate table for Hippocrates testing (Malone, 1948). After a period of 24 hours total of dead was computed and lethal dose (LD_{50} and LD_{10}) was calculated.

Subchronic Toxicity

LD_{10} estimated from the results obtained in acute toxicity was used in the study of subchronic toxicity. In this study, 24 rats were divided into the following groups (n = 8): G1 - received distilled water; G2 - 3% DMSO; G3 - LD_{10} of LGCHF; G4 - LD_{10} of butanol extract. The animals were treated for 30 consecutive days orally. For hematological and biochemical analysis, blood samples were collected in beginning (day 0) and at the end of the experiment (day 30). The weight gain of animals was assessed by weighing the animals on day 0 and day 30 of the experiment. At the end of the experiment, animals were sacrificed and their organs (kidney, heart, liver, spleen and lung) were collected and processed for histological analysis.

Chronic Toxicity

In this study, 24 rats were divided into the following groups (n = 8): G1 - received distilled water; G2 - 3% DMSO; G3 - LD_{10} of LGCHF; G4 - LD_{10} of butanol extract. The animals were treated for 60 consecutive days by oral route. Blood samples were collected at the beginning (day 0) and at the end of the experiment (day 60) for hematological and biochemical analysis. For evaluation of weight gain, the animals were weighed on day 0 and day 60 of experiment. At the end of the experiment, animals were sacrificed and their organs (kidney, heart, liver, spleen and lung) were collected and processed for histological analysis.

Hematological and biochemical analysis

For hematological tests, blood samples were collected through retro-orbital plexus puncture and then added into tubes with EDTA.

Hematological analysis was performed using an automatic hematological analyzer (CELL-DYN3700, Abbott Laboratories, Santa Clara, CA, USA). The parameters evaluated were: red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets count and white blood cell (WBC) count and differential leukocyte.

For biochemical analysis, blood was collected without anticoagulant with tab gel. The tubes were centrifuged at 3000 rpm for 5 min to obtain serum, which was stored at -20°C until determination of the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, triglycerides and electrolytes (Na^+, K^+ and Cl^-).

Histopathological analysis

The organs selected were removed, analyzed macroscopically and preserved in 10% formalin for histological analysis. Tissues were embedded in paraffin, sectioned, stained with

hematoxylin-eosin and examined microscopically.

Statistical analysis

Results are expressed as mean ± standard error of the means (SEM). Statistical comparisons between data for the control and treated groups were performed using Tukey test. P values less than 0.05 were set as the level of significance. The statistical program used for the analysis was the Graph Pad Prism 3.0.

RESULTS

Acute Toxicity

The animals treated with both extracts showed no clinical symptoms and mortality by oral route. Thus it was not possible to calculate lethal dose for this route of administration.

Intraperitoneally the LGCHF and butanol extract were responsible for deaths of all animals at doses of 500 and 700 mg/kg, respectively. DL_{10} and LD_{50} for LGCHF were 199.63 (101.71 - 249.52) mg/kg and 305.02 (240.79 - 368.20) mg/kg, respectively. DL_{10} and LD_{50} for LGCHF butanol extract were 247.32 (108.6 - 295.1) mg/kg and 321.3 (243.8 - 381.2) mg/kg. Both extracts were responsible for an increase in respiratory rate soon after the intraperitoneal administration. This change disappeared in minutes.

Subchronic Toxicity

Hematological Parameters

Hematological tests in animals treated with LGCHF are presented in Tab. 1. The parameters with values significantly higher were: WBC, RBC, neutrophils, platelets and HCt.

Animals treated with butanol extract showed no significant changes in the hematological parameters evaluated when compared with animals that received the vehicle (Tab. 2).

Table 1. Effect of the liquid of green coconut husk fiber (LGCHF) on the hematological parameters of rats treated for 30 days.

Parameters	Control Group		Treated Group	
	Day 0	Day 30	Day 0	Day 30
WBC (x 10^3cels/µL)	4.64 ± 0.48[a]	4.33 ± 0.39[a]	3.64 ± 0.44[a]	5.73 ± 0.45[b]
Neutrophils (x 10^3cels/µL)	1.41 ± 0.20[a]	1.08 ± 0.11[a]	0.93 ± 0.14[a]	2.01 ± 0.48[b]
Lymphocytes (x10^3cels/µL)	3.08 ± 0.34[a]	2.90 ± 0.26[a]	2.59 ± 0.29[a]	3.28 ± 0.22[a]
Monocytes (x 10^3cels/µL)	0.01± 0.0[a]	0.02 ± 0.0 [b]	0.01 ± 0.0[a]	0.02 ± 0.01[a]
Eosinophils (x 10^3cels/µL)	0.10 ± 0.03[a]	0.29 ± 0.11[a]	0.14 ± 0.09[a]	0.36 ± 0.10[a]
Basophils (x 10^3cels/µL)	0.05 ± 0.01[a]	0.05 ± 0.0[a]	0.14 ± 0.09[a]	0.07 ± 0.01[a]
RBC (x 10^6cels/µL)	6.66 ± 0.08[a]	6.42 ± 1.00[a]	6.69 ± 0.06[a]	7.61 ± 0.07[b]
Hb (g/dL)	13.38 ± 0.14[a]	14.86 ± 0.57[b]	13.26 ± 0.14[a]	15.35 ± 0.17[b]
HCt (%)	36.01± 0.39[a]	38.24 ± 5.99[a]	36.14 ± 0.45[a]	44.72 ± 0.58[b]
MCV (fL)	54.13 ± 0.47[a]	59.14 ± 0.62[b]	54.03 ± 0.34[a]	58.87± 0.26[b]
MCHC (g/dL)	37.14± 0.17[a]	34.10 ± 0.23[b]	36.69 ± 0.20[a]	34.10 ± 0.19[b]
Platelets (x10^3cels/µL)	835.13 ± 36.68[a]	715.75 ± 91.77[a]	819.63 ± 22.12[a]	920.67± 44.47[b]

Values are expressed as mean ± SEM. RBC: red blood cell count, Hb: hemoglobin, HCt: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell count. Small letters compare means between columns (P <0.05).

Table 2. Effect of butanol extract obtained from the liquid of green coconut husk fiber (LGCHF) on the hematological parameters of rats treated for 30 days.

Parameters	Control Group		Treated Group	
	Day 0	Day 30	Day 0	Day 30
WBC (x 10^3cels/µl)	5.42 ± 0.37[a]	2.41 ± 0.20[b]	5.07 ± 0.60[a]	2.43 ± 0.29[b]
Neutrophils (x 10^3cels/µl)	1.48 ± 0.33[a]	1.20 ± 0.15[a]	1.13 ± 0.17[a]	0.95 ± 0.08[a]
Lymphocytes (x10^3cels/µl)	3.68 ± 0.47[a]	1.06 ± 0.14[b]	3.70 ± 0.44[a]	1.15 ± 0.16[b]
Monocytes (x 10^3cels/µl)	0.08 ± 0.04[a]	0.07 ± 0.02[a]	0.03 ± 0.01[a]	0.15 ± 0.07[a]
Eosinophils (x 0^3cels/µl)	0.12 ± 0.02[a]	0.06 ± 0.01[a]	0.17 ± 0.02[a]	0.10 ± 0.03[a]
Basophils (x 10^3cels/µl)	0.05 ± 0.01[a]	0.02 ± 0.01[a]	0.05 ± 0.01[a]	0.07 ± 0.02[a]
RBC (x 10^6cels/µl)	7.10 ± 0.11[a]	6.74 ± 0.15[ab]	7.05 ± 0.12[a]	6.49 ± 0.09[b]
Hb (g/dl)	14.60 ± 0.17[a]	14.25 ± 0.09[a]	14.39 ± 0.17[a]	13.84 ± 0.19[a]
HCt (%)	39.62 ± 0.73[a]	37.53 ± 0.39[ab]	38.96 ± 0.59[a]	36.47 ± 0.44[b]
MCV (fl)	54.58 ± 0.84[a]	55.76 ± 0.85[a]	55.29 ± 0.26[a]	56.26 ± 0.33[a]
MCHC (g/dl)	36.52 ± 0.32[a]	38.00 ± 0.25[b]	36.96 ± 0.27[a]	37.94 ± 0.08[b]
Platelets (x10^3cels/µl)	805.9 ± 79.57[a]	755.6 ± 41.72[a]	837.6 ± 33.72[a]	877.1 ± 35.73[a]

Values are expressed as mean ± SEM. RBC: red blood cell count, Hb: hemoglobin, HCt: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell count. Small letters compare means between columns ($P < 0.05$).

Biochemical parameters

Tab. 3 and 4 show, respectively, the results of biochemical parameters examined for animals treated with LGCHF and butanol extract. The parameters showed no significant changes in relation to control post-administration of extracts.

Histopathological analysis and weight of animals

The analyzed organs in animals treated with extracts and their controls showed no morphological changes and structural related toxicity.

There was no significant difference in

Table 3. Effect of the liquid of green coconut husk fiber (LGCHF) on the biochemical parameters of rats treated for 30 days.

Parameters	Control Group		Treated Group	
	Day 0	Day 30	Day 0	Day 30
AST (UI/L)	89.63 ± 5.17[a]	67.38 ± 3.80[b]	103.75 ± 9.92[a]	78.17 ± 8.50[ab]
ALT (UI/L)	69.75 ± 3.49[a]	23.88 ± 1.82[b]	57.88 ± 3.82[a]	27.50 ± 2.59[b]
Creatinine (mg/dL)	0.71± 0.01[a]	0.89 ± 0.03[b]	0.73 ± 0.02[a]	0.83 ± 0.04[b]
FA (UI/L)	12.25 ± 3.76[a]	17.63 ± 1.19[b]	11.75 ± 5.97[a]	17.17± 2.12[b]
Triglycerides (mg/dL)	-	141.25 ± 22.86[a]	-	151.0 ± 26.50[a]

Values are expressed as mean ± SEM. AST: aspartate aminotransferase, ALT: alanine aminotransferase; FA: alkaline phosphatase. Small letters compare means between columns ($P < 0.05$).

Table 4. Effect of butanol extract obtained from the liquid of green coconut husk fiber (LGCHF) on the biochemical parameters of rats treated for 30 days.

Parameters	Control Group		Treated Group	
	Day 0	Day 30	Day 0	Day 30
AST (UI/L)	84.29 ± 18.65[a]	62.29 ± 4.90[a]	56.43 ± 4.62[a]	49.43 ± 2.04[a]
ALT (UI/L)	30.57 ± 11.58[a]	28.00 ± 3.93[a]	17.71 ± 1.15[a]	20.00 ± 0,65[a]
Creatinine (mg/dL)	0.43 ± 0.02[a]	0.61 ± 0.03[b]	0.48 ± 0.01[a]	0.60 ± 0.02[b]
FA (UI/L)	15.14 ± 1.56[a]	17.57 ± 1.84[a]	19.43 ± 2.30[a]	19.57 ± 3.24[a]
Na+ (mg/dL)	142.60 ± 0.97[a]	144.70 ± 0.52[a]	144.10 ± 0.40[a]	144.40 ± 1.31[a]
K+ (mg/dL)	4.43 ± 0.20[a]	4.29 ± 0.18[a]	4.14 ± 0.14[a]	4.14 ± 0.14[a]
Cl- (mg/dL)	106.30 ± 2.48[a]	112.70 ± 3.44[a]	109.10 ± 0.51[a]	112.00 ± 3.28[a]
Triglycerides (mg/dL)	128.90 ± 38.80[a]	156.60 ± 20.39[a]	69.71 ± 11.42[a]	85.57 ± 4.73[a]

Values are expressed as mean ± SEM. AST: aspartate aminotransferase, ALT: alanine aminotransferase; FA: alkaline phosphatase, Na+: sodium, K+: potassium Cl-: chlorine. Small letters compare means between columns (P <0.05).

weight gain comparing to treated and control groups (Tab. 5 and 6).

Tab. 7 shows hematological analysis of rats treated with LGCHF for 60 days. WBC, neutrophils, basophils and platelets of treated group had higher results (P<0.05). But the MCV was minor compared with the control (P<0.05). The other parameters did not differ statistically between the groups.

The butanol extract did not significantly change any hematological parameter (Tab. 8).

Biochemical parameters

Tab. 9 and 10 show, respectively, the results of biochemical parameters examined for animals treated with LGCHF and LGCHF butanol extract. The results with higher triglycerides (P<0.05) in the group treated with LGCHF was the only parameter that differed statistically when compared with control. The treated animals with butanol extract showed no change in the parameters examined in relation control (P>0.05).

Histopathological analysis and weight of animals

All the organs examined of rats treated with the extracts showed no morphological and structural changes that would indicate toxicity of extracts.

Tab. 11 and 12 show rat weight gains in groups treated with LGCHF and butanol extract respectively. Rats treated with both extracts showed no significant change in weight gain (P>0.05).

Table 5. Effect of the liquid of green coconut husk fiber (LGCHF) on rats weight treated for 30 days.

Weight (g)	Control Group		Treated Group	
	Day 0	Day 30	Day 0	Day 30
	183.13 ± 9.73[a]	201.25 ± 5.32[ab]	185.0 ± 5.09[a]	213.33 ± 5.58[b]

Values are expressed as mean ± SEM. Small letters compare means between columns (P<0.05).

Table 6. Effect of butanol extract obtained from the liquid of green coconut husk fiber (LGCHF) on rats weight treated for 30 days.

	Control group		Treated group	
Weight (g)	Day 0	Day 30	Day 0	Day 30
	217.40 ± 5.59[a]	222.50 ± 5.59[a]	204.80 ± 4.41[a]	211.30 ± 4.28[a]

Values are expressed as mean ± SEM. Small letters compare means between columns (P<0

DISCUSSION

In popular culture Brazilian says that "what is natural it does not matter", which leads many people to use the plants as drugs of choice, replacing the conventional medicines or as adjuvants, in a complementary therapy without the guidance adequate (Tavares, 2005). However we must not forget that the plants have active molecules that may have therapeutic efficacy, but also many adverse effects (Pereira, 1992). Moreover, it is not known due to dosage. So when the plants used in the wrong order may aggravate the state of health of the patient, or by not showing effectiveness in the light of adverse effects have not yet explained (Petrovick et al., 1997).

The importance of evaluating the toxicological activity of *C. nucifera* was due to the good results obtained in our laboratory relationship with the *in vitro* anthelmintic activity and the immunomodulatory activity in mice (data not shown).

In acute toxicity, the extracts administered by the oral route were not toxic at the doses used, indicating low toxicity.

Table 7. Effect of the liquid of green coconut husk fiber (LGCHF) on hematological parameters of rats treated for 60 days.

Parameters	Control Group		Treated Group	
	Day 0	Day 60	Day 0	Day 60
WBC (x 10^3cels/µl)	3.80 ± 0.38[a]	4.92 ± 0.37[a]	3.70 ± 0.44[a]	6.08 ± 0.79[b]
Neutrophils (x 10^3cels/µl)	1.65 ± 0.23[ab]	0.91± 0.07[a]	1.48 ± 0.16[ab]	2.03 ± 0.45[b]
Lymphocytes (x10^3cels/µl)	1.99 ± 0.33[a]	3.78± 0.38[b]	2.06 ± 0.35[a]	3.56 ± 0.31[b]
Monocytes (x 10^3cels/µl)	0.01± 0.00[a]	0.01± 0.00[b]	0.01± 0.00[a]	0.02 ± 0.00[b]
Eosinophils (x 10^3cels/µl)	0.11± 0.03[a]	0.15± 0.04[a]	0.10 ± 0.07[a]	0.38 ± 0.19[a]
Basophils (x 10^3cels/µl)	0.05 ± 0.01[a]	0.07± 0.01[a]	0.05 ± 0.01[a]	0.09 ± 0.01[b]
RBC (x 10^6cels/µl)	5.94 ± 0.18[a]	7.09 ± 0.07[b]	5.75± 0.16[a]	6.96 ± 0.08[b]
Hb (g/dl)	12.83 ± 0.52[a]	15.71± 0.16[b]	12.36 ± 0.52[a]	15.34 ± 0.12[b]
HCt (%)	33.18 ± 1.26[a]	41.25± 0.50[b]	32.10± 1.10[a]	40.0 ± 0.26[b]
MCV (fl)	55.79± 0.65[a]	58.15 ± 0.61[b]	55.79 ± 0.57[a]	57.47± 0.47[a]
MCHC (g/dl)	38.66 ± 0.24[a]	38.10 ± 0.19[a]	38.40 ± 0.30[a]	38.37± 0.20[a]
Platelets (x10^3cels/µl)	947.63±49.07[a]	731.50±43.29[b]	922.88 ± 50.74[a]	811.86 ± 25.13[a]

Values are expressed as mean ± SEM. RBC: red blood cell count, Hb: hemoglobin, HCt: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell count. Small letters compare means between columns (P <0.05).

Table 8. Effect of butanol extract obtained from the liquid of green coconut husk fiber (LGCHF) on hematological parameters of rats treated for 60 days.

Parameters	Control group		Treated group	
	Day 0	Day 60	Day 0	Day 60
WBC (x 10^3cels/µl)	4.91 ± 0.52^a	3.10 ± 0.20^b	5.20 ± 0.76^{ab}	3.87 ± 0.71^{ab}
Neutrophils (x 10^3cels/µl)	1.07 ± 0.24^a	1.19 ± 0.13^a	1.10 ± 0.24^a	1.73 ± 0.46^a
Lymphocytes (x10^3cels/µl)	3.56 ± 0.32^a	1.49 ± 0.13^b	3.78 ± 0.51^a	1.76 ± 0.28^b
Monocytes (x 10^3cels/µl)	0.02 ± 0.01^a	0.19 ± 0.06^a	0.05 ± 0.03^a	0.15 ± 0.12^a
Eosinophils (x 10^3cels/µl)	0.20 ± 0.04^a	0.13 ± 0.02^a	0.20 ± 0.09^a	0.17 ± 0.06^a
Basophils (x 10^3cels/µl)	0.05 ± 0.01^a	0.10 ± 0.03^a	0.06 ± 0.02^a	0.07 ± 0.04^a
RBC (x 10^6cels/µl)	7.02 ± 0.21^a	6.62 ± 0.09^a	7.33 ± 0.18^a	7.00 ± 0.14^a
Hb (g/dl)	14.40 ± 0.27^a	14.38 ± 0.11^a	14.58 ± 0.19^a	14.65 ± 0.17^a
HCt (%)	39.28 ± 0.90^a	37.98 ± 0.23^a	40.50 ± 0.59^a	38.95 ± 0.81^a
MCV (fl)	56.05 ± 0.57^a	57.39 ± 0.61^a	55.28 ± 0.69^a	55.68 ± 0.62^a
MCHC (g/dl)	36.68 ± 0.27^a	37.84 ± 0.12^b	36.00 ± 0.07^a	37.68 ± 0.30^b
Platelets (x10^3cels/µl)	715.9 ± 75.94^a	813.4 ± 28.51^a	857.3 ± 62.32^a	797.8 ± 22.72^a

Values are expressed as mean ± SEM. RBC: red blood cell count, Hb: hemoglobin, HCt: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell count. Small letters compare means between columns ($P < 0.05$).

Substances are considered of low toxicity and safe when the LD_{50}, orally, is 1.000 mg/kg (Clarke & Clarke, 1977). In this work the LD_{50} cannot be calculated for the oral route, as the largest dose, 3.000 mg/kg, no deaths occurred from any animal, indicating that the LD_{50} is greater than 1.000 mg/kg. The same did not happen when the extracts were administered by the intraperitoneal route, which had LD_{50} less than the oral, therefore representing greater toxicity. This is because the oral administration of a substance is less toxic than its administration by intraperitoneal route, since orally may occur a poor absorption or detoxication through of passage by liver, whereas intraperitoneally there is the systemic absorption and thus the toxic effects appear to be more intense and early (Loomis, 1996; Obici et al., 2008).

Subchronic and chronic toxicity in the extracts induced no serious changes in the blood cells counting. Rather, the values of WBC, neutrophils, RBC, HCt and platelets had higher values in the group treated with LGCHF. These results are important because the blood is an important parameter of the toxicology studies, as the hematopoietic system is extremely sensitive to activities of toxic agents, particularly those with potential mutagenic or cytotoxic, resulting in qualitative or quantitative changes, ie, leukopenia , leukocytos temporary or permanent and can limit the use of drugs (Leão et al., 2005). Hematological changes may reflect, too, in immune activity by lymphopenia and neutropenia, for example (Oliveira et al., 2001). The results demonstraded that there is a possible immunomodulatory activity since the animals treated with the extracts showed count of neutrophils and lymphocytes higher than the control group, but the why of the changes, is still unknown.

Biochemical parameters in subchronic toxicity have not changed with the administration of the extracts, indicating absence of toxicity during the period of administration. The lack of significant changes in liver parameters (AST, ALT) indicates that the liver function was preserved. The same happened with normal levels of creatinine indicate that the extracts did not affect the renal function and integrity (Kaneco, 1989).

The changed amount of triglycerides indicates the interference of LBGC in lipid metabolism or transport. The possible mechanisms involved can be the change in the composition of lipoproteins, changes in the capture of LDL, change in the rate of liver production of HDL/VLDL, or changes in the conversion of VLDL/LDL (Rodrigues et al., 2006).

The monitoring of the animal's body mass is an important indicator for assessing the toxicity of a substance (Jahn & Günzel, 1997; Teo et al., 2002). The lack of statistical difference between the weights of the groups is another parameter that indicates low toxicity of the two extracts studied.

In conclusion, the extracts were, within the parameters, low toxicity and can be used in experiments with the aim of finding potential clinical and medical applications.

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