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Full Length Research Paper

Acute and sub-chronic toxicity study of the extract and powder of *Operculina macrocarpa* (L.) Urb. in mice

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The aim of the study was to evaluate the experimental acute and sub-chronic toxicities of *Operculina macrocarpa* with plant extract and powder, respectively, in male and female mice. Phytochemical prospection was performed with extract and administration in single doses by intraperitoneal route to six groups and control at 1230, 970, 700, 350, 120 and 30 mg/kg doses and distilled water (0.1 ml/10 g), respectively. Sub-chronic doses of 1230, 700, 30 mg/kg/day and satellite (1230 mg/kg/day) were administered orally in feed. Major endpoints included alterations in the central and autonomic nervous system, water and food intake, body weight, hematological and biochemical parameters. Phytochemical screening identified compounds: Alkaloids, flavonoids, xanthones, leucoanthocyanidins and tannins condensate. In the acute study, mortality was observed with toxicity signs to the central nervous system (CNS) at LD_{50} of 270 mg/kg. There were no significant changes in water and food intake, body weight, haematological and biochemical parameters, and histopathological examination in the sub-chronic study (p value). Results indicate that the oral administration of *O. macrocarpa* powder in feed is less toxic and relatively safer.

Key words: Mice, *Operculina macrocarpa*, hematological parameters, toxicity.

INTRODUCTION

Operculina sp. belongs to Convolvulaceae family, including 55 genera and approximately 1930 species (Austin, 1997) and spread worldwide (Heywood, 1993). In Brazil, *Operculina* sp. is found in several states with many different popular names, such as Jalapa do Brasil, Batata de Purga (a reference to one of its popular uses, as a laxative), Ipu, Purga de Amaro Leite, Briônia da América, Jalapa de São Paulo, Escamonéia da América

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and Xalapa (Planchon et al., 1937). The name of this family is derived from the Latin word *convolvo*, meaning intertwine, and it refers to the way this plant grows because a large number of these plant species are fickle climbing plants that grow and tangle as a support (Pereda-Miranda and Bah, 2003). One of the most remarkable features of Convolvulaceae is the presence of rows of cells that secrete resin glycosides in foliar tissues and in the roots. These resins are one of the chemotaxonomic characteristics of this family, and the use in traditional medicine of some of its genera (*Convolvulus*, *Exogonium*, *Ipomoea*, *Merremia* and *Operculina*) is associated with the purgative properties of their resins (Pereda-Miranda et al., 2006).

Popularly known as batata de purga, *Operculina macrocarpa* (L.) Urb. is commonly found in northeastern Brazil (Matos, 1982). It presents tuberous large starchy milky roots, and it can be easily purchased for medicinal purposes. This species is a climbing ornamental plant mainly because of its fruits. Each fruit contains one to four black hard seeds. *O. macrocarpa* is a biennial species, that is, its aerial part dies every two years, presenting palmatiforme leaves, white flowers and rounded fruits (Lorenzi and Matos, 2002). Batata de purga is widely used by the local populations because of its laxative, purgative and depurative effects on skin diseases and in the treatment of leucorrhea (Belizário, 2012). Despite being listed in pharmacopoeias (Brazilian Pharmacopoeia 1929 and 1959), its phytochemical study is still incomplete.

It contains starch and 12% resin, which is formed by a complex mixture of substances such as glycoside polymers, of purgative effect, and it is known for its laxative property or, in higher doses, for its drastic purgative and anthelmintic effects (Araujo et al., 2012). All homemade or pharmaceutical preparations of batata de purga should be used with caution because in doses higher than the recommended, they can cause severe intoxication, translate into strong cramps and intense diarrhea with risk of rapid dehydration (Lorenzi and Matos, 2002).

The use of medicinal plants and their derivatives have been quite significant in recent years. However, popular and even traditional uses are not sufficient to ethically validate medicinal plants as effective and safe medicines (Agra et al., 2008). Toxicological studies aims to assess the erroneous idea that herbal products, because they are natural, are devoid of toxic or adverse effects, and the popular use of medicinal plants serves as validation of the effectiveness of these medicines (Silveira et al., 2008). Thus, considering the ethnopharmacological reasons and toxicological information available, the toxicological study of medicinal plants is essential regardless of the pharmacological results. Therefore, the purpose of this study was to assess the experimental acute toxicity of the extract and sub-chronic toxicity of the powder of *O. macrocarpa* in mice.

MATERIALS AND METHODS

Plant material and phytochemical extraction

The tubercles of *O. macrocarpa* were collected in the municipality of Patos, Paraiba State, northeastern Brazil, located at the geographic coordinates S37°17'36.4" W07° 04'03.0". The plant material was identified by D.Sc. Maria Teresa Buril, Federal University of Pernambuco, and deposited at the Center for Rural Health and Technology Herbarium, Federal University of Campina Grande (UFCG) authenticated under specimen number 3106.

Phytochemical prospection was carried out with the ethanol extract of *O. macrocarpa* submitted to a series of tests using specific reagents aiming at the elucidation of classes of secondary metabolites. This method is based on the visual observation of colorimetric variation and/or intensification and/or precipitate formation, after addition of specific reagents in the sample solutions. For assay performance, 300 mg of the ethanol extract of *O. macrocarpa* were diluted in 30 ml of ethanol 70%. After that, 3 ml aliquots were distributed in test tubes and the assay was carried out according to the method described by Matos (1997).

Acute toxicity study in mice

The experimental protocol for the assessment of acute toxicity was guided mainly by the Specific Resolution (RE 90/2004) of the National Agency for Sanitary Surveillance (Brazil, 2004) and by Michelin (2008). This study was in accordance with the accepted guidelines for animal experimentation under number 64/2012.

For the preparation and quantification of the extract, the tubercles were collected, cut in approximately 1 cm thick slices, shade dried and then ground in knife mills to obtain the powder. Two hundred grams of the obtained powder was weighed and dried in forced ventilation oven at 55°C for 24 h, it was then macerated in 1 L of ethanol 96°GL (C_2H_6O) for 72 h with sporadic homogenization before filtration with filter paper (Matos, 1997).

After this process, 200 ml of the liquid obtained through filtration was placed in a rotary evaporator for concentration, where 16 ml of ethanol extract (EE) was obtained and concentration was determined according to Matos (1997). A concentration of 3.52 mg/ml was found, which was later used for the calculation of the dose to be administered to the mice by intraperitoneal route.

All animals used in this study were healthy 45-day-old outbred Swiss mice (*Mus musculus*), of either sex, weighing between 22 and 44 g. Animals were segregated according to gender, housed in polypropylene cages, and kept in a room with dark/light cycle (12/12 h) and 28±3°C average temperature with water and food *ad libitum* at the Center for Breeding and Experimentation with Laboratory Animals, Federal University of Campina Grande (UFCG). The ethanol extract of *O. macrocarpa* was administered by intraperitoneal route to groups of 12 animals each (6 male and 6 female mice), which were initially five groups, with standardized volume for dose calculation of 10 ml/kg (0.1 ml/10 g) for all groups. The average doses of 1230, 970, 700 and 350 mg/kg were administered to groups G1, G2, G3 and G4, respectively, and the control group (CG) was treated with distilled water (0.1 ml/10 g), all in single doses. Subsequently lower concentrations of the extract were used for two groups: G5 and G6 received average doses of 120 and 30 mg/kg, respectively.

Animals were examined at times 0, 15, 30 and 60 min; 4, 8, 12 and 24 h; and daily for 14 days post-treatment. Animals were observed for general behavioral changes regarding signs of toxicity according to the methodology adapted from Mariz (2007) and Atsamoa et al. (2011) aiming to identify possible alterations in the central nervous system (CNS) and autonomic nervous system (ANS) using the following parameters: general behavior, response to touch and tail squeezing, twisting, straightening, body tone,

ataxia, tremors, hypnosis, lacrimation, ptosis, micturition, diarrhea, piloerection, respiration, cyanosis and drooling. Animal mortality was recorded to determine the lethal dose (LD_{50}) according to Litchfield and Wilcoxon (1949).

Sub-chronic toxicity study in mice

All procedures used for sub-chronic toxicity assessment followed the same protocol mentioned for the acute toxicity evaluation as well as that by Lagarto et al. (2011). Animals were acclimatized for at least one week prior to toxicity testing. Before feed formulation, the average daily food consumption of each group was calculated for determination of each component percentage. The components of feed formulation were distributed as follows: G1 (control) received commercial feed (Presence® animal nutrition, Paulínia, SP, BRA) *ad libidum*; G2, G3 and G5 received 245.7, 255.3 and 244.5 g of commercial feed; 24.3, 14.7 and 25.5 g of *O. macrocarpa* powder; and 30, 30 and 30 g of corn starch, totaling 300 g of feed; G4 received 349 g of commercial feed; 1 g of *O. macrocarpa* powder; and 150 g of corn starch, totaling 500 g of feed. Manual preparation of feed was carried out by previously grinding the commercial feed in a knife mill in the amounts calculated according to the doses established for each group, *O. macrocarpa* powder was then proportionally added so that the animals could consume daily the previously calculated doses. This mixture was homogenized in a manual mixer, adding 200 ml of water and the corn starch to obtain proper consistency, and was then pelleted by hand using 20 ml disposable syringes. After preparation, the feed was shade dried at room temperature (Benício, 2008). Fifty healthy 2-month-old Swiss (*Mus musculus*) mice, of either sex, weighing 22 to 44 g were previously acclimatized for five days before the conduction of the biological assays and kept as cited for the acute toxicity study. Animals were divided into five groups of 10 (5 males and 5 females). The groups were named and received treatments as follows: G1 (control)– only commercial feed; G2- addition of 24.3 g of *O. macrocarpa* powder to obtain the expected average daily consumption of 1230 mg/kg; G3- addition of 14.7 g of plant powder for the expected average daily consumption of 700 mg/kg; G4- addition of 1 g of plant extract for the expected average daily consumption of 30 mg/kg and Group G5 (satellite) had 25.5 g of *O. macrocarpa* powder added to the feed to obtain the expected average daily consumption 1230 mg/kg. The satellite group remained under study for additional 15 days after the end of the 30-day experiment period for assessment of eventual reversibility of the effects of toxicity and emergence of late effects produced by the powder administered with highest dose. The doses used for the sub-chronic study were obtained from the acute testing: the lowest, the highest, and an approximate intermediate dose (Brasil, 2004). The parameters to be evaluated were distributed among those assessed daily and weekly every five days. Animals were observed for possible changes in the CNS and ANS by the same methodology used for the acute toxicity study.

Water and food was provided as pellets from the first testing day (day 0), providing graduated filled bottles. The average volume consumed by the mice was registered on the following day and the feed was weighed daily and accounted for on the day after. The weight gain curve was drawn weekly using the calculated 5-day weekly total weight of each group. After 30 days of experiment, animals from the treated and control groups fasted for 12 h prior to blood collection. Blood samples were then collected from 40% of the surviving animals (Mariz, 2007), by decapitation and cervical traction (Atsamoa et al., 2011). Blood from each animal was placed into properly identified tubes (Labor Import, São Paulo, SP, BRA) containing ethylenediaminetetraacetic (EDTA) acid for assessment of haematological parameters: red blood cell (RBC) count; determination of hematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC); leukocyte, lymphocyte and neutrophil counts (Jain, 1993). For biochemical analysis, serum was obtained for determination of alanine aminotransferase (ALT), total proteins (TP), and albumin (Jain, 1993). Analyses were carried out immediately after collection in commercial veterinary laboratory according to the protocol of specific commercial kits (Labtest® Diagnóstica S.A. Lagoa Santa, MG, BRA) and semi-automatic analyzer (BIO-200® Products Laboratories Ltda, SP, BRA).

For histopathological analysis, blood was collected from the same animals (40% of each group) randomly chosen (Mariz, 2007). They were subjected to necropsy and had their viscera: heart, lung, kidney, liver, stomach, large and small intestines, collected (Brazil, 2004). The viscera were immersed in fixative solution of 10% buffered formaldehyde; after fixation, they were subjected to histological processing and staining with hematoxylin-eosin. The satellite group animals were euthanized 15 days after the sub-chronic treatment for evaluation of hematological, biochemical and histopathological findings.

Statistical analysis

All results are presented as mean ± standard deviation. Before selecting the statistical test for intergroup comparison, the Shapiro-Wilk normality test was performed. The variables presenting normal distribution were used in the analysis of variance (ANOVA), with multiple comparisons assessed by the Tukey test; and the variables presenting abnormal distribution were analyzed by the Kruskal-Wallis test, with multiple comparisons by the Dunn test (Zar, 1999). Comparison of treatment times (weeks) was performed using the Friedman test. Differences were considered statistically significant at $P \leq 0.05$ using the SPSS 13.0 for Windows and BioEstat 5.03 software.

RESULTS

Phytochemical prospection

The phytochemical prospection of the ethanol extract of *O. macrocarpa* revealed some classes of compounds such as alkaloids, flavonoids (flavanones, flavones and flavonoid), xanthones, leucoanthocyanidins and tannins condensate.

Acute toxicity study

Intraperitoneal administration of EE at 1230, 970 and 700 mg/kg doses produced treatment related effects from the first minutes, and after one hour at the dose of 350 mg/kg, all followed by mortality and with no sign of effects over time. The dose of 120 mg/kg caused two deaths while the dose of 30 mg/kg and the control caused neither death nor alterations (Table 1). The median lethal dose (LD_{50}) was 270 mg/kg, varying from 160 to 470 mg/kg.

Sub-chronic toxicity study

Oral administration of *O. macrocarpa* powder in feed in

Table 1. Acute toxicity of the ethanol extract of *Operculina macrocarpa* (L.) Urb. administered by intraperitoneal route in mice.

Dose (mg/kg)	M/T	Latency	Signs (parameters)
G 1= 1230	12/12	< 1 h	↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone
G2 = 970	12/12	< 1 h	↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone
G3 = 700	12/12	< 1 h	↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone
G4 = 350	8/12	>1 h <24 h	↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone
G5 = 120	2/12	>12 h<24 h	Sudden mortality
G6 = 30	0/12	-	None
G control l	0/12	-	None
LD_{50} = 270 (mg/kg); Confidence limit of 95% = 470 - 160 (mg/kg)			

The ethanol extract of *O. macrocarpa* tubercle was administered by intraperitoneal route to groups of 12 mice (6 males and 6 females). All treated animals were observed for 14 days for signs of toxicity (changes in behavior and mortality). M/T, mortality/treated mice; none, no signs of toxicity were observed during the study period; latency, time period within which signs of toxicity are shown; ↓, reduction.

Table 2. Water intake (ml) of mice treated orally with *O. macrocarpa* powder added to feed for 30 days.

Weeks	Control	1230 (mg/kg/day)	700 (mg/kg/day)	30 (mg/kg/day)	Satellite - 1230 (mg/kg/day)
Males					
1	46 ± 16.0	38 ± 16.0	54 ± 28.4	56 ± 30.0	88 ± 33.4
2	44 ± 17.0	40 ± 19.0	46 ± 15.0	42 ± 17.0	84 ± 23.2*
3	46 ± 14.0	38 ± 12.0	38 ± 12.0	47 ± 30.0	89 ± 7.30*
4	37 ± 15.4	31 ± 20.0*	35 ± 15.1*	38 ± 17.3	76 ± 25.2*
Females					
1	57 ± 23.2	40 ± 35.1	35 ± 20.0	66 ± 16.2	60 ± 23.0
2	95 ± 12.5	63 ± 34.0	36 ± 31.0*	61 ± 17.4	113 ± 35.0
3	62 ± 30.1	37 ± 19.0	28 ± 16.4	69 ± 30.0	68 ± 27.0
4	62 ± 16.3	32 ± 20.0*	41 ± 17.3*	53 ± 8.0	69 ± 27.0

Values represent the mean ± standard deviation of 10 animals (5/gender). *p <0.05, significantly different from the control.

the orm of pellets did not produce behavioral changes or mortality in the groups studied.

There was no statistically significant difference ($p > 0.05$) in water and food intake in the course of time for all the groups assessed, for both male and female mice (Tables 2 and 3). Comparison between the treated and control groups over time showed significant differences in the water intake of male mice during the second and third weeks between the satellite (1230 mg/kg/day) and the control, and in the fourth week between the two highest doses (1230 and 700 mg/kg/day) and between the satellite and the control. With respect to the female mice, there were statistical differences in the second week between the dose of 700 mg/kg /day and the control and in the fourth week between the two highest doses when compared with the control (Table 2).

Statistically significant differences ($p<0.05$) were observed in the food intake of male mice in the second week between the 700 mg/kg/day dose and the satellite when compared with the control; in the third week between the 700 and 30 mg/kg/day doses when compared with the control; in the fourth week between the 700, 30 mg/kg/day and the satellite when compared with the control. Regarding the female mice, there were differences only in the second week between the dose of 1230 mg/kg/day and the satellite when compared with the control (Table 3).

The body weight of mice presented variations between the groups observed along the four weeks. The final assessment at the end of the fourth week showed statistically significant difference ($p < 0.05$) of total weight between the 700 and 30 doses and the satellite (1230

Table 3. Food intake (g) of mice treated orally with *O. macrocarpa* powder added to feed for 30 days.

Weeks	Control	1230 (mg/kg/day)	700 (mg/kg/day)	30(mg/kg/day)	Satellite - 1230 (mg/kg/day)
Males					
1	23 ± 13.2	22 ± 12.5	21 ± 6.0	26 ± 6.2	26 ± 13.0
2	15 ± 2.1	13 ± 2.5	27 ± 5.0*	21 ± 12.0	22 ± 5.0*
3	16 ± 4.0	14 ± 5.4	23 ± 2.0*	28 ± 9.0*	21 ± 5.0
4	14 ± 2.1	15 ± 2.0	23 ± 1.3*	21 ± 3.0*	22 ± 5.0*
Females					
1	24 ± 6.3	20 ± 12.0	25 ± 15.0	35 ± 19.1	33 ± 11.3
2	29 ± 6.0	18 ± 2.3*	20 ± 8.4	34 ± 15.2	36 ± 11.0*
3	23 ± 3.5	19 ± 3.3	21 ± 6.0	34 ± 19.1	34 ± 12.1
4	22 ± 1.3	18 ± 3.0	18 ± 4.0	20 ± 12.0	27 ± 11.0

Values represent the mean ± standard deviation of 10 animals (5/gender). *p <0.05, significantly different from the control.

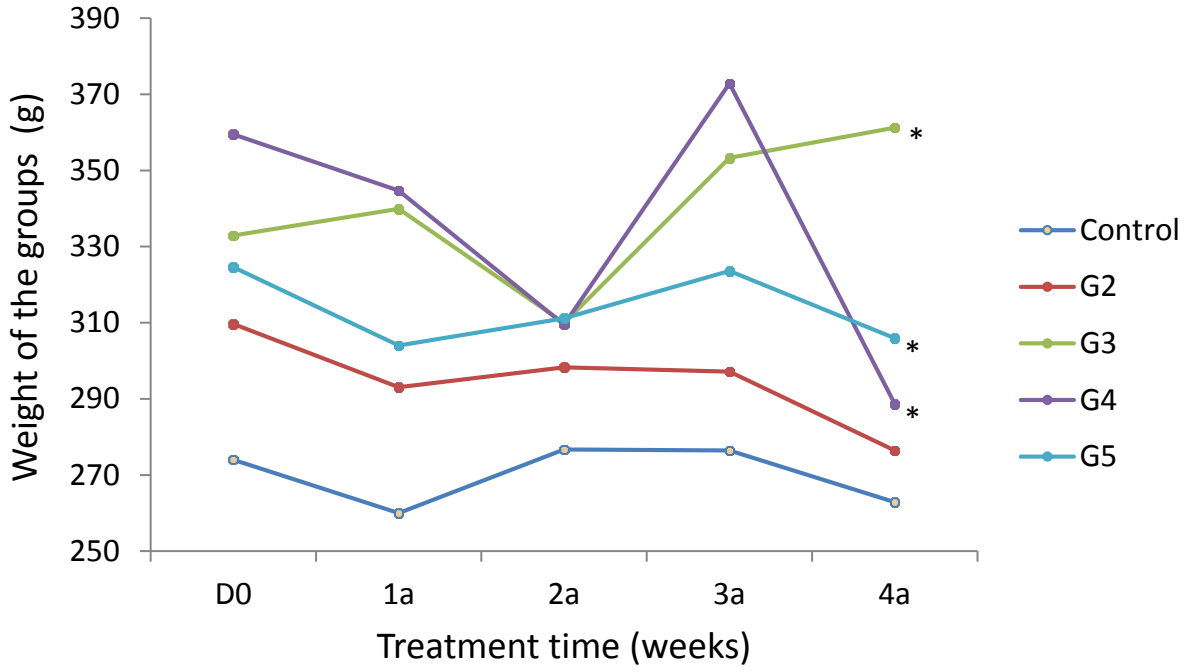


Figure 1. Relative weight of the mice treated orally with *O. macrocarpa* powder added to feed (30-1230 mg/kg/day) for four weeks. Each point represents the total weight of the group at the end of each week, n=10 (5/gender). *p <0.05, significantly different from the control group.

mg/kg/day) in comparison with the control, mainly in the males with no significant difference in the female mice (Figure 1).

Oral sub-chronic administration of *O. macrocarpa* powder to feed for 30 days did not cause any change in the haematological profile (erythrocytes, hematocrit, haemoglobin, MCV, MCH, MCHC, leukocytes, lymphocytes and neutrophils) of the treated groups; all parameters were within the physiological range of reference according to Jain (1993) throughout the treatment period (Table 4).

There were no statistically significant differences ($p > 0.05$) between treatments regarding the biochemical parameters evaluated. Parameters were within the physiological range of reference according to Thrall et al. (2006) for alanine aminotransferase (ALT). They showed a slight increase in relation to the 7 g/dl limit according to Thrall et al. (2006), reaching 8 g/dl in the treatment with the smallest dose (30 mg/kg/day) for total proteins (TP). They presented a small reduction in relation to the inferior

Table 4. Selected haematological and biochemical parameters of the sub-chronic study of groups treated with *O. macrocarpa* powder added to feed for 30 days.

Parameter	Control	1230 (mg/kg/day)	700 (mg/kg/day)	30 (mg/kg/day)	Satellite
Erythrocytes (×10^6/µl)	8.38 ± 0.85	8.68 ± 0.70	8.73 ± 0.22	9.00 ± 1.10	8.88 ± 0.83
Haemoglobin (g/dl)	13.3 ± 0.96	13.0 ± 0.62	13.9 ± 0.42	14.4 ± 1.25	14.8 ± 1.34
Hematocrit (%)	39.1 ± 0.64	38.9 ± 1.03	40.3 ± 1.32	41.6 ± 5.10	41.3 ± 3.86
MCV (fl)	45.6 ± 1.11	45.8 ± 1.38	46.6 ± 1.14	46.3 ± 1.00	46.4 ± 0.46
MCH (pg)	16.0 ± 0.31	15.4 ± 0.52	15.9 ± 0.43	15.7 ± 0.50	15.9 ± 0.47
CHCM (%)	33.1 ± 2.85	33.6 ± 0.88	33.7 ± 1.24	34.0 ± 1.00	34.1 ± 0.81
Leucocytes (×10^3/µl)	7.50 ± 0.49	7.00 ± 1.83	7.58 ± 1.64	7.30 ± 2.70	8.50 ± 1.91
Neutrophils seg. (%)	23.3 ± 5.38	25.0 ± 3.56	30.3 ± 3.70	35.0 ± 12.4	26.0 ± 4.12
Lymphocytes typ (%)	77.0 ± 6.10	75.5 ± 4.20	71.0 ± 5.00	63.8 ± 14.2	70.0 ± 5.60
ALT (Ul/L)	94 ± 65.0	181 ± 108	104 ± 62	90 ± 39	116 ± 23
TP (g/dL)	7.23 ± 0.50	7.95 ± 0.82	7.43 ± 0.68	8.00 ± 0.78	6.83 ± 0.94
Albumin (g/dl)	2.41 ± 0.56	2.37 ± 0.61	2.78 ± 0.17	2.59 ± 0.25	2.47 ± 0.30

Values represent the mean ± standard deviation of 40% of the group (10 animals/group). MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; ALT, alanine aminotransferase; TP, total protein; seg., segmented; typ, typical. There was no statistically significant difference between treatments.

limit of 3 g/dl according to Thrall et al. (2006), reaching 2.37 g/dl in the group treated with the highest dose (1230 mg/kg/day) for albumin (Table 4). Histopathological assessment of tissue samples collected showed no abnormality for all the treated groups.

DISCUSSION

O. macrocarpa popularly known as batata de purga, is widely used by the local populations because of its laxative, purgative and anthelmintic effects (Lorenzi and Matos, 2002), and the latter has been proved by studies to be effective in the control of gastrointestinal parasites of goats, *in vitro* using the extract (Gomes et al., 2010) and *in vivo* using the powder (Silva et al., 2010). However, in high doses, its use can cause severe toxicity, translated into strong cramps and intense diarrhea with risk of rapid dehydration (Lorenzi and Matos, 2002). Various therapeutic applications are attributed to the metabolites found in the phytochemical prospection of *O. macrocarpa* such as tannins, which provide antimicrobial and antifungal effects by protein precipitation (Vieira et al., 2015), and several antioxidant activities are conferred to flavonoids (Karimi and Moradi, 2015).

The Convolvulaceae family presents a great diversity of metabolites, but the glycoresins and phenolic substances are characteristic of this family. This family also presents secondary metabolites of low molecular weight containing nitrogen groups such as: ergolines, pyrrolidines, lipophilic and hydrophilic tropanes, indolizidine and pyrrolizidine alkaloids, cyanogenic glycosides, and different types of flavonoids and amides (Michelin, 2008).

In the acute toxicity study, the extract of this plant administered by intraperitoneal route caused mortality at higher doses with signs of toxicity that affect the central nervous system (CNS), presenting a LD_{50} of 270 mg/kg, of very high toxicity by this route according to Stacey (1993). The intraperitoneal route (which cannot be used in humans) is usually selected to determine the inherent toxicity of chemicals because the effects of an oral dose are subject to systemic bioavailability and hepatic detoxification, and acute dose study provides guidance for the selection of doses for the study of sub-chronic doses, which may be more clinically relevant (Li et al., 2010).

O. macrocarpa seems to be less toxic when administered orally, as demonstrated by Michelin (2004) using the gross extract at the dose of 3800 mg/kg, presenting moderate toxicity; and by Stacey (1993) using the powder >5000 mg/kg, presenting slight toxicity; and yet by the present sub-chronic study using the powder added to feed at the dose of 1230 mg/kg/day obtained by the acute study for thirty days, not presenting mortality or clinical signs of toxicity. According to toxicology, any substance can be considered a toxic agent depending on the exposure conditions, such as the dose administered or absorbed, time and frequency of exposure, and the process by which it is administered. The toxicity of a substance can be considered as the ability to cause serious injury or death (Ruppenthal, 2013).

In the evaluation of water and food intake, no statistically significant differences ($p>0.05$) were observed in the groups treated over time, even with the highest dose, confirming that, orally and in the form of powder added to the feed, *O. macrocarpa* did not produce toxic effects that could reduce consumption during the 30-day study. Nevertheless, there were some differences between the treated groups when compared

with the control for both males and females, and between the high, intermediate and low doses. This probably occurred because experimental animals exhibit variations depending on environmental factors, diet, and biotherium conditions, according to Harkness and Wagner (1993).

Variations occurred over time in the assessment of the total body weight (n = 10) of treated groups; however, after four weeks, the weights of the groups that received the powder added to the feed, mainly the dose of 1230 mg/kg/day and the satellite (1230 mg/kg/day), were higher than the control. This corroborates the results of Michelin (2004), who found no significant difference in the weight of animals treated with the powder. The body weight changes have been used as an indicator of adverse effects of drugs and chemicals and Li et al. (2010) demonstrated the non-toxicity of *O. macrocarpa* considering that it did not affect the body weight of treated animals after 30 days of daily consumption.

According to Li et al. (2010), the hematopoietic system is one of the most sensitive targets of toxic chemicals and an important indicator of physiological and pathological state in humans and animals. In the present study, the data of hematological parameters showed no differences ($p > 0.05$) between the treated and control groups. These results indicate that the powder of *O. macrocarpa* added to the feed for 30 days had no effect on the circulating blood cells, or on their production.

In most rodents, the serum activity of ALT increases with hepatocellular damage, where it seems to be specific for rat and mice liver (Thrall et al., 2006) and as a good indicator of hepatic function as biomarkers predicting possible toxicity (Li et al., 2010). In general, any damage to the parenchymal liver cells results in the increase of this transaminase in blood. In this study, ALT remained within the physiological range for mice, suggesting that the sub-chronic administration of *O. macrocarpa* powder added to the feed for 30 days did not influence the function and metabolism of hepatocytes.

In other chemical parameters such as TP and albumin, a small increase was observed in relation to the superior TP reference limit for mice with the lowest dose (30 mg/kg/day) and a small reduction with respect to the inferior albumin limit in the group treated with the highest dose (1230 mg/kg/day). According to Thrall et al. (2006), in mice, the normal plasma protein concentration varies between different strains.

However, no significant differences were observed in either parameter when compared with the control, giving an indication that there is no presence of hepatic disease, which would lead to decreased levels of total protein by reducing the production; and in principle, the presence of kidney disease that could lead to excessive protein loss can also be discarded. These findings confirm that the sub-chronic administration of *O. macrocarpa* powder added to the feed for 30 days did not influence the biochemical profile of the treated animals. This hypothesis is consolidated by histopathological analyses, especially those of the heart, lung, kidney, liver, stomach and intestines, which showed normal morphology.

Conclusion

This study provides valuable data on the acute toxicity profile of the intraperitoneal administration of ethanol extract and the sub-chronic toxicity of the powder of *O. macrocarpa* (batata de purga) added to the feed. These data can be very useful for any further study *in vivo*, especially the powder administered orally. It is because it did not cause mortality and did not significantly alter body weight gain, hematological and biochemical parameters, and caused no histopathological damage to the organs analyzed for 30 days. Also, it seemed less toxic and relatively safer by this route, with new studies with different formulations to be developed.

Conflict of Interests

The authors have not declared any conflict of interests.

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