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Original Article

THE STUDY OF THE ACUTE AND SUB-ACUTE ORAL TOXICITIES OF THE NEBULIZED EXTRACT OF *MYRACRODRUON URUNDEUVA* ALLEMÃO IN RABBITS

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ABSTRACT

Objective: The aim of this research was to evaluate the acute and sub-acute oral toxicities of the nebulized dried extract of *Myracrodruon urundeuva* (NDEMU) leaf obtained by the spray drying technique on rabbits.

Methods: In the acute toxicity study, the amount of nebulized dried extract (NDE) administered was adjusted to a dose of 2000 mg/kg of leaf powder of *M. urundeuva* to 6 rabbits once orally and were observed for 14 days. In the sub-acute study, the amount of NDEMU administered was adjusted to a dose of 2000 mg/kg/day of to 6 rabbits once daily for 30 day, orally. The appearance of toxic symptoms was observed every day, followed by each rabbits' food and drink intake. Haematological and biochemical analysis were observed and statistical analysis was performed on them. The rabbits were killed at the end of the study, and their organs were weighed and examined before organ histology were evaluated.

Results: No toxic signs and no mortality were observed in the acute and sub-acute study. In the sub-acute study, the amount of dried extract administered was adjusted to a dose of 2000 mg/kg of leaf powder of *M. urundeuva* to 6 rabbits once daily for 30 days, orally. No toxic signs and no mortality were observed. There were no significant changes ($p < 0.05$) in the body weights, organ weights and haemato-biochemical parameters in any of the dose levels. No related histo-pathological lesions were observed.

Conclusion: The results indicate that the treatment of repeated doses with the dried NDEME showed low toxicity in rabbits.

Keywords: *Myracrodruon urundeuva*, Quercetin, Nebulized dried extract.

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INTRODUCTION

Myracrodruon urundeuva allemão (MUA) belongs to the tree family Anacardiaceae, commonly found in the northeast of Brazil [1]. Every plant contains a certain unique type of chemical substances/ compounds, which are produced during the normal growth and development of the plant body [2, 3]. In folk medicine, the plant is used to treatment of bleeding, respiratory and urinary infections, gastritis, gastric ulcers, cervicitis, vaginitis and hemorrhoids [2].

Studies with extracts of the stem of MUA identified dimeric chalcones and tannins that have analgesic and anti-inflammatory activity [4]. It has been reported that ethanol extracts of the stem bark of MUA possess potential activity against rotaviruses [5]. Other studies have demonstrated healing activity, antioxidant and antiulcerogenic effects [6-10]. The dry extract MUA showed anti-inflammatory activity in mice orally [11].

Phytochemical studies of the stem and leaf of MUA shown that have similar compounds which were analyzed in tannins, flavonoids, mono and sesquiterpenes, triterpenes and steroids, and leucoanthocyanidins condensed proanthocyanidins, and sugars [12]. Quercetin is a flavonoid identified in MUA [13].

Almeida *et al.* [14] demonstrated that MUA is highly toxic when administered intraperitoneally 8 mg/kg. However [4] demonstrated that aqueous and hydroalcoholic extracts of MUA exhibited low toxicity when administered orally in mice.

The production of herbal medicines has developed new technology for obtaining a dry extract. An advantage of new technologies is that it has allowed for lower storage costs and higher concentration and stability of the active substances, which allow longer storage periods and reducing shipping weights [15-17].

The present study was conducted to evaluate acute toxicity (14 d) and sub-acute (30 d) toxicity of the oral administration in rabbits of nebulized dry extracts of *M. urundeuva* leaves obtained by spray drying technique.

MATERIALS AND METHODS

Plant material and chemicals

Myracrodruon urundeuva (Anacardeaceae) leaves were collected on the farm Cacimbas in Caraúbas in the state of Paraíba, in Brazil. Entire plants were collected during the flowering stage, in May 2013. A representative sample of this species was deposited in the Lauro Pires Xavier herbarium of the federal university of paraíba (UFPB) registration no. NC240. The botanic material was dried at 50+2 °C in circulating air oven and reduced at powder.

Solvents high-performance liquid chromatography (HPLC) grade were purchased from Tedia Co. (Phoenix, AZ-USA). The standard employed in the analyses was quercetin dehydrate CAS–117-39-5 (97% pure) that had been acquired by Merck, Brazil.

Preparation of dried extract

The leaves of the MUA were air-dried in shade and finely powdered. The leaves fluid extract were prepared by the maceration method using a proportion of 20% of leaves powders for solvent system ethanol-water (1:1) at 25 °C for 120 h. The extract was filtered with Whatman filter paper no 1 (Millipore, Malaysia) and adjuvant colloidal silic on dioxide (SiO_2) were added to the dried residue at a proportion of 10% to yield the fluid mixture extract which were used to prepare dried extract. The spray-dried extracts were obtained in a spray drier (model SD-05 of LabPlant®) following operating conditions: flow of 8 ml/min; inlet temperature of 180 °C; spraying pressure of 2 bar; air flow of 62 m^3/h.

Identification and quantification of quercetin by HPLC

The quercetin (Sigma-Aldrich) biomarker was monitored in hydroethanolic and nebulized dried extract (NDE) of MUA. The analysis of quercetin in extracts was carried out using an HPLC system (Shimadzu, Tokyo, Japan) consisting of a model LC-20AD, a model SIL-20A autosampler, a model SPD-M20A diode array detector, DGU-20A5 in-line degasser and software Class VP (version6.14) were used for data acquisition and analysed.

The injections 20 µl were carried out on a Phenomenex (Torrance, California,USA) Luna C18 5 mm (250-4.6 mm) conditioned in a Shimadzu CTO-10AS VP column oven equilibrated at 40 °C, with detection at 370 nm. Solvent systems were assayed in isocratic conditions using a mixture of methanol/phosphoric acid 1% (47:53, v/v). The flow rate was 1.2 ml/min at 30 min. The identification of quercetin was compared the retention time and UV-Vis spectra of the peaks with those previously obtained by the injection of standards.

Quercetin quantitative determination were based on the external standard method by comparison with the standard retention time of pure quercetin ($y = 56948x–6354.0$, $R^2 = 0.99$) (Sigma-Aldrich). Parameters of validation such as selectivity, linearity, detection (LOD) and quantification limits (LOQ) and precision or relative standard deviation (RSD, %) were established [18-20]. The LOD and LOQ were evaluated on the basis of the noise obtained with analysis of non-spiked blank samples for quercetin n = 3. LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively [18]. The total quercetin the LOD and LOQ were estimated by the slope and mean standard deviation of quercetin concentrations used in the standard curve [19, 20]. The LOD for the quercetin was of 0.18 µg/ml and the LOQ ranged 0.56 µg/ml. Results of six parallel experiments indicated that precision or RSD were all<5%.

Experimental animals

Adult healthy male and female *Cuniculus orytolagus*, New Zealand rabbits (8 w, 1.4 and 1.3 kg, respectively) were used for the repeated doses toxicity experiments. They were come from the animal house of the Research Institute for Drugs and Medicines of UFPB and housed in plastic cages under normal laboratory conditions (12h light/dark cycle: 22±2 °C) for an acclimatization period of 7 d prior to the experiments. All the animals were given food and water *ad libitum*. The bioassay was conducted in accordance with the internationally acceptable guidelines for evaluating the safety and efficacy of herbal medicines [21-23]. All experiments were performed in accordance with the protocol approved by the animal experimentation committee of the UFPB (number 0207/10).

Acute oral toxicity study

In order to study any possible toxic effect or changes in normal behaviour, two groups of 6 rabbits (3 males and 3 females) were used in this experiment. The control group received distilled water, and test groups received the NDEMU dissolved in water by the oral route. The amount of NDE administered was adjusted to a dose of 2000 mg/kg of leaf powder of MUA. This dose was equivalent to a concentration of quercetin of 142.6 µg/ml. Those doses were chosen after several screenings on mice.

The experimental animals were deprived of food for 2h prior to extracting administration. They were continuously monitored after administration in 0, 15, 30 and 60 min and every 4 h to 12 h and daily for 14 d thereafter for any signs of toxicity such as changes: in behavior, breathing, piloerection, diarrhea, excessive salivation, hyperexcitability, reduced mobility, aggressiveness, reaction to stimuli, weight loss, ataxia and mortality. During that period, the animals were supplied food and water *ad libitum*.

Repeated-doses toxicity study

Healthy male and female rabbits were divided into two groups of 6 rabbits (3 males and 3 females). The control group received distilled water, and test groups received the NDEMU dissolved in water by oral route for 30 consecutive days. The amount of NDE administered was adjusted to a dose of 2000 mg/kg/d of leaf powder of MUA.

During the treatment, the food consumption and water intake of the animals were recorded on an alternate day. Animals were observed twice daily for signs of toxicity, such as piloerection, diarrhoea, and changes in locomotor activity, reaction to stimuli, ataxia, loss of reflex and mortality. At the end of the 30-day treatment, they were then anesthetized with thiopental 35 mg/kg, and blood samples were obtained and collected in two tubes: one tube containing the anticoagulant ethylene diamine tetra acetic acid (EDTA) and one tube without anticoagulant for haematological and biochemical parameters, respectively. This work was carried out following the welfare of animals as recommended [21].

Hematological and biochemical analysis

Hematological analyses were carried out immediately after collection using an automatic hematology analyzer BC-3000 plus, Mindray®. Parameters included red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), globular volume (GV) and platelet count. For biochemical analysis, blood was centrifuged at 3000 rpm for 5 min to obtain serum, and the following parameters were determined: glucose, uric acid, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglycerides, total protein, total bilirubin, potassium, sodium and alkaline phosphatase. Dosages were made using an automatic biochemical analyzer BS 300 Mindray®.

Morphological study

After blood collection, the animals were euthanized with an excess of thiopental (140 mg/kg), and a necropsy was performed for macroscopic external evaluation of the heart, lungs, liver, kidneys and spleen. These organs were carefully removed and weighed individually. Organ weights were expressed in and relative terms (g/100 g of body weight).

Histological analysis

The organs described previously of each group were fixed in 10% formalin for one month and then, embedded in paraffin (Sigma-Aldrich. Sections of 5–6 µm were routinely stained with haematoxylin (Sigma-Aldrich) and eosin (Sigma-Aldrich) and examined under a light microscope (Olympus CH02).

Statistical analysis

The values were expressed as mean±standard error of the mean (SEM). Data were analyzed by comparison between two groups used the test "t" Student using the software Graph Pad Prism 6.0 (Graph Pad Software Inc. San Diego CA, USA), and the results were considered significant when presented values of $^*p \leq 0.05$.

RESULTS

Identification and quantification of quercetin by HPLC

HPLC analyses were performed to assess the extract composition after the drying process by a spray dryer, and flavonoid quercetin was monitored biomarker, this substance was identified and quantified on hydroethanolic and NDEMU. Chromatograms of the sample of standard chemical quercetin of the hydroethanolic extract and NDE of *M. urundeuva* are illustrated in fig. 1. Peak retention times of quercetin in the standard sample, hydroethanolic extract and dry extract were 10.1, 9.7 and 9.7 min respectively.

From the spectra of UV, absorption of quercetin was obtained purity curves of the quercetin peak for extracts samples, and peaks purities indexes were 0.99 for the hydroethanolic extract and for dry extract. The concentrated hydroethanolic extract and NDEMU presented values for quercetin of 14.82±0.25 and 15.87±0.12 µg/ml, respectively.

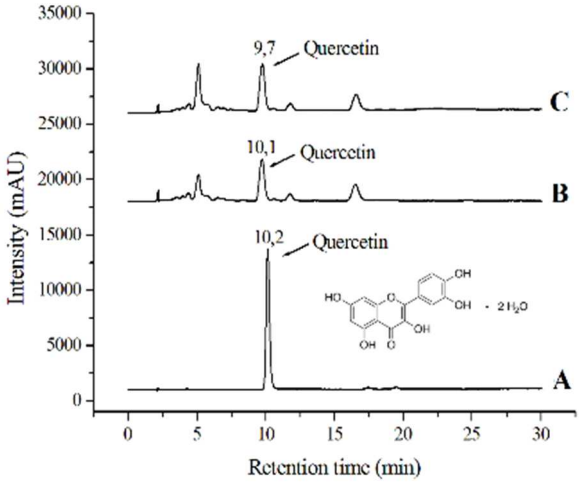

Fig. 1: Chromatograms for sample chemical standard quercetin (A), hydroethanolic extract (B) and dry extract (C) of *M. urundeuva*. For chromatographic conditions, see Section 2

Acute toxicity

No clinical toxicity signs were observed in the extract treated group compared to the control group.

Table 1 show that oral administration of NDEMU at a dose 2000 mg/kg did not cause a significant change in water and food consumption of the test rabbits when compared with their respective control group (P≤ 0.05).

Table 1: **Effect of NDEMU treatment on the water intake and feed consumed by groups of rabbits**

Parameters	Male			Female		
	Control	Test	p*	Control	Test	p*
Water (ml)	207.9±3.841	203.6±4.006	0.447	212.9±4.621	206.8±4.435	0.351
Food (g)	228.2±13.05	220.5±13.20	0.681	231.8±13.24	224.9±13.92	0.721

Values are expressed as mean±SEM (n = 3/group). *P≤0.05, when compared to control group treated with NDEMU (Analyzed by Student's t-test)

Repeated-doses toxicity

Oral administration at repeated doses of the NDEMU in rabbits of both sexes did not cause death or any clinical signs of toxicity. The oral ingestion of NDEMU over 30 d caused no significant changes in weight of the organs in the treated as compared to the control animals (P≤0.05) the results are shown in table 2. The intake of NDE of *M. urundeuva* in the rabbits studied for 30 d did not cause significant changes in haematological parameters when compared to the control group (P≤0.05) the results are shown in table 3.

Table 2: Effect of the NDEMU on relative organ weight (g/100g of animal body weight) rabbits treated orally for 30-day

Parameteres	Male			Female		
	Control	Test	p*	Controle	Test	p*
Heart (g)	4.367±0.1856	4.933±0.260	0.151	5.900±0.404	4.767±0.133	0.056
Liver(g)	51.67±2.576	52.50±3.980	0.869	73.60±3.329	69.17±3.282	0.396
Spleen (g)	0.6680±0.011	0.7700±0.100	0.370	1.143±0.307	0.7954±0.024	0.322
Kidney (g)	9.967±0.497	10.13±0.463	0.818	13.00±0.360	11.85±0.444	0.114
Lung (g)	9.600±0.472	11.53±0.648	0.073	9.067±0.633	8.050±0.259	0.211

Values are expressed as mean±SEM(n = 3/group). *P≤0.05, when compared to control group treated with NDEMU (Analyzed by Student's t-test)

Table 3: Effect of NDEMU treatment on the haematological parameters of rabbits treated orally for 30-day

Parameters	Male			Female		
	Control	Test	p*	Control	Test	p*
Erythrocytes (10^6/µL)	4.900±0.115	4.300±0.608	0.387	4.433±0.284	4.700±0.264	0.530
Hemoglobin (g/dL)	12.10±0.6351	11.97±0.902	0.909	11.53±0.176	12.32±0.400	0.147
Hematocrit (%)	39.30±0.115	38.60±0.916	0.490	37.27±1.291	37.33±1.133	0.970
MCHC g/dL	34.00±0.568	33.63±0.318	0.603	34.60±0.750	34.57±0.348	0.969
GV (10^6/µL)	5.157±0.293	5.170±0.346	0.978	5.333±0.255	4.880±0.242	0.267
MCH (pg)	23.50±0.378	22.87±0.272	0.246	23.37±0.584	23.07±0.484	0.712
Platelet (10^3/µL)	113.0±10.58	133.3±17.75	0.380	127.3±20.28	134.7±8.090	0.753
MCV (fL)	65.70±3.381	67.30±1.229	0.679	67.23±1.105	67.65±1.146	0.806

Values are expressed as mean±SEM (n = 3/group). *P≤0.05, when compared to control group treated with NDEMU (Analyzed by Student's t-test), MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, platelet and GV: globular volume.

The serum biochemical results in rabbits are presented in table 4. Results showed that the treatment did not affect the biochemical parameters of test rabbits when compared with the control group (P≤0.05).

The histopathology results were shown in fig. 2 and fig. 3. It was not observed morphological changes in kidney, heart, lung, spleen and liver in all rabbits from all groups of study.

Table 4: **Effect of NDEMU treatment on the biochemistry parameters of rabbits treated orally with for 30-day**

Parameters	Male			Fêmea		
	Control	Test	p*	Control	Test	p*
Glucose mg/dL	116.5±2.598	121.3±14.72	0.762	122.3±13.59	145.7±3.383	0.171
Cholesterol mg/dL	38.00±0.577	34.67±3.180	0.360	42.00±5.859	58.00±2.887	0.070
Uréia mg/dL	39.00±5.196	35.00±2.646	0.530	44.67±3.283	44.50±1.607	0.965
Creatinine mg/dL	1.287±0.219	1.267±0.240	0.954	1.373±0.073	1.552±0.075	0.166
Alkalinephosphatase mg/dL	61.50±12.99	32.67±2.333	0.094	62.33±15.21	51.13±9.442	0.565
Uricacid mg/dL	0.3267±0.079	0.4433±0.084	0.370	0.3067±0.035	0.3233±0.024	0.716
Potassium (mmol/l)	8.027±0.433	7.190±0.335	0.201	5.963±0.655	6.680±0.473	0.425
Sodium(mmol/l)	151.0±2.887	149.0±2.517	0.629	146.0±4.583	145.5±0.288	0.918
AST U/l	135.0±0.577	104.7±15.39	0.120	106.0±5.508	120.8±5.183	0.121
ALT U/l	113.0±25.11	114.7±21.11	0.961	133.0±11.79	124.2±1.878	0.500
Total protein g/dL	5.723±0.413	6.097±0.407	0.554	5.727±0.539	5.937±0.218	0.736
GGT U/l	14.67±3.180	11.67±1.453	0.439	15.33±1.856	14.17±1.302	0.633
Triglycerides mg/dL	62.00±13.05	65.67±23.25	0.897	68.00±18.36	81.83±15.44	0.595
Total bilirubin mg/dL	0.0500±0.028	0.02667±0.012	0.497	0.0200±0.015	0.02333±0.014	0.882

Values are expressed as mean±SEM (n = 3/group). P≤0.05, when compared to control group treated with NDEMU (Analyzed by Student's t-test), AST: aspartate aminotransferase, ALT: alanine aminotransferases and GGT: gamma glutamyltransferase.

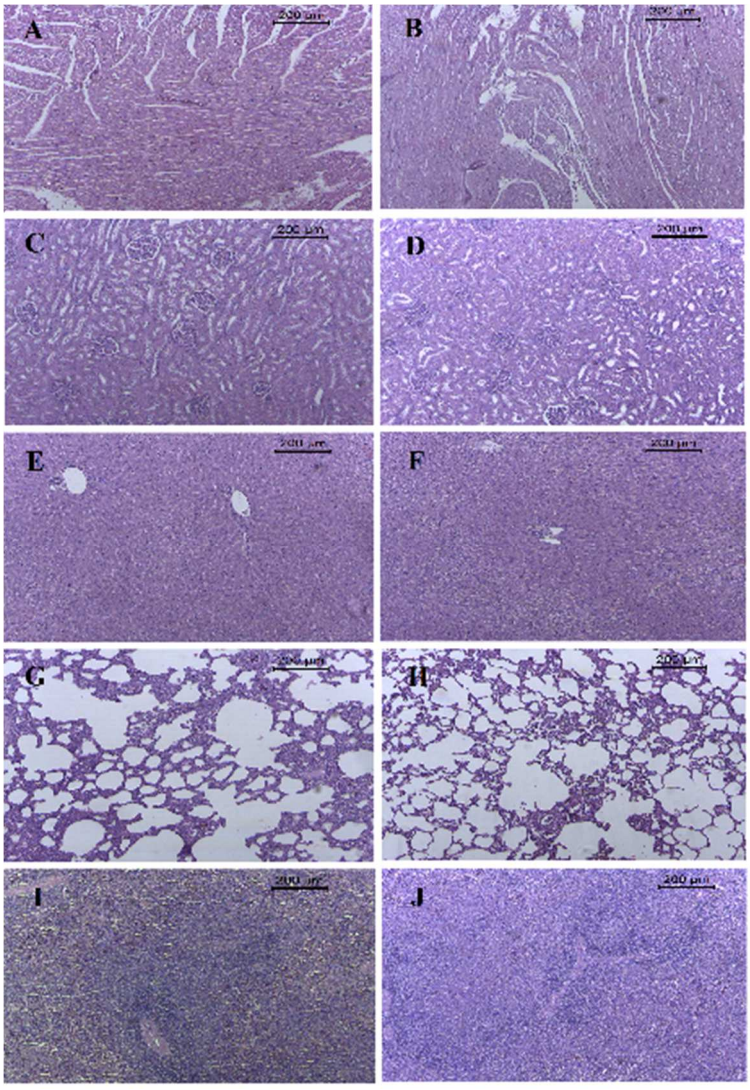

Fig. 2: Histological examination revealed that there were no changes observed of male rabbits due to the 30-day NDEMU administration in heart (A, B), kidneys (C, D), liver (E, F), lung (G and H) and the spleen (I and J)

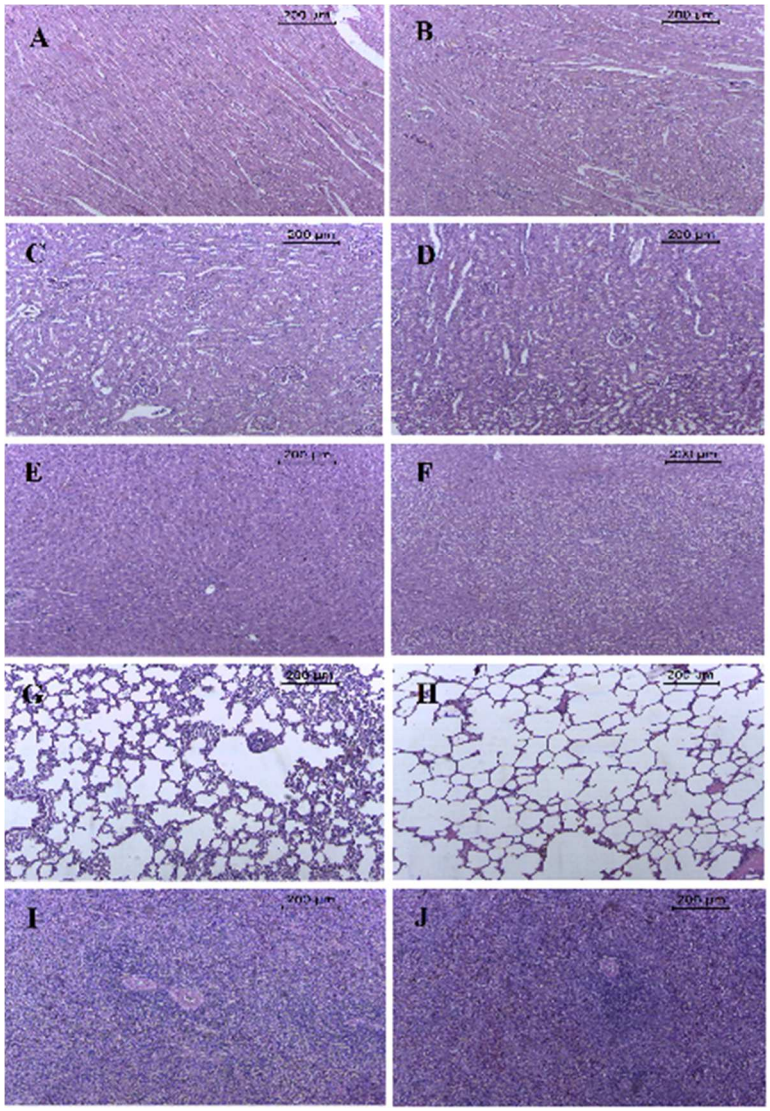

Fig. 3: Histological examination revealed that there were no changes observed of female rabbits due to the 30-day NDEMU administration in heart (A, B), kidneys (C, D), liver (E, F), lung (G and H) and the spleen (I and J)

DISCUSSION

Identification and quantification of quercetin by HPLC

Fig. 1 showed the chromatograms of the sample of standard quercetin of the hydroethanolic extract and NDEMU. Quercetin identification was based on comparing chromatographic behaviour (retention time) and UV–visible with an external standard. The concentrated hydroethanolic extract and NDEMU and the chromatographic profile remained similar after the drying process.

The standardization of plant extracts, through the identification and quantification of a substance for follow-up during the processes, identification of the plant drug or to verify the presence of substances responsible for the pharmacological action is an indispensable parameter of evaluation for the quality control of plant products [24, 25]. The analytical method developed for the identification and quantification of quercetin by HPLC in the extracts of *M. urundeuva* was efficient, fulfilling satisfactorily with the required quality parameters [26-28].

Acute toxicity

During the development of herbal medicine toxicology studies of pre-formulated products plant in animals are needed to ensure the use in humans. In the present study, there was the absence of observed toxic effects of NDEMU administered to rabbits making possible its use for toxicological and pharmacological studies in humans by the oral route.

In the present work, the NDEMU did not induce changes in the water consumption of the females and food consumption of the males when compared with their respective control group (table 1). Acute intragastric administration of NDEMU at a dose equivalent to 2000 mg/kg of leaf powder weight to male and female rabbits caused no animal deaths and, therefore, it was not possible to determine LD_{50}.

Moreover, there were not observed signs of toxicity, including changes in behaviour, locomotion, respiration, piloerection, diarrhoea, drooling, altered muscle tone, hypnosis, convulsions, hyper-excitability and writhing. There were no significant differences in the body weight gain between animals of the control and mate groups for 14 d monitoring. Menezes *et al.* [29] while working with of alcoholic and aqueous extracts of *M. urundeuva* found no signs of toxicity in rats after acute administration (5000 mg/kg) or throughout a 20-day treatment (500 mg/kg).

Substances with LD50 of 1000 mg/kg given orally are considered safe or of low toxicity [30]. Similarly, the chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the organization for economic cooperation and development is considerate relatively low acute toxicity LD_{50} values between 2000 and 5000 mg/kg [31].

Repeated-doses toxicity

Oral administration at repeated doses 30 d of the NDEMU in rabbits of both sexes did not cause death or any clinical signs of toxicity.

In studies of repeated dose toxicity body weight gain and organ weight are considered important parameters and changes in these parameters can indicate a toxic effect of the drug [31]. Sub-acute toxicity study gives valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at a low dose on prolonged exposure. A wide variety of adverse effects can be detected with sub-acute toxicity studies [32]. The oral ingestion of NDEMU over 30 d caused no significant changes in weight of the organs (i.e. liver, kidneys, heart, lungs and spleen) in the treated as compared to the control animals (g/100 g of animal body weight) (table 2). Chronic toxicity studies with hydroethanolic bark extract of *M. urudeunva* stem in rats at doses of 200 mg/kg and 400 mg/kg orally for 90 d showed mortality 20 and 30% and there were no changes in the histopathological and hematological parameters of animals [14].

The intake of NDEMU in the dose studied rabbits for 30 d did not cause significant changes in haematological parameters when compared to the control group (table 3). These data indicate that NDEMU had no effects on the circulating blood cells or on their production. The analysis of blood parameters is important for risk evaluation, as any changes in the haematological system have a higher predictive value for human toxicity when data are translated from animal studies [4].

The assessment of pathological changes in the organs of treated animals, both macro and microscopically, is the basis of a safety assessment [33]. The histopathology results were shown in fig. 2 and fig. 3. There were no observed morphological changes in kidney, heart, lung, spleen and liver in all rabbits from all groups of study. According to the histopathological findings of the organs, significant morphological changes were not observed, indicative of cell or tissue damage in male and female rabbits compared to the control group animals indicating no toxicity in the organs studied. The absence of aspects and pathogenic mechanisms in tissues justified the values found in behavioural, haematological and biochemical testing when compared the control and the experimental groups.

Analysis of the biochemical parameters for the rabbits showed that the treatment did not affect serum levels of glucose, triglycerides, urea, total bilirubin, potassium, sodium, alkaline phosphatase, creatinine, AST, ALT, GGT, total cholesterol, VLDL, and total protein when compared with the control group indicating no change in the overall metabolism of the test animals (table 4). Since the enzyme AST was also found in a large number of tissues, such as heart, lung, skeletal muscle, and kidney, whereas ALT is primarily limited to hepatocytes, the latter is considered a highly sensitive indicator of hepatotoxicity [34]. Therefore the fact that the administration of *M. urundeuva* did not produce changes in these biomarkers suggests absence of renal and hepatic toxicity [35–37]. There was an increase of triglycerides and decreased alkaline phosphatase but the results are still within the range described for the species observed in other studies [36, 37].

Histopathological examination of the liver revealed the absence of congestion; cellular infiltrates that characterize an inflammation and absence of degenerations [38, 39]. The surface and staining the cellular composition of the kidneys did not have change; there was no presence of focal or multifocal hemorrhages. In the cortical and medullary layers were not observed congestion or degeneration and nephrosis [39–41]. The heart showed no congestion, the tumescent cardiac fibers, no eosinophilia or degeneration of bundles of cardiac fibers [40]. It was not observed in the lung congestion, pneumonia, edema, disappearance of ciliated cells of the bronchioles or deposition of refractive material, amorphous and eosinophilic [38].

CONCLUSION

The data suggest that oral administration of the NDEMU showed low toxicity in rabbits. The results showed an absence of acute oral toxicity of the NDEMU at the dose of 2000 mg/kg/day in rabbits. Based on the measurement of blood biochemical and haematological parameters, and histological examinations of main organs that could eventually be affected by long-term administration of MUA indicate that administration of NDEMU not promoted toxic effects for the rabbits. However further studies are needed to fully assess the safety of this product such as studies of chronic oral toxicity, reproductive toxicity, genotoxicity and carcinogenicity studies.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Santin DA, Leitão Filho HF. Restabelecimento e revisão taxonômica do gênero *Myracrodruon* freire allemão (Anacardiaceae). Rev Bras Bot 1991;14:133-45.
2. Lorenzi H, Matos FJA. Plantas medicinais no Brasil-nativas e exóticas. 1st ed. Nova Odessa: Instituto Plantarum; 2002.
3. Diorge JM, Shanna B, Amanda CS, Márcia IG, Claudete R. Medicinal plants of *Renisus* with analgesic activity. J Crit Rev 2016;3:1-4.
4. Viana GSB, Bandeira MAM, Matos FJA. Analgesic and antiinflammatory effects of chalcones isolated from *Myracrodruon urundeuva Allemão*. Phytomedicine 2003;10:189-95.
5. Cecilio AB, Faria DB, Oliveira PC, Caldas S, Oliveira DA, Sobral MEG, *et al.* Screening of Brazilian medicinal plants for antiviral activity against rotavirus. J Ethnopharmacol 2012;141:975-81.
6. Carlini EA, Duarte-Ameida JM, Rodrigues E, Tabach R. Antiulcer effect of the pepper trees *Schinustere binthifolius* Raddi (aroeira-da-praia) and *Myracrodruon urundeuva* Allemão, Anacardiaceae (aroeira-do-sertão). Rev Bras Farmacogn 2010;20:140-6.
7. Desmarrchelier C, Romão RL, Coussio J, Ciccia G. Antioxidant and free radical scavenging activities in extracts of medicinal trees used in the Caatinga region in northeastern, Brazil J Ethnopharmacol 1999;67:69-77.
8. Cavalcante ARSM, Rodrigues LV, Menezes DB, Cunha MPSS, Goes ACAM. Tension and morphological analysis of colonic anastomosis in 10% acetic acid-induced colitis in wistar rats treated with 10% aroeira-do-sertão aqueous extract (*Myracrodruon urundeuva* fr. All.). Acta Cir Bras 2005;20:180-6.
9. Nobre-Júnior HV, Oliveira RA, Maia FD, Nogueira MAS, Moraes MO, Bandeira MAM, *et al.* Neuroprotective effects of chalcones from *Myracrodruon urundeuva* on 6-Hydroxydopamine-Induced cytotoxicity in rat mesencephalic cells. Neurochem Res 2009;34:1066-75.
10. Sá RA, Argolo ACC, Napoleão TH, Gomes FS, Santos NDL, Melo CML, *et al.* Antioxidant, *Fusarium growth* inhibition and *Nasutitermes corniger* repellent activities of secondary metabolites from *Myracrodruon urundeuva*. Int Biodeterior Biodegrad 2009;63:470-7.
11. Renata S, Valmir GS, Agna HO, José VCJ, Islaine SS, Rui OM, *et al.* Standardization and stability evaluation of dry extracts of *Myracrodruon urundeuva* Allemão obtained by a spray drier. Int J Pharm Pharm Sci 2017;9:154-9.
12. Monteiro JM, Lins Neto EMF, Amorim ELC, Strattmann RR, Araújo EL, Albuquerque UP. Tannin content in three sympatric medicinal tree species of the caatinga. Tree Magazine 2005;29:999-1005.
13. Jandú JJB, Silva LCN, Pereira APC, Souza RM, Silva Júnior CA, Figueiredo RCBQ. *Myracrodruon urundeuva*bark: anantimicrobial, antioxidantand non-cytotoxicagent. J Med Plants Res 2013;7:413-8.
14. Almeida AC, Sobrinho EM, Pinho L, Souza PNS, Martins ER, Duarte ER, *et al.* Acute toxicity of leaf hydroalcoholic extracts of *Lippia sidoides, Myracroduon urundeuva, Stryphnodendro nadstringens* and of *Caryocar brasilliense* administered by intraperitoneal route. Ciência Rural 2010;40:200-3.
15. Andrade F, Albuquerque CAC, Maraschin M, Silva EL. Safety assessment of yerba mate *(Ilex paraguariensis*) dried extract:

results of acute and 90 d subchronic toxicity studies in rats and rabbits. Food Chem Toxicol 2012;50:328-34.

16. Shahina P, Anwar S, Anamica U, Vikas Y. Gas chromatography-mass spectrometry analysis of methanolic leaf extract of *Cassia angustifolia* Vahl. Asian J Pharm Clin Res 2016;9:111-6.
17. Gallo L, Llabot JM, Allemandi D, Bucalá V, Piña J. Influence of spray-drying operating conditions on *Rhamnus purshiana* (Cáscarasagrada) extract powder physical properties. Powder Technol 2011;208:205-14.
18. American Chemical Society (ACS). Subcommittee on Environmental Analytical Chemistry. Guidelines for data acquisition and data quality evaluation in environmental chemistry. Anal Chem 1980;52:2242-9.
19. ICH Q2B. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation Research. Guidance for industry: ICH Q2B Validation of analytical procedures: methodology. Rockville; 1995.
20. Megha S, Neeraj M. Development and validation of stability indicating a RP-HPLC method for determination of β-acetyldigoxin. Int J Appl Pharm 2017;9:54-9.
21. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. 1st ed. Geneva: World Health Organization; 2000.
22. Organisation for economic cooperation and development (OECD). Repeated dose 28-day oral toxicity test method guideline 407. In: OECD, Guidelines for testing of chemicals. Paris: Organisation for Economic Cooperation and Development; 1995.
23. Organisation for Economic Cooperation and Development (OECD). Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 423: Acute oral toxicity-acute toxic class method. Paris: Organization for Economic Cooperation and Development; 2002.
24. Koo MH, Park YK. Investigation of flavonoid aglycones in propolis collected by two different varieties of bees in the same region. Biosci Biotechnol Biochem 1997;61:367-9.
25. Ministério da Saúde, Anvisa-Brasil. Resolução n ° 899-Guia para Validação de Métodos Analíticos e Bioanalíticos, Anvisa: Brasília; 2003.
26. Agência Nacional de Vigilância Sanitária. Anvisa-Brasil). Farmacopéia brasileira. 5nd ed. Anvisa: Brasília; 2010.
27. Yuangang Z, Chunying L, Yujie F, Chunjian Z. Simultaneous determination of Catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. J Pharm Biomed Anal 2006;41:714-9.
28. Marina S, Svetlana K, Trajce S. Assay of flavonols and quantification of quercetin in medicinal plants by HPLC with UV-DIODE array detection. J Liq Chromatogr Relat Technol 2001;24:2283-92.
29. Menezes MAS, Rao VSN, Fonteles MC. Antiinflammatory activity of *Astronium urundeuva* Fr. All. possible mechanisms involved. Braz J Med Biol Res 1985;18:861-4.
30. Clarke EGC, Clarke ML. Veterinary toxicology. 1st ed. London: Bailliere Tindall; 1975. p. 438.
31. Jahn AI, Gunzel PKH. The value of spermatology in male reproductive toxicology: do spermatologic examinations in fertility studies provide new and additional information relevant for safety assessment. Reprod Toxicol 1997;11:171–8.
32. Kayarohanam S, Kavimani S. Acute and sub-acute toxicity study of aqueous methanolic leaf and bark extract of *dolichandrone atrovirens*. Int J Pharm Sci 2015;7:63-5.
33. Al-Habori M, Al-Aghbari A, Al-Mamary M, Maker M. Toxicological evaluation of *Catha edulis* leaves a long-term feeding experiment in animals. J Ethnopharmac 2002;83:209-17.
34. Emanuelli MP, Lopes STA, Macel RM, Garmatz BC, Tavares MO. Toxicological evaluation of *Catha edulis* leaves a long-term feeding experiment in animals. J Ethnopharmac 2002;83:209–17.
35. Havel RJ, Kita T, Kotite L, Kane JP, Hamilton RL. Serum concentration of alkaline phosphatase, gama-glutamyl transferase, urea and creatinin in rabbits (*Oryctolagus cuniculus*). Ciência Animal Bras 2008;9:251-5.
36. Kaneko JJ, Harvey JW, Bruss LM. Clinical biochemistry of domestic animals. 5nd ed. San Diego: Academic; 1997. p. 895-9.
37. Prabu PC, Panchapakesan S, Raj CD. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somniferaroots* in Wistar rats. Phytother 2013;27:1169–78.
38. Carlton WW, McGavin MD. Patologia Veterinária Especial de Thonson. 2nd ed. Artmed; 1998. p. 635.
39. Dobereiner J, Peixoto PV, Tokarnia CH. Experimental poisoning by *Arrabidae abilabiata* (Bignoniaceae) in rabbits. Pesq Vet Bras 1984;4:89-96.
40. Maxie MG. The urinary system. In: Jugg KVF, Kennedy PC, Palmer N. Pathology of domestic animals. 4nd ed. San Diego: Academic Press; 1993. p. 447-538.
41. Jones TC, Hunt RD, King NW. Patologia Veterinária. 1nd ed. São Paulo: Malone; 2000. p. 586.

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