WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 30-42 Research Article ISSN 2278 – 4357

ACUTE AND SUB-ACUTE TOXICITY STUDIES OF AQUEOUS DECOCTION OF THE TRUNK BARKS FROM *LANNEA MICROCARPA* ENGL. AND K. KRAUSE (ANACARDIACEAE) IN RODENTS

***[1,2]Nitiéma Mathieu, [1,3]Ilboudo Sylvain, [1]Belemnaba Lazare, [1]Ouédraogo Geoffroy Gueswindé, [1,2]Ouédraogo Salfo, [1,3]Ouédraogo Noufou, [1]Ouédraogo Sylvin and [1,2]Guissou Innocent Pierre**

[1]Département Médecine, Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé (MEPHATRA-PH/IRSS)/CNRST, 03 BP 7192 Ouagadougou 03, Burkina Faso.
[2]Laboratoire du Développement du Médicament, Université Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
[3]Unité Mixte de Recherche Internationale - Environnement, Santé, Sociétés (UMI 3189, ESS) CNRS/CNRST/UCAD/UGB/USTTB.

Article Received on
24 June 2018,
Revised on 14 July 2018,
Accepted on 04 August 2018
DOI: 10.20959/wjpps20189-12185

***Corresponding Author**
NITIEMA Mathieu
Département Médecine, Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé (MEPHATRA-PH/IRSS)/CNRST, 03 BP 7192 Ouagadougou 03, Burkina Faso.

ABSTRACT

Lannea microcarpa Engl. & K. Krause (Anacardiaceae) is a multipurpose plant mostly distributed in various tropical African countries, including Burkina Faso and commonly known as African grape in French. To ensure safe use of the plant, acute and sub-acute toxicity studies were undertaken with the trunk barks aqueous extract of *Lannea microcarpa* (LMaq) in NMRI male mice and Wistar rats respectively, in accordance with Organization for Economic Cooperation and Development (OECD) test guidelines. The acute toxicity was performed with the single dose of 2000 mg/kg of body weight (bw). Observations were made and recorded for 24 h, and once daily further for a period of 14 days. For sub-acute study, four groups of 10 animals (5 per sex) received orally distilled water (control), and 100, 500, and 1000 mg/kg of LMaq, respectively, every 24 h for 28 days. At the end of sub-acute study, biochemical parameters were evaluated. No mortality was observed at single dose of 2000 mg/kg bw. The LD_{50} value was estimated to 5000 mg/kg bw. In the 28 days sub-acute oral toxicity study in rats, there was no mortality observed for all tested doses in both sex. Furthermore, there was no significant difference observed in food and water consumption, relative organs, body weights,

biochemical profile in animals, compared to the control. Therefore, analysis of results may lead to the conclusion that oral administration of LMaq for 28 days does not cause toxicity. These results suggest a safe use of *Lannea microcarpa* trunk barks based remedies for human or veterinary medicines.

KEYWORDS: *Lannea microcarpa*, Biochemical parameters, Traditional medicine, Wistar rats, NMRI mice.

INTRODUCTION

Medicinal plants are an immense source of bioactive molecules. Thus, these natural molecules contribute to the fight against the multiplicity of diseases that modern medicine cannot always cope with. Indeed, traditional herbal medicine is attracting growing interest. It was estimated that approximately 80% of the rural populations living in developing countries depend on traditional medicine for their needs health care.[1] In Burkina Faso, the practice of traditional medicine is based largely on the use of medical plants. Among these plants, figure *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) commonly known as African grape in French. This plant is widely distributed in the sub-Saharan region from Senegal to Cameroon. Traditional remedies prepared from its leaves, barks, roots and fruits are used to treat many diseases such as mouth blisters, rheumatism, sore throats, dysentery, conjunctivitis, stomatitis, skin eruptions, ulcers[2-4] and high blood pressure [5]. The phytochemical studies have shown the presence of steroids, saponosides, triterpens and tannins in the barks of *Lannea microcarpa.*[6] Also, anthocyanins and tannins were found in the fruit's epicarp extracts of the plant.[7, 8] Other authors have identified the polyphenol and tocopherol in the seed oil of *Lannea microcarpa.*[2] Pharmacologically, bark extracts from *Lannea microcarpa* have shown antioxidant activity and may attenuate the harmful effects of oxidative stress on cells.[9-11] In addition, the antihypertensive effect of the extracts via the inhibition of phosphodiesterases and alpha-adrenergic receptors as well as the anti-inflammatory effect have also been demonstrated.[5,12,13] Although *Lannea microcarpa* has many pharmacological properties, there is few data on the toxicity of extracts from this plant. The objective of this study was to evaluate the acute and sub-acute toxicity of *Lannea microcarpa* trunk barks for safe use in traditional medicine.

MATERIALS AND METHODS

Plant material

The trunk barks from *Lannea microcarpa* (Engl and K. Krause) were collected on January 2015 in Loumbila (savannah zone), located at 20 km in the Northeast of Ouagadougou (Burkina Faso). The plant sample was authenticated at "Herbier National du Burkina (HNBU)" located at Centre National de Recherche Scientifique et Technologique (CNRST), Ouagadougou (Burkina Faso) where the voucher specimen has been deposited under number HNBU 361. The collected sample was air-dried deprived of solar light, dust and was then powdered using a mechanical grinder. The powder obtained was used for preparation of extract for toxicity tests.

Preparation of the lyophilized aqueous decoction

One hundred grams (100 g) of powder of the trunk barks from *Lannea microcarpa* were mixed with distilled water (500 mL) and a decoction was carried out during 30 min. After cooling, the aqueous decoction extract was filtered and then centrifuged (2000 trs/min, 5 minutes). The supernatant was collected, concentrated and frozen before lyophilised to obtain the aqueous extract powder form *Lannea microcarpa* (LMaq).

Animals

Healthy male NMRI mice (6-8 week-old and weighing 30-36 g) and Wistar rats (8-10 week-old and weighing 200-250 g) were used in the study. They were obtained from the pet Shop of the "Institut de Recherche en Sciences de la Santé" (IRSS), Ouagadougou, Burkina Faso. The animals were randomly selected, marked for individual identification and housed in animal cages with free access to water and standard laboratory pellet enriched with proteins (29%). All animals were maintained in controlled temperature room of 22-25°C with a 12 h light/dark cycle. The experimental protocol was carried out in accordance with international standard protocols [Guidelines set by the European Union on the protection of animals (CEC Council 86/609)] and adopted by IRSS, Burkina Faso.[14]

Acute toxicity test

The acute oral toxicity test of LMaq was performed on male NMRI mice according to Organization for Economic Co-operation and Development (OECD) test guideline 423, the acute toxic class method.[15] After a 4-hour fastening period, the mice were weighed and the dose of LMaq was calculated in reference to the body weight. The extract was administered orally by gavage in single dose to the mice according to the sequential procedure.

In the conducting of test, 2000 mg/kg bw of extract was chosen as the starting dose. After 2 hours post-treatment observation of all animals, feeding was restored. They are then observed at least once daily for 14 days for mortality and signs of toxicity such as changes in skin and fur, eyes, mucus membranes, salivation, convulsion, diarrhea, lethargy, sleep, and coma.[14,16]

Sub-acute toxicity study

The sub-acute oral toxicity study was carried out in accordance with the OECD Guidance Document on Sub-acute Oral Toxicity Testing 407.[17] Twenty (20) rats of either sex were randomly selected for that purpose. Females involved were nulliparous and nonpregnant. The rats were divided into four groups of 10 animals each (5 males and 5 females); males and females were kept in separate polypropylene cages. Group 1, which served as the control received the vehicle (distilled water), while rats in groups 2, 3 and 4 were respectively received daily doses of 100, 500 and 1000 mg/kg bw of LMaq for 28 days at the same hour. All animals were closely observed for the first 1 and 4 hours of dosing to examine any adverse toxic signs, behavioural changes and at least twice a day for morbidity and mortality. Body weight and food consumption were recorded once weekly. Water consumption was monitored daily for each cage (5 rats per cage) up to 4 weeks. On the 29th day, after over-night fastening, all the rats were anaesthetized using ketamine. Blood samples were collected via cardiac puncture into dry vacutainers for each animal.[16]

Effect of LMaq on vital organs

At the end of treatment, after overnight fastening all animals were sacrified and vital organs such as heart, kidneys, liver, lung, gonads (testis or ovaries) and spleen were isolated and observed macroscopically for any lesions. After that, all organs were dried with toilet paper and then weighed on a sensitive balance (Sartorius; precision 0.1 mg). The relative organ weight ratio (ROW) of each rat was calculated as follows:

$$\text{ROW}\ (\%) = 100 \times \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}}$$

Effect of LMaq on serum biochemical parameters

The blood samples collected in dry vacutainers were centrifuged at 3000 rpm for 10 min using a table centrifuge (ROTOFIX 32A, Mettich Zenfrifugen, Germany) ; the sera obtained were used for biochemical assays. Blood chemicals tests were carried out using an automatic biochemistry analyzer (Mindray BS-300, China). Sera biochemical parameters including total proteins, aspartate aminotransferase, alanine aminotransferase, uric acid, creatinine,

cholesterol, tryglicerid, fasting blood glucose, chlorid, phosphorus, and magnesium were determined.

Statistical analysis

Results were presented as mean ± standard deviation SD (n = 5). Data were calculated separately for males and females. The statistical significance of difference between treated and control groups were analyzed by one-way analysis of variance (ANOVA) using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) followed by Dunett's multiple comparison tests. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Acute toxicity study of *Lannea microcarpa* trunk barks aqueous extract in mice

In acute oral toxicity study, a single administration of aqueous extract of *Lannea microcarpa* at dose of 2000 mg/kg bw did not show any remarkable behavioural changes and mortality of mice in the first and second step (Table 1). According to the acute toxic class method, the aqueous extract of the plant tested is classified to the 5^{th} toxicity class with a LD_{50} value estimated to 5000 mg/kg bw.

Table 1: Mortality of male mice in acute oral toxicity study.

	Aqueous extract of *L. microcarpa* trunk barks	
Dose of administration (2000 mg/kg)	First test	Second test
Number of death / Number of mice using (Mortality: 72 hours)	0/3	0/3

Sub-acute toxicity

During the period of sub-acute toxicity study (28 days), any death was not recorded. Also, there was not observed clinical behavioural change between control and treated groups.

Body weight

The mean weekly body weight gains of control and daily treated rats with LMaq during 28 days are illustrated in figures 1 and 2. As shown in these figures, there was no significant statistical difference in body weight gain between treated and control groups in both sex.

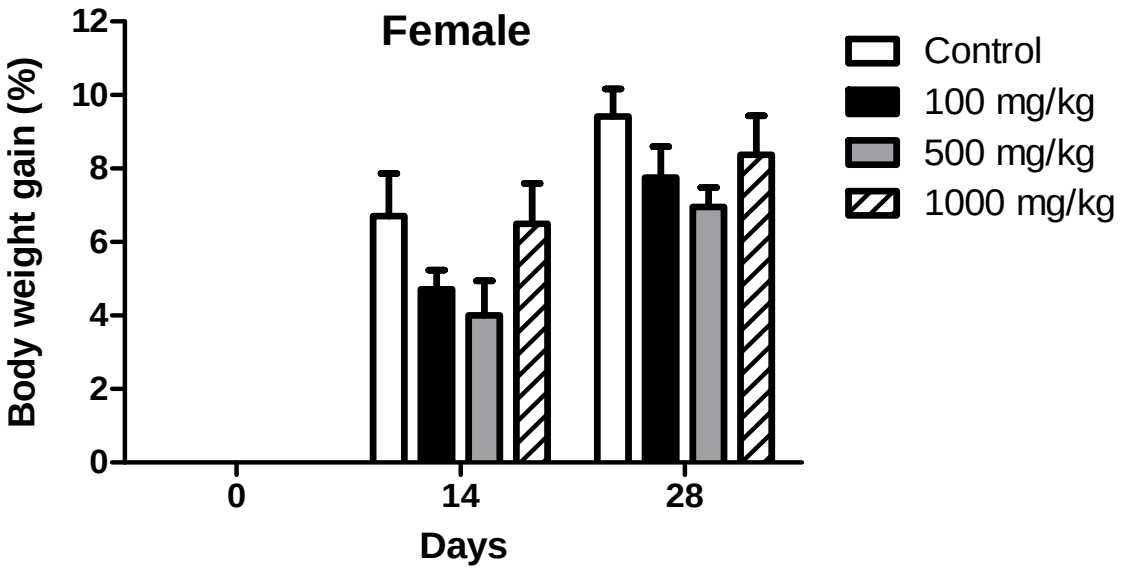

Figure 1: Mean body weight gain (%) of control and treated females rats with different doses of LMaq. Mean and Standard deviation are presented (n = 5).

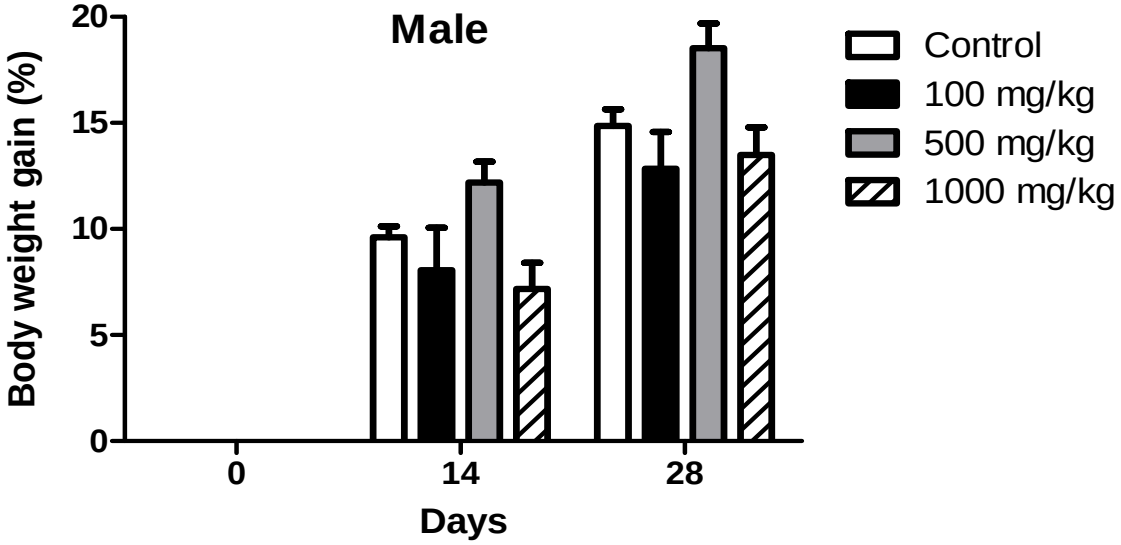

Figure 2: Mean body weight gain (%) of control and treated male rats with different doses of LMaq. Mean and Standard deviation are presented (n = 5).

Water intake and food consumption

The results of daily water intake are presented in table 2. The averages water consumption of treated groups were found to be similar to the control groups. Table 3 summarize the mean weekly food consumption of the different groups during study period (28 days). As shown in this table 3, the food consumption of treated groups was found to be unaffected by the

treatment as there were no significant changes in the average food consumption when compared with the control groups.

Table 2: Mean daily water consumption (mL/day/rat) during 28 days treatment with *Lannea microcarpa* trunk barks aqueous extract.

Dose of LMaq	Sex	Week 1	Week 2	Week 3	Week 4
Control	F	44.72 ± 3.90	32.92 ± 3.02	34.83 ± 4.65	29.52 ± 3.90
	M	48.20 ± 2.43	47.36 ± 4.63	48.83 ± 2.17	46.94 ± 2.28
100 mg/kg	F	44.58 ± 8.84	31.39 ± 1.94	30.83 ± 1.83	27.08 ± 2.92
	M	48.19 ± 1.70	43.89 ± 4.40	47.78 ± 3.48	43.83 ± 2.47
500 mg/kg	F	43.61 ± 2.56	31.25 ± 2.51	29.72 ± 1.01	26.25 ± 2.45
	M	47.92 ± 2.16	44.17 ± 3.25	45.17 ± 3.03	43.33 ± 1.32
1000 mg/kg	F	37.08 ± 4.87	34.58 ± 3.15	33.06 ± 2.34	28.47 ± 3.22
	M	49.30 ± 2.91	47.92 ± 2.02	47.36 ± 2.86	46.33 ± 2.67

Mean and standard deviation are presented (n = 10; 5/sex). M = Male; F = Female

Table 3: Mean weekly food consumption (g/day/rat) during 28 days treatment with *Lannea microcarpa* trunk barks aqueous extract.

Dose of LMaq	Sex	Week 1	Week 2	Week 3	Week 4
Control	F	22.02	18.88	20.55	21.81
	M	24.29	26.60	23.69	21.57
100 mg/kg	F	19.12	18.57	17.26	16.93
	M	21.71	22.90	22.02	19.40
500 mg/kg	F	18.48	17.52	17.69	18.81
	M	25.19	25.17	24.71	21.29
1000 mg/kg	F	16.98	18.57	17.98	17.71
	M	25.07	25.26	24.40	21.17

Mean and standard deviation are presented (n = 10; 5/sex). M = Male; F = Female

Macroscopic effects of LMaq on vital organs

Macroscopic examination of vital organs such as heart, lung, liver, kidney, gonads and spleen of control and treated animals with LMaq show that extract doesn't affect vital organs as there was no change in color and aspect of different organs.

Effect of LMaq on biochemical parameters of rats

The results of biochemical tests of treated animals with LMaq and control groups are presented in table 4. These results indicated that *per os* administration of LMaq at doses of 100, 500 and 1000 mg/kg bw on rats for 28 consecutive days did not cause significant changes in blood serum biochemical parameters such as total proteins, chlorid, phosphorus, aspartate aminotransferase, alanine aminotransferase, magnesium**,** fasting blood glucose,

creatinine, triglycerid, uric acid, and total cholesterol levels when compared to control groups. However, significant difference in chlorid level were observed in group treated at dose of 100 and 500 mg/kg bw when compared to control groups ($p < 0.01$).

Table 4: Biochemical parameters for rats after 28 days treatment with *Lannea microcarpa* trunk barks aqueous extract.

	Sex	Control	Doses of LMaq		
			100 mg/kg	500 mg/kg	1000 mg/kg
Total protein (g/L)	F M	83.18±2.90 78.84±2.03	78.50±1.36 74.60±2.63	79.82±5.29 75.50±2.01	79.80±4.36 77.06±2.48
Chlorid (mmol/L)	F M	112.00±2.12 110.00±1.87	106.60±1.95** 105.00±1.00**	106.40±2.30** 105.80±1.10**	110.60±0.55 111.60±1.67
Phosphorus (mmol/L)	F M	2.28±0.20 2.62±0.18	2.62±0.43 2.62±0.29	2.82±0.61 2.48±0.08	2.52±0.27 2.48±0.33
Aspartate aminotransferase (U/L)	F M	79.51±3.21 84.56±15.74	97.54±17.11 77.28±17.14	96.19±33.43 89.51±9.43	106.87±18.97 79.59±6.98
Alanine aminotransferase (U/L)	F M	21.19±3.65 29.78±2.94	24.55±4.33 36.12±7.34	30.36±11.08 31.12±2.08	23.54±5.59 23.66±1.90
Magnesium (mmol/L)	F M	2.61±0.85 2.33±0.61	2.81±1.37 3.07±1.41	3.47±1.20 3.19±0.96	3.25±1.22 2.30±0.61
Fasting blood glucose (mmol/L)	F M	4.98±0.79 4.84±0.80	4.10±0.76 4.10±1.27	4.34±1.24 4.72±0.54	5.32±0.72 4.50±1.01
Creatinine (µmol/L)	F M	77.40±3.92 65.82±2.63	73.31±2.71 64.21±3.26	67.18±7.68 63.97±3.37	69.65±1.18 61.21±2.52
Triglycerid (mmol/L)	F M	0.24±0.05 0.24±0.05	0.28±0.08 0.28±0.04	0.28±0.13 0.26±0.09	0.22±0.08 0.26±0.05
Uric acid (µmol/L)	F M	93.70±18.09 89.91±10.57	93.49±19.14 92.42±14.56	89.29±15.17 97.07±12.90	91.92±15.32 85.57±8.37
Total cholesterol (mg/dL)	F M	25.18±4.19 30.86±3.08	23.06±2.69 23.34±4.61	25.40±8.87 26.62±4.52	22.44±3.92 30.22±3.78

Mean and standard deviation are presented (n = 10; 5/sex). M = Male; F = Female

Effect of LMaq on organs weights in rats

The relative organs weights (ROW) of control and treated rats with LMaq at dose of 100, 500 and 1000 mg/kg bw are shown in Table 5. No significant change was noticed among different doses of treatment and control groups ($p > 0.05$).

Table 5: Mean relative organs weights (%) of rats after 28 days treatment with *Lannea microcarpa* trunk barks aqueous extract.

Organs	Sex	Doses of LMaq			
		Control	100 mg/kg	500 mg/kg	1000 mg/kg
Liver	F	2.54±0.08	2.83±0.32	2.80±0.34	2.83±0.08
	M	2.41±0.11	2.40±0.15	2.46±0.09	2.43±0.17
Heart	F	0.37±0.02	0.38±0.05	0.36±0.02	0.35±0.05
	M	0.34±0.05	0.34±0.03	0.34±0.02	0.36±0.03
Kidney	F	0.64±0.06	0.64±0.04	0.65±0.02	0.65±0.04
	M	0.59±0.04	0.60±0.05	0.61±0.03	0.63±0.06
Lung	F	0.62±0.05	0.64±0.03	0.57±0.05	0.56±0.10
	M	0.49±0.06	0.47±0.05	0.47±0.02	0.48±0.04
Spleen	F	0.24±0.02	0.24±0.01	0.26±0.03	0.26±0.05
	M	0.20±0.01	0.19±0.02	0.20±0.03	0.21±0.02
Gonads	F	0.07±0.01	0.07±0.02	0.07±0.01	0.07±0.01
	M	1.04±0.08	1.16±0.10	1.07±0.12	1.14±0.06

Mean and standard deviation are presented (n = 10; 5/sex). M = Male; F = Female

DISCUSSION

The use of the natural products as a source of cure for many diseases goes back to ancient times.[18] Herbal medicines are often mistakenly considered as non-toxic drugs because they are "natural". These products contain a multitude of bioactive principles that can cause damage. It is therefore necessary that all these herbal drugs be subjected to safety tests by the same methods as those used for modern drugs.[19-21] Products safety tests include acute general toxicity, which refers to adverse events occurring during the single dose exposure of a chemical, and sub-acute toxicity which monitored adverse effects over a long period of exposure repeated to a test substance.[22, 23] In this present study, acute toxicity of LMaq was assessed. The results obtained indicate that a single administration of plant extract at dose of 2000 mg/kg bw to mice did not cause mortality or any behavioral change during 14-days observation period. Based on OECD guideline 423, the LD_{50} of LMaq is estimated to 5000 mg/kg. This LD_{50} value suggest that plant extract tested is classified to the 5th toxicity class i.e. products which have relatively a low oral acute toxicity.[15,24]

Our results are similar to those of other authors which found that the aqueous infusion of the powder of the stem barks of *Lannea microcarpa* was nontoxic in rats up to the dose of 3000 mg/kg bw by oral administration.[23]

In addition to acute oral toxicity assessment we sought to determine sub-acute toxicity of LMaq as most of the medicinal herbal recipes are repeatedly taken. Thus, the daily doses of

100, 500 and 1000 mg/kg bw were administered to the groups of animals during four (04) weeks and a control groups which received only the distilled water. In this study, the daily oral administration of LMaq at different doses during 4 weeks did not cause any mortality and clinical sign of toxicity. The mean weekly body weight gain and relative organ weights of treated groups were similar to control ones. Body weight is known to be one of the most sensitive indicators of adverse effects of drugs and chemicals.[16] Weight loss is a sign of toxicity resulting from a loss of appetite or a metabolism problem of food consumed by animals.[14] The results obtained during the 4 weeks of study concerning the weight gain suggest that LMaq did not influence the animals weight gain up to dose 1000 mg/kg bw because an increase in body weight in treated groups similar to control groups was observed. In addition, the consumption of water and food in different treated groups was similar to control groups.

Levels of biochemical parameters determine the physiological conditions. Increasing and decreasing in biochemical parameters may give indications of specific organ toxicity [25]. In preclinical toxicity studies, biochemical parameters evaluation is important to know if some organs don't affect by test substance. Indeed, several studies have reported that herbal products can cause for example hepatic and renal toxicity.[21,26] In our study the major biochemical parameters were not modified in the different treated groups when compared to the control with the exception of chlorid. There were statistical significant differences in serum levels of chlorid in the LMaq-treated groups (100 and 500 mg/kg bw) compared to controls, but no significant difference for the last group with high dose (1000 mg/kg bw). This variation in chlorid values is not dose dependent as the rats administered at the high dose of 1000 mg/kg bw had similar values to the control groups. Moreover, the non-modification of the creatinine values does not allow to say that there were really harmful effects of LMaq on the kidneys. Referring to the results of some authors, a daily 14 day's oral administration of infusion of the plant trunk barks at dose up to 3000 mg/kg bw on rats doesn't cause change in biochemical parameters.

Also macroscopic examination of vital organs such as liver, heart, kidney, lungs, spleen and gonads as well revealed no treatment-related changes due to the administration of plant extract. This result confirms the no change in relative organs weight in treated and control groups.

These all results suggest that LMaq did not negatively affect the animals after 4 weeks of treatment indicating that this extract is relatively safe.

CONCLUSION

Oral acute and sub-acute toxicity of the aqueous extract from the trunk barks of *Lannea microcarpa* respectively in mice and rats were studies. The results obtain in this study suggest that aqueous extract of *Lannea microcarpa* trunk barks are relatively safe when administered orally and could justify the use of this part of the plant in traditional medicine for the treatment of various diseases.

REFERENCES

1. Organization WH: **Promoting the Role of Traditional Medicine in the Health System: A Strategy for the African Region AFR/RC50/9.** *Harare: Regional Office for Africa,* 2001.
2. Bazongo P, Bassolé IHN, Nielsen S, Hilou A, Dicko MH, Shukla VK: **Characteristics, composition and oxidative stability of *Lannea microcarpa* seed and seed oil.** *Molecules,* 2014; **19:** 2684-2693.
3. Picerno P, Mencherini T, Loggia RD, Meloni M, Sanogo R, Aquino R: **An extract of *Lannea microcarpa*: composition, activity and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis.** *Journal of pharmacy and pharmacology,* 2006; **58:** 981-988.
4. Marquet M, Jansen P: ***Lannea microcarpa* Engl. and K. Krause.** *Prota 3: Dyes and Tannins/Colorants et Tanins,* 2005.
5. Belemnaba L, Nitiema M, Traoré S, Somé N, Traoré A, Ouédraogo S, Guissou I: **Recherche de plantes à potentialités antihypertensives dans la biodiversité du Burkina Faso.** *Pharmacopée et médecine traditionnelle africaine,* 2014; **17:** 33-40.
6. Hilou A, Bougma A, Dicko MH: **Phytochemistry and Agro-Industrial Potential of Native Oilseeds from West Africa: African Grape (*Lannea microcarpa*), Marula (*Sclerocarya birrea*), and Butter Tree (*Pentadesma butyracea*).** *Agriculture,* 2017; **7:** 24.
7. Pale E. **Contribution to the study of anthocyanin compounds in plants: The case of *Hibicus sabdariffa, Lannea microcapa* and *Vigna sorghum caudatum* from Burkina Faso.** *PhD.* University of Ouagadougou, 1998.
8. Duponchel P. **Textiles bògòlan du Mali:** *Musée d'ethnographie de Neuchâtel,* 2004.

9. Peters BSE, Martini LA: **Nutritional aspects of the prevention and treatment of osteoporosis.** *Arquivos Brasileiros de Endocrinologia & Metabologia,* 2010; **54:** 179-185.
10. Ouattara L, Koudou J, Zongo C, Barro N, Savadogo A, Bassole I, Ouattara A, Traore AS: **Antioxidant and antibacterial activities of three species of *Lannea* from Burkina Faso.** *Journal of Applied Sciences,* 2011; **11:** 157-162.
11. Ajiboye T, Raji H, Muritala H, Ojewuyi O, Yakubu M: **Anthocyanin extract of *Lannea microcarpa* fruits stall oxidative rout associated with aflatoxin B 1 hepatocarcinogenesis.** *Food Bioscience,* 2013; **4:** 58-67.
12. Ouédraogo S, Belemnaba L, Zague H, Traore A, Lompo M, Guissou IP, Lugnier C, Bucher B: **Endothelium-independent vasorelaxation by extract and fractions from *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae): Possible involvement of phosphodiesterase inhibition.** *International Journal of Pharmacology and Biological Sciences,* 2010; **4:** 9-16.
13. Antwi-Adjei M, Owusu G, Ameade EP: **Aqueous extract of *Lannea microcarpa* attenuates dextran sulphate-induced paw oedema and xylene-induced ear oedema in rodents.** *International Journal of Basic & Clinical Pharmacology* 2017, **6:**1048-1053.
14. Almamy Konate, Geoffroy G. Ouedraogo, Sylvain Ilboudo, Noufou Ouedraogo, Adama Kabore, Amadou Traore, Guissou IP, Tamboura HH: **Acute and sub-acute oral toxicity studies of aqueous extract of *Securidaca longepedunculata* Fresen (Polygalaceae) root barks in rodents.** *International Journal of Advanced Research,* 2016; **4:** 550-558.
15. OCDE: **Toxicité orale aiguë - Méthode par classe de toxicité aiguë. Lignes directrices de l'OCDE pour les essais de produits chimiques.** vol. 2001.
16. Ouedraogo GG, Ouedraogo M, Lamien-Sanou A, Lompo M, Goumbri-Lompo OM, Guissou PI: **Acute and Subchronic Toxicity Studies of Roots Barks Extracts of" *Calotropis procera*"(Ait.) R. Br Used in the Treatment of Sickle Cell Disease in Burkina Faso.** *British Journal of Pharmacology and Toxicology,* 2013; **4:** 194-200.
17. OECD: **Guidance Document on Subacute Oral Toxicity Testing 407; Organization for Economic Co-operation and Development: Paris, France,** 2008.
18. Baliga MS, Jagetia GC, Ulloor JN, Baliga MP, Venkatesh P, Reddy R, Rao KM, Baliga BS, Devi S, Raju SK: **The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (*Alstonia scholaris*) in mice and rats.** *Toxicology letters,* 2004; **151:** 317-326.

19. Talalay P, Talalay P: **The importance of using scientific principles in the development of medicinal agents from plants.** *Academic Medicine,* 2001; **76:** 238-247.
20. Bent S, Ko R: **Commonly used herbal medicines in the United States: a review.** *The American journal of medicine,* 2004; **116:** 478-485.
21. Abotsi WK, Ainooson GK, Gyasi EB, Abotsi WKM: **Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt.(Phytolaccaceae) in rodents.** *West Afr J Pharma,* 2011; **22:** 27-35.
22. Nath P, Yadav AK: **Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of *Hibiscus rosa-sinensis* L. in mice.** *Journal of intercultural ethnopharmacology,* 2015; **4:** 70.
23. George Owusu, Antwi-Adjei M: **Acute and sub-acute oral toxicity studies of the aqueous extract of *Lannea microcarpa* stem bark on rats.** *International Journal of Pharmacy & Pharmaceutical Research,* 2017; **9:** 17-30.
24. Unies N: **Système Général Harmonisé de classification et d'étiquetage des produits chimiques (SGH). Quatrième édition révisée. New York et Genève. ST/SG/AC.10/30/Rev4.** 2011.
25. Das N, Goshwami D, Hasan MS, Raihan SZ: **Evaluation of acute and subacute toxicity induced by methanol extract of *Terminalia citrina* leaves in Sprague Dawley rats.** *Journal of Acute Disease,* 2015; **4:** 316-321.
26. Greaves P. **Histopathology of preclinical toxicity studies***: interpretation and relevance in drug safety evaluation.* Academic Press: 2011.