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Acute and Sub-Acute Oral Toxicity Studies of the Aqueous Extract of *Lannea microcarpa* Stem Bark on Rats

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ABSTRACT

The aqueous stem bark of *Lannea microcarpa* is used traditionally for the treatment of swellings, wounds, rheumatism, pains, and infections. **Aim:** To evaluate the safety of the aqueous extract of the stem bark of *Lannea microcarpa*. **Materials and Methods:** Male Sprague-Dawley rats (200-250 g, n=5) received oral administration of 300, 1000 and 3000 mg kg^{-1} of aqueous extract of *Lannea microcarpa* and were monitored for 24 hours and 14 consecutive days for acute and sub-acute toxicity effects respectively. Signs of treatment-related toxicity such as behavioral changes, mortality and clinical effects were observed and evaluated. Control rats received 1 ml of normal saline. **Results:** In acute toxicity study, there were no behavioral changes as well as mortality in the LME (300-3000 mg kg^{-1}, *p.o*)-treated rats. Similarly, in the sub-acute studies, LME (300-3000 mg kg^{-1}, *p.o*)-treated rats showed no significant differences in the body weight, organ weight, hematological and biochemical parameters that were assessed ($P \leq 0.05$). **Conclusion:** Oral administration of aqueous extract of *Lannea microcarpa* revealed no signs of toxicity.

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INTRODUCTION

Traditional medicine has been practiced by man since the advent of a human race. It has a long standing history that while ancient humans were searching for food, they discovered that some plants had the potential of treating both human and animal diseases [1]. Today, every culture has evolved the indigenous system of traditional healing [2]. In addition to providing the potential remedy to many ailments, traditional medicine also serves as a gateway to the discovery of many substances with active therapeutic effects used in the commercial production of drugs [3]. Thus, plants remain the main source of active natural drugs from natural sources and are imperative in traditional medicine for treating numerous diseases [4].

Lannea microcarpa Engl. and Krause (Family Anacardiaceae) is a tropical deciduous tree that grows up to about 15 m tall and 70 cm in diameter. It is located in most regions of West Africa and largely distributed in the dry forest regrowth areas. The leaves usually alternate and imparipinnate up to about 25 cm long with 2-3 pairs of leaflets. The flowers are green-yellowish, unisexual and regular; and the fruits are ellipsoid and glabrous drupes containing a single seed [5]. Most parts of the plant such as the bark and leaves are used traditionally in treating numerous medical disorders including swellings, wounds, blisters, sore throat and rheumatism [5]. The leaves are used to treat diarrhea, gastroenteritis, malaria, bacterial infections, toothaches, swellings, wounds [6,7] and also to manage mouth blisters, sore throat, dysentery and as dressing on boils [8,9]. The fruits are used to treat scurvy, rickets, and cough. The bark and roots are used to treat stomach pain, rheumatism, gonorrhea, diarrhea, chest pain, gastric ulcer, skin and respiratory tract infections [9] and also possess antibacterial and antihypertensive effects [10,11,12]. In Mali, the plant is used traditionally to treat wounds [13]. Isolated and characterized of some phytochemical constituents of the plant [14] and its antioxidant activity [15] have also been reported. We recently reported on the anti-inflammatory effect of the aqueous extract of the plant on dextran sulphate-induced edema and xylene-induced pineal edema in murine models [16]. Though the folkloric use of the plant is being established and supported with scientific data, there is no report on its safety in animals or humans. Furthermore, a plant or any herbal formulation containing certain active organic constituents to be identified for traditional medicine, a systematic approach is required for the investigations of safety and efficacy through experimental and clinical discovery [17]. Hence, this study is aimed to evaluate the safety or otherwise of this time-tested plant, *Lannea microcarpa*.

MATERIALS AND METHODS

Materials

Plant collection and extraction

The stem bark of *Lannea microcarpa* was collected from New Eduabiase, Ashanti Region of Ghana in January 2017 and it was authenticated by Dr. Henry Sam of Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A voucher specimen (KNUST/HM1/2017/L005) was prepared and kept in the herbarium of the same department. The extract was prepared as we previously described [16]. Briefly, the stem bark was chopped into pieces and air-dried for 14 days under room temperature. The dried material was pulverized with a hammer-mill mill (DF-19, DADE, 20 kg/h-101v, HXJQ, China) to a coarse powder. Five hundred grams (500 g) of the powder was infused with 2.0 L of distilled water and warmed for 60 minutes at 90 ^{0}C. The infusion was filtered to obtain a dark-brown filtrate which was evaporated over a hot water-bath and later dried in an oven at 55 ^{0}C to obtain a solid extract. The yield was 10.8 % W/W. The extract was labeled as LME and kept in a refrigerator for use. The extract was reconstituted as an emulsion in 2 % tragacanth mucilage. LME in this report refers to *Lannea microcarpa* extract.

Animals

Male Sprague-Dawley rats (200 -250 g; 8-10 weeks old) were used in both acute and sub-acute protocols. The animals were obtained from the animal house of Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and were kept at the same facility until used. The animals were randomly selected, grouped (n=5) and kept in stainless cages (34×47×18 cm) with soft wood shavings as bedding. The animals were maintained under laboratory conditions (temperature 24-25 ^{0}C, 12-hrs light-dark cycle) with free access to pellet diet (GAFCO, Tema, Ghana) and water *ad libitum*. All animals were handled and ethically in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 88-2959, revised 1996). All experiments were examined and approved by the ethics committee of the Department of Pharmacology, KNUST. Each animal was used once and properly euthanized at the end of the experiment.

Methods

Acute toxicity test of LME

Acute toxicity effect of aqueous extract of *Lannea microcarpa* was evaluated as previously described [18]. Briefly, rats were randomly put into 4 groups (n = 5) and denied food over 24 hrs before the experiment. Group I (control) 1 ml of normal saline orally whereas groups II-IV received a single dose oral administration of 300, 1000 and 3000 mg kg^{-1} of LME respectively. The body weight of each rat was determined before dosing and the doses were calculated based on the body weight of rats. Animals were observed continuously for 24 hrs to detect any signs of behavioral or physiological changes, mortality, and toxicity.

Sub-acute toxicity test of LME

The rats were put into 4 groups (n=5). Group 1 (control) received normal saline (1 ml kg^{-1} *p.o*) daily for 14 days. Groups II-IV were received oral administration of 300, 1000 and 3000 mg kg^{-1} of the extract daily for 14 consecutive days. Animals were then weighed every other day, from the start of treatment, to note weight variation. Animals were monitored closely for signs of toxicity. Changes in appearance and daily behavior such as food and water intake were also assessed.

Serum preparation

The rats were sacrificed on day 15 by cervical dislocation. Blood sample (1.5 ml) was collected from a severed jugular vein into EDTA tubes for hematological assessment. About 3.0 ml of blood was collected into vacuum container gel and activator tubes which contained no anticoagulant. A blood sample was allowed to clot and then centrifuged (Denley BS-400, Milton Keynes, England) at ×4000 g for 10 min and the serum obtained was stored at -20 °C and later assayed for biochemical parameters.

Effect of LME on hematological parameters

Blood samples collected were screened and the levels of concentration of the various hematological parameters were determined using an automatic haem analyzer (Cell Dyne: Model 331430- Abbott Laboratories, IL, USA).

Effect of LME on biochemical parameters

The serum obtained was analyzed for aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein, albumin, globulin, total bilirubin, direct bilirubin, indirect bilirubin, urea and creatinine levels using an automated blood chemistry analyzer (model: ATAC 8000, Elan Diagnostics, CA, USA).

Relative organ weight

After blood collection, rats were quickly dissected and vital organs such as the spleen, liver, heart, kidney, and stomach were removed, freed of extraneous tissues and washed. Organs were then weighed on a balance and the relative organ weight of each animal was calculated as;

$$\text{Relative organ weight} = \frac{Absolute\ organ\ weight\ of\ rat\ (g)}{Rat\ body\ weight\ on\ the\ day\ of\ sacrifice(g)} \times 100$$

STATISTICAL ANALYSIS

Data obtained was presented as mean ± SEM. Significant differences were determined using one-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test. All statistical analyses were done using GraphPad Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA). Differences between means of treated groups and the control were considered as statistically significant at $P \leq 0.05$.

RESULTS

Acute toxicity test of LME

There was no mortality or any observable related signs of behavioral changes or symptoms of toxicity after oral administration of LME (300-3000 mg kg^{-1}) in the rats at all doses (Table 1.0).

Table 1.0. Acute toxicity effect of LME on rats.

Observation	Group I Control	Group II 300 mg kg^{-1}	Group III 1000 mg kg^{-1}	Group IV 3000 mg kg^{-1}
Body weight	unchanged	unchanged	unchanged	unchanged
Food & water intake	normal	normal	normal	normal
Urination	regular	regular	regular	regular
Skin & eye color	normal	normal	normal	normal
General physique	normal	normal	normal	normal
Diarrhoea	nil	nil	nil	nil
Stool	solid	solid	solid	solid
Coma	nil	nil	nil	nil
Mortality	0/5	0/5	0/5	0/5

Rats orally received single doses of LME (300-3000 mg kg^{-1}) and were closely observed 24 hrs for signs of treatment-related toxicity. Control group received 1 ml kg^{-1} of normal saline.

Subacute toxicity study of LME

Effect of LME on the body weight of rats

Administration of LME (300, 1000 and 3000 mg kg^{-1} *p.o*, daily) did not cause any significant treatment-related changes in the mean maximum and total body weights of the rats at end of the study period as compared to the control ($P \leq 0.05$) (Fig. 1).

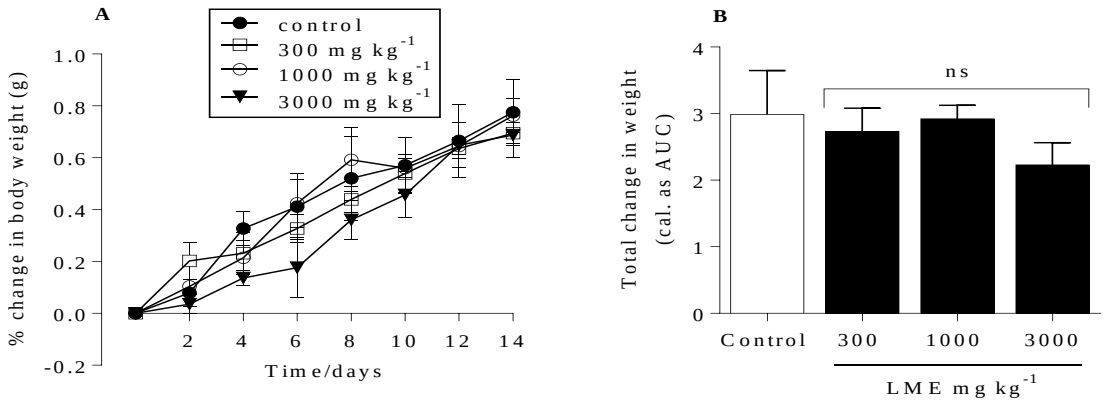

Figure 1.0. Effect of LME (300-3000 mg kg^{-1}, *p.o*, daily) on the maximum (A) and total (B) body weights (g) of rats (n = 5). ns denotes non-significance.

Effect of LME on the organ weight of rats

There were no significant differences in the relative organ weights of the rats after oral administration of LME (300, 1000 and 3000 mg kg^{-1}, daily) for 14 days of study as compared to the control ($P \leq 0.05$) (Table 2.0).

Table 2.0. Sub-acute toxicity effect of LME on organ weights of rats.

	Relative Organ Weight (g/100g)			
Organs	Group I Control	Group II 300 mg kg^{-1}	Group III 1000 mg kg^{-1}	Group IV 3000 mg kg^{-1}
Liver	3.16±0.43	3.20±0.64	2.72±0.56	3.00±0.47
Lungs	3.03±0.42	3.09±0.58	3.33±0.66	2.86±0.39
Heart	2.12±0.38	2.12±0.38	2.09±0.33	2.49±0.41
Kidney	1.18±0.21	1.35±0.23	1.28±0.28	1.10±0.22
Stomach	1.18±0.31	1.13±0.24	0.99±0.12	1.02±0.15
Spleen	0.54±0.14	0.62±0.20	0.51±0.16	0.51±0.16

Values are expressed as mean ± SEM. Comparisons were made between control and LME (300-3000 mg kg^{-1}, *p.o*, daily). The statistical interval at $P \leq 0.05$ was considered as significant.

Effect of LME on hematological parameters of rats

LME (300 -3000 mg kg^{-1}, *p.o*, daily) did not cause any significant treatment-related changes in the hematological parameters that were measured when compared to the control ($P \leq 0.05$) (Table 3.0).

Table 3.0. Sub-acute toxicity effect of LME on hematological parameters of rats.

Parameters	Group I Control	Group II 300 mg kg^{-1}	Group III 1000 mg kg^{-1}	Group IV 3000 mg kg^{-1}
RBC ($\times 10^{12}$/L)	9.02±1.31	8.32±2.47	7.05±1.87	9.21±4.22
WBC ($\times 10^{9}$/L)	5.93±0.26	6.44±3.71	5.82±3.67	5.29±4.78
HGB (g/dL)	13.76±0.52	12.88±0.33	14.76±1.21	13.91±0.66
MCV (fL)	59.28±0.32	61.02±4.45	58.26±0.71	60.27±4.55
MCH (pg)	21.42±0.66	21.09±0.31	20.53±0.44	21.21±0.41
MCHC (g/dL)	41.19±1.03	39.16±0.81	40.05±1.41	41.03±0.55
PLT ($\times 10^{9}$/L)	388.78±45.78	391.22±67.00	374.72±88.02	377.92±34.04
HCT (%)	46.22±0.81	47.01±0.25	45.60±0.43	47.34±0.03
PCV (%)	40.45±0.22	40.62±1.12	39.22±0.07	41.91±0.01
LYM (%)	57.17±2.15	61.01±2.30	56.38±0.26	58.33±2.11
Neutrophil (%)	23.32±0.17	22.87±0.01	21.91±0.33	24.92±0.35
Monocyte (%)	4.32±0.60	3.38±0.33	5.06±0.04	3.27 ±0.07

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Rats (n = 5) were treated with LME (300-3000 mg kg^{-1}, *p.o*, daily) for 14 days. Blood samples were collected into EDTA tubes for analysis. Values are presented as mean ± SEM.

Effect of LME on biochemical parameters of rats

There were no significant differences in the biochemical parameters in the sera after the rats received LME (300-3000 mg kg^{-1}, *p.o*, daily) for 14 consecutive days as compared to the control ($P \leq 0.05$) (Table 4.0).

Table 4.0 Sub-acute toxicity effect of LME on biochemical parameters of rats.

Parameters	Group I Control	Group II 300 mg kg^{-1}	Group III 1000 mg kg^{-1}	Group IV 3000 mg kg^{-1}
Total protein (g/dL)	4.01±2.10	5.21±3.06	3.91±0.22	3.72±3.01
Albumin (g/dL)	2.99±0.03	3.62±1.12	1.97±0.06	2.74±2.11
Total bilirubin (mg/dL)	0.39±0.57	0.37±0.02	0.221±1.03	0.32±0.09
Direct bilirubin (mg/dL)	3.17±0.32	2.91±0.77	3.21±0.01	2.89±0.44
Indirect bilirubin (mg/dL)	2.75±0.27	3.62±0.12	2.59±0.45	3.57±0.22
Urea (mg/dL)	31.34±2.38	28.27±2.31	33.2±03.07	29.41±1.42
Creatinine (mg/dL)	0.94±0.28	1.03±0.27	0.86±0.78	0.89±0.24
Uric acid (mg/dL)	0.38±0.39	0.21±0.77	0.28±0.81	0.32±0.29

Rats (n = 5) were treated with LME (300-3000 mg kg^{-1}, *p.o*, daily) for 14 days. Serum obtained from blood sample was analyzed. Values are presented as mean ± SEM.

Effect of LME on liver enzymes of rats

Levels of AST and ALT concentration increased slightly at 300 mg kg^{-1} dose of LME when compared to the control rats (Fig. 2[A, B]). Similarly, levels of ALP and GGT concentration increased marginally at 1000 mg kg^{-1} dose of LME when compared to the control (Fig. 2[C, D]). However, there were no significant differences observed in the levels of the liver enzymes of LME (300-3000 mg kg^{-1}, *p.o*, daily) as compared to that of the control ($P \leq 0.05$) (Fig. 2)

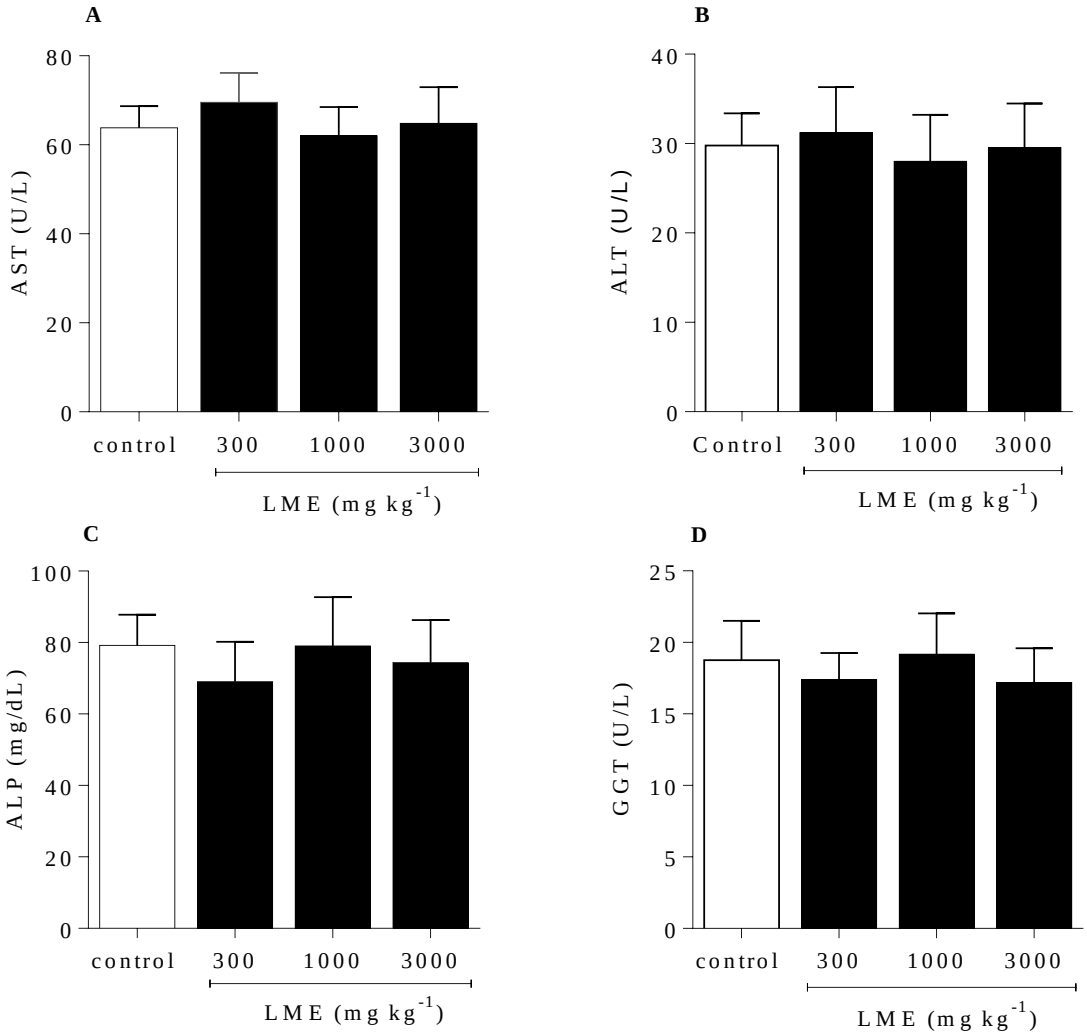

Figure 2.0. Effect of LME (300-3000 mg kg^{-1}, *p.o*, daily) on the levels of AST (A), ALT (B), ALP (C) and GGT (D) of rats respectively.

DISCUSSION

The use of the natural product as a source of remedy for many diseases dates back in the ancient time. Natural products are known to provide potential pharmacological effects in the management of various acute and chronic diseases, especially in developing countries [19]. The use of herbal medicines has received greater attention as an alternative to clinical therapy leading to the high increase in demand [17]. Though the use of herbal medicines is receiving popularity, there is still a misconception among practitioners on their safety as compared to the conventional therapy. It is therefore imperative for experimental screening method to be conducted in order to ascertain the safety and efficacy of the herbal medicines as well as to establish the active constituents of these herbal medicines [17]. This research is aimed to

evaluate the toxicity profile of aqueous extract of *Lannea microcarpa* and also to provide a valid scientific data as a repository for further scientific research.

Acute toxicity refers to the adverse effects that occur on the first exposure to a single dose of a chemical [20]. In our acute toxicity study, there were no mortality and behavioral changes observed in the animals after doses of LME (300-3000 mg kg^{-1}) were administered orally. Thus, our study demonstrates that LME did not induce any apparent acute toxicity and can be considered to be reasonably safer on acute exposure [21].

While the acute effect is usually observed soon after single dose exposure of a test agent, the subacute effect is monitored over an extended period of repeated exposure to a test substance [22]. The changes in body weight are used as an indicator of adverse effects of substances [23] but weight loss is a sign of toxicity as results of fluid loss, loss of appetite, poor metabolism or food utilization. In this study, there were no significant differences observed in the body and vital organ weights of the animals at all doses used indicating that LME did not have any adverse effects on body weight and vital organs, which are used to assess the response of therapy to drugs [24] and adverse effects of drugs [23]. Usually, drugs are metabolized and transported to target organs and tissues in the body via the blood; and this makes the blood cells more vulnerable to harmful effects of drugs and other xenobiotic substances. As a result, pro-drugs and their active metabolites have the potential to affect the different blood cells thereby altering the normal blood profile [25]. Since there were no significant changes in the hematological parameters of LME-treated rats, we can, therefore, say that LME may not be toxic and has no adverse effect on blood cells or hematopoiesis [26].

Levels of total proteins, albumin and globulins are used as diagnostic tests for liver function [27]. High levels of globulins are indicative of hemolysis, hepatitis, heart failure, kidney or liver diseases [28] and high levels of direct or unconjugated indirect bilirubin causes liver disorder such as cirrhosis or bile duct blockade [29]. In this study, we have demonstrated that LME (300-3000 mg kg^{-1}) showed no significant differences in the levels of biochemical indices which were analyzed. It is therefore not surprising that LME did not interfere with renal and liver function, and hence supports the claim that LME is non-toxic.

Transaminases (AST, ALT) and *alkaline phosphatase* (ALP) are vital indicators of liver and kidney damage respectively [30,31]. Mostly, GGT and ALP are elevated in bile duct diseases

or hepatic disorders, however, high ALP level only with normal GGT level is a sign of bone disease [32]. Although the liver enzyme assessment showed the marginal increase of AST, ALT, and GGT, ALP at 300 mg kg^{-1} and 1000 mg kg^{-1} of LME-treated rats respectively, there were no significant differences observed in AST, ALT, GGT and ALP levels of LME-treated rats as compared to the control. Hence, from the results, it can be delineated that LME did not induce any deleterious effects on bile duct, bone damage or liver function. However, histological assessment needs to be conducted further to ascertain the organ protective effect of LME.

CONCLUSION

The oral administration of aqueous extract of *Lannea microcarpa* did not cause any acute or sub-acute toxicity effects and could be considered as safe for traditional medicinal use.

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