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Acute and Subchronic (28-Day) Oral Toxicity Studies of Hydroalcoholic Extract of *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae) Stem Bark

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Abstract: In this study we investigated the acute and subchronic toxicity of hydroalcoholic (50-50: v/v) extract of *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae) stem bark in Wistar rats. In the acute test, the dose of 5.000 mg kg^{-1} was used for the test limit. Animals were then observed individually 1 h post dosing and at least once daily for 14 days. The subchronic toxicity was evaluated through biochemical, haematological, body and relative organ weight of rats using daily oral doses of 500 and 1000 mg kg^{-1} b.wt., during 28 days. The limit dose of 5.000 mg kg^{-1} did not cause mortality or any sign of acute toxicity in any of the rats tested in the observatory period. In the subchronic test, *L. kerstingii* at 1000 mg kg^{-1} decreased significantly ($p<0.05$) the increment of body weight of rats from the 2nd to the 4th week. The decrease of the increment was 11, 11 and 10% on the 2nd, 3rd and 4th week, respectively. The relative weight of the spleen in the group treated with 1000 mg kg^{-1} b.wt. (0.19±0.01) showed a significant increase ($p<0.05$) as compared to control group (0.15±0.01). Biochemical and haematological parameters measured were similar between the control and treated groups.

Key words: *Lannea kerstingii*, acute toxicity, repeated dose toxicity, phytotherapy, Wistar rats

INTRODUCTION

Herbal medicines are widely used for the treatment and prevention of various diseases in Africa and other developing countries of the world (Islam *et al*., 2007). These herbs are generally accessible, affordable and acceptable by most of the consumers. However, information on their safety is not usually adequate (Obidah *et al*., 2009). One of these traditional medicines is *L. kerstingii* Engl. and K. Krause (Anacardiaceae) which is widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. In Togo it is used to treat anaemia and malaria (Diallo *et al*., 2009). Traditional communities in Nigeria use *Lannea barteri*, a species very close to *L. kerstingii*, in the treatment of malaria (Adoum, 2008). The fruits of *L. kerstingii* and *L. acida* are eaten raw in the Guinean pre-forest savannas of Ivory Coast (Ambe, 2001). In Benin, *L. kerstingii* leaves are used in the treatment of Buruli Ulcer (Yemoa *et al*., 2008).

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Pharmacological studies of *L. kerstingii* extracts have revealed several properties such as antioxidant effect (Diallo *et al*., 2009), antihelmintic (Assi *et al*., 1985; Maidou *et al*., 2005) and trypanocidal effect (Atawodi *et al*., 2003).

We have undertaken *L. kerstingii* cytotoxicity study on Caco-2 cells and the acute toxicity test in mice (Diallo *et al*., 2009). This study has showed a cytotoxic activity of *L. kerstingii* hydroalcoholic extract against tumour cell lines. The IC_{50} values obtained were 186 and 328 μg mL^{-1} using, respectively MTT and Neutral red uptake assay. The acute test in mice had demonstrated that the LD_{50} of the hydroalcoholic extract of *L. kerstingii* is greater than 5.000 mg kg^{-1}. The extracts of the bark of *L. barteri* exhibited relatively low lethal toxicity (LC_{50}>1000 μg mL^{-1}) on brine shrimp larvae of *Artemia salina* (Adoum, 2008); while with the leaves of *Lannea stuhlmannii*, another species close to *L. kerstingii*, the toxicity was relatively high (Moshi *et al*., 2007). The LD_{50} of *Lannea coromandelica* was greater than 2.000 mg kg^{-1} p.o., in mice (Singh and Singh, 2006).

However, detailed toxicity profile of *L. kerstingii* has not been well studied. In order to develop and establish the safety and efficacy level of a new drug, toxicological studies are very essential in animals like rat, guinea pigs, dog, monkey etc under various conditions of drug. No drug is used clinically without its clinical trial as well as toxicity studies. Toxicological data help to make decision whether a new drug is adopted for clinical use or not (Khurshid Alam *et al*., 2006; Islam *et al*., 2007). This led to the present study where we investigated the acute and subchronic toxicity of *L. kerstingii* hydroalcoholic extract in Wistar rats.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

Lannea kerstingii stem bark was collected in Bagbe at 30 km from Lomé (Togo) in July 2009. It was identified by Professor Kouami Kokou from the Botany department of University of Lome (Togo) and a voucher specimen was kept in the herbarium of the Laboratory of Botany and Plant Ecology (Faculty of Science/University of Lome) under the reference N° 10553 of Akpagana.

The stem bark was washed in running water, then shade dried and ground to a powder. The powder was soaked in ethanol-water (50-50: v/v) for 72 h with manual discontinue agitation. The solution was filtered and evaporated using a rotary evaporator set at 45°C (yield: 12.34). The study was conducted in Animal Physiology Department, Faculty of Sciences, University of Lome, Togo.

Animals

Male and female rats (n = 27) weighing between 150 and 200 g, provided by the department of Animal Physiology were used. They were housed in a standard environmental condition and fed with rodent standard diet and water ad libitum. Animal care and handling were conformed to accepted guidelines (OCDE, 2002, 2008).

Acute Toxicity Test

The limit test dose of 5.000 mg kg^{-1} was used as described by the Organization for Economic Cooperation and Development (OCDE, 2002) guideline. Three female rats each sequentially dosed at interval of 48 h (short term observation period) were used for the test. The general behaviour of rats was observed 1 h post dosing and at least once daily for 14 days.

Subchronic Toxicity Test

The repeated doses for oral toxicity study was carried out according to OCDE guideline 407 (OCDE, 2008). Wistar rats were divided into three groups of 8 animals each (4 males and 4 females). Group 1 received 10 mL kg^{-1} of distilled water and served as control. Group 2 and 3 received *L. kerstingii* hydroalcoholic extract at 500 and 1000 mg kg^{-1} b.wt., respectively. Extract was administered daily for 28 days at similar time. Toxic manifestations and mortality were monitored daily and body weight changes of animals were recorded every week.

On the 29th day, after an overnight fast, rats were anaesthetized with ether and blood sample for haematological and biochemical analysis were collected into tubes with or without EDTA, respectively. Haemoglobin, haematocrit, red blood cells count, white blood cells count, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and platelet count were determined using automatic counter Sysmex (K21. Tokyo, Japan).

The biochemical parameters were determined in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min. Standardized diagnostic kits (Labkit®) and a Biotron® spectrophotometer were used for spectrophotometrical determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase, total proteins and urea. The blood glucose was measured with a glucometer (One touch®).

Necroscopy of all animals was carried and the organ weights (heart, liver, kidney and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each rat (relative organ weight).

Statistical Analysis

The results are expressed as Mean±Standard Error of the Mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) with Tukey test to evaluated significant differences between groups. Values of $p<0.05$ were considered significant. All statistical analysis were carried out using the Instat Statistical package (Graph Pad software, Inc. USA).

RESULTS AND DISCUSSION

The limit dose of 5.000 mg kg^{-1} did not cause mortality or any clinical sign of acute toxicity in none of the three rats tested in the short term (i.e., 48 h) and long term (i.e., 14 days) observatory period. The limit test is primarily use in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity (OCDE, 2002). The result of the acute oral toxicity study therefore, suggests that the extract of *L. kerstingii* stem bark at the limit dose tested is essentially non-toxic and safe in oral formulation. This result is in line with our previous data (Diallo *et al*., 2009) where we reported that *L. kerstingii* LD_{50} in mice was more than 5.000 mg kg^{-1}.

For the result of the subchronic toxicity test; in clinical evaluation, no behavioural changes and death were observed at the end of the treatment.

Lannea kerstingii at 1000 mg kg^{-1} decreased significantly ($p<0.05$) the increment of body weight of rats from the 2nd to the 4th week (Table 1). The decrease of the increment was the same on the 2nd and 3rd week (11%), but on the 4th week the decrease was 10%.

Table 1: Mean body weight of rats after 28 days treatment with hydroalcoholic extract of *L. kerstingii* stem bark

		Extract of *L. kerstingii*	
Week	Control (vehicle)	500 mg kg^{-1}	1000 mg kg^{-1}
0	142±3.5	145±2.7	142±4.7
1	162±2.6	156±2.8	149±6.0
2	171±4.7	164±3.6	152±4.9*
3	181±5.7	163±2.5	161±5.7*
4	180±6.3	165±3.6	161±6.8*

The results are Mean±SEM with N = 8; *p<0.05 (control group vs. extract)

Table 2: Mean relative organ weight of rats after 28 days treatment with hydroalcoholic extract of *L. kerstingii* stem bark

		Extract of *L. kerstingii*	
Parameters	Control (vehicle)	500 mg kg^{-1}	1000 mg kg^{-1}
Heart	0.39±0.03 (8)	0.39±0.02 (8)	0.34±0.03 (8)
Liver	3.10±0.10 (8)	3.00±0.11 (8)	2.90±0.08 (8)
Spleen	0.15±0.01 (8)	0.15±0.01 (8)	0.19±0.01* (8)
Kidney	0.66±0.02 (8)	0.62±0.02 (8)	0.66±0.03 (8)
Testis	1.18±0.05 (4)	1.20±0.12 (4)	1.24±0.06 (4)

The results are Mean±SEM. n: No. of rats/group; *p<0.05 (control group vs. extract)

The relative weight of the spleen in the group treated with 1000 mg kg^{-1} b.wt. showed a significant increase ($p<0.05$) as compared to control group (Table 2).

The increase in relative organ weight could be attributable to induction of xenobiotic enzymes leading to increased proteins synthesis. The induction of these enzymes frequently results in an increased of mean relative organ weight following an exposure to xenobiotic (Chukwunonso and Irene, 2006). One may also argue that these changes could be toxicologically significant (Dehghani and Panjehshahin, 2006; Souza *et al*., 2010).

Body weight changes are an indicator of side effects, since surviving animals cannot lose more than 10% of the initial body weight (Raza *et al*., 2002; Teo *et al*., 2002). *Lannea kerstingii* at 1000 mg kg^{-1} may reduce appetite in the treated groups. The regulation of appetite is an immensely complex process involving the gastrointestinal tract, many hormones, aging and both the central and autonomic nervous systems (Adam *et al*., 2001; Chittrakarn *et al*., 2008; Wadaan, 2009). It's speculated that the mechanism of side effects of plants such as anorexia and weight loss is related to an inhibition effect of gastric acid secretion (Tsuchiya *et al*., 2002; Taufiq-Ur-Rahman *et al*., 2005).

Haematological analysis showed no significant difference between the control and the animal treated with the extract (Table 3). The result of the biochemical study also showed no significant difference between the control and the animals treated with the extract in all the biochemical parameters tested (Table 4).

The analysis of AST, ALT, glucose and alkaline phosphatise is important since there are several reports of liver toxicity related to the use of phytotherapeutic products (Corns, 2003; Pittler and Ernst, 2003). Creatinine and urea determinations are critical as these substances are markers of kidney function (Obidah *et al*., 2009). Present result suggests that subchronic administration of *L. kerstingii* did not alter the liver and kidneys of rats and, furthermore, the normal metabolism of the animals.

The haematological parameters in the control and the treated groups showed no differences. *L. kerstingii* extract was then not toxic to circulating red cells, nor interfered with their production and that of platelets. The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals (Adeneye *et al*., 2006; Diallo *et al*., 2008).

Table 3: Haematological parameters of rats after 28 days treatment with hydroalcoholic extract of *L. kerstingii* stem bark

		Extract of *L. kerstingii*	
Parameters	Control (vehicle)	500 mg kg^{-1}	1000 mg kg^{-1}
WBC (10^3 μL^{-1})	5.2±0.51 (8)	6.7±0.73 (8)	4.8±0.45 (8)
RBC (10^6 μL^{-1})	7.6±0.18 (8)	7.6±0.12 (8)	7.7±0.16 (8)
Haemoglobin (g dL^{-1})	13.9±0.20 (8)	14.1±0.20 (8)	14.0±0.24 (8)
Haematocrit (%)	38.1±0.63 (8)	38.3±0.63 (8)	38.0±0.74 (8)
MCV (fl)	50.5±1.02 (8)	50.2±0.66 (8)	49.7±0.91 (8)
MCH (pg)	18.4±0.34 (8)	18.5±0.22 (8)	18.2±0.35 (8)
MCHC (%)	36.41±0.22 (8)	36.7±0.30 (8)	36.8±0.34 (8)
Platelet (10^3 μL^{-1})	763.0±50 (8)	913.0±48 (8)	929.0±56 (8)

The results are Mean±SEM. n: No. of rats/group

Table 4: Biochemical parameters of rats after 28 days treatment with hydroalcoholic extract of *L. kerstingii* stem bark

		Extract of *L. kerstingii*	
Parameters	Control (vehicle)	500 mg kg^{-1}	1000 mg kg^{-1}
AST (U L^{-1})	273.0±46 (8)	279.0±27 (8)	218.0±13 (8)
ALT (U L^{-1})	56.0±8.30 (8)	76.0±6.80 (8)	62.0±2.60 (8)
Total proteins (g dL^{-1})	6.2±1.90 (8)	6.3±0.98 (8)	6.3±1.40 (8)
Créatinine (mg dL^{-1})	6.0±0.46 (8)	5.8±0.49 (8)	5.7±0.42 (8)
Urea (mg dL^{-1})	27.0±0.03 (8)	33.0±0.02 (8)	29.0±0.02 (8)
Alkaline phosphatase (U L^{-1})	180.0±21 (8)	192.0±25 (8)	198.0±26 (8)
Glucose	4.7±0.27 (8)	4.8±0.17 (8)	5.0±0.46 (8)

The results are Mean±SEM. n: No. of rats/group

Observation for gross pathological lesion of the organs examined immediately after dissection show no visible lesion.

Present results have demonstrated that the hydroalcoholic extract of *L. kerstingii* could be relatively toxic when administered orally in rats. Further histological study and more specific assays of toxicity could furnish more information regarding the spleen toxicity and the weight loss.

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