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Evaluation of acute oral toxicity induced by n-hexane extract of *Leptadenia hastata* Leaves in wistar rats

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Abstract
Objectives: Acute toxicity is defined as the toxic effects produced by single exposure of drugs by any route for a short period of time. These studies identify a single dose causing adverse effects which cause lethality. The results of the acute toxicity study can also be used to determine dosages in sub-acute toxicity studies. In the present study, modified Lorke's method was used to determine acute toxicity.
Methodology: Seventeen (17) Wistar rats were used for the study. Twelve rats were grouped into two groups consisting of six rats each, which received a dosage of 10mg/kg, 100mg/kg and 1000mg/kg in the first phase. The second phase consisted of three groups of two rats each being administered a dosage of 1600mg/kg, 2900mg/kg and 5000mg/kg. No mortality was recorded in the first and second phases of the toxicity study. Five rats were given 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg of n-hexane extract of *Leptadenia hastata* to determine the effect of the extract on the liver and kidney at these concentrations. These tissues were carefully excised and prepared for histological observation.
Results: no mortality was recorded after both phases of the toxicity study. From the micrographs, it was determined that at a dosage of 3000mg/kg and above, there was heamolysis in the parenchyma of the tissues observed which could signify tissue damage at high concentrations of extract administered.
Conclusion: n-hexane extract of *Leptadenia hastata* did not cause mortality in the Wister rats but it may have a toxic effect on liver and kidney tissue following administration of high doses.

Keywords: acute toxicity, heamolysis, *Leptadenia hastata*, Liver, kidney, n-hexane extraction

Introduction
Acute toxicity is defined as the toxic effect(s) that is produced by a single exposure of drugs by any route for a short period of time [1]. Acute toxicity studies are commonly used to determine the lethal dose (LD50) of drug or chemicals [2]. Acute toxicity studies in animals are considered necessary if there is any intention to utilize the plant pharmaceutically. The main objective of acute toxicity studies is to identify a single dose causing major adverse effects or life threatening toxicity, which often involves an estimation of the minimum dose causing lethality [3]. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering it for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects [4].
In pharmaceutical drug development, this is the only study type where lethality or life threatening toxicity is an endpoint as documented in current regulatory guidelines [5]. To evaluate toxicity of a compound in animals, several routes may be used, but the commonest modes of administration for animals studies are via intra-peritoneal injection (IP) or the oral route [6, 3]. Usually, acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds [3, 7].
Apart from determining the lethal dose, the acute study also provides a guideline for selecting doses for the sub-acute and chronic low dose study, which may be clinically more relevant [8, 9]. Green leafy vegetables constitute an indispensable constituent of human diet in Africa generally [10]. *Leptadenia hastata* commonly known as Yadiya in Hausa belongs to the family Asclepiadaceae (Apocynaceae) and is widely distributed in tropical regions of Africa: from Mauritania and Senegal eastwards to Cameroon, Ethiopia, Northern Kenya, and Uganda and

In Ethiopia. It is also widely spread in Nigeria [11, 12]. Everywhere in its distributed area, leaves, young shoots and flowers of *Leptadenia hastata* are eaten as cooked vegetable and in soups [12].The multiple roles of wild traditional vegetables as both food and medicinal sources have been widely documented [13, 14, 15, 16, 17]. In Uganda, chopped and boiled leaves are eaten when mixed with beans, pigeon peas or cowpeas. In many parts it is a famine food, but poor people also eat this vegetable in normal times [12]. *Leptadenia hastata* is commonly used in Niger republic in day to day nutrition and is considered as hunger food due to its very important content of valuable nutrients [18].

According to [18, 19], *Leptadenia hastata* contains triterpenes, fatty acids, polypregnane, lutein, carotene, selenium and phosphorous [12]. It is widely used traditionally in the management and treatment of many diseases [20]. *Leptadenia hastata* is a characteristic of dry savanna vegetable in semi – arid zones [11]. The leaves are more abundant and fresh during the rainy season [14]. In some parts of Ethiopia the fresh leaf is marketed as an ingredient for making soup. Medicinally, it is used in the northern parts of Cameroon and Nigeria for the treatment of ear infection, blood replenishing, constipation, urethral discharge, gonorrhea, stomachache, diarrhea, milk drying, sexual-impotence, trypanosomiasis, acute rhinpharyngitis, and wounds and as folder for ruminants [21]. *Leptadenia hastata* which has been reported to contain comparatively high amounts of vitamin A and C and other antioxidant micronutrients [22,23], which promote good health by assisting in reducing oxidative stress, preventing cancer and high blood pressure, stimulating the immune system, improving drug metabolism and tissue regeneration by protection of the epithelial layer [24]. Food which is ingested by humans and animals, including *Leptadenia hastata*, contains a variety of nutrients, but there is a little knowledge of the fact that many foods contain small amounts of potentially harmful substances. These are toxins commonly referred to as anti-nutrients [21]. Since anti-nutrients are found in varying levels in almost all foods for a variety of reasons, it is necessary to test for their presence in *Leptadenia hastata* [26].

Methodology

Collection, identification and storage of plant material

Leptadenia hastata was collected from a garden in the University of Maiduguri, Borno State, authenticated by a plant taxonomist, from the Department of Biological Sciences, University of Maiduguri and the specimen voucher was deposited in the herbarium of the same of department. The leaves were collected and shade dried for a period of two weeks and then ground to powder mechanically using a mortar and pestle and then sieved to obtain the fine powder, it was then labeled and stored for use.

Extraction

Maceration technique was used in the current study. The powdered leaves were weighed to determine the dry mass in order to determine the yield. Maceration involved soaking the plant material (coarse or powdered) in a stoppered container with the solvent of choice (n-hexane) and be allowed to stand at room temperature for a period of 3 days at the minimum with frequent agitation. The process softened and broke the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture was pressed or strained by filtration using Whitman's filter paper. The resulting n-hexane filtrate was separated and concentrated to dryness in-vacuo using a soxhlet evaporator and the resulting powder was kept a freezer in an air–tight container.

Preparation of *Leptadenia hastata* Stock solutions

For the first phase of the acute toxicity study, hexane extract of the leaves of *Leptadenia hastata* (1g) was dissolved in 5ml of olive oil which served as the vehicle for the experiment to produce a solution with a concentration of 200mg/ml. From this stock solution, different concentrations of 10, 100 and 1000mg were prepared using olive oil to dissolve the extract.

For the second phase of the acute toxicity study, hexane extract of the leaves of *Leptadenia hastata* (4.5g) was dissolved in 7.5ml of olive oil which served as the vehicle for the experiment to produce a solution with a concentration of 600mg/ml. From this stock solution, different concentrations of 1600, 2900 and 5000mg were prepared using olive oil.

Animal treatment

All experiments were be performed on Wistar albino rats of either sex. A total of seventeen (17) albino rats was used. The rats were obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. They were kept in the Animal house of the Department of Human Anatomy, University of Maiduguri, Borno State for two weeks prior to the start of the experiment to acclimate to the new environment. The rats were weighed and maintained under controlled conditions of humidity and temperature. They were fed with pelletized ECWA (Jos) feed and water *ad libitum*

Acute toxicity study

An oral toxicity study was performed according to the Organization of Economic Co-operation and Development (OECD) guideline 420 for testing of chemicals [26].

The method described by Lorke was employed as described by [27]. The route of administration was oro-gastric. In the first phase, six rats of either sex were divided into three groups containing two rats each. The first, second and third groups received 10 mg/kg, 100 mg/kg and 1000 mg/kg of *Leptadenia hastata* respectively. These animals were monitored for 21 days to observe any mortality. In the second phase, six rats were used and divided into three groups of two rats each. Each of the group received different doses of the extract which were 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. These rats were monitored for 21 days. In addition, 5 animals were administered a dosage of the extract at concentrations of 1000mg/kg, 2000mg/kg, 3000mg/kg, 4,000mg/kg and 5000mg/kg to determine the effect of the extract at microscopic level on the liver and kidney tissues. At the end of 7 days, these animals were sacrificed and tissue samples from the liver and kidney were carefully removed and histologically prepared and stained with Heamatoxylin and Eosin to observe histopathological changes at the above concentrations. The results obtained determined the dosage of the extract to be administered. This method adopted was as described by [28].

Results

In the first phase the test animals lived up to 24 hours after the administration of hexane extract of *Leptadenia hastata* at concentrations of 10mg/kg, 100mg/kg and 1000mg/kg. The behavioural patterns of animals were observed first 6 h after administration and then afterwards for 21 days. After administration, animals in the extract-treated groups were normal and did not display significant changes in behavior, skin effects, and breathing, impairment in food intake and

water consumption and postural abnormalities. No lacrimation, salivation nor diarrhea was also detected in the animals tested.

In the second phase of the acute oral toxicity study, *Leptadenia hastata* was also administered at concentrations of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. No mortality was also recorded in the second phase of the study. The animals in this phase also did not display significant changes in mucous membrane or behavioral patterns. There was also no salivation, lacrimation, tremors, diarrhea or lethargy observed in any of the animals in the groups. No deaths were also recorded in the rats administered with 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg of extract to determine the effect on histological tissues: liver and kidney tissue.

Table 1: Showing the Classification of Rats in Groups in the First Phase for Determination of Median Lethal Dose (LD_{50}) of n-hexane extract of *Leptadenia hastata*

Dose (Mg/Kg)	Number of Rats Used	Mortality
10	2	0/2
100	2	0/2
1000	2	0/2

Table 2: Showing the Classification of Rats in Groups in the Second Phase for Determination of Median Lethal Dose (LD_{50}) of n-hexane extract of *Leptadenia hastata*

Dose (Mg/Kg)	Number of Rats	Mortality
1600	2	0/2
2900	2	0/2
5000	2	0/2

The hexane extract of *Leptadenia hastata* was found to be safe as the highest dose that did not kill any of the experimental animals. Hence, the LD50 value was found to be greater than 5000 mg/kg.

LD50> 5000 mg/kg.

Histological Observations in Kidney and Liver

The micrographs of the rats administered 1000mg/kg and 2000mg/kg of n-hexane extract of *Leptadenia hastata* revealed normal kidney architecture with the glomerulus within the Bowman's capsule and surrounded by Bowman's space. The renal tubules consist of both proximal and distal convoluted tubules and were found surrounding the renal corpuscles. In the micrographs of animals that received 3000mg/kg and 4000mg/kg of extract, there was an observable area of hemorrhaging as well as mildly dilated Bowman's space and renal tubules. The micrograph of the animals that received 5000mg/kg of extract. There was distortion of the renal parenchyma as well as dilated renal tubules.

In the micrograph of the liver of rats that received 1000mg/kg of extract, cords of hepatocytes were observed radiating from the central vein. The central vein was suffused with red blood cells. The sinusoids were located between the hepatic cords and appeared as clear spaces. The micrograph of animals that received 2000mg/kg of extract also revealed normal hepatic architecture. The hepatocytes formed cords that radiated from the central/ hepatic vein. Sinusoids which were found between the hepatocytes appeared clear.

The micrograph of the animal that received 3000mg/kg, revealed a dilation of the portal vein in which there was an accumulation of red blood cells. The cord-like arrangement of the hepatocytes appeared disorganized and the sinusoids also appeared discontinuous. The central vein appeared clear and devoid of blood cells. The micrograph of animals that ingested 4000mg/kg and 5000mg/kg revealed a disrupted hepatic cyto-architecture, with disrupted cords and disrupted sinusoids. The central vein appeared clear in the animals that ingested 4000mg/kg of extract and suffused with blood in the animal that consumed 5000mg/kg.

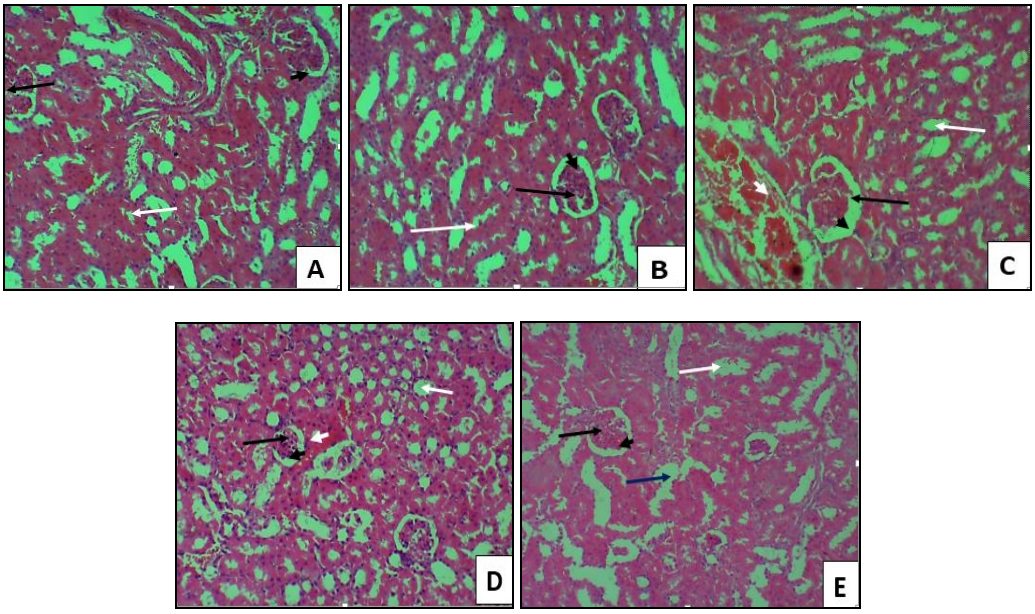

Fig 1: showing micrographs of kidneys of rats that were used in the acute toxicity study. A- 1000mg/kg, B- 2000mg/kg, C- 3000mg/kg, D- 4000mg/kg, E- 5000mg/kg White arrow – Renal Tubule, Black arrow– Glomerulus, Black arrow head– Bowman's space, White arrow head – heamolysis in tissue, Dark blue arrow – distorted tissue. H & E X100

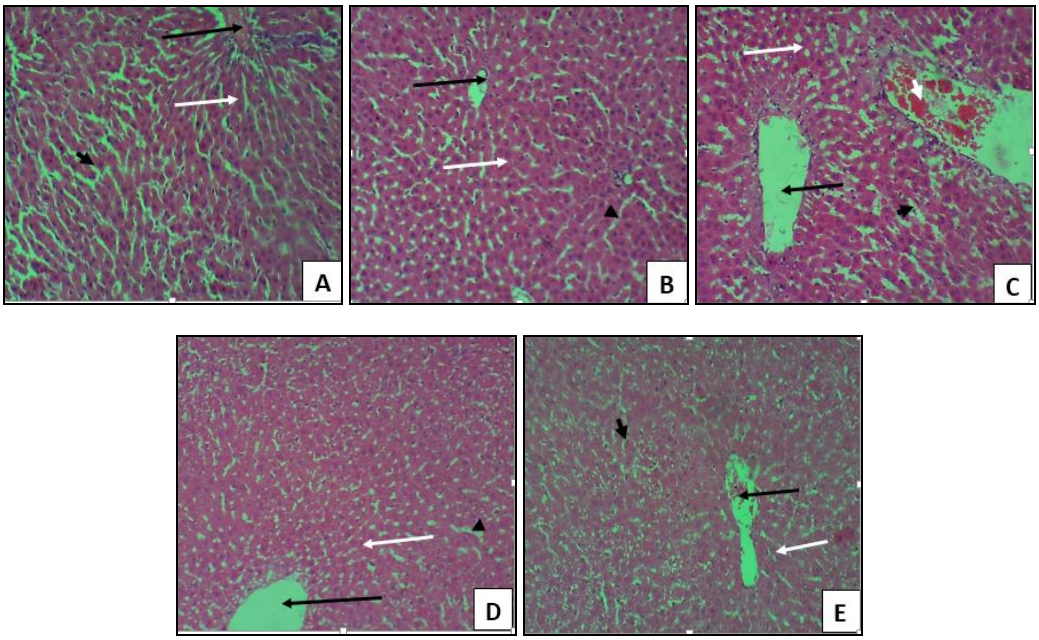

Fig 2: showing micrographs of livers of rats that were used in the acute toxicity study. A- 1000mg/kg, B- 2000mg/kg, C- 3000mg/kg, D- 4000mg/kg, E- 5000mg/kg White arrow – Cords of hepatocytes, Black arrow– Central vein, Black arrow head– Sinusoids, White arrow head – dilated portal vein suffused with blood. H & E X100

Discussion

Acute toxicity studies in animals are considered necessary for any pharmaceutical intended for human use. The main objective of acute toxicity studies is to identify a single dose causing major adverse effects or life threatening toxicity, which often involves an estimation of the minimum dose causing lethality [1].

Usually acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds [7]. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals [2]. The acute study provides a guideline for selecting doses for the sub-acute and chronic low dose study, which may be clinically more relevant [8, 9].

The results of the study suggested that *Leptadenia hastata* is a relatively non-toxic plant as during acute toxicity evaluation, the Wistar rats were not associated with any mortalities. This is in agreement with studies carried out by Maina *et al.*, 2013, who recorded no mortalities after a dosage of 5000mg/kg of *Leptadenia hastata* was administered. However, there was an observed increase in organ weight as dosage was increased. In the present study, the micrographs of various tissues were harvested after the animals were sacrificed to observe histological changes in the tissues and in the kidney, there was heamolysis in the liver parenchyma observed in animals that received 3,000mg/kg and 4000mg/kg of *Leptadenia hastata* extract. The micrograph of the liver in rats that ingested 3000mg/kg of extract, there was also observable heamolysis in the liver parenchyma. The sinusoids of animals that ingested above 3000mg/kg were unclear and discontinuous.

Conclusion

The result of these findings corresponds to the study carried by [25] who determined that the LD50 of *Leptadenia hastata* was found to be as high as 2320 mg/kg body weight. The outcome of his experiment and the present study suggested that though safe, but at a higher dosage, *Leptadenia hastata* may be poisonous or lethal to rats.

Conflict of interests

The authors declare no conflict of interests.

Financial contributions

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