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Acute toxicity, haematological and histopathological assessment of the ethanolic extract of *Lantana camara* leaf

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Abstract
Lantana camara is a noxious weed. This study was conducted to investigate the acute toxicity of the ethanol macerate of *Lantana camara* Linn. leaf in albino rats. Assessment of haematological and histological parameters of major organs following administration of the extract was undertaken. Lorke's method was used for the acute oral toxicity testing. Haematological parameters; whole and differential blood cell counts were determined using automated haematology analyzer. Histological examination of major organs was done using John D Bancroft manual method. The LD_{50} was found to be greater than 5,000 mg/kg body weight of the rats. The white blood cells, red blood cells, haemoglobin, packed cell volume, platelet, and differential cell counts of the albino rats were found to be within normal ranges for rats. Histological organization of the kidney and liver of treated rats at high doses showed structural changes typical of adaptive responses to pathologic stimuli. The results from this study suggest that the seventy percent ethanol macerate of *L. camara* Linn. leaf can be used with some degree of safety for short period of time via oral route.

Keywords: *Lantana camara;* Pathologic stimuli; Toxicity

INTRODUCTION

The history of the use of herbs is entwined with that of modern medicine. A lot of medicinal plants used from early ages as healing remedies have kept their ancient use until present time. Many drugs listed as conventional medications were originally derived from plants. *Lantana camara* Linn, also known as wild sage is a gregarious, erect or half-climbing aromatic shrub of the Verbenaceae family. It is a native of the American tropics and sub-tropics that has become naturalized in suitable habitats in tropical and warm regions worldwide. It is considered an invasive weed in some countries. It releases chemicals in its surroundings preventing germination of the native flora [1,2]. The leaves are used in folklore as gargle for catarrhal infections, toothaches, treatment of wounds, febrifuge, treatment of chest diseases, and high blood pressure in Nigeria [3-5]. *Lantana camara* has many common names including big sage, camara, wild sage, éwon àdele (Yoruba, Western Nigeria), anya nnùnú (Igbo, Eastern Nigeria) and kashin kuda (Hausa, Northern Nigeria). It is listed among the useful plants of west tropical Africa and medicinal plants of Nigeria [4,5]. *L. camara* has been reported to be unpleasant and toxic to livestock. The

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leaves are said to contain poisonous triterpenes, lantadenes A and B which are responsible for its toxicity [6-8]. This study was conducted to investigate the acute toxicity of seventy percent ethanol macerate of *L. camara* leaf. Its effect on haematological parameters and histological examination of specific organs were also examined.

EXPERIMENTAL

Collection of plant material. The leaves of the plant were collected in the month of October and November from Afaka, Igabi Local Government Area of Kaduna, Kaduna State, Nigeria. Its identification was done by comparison with an authentic voucher of *Lantana camara* (voucher, No.5) deposited in the herbarium of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Preparation of extract. The collected leaf samples were air dried at room temperature for 28 days. The dried leaves were then pounded to powdery form using wooden mortar and pestle. 100g of the powdery form of the leaf was extracted with 350ml of 70% ethanol by maceration for 4days. The macerate was filtered using muslin cloth and Whatman filter paper No 1. The filtrate was concentrated over a water bath at temperatures between 50 & 60 ^{0}C.

Experimental animals. Adult male albino rats (Wistar strain) were used in this study. They weighed between 150g & 175g. They were purchased from Animal house unit, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Acute toxicity testing. The acute toxicity test (LD_{50} test) was conducted according to the method of Lorke [9] with modifications via oral route. Rats received orally a single dose of the macerate (70% ethanol) of *L. camara* leaf in two (2) phases of experiment. The rats were observed for physiological and behavioural changes that included feeding behaviour, increased or decreased activity due to stress, drug reaction and rat mortality for 24 hours. They were also monitored further for 14days. At the end of 14 days, all surviving rats were sacrificed.

Determination of haematological parameters. Blood samples collected from albino rats after sacrifice were placed in EDTA-3K anticoagulant bottles. The determination of haematological parameters; white blood cell count, red blood cell count, haemoglobin, platelet, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular haemoglobin concentration, lymphocytes, and middle cell ratio (percentage summation of basophils, eosinophils and monocytes) was done using Sysmex KX-21N automated haematology analyzer, product of Sysmex Corporation, Kobe, Japan. Whole blood mode was selected and 50µl of the blood sample was aspirated via the contact probe into the analyzer.

Histological procedure. Immediately after collection of blood samples from the sacrificed rats. The rats were dissected, the heart, kidneys, liver, lungs, and spleen were removed. The selected organs were fixed in 10% formal saline in labelled and covered containers. The manual processing method of John D Bancroft was used. The tissue samples were sectioned at 6 micron. Staining was done using hematoxylin and eosin stains. Viewing and examination was done at 400X magnification using Olympus binocular microscope (model CHA&CHB, product of Shinju Kutku, Tokyo, Japan) attached to a digital camera (scope photo digital camera for microscope, DCM 35).

RESULTS

The acute toxicity test (lethal dose test) indicated that there were no deaths recorded in any of the group of rats orally administered with a single dose of the 70% ethanol macerate of *L. camara* Linn. leaf at the various concentrations based on Lorke's

method as shown in table 1. Haematological parameters; whole and differential cell counts are reported in table 2. Histological Findings are shown in plates 1-6. The heart and lungs presented normal histological organization as shown in plate 1 and 2. The kidney presented histological distortions as shown in plate 4-6. The liver histological findings are shown in plate 7-8.

DISCUSSION

Rat mortality was not recorded within 24 hours and 14 days, the acute administration of seventy percent macerate of *lantana camara* Linn. leaf may have little or no toxicity. The oral LD_{50} was found to be greater than 5,000 mg/kg body weight. LD_{50} is one way to measure the short term poisoning potential (acute toxicity) of a material.

Table 1: Acute toxicity test of 70% ethanol macerate of *Lantana camara* Linn. leaf.

Experiment Phase	Treatment Dosage mg/kg body weight	Proportion of rats Dead/Rats used	
		After 24hours	After 14days
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Phase 2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

Table 2. Haematological indices of blood samples of rats used for acute toxicity studies.

Dose (mg/kg b.w)	WBC $x10^9$ L	RBC $x10^{12}$ L	HB g/dl	PCV %	PLT $x10^9$ L	MCV fl	MCH Pg	MCHC g/dl	LYM %	MXD %	NEUT %
10	15.9	6.6	12.6	39.4	481	59.7	19.0	31.9	78.9	03.1	18.0
100	18.3	8.5	15.2	48.7	508	57.2	17.8	31.2	79.4	02.7	17.9
1000	20.5	9.2	15.7	51.9	576	56.5	17.1	30.3	58.0	03.9	14.0
1600	23.4	8.4	15.6	49.1	878	58.2	18.5	31.8	83.1	03.3	15.8
2900	24.9	8.0	13.6	45.0	232	56.3	17.0	30.2	66.3	02.9	18.6
5000	26.2	8.7	15.0	48.4	771	55.4	17.2	31.0	79.8	03.2	19.0
Control	24.4	7.6	13.9	45.3	488	59.7	18.3	30.6	80.7	02.4	16.8

WBC (White blood cell), RBC (Red blood cell), HB (Haemoglobin), PCV (Packed Cell Volume), PLT (Platelet), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular haemoglobin), MCHC (Mean Corpuscular haemoglobin Concentration), Lym (Lymphocytes), MXD (Middle Cell Ratio; % summation of basophils, eosinophils & monocytes), NEUT (Neutrophils

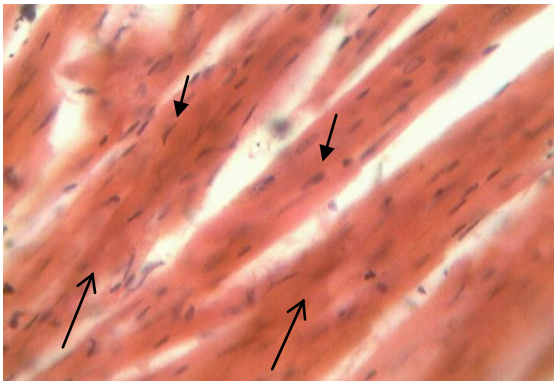

Plate 1. Normal heart muscle (up-pointing arrows) & normal nucleus (down-pointing arrows) of control & treated rats. H&E, 400X.

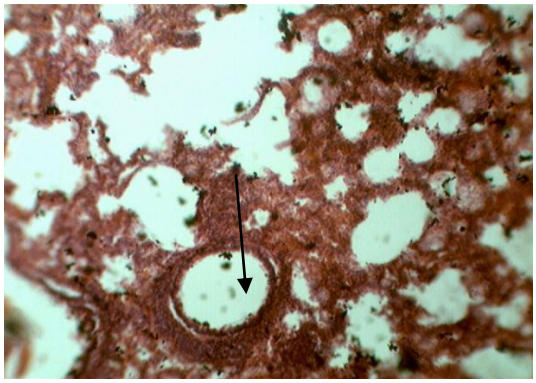

Plate 2. Normal alveoli (down-pointing arrow) of lungs of control and treated rats. H&E, 400X.

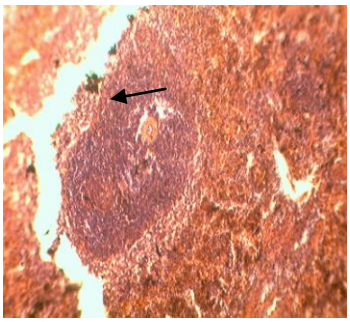

Plate 3. Spleen of normal and treated rats showing normal white pulp (left-pointing arrow). H&E, 400X

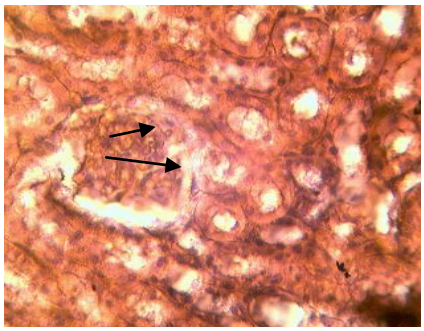

Plate 4. Kidney of rats treated with 1600mg/kg of extract Right-pointing arrows show eroded bowman`s space . H&E, 400X

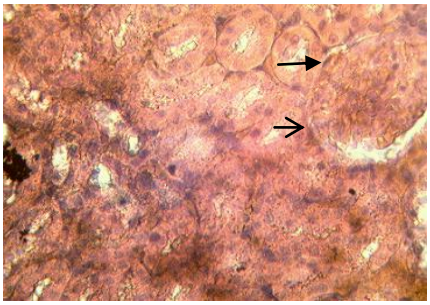

Plate5. Kidney of rats treated with 2900 mg/kg of extract right pointing-arrows show eroded bowman`s space. H&E, 400X.

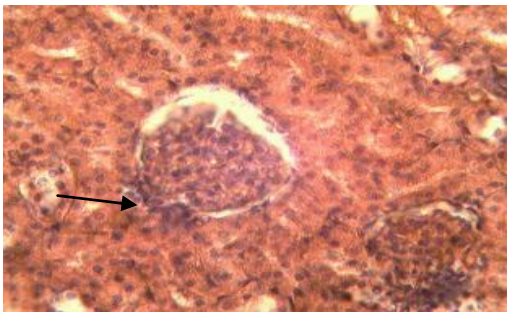

Plate 6. Kidney of rats treated with 500mg/kg extract. Right pointing arrow shows eroded bowman`s space. H&E, 400X.

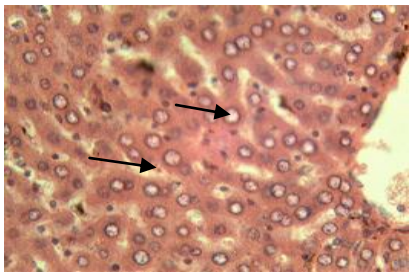

Plate7. Liver of rat treated with 5000mg/kg extract left pointing arrows show mass nuclei enlargement, H&E400X,

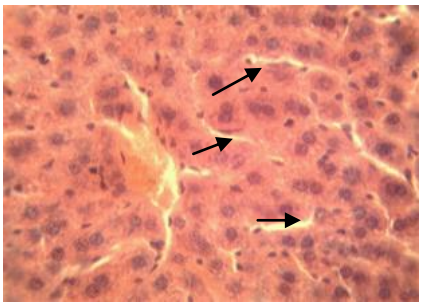

Plate 8. Liver of rat treated with 2900mg/kg extract, left pointing - arrows show massive distortion of sinusoids. H&E, 400X.

According to Hodge and Sterner scale toxicity rating, an oral LD_{50} of between 5,000-15,000 mg/kg body weight in rats reflects that the administered substance is practically non-toxic [10,11]. According to the OECD (organization for economic cooperation and development) guidelines for testing chemicals, animals are clinically observed for a period up to 14 days, hence the observation of the rats for 14 days. Behavioural changes such as cleaning of mouth region, scratching of body and discoloration of ear lobe and loss of sharpness of edge of the ear lobes by the rat given 5,000m/kg of the extract by the seventh day was recorded.

Haematological analysis was performed to assess the physiological status of the rats used for the acute toxicity studies. Examination of the blood is often used to assess physiological status of organisms **[**12]. In this study, the whole blood cell counts and differential counts were examined in order to monitor possible traces of toxicity of administered extract. The haematological indices were found to be within physiological values for rats [13]. The red blood cell counts

at low doses (10, 100, 1000mg/kg) showed increase in number as the dose increased. *L. camara* Linn. extract probably has the ability to improve red blood cellular level. The haemoglobin level increased as well, this is not unusual because as the red blood cell increased, the quantity of haemoglobin should proportionately increase as they are part of constituents of the red blood cells.

The packed cell volume of the rats used in this study increased with the low doses. Packed cell volume is a measure of blood that is made of red blood cells if the blood is allowed to settle [14]. This increase is in agreement with the red blood cell counts that increased proportionately with dose. The white blood cells also recorded an increase with the low doses of the extract given to the rats. White blood cells comprise the immune component of blood, they multiplied with the administration of the extract. The same trend was noticed with the higher doses of 1600, 2900, 5000mg/kg.

Platelets (thrombocytes) are the second most numerous cells in the blood, they are crucial to clotting as they form a primary haemostatic plug in response to vessel damage and provide a surface upon which the coagulation cascade occurs for formation of definitive thrombus [15]. The values obtained in this study showed increase with lower doses of *L. camara* Linn. leaf extract. Same trend with the high doses (1600 and 5000), except 2900 that indicated a low level of platelets. Traditionally, the mashed fresh leaves of *L. camara* Linn. is used to stop bleeding in traumatic injuries, the leaves orally administered to human adults has been reported to check cases of nasal and rectal bleeding, the dried leaves as paste on wounds showed antihaemorrhagic activity [16, 17]. It can be speculated that *Lantana camara* Linn. leaf can improve level of whole blood counts, which are blood parameters used to measure qualitative and quantitative aspects of the blood. The macerate at doses below 1000mg/kg can thus be described as non-haematotoxic, hence can find use as a blood tonic for treatment of anaemia. This finding further gives credence to the traditional use of decoction of the dried leaves of *L. camara* as a tonic in Brazil [18,19].

The histological organization of the heart and lungs of the rats were normal (plates 1 & 2). The spleen also had normal histological outlook (plate 3). The kidney showed atrophic glomeruli tuft which was characterized by eroded bowman's space as shown in plate 4-6. The liver of the rats however had histological changes, these changes seem to be dose dependent, the higher the dose, the more pronounced the changes observed (plates 7 & 8). These changes include enlargement of nuclei, distortion of normal arrangement of sinusoids in the liver. Hypertrophy was noticed in the liver cells, it involved enlargement of organelles (nuclei) which led to enlargement of the cells probably to increase function of individual hepatocytes. The enlargements probably caused the distortion of arrangement of sinusoids. Enlargement is an adaptive response to effect of a pathologic stimuli (such as herbal preparation), which probably put stress on the liver and increased the liver workload. The histological findings of organs (kidney and liver) are adaptive responses, no injury/damage was established.

The result of this study indicates that utilization of the leaf of *L. camara* Linn. at high doses may produce toxicity during chronic administration but may be practically safe during acute administration via oral route. The use of herbal preparations traditionally for the treatment of medical conditions should be with caution due to ineffective dose regulation.

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