Toxicity of Methanol Extract of Lasiosiphon kraussianus Root

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Abstract

The toxicity of a methanol extract of roots of *Lasiosiphon kraussianus* (1g = 20 g dried root) was investigated in mice, rats, rabbits, dogs and isolated rabbit ileum and heart. In acute toxicity studies carried out in mice, LD$_{50}$ of the extract was 27.1 g/kg and 330 mg/kg after oral and intraperitoneal administrations respectively. Clinical signs of peritoneal irritation, tachypnoea and listlessness and post mortem lesions of haemorrhagic enteritis were observed. In chronic toxicity studies with rats fed the extract at 2, 4 and 8 mg/kg/day for six months, no clinical signs of toxicity were observed and no gross lesions were detected at post mortem examinations. Haematological studies carried out in rabbits given 2 to 16 mg/kg of the extract *per os* showed no significant changes in the blood parameters measured. There was also no effect on blood pressure and respiration in the dog. On isolated rabbit ileum and heart however, high concentrations of the extract (2–32 mg/ml) inhibited intestinal rhythmic contractions and had negative inotropic and chronotropic effects on the heart.

It was concluded that a methanol extract of root of *Lasiosiphon kraussianus* when given at the dose of 2 mg (equivalent to 0.4 g dried root) /kg *per os* does not cause any toxic effects in mice, rats and rabbits.

A further study is required to confirm its efficacy in the treatment of leprosy and viral diseases.

Introduction

There have been several reports (Collins and Wild, [4]; Watt and Breyer-Brandwijk, [13]; Nwude, [9, 10] that the leaves of *Lasiosiphon kraussianus* are poisonous when fed to cattle, sheep, goats and donkeys. The toxic signs include listlessness, anorexia, nasal and ocular discharges, dysentery, haemorrhagic enteritis, extensive ulceration and haemorrhages in the gastrointestinal tract, petechial and ecchymotic haemorrhages on
the epicardium and endocardium, congestion of the brain and lymphopenia. Either, chloroform and ethanol extracts of the leaves were also toxic but the ethanol extract was the least toxic [9].

Tubery [12], on the other hand, has reported the use of methanol extract of the root of *L. kraussianus* in the treatment of leprosy. The extract caused lymphocytosis, increased the tonicity of the lymphatic vessels and was effective in the treatment of oedema of lymphatic origin. The extract was also useful in chronic ulcerations of the skin and mucous membranes, post traumatic oedema and neurotropic viral diseases in man. For the treatment of leprosy a dose of 40 mg twice daily was recommended for an adult human, while 2 mg/kg/day was recommended for the treatment of viral diseases [12].

Since this plant is readily available in many localities in Nigeria including Zaria, Katagum, Sokoto, Bauchi Plateau [7], the possibility of using it as a cheap and readily available drug for the above named diseases has to be considered.

As part of the safety evaluation of the extract, this study was undertaken to determine the LD$_{50}$ of the methanol extract of *L. kraussianus* in mice, chronic toxicity in rats, haematological changes in rabbits, effect of the extract on isolated organs, and effect of the extract on blood pressure and respiration in dogs.

**Material and Methods**

*Preparation of the Methanol Extract*

Roots of *L. kraussianus* collected from Zaria area during the dry season (in January) were spread out and left in the laboratory for 6–8 weeks to dry. They were then cut into small pieces and milled to a fine powder. The pulverized plant material was sealed in plastic bags and stored at 25°C. The extract was prepared essentially as described by Tubery [12]. The ground roots were mixed with methanol (1:10 w/v). The mixture was left for 24 hours at 25°C. It was filtered and the filtrate mixed with ether (1:2 v/v). The precipitate formed was separated by filtration, left in a CaCl$_2$ (anhydrous) desiccator to dry and was used for the experiments. Yield 20 g dried root = 1 g extract.

**Acute Toxicity**

Administration of the extract to mice

Swiss white mice three to four weeks old and weighing 20–25 gm were used for this experiment. The extract was dissolved in physiological saline solution and administered by oral and intraperitoneal routes. Ten mice (five males and five females) were used for each dose level. For the oral route the doses were 15.90, 20.00, 25.20, 30.00, 31.70 and 39.82 mg/kg and for the intraperitoneal route the doses were 250, 295, 322, 350, 410 and 512 mg/kg. The doses for the oral route were divided into two and given at 12 hourly interval. Ten control mice (five males and five females) each received 0.6 ml physiological saline, orally and another 10 received 0.4 ml intraperitoneally. The volumes of physiological saline correspond to the largest volume of solvent used in treated animals. The animals were observed for 24 hours for acute signs of toxicity. Necropsy was performed on any dead animal.

**Determination of LD$_{50}$**

The LD$_{50}$ of the extract was determined by plotting the percentage mortality against dose on log dose probability graph paper [2]. Standard error was estimated according to the method of Miller and Tainter [8]. Regression lines were fitted as described by Snedecor and Cochran [11].

**Chronic Toxicity**

Administration of extract to rats

20 male albino rats weighing between 200–250 gms were used for the experiment. The animals were divided into four groups of 5 rats each. Groups 1, 2 and 3 received 2, 4 and 8 mg/kg/day respectively.

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Toxicity of Lasiosiphon

ly of the extract per os for six months. Group 4 served as control and received 0.12 ml/day of physiological saline (largest volume used in treated animals) orally for six months. Animals in each group were kept together in a cage and were fed commercial rat pellets for the period of the experiment. The rats were observed daily for signs of intoxication.

Post mortem examination

The surviving animals were euthanatized at the termination of the experiment by exposing them to ether fumes in a chamber. Post mortem examination was performed on all the animals. For histopathologic examination, the lungs, liver, heart, kidney and stomach were collected from each animal. These were fixed in 10 percent buffered neutral formalin, trimmed and processed in a tissue maton, embedded in paraffin, cut at six microns and mounted and stained with haematoxylin and eosin [5].

Haematological Studies

Experimental animals

30 albino rabbits of both sexes weighing between 1.3 kg to 1.8 kg were used for the experiment. The rabbits were purchased locally and housed in rabbit pens in the Faculty. They were maintained on commercial rabbit pellets and were observed for three weeks prior to use.

Feeding experiment

The experiment was carried out in two batches. Three groups of five rabbits each were used for each batch.

In the first batch, groups 1 and 2 received 2 and 4 mg/kg/day, respectively, of extract per os, for 16 days. Group 3 served as control and received 0.6 ml water for the same period.

In the second batch, groups 4 and 5 received 8 and 16 mg/kg/day, respectively, of extract per os, for 16 days. Group 6 served as control and received 2.6 ml of water for the same period.

Blood sampling

Blood samples (0.5 ml) were taken from the marginal ear vein of the rabbits using a 25 Gauge needle and a 1 ml syringe. Blood was withdrawn four days before and on the day of administration of the extract. Subsequently blood samples were collected once a day at four-day intervals for 16 days. Heparin was used as anticoagulant (4 drops/ml from a 25G needle).

Haematological examination

The haematological examinations performed were according to standard methods. Haemoglobin (Hb) was determined by cyanmethaemoglobin method, red and white blood cells were enumerated using the Coulter Counter-(2B)1 and haematocrit was determined by the microhaematoctrit method. Blood smears were stained with Giemsa stain and differential counts were based on 100 cells [3].

The haematological values were analyzed statistically for significance of differences by Student's t-test.

Isolated Organs

Thirty adult rabbits of either sex, purchased locally and weighing between 2.0 and 3.0 kg were used for the experiment. Each rabbit was euthanatized by a blow on the head and bled out completely before the heart or the ileum was removed.

The isolated rabbit ileum.

A segment of the ileum (5-6 cm long) was removed and suspended in Tyrode solution (composition in gm/litre: NaCl, 8.0; KCl, 0.2; MgCl2, 0.1; CaCl2, 0.2; NaH2PO4, 0.5; NaHCO3, 1.0; glucose 1.0). The tissue was suspended in a 25 ml organ bath at 37° C and aerated with oxygen (95 %) and CO2 (5 %). The recordings of the muscular contractions were made on a Four A physiograph1 using a microdisplacement myograph transducer (Linear cone)2. Tissues were allowed to stabilize for 15 minutes before the extract was tested. Doses from 0.1 mg to 32 mg/ml (concentrations of extract in the perfusing fluid) were tested and their effects observed for 60 seconds. After washing off each dose the tissue was allowed to recover for three minutes before testing other doses.

The isolated rabbit heart

The experiment was set up as described by AN-ON. [1], Ringer Locke's solution (composition in g/litre: NaCl, 9.0; KCl, 0.42; CaCl2, 0.24; NaHCO3, 0.15; glucose, 0.15) was used to perfuse the heart. The solution was maintained at 37° C, and aerated with oxygen (95 %) and CO2 (5 %). The ventricular contractions were recorded on a four A

1 Coulter Electronics Inc. Hialeah, Florida.
1,2 E & M Instrument Co., Inc. Houston, Texas 77021, U.S.A.
Physiograph\(^1\) using the microdisplacement myograph transducer (Linear Cole)\(^2\). The effect of the extract at 0.01 mg/ml to 32 mg/ml were tested. After each dose the heart was allowed to recover before testing subsequent doses.

**Blood pressure and Respiration**

Five Mongrel dogs of either sex, purchased locally and weighing between 8 to 12 kg were used for the experiment. They were kept in the Faculty kennels for four weeks prior to use. Each dog was anaesthetized by intravenous injection of 30 mg/kg pentobarbitone sodium. The procedure was essentially that described by ANON. [2]. The trachea was exposed and a tracheal cannula inserted. The right femoral vein was cannulated for administration of the extract and other drugs. The left carotid artery was cannulated for blood pressure recording as described by HOFF and GEDDES [6].

For recording respiration, chest electrodes connected to the impedance pneumograph (Mk IV)\(^1\) were used. Doses of 0.32 mg/kg to 32 mg/kg of the extract were injected intravenously into the dog. Extract was injected at five-minute intervals.

**Results**

**Extraction**

5 gms of brownish hygroscopic powder was obtained from 100 gms of the root.

**Acute Toxicity**

**Clinical signs and necropsy findings**

Mice injected intraperitoneally (128–512 mg/kg) had signs of peritoneal irritation, listlessness and tachypnoea. Death started to occur three hours after administration of extract from 295 mg/kg. At necropsy, haemorrhagic enteritis, was observed.

Those mice that received the extract orally (20.00–39.82 gm/kg) had signs of initial listlessness followed by tachypnoea and died within 6 to 12 hours. Necropsy findings showed non haemorrhagic enteritis.

\[ \text{LD}_{50} \]

The \text{LD}_{50} of the extract by the oral route is 27.10 ± 1.68 gm/kg while by the intraperitoneal route \text{LD}_{50} is 330 ± 12 mg/kg.

**Chronic Toxicity**

**Clinical signs and necropsy findings**

No clinical signs of intoxication were observed in the experimental animals throughout the period of the experiments. However, one rat that had received 2 mg/kg of extract died after 4 months. No gross lesions were observed at necropsy.

At the termination of the experiment, both treated and control rats showed congestion of the lungs and the liver when posted.

**Histopathologic findings**

GROUP 1 (2 mg/kg): – One rat had perivascular and peribiliary infiltrations with round cells (lymphocytes and plasma cells) in the liver. No lesions were observed in the rest of the animals.

GROUP 2 (4 mg/kg): One rat had perivascular and peribiliary infiltrations with round cells in the liver. Another had foci of lymphocytic accumulation in the parenchyma of the liver and around the portal vessels. The kidney of the latter animal showed periglomerular and interstitial infiltration with mononuclear cells (interstitial nephritis). No changes were observed in the remaining three animals.

\(^{1,2}\) E & M Instrument Co., Inc. Houston, Texas 77021, U.S.A.

\(^1\) Narco Bio Systems Inc. Houston, Texas 77021, U.S.A.
GROUP 3 (8 mg/kg): One rat had foci of hydropic degeneration of hepatocytes. No lesions were observed in the rest of the rats.

GROUP 4 (Control): One rat had perivascular and peribiliary infiltration with round cells and a few eosinophils in the liver. Another had a focal area of round cell infiltration in the renal cortex. No lesions were observed in the remaining three animals.

Haematological Studies
There were no significant changes (P<0.05) in any of the haematological values measured in the treated as well as in the control animals for the duration of the experiment.

The Isolated Organs

Isolated rabbit ileum
The effect of the methanol extract of the root of L. kraussianus on the contraction of isolated rabbit ileum is shown in Figure 1. At concentrations of 0.5–1 mg/ml, the extract had no effect on the contraction of the ileum. Rhythmic contraction was slightly inhibited at a concentration of 2 mg/ml; at concentrations of 4, 8, 16 and 32 mg/ml the rhythmic contractions were completely abolished (Fig. 1). The effect of the extract was immediate; and at concentrations above 4 mg/kg the effect lasted until the extract was washed off.

Fig. 1. Effect of methanol extract of roots of L. kraussianus (32 mg/ml in Tyrode solution) on rhythmic contraction of a portion of the isolated ileum. Arrows indicate addition of extract to organ bath and washing off (W).

Fig. 2. Effects of methanol extract of root of L. kraussianus (16 mg/ml in Locke'solution) on contraction of the isolated rabbit heart.
**Isolated perfused rabbit heart**

The extract had no significant effect on isolated rabbit heart at concentrations below 16 mg/ml. At 16 and 32 mg/ml there was progressive decrease in the force and rate of contractions (Fig. 2). After 2–8 minutes both the mechanical and electrical activities of the heart were completely stopped. The heart recovered after perfusion with plain Locke’s solution for about one minute.

**Blood Pressure and Respiration**

The intravenous administration of the methanol extract of the root of *L. kraussianus* into the dog produced no noticeable effect on the blood pressure and respiration at the doses (0.32 to 32 mg/kg) tested.

**Discussion**

The results of the acute toxicity tests with methanol extract of the root of *L. kraussianus* show that the LD₅₀ for oral and intraperitoneal routes in mice are 13,500 and 165 times respectively the dose of 2 mg/kg recommended by Tubery [12] for use in humans. This indicates a wide margin of safety especially for oral route. The difference between the LD₅₀ for intraperitoneal and oral routes may be due to rate and possibly the amount of extract absorbed via the two routes. The lesions observed in the gastrointestinal tract indicate that part of the toxic principles reported by Nwude [9] are contained in the drug.

The results of the chronic toxicity test in rats indicate that the drug could be given for up to six months in the recommended dose without any adverse effects. Congestion of the liver and lungs observed at post mortem are probably due to euthanasia with ether since it was found in both treated and control animals. The lymphocytic and plasma cell infiltration found in the liver suggest that some of these rats might have been exposed to subclinical bacterial infection during the course of the experiment.

The results of the haematological studies did not confirm the report by Tubery [12] that the drug causes lymphocytosis. He reported lymphocytosis after 10 days of dosing with the extract orally. In the present study the animals were dosed and blood parameters monitored for 16 days; no significant changes were observed in haematological values of the rabbits fed the extract at various levels.

On the isolated organs, the extract caused negative chronotropic and inotropic effects on the heart and inhibited rhythmic contractions of the intestines. These effects were observed only at higher concentrations. Since these high doses relaxed the intestines and reduced the force of contraction of the heart it could be suggested that the toxic effect of the extract in intact animal would include constipation and a fall in blood pressure. However, the studies on the effect of the extract on the blood pressure of dog did not substantiate the latter deduction. It may be due to the fact that the high level attained in isolated tissue could not be attained in the tissues of the dog with the doses given.

**References**


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