Haematological and Biochemical changes in Rats Given water extract of Calotropis procera

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Abstract

The toxic effects of the leaves of Calotropis procera were evaluated by observing abnormal changes in haematological, biochemical parameters and gross post-mortem changes in rats. The leaf extract produced significant increase ($P<0.05$) in packed call volume (PCV) but did not influence coagulation time. The extract also produced hypoproteinaemia reflected as hypoalbuminaemia. Similarly the leaf extract also caused elevation in the activities of aspartate aminotransferase and alanine aminotransferase. Although the extract did not produce lesions in the heart, spleen and liver examined, the increase in liver enzyme activities could be due to early liver damage.

Key words: Calotropis procera, toxicity, haematological, biochemical parameters rats

Introduction

Poisonous plants are among the important causes of economic losses to livestock and so it is considered when evaluating illnesses and decreased productivity (Abatan and Arowolo 1989).

In fact many instances of general malaise, inappetence and dullness in grazing animals which practitioners are called in to deal with and the cause of which is seldom diagnosed are due to consumption of sub-clinical dose of some harmful plants (Clarke and Clarke 1977).

This is usually dependent on the environment. The nomadic system of animal management in Nigeria constantly expose livestock to plant poisoning. Sometimes these poisonous plants are not widespread but are poisonous only at certain seasons. Animals are exposed most especially, during periods of starvation or when livestock is moved from place to place in search of better pasture (Hall 1977).

Calotropis procera is a flowering plant of the family Asclepiadaceae; it is a perennial woody shrub with broad grey leaves, the plant is commonly grown in the tropics and found abundantly in the extreme Northern part of Nigeria especially Sokoto (Daziel 1937).

This plant is therefore easily accessible to livestock which roam around for pasture especially in the dry season. Calotropis procera contain some toxic principles such as calactric acid calotoxin, calotropain, calotropagenin etc. (Hesse *et. al.* 1938, Seiber et al 1982), Toxicity as a result of the plant has been reported in literature particularly in sheep and include anorexia and diarrhoae in the animal (Mahmoud *et. al.* 1979). Locally in our environment here, inappetence has also been observed in some animals that fed on the plant (unpublished information).

The study of Calotropis procera for its toxicological effect is one of the efforts to classify the plants, determine their mode of poisoning and isolate the active component involved in the poisoning.

Materials and Methods

Animals:

Wister-Lewis breed of rats of both sexes weighing between 130 – 260 were used. They were fed chick mash (Bendel Feeds Nigeria Plc.) and allowed access to fresh water ad-libitum in rat cages. The animals were housed in cages in groups of five and allowed two weeks to aclimatise before commencement of experiments. They were weighed at intervals of 5 days initially and later at 2 days during the study.

Preparation of plant materials:

Fresh leaves of Calotropis procera were collected around the temporary site of Usmanu Danfodiyo University Sokoto with the help of a traditional medical practitioner and identified by a botanist of the same University.

A voucher specimen is kept at the College Herbarium for reference purpose. The plant was ground to a pulp in a mortar. The pulp was then filtered to obtain the water extract. The extract was concentrated and dried in the oven at 60°C to obtain a semi-solid substance. The extract was weighed into the various doses for each group of rats and administered by stomach tube after dissolving in distilled water. The doses were 25, 50 75mg/kg daily. The highest volume of extract administered was 2ml. Control group received 2ml of distilled water only per day.

Collection of Blood for Analysis:

Blood was collected from the rats by cardiac puncture under diethyl ether anaesthesia on the fifteenth day from the commencement of administering the extract. Whole blood was collected into bottles containing the anticoagulant, ethylene diamine tetraactic acid.

The haemoglobin concentration (Hb) was determined by cyanmethaemoglobin method using the Bauch and Lamb model spectrophotometer.

Total erythrocyte (RBC) and total white blood cell (WBC) counts were made from blood smears stained with Giemsa (Schlam *et. al.* (1975). Blood coagulation times were estimated by breaking bits of a non-heparinized capillary tube filled with blood.

The plasma activities of liver enzyme alkaline phosphatase was estimated by 4-Nitrophenol method, while those of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) were estimated by colometric method (Sigma Diagnostics 1985). Sodium and Potassium were estimated by flame photometer while urea was estimated by the Diacetylmonoxime method. The total blood protein was estimated by Biuret method while that of albumin was determined by bromocresol green

method, the globulin fraction calculated as the difference between the total protein and albumin determined. The total bilirubin as well as conjugated bilirubin were determined by Jendrassik-Grof method (Spencer and Price (1977).

Results are expressed as mean ± S.E.M. significance between control and treated groups were determined by students test. A probability level of ($P<0.05$) was taken as significant.

Results:

Extraction:

Extraction of the fresh leaves was done without any solvent being added and the percentage yield was approximately 18%.

Effects of extract on weight gain:

The fresh extract used in this study did not affect the weight of the animals significantly ($P>0.05$). The weight gain observed in the study for the controls is significantly ($P<0.05$) greater than that observed in the treated group (Table 1)

Effects of the extract of C. Procera on haematology:

The extract significantly ($P<0.05$) elevated the PCV of rats (Table 2).

It also elevated WBC of rats (Table 3).

It however, did not affect the coagulation time, the Hb and RBC.

Effects of the extract on plasma electrolytes and urea:

The extract elevated the plasma potassium dose dependently, this was significant ($P<0.05$) at 50 and 75mg/Kg, while no significant increase occurred in the level of sodium. The plasma urea level was also elevated by the extract (Table 4).

Effect of the extract C. procera on plasma protein and bilirubin:

The extract reduced plasma total protein due to changes in albumin.

The total bilirubin level remained unchanged (Table 5).

Effect of the extract of C. procera on liver enzymes:

The extract elevated the alanine aminotransferase and asparatate aminotransferase but decreased alkaline phosphatase (Table 6).

Other Effects of C. procera

Paleness and some petechial haemorrhages in the liver were observerd.

There were no gross lesions in other organs examined.

Discussion

The water extract of Calotropis procera used in this study showed no significant reduction in the weight of the rats receiving the extract; there was continued increase in the weight of both control and treated groups of rats during the period of administration of the extract. Therefore, within the time limit used in this study, we can conclude that the extract does not cause reduction in the weight of the rats.

The extract caused increase in the erythrocyte parameters particularly the packed cell volume. It has been observed in previous studies on poisonous plants that haemolytic poisons could induce the proliferation of reticulocytes which pass into the blood stream and thus give an increased erythrocyte parameter (Schlam *et. al.* 1975). In this study, reticulocytes were not observed in blood smears, so the increase in PCV may be due to other factors.

The coagulation time was not

affected by the extract. A similar study by Abatan and Arowolo (1989) with another poisonous plant, *Eugenia uniflora* showed no changed in coagulation time of both control and treated rats.

The extract of *C. procera* in this study also elevated the leucocyte counts, the increased leucocyte count could probably be due to stimulation of haemopoietic system by the presence of the poison in the extract that led to increased synthesis of white blood cells that function as body defence mechanism.

Sodium, potassium and chloride are the three ions most commonly considered in electrolyte studies. The extract in this study elevated plasma potassium significantly ($P<0.05$), but this is not the case with the slight increase with sodium.

Potassium is primarily an intracellular ion and concentration in the extracellular fluid are low. It is less connected with water balance than sodium. It has been suggested that increased level of potassium can be due to a failure of the kidney to excrete potassium (Morag, 1989). However, in this study the kidney functional test was not carried out, therefore we cannot conclude that increased level of potassium was only due to kidney failure since potassium is primarily intracellular.

The extract elevated the plasma urea level significantly ($P<0.05$), the elevation could probably be due to the increase in activities of urea enzymes, ornithene carbomoyl transferase and orginase can provide evidence of liver damage in many animal species, since urea cycle is confined to the liver (Woodman, 1988). Elevated blood urea in this study may also indicate kidney damage, however, no gross lesion was seen on the kidneys examined from the test animals.

The extract produced hypoproteinaemia which is reflected by hypoalbuminaemia. Previous studies indicated that common pattern seen following significant hepatocellular damage is reduction in albumin (Woodman, 1988) but since gross pathological examination here only revealed pale liver, and petechial haemorrahges, the hypoproteinaemia may be as a result of early cell damage, though hypoproteinaemia is also said to follow excessive loss of protein into the urine in case of severe kidney damage (Kaneko and Cornelius 1980). There was no gross lesion of the kidney in this study.

The extract of *C. procera* also elevated the liver enzymes, (GOT) and (GPT) activities with the exception of alkaline phosphatase. The alkaline phosphatase has long been the standard enzyme marker of tissue damage despite its short-comings. Its low activity in the rat and cat liver does not make it an in ideal choice, therefore its significantly low activity in this study does not necessarily mean that the extract has no effect on the enzyme activity. The elevation of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities suggested liver tissue damage.

Previous study by Kaneko and Cornelius (1980) indicated that GPT is found in high concentrations in hepatic tissues of dogs, cats and primates and elevation of its activity in plasma indicates hepatocellular damage (Sigma Diagnosic 1985). Similarly another study (Woodman 1988) indicated that the increase in plasma enzyme activities often seen following liver damage does not indicate an increase in liver ability to

synthesize that enzyme but rather a loss of material from damaged hepatocytes. This means that the plasma enzymes usually offer an indirect reflection of tissue damage appearing in the plasma at increased activities following their loss from damaged cells. Thus elevation of GOT and GPT in this study suggest tissue damage with loss of materials.

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TABLE 1: Body weight changes in rats given water extract of Calotropis procera (grams)

Dose mg/kg	No. of animals	Day 0	Day 5	Day 10	Day 12	Day 14
0	5	132.9±1.4	152 ± 2.5	156.8 ± 3	160 ± 3	165 ± 6.5
25	5	173 ± 2.5	185±12.5	187 ± 11	190 ± 11	185 ±12.3
50	5	183 ± 17	204 ±15	206.9 ±14	207 ± 13	205 ±13.4
75	5	217 ±15	219 ±12	233 ± 10	234 ±10	230 ±11.6

TABLE 2: Heamatological responses of rats extract Calotropis procera

Dose mg/kg	No. of animals	PCV %	HB g/100ml	RBC ml	MCV U^3	MCHU %	MCH Pg	Coagulation time (mins)
0	5	45.8±13.3	12.5± 0.3	6.0± 0.1	73.9	27.3	20.2	1.50
25	5	58.6± 1.9*	13.6± 0.4	7.2± 0.1	81.4	23.2	18.9	1.63
50	5	67.2± 2.7*	13.9± 0.4	6.7± 0.1	100	20.9	20.7	1.53
75	5	70.8± 2.8*	14.7± 0.2	6.6± 0.2	106	20.8	23.0	1.75

*Represents P<0.05.

TABLE 3: Total and leucocyte counts of control and rats given water Extract of *Calotropis procera*

Dose mg/kg	No. of animals	WBC 10^3/ml	Lympho-cyte	Neu-Trophillis	Eosinophils	Basophils	Monocytes
0	5	11.5 ± 0.3	6440	3910	644	391	115
25	5	14.2 ± 0.4	8236	5112	426	284	142
50	5	14.4 ± 0.2	7810	5396	568	341	85
75	5	14.0 ± 0.1	6076	4588	864	744	124

TABLE 4: Effects of extract *C. procera* on electrolytes and urea

Dose/mg/kg	No. of animals	Na mmol/l	K mmol/l	Urea mmol/l
0	5	148 ± 0.9	5.8 ± 0.6	2.7 ± 0.6
25	5	142 ± 1.4	6.1 ± 0.5	5.0 ± 0.8*
50	5	151 ± 3.0	6.3 ± 0.6*	4.9 ± 0.5*
70	5	150 ± 2.8	8.8 ± 0.5*	4.2 ± 0.6*

*Represents P<0.05.

TABLE 5: Effect of the extract of *C. procera* on plasma protein and Bilirubin in rats

Dose mg/kg	No. of animals	Total protein (g/dl)	Albumin g/dl	Globulin g/dl	Total Bilirubin	Conjugated Bilirubin
0	5	7.7 ± 0.3	3.9 ± 0.3	3.8± 0.2	9.5 ± 1.1	3.0 ± 0.8
25	5	7.5 ± 0.2	3.5 ± 0.2	4.0 ± 0.3	8.6 ± 0.1	4.6 ± 0.8
50	5	6.0 ± 0.4*	2.2 ± 0.3*	3.8 ± 1.0	8.3 ± 0.3	1.5 ± 0.2
75	5	4.7 ± 1.0*	1.4 ± 0.1*	3.3 ± 0.2	7.0 ± 0.2	1.5 ± 0.3

*Represent P<0.05

TABLE 6: Effect of the Extract of *C. Procera* on liver enzymes

Dose mg/kg	No. of animals	Alkaline Phosphatase u/l	(GOT) u/l	(GPT) u/l
0	5	20.2 ±1.1	41 ± 5.0	23 ± 1.0
25	5	15.8 ± 1.1	48.3 ± 4.5	43.3 ± 1.9
50	5	17.1 ± 2.5	53 ± 0.8*	36.7 ± 4.7*
75	5	13.7 ± 0.1	52 ± 0.7*	36.8 ± 3.7*

*Represents P<0.05