

TOXIC EFFECTS OF THE EXTRACTS OF *EUGENIA UNIFLORA* LINN IN RATS

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ABSTRACT

The toxic effects of the leaves of *Eugenia uniflora* Linn on rats was evaluated by observing abnormal changes in the haemogram including erythron and leukogram, serum biochemical parameters, histopathology, and hexobarbital sleeping time. The leaf extract produced significant increases in the packed cell volume (PCV) haemoglobin concentration and total red blood cell counts (P.05) but did not influence the blood coagulation time. Similarly the leaf extract of *Eugenia uniflora* produced significant increases (P.05) in the serum activities of alanine aminotransferase and aspartate aminotransferase. Although the extract did not produce histological lesions of the liver, the increases in liver enzyme activities could be due to incipient liver damage.

Key words:- *Eugenia uniflora*, Erythron, Leucogram, Aminotransferase.

INTRODUCTION

Almost any edible plant may be judged as questionable depending upon the reference consulted. Plants in fact comprise the third largest category of poisons.

There can be little doubt that 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" doses of some harmful plants (Clarke and Clarke 1977).

The nomadic system of animal management in Nigeria, constantly exposes livestock to plant poisoning. Sometimes these poisonous plants are not widespread because they are poisonous only at certain seasons. Often a poisonous plant may be ingested by accident if it is growing in close association with grazing plants or during periods of starvation or drought when livestock is moved on hoof from the place to place in search of better pasture. Obviously, plants which are normally grazed or browsed will be eaten and it is a curious fact that often an animal having tasted such a plant and even having been poisoned and recovered, will return and eat more of it (Hall 1977).

Eugenia uniflora Linn is a plant of the family Myrtaceae. It is a small shrubby tree up to 2.5 metre high and is widely grown for its sourly sweet fruit (Hutchinson and Dalziel 1954, Mclean and Ivimey-Cook, 1964). Locally the leaves were observed to poison livestock which browsed on them, although the fruits are said to be crisp and apple-like with a refreshing sub-acid flavour (Mclean and Ivimey-Cook 1964). Therefore, the fruits may not be poisonous to humans.

The study of *E. uniflora* for its toxicological effects is one of the efforts to group plants which are locally observed to be poisonous to livestock, determine their mode of poisoning and isolate the active components involved in poisoning.

MATERIALS AND METHODS

Animals

Sprague Dawley rats of both sexes weighing between 150 - 200 gm were used. They were fed on rat cubes (Ladokun and Sons Livestock Feeds Nigeria Limited) and allowed access to fresh water ad-libitum in rat cages.

Preparation of plant material

The leaves of *E. uniflora* were collected and dried under shade with a good air draft. The dried leaves were crushed and extracted with absolute ethanol using the Soxhlet extractor. The extract was concentrated and dried in the oven to obtain a semi-solid substance. The extract was weighed into the various doses for the groups of the rats and administered by gavage after dissolving in propylene glycol for a period of seven days daily.

The doses were 100, 150 and 200 mg/kg with the control group of rats receiving only propylene glycol.

Collection of blood for analytical procedures

Blood was collected from the animals by cardiac puncture under diethyl ether anaesthesia on the eighth day from the commencement of administering the extract. Serum was collected for biochemistry, while whole blood was collected

Table 1: Haematological responses of rats to ethanolic extract of *Eugenia uniflora*

Dose mg/kg	No. of Animals	PCV %	Hb g/100mL	RBC 10^6 /mL	MCV U ³	MCHC %	MCH pg	Coagulation Time(mins)
0	10	45 ± 2.7	14.1 ± 2.3	6.8 ± 1.9	66.2	31.3	20.7	1.95
100	10	58 ± 2.0	18.6 ± 3.5	8.2 ± 0	70.2	32.1	22.6	2.80
150	10	57 ± 2.5	19.3 ± 2.2	9.7 ± 2.	58.8	33.9	19.9	2.0
200	10	67 ± 0	17.7 ± 1.3	7.7 ± 1.	86.8	22.9	22.9	2.0

into bottles containing the anticoagulant, the sodium salt of ethylene diamine tetracetic acid (EDTA).

The haemoglobin concentration (Hb) was determined by cyanmethaemoglobin method using the Beckman model spectrophotometer. Total erythrocyte (RBC) and total white blood cell (WBC) count were by the haemocytometer method, the haematocrit (PCV) by the microhaematocrit method. The differential WBC counts were made from blood smears stained with Giemsa (Schaim et al., 1975).

Blood coagulation times was estimated by breaking bits of a non-heparinized capillary tube filled with blood.

Serum activities of alanine aminotransferase (GPT; EC. 2.6.1.2) and aspartate aminotransferase (GOT; EC 2.6.1.1) were estimated according to the Sigma diagnostic methods (1985). Calcium was determined by the Sigma diagnostic method (1988) while bicarbonate and chloride were determined as

The total blood protein and albumin were determined by Sigma diagnostic method (1987). The globulin fraction was calculated as the difference between the total protein and albumin determined.

Statistic

Results are expressed as mean ± S.E. Significances between control and treated groups were determined by students't test.

RESULTS

Effects of the extract of *E. uniflora* on haematology

The ethanolic extract of *E. uniflora* significantly elevated the PCV, Hb, and RBC of rats (Table 4).

The extract did not affects the coagulation time

Effect of the extract of *E. uniflora* on serum enzyme activities.

The ethanolic extract of *E. uniflora* significantly elevated the alanine aminotransferase

TABLE 2: TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS OF CONTROL AND TREATED RATS

Treatment	Dose	WBC 10^3	Lympho-	Neutro-	Eosino-	Baso-	Monocyte
Control	0	12.4 ± 12.1	6200	4960	620	496	124
<i>E. uniflora</i>	100	14.2 ± 3.4	7526	5822	426	426	0
<i>E. uniflora</i>	150	26.7 ± 7.2	14418	1068	801	534	267
<i>E. uniflora</i>	200	10.6 ± 5.8	5088	4028	848	636	0

decribed by Skeggs andHochstrasser (1962). Sodium and potassium were estimated by the flame photometer.

levels at the 100 mg/kg dose and for all the doses in the treatment group for the enzyme aspartate aminotransferase (P.05).

Effect of the extract of *E. uniflora* on serum electrolytes and protein

TABLE 3: THE EFFECT OF THE EXTRACT OF *E. UNIFLORA* ON SERUM ELECTROLYTES AND PROTEIN (MEAN "S.E.)

Treatment & Dose	No. of animals	Tot. Prot. g/dl	Albumin g/dl	Globulin g/dl	Ca mmole/L	K mmole/L	Na mmole/L	HCO ₃ mmole/L	Cl ⁻ mmole/L
Control									
0 mg/kg	10	5.6 ± 0.6	2.6 ± 0	3.0 ± 0.4	7.9 ± 0.9	4.3 ± 0.3	148 ± 0	19.2 ± 0.6	85 ± 1.1
<i>E. uniflora</i>									
100 mg/kg	10	4.7 ± 3.4	3.6 ± 1.9	3.1 ± 1.9	8.5 ± 2.7	5.2 ± 1.0	141 ± 1.5	20.1 ± 0.9	86 ± 0.7
<i>E. uniflora</i>									
150 mg/kg	10	4.1 ± 1.9	0.6 ± 1.4	3.5 ± 1.2	9.1 ± 0	6.3 ± 1.0	145 ± 0.2	21.6 ± 2.0	84.1 ± 1.8
<i>E. uniflora</i>									
200 mg/kg	10	3.4 ± 0.7	0.2 ± 0.9	3.2 ± 1.6	9.6 ± 0.7	7.4 ± 0.4	149 ± 1.3	18.9 ± 1.3	85.4 ± 0.09

The extract significantly reduced ($P < 0.05$) serum total protein due to changes in albumin level. The globulin level remained unchanged (Table 3).

Serum calcium and potassium increased slightly with increasing dose of the extract administered to the rats. The increase in serum potassium is only significant with the 200 mg/kg dose group ($P < 0.05$) while no significant changes occurred in the levels of sodium, bicarbonate or chloride (Table 3).

TABLE 4: THE EFFECT OF THE EXTRACT OF *EUGENIA UNIFLORA* ON SERUM ENZYMES

Dose mg/kg	Activities of serum enzymes in mmole of substrate converted/min/mL serum	
	GPT	GOT
0	27.5 ± 4.2	38.8 ± 3.5
100	40.2 ± 3.8	59.2 ± 7.1
150	47.5 ± 9.2	58.1 ± 4.1
200	44.3 ± 8.4	53.5 ± 3.2

Other effects of *E. uniflora*

The extract prolonged the sleeping time of the rats receiving them as compared with the control animals.

Except for petechial haemorrhages in the ileum there were no gross lesions in the other organs examined.

Histologically, there was haemorrhagic enteritis with erosion of the epithelial lining of

the villus of the small intestine. The sinusoids of the liver were dilated.

DISCUSSION

The presence or absence of poison plants is most dependent on the environment. Certain ecological conditions favour certain plant

species including poisonous plants. Variations in these conditions will cause variations in prevalence of poison plant and in the likelihood of these plants poisoning livestock.

E. uniflora is often grown as a hedge (Irvine 1961). Therefore it is easily accessible to livestock which roam about in search of pasture. The ethanolic *E. uniflora* used in this study caused increases in the erythrocyte parameters (Table 1), though text indicate that haemolytic poisons could induce the proliferation of reticulocytes which pass into the blood stream (Schalm, Jain and Carroll 1975; Swenson 1975) and thus give increased erythrocyte parameter, this is not so in this study. The extract was not haemolytic in this study and reticulocytes were not observed in blood smears.

The extract produced hypoproteinaemia which is reflected as hypoalbuminaemia. Gross and histopathological examinations didn't reveal any liver damage therefore this could be the result of early cell damage, though hypoproteinaemia is also said to follow upon ex-

cessive loss of protein into the urine in case of severe kidney damage (Kaneko 1980). There was no lesion of the kidney.

Apart from increases in the serum calcium level of the rats receiving the extract, the serum potassium, sodium, bicarbonate and chloride levels showed no significant changes. Forty-five to fifty percent of the blood calcium is in the soluble, ionized form, while 40 to 45 percent is bound with protein primary albumin and other blood proteins. They hypoalbuminaemia may therefore explain the increase in serum calcium.

The serum enzyme activities of GOT and GPT were significantly elevated which suggests tissue damage. Alanine aminotransferase is found in high concentrations hepatic tissue in dogs, cats, and primates (Kaneko 1980) and elevations of its activity in serum indicates hepatocellular damage (Cysewski et al., 1968). Since GOT is found in other tissues apart from the liver, it cannot be concluded here that the serum activities of GOT observed with the intoxication of rats with extract of *E. uniflora* is solely derived from the liver.

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