

Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats

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ADEDAPO, A. A., M. O. ABATAN, O. O. OLORUNSOGO: Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats. Vet. arhiv 74, 53-62, 2004.

ABSTRACT

The toxic effects of 5 suspected poisonous plants of the genus *Euphorbia* (*Euphorbia balsamifera* Aiton, *E. heterophylla* L., *E. hirta* L., *E. hyssopifolia* L., and *E. lateriflora* Schum and Thonn), which are commonly found in the Nigerian pasture, were evaluated in albino rats using crude aqueous extracts for 14 days. Changes in haematological as well as biochemical parameters were used as indices of toxicosis. Clinical signs were also noted. The extracts of all the plants caused dullness, anorexia, starchy haircoat, and 20% mortality rate in *E. hirta* and *E. hyssopifolia*. Animals that received the extracts of *E. balsamifera*, *E. hyssopifolia*, *E. heterophylla*, and *E. lateriflora* appeared to show an excitatory effect. However, it was noted that the animals became dull afterwards. *E. hirta* produced the highest mortality rate. The crude extracts that were administered orally produced anaemia in the animals. While *E. heterophylla*, *E. hyssopifolia* and *E. lateriflora* caused leucopaenia, *E. hirta* and *E. balsamifera* caused leucocytosis. Three of the plants caused a significantly increased ($P < 0.05$) level of total protein; the remaining 2 (*E. balsamifera* and *E. heterophylla*) caused an insignificant increase. While all the plants caused a significant increase in the level of albumin, the reverse was the case for globulin. All the plants also caused significant increase in levels of ALT and AST.

Key words: *Euphorbia*, haematology, biochemistry, rats, toxicity

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Introduction

The genus *Euphorbia* produces caustic lattices, which constitute a health hazard to humans and livestock. Direct contact of the irritant latex with the eye can cause blindness (UPADHYAY et al., 1980). Members of this genus are known to contain substances which are inhibitory to seed germination and seedling growth as well as to bacteria (RICE, 1965; RICE, 1974). This inhibitory action has been attributed to the presence of large amounts of phenolic compounds (RICE, 1965; RICE, 1974). The presence of lactone-forming acids has also been reported (NORDAL and BENSON, 1969; KRINGSTARD, 1980). Several groups of secondary metabolites, such as alkaloids, diterpenes, glucosinolates, tannins, and triterpenes have been reported in this genus (SEIGLER, 1994).

E. balsamifera is commonly grown as a hedge and field boundary marker (BURKILL, 1994). Its succulent branches carry a copious amount of latex which is generally reported to be toxic (KERHARO and ADAM, 1974).

The sap of *E. hyssopifolia* is applied to warts, corns and indurations of cornea (HARTWELL, 1969). The latex is used as a purgative and as a caustic agent on skin-lesions (WATT and BREYER-BRANDWIJK, 1962).

E. heterophylla is a fast-growing weed that can form a dense canopy over soybean crop, making it difficult if not impossible to harvest (NESTER et al., 1979). The toxicity of the plant, especially of the root and latex, is recognized in East Africa (BURKILL, 1935).

E. hirta has a diuretic and purgative action. It is known to have a remedial effect for inflammation of the respiratory tract, while for asthma it has a special reputation for inducing bronchial relaxation (DALZIEL, 1937; KERHARO and ADAM, 1974).

The latex of *E. lateriflora* is taken in Northern Nigeria along with milk, cereals or liver causing purging and sometimes vomiting. Because the latex is a drastic purge it is used in the treatment of syphilis and also as a remedy for head lice and ringworm on the scalp (BURKILL, 1994).

The plants which have been chosen for this study all belong to the family Euphorbiaceae, which is a large family of trees, shrubs and herbs of rainforest, Guinean, Sudanian, and xerophylactic habitats. Most members of this family are poisonous and some are of economic and medicinal

value (GARNER, 1957; BURKILL, 1994). The leaves of the plants under study are of medicinal value, hence the need to evaluate their toxicity, especially as it has been shown that the latex from these plants are toxic (BURKILL, 1935).

Materials and methods

Animals and experimental design. The animals used in this study were rats of the Sprague Dawley strain weighing between 100 and 190 grams, of both sexes and maintained at the Animal House of the Faculty of Veterinary Medicine, University of Ibadan. They were kept in rat cages and fed rat cubes (Ladokun and Sons Livestock Feeds Nigeria Ltd.) and allowed free access to clean fresh water in bottles *ad libitum*.

Thirty animals divided into 6 groups of 5 animals per group were used in this study. While the first 5 groups corresponded to the plants under study (*E. balsamifera*, *E. heterophylla*, *E. hirta*, *E. hyssopifolia*, and *E. lateriflora*) the sixth group served as control.

Preparation of the aqueous crude extracts of plants. The leaves of the plants were always harvested freshly for preparation of the extract. The leaves were weighed and macerated using mortar and pestle. A specific quantity of water was added to ensure proper maceration. Thereafter, the solution was filtered using filter paper. The filtrate was administered to the rats per os using stomach canula for 14 days. Dosage of the extract was 1g/100g body mass of rats. The control group received water instead of extract.

Technique for obtaining blood and serum samples. Blood was collected by cardiac puncture from chloroform-anaesthetized rats into heparinised bottles for haematological studies. A further blood sample was collected into a clean bottle (non-heparinised) and allowed to clot. The serum was separated from the clot and centrifuges according to groups into clean bottles for biochemical analysis.

Determination of haematological parameters. Determination of haemoglobin concentration is as described by JAIN (1986) using the cyanomethaemoglobin method. Packed cell volume (PCV) was carried out using the conventional method of filling the capillary tubes with blood

as described by SCHALM et al. (1975). Erythrocyte count was determined by the haemocytometer method as described by JAIN (1986). Total leukocyte and leukocyte differential counts were also determined. Erythrocyte indices were determined from values obtained from RBC, haemoglobin concentration, and PCV values.

Determination of serum biochemical parameters. Total protein was measured using biuret reaction while albumin was measured by colorimetric estimation using the sigma diagnostics albumin reagent (Sigma Diagnostic, U.K.) which contained bromocresol green (BCG). Globulin was obtained from the difference between total protein and albumin. Aspartate aminotransferase (AST) and alanine amino transferase (ALT) were also measured. While AST was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine, ALT was determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine.

Results

The results of this study with respect to the haematological changes showed that *E. balsamifera* and *E. hirta* caused a significant reduction ($P < 0.05$) in PCV level. Extracts of all the plants caused a significant reduction in the levels of red blood cell counts and haemoglobin concentration. The erythrocyte indices showed that *E. balsamifera* and *E. lateriflora* caused normocytic hypochromic anaemia. *E. heterophylla* and *E. hirta* caused macrocytic hypochromic anaemia, while *E. hyssopifolia* caused macrocytic normochromic anaemia. While *E. heterophylla*, *E. hyssopifolia* and *E. lateriflora* caused a significant reduction in TWBC, only *E. lateriflora* caused a significant reduction in lymphocyte level (Table 1).

Extracts of *E. hirta*, *E. hyssopifolia*, and *E. lateriflora* all caused a significant increase ($P < 0.05$) in the level of total protein while *E. balsamifera* and *E. heterophylla* caused an insignificant increase ($P > 0.05$). All plant extracts caused a significant increase in the level of albumin but a significant decrease in the level of globulin. For the enzymes AST and ALT, all the plants caused a significant increase in their levels (Table 2).

Table 1. Effects of the aqueous crude extracts of suspected poisonous plants on haematological parameters of rats (n = 5).

Plants	PCV	Hb	RBC	MCV	MCH	MCHC
<i>E. balsamifera</i>	31.7 ± 3.1 ^a	8.8 ± 1.3 ^b	4.6 ± 0.9 ^c	61.2 ± 0.2	15.5 ± 1.6 ^d	26.6 ± 2.1 ^c
<i>E. heterophylla</i>	34.8 ± 1.1	8.6 ± 0.6 ^b	5.5 ± 0.3 ^c	64.2 ± 1.5 ^f	15.8 ± 1.4 ^d	24.8 ± 2.9 ^c
<i>E. hirta</i>	30.5 ± 2.9 ^a	8.7 ± 0.6 ^b	5.4 ± 0.3 ^c	67 ± 3.1 ^f	16.1 ± 1.4 ^d	26 ± 2.9 ^c
<i>E. hyssopifolia</i>	37.3 ± 1.4	9.4 ± 0.4 ^b	4.9 ± 0.4 ^c	79.0 ± 9.1 ^f	19.6 ± 0.3	29.0 ± 3.1 ^c
<i>E. lateriflora</i>	34.8 ± 1.1	8.6 ± 0.6 ^b	5.5 ± 0.3 ^c	63.0 ± 1.0	17.2 ± 0.8	28 ± 1.4 ^e
Control	36.6 ± 2.2	11.4 ± 0.5	6.0 ± 0.3	60.8 ± 3.7	19.0 ± 1.6	31.2 ± 2.4

Plants	TWBC	Lymph.	Neut.
<i>E. balsamifera</i>	4.8 ± 0.6	2.4 ± 1.2	2.3 ± 1.2
<i>E. heterophylla</i>	3.7 ± 0.3	1.8 ± 1.2	1.8 ± 1.3
<i>E. hirta</i>	5.4 ± 0.4	2.7 ± 1.2	2.6 ± 1.3
<i>E. hyssopifolia</i>	2.8 ± 0.1	1.4 ± 0.6 ^g	1.4 ± 0.6
<i>E. lateriflora</i>	3.7 ± 0.3	1.8 ± 1.2	1.8 ± 1.3
Control	4.7 ± 0.4	2.7 ± 0.8	2.0 ± 0.9

Superscripted items = Statistically significant; PCV = Packed cell volume, unit is %; Hb = Haemoglobin concentration, unit is g/dl; RBC = Red blood cell, unit is U/L; MCV = Mean corpuscular volume, unit is U/3; MCH = Mean corpuscular haemoglobin, unit is pg; MCHC = Mean corpuscular haemoglobin concentration, unit is %; TWBC = Total white blood cells, unit is 10³/ml; Lymph = Lymphocyte, unit is 10³/ml; Neut = Neutrophil, unit is 10³/ml

Table 2. Effects of the aqueous crude extracts of suspected poisonous plants on serum biochemical parameters of rats

Plants	Total Protein	Albumin	Globulin	ALT	AST
<i>E. balsamifera</i>	39 ± 2 ^a	29 ± 2 ^b	10 ± 1 ^c	40 ± 1.4 ^d	50 ± 1.4 ^e
<i>E. heterophylla</i>	38 ± 2 ^a	28 ± 2 ^b	11 ± 3 ^c	41.2 ± 2.2 ^d	50.8 ± 2.6 ^e
<i>E. hirta</i>	45 ± 2 ^a	36 ± 1 ^b	9 ± 2 ^c	45 ± 2.4 ^d	47.5 ± 1.7 ^e
<i>E. hyssopifolia</i>	43 ± 5 ^a	32 ± 3 ^b	11 ± 3 ^c	39 ± 1.3 ^d	45.5 ± 1.7 ^e
<i>E. lateriflora</i>	42 ± 2 ^a	35 ± 2 ^b	7 ± 1 ^c	48 ± 2.2 ^d	48 ± 2 ^e
Control	36 ± 1	22 ± 2	14 ± 1	34.4 ± 0.6	41.6 ± 0.7

Superscripted items indicate significant values. Total protein, albumin and globulin were measured in g/l. AST and ALT were measured in U/L.

Discussion

In this study, the aqueous crude extracts of *E. balsamifera* caused a statistically significant decrease in the levels of PCV, haemoglobin concentration and red blood cell counts. Thus, this shows that the leaves of this plant could cause anaemia in animals that browse on them. The anaemia may, however, be of regenerative type since the MCHC is low and MCV is normocytic (RADIN et al., 1986; CLARK, 1988; BAUER and KURTZ, 1989; KRANTZ, 1991; JELKMAN, 1992). The same situation applies with the extract of *E. hirta*. For *E. heterophylla* and *E. lateriflora*, although their extracts caused an insignificant decrease in PCV level but a significant decrease in the levels of RBC and haemoglobin concentration, this indicates that the leaves of these plants could also cause anaemia in animals that browse on them. For *E. hyssopifolia*, the extract caused an insignificant increase ($P < 0.05$) in PCV level but a significant decrease in RBC and haemoglobin, thus indicating that the leaves of this plant could also produce anaemia if browsed on by animals (Table 1).

This observation of decreased levels of erythron may support the claim that many members of the spurge family, to which these plants belong, are poisonous (BURKILL, 1994). For instance, *Mercurialis perennis* (dog's mercury) and *M. annua* (annual mercury), which also belong to the spurge family, are poisonous. *M. perennis* gives rise to two distinct syndromes, the first, and the one usually encountered in field case, is a haemolytic anaemia, the second an acute oedematous gastroenteritis (CLARKE and CLARKE, 1975). In poisoning by *M. annua*, haematuria is also the most obvious clinical sign. In fact, DEPPEZ et al. (1996) reported on 2 cattle farms that animals demonstrated constipation or diarrhoea, dullness, haemolytic anaemia and red urine after ingestion of *M. annua*. WELCHMAN et al. (1995) earlier reported that 11 lambs in a flock of 400 eight-month-old Romney lambs died from grazing *M. annua*. Pathological findings, which included haemolytic anaemia, were indicative of annual mercury poisoning. OLUWOLE and BOLARINWA (1997) in a study on the extract of *Jatropha curcas*, another member of the spurge family, showed that the extract causes a progressive reduction in the measured haematological values (packed cell volume, haemoglobin concentration and red blood cell counts). All the foregoing shows that these plants could produce toxic effects on haematological values.

This study also showed that *E. heterophylla*, *E. hyssopifolia* and *lateriflora* caused a significant decrease in the level of total white blood cells. Particularly, *E. hyssopifolia* caused a significant decrease in the level of lymphocytes. It therefore showed that with continuous administration of these plant extracts to animal the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes, may be compromised (FORMAN and THOMAS, 1986; HOGG, 1987; YOUNG, 1989; PAUL, 1993; STAUB, 1994). It should be noted that while some plant extracts caused an insignificant decrease in the level of lymphocytes, others cause an insignificant increase in the level of neutrophils. Continuous exposure to these plants may then lead to lymphopaenia, which may have an immunosuppressive effect. On the other hand, neutrophilia may then account for the use of these plants for medicinal purposes (KEENWE and BEKALO, 1996).

The aqueous crude extracts of these five plants all caused elevation in the levels of total protein and albumin, with decreased globulin. The increase noted for total protein and albumin may be due to the refusal of animals to drink water as a result of inclusion of these extracts. One should then note that this in itself may lead to dehydration. In the case of the decreased level noted for globulin, it may mean that the immune competence of the animals will be easily compromised. As a matter of fact, lymphopaenia accompanied by low globulin level, may lead to immunosuppression. In cases of decreased globulin level, diseases characterized by deficiency of immunoglobulin, such as agammaglobulinaemia selective IgM, IgA and IgG deficiencies, and transient hypogammaglobulinaemia, may lead to low-level globulin (DUNCAN et al., 1994). For instance, *E. hirta* is said to possess immunosuppressive properties, as well as causing inflammatory effects (ABO, 1994; GU et al., 1999). Results of this study may then lend credence to this observation.

The aqueous crude extracts of these plants also caused a significant increase in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Elevation in the extract of AST can be associated with cell necrosis of many tissues. For example, pathology involving the

skeletal or cardiac muscle and/or the hepatic parenchyma, allows for the leakage of large amounts of this enzyme into the blood (KANEKO, 1980). The elevation in AST produced by these plants is an indication of tissue necrosis. ALT, on the other hand, is present in liver and other cells. It is particularly useful in measuring hepatic necrosis, especially in small animals (CORNELIUS, 1989). Since it is one of the specific assayable liver enzymes, its elevated level in this study may indicate hepatic damage caused by these plants.

One should then conclude by stating that although these plants possess medicinal properties, this study has shown that: *E. balsamifera*, *E. heterophylla*, *E. hirta*, *E. hyssopifolia*, and *E. lateriflora* have toxic potentials. Caution should therefore be exercised in their use for medicinal purposes. More important, however, is the fact that continuous exposure of livestock animals to these plants may lead to morbidity and mortality with dire consequences for livestock production and management.

Acknowledgements

This study was supported by the Basir-Thomas Biomedical Foundation and University of Ibadan Senate Research Grant.

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Received: 4 August 2002

Accepted: 23 January 2004

ADEDAPO, A. A., M. O. ABATAN, O. O. OLORUNSOGO: Toksični učinci nekih biljaka roda *Euphorbia* na hematološke i biokemijske pokazatelje u štakora. *Vet. arhiv* 74, 53-62, 2004.

SAŽETAK

Istraženi su toksični učinci pet na otrovnost sumnjivih biljaka roda *Euphorbia* (*Euphorbia balsamifera* Aiton, *E. heterophylla* L., *E. hirta* L., *E. hyssopifolia* L. i *E. lateriflora* Schum et Thonn), koje se često nalaze na pašnjacima Nigerije. Istraživanje je provedeno na albino štakorima upotrebom sirovog vodenog iscrpka u tijeku 14 dana. Hematološki i biokemijski poremećaji uzeti su kao pokazatelji toksičnosti. Promatrani su i klinički znakovi trovanja. Iscrpci svih biljaka uzrokovali su potištenost, anoreksiju i neuredan dlačni pokrivač, a iscrpci *E. hirta* i *E. hyssopifolia* 20%-tni pomor štakora. Životinje koje su dobivale iscrpak *E. balsamifera*, *E. hyssopifolia*, *E. heterophylla* i *E. lateriflora* pokazivale su najprije razdražljivost, a potom potištenost. *E. hirta* uzrokovala je najveći stupanj pomora. Sirovi iscrpci davani peroralno uzrokovali su anemiju. Dok su *E. heterophylla*, *E. hyssopifolia* i *E. lateriflora* uzrokovale leukopeniju, *E. hirta* i *E. balsamifera* uzrokovale su leukocitozu. Tri pretraživane biljke uzrokovale su značajno ($P < 0,05$), a preostale dvije (*E. balsamifera* i *E. heterophylla*) neznatno povećanje razine ukupnih proteina. Za razliku od razine globulina, sve su biljke uzrokovale značajno povećanje razine albumina. Sve su također dovele do značajnog povećanja razine ALT i AST.

Ključne riječi: *Euphorbia*, hematologija, biokemija, štakor, toksičnost
