

The Toxicity Material Extraction From Euphorbia Species

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ABSTRACT- Euphorbia is a genus of flowering plants belonging to the family Euphorbiaceae. Consisting of 2008 species. The genus Euphorbia produces an irritant, which constitute a health hazard to humans and livestock. The genus Euphorbia is one of the largest and most complex genera of flowering plants, however, several botanists have made unsuccessful attempts to subdivide it to smaller genera. Many Euphorbias are used for medicinal and other purposes. But it is interesting to note that the plant can be both helpful and toxic. In this study, the toxicity of Euphorbia. sp. was examined. The toxic effects of some 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), commonly found in the Iran pastures. Changes in haematological as well as biochemical parameters were used as indices of toxicosis. Experiment showed the important constituents of the aerial parts are terpenoids, including triterpenes: α -amyrin and β -amyrin.

Keywords: Biochemistry, Euphorbia, Toxicity, α -amyrin, β -amyrin

INTRODUCTION

Few toxic effects have been documented for Euphorbia hirta(1 and 5). An ether extract was found to be toxic in a brine shrimp lethality test, whereas ethyl acetate and aqueous extracts were within safe limits. In another test, however, an aqueous crude extract was found to cause testicular degeneration in sexually mature male rats as well as a reduction in the mean seminiferous tubular diameter. Several other extracts, given to rats orally, caused dullness and anorexia and induced a 20% mortality rate. Some fractions from the ethanolic extract showed potentially deleterious effects on the blood serum chemistry of rats. In feeding experiments with rats, however, no difference in the blood serum was found after a prolonged period of adding Euphorbia hirta to the diet. It was also found that drying Euphorbia hirta prior to extraction considerably reduces the cytotoxic activity of certain extracts (11). Several traditional medicinal uses of Euphorbia hirta have been supported by in-vitro studies. An aqueous extract of the whole- plant acts as an antidiarrhoeic agent by anti-amoebic, antibacterial and antispasmodic activities. The antidiarrhoeal activity is

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attributed to quercitrin through the release of the aglycone quercetin in the intestine. Quercitrin showed antidiarrhoeic activity at doses of 50 mg/kg in mice.

A crude plant extract and an ethanolic extract had significant anti-amoebic activity against *Entamoeba histolytica* in vitro at 35 mg/mL. An aqueous lyophilysate of the whole-plant showed higher activity against *Entamoeba histolytica* than either the ethyl acetate or methanol extracts, at 30 mg/mL. An aqueous plant extract showed concentration-related activity against non-pathogenic amoebae of the *Amoeba proteus* type. Different extracts from the aerial parts showed antibacterial activity against a wide spectrum of both gram-positive and gram-negative bacteria. Extracts of the aerial parts showed strong antibacterial activity against *Shigella dysenteriae*, a causal agent for dysentery in humans. The active compound was found to be ethyl gallate, which has a broad spectrum of antibiotic activity at non-toxic doses. A crude ethanol extract of the whole plant showed dose-dependent activity against *Candida albicans*, but not against several other pathogenic fungi. Some of the isolated antibacterial compounds were taraxerone and 11 α ,12 α -oxidotaraxerol, which showed low cytotoxicity.

The genus *Euphorbia* produces caustic lattices, constituting a health hazard to humans and livestock. Direct contact of the irritant latex with the eye can cause blindness (33). Members of this genus are known to contain substances which are inhibitory to seed germination and seedling growth as well as to bacteria (28 and 29). This inhibitory action has been attributed to the presence of large amounts of phenolic compounds (28 and 29). The presence of lactone-forming acids has also been reported (22 and 24). Several groups of secondary metabolites, such as alkaloids, diterpenes, glucosinolates, tannins, and triterpenes have been reported in this genus (31). *E. balsamifera* is commonly grown as a hedge and field boundary marker (4). Its succulent branches carry a copious amount of latex which is generally reported to be toxic (20). The sap of *E. hyssopifolia* is applied to warts, corns and indurations of cornea (14). The latex is used as a purgative and a caustic agent on skin-lesions (34). *E. heterophylla* is a fast-growing weed that can form a dense canopy over soybean crop, making it difficult if not impossible to harvest (23). The toxicity of the plant, especially of the root and latex, is recognized in East Africa (3). *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 (8 and 20). 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 (4).

The plants chosen for this study all belong to the family Euphorbiaceae, which is a large family of trees, shrubs and rainforest herbs of Guinean, Sudanian, and xerophylactic habitats. Most members of this family are poisonous, but some are of economic and medicinal value (4 and 12). The leaves of the plants under study are of medicinal value, however, since the latex from these plants has shown to be toxic (3), evaluating their toxicity gains significance.



Fig. 1. Euphorbia hirta

MATERIALS AND METHODS

The leaves of the plants were harvested freshly for preparation of the extract. The leaves were weighed and macerated using mortar and pestle. A certain amount of water was added to ensure proper maceration. Thereafter, the solution was filtered by filter paper. The 1 mL filtrate put to the rats stomach canula for 14 days.

Blood sampling

Blood was collected by cardiac puncture from chloroform-anaesthetized rats into heparinised bottles for haematological studies. Another blood sample was collected into a clean bottle (non-heparinised) and allowed to clot. The serum was separated and centrifuged according to groups into clean bottles for biochemical analysis. Determination of haemoglobin concentration was performed by using the cyanomethaemoglobin method. Packed cell volume (PCV) was carried out using the conventional method of filling capillary tubes with blood as described by Schalm et al. (30). Erythrocyte count was done using the haemocytometer method as described by Jain (13 and 16). Total leukocyte and leukocyte differential counts were also determined. Erythrocyte indices were determined from values obtained from RBC, haemoglobin concentration, and PCV values. Total protein was measured using biuret reaction while albumin was measured by colorimetric estimation using 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 pyruvatehydrazone, formed with 2, 4-dinitrophenyl hydrazine.

The Anthelmintic activities of different extracts of aerial parts of *Cynodon dactylon* Pers were evaluated separately on adult Indian earthworm (*Pheritima posthuma*).

A lyophilized aqueous of extract of aerial parts of *Euphorbia* were evaluated separately for analgesic, antipyretic and anti-inflammatory properties. The extract

exerted central analgesic properties at doses of 20 and 25 mg/kg, and antipyretic activity at doses of 100 and 400 mg/kg, whereas anti-inflammatory effects against carrageenan-induced oedema in rats were observed at a dose of 100 mg/kg. The aqueous extract of the aerial parts was found to strongly reduce the release of prostaglandins, and thus depress inflammation. An ethanolic extract of the aerial parts was found to possess a prominent anti-anaphylactic activity and also showed significant antihistaminic, anti-inflammatory and immunosuppressive properties in various animal models. Water and ethanolic leaf extracts produced a time-dependent increase in urine output in rats. A methanol extract of leaves and stems inhibited the activity of the angiotensin-converting enzyme by 90% at 500 µg and 50% at 160 µg. The extract (10 mg/100 g, intraperitoneally) significantly decreased the amount of water consumed by rats. An ethanolic extract of the whole-plant showed a dose-dependent ulcer protective effect in rats. The active compound was found to be quercetin, which had an anti-ulcer activity ranging from 48–64% comparable to 61–80% of the standard drug ranitidine. An ethanolic extract of the aerial parts showed significant hepatoprotective activity in rats.

RESULTS

The results of this study with respect to the haematological changes showed that *E. balsamifera* and *E. hirta* caused significant reductions ($P < 0.05$) in PCV levels. Extracts of all plants caused significant reductions in the levels of red blood cell counts and haemoglobin concentrations. 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. In addition, while *E. heterophylla*, *E. hyssopifolia* and *E. lateriflora* caused a significant reduction in TWBC, only *E. lateriflora* caused significant reductions in lymphocyte levels (Table 1).

Table 1. Effects of the aqueous crude extracts of suspected poisonous plants on haematological parameters of rats

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 ^e
<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 ^e
<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 ^e
<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 ^e
<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

Table 1- continued			
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

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

Extracts of *E. hirta*, *E. hyssopifolia*, and *E. lateriflora* all caused significant increases ($P < 0.05$) in the level of total proteins 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 globulin levels. Finally, all the plants caused significant increases in enzyme AST and ALT levels (Table 2).

Table 2. Effects of the aqueous crude extracts of suspected poisonous plants on serum biochemical parameters of rats. Descriptions under the table are as Table 1 Total protein, albumin and globulin were measured in g/L. AST and ALT were measured in U/L

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	39±1	22±2	14±1	34.4±0.6	14.6±0.7

DISCUSSION

In this study, aqueous crude extracts of *E. balsamifera* caused a statistically significant decrease in the levels of PCV, haemoglobin concentrations and red blood cell counts, showing that the leaves of this plant could cause anaemia in animals that browse on them. The anaemia may, however, be of a regenerative type since the MCHC is low and MCV is normocytic (2, 6, 17, 21, and 27). The same situation also applies to the extract of *E. hirta*. Although extracts caused an insignificant decrease in PCV levels for *E. heterophylla* and *E. lateriflora*, a significant decrease in the levels of RBC and haemoglobin concentrations were observed, indicating 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 levels 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 (4). 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 cases being a haemolytic anaemia, and the second an acute oedematous gastroenteritis (6). In poisoning by *M. annua*, haematuria is also the most obvious clinical sign. In fact, Deprez et al. (9) reported on 2 cattle farms where animals demonstrated constipation or diarrhea, dullness, haemolytic.

Anaemia and red urine after ingestion of *M. annua*. Welchman et al. (35) had reported earlier that 11 lambs in a flock of 400 eight-month-old Romney lambs died from grazing on *M. annua*. Pathological findings, which included haemolytic anaemia, were indicative of annual mercury poisoning. In a study on the extract of *Jatropha curcas*, another member of the spurge family, Oluwole and Bolarinwa (25) 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 above findings show that these plants could produce toxic

effects on haematological values. The present study also showed that *E. heterophylla*, *E. hyssopifolia* and *lateriflora* caused significant decrease in the total number of white blood cells. *E. hyssopifolia*, particularly caused a significant decrease in the level of lymphocytes, showing that with continuous administration of these plant extracts to animals, 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 (11, 15, 26, 32, and 36). It should be noted that while some plant extracts caused an insignificant decrease in the level of lymphocytes, others caused an insignificant increase in the level of neutrophils. Continuous exposure to these plants may therefore, 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 (19). 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, which in turn may lead to dehydration. In case of the decreased globulin level, it may mean that the immune competence of the animals can be compromised easily. In fact, lymphopaenia accompanied by low globulin levels may lead to immunosuppression. In cases of decreased globulin levels, diseases characterized by immunoglobulin deficiency such as agammaglobulinaemia selective IgM, IgA and IgG deficiencies and transient hypogammaglobulinaemia, may lead to low-level globulin (10). For instance, *E. hirta* is said to possess immunosuppressive properties, as well as causing inflammatory effects (1). The results of this study may hence lend credibility 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 muscles and/or the hepatic parenchyma, allows for the leakage of large amounts of this enzyme into the blood (18). 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. This is particularly useful in measuring hepatic necrosis, especially in small animals (7). Since one of the specific assayable liver enzymes its elevated level in this study may indicate hepatic damage caused by these plants.

In conclusion, although these plants possess medicinal properties, the present 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 to these plants may lead to morbidity and mortality with dire consequences for livestock production and management.

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استخراج مواد سمی از گونه‌های متعدد فرفیون

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چکیده - فرفیون از گیاهان گلدار متعلق به خانواده شیرسگ یا شیرشیرکان (Euphorbiaceae) است. جنس فوق دارای ۲۰۰۸ گونه متعدد است. فرفیون یکی از بزرگترین و پیچیده‌ترین جنس از گیاهان گل دار بوده و گیاه شناسان چندین تلاش ناموفق برای کوچکتر نمودن جنس ها انجام داده‌اند. تعدادی از جنس‌ها برای مصارف دارویی و تعدادی برای مقاصد دیگر بکار برده می‌شوند. اما جالب است که یک گیاه می تواند هم مفید و هم اثرات سمی داشته باشد. در این مقاله، سمیت فرفیون مورد بررسی قرار گرفته و اثرات سمی جنس فرفیون در گونه‌های *E. balsamifera*, *E. heterophylla*, *E. hirta*, *E. hyssopifolia*, and *E. lateriflora* ثابت شده است. تغییر در جریان خون و همچنین پارامترهای بیوشیمیایی به عنوان شاخص اصلی از مسمومیت مورد مشاهده بوده است. نتایج آزمایشات ما نشان می‌دهند که ترکیبات مهم اندام هوایی فرفیون ترپنوئید بوده که شامل α -amyrin و β -amyrin است.

واژه‌های کلیدی: بیوشیمی، سمیت، فرفیون، β -amyrin، α -amyrin

* به ترتیب استادیار و استادیار

** مکاتبه کننده