

## Effects of some plants of the spurge family on haematological and biochemical parameters in rats

Adeolu Alex Adedapo<sup>1\*</sup>, Matthew Oluwole Abatan<sup>1</sup>,  
and Olufunso Olabode Olorunsogo<sup>2</sup>

<sup>1</sup>Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan, Nigeria

<sup>2</sup>Department of Biochemistry, University of Ibadan, Ibadan, Nigeria

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### ABSTRACT

The effects of five suspected poisonous plants of the spurge family (*Euphorbiaceae*) i.e. *Alchornea cordifolia* Schum and Thorn, *Cnidocolus acantifolius* Mill, *Phyllanthus amarus* Schum and Thorn, *Phyllanthus muellerianus* Exell and *Securinega virosa* Baill, which are commonly found in Nigerian pasture were evaluated in albino rats using crude aqueous extracts for 14 days. All the extracts were administered orally. Changes in haematological and biochemical parameters were used as indices of toxicosis. The extracts of the plants caused a significant reduction ( $P < 0.05$ ) in the levels of PCV and haemoglobin concentration. All except *C. acantifolius* caused a significant reduction in RBC level. The extract of four plants (*A. cordifolia*, *C. acantifolius*, *P. amarus* and *P. muellerianus*) caused significant changes of the total white blood cells when compared to that of the control. The extracts also caused a significant increase in the levels of total protein, albumin and AST activity. The extracts of *A. cordifolia*, *P. muellerianus* and *S. virosa* caused a significant increase in the level of ALT. Only *P. muellerianus* and *S. virosa* produced significant changes in the globulin level.

**Key words:** *Euphorbiaceae*, haematology, biochemistry, rats

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### Introduction

Nigeria is blessed with luxuriant pasture, among which grow many plants that are potentially dangerous to livestock (ABATAN, 1992). In Nigeria, conditions which favour the occurrence of plant poisoning in livestock are prevalent. These conditions include nomadism, which is movement to obtain grazing on the hoof from North to South, luxuriant

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\*Contact address:

Dr. Adeolu A. Adedapo, Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan, Nigeria, Phone: +80 2392 8512; E-mail: adedapo3a@yahoo.co.uk

growth of pasture, or when poisonous plants are harvested with hay or when their seeds become mixed with grain (NWUDE and PRASON, 1977; ADEDAPO and ABATAN, 2005). Most of the toxic plants in Nigeria have to be scientifically investigated (NWUDE and PRASON, 1977; ABATAN, 1992; ADEDAPO, 2002). These toxic plants occur generally all over the country, although some occur only in restricted areas.

The plants which have been chosen for this study belong to the family *Euphorbiaceae*. Most members of this family are poisonous while some are economically important (BURKILL, 1994). For instance, *Mercurialis perennis* (dog's mercury) and *M. annua* (annual mercury), which also belong to the same family, are poisonous. *M. perennis*, for instance gives rise to two distinct syndromes: the first is haemolytic anaemia, and the second acute, oedematous gastroenteritis (ADEDAPO and ABATAN, 2005). OLUWOLE and BOLARINWA (1997) in a study on the extract of *Jatropha curcas*, another member of spurge family, showed that the extract caused a progressive reduction in the measured haematological parameters (PCV, RBC, and haemoglobin concentration).

*Alchornea cordifolia* Schum and Thorn is a sprawling, multi-branched scandent tree, or shrub which grows to about 8 cm in height. Although the leaves are not eaten, they are freely used in traditional medicine (BURKILL, 1994).

*Cnidioscolus acotifolius* Mill is a multi-branched shrub, 3-4 m high, stems thick and fleshy, leaf 10-15 cm wide, palmately lobed, a native of Central America but now introduced and cultivated in Ghana and Nigeria. The leaf can be cooked and eaten as spinach (BURKILL, 1994).

*Phyllanthus amarus* Schum and Thorn is a weed that grows in both cultivated and uncultivated land and which has a sand-binding property. A plant with general medical application it is an ingredient of the "agbo" [medicinal herb] prescription in Lagos (BURKILL, 1994). The plant has an inhibitory effect on endogenous hepadnavirus DNA polymerase (UNANDER et al., 1993). Aqueous extracts of the leaves have been found to have a hypoglycemic effect comparable to that of tolbutamide (RAMAKRISHNA, 1969).

*Phyllanthus muellerianus* Exell is widespread in tropical Africa. The root is cooked with maize meal for severe dysentery in Ghana. It was also reported that the root of this plant is cut into small pieces with those of *Psychotria calva* and *Harrisonia abyssinica* and decocted and the liquid drunk for the treatment of cough in Ghana (BURKILL, 1994).

*Securinega virosa* Baill is a shrub or small tree, up to 4 m high and with numerous branches. It is widespread from tropical Africa to India and Australia (BURKILL, 1994). The leaves are considered to have laxative properties and are used in decoction in Nigeria (SOFOWORA, 1993).

The study was thus carried out in order to assess the toxic potential of these 5 plants, which are of medicinal value and are often found among Nigerian pasture, using changes in haematological and biochemical parameters as indices of toxicosis.

## Materials and methods

*Preparation of the aqueous crude extracts of the plants.* The leaves of the plants were always harvested freshly for preparation of the extract. The plants were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN).

The leaves were weighed (200 g) and blended into liquefaction in 400 mL of distilled water. The mixture was then centrifuged at 1500 rpm. The supernatant was filtered through sterile filter papers into a conical flask as the study extract. The filtrate was administered to rats per os once daily, using a stomach canula, for 14 days. The dose was 2 mL/100g body mass of rats. The control group received water instead of extract.

*Animals and experimental design.* The animals used in this study were rats of the Sprague Dawley strain, weighing between 100 and 190 grams. They were of both sexes and maintained at the Animal House of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. 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 a bottle *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 5 plants under study (*A. cordifolia*, *C. acontifolius*, *Phyllanthus amarus*, *Phyllanthus muellerianus* and *S. virosa*), the sixth group served as the control.

*Techniques for obtaining blood and serum samples.* Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into heparinised test tubes for haematological studies. Blood samples were also collected into clean non-heparinised test tubes and allowed to clot. The serum was separated from the clot and centrifuged according to groups into clean test tubes for biochemical analysis.

*Determination of haematological parameters.* Determination of haemoglobin concentration was performed according to JAIN (1986), using the cyanomethaemoglobin method. Packed cell volume (PCV) was done by conventional means.

*Method of filling capillary tubes with blood.* 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 count, 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 Sigma Diagnostics albumin reagent (Sigma Diagnostic, U.K.), which contained bromocresol green (BCG). Globulin was obtained from the difference between total protein and

albumin. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was also measured (DUNCAN et al., 1994).

*Statistical analysis.* Results were subjected to the Student's *t*-test and were considered significant at  $P < 0.05$  (ESSEX-SORLIE, 1995).

## Results

The results of this study showed that *A. cordifolia* caused a significant reduction ( $P < 0.05$ ) of PCV, Hb concentration and RBC. The extracts of all plants except *P. muellerianus* also caused significant changes of white blood cells (WBC) count ( $P < 0.05$ ). For *C. acontifolius*, all the parameters except RBC significantly decreased ( $P < 0.05$ ). The results of this study also showed that both *P. amarus* and *P. muellerianus* caused a significant reduction ( $P < 0.05$ ) in the levels of PCV, RBC, WBC and Hb concentration. In the case of *S. virosa* there was a significant decrease ( $P < 0.05$ ) in the levels of PCV, RBC, Hb concentration and total WBC count (Table 1).

Table 1. Effects of the aqueous plant extracts on the haematological parameters of rats (n = 5)

Parameters	Control	<i>A. cordifolia</i>	<i>C. acontifolius</i>	<i>P. amarus</i>	<i>P. muellerianus</i>	<i>S. virosa</i>
PCV (%)	36.6 ± 2.2	32.4 ± 1.4 <sup>a</sup>	26.4 ± 3.2 <sup>a</sup>	22.8 ± 1.8 <sup>a</sup>	28.0 ± 2.1 <sup>a</sup>	29.8 ± 1.7 <sup>a</sup>
Hb (g/L)	114 ± 5	104 ± 5 <sup>a</sup>	85 ± 11 <sup>a</sup>	74 ± 7 <sup>a</sup>	91 ± 5 <sup>a</sup>	100 ± 5 <sup>a</sup>
RBC (10 <sup>6</sup> /μL)	6.0 ± 0.3	5.34 ± 0.2 <sup>a</sup>	5.5 ± 0.6	4.4 ± 0.2 <sup>a</sup>	4.9 ± 0.2 <sup>a</sup>	4.9 ± 0.3 <sup>a</sup>
MCV (fl)	60.8 ± 3.7	60.6 ± 0.1	49.4 ± 5.1 <sup>a</sup>	52.0 ± 4.4 <sup>a</sup>	57.5 ± 1.7 <sup>a</sup>	60.8 ± 2.6
MCHC (g/L)	31.2 ± 2.4	32 ± 0.5	32.0 ± 0.4	33.0 ± 0.7	33.0 ± 0.8	33.0 ± 0.8
MCH (pg)	19.0 ± 1.6	20.2 ± 0.6	15.8 ± 1.4 <sup>a</sup>	16.9 ± 1.0 <sup>a</sup>	18.8 ± 0.1	20.3 ± 0.6
WBC (10 <sup>3</sup> /μL)	4.7 ± 0.4	5.8 ± 0.5 <sup>a</sup>	5.5 ± 0.3 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>	4.4 ± 0.8 <sup>a</sup>	4.5 ± 0.1 <sup>a</sup>

Mean ± SD. Superscripts indicate significant difference from control at  $P < 0.05$ .

The extract of *A. cordifolia* caused a significant increase ( $P < 0.05$ ) of all the biochemical parameters investigated, except globulin. The extract of *C. acontifolius* also caused a significant increase in the levels of the parameters, except for globulin, and ALT. *P. amarus* caused a significant increase ( $P < 0.05$ ) of total protein and albumin, although no significant change was observed with globulin. The extract of *P. amarus* caused a significant increase in the activity of AST but not that of ALT. For *P. muellerianus*, there was a significant increase in the levels of total protein and globulin, but no significant change in the level of albumin. The extract of *P. muellerianus* caused a significant increase

in the activities of ALT and AST. In the case of *S. virosa*, all the parameters significantly increased, except globulin, which significantly decreased (Table 2).

Table 2. Effects of the aqueous plant extracts on the serum biochemical parameters of rats (n = 5).

Parameters	Control	<i>A. cordifolia</i>	<i>C. acontifolius</i>	<i>P. amarus</i>	<i>P. muellerianus</i>	<i>S. virosa</i>
Total prot. (g/L)	36 ± 1	43 ± 2 <sup>a</sup>	46 ± 2 <sup>a</sup>	44 ± 1 <sup>a</sup>	44 ± 3 <sup>a</sup>	42 ± 1 <sup>a</sup>
Albumin (g/L)	22 ± 2	28 ± 4 <sup>a</sup>	33 ± 1 <sup>a</sup>	32 ± 2 <sup>a</sup>	25 ± 4	30 ± 1 <sup>a</sup>
Globulin (g/L)	14 ± 1	15 ± 2	13 ± 1	12 ± 3	19 ± 1 <sup>a</sup>	12 ± 1 <sup>a</sup>
ALT (IU)	34.4 ± 0.6	38.8 ± 1.0 <sup>a</sup>	34.5 ± 1.7	34.0 ± 1.8	48.0 ± 2.9 <sup>a</sup>	40.3 ± 0.9 <sup>a</sup>
AST (IU)	41.6 ± 0.7	48.0 ± 1.4 <sup>a</sup>	51.0 ± 1.3 <sup>a</sup>	49.5 ± 1.0 <sup>a</sup>	53.5 ± 2.6 <sup>a</sup>	48.4 ± 0.7 <sup>a</sup>

Mean ± SD. Superscripts indicate significant difference from control at P<0.05.

## Discussion

This study showed that the extracts of the 5 plants caused a significant reduction in the levels of haematological parameters i.e. PCV, RBC and haemoglobin. It thus showed that animals that browse the leaves of these plants might develop anaemia. Anaemia is a reduction in the number of erythrocytes, haemoglobin, or both, in the circulating blood. It results from excessive red blood cell (RBC) destruction, RBC loss, or decreased RBC production and is a manifestation of an underlying disease process; therefore, the response to treatment of anaemia is transient unless the underlying disease process is addressed (STRAUS, 1998).

This observation of decreased level of erythron may support the claim that many members of the spurge family (*Euphorbiaceae*) to which these 5 plants belong are poisonous (BURKILL, 1994).

In a study on the phytochemical constituents of aqueous extract of *P. amarus*, the following were detected: alkaloids, saponins, tannis, anthraquinones, flavones, carotenoids, reducing compounds, cardiac glycoside, steroids and triterpenes, coumarins, volatile oils, etc. (ODETOLA and AKOJENU, 2000). The leaves and bark of *A. cordifolia*, for instance, are reported to contain 10-11 % of tannins (BURKILL, 1994). Tannins, the constituents of the pods of *Acacia nicotica*, have been indicated as the cause of death in goats in South Africa, giving rise to tachycardia, anorexia, ruminal stasis, anaemia, dyspnoea and recumbency (ADEDAPPO and ABATAN, 2005). The hydrolysable tannis are astringents and apparently bind the proteins in plasma and organs causing coagulation and necrosis (SPIER et al., 1987). Saponins are also known to cause poisoning in animals. For instance, plants such as *Tribulus terrestris*, *Panicum coloratum*, *Nolina texana*, etc., may contain toxic levels of hepatotoxic steroidal saponins (SHUMAIK et al., 1988;

MILES et al., 1993). Saponinins are metabolized in animals to glucuronide conjugates of epsimilagenin, which crystallize in bile, leading to biliary blockage, cholangitis, and secondary photosensitization (MILES et al., 1991; MILES et al., 1993). In fact, herbivores are affected with signs of toxicity involving liver damage, with anorexia, mass loss, icterus, hepatoencephalopathy, and photosensitization (CAMP et al., 1988; CORNICK et al., 1988; LOW et al., 1993; SMITH and MILES, 1993). It may then suggest that the presence of tannins and saponins in the aqueous extract of *P. amarus* and *A. cordifolia* may be responsible for the significant reduction in the levels of red blood cells and haemoglobin, leading to the observed anaemia. It should be noted that a wide variety of poisonous plants are capable of producing anaemia in domestic animals.

The effects of the extracts of plants in this study on total white blood cells showed that there is a significant difference when compared with those of the control. Although it was stated that toxic plants do not produce a direct effect on white blood cells, such as neutrophils, lymphocytes, eosinophils, and monocytes (SWENSON and REECE, 1993), the results of this study showed otherwise. Excessive ingestion of a wide variety of plants or their products has been found to cause hypoproliferative or non-regenerative anaemia, which is a stem cell disorder characterized by reduced bone marrow production of all blood components in the absence of a primary disease process infiltrating the bone marrow or suppressing haematopoiesis (OLSON et al., 1984). It shows that continuous administration of these extracts or ingestion of the leaves of these plants may produce these effects in animals. It may also mean that the principal function of white blood cells, which is to defend against invading organisms, will be compromised (SWENSON and REECE, 1993; GANONG, 1997; STRAUS, 1998).

The aqueous crude extracts of the 5 plants used in this study caused a significant increase in the levels of total protein and albumin, although the extract of *P. muellerianus* did not cause any significant increase in the level of albumin. The increase noted for total protein and albumin may be the result of animals' refusal to drink water, and this may lead to dehydration. *S. virosa* extract actually caused a significant decrease in the globulin level, and this may mean that the immune competence of the animals may be easily compromised. The continuous exposure of animals to this plant may then mean that the immune diseases characterized by deficiency of immunoglobulin, such as agammaglobulinaemia, selective IgM, IgA and IgG deficiencies, and transient hypogammaglobulinaemia, may lead to low globulin (DUNCAN et al., 1994). Reduced volume of extracellular fluids could also contribute to changes in other parameters measured (DUNCAN et al., 1994).

The aqueous crude extracts of all the plants in this study caused a significant increase in the levels of aspartate aminotransferase AST. Elevation in the activity of AST can be associated with cell necrosis of many tissues. Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma, allows leakage of large amounts of this enzyme

into the blood (BUSH, 1991). The elevation in AST activity induced by these plants is an indication of tissue necrosis.

All the plants except *C. acontifolius* and *P. amarus* induced a significant increase in ALT activity ( $P < 0.05$ ). ALT is present in liver and other cells and is particularly useful in measuring hepatic necrosis, especially in small animals (DUNCAN et al., 1994). Since ALT activity is one of the specific assayable liver enzymes, its elevated level in this study may indicate hepatic damage by these plants. Since *P. amarus* extract did not cause a significant increase in ALT activity, it thus means that the aqueous crude extract of this plant may not be hepatotoxic, because some studies have observed that the plant has been traditionally used to promote liver functions, which demonstrate the beneficial effects of the plant extract on liver functions (THYAGARAJAN et al., 1990; BERK et al., 1991; DOSHI et al., 1994; WANG et al., 1995; CALIXTO et al., 1998; NARENDRANATHAN et al., 1999).

This study shows that the five investigated plants have toxic potential and their continued presence in Nigerian pasture could lead to plant toxicity, especially if these plants are consumed in pasture. The effect of this toxicity in livestock production, especially in Nigeria, could be direct and indirect losses. Direct losses (death) and indirect losses, which include reduced weight gains, decrease reproductive performance, fencing and management expenses, may have high economic consequences on livestock production for farmers (NIELSEN and JAMES, 1992).

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**ADEDAPO, A. A., M. O. ABATAN, O. O. OLORUNSOGO: Učinci nekih biljaka porodice mlječika na hematološke i biokemijske pokazatelje u štakora. Vet. arhiv 77, 29-38, 2007.**

**SAŽETAK**

Učinci sirovih vodenih iscrpaka pripremljeni od pet vrsta biljaka porodice mlječika (*Euphorbiaceae*) sumnjivih da su otrovne: *Alchornea cordifolia* Schum i Torn, *Cnidioscolus acontifolius* Mill, *Phyllanthus amarus* Schum i Torn, *Phyllanthus muellerianus* Exell i *Securinega virosa* Baill, koje se često nalaze na nigerijskim pašnjacima, istraženi su na albino štakorima u tijeku 14 dana. Svi iscrpci davani su oralno. Promjene u hematološkim i biokemijskim pokazateljima uzete su kao znakovi otrovanja. Davanje iscrpaka

prouzročilo je značajno smanjenje ( $P < 0,05$ ) vrijednosti hematokrita i koncentracije hemoglobina. Svi su, osim iscrpka *Cnidoscolus acontifolius*, uzrokovali značajno smanjenje broja crvenih krvnih stanica. Iscrpci biljaka *Alchornea cordifolia*, *Cnidoscolus acontifolius*, *Phyllanthus amarus* i *Phyllanthus muellerianus* uzrokovali su značajne promjene ukupnog broja bijelih krvnih stanica u usporedbi s kontrolom. Iscrpci su također doveli do značajnog povećanja razina ukupnih bjelančevina, albumina i aktivnosti AST. Iscrpci *Alchornea cordifolia*, *Phyllanthus muellerianus* i *Securinega virosa* uzrokovali su značajno povećanje razine ALT. Samo *Phyllanthus muellerianus* i *Securinega virosa* uzrokovali su značajne promjene u razini globulina.

**Ključne riječi:** *Euphorbiaceae*, hematologija, biokemija, štakori

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