IN VITRO EVALUATION OF THE EFFECT OF *Euphorbia kamerunica* LATEX ON THE BLOOD OF ALBINO RAT (*Rattus norvegicus*)

KONYA, Roseline Sonayee, ZITTE, Leelee Famii and CHUKWU, Kenneth Obinna
Department of Animal and Environmental Biology, Faculty of Biological Sciences, College of Natural and Applied Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Corresponding Author: Zitte, L. F. Department of Animal and Environmental Biology, Faculty of Biological Sciences, College of Natural and Applied Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. **Email:** leelee.zitte@uniport.edu.ng **Phone:** +234 8037971401

ABSTRACT

The effect of Euphorbia kamerunica latex on the blood of albino rat (Rattus novergicus) was studied. Two different samples were prepared, one with 10% E. kamerunica latex in blood of albino rat and the other, whole blood. Blood from each of the two samples was analyzed haematologically for the packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and neutrophils. The results of the study showed that there was a drop in all the blood parameters in the blood sample with the latex as compared with the whole blood. The PCV dropped from 17% to 9%, (47.06% reduction), Hb reduced from 5.1g/dl to 3.2g/dl (37.25% reduction), RBC had a three log reduction from $3.7x10^{12}$ to $2.7x10^{9}$ (99.88% reduction), WBC fell from $4.2x10^{9}$ to $3.2x10^{7}$ (99.24 % reduction) while neutrophils dropped from 20% to 15% (25.00% reduction) in the blood with latex. The proximate analysis of the latex revealed that it contains 62.13 ± 0.48 % lipid, 0.16 ± 0.01 % carbohydrate, 3.69 ± 0.05 % protein and 3.86 ± 0.24 % ash. The result of this in vitro study demonstrated that Euphorbia kamerunica has some toxic effects on the blood of Rattus novergicus.

Keywords: *Euphorbia kamerunica,* Latex, *Rattus norvegicus,* Packed cell volume, Haemoglobin, Red blood cell, White blood cell, Neutrophils

INTRODUCTION

In human adults, about 5 liters of blood contribute 7 – 8% of the body weight. The contribution of red blood cells (erythrocytes) to the total volume of the blood (haematocrit) is about 43%. Erythrocytes are the dominant cell in the blood, measuring 99% of the blood cells. They are not the only type of cells in the blood. There are also leukocytes and blood platelets. Erythrocytes are important to the blood as the carrier of oxygen (with the help of the pigment haemoglobin), functionality of the blood could be traced to the healthy state of the erythrocytes. Pathological states of the blood generally affect the physiology of the other components of the body. Thus investigation of the effects of toxic compounds on blood parameters is of enormous physiological importance as such toxins can offset the normal state of the body.

Euphorbia kamerunica is a species of plant belonging to the family Euphorbiaceae, consisting of about 2008 species (WCLSP, 2011). It is very diverse and sometimes it is referred to as spurges. The genus is mostly found in the tropical and sub-tropical Africa and America. The plants are annual or perennial woody shrubs or trees with a caustic poisonous milky sap (latex). The latex acts as a deterrent for herbivores. It also acts as a wound healer (Yang *et al.,* 2005).

The components parts are mainly of di-terpene ester, the terpene ester composition determine how caustic and irritating the latex will be. Post mortem examination of those who mistakenly eat *Euphorbia* reveals severe inflammation of the walls of the stomach, in some cases, the wall of the stomach was perforated. Exposure to the sap has been implicated with severe eye damage including permanent blindness (Eke *et al.,* 2000). *Euphorbia abyssinica,* one of the species of *Euphorbia* is considered poisonous and it has been used for homicidal purposes. The latex is used as purgative and it is caustic on skin lesion. On the other hand, neither the latex nor the water extract was reported to be toxic to guinea pig when administered orally (Silva *et al.,* 2007).

Plants from the *Euphorbiaceae* family, mainly from the *Euphorbia* genus, have also been used by some Brazilian folk communities for the treatment of injuries, infectious illnesses, viral, tumors and inflammatory diseases (Amirghofran *et al.,* 2006; Zhang *et al.,* 2008; Uzair *et al.,* 2009; Fernandez-Arche *et al.,* 2010). Regarding the high concentrations of terpenoid compounds in some specific plants within this family, it is possible that some species have medicinal properties that can be exploited for therapeutic purposes (Pusztai *et al.,* 2007; Lage *et al.,* 2009).

They have also been reportedly used as medicinal plants in humans because of their antiviral and antitumor properties (Yang *et al.,* 2005; Amirghofran, 2006; Kuo *et al.,* 2006; Yan *et al.,* 2008; Lage *et al.,* 2009). Pre-clinical studies indicated that the plant extract had low toxicity in mice (Silva *et al.,* 2007). Kumar *et al.* (2011) reported that the latex of *Euphorbia tirucalli* reduced blood calcium and blood phosphorous in catfish (*Heteropneustes fossillis*) after 72 hours. In line with the above background, this in vitro study was carried out to investigate the effect of *E. kamerunica* latex on the blood of albino rat. This study will provide useful base line data for enthnomedicinal practitioners since *E. kamerunica* has been implicated in herbal medicinal cure of various ailments.

MATERIALS AND METHODS

Latex: The plant *Euphorbia kamerunica* used in this study was obtained from the botanical garden of University of Port Harcourt, Choba, Port Harcourt, Rivers State of Nigeria. The stems of the plant were cut and taken to the laboratory and latex extracted from the freshly collected plant by allowing the latex to drip from a cut in the plant into a clean a 10 ml test tube. The proximate analyses were carried out (AOAC, 2005) to determine the nutritive composition of the *Euphorbia kamerunica* latex. The latex was used fresh for the *in vitro* studies.

In Vitro Assay: Ten (10 ml) of blood was taken from albino rats and stored in heparinised bottle in the refrigerator at a temperature of 4^0C pending use. A Pasteur pipette was used to collect 1ml of the blood into a test tube. Red blood cell diluents were prepared by dissolving 3g of sodium citrate and 1ml of 40% formaldehyde in 100 ml of distilled water. 0.1 ml of the latex was measured into a test tube containing 1ml of whole blood. The mixture was gently stirred and with the aid of Pasteur pipette and 0.55 ml (equivalent to 0.5ml of whole blood) was measured into a test tube containing 9.45 ml of diluents to have 1:20 dilution. 1ml of the 1:20 was measured into 9 ml diluents to have 1:200 dilutions. One drop of the 1:200 dilution was introduced into the chamber of the Neubauer slide. With the aid of x10 and x40 microscope objective lenses, the red blood cells were counted. The experiment was repeated three times to authenticate the results (Sood, 2006).

Estimation of haemoglobin concentration was carried out using the Drabkin's method (Sood, 2000). 5ml of Drabkin's solution was pipette into each of six different test tubes. Then 0.02 ml (20 µl) of blood with latex was added to three of the test tubes while the other three were with 0.02ml of whole blood each. The test tubes were then covered and mix by swirling. The mixtures were allowed to stand for 10 minutes. Thereafter, the absorbance was read at 540 nm using Drabkin's solution as blank.

Packed cell volume (PCV) also known as haematocrit was determined by filling heparinised capillary tube with blood samples from the whole blood and the blood with latex. One end of the capillary tubes was sealed with a small piece of plasticine and the capillary tubes placed in a centrifuge and revolved at 12,000 rpm for five minutes. After that, the PCV was read with the graphic reader and the result expressed in percentage.

During neutrophil analysis, blood film was prepared by spreading a small drop of fresh blood and the blood with latex over the surface of the Improved Neubauer slide. After drying, the film was stained in order to show the structure and nature of the white blood cells. The number of the neutrophils were counted (neutrophils has a multi-lobed nucleus with purple cytoplasm containing pale pink, small pink or purple granules) in each 0.0025 mm^2 size of the improved Neubauer slide With the aid of x10 and x40 microscope objective lenses. The result was multiplied by the total number of square boxes and the volume of blood in it (Sood, 2006).

RESULTS

The proximate analysis of the latex revealed that it contained 62.13 ± 0.48 % lipid, 0.16 ± 0.01 % carbohydrate, 3.69 ± 0.05% protein and 3.86 ± 0.24 % ash. The fresh sample had 76.70% moisture content (Table 1). The results of the blood parameters with the latex and without the latex revealed that there was a drop in all the blood parameters as the latex was added to the blood. The packed cell volume (PCV) dropped from 17.0 ± 1.0 % in the whole blood to 9.0 ± 0.58 % in blood with latex (47.06% reduction). The haemoglobin (Hb) reduced from 5.10 ± 0.37 g/dl to 3.20 ± 0.12 g/dl (37.25% reduction). Red blood cell (RBC) changed from $3.7x10^{12}$ to $2.7x10^{9}$ (99.88% reduction), while blood cell (WBC) value also fell from $4.2x10^{9}$ to $3.2x10^{7}$ (99.24% reduction) and neutrophils dropped from 20% to 15% (25.00% reduction) in the blood with latex (Table 2).

Table 1: Proximate analysis of *Euphorbia kamerunica* latex

Constituents	Percentage composition	
	Fresh latex	Dry latex
Moisture	76.70 ± 0.18	0.00 ± 0.00
Ash	0.90 ± 0.06	3.86 ± 0.24
Carbohydrate	0.04 ± 0.02	0.16 ± 0.01
Lipid	14.48 ± 0.11	62.13 ± 0.48
Protein	0.86 ± 0.01	3.69 ± 0.05

Table 2: Effect of *E. kamerunica* latex on blood of albino rat under in vitro evaluation

Blood Parameters	Without latex	With latex
PCV (%)	17.0 ± 1.00	9.0 ± 0.58
Haemoglobin (g/dl)	5.10 ± 0.37	3.2 ± 0.12
RBC	3.70 ± 0.12 $x10^{12}$	2.1 ± 0.12 $x10^{9}$
WBC	4.20 ± 0.06 $X10^{9}$	3.2 ± 0.18 $X10^{7}$
Neutrophils (%)	20.0 ± 1.86	15.0 ± 1.20

The percentages reduction in haematological parameters between blood samples with latex and blood sample without latex is presented in Figure 1.

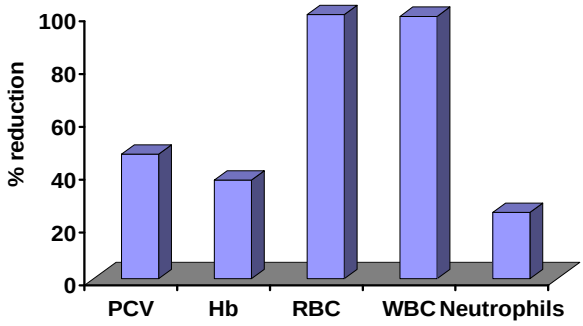

Figure 1: Percentage reduction of blood parameters of albino rat by *Euphorbia kamerunica* under an in vitro evaluation. Key PCV: Packed cell volume, HB: Haemoglobin, RBC: Red blood cell, WBC: White blood cell

DISCUSSION

Though the analysis did not include a more detailed chemical analysis of the constituents of the latex, it showed a gross overview of the composition. It was observed that on the dry matter basis, the latex contains a high percentage of lipid (62.13%). The value which is more than the lipid constituent of most food items.

The relative constituent of the latex can be a good indication that the latex will have some physiological effects on animals even as it is reported that it has some antiviral, antitumor and other therapeutic functions (Yang *et al.,* 2005; Amirghofran *et al.,* 2006; Zhang *et al.,* 2008; Uzair *et al.,* 2009; Fernandez-Arche *et al.,* 2010). The carbohydrate and protein were low. From the result, lipid was more than 360 times that of carbohydrate, and more than 16 times of ash and protein.

The latex grossly reduced blood parameters. The PCV and RBC had the highest percentage reduction. All percentage reductions were significantly above 35% reduction. The RBC had a three log reduction, while WBC had a two log reduction. The percentage reductions were 99.88 and 99.24, respectively. The PCV and Hb concentrations had percentage reductions of 47.06 and 37.25, respectively. The reductions observed could be as a result of haemolysis caused by disruption of the integrity of the blood cells (Eno *et al.,* 1999). In another study, Kumar *et al.* (2011) reported that *Euphorbia* latex reduced the calcium and phosphorous contents of the blood of a freshwater catfish. However Avelar *et al.* (2011) reported immunomoduratory effects of low doses of the Euphorbia latex. From the above cited studies, *Euphorbia latex* greatly affected the blood parameters. Its therapeutic effects as reported by other researchers could be as result of doses differences.

Conclusion: *Euphorbia kamerunca,* one of the many species of the family *Euphorbiaceae* is observed to have some disruptive toxic effects on blood parameters. It is therefore of great importance to handle it with care. Any unguarded handling of the plant's latex should be discouraged. There should also be some more research into the therapeutic effects and the doses that will not be injurious to animals and humans. Use of this plant for therapeutic purposes in humans has to be managed carefully since it has disruptive effects on the blood cells.

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