Journal of Hematology & Thromboembolic Diseases

Jimmy et al., J Hematol ?rombo Dis 2016, 4:6
DOI: 10.4172/2329-8790.1000256

Research Article Open Access

Upgrading of Lethal Dose of *Tetrapleura tetraptera* Extract Enhances Blood Cell Values

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Received date: November 01, 2016; **Accepted date:** November 28, 2016; **Published date:** December 01, 2016

Abstract

Effects of aqueous extract of *Tetrapleura tetraptera* (Uyayak) on blood cells were investigated in 28 albin rats for the period of 28 days. The acute toxicity LD50 on 30 albino mice was 244.94 mg/kg. The red blood cells counts increased at 40% of the LD50 extract on day 14, 30% of LD50 on day 21 and at 10% of the LD50 on day 28, ($P<0.05$). Platelets counts increased at 20% of LD50 on day 14, 40% LD50 on day 21 and 20% of LD50 on day 28 ($P<0.05$) total white blood cells counts increased at 40% of the LD50 on day 14, at 10% of LD50 on day 21 and at 20% of the LD50 on day 28, ($P<0.05$). The lymphocytes differential counts increased at 40% of the LD50 extract on day 14, at 10% of the LD50 on day 21 and at 20% of the LD50 on day 28 ($P<0.05$). Neutrophil different counts increased at 20% of the LD50 extract on day 14, at 40% of the LD50 on day 21 and at 30% of the LD50 on day 28. Mean corpuscular haemoglobin concentration (MCHC) increased at 20% LD50 on day 14, at 10% LD50 on day 21 and at 20% LD50 on day 28. Also the mean corpuscular haemoglobin, (MCH) increased at 20% LD50 on day 14, at 10% LD50 on day 21 and at 20% LD50 on day 28. The mean corpuscular volume, (MCV) increased at 20% LD50 on day 14, at 10% LD50 on day 21 and at 20% LD50 on day 28. It is shown in the study that extract of *Tetrapleura tetraptera* is a potent stimulant for blood cells development and should be adopted as haematinic.

Keywords: *Tetrapleura tetraptera*; upgrading dose; blood cells

Introduction

Tetrapleura tetraptera is a legume in the family Fabaceae. It is one of the plants that is often used in African traditional medicine as anti-contraceptive, [1,2]. It is a tropical climbing woody botanical constructors agent, [3]. It has bat pollinated flowers. The pods are produced on the stems that hang from the forest canopy. The seed pods are covered with hairs called trichomes which can be very painful in contact with the eyes. At maturity each pod produces several hard brown, marble-like seeds which resemble the eye of a bull otherwise called sea beans. The plant is rich in crude protein, iron, fat, crude fibre, energy, phosphorus, calcium, sodium, magnesium, manganese, copper, zinc, it consists of certain amino acids e.g cysteine and methionine. However, it has some antinutrients e.g L.Dopa, tamin, oxalate, saponin, phytate [4]. *Tetrapleura* is popularly used in South Nigeria as soup spices particularly in pounded yam soup and in palm fruits soup with pumpkin leaves (Abak). It has been recommended as proteinous meal, [9]. It is also used as antiparasitic dispelling agents when its hairs are made into honey or syrup in the Caribbean region of Central America. The seeds are used in making beads as part of dressing. The oil from the fruit is used in the preparation of soap. Despite all these good attributes of *Tetrapleura*, it has been incriminated in causing ill-health. For example; it is reported to cause sperm abnormalities e.g unusual head with large acrosome, looped tail piece and mid piece with distal droplet, pin head; pyriform head which may affect male fertility. But the haematinic potentials of this plant has not been well documented and hence this study. Most of the endemic diseases in the tropics affect the blood cells particularly the red blood cells leading to anaemia e.g malaria cause by female anopheles mosquitoes. Supplements and orthodox blood syrups and tablets are not very active in restoring normalcy in anaemic situation. Moreover, there is often revisitation of the parasites to cause more health hazard. The fact that this plant species also possess antiparasitic properties point to its good medicinal health attributes and require thorough exploitation for its potential as insectides against mosquitoes. The usefulness of tetrapleum as haematinic to alleviate problem of anaemia particularly in the rural area with its high availability and affordability. This could be used in the formulating haematinic drugs and will go a long way in increasing the number of such drugs in pharmaceutical shops for the management of anemia.

Materials and Methods

Collection of plant material

The fruit of *Tetrapleura tetraptera* was purchased from the market in Akwa Ibom State.

Preparation of extract

It was washed, sliced into pieces, air dried and pulverized into fine coarsive form with mortar and pestle. It was weighed 617 g. This was macerated in distilled water and allowed to stand for 24 hours. It was filtered and the filtrate concentrated at 45˚C in the water bath and left to dry. It was further weighed to be 19.88 g.

Acute toxicity studies (LD50)

The Methods of Lorke was used for the extraction [5].

Preparation of stock solution administration of drugs

A weight of 1 g of aqueous extract of *Tetrapleura tetraptera* was dissolved in 10 ml of distilled water as follows; (1 g/10 ml)=1000 mg.

1000 mg=1 g

Stock solution=100 mg/ml

$$\text{Therefore Dosage} = \frac{\text{weight of animal (kg)} \times \text{dose of drug mg/kg}}{\text{Stock solution (mg)ml}}$$

Animal

A total of 30 mice and 28 rats were kept in a ventilated University of Uyo Pharmacology animal house they were fed with pullets and water. The administration of 30 mice was done intraperineally (IP) as administration of extraction mice: This was done by intraperitoneal route. Group 1; administered 5000 mg/kg, group 2; 4000 mg/kg group 3; 3000 mg/kg, group 4; 2000 mg/kg, group 5; 1000 mg/kg group 6; 500 mg/kg, group 7; 400 mg/kg, group 8; 300 mg/kg; group 9; 200 mg/kg, group 10; 100 mg/kg. The LD50 was established as geometric mean square root of the maximum dose that produced 0% mortality (A) and the minimum dose that produced 100% mortality (B).

$$LD50 = \sqrt{A \times B}$$

Where A=200 mg/kg

B = 300 mg/kg

LD50=200 × 300=6000

=244.95 mg/kg.

Therefore LD50=244.95 mg/kg, this form the dosage for administration into the rats.

Administration of extract in rats

A total of 28 rats were used they were divided into 4 groups with 7 rat in each group including control. Group 1 was administered with distilled water, group 2; 24.50 mg/kg, group 3; 49.00 mg/kg; group 4; 73.50 mg/kg. The crude extract was administered using canula – by-passing the oesophagus and delivered into the stomach, [6,7]. Actual doze was calculated as follows;

$$\frac{\text{Weight of rat (kg)} \times \text{dose of drug (LD50)}}{\text{Stock solution}}$$

Blood collection and analysis

The rats were observed for 28 days after administration and 5 ml of blood collected by cardiac puncture into EDTA bottles by anesthetic of the rats with chloroform. The sacrifice was done for 7, 14, 21 and 28 days for red blood cells, white blood cells, packed cell volume, platelets, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin, (MCH) values using methods of Dacie and Lewis 2007 and automated haematological analyzed CBC – 2300 Mindray haematological analyzed-hambing-German.

Statistical analysis

Statistical analysis applied was student t-test with SSPS package [5].

Results

The study showed enhanced blood cells elevation at increase dosage of the extract of *Tetrapleura tetraptera*. Red blood cells showed increase at 14 and 28 days of extract administration, table 1. In WBC, increase was observed in day 14 and same results in platelet counts. The hymphocyte counts increased as the period of administration increased. MCV, MCHC and MCH were enhanced increased, Table 1.

Period	RBC × 10^{12}	WBC × 10^{9}	Platelets × 10^{9}	MCV N3	MCHC %	MCH pg.	Neutrophil (%)	Lymphocyte (%)
14 days	8.77	5.2	722	56.6	28.4	16.1	58	58
21 days	6.68	9.43	735	56.3	40.5	22.8	19	64
28 days	8.91	3.9	652	54.4	28.9	15.7	32	68

Table1: Effects *Tetrapleura tetraptera* on blood cells.

Discussion

Variation in the effects of *Tetrapleura tetraptera* on the various red blood cells have been observed as per the dosage of the extract i.e percentage dosage of the LD50 and the period of extract administration particularly the red blood cells had increased with increased in percentage of extract. This means that increase consumption of the extract will increase red blood cells count. This is shown in such increase in days 14 and 28 of the administration. The normal red blood cells development takes 3-4 days i.e from the erythroblast to erythrocyte stage. This rate of production is a function of the rate of destruction and the oxygen content and stimulation by erythropoietin [8,9]. The boosted effect of *Tetrapleura tetraptera* may be the fact that it has iron content which is an important constituent of the haem. Other constituents include amino acid for globin chain, copper and zinc and which are very essential in haemopoiesis. However, phytate is reported in *Tetrapleura tetraptera* which is iron chelator which may reduce its concentration [10-12]. The red cell number is very important in its oxygen carrying capacity and it is very relevant that adequate number be present to avoid increase cardiac activity in low availability. All the haemoglobin indices increased at increase concentration of the extract. Low values of these parameter suggest anaemia. The increase in these values goes to confirm its positive effect on red blood cells particularly the packed cell volume. The positive impacts on mean corpuscular volume (MCV) mean

corpuscular haemoglobin concentration; (MCHC) and mean corpuscular haemoglobin (MCH) correlate the normal value of haemoglobin concentration in *Tetrapleura tetraptera*. The platelet counts also increased appreciably on day 21 and at highest dosage of the extract. This is very remarkable as this extract could be very useful in thrombocytopenia associated with blood loss and infection and blood clothing. The calcium content in tetrapleura indicates its likely contribution in blood clotting. The increase in white cell count indicates that *Tetrapleura tetraptera* may cause leucocytosis due to its contents of tannin saponin which are antinutrient. But further study on this aspect is necessary. The increase in white blood cells is also beneficial for people with leucopenia infection induced as in HIV/AIDs. And in the study the lymphocytes and neutrophils were enhancedly elevated.

Recommendation

Tetrapleura tetraptera extract has proven its haematnic potentials and so is recommended as a good alternative or combine therapy in anaemia treatment and in quantitative platelet disorders. But caution must be taken in dosage intake particularly in males with infertility disorders.

Conclusion

The extract of *Tetrapleura tetraptera* has haematopoietic stimulating potentials blood cells friendly at upgrading dosages.

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