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Investigation of the Haematotoxicity of Ethanol Stem Bark Extract of Dialium Guineense in Rats

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Abstract

Dialium guineense is a medicinal plant used in Traditional Medicine for the treatment of different disease conditions, such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids. The present study investigated the haematotoxicity of ethanol stem bark extract of D. guineense in rats. Thirty-five (35) adult male Wistar rats weighing 160 to 180 g were randomly assigned to seven groups (5 rats per group). Group I served as control, while rats in the other groups received varied doses of extract (200 - 5000 mg/kg body weight, bwt) orally for 28 days. Haematological indices of rat blood were analysed using haematological Swelab auto counter 920E+ (UK) system. The results revealed that graded doses of ethanol extract of D. guineense stem bark did not significantly alter the concentrations of the measured haematological parameters ($p > 0.05$). These results suggest that ethanol extract of the medicinal plant has no obvious deleterious effect on the haematopoietic system of rats.

Keywords: Blood, Dialium Guineense, Haematology, Haematotoxicity, Haematopoietic System

Introduction

Besides liver and kidney toxicities, haematotoxicity is a major consideration in the assessment of adverse effects caused by pharmaceuticals as well as occupational and environmental chemical exposures [1]. In humans, the blood makes up about 7% of the body weight of a typical adult. Some of the key functions of the blood include oxygen delivery to tissues, vascular integrity maintenance, and immunity. The haematopoietic tissue is highly sensitive to drugs used for the treatment of cancer, infection, and immune-mediated disorders [2]. This tissue is also susceptible to secondary effects of substances that hinder nutrients availability (for example, iron); the clearance of toxicants; or the synthesis of growth factors [for example, erythropoietin and granulocyte colony-stimulating factor (G-CSF)]. It is almost possible to predict the consequences of direct or indirect damage to blood cells and their precursors [3, 4]. They include hypoxia, hemorrhage, and infection [5]. These effects may be subclinical or acute. In cancer treatment and other clinical settings, possible toxic responses of the blood may be used to screen for treatment dosage [6].

As sources of therapeutically active compounds medicinal plants have gained wide acceptability [7]. The isolation and characterization of pharmacologically important compounds from natural sources is the present focus of most researches in Ethnomedicine [8-10]. Dialium guineense is a medicinal plant used locally to treat diverse kinds of diseases [11-13]. A substantial tropical fruit tree of the family Leguminosae, it bears tiny, frequently grape-sized edible fruits that are coated in brown, inedible shells. At the southernmost border of the Sahel in Africa, it grows in thick woods [12]. The Central African Republic, Sudan, and West Africa are the original home of this plant. In Nigeria, it is referred to by a variety of names, including “Icheku (Igbo), Awin (Yoruba), Tsamiyarkurm (Hausa), and Amughen (Bini) [13]. According to reports, the plant's extracts are rich in phytochemicals [14, 15]. As yet not much is known about the responses of the blood to extracts of the plant. The aim of this study was to investigate the haematotoxicity of ethanol stem bark extract of D. guineense in rats

Materials and Methods

Chemicals

The chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

Collection of Plant Material

The stem barks of D. guineense were collected from Auchi, Edo State, Nigeria and authenticated at the herbarium of the University of Benin, domiciled in the Department of Plant Biology and Biotechnology (No. UBHD330).

Plant Extract Preparation

The plant stem bark was washed and shade-dried at room temperature for 14 days and thereafter pulverized. Approximately 500 g of the ground plant material was soaked in absolute ethanol (5 L) with intermittent stirring for 72 h. The resultant extract was filtered with a muslin cloth and freeze dried via lyophilization [16].

Experimental Rats

Male Wistar rats (n = 35) weighing between 160 and 180 g (mean weight = 170 ± 10 g) were bought from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions (25 oC, 60 ± 5 % humidity and 12-h light/12-h dark cycle). They were allowed unrestricted access to feed and drinking water. The rats were acclimatized to the laboratory environment for 7 days just before commencement of the study. Standard experimental procedure was adopted for this study.

Experimental Design

The rats were randomly assigned to 7 groups (5 rats per group). One group served as control, while rats in the treatment groups received varied doses of the extract (200 - 5000 mg/kg bwt) for 28 days. Blood samples were collected for haematological analysis at the end of the treatment period.

Haematological Analysis

Haematological parameters of rat blood were analysed using haematological Swelab autocounter 920E+ (UK) system.

Statistical Analysis

Data are presented as mean ± standard error of mean (SEM, n = 5). Statistical analysis was performed using SPSS version 20. Mean differences among the groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

Results

Effect of Ethanol Stem Bark Extract of D. Guineense on Rat Weight

As shown in Table 1, percentage increases in body weight of rats treated with ethanol extract of D. guineense stem bark were significantly reduced, relative to the control group ($p < 0.05$).

Table 1: Comparison of Rat Body Weight

Groups	% Increase in weight
Control	61.35 ± 4.11
200 mg/kg bwt	52.60 ± 2.92[a]
500 mg/kg bwt	22.63 ± 1.56[ab]
1000 mg/kg bwt	21.00 ± 1.00[ab]
2000 mg/kg bwt	18.30 ± 1.06[ab]
3500 mg/kg bwt	17.73 ± 0.92[ab]
5000 mg/kg bwt	16.80 ± 1.10[ab]

Expressed as mean ± SEM (n = 3), data are percentage weight increases. ap < 0.05, when compared with control group; bp < 0.05, when compared with 200 mg/kg bwt group.

Concentrations of Haematological Parameters in Extract-Treated Rats

Graded doses of ethanol extract of D. guineense stem bark did not significantly alter the concentrations of the measured haematological parameters ($p > 0.05$; Tables 2 to 4).

Table 2: Concentrations of Haematological Parameters in Extract-Treated Rats

Groups	Hg (g/100 mL)	PCV (%)	MCV (fi)	MCH (p.g)
Control	16.65 ± 1.85	45.95 ± 6.75	71.55 ± 1.45	22.15 ± 0.65
200 mg/kg bwt	16.70 ± 1.00	51.85 ± 1.25	72.75 ± 3.75	21.80 ± 0.80
500 mg/kg bwt	15.85 ± 0.25	48.06 ± 1.35	69.20 ± 4.30	21.20 ± 0.80
1000 mg/kg bwt	16.95 ± 0.75	48.06 ± 1.35	70.65 ± 0.85	21.30 ± 0.60
2000 mg/kg bwt	16.40 ± 0.20	50.15 ± 0.05	72.25 ± 2.55	22.10 ± 0.90
3500 mg/kg bwt	17.75 ± 1.85	52.65 ± 3.05	80.15 ± 2.65	23.70 ± 0.20
5000 mg/kg bwt	15.70 ± 0.00	52.55 ± 2.55	77.95 ± 5.05	24.05 ± 1.45

Expressed as mean ± SEM (n = 3), data are concentrations of haematological indices.
Hg = haemoglobin; PCV = packed cell volume; MCV = mean cell volume; and MCH = mean corpuscular haemoglobin

Table 3: Concentrations of Some Haematological Parameters in Extract-Treated Rats

Groups	RBC (106/µL)	WBC (× 103/µL)	MCHC (g/dL)	RETICS (%)
Control	7.48 ± 0.61	7.75 ± 1.65	30.00 ± 1.60	0.90 ± 0.10
200 mg/kg bwt	7.02 ± 0.03	7.85 ± 0.50	29.75 ± 0.25	2.75 ± 1.25
500 mg/kg bwt	6.55 ± 0.22	6.35 ± 0.55	30.70 ± 0.80	1.00 ± 0.05
1000 mg/kg bwt	7.04 ± 0.57	6.05 ± 1.25	30.15 ± 0.45	3.55 ± 1.25
2000 mg/kg bwt	8.25 ± 0.63	10.40 ± 2.40	29 85 ± 0.65	3.40 ± 0.40
3500 mg/kg bwt	7.55 ± 0.20	12.00 ± 2.60	29.60 ± 1.60	2.20 ± 0.70
5000 mg/kg bwt	6.05 ± 0.97	5.40 ± 0.10	30.85 ± 1.50	2.65 ± 0.85

Data are concentrations of haematological indices and are presented as mean ± SEM (n = 3).
RBC = red blood cells; WBC = white blood cells; MCHC = mean corpuscular haemoglobin concentration; RETICS = reticulocytes.

Table 4: Concentrations of monocytes and platelets in Extract-Treated Rats

Groups	NEUT (%)	LYMPH (%)	MO (%)	PLT (× 105/µL)
Control	49.0 ± 1.00	45.00 ± 1.00	6.00 ± 0.00	4.82 ± 0.44
200 mg/kg bwt	45.50 ± 4.50	52.50 ± 4.50	3.00 ± 0.00	6.17 ± 1.31
500 mg/kg bwt	35.00 ± 4.50	59.00 ± 1.00	6.00 ± 1.00	5.79 ± 0.62
1000 mg/kg bwt	57.0 ± 18.0	36.00 ± 2.20	7.00 ± 4.00	7.02 ± 0.85
2000 mg/kg bwt	56.00 ± 21.00	38.50 ± 1.85	7.00 ± 4.00	5.66 ± 0.50
3500 mg/kg bwt	43.50 ± 3.50	52.00 ± 3.00	4.50 ± 0.50	6.93 ± 0.40
5000 mg/kg bwt	47.50 ± 2.50	47.50 ± 0.50	5.00 ± 2.00	4.01 ± 0.13

Data are concentrations of haematological indices and are expressed as mean ± SEM (n = 3).
NEUT = neutrophils; LYMPH = lymphocytes; MO = monocytes; and PLT = platelets

Discussion

Haematotoxicity is the study of blood and blood-forming tissues as a target organ for drugs, chemicals and factors such as stress, exercise, and ionizing radiation. In preclinical and clinical safety assessments, blood and haematopoietic tissue are given careful attention besides liver and kidney. This is necessary because of the high mitotic rate of haematopoietic tissue, the direct contact blood cells have with substances administered systemically, and the severity of haematotoxicity. In normal individuals, red cells, platelets and neutrophils are synthesized at a rate of 1 – 3 million/s. Like other rapidly dividing tissue (intestine and gonads), bone marrow is highly sensitive to the toxic effect of drugs and other agents. It is also a key target for molecules engineered to stimulate the synthesis of blood cells or prevent myelotoxicity [1, 5]. Bone marrow impairment or direct damage to blood cells which causes cytopenia or dysfunction can be life-threatening. The obvious sequelae include anoxia (due to anaemia), infection and sepsis following leukopenia, and haemorrhage. These changes can be dramatic or subtle and present with a host of secondary and compensatory changes in haematopoietic or extra-medullary tissues. Although generally regarded as being among the most common serious adverse effect of drug therapy, primary iatrogenic (drug-induced) blood dyscrasias is difficult to assess and its pathogenesis is not well understood. About 61 % of drug-induced haematotoxicity is caused by anticancer drugs [3, 4].

Haematological parameters such as haematocrit, haemoglobin, erythrocytes and white blood cells are used to evaluate toxicity. They find application in environmental and occupational monitoring. The normal ranges of these parameters are altered by the ingestion of some toxic substances. It has been reported that alterations in haematological parameters by medicinal compounds could either be positive or negative [17, 18].

This study investigated the haematotoxicity of ethanol stem bark extract of Dialium guineense in rats. The results showed that graded doses of ethanol extract of the medicinal plant stem bark did not significantly alter the concentrations of the measured haematological parameters, an indication that it may not be toxic to the blood and blood-forming tissues. The relative safety as well as the protective properties of extracts of the medicinal plant have been reported [19-23]. Extracts of D. guineense stem bark have been reported to possess different pharmacological and biological activities [24-29].

Conclusion

The results obtained in this study indicate that ethanol extract of D. guineense stem bark does not produce any toxic response in rats' blood. It produces no deleterious effect on haematopoietic system of rats.

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