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EFFECTS OF *ALSTONIA BOONEI* EXSTRACT ON HEMATOLOGICAL INDICES OF MALE WISTER RAT

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Abstract

DDVP (O, O-dimethyl-O- 2, 2-dichlorovinyl phosphate) is an organo-phosphate insecticide used in crop and food storage areas, greenhouses, barns and in workplaces. The present study examined hematology of *Alstonia boonei* stem bark extract in DDVP-induced experimental rats. Twenty male rats' weight ranges 100-190 g divided into four groups of five rats each. Group A were control rats, group B received DDVP only, group C received DDVP and 200 mg/kg dosage of extract and group D received DDVP and 400 mg/kg dosage of extract. Increasing doses (200 and 400 mg/kg body weight) of *Alstonia boonei* ethanol stem bark extract were administered by oral gavage to the other two treatment group C and D for 21 days. The animals were sacrificed using diethyl ether, and their blood sample collected into EDTA bottles, for assessing hemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), lymphocyte (L), monocytes (M), eosinophils (E), neutrophils (N), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Statistical analyses of the results shows significant decrease in the hemoglobin and reverse all abnormalities in the hematological parameters when compared DDVP control with the extracts treated groups. In conclusion, *Alstonia boonei* ethanolic stem bark has a protective effect on blood profile against the DDVP toxicity.

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Keywords: Hematology, *Alstonia boonei*, DDVP

Introduction

Alstonia boonei De Wild is a large deciduous evergreen tree, of about 45 m high and 1.2 m in diameter, belonging to the family Apocynaceae which consists of about 40-60 species. It is a native of tropical and subtropical Africa, Southeast Asia, Central America and Australia. 'Alstonia' is named after Dr C. Alston (1685-1760). It's called 'devil tree' in tropical and subtropical Africa; Central America and Australia. It is reportedly used as treatment of malaria, intestinal helminthes, rheumatism, muscular pain, insomnia, and hypertension. It is known to contain phytochemicals such as saponins, alkaloids, tannins and steroids [1, 2]. In folk medical practice, an infusion of the stem bark extract serves as antisnake venom and as antidote to some arrows poisons. Antimalarial potentials of various fractions of the stem bark extract of *Alstonia boonei* was reported by [3, 4] while [5, 6, 7] confirmed indigenous medicinal usefulness of *Alstonia boonei* for malaria therapeutic use in Southwestern part of Nigeria. The erythrocytes (red blood cell) are a nucleate packed with the oxygen carrying protein-hemoglobin; the cell survive in the circulation for about 120 days, worn out erythrocytes removed from circulation by the macrophages of the spleen and bone marrow. The signal for removal is by complex oligosaccharides attached to integral membrane protein of the plasmalemma [8]. Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (Trade Names: DDVP, Vapona, etc.) Is a volatile organophosphate, used as an organo-phosphorus insecticide to control household pests, in public health, and protecting stored product from insects.]. Dichlorvos has high to extreme acute toxicity from oral or dermal exposure, and extreme acute toxicity from inhalation. Taking in large doses may cause nausea and vomiting, restlessness, sweating, and muscle tremors. Large doses may cause coma, inability to breathe, and death [9]. Because of the widespread use of *Alstonia boonei*, it is necessary to study its effect on blood, the [8]. To further carry out scientific scrutiny on this plant, this study examined the hematological responses of the stem bark extract of A. boonei in rats since blood are associated with the development of several diseases.

Materials and Methods

Plant material

Fresh stem bark of *Alstonia boonei* was collected on the 16th March, 2014 from a local garden at Ekiti State, Nigeria. The plant was identified and authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Preparation of Alstonia boonei stems bark extract

Fresh stem bark of *A. boonei* was cut into small pieces and air-dried at room temperature. Five hundred gram (500 g) of the air-dried stem bark of the plant was milled into fine powder using commercial blender. The powdered stem bark was extracted with one liter (1L) of 70% ethanol for 12hours by maceration method. The ethanolic extract was concentrated to dry under reduced pressure at 50±1°C in a rotator evaporator. The resulting crude dark-brown powdered residual extract gave a percentage yield of 5.01%.

Animals

Twenty (20) inbred albino male rats (*Rattus norvegicus*) weighing between 100-190 g was used in the study. The animals were obtained from the animal house of the Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The animals divided into cages and allowed to acclimatize for 7 days in a well-ventilated room at a room temperature of 25.0±2.0°C under natural lighting condition. The animals were allowed free access to standard rat chow (Topfeeds Ltd., Ado-Ekiti, Nigeria) and distilled water ad libitum. All animals in this study follow the international, national and institutional guidelines for Care and Use of Laboratory Animals as published by the [10].

Induction of DDVP

DDVP [O,O-dimethyl O-(2,2-dichlorovinylphosphate) induced in groups B and C. Briefly, DDVP dissolved in distilled water and after that managed by intravenous injection (through tail vein) at a dose of 50 mg/kg body weight.

Experimental Design

Twenty rats with fifteen DDVP induced rats and four normal rats, divided into four groups with five rats each.

Group A: Control rats
Group B: DDVP rats
Group C: DDVP rats 200 mg/kg body weight of *Alstonia boonei* stem bark extract
Group D: DDVP rats 400 mg/kg *body* weight of *Alstonia boonei* stem bark extract according to method of [11].

Blood sample collection
Whole blood was collected by cardiac puncture from each experimental rat into a tube containing Ethylenediaminetetraacid (EDTA).

Hematological parameters determination
The parameters such as packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocytes, eosinophils, monocytes, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) analyzed using an automated analyzer (Sysmex K-2 IN, Japan)

Statistical analysis
The data were analyzed statically using students' T-test and one-way ANOVA. Values of $p < 0.05$ considered significant.

Result
Table 1 shows significant decrease in the haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), red blood cell (RBC) in DDVP-induced untreated group when compared to control, 200 mg/kg and 400 mg/kg treated groups.
group fed with normal diet as depicted in figure 2.

Discussion
The present decade witnessed a great and intense resurgence in the interest and use of medicinal plant and medicinal plant products, especially in Africa and North America. The useful effects of these plant materials ascribed to the combinations of secondary metabolites present in the plant [12]. Botanical medicine has been recognized has one of the oldest practiced professions by humanity [13]. Apart from the lack of information about the adverse or toxicity effect of this plant; its widespread use in folk medicinal or traditional practice provides lack of information underlying the biochemical mechanism responsible for some of the observable and reported properties of this plant. The result of the present study suggests that *A. boonei* stem bark extract caused a significant decrease in hemoglobin of the rats. However, hematological parameters provide information on the status of bone marrow and hemolysis [14]. Also, it's revealed by the present study that hematological parameters in DDVP control (untreated group) showed abnormalities. This might results in glycosylated hemoglobin with decrease in red blood cell (RBC). This suggests an imbalance between its synthesis and destruction and packed cell volume (PCV) normally affected by DDVP-induced toxicity, a sign of anemia [15, 16]. Lowered RBC count, decreased MCH and MCV are other hematological changes found in the group which DDVP administrated [15, 17]. Anemia was in the form of microcytic and hypochromic. This might be because of effects of dichlorvos in cell metabolism, changes of the enzyme, with reactions in which calcium is their secondary mediator. In addition, decrease in the MCV, MCHC and MCH relates al red blood cells while decrease in WBC and its indices (lymphocytes, neutrophil, monocytes) DDVP control (untreated group) might suggests decrease in immune in fighting foreign substances. The performance of the ethanolic extract (especially at 400 mg/kg) in reversing this irregularities in the hematological parameters may be due ascribes to the presence of iron in the plant extract as reported by [18], an essential part of many enzymes in cells and parts of heme group in hemoglobin. Most iron in the body stored within the red blood cells where iron is critical for hemoglobin synthesis [14]. The presence of other antioxidant vitamins (vitamin B, and C) and mineral (Zn, Ca, Fe, K, P, Cu etc), total flavonoids and total phenol [18] might be responsible in improving the immune being weak because of the generation of reactive oxygen species because of DDVP-induction and shows the ethanolic extract may not have negative effect on the bone marrow and hemoglobin metabolism.

Conclusion
In conclusion, *Alstonia boonei* ethanolic stem bark has a protective effect on blood profile against the DDVP toxicity.

Conflict of Interest
The authors declare no conflict of interest. No external funding.

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Table 1: Effects of *A. boonei* stem extract on hematological parameters in Dichlorvos induced Wister rats.

Groups	Hb (g/dl)	PCV (%)	MCHC ($X10^2$g/l)	MCH (pg)	MCV (fl)	RBC ($X10^{12}$/L)
Control	15.6 ± 0.68^a	37.8 ± 1.07^{ab}	3.12 ± 0.05^a	29.60 ± 0.68^a	78.60 ± 2.16^a	5.34 ± 0.24^b
DDVP untreated	11.1 ± 0.05^b	30.23 ± 0.40^c	1.21 ± 0.02^b	20.14 ± 0.23^b	48.45 ± 0.02^b	2.50 ± 0.02^c
200mg/kg treated	14.8 ± 0.58^a	39.8 ± 0.58^b	3.18 ± 0.03^a	28.60 ± 0.51^a	74.80 ± 2.18^a	4.74 ± 0.12^a
400mg/kg treated	15.00 ± 0.71^a	35.00 ± 1.00^a	3.12 ± 0.03^a	29.20 ± 0.73^a	78.20 ± 1.56^a	4.26 ± 0.05^a

g/dl = gram per deciliter, MCH = mean corpuscular hemoglobin, pg = Picogram, MCV = mean corpuscular volume, fm =femtoliter. Values are expressed as mean of five replicates ± SEM. Values with different superscripts (^{abc}) along the row are significantly different ($p<0.05$)

Table 2: Effects of *A. boonei* stem extract on White blood cell indices in Dichlorvos induced Wister rats.

Groups	WBC ($X10^3mm^2$)	Neu (%)	Lym (%)	Eos (%)	Mon (%)
Control	4.16 ± 0.12^b	49.40 ± 0.75^{ab}	41.80 ± 0.80^b	2.80 ± 0.37^a	6.00 ± 0.63^a
DDVP untreated	1.2 ± 0.01^c	31.02 ± 0.03^c	32.10 ± 0.01^c	0.2 ± 0.31^b	3.2 ± 0.31^b
200mg/kg treated	4.30 ± 0.25^b	47.80 ± 0.20^a	42.60 ± 1.12^b	2.2 ± 0.20^a	7.4 ± 1.12^a
400mg/kg treated	2.96 ± 0.12^a	50.40 ± 0.51^b	39.00 ± 0.45^a	3.4 ± 0.51^a	7.2 ± 0.66^a

WBC = White blood cell, Neu = Neutrophils, Lym = Lymphocytes, Eos = Eosinophils, Mon = Monocytes. Values are expressed as mean of five replicates ± SEM. Values with different superscripts (^{abc}) along the row are significantly different ($p<0.05$)