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Research Article

Levels of Haematological Parameters in Normal Rats after Prolonged Exposure to Aqueous Extract of *Dialium guineense* Stem Bark

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Abstract

Background and objective: *Dialium guineense* is commonly used in traditional medicine for the treatment of various ailments. However, there is limited information regarding its potential hematological toxicity upon prolonged use. This study aimed to evaluate whether long-term administration of aqueous extract of *D. guineense* (AEDG) stem bark has any effect on the haematological parameters of healthy rats.

Materials and methods: Ten male Wistar rats (150–180 g; mean weight = 165 ± 15 g) were randomly divided into two groups (n = 5 per group): control and observation. The observation group received 1000 mg/kg body weight of AEDG orally for 12 weeks. Haematological parameters were analyzed using a Swelab Autocounter 920E+ (UK). Statistical analysis was performed to compare values between groups, with significance set at $p < 0.05$.

Results: There were no statistically significant differences ($p > 0.05$) in red and white blood cell counts, haemoglobin concentration, haematocrit, or platelet levels between the control and treated groups after 12 weeks. These findings suggest that prolonged administration of AEDG stem bark at the tested dose does not adversely affect blood composition.

Conclusion: The aqueous extract of *D. guineense* stem bark appears hematologically safe in normal rats at 1000 mg/kg body weight for up to 12 weeks. Further studies are recommended to evaluate potential long-term effects and explore other systemic toxicities.

Introduction

The adverse effects produced by pharmaceuticals and environmental/chemical agents have received huge attention in recent times. Making up approximately 7% of the body weight of a typical adult human, the blood aids oxygen delivery to tissues, maintains vascular integrity, and supports immunity. A large number of substances have a direct or indirect effect on blood tissue [1-3]. The effects of hypoxia, hemorrhage, and infection are also remarkable [4]. These effects may be subclinical or acute [5].

At present, the whole world is turning to medicinal plants as sources of therapeutically/pharmacologically active compounds [6-9]. *Dialium guineense* is a medicinal plant used locally to treat diverse kinds of diseases [10,11]. As a tropical fruit tree of the Leguminosae family, it produces tiny, grape-sized edible fruits that are coated in brown, inedible shells. Found at the southernmost border of the Sahel in Africa, the plant grows in thick woods. The Central African Republic, Sudan, and West Africa are the original homes of this plant. In Nigeria, it is known by different names: "*Icheku* (Igbo), *Awin* (Yoruba), *Tsamiyarkurm* (Hausa), and *Amughen* (Edo) [11]. Studies have demonstrated that extracts of the plant are rich in phytochemicals and other bioactive compounds [12-30]. Currently, not much is known about the responses of

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blood components to extracts of the plant. This study aimed to investigate whether prolonged exposure of normal rats to AEDG stem bark can affect the composition of their blood.

Materials and methods

Study area and duration

This study was carried out at the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, and it lasted five months from the time of materials gathering/literature review to the end of assays (January to May, 2024).

Chemicals and reagents

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 stems of *D. guineense* were collected from Auchi, Edo State, Nigeria, and authenticated at the herbarium of the University of Benin, Nigeria, domiciled in the Department of Plant Biology and Biotechnology (No. UBH_b330).

Extract preparation

The plant stem bark was washed and shade-dried at room temperature for 30 days and thereafter pulverized. Exactly 500 g of the ground plant material was macerated in distilled water (5 L) with intermittent stirring for 72 h. The resultant extract was filtered with a muslin cloth and consequently freeze-dried via lyophilization [31-34].

Experimental animals

Male albino rats (Wistar strain, n = 10) weighing between 150 and 180 g (mean weight = 165 ± 15 g) were bought from the Department of Anatomy, University of Benin, Nigeria. The rats were housed in metal cages under standard laboratory conditions (25 °C, 60 ± 5% humidity, and 12-h light/12-h dark cycle). They were acclimatized for fourteen days before commencement of the study, and had free access to feed and water.

Experimental design

The experimental rats were divided into two groups (5 rats/group): control and observation groups. The observation group rats were administered 1000 mg/kg bwt AEDG stem bark orally for 12 weeks.

Haematological analysis

Haematological parameters of rat blood were analysed using the haematological Swe lab auto counter 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 the Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

Ethical statement

All procedures involving animals were conducted according to international guidelines for the care and use of laboratory animals (CPCSEA Guidelines). Ethical clearance was obtained from the Animal Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria.

Results

Effect of AEDG stem bark on rat weight

As shown in Figure 1, percentage increases in body weight of rats treated with aqueous extract of *D. guineense* stem bark were significantly and time-dependently increased ($p < 0.05$).

Levels of haematological parameters in normal rats on prolonged exposure to AEDG stem bark

There were no significant differences in the haematological parameters after 12 weeks of exposure to AEDG stem bark ($p > 0.05$; Figures 2 - 6). Comparing control *vs.* test means at 12 weeks, the values were: haemoglobin (Hg) (15.45 ± 0.53 *vs.* 16.70 ± 0.50 g/100 mL), packed cell volume (PCV) (50.60 ± 2.40 *vs.* 51.15 ± 2.15%), mean corpuscular volume (MCV) (62.55 ± 2.45 *vs.* 62.25 ± 2.35 fi), mean corpuscular haemoglobin (MCH) (19.05 ± 0.57 *vs.* 20.60 ± 0.10 p.g), mean corpuscular haemoglobin concentration (MCHC) (34.70 ± 2.06 *vs.* 33.10 ± 1.10 g/dL), red blood cells (RBC) [(8.09 ± 0.40 *vs.* 8.21 ± 0.03) x 10^6/µL], white blood cells (WBC) [(9.90 ± 0.71 *vs.* 11.20 ± 1.40) x 10^3/µL], granulocytes (GR) [(2.50 ± 0.95 *vs.* 1.75 ± 0.35) x 10^3/µL], percentage GR (30.20 ± 8.72 *vs.* 29.75 ±

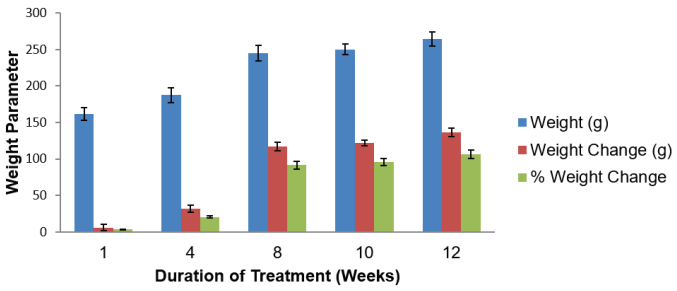

Figure 1: Body Weight of Rat.

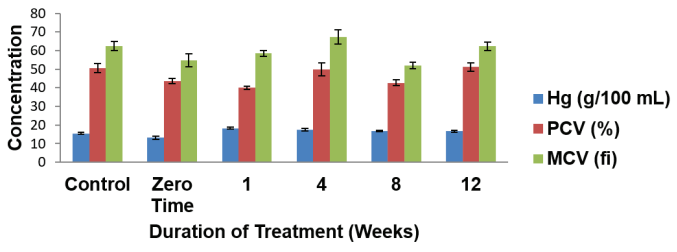

Figure 2: Concentrations of Haemoglobin, Packed Cell Volume, and Mean Corpuscular Volume.

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0.95%), lymphocytes (LYMPH) [(5.45 ± 0.25 *vs.* 7.750 ± 0.75) x 10^3/µL], monocytes (MO) [(1.25 ± 0.15 *vs.* 1.70 ± 0.30) x 10^3/µL], percentage lymphocytes (59.00 ± 2.55 *vs.* 69.50 ± 1.80%), percentage MO (12.35 ± 1.65 *vs.* 14.75 ± 0.85%), and platelets (PLT) [(6.80 ± 1.01 *vs.* 7.23.± 2.52) x 10^5/µL].

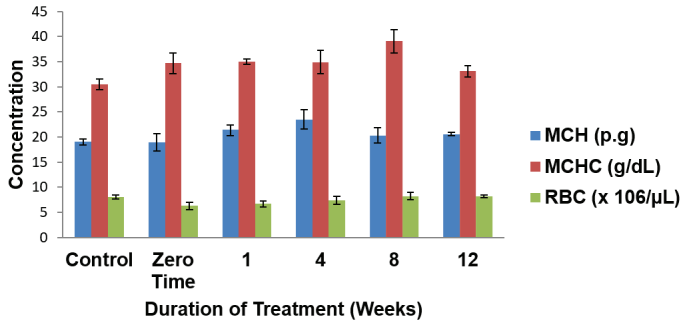

Figure 3: Concentrations of Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, and Red Blood Cell.

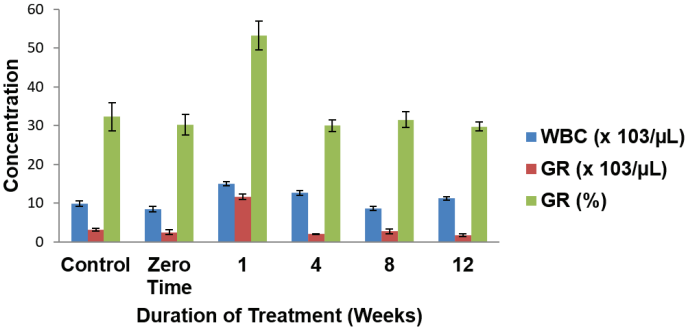

Figure 4: White Blood Cells and Granulocyte Concentrations.

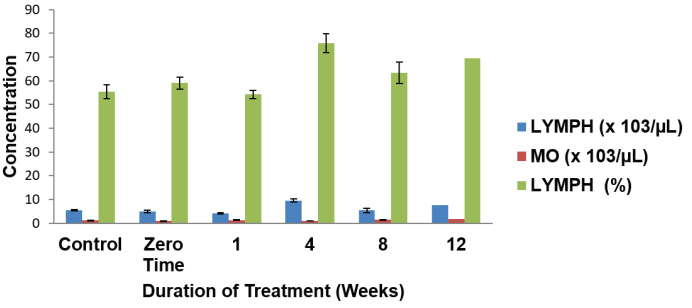

Figure 5: Concentrations of Lymphocytes and Monocytes.

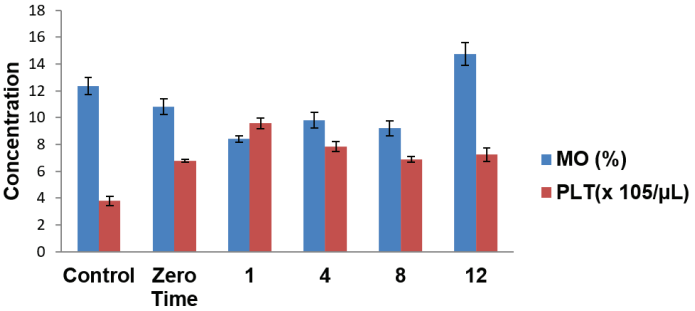

Figure 6: Percentage Monocytes and Concentration of Platelets.

Discussion

This study attempted to determine whether prolonged exposure of normal rats to AEDG stem bark can affect the status of their blood. The results showed that there were no significant differences in the concentrations of haematological parameters throughout the 12 weeks, an indication that the medicinal plant extract is not toxic to rat blood at the dose used. A number of studies have demonstrated the relative safety of extracts of *D. guineense* stem bark [35,36]. In a previous study, it was reported that the extract of *D. guineense* stem bark was safe at concentrations not exceeding 5000 mg/kg bwt [36,37]. A study revealed that exposure of normal Wistar rats to sub-chronic doses of the plant extract did not elicit any toxic response/effect on their blood, but instead, it activated the immune system of the rats [38]. Its potential in herbal formulation for the treatment of diseases has been stressed [39]. Graded doses of ethanol extract of *D. guineense* stem bark did not elicit any deleterious effects on liver function indices [40]. In one study involving total saponins isolated from *D. guineense* stem bark, it was concluded that the isolated phytochemical demonstrated therapeutic effect at a relatively good dose [41]. The graded and quantal dose response curves showed that 1000 mg/kg bwt was effective in reducing the blood glucose of rats (produced the best hypoglycemic effect) [42]. The data indicated that the stem bark extract can potentiate antioxidant defense in diabetic rat liver and heart, as well as ameliorate kidney dysfunction [43-45]. Plants rich in important bioactive compounds have been shown to possess medicinal properties [46-52]. Extracts of *D. guineense* stem bark have been reported to possess different pharmacological and biological activities [53,54].

The high mitotic rate of blood cells and the direct contact they have with substances administered systemically make them highly susceptible to toxicity [55]. Under normal physiological conditions, red cells, platelets, and neutrophils are synthesized at a very high rate (1 – 3 million/s). The hematopoietic tissue is highly sensitive to the toxic effects of drugs and other agents. Bone marrow impairment or direct damage to blood cells can be life-threatening. The secondary effects are anoxia, infection, and sepsis. These alterations may be dramatic or subtle, and are accompanied by several secondary/compensatory changes in haematopoietic or extra-medullary tissues [3,4].

Haematotoxicity can be evaluated using haematological parameters (haematocrit, haemoglobin, erythrocytes, and white blood cells). The normal ranges of these parameters are altered by exposure to certain toxic compounds. Studies have shown that alterations in haematological parameters by medicinal compounds could either be beneficial or deleterious [56]. The major limitation of this study is that, whereas it lasted 12 weeks, assays were not performed every week to assess weekly variations in measured parameters.

Conclusion

The results obtained in this study suggest that the medicinal plant extract is non-cytotoxic (non-toxic to blood cells) at the dose used and duration of exposure. The extract is relatively

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safe for use and consumption, as it does not cause harm or damage to blood components.

The results of this study have further given credence to the use of *D. guineense* in folklore medicine to treat systemic diseases. However, studies aimed at unravelling the precise molecular mechanism(s) underlying the effectiveness of the plant extract in the amelioration of various health conditions are warranted. In addition, attempts should be made to identify and characterize the major class of bioactive compounds in the stem bark extract.

Author's contribution

Abu OD designed the work and analyzed the data; Ekugum, E., Ogbe, O.M., and Hassan, R.T. conducted the literature search. All the authors performed the experiment and assays, and approved the initial draft of the manuscript.

Significance statement

"This study identified the non-toxic effect of a moderate dose (1000 mg/kg bwt) of aqueous Dialium guineense stem bark extract on haematological indices in rats, which could be beneficial for validating its safe use in traditional medicine. This study will assist researchers in uncovering critical areas of long-term haematological safety and pharmacological profiling that have remained unexplored by many. Consequently, a new theory on the therapeutic window and systemic tolerance of D. guineense extract may be developed."

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