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Full Length Research Article

Protection by *Dialium indium* (Fruit) against Pb²⁺-Induced Microcytic Anemia and Chromosome Aberration in Albino Rats *In Vivo*

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The chelating ability and anticlastogenic potential of *Dialium indium* aqueous extract on lead-induced microcytic anemia and chromosome aberration was investigated in albino rats. Twenty rats were randomly grouped into four where rats in group B were treated daily with oral administration of 2.5mg/kg lead acetate, animals in C were fed simultaneously with 2.5mg/kg lead acetate and 200mg/kg *Dialium indium* extract while group D animals were fed 200mg/kg *Dialium indium* extract only and animals in group A served as control. The animals were sacrificed after four weeks of exposure and the toxicological effect of lead and the plant extract on hemoglobin-enzymes and chromosomes were equally assessed via micronucleus assay. The results from hematological and micronucleus assay suggest that lead could possibly induce microcytic-anemia and as well initiate the development of chromosome aberration in albino rats as observed in group B animals, while the extract offered protective effect to animals in group C. However, the extract anticlastogenic and chelating potential was in synergy with the activity of hemoglobin-enzymes as the frequency of chromosome aberration was significantly ($P<0.05$) low in group D animals compared to those in group B.

Keywords: Microcytic anemia, *Dialium indium*, lead acetate, anticlastogenic, hemoglobin-enzymes.

INTRODUCTION

Heavy metals are mostly clastogens in the environment causing oxidative burst in the exposed individuals leading to tissue damage. Damage to DNA and other body tissues by these metals is likely to be a major cause of cancer and genetic birth defects and may as well contribute to aging and cardiovascular diseases

(Tugbobo et al., 2014). The metals are mostly chemicals present in the diets as complex mixture, or as contaminants as well as e-waste. Large amount of these chemicals were tested on their ability to cause damage with newly developed short and long-term tests that accounts for mutagenicity, clatogenicity and carcinogenicity among which lead, cadmium, arsenite, mercury are notable (Stohs and Bagchi, 1995). Studies conducted about a decade ago by Environmental Protection Agency and other associated International Regulatory agencies showed that low level exposure to

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lead is associated with societal problems such as brain dysfunction, neurobehavioural changes as well as kidney and liver diseases (Rimbach et al., 1999). Indiscriminate exposure to lead has been reported to induce renal disorders, reduce sperm counts or increase abnormal sperm frequencies, and increase risk of hypertension (Humphrey, 1991). Medicinal plants from time immemorial have been used in virtually all cultures for healing purposes (Sofidiya et al., 2006). They are considered to be the backbone of traditional medicine and are widely used to treat acute and chronic diseases. The World Health Organization estimated that about 80% of the world population relies mainly on traditional medicines. *Dialium indium* is

grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose, mostly in type 2 diabetes levels (Semiz and Sen, 2007). The plant has been reported to be rich in volatile essential oils of therapeutic importance (Fejes et al., 2000). Its anticlastogenic and chelating potentials against lead-induced microcytic anemia as well as possible effect on activities of hemoglobin-enzymes would be investigated in this study.

MATERIALS AND METHODS

Preparation of Extract

50g of the powdered *Dialium indium* were extracted with distilled water (500ml) via maceration for 48hrs using method of Agugwa and Mittal (Aguawa and Mittal, 1981). The mixture was decanted and filtered using sterile whatman paper No 1. The filtrate measured up to 425ml and evaporated to dryness using a freeze dryer to obtain 8% yield. The crude extract was later subjected to bioassay analyses. From the stock solution, concentrations of (1.0, 2.0, 3.0, 4.0 and 5.0) mg/ml were obtained via serial dilution which were used for the assessment of anticlastogenic and chelating potential of the fruit.

$$\text{Percentage yield} = \frac{\text{Weight of dry cloves} \times 100\%}{\text{Weight of powdered cloves}}$$

Experimental Animals

The *in vivo* experiment was performed using twenty albino rats, weighing between 150 - 180g housed in stainless cages with temperature maintained $25 \pm 2^{\circ}\text{C}$ and 12hr alternating day/night cycle. The rats were fed

standard pellets and water *ad libitum*. The handling and use of the animals were in compliance with Organization for Economic Co-operation and Development (OECD) and NIH guidelines No 423 (2001) for the care and use of laboratory animals.

Experimental Design

The animals were divided into four groups with five rats in each group; rats in group **A** serve as control and were treated with distilled water only. Those in group **B** received 2.5mg/kg lead acetate, group **C** rats were fed simultaneously with 2.5mg/kg lead acetate and 200mg/kg *Dialium indium* extract (1:1), while rats in group **D** were administered 200mg/kg *Dialium indium* extract only. The concentration of lead salt was made equivalent to $1/10$ of the LD_{50} (Preston et al., 1987). The dose of the extract was equivalent to the exact concentration used for beneficial effect against specific disease conditions (Das et al., 1993). Each dose was administered to the animals on daily basis for four weeks.

Hematologic Analysis

Blood samples were collected by cardiac puncture and immediately transferred into tubes containing EDTA (BD Diagnostics, Pre-analytical Systems, Midrand, USA) for analysis of hematological parameters such as hemoglobin, total red blood cells (RBC), packed cell volume (PCV), total white blood cells (W.B.C.), neutrophils, lymphocytes, eosinophils, monocytes, basophils using hematology analyzer Sysmex XS800i (Sysmex Corporation, USA).

Micronucleus Assay

Chromosomes were studied from bone marrow cells using micronucleus assay (Sharma and Sharma, 1994). Bone marrow were flushed out in 75mM KCl hypotonic solution, incubated for 20min at 37°C and fixed in methanol glacial acetic acid (3:1). Chromosome preparations were made, stained in 7% Giemsa solution while slides were coded and scored blind.

Statistical Analysis

The data from the groups were pooled and analyzed statistically using one-way analysis of variance ANOVA (Sokal and Rohlf, 1987). This was followed by Duncan's multiple range test in order to compare the significance of differences among different experimental rats.

RESULTS

Table 1. Hematological parameters of rats following the administration of aqueous extract of *Dialium indium* and lead acetate

Treatment Groups →	A	B	C	D
White blood cell count ($\times 10^9$ /L)	5.76 \pm 1.02 ^a	2.80 \pm 0.49 ^{ab}	9.25 \pm 1.77 ^{bc}	10.60 \pm 1.85 ^c
Heamoglobin (g/dl)	12.53 \pm 4.38 ^a	6.78 \pm 6.74 ^a	13.73 \pm 1.30 ^a	15.70 \pm 8.24 ^a
Red blood cell ($\times 10^{12}$ /L)	4.63 \pm 0.59 ^a	1.38 \pm 2.02 ^a	4.70 \pm 1.70 ^a	6.08 \pm 2.27 ^a
Packed cell volume (%)	32.67 \pm 2.09 ^a	18.50 \pm 5.02 ^a	30.00 \pm 4.41 ^a	33.50 \pm 4.95 ^a
Neutrophils (%)	32.07 \pm 3.06 ^a	12.00 \pm 2.83 ^a	30.18 \pm 2.57 ^a	36.40 \pm 4.73 ^a
Lymphocytes (%)	44.00 \pm 0.23 ^a	25.00 \pm 9.89 ^a	56.50 \pm 4.83 ^{ab}	54.00 \pm 3.46 ^b
Monocytes (%)	10.00 \pm 2.00 ^a	5.01 \pm 0.80 ^b	11.03 \pm 1.41 ^{ab}	13.67 \pm 1.15 ^b
Eosinophils (%)	5.23 \pm 0.58 ^a	0.02 \pm 0.05 ^b	6.18 \pm 0.64 ^b	9.39 \pm 0.55 ^b
Basophils (%)	2.30 \pm 0.03 ^a	0.37 \pm 0.02 ^a	4.29 \pm 0.05 ^a	5.26 \pm 0.13 ^a

Results are expressed as means \pm standard deviation. Values along the horizontal row with different superscripts indicate significant difference at ($P < 0.05$).

Table 2. Chromosome aberration following treatment of rats with aqueous *Dialium indium* extract and lead acetate

Group	G	B	RR (%)	C.A/cell (%)	Mean \pm Standard Deviation
A	0	0	0	0	0.001 \pm 0.003
B	32	37	25	25	0.013 \pm 0.010
C	7	10	12	4	0.025 \pm 0.053
D	1	4	2	2	0.014 \pm 0.002

Total of 300 cells per treatment; G = chromosome gap, B = chromosome break, RR = chromosome re-arrangement, C.A = chromosome aberration per cell

DISCUSSION

The results from Table 1 show the effect of lead acetate and *Dialium indium* aqueous extract on the hematological parameters of test animals in group A, B, C and D. The hematological parameters were significantly ($P < 0.05$) reduced with lowest values obtained mainly in group B animals fed lead acetate only. There was a marked increase in values of the parameters observed in group C animals fed with lead acetate and the extract simultaneously, while in group D animals fed with the extract, the values of hematological parameters obtained were significantly high. The deleterious effect of lead and protective potential of the extract could be assessed from the results. From Table 1 above, red blood cells had (6.08) highest and (1.38) lowest concentrations which were obtained in group D and B animals respectively, while hemoglobin had (15.70) highest and (6.78) lowest concentrations obtained from group D and B animals respectively. This is a clear indication that lead is a pro-oxidant or clastogen capable of inducing microcytosis in animal's circulatory system leading to the development of microcytic anemia as observed in group B animals. Besides, the significant increase in hematological values observed in group D animals further justifies the inherent antioxidant potential of the *Dialium indium* fruit extract.

The toxicity of lead ions in animal and human is caused by its affinity of binding to thiol ions, thus, inhibiting some essential enzymatic reactions (Leonard, 1991). However, the development of microcytic anemia in the test animals in group B could be due to the inhibitory effect of lead on delta-aminolevulinic acid dehydratase and ferrochelatase enzymes which reduces the heme synthesis that is essential for cellular respiration and cytochrome-system (Leonard, 1991). Hence, aminolevulinic acid cannot be converted to porphobilinogen, nor can iron be incorporated into protoporphyrin ring and thus, microcytes, unusual small red blood cells are produced in form of anemia known as microcytic anemia. Besides, the significant ($P < 0.05$) reduction of % chromosomal aberration in group C and D animals could be attributed to the anticlastogenic effect of *Dialium indium* extract. This could be due to the interaction between the lead ions and the bioactive components of the extract (Sharma and Talukder, 1987) which further indicates that the extract could be a dependable metal chelator. Hence, the anticlastogenic effect demonstrated by this extract in this study suggests that *Dialium indium* fruit is a viable protective dietary supplement against lead-induced DNA damage, hematologic disorders as well as other related genetic birth defects.

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