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Anticlastogenic potential of euphorbia heterophylla leaf extract against lead-induced polychromatic erythrocytes and tissue atrophy

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Abstract

Myriads of research reviews have justified that unrestricted and uncontrolled exposure to heavy metal contamination through occupation and environmental pollution generally result in health risks with attendant health burdens including genetic birth defects. This study evaluates the anticlastogenic potential of Euphorbia heterophylla leaf extract against lead-induced polychromatic erythrocytes and the damaged tissues of the albino rats. A total of sixty (60) albino rats were segregated into three sets namely Set I, II and III. Each set contains twenty albino rats which were then divided into four groups A, B, C and D containing five rats each. Group A serves as control and were treated with distilled water only. Group B rats received 2.5 mg/kg lead acetate while group C rats were simultaneously fed 25, 50 and 100 mg/kg Euphorbia heterophylla leaf extract concentrations and 2.5 mg/kg lead acetate at ratio 1:1. Rats in group D were administered 25, 50, and 100 mg/kg Euphorbia heterophylla leaf extract. The schedule of the animal treatment was divided into three set I, II and III while micronucleus assay and histopathological examination were carried out on the test animals. Results show that polychromatic erythrocytes induced by lead acetate was significantly ($P<0.05$) reduced in group C animals (6%), while lead acetate administered to animals in group B was highly clastogenic. Besides, the histopathological architecture of the rat tissues indicates protection offered by the extract against tissue atrophy.

Keywords: Anticlastogenic; Lead acetate; Histopathological; Polychromatic-erythrocytes

1. Introduction

Report from environmental protection agency as well as other environmental regulatory agencies have shown that chronic low level exposure to lead is associated with societal problems such as brain dysfunction in children exposed to lead, neurobehavioral changes in adults, hypertension and chronic liver and kidney diseases (Brautbar, 2005). Euphorbia heterophylla leaf has been in use as medicinal plant for over two hundred years and its roles in diet has been reported to reduce cholesterol levels (Semiz and Sen, 2007). Some medicinal plants have been investigated for their antioxidant properties and many of the metabolites from these plants are justified to be of high medicinal values especially flavonoid which exhibits potent antioxidant activity (Usoh et al., 2005). Most of the free radical scavenging potential in herbs and spices is due to redox properties of phenolic compounds that allow them to act as reducing agents (Caragay, 1992). Research from various quarters has revealed that oxidation of lipids is crucial in the pathogenesis of several disease states, especially in adult and infant patients (Flora, 2013). Myriads of pro-oxidants have been identified to cause oxidative damage to biomolecules such as proteins, lipids and DNA (Halliwell and Gutteridge, 2015). The present investigation was designed to examine the protection afforded by Euphorbia heterophylla leaf extract against lead-induced polychromatic erythrocytes and tissue atrophic changes in albino rats since it had been reported that lead is a potential clastogen capable of inducing chromosomal aberrations in man (Sharma and Talukder, 2000). Besides,

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induction of aneuploidy in germ cells is a potential cause of genetic birth defects, fetal deaths and infertility in man (Tugbobo et al., 2014).

2. Material and methods

2.1. Collection of Plant

Fresh leaves of Euphorbia heterophylla were fetched from different forest within Ekiti State, Nigeria. The leaves were identified and authenticated by the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti-State, Nigeria.

2.2. Experimental Design

The in vivo experiment was conducted using total of 60 laboratory bred Swiss albino rats with average weight of 130g, housed in stainless cages with temperature maintained at 25^0C and 12hrs alternating day/night cycle in accordance with NIH guidelines No 423 (2001) for the care and use of laboratory animals. The rats were divided into four groups A, B,C and D with five rats in each group. Rats in group A serves as control and were treated with distilled water only. Group B received 2.5 mg/kg lead acetate while group C rats were simultaneously fed 25, 50 and 100 mg/kg Euphorbia heterophylla leaf extract concentrations and 2.5 mg/kg lead acetate at ratio 1:1. Rats in group D were administered 25, 50, and 100 mg/kg Euphorbia heterophylla leaf extract. The lead acetate concentration was made equivalent to $1/10^{th}$ of the LD50 while highest extract concentration corresponds to exact concentration used for treating specific diseases (Mansell and Reckless, 1991).

2.3. Micronucleus Assay

2.3.1. Bone Marrow Preparation

The rats were sacrificed 24hrs after the last treatment 1hr prior to sacrifice, each animal was injected with 0.04% colchicine (1 ml/100 g b.wt Sigma USA). Femurs of the rats were excised and bone marrow cells were flushed out into 75mM KCl-hypotonic solution incubated for 20mins at 37 °C and fixed in methanol-glacial acetic acid (3:1). Chromosome preparations were made following standard procedure of air drying and then stained in 7% Giemsa solution (Sharma and Sharma, 1994). The slides were coded, mounted on microscope coupled with chromosome tally counter and were scored blind. Thereafter, the histopathological examination of the rat liver tissue was conducted.

2.4. Statistical Analysis

The data from each group was pooled and analyzed using one-way analysis of variance (Sokal and Rohlf, 1987). This was followed by Duncan multiple range test in order to compare significance of difference amongst different experimental animals.

3. Results

3.1. SET I

Table 1 Total polychromatic erythrocytes (PCEs) following treatment with 25 mg/kg Euphorbia heterophylla (E.h) leaf extract and 2.5 mg/kg lead acetate

Group	Treatment (mg/kg)	PCEs (%)	Mean ± S.D.
A	Distilled water only	0	0.01±0.03
B	2.5 lead acetate	25	0.17±0.10
C	2.5 lead acetate + 25 E.h	6	0.03±0.21
D	25 E.h extract only	1	0.01±0.11

3.2. SET II

Table 2 Total polychromatic erythrocytes (PCEs) following treatment with 50 mg/kg Euphorbia heterophylla (E.h) leaf extract and 2.5 mg/kg lead acetate

Group	Treatment (mg/kg)	PCEs (%)	Mean ± S.D.
A	Distilled water only	0	0.01±0.03
B	2.5 lead acetate	24	0.17±0.12
C	2.5 lead acetate + 50 E.h	10	0.13±0.31
D	50 E.h extract only	2	0.01±0.11

3.3. SET III

Table 3 Total polychromatic erythrocytes (PCEs) following treatment with 100 mg/kg Euphorbia heterophylla (E.h) leaf extract and 2.5 mg/kg lead acetate

Group	Treatment (mg/kg)	PCEs (%)	Mean ± S.D.
A	Distilled water only	0	0.01±0.03
B	2.5 lead acetate	25	0.17±0.10
C	2.5 lead acetate + 100 E.h	10	0.15±0.19
D	100 E.h extract only	2	0.01±0.11

Figure 1 Normal liver of rat in group A

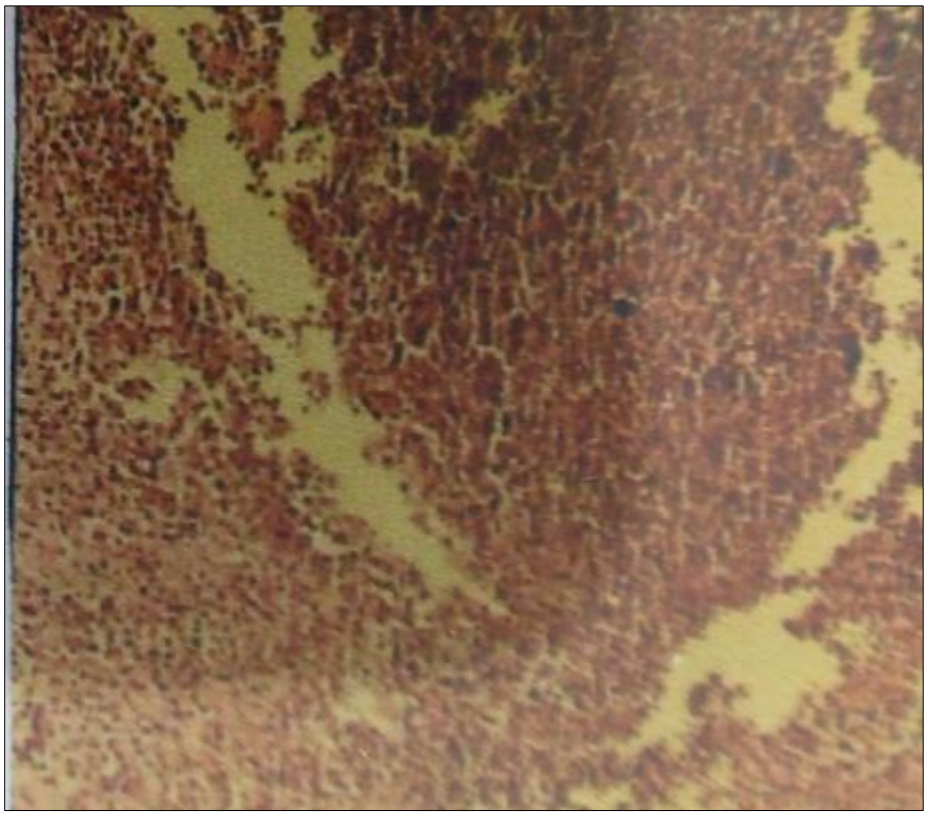

Figure 2 Necrotic liver of rat in group B

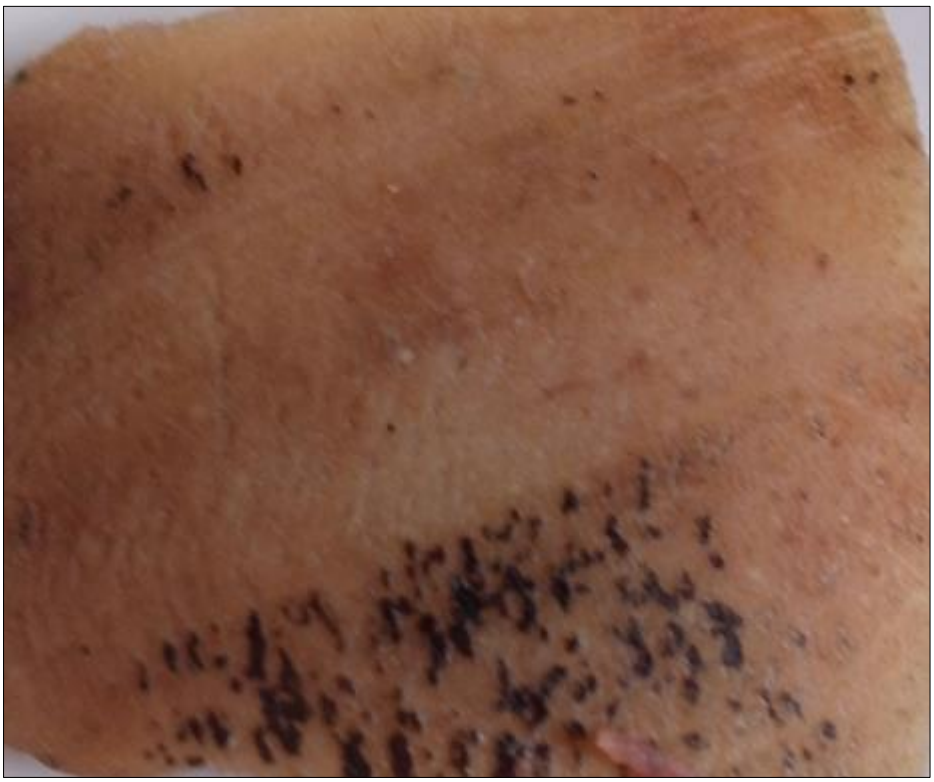

Figure 3 Ameliorated necrotic liver of rat in group C

4. Discussion

Results from micronucleus assay in the three set of tables indicate significant ($P<0.05$) increase in the frequency of polychromatic erythrocytes mainly in group B animals fed with lead acetate only. This further buttresses the fact that lead is a heavy metal with very high propensity of inducing clastogenicity and mutagenicity in man. This also corroborates the findings that lead is a clastogen judging from results obtained in previous research on genomic modification by Ocimum canum against lead-induced chromosome aberration in mice in vivo (Tugbobo et al., 2017). On the contrary, significant ($P<0.05$) reduction in the frequency of polychromatic erythrocytes was observed in group C animals fed simultaneously with lead acetate and Euphorbia heterophylla leaf extract. The highest reduction (6%) in polychromatic erythrocytes against lead acetate was observed in group C animals in set I and table 1 where the least or minimum (25mg/kg) extract concentration of Euphorbia heterophylla leaf was fed to the test animals. The remarkable reduction in frequency of polychromatic erythrocytes in group C animals may be attributed to the chelating potential of Euphorbia heterophylla extract while the frequency of polychromatic erythrocytes observed in group D animals were insignificant. Results from the histopathological examination of the gross appearances of the liver tissue indicate that livers of group A animals were normal, well vascularized with intact size and weight, whereas livers of group B animals treated with lead acetate only appeared pale, showing high resistance to cutting with tough texture and reduced size (Tugbobo et al., 2012). These are indicative atrophic changes induced by lead with gross necrosis. However, livers of group C animals fed simultaneously with lead acetate and the extract exhibit mild or fair necrosis an indication that the necrotic plaque has been ameliorated with less atrophic changes which could possibly improve overtime.

5. Conclusion

The results obtained from this study indicate that lead is a potential clastogen with high propensity towards inducing polychromatic erythrocytes as well as capable of causing immense tissue atrophy. The study also emphasizes on Euphorbia heterophylla leaves as vital needful dietary supplement against clastogenic toxicity of lead because the significant reduction in the percentage of polychromatic erythrocytes by the administration of the extract may be attributed to it metal chelating potential due to the protective activity of its inherent bioactive components.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors hereby declare no conflict of interest in this research work.

Statement of ethical approval

The research work was approved under the authority of research ethical body management of Centre for Research Innovation and Development (CRID), Federal Polytechnic, Ado-Ekiti, Nigeria.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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