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Effect of dimercaptosuccinic acid against lead-induced clastogenicity and enzyme activity in mice *in-vivo*

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Abstract

Indiscriminate exposure of humans to heavy metal contamination via occupation and enviro nmental pollution has been reported with its attendant health burdens including genetic mutation and cancer. This study examined effect of dimercaptosuccinic acid (DMSA) a metal chelator against lead-induced mutagenicity in mice and resultant effect on proper formation of erythrocyte cells. Lead acetate (2.5 mg/kg b.wt) and DMSA (25 mg/kg b.wt) were fed to the mice via oral gavage for 14 consecutive days. Clastogenic effects were observed in the bone marrow cells using micronucleus assay while activity of gamma glutamyl transferase (ý-GT) in both serum and liver was evaluated and the hematologic state of the mice was also monitored. The results obtained indicate that group B animals fed with lead acetate only, significantly ($P<0.05$) induced the formation of micronucleated polychromatic erythrocytes (mPCEs) in bone marrow of mice compared with control. However, in group C animals fed simultaneously with lead acetate and DMSA, the frequency of micronucleated polychromatic erythrocytes was significantly ($P<0.05$) reduced while there was marked reduction in levels of hematologic parameters in group D animals. Results from enzyme assay showed that treatment with DMSA resulted in decrease activities for both liver and serum gamma glutamyl transferase with highest mean values of 5.02 U/L and 4.38 U/L respectively. This study underscores the cytotoxicity of lead acetate and ameliorative effect of DMSA with great respite from organic alternative therapy.

Keywords: Dimercaptosuccinic acid; Lead acetate; Enzymes; Micronucleus; Hematologic

1. Introduction

The world nations either developed or underdeveloped are constantly being exposed to different enviro nmental hazards from various sources. Most of these chemicals are toxic, carcinogenic and mutagenic where more than one thousand different chemicals have been implicated in carcinogenicity (Hassan, 1991) Lead (Pb) is a well-known enviro nmental toxicant (Elias, 1985). Exposure to lead through consumption of contaminated water has been associated with certain forms of cancers and non- cancer health burdens such as neurological disorders, renal disorders as well as impaired cognitive development in children (Parvez et al., 2006). Besides, indiscriminate exposure to lead especially through inhalation has been linked to induction of chromosomal aberrations, cancers and birth defects (Valverde et al., 2002). Dimercaptosuccinic acid (DMSA) is a chemical chelator which is capable of binding to substances and forming a stable water soluble compound that may be easily excreted from the body system (Campbell, 1999). Also lead toxicity is deleterious to the body system in which its predisposition to degenerative diseases results from accumulation of contaminants and as pollutant in the biosphere. The toxicity is largely due to its capacity to mimic calcium and substitute it in many of the functional cellular processes that depend on calcium (Kim and Leonard, 2006). Lead penetrates and replaces intracellular calcium binding proteins such as Calmodulin, Ca-ATPase and protein kinase C (Ouyang and Vogel, 2008) which are vital in numerous physiological functions. Hence, this study was designed to investigate the protective effect of dimercaptosuccinic acid against lead-induced clastogenicity in mice.

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2. Material and methods

2.1. Test Chemicals and Concentration

Lead acetate [Pb $(CH_3COO^-)_2$] 1.5 mg/kg b.wt and dimercaptosuccinic acid (DMSA) 25 mg/kg b.wt made by BDH chemicals Ltd., U.K were used. The salts were dissolved in distilled water and concentrations were made equivalent to $1/10^{th}$ of the LD_{50} of each salt (Das *et al,* 1993).

2.2. Experimental Design

Sixteen (16) male albino mice weighing between (40-60) g were obtained from animal house, Science Technology Department, Federal Polytechnic, Ado-Ekiti. They were caged and fed with normal laboratory chow *ad libitum* and were distributed into four groups each containing four mice. Group A serves as control and the mice were treated with distilled water only. Those in group B, C and D were respectively treated with 1.5 mg/kg b.wt lead acetate, 25 mg/kg b.wt DMSA, and 1.5 mg/kg b.wt lead acetate + 25 mg/kg b.wt DMSA. The chemicals were administered via oral gavage using cannula to the animals consecutively for 14-days. Animals were sacrificed 24hr after the last treatment.

Table 1 Group of mice and treatment administered

Group	Treatment
A	distilled water
B	1.5 mg/kg lead acetate
C	25 mg/kg DMSA
D	1.5 mg/kg Lead acetate + 25 mg/kg DMSA

2.3. Micronucleus assay

Mice were sacrificed 24hr after last treatment where femurs were removed and bone marrow cells flushed out in 75mm KCl-hypotonic solution, incubated for 20min at 37^0C and fixed in methanol glacial acetic acid [3:1]. Chromosome preparation was made following the standard procedure of air-drying and then stained in 7% Giemsa solution (Preston *et al.*, 1987). Slides were coded and scored blind (Sharma and Sharma, 1994) where polychromatic erythrocytes were scored per animal for the mean frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice relative to test substances in each group.

2.4. Enzyme assay

2.4.1. Determination of liver gamma glutamyl transferase activity

This was determined using reagent kits of the enzyme produced by Randox Lab. Ltd. U.K and with procedure described by (Szasz, 1995). Mice were sacrificed and liver excised, rinsed, weighed and placed in a beaker containing chilled saline solution (1.15 KCl) and homogenized. The homogenate was centrifuged at 9000g for 20min. at 40 °C. The supernatant was collected and readings taken at 540 nm using spectrophotometer.

2.4.2. Determination of serum gamma glutamyl transferase activity

This was determined using reagent kits of the enzyme produced by Randox Lab. Ltd. U.K and with procedure described by (Szasz, 1995). After sacrifice, blood was collected from the heart and quickly stored in heparinized bottles to prevent coagulation and ice blocks to prevent enzymes from being active. The blood samples were then centrifuged at 1000xg for a period of 30min. The serum was collected and readings taken at 540 nm using spectrophotometer.

3. Results

Table 2 Frequency of polychromatic erythrocytes in bone marrow of treated mice

Group >	**A**	**B**	**C**	**D**
No of slides				
1	0	7	2	3
2	1	5	4	5
3	1	6	1	3
Total cell	2	18	7	11
Mean	1.13	6.47	2.91	4.02
Mean±SD	1.13±0.37	6.47±0.31	2.91±0.11	4.02±0.08

Table 3 Gamma glutamyl transferase activity in liver and serum of mice

Group	**mean liver Ý-GT**	**mean serum Ý-GT**
A	1.09±0.71	1.36±0.87
B	16.14±1.68	20.12±4.58
C	3.12±0.90	2.44±0.77
D	6.76±2.21	4.80±1.55

4. Discussion

Results from the micronucleus assay in Table 2 indicate significant ($P<0.05$) increase in the frequency of polychromatic erythrocytes in group B animals with mean frequency of polychromatic erythrocytes (6.47) compared to control (1.13). This is a clear evidence that lead is a heavy metal that has high propensity of inducing clastogenicity in mice in vivo. This also corroborate the findings that lead is a clastogen from results obtained in previous study 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 (4.02) was observed in group D animals fed simultaneously with lead acetate and dimercaptosuccinic acid. The remarkable reduction in frequency of polychromatic erythrocytes of group D animals may be attributed to the chelating ability of heavy metals by DMSA. However, subtle increase in the mean frequency of polychromatic erythrocytes (2.91) of animals in group C fed only with the chemical chelator (DMSA) was observed. This further buttresses the fact that DMSA is synthetic chemical chelator of heavy metals which perhaps could possibly present along with its efficacy side effects. Besides, results from Table 3 show that control animals treated with distilled water only in group A produced slight increase in both liver and serum gamma glutamyltransferase (ý-GT) activity which could have emanated from contaminant present in the feeds fed to the animals. Animals in group B fed with lead acetate only indicate significant ($P<0.05$) increase in both liver and serum mean ý-GT activity (16.14) and (20.12) respectively. This development could be attributed to enzyme induction due to the presence of lead as clastogen and could as well result from hepatic damage by lead since it is a potential toxin capable of damaging body tissues (Fails and Richards, 1996). Comparatively, mean ý-GT activity in group D animals for both liver and serum was higher than that of group C animals. This could be due to possible synergy between lead and DMSA in contribution to clastogenicity in the body.

5. Conclusion

The results from this study demonstrate that lead acetate is a potential clastogen capable of inducing polychromatic erythrocytes in body system. However, further work is required to really ascertain the clastogenic properties of dimercaptosuccinic acid as it is usually used as metal chelators.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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.

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