



AMELIORATIVE EFFECT OF ZINGERONE AGAINST LEAD INDUCED SPLEEN TOXICITY OF SWISS ALBINO MICE (*MUS MUSCULUS*)

Zoology

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ABSTRACT

Lead is a toxic heavy metal found in earth crust. Poisoning of lead is a serious global problem for a long time. The experiment was conducted to explain the preventive effect of Zingerone against lead poisoning. Toxicity of lead was assessed by applying lead acetate to healthy Swiss albino mice (*Mus musculus*) for 12 weeks continuously followed by administration of Zingerone along with lead by oral gavage at selected doses for 4 weeks. Morphological, biochemical and histological parameters were done to show the ill effects of lead on spleen in dose dependent manner. Application of Zingerone at a specific dose resulted in a significant reduction of the above parameters. Thus this herbal drug offers a significant ameliorating effect against lead toxicity.

KEYWORDS

Lead, Zingerone, Toxicity-amelioration, Swiss albino mice.

INTRODUCTION:

The heavy metal lead is one of the most hazardous environmental pollutant [1],[2]. Important sources of lead contamination include mining, smelting, manufacturing and recycling activities and the continued use of leaded paint, leaded gasoline, and leaded aviation fuel. Drinking water delivered through lead pipes or pipe joined with lead solder may contain lead [4],[2]. This metal is distributed throughout the body and affects gastrointestinal and hematopoietic system [2]. Most of the lead which enters through systemic circulation invades the reticulo-endothelial system like bone marrow, liver and spleen. Spleen is one of the target organ for lead toxicity [3].

The mechanism of lead related pathologies many of which are a direct result of the oxidant effect of lead on tissues and cellular components may be minimized by cellular availability of antioxidants. One of the major component of ginger – Zingerone (4-(4-hydroxy-3methoxyphenyl)-2-butanone) is a non-toxic and inexpensive compound with potent anti-inflammatory, anti-diabetic, anti-diarrhoeic, anxiolytic, antithrombotic, radiation protective and so forth properties [5],[6],[7]. Lead toxicity and its detrimental effects on various organs are illustrated by many researchers but our objective is to study the perilous effects of lead on spleen tissue and trials to lower these effects by using the natural drug Zingerone.

MATERIALS AND METHODS:

Female healthy Swiss albino mice (*Mus musculus*) of 15 days old were taken as model animal. They were maintained in plastic cages under normal light and temperature (25°C±5°C) conditions. Normal and standard diet and distilled water were given to the animals. Animals were randomly divided into six groups with 5 mice in each group. The experiment was carried on for 16 weeks continuously and once daily exposure through oral gavage was given to the animals.

Group I mice were treated as normal control and were given distilled water throughout the experimental period. Group II mice were treated with Zingerone (100mg/kg bw) along with distilled water for last 4 weeks. Both group III and group IV mice were administered lead acetate (40 mg/kg body weight) for 12 weeks continuously. Group V and group VI mice were also exposed with lead acetate (60mg/kg body weight) through for 12 weeks continuously. After 12 weeks mice of group IV and group VI were treated with Zingerone (100mg/kg body

weight) for last 4 weeks along with their respective lead acetate doses. Group III and V were exposed with lead acetate as usually for last 4 weeks.

After 16 weeks the animals were sacrificed after anesthesia with chloroform. The spleen was quickly removed and blood was collected from heart of each mice. It was stored in an eppendorf containing anticoagulant heparin (20mg/ml). Plasma was separated from each of the blood sample and was used for biochemical estimation. A portion of spleen of each set of mice was fixed in bouin's solution for 24 hours which was used for histological analysis. Rests of the spleens were used for further biochemical analysis.

All experiments were performed at Vidyasagar College, University of Calcutta, India, following the guidelines of animal ethical rules.

RESULT:

Morphometric analysis:

In our present investigation, effect of lead toxicity on spleen and the ameliorating effect of Zingerone over it was studied. Initially, from the morphometric analysis of spleen weight and body weight ratio, splenomegaly is clearly evident. Fig. 1a shows the images of spleen of mice of different sets. Fig. 1b depicts the increased weight of spleen with increasing level of lead which comes down near to control after treatment with the Zingerone (100mg/kg bw).



FIG: 1a.

Morphometric changes of spleen after chronic lead exposure and co-treatment with Zingerone in different groups of mice (*Mus musculus*)

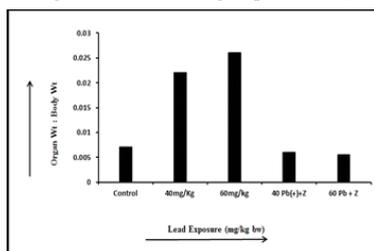


FIG: 1b. Analysis of organ wt. and body wt. ratio of different groups of mice (*Mus musculus*). Values are mean of 3 independent experiments with 5 animals in each group.

BIOCHEMICAL ANALYSIS:

Thiobarbituric acid reactive substances are the marker of lipid peroxidation. The level of TBARS was estimated after last 4 weeks of consecutive treatment with 100mg/kg body weight of Zingerone in the control animal. Values do not display any significant changes in the TBARS content both in control and drug + control co-treated groups (Fig. 2a).

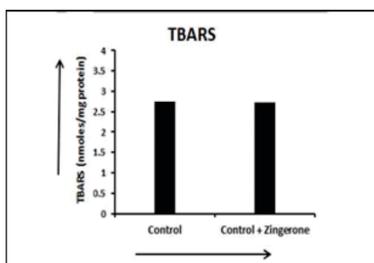


FIG: 2a: Measurement of TBARS activity in control and control + Zingerone co-treated groups. Values are mean of 3 independent experiments with 5 animals in each group.

TBARS level were also estimated in different lead exposed as well as lead and Zingerone co-treated groups. Recorded data shows an increase of TBARS level with increasing level of lead concentration which decreases near the control level after administration of Zingerone (Fig. 2b).

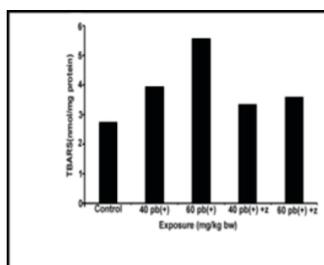


FIG: 2b: Graphical representation of TBARS activity in control, different lead induced and Zingerone co-treated groups. Values are mean of 3 independent experiments with 5 animals in each group.

Reduced Glutathione (GSH) is readily oxidized upon oxidative stress. Our experiment shows a lowering level of GSH upon administration of different concentrations of lead whereas co - treatment of Zingerone regulates to raise the level of GSH (Fig 2c).

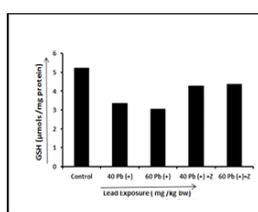


FIG: 2c:

Analysis of GSH activity in control, different lead induced and Zingerone co-treated groups. Values are mean of 3 independent experiments with 5 animals in each group.

HISTOLOGICAL ANALYSIS:

Histological study reveals that in lead treated group enlargement of trabeculae, formation of necrotic lesions and hyperplasia of the lymphoid follicle in the white pulp which ultimately leads to loss of tissue interiority in lead exposed spleen. Application of drug effectively maintained the cellular arrangement more or less similar to control animal (Fig: 3).

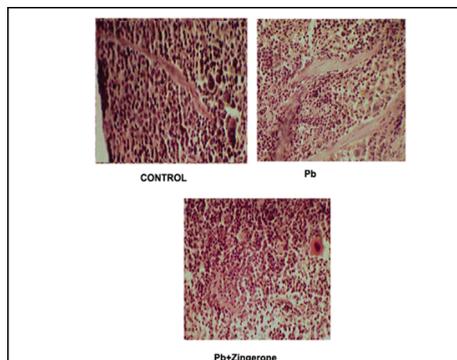


FIG: 3: Photomicrograph of representative section of spleen from control, lead acetate (60mg/kg body weight) treated, lead and Zingerone co-treated mice showing enlarged trabeculae, hyperplasia in lead exposed group which comes towards normal condition after co-treatment. (H&E, magnification x40)

DISCUSSION:

In this age of industrialization lead poisoning is one of our major matter of concern as this heavy metal is used by human beings for many years. According to our ancient system, there are many plants with great medicinal properties which may be used as herbal drugs for treatment of many diseases as they show very less toxicity and side effects as well as cost effective as compared to chemical and artificial drugs.

Our present study is to investigate the ameliorative effects of Zingerone, one of the active component of ginger on lead induced spleen of Swiss albino mice. It has been shown that this dose of Zingerone has no toxic effect on the target organ of the animal as the TBARS data shows. Morphometric study shows the increasing values of lead induced spleen clearly indicating splenomegaly which are reduced by co-treatment with Zingerone.

In our present investigation the biochemical parameters indicate that there was a stress induced by the application of lead on the animals. Data suggest an increase in accumulation of TBARS on spleen tissue along the increasing dose of lead which is minimized by Zingerone. Also here we observed an accountable decrease of data of GSH according to the increasing level of lead concentrations which is increased by application of this active component of ginger. Glutathione are readily oxidized upon stress. So it is evident that a stress was generated by this heavy metal and Zingerone minimizes the stress.

Finally our study shows that Zingerone was able to reduce histopathological damage of the spleen tissue and helps the tissue to regain its normal structure.

CONCLUSION:

From the present study it is clearly evident that treatment of Zingerone, a herbal drug reduces the stress caused due to lead toxicity in spleen. It also propels the tissue towards its normal condition. Further studies will be needed to find out its mechanism against lead poisoning.

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