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Effect of aqueous extract of *Talinum triangulae* (water leave) in lead- induced chromosomal aberration

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Abstract

Phytomedicine is a subject that is now gaining relevance worldwide aiding treatment for diseases that were once incurable among folks in which genotoxicity is a prominent one. The present study was designed to examine the ability of Talinum triangulare (water leaf) to protect the genomic integrity of swiss albino rat from lead-induced chromosomal damage in the bone marrow red blood cells. 20 eight week old rats were divided into five groups with group 1 as the negative control and group 2 is the positive control and the other groups, all treated by gavage once per day with a single dose of 2.5 mg/ml lead acetate. The frequency of micronuclei formed was examined in the bone marrow erythroblast of the treated and untreated groups through standard micronuclei assay method. The plant shows significant ($p<0.05$) difference in genoprotective ability in the group treated with 200 mg/kg body weight of the aqueous extract of the plant over 100 and 400 mg/kg b.w when the level of the micronucleated proerythroblast formed in the bone marrow was considered and a substantial decrease in the number of polychromatophilic erythroblasts (PCEs) scored from the stained blood cells in the groups respectively. It is shown in the present study that lead-induced chromosomal damage could be averted measurably by dietary consumption of Talinum triangulare.

Keywords: *Talinum triangulare*, Genotoxicity, Micronuclei, Polychromatophilc, Erythroblast.

Introduction

Talinum triangulare is an herbaceous perennial plant that is a native of tropical America to Mexico, the Caribbean, Central America, and much of South America. *T. triangulare* is one of the most important vegetables, an erect herb with swollen roots and succulent stems.[1] *Talinum triangulare* is one of the plants that often exhibit a wide range of biological and pharmacological activities such as anti-inflammatory.[2] Plants are rich in numerous endogenous antioxidants such as polyphenols, carotenoids, ascorbic acids, tocopherol and flavonoids.[3] The consumption of dietary antioxidants from vegetables and fruits is beneficial in preventing common neurodegenerative diseases including; Parkinson's and Alzheimer's diseases and it has been proven to substantially reduce the risk of cardiovascular diseases and cancers.[4]

Lead compound is the most universally available toxic metals, is detectable practically in all spheres ecosystems. It's reasonably present in the air in the vicinity of factories. [5] Vegetables are polluted by lead from the air and a considerable amount of lead contamination is found in cereals and broad-leafed vegetables. In the general public, exposure to lead occurs primarily through the oral route, with some contribution from

inhalation. In contrast, in the occupational setting, inhalation of inorganic lead in the form of fumes, mists, dusts and vapors is a major route of exposure but specific concern varies with the age and circumstances of the host, and the major risk is toxicity to the nervous system.[6, 7] However, the toxicological effects are the same in living things regardless the route of exposure and there is no demonstrated biologic need, the major issue regarding lead is at what dosage it becomes toxic.[8] In eukaryotic cells, this metal is usually genotoxic through a mechanism that until now has not been well pigeonholed and possibly involves indirect damage to DNA, either by affecting the stabilization of chromatin or by interacting with repair processes.[9, 10]

The production of red cells is regulated by the demands of oxygen delivery to the tissues. In response to reduced tissue oxygenation, the kidney releases the hormone erythropoietin, which stimulates the multiplication and maturation of erythroid progenitors.[11, 12] Erythropoiesis takes place within the bone marrow in units composed of macrophages surrounded by erythroblasts and progression along the erythroid pathway beginning with the stem cell and passes through the mixed myeloid progenitor cell, (CFU-GEMM, colony-forming unit–granulocyte, erythroid, monocyte and megakaryocyte), burst-forming unit–erythroid (BFU-E), colony-forming unit–erythroid (CFU-E), and to the first recognizable red cell precursor, the normoblast. Each normoblast undergoes four more cycles of cell division. When the erythroblast develops into an erythrocyte the main nucleus is extruded and may leave a micronucleus in the cytoplasm. The visualization of micronuclei is facilitated in these cells because they lack a nucleus.

Chromosomal breakage in any population of dividing cells will lead to loss of acentric fragment and thus to micronuclei, the micronucleus frequency may increase for several cell division after treatment.[13] As further division occurs, cell containing micronuclei are diluted out of the population.[14] The maximum frequency of micronucleus occurs in treated subjects between the third and the fourth mitoses, the absence of the micronuclei can be attributed to a lack of chromosomal aberrations rather than to a failure of cells to division.[14]

Materials and Methods

All chemicals were of analytical grades and prepared in all-glass apparatus using sterilized distilled water.

Preparation of Aqueous extract

The leaves (edible) parts of the plant were air-dried in a ventilated place at ambient temperature of 30 ± 2°C for 15days, pulverized, using a laboratory blender and the fine powders obtained and stored at moderate temperature of 37°C until further use. 50 g of the powdered sample was soaked in 500 ml distilled water for 48hrs. The mixture was filtered using sterile whatman paper no 1. The filtrate measured up to 410 ml, which was then evaporated to dryness to obtain a total yield of 20.9 g. The crude extract was later subjected to bioassay analyses. From the stock solution, a concentration of 10 mg/ml was obtained.

$$\% \text{ yield} = \frac{\text{Extract obtained in mg}}{\text{Crude plant sample in mg}} \text{ x100}$$

Animal Treatment/Grouping

Albino rats (*Rattus norvegicus*) of weight ranging between 90-150 g were used for these experiments and the principles of laboratory animal care (NIH) were followed. The environment was kept clean and disinfected, the rats were acclimatized normally giving them the standard rodent diet and water *ad libitum*. Thereafter, the individual weight of the rats was taken prior the commencement of the experiment and this was observed at three day intervals throughout the experimental window.

The rats were randomly distributed into five treatment groups of four rats each as follows:

Group 1: (Negative control group) consists of animals fed with a standard diet.

Group 2: (Positive control group) consists of animal administered with 2.5 mg/ml of lead- acetate and standard diet and water *ad libitum*

Group 3: consists of animals administered with 100 mg per kg body weight dosage of the aqueous extract of *Talinum triangulare* and 2.5 mg/ml lead acetate.

Group 4: consists of animals administered with 200 mg per kg body weight dosage of the aqueous extract of *Talinum triangulare* and 2.5 mg/ml lead acetate.

Group 5: consists of animals administered with 400 mg per kg body weight dosage of the aqueous extract of *Talinum triangulare* and 2.5 mg/ml lead acetate.

Micronuclei test

The assay is based on an increase in the frequency of micronucleated polychromatophilic erythroblast in bone marrow of treated animals as an indication of induced chromosomal damage; micronuclei (MN) are formed from various types of chromosomal damages.[14, 15] Following the last day of administration of lead-acetate alongside extract, the rats were sacrificed through cervical dislocation and the femur removed and stripped clean of muscle. Thereafter, slides with film of blood smear were prepared and evenly mixed with a drop of fetal bovine serum to form a homogenous mixture. The slide were air-dried and fixed in absolute methanol and stained in 5% Giemsa at pH 6.8. The frequency of polychromatophilic erythroblasts (PCEs) per total erythroblast was determined using a sample size of 1000 erythrocytes per slide and also the number of MNPCEs was determined using 1000 PCE per slide.

Data Analysis

The results of replicate readings were pooled and expressed as mean ± standards deviation. One way analysis of variance was used to analyze the results and Duncan multiple tests was applied for the post hoc.[16] Statistical package for Social Science (SPSS) 10.0 for Windows was used for the analysis. The p value < 0.05 was considered statistically significant in the analytical data.

Results and Discussion

Figure 1 and 2 shows the frequency of polychromatophilic erythroblasts (PCEs) per total erythroblast that were scored using a sample size of 1000 erythroblasts per animal and the number of micronucleated polychromatophilic erythroblasts (MNPCEs) was determined in 1000 PCE per slide. The %PCEs and %MNPCEs in rats treated by gavage with a single dose (2.5 mg/ml) of lead acetate are thereby shown above. In the lead-acetate treated animals, significant micronuclei frequency was observed, showing the potential ability of the compound to induce aberration in the animals' blood cell. However, damages were virtually observed in all the tested groups, a group with 200 mg/kg body weight of the extracts producing significant ($p<0.05$) reduction in the frequency of MNPCEs compared to the group administered with lead acetate alone. The responses produced by the rats administered with 100mg/kg body weight and that of 400 mg/kg body weight along with a single dose of lead acetate (2.5 mg/ml) produced the highest frequency of MNPCEs, and there was significant ($P>0.05$) difference between the MNPCEs frequency induced compared to that of lead acetate alone. Potentially and effectively enough, group four administered with 200 mg body weight was actively effective in reducing lead-induced chromosomal damage to a lesser ($p<0.05$) level than that of lead acetate alone, however, there was significant ($P<0.05$) different, but lesser than that of lead acetate treated group, hence the active dose per kg body weight against lead-induced aberration. As a matter of fact, the high frequencies of MNPCEs produced by 100 and 400 mg/kg body weight of the extract could be traced to the fact that; at 100 mg/kg, the potency of the extract was quite ineffective enough to cushion the progressive effect of lead acetate-induced aberration and at higher dose (400 mg/kg body weight) of the extract became somewhat toxic, causing reduction in the defensive mechanism synthesis and thereby synergistically aid lead acetate to promote aberration in the animals system, with this, it prevent the ability to reduce chromosomal aberration in the animal examined.

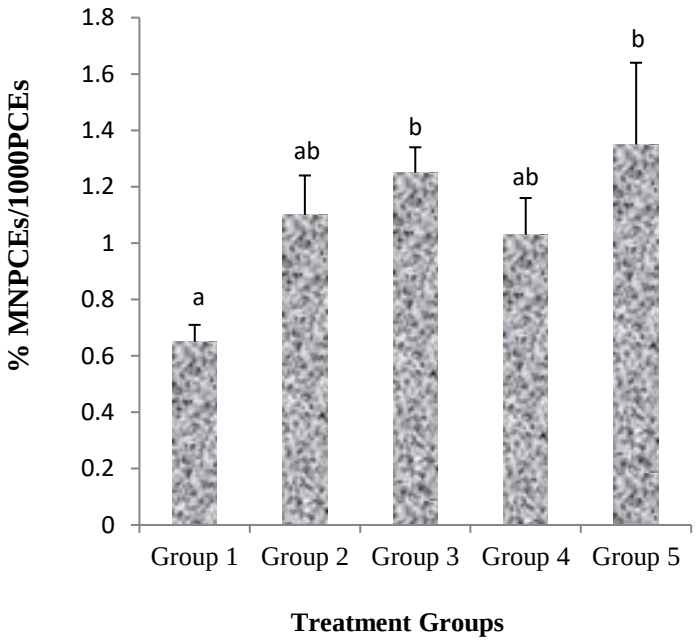

Figure 1: Frequency of micronuclei formation in the bone marrow erythroblast of rats treated with lead acetate only, lead acetate (2.5 mg/ml) with 100, 200, 400 mg/kg body weight of *Talinum triangulare* and control group, where [a, b] are the significance values along the treatment groups (p= 0.05).

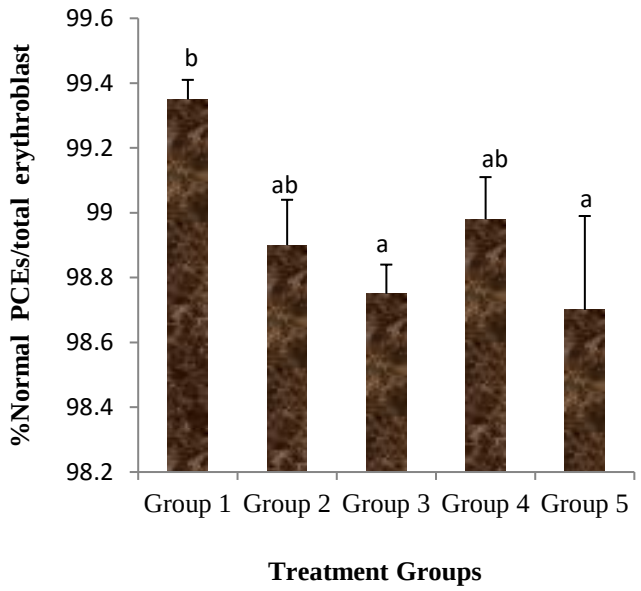

Figure 2: Frequency PCEs in the bone marrow erythroblasts of rats treated with lead acetate only (2.5 mg/ml), lead acetate (2.5 mg/ml) with 100, 200, 400 mg/kg body weight of *Talinum triangulare* and control group, where [a, b] are the significance values along the treatment groups (p= 0.05).

Conclusion

Based on the research work it could be deduced that at lower concentration, *Talinum triangulare* is genoprotective and can reasonably avert chromosomal disordered-related diseases owe to the effect of long-term lead exposure.

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