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Genotoxic Effect of Lead Acetate on *Drosophila melanogaster*

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ABSTRACT

Lead is a pollutant heavy metal .An experiment was conducted to study the genotoxicity of lead acetate on *Drosophila melanogaster* of either sex in different doses. Viz 1M, 0.5M, 0.05M and its external morphology were examined at 24hr and 48hr post treatment. DNA Fragmentation assay was performed to study the extent of DNA damage. It was observed that lead acetate brought about various morphological abnormalities in both male and female flies. Morphological changes such as orange discoloration of head, thorax, abdomen, curling of abdomen, bulging of abdomen were seen in adult flies. The results of phenotypic changes and DNA fragmentation assay reveal the genotoxic effect of lead acetate

Keywords: Lead Acetate, genotoxicity, DNA fragmentation assay, Phenotypic changes

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INTRODUCTION

Lead is a pollutant heavy metal. It is a white crystalline chemical compound with the molecular formula: $Pb(C_2H_3O_2)_2$ and has a sweetish taste. It is toxic and is soluble in water and glycerol. It appears as white or colourless powder or as efflorescent crystals and slightly acetic in odour. Lead acetate is soluble in water and glycerol. Anhydrous lead acetate is soluble in alcohol whereas hydrous lead acetate is insoluble in alcohol. It is a stable compound, becomes unstable with excessive heat and, incompatible compounds like chlorides, carbonates, sulfates etc.[1,2]

Toxicity of Lead Acetate

Lead, a pollutant heavy metal is absorbed by the gastrointestinal system and is capable of releasing free radicals which are capable of causing breaks in DNA and adducts. An over dose of lead absorption results in its competitive binding with calcium and turn into a poison and also inhibit heme group synthesis and result in cell death.[2,3,4] The other routes of entry into humans include inhalation and cutaneous absorption. The clinical symptoms of lead intoxication include colic, constipation, muscle weakness and may finally extend to paralysis. Ingestion of higher doses results in cramps, depression, coma and death [5,6,7].

Genotoxicity

Evaluation of toxicity of chemicals/materials to the genetic material has helped evolve the science of genotoxicity and agents that have genotoxic potential are referred to as genotoxins.[8,9] Genotoxins can induce cancer(carcinogens), mutations(mutagens) or birth defects(teratogens)(10,11,12) The mechanisms of genotoxicity are diverse and complex and many in vitro and in vivo tools are employed to evaluate the degree of genotoxicity.[13]

MATERIALS AND METHODS

The flies (Canton Sp) were reared in bottles containing corn meal medium. The flies were exposed to 3 concentrations of lead acetate (1M, 0.5M and 0.05M). The defined concentration was mixed with the food and 50 flies (3:1 – Female: male) were exposed for 24 hr and 48 hr time frames and the exposure was conducted in duplicates along with control. In each time frame 100 µl and 500µl of the defined concentrations were chosen as volume of exposure. Post exposure, the flies were subjected to phenotypic analysis under stereo zoom microscope and documented the changes following which DNA was isolated from the exposed flies and the genotoxic effect was evaluated quantitatively using DNA fragmentation assay.

RESULTS

Phenotypic changes: The exposed population showed marked phenotypic changes. [Figures 1, 2, 3, 4]

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Figure 1: Phenotypic changes observed in exposed population post 24 hour exposure (100µl)

Control: No changes observed

0.05M Lead Acetate: 100 µl

Posterior tip of abdomen was found to be curled in all males

0.5M Lead Acetate: 100 µl

Orange discoloration of thorax was seen in both males and females Males appeared shorter and shrunken

1M Lead Acetate: 100 µl

Orange discoloration of thorax was seen in both males and females Distinct curling of the abdomen was seen in males

Figure 2: Phenotypic changes observed in exposed population post 48 hour exposure (100µl)

Control: No change

0.05M Lead acetate: 100µl

Orange discoloration of thorax was seen in both males and females. Females appeared stout on the abdomen

0.5M Lead acetate: 100µl

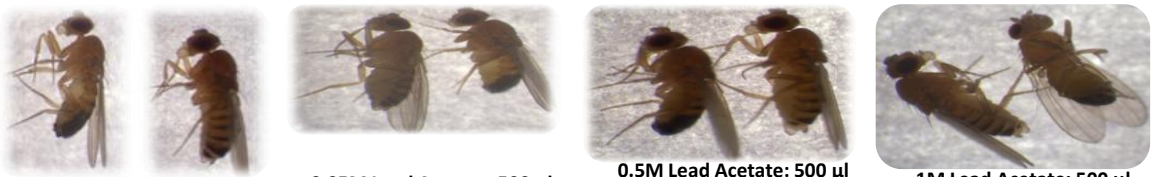
Males appeared shorter and mild curling of the abdomen was seen

Orange discoloration of thorax was seen in both males and female

1M Lead acetate: 100µl

Orange discoloration of thorax and curling of abdomen was seen in both males and females

Figure 3: Phenotypic changes observed in exposed population post 24 hour exposure (500µl)



Control: No changes observed

0.05M Lead Acetate: 500 µl

Orange discoloration of thorax and was seen in both males and females.Males and females appeared stout On the abdomen

0.5M Lead Acetate: 500 µl

Orange discoloration of head, thorax and abdomen was seen in both males and females

Males appeared smaller, shrunken and abdomen was found to be curled

1M Lead Acetate: 500 µl

Orange discoloration of head, thorax and abdomen was seen in both males and females

Males appeared short and shrunken Abdomen was found to be curled in both males and females

Figure 4: Phenotypic changes observed in exposed population post 48 hour exposure (500µl)

Control: No changes observed

0.05M Lead Acetate: 500 µl

Orange discoloration of the head and thorax was seen in males

Males were found to have a curled and shrunken abdomen whereas females were stout on the abdomen

0.5M Lead Acetate: 500 µl

Orange discoloration of thorax and abdomen was seen in both males and females

Males were found to have curled abdomen whereas stout abdomen was seen in females

1M Lead Acetate: 500 µl

Orange discoloration of thorax and curling of abdomen was seen in both males and females

DNA fragmentation assay (Figure 5& 6): Patterns of shearing was observed in DNA isolated from flies exposed to 100µl and 500µl of 3 molar concentrations – namely 0.05M,

0.5M and 1M at 24 and 48 hour intervals. However, the shearing was observed to be intense at 0.05M and 1M (24 and 48 hours) on comparison with to the control.

Figure 5: DNA fragmentation assay of all the three molar concentrations (100µl)

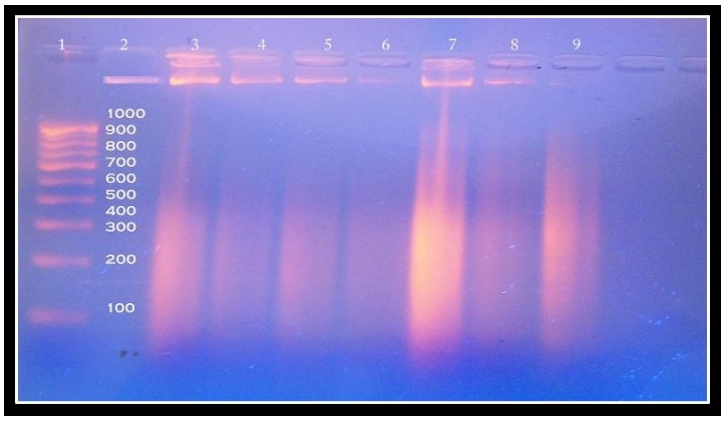
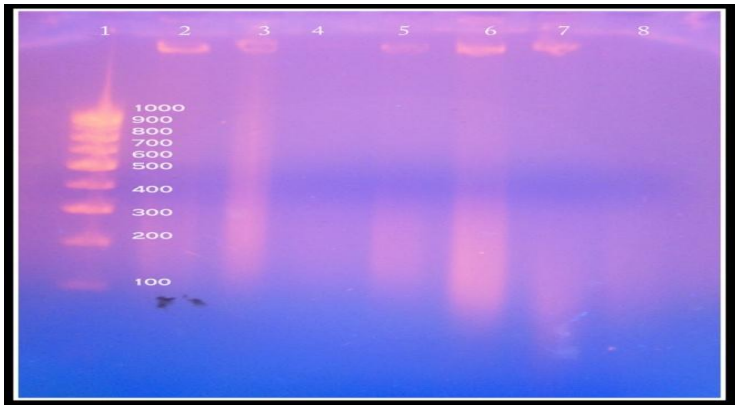


Figure 6: DNA fragmentation assay of all the three molar concentrations (500µl)



DISCUSSION

The present study was designed to evaluate the 'in-vivo' genotoxicity of lead acetate on *Drosophila melanogaster*. Three molar concentrations of lead acetate, viz 0.05M, 0.5M and 1M were chosen as doses of exposure and flies were exposed to 100 µl and 500 µl of the defined concentration for 24 and 48 hours respectively. Post exposure, flies were evaluated under the microscope for phenotypic changes. The exposed flies were subjected to a protocol for DNA isolation by phenol-chloroform method and the DNA thus isolated was analyzed by DNA fragmentation assay on a 3% gel.

The phenotypic changes observed in exposed population included orange discoloration of thorax and abdomen in both males and females. The abdomen of the male flies appeared curled and shrunken whereas the abdomen of the female flies appeared stout and curled.

The DNA fragmentation assay revealed distinct patterns of shearing in all 3 molar concentrations at 24 and 48 hours of duration.

The results of phenotypic changes and DNA fragmentation assay reveal the genotoxic effect of lead acetate. However evaluation of the exact mechanism of

genotoxicity and degree of genotoxicity is beyond the scope of the present study. Specialized molecular tools can be employed in the future to extend the study[14,15].

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