Effect of Lead Acetate on the Chromosome Structure in Grasshopper *(Gesonula punctifrons)*

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Abstract :

Lead is widely used heavy metal that is released into the environment from different sources. Their accumulation in the soils can become harmful to all kinds of organisms, including plants, animals and human life, causing many adverse effects. Lead accumulates in the bodies of water and soil organisms and it is a bio-persistent pollutant that accumulates at the top of the food chain. The action of lead acetate was investigated on the meiosis of male animal model, *Gesonula punctifrons* (Stal). Grasshopper species may provide good indicator in ecosystem to evaluate the toxic effects of some environmental contaminants. Due to the toxic effect of lead acetate abnormal cell division occur during meiosis which then results in polyploidy. Different types of qualitative changes are seen in the treated organism like chromosome stretching, breakdown of chromosome, polyploidy, clumping, misdistribution of chromosome.

Keywords : Chromosomes, Meiosis, Lead Acetate, Testis, Polyploidy.

INTRODUCTION

Heavy metals cause different genetic and biochemical effects on organisms. Lead (Pb) is the most abundant toxic metal in the environment (Patra et al., 2011). It occurs naturally in the environment. However, accumulation of Lead found throughout the environment due to human activities. It has an adverse effect on sperm count, sperm motility and retarded the activity of spermatozoa (Chowdhury, 2009). Genetic and biochemical effects of pollutants on organisms are important in establishing species as bioindicators for environmental hazards (Michailova et al., 2000). Arsenic and lead are widespread heavy metals that are released into the environment from different sources. Their accumulation in the soils can become harmful for all kinds of organisms, including plants, animals and human, causing many adverse effects (Panda and Panda, 2002; Arcadio and Gregoria, 2002). Lead accumulates in the bodies of water and soil organisms and it is a bio-persistent pollutant that accumulates at the top of the food chain (Scheifler et al., 2006).

In a terrestrial ecosystem grasshopper occupied a significant position in food chain, representing 20-30% of arthropod biomass (Schmidt, 1986). As herbivorous (primary consumer) and being preyed upon by other insectivorous vertebrates, they play a significant role in accumulating and further transfer. The studies on the transfer of heavy metals in an aquatic ecosystem by Jamil and Hussain (1992) and in copper (Cu) and cadmium (Cd) contaminated grassland by Hunter et al. (1987), showed that the accumulation and biotransfer of anthropogenic heavy metals can be very high. The grasshopper chromosomes, because of their extreme clarity and readily available divisional stages in their testes are known to be a classical material for cytological studies (Chatterjee et al., 1971). Grasshopper species may provide good indicator in ecosystems to evaluate the toxic effects of some environmental contaminants (Barsyte, 1999; Warchalowska–Sliwa et al., 2005). Methyl mercury hydroxide showed also impact on meiotic chromosomes of the grasshoppers, *Stethophyma grossum* (Klasterska and Ramel, 1978). Mercuric chloride exposed monkey *Macaca fascicularis* showed changes in spermatogenesis (Mohamed et al, 1986). Effect of arsenic on haemolymph cells of short horned grasshopper was studied by Nath et al. (2012).

Accumulation of mercury in the gut and testes of a species of grasshopper were observed by Schmidt et al. (1992) and Schmidt & Ibrahim (1994). The male grasshoppers, *Gesonula punctifrons* (Stal) is an ideal model to study the various meiotic stages of spermatogenesis due to their easy availability, fairly large chromosomes, and fewer numbers of chromosomes (Liu et al., 2012). Being a component of ecological food chain, this arsenic and lead affected grasshopper may play a significant role in accumulating and further transferring toxic metals to higher trophic levels

in the food chain (Meharg, 2003). Recent reports proclaim that methyl mercury induces free radical stress and results in early aging (Zahir et al., 2006). The present study deals with the changes in the testicular activity of a model organism, grasshopper belonging to species *Gesonula punctifrons* and to evaluate its potential as a bio monitor for detecting a heavy-metal polluted environment. This study incorporate the effects of lead acetate on the chromosome structure during meiosis of male individual of *Gesonula punctifrons* (2n=23) grasshopper species.

MATERIALS AND METHODS

Grasshoppers *(Gesonula punctifrons)* were collected from the East Calcutta Wetland (22 0 27' N 88 0 27' E), lying east of the city of Calcutta (Kolkata), of West Bengal in India on August 2018.

Treatment:

The food was supplied to the adult male grasshoppers for seven days' observation. They were fed on treated plants (their stems were previously immersed for 24 hours in distilled water containing 50mg/lit and 100mg/lit of lead acetate to allow the plant to absorb contaminated water) and on untreated plants. They were supplied with food and water every day and the survivors were collected on the seventh day.

Testis collection:

The testes were taken out from both the groups by dissecting their abdomen and testis-lobes were collected from both treated and untreated grasshopper.

Fixation and staining of the testis:

The collected tissues were shifted quickly to a closed cavity block containing Aceto-Ethanol and kept for 1 minute for fixation. The tissue was then transferred to 2% Aceto-Orcein and stained for at least 30 minutes.

Washing and squashing of the testis lobes:

The stained tissue was then washed properly in 50% acetic acid to remove excess stain and to make the tissue soft. The tissue was then placed in a drop of 2% Lacto-Aceto-Orcein on a clean glass slide. A clean cover slip was placed on the tissue and the tissue was squashed by tapping with the help of a rubber tipped pencil or just by thumb. Then the slide was sealed with a transparent nail polish and observed under microscope to study the chromosomal features in the testis.

RESULT AND DISCUSSION

Family Acrididae encompasses the short-horned grasshoppers and locusts, phytophagous insects that are widely distributed throughout the world (Watts et al., 1982). Heavy metals are important among the most harmful pollutants, causes of water, soil and plant pollution. Lead acetate is a widespread heavy metal, known to its historical use as a sugar substitute, as it has a sweet taste. Because of its toxic effects lead acetate is no longer used in the production of sweeteners. But it has been used in cosmetics throughout the history; it is still used in men's hair coloring products in USA. The testicular tissue stained with aceto-orcein showed details of the process of meiotic cell division. A comparison of the tissues from the untreated grasshopper and that of the treated ones indicated some landmark observations. Many cytological changes were noticed in the treated grasshopper's testis. These changes include qualitative effect on the nucleus and chromosome. Improper distribution of chromosome, polyploidy, stretching of chromosome, chromosome breakdown, fragmentation, undivided chromosome, clumping are all evident at different stages of the treatment.

Control series:

In case of control organism no perceptible changes from the normal meiotic behavior of the chromosome was observed. Different stages of meiotic cell division that is, prophase, metaphase, anaphase and telophase are seen clearly in ideal state in the control plate (Plate-1).

Treated series:

Schmidt et al. (1992) and Schmidt & Ibrahim (1994) observed accumulation of mercury in the gut and testes of a species of grasshopper when they were fed with food treated with mercuric chloride and they also observed chromatin fragmentation in the treated animals.

One of the most profound effects of lead acetate is the stretching of chromosome. From the observation and progressive manner in which lead acetate effects the chromosome, it seems that there is considerable change taking place. As a result, the individuality and discreteness of the chromosome are gradually lost and they massed together to form irregularly shaped clumps, from which few chromosome strands projected.

Chromosome breakage and fragmentation also occur as a result of treatment with lead acetate. Fragmentation is noticed for the first time after 36 hours of injection. It is a progressive phenomenon. It is noticed extensively in the meiosis. Extensive fragmentation of the chromosomes is seen in material fixed 72 hours after injection. **Polyploidy** is another change induced as a result of lead acetate treatment. Polyploidy expresses itself in more than one form and affects different types of cells and stages in *Gesonula punctifrons*. Due to the toxic effect of lead acetate abnormal cell division occur during meiosis which then results in polyploidy. Apart from these different kinds of structural abnormalities are distinct in the treated grasshopper's testis. **Sex chromosome** (XO) changes its shape and forms a comma like structure. (Plate 2)

Continued action of the chemical over a period of time causes very severe changes and ultimately the death of the grasshoppers. After 6-7 days the maximum casualty was observed in survived animals after injecting it with the lead acetate. After 7 days the chromosomal anomalies are extensive and cause maximum death.

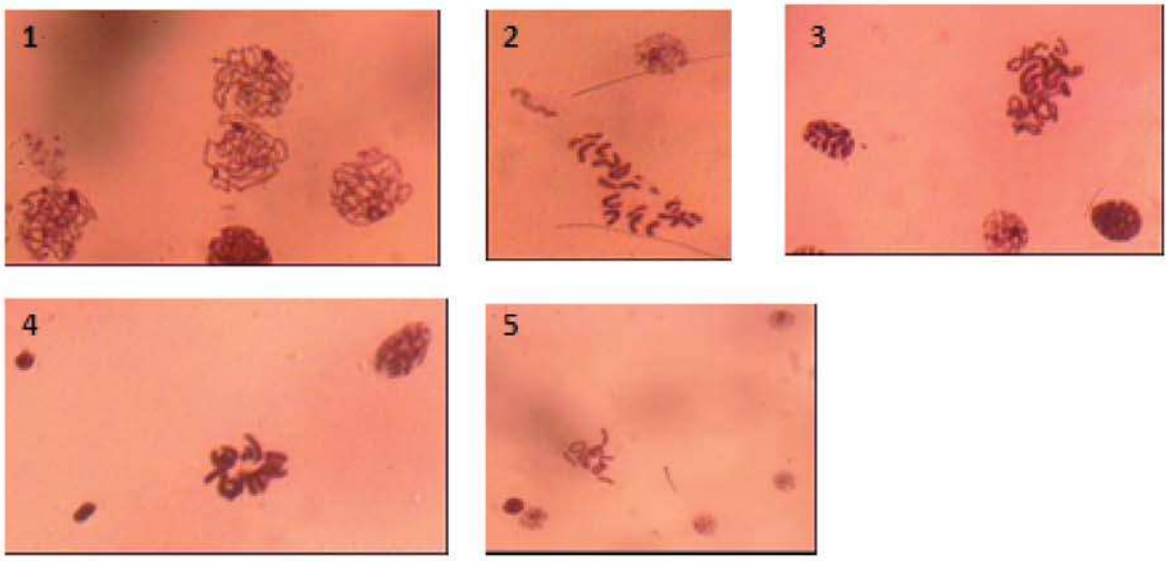

Plate I: Fig. 1-5 Different stages of mitotic cell division in control series. 1, Pachytene stage. 2, Metaphase stage. 3, Diplotene stage. 4, Metaphase 1 stage. 5, Diakinesis stage.

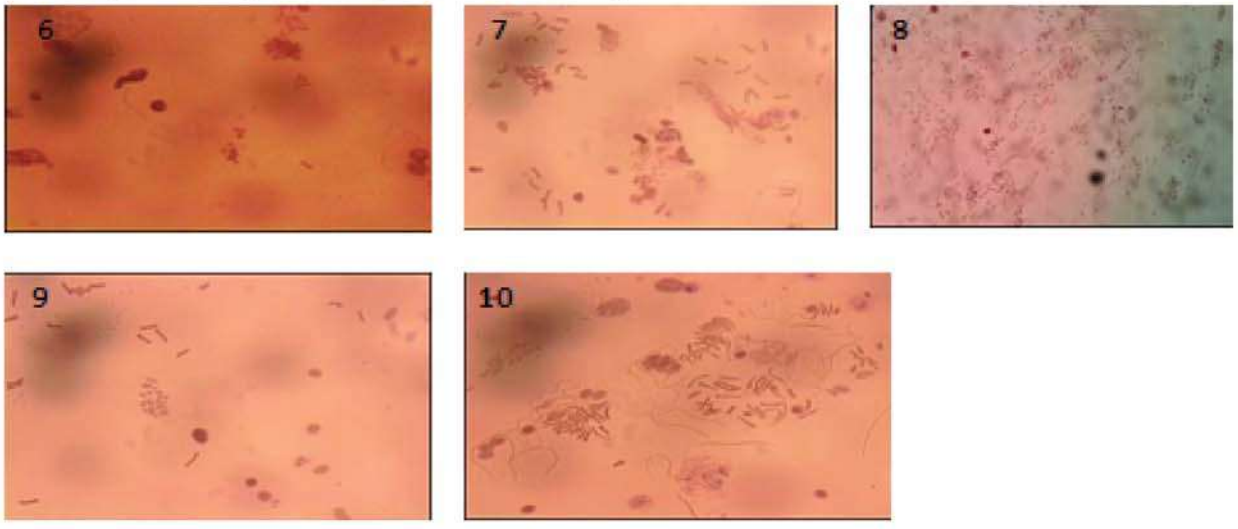

Plate 2: Fig. 6-10 Different stages of mitotic cell division in treated series. 6, Stretching of chromosome. 7, Chromosome breakage. 8, Fragmentation of chromosomes. 9, Polyploidy of chromosomes, clumping of chromosome. 10, Change in sex chromosome, chromosome breakage.

The accumulation of lead in soils can become risky to all kind of organisms including plant, animal and human and causes many adverse effects. Researchers apprehend such scenario in the natural environment may lead to a decline in the population of insects and loss of grassland biodiversity. Being herbivores and being preyed upon by other insectivorous vertebrates and they play a significant role in accumulating and further transferring toxic metal to higher tropic levels. Grasshopper species may provide good indicator in ecosystem to evaluate the toxic effects of some environmental contaminants.

CONCLUSION

Lead is widespread heavy metal that is released into the environment from different sources. Analysis of the division stages reveals that lead acetate has changed the chromosomes structure during meiosis stages extensively. Present study reflects the toxic effect of lead acetate has extensive damaging effect on chromatin material and lead animals to die. Further studies will enlighten how chromatin organization was affected by accumulation of Lead acetate.

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