Research Article

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STUDY OF THE MITOTIC ABNORMALITIES DUE TO MERCURIC CHLORIDE ON *ALLIUM CEPA* AT DIFFERENT CONCENTRATION AND TIME EXPOSURE

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ABSTRACT

Mercury is a bio accumulative, persistent, toxic substance that threatens the health of humans and wildlife. Mercury was traditionally used in agricultural chemicals as a fungicide or pesticide or in dental amalgum. In the present investigation the effect of Mercuric chloride at different concentration (1% and 3%) and varied time exposure (3h and 5h) on root tip of *Allium cepa* were studied. It was found that the affect of this agent increases with increasing concentration and time exposure. Various abnormalities observed mostly in metaphase with separation of chromosome that leads chromosomal anomalies. Anaphase also affected when roots were exposed for 5h and at both concentration.

Keywords: *Allium cepa*, Mercuric chloride, Chromosomal anomalies

INTRODUCTION

Carcinogenic agents are chemical compound which affect the cell at genetic level. The genetic information of all organisms resides in the individual DNA molecules or chromosomes. An onion cell possesses eight chromosomes like forty six chromosomes of human cells. Carcinogenic substances chemically react with DNA and cause mutations. Mutation due to DNA damage leads the process of tumour formation [1]. Different carcinogenic substances can be used as pesticides, fungicides. Mercury was also traditionally used in agricultural chemicals as a fungicide, or pesticide. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. Some of the mercury of these pesticides and fungicides may volatilized to the atmosphere and then be deposited into lakes and streams, other water bodies. Through the water mercury can be ingested by fish, eventually reaching wildlife and humans. The toxicity of mercury has long been known to humans[2]. Mercury may harm bird reproduction and behaviour. Some seals and whales in the Arctic and some predatory marine mammals in warm waters may be at risk. The hat makers during the 19th century were suffering from neurological damage from the inhalation of mercury fumes. Exposure of mercury vapors can cause acute respiratory problems, neurologic disturbances and general systemic effects and ingestion of inorganic mercury may also cause gastrointestinal disturbances and affect the kidneys[3]. Mercury is also used in Indian system of Ayurvedic medicine like bhasm but vapors of mercury affect the lungs[4]. It is therefore very important to keep these materials out of the environment and avoid the excessive use in medicine. The present study was shown the different chromosomal abnormalities due to the exposure of mercury by analysing the different stages of cell division.The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains like Feulgen stain and Acetocarmine stain. A carcinogen is any substance, radionuclide, or radiation that is an agent directly involved in causing cancer. This may be due to the ability to damage the genome or to the disruption of cellular metabolic processes.

METHODOLOGY

Root growing:- *Allium cepa* is grown in a small plastic flask, in which lower portion of onion was connected with water.

Treatment with chemicals:- After 3-4 days onion becomes rooted, then transferred it into a flask containing 50ml water and $HgCl_2$. Roots were allowed to grow for 5, 3 & 1h with two respective concentration 1% & 2%.

Root tip cutting:- Root tips were cut between 9 to 10 AM. The time of cutting of root tip is the critical factor here since the rate of nuclei division is not constant throughout the day, in the morning its being highest as this time specification is too vague[5]. After cutting, the root was transferred in to fixative so that cell division stopped at respective phases.

Stain Preparation:- For 100 ml:- 2gm of orcine was added in 45 ml of glacial acetic acid and boiled until it completely dissolved. Then after cooling 55ml of distilled water was added.

Washing:- Firstly the root tip were washed with distilled water and then treated with 1N HCl for 5 minutes to remove pectin and thus softening the tissue.

Staining:- After that root tip was stained with aceto orcine (for 10 min).

Slide preparation:- Root cap was removed and the tissue just above it was taken for study.1 drop of glacial acetic acid (1%) was applied on the tissue and then cover slip was placed. It was then tapped lightly with a pencil or a pen. Slide was observed under the microscope at 40X and 100X (with oil emulsion).

OBSERVATION

Control

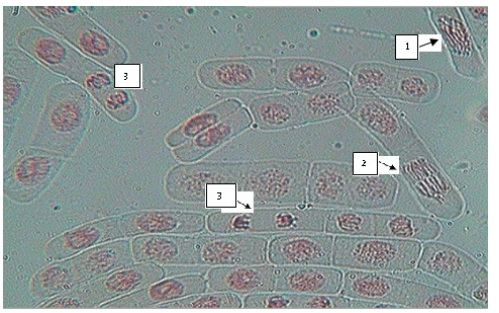

Figure 1: Stages- Prophase(1), Anaphase(2), Telophase(3)

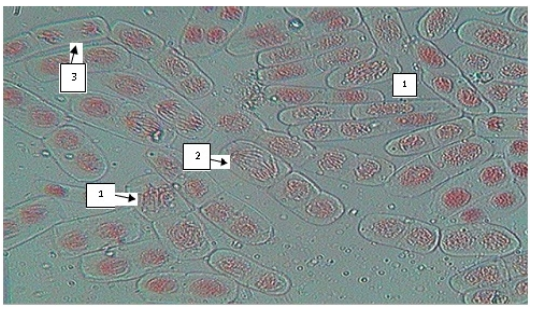

Figure 2: Stages- Metaphase(1), Anaphase(2), Telophase(3)

Abnormalities due to $HgCl_2$

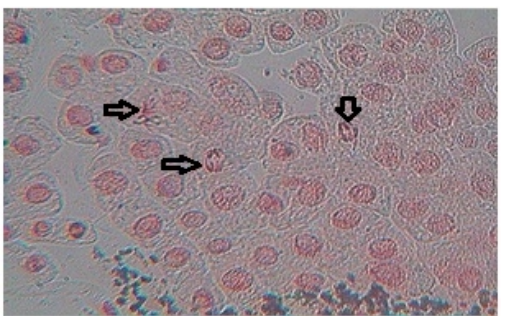

Figure 3: 1%, 5h exposure Condensed metaphase & Anaphase

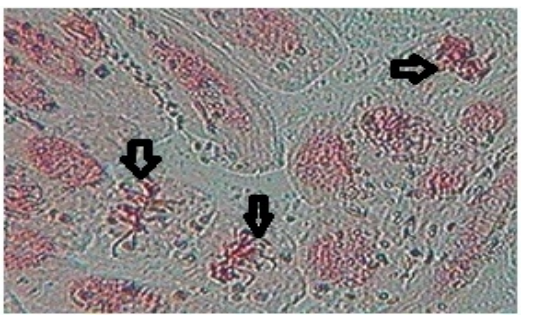

Figure 4: 2%, 5h exposure Abnormal metaphase

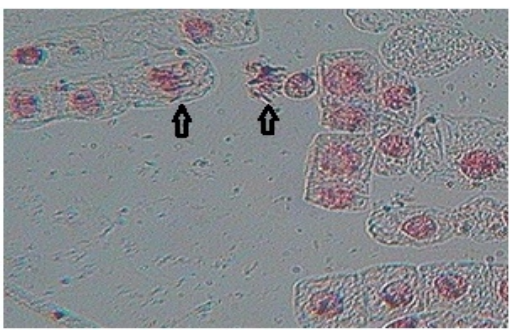

Figure 5: 1%, 3h exposure Affected metaphase

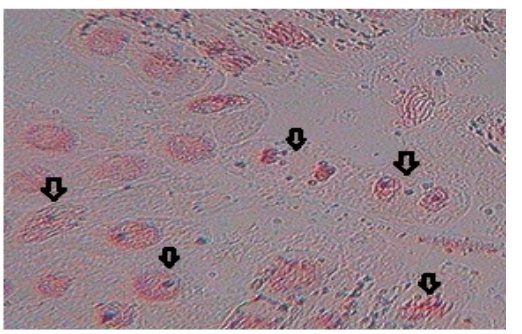

Figure 6: 2%, 3h exposure Abnormal metaphase

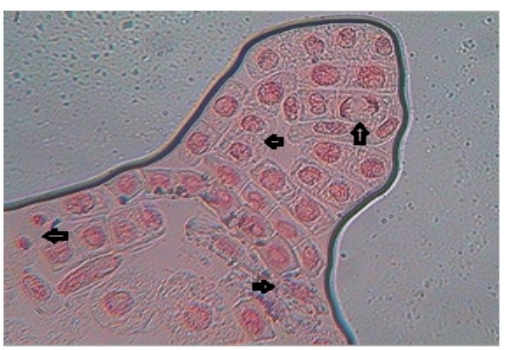

Figure 7: 1%, 1h exposure

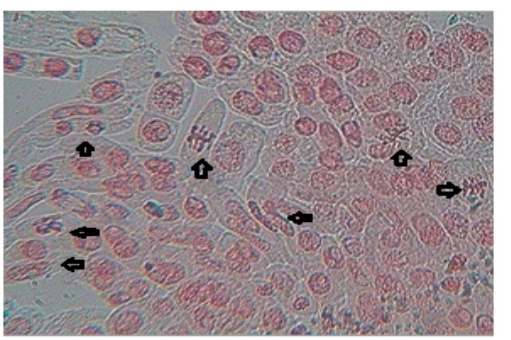

Figure 8: 2%, 1h exposure Splitted chromosome

RESULT AND DISCUSSION

Carcinogen affects the cell differently depending upon the concentration & time exposure. Cell division also becomes abnormal in the presence of some chemicals which are carcinogenic. The present investigation is to study the effect of mercuric chloride at different concentration and time exposure on root tip of *Allium cepa.* Arunima Karkun and her coworker studied the effect of lead and mercury on germination of gram seed and conclude that lead and mercury are toxic and alter the normal germination. Tartar Gul had studied cytogenetic effect of herbicide at varied conc. (0.1, 0.2, 0.4%) for varied time exposure 3, 6 & 12h. on both *Allium cepa* & *Allium sativum.* They found that following treatment significantly induced abnormalities such as c-Mitosis, chromosome stickiness, bridges, laggards multiple cells & decreased mitotic index. They also found the inhibition of mitotic index depending upon conc. & time of treatment. Kayamak Fisun and coworker had studied the genotoxic effects of fungicides (raxil) on *Allium cepa* at different concentration (1800ppm, 2400ppm) for different time exposure (3,6 ,12,24h). They found that all conc. and treatment periods of raxil induced a number of chromosomal aberrations, decrease the frequency of mitotic index & cause pollen fertility. In the present investigation, results are similar to tartar, kaymak that is conc. and time exposure affect the chromosome differently. It was found that chromosome aberration increase with increased concentration (Figure 3-8) and time exposure. Maximum abnormalities was observed in metaphase and anaphase (Table 1). The bridges (Figure.8), breaks (Figure 5-8), lagging and multiple anaphase chromosomes were observed at higher concentrations, except at the lowest tested concentration. The results showed that higher concentrations were able to inhibit significantly cell division. The presence of fragmented nuclei and poly nuclear cells can indicate a cell death process and this may lead to aneuploidy and then to cell death. The number of cytological aberrations increased with increasing concentration that cause the chromosomal anomalies (Table 1).

Table 1: Effects of mercuric chloride with different hours of exposure and concentration

Carcinogen	Percentage of concentration	Effect on mitosis in different Hours		
		5h	3h	1h
Mercuric chloride	1%	Scattered, fragmented chromosome,	Conjugated chromosome,breakage of chromosome at metaphase	not much effected
	2%	condense chromosome, breakage of chromosome at metaphase	Laggard chromosome, chromosome not properly separated	adhered chromosome,

CONCLUSION

Mercury is a toxic heavy metal. It is also considered as carcinogenic substance that cause chromosomal abnormalities. It affects the normal division of cell that ultimately leads anomalies.

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