# Toxic effect of Chromium on genotoxicity and Cytotoxicity by use of *Allium Cepa* L.

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## Abstract:

The genotoxicity and cytotoxicity of chromium was investigated using both morphological , root growth inhibition and chromosomal assays. The *Allium cepa* L. were exposed to different concentration of Chromium in different time interval ( 0.2, 2,4, 8, 12.5 mg/l and control) and mean root length of onion were measured after 24h, 48h, 72h and 96h. EC-50 value were determined from the growth curve as 47%. The result indicate that EC-50 of chromium for 24h,48h,72h and 96h of exposure are 0.8, 5.4, 4.9 and 4.4 mg/l respectively. The results of mean root length was statistically evaluated by the analysis of least significant difference. There was significant decrease in root length as concentration increased in the experiment. Also the mitotic index decreased as concentration increased.Total chromosome aberrations increased significantly as concentration increased. The result demonstrate that *Allium* test is a useful screening test for the evaluation of toxicity of heavy metal.

**Key words:** Chromium toxicity, genotoxic, *Allium cepa* L.

## Introduction:

Heavy metal pollution is one of the serious problem of fresh water bodies which are highly vulnerable to natural and anthropogenic interferences ( Sanders,1997). Due to some properties like high solubility, long half life period, non biodegradable nature and tendency of bioaccumulation and bio-magnification they cause deleterious effects on aquatic flora and fauna as well as directly and indirectly affect human health ( Chopra *et.al*.,2009). The elevated levels of heavy metals in plants may suppress the metabolism and translocation of reserve materials to the growing regions and their subsequent utilization. Heavy metals like chromium, cadmium, nickel, iron and lead are carcinogenic in human and epidemiological studies on genotoxic effects of these heavy metals have been reported in various biological systems ( Sik *et a*l.,2009, Samuel *et. al*., 2010, Olorunfemi *et.al*., 2011, Sharma and Vig, 2012). Toxicity through heavy metals including arsenic, cadmium , lead , mercury, chromium , nickel , manganese and iron, is a widespread problem in India and other developing countries (Ganesan and Panneerselvam,2013, Leme and Marin,2009). Excess heavy metal stress causes oxidative damage, but some reactive oxygen species can participate in signal transduction pathway. Chromium is unique among the heavy metals because it naturally occurs in several oxidation states ranging from Cr2+ to Cr6+ , with trivalent and hexavalent states being the most stable and common in the environment ( Chandran et.al.,2012). Toxic effect of Cr in biota have been widely reported ( Akinci and Akinci,2010 , Santos and Rodriguez, 2011). Plants do not possess specific mechanisms for Cr (VI) uptake and transport, so it is considered the highest mobile and toxic form of Cr. The toxicity of Cr(VI) compounds is most probably based on an oxidative DNA impairment ( Pandey, 2012, Fargasova and Listiakova, 2009). Hyper accumulation of toxic heavy metal ions by plants is thought to be dependent on three physiological mechanisms, high rates of uptake from soil, efficient translocation from the roots to the shoot and safe deposition of heavy metals in appropriate compartments of the shoot ( Lasat et.al., 2000). The elevated levels of heavy metals in plants may suppress the metabolism and translocation of reserve material to the growing region and their subsequent utilization thus heavy metals at supra. Optimal concentrate affect the

agronomic trait of plants ( Sinha and Gupta, 2005). Chromium is an essential element for plants and in small quantities has been reported to improve crop yield and quality ( Panda and Choudhury,2005)

Though several report suggest deleterious effects of heavy metals, simple assays and specific mechanism to ascertain the level of genotoxicity of these metals in occupationally exposed population are inadequate. Therefore, in the present study an assessment was made to compute the level of genotoxicity in the root meristem of ***Allium cepa*** L. exposed with $K_2Cr_2O_7$ through chromosomal assays.

**Materials & Methods:** Clean and healthy onions bulbs were allowed to produce roots in distilled water. After 2, 3 days, the onion bulbs with freshly emerged roots were placed on coupling jar filled with different concentration of chromium ( 0.2 , 2, 4, 8, 12.5 mg/L and control) for 5 days. The root length from the control and experimental sets were measured (length of 10 roots from each bulbs) at the end of exposure time. The relative reduction of root length was calculated as the percentage of the deviation from the control. The effective concentration i.e. EC-50 value was determined as the effective concentration for 50% growth inhibition. Experiments were carried out in different concentration of chromium (0.2, 2, 4, 8, 12.5 and control) were used in the treatment of *Allium cepa* by using $K_2Cr_2O_7$ salt and a preliminary root growth inhibition test for 96 hour. The five bulbs which have approximately same root length were transferred to each test solution. After the completion of 24 hr, 48 hr, 72 hr and 96 hr treatment, the root tip of each bulb were cut carefully and fixed in fixative FAA ( Formalein acetic alcohol) in the ratio of (85ml: 10ml: 5 ml) for 24 hour. After fixation, the roots were stored in 70% alcohol. For cytological studies the roots were hydrolysed in 1N-HCl for 10 minutes and squashed in aceto-orcein stain after intermittent heating for 3-5 minutes. After removing the root caps from well stained root tips, they were immersed in a drop of 45% acetic acid on a slide, squashed under a cover slip and sealed with nail polish and examined microscopically. Ten root tip squashes were prepared for each treatment and a minimum 400 cells were examined for each concentration. The mitotic index was calculated using the method of ( Fiskesjo, 1977 &1985). Chromosomal aberrations in each treatment were also recorded.

**Mitotic index= ( Number of dividing cells/ Total number of cells)x 100**

**Statistical analysis**: Mitotic index (MI) was calculated by scoring dividing cells. The experirmental data is presented as mean ± S.E. of triplicates.

**Fig.-1: Developing roots of *Allium cepa* L. were treated with different concentration of chromium ( $K_2Cr_2O_7$). Right to left control, 0.2, 2, 4,8,12.5 mg/l for determination of EC-50**

**Determination of EC-50 of Chromium:**

EC-50 concentration of chemical permitting 50% growth of the sample under study in relation to control. All samples were assessed by using $K_2Cr_2O_7$ salt and a preliminary root growth inhibition test for 96 h. Concentration between the higher concentration that inhibited root growth and the lowest concentration that rarely inhibited the root growth were assessed. As a result five concentration of chromium (0.2, 2, 4, 8,12.5 and control) were chosen to evaluate EC-50 concentration. A set of 1000 mg/l $K_2Cr_2O_7$ standard solution was prepared in the laboratory. EC-50 concentration was statistically evaluated by plotting of graph mean root length The trendline equation with the biggest $R^2$ value were chosen for this evaluation.

## Results and discussions:

Mean root length values of *Allium cepa* roots, exposed with preliminary five different concentration of $K_2Cr_2O_7$ for 96 hour were plotted on graph as percentage to control. EC-50 value were statistically evaluated by polynomial equation, which had the highest R2. After 24h, 48h, 72h and 96 h EC-50 estimated 0.8, 5.4, 4.9 and 4.4 mg/l respectively . The mean root length at different concentration are shown in table-1. Generally growth retardation was observed in all concentration. There was concentration dependent decrease in root growth were in the decreasing order at all concentration. Results of microscopic analysis of the treated root tips are summarized in Table-2,3,4 and plate-1. Different mechanisms have been proposed to deal with the tolerance to heavy metal stress in plants, for instance heavy metal immobilization and synthesis of stress proteins (Li and Xiong,2004). In the present studies, the cytotoxicity and genotoxicity of Cr was investigated by observing the decrease in the mitotic index. The current findings agreed well with other reports which showed cytotoxicity and genotoxicity of Cr in plant cells ( Panda & Patra,1997, Panda et.al,2002). Kwankua et.al.( 2012) suggested that exposure to heavy metals prevented cells entering cell division phases which then resulted in a decrease in the MI. Additionally, the primary action of heavy metal on the mitotic spindle promoted spindle related abnormalities such as

laggard chromosome and bridges during cell division. Chromosomal aberration were induced at all the concentration were statistically significant. However similar to other microelement, at excess concentration this metal become toxic for most of the plant species (Cary *et al,*1997). Phytotoxicity of Cr in aquatic enviroment has not been studied in detail. The mechanism of injury in terms of ultrastructural organization, biochemical change and metabolic regulation has not been elucidated . It has been pointed out that, while considering the toxicity of heavy metal a distinction should be made between elements essential to plants and metals that have no proven beneficial biochemical effect ( Cary *et.al*.,1997, Gauglhofer & Bianchi,1991). Heavy metal induced genotoxic effects on plants and other biological systems depend on the oxidation state of metal, its concentration and duration of the exposure. In general, effects are more pronounced at higher concentration and at longer duration of exposure.

Concerning cyto/genotoxic potency of Cr(VI) metal doped drinking water showed a strong phytotoxic effect, affecting *A.Cepa* at the concentration 4.0 mg/l ( EC-50 of below 50% inducts sublethal effect and below 20% cause lethal effect on test organism. Reduction in cell division due to Cr is well documented effect in many plants ( Santos & Rodriguez, 2011), assuming that root growth inhibition and impaired penetration of roots into the soil due to Cr toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle. Ditika and Anila ( 2013) suggested that the decreased mitotic index in *Allium* roots treated with metal is probably due to either disturbances in the cell cycle or chromatin disfunction induced by metal-DNA interaction. The result of this investigation showed a significant reduction increase of mitotic index in onion root meristematic cells with the addition of Cr(VI) salt. The results demonstrated an inhibition of mitosis or extension of cell cycles. This may attributed to the blocking of cell division by Cr(VI) at the end of the prophase. In this case, this metal may be considered as pre mataphase inhibitor. Mito depressive action of Cr(VI) on the cell division may be the reason of MI reduction in *A.cepa* root.

Data of mitotic index were presented in table-2,3 and 4. In the present study, $K_2Cr_2O_7$ was exposed to onion root in different concentration and time intervals to evaluate Cr induced genotoxicity. Mitotic index was higher 19.5,18.2, 17.8 and 17.2 at initial concentration of 0.2 mg/l in all time interval ( 24h,48h,72h and 96h) tested and decreased progressively when Cr concentration ( 0.2, 2,4 , 8, 12.5 mg/l) and exposure time was increased ( table-4). In general, effects are more pronounced at higher concentration and at longer duration of exposure. Chromosomal aberrations were induced at all the tested concentration. The most frequent aberration were bridges and sticky chromosome. Chromosome with disturbed spindles and fragments were also present in appreciable amounts. In *Allium cepa* whenever chromosome aberration occurred, there were almost always certain growth restrictions. Sticky chromosome represent poisoned chromosome with sticky surface and probably lead to cell death. Sticky chromosome at metaphase and anaphase stage were abundant in the *Allium* test at high concentration indicating its toxicity. Genotoxicological effect is developed than the concentration is low order than that for phytotoxicity effect (Walker et.al.,2012). As a potent oxidant, the ability of Cr(VI) to induce oxidative stress and the formation of stable Cr-DNA adducts contribute to its cytotoxic and genotoxic effects. Chromiume salts easily penetrate the cell membrane and are reduced inside the cell to their trivalent form, which unlike other metal react directly with DNA, causing DNA damage including : base modification, single-strand breaks, double strand breaks, Cr-DNA adducts, DNA-Cr-DNA adducts and protein-Cr-DNA adducts ( Wise et.al.,2008). By this, while other metal are considered weakly mutagenic, mostly acting through the inhibition of DNA repair machinery, Cr acts directly on DNA causing genotoxicity. In the present investigation showed a significant increase of the frequency of abnormal cells with chromosomal aberration in *A.cepa* root meristematic cell with the addition of chromium salt.

**Conclusion:** The present study provide additional and valuable information about the toxic effects of Chromium by evaluating genetic and cytological end point on *A.cepa* roots, grown in different concentration of Cr. *Allium cepa* test demonstrated different sensitivities showing correlation to river water quality. The result should be considered a warning of risk the environment, biota and human health may incur by natural and anthropogenic chromium discharge in water bodies. This study showed the necessity of combining physical-chemical and sapro-biological analysis with cytogenetic approaches to better understand the toxicity of water chemical pollutant and their influence on health. In general the *Allium cepa* test bioassays should be an integral tool in quality monitoring of water bodies, soil and air.

**Table-1: Root length of *Allium cepa* L. treated with different concentrationof Chromium at different time interval**

| **Treatment** $K_2Cr_2O_7$ | Mean root length±S.E.(cm) | Mean root length±S.E.(cm) | Mean root length±S.E.(cm) | Mean root length±S.E.(cm) |
|---|---|---|---|---|
| | 24 hour | 48 hour | 72 hour | 96 hour |
| Control | 1.8±0.12 | 3.7±0.13 | 4.1±0.23 | 5.3±0.21 |
| 0.2 | 1.2 ±0.22 | 2.9±0.19 | 3.2±0.21 | 3.4±0.17 |
| 2.0 | 0.8±0.16 | 1.9±0.20 | 2.5±0.20 | 3.1±0.14 |
| 4.0 | 0.6±0.19 | 1.7±0.16 | 2.0±0.07 | 2.8±0.17 |
| 8.0 | 0.3±0.18 | 1.2±0.21 | 1.8±0.14 | 3.3±0.15 |
| 12.5 | 0.2 ±0.12 | 1.9±0.18 | 2.5±0.09 | 3.7±0.18 |

Values are mean of three replicates ± SE

**Table-2: Cytological effects of on cells of *Allium cepa* L. at different concentration of Chromium at different time interval**

| Treatment | 24 hour | | 48 hour | | 72 hour | | 96 hour | |
|---|---|---|---|---|---|---|---|---|
| $K_2Cr_2O_7$ | No. of dividing cells | Mitotic index % | No. of dividing cells | Mitotic index % | No. of dividing cells | Mitotic index % | No. of dividing cells | Mitotic index % |
| Control | 214 | 21.4 | 222 | 22.2 | 232 | 23.2 | 209 | 20.9 |
| 0.2 | 195 | 19.5 | 182 | 18.2 | 178 | 17.8 | 172 | 17.2 |
| 2.0 | 170 | 17.0 | 167 | 16.7 | 168 | 16.8 | 167 | 16.7 |
| 4.0 | 158 | 15.8 | 159 | 15.9 | 154 | 15.4 | 152 | 15.2 |
| 8.0 | 128 | 12.8 | 132 | 13.2 | 115 | 11.5 | 118 | 11.8 |
| 12.5 | 98 | 9.8 | 112 | 11.2 | 97 | 9.7 | 96 | 9.6 |

*1000 cells per water samples and the control

**Table-3: Chromosomal aberration on cells of *Allium cepa* L. at different concentration of Chromium after 24 h and 48 h**

| Treatment $K_2Cr_2O_7$ | 24 hour | | | | | | 48 hour | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No.of dividingcells | Chromosomal aberration | | | | Total abe. Ratio % | No.of dividingcells | Chromosomal aberration | | | | Total abe. Ratio % |
| | | Lg | Vg | Sc | Bg | | | Lg | Vg | Sc | Bg | |
| Control | 214 | 2 | 0 | 0 | 1 | 1.40 | 222 | 2 | 2 | 0 | 1 | 2.25 |
| 0.2 | 195 | 0 | 0 | 0 | 1 | 0.51 | 182 | 0 | 2 | 0 | 2 | 2.08 |
| 2.0 | 170 | 1 | 0 | 0 | 1 | 0.83 | 167 | 0 | 2 | 0 | 3 | 2.31 |
| 4.0 | 158 | 2 | 0 | 1 | 0 | 1.19 | 159 | 3 | 1 | 1 | 0 | 1.98 |
| 8.0 | 128 | 3 | 0 | 1 | 2 | 2.14 | 132 | 0 | 0 | 2 | 1 | 1.28 |
| 12.5 | 98 | 2 | 0 | 2 | 2 | 2.58 | 112 | 1 | 1 | 3 | 0 | 2.63 |

**Note:** Lg-Laggard, Vg- Vagrant, Sc- Stickness, Bg- Chromosomal bridge

**Table-4: Chromosomal aberration on cells of *Allium cepa* L. at different concentration of Cr after 72 h and 96 h**

| Sites | 72 hour | | | | | | 96 hour | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No.of dividingcells | Chromosomal aberration | | | | Total abe. Ratio % | No.of dividingcells | Chromosomal aberration | | | | Total abe. Ratio % |
| | | Lg | Vg | Sc | Bg | | | Lg | Vg | Sc | Bg | |
| Control | 232 | 2 | 2 | 0 | 0 | 1.72 | 209 | 2 | 1 | 0 | 0 | 1.43 |
| 0.2 | 178 | 1 | 2 | 0 | 0 | 1.66 | 172 | 0 | 0 | 2 | 1 | 1.71 |
| 2.0 | 168 | 1 | 2 | 0 | 0 | 1.61 | 167 | 1 | 0 | 0 | 2 | 2.43 |
| 4.0 | 154 | 1 | 0 | 1 | 2 | 2.29 | 152 | 0 | 1 | 0 | 3 | 2.22 |
| 8.0 | 115 | 0 | 0 | 2 | 1 | 1.39 | 118 | 0 | 0 | 2 | 1 | 1.59 |
| 12.5 | 97 | 0 | 0 | 3 | 1 | 2.00 | 96 | 0 | 0 | 2 | 0 | 1.96 |

**Note:** Lg-Laggard, Vg- Vagrant, Sc- Stickness, Bg- Chromosomal bridge

## References:

Akinci,I.F. and Akinci, S.,(2010). Effect of chromium toxicity on germination and early seedling growth in melon ( Cucumis melo L.). Afr. J.Biotech.,9(29),4589-4594.

Cary, E.E.,Allaway W.H. and Olson O.E.(1997). Control of Cr concentration in food plants, Absorption and translocation of Cr by plants.J.Agric.Food Chem.25(2):300-304.

Chandran S., Niranjana V. and Joseph Benny (2012). Accumulation of heavy metals in waste water irrigated crops in Mudurai India,J.Environ. Res Develop. 6(3),432-438.

Chopra,A.K., Pathak,C and Prasad G. (2009). Scenario of heavy metal contamination in agricultural soil and its management. J. Appl.& Nat.Sci.,1(1):99-108.

Ditika Kopliku and Anila Mesi (2013). Assessment of cytotoxic and genotoxic potency of Cr(VI)-Doped river water of Nen-Shkodra Lowland, Albania, on *Allium cepa* L. J.of Enviromental Research and Development. Vol.7 No.4, April-June.

Fargasova A. and Listiakova J.(2009). Cr and Ni simultaneous phytotoxicity and mutagenicity assay, Nova Biotech., 9(2), 107-112.

Fiskesjo,G. (1985). The Allium test as a standard in environmental monitoring.Hereditas 102: 99-112.

Fiskesjo,G.(1997). Allium test for screening chemicals: Evaluaton of cytologic parameters.In plants for environmental studies,Wang,W,J.W. Gorsuch and J.S. Hughes (eds). CRC Lewis publisher,Boca,Raton,New York pp: 308-333.

Gauglhofer,J & Bianchi,V.(1991). Chromium.pp 853-861 in E.Merian(ed), Metals and their compound in the environment,VCH,Weinheim, Germany and Newyork.

Ganesan A. and Panneerselvam N. ( 2013). Analysis of Ni induced genotoxicity in root meristem of Allium cepa. Inter.J. Biological technology.Vol.No.4(2): 19-22.

Kwankua W., Sengsai,S.,Muangphra, P.,and Euawong,N. (2012). Sccreening of plants sensitive to heavy metals using cytotoxic and genotoxic biomarkers. Kasetsart J.( Nat.Sci.) 46: 10-23.

Lasat, MM., Pence, N.S., Garvin, D.F.,Ebbs S.D. and Kochian, I.V.(2000). Molecular physiology of the Zn transport in the hyper accumulation thlaspi caerulescens. J. Of Experimental Botany,51: 71-79.

Leme D.M. and Marin-Morales M.A. (2009). *Allium cepa* test in environmental monitoring : A review in its application, Mut.Res./Rev.,682(1),71-81.

Olorunfemi, DI.,Okoloko, G.E., Bakare, A.A. and Akinboro,A. (2011). Cytotoxic and genotoxic effects of cassava effluents using the Allium cepa test. Research journal of Mutagenesis.1:1-9.

Panda S.K. and Choudhury S. (2005). Chromium stress in plants. Braz.J.Plant Physiol. Vol.17.No-1 Londrina Jan/ Mar .

Panda SK and Patra HK (1997). Physiology of Cr toxicity in plants-A Review. Plant Physiol.Biochem,24 (1): 10-17.

Panda SK, Mahapatra S, Patra, HK (2002). Chromium toxicity and water stress simulation effects in intact senescing leaves of greengram,In: Panda SK (ed), Advance in stress physiology in plants,pp. 129-136.Scientific publishers,India.

Pandey Ambuj (2012). Arsenic contamination of ground water in South and east Asia. J.Environ. Res.Develop.,7(1), 70-74.

Rank,J. ; Nielson,MH (1998). Genotoxicity testing of waste water sludge using the allium cepa. Anaphase-telophase chromosome aberration assays. Mutation Research 418:113-119.

Samuel,O.B., Osuala,F. and Odeigah,P.G.C.(2010). Cytogenotoxicity evaluation of two industrial effluents using *Allium cepa* assay. African J. of Enviromental Science and Technology.4(1):21-27.

Sanders,MJ (1997). A field evaluation of the fresh water river crab,*Potamonantes warren* as a bioaccumulation indicator of metal pollution. Thesis,Rand Afrikaans University, South Africa.

Santos S.and Rodriguez E.(2011). Review on some emerging endpoints of Cr and Pb phtotoxicity, In Botany,Ed.Mworia J., In tech, available at :www.intechopen.com/book/botany/ review on emerging endpoint of chromium vi-and-leadphytotoxicity.

Sharma,S and Vig,A.P.,(2012). Genotoxicity of Atrazine, avanoxan, diuron, quisolofor-p-ethyl-herbicides using Allium cepa root chromosomal aberration assay. Terrestrial and Aquatic Enviromental Toxicology,6(2):90-95.

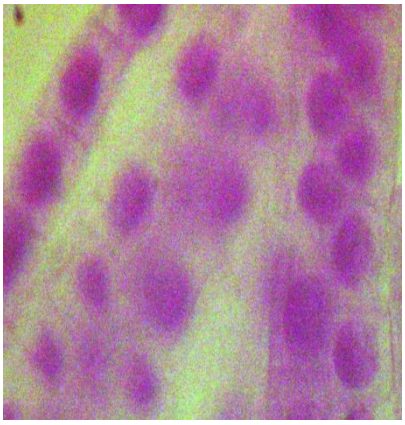
Sik,L., Acar,O. and Aaki,C.(2009). Genotoxic effects of industrial waste water on Allium cepa L.African J. of Biotechnolgy,8(9): 1919-1923.

Sinha,S. and Gupta A.K.(2005). Assessment of metal in leguminous green manuring plant of Sesbania cannabina L. grown on Xy ash amended soil: Chemosphere,61: 1204-1214.

Walker C.H., Sibly R.M., Hopkin S.P. and Peakall D.B.(Eds), 2012 .Principal of Ecotoxicol. 4th Ed.,CRC Press,978.

Wise S.S.,Holmes A.L. and Wise J.P.S.,(2008). Hexavalent Cr-induced DNA damage and repair mechanism .Rev.Environ.Health,23 (1), 39-57.

**PLATE-1**



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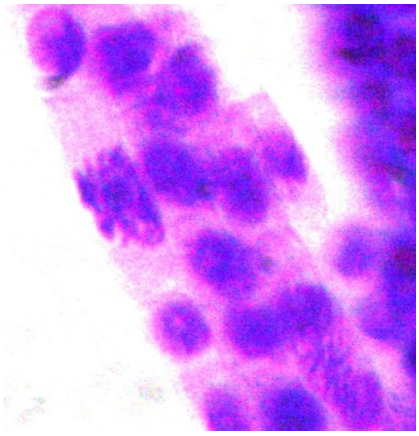

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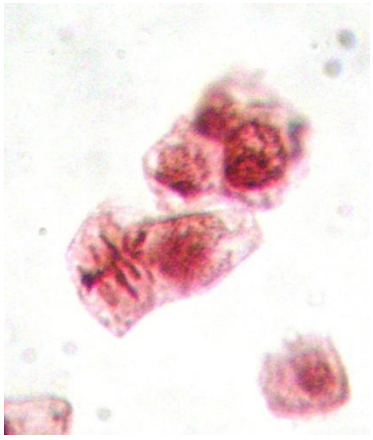

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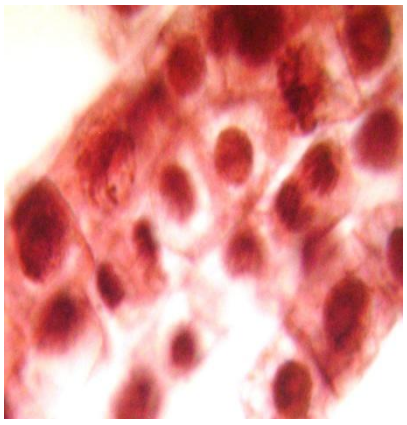

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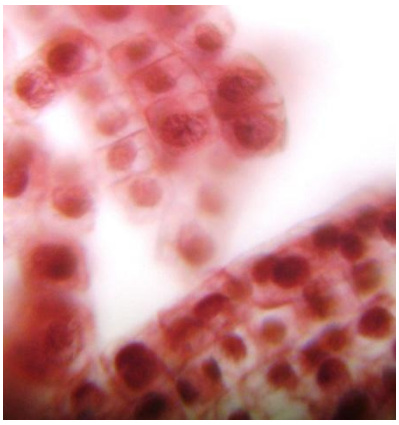

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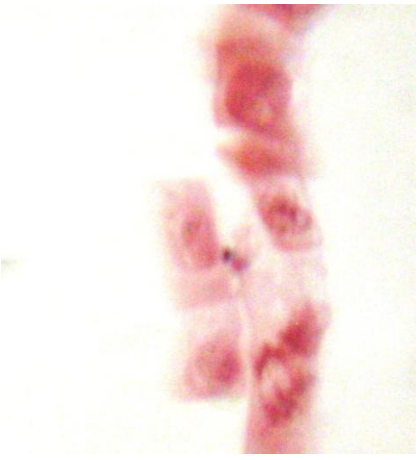

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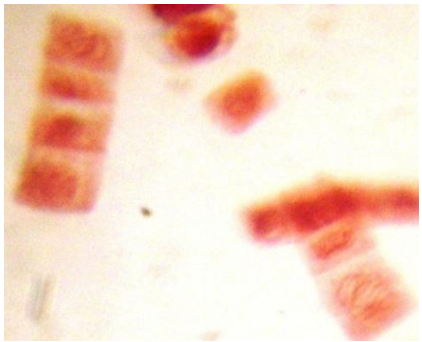

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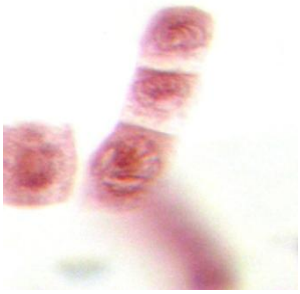

8

9

**Stages of mitosis and chromosomal aberrations induced in the cells of onion in different concentration of Cr after 24h, 48h, 72h, 96h time interval abnormalities were observed 1.Disturbed metaphase 2. prophase3. Metaphase 4.Stickymetaphase 5.Disturbedspindles 6.anaphasebridge 7.disturbed metaphase 8.telophase 9.metaphase**