GENOTOXICITY AND HISTOPATHOLOGICAL CHANGES DUE TO TOXIC EFFECT OF CADMIUM IN ALBINO MICE

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Abstract:

Heavy metal toxicity is great life threatening problem for every organism including human. Heavy metal becomes toxic when they are not metabolized by the body and accumulate in soft tissue. Cadmium is a heavy metal that causes several diseases in human and has been classified as human carcinogen. Cadmium not only effect human but also other mammals. it effect the soft tissue of lung, kidney, liver, testis and also it effect in genomic level. The present study aimed to investigate that toxicological effect of heavy metal Cd (cadmium)on mammalian system(Albino mice). Histopathological changes in tissue of mice as well as chromosomal aberration and change in protein have been noticed by inducing the Cd. For this purpose mice were treated as different concentration of Cd for 90 days. Tissue were collected from both control and treated animals and studied mice exposed to Cd showed Histopathological changes in the liver, kidney, lungs and testicular tissue as well as chromosomal abnormality such as break, ring, centromeric separation were observed.

Key ward: Heavy metal, genotoxicity, histopathology, cadmium, Albino mice

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INTRODUCTION:

The exploration and exploitation of natural resources using modern technology and the exponential growth of population have inadvertently resulted in the release of varied types and amounts of industrial wastes into the environment. These industrial wastes are complex mixtures of several classes of pollutant such as hydrocarbons and heavy metals. These have contributed immensely to the heavy metal load in the environment. Heavy metals also bio accumulates in one or several compartments across food webs as shown by several scientific observations. Among several elements of periodic table, there are 35 metals are associated with commonly and occupational exposure. Out of these 23 are described as heavy metals. Presently, there is steady increase in their concentration in all habitats owing to mining, electroplating, plants and dye, battery making industries etc. are the release is rapid in rapidly growing technology and heavy metal application in these industries. .Out of the several heavy metal in the industries waste streams, cadmium is reported to be associated with the effluents of battery, electroplating and metal finishing, mining and meteorology and paints and dye industries. Most of the heavy metal exhibit toxicity through the formation of coordination complex and cluster in the animal cells. Cadmium is considered as one of the most toxic heavy metals. It is a non essential element to living organism.

Water is mainly effect by diluted cadmium due to contamination of river and other water sources. I t is important to note that cadmium is highly toxic element for all mammals. Cadmium levels have constantly been increasing, and consequently, the research on cadmium has become quite topical and urgent.

In mammals cadmium can cause a number of structural and morphological changes in various organ as well as genomic level. The present study aimed to investigate that toxicological effect of heavy metal (Cd) on mammalian system (albino mice)

Materials and method:

CHEMICALS

1. Bouin's fluid, 2. Mayer's albumin, 3. Stains- Acetocarmine , Haematoxylin Eosin, Leishman's stain ,4. Cholsicin solution

Study design

The genotoxicity assays were performed at the department laboratory. Animals were kept in the cage and were fed regularly with balanced diet. Cytogenetic analysis was performed by bone-marrow chromosome preparation and protein assay method. All animals were acclimatized for 15 days before starting the experimental procedure. The mice were assigned randomly to either a control or test group and were housed in individual cages (19 × 19 × 12 cm) with solid plastic side and stain less steel grid tops and floors in a room with temperature of approximately 30 ± 1℃, 60-75% humidity and 12h light/dark cycle. Mice were feed with balanced diet. Sanitation was maintained properly inside the cages. 20 mice were divided to four groups each groups contain 5 numbers of mice 1st group which contain 5 mice were taken as control was given only distilled water. The second group received distilled water supplemented with 5mg Cd/Kg body weight per day orally, 3rd group received 10mg Cd/Kg fourth group received distilled water supplemented with15mg Cd/Kg body weight orally per days for 90 days .Tissue were collected from both control and treated animals by sacrificing the mice.

TABLE-1 :

Sl NO Of MICE	SEX: Mice Male/Female	Weight(gm)	Dose for 1st group (control)	Dose for 2nd group mg/Kg bw	Dose for 3rd group mg/Kg bw	Dose for 4th group mg/kg bw
1	Female	75	No treat			
2	Female	80	No treat			
3	Male	80	NO treat			
4	Male	85	No treat			
5	Male	85	No treat			
6	Female	74		5mg		
7	Female	78		5mg		
8	Female	74		5mg		
9	Male	75		5mg		
10	Male	80		5mg		
11	Female	76			10mg	
12	Female	78			10mg	
13	Male	75			10mg	
14	Male	80			10mg	
15	Male	83			10mg	
16	Female	80				15 mg
17	Female	80				15mg
18	Female	75				15mg
19	Male	78				15mg
20	Male	80				15mg

Bone marrow chromosome assay

The method of tissue collection was followed by ford and Hamerton (1956). Each individual was pre treated with colchicines @ 4 mg/Kg .b.w. 90 minutes before sacrifice. The animals were made unconscious by cervical dislocation and the long bones such as humorous and femur was removed quickly. With the help of hypodermic syringe marrow cell were flushed out with warm water (37℃) sodium citrate (1%) solution to centrifuge tube. Then the material was centrifuged for 5 minutes at1000 r.p.m. and the supernatant fluid was discarded.

The fixation was done in freshly prepared acetic acid-methanol mixture 1:3. After 15-20 minutes, the material was centrifuge and supernatant was discarded. Then fresh fixative was added and kept for 30 minutes. Again the material was centrifuge after discarding the supernatant fluid, the required amount of fixative was added finally for cytological preparation of slides. Slides were prepared according to air drying technique of Rothfels and Siminovitech (1959) with some modifications.

Before use, grease-free slides were preserved in 50% alcohol for half an hour for chilling. Then the materials were dropped on the chilled slides with the help of fine Pasteur pipette, after which, the slides were shown to the spirit lamp flame for a while. Then the flame was extinguished immediately and the slide was allowed to dry in air. On the following day, the slides were stained for90 minutes in 10-15% Gimsa stain (BDH). After staining, the slides were coded for analysis of chromosomal aberration. From each individual 60 good and well spread Meta phage stage were examined at 1000x magnification. Thus for every exposure time 300 cells were studied.

Protein Assay

The method of SDS PAGE. It was performed to identify the protein bands of the blood serum.

Procedure:

Preparation of resolving gel

- 4.6m.lofdistilled water ,2.7 ml. of 30% acrylamid3 and water,2.5 ml of 1.5 molar of tris HCL PH-8.8,0.1m.lof 10% SDS,0.1mlof 10% APS

After addition of APS the solution was shaken gently and0.006ml of TEMED was added .Again the solution was shaken then poured into the gel constantly and left for 10 minutes.

Preparation of staking gel:

5m.l of staking gel was prepared on test tube by adding the following, 3.4m.l of distilled water, 0.83ml of 30% acrylamide,0.63 m.l 1 molar tris HCL PH-6.8, 0.05m.l of10%SDS,
0.5 m.l of 10% APS

After addition of APS the solution was shaken gently. Then 0.005ml of TEMED was added and again solution was shaken well gently. Allow the solution was poured to gel casting plate above the resolving gel. Immediately, a comb was inserted to it for making well for 10 for 10 minutes. After 10 minutes comb was removed and the well was ashes properly with distilled water.

Preparation of sample:

50 µl of gel loading dye was taken in an appendrof. Then the sample was taken away from the water bath and it was brought to normal temperature.

Gel running:

At 1st sample was loaded and allowed to run on electroforetic apparatus contain gel with electric supply after run the gel was removed and Staining and de staining was performed:

The gel was removed carefully from gel casting plate to a gel staining box containing solution now the staining solution was removed from the gel staining box containing gel and placed in distaining solution and left for 30 minutes. This process was replaced for few times for complete removed of excess stacking. After complete distaining, the spread protein band was visible.

Observation:

After de staining, it was observed that the well containing more quantity of sample gives thin bands, the bands thickness increase in sample concentration.

Results and Discussion

Results:

The oral administration at different concentration of cadmium to Mice shows many symptoms of toxicity such as cellular abnormalities, deposition of heavy metals on tissues, tumor formation, paralysis and death.

Chromosome Assay:

From the normal bone marrow chromosome preparation with giemsa stain, a good number of metaphase stages were observed. The chromosomes have been observed to be of acrocentric in nature. In the control sets, the percentage of abnormal cells having chromosomal aberrations such as stickiness, clumping, breaks and stretching and woolly appearance are not found. It is

evident that the percentage of abnormal cells was increases in dose and duration of exposure to cadmium. From this it was clear that Cadmium has genotoxic effect in 90 days genotoxic test.

The chromosomal study with no genotoxic effect is shown in fig.1.A, which is control. But genotoxic effect is shown in fig.1.B.

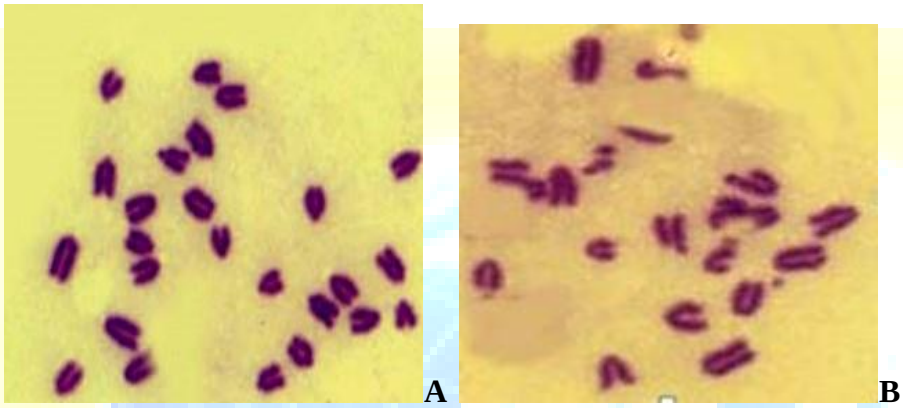
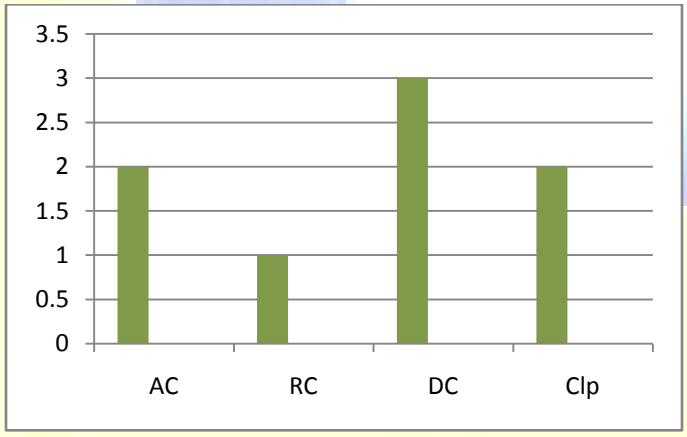


Fig.I.1.A (control), B (Treated with $CdCl_2$)



X- Type of abnormality, Y- no. Of abnormality

Protein Assay:

The obtained results showed that there is change in the serum proteins in treated animals in comparison with controlled one. The protein bands which were observed in controlled and treated ones are different. The 1st lane is Marker, 2nd lane is controlled, 3rd lane is treated with 2mg $CdCl_2$, 4th lane is treated with 5mg $CdCl_2$ and 5th lane is treated with 10mg $CdCl_2$ /kg bw .

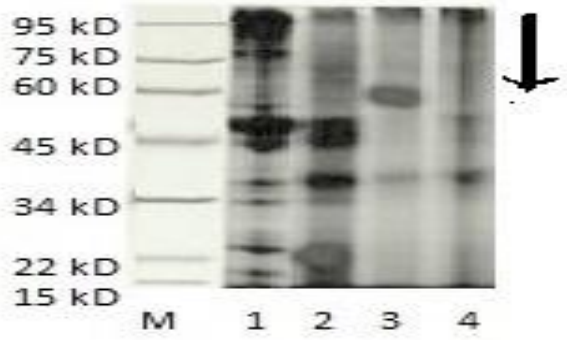


Fig.II.2-Protein gel electrophoresis, 10% SDS (1-mw Marker, 2-control, 3&4,-treated with 5,&10 mg $CdCl_2$/ kg bw).

Histological preparation:

For sectioning tissue (liver, kidney &testis) were collected from mice after sacrifice. Then the tissue were cut into small pieces and washed with physiological solution and immerged in fixative (Bouin's fluid) for 24 hours. Then the tissue was washed in running tap water for 24 hours till removal of fixative. Then tissue were dehydrated through upgrade of alcohol, Viz, 30%, 50%, 75%, 90% and 100% then the tissue were transfer to malted paraffin wax and section cutting was done by using microtome in thickness of 6µm, grease free slide were taken, one surface of slide was marked with diamond pencil. A droplet of Mayer's albumin was smeared on the marked surface, a thin film of water was put on the slide and paraffin ribbons with section were placed on it. Then the slide was heated on a hot plate and the sections were properly stretched. The water was drained off and the slides were dried on hot plate.

Then the slide was transferred to absolute alcohol 100%, 90%, 70%, 50%, 30% each for 15-20 minutes. Then transferred to distilled water. Section of tissue were usually stained with two dyes, means double staining haematoxylin and eosin. At first the slides were stained with haematoxylin then put on running tap water for removal of excess stain. Then the slides were transferred to acetic water, then from acid water to distilled water and finally transferred to alcohol of different concentration (30%, 50%, 70%, 90%). After this the slides were stained with another stain i.e. eosin from this stain the slides were transferred to absolute alcohol 100%. Then a small amount of DPX as putted on the slide, depending on the size of cover slip.

Results and Discussion

Histopathological changes

Histopathological changes were observed in the liver, kidney & testis for different treated groups. Lesions were essentially similar for all the treatment & the exposure time, although the intensity of cell damage increases with the increasing concentration & the time of exposure.

Exposure of CdCl2 to liver:-

Normal liver cell of climbing perch no pathological lesion observed in control &2mg/ kg bw $CdCl_2$ in Albino mice. There are disarrangement of hepatic cell exposed to concentration of 5mg/ kg bw. Necrosis & thickness of hepatic cell was observed in 10 mg/kg bw which is highest dose given to the climbing perch, showed the necrosis & faded hepatic cell in the liver.

Exposure of $CdCl_2$ to kidney:-

In 2 mg/ kg bw of $CdCl_2$ to kidney cells does not show any changes, but in 5mg & 10 mg/kg bw , the cell shrinkage takes place, black patches & degeneration of cell was observed.

Exposure of $CdCl_2$ to testis:-

Exposure of 5mg, 10mg/ kg bw & 15 mg /kg bw show hemorrhagic, interstitial edema degeneration of somniferous tubules of testis, but no changes occur in control Albino mice.

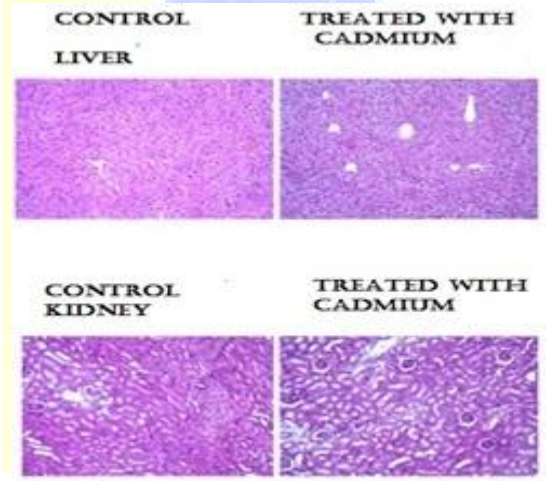


Figure 3: A &B control of liver & kidney tissue, C & D 5 mg & E,F 10mg / kg bw CdCl2 liver & kidney

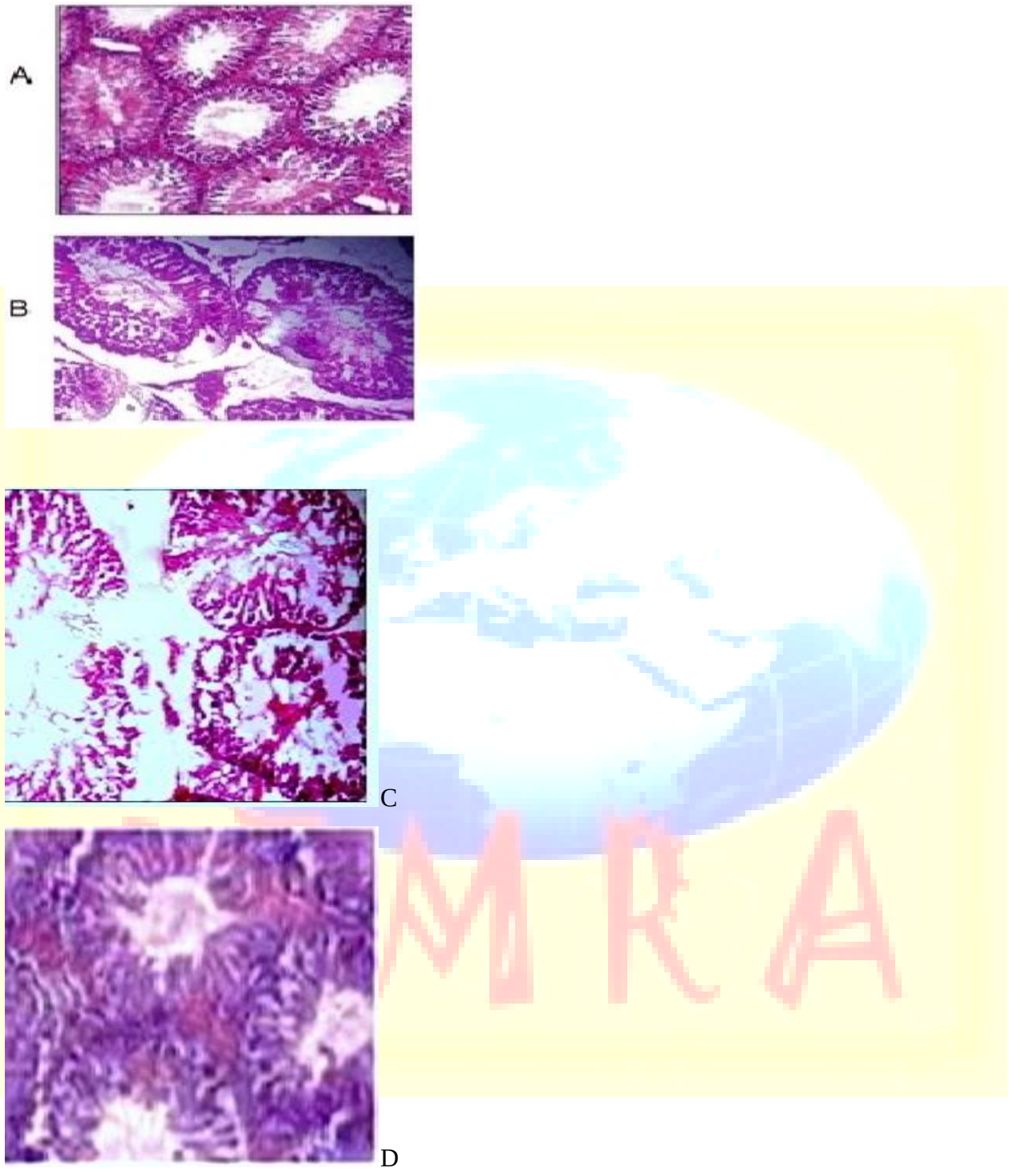


Figure 4: A control of testis & B,C,D treated with 5 , 10 and 20 mg / kg bw CdCl2, fig B , C&D shows hemorrhagic, interstitial edema degeneration of somniferous tubules, but control is almost normal.

Discussion

Exposure of heavy metal (Cd) is common, because exploration and exploitation of natural resources using modern technology and the exponential growth of population have inadvertently resulted in the release of varied type and amounts of industrial wastes and heavy metals into the environment. The heavy metals are release into environment by process like weathering of rocks, volcanic eruption mining and exposure through water, air and food. Hence their exposure to population is inescapable consequences exposure of Cd shows genotoxicity like chromosomal aberration, chromatid breaks, chromatid deletion, acentric fragments, ring and dicentric chromosome and histopathological changes like cell shrinkage, cell necrosis etc. in mice from the above analysis it was observed that long term exposure to Cd shows chromosomal abnormalities, cellular abnormalities, tumour formation and death of the animals. The 90 days study in rats provided information that there is health risk from repeated exposure of Cd .

CONCLUSION

Prolonged exposure of the heavy metals like $CdCl_2$, even at low levels lead to chromosomal aberration including gene changes in Albino mice. Exposure to sub lethal concentration of $CdCl_2$ cause changes in both Histopathological as well as genotoxicity. Therefore, Cadmium chloride exposure to even sub lethal concentration may pose serious threat. The chromosomal abnormality and Histopathological changes increase with increase concentration of the cadmium chloride. Thus the present study suggests that Cd interferes with both cellular and genomic activities in Albino mice.

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