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EFFECT OF HEAVY METAL CONTAMINATED MAIZE ON MAMMALIAN SYSTEM

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ABSTRACT

The result of anthropogenic activity has resulted in the entry of toxic heavy metals into the environment at any stage from mining to final use. During recycling and use it contaminates soil, crops, water, air, dust and food crops. The East Kolkata Wetland has high environmental concentrations of toxic pollutants including heavy metals in the soil because it receives the untreated sewage of Kolkata; different crops and vegetables including maize are grown here in this place. In the present investigation, the plant and animal models selected were maize and rats. The maize collected from the contaminated soil of East Kolkata wetlands was found to contain 12.5µg/mg dry-weight of lead. Cadmium was also tested but it was not present. Sixteen albino rats were divided into four groups: Group I served as control and was fed with normal diet; group II was fed with normal diet mixed with normal maize; group III was given contaminated maize from the East Kolkata wetlands mixed with normal diet and group IV was fed with a combination of 12.5µg/mg of lead chloride and normal diet. After 6 weeks, the rats were anaesthized with chloroform and then dissected. Changes in organ weight, body weight, haemoglobin percentage and lead content in various organs were determined. Short-term lead exposure showed enlargement of the kidney. In the liver, accumulation of fatty acids and lesions present were pronounced. Spleen mass had visibly increased and, in general, white patches or globules were observed. The SGPT count did not show any major changes. The body weight was found to reduce in the groups administered with lead salts and contaminated maize. But the one fed with lead contaminated maize showed partial increase. Analysis of lead in the organ samples showed the expected dose-dependent accumulation of the metal even when fed with a low concentration. There was a decrease in the weight of the liver from 2.5g to 1.9g, and increase in the weight of the spleen from 0.75g to 1g, and of kidney from 0.1g to 0.6g. There was also a notable decrease in the haemoglobin percentage of the third and fourth groups. The rats belonging to the first and second group were fed with normal food and they showed a haemoglobin percentage of 13.8% and 14.4% respectively. The third and fourth groups of rats showed a considerable fall in the haemoglobin percentage of 12.2% and 11.8% respectively.

INTRODUCTION

The East Kolkata Wetland is a highly complex and poorly understood system. It processes the city's waste, both garbage and sewage, a service that would be expensive to replace. It is a home to numerous people and animals, and plants are also grown in modified agricultural land. Further study of the wetland system and efforts towards its conservation are vital to understand the effect of presence of toxic heavy metals like lead, cadmium, copper etc. in the soil of farmlands that directly enter the food chain.

The land was recognized as a wetland protected under the Ramsar Convention but because of dumping of solid waste for years together the place has lost its characteristic feature and to a great extent it serves as a granary of Kolkata. But because of mixed pollution in that area the food crop and vegetables are getting increasingly contaminated. The present investigation was undertaken with the following objectives in view.

1. To ascertain the level of heavy metals in the crop grown (maize).
2. To test the effect of feeding contaminated maize in mammalian system (albino rats).
3. To study the changes in various biochemical parameters like haemoglobin %, S.G.P.T
4. To measure the level of heavy metals in blood and soft tissues.

MATERIALS AND METHODS

1. Rearing of albino wister rats: Sixteen, two and half weeks old male albino wister rats were obtained from the breeder. The rats were housed in stainless steel cages under conventional conditions of temperature (21° ± 0.5°C) and relative humidity of 50% ± 10%. They had access to daily fluid of 50 mL and standard diet of 10g once in 24 hours for the first seven days to create a conditioned reflex. The experimental period was for six weeks. The sixteen albino rats were divided into four rats each in four cages. Cage I consisted of four rats which were fed with 10 g of standard diet once in 24 hours. Cage II consisted of four rats that were fed with standard diet and normal maize powder (10 g in 1:1 proportion). Cage III consisted of four rats which were fed with 5 g of standard diet and 5 g of contaminated maize powder. Cage IV consisted of four rats that were fed with 10 g of standard diet containing 12.5mg/g of lead chloride ($PbCl_2$). The rats were fed once daily to prevent wastage. Each rat was weighed separately every week on a fixed day.

2. Autopsy and determination of organ weight: At the end of six-week experimental period, the rats were sacrificed under anaesthized condition. They were dissected open, visceral organs were displayed and observed for any irregularity. Liver, spleen and kidney were collected and stored in 0.9% brine solution after weighing. Blood was collected from the heart using sterile syringe and stored in vials containing oxalate.

3. Determination of haemoglobin percentage: The haemoglobin percentage of the collected blood was determined using titration method.

4. Determination of serum glutamate pyruvate transaminase (SGPT using standard protocol): SGPT of whole blood was determined using standard kit by Kind and King's method. SGPT catalyses the reaction to form pyruvate. Pyruvate formed is coupled with 2, 4 dinitrophenylhydrazine (or DNPH) to give the corresponding hydrazone which is brown in colour in alkaline solution. This can now be measured with the help of the colorimeter at a wavelength of 510 nm.

5. Acid hydrolysis of organs and determination of heavy metals: After autopsy, the rat organs of liver, spleen and kidney were isolated and a section of them were separated and refrigerated to carry out acid hydrolysis. For the hydrolysis, the organs were taken in a conical flask and 5 mL of conc. HNO_3 was added and digested. The setup was cooled and the acid vapours got condensed after the cooling took place. It was filtered in a volumetric flask of 10 mL the volume was made up to 10 mL using double distilled water. The samples were analysed in an Atomic Absorption Spectrophotometer (Perkin Elmer). The metal concentrations of lead and cadmium were determined using standard curve.

RESULTS

The results of the study are given in Tables 1, 2, 3, 4, 5 and Figs. 1, 2 and 3.

Effect of lead contaminated diet on body weight of rat: A general account and check on the body weight of rats over six weeks show a general trend of increase and decrease of weight. Rats belonging to groups I and II, which were fed with normal diet showed an increase in weight. Rats fed with

contaminated maize show an overall decrease in weight. Rats belonging to Group IV fed with Pb salts mixed with food show a rapid decrease in weight. Therefore, we can infer that Pb salts present in the nutritional intake of rats lead to a considerable loss of body weight. The difference is significant between the first and the sixth week.

Effect of lead on organ weight: In this experiment the Group I rats served as control. In Group II lesions were pronounced in the liver. Group III and Group IV rats showed swelling or bulb like structures in the liver. In comparison to the first 3 groups, the Group IV rats showed reduced organ weight : body weight ratio. The enlargement of kidney and spleen was pronounced in the Group IV rats.

Lead uptake by internal organ of rat: Acid hydrolysis of the organs like liver, kidney and spleen followed by atomic absorption spectroscopy showed high doses of lead accumulation in liver and spleen. However, in the kidney the amount of lead accumulated was significantly less in Group III rats but in group IV rats the accumulation was almost same.

Effect of lead on SGPT of rat: The results do not show much difference, therefore, we can infer that

Table 1: Change in body weight of rats.

		Change in Body Weight (g)					
Treatment	GroupNo.	Week-I	Week-II	Week-III	Week-IV	Week-V	Week-VI
Normal diet	I	50.00 ± 4.55	52.25 ± 3.7	41.66 ± 3.38	40.00 ± 5.77	49.00 ± 5.00	53.50 ± 5.50
Normal diet+ normal maize	II	38.50 ± 2.26	43.50 ± 4.92	41.25 ± 4.13	38.5 ± 3.77	42.83 ± 3.35	42.67 ± 3.35
Normal diet+ contaminated maize	III	40.25 ± 11.41	69.75 ± 12.06	69.00 ± 18.00	68.00 ± 18.00	69.50 ± 8.36	70.50 ± 22.5
Normal diet+ Pb salts	IV	65.50 ± 4.17	48.75 ± 3.04	50.00 ± 3.24	52.25 ± 3.88	50.00 ± 4.26	47.25 ± 5.41

Table 2: Effect of lead on organ weight of rats.

Group	Weight of Liver (in g)	Weight of Spleen (in g)	Weight of Kidney (in g)
I	2.50 ± 0.50	0.75 ± 0.25	1.00 ± 0.00
II	2.70 ± 0.17	0.83 ± 0.17	1.50 ± 0.29
III	2.50 ± 0.50	0.75 ± 0.25	1.00 ± 0.00
IV	1.88 ± 0.13	1.00 ± 0.25	1.00 ± 0.00

Table 3: Lead content of internal organs of rats.

Group	Liver (10^{-6}) g/g	Kidney (10^{-6}) g/g	Spleen (10^{-6}) g/g
I	1.04 ± 0.05	1.29 ± 0.00	1.08 ± 0.16
II	0.92 ± 0.25	0.53 ± 0.18	0.32 ± 0.14
III	0.57 ± 0.04	1.22 ± 0.06	0.21 ± 0.00
IV	0.86 ± 0.30	1.78 ± 0.16	1.01 ± 0.06

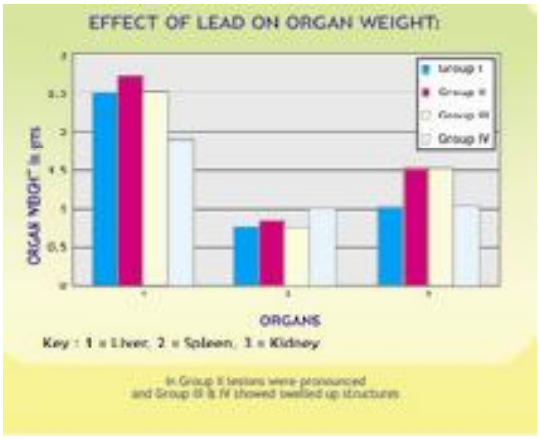

Fig. 1: Effect of lead contaminated diet on body weight of rats.

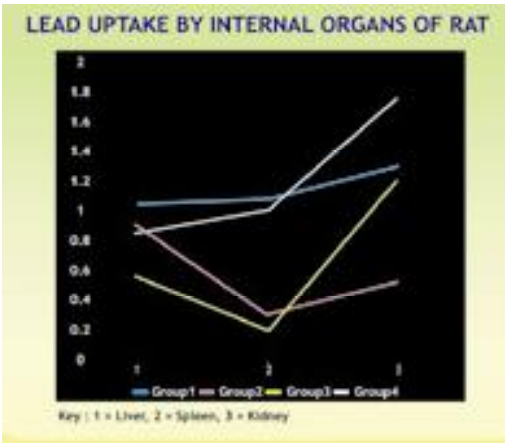

Fig. 2: Effect of lead on organ weight.

small amounts of Pb salts do not result in considerable changes in the SGPT count of rats.

Impact of lead toxicity on total haemoglobin percentage: The percentage of haemoglobin has shown a rapid decrease in the group of rats which were fed with contaminated maize and lead salts mixed with food. This indicates that the presence of Pb in the blood stream decreases the haemoglobin count and leads to blood toxicity even when present in small amounts. This is probably due to interference with the Hb biosynthesis pathway.

Table 4: Variation of SGPT level due to lead exposure.

	% Transmittance	Optical Density
Standard-1	59	
Standard-2	42	
Standard-3	34	
Standard-4	24	
Standard-5	17	0.498
GROUP I	87.50 ± 5.50	0.06 ± 0.03
GROUP II	74.00 ± 3.46	0.15 ± 0.03
GROUP III	77.50 ± 3.50	0.14 ± 0.05
GROUP IV	68.67 ± 0.66	0.16 ± 0.00

Table 5: Effect of lead toxicity on haemoglobin.

Group number	Haemoglobin percentage
I	13.8 %
II	14.4 %
III	12.2 %
IV	11.8 %

DISCUSSION

Heavy metals are toxic to plant and animal systems (Bringezu et al. 1999, Brune et al. 1994, Grill et al. 1989, Keltjens et al. 1998). Toxicity in animals is pronounced because of the complex system and a central circulation of fluids. In this investigation, a unit effect of heavy metal has been shown through a plant system. The metal uptake by maize plant from contaminated soil was investigated, and in order to remove the matrix effect of the heavy metal, the heavy metal was given in inorganic form in diet to mammalian system. The toxicity at every level was more in the Group III and Group IV rats exposed to lead in comparison to the untreated groups showed a differential toxicity. The uptake is correlated with different toxicity parameters like haemoglobin % and S.G.P.T count, though the haemoglobin % was reduced significantly, S.G.P.T toxicity was not accurate.

Heavy metal toxicity was denoted by Cha (1987) who showed that heavy metals like Cd and Hg

Fig. 3: Lead uptake by internal organs of rats.

in rats are reduced by garlic. Dhir et al. (1990, 1993) showed similar findings in mouse where clustogenecity of Pb and Al is reduced by extracts of *Phyllanthus emblica.* Similarly in this case metal toxicity is apparent, both in third and fourth groups of rats, and comparatively less in the fourth group probably because of partial detoxification by maize tissue. In accordance with the findings of Lewis et al. (2001), deposition of Pb was found to be mainly in skin and soft tissues and primarily it affected the Hb% of albino rats.

So it is obvious that there is adequate possibility of heavy metal toxicity through food chain in mammalian system. Only, it can be reduced by using antidotes or some matrix that reduces metal dispersal.

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