PROTECTIVE EFFECT OF POLYBION AGAINST RADIATION AND CADMIUM INDUCED HISTOPATHOLOGICAL CHANGES IN THE LIVER OF SWISS ALBINO MICE.

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Summary

The extensive use of atomic energy now a days in various branches of natural economy, science and technology, radiodiagnosis, radiotherapy, industries, agriculture, nuclear researches etc. has made radiation injury an urgent problem attracting the attention not only of specialists in a variety of clinical disciplines but also of a vast army of theoretical scientists. Metals like cadmium have always been an intrinsic components of earth crust with the continuing trends towards and increasing human activities involving man may become exposed to concentration of toxic metals presenting a potential threat for survival. The severity of the damage can be modulated by treated the animals with antioxidants. In view of the potential for practical application, a variety of compounds are being tested for their radioprotective activities. Among these, vitamins hold a great promise.

Polybion is a vitamin B-complex mixture that is easily available in the market in the form of tablets, syrup, capsules and injection. The drug is prescribed in India for the treatment of various types of body dysfunctions.

In light of the above, the present study was aimed to evaluate the protective effect of polybion against radiation and cadmium induced histopathological changes in the liver of Swiss albino mice.

The animals from all the experimental groups were sacrificed by cervical dislocation at each post-treatment intervals of 1,2,4,7,14 and 28 days. After sacrificing the animals, pieces of the liver were taken out and some of them were immediately fixed in Bouin's fluid for histological observations by routine procedure.

The changes observed were distortion of hepatic architecture, intracellular oedema, narrower sinusoids, cytoplasmic degranulation, vacuolation, hyperaemia, pycnotic and crenated nuclei.

In the combined treatment of radiation and cadmium chloride synergistic effects were observed. The liver of polybion treated animals exhibited less severe damage as compared to non-drug treated animals at all the corresponding intervals. An early and fast recovery was also noticed in polybion pretreated animals. Thus, it appears that polybion is potent enough to check cadmium and radiation induced hepatic lesions in Swiss albino mice.

Key Words - Polybion, Antioxidant, Liver, Radioprotection

Introduction

All high energy radiation, weather particulate or electromagnetic, are capable of inducing excitation or ionization of atoms or molecules of both living and non living matter, called ionizing radiation. Ionizing radiations are a double-edged sword; they can both cure cancer as well as cause it. It depends on the way we use them.

Radiations cause biological damage by depositing energy in the system. Different types of ionizing radiations cause similar kind of damage[1-2].

Interesting histological and clinical evidences of hepatic radiation injury have appeared in the last 40 years. Radiation induced changes were observed in hepatic parenchymal and reticulo-endothelial tissue, arterioles and capsule in three patients who had received high levels of Cobalt-60 radiations for carcinoma of the lower zones of the lungs. The gross pathological changes confined to the irradiated superior portion of the liver[3].

Histological changes observed in the liver of *Uromastix hardwickii* gray after exposure to three doses (i.e. 2.25, 4.50 and 9.00 Gy) of gamma radiations from Cobalt-60 source included cytoplasmic degranulation, swollen hepatocytes, pycnosis and increase in bile pigmentation. On day-14 the signs of recovery were noticed[4].

Apart from ionizing radiations, human beings are continuously exposed to a wide range of metallic pollutants from the environment. Man releases lots of chemicals in the environment by mining, smelting, discharging industrial wastes, agricultural and domestic waste, burning fossil fuel (coal) and by use of pesticides. These pollutants cannot be easily and readily detoxified by metabolic activities; as a result they accumulate and cause severe toxic effects. These toxic chemicals get incorporated in food chain and they enter in the biological system. Cadmium is reported as one of the most toxic elements in the environment, which caused severe effect to the biological system.

No systematic work has appeared so far on the use of such Vitamins as chelators for their protective role against heavy metal like Cadmium intoxication or the combined use of such metal and radiation. Therefore, in the present study an attempt has been made to investigate the possible prophylatic role of Polybion (Vitamin B-complex mixture) against the cadmium and radiation induced histopathological changes in the liver of Swiss albino mice.

Modulation of radiation and cadmium induced biochemical changes in the jejunum and liver of Swiss albino mice by polybion have also been studied. The alterations in the biochemical parameters were noticed in the form of increase or decrease in the values of total proteins, glycogen, cholesterol, acid & alkaline phosphatase activity, DNA and RNA [5-6].

Materials and Methods

Animals

Six to seven weeks old male Swiss albino mice were procured from CCS University, Hissar and maintained at 20-25^{0}C. The animals were provided with standard mice feed and tap water *ad libitum.*

Drug (Polybion) :

Polybion syrup was procured from Merck Co. India. The drug was fed orally at the dose rate of 0.01 ml/animal/day. The drug was given from seven days prior to cadmium chloride treatment or irradiation.

Source of Irradiation :

Cobalt ^{60} gamma radiotherapy source (Theratron) of AECL make obtained from Canada was used to irradiate the animals. This facility was provided by the radiotherapy department of prince Bijay Singh Memorial hospital, Bikaner (Rajasthan), India. The animals were irradiated at the dose rate of 0.85 Gy/min.

Cadmium

The cadmium salt in the form of $CdCl_2$ (cadmium chloride) was used for the present study. It was purchased from SDS chemicals, india.

Plan of Experimentation:

The animals were divided into following groups :-

GROUP-I –(Sham-irradiated animals-normal)

GROUP-II- Only cadmium chloride treated animals.

GROUP-III- 4.0 Gy of gamma irradiated animals.

GROUP-IV -$CdCl_2$ + 4.0 Gy of gamma radiation.

GROUP-V - $CdCl_2$ + Polybion

GROUP-VI - 4.0 Gy gamma radiation + Polybion

GROUP-VII- $CdCl_2$ + 4.0 Gy gamma radiation + Polybion

Autopsy of Animals -

Three animals from each group were autopsied after 1, 2, 4, 7, 14 and 28 days of treatment. The animals were sacrificed by cervical dislocation.

Results and Discussion

In the present experiments histopathological changes were found in the liver of Swiss albino mice exposed to 4.0 Gy gamma rays with or without cadmium chloride treatment. The changes observed on day-1 after exposure were distortion of hepatic architecture, intracellular oedema, narrower sinusoids, cytoplasmic degranulation, vacuolation and pycnotic nuclei. The changes were more marked on day-4 and continued up to day-14. But on day-28 the sign of recovery was observed. In the combined treatment of radiation and cadmium chloride synergistic effects were observed. The liver of polybion treated animals exhibited less severe damage as compared to non-drug treated animals at all the corresponding intervals. An early and fast recovery was also noticed in polybion pretreated animals.

Liver, kidney, testes and severe gastrointestinal disorders have been reported due to cadmium ingestion in experimental animals. Most of the cadmium absorbed from the lung and intestine initially is deposited in the liver binding to a low molecular protein, metallothioneine, which has a high binding capacity for cadmium, zinc and copper. Later on, transferred as storage protein for cadmium. Cadmium is an integral part of metallothioneine. It can induce metallothioneine synthesis in many organs including liver and kidney. The binding of intracellular cadmium to metallothioneine in tissues protects against the toxicity of cadmium.

Gastrointestinal absorption of cadmium was reported by many scientists[7-11]. They also reported the role of metallothioneine in the absorption of cadmium through intestine. According to some others more cadmium is absorbed in the proximal part of intestine in natural physiological conditions[12].

Cadmium is a potent pollutant and enters the biological systems through air, water and food[13]. The living beings are also simultaneously exposed to radiation which may be man made (research labs, diagnostic clinics, nuclear reactors, radiotherapy etc.) or high background radiations. The polybion might have protected the animals from radiation by more than one mechanism due to multiplicity of these properties.

It has been shown that the exogenous application of vitamin B complex and vitamin E and GSH, increase glutathione levels in the tissues on one hand and maintains -SH groups and increases protein synthesis on the other hand [14]. Studies have also indicated that amount of certain vitamins decreases during heavy metal intoxication and recovery takes place during their exogenous application either alone or in combination [15].

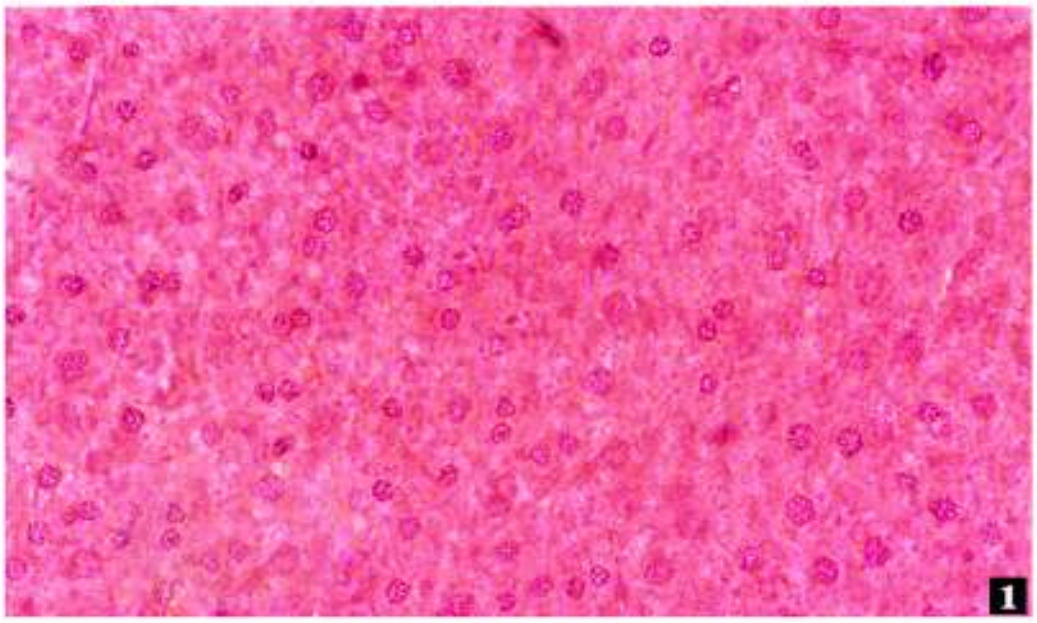

Fig. 1. Normal hepatic architecture

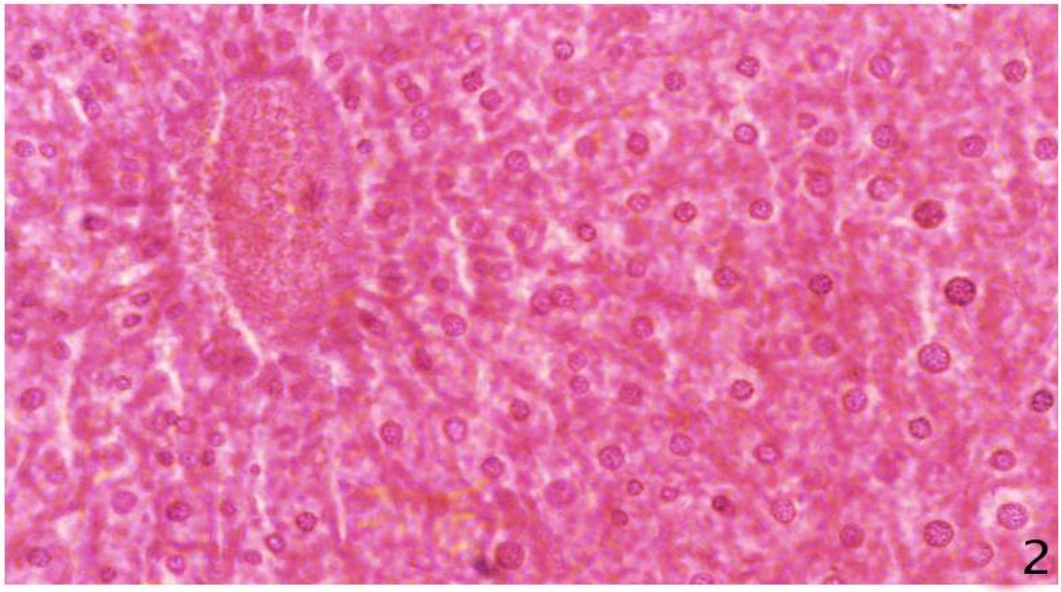

Fig. 2. Showing intracellular oedema, narrower sinusoids and hyperaemia of blood vessels

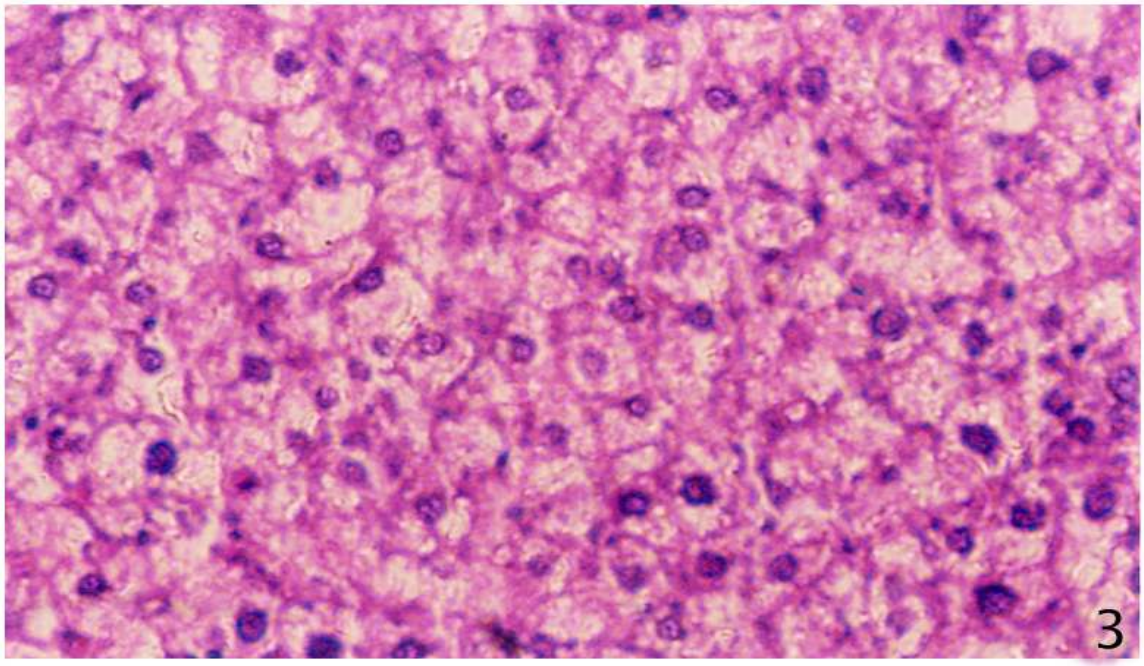

Fig. 3 Showing cytoplasmic deregulation vacuolation and many cells devoid of nuclei.

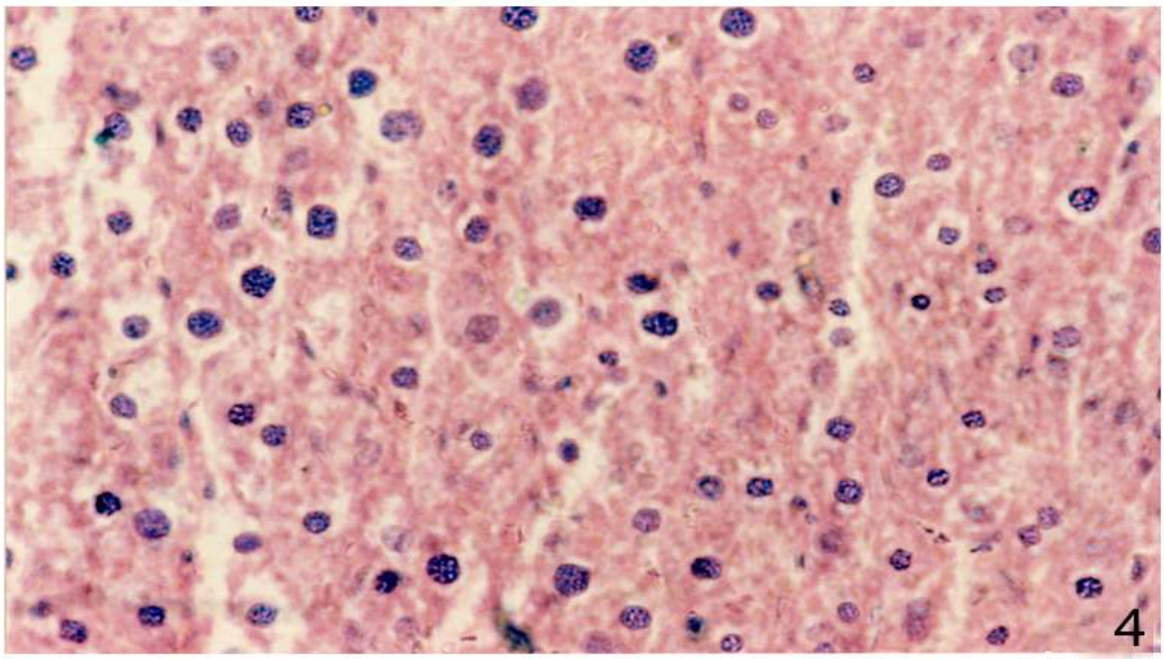

Fig. 4 Showing distorted hepatic architecture, pycnotic nuclei and necrotic cells.

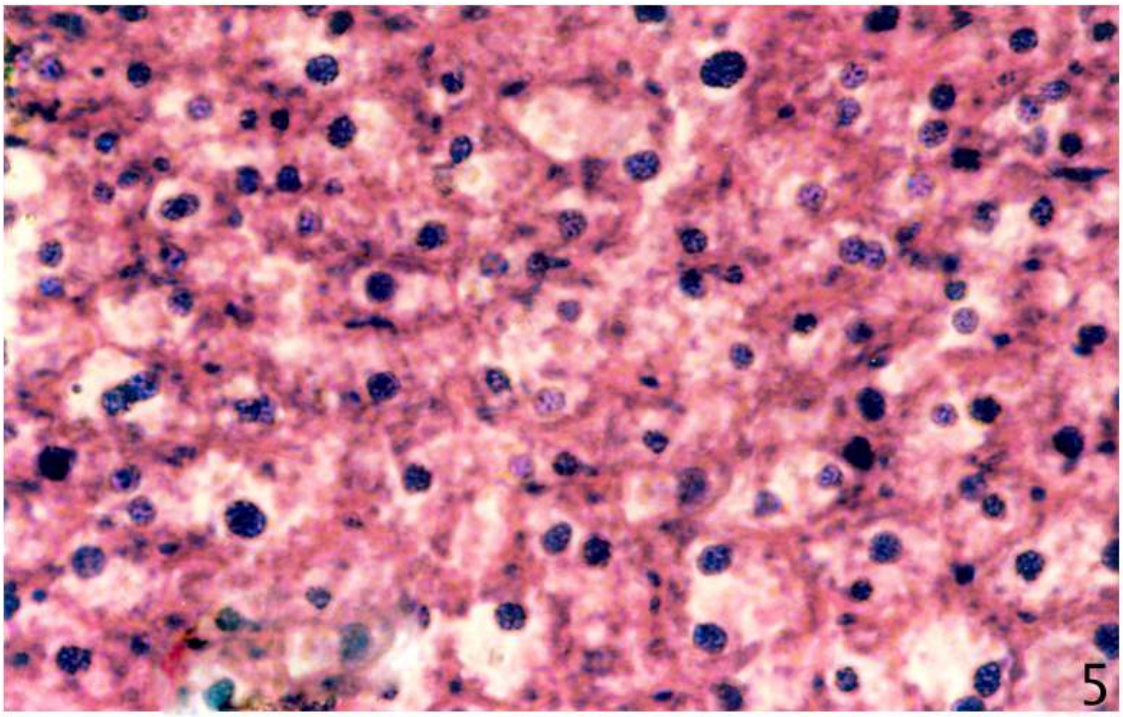

Fig. 5. Showing cytoplasmic degranulation vacuolation and pycnotic nuclei.

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References

1. Desjardins, A. V.: Action of roentgen rays and radium on the gastrointestinal tract: Experimental data and clinical radiotherapy. Am. J. Roentgenol. Radium Therapy, 1931; 26: 335.
2. Casarett, G.W.: pathology of single intravenous doses of polonium. Radiat. Res. (Suppl.), 1964; 5 : 246.
3. Ojata, K., Hizawa, K., Yashida, M., *et al.* : Hepatic injury following irradiation, A morphologic study, Tokushima, J. Expt. Med., 1963; 9 : 240.

4. Gupta, M.L. and Uma Devi, P. : Response of Piscine liver to external gama irradiation. Radiobiol, Radiother, 1990; 31 : 289.

5. Purohit, R. K.; Purohit, R., Bhartiya, K.M., Chakarwarti, A. and Gupta, M.L. : Modulation of radiation and cadmium induced biochemical changes in the jejunum of Swiss albino mice by polybion. Indian Journal of Radiation Research Varansi (India) 2006; Vol-3 No.-4 : P. 101.

6. Joshi, R., Purohit, Ramesh, Purohit, R.K. : Protective effect of polybion against radiation and cadmium induced biochemical changes in the liver of Swiss albino mice. NCETMB, DTMC, Bikaner. Rajasthan (India) 2007.

7. Cherian, M.G., Goyer, R. A. and Vlberg, L.S.: Gastrointestinal absorption and organ distribution of oral cadmium cloride and cadmium-metallotionein in mice. Toxicol. Environ. Health, 1978; 4 : 861.

8. Kello, D., Sugawara, N., Voner, C. and Foulkes, E. C. : On the role of metallothionein in cadmium absorption by rat jejunum in situ. Toxicology, 1979; 14:199.

9. Chertok, R. J., Sasser, L. B., Callaham, M. F. and Jorboe, G. E. : Influence of cadmium on the intestinal uptake and absorption of calcium in the rat. J. Nutr., 1981; 111:631.

10. Foulkes, E.C. and McMullen, D.M. : Endogenous metallothionein as determinant of intestinal cadmium absorption : A re-evaluation. Toxicology, 1986; 38 : 285.

11. Sugawara, N. and Sugawara, C. : The effect of aluminum ingestion on intestinal cadmium absorption, lipid peroxidation and alkaline phosphatase activity. Toxicol. Lett., 1992; 61 : 225.

12. Sorensen, J. A., Neilsen, J. B. and Andersen, O. : Identification of the gastro-intestinal absorption site for cadmium chloride in vivo. Pharmacol. Toxicol.,1993; 73:169.

13. IRPTC : IRPTC legal file 1986, Geneva International Register of potentially toxic chemicals, United Nations Environment Programs, 1987; Vol-1.

14. Bapu, C. : Protective effects of monothiols and Vitamins in methylemercury detoxication : A histopathological and biochemical study. Ph.D. thesis submitted to University of Saurashtra, Rajkot, 1993; 1-203.

15. Tandon, S.K. and Jain, V.K. : Efficacy of adjuvants in prevention or therapy of lead intoxication. Ind. J.Toxicol.,1994; 1 (1 & 2) : 25-32.