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PROTECTIVE EFFECT OF POLYBION AGAINST RADIATION AND CADMIUM INDUCED CHANGES IN TOTAL PROTEINS AND CHOLESTEROL CONTENT IN THE LIVER OF SWISS ALBINO MICE

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ABSTRACT

More uses of radiation and its concern technology is increasing more danger of its acute as well prolonged effects. As we all know that for the global changes in the climate is due to human activities which resulting in the radiation pollution turning in the many severe environmental problems or hazards and human exposure is one of the most common incident in the same. On the other side of the coin heavy metals are also toxic and present in the food chain in many ways. If a reaction occur between radiation and heavy metal it is going to be very severe concerning to our health. In the present study six to eight weeks old male Swiss albino mice were exposed to 3.0 and 6.0 Gy of gamma rays with or without cadmium chloride treatment. The animals of experimental groups were administered *Polybion* for seven days prior to radiation or cadmium chloride or combined treatment. The total proteins and cholesterol content were observed in the liver of Swiss albino mice.

KEYWORDS: *Polybion*, Radiation hazards, Total proteins, Cholesterol, Cadmium chloride.

INTRODUCTION

Our modern environment is beset with various kinds of artificial factors most of which are pollutants, arising from an ever-increasing culture. It stands to reason that in such a complex system various factors like radiation (both natural as well as artificial), metals (especially the heavy metals like mercury, lead, cadmium), various inorganic compounds and organic agents continue to produce synergistic effects. Due to such a situation pathological conditions like cancer and other diseases have arisen. To counteract such deleterious conditions, use of

ionizing radiations to treat such diseases has increased rapidly, in the field of radiodiagnosis radiotherapy, research or agriculture.

Ionizing radiations can penetrate the tissues and impart their energy to the atomic and subatomic structures, which build the cells, thereby exciting those causing consequent alterations in important metabolic chemical reactions which eventually become apparent as radiation generated damage.

An ever increasing use of various types of electromagnetic radiations in the fields of scientific research, applied technology and medicine has produced many instances of radiation damage which has increased the concern of scientific workers, both in field clinical medicine and theoretical biology.

Gupta *et al*. (1989)[1] opined that cadmium ranks upon the most hazardous heavy metals being used in modern society and the United Nations have rightly included cadmium in the list of chemical substances and processes which have been found to have deleterious effects globally (IRPTC, 1987).[2]

Oral intake of cadmium produces deleterious gastrointestinal syndrome while its inhalation causes edema in lungs, eventually leading to its fibrosis. Workers involved in industries related to manufacturing of vapor lamps, alloys, Cd-batteries and glass blowing have experienced dyspnoea, headache, cough and vomiting after inhaling fatal concentration of cadmium. On the cellular level, cadmium has high affinity for Sulphydryl (-SH) groups of enzymes and other important biological compounds.

In this regard, very little amount of work has been done to enumerate the effects of administration of metals, in combination with radiation. It is expected that radiation and metallic compounds, acting together may have a supra additive (synergistic) influence and thus it is pertinent to investigate the combined effects of ionizing radiation and metallic pollutants like cadmium.

It has been suggested that polybion is good for almost everyone on a regular basis. It reduces or eliminates the risk of environmental pollutants, normalizes cholesterol, reduces unwanted fat, cures ulcers, reduces or prevents cancer, has the highest content of vitamin C among natural sources, detoxifies the body, regulates digestion, has inhibiting effects against the

HIV virus, promotes metabolic functions and can produce these results in a dried, natural and unprocessed form.

In the past investigations regarding toxicity of cadmium in tissues of various organisms including those of man have been conducted, shedding light on its long biological life period of 30 years in men (Nordberg and Kjellstrom, 1979).[3] Cadmium is easily absorbed by the organism body after which it gets concentrated in the tissues where it readily associates with low molecular weight metallothionein proteins, particularly in the kidney, liver and gonads. Among other tissues, lungs, prostrate, bones and central nervous system have been found to be affected by cadmium. In recent years uses of cadmium in industries related to mining, glass blowing, nickel-cadmium battery production have grown rapidly, making its biological encounter with our biological systems more and more common. In such a situation it has been estimated that our body can tolerate a maximum amount of 60-70µg/day and over dose of cadmium causes severe pathological complication in the body which has been exemplified by occurrence of the well-known Itai-Itai disease related to the stiffness of joints in the body and associated pain in elderly Japanese people (Friberg*et al.*, 1974).[4]

Cadmium is known to cross the blood-liver barrier and is retained in the liver tissue and studies (Stowe *et al.*, 1972)[5] have shown that cadmium inhibits the liver enzymes containing sulphahydryl (-SH) groups. Anatomically, cadmium exposure causes lesions in the Gasserian ganglia, spinal sensory ganglia of spinal cord, cerebrum and cerebellum of adult rat livers (Gabbaini*et al.*, 1967[6] Nomiyama*et al.*, 1973).[7] Studies have shown that administration of cadmium chloride at a dose level of 20ppm induces alterations in various organs. Cadmium salts have also been implicated in generating chromosomal aberrations (Mukherjee *et al.*, 1988).[9]

MATERIALS AND METHODS

For the study, adult healthy male Swiss albino mice (6-8 weeks old) were procured from LalaLajpat Rai University of Veterinary and Animal Sciences, Hisar (India). The animals were kept in polypropylene cages. They were fed with standard mice pellet diet (Hindusthan Liver Limited, India) and water was given *ad libitum*. The cages were cleaned daily. The temperature of the room was maintained between 22-27ºC.

The Govt. Dungar College, Bikaner is registered under CPSCEA, Chennai, India (Registration no. 1066/ac/07/CPCSEA) and has its own Institutional Animal Ethics

Committee (IAEC). The animals used for the present investigation were sacrificed strictly under the supervision of IAEC of the college.

Cadmium chloride treatment

Cadmium, in the form of cadmium chloride was administered orally in drinking water. Cadmium chloride was procured from S.D. Fine Chemicals Private Limited, Boisar (Mumbai), India. 20 ppm aqueous solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloridein 1000 ml of distilled water.

Source of irradiation

The animals used in the experiment were irradiated at the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan) by Theratron, a Cobalt-60 beam therapy unit, procured from Atomic Energy Agency Limited, Canada.

Mode of irradiation

All the mice were exposed to Co^{60} γ-radiation simultaneously in a well-ventilated wooden box of size 30 cm x 30 cm x 5cm having a glass lid. The box was placed at a distance of 75cms from the radiation source.

During experimentation, the dose rate varied from 0.97 Gy/min to 1.97 Gy/min. The dose was calculated at the midpoint by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Amla*(Polybion)*

Fresh fruits of the *E. officinalis* were cleaned, cut into small pieces, air dried, powered and extracted with double distilled water (DDW) by refluxing for 36 hrs. (12 hrs.x 3). The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in DDW just before oral administration. An approximate 38 percent yield of the extract was obtained. The drug was given from seven days prior to cadmium chloride treatment or irradiation or combined treatment.

Experimental design

The animals were grouped as under

Group - I (Sham Irradiated Animals-Normal)

Animals of this group were sham-irradiated and served as normal group.

Group - II (Cadmium chloride treated animals)

The animals of this group were orally fed with cadmium chloride solution at the dose of 20 ppm *ad libitum* in drinking water continuously till the last autopsy day.

Group - III (Only irradiated animals)

Animals of this group were exposed to sub-lethal doses of gamma radiation from Cobalt 60 source. This group was divided into two sub-groups, each of which was exposed to a different dose of radiation:-

Sub group IIIa: 3.0 Gy

Sub group IIIb: 6.0 Gy

Group - IV (Animals treated with Radiation and Cadmium chloride)

Mice of this group were administered cadmium chloride orally at a dose of 20 ppm and were also exposed to different doses of radiation. This group was further divided into two sub groups on the basis of radiation dose received:

Sub group IVa: 3.0 Gy + $CdCl_2$

Sub group IVb: 6.0 Gy + $CdCl_2$

Group - V (Cadmium chloride and *Polybion* treated animals)

The mice of this group were orally fed with cadmium chloride at a dose of 20 ppm and were administered *Polybion* orally at a dose of 1000mg/kgb.wt./animal/day, from seven days prior to cadmium chloride treatment and this was continued up to last day of autopsy.

Group - VI (Radiation and *Polybion* treated animals)

The animals of this group were irradiated with a sub lethal dose of gamma rays from a cobalt-60 source. *Polybion* was provided orally, from seven days prior to the irradiation and continued till the 28^{th} day.

This group was further divided into two sub-groups on the basis of radiation dose administered:

Sub group VIa: 3.0 Gy + *Polybion*

Sub group VIb: 6.0 Gy + *Polybion*

Group - VII (Radiation, Cadmium Chloride and Drug treated animals)

Mice of this group were given cadmium chloride orally at the dose of 20 ppm and were also administered *Polybion (*1000mg/kgb.wt./animal/day) from seven days prior to cadmium

chloride treatment and irradiation and continued till the last day of autopsy interval (i.e. 28th day). This group was further divided into two sub-groups, each of which was irradiated with a different dose of radiation:

Sub group VIIa: 3.0 Gy + $CdCl_2$ + *Polybion*

Sub group VIIb: 6.0 Gy + $CdCl_2$ + *Polybion*

Autopsy

Five animals of each group (groups II to VII) were autopsied after cervical dislocation at each post-treatment intervals of 1, 2, 4, 7, 14 and 28 days. In addition, five sham-irradiated (normal) mice were also autopsied in a similar manner.

Immediately after the autopsy, the liver was taken out and weighed. Later on, the width and length of liver were also recorded. Afterwards, part of liver was kept at -20^{0}C for biochemical investigation.

Parameters selected for study

A. Total proteins [Lowry *et al.*, 1951][9]

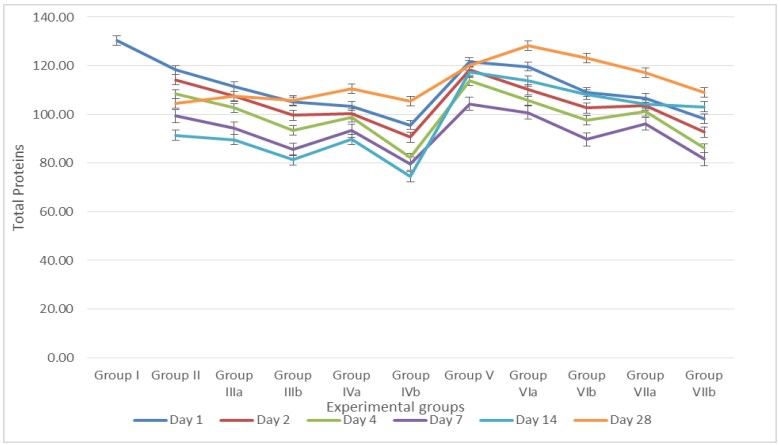
B. Cholesterol [Oser, 1965][10]

RESULTS

Total Proteins

In the present investigation, the total proteins content decreased in the non-drug treated groups II, III and IV as well as *Polybion* treated groups V, VI and VII respectively. After combined treatment of radiation and cadmium chloride the changes observed were more severe showing synergistic effect of both the agents. The value decreased up to day-14 in the group II, III and IV and up to day-7 in the *Polybion* treated groups V, VI and VII respectively. In the *Polybion* treated groups, an early and fast recovery observed showing protection provided by *Polybion*.

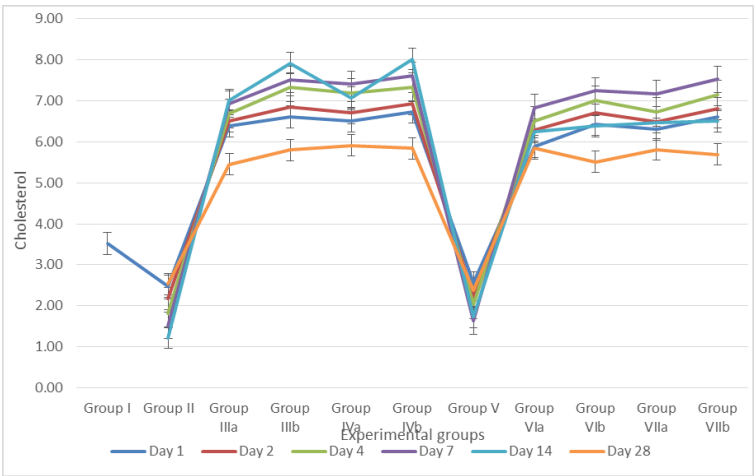
Histogram- 01 Variations in the values of Total Proteins (mg/gm of tissue weight) in the liver of mice in various experimental groups (Mean ± S.E.)



Cholesterol

In the present investigation, the cholesterol content declined up to day-14 in the cadmium chloride treated group II and day-7 in the cadmium chloride and *Polybion* treated groups V. Thereafter, an increase in the value was seen up to day-28 in both the groups. Similarly, an increase in the value was noted up to day-14 in non-drug treated groups III, IV and day-7 in the *Polybion* treated groups VI and VII, thereafter value declined up to day-28 in all the groups. After combined treatment of radiation and cadmium chloride synergistic changes were observed. In the *Polybion* treated groups the decrease or increase were less severe showing protective effects of the *Polybion*.

Histogram- 02 Variations in the values of Cholesterol (mg/gm tissue weight) in the liver of mice in various experimental groups (Mean ± S.E.)



DISCUSSION

The liver cells are active sites of protein synthesis. Normally rat cerebellum exhibits significantly elevated values as compared to pig and mouse. With 72 R and 240 R irradiation protein concentration exhibits a pronounced decrease in all the animals excluding guinea pig cerebellum after 72R irradiation where there was noticed a slight increase in protein amount. Biochemical investigations showed that X-irradiation inhibited protein concentration (DeVellis*et al.*, 1967).[11]

The synthesis of proteins also depends upon the activity of DNA and RNA (Monesi, 1967).[12] Similarly, the protein content of the liver decreases more with the increase in the dose of irradiation. The reasons behind the decrease in the protein content of the liver in various groups may be either the decline in the rate of protein synthesis or increase in the protein consumption. Reduced rate of the protein synthesis may be due to unfavorable conditions like unavailability of one or more essential enzymes and/or reduction in the sites of protein synthesis (Bacq and Alexander, 1961).[13] Increase in protein consumption with the increase in the dose of irradiation may be due to the increased demand of protein in repair processes or increased activity of lysosomal enzymes (Bacq and Alexander, 1961,[13] Wills and Wilkinson, 1967.[14]

Sulphydryl groups (-SH groups) of nuclear proteins play an important role in forming the mitotic apparatus (Mazia, 1961).[15] This group belongs to the most sensitive configuration, which becomes oxidized by ionizing radiation (Okada, 1970).[16] Ord and Stocken (1968).[17] demonstrated that oxidation of –SH group is capable of blocking the transition of cells from G_1 phase to S phase by disturbing phosphorylation in nuclei, which in turn disturbs DNA synthesis.

DeVellis and Schjeide (1969)[11] irradiated (100 to 1500R) the heads of two days old rats and observed inhibition in increase of nucleic acids, individual enzymes, total proteins, and myelin in rat liver, stems and cerebrum. When irradiation was administered at later ages a decrease in inhibition was observed with increasing age.

According to Grant (1969)[18] the protection of protein is due to hydrogen atom donation by the protector. Irradiation of the biological material produces unpaired electrons which can migrate frequently to reach location of minimum energy. In the present study, availability and

accessibility of *Polybion* causes many of these unpaired electrons to be scavenged due to a good antioxidant activity of *Polybion*.

The protein levels may decrease its lysis caused by X-irradiation or may be at the synthesis level or the depression may be due to inhibition of enzymes participating in the activation of amino acid and transferring them to t-RNA (Wender and Zgorzalewiez, 1970)[19] or it may be due to the inhibition of the release of synthesized polypeptides from polysomes (Kim *et al.*, 1970).[20]

Shah and Gadhia (1977)[21] reported effects of total body gamma-irradiation on pigeon with 400 rads and found that protein content was lowered in liver after 1 hour, 24 hours, 48 hours and 72 hours, post irradiation that is in agreement with the present investigation.

The present results showed that the change in protein concentration in the liver depends upon the dose of irradiation provided and the resultant cell death. Due to irradiation, there is an alteration in protein synthesis pattern, which in some tissues has been reported to be retarded, whereas, in others elevation in protein synthesis is discernible. Shah and Ghadia (1977)[21] reported that the whole body exposure at sub lethal and near lethal doses step up protein synthesis capacity of liver.

Saraswat (1986)[22] reported that total protein content remained at normal values at 18 days of prenatal age, and at 2,23 and 44 days post natally, when Long-Evans rats were exposed to 100 R X-radiation at the 14^{th} day of gestation. But it was reported that the intensity of protein synthesis, especially the synthesis of acid soluble nuclear proteins, was inhibited by irradiation less than 10 per cent decrease was recorded in the amino acid pool which was probably due to the slightly disturbed permeability of blood barrier after irradiation on the basis of reduced amounts of histones after neutron radiation *in utero*, one can assume that the replacement process variants during embryo genesis might be a radiosensitive process. Shore *et al.,(*1969)[23] showed that amino acid incorporating capacity of post mitochondrial supernatant of rat liver decreased by one third after X-irradiation of the fetus with 180 R on the 13^{th} day of pregnancy. This is clue to decreased protein synthesis in post treatment groups.

Combined exposure of radiation and cadmium chloride showed the similar pattern of decline but this decline was more severe as compared to the individual exposure because both

radiation and cadmium chloride have strong affinity to the sulphydryl groups (-SH) of proteins, may bind with the carrier molecules and decreased the rate of transport. In the *Polybion* treated groups, the decrease in value was of lesser magnitude. Gajawat*et al.* (2001)[24] also reported decreased concentration of proteins till day-2 but elevated further up to last autopsy interval without reaching normal after the treatment of Cadmium chloride and radiation. Cadmium chloride and radiation attack –SH groups of proteins and the level of blood and liver glutathione (GSH) was decreased. This lowered GSH level may be responsible for the biochemical alterations.

Schjeide*et al.* (1966)[25] observed that in animals exposed at 2 days of age and assayed at 9-10 days of age (prior to the extensive myelination beginning at 2 weeks), there occurs a specific increase in the proportion of cholesterol seems to be synthesized at a relatively more rapid rate in irradiated liver and since it apparently cannot be broken down in this tissue, the increase in readily detected.

Cholesterol is present in the liver in greater quantity than any other single constituent apart from water, comprising 4-5 per cent of the fresh weight of cerebral white matter. In the normal adult liver is it exists almost entirely as free cholesterol. During development of liver in growing animals, however, the proportion of cholesterol esters can rise to 20 per cent of the concomitant cholesterol. This maximum value in man and chick was at the commencement of myelination. It is supposed that the normalizing effect of *Polybion* on exchange of lipids across plasma membrane in the event of chronic irradiation of animals and permanent introduction of the drug are associated with radioprotective effect of *Polybion*. A significant increasefound in cholesterol level at days 14 and 28 in the present investigation may be due to enhanced glycogen accumulation at this age because more substrate for these metabolic processes is made available as a result of tissue breakdown. Substrate utilization for synthesis rather than for oxidation predominates during the post-exposure period because adaptation to the state of starvation in irradiated animals is restricted to non-oxidative enzymes (Shah and Bhatvdekar, 1979).[26]

Exposure to radiation produces a stress reaction in the animal body and stimulates steroid hormone synthesis through the hypothalamic pituitary axis. At six weeks of age there occurs an increase in cholesterol which is probably due to increased lipogenesis because more substrate for this is made available due to tissue breakdown after irradiation (Shah and Bhatvdekar, 1979).[26]

When animals at the two days of age were irradiated and then assayed at 9 to 10 days of age (before extensive myelination starting at two weeks) it was found a particular increase in levels of cholesterol. It seems that cholesterol is synthesized at a comparatively more rapid rate in the irradiated liver and because it is apparently not degraded in this tissue, the increase is readily detected (Schjeide*et al.*, 1966).[25]

In present study, groups IV and VI exhibited similar pattern of decline in cholesterol level. But here decline was serving due to the synergistic or additive effect of cadmium chloride and gamma radiation. This is in good agreement with the results of Basu (2010)**27** who also reported that decline in the value of cholesterol was more severe after the combined treatment of radiation and cadmium chloride.

Vyas (2003)[28] also studied a decrease in cholesterol content in the liver of Swiss albino mice after exposure of 5.0 Gy of gamma rays with or without cadmium chloride treatment. After combined treatment synergistic changes were observed. In the Liv.52 treated experimental animals a less severe decline was seen showing protection by Liv.52. Yadav (2008)[29] also observed similar changes with polybion in the liver of Swiss albino mice. These findings are in accordance with our present investigation with *Polybion*.

Machanism of protection provided by *polybion*

The possible mechanisms of action of *Polybion* may be as under:

1. Radiation shows changes includes mutation due to breakage of DNA strands and this activity induced significant changes in the lipid as well as proteins. Though Polybion is showing antioxidant properties show it reduce oxidative consequences produced by radiation.
2. Polybion inhibit carcinogenic activities by inhibing mutagenesis.
3. It prevent mucosal damage by making a protective layer when irradiate.
4. Zhang *et al*[30] reported that polyphenols present in the *Polybion* is concerning to the protection of the body as it is a wonderful scavenger of the oxygen radicles.
5. Jeena and Kuttan 1995[31] reported the property of the Polybion that it increases GSH level and having antioxidant potential also.
6. Hari Kumar *et al.*, 2004[32] reports its potential as a wonderful radio-protector as its easy availability as well as its nontoxic impact even at high dose level.

CONCLUSION

1. The liver of Swiss albino mice suffered with radiation and cadmium induced changes at histological and biochemical levels.
2. Alterations in the histological structures followed the biochemical changes.
3. The combined treatment of radiation and cadmium chloride showed synergistic changes.
4. The liver of *Polybion* treated animals showed less severe radio lesions and an early and fast recovery in comparison to non-drug treated animals. Thus, it seems that *Polybion* has protected the liver at both the dose levels with and without cadmium chloride treatment.
5. The *Polybion* might have protected the animals from radiation by more than one mechanism due to multiplicity of its properties.
6. Thus, *Polybion* has the ability to impart appreciable amount of radioprotection and can be tried on cancer patients undergoing radiotherapy in order to minimize the adverse effects of radiation.

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