

PROTECTIVE EFFECT OF POLYBION AGAINST RADIATION AND CADMIUM INDUCED CHANGES IN ACID PHOSPHATASE CONTENT IN THE LIVER OF SWISS ALBINO MICE

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Article Received on
28 Feb. 2022,

Revised on 18 March 2022,
Accepted on 08 April 2022

DOI: 10.20959/wjpr20225-22932

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ABSTRACT

Man has always been exposed to ionizing radiation from natural sources. The natural background exposure varies with the locations. No ill effects have been uniquely correlated. Either no deleterious effects are produced at these levels of exposure or their frequency is too low to be statistically observable. Direct source of information on radiation hazards in man is obviously based on follow up of population groups exposed to certain levels of radiation. Harmful effects of ionizing radiations recorded from exposed radiologists during 1920s and 30s, miners exposed to airborne radioactivity, workers in the radium industry, follow-up data of Japanese nuclear bomb survivors of Hiroshima and Nagasaki, the Marshallese accident in 1954, and the

victims of the limited number of accidents at nuclear installations including Chernobyl form the basis of data to develop various recommendations. From these various sources, the pattern of events that follows a total body exposure to a dose of ionizing radiation have been well documented. Mostly this information is from situations involving higher doses and dose rates. In the present study biochemical parameters of acid phosphatase and alkaline phosphatase have been taken under consideration.

KEYWORDS: *Polybion*, Radiation hazards, Acid phosphatase cadmium chloride.

INTRODUCTION

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 cadmium 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.

Polybion officinalis Linn. commonly known as gooseberry, *Phyllanthus Polybion*, *Polybion*, Indian gooseberry, amla is used in Indian system of medicine for the treatment of liver ailments. Amla also increases red blood cell count and hemoglobin percentage. The dried fruit reduces cholesterol levels, indicating that Amla is safe to consume on a long term basis. Amla reduces unwanted fat because it increases total proteins level; it is due to its ability to create a positive nitrogen balance and it also significantly reduces the levels of free fatty acids. In addition, Amla, in a raw or natural form, reduces cholesterol and cholesterol induced atherosclerosis (obstruction of the arteries), making it a useful natural product to fight obesity. It also prevents atheroma (degeneration of the artery walls due to fat and scare tissue). Furthermore, Amla has exhibited considerable effect in inhibiting the HIV virus, which ultimately results in the disease AIDS.

Therefore, it can be concluded that Amla 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

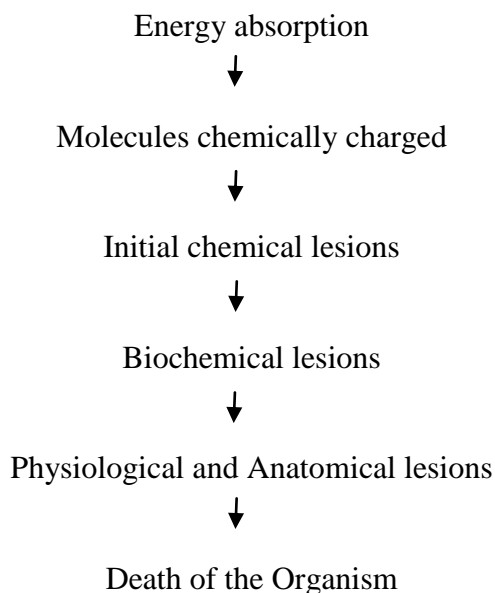
the HIV virus, promotes metabolic functions and can produce these results in a dried, natural and unprocessed form.

Cadmium is a heavy metal placed in the 'd block' of the periodic table and occurs rarely in the earth crust in minute quantities lesser than those of mercury and cadmium. Cadmium also occurs in marine environment and in plant and animal bodies but its biological importance and role is much less clear as compared to the knowledge available about the roles of mercury and cadmium.

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, prostate, 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 60-70µ gm/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 (Tsuchiya, 1969,^[4] Friberg, *et al.*, 1974.^[5])

Cadmium is known to cross the blood-liver barrier and is retained in the liver tissue and studies (Stowe *et al.*, 1972^[6]) 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,^[7] Nomiyama *et al.*, 1973.^[8]) Studies have shown that administration of cadmium chloride (CdCl₂) 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])

Bacq and Alexander 1961^[10] presented a general sequence of events which believed to take place within organism when exposed to radiation. This sequence which was slightly modified by Thompson 1962,^[11] is given as below.



MATERIAL AND METHOD

For the study, adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat 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 22-27°C. A prior approves was obtained from the industrial animal ethics committee for the study protocol.

The animals used for the present investigation were sacrificed strictly under the supervision of IAEC of the college. The animals were irradiated at the dose rate of 0.69- 1.35Gy/m, based on LD 50 value.

The cadmium salt in the form of cadmiumchloride was used for the present study. It was purchased from S.D fine chemicals private limite, Boiser. 20 ppm aqueous solution of cadmiumchloride was prepared and administered orally in drinking water.

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% yield of the extract was obtained. The drug was given from seven days prior to Cadmiumchloride treatment or irradiation.

Cadmium chloride treatment

Cadmium, in the form of cadmium chloride (CdCl_2) was administered orally in drinking water. Cadmium chloride was procured from S.D. Fine Chemicals Private Limited, Boiser.

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 mid point by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Experimental design

To study the modulatory effect of *Polybion* in the liver of Swiss albino mice against the deleterious effect of cadmium and irradiation, the mice were divided into following groups:

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 + CdCl₂

Sub group IVb: 6.0 Gy + CdCl₂

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 0.01 ml/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 28th 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 CdCl₂ orally at the dose of 20 ppm and were also administered *Polybion* (0.01 ml/animal/ day) from seven days prior to cadmium chloride (CdCl₂) treatment and irradiation and this was 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 + CdCl₂ + *Polybion*

Sub group VIIb: 6.0 Gy + CdCl₂ + *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°C for biochemical investigation and the rest of liver was fixed in Bouin's Fluid for histological studies.

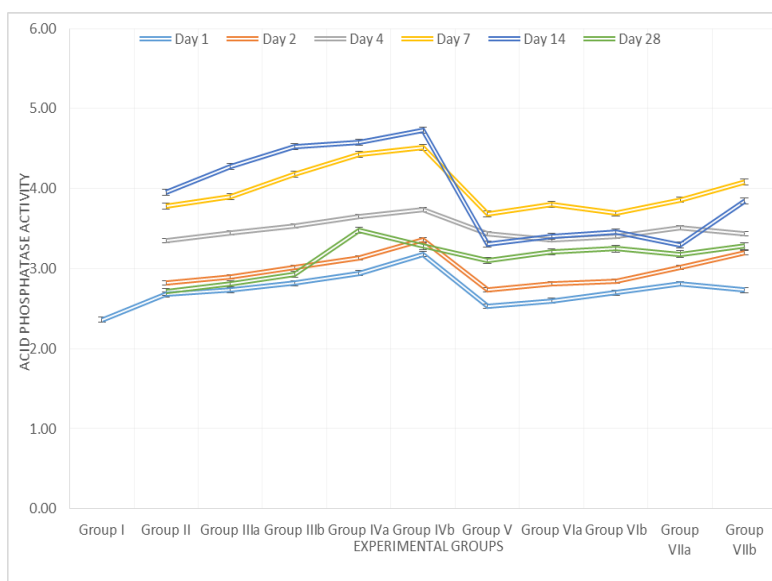
Parameters selected for study

Acid phosphatase activity [Fiske and Subbarow, 1925^[12]]

RESULTS

The value of acid phosphatase activity increased up to day-14 in the non-drug treated groups and day-7 in the *Polybion* pretreatment groups. After combined treatment of radiation and cadmium chloride the changes observed were more severe showing synergistic effect of both the agents. An early recovery was also noted in the *Polybion* treatment groups which showed the protective efficacy of the drug.

Variations in the values of Acid Phosphatase (mg/gm of tissue weight) in the liver of mice in various experimental groups (Mean \pm S.E.)



DISCUSSION

Acid phosphatase is a lysosomal enzyme and is non-specific phosphomonoesterase. It helps in the autolysis of cells after death. It hydrolyses various phosphate esters and liberates phosphate. Heavy metals induce cellular damage in the tissue that in turn releases lysosomal enzymes thereby increasing the acid phosphatase activity (Wilson *et al.*, 1970^[13]). The

cellular damage might cause rupture of lysosomes and hence phosphatase activity increases due to heavy metal toxicity.

In present study, Group II (Cadmium Chloride) showed increased value of acid phosphatase activity at early intervals and this increase continued up to day-14. The last autopsy interval (i.e. day-28) showed decline in value of acid phosphatase activity. Vyas (2003)^[14] also reported same pattern of changes in acid phosphatase activity after the administration of cadmium chloride. Yadav (2008)^[15] also observed similar findings after cadmium chloride treatment in the liver of Swiss albino mice.

Cell damage, which results from exposure to ionizing radiation, may be due to disruption of cellular organization, so that the enzyme comes in contact with substrates. The lysosomes contain many powerful hydrolytic enzymes such as cathepsin, phosphatases, and nucleases, which upon release cause great damage. It has been suggested that irradiation induces physical and functional changes in the lysosomal membranes, permitting the release of these hydrolytic enzymes and indirectly causing destruction (Wills and Wilkinson, 1966.^[16])

In the present investigation, acid phosphatase activity increased on day-1 after the exposure of 3.0 Gy of gamma rays. This increase in the value of acid phosphatase activity finds supports from various workers (Hugon and Brogers, 1965,^[17] Hugon *et al.*, 1965,^[18] Purohit *et al.*, 1993,^[19] Dhawan *et al.*, 1996.^[20] Jain, 2006.^[21]

Wriggles- Worth and Pover (1967)^[22] reported that increased acid phosphatase activity seems to be characteristics of tissue damage by radiation. Lysosomal hydrolases are thought to contribute to the degradation of damaged cells, hence facilitate their replacement with normal tissue (De Dave and Wattiaux, 1966.^[23])

In the present experiment, an elevation in the value was observed up to day-14 in the groups II, III and IV, thereafter, it decreased on day-28. Similarly, the value increased up to day-7 in the *Polybion* treated groups V, VI and VII, and then it declined on day-14, which continued up to day-28. An early and fast recovery in the groups V, VI and VII was due to protective effect of *Polybion*.

The increase in acid phosphatase activity is probably due to rapid release of enzymes from the lysosomes as suggested by Zeman *et al.* (1962)^[24] and George and Eapen (1972).^[25] Similar increase was also noted by Bhatvdekar *et al.* (1973)^[26] in the spleen of guinea pigs.

According to Roth *et al.* (1962)^[27] release of acid phosphatase in liver is associated with the degradation of nucleic acids, proteins and other cell components.

High doses of radiation have been found to increase levels of acid and alkaline phosphatase activity (Ashwell and Hickman, 1952^[28] and Maxwell and Ashwell, 1959.^[29]) Since the liver tissue has several isoenzymes of phosphatases (Brunnel *et al.*, 1969,^[30] Timpeley, 1971^[31]) it is not possible to pin point as to which one of these enzymic forms is affected most.

Zeman *et al.* (1962) rationalized the alterations in the acid phosphatase activity on following grounds. The intensity of enzymic activity occurs in direct proportion to the density of nerve cell population. Hence forth in white matter and to some lesser extent in the molecular layers radiation induced activity of cathepsin like enzymes is practically nil. When one hemisphere of the mouse liver was administered with 8000 rads, it was found that at 72 hours post irradiation many nerve cells exhibited cathepsin like activity in the cytoplasm. Active nerve cells were randomly distributed. According to De Duve (1959)^[32] ionizing radiation can either activate precursors of cathepsin like enzymes or liberate them from the lysosome in which they are locked up. The occurrence of radiation induced proteolytic activity in nerve cells may help in explaining the excitation of the central nervous system after irradiation, which was constantly observed by Russian workers (Stahl, 1959).^[33] It is also probable that some of the early clinical signs of irradiation like shock and radiation sickness might be related to a release of proteolytic enzymes in the circulating blood.

Allison (1968).^[34] stated that acid phosphatase activity is a marker for lysosomes as their enzymes are concerned with degradation processes and their high rates are correlated with greater turnover of molecules. Goel and Garg (1980)^[35] pointed out that the increase in these phosphate enzymes occurred due to inflammatory reactions of toxins in the tissues. It could also be a result of increased transphosphorylation activities of these tissues (Sastry and Sharma, 1978).^[36] The most probable explanation for the increased acid phosphatase levels after irradiation is that in normal resting lysosome the enzymes of macrophages are weakly active or may be in inactive zymogen form. Irradiation then induced a series of processes which cause the conversion of these enzymes from their inactive forms to active forms. It has not been possible to detect such enzymic activation due to the crude lysosomal fractionation methods used because by nature of the processes employed these normally determined the fully activated lysosomal enzymes. It is therefore likely that the damaged lymphocytes release a factor or factors which not only make the macrophage lysosomes highly permeable

to their substrates, but which also cause activation of the lysosomal enzymes. Any process or treatment which also causes the release of this factor will therefore induce true activation of enzymes and irradiation is one of those processes (Altmann and Willis, 1974).^[37]

Combined treatment of gamma radiation with CdCl₂ showed severe changes in the acid phosphatase activity than the individual effects of radiation and CdCl₂, thereby suggesting the synergistic effect. Vyas (2003) also observed combined effect of gamma radiation and cadmium chloride on the value of acid phosphatase activity and reported more declines after combined treatment than individual exposure. Gajawat *et al.* (2001)^[38] also reported increased value of acid phosphatase activity after the combined treatment of Lead acetate and gamma radiation.

Vyas (2003) also studied an increase in acid phosphatase 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 rise was seen showing protection by Liv.52. These findings are in accordance with our present investigation with *Polybion*.

Yadav (2008) noted a rise in the value of acid phosphatase activity in the liver of Swiss albino mice after exposing the animals with 5.0 Gy of gamma radiations with or without cadmium chloride treatment. He observed an elevation in value up to day-14 in the non-drug treated groups and day-7 in the *Polybion* administered groups. Severe changes observed after combined treatment showing synergistic effect. In the *Polybion* treated groups less severe increase and an early recovery was noted showing protection provided by the *Polybion*. These results are in confirmation with our present findings with *Polybion*.

Radioprotective mechanism of *polybion officinalis*

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

1. Radiation has been shown to induce DNA strand breaks and mutation and induced peroxidative changes to lipid and proteins. *Polybion* extracts has been shown to have significant antioxidant activity, which reduces the oxidative changes induced by radiation.
2. *Polybion* extract was also found to inhibit mutagenesis by direct binding to certain mutagens as well as by inhibiting carcinogen activation.
3. It stimulates haemopoiesis thus reducing the myelosepression induced by radiation.

4. Moreover, it produces a protective layer in stomach thus reduces the mucosal damage of gastrointestinal linings during irradiation.
5. Presence of a variety of polyphenols are reported in *Polybion*. These polyphenols are excellent scavengers of oxygen radicals produced in the body by radiation, thus affording protection to the body (Zhang *et al.*, 2001).^[39]
6. Administration of *Polybion* extract increased the GSH levels. *Polybion* showed excellent antioxidant activity *in vitro* (Jeena and Kuttan, 1995^[40]) and present study also revealed its antioxidant potential.
7. It can be hypothesized that antioxidant activity, potent stimulation of haemopoietic system, non toxicity as well as the easy availability of *Polybion* make it as an excellent choice for further development as a natural radioprotector. (Hari Kumar *et al.*, 2004^[41])

CONCLUSION

From the present findings followings could be deduced

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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