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Neuroprotective Effect of Polybion Against Radiation and Cadmium Induced Biochemical Changes in Mice

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Abstract

The adult healthy Swiss albino mice were procured and maintained at animal house of Govt. Dungar college Bikaner (registration no.1066/go/re/s/07/CPSEA) and provided balanced mice feed and water *ad libitum*. As to investigate protective effect of Polybionin mice brain against gamma radiation and cadmium chloride alone and in combination also, the animals were divided into seven groups, (control and experimental drug treated and non drug treated).Group I To IV were treated with doses of radiation and Cadmium chloride and group V to VII were treated with .Polybion besides the doses of radiation and Cadmium,seven days prior to irradiation. A minimum of five animals from each group were sacrificed by cervical dislocation and autopsied at each post treatment intervals of 1,2,4,7,14,28 days. Brain is taken out and was kept at -20^{0}c.The biochemical estimation of Phospholipids,Total protein, Glycogen ,Cholesterol, Acid Phosphatase activity ,Alkaline phosphatase activity ,DNA and RNA was done in laboratory. All parameters showed an increasing or decreasing trend up to 14-day in non drug treated group and up to 7 days in drug treated groups thereafter all parameters showed signs of recovery after 28 days in non-drug treated group and after 14 days in drug treated group. Early and fast recovery in Polybion treated groups were noticed, Hence Polybion could be used as potent radioprotector during oxidative stress and protect brain from neurological disorders.

Introduction

Exposure to ionizing radiation can be classified into three exposure situations. The first, planned exposure situations, as is the case with the medical use of radiation for diagnosis or treatment of patients, or the use of radiation in industry or research. Medical use of radiation accounts for 98 % of the population dose contribution from all artificial sources, and represents 20% of the total population exposure.

The second type of situation, existing exposures, exposure to radon in homes or workplaces or exposure to natural background radiation from the environment. The last type, emergency exposure situations, result from unexpected events requiring prompt response such as nuclear accidents or malicious acts.

Estimated 70% of tissue destruction that happen during irradiation is due to free radicals, therefore it is necessary to find agents that could neutralize or eliminate free radicals. Antioxidants can stop or slow down oxidation in tissue and so reduce DNA damage that results from ionizing radiation.

Heavy metals enter the surroundings by natural means and through human activities. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops.

Free radicals are responsible for perpetuating a large amount of the damage cause by ionizing radiation. Therefore, for an agent to protect cells from primary free radical damage, the agent needs to be present at the time of radiation and in sufficient concentration to compete with radicals produced through radical-scavenging mechanisms. Many radical scavengers and antioxidants exist that can limit the oxidative stress induced by free radicals. Superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase are a few examples of naturally occurring antioxidants that defend against free radical–mediated damage, where the substrates are specific to each enzyme. General antioxidant defense is also provided by low molecular weight antioxidants, which are hydrogen atom–donating reducing agents such as ascorbic acid, tocopherols, polyphenols, and thiols such as glutathione. In this situation, the oxidants are neutralized by hydrogen atom donation, resulting in a less reactive or nonreactive product from the original oxidant and a radical product from the antioxidant, which no longer can exert detrimental effects.

Therefore, antioxidants have gained extensive attention as promising therapeutic agents forprotection against deleteriousneuronal changes caused by ionising radiation . Therefore present study was taken into consideration to analyse the cerebroprotective effect of Polybionagainst radiation and cadmium induced changes in Swiss albino mice.

Material and methods

The present investigation is an attempt to study the protective action of Polybion against the effects produced by radiation and cadmium in the brain of Swiss albino mice.

PROCUREMENT OF ANIMALS

Six to eight weeks old male Swiss albino mice were obtained from animal house of CCS University, Hissar and maintained in an air cooled room under controlled conditions of temperature and light. The animals were given standard mice feed procured from Brook Bond Lipton India Limited, Kolkata. Occasionally germinated gram was also given along with tap water ad libitum. In addition, tetracycline water, once in a fortnight, was also provided orally as a precautionary against infection.

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 beamtherapy unit which was a source, procured from Atomic Energy Agency Ltd., Canada.

MODE OF IRRADIATION

All the mice were exposed to Co60 y-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.

POLYBION

Polybion syrup was procured from Merck Company, Gujrat, India. Polybion was administered orally at a dose rate of 0.01 ml/animal/day. The drug was given from seven days prior to the commencement of cadmium chloride treatment or irradiation.

EXPERIMENTAL DESIGN

To study the modulatory effect of polybion in the brain 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: 2.0 Gy

Sub group IIIb: 4.0 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: 2.0 Gy + $CdCl_2$

Sub group IVb: 4.0 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 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: 2.0 Gy+ Polybion

Sub group VIb: 4.0 Gy + Polybion

GROUP VII (Radiation, Cadmium chloride and drug treated animals)

Mice of this group were given CdCl, orally at the dose rate of 20 ppm and were also administered polybion (0.01 ml/animal/day) from seven days prior to cadmium chloride ($CdCl_2$) 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: 2.0 Gy + $CdCl_2$ + Polybion

Sub group VIIb: 4.0 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 brain was taken out and weighed. Later on, the width and length of brain were also recorded. Afterwards, part of brain was kept at -20° C for biochemical investigation and the rest of brain was fixed in Bouin's Fluid for histological studies.

PARAMETERS SELECTED FOR STUDY:

The following parameters were taken into consideration

BIOCHEMICAL STUDIES

A. Total proteins
B. Glycogen
C. Cholesterol
D. Phospholipids
E. Acid phosphatase activity
F. Alkaline phosphatase activity
G. DNA
H. RNA

Result

The value of total proteins, cholesterol, phospholipids and DNA declined up to day-14 in the non drug treated groups and day-7 in the drug treated groups.

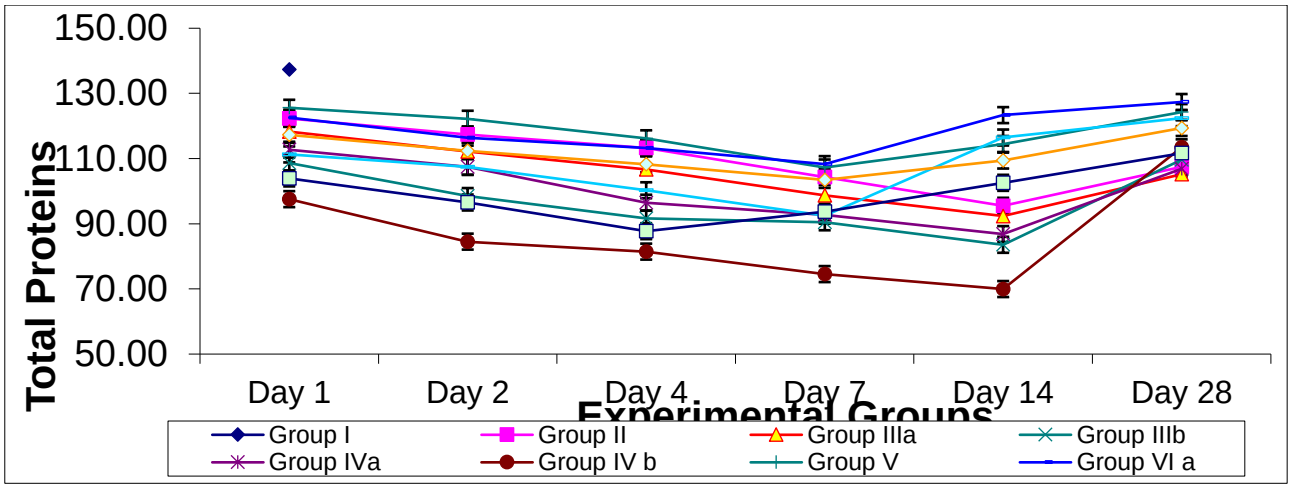
Thereafter a rise in the value was observed up to last autopsy interval i.e. day-28.

Whereas the value of glycogen, acid phosphatase activity, alkaline phosphatase activity and RNA increased up to day-14 in the non drug treated groups and day-7 in the Polybion pretreatment groups.

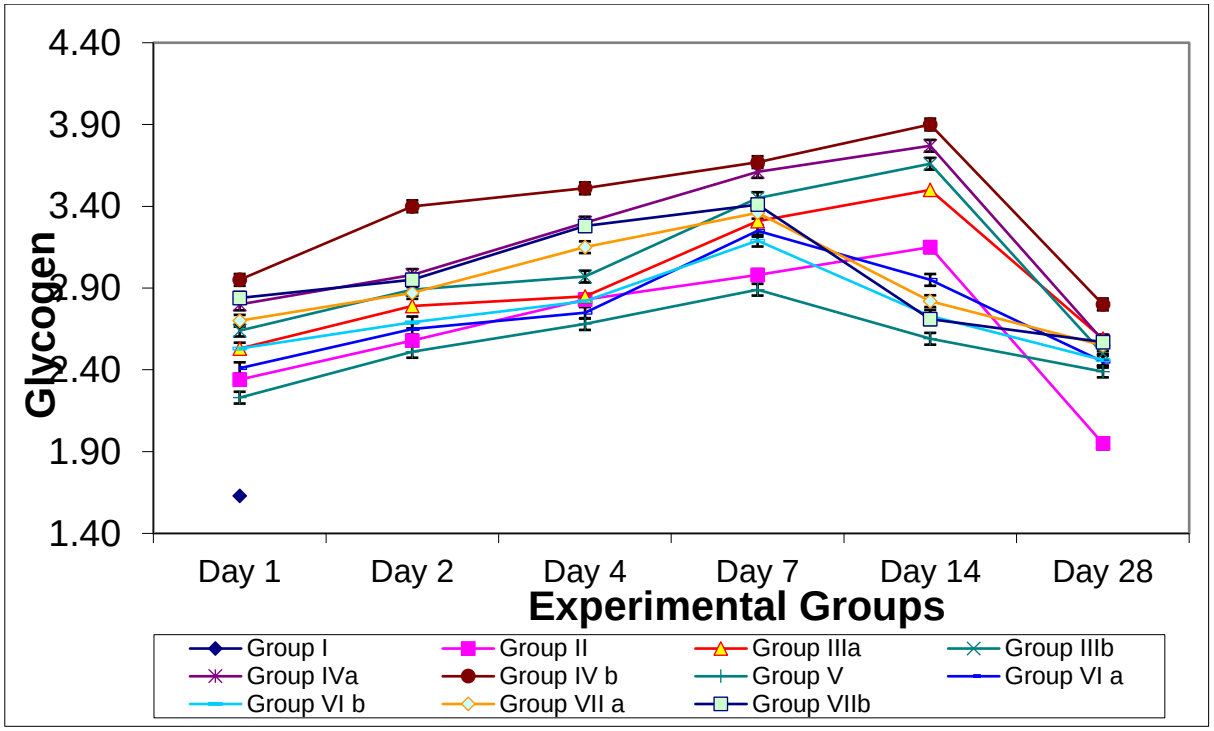
After combined treatment of radiation and Cadmium chloride the changes observed were more severe showing synergistic effect of both the agents.

An early recovery was also noted in the Polybion treatment groups which showed the protective efficacy of the drug.

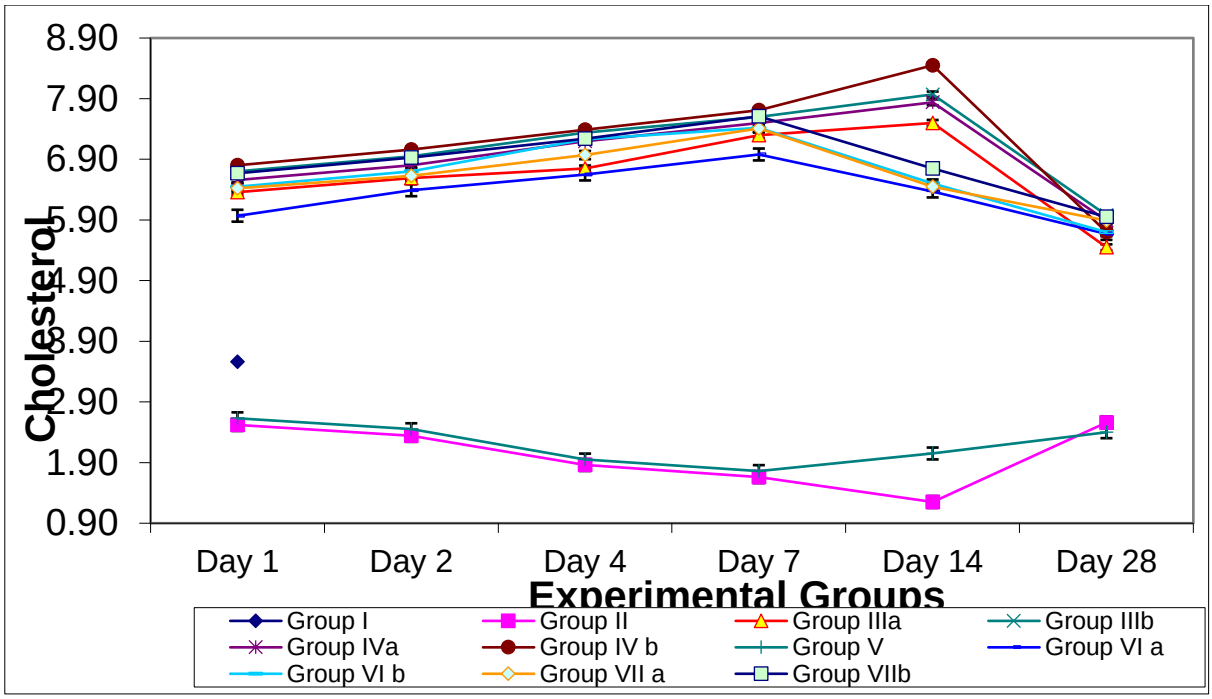
Total Proteins (mg/gm of tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



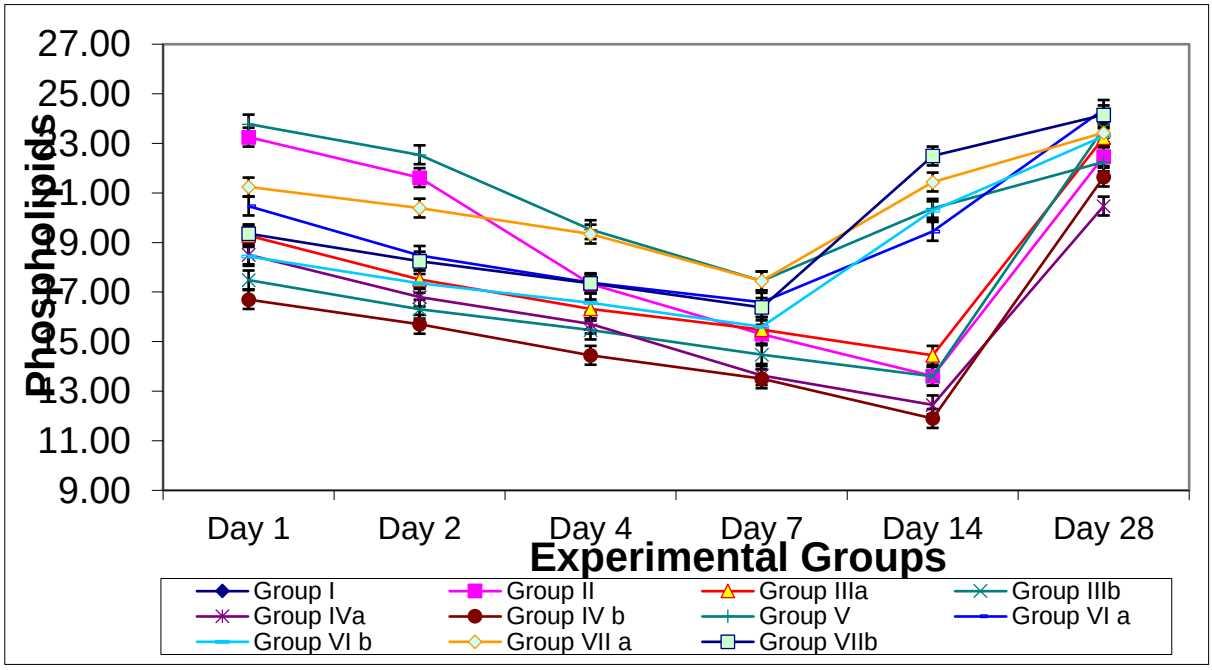
Glycogen (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



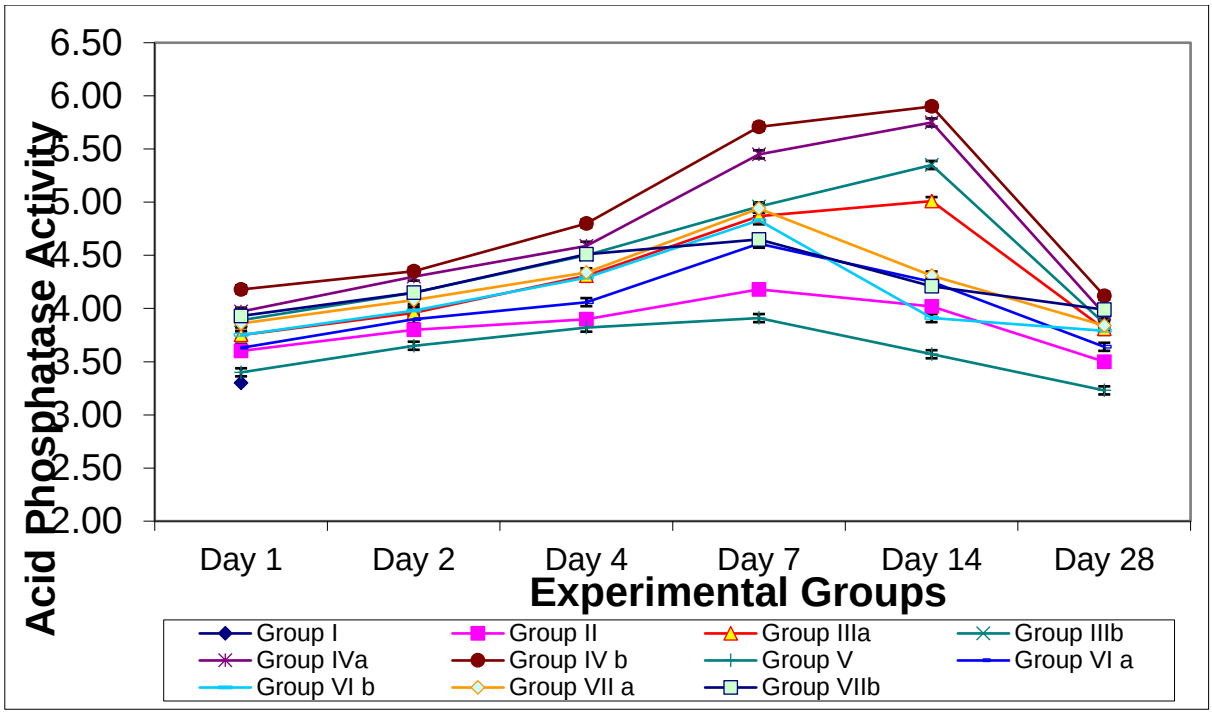
Cholesterol (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



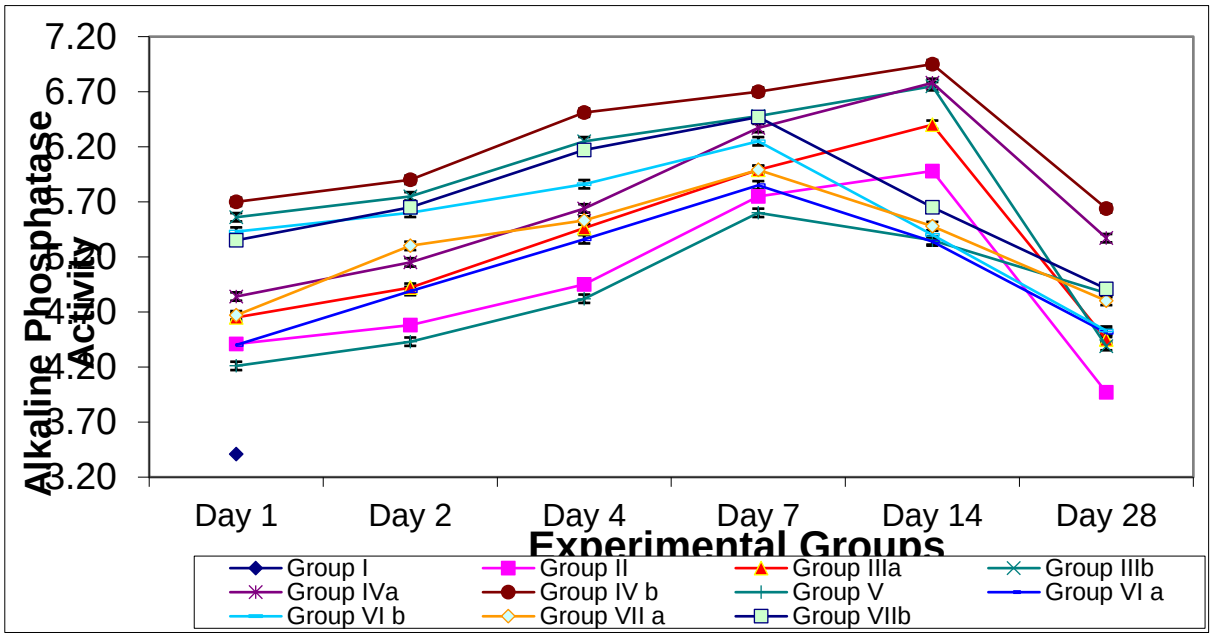
Phospholipids (mg/gm of tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



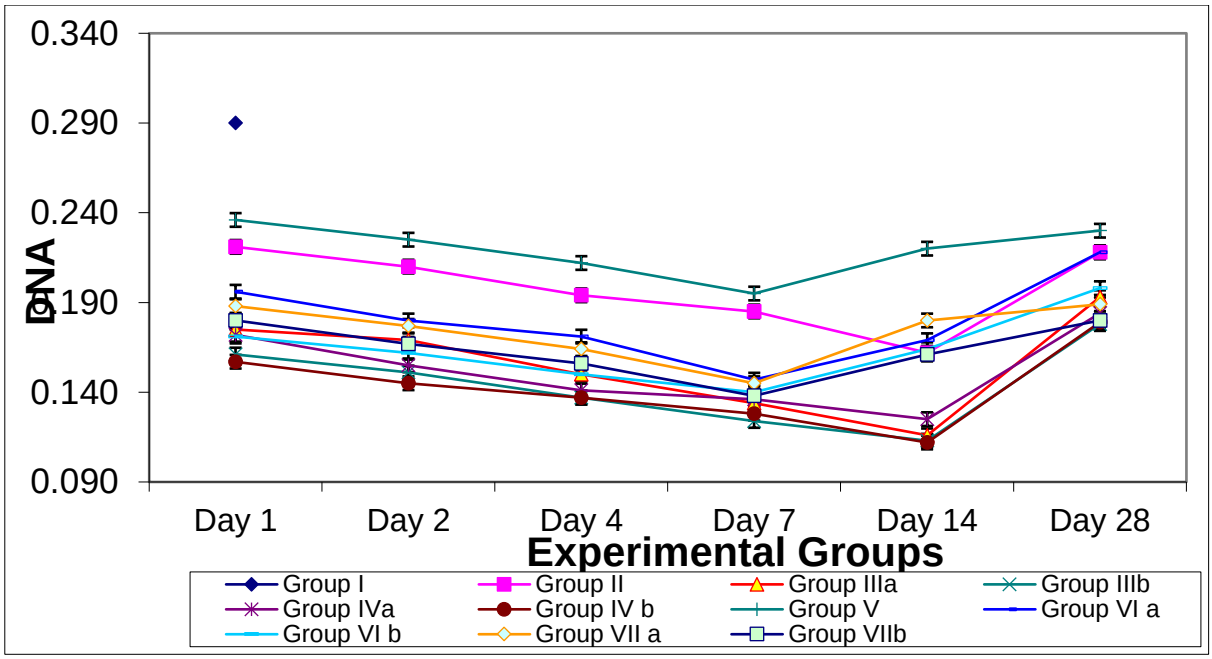
Acid Phosphatase activity (mg pi/gm/hr) in the brain of mice in various experimental groups (Mean ± S.E.)



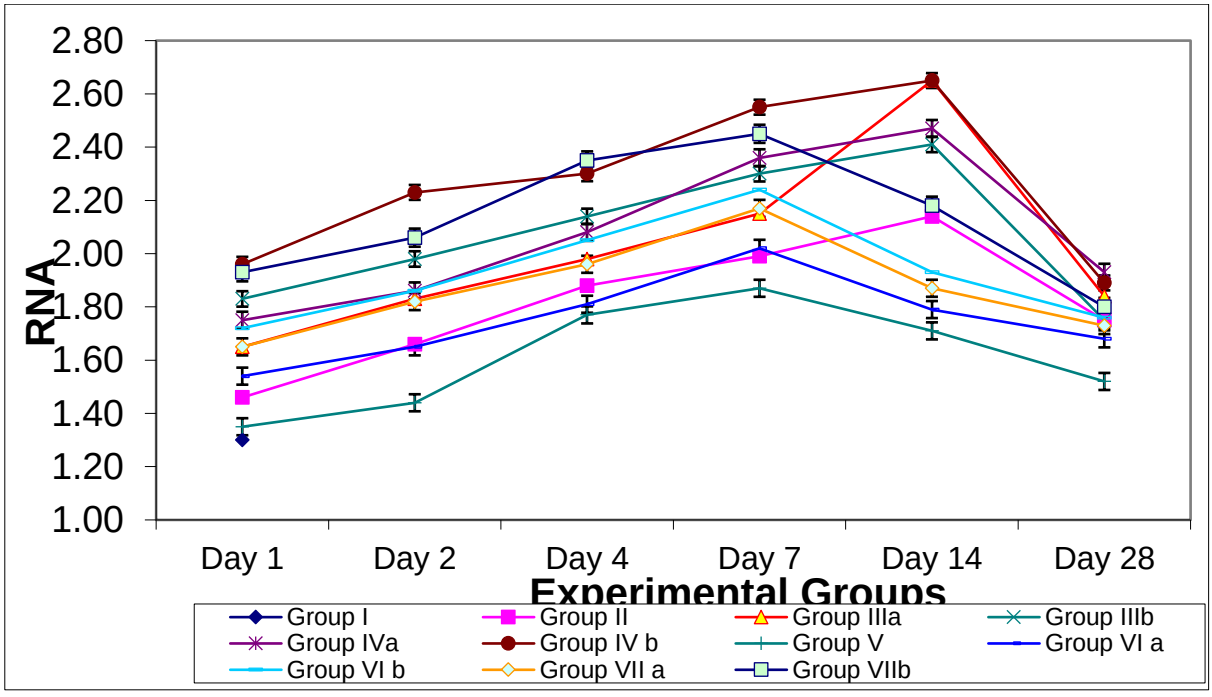
Alkaline Phosphatase activity (mg pi/gm/hr) in the brain of mice in various experimental groups (Mean ± S.E.)



DNA (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



RNA (mg/gm tissue weight) in the brain of mice in various experimental groups (Mean ± S.E.)



Discussion

Historically, radiation-induced injury to normal tissue has been attributed to DNA damage and the subsequent death of replicating cells (Puck and Marcus,1956). As such cognitive impairment following brain radiation was viewed as irreversible. Alternative lines of evidence now suggest that surviving cells play an important role in long-term cognitive dysfunction following radiotherapy. Recent work in a rodent model has shown that transient hypoxia after whole brain radiation may reverse learning deficits (MacDonald et. al,2006). Early phase clinical studies also suggest that when agents normally used to treat Alzheimer's type dementia are administered following cranial radiation, they improve cognitive function (Lau and Zukin, 2007). These observations suggest that radiation-induced brain damage is perpetuated by surviving cells and potentially reversible.

Chronic accumulation of cadmium results in multiorgan toxicity, primarily targeting the kidney, skeleton, liver, and nervous system (Ma et.al 2022). Among these, the nervous system is a particularly vulnerable target for cadmium toxicity. Cadmium can increase risk of peripheral neuropathy, altered equilibrium, and poor performance on visuomotor tasks. Exposure to cadmium is correlated with reduced concentration, poorer cognitive function in older adults, and adverse learning outcomes in children. (Viaene et.al,2000, Li et.al, 2018.Ciesielski et.al, 2012, Kippler et.al,2012)

Under normal conditions, Cd barely reaches the brain in adults due to the presence of the blood brain barrier (BBB); however, this structure is not fully developed in young animals ((Pal et.al,1993). The anatomical and physiological bases on which the choroids plexus becomes the target of xenobiotics have been examined. Cd tends to accumulate in the choroids plexus at concentrations much greater than those found in the cerebrospinal fluid (CSF) and elsewhere in brain tissues. A postmortem human study revealed that the Cd concentration in the choroids plexus was about 2-3 times higher than that found in the brain cortex (Manton and Cook ,1984). As a general choroids plexus toxicant, Cd can directly damage the choroids plexus ultrastructure. Due to differences in the BBB integrity, (Antonio et.al, 2003) Cd is thus more toxic to newborn and young rats than to adult rats. Cd can increase permeability of the BBB in rats (Shukla and Chandra, 1987) to penetrate and accumulate in the brain of developing and adult rats, leading to brain intracellular accumulation, cellular dysfunction, and cerebral edema.(Gonçalves et.al, 2010, Mendez-Armenta and Rios ,2007).

Vitamin B12, or cobalamin, is a water-soluble and vital micronutrient essential for cell homeostasis, hematopoiesis, and immunity. Vitamin B12 is involved in cell growth, myelin, DNA, and erythrocyte synthesis] (Karabulut et.al. 2020 Renata et.al, 2022) Cobalamins have been shown to regulate inflammatory cytokines and act as antioxidants] (Pisoschi et.al, 2022. Birch et.al, 2022, van de Lagemaat et.al,2009, Akbari et.al. 2022) Vitamin B12 directly scavenges ROS, particularly superoxide, and indirectly stimulates ROS scavenging by maintaining spare GSH and enhancing cytosolic antioxidant bioavailability] (. Moreira et.al,2011 Bito et.al,.2017). Moreover, Vitamin B12 controls the synthesis of anti-inflammatory cytokines and growth factors, reducing systemic inflammation (Batistaet.al, 2022).

Hence present study shows the protective action of Polybion against radiation and cadmium induced neuronal changes in Mice.

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Conclusion

Radiation and heavy metals cause deleterious changes in neuronal tissues and biochemical parameters. In above experiment experimental drug Polybion Treated groups depicted early onset of recovery in all the parameters. Thus it may conclude that Drug Polybion act as Antioxidant and protect the brain from side effects of radiation and cadmium. In future the drug may be used as free radical scavenger during cancer treatment.