



PROTECTIVE ROLE OF POLYBION AGAINST RADIATION AND CADMIUM INDUCED HAEMATOLOGICAL CHANGES IN THE SWISS ALBINO MICE

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ABSTRACT

The present study was aimed to evaluate the protective effect of polybion against radiation and cadmium induced haematological changes in the Swiss albino mice. The five animals from each experimental group were sacrificed by cervical dislocation at post-treatment interval of 1,2,4,7,14 and 28 days. After sacrificing the animals, the blood was collected by cardiac puncture in heparinized tubes for various haematological studies. The values of RBC, WBC, Haemoglobin, PCV and MCV were found to decrease in all the groups as compared to normal groups, but the decrease in these values was lesser in polybion treated groups. On the other hand the value of MCH

and MCHC increased in all the groups at various intervals. After combined treatment of radiation and cadmium chloride simultaneously synergistic effects were observed. The polybion treated animal 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 to enough to check cadmium and radiation induced haematological changes in Swiss albino mice.

KEY WORDS: Polybion, Anti oxidants, Heavy metal, Blood, Radioprotection.

INTRODUCTION

The extensive use of atomic energy now-a-days in various branches of natural economy, science and technology, radio diagnosis, 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 intrinsic components of earth crust with the continuing trends towards increasing human activities. Exposure of man to toxic metals presented a potential threat for health hazard. The severity of damage can be modulated by treatment with antioxidants. In view of the potential for practical application, a variety of compounds are being tested for their radio protective activities. Among these vitamins hold a great promise. Polybion is a vitamin B-complex mixture that easily available in the market in the form of tablet, syrup, capsule and injection. The drug is prescribed in India for the treatment of various types of body dysfunctions. The blood is of great importance in clinical medicine because it is easy to examine and provide vital information for the diagnosis and treatment of many diseases. The haematopoietic tissues due to their proliferative activity have received special attention.

Various cells of haematopoietic tissue show differential sensitivity. The erythroblasts and haemocytoblasts are highly sensitive followed by myeloblast and finally megakaryocytes, but the most sensitive of all the blood cell are the lymphocytes and their precursors.

After whole body exposure, manifestations of injury to mammalian tissue are well reflected in peripheral blood. Changes in the blood cell counts are still considered as the most reliable biological evidences of acute exposure to both external and internal irradiation. This is because of the high sensitivity of blood forming tissue to ionizing radiation.

A part from ionizing radiation, human beings are continuously exposed to a wide range of metallic pollutant from the environment. Many chemicals are released in the environment by mining, smelting, discharging industrial, agricultural and domestic waste, burning fossil fuel (coal) and using 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 the blood chain and enter in the biological system. Cadmium is reported as one of the most toxic elements in the environment, which causes severe effect on the biological system.

No systematic work has appeared so far on the use of 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

prophylactic role of polybion (Vitamin B-complex mixture) against the cadmium and radiation induced haematological changes in the Swiss albino mice.

MATERIALS AND METHODS

ANIMALS

Six to eight weeks old male Swiss albino mice were procured from an inbred colony maintained in animal house of HAU, Hissar. The animals were kept in the polypropylene cages in the departmental animal house of Govt. Dungar College Bikaner. The standard mice feed and water were provided *ad libitum*. The temperature of the animal house was maintained between 20-25°C.

SOURCE OF IRRADIATION

A cobalt-60 gamma radiotherapy source (Theratron) of AECL make obtained from Canada was used for irradiating the animals in the present investigation. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate of 0.97 Gy/minute. The dose was calculated at mid point by multiplying dose rate and tissue air-ratio. The tissue of Swiss albino mice was assumed to be equivalent to human soft tissues.

CADMIUM CHLORIDE TREATMENT

Cadmium salt in the form of cadmium chloride (SDS Chemicals, India) was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20ppm and then administered orally in drinking water.

POLYBION

Polybion syrup was procured from Merck K. 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.

Plan of experimentation

The animals for the experiments were divided into the 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 rate of 20 ppm *ad libitum* in drinking water continuously till the last autopsy day.

Group III: (Only irradiated animals)

The animals of this group were exposed to sub-lethal doses of gamma radiation from cobalt-60 source. This group was further divided into two sub groups on the basis of radiation dose received: **Sub- group III a: 2.0 Gy.**

Sub- group III b: 4.0 Gy.

Group IV: (Animals treated with radiation and cadmium chloride)

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

Sub- group IV a: 2.0 Gy+CdCl₂.

Sub- group IV b: 4.0 Gy+CdCl₂.

Group V: (Animals treated with cadmium chloride and polybion)

The animals of this group were orally fed cadmium chloride solution at the dose of 20 ppm and were also administered polybion orally for seven days at a dose of 0.01ml/animal/day prior to cadmium chloride treatment and continued up to the last autopsy interval.

Group VI: (Animals treated with radiation and polybion)

The animals of this group were exposed to sub lethal dose of gamma radiation from cobalt-60 source. The polybion was given seven days prior to irradiation and continued up to the last autopsy interval. This group was further divided into two sub groups on the basis of radiation dose received:

Sub- group VI a: 2.0 Gy+Polybion.

Sub- group VI b: 4.0 Gy+ Polybion.

Group VII: (Animals treated with radiation, cadmium chloride and polybion)

The animals of this group were orally fed cadmium chloride at the dose of 20 ppm and received polybion orally for seven days at a dose of 0.01 ml/animal/day prior to irradiation and cadmium chloride till the last autopsy day of experiment. This group was further divided into two sub groups on the basis of radiation dose received:

Sub -group VII a: 2.0 Gy + CdCl₂ + Polybion.

Sub –group VII b: 4.0Gy + CdCl₂ + Polybion.

AUTOPSY

Five animals from each group were autopsied by cervical dislocation at each post-treatment interval of 1, 2, 4, 7, 14 and 28 days. The weight of animals was recorded before the autopsy. Five normal mice were also autopsied.

Immediately after the autopsy the blood was collected by cardiac puncture in heparinized tubes for various haematological studies.

Haematological Parameters

The various haematological parameters estimated were as follows:

1. Red blood corpuscles (R.B.C.).
2. White blood corpuscles (W.B.C.).
3. Haemoglobin (Hb).
4. Packed cell volume (PCV).
5. Mean cell volume (MCV).
6. Mean corpuscular haemoglobin (MCH).
7. Mean corpuscular haemoglobin concentration (MCHC).

RESULT AND DISCUSSION

All these parameters exhibited modulations in the form of increase or decrease following treatment of cadmium chloride and radiation exposure independently as well as in combination with or without polybion.

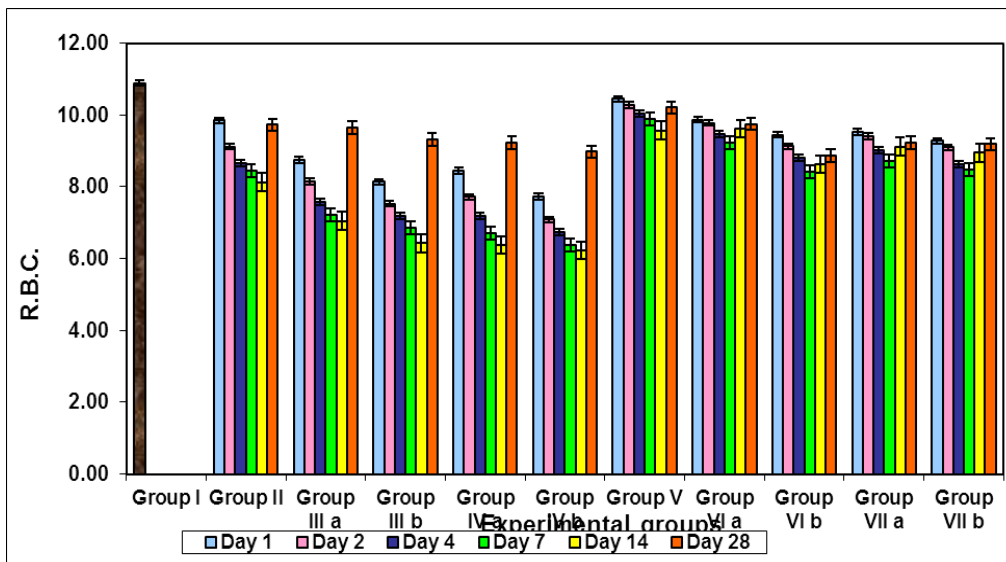
The values of RBC, WBC, Hb and PCV were found to decrease in all the groups as compared to normal group, but the decrease in these values was lesser in polybion treated groups (V to VII) as compared to non-drug treated groups (II to IV). The values of MCV were also found to decrease but the difference from normal value was significant at previous intervals and it was significant on later intervals.

The values of MCH increased in all the groups as compared with normal group after 1, 2, 4, 7, 14 and 28 days of post-treatment intervals. The increase in the value of MCH was lesser in polybion treated groups (V to VII) as compared to non drug treated groups (II to IV). Besides this values of MCHC increased in all the groups at various intervals but the values were lower

in the polybion treated groups (V to VII) as compared to non-drug treated groups (II to IV). The difference from the normal was non-significant in all the groups.

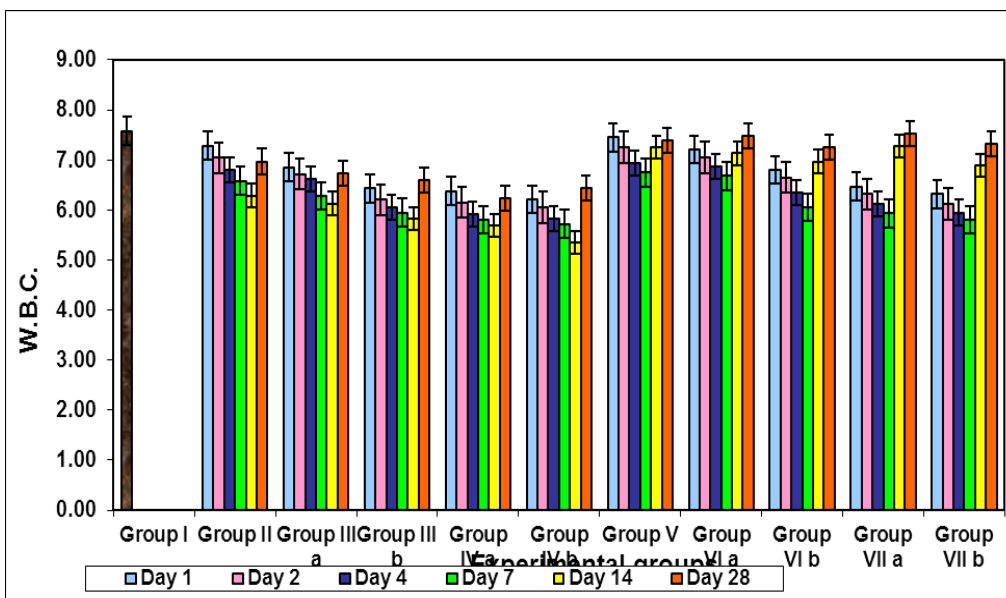
HISTOGRAM- 1

Variation in the values of RBC (thousand/Cu.mm) of mice in various experimental groups (Mean \pm S.E.).



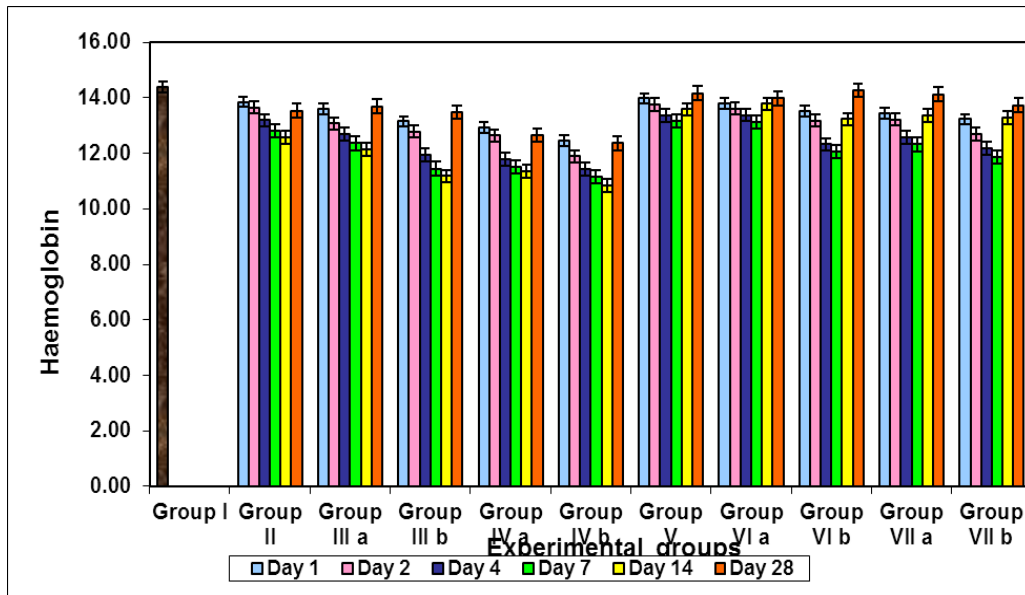
HISTOGRAM- 2

Variations in the values of W.B.C. (thousand/Cu.mm) of mice in various experimental groups (Mean \pm S.E.).



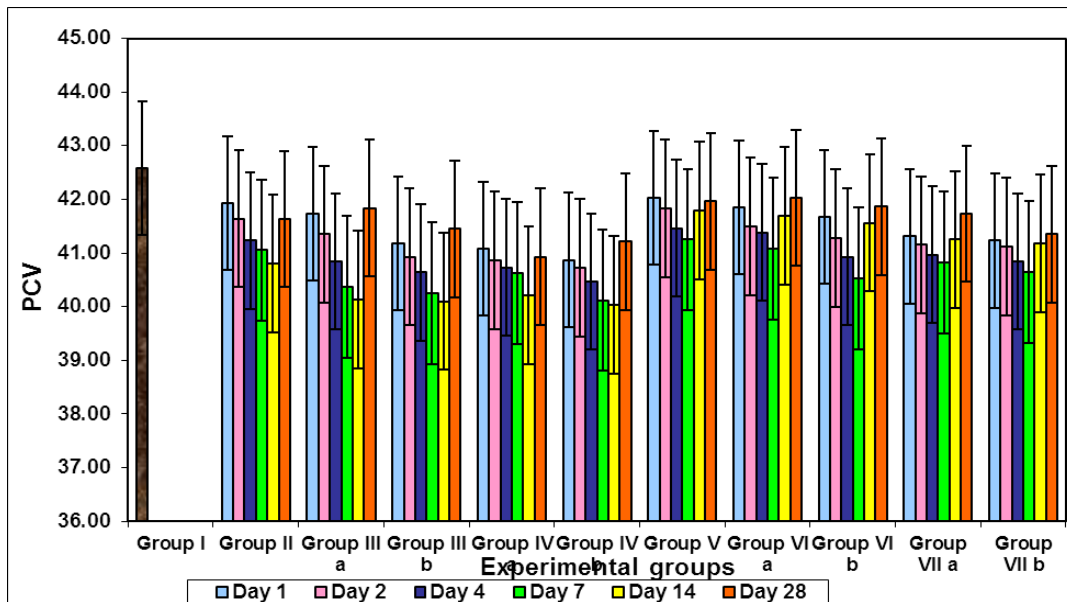
HISTOGRAM- 3

Variations in the Haemoglobin content (gms/100ml.of blood) of mice in various experimental groups (Mean \pm S.E.).



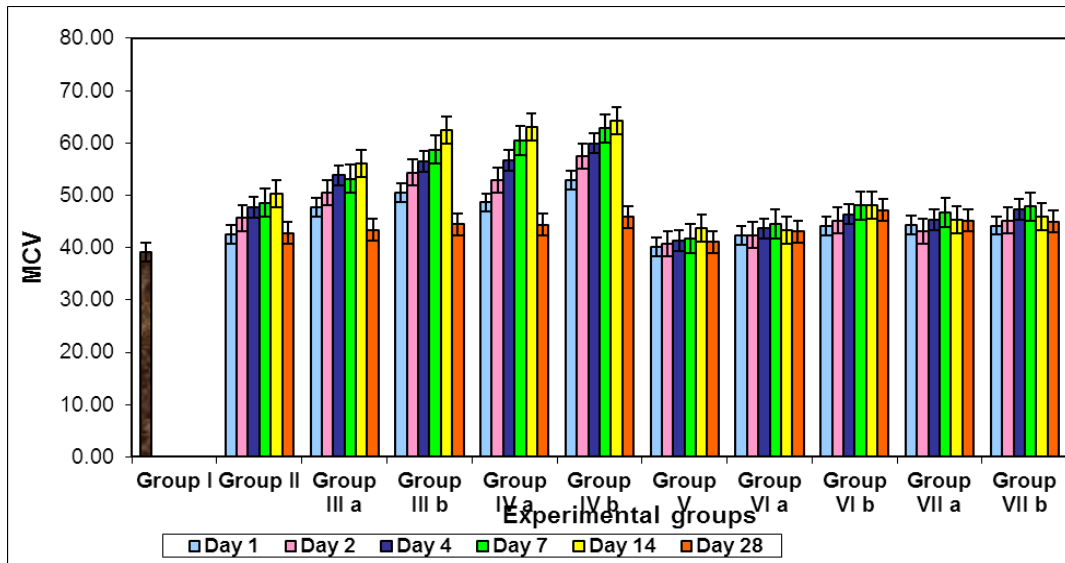
HISTOGRAM- 4

Variations in the values of PCV (%) of mice in various experimental groups (Mean \pm S.E.).



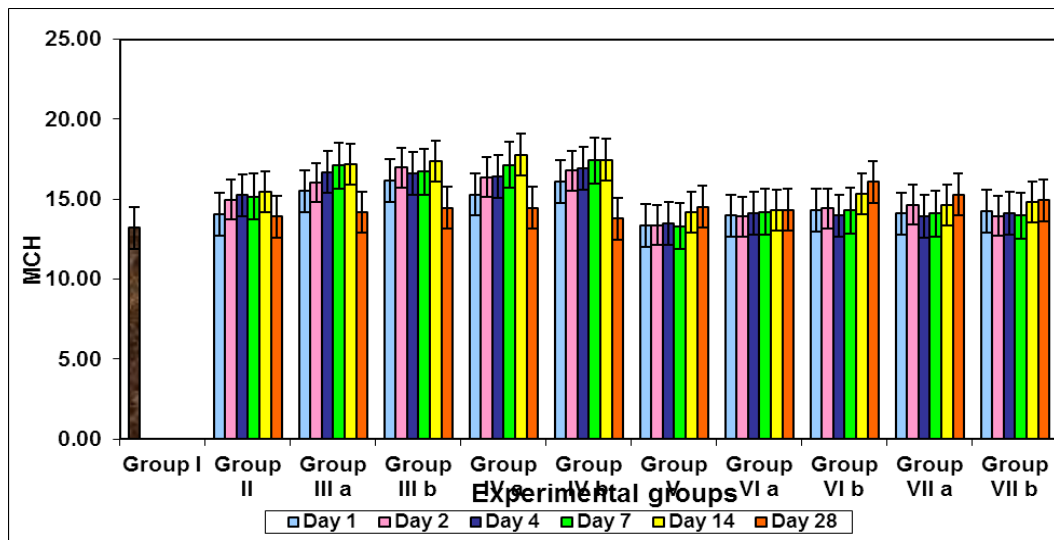
HISTOGRAM- 5

Variations in the values of MCV (cubic micron) of mice in various experimental groups (Mean ± S.E.).



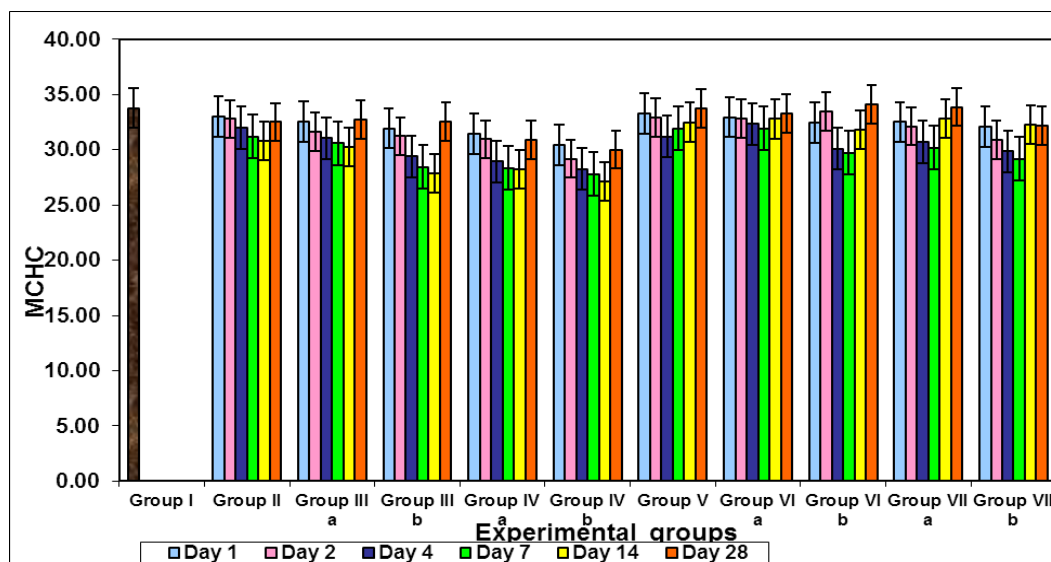
HISTOGRAM- 6

Variations in the MCH (micro micro gms) of mice in various experimental groups (Mean± S.E.).



HISTOGRAM- 7

Variations in the MCHC (%) of mice in various experimental groups (Mean \pm S.E.).

**DISCUSSION****RBC**

The change in values of RBC (million/cubic mm.) In various groups are mentioned in histogram-1.

Group I (Sham-irradiated)

The value of RBC in sham irradiated mice was 10.89 ± 0.08 .

Group II (CdCl₂)

A fall in the value of RBC was noted on day -1 (9.85 ± 0.08). This decreasing trend continued upto day-14 (8.13 ± 0.08) significantly ($p < 0.001$). The value rose on day -28 (9.73 ± 0.05) which was highly significant when compared with the normal value ($p < 0.001$).

Group IIIa (2.0 Gy)

The value of RBC declined significantly ($p < 0.001$) on day -1 (8.75 ± 0.07). The value further declined on days 2 and 4 and continued to decrease up to day-14 (7.05 ± 0.25). On day-28 value increased (9.65 ± 0.28) without reaching to the normal ($p < 0.001$).

Group IIIb (4.0 Gy)

The value of RBC declined significantly ($p < 0.001$) on day-1 (8.14 ± 0.04). The value further declined on days 2 and 4 and continued to decrease upto day-14 (6.43 ± 0.05). On day-28 value increased (9.32 ± 0.28) but still it could not reach to the normal ($p < 0.001$).

Group IV a (2.0 Gy+ CdCl₂)

The value of RBC declined significantly ($p < 0.001$) on day-1 (8.45 ± 0.08). This decreasing trend in the values was continued to upto day-14 (6.38 ± 0.15). The value increased day-28 (9.23 ± 0.17) which was statistically significant ($p < 0.001$) as compared to the normal.

Group IVb (4.0Gy+CdCl₂)

The value of RBC decreased significantly ($p < 0.001$) on day-1 (7.73 ± 0.014). This decreasing trend in the values was continued to upto day-14 (6.23 ± 0.35). The value increased day-28 (8.98 ± 0.14) which was statistically significant ($p < 0.001$) in compared to the normal.

Group V (CdCl₂+Polybion)

The value of RBC dropped down on day -1 (10.45 ± 0.07). The Value further declined on days 2 and 4 (10.28 ± 0.06) and (10.03 ± 0.007) and continued to decrease up to day-7 (9.57 ± 0.08). On day -14 value increased (9.89 ± 0.07) significantly ($p < 0.001$) and continued so up to day-28 (10.21 ± 0.08).

Group VIa (2.0Gy+Polybion)

The value of RBC declined significantly ($p < 0.001$) on day -1 (9.87 ± 0.08). This trend of decrease was continued up to day-7 (9.23 ± 0.06). On day -14 value increased (9.62 ± 0.15) and continued so upto day -28 (9.75 ± 0.16). The difference in the value was still significant ($p < 0.001$) and continued to the normal.

Group VIb (4.0 Gy+Polybion)

The value of RBC declined significantly ($p < 0.001$) on day -1 (9.45 ± 0.05). This trend of decrease was continued up to day-7 (8.43 ± 0.05). On day -14 value increased (8.63 ± 0.17) and continued so upto day-28 (8.87 ± 0.13). The difference in the value was still significant ($p < 0.001$) and continued to the normal.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of RBC decreased on day-1 (9.53 ± 0.06) significantly ($p < 0.001$) as compared to normal value. This decrease in the value was continued up to day-7 (8.73 ± 0.13) but showed a rise on day-14 (9.12 ± 0.06) and continued up to day-28 (9.23 ± 0.08) but it was still lower than the normal value. The difference in the value was still significant ($p < 0.001$) and continued to the normal.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of RBC decreased on day-1 (9.28 ± 0.16) significantly ($p < 0.001$) as compared to normal value. This decrease in the value was continued up to day-7 (8.49 ± 0.06) but showed a rise on day-14 (8.95 ± 0.08) and continued up to day-28 (9.19 ± 0.07) but it was still lower than the normal value. The difference in the value was still significant ($p < 0.001$) in comparison to the normal.

WBC

The change in values of RBC (million/cubic mm.) In various groups are mentioned in histogram-2

Group I (Sham-irradiated)

The value of WBC in sham irradiated mice was 7.58 ± 0.17 .

Group II (CdCl₂)

The value of WBC decreased significantly ($p < 0.005$) from the normal on day-1 (7.28 ± 0.93). This decreasing trend was continued upto day-14 (6.29 ± 0.15). On day-28 (6.97 ± 0.14) the value increased significantly ($p < 0.001$) but still it was below the normal value.

Group IIIa (2.0 Gy)

The value of WBC declined significantly ($p < 0.005$) from the normal on day -1 (6.85 ± 0.13) after irradiation. The value further decreased on days 2 (6.72 ± 0.16) and continued to decrease up to day-14 (6.13 ± 0.16). On day-28 (6.73 ± 0.15) value of WBC rise significantly ($p < 0.002$).

Group IIIb (4.0 Gy)

The value of WBC declined significantly ($p < 0.001$) from normal on day-1 (6.43 ± 0.12) after irradiation. The value further decreased on days 2 (6.20 ± 0.18) and continued to decrease upto day-14 (5.82 ± 0.16). On day-28 value of WBC rise significantly increased ($p < 0.001$) to 6.59 ± 0.13 with out reaching to the normal value.

Group IV a (2.0 Gy+ Cdcl₂)

The value of WBC decreased significantly on day-1 (6.58 ± 0.16) from the normal value. The value lowered down on day-2 (6.15 ± 0.22) and continued to decline upto day-14 (5.69 ± 0.23). On day-28, the value rose to (6.23 ± 0.25) which was highly significant ($p < 0.001$) as compared to the normal value.

Group IVb (4.0Gy+CdCl₂)

The value of WBC decreased on day-1 (6.21 ± 0.23) from the normal value. The value lowered down on day-2 (6.05 ± 0.31) and continued to decline upto day-14 (5.35 ± 0.15). On day -28 the value rose to 6.43 ± 0.23 which was highly significant ($p < 0.001$) as compared with normal value.

Group V (CdCl₂+Polybion)

The value of WBC declined significantly ($p < 0.05$) on day -1 (7.45 ± 0.18). This trend of decrease was continued upto day-7 (6.75 ± 0.028). On day -14 (7.25 ± 0.35) the value increased which continued upto day-28 (7.38 ± 0.19). But still difference in the value was significantly ($p < 0.05$) in comparison to the normal.

Group VIa (2.0Gy+Polybion)

The value of WBC declined on day -1 (7.21 ± 0.27) which continued to decline upto day-7 (6.68 ± 0.29). The value of WBC showed a rise on day -14 (7.13 ± 0.20) and continued to rise upto day-28 (7.45 ± 0.13).

Group VIb (4.0 Gy+Polybion)

The value of WBC declined on day -1 (6.80 ± 0.23) and continued to decline upto day -7 (6.05 ± 0.20). The value of WBC showed a rise on day -14 (6.97 ± 0.23) and continued rise upto day-28 (7.25 ± 0.23).

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of WBC declined significantly ($p < 0.001$) on day-1 (6.47 ± 0.17). The value lowered down continuously upto day-7 (5.93 ± 0.17) significantly ($p < 0.01$). The value increased on day-14 (7.27 ± 0.19) which rose further upto day-28 (7.53 ± 0.28) but it was still below the normal value.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of WBC declined significantly ($p < 0.001$) on day-1 (6.32 ± 0.28). The value lowered down further upto day-7 (5.80 ± 0.29) significantly ($p < 0.01$). The value increased on day-14 (6.89 ± 0.32) which rose further upto day-28 (7.32 ± 0.32) but it was still below the normal value.

HAEMOGLOBIN

The change in values of haemoglobin (gms/100 ml.of blood) in various groups are expressed in histogram-3.

Group I (Sham-irradiated)

The value of haemoglobin in sham irradiated mice was 14.38 ± 0.25 .

Group II (CdCl₂)

The value of decreased from the normal on day-1(13.85 ± 0.18). This decreasing trend was continued upto day-14(12.58 ± 0.15). On day-28, the value rose to (13.53 ± 0.12) which was significantly ($p < 0.001$) as compared with the normal value.

Group IIIa (2.0 Gy)

The value of haemoglobin declined on day -1 (13.59 ± 0.19). The value further decreased on days 2, 4 and 7 and continued to decrease up to day-14 (12.13 ± 0.17) which was significant in comparison to the normal value ($p < 0.001$).

Group IIIb (4.0 Gy)

The value of haemoglobin declined on day-1 (13.15 ± 0.23). The value further decreased on days 2, 4 and 7 and continued to decrease upto day-14 (11.18 ± 0.23). On day-28, the value rose to (13.48 ± 0.26) but it was still below the normal value.

Group IV a (2.0 Gy+ CdCl₂)

The value of haemoglobin decreased significantly on day-1 (12.93 ± 0.25). The values further declined on days 2, 4, 7 and continued to decline upto day-14 (11.35 ± 0.14). The value rose on day-28 (12.65 ± 0.25) which was significant as compared to the normal value ($p < 0.001$).

Group IVb (4.0Gy+CdCl₂)

The value of haemoglobin decreased on day-1 (12.45 ± 0.19). The value further declined on days 2,4 and 7 and continued to decline upto day-14 (10.85 ± 0.27). The value rose on day -28 (12.37 ± 0.32) which was highly significant as compared with normal value ($p < 0.001$).

Group V (CdCl₂+Polybion)

The value of haemoglobin declined on day -1 (13.98 ± 0.18). This trend of decrease continued upto day-7 (13.17 ± 0.30). On day-14, the value rose to (13.58 ± 0.23) and continued upto day-28 (14.17 ± 0.12) but it was still below the normal.

Group VIa (2.0Gy+Polybion)

The value of haemoglobin decreased on day-1 (13.79 ± 0.14) from the normal value. This decreasing trend continued upto day-7 (13.12 ± 0.24). The value showed a rise on day-14 (13.78 ± 0.15) and rose further upto day-28 (13.98 ± 0.14) but it was still below the normal value.

Group VIb (4.0 Gy+Polybion)

The value of haemoglobin decreased on day-1 (13.52 ± 0.17) from the normal value. This decreasing trend continued upto day-7 (12.05 ± 0.17). The value showed a rise on day-14 (13.23 ± 0.18) and rose further upto day-28 (14.27 ± 0.15) but still it could not reach to the normal value.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of haemoglobin decreased on day-1 (13.43 ± 0.14) significantly ($p<0.05$). This decreasing continued upto day-7 (12.32 ± 0.18). The haemoglobin content showed a rise on day-14 (13.35 ± 0.18) and rose further upto day-28 (14.13 ± 0.18) without reaching to the normal value.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of haemoglobin decreased on day-1 (13.23 ± 0.15) from the normal value significantly ($p<0.2$). This decreasing trend continued upto day-7 (11.86 ± 0.15). The haemoglobin content showed a rise on day-14 (13.28 ± 0.15) and rose so upto day-28 (13.73 ± 0.23) without reaching to the normal value.

PCV

The change in values of PCV in various groups are mentioned in histogram-4.

Group I (Sham-irradiated)

The value of PCV in sham irradiated mice was 42.58 ± 1.25 .

Group II (CdCl₂)

The value of PCV declined non-significantly from the normal on day-1 (41.73 ± 0.18). The value lowered down further upto day-14 (40.13 ± 1.25). On day-28, the value was non-significant at all the intervals in comparison to the normal.

Group IIIa (2.0 Gy)

The value of PCV declined non-significantly on day-1 (41.73 ± 1.18) and continued to decline upto day-14 (40.13 ± 1.25). On day-28, the value rose to (41.84 ± 1.08). All these values were non-significant as compared to the normal.

Group IIIb (4.0 Gy)

The value of PCV declined on day-1 (41.18 ± 1.25) and continued to decline upto day-14 (40.10 ± 1.38). On day-28, the value rose to (41.45 ± 1.28). All these values were non-significant as compared to the normal.

Group IV a (2.0 Gy+ CdCl₂)

The value of PCV declined (41.08 ± 1.32) non-significantly on day-1. This decrease was continued upto day-14 (40.21 ± 1.42). The value showed a rise on day-28 (40.93 ± 1.37), which was non-significant as compared to normal value.

Group IVb (4.0Gy+CdCl₂)

The value of PCV declined (40.87 ± 1.25) non-significantly on day-1. This decrease was continued upto day-14 (40.03 ± 1.37). The value showed a rise on day-28 (41.21 ± 1.27), which was non-significant as compared to normal value.

Group V (CdCl₂+Polybion)

The value of PCV decreased non-significantly on day-1 (42.03 ± 1.33), reducing further till day-7 (41.25 ± 1.05) but again increased non-significantly on day-14 (41.79 ± 1.28) and rose further upto day-28 (41.98 ± 1.35), but still difference in the value was non-significant as compared to normal value.

Group VIa (2.0Gy+Polybion)

The value of PCV decreased from the normal value on day-1 (41.85 ± 1.32) which decreased further non-significantly upto day-7 (41.08 ± 1.17). On day-14, the value of PCV increased (41.69 ± 1.08) and rose further upto day-28 (42.03 ± 1.13) but it was still below the normal value. All these values were non-significant.

Group VIb (4.0 Gy+Polybion)

The value of PCV decreased from the normal value on day-1 (41.67 ± 1.16) which decreased further non-significantly upto day-7 (40.53 ± 1.16). On day-14, the value of PCV increased

(41.56±1.43) and rose further upto day-28 (41.86±1.28) but it was still below the normal value. All these values were non-significant.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of PCV lowered down non-significantly on day-1 (41.31±1.23), which decreased further till day-7 (40.82±1.11) non-significantly. The value of PCV increased non-significantly on day-14 (41.25±1.25) and rose further upto day-28 (41.73±1.36) but still difference in the value was non-significant as compared to the normal value at all the corresponding intervals.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of PCV lowered down non-significantly on day-1 (41.23±1.17), which decreased further till day-7 (40.65±1.30) non-significantly. The value of PCV increased non-significantly on day-14 (41.18±1.43) and rose further upto day-28 (41.35±1.38) but still difference in the value was non-significant as compared to the normal value at all the intervals.

MCV

The change in values of MCV (cubic micron) in various groups are mentioned in histogram-5.

Group I (Sham-irradiated)

The value of MCC in sham irradiated mice was 39.10±1.78.

Group II (CdCl₂)

The value of MCV rose from normal on day-1(42.56±1.78). The value further increased on day-2(45.65±1.86) and continued to increase upto day-14(50.23±2.12) significantly (p<0.02). On day-28, Value decreased (42.78±1.89) but still it could not reach to the normal.

Group IIIa (2.0 Gy)

The value of MCV increased significantly (p<0.05) from normal on day-1(47.69±2.13). The value further declined on day-2(50.47±1.93), day-4(53.80±2.18) and continued so upto day-14 (56.02±1.76). On day-28, Value rose (43.35±2.23) but difference in the value was non-significant as compared with normal value.

Group IIIb (4.0 Gy)

The value of MCV elevated significantly ($p < 0.05$) from normal on day-1 (50.58 ± 2.58). The value further increased on day-2 (54.35 ± 1.80), day-4 (56.44 ± 1.97) and continued so upto day-14 (62.36 ± 2.59). On day-28 value rose (44.47 ± 1.67) without reaching to the normal.

Group IV a (2.0 Gy+ CdCl₂)

The value of MCV increased significantly ($p < 0.02$) from normal on day-1 (48.61 ± 1.28) and continued to increase so up to day-14 (63.02 ± 2.13). On day-28, value decreased (44.34 ± 2.28) non significantly without reaching to the normal value.

Group IVb (4.0Gy+CdCl₂)

The value Of MCV increased significantly ($p < 0.02$) from normal on day-1 (52.87 ± 2.63) and continued to increase so upto day-14 (64.25 ± 2.50). On day-28, value decreased (45.89 ± 1.49) significantly ($p < 0.05$) without reaching to the normal value.

Group V (CdCl₂+Polybion)

The value of MCV rose non-significantly from normal on day-1 (40.22 ± 2.87) thereafter rose on day-2 (40.69 ± 2.47). The value increased on day-4 (41.33 ± 1.83), day-7 (41.70 ± 1.94) and continued to increase upto day-14 (43.66 ± 1.84). On day-28 (41.11 ± 2.08) the value slightly decreased but it was still above the normal value.

Group VIa (2.0Gy+Polybion)

The value of MCV increased non-significantly from normal on day-1 (42.40 ± 2.87), day-2 (42.43 ± 3.21), and continued so upto day-7 (44.50 ± 2.81). The value declined on day-14 (43.33 ± 1.85) which continued declining upto day-28 (43.10 ± 2.38). All the value were non-significant as compared to the normal.

Group VIb (4.0 Gy+Polybion)

The value of MCV increased non significantly from normal on day-1 (44.09 ± 2.43), day-2 (45.21 ± 2.69), day-4 (46.40 ± 2.77) and continued to increased upto day-14 (48.15 ± 2.03). The value increase further upto day-28 (47.19 ± 2.24) significantly ($p < 0.05$) as compared to the normal value.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of MCV increased non-significantly from normal on day-1 (43.34 ± 2.93) and continued to increase upto day-7 (46.75 ± 1.58) significantly ($p < 0.05$). The value decreased on

day-14 (45.23 ± 2.64) which continued upto day-28 (45.21 ± 2.58). But it was still above the normal value.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of MCV increased non-significantly on day-1 (44.42 ± 2.53) and further increase on day-4 (47.32 ± 2.32) significantly ($p < 0.05$). The value continued to rise on day-7 (47.87 ± 3.13). Thereafter a fall in the value was observed on day-14 (46.01 ± 3.20) which continued upto day-28 (44.99 ± 2.46).

MCH

The change in values of MCH content (micro micro gms) in various groups are mentioned in histogram-6.

Group I (Sham-irradiated)

The value of MCH in sham-irradiated mice was 13.20 ± 0.58 .

Group II (CdCl₂)

The value of MCH decreased non-significantly ($p < 0.01$) on day-1 (14.06 ± 0.56). The value further increased on day-2, 4 and 7 and continued to decline upto day-14 (15.47 ± 1.16). On day-28, the value rose (13.90 ± 0.76) without reaching to the normal. All these value were non-significant.

Group IIIa (2.0 Gy)

The MCH level rose non-significantly from the normal value on day-1 (15.50 ± 1.32). The value rose further upto day-7 (17.20 ± 1.29) and continued to rise upto day-14 (17.20 ± 1.29). The value declined on day-28 (14.18 ± 1.57) which was still higher than the normal value.

Group IIIb (4.0 Gy)

The MCH level rose non-significantly from the normal value on day-1 (16.15 ± 1.25) and continued to rise upto day-14 (17.38 ± 1.43). The value declined non-significantly on day-28 (14.46 ± 0.86) which was still higher than the normal value.

Group IVa (2.0 Gy+CdCl₂)

The value of MCH increased on day-1 (15.30 ± 0.93) day-2 (16.38 ± 1.35), day-4 (16.42 ± 1.27) and continued to increase upto day-14 (17.78 ± 1.40). On day-28, the value of MCH decreased non-significantly (13.70 ± 1.63) but it was still above the normal level.

Group IVb (4.0 Gy+CdCl₂)

The value of MCH increased non significantly on day-1 (16.10±1.83) then increased further significantly ($p<0.05$) on day-7 (17.41±1.28) and continued so upto day-14 (17.46±1.20). On day-28 (13.77±1.33) the value of MCH decreased non-significantly which was still higher to the normal value.

Group V (CdCl₂+Polybion)

The value of MCH slightly increased on day-1 (13.37±1.45) to day-4 (13.50±0.86) and the value decreased non-significantly on day-7 (13.31±1.35). The value increased non-significantly on the day-14 (14.19±1.47) and continued so upto day-28 (14.53±1.50) which was still above the value.

Group VIa (2.0 Gy+Polybion)

The value of MCH increased non-significantly on day-1 (13.97±1.44) decreased on day-2 (13.91±1.53) and further increased non-significantly on day-4 (14.11±0.76) and continued rising upto day-14 (14.32±1.18). on day-28 (14.33±1.23) value slightly increased but it was still higher then the normal values.

Group VIb (4.0 Gy+Polybion)

The value of MCH increased significantly ($p<0.01$) on day-1 (14.30±1.33) and than non-significantly increased on day-2 (14.43±0.89). The value declined non-significantly on day-4 (13.96±0.97) and increased on day-7 (14.29±1.13) to day-28 (16.08±1.52). But it was still higher than the normal value.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The value of MCH rose non-significantly on day-1 (14.09±1.53) to day-2 (14.64±0.84) and than value decreased non-significantly on day-4 (13.93±0.93). Thereafter the value increased non-significantly on day-7 (14.11±0.68) to day-28 (15.30±1.30) which was higher than the normal value.

Group VIIb (4.0 Gy+CdCl₂+Polybion)

The value of MCH rose non-significantly on day-1 (14.25±1.36) and decreased significantly on day-2 (13.94±1.24). The value further increased non-significantly on day-4 (14.12±1.33) and decreased on day-7 (13.96±1.43). On the day-14 (14.83±1.56) the value increased on non-

significantly and continued to increase upto day-28 (14.74 ± 1.18). But it was still higher than the normal value.

MCHC

The change in values of MCHC (%) in various groups are expressed in histogram-7.

Group I (sham-irradiated)

The value of MCHC in normal mice was 33.77 ± 1.37 .

Group II (CdCl_2)

The Value of MCHC decreased on day-1 (33.03 ± 1.42) and day-2 (32.78 ± 1.78) non-significantly. The value decreased further non-significantly on day-4 (31.99 ± 2.13) which continued upto day-14 (30.80 ± 2.14). The value of MCHC increased on day-28 (32.50 ± 2.60) non-significantly but it was still below the normal value.

Group IIIa (2.0 Gy)

The value of MCHC dropped rise non-significantly on day-1 (32.56 ± 1.82) which continued so upto day-14 (30.22 ± 1.36). The value of MCHC increased non-significantly on day-28 (32.71 ± 1.56) but still it was below the normal value.

Group IIIb (4.0 Gy)

The value of MCHC reduced non-significantly on day-1 (31.93 ± 2.32) which continued so upto day-14 (27.38 ± 1.79). The value of MCHC increased on day-28 (32.52 ± 1.72) which was still below the normal value.

Group IVa (2.0Gy+ CdCl_2)

The value of MCHC decreased non-significantly on day-1 (31.47 ± 1.65), day-2 (30.95 ± 1.73) and continued so upto day-14 (28.22 ± 1.90). The value of MCHC increased non-significantly on day-28 (30.90 ± 2.13) which was still below to the comparison of normal value.

Group IVb (4.0Gy+ CdCl_2)

The value of MCHC decreased non-significantly on day-1 (30.46 ± 0.89) which decreased significantly ($p < 0.01$) on day-2 (29.19 ± 0.67) to upto day-14 (27.10 ± 1.76). The value of MCHC increased non-significantly on day-28 (30.01 ± 2.10) which was still below to the normal value.

Group V (CdCl₂+Polybion)

The value of MCHC decreased non-significantly from normal on day-1 (33.26±1.35) and continued so upto the day-4 (31.19±1.47). On day-7 the value increased (31.92±1.38) non-significantly and continued to increase upto day-28 (33.75±1.63).

Group VIa (2.0Gy+Polybion)

The value of MCHC decreased non-significantly on day-1 (32.95±1.82) and continued to decrease non-significantly on day-7 (31.93±1.63). The value of MCHC increased on day-14 (32.78±1.68) and continued to decrease upto day-28 (33.26±1.70).

Group VIb (4.0 Gy+Polybion)

The value of MCHC decreased non-significantly from the normal on day-1 (32.44±1.35) and continued to decrease upto day-14 (31.83±1.03). The value increased non-significantly on day-28 (34.08±1.08) which was slightly higher than the normal value.

Group VIIa (2.0 Gy+CdCl₂+Polybion)

The values of MCHC decreased non-significantly from the normal on day-1 (32.51±2.13) and continued decreasing upto day-7 (30.18±1.68). On day-14 (32.84±1.58) the value rose and continued so upto day-28 (33.86±1.73) non-significantly.

Group VII b (4.0 Gy+ CdCl₂+Polybion)

The value of MCHC decreased on day-1 (32.08±1.82). The value further declined on day-2 (30.86±1.93) and continued so upto day-7 (29.17±2.03). On day-14 the value rose (32.24±2.16) and declined slightly on day-28 (32.20±1.83).

Radio Protective Mechanism of Vitamins

The exact mechanism by which vitamins prevents the animals from radiation induced damage is not known and secondly, it may not have a single mechanism of radio protection. It seems that vitamins may protect by different mechanisms because of their various physiological and biochemical properties which are as follows:

1. It is speculated that vitamin E can be regenerated from tocopheroxy radicals either by reduced glutathione (Pryor, 1976) or ascorbic acid (Packer, 1979). Under such conditions, exogeneous application of GSH in association with vitamins also maintains the vitamin E level as observed by Sood and Vijaylakshmi (1995).

2. Vitamins, a group of complex organic compounds present in minute amounts in natural foodstuffs, are essential to normal metabolism. They act as co-factors in enzymes systems and anti-oxidants, or antagonists. Suzuki and Yoshida (1979), Cerklewski (1984) and Tandon and Jain (1994) have reported that Zn or/and ascorbic acid (Vitamins C) administration decreased lead intoxication by interfering in the absorption of lead than by mobilizing lead from the site of deposition.

3. **Vitamin E** is believed to interrupt the chain of membrane **lipid peroxidation** (Packer, 1994). It is the major antioxidant in the membrane of cells and subcellular particles.

4. 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 (Bapu, 1993). 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 (Tondon and Jain, 1994).

5. **Vitamin C** and **E** are found to be promising as good **antimutagenic** and **anticarcinogenic** against a number of chemical mutagens in adults (Henson *et al.*, 1991 and Packer, 1991). The protection offered by vitamins has been explained by scavenging of oxidizing free-radicals (Ames, 1983 and Micozzi, 1989). It is evident that both Vitamins C and E are good antioxidant *in vivo* (Burton and Ingold, 1989 and Niki, 1987).

CONCLUSION

Thus it can be concluded that Polybion may inhibit the Lipid peroxidation by:

- (i) reducing the formation of free radicals,
- (ii) destroying the free radicals already formed,
- (iii) by supplying a competitive substrate for unsaturated lipids in the membrane, and
- (iv) exuadating the repair mechanism of damaged cell membrane.

From the present findings followings could be deduced –

1. The blood 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 blood of polybion treated animals showed less severe radio lesions and early and fast recovery in comparison to non-drug treated animals. Thus, it seems that polybion has protected the blood 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.

Thus, polybion is a good radio protector and can be given to cancer patients during radiotherapy to minimize the side effects of exposure.

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