IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Entero Protective Action Of Cadmium And Radiation On Some Biochemical Markers And Its Modification By *Moringa Oleifera*.

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ABSTRACT

The Ayurveda *Moringa oleifera* is perhaps the most important medicinal plant in the Indian traditional system of medicine. Leaf is the most important part of the plant are used to treat a variety of diseases. It is rich in vitamins A, C, E (as antioxidant) minerals enzymes and amino acids. Herbal plants are potential source of phytochemicals of pharmaceuticals interest, act as antioxidant, anticarcinogenic, free radical scavenging, immunomodulatory activity and antimicrobial preclinical studies, The exposure of living system to ionizing radiations causes a variety of damages to various system due to generation of free radicals and reactive oxygen species. Cadmium is one of the most toxic elements in geological cycles, Various studies showed the prevention of radiation induced suppressions of immunity by Moringa. Having this unique properties Moringa could be used as protector against radiation arid cadmium. For this purpose six to eight weeks old mice were selected and divided into seven groups, biochemical parameters of control groups were compared with the respective experimental groups. The biochemical findings indicated the drug treated of living tissue i.e. jejunum showed slightly no degenerative changes. The drug treated groups demonstrated the ability of Moringato inhibit oxidative stress thus preventing tissue injury.

Key words: Mice, *Moringa oleifera,* Radiation and cadmium.

INTRODUCTION

Human beings are continuously exposed to natural radiation sources as well as to ionizing radiation nuclear weapons testing, occupational consumer products and medical procedures. Ionizing radiations damages biological tissues by ionizing their atoms and molecules. Depending on the exposure to radiation dose and the biochemical processes (Saini et.al.2024) Apart from ionizing radiation human beings are continuously exposed to a wide range of metallic pollutants from the environment. Man, releases lots of chemical in the environment in the gastrointestinal tract, cadmium compromises the integrity of the microbiota, thus reducing the efficiency of this intestinal barrier and increasing the risk of inflammation, allergies, and metabolic disorders (Tinkov *et.al.*2018 and Chang X *et.al.*2019) Cadmium has been reported to interfere with the gut mucosa. Degenerated nuclei, apoptosis, and altered lamina propria infiltrated with leukocytes have been reported. Metallothionein (MT) is a multifunctional protein with cytoprotective properties against heavy metals and oxidative damage also showing a cytoprotective role on the gastroduodenal mucosa a significant interference of cadmium, in a dose- and site-dependent manner, with mucosal efficiency. This effect could be a direct health risk for the organism exposed to the contamination and indirectly a risk for the trophic chain (Takano et.al. 2000). *Moringa oleifera* significantly regulated not only gastrointestinal hormones and neurotransmitters in serum but also important gastrointestinal motility factors in the enteric nervous system (ENS)-interstitial cells of Cajal (ICCs)-smooth muscle cell (SMC) network. Isoquercitrin, astragalin, vitexin and rutin are the four flavonoids with relatively high abundance in MOE. These four flavonoids have biological activities such as antioxidant, anti-inflammatory and gut microbiota regulation (Muvhulawa et.al.2022). Intestine is the most sensitive tissue in the gastrointestinal tract mainly because of cell population in the villi is dynamic and under normal condition it is in a steady state. Intestine plays very important physiological role in the survival of an organism when individual system is affected by radiations (Purohit et.al.2015 and Arti, 2024).

MATERIALS AND METHODS

Six to eight weeks old male Swiss Albino Mice were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and maintained at 20-25^{0}C. The animals were provided with standard mice feed and tap water *ad libitum*.

DRUG (MORINGA OLEIFERA)

The dried powder of *Moringa oleifera* was procured from the Umalaxmi Organics Private Limited, Jodhpur and aqueous extract of the same was obtained in the department. The plant extract of Moringa was fed orally at the dose of 150 mg/kg body weight. The drug was given seven days prior to radiation or cadmium chloride or combined treatment.

SOURCE OF IRRADIATION

A Cobalt^{60} gamma radiotherapy source (Theratron) of AECL make obtained from Canada was used to irradiate the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial hospital, Bikaner (Rajasthan), India. The animals were irradiated at the dose rate of 0.82 Gy/min during the first year and 1.15 Gy/min for the subsequent year.

CADMIUM

Cadmium salt, in the form of cadmium chloride of analytical grade was used for the present study. It was purchased from Ranbaxy Laboratories Limited, India. It was administrated orally in drinking water at the dose of 20 ppm.

PLAN OF EXPERIMENTATION

The animals were divided into the following groups
Group – I: Sham-irradiated animals (normal)
Group – II: Cadmium chloride treated animals
Group – III: Only irradiated animals
Sub-group III a: 2.0 Gy
Sub-group III b: 4.0 Gy
Group – IV: Radiation + Cadmium chloride
Sub-group IV a: 2.0 Gy + Cadmium chloride
Sub-group IV b: 4.0 Gy + Cadmium chloride
Group – V: Cadmium chloride + *Moringa*
Group – VI: Radiation + *Moringa*
Sub-group VI a: 2.0 Gy + *Moringa*
Sub-group VI b: 4.0 Gy + *Moringa*
Group – VII: Radiation + Cadmium chloride + *Moringa*
Sub-group VII a: 2.0 Gy + Cadmium chloride + *Moringa*
Sub-group VII b: 4.0 Gy + Cadmium chloride + *Moringa*

AUTOPSY OF ANIMALS

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

PARAMETERS

Biochemical studies

The following biochemical parameters were taken into consideration:
Total proteins
Cholesterol
Acid phosphatase activity
Alkaline phosphatase activity
DNA
RNA

RESULTS AND DISCUSSION

BIOCHEMICAL

The value of total proteins, Cholesterol, DNA and RNA decreased up to day-14 in the non-drug treated groups II, III and IV and day-7 in the *Moringa* treated groups V, VI and VII. This observation indicates that the number of total proteins is adversely affected by cadmium chloride. Toxic chemicals may impair the protein synthesis pattern. According to Sunita (2006) and Purohit *et.al*. (2020) the decline in protein values reflects the combined effect of a decreased of mature cells entering the villi and presence of the damaged cells on the villi. Low values of total proteins corresponded to a decrease in the cellularity of intestinal epithelium. Decrease in protein synthesis also caused a decrease in mitotic index of tissues (Baijal, 1978). The change in protein concentration in the intestine depend 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 other elevation in protein synthesis in discernible. The synthesis of proteins also depends upon the activity of DNA and RNA content, reduced rate of protein synthesis may be due to unavailability of one or more essential enzymes or reduced in the sites of protein synthesis and 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). Combined exposure of radiation and cadmium 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 decrease the rate of transport. Gajawat *et.al*. 2001also reported cadmium chloride and radiation attack -SH groups of protein and the level of blood and liver glutathione (GSH) was decreased, and this lowered GSH level may be responsible for the biochemical alterations. Gould *et.al.*1(956) and Gould and Cook (1958) stated that the total cholesterol decreases after whole body X-irradiation in rats, there is a great increase in the hepatic cholesterol biosynthesis hence cholesterol is either excreted or destroyed by any other means causing a decrease in tissue cholesterol level. DNA is the critical target of radiation damage in the living cells, which may lead to alteration in the functional state of the cell and further to the cell death. Irradiation insults to a single DNA molecule within the nucleus alters the normal process of differentiation of the cell by preventing the formulation of proteins of the cytoplasm, which bring about the differentiation. Damage caused to the DNA molecules by irradiation causes other metabolic alterations also. The prolonged interphase or delayed onset of DNA synthesis after irradiation also could lead to decreased content of DNA or DNA fragmentation (Purohit *et.al*. 1998 and Mylonas *et.al.* 2000). Irradiation disturbs RNA metabolism; it is less sensitive in comparison to DNA metabolism and affect synthesis of nucleic acids in a radiosensitive tissue depends upon cytolysis of large proportion of cells and change in population of cells after irradiation. The single dose may inhibit the RNA enhancing activities thus causing decreased value of RNA. The increase value in later intervals may be due to recovery action attained by intestinal cell against irradiation.

Thereafter, the value increased on day-28 in non-drug treated groups and on day-14 in *Moringa* treated groups because of -OH scavenging by GSH elevated by Moringa.

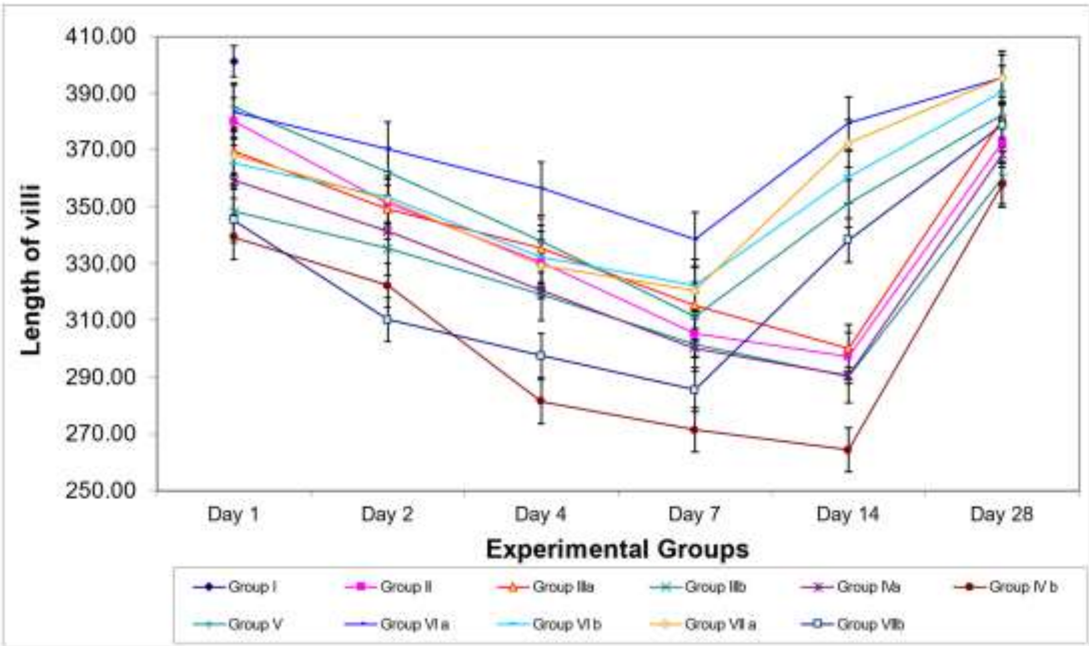
The value of Acid and Alkaline phosphatase activity increased up to day-14 in non-drug treated groups and day-7 in Moringa treated groups then declined in all the groups.

The severe changes were observed after combined exposure showing synergistic effect. The increase was found dose dependent.

The reason for the decrease in the value may be due to the replacement of damaged cells by new cells arising from the division of intestinal stem cells (Jain *et.al.* 2017). In intestine alkaline phosphatase activity is located mainly on the brush border and Golgi zone of the villi epithelial cells. The increased activity may be due to the increased phosphorylation on tissue damage caused by excess amount of heavy metals (cadmium chloride). An early recovery and less severe changes in the drug treated groups indicate protection by *Moringa oleifera*. (Chakrawarti *et.al.,*2010, 2013,2015, 2017, 2022, 2024) stated that ionizing radiation generates both direct and indirect damage to biological molecules. Free radicals cause single or double stranded DNA breaks that, in rapidly dividing tissue such as the intestinal epithelium, trigger apoptosis. A therapeutic action of Moringa may be due to its antioxidant activity that antagonizes alterations in the oxidizing systems that resulted from irradiation.

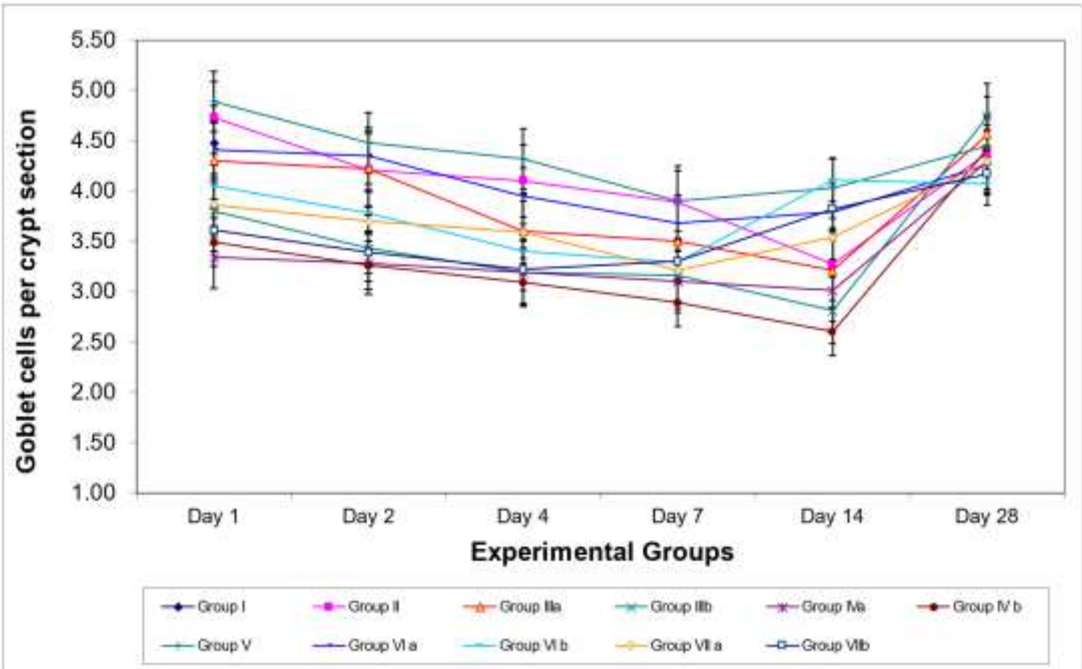
GRAPH- 1

Changes in the length of villi (µm) in the jejunum of mice in various experimental groups (Mean ± S.E.)



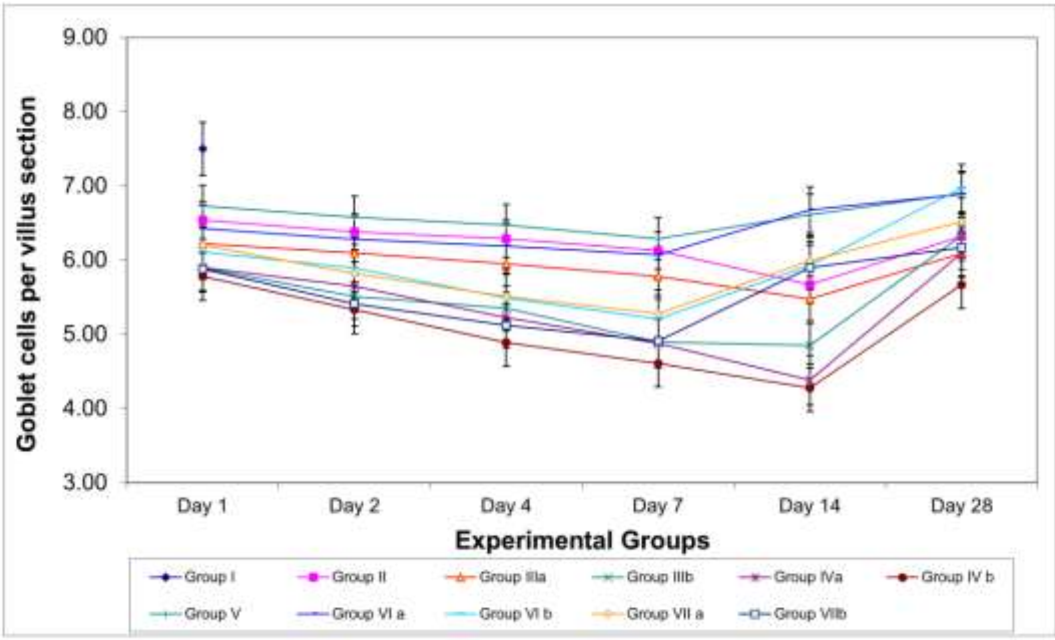
GRAPH - 2

Changes in the number of goblet cells per crypt section in the jejunum of mice in various experimental groups (Mean± S.E.)



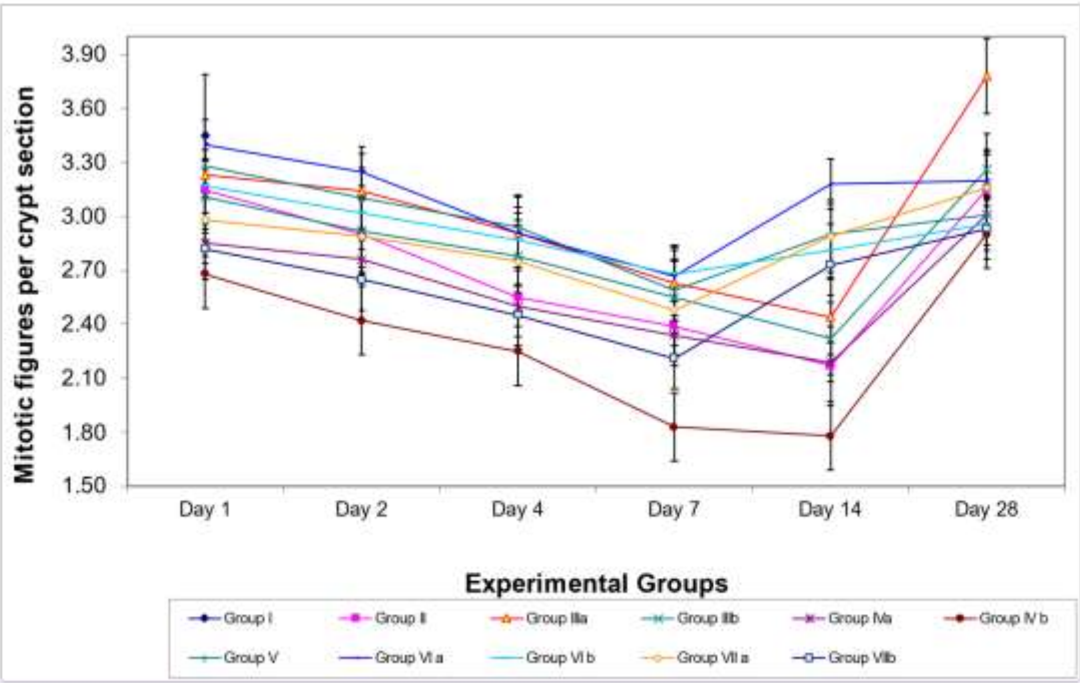
GRAPH - 3

Changes in the number of goblet cells per villus section in the jejunum of mice in various experimental groups (Mean ± S.E.)



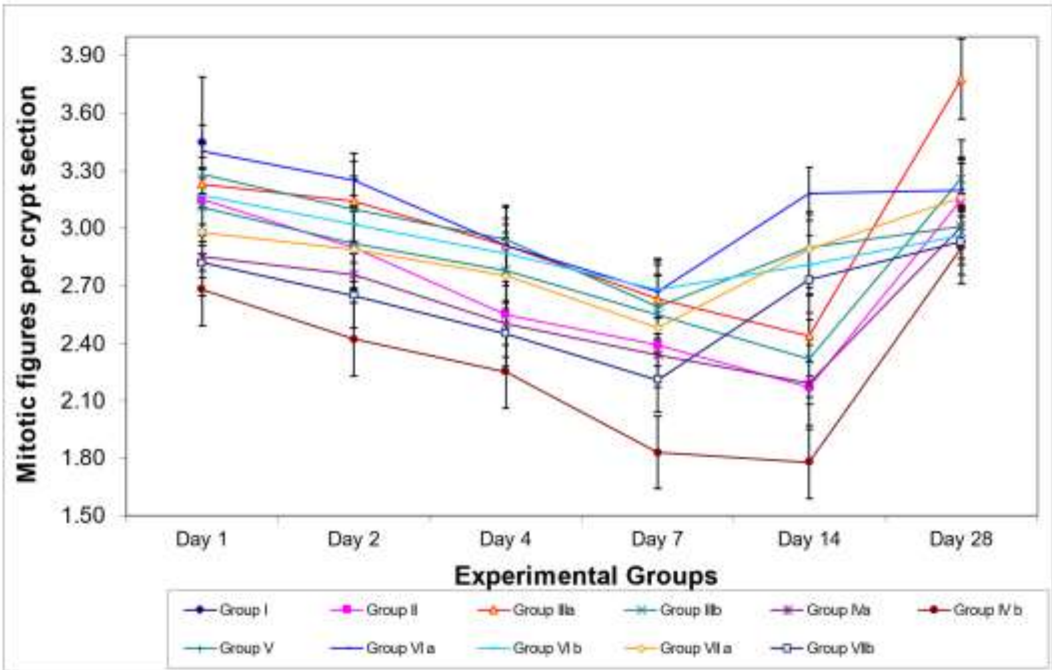
GRAPH - 4

Changes in the number of mitotic figures per crypt section in the jejunum of mice in various experimental groups (Mean± S.E.)`



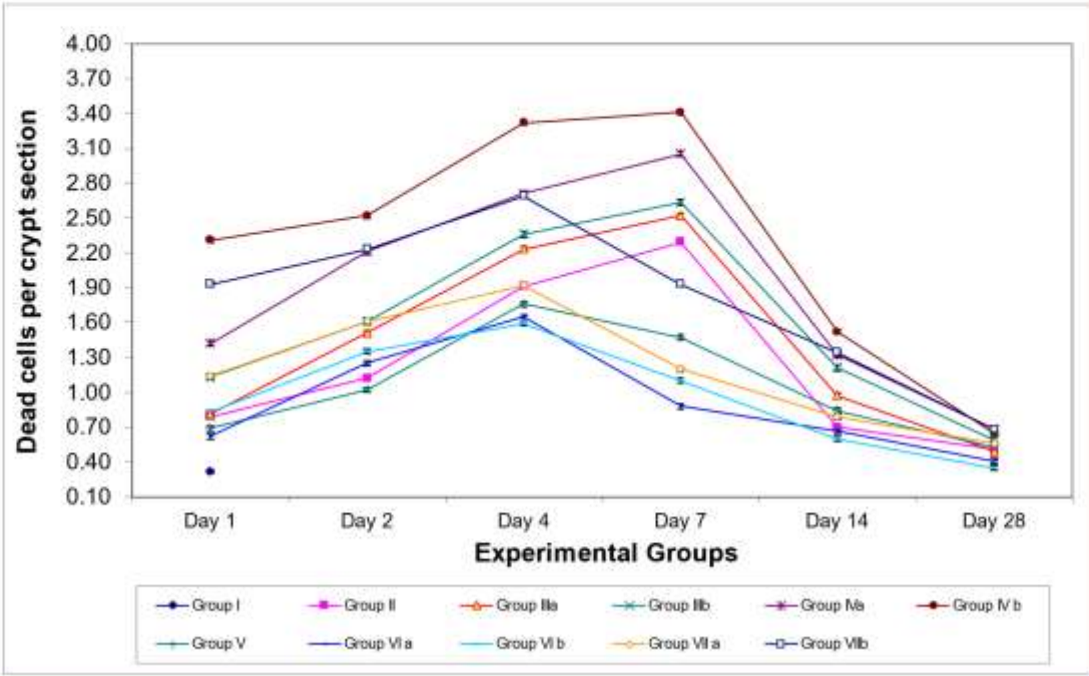
GRAPH - 5

Changes in the number of abnormal mitotic figures per crypt section in the jejunum of mice in various experimental groups (Mean± S.E.)



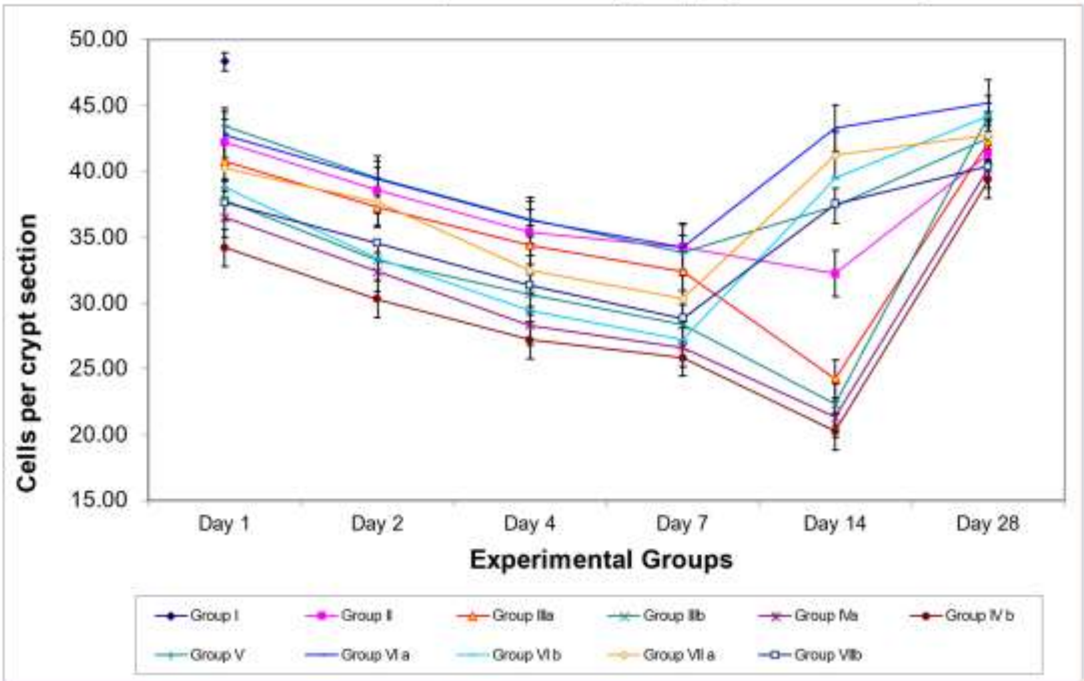
GRAPH - 6

Changes in the number of dead cells per crypt section in the jejunum of mice in various experimental groups (Mean± S.E.)



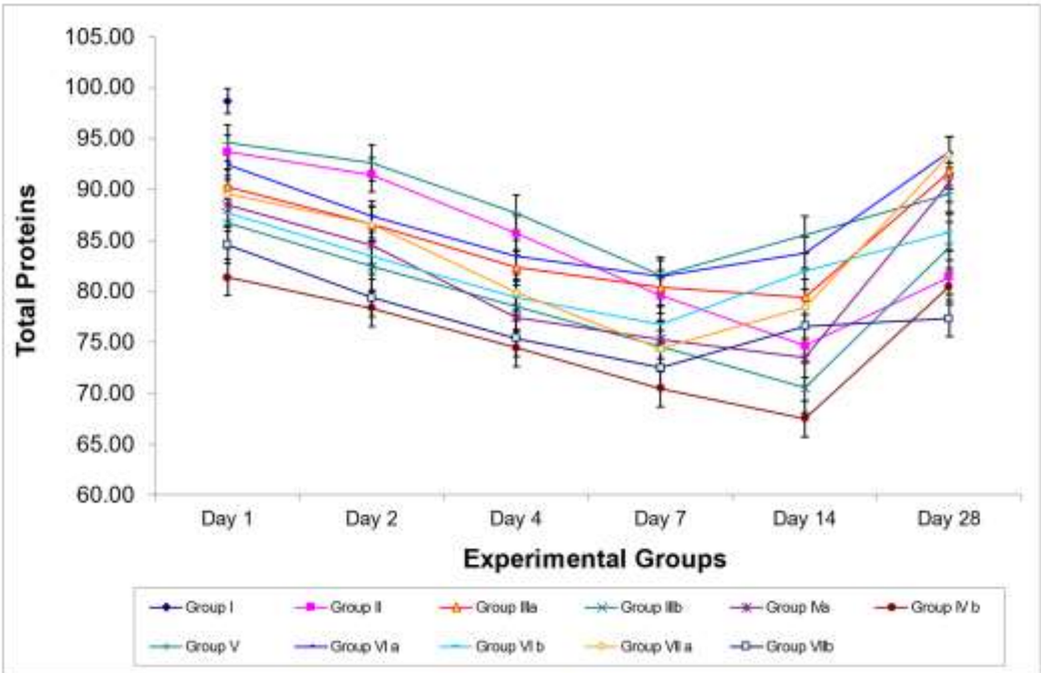
GRAPH - 7

Changes in the number of cells per crypt section in the jejunum of mice in various experimental groups (Mean± S.E.)



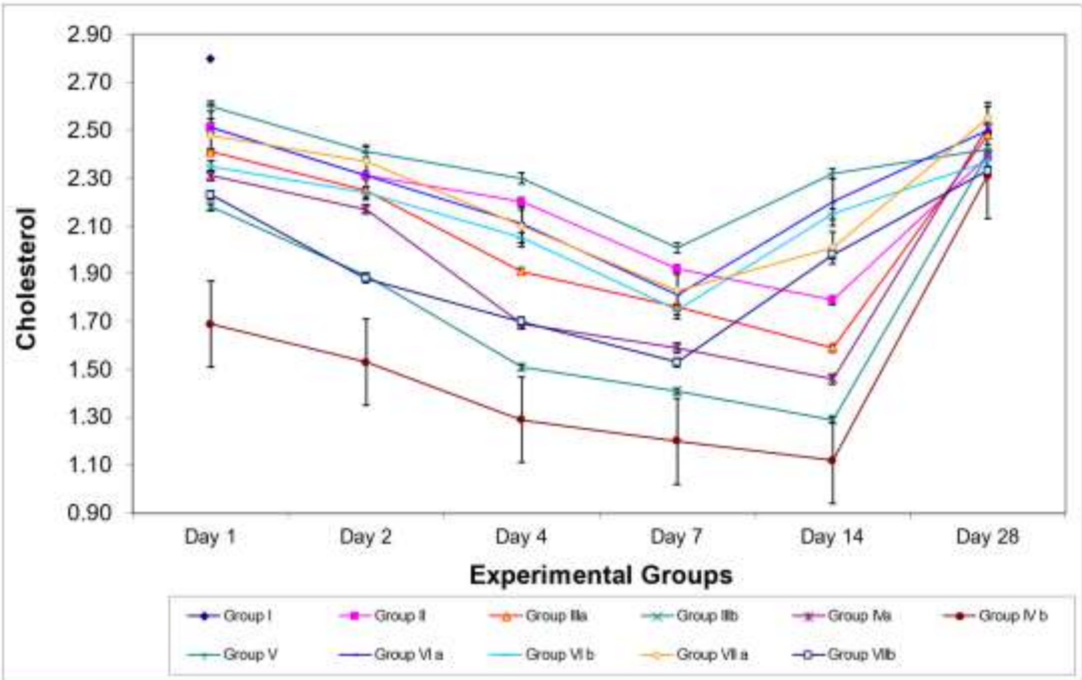
GRAPH - 8

Changes in the values of total proteins (mg/gm of tissue weight) in the jejunum of mice in various experimental groups (Mean± S.E.)



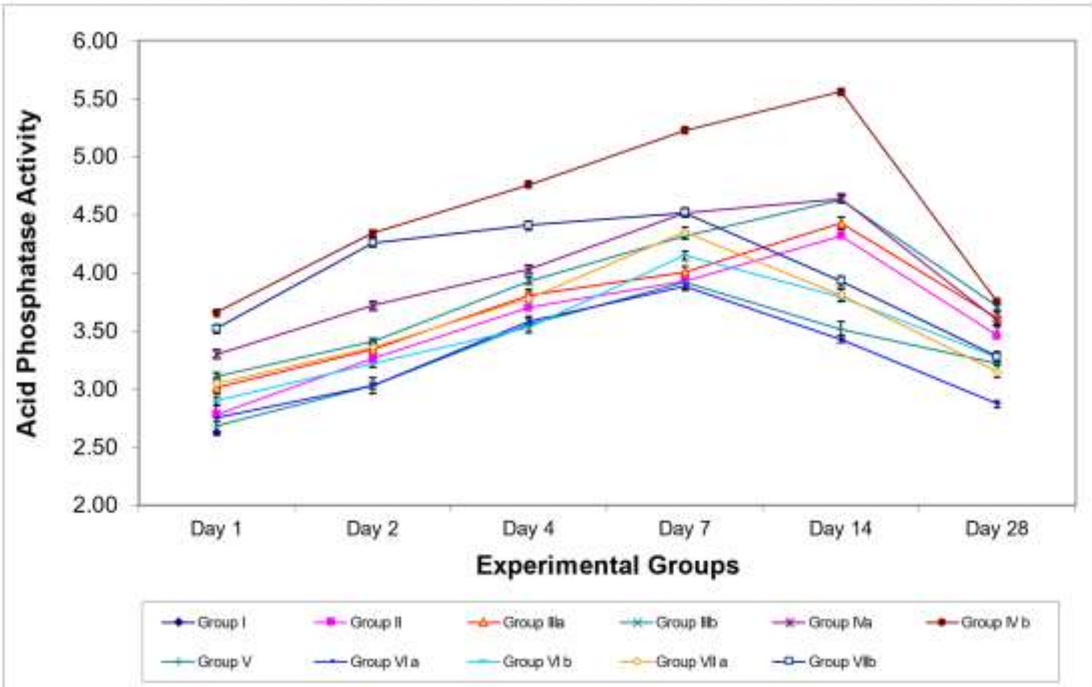
GRAPH - 9

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



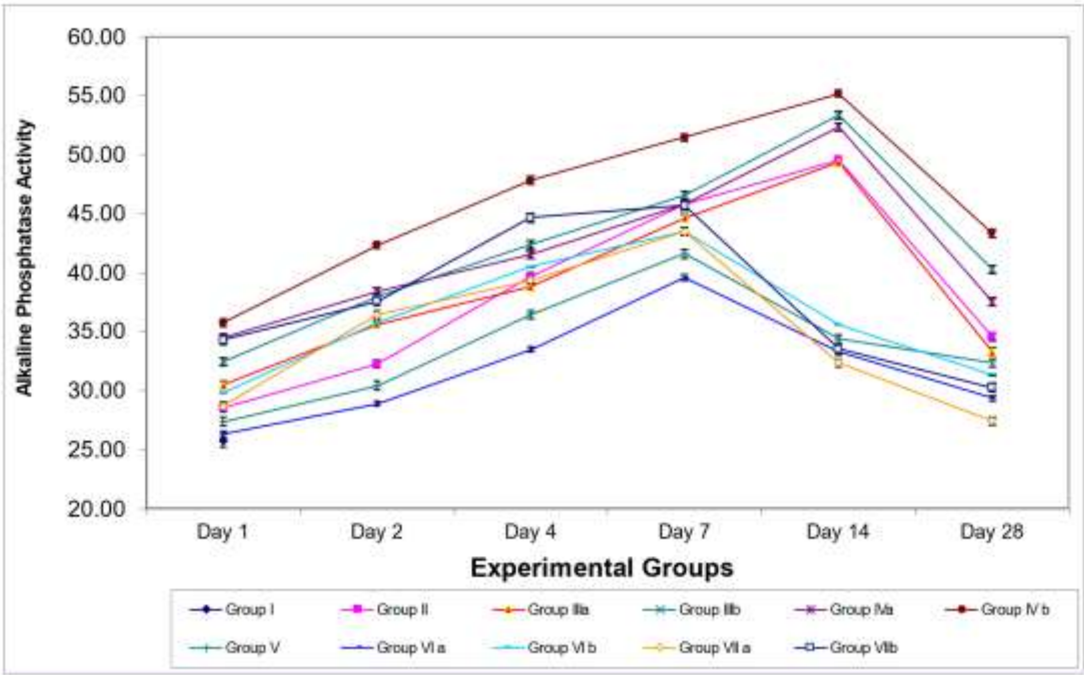
GRAPH - 10

Changes in the values of acid phosphatase activity (mg pi/gm/hr.) in the jejunum of mice in various experimental groups (Mean± S.E.)



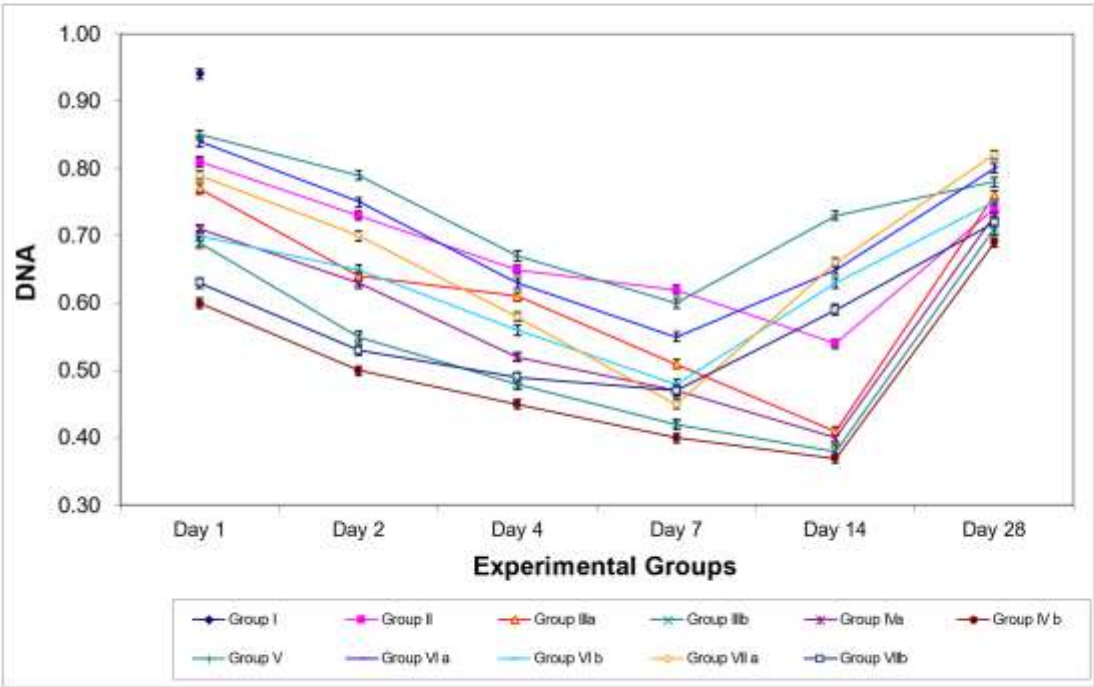
GRAPH - 11

Changes in the values of alkaline phosphatase activity (mg pi/gm/hr.) in the jejunum of mice in various experimental groups (Mean± S.E.)



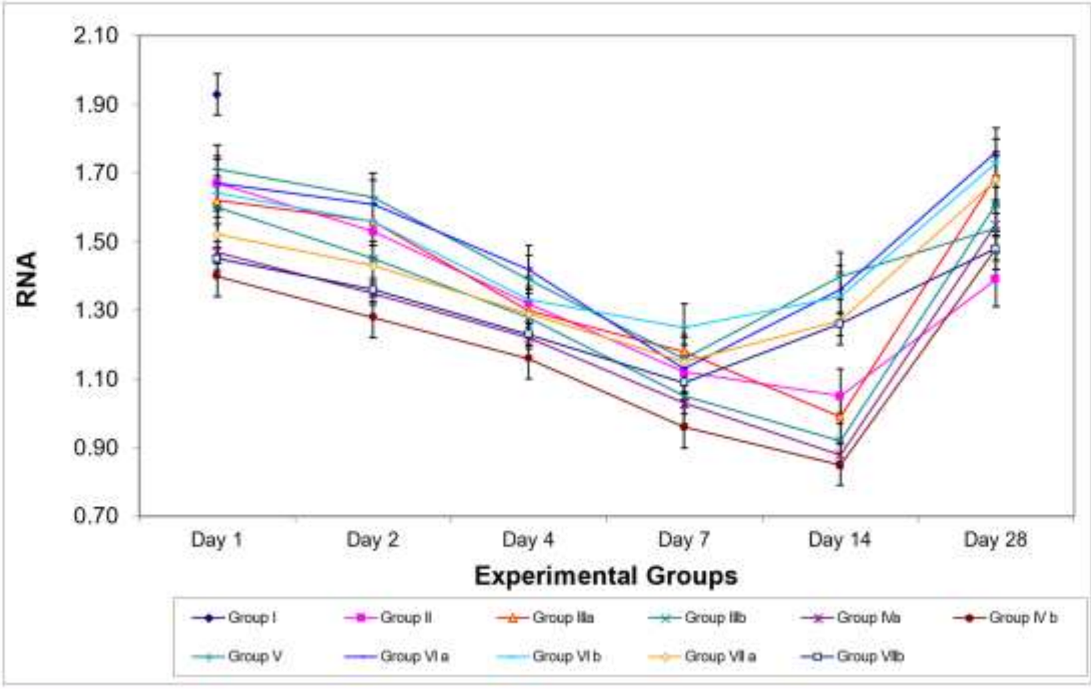
GRAPH - 12

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



GRAPH - 13

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



CONCLUSIONS

From the present findings the following conclusions could be drawn:

1. The jejunum 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 mitotic figures show parallel behavior with the total cell population and inverse relationship with the number of dead cells.

4. The abnormalities in mitoses related to cell death.

5. The combined treatment of radiation and cadmium chloride showed synergistic changes.

6. The jejunum of *Moringa* treated animals showed less severe radio lesions and an early and fast recovery in comparison to non-drug treated animals. Thus, it seems that *Moringa* has protected the jejunum at both the dose levels with and without cadmium chloride treatment.

7. The *Moringa* might have protected the animals from radiation by more than one mechanism due to the multiplicity of its properties.

8. *Moringa* protects the animal by increasing the level of glutathione.

9. It also neutralizes the peroxides formed from water molecules after irradiation.

10. Thus, *Moringa* is a good herbal radioprotector and can be given to cancer patients during radiotherapy to minimize the side effects of exposure.

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