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ORIGINAL ARTICLE

Modifying Effect of β -Carotene against Radiation Stress in Intestinal Crypt Cells Injury

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ABSTRACT

The importance of radiobiological studies could be highlighted as there is no environment which is absolutely devoid of radiation. Most of the body tissues are radiovulnerable. However, all cells do not respond to radiation in the same way. The response of a tissue or organ to a dose of ionizing radiation depends primarily upon two factors- The inherent sensitivity of the individual cell and the kinetics of the population. Small intestine has a cell renewal system. Crypts of Liberkuhn constitute its proliferative compartment, consisting of undifferentiated cells. The function of which is to produce cells for another population. A feedback mechanism can speed up or slow down the production of new cells according to demand. As crypt posses all the characteristics required for a tissue to be radiosensitive. Any radiation exposure will disrupt the normal equilibrium of intestinal mucosa which in turn will have direct bearing on its functions. Thus β- Carotene is a potent radio-protector which neutralizes free radicals and mitigates oxidative stress and stimulates inherent antioxidant defense system. The present study was therefore planned to investigate radidoprotective Efficacy of β-Carotene against radiation stress apoptosis, for this swiss albino mice of 1, 2, 3, and 6 weeks were selected and supplemented β -Carotene (1mg/ml of olive oil) is supplemented for 14 days. An inverse relationship between survival fraction of crypt cellularity and apoptotic index was noticed. The radio sensitivity changes rapidly during the period of growth to maturity and remains constant during adulthood. Post irradiation reduction in crypt cell population can be attributed to (i) an early and marked decline in DNA synthesis (ii) cell death (iii) the movements of cells up to villi in the absence of replacement by cellular proliferation. At early degenerative intervals crypt cell population register a decrease which can be attributed to inhibition of mitosis and increase in apoptotic phenomenon.

***Key words**: β–Carotene, Crypt cell, apoptosis, Radioprotection*

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INTRODUCTION

Life on earth has evolved with an inherent background of radiation. It has always played an important role in natural selection and survival of all biological species. It is not something new invented by the wit of man, radiation has always been there. It is only extra addition to the pre-existing natural background radiation from manmade devices that causes an imbalance. Ionizing irradiation induces severe damage to the intestinal crypt cells which are responsible for renovation and maintenance of the intestinal cellular architecture. (Patel, *et al*., 2012) Recently, radiobiologists have begun to acknowledge the importance of apoptosis or programmed cell death in radiation response. This acknowledgment may be more a rediscovery. However, because in many situations apoptosis is synonymous with "interphase death" an older term used to describe cells that die before their first division after irradiation. The recent interest in apoptosis among radiobiologists has been stimulated by reports of its occurrence after therapeutic doses in

irradiated tumor (Gazda, *et al*., 1992; Potten, 1992 and Stephans *et al*., 1991) and as well as normal tissues (Falkvell, 1991; Meyn *et al*., 1993).

Apoptosis can be induced by a variety of stimuli such as ionizing radiations, gluco-corticoids, chemotherapeutic agents, lymphokines deprivation and various oxidants (Sies, 1993). Although the stimuli which induce apoptosis vary markedly, the morphological features of the process are however conserved in different cell types (Wyllei *et al*., 1980). Exposure of various cell types to oxidative stress-causing agents can directly induce apoptosis which can be blocked by a wide range of antioxidants. It is now believed that oxidants may be essential biochemical intermediates in the progression of many forms of apoptosis induced by different stimuli and the presence of antioxidant in body at the time of radiation exposure mitigate the damage caused by free radicals. It is essential to identify efficient, safe, and affordable radioprotectors for the prevention of radiation-induced damage in intestinal cells. Recently, several studies have been carried out to discover radioprotective agents from natural resources such as fruit, vegetables, and medicinal plants (Park and Pezzuto 2002; Stan et al. 2008), previous studies stated that beta-carotene treatment may protect the impairment of oxidative stress and ameliorate intestine damage at biochemical and histological levels. (Vardi *et al* 2008). In the present study, β-carotene through its antioxidative mechanism (free radical scavenger) prevent the radiation induced damage to intestinal cells.

MATERIAL AND METHOD

The present investigation has been designed in two phases:

The first phase of experiments was conducted to observe and evaluate the effect of β carotene administration in developing (1-3 weeks) and adult (6 weeks old) mice. In the second phase of experiments, the protective efficacy of β carotene has been examined in the Intestine.

Animals: Swiss albino mice (*Mus musculus*) originally procured from AIIMS, New Delhi were maintained and bred in ventilated and constant temperature (18-22°C) housing and were fed with balanced food manufactured by Hindustan Lever Ltd. Water was given ad libitum.

Source of Irradiation: Mice were irradiated at the Radiotherapy Unit of S.M.S. Hospital, Jaipur, India. by a Theratron model B 60Co beam therapy unit supplied by the AER, Canada Ltd., Canada. Mice were exposed to a total dose of 4.5 Gy.

Radio-protective Drug: β-CAROTENE: β carotene was procured from Sigma Chemicals, USA in powder form. It was dissolved in olive oil and solution containing 1 mg β carotene /ml of olive oil is prepared. This solution was given to animals orally for two weeks at the dose of 30 mg/kg of their body weight.

EXPERIMENTAL DESIGN

Swiss albino mice of 1, 2, 3 and 6 weeks age were selected from the colony and supplemented β carotene for 14 days to animals of each age group as per the following protocol–

Animals of belonging to each age group were divided into four groups as per the following protocol-

1. **Group 1 (Normal):** Mice of this group did not receive any treatment and served as normal.
2. **Group 2 (Beta-Carotene):** Mice of this group received oral doses of beta-carotene (30mg/kg b.wt.)
3. **Group 3 (Beta-Carotene + Irradiation):** In order to examine radio-protective efficacy of beta carotene mice were exposed to 4.5Gy of gamma radiation after treatment with beta carotene.
4. **Group 4 (Irradiation):** To evaluate the direct effect of radiation, mice were exposed to 4.5 Gy of gamma radiation.

The jejunum (a portion of small intestine next to duodenum) was removed carefully and fixed in Bouin's fluid. After routine dehydration and paraffin embedding, blocks were prepared. 5 m thick sections were cut, fixed and stained with Eosin and Harris Haematoxylin. Stained sections of jejunum were observed for the crypt cellularity parameters.

SELECTION OF CRYPTS

Crypts cut longitudinally and centrally (as evidenced by a continuous lumen for the base of the villus to paneth cells at the bottom and which had a neck) were considered for the scoring. Cells were counted on either side of the crypt starting from the base at a point where the lumen axis touches the epithelium up to the crypt villus junction. The latter was identified by either of the following two criteria:

(i) The crypt epithelium forming a genu where it continuous as villus epithelium; and

(ii) From the base of the crypt where the nucleus of each succeeding cell gradually gets centrally placed. The epithelial cells with a typically centrally placed nucleus were thus considered to be the crypt villus junction. Those crypt cells were counted in which the nucleus was visible in the section and cytoplasm was in contact with the basement membrane.

OBSERVATION

Histopathological studies of jejunum revealed that the pattern of radiolesions produced in different age groups (1, 2, 3 and 6 weeks of age) was of similar nature. However, it differed in the degree. In early age groups it was more severe and pronounced.

β -Carotene Supplementation Initiated From1, 2, 3 and 6 Weeks of Age

β -Carotene Vs Normal

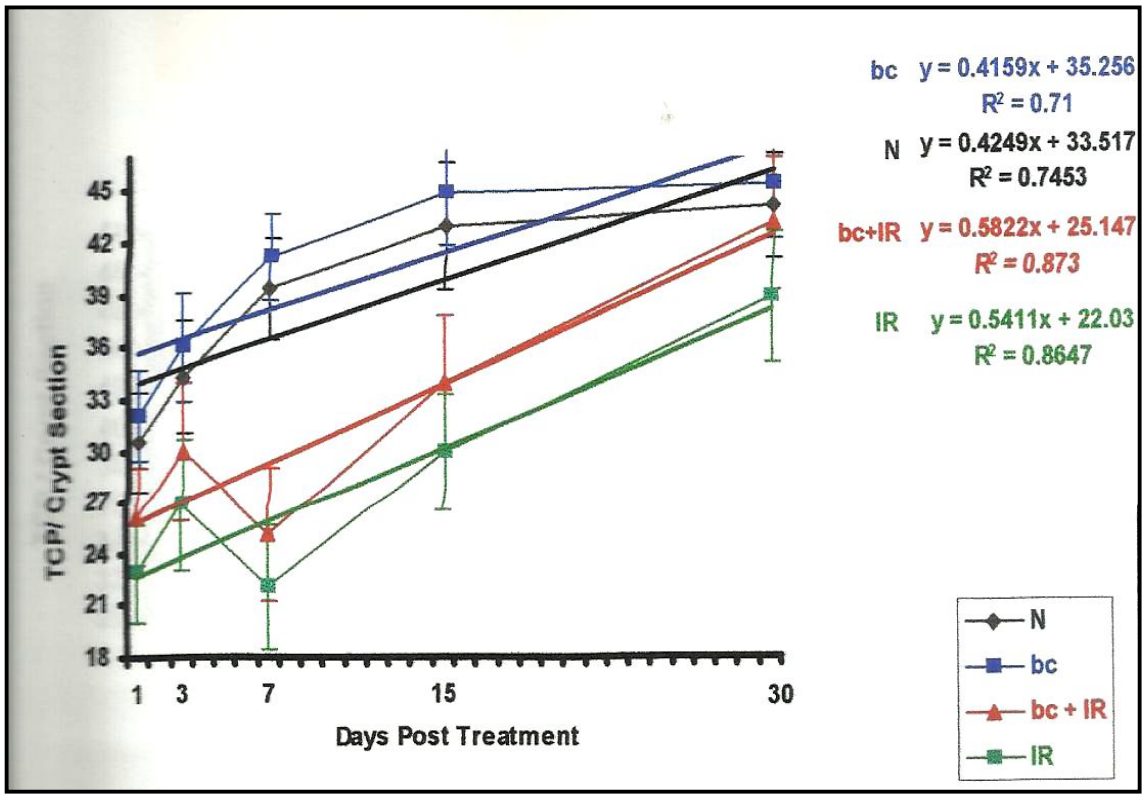
The graphs A, B, C and D showed that the count of Total cell population per crypt was raised from day 1 to the last interval day 30 in both normal and β -Carotene supplemented group. In one week age group, the crypt cell population of β -Carotene fed animals was found to be non significant except on day 7 (statistically significant at $P < 0.05$).When β -Carotene supplementation is initiated from 2 week of age, significant difference from normal could be observed only on day 30 p.i. When β -Carotene treatment is initiated from 3 and 6 weeks of age, the difference was non significant at all the observation intervals but the magnitude of count was higher in β -Carotene supplemented groups.

EXPERIMENTAL CONTROL

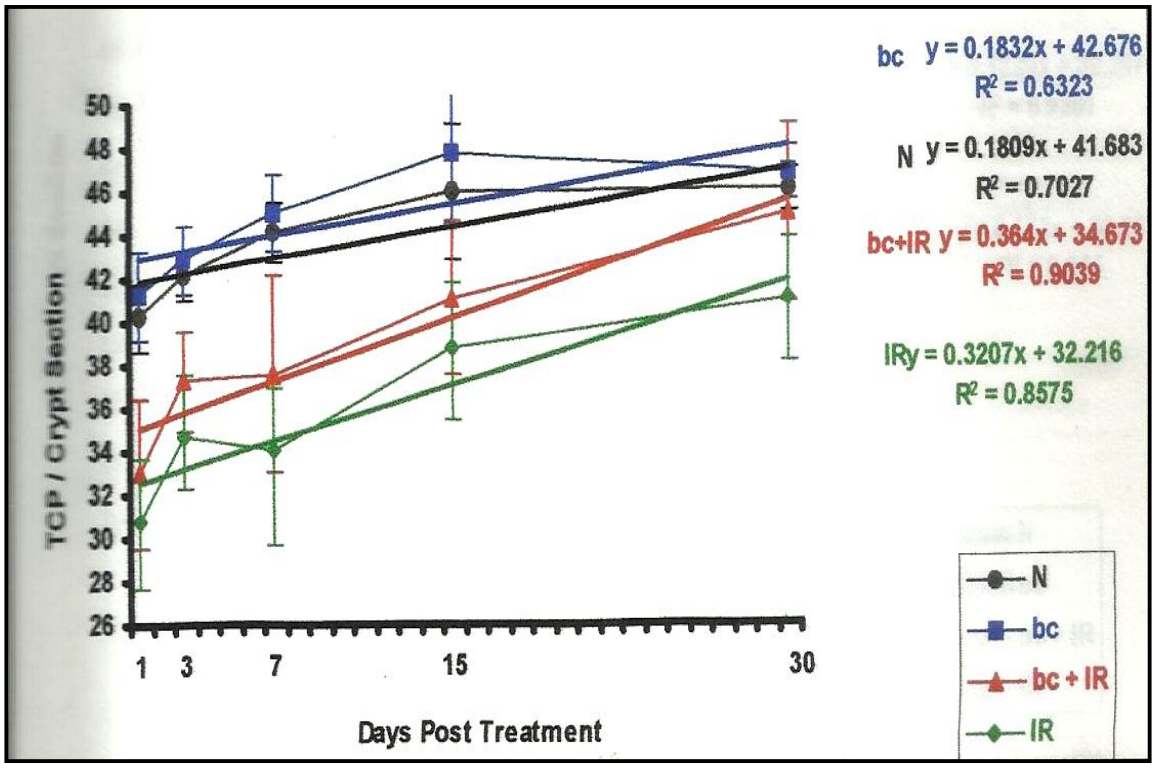
One Week of Age Group: After exposure to 4.5 Gy gamma radiations, the count of crypt cell population show a profound decrease at day 1 and coefficient value showed a strong correlation between both control and experimental group in similar manner. However, the experimental group shows statistically higher count ($P < 0.001$). Relapse of damage was observed at post exposure day 7 and percentage decreased by approx. 36% and 44% in experimental and controls groups respectively. This minimum value is followed by an increase on 15 day, which marks the beginning of the recovery phase. This increase in crypt cell population continues during subsequent intervals and it approaches to normal value by day 30. (Graph A)

Two Weeks of Age Group: In this age group, the biphasic trend of crypt cell population is similar as one week old age group. Severe damage is observed at day 1 where the damage was maximum by approx. 24% in control and 18% in experimental animals At post exposure day 7 percentage again register a fall in both the groups thereafter the count progressively increases and by day 30 near to normal values are approached. At each post exposure interval, the behavior of curve was obtained similar in both control as well as experimental group. This was strongly supported by correlation coefficient. (Graph B)

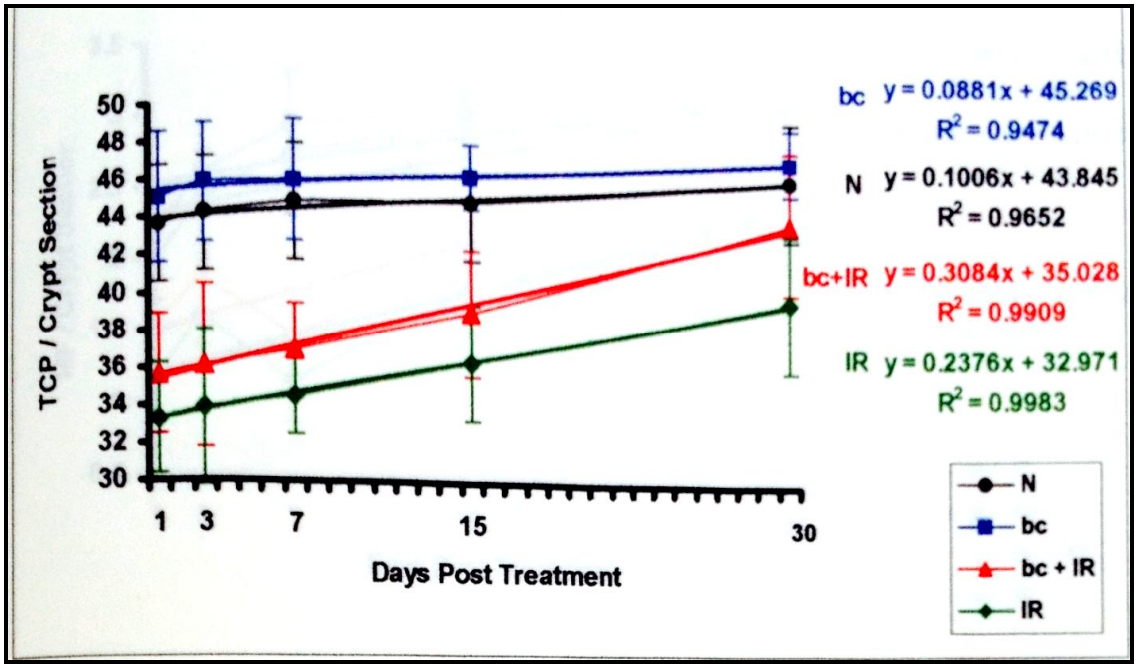
Graph A: Variation in the count of Total Cell Population / Crypt Section (±SD) of One Week Old Mice at Various Post Treatment Days, in the presence and Absence of β- Carotene



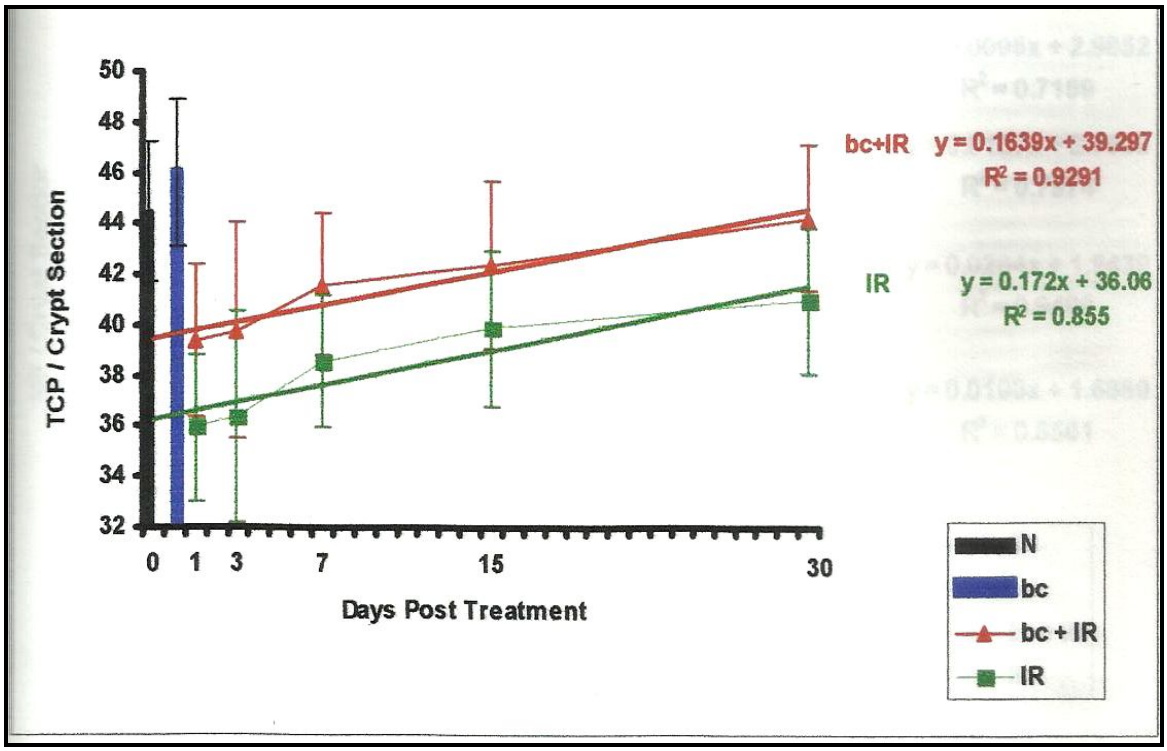
Graph B: Variation in the count of Total Cell Population / Crypt Section (±SD) of Two Week Old Mice at Various Post Treatment Days, in the presence and Absence of β-Carotene



Graph C: Variation in the count of Total Cell Population / Crypt Section (±SD) of Three Week Old Mice at Various Post Treatment Days, in the presence and Absence of β-Carotene



Graph D: Variation in the count of Total Cell Population / Crypt Section (±SD) of Six Week Old Mice at Various Post Treatment Days, in the presence and Absence of β-Carotene



Three Weeks of Age Group: Crypt cell population showed a post irradiation reduction at day 1, which was maximum in control by 24% and by 12% in experimental animals. The pattern of decrease or increase in the values after gamma irradiation was similar in both

the control as well as experimental group. As it is evident from the analysis of correlation coefficient. This minimum count of crypt cells is followed by an increase on day 3, which marks the beginning of recovery phase. This increase in crypt cell population continues till the last post irradiation interval. Difference between both the groups was statistically higher in experimental group except day 3, which was found to be non-significant. (Graph C)

Six Weeks of Age Group: The control and experimental animals of this age group have shown the variation in crypt cell population as similar as in the variation of crypts cell population of 3 weeks of age. After exposure maximum percentage fall was register on day 1 by approximately 21% in control animals. When we supplemented b-Carotene before irradiation approximately 87% protections was observed at the same interval. There was strong correlation between both the groups. As it is evident from the correlation analysis. Recovery was observed from day 3 p.i. to the last autopsy interval in both experimental and control animals but the magnitude of recovery was statistically higher in experimental animals. (Graph D)

RESULT AND DISCUSSION

Radiation injuries result from the increased production of reaction oxygen species (ROS) such as superoxide anions, hydroxyl radicals, hydrogen peroxide, peroxyls, and alkoxy radicals. These reactive molecules may play an important role in the initiation and propagation of free radical chain reactions, inducing potentially severe damages to cells (Maharwal *et al* 2005). The intestinal epithelium is markedly sensitive to ionizing radiations and the jejunum constitutes the most radiosensitive segment of the gastrointestinal tract. Radiation interferes with the proliferative activity of the crypt by inhibiting mitosis of its very sensitive stem cells or by killing them. With the cessation of cell division there was a complete loss of cell renewal for the other areas of the epithelium. Radiation induced insult of jejunal epithelium include suppression of mitosis, vacuolation, chromatolysis, karyorrhexis, abnormal mitosis and appearance of apoptotic cells in the crypt. Immediately after irradiation, intestinal crypt cells undergo apoptosis, and stem cells cannot recover as rapidly as necessary to restore intestinal villi. As a consequence, the heights of small intestinal villi are reduced, and blunting as well as subsequent functional incapacity occurs immediately after ionizing radiation (Somosy *et al*. 2002). These intestinal damages following an exposure to ionizing radiation are collectively called gastrointestinal (GI) syndrome. (Danbee Ha *et al* 2013). Villus epithelium showed swollen or empty cells, broken tips and hydropic degeneration in lamina propria. Tsubouchi and Matsuzawa (1974) reported that the tendency for the cells to develop into pyknotic cells was high in G2 phase, intermediate in S phase and low in G1 phase.

In all the experimental animals belonging to different age groups after initial damage at day 1,clear signs of recovery were noticeable at day 3 However, after this the jejunum epithelium of different age groups shows a different trend. In one week age group animals after beginning of recovery, a phase of relapse of damage was observed at day 7 in both control and experimental animals. Damage was less severe in drug treated animals than control ones. In two weeks animals the pattern of post irradiation damage and recovery was found to be similar to one week old animals. In the control and experimental animals belonging to 3 and 6 weeks of age groups the maximum damage was evident on 1 day pi. It was followed by initiation of recovery on day 3 which continued till last autopsy interval and normal values were approached. Thus, no second phase of damage was evident in these animals.

The survival fraction of crypt cellularity exhibits an inverse relationship with apoptotic index. It is now almost an established fact that there exists a correlation between age and sensitivity to ionizing radiation. The radiosensitivity changes rapidly during the period of growth to maturity and remains almost constant during adulthood. In the present work as

a result of exposure with gamma rays the percentage of apoptotic bodies was found to increase in jejunal crypts of control animals in comparison to coeval normal animals in all the age groups. This is indicative of the stressed homeostatic system of jejunum leading to the rapidaly of removing damage from the tissue. In the present study b-carotene is particularly efficient as a singlet oxygen quencher but also acts as a free radical scavenger. The metabolic fate of carotenoids in the human organism is virtually unknown apart from the conversion of provitamin A carotenoid to vitamin A (retinol, retinol esters) in the intestinal mucosa and to a lesser extent in the liver. It has been found that retinoic acid which is vitamin A metabolite have anticarcinogenic activity Thus, the chemoprevention and anti-cancer effects of retinoid may also play an important role in the preservation of organs (Yamamoto, 2001).

CONCLUSION

From the above finding and in the light of discussion the following conclusion can be drawn.

1. β-carotene administration prior to irradiation reduces the destructive effect of gamma rays on developing jejunal epithelium and augments the process of recovery and reparation.
2. β-carotene offers higher protection in experimental animals of one week age group as compared to other ages. In one week age group animals, maximum susceptibility to apoptotic death was noticed at post exposure day 7.
3. On the basis of radioresponse jejunal epithelium of different postnatal ages can be arranged in a sequence of decreasing radiosensitivity as per the order 1>2>3>6.

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