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THERAPEUTIC POTENTIAL OF CUCURBITS AGAINST RADIATION INDUCED INFLAMMATION AND DAMAGE IN MICE GUT

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Radiation damage, Gut-inflammation, Cytokines, Gut-microbiota, Villi, Dietary-cucurbits

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ABSTRACT: Whole body or local irradiation to gut is known to induce significant damage to gut epithelium and crypt stem cells. A large population of microbes in the gut remains unaffected. We hypothesize that the modifications in diet and enteric microbial population may provide a non-toxic mechanism to counter radiation induced health deficit, especially in the gut and therefore investigated the effect of dietary cucurbits in this context. Radio-protective effects of *Lagenaria* (Ls), *Cucurbita* (Cp) and *Luffa* (Lc) were evaluated in mice for cytokines (in jejunum tissues) through ELISA and intestinal villi microscopically. Irradiation of mice increased pro-inflammatory biomarkers NAP-3, IFN-γ and IL-17 and decreased anti-inflammatory biomarkers IL-10, IL-4 and TGF-β. Post-irradiation treatment with cucurbit extract (Ls, Cp or Lc) decreased pro-inflammatory cytokines NAP-3, IFN-γ and IL-17 and increased anti-inflammatory cytokines IL-10, IL-4 and TGF-β in mice. Treatment with cucurbits restored structural integrity of villi in irradiated mice. Various antioxidants and anti-inflammatory molecules present in cucurbits, their positive effects on probiotics and host tissues and microbial fermentation of dietary fibers may mitigate radiation induced damage. The cucurbits, as non-toxic anti-inflammatory agents and nutrition enhancer can be helpful in irradiated and inflamed systems.

INTRODUCTION: Whole body exposure to ionizing radiations results in production of reactive oxygen species, which initiate a series of signalling cascades and lead to the genesis of several pro-inflammatory responses in various tissues and systems leading HP and GI syndrome, depending on the dose received [1, 2]. Radiation induced inflammation in the gut is one of the major events during radiotherapy and radiation accidents.

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The gut mucosa, under normal conditions maintains a quiescent state against a diverse array of dietary antigens and luminal microbes. However exposure to ionizing radiation leads to gut inflammation and stem cell necrosis [3, 4]. The inflammatory manifestations of the gut are therapeutically managed in palliative and symptomatic manner through analgesic, anti-emetic and anti-diarrheal agents, though most of them induce adverse effects [5].

In view of this, the search for newer, less toxic and more effective anti-inflammatory agents becomes inevitable. In fact, a large number of agents of natural and synthetic origin have been attempted against gastro-intestinal (GI) inflammation [6]. The

human colonic microbiota is very large, about 10 - 100 times the total number of human cells in an individual, comprises a consortium of a large number of bacterial species (450 - 500), and out of this only about 45 - 50 spp. are present in an individual [7]. The triangular interaction between host, the enteric microbiota and the diet, leads to the generation of metabolome which remains central to all the organs and systems of the body for nutrition, growth and the repair / recovery. The modification of metabolomic contents can be manipulated through diet and exploited for alleviation of the radiation induced health deficits. The recent literature extensively reveals about the influence of diet on host and microbiota and their subsequent implications in inflammatory and immune responses [8, 9].

The inflammatory response is coordinated by a wide range of signalling molecules and pathways that constitute complex regulatory networks like NFkB and MAPK signalling pathways. Nuclear Factor-kappaB (NF-κB) is the gene transcription factor, ubiquitously present in every cell and has a key role in enhancing immune and inflammatory responses [10]. NF*k*B upregulate expression of genes for pro-inflammatory cytokines, chemokines, enzymes, receptors, apoptotic regulators, growth factors and adhesion molecules that play a key role in the inflammatory processes and regulate several aspects of innate and acquired immune responses [10]. A large number of the immune-competent cells are located in the intestinal epithelium, Peyer's patches, lamina propria and isolated lymphoid follicles. Within the lymphocyte subsets that populate mucosa and gut-associated lymphoid tissues (GALT), T helper cell populations (Th-1, Th-2, and Th-17) and a wide variety of regulatory T cells (T_{regs}) such as Th_3 (primarily secrete TGF-β) and Tr_1 (secrete high levels of IL-10) are noteworthy [11]. Th-17 cells have been identified as major mediators of pathology in the inflammatory disease models. The balance between Th-17 and T_{reg} populations is important for maintaining a healthy immune status.

A number of medicinal plants evaluated for their radio-protective efficacy have shown protective effects against the damaging effects of ionizing radiation. An urge to consume cucurbit diet during dysbiosis may be an indication of signalling by gut-brain axis and this led to the selection of cucurbit plants for this study. Cucurbits were investigated for their effect on various inflammatory biomolecules involved in intestinal inflammation. Since ancient times, the cucurbits have been used as prebiotics for treating pathological conditions of GI tract [12, 13]. A wide range of chemical compounds including sterols, terpenoids (cucurbitacins), flavonoids, bryonolic acid and saponins have been isolated from various cucurbit plants and have been reported to exhibit analgesic, anti-inflammatory, immunomodulatory and antioxidant [12, 14, 15].

To counter or mitigate the inflammatory action in GI tract several prebiotics have been demonstrated to suppress pro-inflammatory mediators like tumor necrosis factor-α (TNF- α), interferon-γ (IFN-γ) and IL-8 [9, 16], which are found in high levels in intestinal mucosa of patients suffering of inflammatory bowel syndrome (IBS). Prebiotics also modulate immune responses by down-regulating T helper population but little information is available on the direct effect of prebiotics on various populations of T-helper cells like Th_1, Th_2, Th_{17} and T_{regs} and warranted investigation. The damage to GI system is also reflected in the shortening of intestinal villi through destruction of the stem cells (crypt cells). The intestinal villus is a self-renewing organ and the mucosa overlying it is replaced entirely every 3 - 5 days. Stem cells present at the base of each villus are responsible for self-renewal of intestinal villi. During radiotherapy of GI tumors, the highly radiosensitive stem cells of the crypts get damaged and subsequently, inhibition of stem cell proliferation leading to shortening of villi height.

Cucurbits (*Lagenaria siceraria*, *Luffa cylindrica* and *Cucurbita pepo*) have already been shown to display potent anti-inflammatory and radio-protective activities *in-vitro* [17]. *Lagenaria* extract has been reported to improve the tight junction integrity and length of the villi in the mice which were subjected to radiation exposure (10 Gy) *in-vivo* [18].

In view of the sparse information regarding the effect of cucurbits as prebiotics on modulation of inflammatory response, we investigated the effect of cucurbit extracts (Ls, Cp, and Lc) on various inflammatory (NAP-3, IFN-γ and IL-17) and anti-

inflammatory (IL-10, TGF-β and IL-10) cytokines and intestinal villi morphology (height and width).

MATERIALS AND METHODS:

Reagents: Various chemicals and reagents were procured from different sources: Formaldehyde, Ethanol, Xylene, Paraffin, Canada balsam from Merck (Mumbai); Hematoxylin and Eosin from M/S Fisher Scientific, Mumbai. Protease inhibitor cocktail from M/s Sigma Aldrich (USA). Bradford reagent from Bio-Rad Laboratories (USA). ELISA kits for NAP-3. IL-17, IFN-γ, IL-10, IL-4, and TGF-β from Cloud-Clone Corp., Houston.

Preparation of Plant Extracts: Fresh fruits of cucurbits, *Lagenaria siceraria* (Ls), *Luffa cylindrica* (Lc), *Cucurbita pepo* (Cp) were procured from local market, identified by the botanist at Department of Biosciences, Jamia Millia Islamia, New Delhi and were thoroughly washed with distilled water. Known quantity of a plant material was homogenized in distilled water and kept for 24 h at 25 °C and was filtered thereafter through a fine strainer having a spread of muslin cloth. The filtrate was further passed through membrane filter of 0.22µ, lyophilized and stored at 4 °C in air tight bottles.

Animals: Balb/c female mice (14 weeks old) weighing about 25g were bred, housed and treated under specific pathogen free laboratory environment (25 ± 2 °C, photoperiod - 12 h) and were given standard animal diet (Lipton, India) and tap water *ad libitum*. The experimental protocol was approved by institutional animal ethics committee of Amity University, Noida.

Irradiation: The Tele-therapy cobalt machine (Bhabhataron II) at 'Institute of Nuclear Medicine and Allied Sciences' Delhi, was used for whole body gamma irradiation (6 Gy) of experimental animals. The dose rate during these experiments ranged between 1.278 to 1.250 Gy / Minute and each mouse was irradiated individually.

Experimental Design: 66 mice were randomly assorted to 11 groups, each containing 6 animals. Cucurbit extract at the rate of 2 mg/kg body weight was administered to each mouse orally through a catheter. For sample collection, the animals were sacrificed by cervical dislocation on 8th day after irradiation. The animals were grouped as under:

- **Group 1 (n = 6):** Control, no treatment.
- **Group 2 (n = 6):** Irradiated control, received 6Gy (single dose) whole body gamma irradiation.
- **Group 3, 4, 5 (n = 6 in each group):** Post-irradiation cucurbit treatment (Protocol A), each mouse received 6 Gy WBI and one hour later received a single dose of cucurbit extract (Ls, Cp or Lc) for 7 post irradiation days.
- **Group 6, 7, 8 (n = 6 in each group):** Pre-irradiation cucurbit treatment (Protocol B), each mouse received a single dose of a cucurbit extract (Ls, Cp or Lc) for 7 days and irradiated on 8th day.
- **Group 9, 10, 11 (n = 6 in each group):** Pre + Post-irradiation cucurbit treatment (Protocol C), each mouse received cucurbit extract (Ls, Cp or Lc) for 7 days before and 7 days after irradiation.

Cytokine Analysis: The jejunal section (2cm long) about 3cm away from duodenal end was carefully taken out from each mouse, and was washed with cold PBS, pH 7.4, and fresh frozen immediately in liquid nitrogen for analysis of different cytokines (NAP-3, IFN-γ, IL-10, IL-4, IL-17, TGF-β). Samples were prepared following the method described by Liu *et al*., [19] Briefly, frozen tissues were homogenized in lysis buffer containing protease inhibitors. The homogenates were then centrifuged at 12,000g for 10 min at 4 °C after incubation on ice for 30 min. The supernatant from each sample was collected and stored at - 20 °C. In each sample total protein concentration was estimated sing Bradford Reagent. Cytokines level in each sample was estimated through ELISA following manufacturer's instructions.

Histological Study: About 2cm long pieces of jejunum were taken out from each mouse and were fixed in 10% neutral formalin (pH 7.0–7.6) for 24 hours. The tissue was processed for dehydration and paraffin block making following standard procedure. Microtomy was done to get 5µ thick sections which were stained using Haematoxylin and Eosin (H and E) following standard method [20]. The sections were studied under the light microscope (40X) for various villar parameters

(height and width). Data for 10 villi per section was collected.

Statistical Analysis: The data from animals of each group was pooled and has been presented as mean ± SE. Student's t test was applied to estimate the statistical significance difference between different experimental groups at $P < 0.05$.

RESULTS AND DISCUSSION: Exposure to ionizing radiation is known to generate oxidative stress, a pro-inflammatory response and modulation of the expression of pro- and anti-inflammatory cytokines. The inflammatory manifestations in the gut have been reported to be mediated *via* pro-inflammatory cytokines like TNF-α, IFN-γ, IL-17, IL-6 and NAP-3 [21, 22]. In this context, the immuno-suppressive responses are mediated by anti-inflammatory cytokines such as IL-10 [23, 24], TGF-β [25] and IL-4 [26].

Effects of Cucurbits on Expression of NAP-3 *in-vivo*: Daily administration of cucurbit extract (Ls, Cp or Lc) orally to mice for 7 days post irradiation (Protocol-A) significantly decreased the expression of NAP-3 to 91, 89 and 83% of irradiated control, 100% (**Fig. 1**). Pre-treatment of mice with Ls for 7 days before irradiation (Protocol-B) also decreased NAP-3 expression significantly, 85% of irradiated control; while Cp, or Lc remained insignificant at decreasing the NAP-3, 92 and 91% of irradiated control respectively. Mice under protocol C received cucurbit extracts Ls, Lc or Cp for 7 days before and 7 days after irradiation and NAP-3 expression decreased to 82, 81 and 87% of irradiated control (100%) respectively. Cp when administered under protocol C (Pre- and post-irradiation combined treatment) rendered maximum decrease in NAP-3 when compared to control.

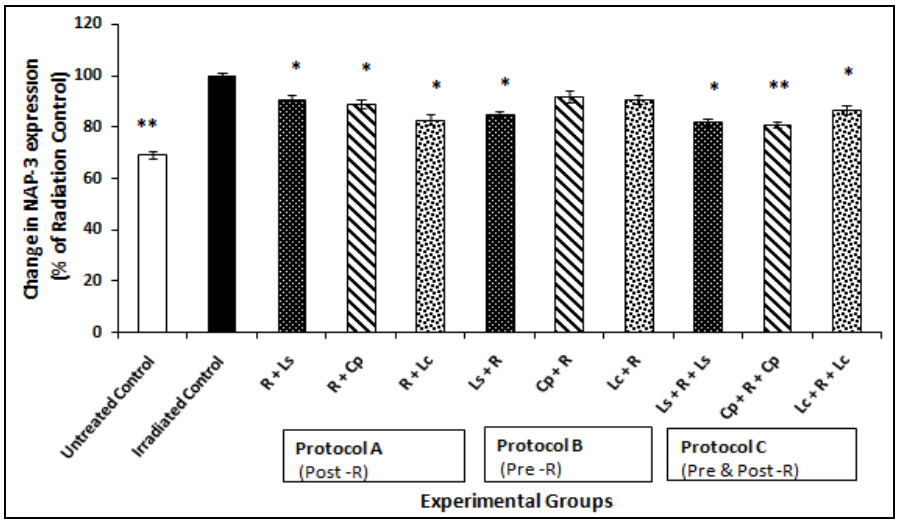

FIG. 1: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF NAP-3 IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in NAP-3 were evaluated with respect to the irradiated control, considered as 100%. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, **p<0.01).

Effects of Cucurbits on Expression of IFN-γ *in-vivo*: Daily administration of cucurbit extract Ls, Cp or Lc to mice orally for 7 days (post irradiation, Protocol-A) significantly decreased the expression of IFN-γ to 63, 64 and 60% respectively when compared with irradiated control, considered as 100% (**Fig. 2**). Pre-treatment of mice with cucurbit extracts Ls, Cp, or Lc for 7 days before irradiation (Protocol-B) also significantly decreased IFN-γ expression to 72, 68 and 64% respectively as compared to irradiated control. Mice under protocol C received cucurbit extracts Ls, Lc or Cp for 7 days before and 7 days after irradiation and IFN-γ levels were 67, 64 and 64% of the control value. Post irradiation treatment of mice with cucurbit extract (Lc) rendered maximum decrease in IFN-γ levels as compared to control.

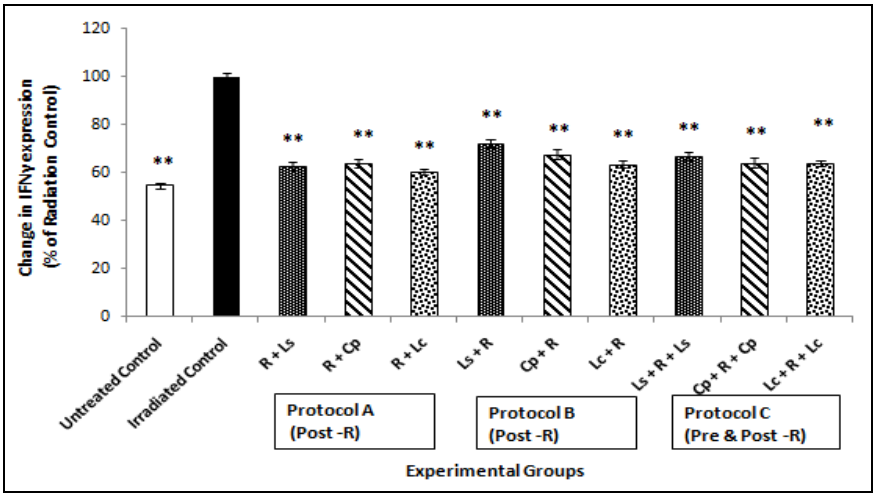

FIG. 2: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF IFN-γ IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in IFN-γ were evaluated with respect to the irradiated control, considered as 100%. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, **p<0.01).

Effects of Cucurbits on Expression of IL-17 *in-vivo*: Daily administration of cucurbit extract Ls, Cp or Lc to mice orally for 7 days (post irradiation, Protocol-A) significantly decreased the expression of IL-17 to 54, 53 and 54% respectively when compared to irradiated control, 100% (**Fig. 3**). Pre-treatment of mice with cucurbit extracts Ls, Cp, or Lc for 7 days before irradiation (Protocol-B) also significantly decreased IL-17 expression to 73, 57 and 52% respectively as compared to irradiated control. Mice under protocol C received cucurbit extracts Ls, Lc or Cp for 7 days before and 7 days after irradiation and IL-17 levels were 65, 67 and 55% respectively with respect to irradiated control. Among the three protocols, post irradiation treatment (Protocol-A) of mice with cucurbit extract (Ls, Cp or Lc) rendered maximum decrease in IL-17 as compared to control.

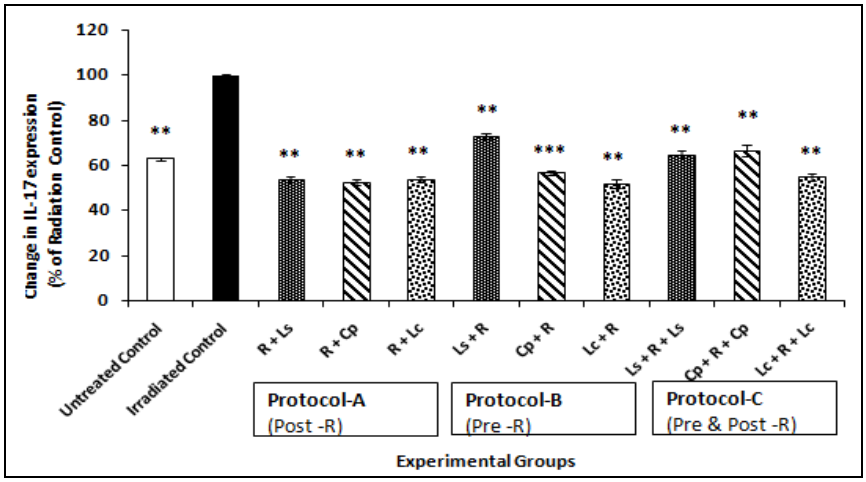

FIG. 3: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF IL-17 IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in IL-17 were evaluated with respect to the irradiated control, considered as 100%. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, ** p<0.01, *** p<0.001).

Effects of Cucurbits on Expression of IL-10 *in-vivo*: Daily administration of a cucurbit extract (Ls, Cp or Lc) to mice orally for 7 days post irradiation (protocol A), significantly increased the expression of IL-10 to 124, 114 and 132% respectively *vs* irradiated control considered as 100% (**Fig. 4**). Treatment of mice with cucurbit extract (Ls) for 7 days before irradiation (Protocol B) significantly increased IL-10 expression to 119%; however treatment of mice with Cp or Lc for 7 days before irradiation insignificantly modified IL-10 levels to 106 and 103% respectively *vs* irradiated control. Mice under protocol C received cucurbit extracts Ls, Lc or Cp for 7 days before and 7 days after irradiation and the IL-10 expression increased to 114, 109 and 127% respectively with respect to irradiated control. Lc when administered under protocol A (post-irradiation treatment) rendered maximum increase in IL-10 when compared to control.

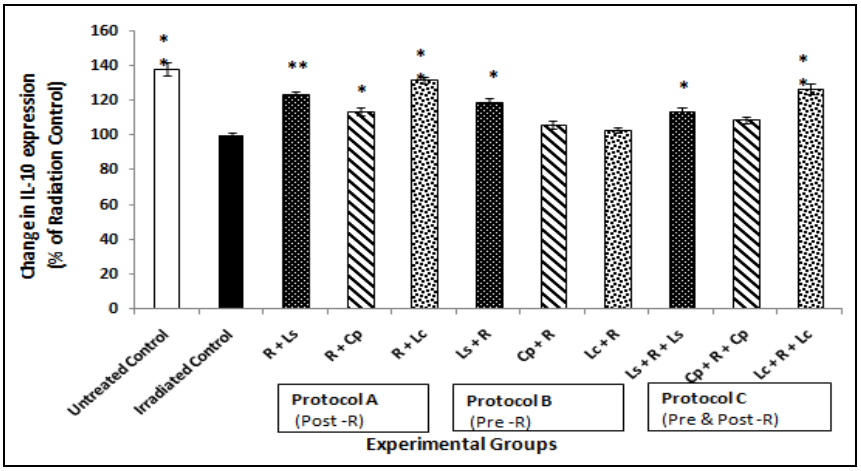

FIG. 4: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF IL-10 IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in IL-10 were evaluated with respect to the irradiated control, considered as 100 %. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, **p<0.01).

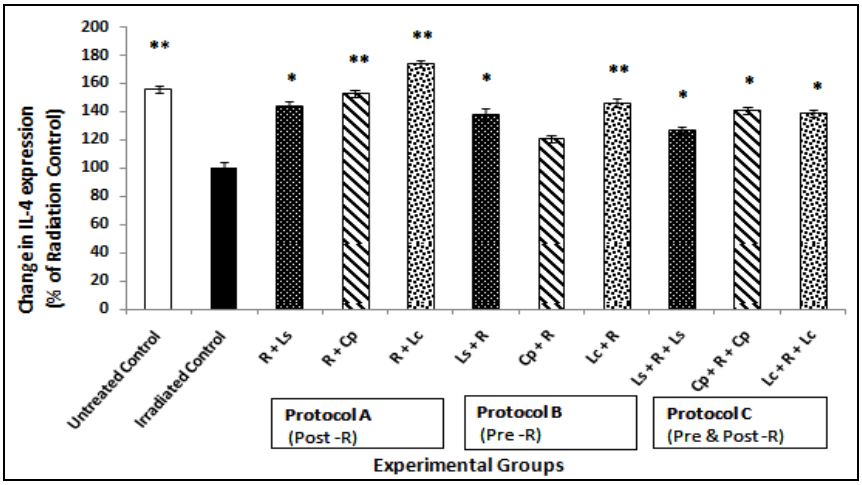

FIG. 5: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF IL-4 IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in IL-4 were evaluated with respect to the irradiated control, considered as 100%. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, **p<0.01).

Effects of Cucurbits on Expression of IL-4 *in-vivo*: Daily administration of a cucurbit extract (Ls, Cp or Lc) to mice orally for 7 days post irradiation (protocol A), significantly increased the expression of IL-4 to 144, 153 and 174% respectively *vs* irradiated control considered as 100% (**Fig. 5**). Treatment of mice with cucurbit extracts Ls, Cp or Lc for 7 days before irradiation (Protocol B) significantly increased IL-4 expression to 138, 121 and 146% respectively *vs* irradiated control. Mice under protocol C received cucurbit extracts Ls, Lc or Cp for 7 days before and 7 days after irradiation and IL-4 expression increased to 127, 141 and 139 % respectively with respect to irradiated control. Lc when administered under protocol A (post-irradiation treatment) rendered maximum increase in IL-4 when compared to control.

Effects of Cucurbits on Expression of TGF-β *in-vivo*: Daily administration of Ls orally to mice for 7 days post irradiation (protocol A) significantly increased the expression of TGF-β to 121% with respect to irradiated control (100%); while the increase in TGF-β expression remained in significant (113%) with Cp or Lc (**Fig. 6**). Similarly treatment of mice with Ls for 7 days before irradiation (Protocol B) significantly increased TGF-β expression to 126% in comparison to irradiated control; while the increase in TGF-β expression remained insignificant (111% and 112%) with Cp or Lc respectively. Pre-treatment of mice with Ls or Cp for 7 days followed by irradiation after 24 h and again treatment with Ls or Cp for 7 days (Protocol C) rendered significant increase in TGF-β expression (125 and 117% respectively) with respect to irradiated control while Lc remained insignificant at increasing TGF-β (113%). Lc when administered under protocol A (post-irradiation treatment) rendered maximum increase in IL-10 when compared to control.

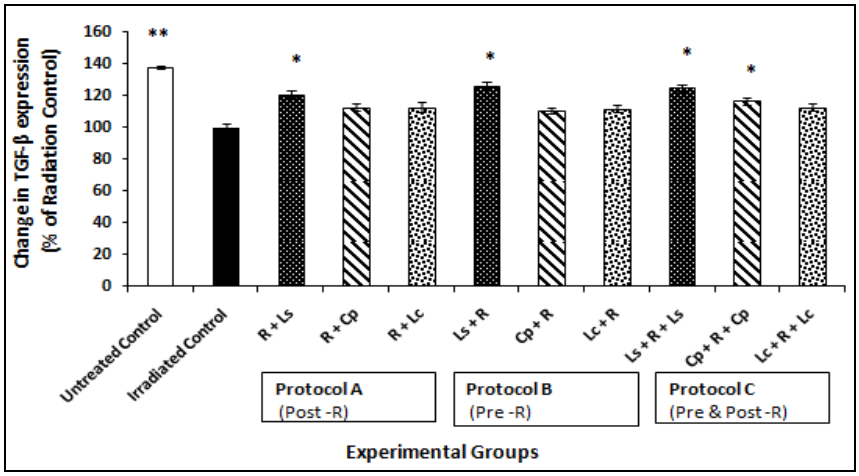

FIG. 6: RADIO-MODIFYING EFFECT OF CUCURBITS, *LAGENARIA SICERARIA* (LS), *CUCURBITA PEPO* (CP) AND *LUFFA CYLINDRICA* (LC) ON THE EXPRESSION OF TGF-β IN THE HOMOGENATE OF JEJUNUM OF BALB/C MICE.

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. The changes in TGF-β were evaluated with respect to the irradiated control, considered as 100%. Each bar represented the Mean ± SE of three experiments; asterisk above a bar indicated significance of difference with respect irradiated control (*p<0.05, **p<0.01).

In the present study, irradiation of mice rendered an increase of pro-inflammatory biomarkers NAP-3, IFN-γ and IL-17 (**Fig. 1, 2** and **3** respectively) and concurrent decrease of anti-inflammatory biomarkers IL-10, IL-4 and TGF-β (**Fig. 4**, **5** and **6** respectively). This is in agreement with other studies reporting the increase of radiation induced pro-inflammatory cytokines and growth factors [27, 28] and decrease of anti-inflammatory biomarkers [29].

Oral administration of cucurbit extract (Ls, Cp or Lc) to mice under all the three experimental protocols countered the inflammatory effects of radiation, by decreasing pro-inflammatory cytokines NAP-3 (**Fig. 1**), IFN-γ (**Fig. 2**) and IL-17 (**Fig. 3**) and increasing anti-inflammatory cytokines IL-10 (**Fig. 4**), IL-4 (**Fig. 5**) and TGF-β (**Fig. 6**) concurrently as compared to irradiated control (no cucurbit treatment).

Post-irradiation administration of cucurbit extracts was most effective in decreasing the IL-17 or IFN-γ and increasing the IL-10 or IL-4. However, NAP-3 was maximally decreased by Cp and TGF-β was maximally increased by Ls when administered under combined pre + post-irradiation cucurbit treatment protocol. Anti-inflammatory effects of cucurbits have also been demonstrated by other workers; Ls decreased cyclooxygenase and lipoxygenase [30], Cp down regulated IFN-γ [31] and Lc inhibited IL-6 and ROS generation [32]. Similarly, anti-inflammatory effects of other prebiotics via induction of IL-10 and down regulation of Th-1 and Th-17 cytokines have also been reported [33].

Anti-inflammatory effect of Ls, Cp and Lc may be attributed to various antioxidants present in cucurbits like, polyphenols, carotenoids, and Vitamins C and E [34 - 36] which act as singlet oxygen quenchers and hydrogen ion donators to the free radicals and provide protection against oxidative damages. TGF-β, IL-10 and IL-4 have been known to inhibit ROS/RNS-mediated damage [27]. Our previous studies on *in - vitro* system [37, 38] have already demonstrated the antioxidant activities of cucurbit extracts. Flavonoid (epigallocatechin-3-gallate) from other dietary sources have also been reported to decrease inflammatory cytokines like IL-6, IL-17, IFN-γ and TNF-α through decrease in Th-1 and Th-17 population and increase in Tregs population enhancing IL-10 production [39]. Immuno-modulatory effect of cucurbits may also be attributed to various alkaloids, sterols and saponins [40, 41]. Cucurbitacin, a flagship alkaloid of family cucurbitaceae also rendered anti-oxidant, anti-inflammatory and immunomodulatory effect both *in-vitro* and *in-vivo* [15].

Anti-inflammatory and immuno-modulatory activity of Ls, Cp and Lc may also be attributed to short chain fatty acids (SCFAs) which are produced by metabolism of dietary fiber [42] and are considered as enactors for prebiotics effects [43]. SCFAs have been reported to modulate immune responses by inducing both T-cell and B-cell hyporesponsiveness and down-regulating Th_1, Th_2 and Th_{17} cytokines [42, 44]. Administration of B-GOS (5.5g daily for 10 weeks) has been reported to lower circulating levels of inflammatory cytokines such as IL-6, IL-1β, and TNF-α and to enhance the level of IL-10 [45, 46]. Dietary fiber has been shown to enhance TGF-beta signalling in the rat intestine through SCFAs [47]. Butyrate (a SCFA) enemas have also been reported to reduce inflammation and oxidative stress in colonic mucosa of patients with ulcerative colitis by increasing colonic IL-10/IL-12 ratio [48].

Anti-inflammatory and immuno-modulatory effect of Ls, Cp and Lc may also be credited to their positive effects on probiotics growth. Our earlier studies have shown that cucurbits enhanced the activity of Lrh, a probiotics [37]. Intragastric administration of IL-10 producing *Lactococcus lactis* protected mice from DSS-induced colitis and prevented development of colitis in IL-10-deficient mice [49]. Probiotics have been reported to increase the production of an anti-inflammatory cytokine, IL-10 [50]. IL-17 production by lymphocytes has been reported to be decreased by probiotics in the gut [51, 52]. Our study can be exploited for developing strategies to mitigate radiation induced damage at GI syndrome level.

Effects of Cucurbits on Height of Villi in Jejunum: The changes in the height of villi in jejunum of different mice have been displayed in **Fig. 7** and the microscopic images displaying normal, disrupted and recovering villi have been displayed in **Fig. 9**. Daily administration of a cucurbit extract (Ls, Cp or Lc) orally to mice for 7 days post irradiation (protocol A), significantly increased the villi height from 214 ± 24μm (irradiated control) to 292 ± 32, 264 ± 29 and 307 ± 34μm respectively. Treatment of mice with cucurbit extract (Ls or Lc) for 7 days before irradiation (Protocol B) significantly increased villi length to 271 ± 30 and 281 ± 31μm respectively as compared to irradiated control (214 ± 24μm) while increase with Cp remained insignificant at $p < 0.05$ (226 ± 25μm). Pretreatment of mice with Ls, Lc or Cp for 7 days followed by irradiation after 24 h and again treatment with Ls, Cp or Lc for 7 days (Protocol C) did not display significant recovery in villi length which remained at 220 ±24, 224 ±25 and 232 ±26μm.

Ls was most effective at increasing the villi height when administered under protocol A, where the increase was 21 and 72 μm more than protocol B and C respectively. Similarly, Cp rendered maximum increase in villi height under protocol A where the increase was 38 and 40μm more than

protocols B and C respectively. Lc also displayed maximum increase in villi height under protocol A where the increase was 26 and 75µm more than protocols B and C respectively.

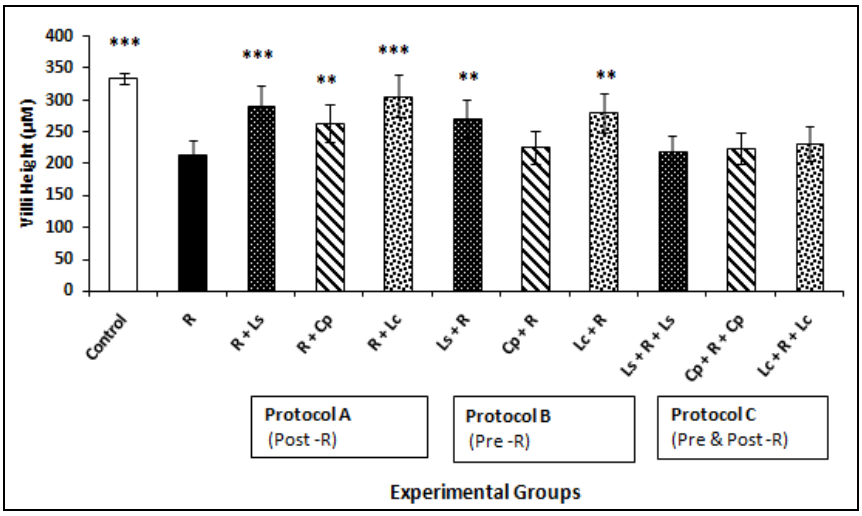

FIG. 7: EFFECT OF CUCURBITS *LAGENARIA SICERARIA* (LS), *LUFFA CYLINDRICA* (LC) AND *CUCURBITA PEPO* (CP) ON THE CHANGES IN HEIGHT OF VILLI IN JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. Each bar represented the Mean ± SE of three experiments. Asterisk above a bar represented significance of difference with irradiated control at *p<0.05, **<0.01 and ***<0.001 respectively.

Effects of Cucurbits on Width of Villi in Jejunum: The changes in the width of villi in jejunum of different mice have been displayed in **Fig. 8** and the microscopic images displaying normal, disrupted and recovering villi have been displayed in **Fig. 9**. Daily administration of a cucurbit extract (Ls, Cp or Lc) orally to mice for 7 days post irradiation (protocol A), significantly increased the villi width from 39 ± 1.1µm (irradiated control) to 45 ± 2.1µm, 43 ± 2.4µm and 46 ± 1.3µm respectively.

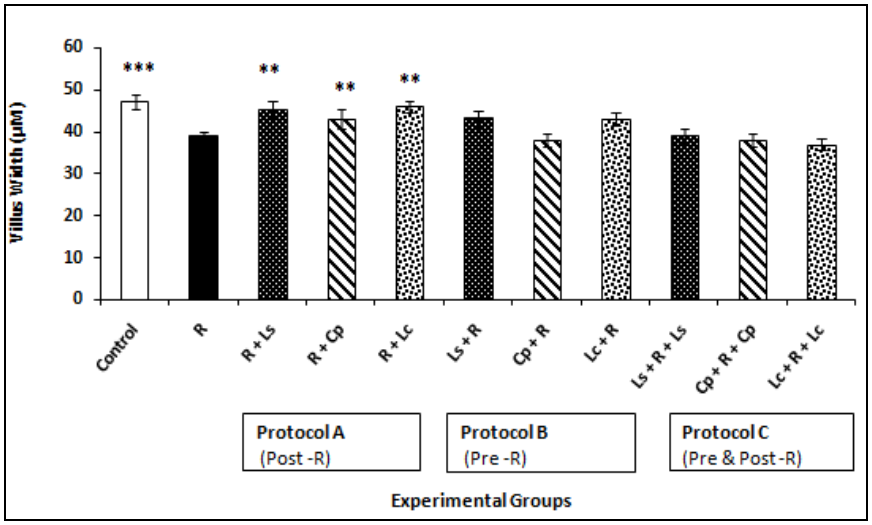

FIG. 8: EFFECT OF CUCURBITS *LAGENARIA SICERARIA* (LS), *LUFFA CYLINDRICA* (LC) AND *CUCURBITA PEPO* (CP) ON THE CHANGES IN WIDTH OF VILLI IN JEJUNUM OF BALB/C MICE

Each animal in protocol A (Post -R) received a single whole body dose of 6 Gy and cucurbit extract was given each day for 7 days; Protocol B (Pre-R) received cucurbit extract each day for 7 days and 6 Gy dose on 8th day; Protocol C (Pre and Post -R) received a cucurbit extract each day for 7 days, and 6 Gy dose on 8th day and cucurbit extract each day for 7 days. Each bar represented the Mean ± SE of three experiments. Asterisk above a bar represented significance of difference with irradiated control at *p<0.05, **<0.01 and ***<0.001 respectively.

Treatment of mice with cucurbit extract (Ls, Cp, or Lc) for 7 days before irradiation (Protocol B) also revealed an increase in villi width (43 ± 1.9µm, 38 ± 1.4µm and 43 ± 1.4µm respectively) as compared to irradiated control (39 ± 1.1µm) though the increase was insignificant at $p < 0.05$. Pretreatment of mice with Ls, Lc or Cp for 7 days followed by irradiation after 24 h and again treatment with Ls, Cp or Lc for 7 days (Protocol C) did not display significant increase in villi width with respect to the irradiated group and remained at 39 ± 1.6, 38 ± 1.4 and 37 ± 1.5µm.

Ls was most effective at increasing the villi width when administered under protocol A, where the increase was 2 and 6 µm more than protocol B and C respectively. Similarly, Cp rendered maximum increase in villi width under protocol A where the increase was 5µm more than both the protocols B and C. Similarly, Lc displayed maximum increase in villi width under protocol A where the increase was 3 and 9µm more than protocols B and C respectively.

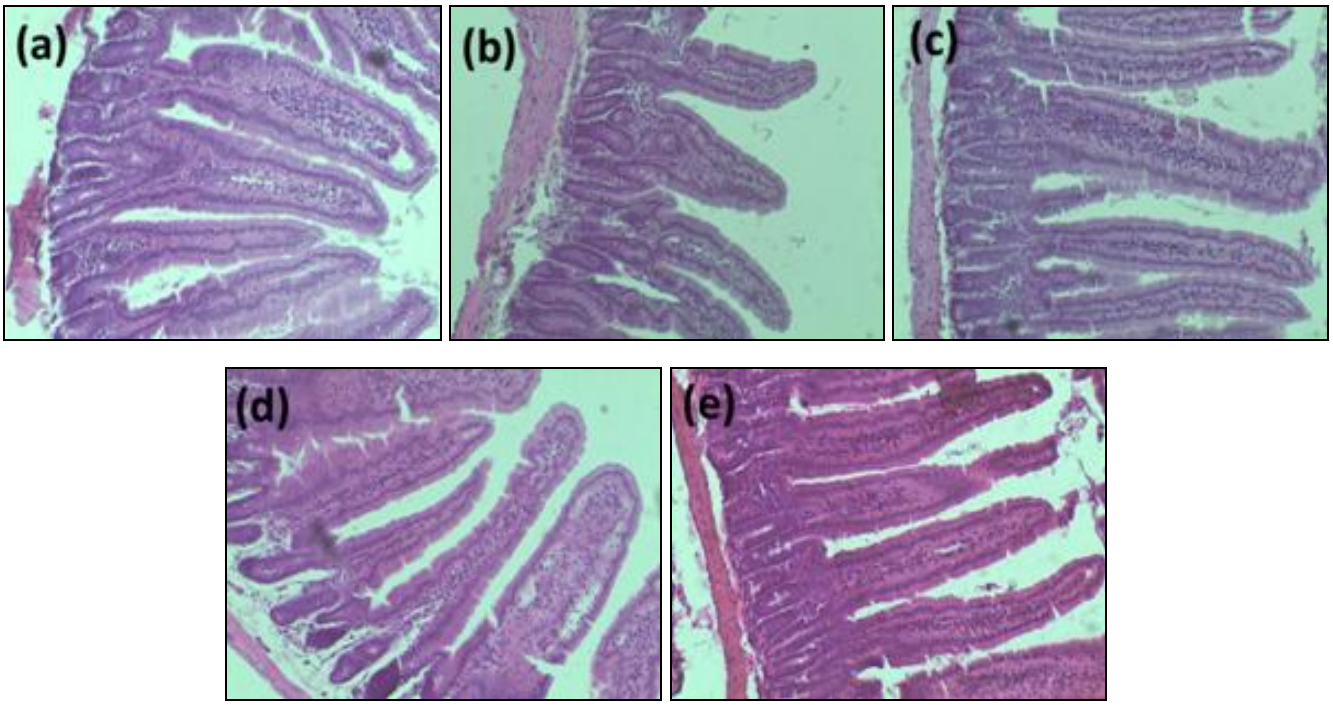

FIG. 9: MICROSCOPIC IMAGE (40X) OF JEJUNUM OF MICE STAINED WITH HEMATOXYLIN AND EOSIN

a) Un-irradiated control showing healthy villi.
b) Irradiated control showing disrupted villi.
c) R + Ls: Irradiation followed *Lgenaria siceraria* once a day for 7 days showing, recovering villi.
d) R + Cp: Irradiation followed *Cucurbita pepo* once a day for 7 days showing, recovering villi.
e) R + Lc: Irradiation followed *Luffa cylindrical* once a day for 7 days showing, recovering villi.

The newly formed cells produced by the stem cells, the crypts of Lieberkuhn have been reported to migrate towards the tip of villi reaching the top within about 72 h in mice and thereafter denudate in the gut lumen. Irradiation of mice revealed a decrease in villi height (**Fig. 7** and **9**) and width (**Fig. 8** and **9**). Radiation damage has been reported to induce apoptosis in crypt cells of the intestine [53] and also affected the proliferation of crypt cells adversely [54, 55]. Daily administration of cucurbit extracts (Ls, Cp or Lc) to mice countered the radiation induced injury to villi by increasing the height (**Fig. 7**) and width (**Fig. 8)** in all the three experimental protocols with respect to irradiated control, however post-irradiation cucurbit treatment rendered maximum effect. Microscopic images of jejunum of mice displaying normal, disrupted and recovering villi have been displayed in **Fig. 9**. Ls has been reported to counter the radiation induced injury to villi in our previous study [18]. The Ls, Cp and Lc have been reported to contain anti-oxidants like flavonoids and other polyphenolic compounds [30, 36, 56]. Hence, the anti-oxidative property of cucurbit extracts might have helpful in ameliorating ROS induced damage to crypts and hence were able to restore villi structural integrity.

CONCLUSION: The dietary cucurbits reduced the pro-inflammatory cytokines and increased the anti-inflammatory cytokines and the differences in the biochemical constituents of three cucurbits investigated here may be responsible for differential efficacy among themselves. The pre-irradiation administration was studied for prophylactic action against radiation induced inflammatory processes, but the anti-inflammatory molecules in the circulation dwindle with time and only little mitigation of radiation damage occurred. Post-irradiation treatment rendered maximum repair and recovery after radiation damage. Combined modality of pre- and post-irradiation administration of cucurbits yielded still better results in NAP decrease and TGF-β increase. Further studies are necessary to unravel these points. This study revealed the non-toxic anti-inflammatory potential of cucurbits in the therapy of chronic inflammatory disorders.

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