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Original Article

The Possible Protective Role of Ginger in Gamma Radiation-Induced Jejunal Enteropathy in Adult Male Rats. A Light and Electron microscopic study

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ABSTRACT

Background: The intestine is one of the most sensitive organs affected by radiation toxicity, that is why mortality due to radiation enterocolitis stays a challenge. Recent studies had stated that ginger might possess, anti-inflammatory, and antioxidant properties. The study aimed to explore the possible role of ginger as a protective and as a therapeutic agent on the gamma irradiation induced enteropathy.

Material and Methods: Thirty six adult male albino rats were used in this study. The animals were divided randomly into three groups: group I, control, group II; (gamma irradiated) each rat was exposed to single exposure of 0.739 (11Gray/min) gamma radiation, group III; irradiated and given ginger extract, it was further subdivided into two subgroups each of them was irradiated as in group II and given ginger extract orally once daily in a dose of 120mg/kg. Subgroup IIIa: received ginger extract seven days before radiation while subgroup III b: received ginger extract fourteen days before and after radiation. Jejunal specimens were collected from sacrificed animals and examined by light and scanning electron microscopes. Morphometric study and statistical analysis were done.

Results: Group II as compared to control showed distortion and fusion of some villi. While the crypts showed focal proliferation of its lining cells and invasion with inflammatory cells. Moreover, subgroup IIIa showed focal disruption of the crypts' wall. Significant decrease in mean number of goblet cells and significant increase in collagen fibers mean percentage area were noticed in group II compared to the control. However, subgroup IIIb showed restoration of most of the structure of the jejunal mucosa to become comparably similar to control.

Conclusion: Ginger seemed to be a promising agent for amelioration of intestinal injury induced by radiation.

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Key Words: Electron microscopy, ginger, jejunum, radiation, rats.

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INTRODUCTION

Radiotherapy is considered to have an important role in cancer treatment. Radiation-induced enteropathy is a common and dangerous problem with subsequent symptoms such as diarrhea, and vomiting. The injury is progressive and often reduces the quality of life for patients[1]. Radiotherapy damage intestinal barrier function and modifies its bacterial flora, intestinal motility and vascular permeability of mucosal cells. Hence, the intestine is one of the most sensitive organs affected by radiation toxicity[2]. Complications of abdominopelvic radiotherapy remain major problems for clinicians and accounts for the high morbidity and mortality rates among these patients. These complications were treated with medications, antibiotics, anti-inflammatory drugs, and hyperbaric oxygen which can lead to interruption of the therapy[3].

The evolution of effective radio-protectors and radio-recovery drugs is of great significance putting into consideration their possible application during exposure to both (radiotherapy) and (nuclear industry)[4].

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The herbs use in cancer patients is uprising as they are non-toxic, improve the immune system, and can attack the cancer itself. Ginger has a long history of medicinal use in various cultures for treating common colds, nausea, diarrhea, fever, stomach upset, to aid digestion, rheumatic disorders, and dizziness[5]. Recent studies had stated that ginger might possess anti-inflammatory, antimicrobial, antioxidant and anti-cancer properties[6].

Aim of the present work was to declare the structural changes that might occur in the jejunal mucosa after exposure to gamma radiation and the possible ameliorative role of ginger as a protective and as a therapeutic agent on these changes in a rat model that might yield hopeful results.

MATERIAL AND METHODS

The experiment was carried out in the Animal Research Center, Faculty of Medicine, Ain Shams University, and the National Centre for Radiation Research and Technology, Cairo, Egypt.

2.1 Animals:

Thirty six male albino rats weighing 230-250gm and aged about 2-3 months were used in the current study. They were housed in clean wire mesh cages, they had free access to water and standard laboratory diet and were kept under proper conditions of light, temperature and humidity. The procedures were carried out according to the guideline of Animal Care and the Scientific Research Ethical Committee of the Faculty of Medicine, Ain Shams University.

Acclimatization of the animals were performed by keeping them for one week before the beginning of the experiment.

2.2 Experimental protocol:

Rats were randomly divided into three groups:

Group I (Control): this group comprised 18 rats that were further subdivided into three subgroups six rats each:

Subgroup Ia: the untreated control group they were sacrificed after 14 days.

Subgroup Ib: rats were given ginger extract (Sigma -Aldrich, St. Louis, Missouri, USA) by oral gavage once daily at a dose of 120mg/kg dissolved in 1ml distilled water[7] for 7 days and then they left for another 7 days without any treatment and then they were sacrificed.

Subgroup Ic: rats were given ginger extract the same dose as subgroup Ib for 14 days then they were sacrificed.

Group II (Gamma irradiated): this group comprised 6 rats , each rat was given 100 mg/kg of ketamine hydrochloride as anesthetic (Pfizer, New York, USA), positioned supine and then irradiated with single exposure of gamma radiation to the abdominopelvic area while the other parts of the body were carefully shielded. It was performed by cesium-137 ventilated gamma cell 40 with a dose rate 0.739 (11Gray/min). After radiation rats were recovered from the anesthesia[8]. The irradiated rats were sacrificed after 7 days. The irradiation was conducted in the National Centre for radiation research and Technology.

Group III (Irradiated and Ginger): this group comprised 12 rats they were further subdivided into two subgroups each of them was irradiated as in group II and given ginger extract in a dose as in subgroup Ib.

Subgroup III a six rats: (ginger pre-radiation): received ginger extract 7 days before radiation and then they left for another 7 days without any treatment and then they were sacrificed.

Subgroup III b six rats: (ginger pre and post-radiation): received ginger extract 7 days before radiation and 7 days after radiation and then they were sacrificed.

2.3 Sample Collection:

At the end of the experiment all animals were anesthetized with 40mg / kg sodium pentobarbital intraperitoneally. Cardiac perfusion with 20ml of 4% paraformaldehyde fixative in phosphate-buffered saline was done[9]. Small pieces of jejunal specimens were taken and cut longitudinally to wash the mucosa with saline to remove the debris. The specimens were then fixed for the following histological studies:

2.4 Light microscopic study (LM):

The specimens were fixed in 10% neutral buffered formalin. Serial sections of 5µm thickness were stained with Hematoxylin and Eosin stain (H&E), Periodic Acid- Schiff stain (PAS) and Masson's trichrome stain[10]. Sections were examined and photographed with Olympus BX 40 light microscope (Olympus, Hamburg, Germany) connected to a digital camera power shot A640 (Canon Inc., Tokyo, Japan) at Histology and Cell Biology Department Faculty of Medicine, Ain Shams University.

2.5 Scanning electron microscopic study (SEM):

Small pieces of the jejunum were fixed in 2.5% glutaraldehyde in phosphate buffered saline (pH=7.4) for 24 hours. Tissues were then dried using Bal-Tec CPD with liquid carbon dioxide, and then sputter coated with gold using Bal-Tec SCD/005. Samples were then mounted on copper stub and viewed using scanning electron microscope (Philips XL30) in the Anatomy Department, Faculty of Medicine, Ain Shams University[11].

2.6 Morphometric and Statistical studies:

The readings of five different randomly selected non overlapping fields from five different sections of five different rats were examined in each group using the image analyzer Leica Q win V.3 program installed on a computer in the Histology & Cell biology department Faculty of Medicine Ain Shams University. the following parameters were measured

a) Number of goblet cells in jejunal villi in PAS-stained sections.

b) Collagen fibers percentage Area in Masson's trichrome stained sections.

All measurements were taken at high-power fields of magnification (×400). The collected data were revised and then statistically analyzed using one-way ANOVA performed by SPSS 21program (IBM Inc. Chicago, Illinois, USA). The significance of the data was determined by *P value P>0.05* non-significant (NS), *P≤0.05* significant (S). the data was summarized and expressed as mean ± standard deviation (SD).

RESULTS

The structure of jejunum of rats of all subgroups of (group I) revealed nearly similar structure in all different histological methods.

3.1 Light microscopic results:

The H&E stained sections of group I (control) showed the jejunal mucosa formed of long finger-like villi with a connective tissue core extending from the lamina propria. Invaginated crypts of Lieberkühn were noticed spanning the lamina propria. The submucosa was also formed of loose connective tissue surrounded by muscularis externa arranged as inner circular and outer longitudinal smooth muscle fibers (Fig. 1a). The villi were lined by columnar acidophilic enterocytes with prominent apical brush borders with basal oval vesicular nuclei. Goblet cells with expanded pale apical part distended with foamy secretions were also seen interspersed between the enterocytes and few intraepithelial lymphocytes. Mononuclear cells were noticed in the core of the villi (Fig. 1b). Moreover, crypts of Lieberkühn were noticed lined by enterocytes with basal vesicular nuclei and few scattered goblet cells. Some Paneth cells were seen lining the base of the intestinal crypts with many apical eosinophilic granules and basal vesicular nuclei. Moreover, few mitotic figures in some crypt base columnar cells with deeply basophilic cytoplasm were also detected lining the base of the crypts (Fig. 1c).

Meanwhile, examination of group II (gamma irradiated) showed distortion and fusion of some villi while the apical parts of some villi were sloughed. Infiltration of lamina propria with mononuclear inflammatory cells were also seen (Figs. 2a & 2b). The crypts showed focal proliferation of its lining cells that appeared with darkly stained nuclei. Many mitotic figures were detected at the base of the crypts. Focal disruption of the crypts' wall which had been invaded with inflammatory cells were detected. Infiltration of the lamina propria with many mononuclear inflammatory cells such as lymphocytes and eosinophils were seen. Paneth cells with few eosinophilic granules, vacuolated cytoplasm and darkly stained nuclei were also noticed (Fig. 2c).

Examination of subgroup IIIa (ginger pre-radiation) showed distortion and fusion of some villi. Apical tips of few villi were sloughed. Some scattered goblet cells were detected. Infiltration of lamina propria with mononuclear inflammatory cells was also noticed (Figs. 3a & 3b). Focal disruption of the crypts' wall and congested blood capillaries in the lamina propria were seen. Paneth cells with few eosinophilic granules and basal vesicular nuclei were also detected (Fig. 3c). However, examination of subgroup III b (ginger pre and post-radiation) showed the restoration of most of the structure of the jejunal mucosa to become nearly comparable to the control group. The villi were long and regular, while few villi appeared with partially sloughed tips (Fig. 4a). The enterocytes lining the villi showed basal oval vesicular nuclei while, the apical brush border in some focal areas appeared distorted. Few intraepithelial lymphocytes were detected, and some mononuclear cells were also noticed in the core of few villi (Fig. 4b).

The crypts were lined by enterocytes that had basal vesicular nuclei and few scattered goblet cells. Paneth cells with many apical eosinophilic granules and basal vesicular nuclei lining the crypt base were also detected (Fig. 4c).

Examination of Periodic Acid–Schiff (PAS) stained section of group I (control) showed the villi having regular intact brush border of enterocytes and many PAS positive goblet cells scattered among the enterocytes (Fig. 5). However, group II (gamma irradiated) and subgroup IIIa (ginger pre-radiation) showed disruption of the brush border in most of the enterocytes and significant decrease in the mean number of PAS positive goblet cells ($P \leq 0.05$) with values of (4.6±1.14), (22.2±2.28) respectively in comparison to the control group (49±2.23). In addition, there was also apparent decrease in their mucin content in the villi compared to control group (Figs. 6 & 7, Table 1). Meanwhile, examination of subgroup III b (ginger pre and post - radiation) showed enterocytes with intact brush border in most of the villi and significant increase in the number of PAS positive goblet cells (32.4±2.7) ($P \leq 0.05$) in comparison to group II (4.6±1.14) & subgroup IIIa (22.2±2.28). There was also an apparent increase in the mucin content of goblet cells in the villi compared to group II and subgroup III-a (Fig. 8, Table 1).

Sections stained with Masson's trichrome of group I (control), and subgroup III b (ginger pre and post-radiation) showed few collagenous fibers (3.09±0.4), (5.08±0.59) respectively within the lamina propria (mostly between the jejunal crypts) and submucosa (Figs. 9 & 12, Table 1). Meanwhile, examination of group II (gamma irradiated) and subgroup IIIa (ginger pre-radiation) showed significant increase in the percentage area of collagen fibers with values of (20.72±0.81), (11.1±0.69) ($P \leq 0.05$) respectively in the submucosa and lamina propria of jejunum compared to the other groups (Figs. 10&11, Table 1).

3.2 Scanning electron microscopic results:

Examination of sections of jejunum of group I (control) showed regular villi covered by enterocytes with hexagonal appearance and their apical surfaces covered by a carpet of microvilli. The mucous secretion of many goblet cells on villus surface was noticed while few goblet cells were seen devoid of their secretions (Figs. 13 a &b). Meanwhile, examination of sections of group II (gamma irradiated) Jejunum showed many distorted villi with complete shedding of their epithelial covering and exposure of their underlining connective tissue core with infiltration of some RBCs. Columnar enterocytes were seen surrounding the defects with their apical surfaces covered with microvilli. Moreover, the number of goblet cells were apparently decreased compared to the control group and depletion of their mucin content was also noticed (Figs. 14 a &b). The examination of sections of subgroup IIIa (ginger pre- radiation) showed partially distorted tips of some villi and the epithelial cells covered with microvilli. Meanwhile, other focal areas showed lack of such a cover with no cellular loss. The mucin secretion of some goblet cells was also detected (Figs. 15 a &b). However, examination of subgroup III b (ginger pre and post-radiation) showed regularly arranged villi with hexagonal enterocytes that was covered by microvilli. Some goblet cells with depleted mucin content were detected while others appeared with their mucin secretion (Figs. 16 a &b).

3.3 Morphometric and statistical results (Table 1):

Non-significant changes were detected between subgroups of group I (control) (*P>0.05*) in all the statistical parameters measured in this study.

1- The mean number of goblet mucous secreting cells stained by PAS showed significant decrease (*P≤0.05*) in group II & subgroup IIIa in comparison to the other groups. while, there was a significant increase in subgroup III b compared with group II & subgroup III a (*P≤0.05*). Meanwhile, there was significant increase in subgroup IIIa compared with group II (*P≤0.05*).

2- The mean percentage area of collagen fibers in sections stained with Masson's trichrome showed significant increase in group II and subgroup IIIa in comparison to the other groups (*P≤0.05*). Meanwhile, there was a significant decrease in subgroup III b compared with group II & subgroup IIIa (*P≤0.05*). While, there was significant decrease in subgroup IIIa in comparison to group II (*P≤0.05*).

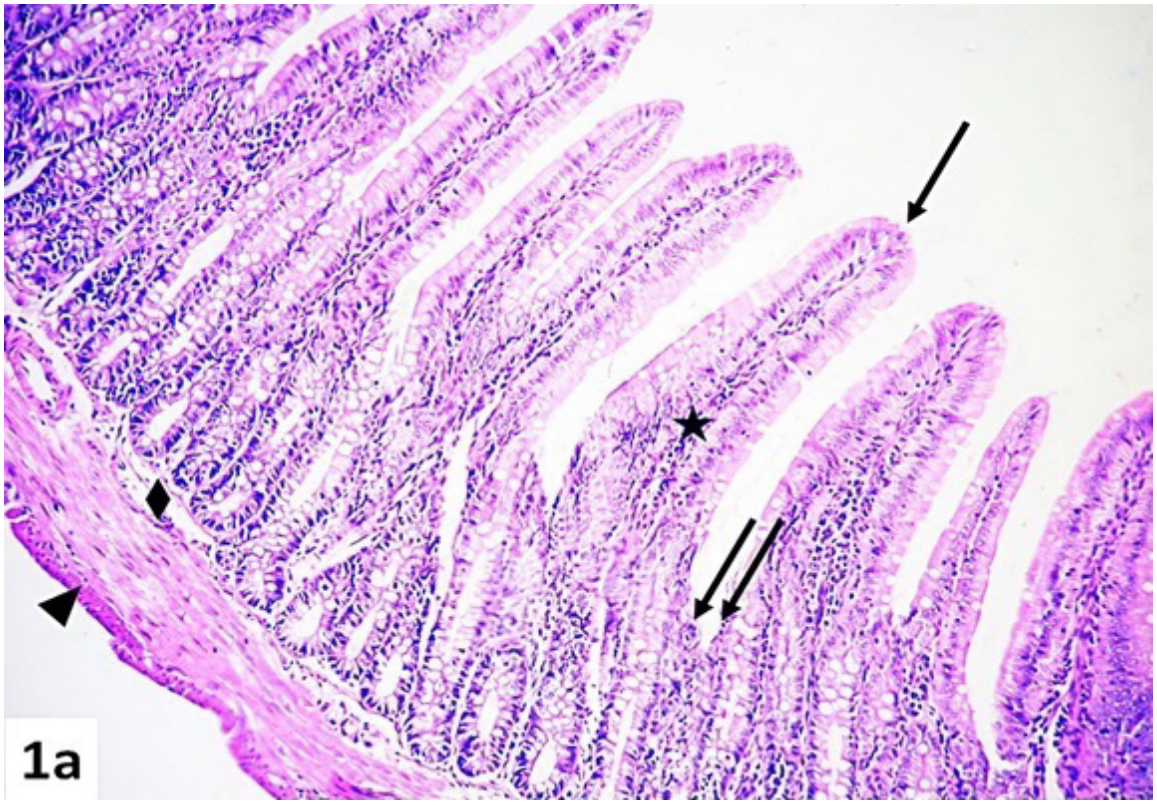

Fig. 1a: A photomicrograph of a section of the jejunum from group I (control) showing jejunal mucosa formed of long finger-like villi (↑) with a core of connective tissue extending from the lamina propria (*). Invaginated crypts of Lieberkühn (↑↑) spanning the lamina propria were seen. Notice the submucosa (♦) and the muscularis externa (▲) layers. H&E X 100

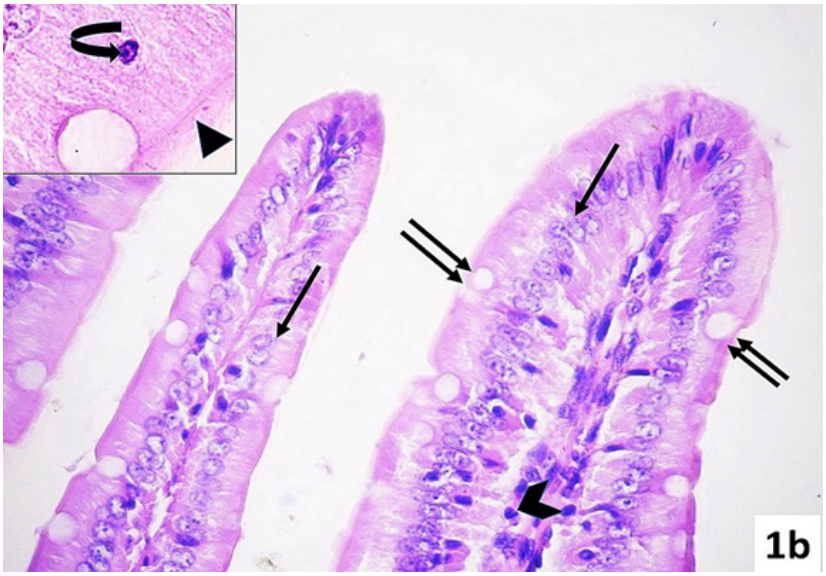

Fig. 1b: A photomicrograph of a section in the jejunum from group I (control) showing the villi lined by columnar acidophilic enterocytes with basal oval vesicular nuclei (↑). Goblet cells with expanded apical part containing pale foamy secretions (↑↑) are seen interspersed between the enterocytes. Notice some mononuclear cells in the core of the villi (arrowhead). Inset: the apical brush border of the enterocytes (▲) and few intraepithelial lymphocytes (curved arrow) can be detected H&E X 400 Inset: X 1000

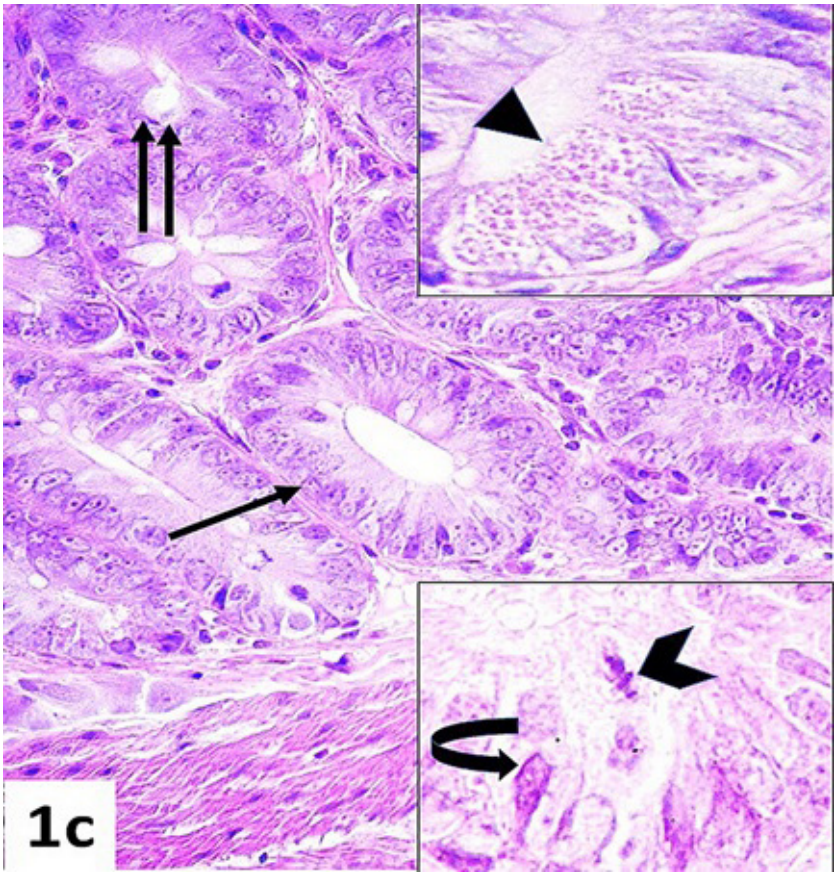

Fig. 1c: A photomicrograph of a section in the jejunum from group I (control) showing crypts of Lieberkühn lined by enterocytes with basal vesicular nuclei (↑) and few scattered goblet cells (↑↑). Upper inset: some Paneth cells with many apical eosinophilic granules (▲) and basal vesicular nuclei lining the base of the crypts are noticed. Lower inset: few mitotic figures (arrowhead) and crypt base columnar cells with deeply basophilic cytoplasm (curved arrow) were also detected lining the base of the crypts. H&E X 400 Upper inset: X 640, lower inset: X 1000

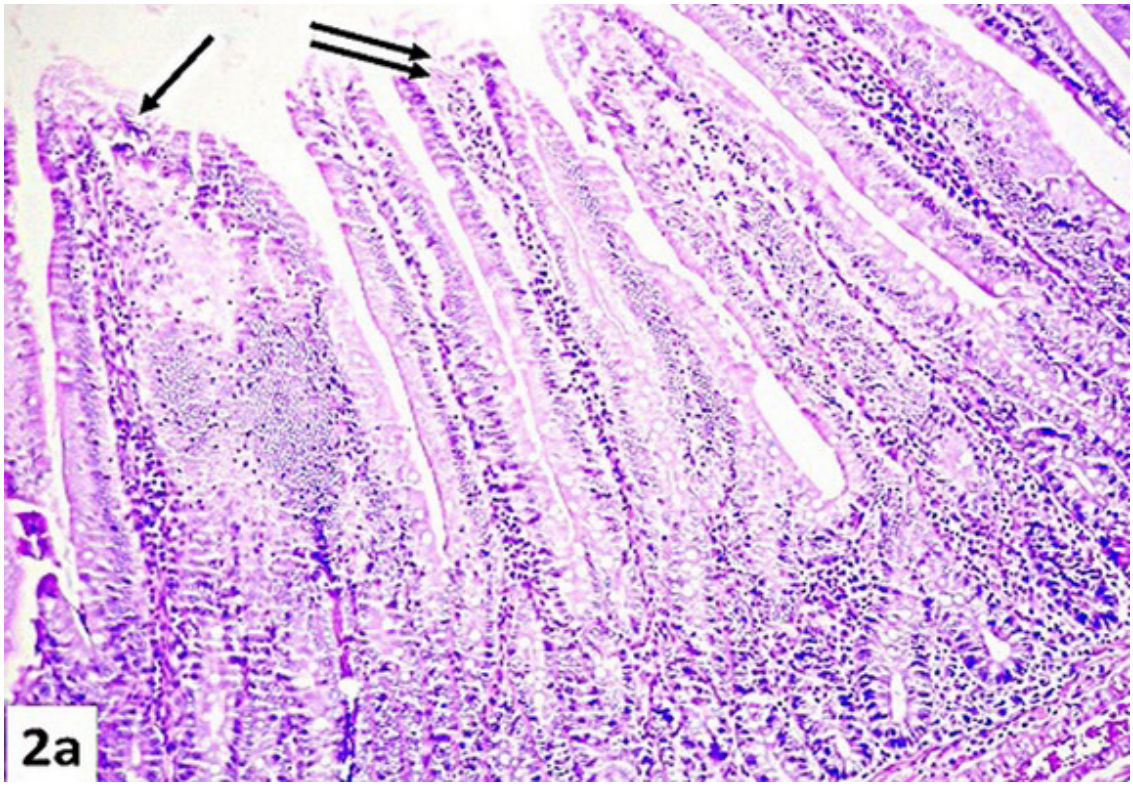

Fig. 2a: A photomicrograph of a section in the jejunum from group II showing distortion and fusion of some villi (↑). Apical part of some villi is sloughed (↑↑). H&E X 100

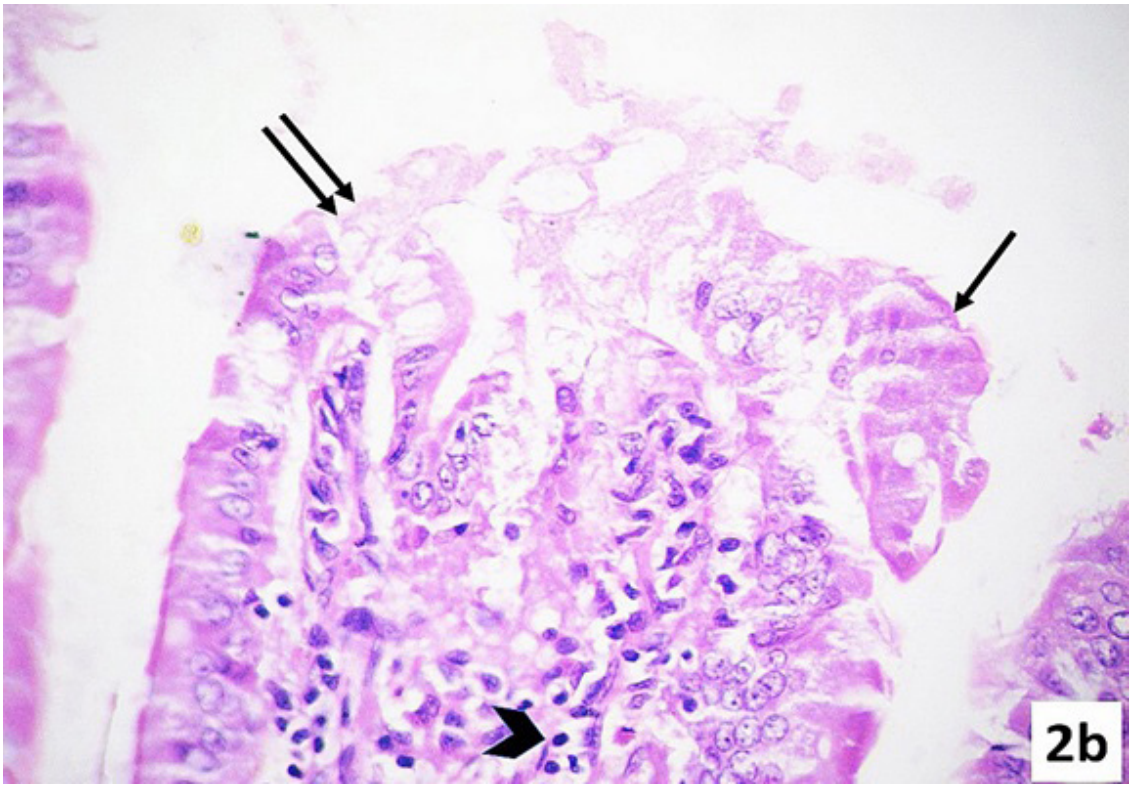

Fig. 2b: A photomicrograph of a section in the jejunum from group II showing broadening of the villus, shedding of some epithelial cells (↑) and apical epithelial cell erosion (↑↑). Notice infiltration of lamina propria with mononuclear inflammatory cells (arrowhead). H&E X 400

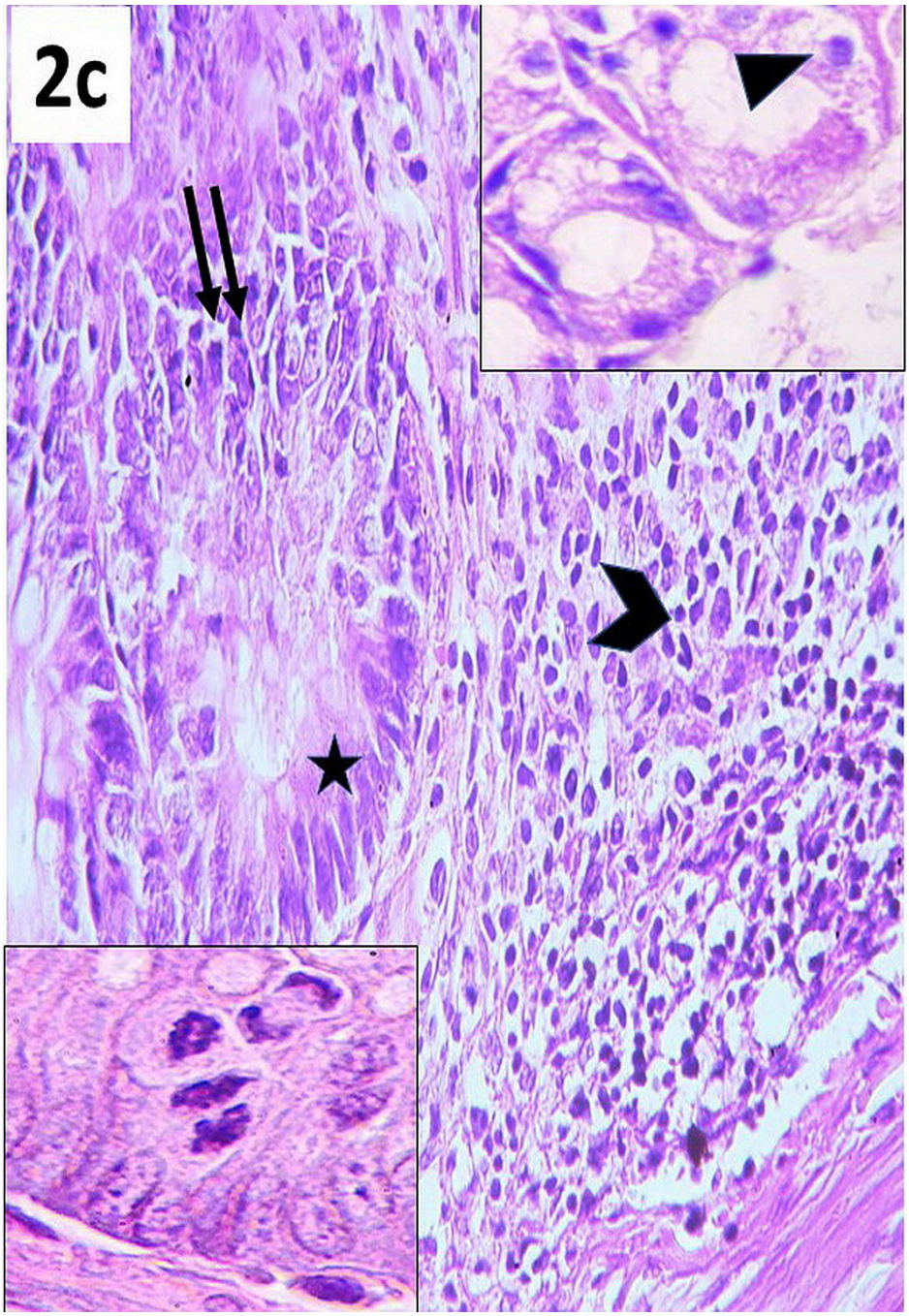

Fig. 2c: A photomicrograph of a section in the jejunum from group II showing focal proliferation of the cells lining the crypts and they appear with darkly stained nuclei (*). Focal disruption of the wall of the crypt and invasion with inflammatory cells (↑↑) are seen. Notice infiltration of the lamina propria with many mononuclear inflammatory cells (arrowhead). Upper inset: Paneth cells with few eosinophilic granules, vacuolated cytoplasm and darkly stained nuclei (▲) are noticed. Lower inset: many mitotic figures are detected at the base of the crypts. H&E X 400 Upper inset: X 640, lower insets: X 1000

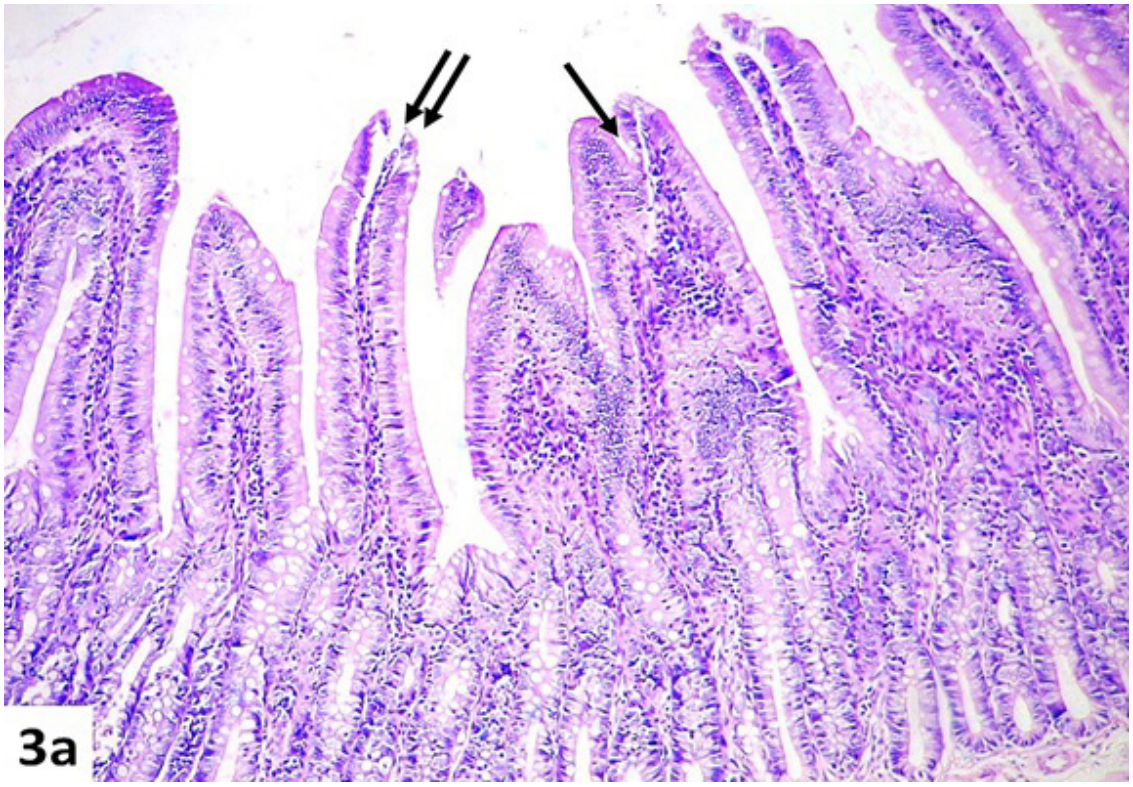

Fig. 3a: A photomicrograph of a section in the jejunum from subgroup IIIa showing distortion and fusion of some villi (↑). Apical tips of few villi are sloughed (↑↑). H&E X 100

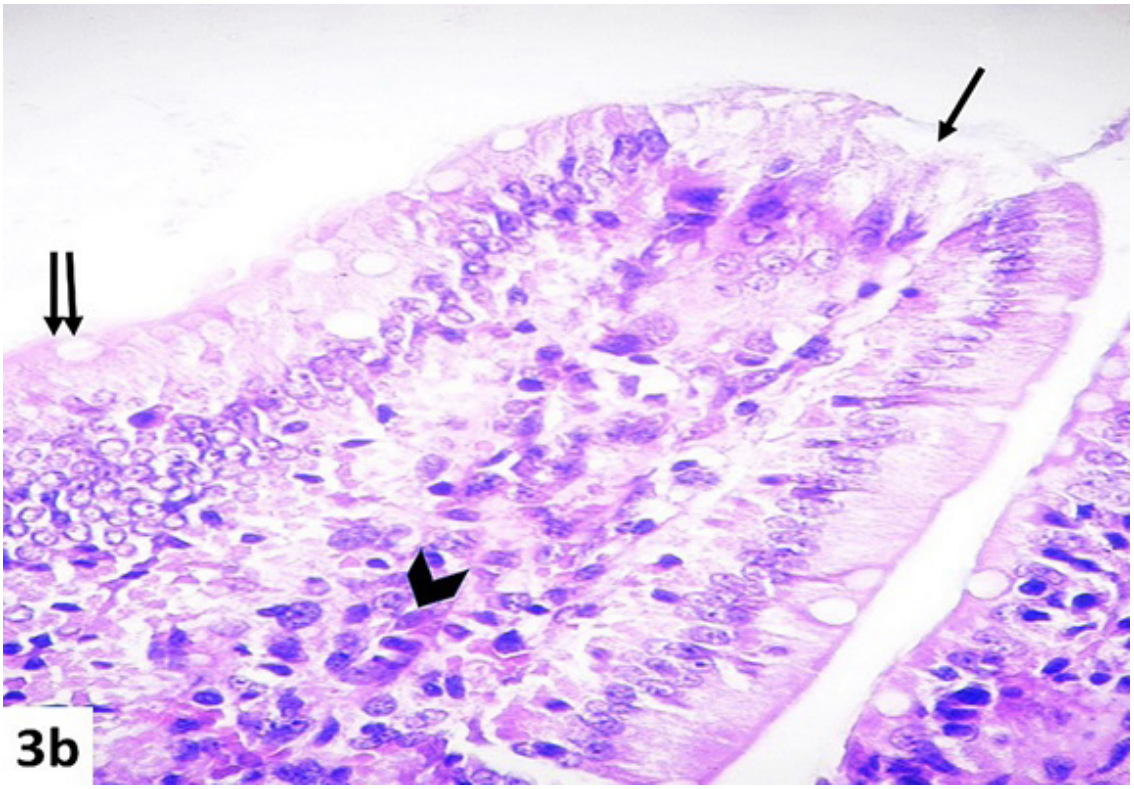

Fig. 3b: A photomicrograph of a section in the jejunum from subgroup IIIa showing broadening of the villus and sloughing of the epithelial cells at its apex (↑). Some scattered goblet cells (↑↑) are detected. Notice infiltration of lamina propria with mononuclear inflammatory cells (arrowhead). H&E X 400

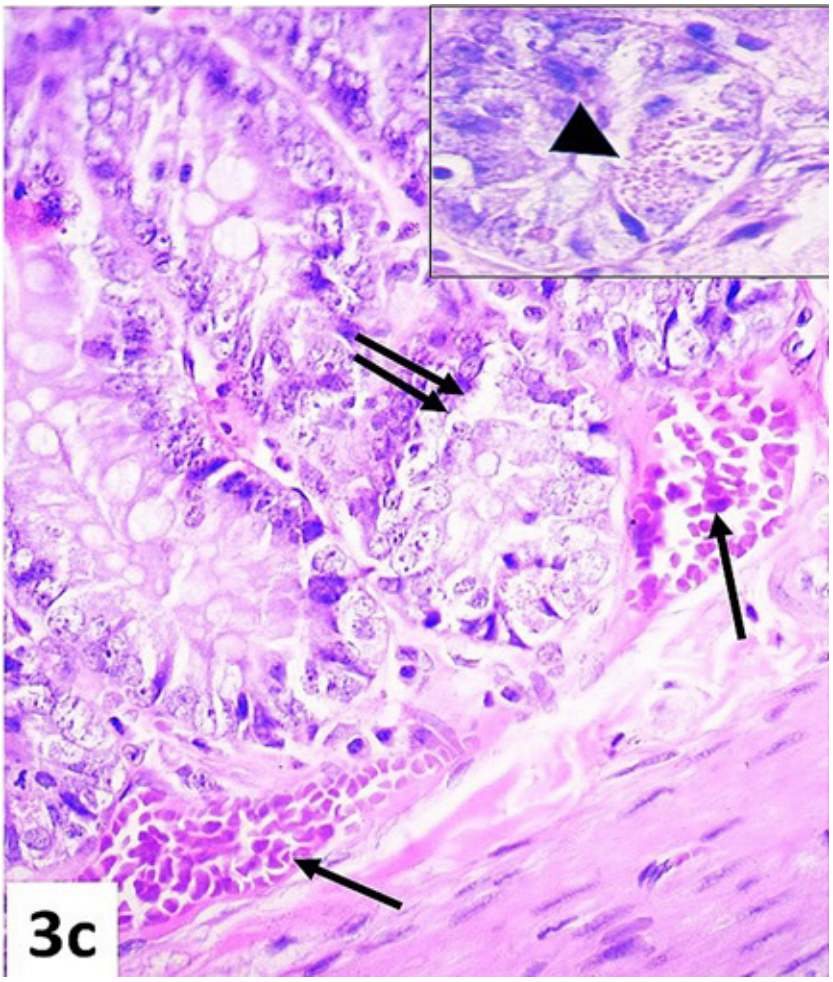

Fig. 3c: A photomicrograph of a section in the jejunum from subgroup IIIa showing focal disruption of the wall of the crypt (↑↑). Notice congested blood capillaries in the lamina propria (↑). Inset: Paneth cells with few eosinophilic granules and basal vesicular nuclei (▲) are also noticed. H&E X 400 Inset: X 640

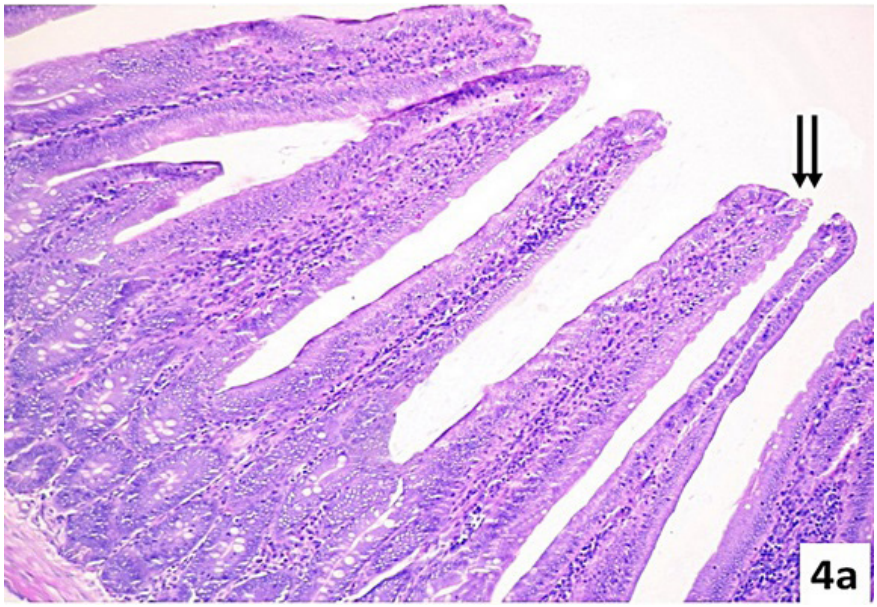

Fig. 4a: A photomicrograph of a section in the jejunum from subgroup IIIb showing most of the villi are long and regular. Few villi with partially sloughed tips (↑↑) can be noticed. H&E X 100

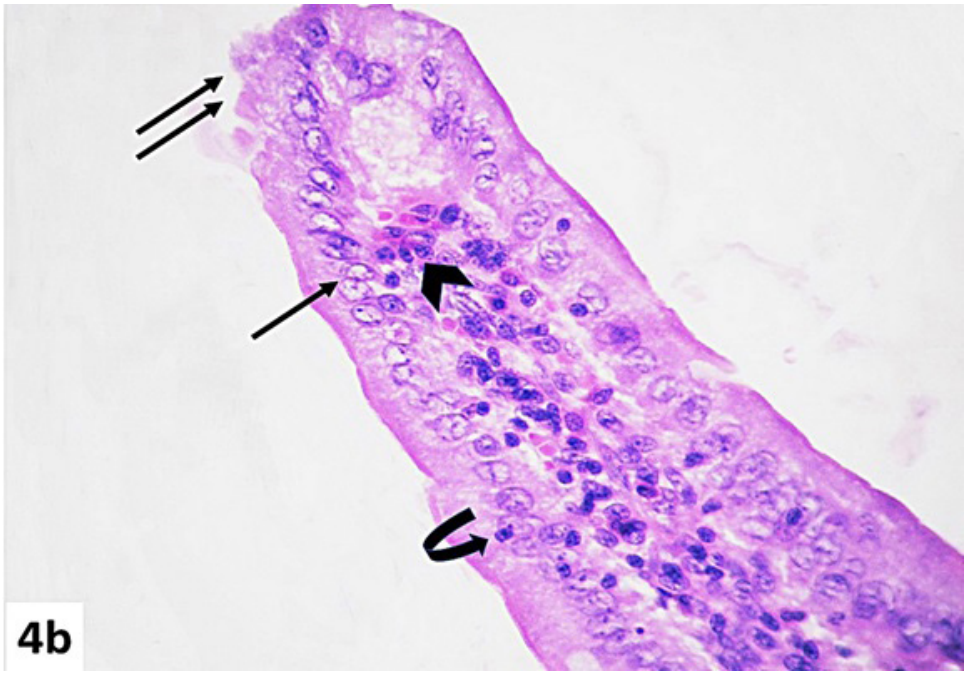

Fig. 4b: A photomicrograph of a section in the jejunum from subgroup IIIb showing the villus lined by enterocytes with basal oval vesicular nuclei (↑). The apical brush border in some focal arears appear distorted (↑↑). Few intraepithelial lymphocytes can be detected (curved arrow). Notice some mononuclear cells in the core of the villus (arrowhead). H&E X 400

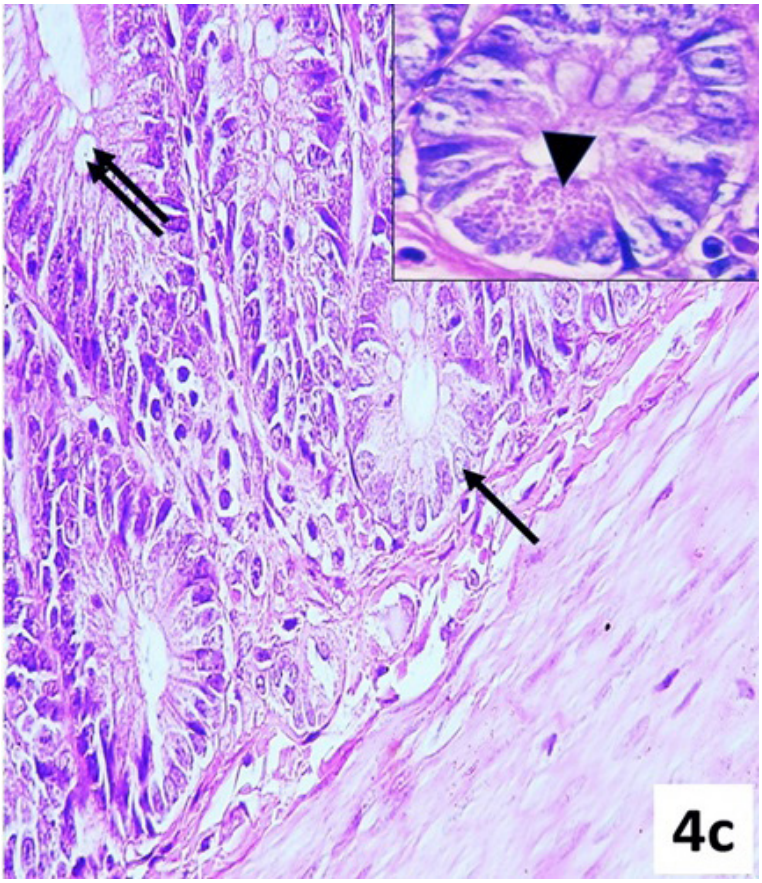

Fig. 4c: A photomicrograph of a section in the jejunum from subgroup IIIb showing the crypts lined by enterocytes with basal vesicular nuclei (↑) and few scattered goblet cells (↑↑). Inset: Paneth cells with many apical eosinophilic granules and basal vesicular nuclei (▲) lining the base of the crypts are also noticed. H&E X 400 Inset: X 640

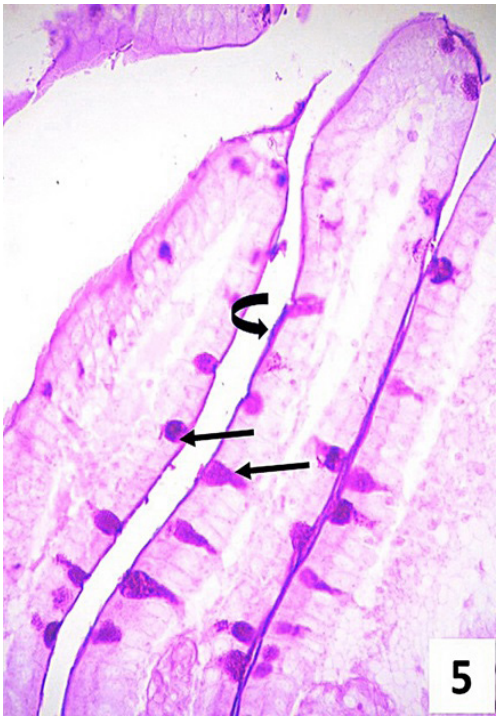

Fig. 5: A photomicrograph of a section in the jejunum from group I (control) showing the villi having regular intact brush border of enterocytes (curved arrow). Notice many goblet cells with periodic acid–Schiff (PAS) positive magenta reaction (↑). PAS X 400

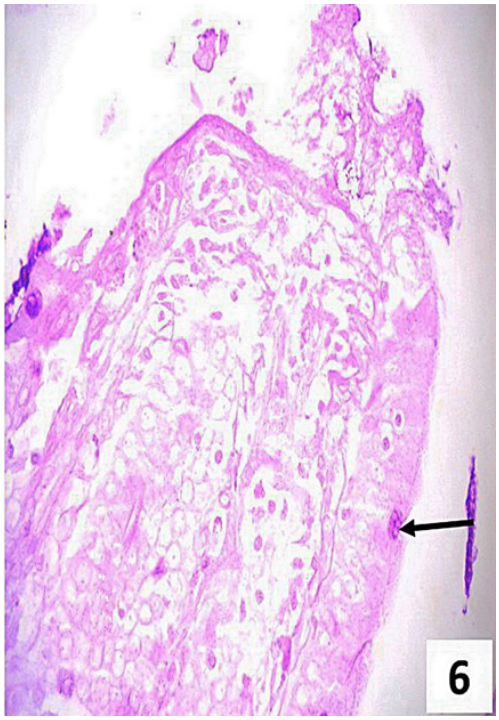

Fig. 6: A photomicrograph of a section in the jejunum from group II showing disruption of the brush border in most of the enterocytes. Sloughing of the apical part of the villus is noted. Few goblet cells with decreased mucin content can be seen (↑). PAS X 400

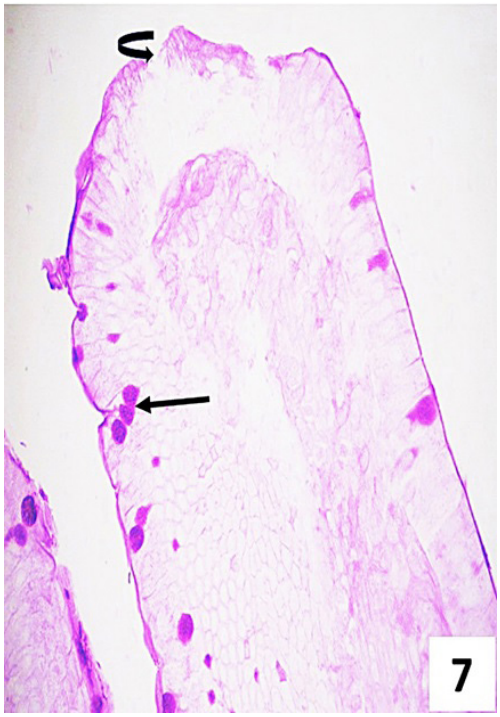

Fig. 7: A photomicrograph of a section in the jejunum from subgroup IIIa showing focal disruption of the brush border of enterocytes at the tip of the villus (curved arrow). Few goblet cells with decreased mucin content can be noticed (↑). PAS X 400

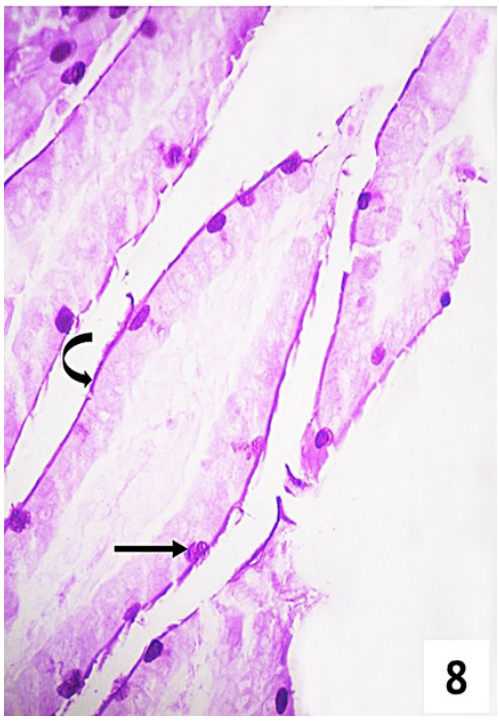

Fig. 8: A photomicrograph of a section in the jejunum from subgroup IIIb showing intact brush border (curved arrow) of enterocytes. Note the increased number of goblet cells with increased mucin (↑) compared to group II and subgroup IIIa. PAS X 400

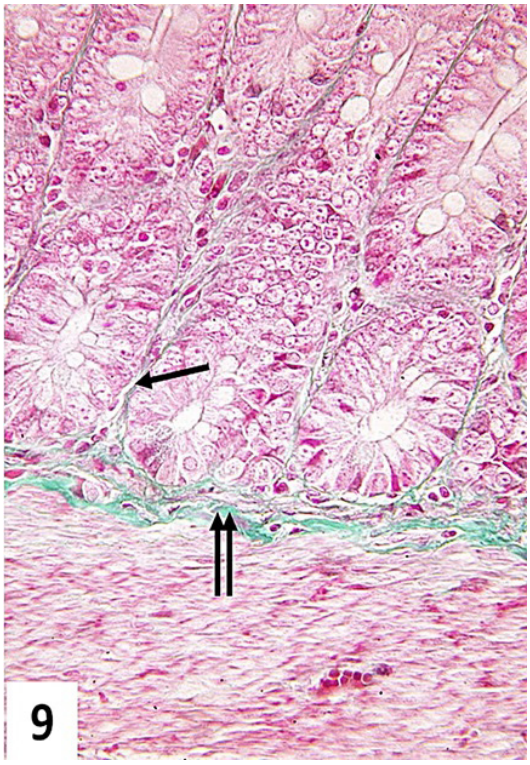

Fig. 9: A photomicrograph of a section in the jejunum from group I (control) showing minimal collagen fibers in the lamina propria (↑) and submucosa (↑↑). Masson's trichrome stain X 400

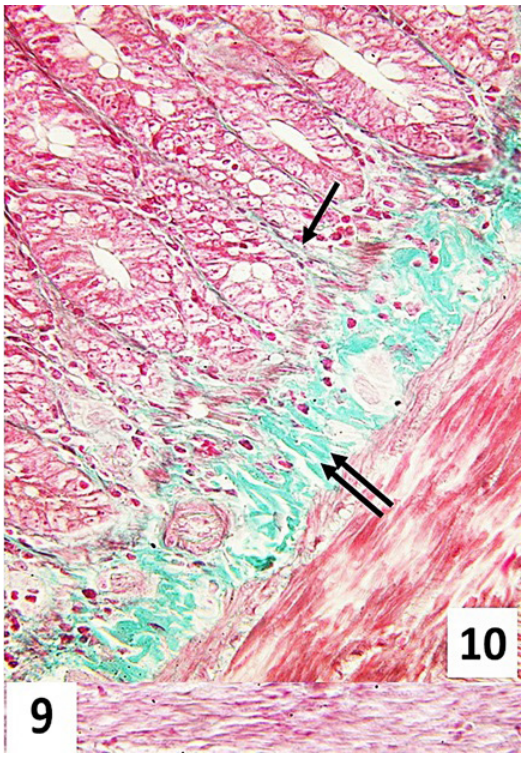

Fig. 10: A photomicrograph of a section in the jejunum from group II showing extensive collagen fibers in the lamina propria (↑) and submucosa (↑↑). Masson's trichrome stain X400

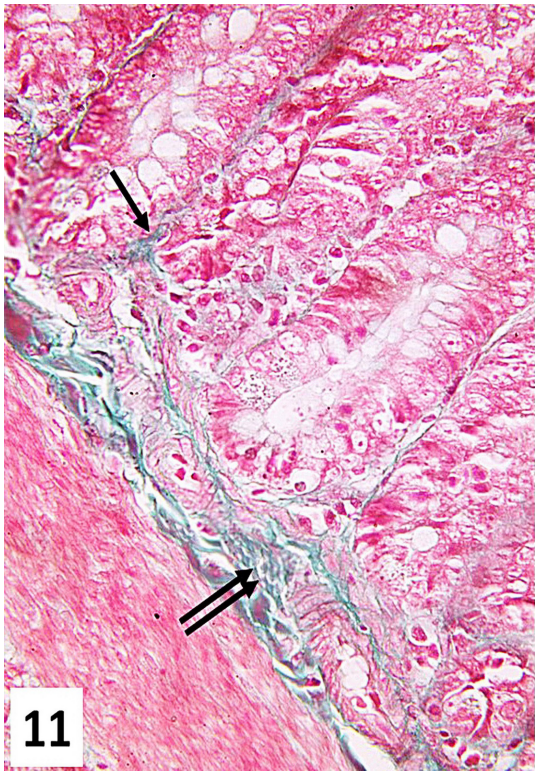

Fig. 11: A photomicrograph of a section in the jejunum from subgroup IIIa showing some collagen fibers in the lamina propria (↑) and submucosa (↑↑). Masson's trichrome stain X400

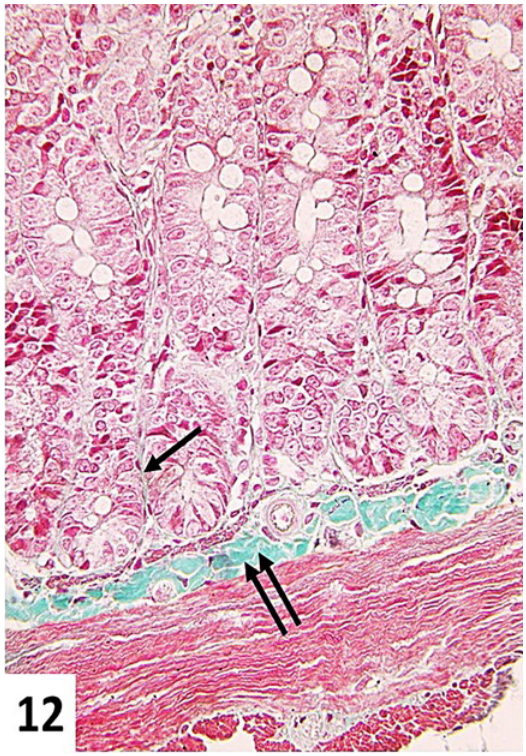

Fig. 12: A photomicrograph of a section in the jejunum from subgroup IIIb showing few collagen fibers in the lamina propria (↑) and submucosa (↑↑). Masson's trichrome stain X400

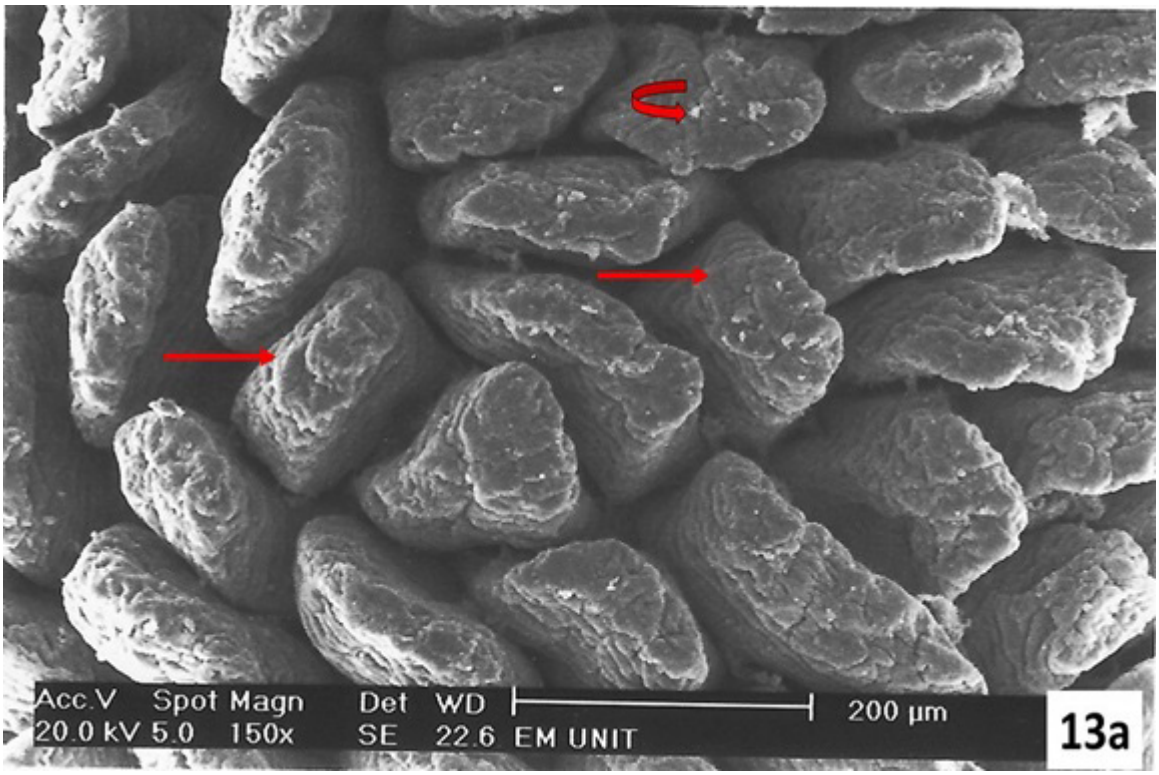

Fig. 13a: A scanning electron micrograph of a section in the jejunum from group I (control) showing regular villi lined by enterocytes with hexagonal appearance (↑). Notice the mucous secretion on villus surface (curved arrow). SEM X 150

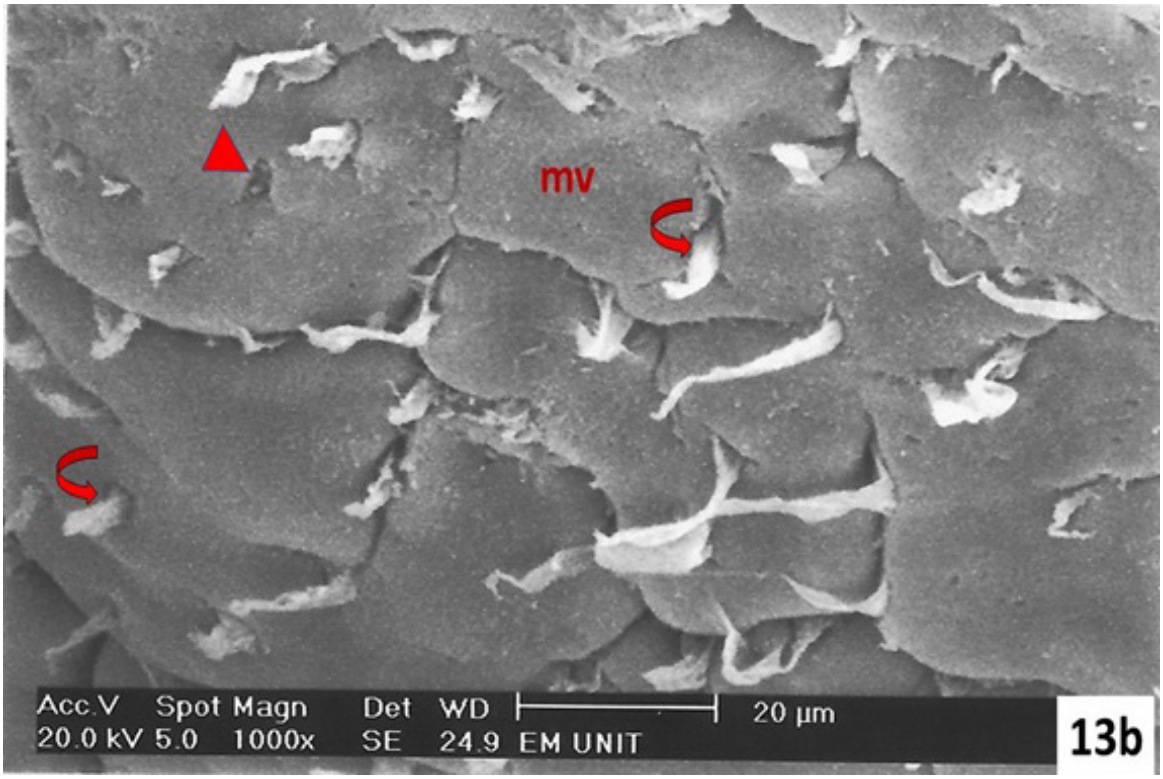

Fig. 13b: A scanning electron micrograph of a section in the jejunum from group I (control) showing apical surface of the enterocytes covered by carpet of microvilli (mv). Notice most of goblet cells' orifices are protruding secretion (curved arrow) while others are devoid of secretion (▲). SEM X 1000

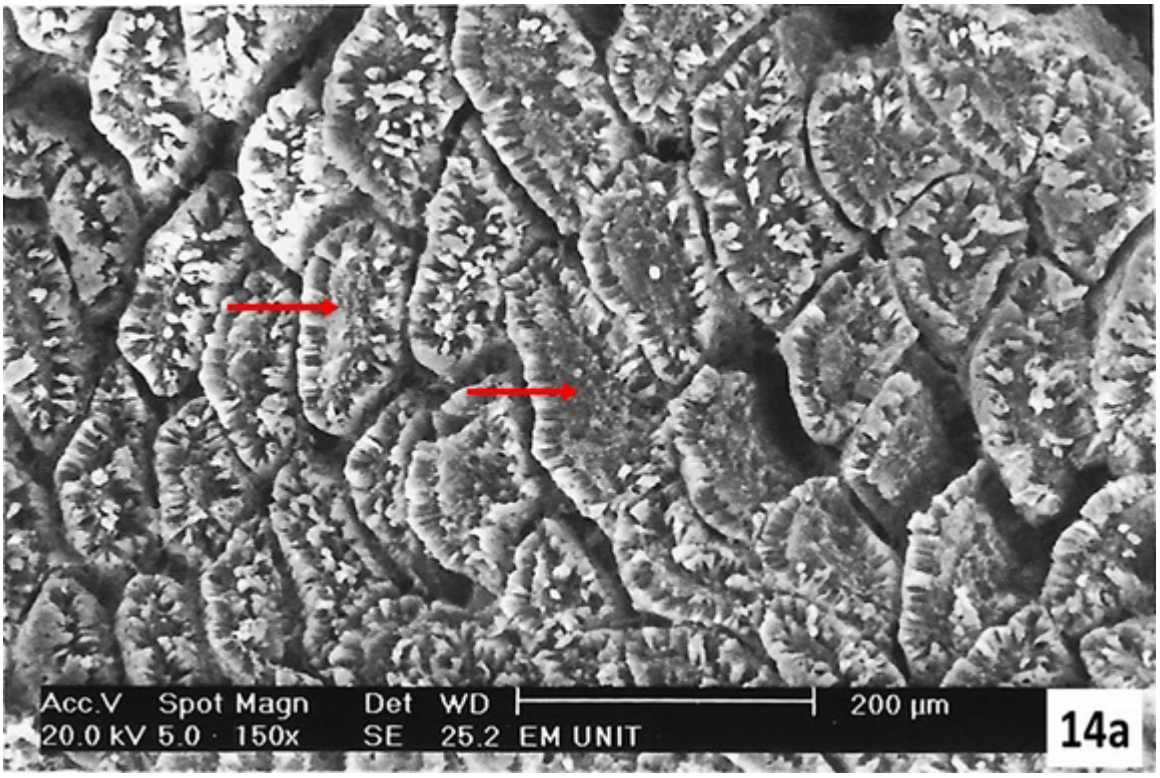

Fig. 14a: A scanning electron micrograph of a section in the jejunum from group II showing many distorted villi with complete shedding of the epithelial covering of the villi with exposure of the underlining connective tissue core (↑) . SEM X 150

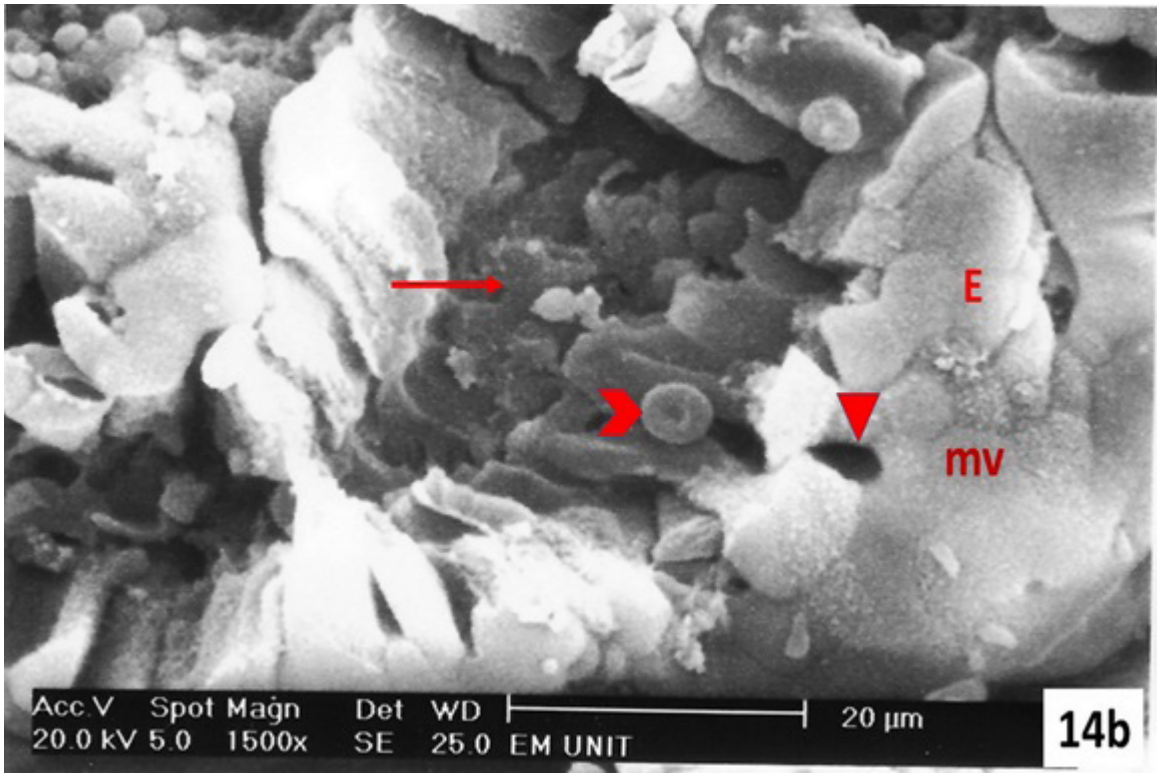

Fig. 14b: A scanning electron micrograph of a section in the jejunum from group II showing tip of a villus with an area of epithelial cells loss (↑), and infiltration of some RBCs (arrowhead). Columnar enterocytes (E) surrounding the defect are seen covered with microvilli (mv). Notice apparently decreased number of goblet cells with depletion of their mucin content (▲). SEM X 1500

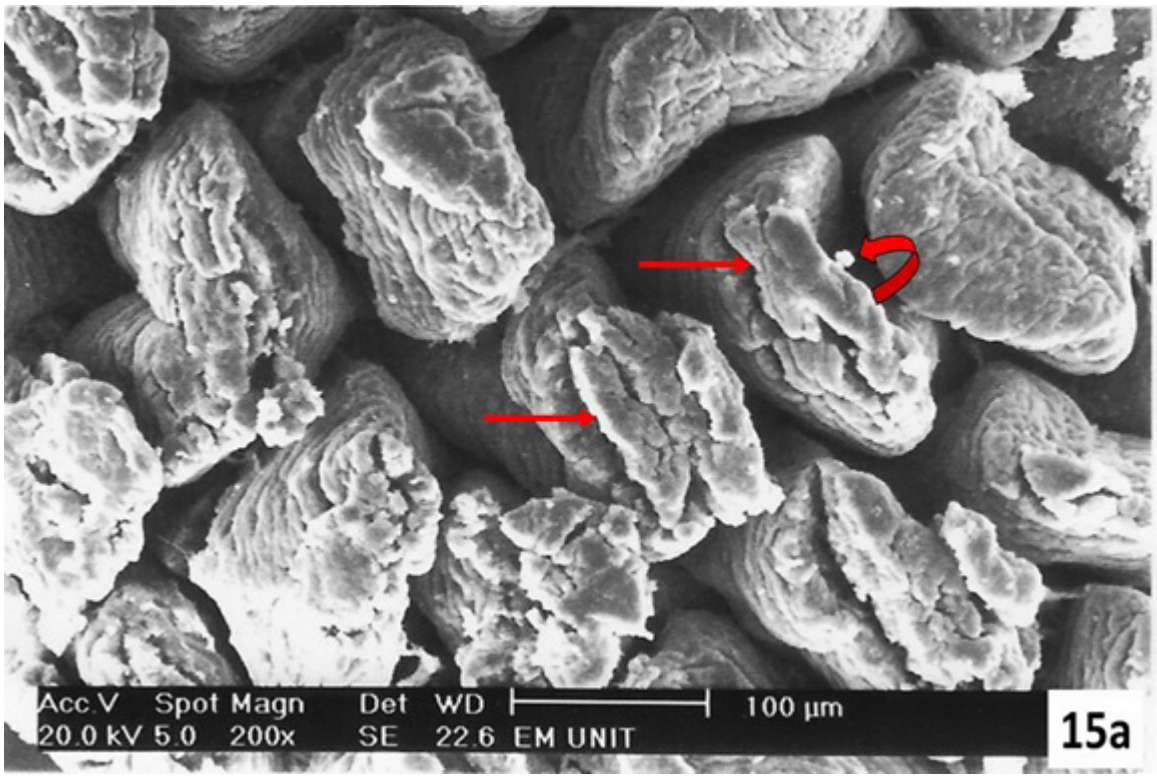

Fig. 15a: A scanning electron micrograph of a section in the jejunum from subgroup IIIa showing partially distorted tips of some villi (↑). Notice the mucous secretion on villus surface (curved arrow). SEM X 200

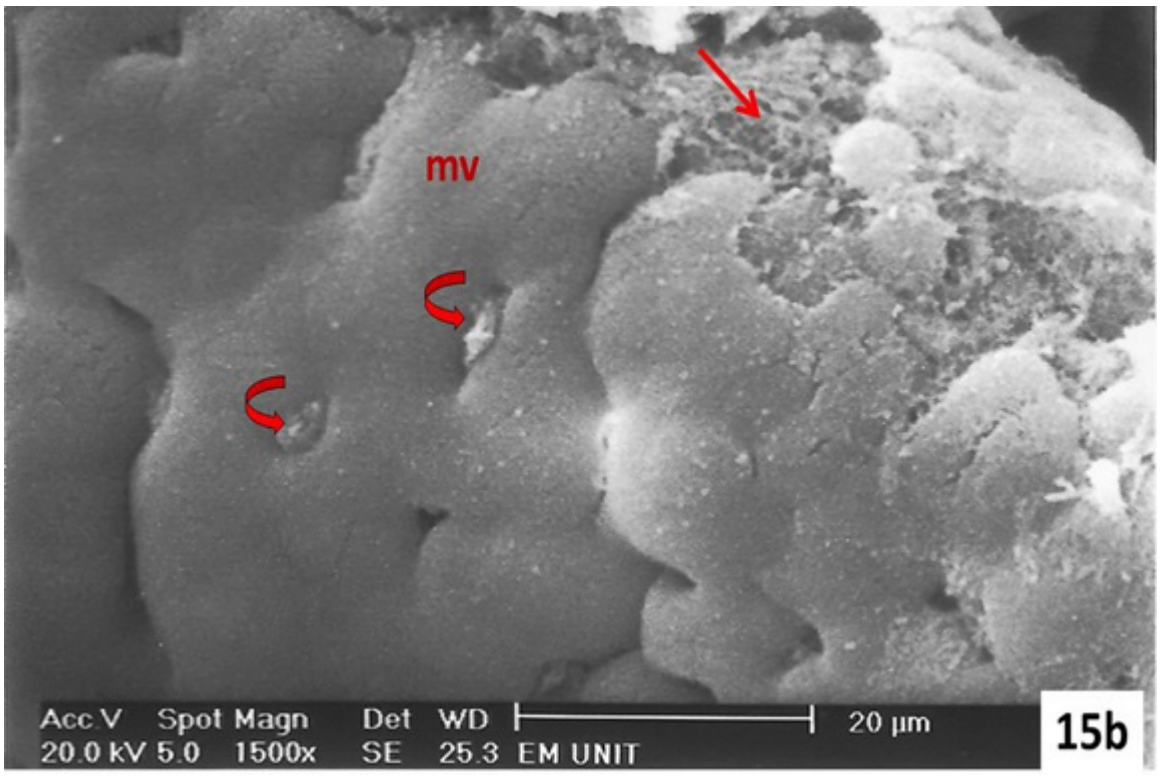

Fig. 15b: A scanning electron micrograph of a section in the jejunum from subgroup IIIa showing the apices of the epithelial cells are covered with microvilli (mv) while other focal areas lack such a cover (↑) with no cellular loss. Notice the mucin secretion of some goblet cells (curved arrow). SEM X 1500

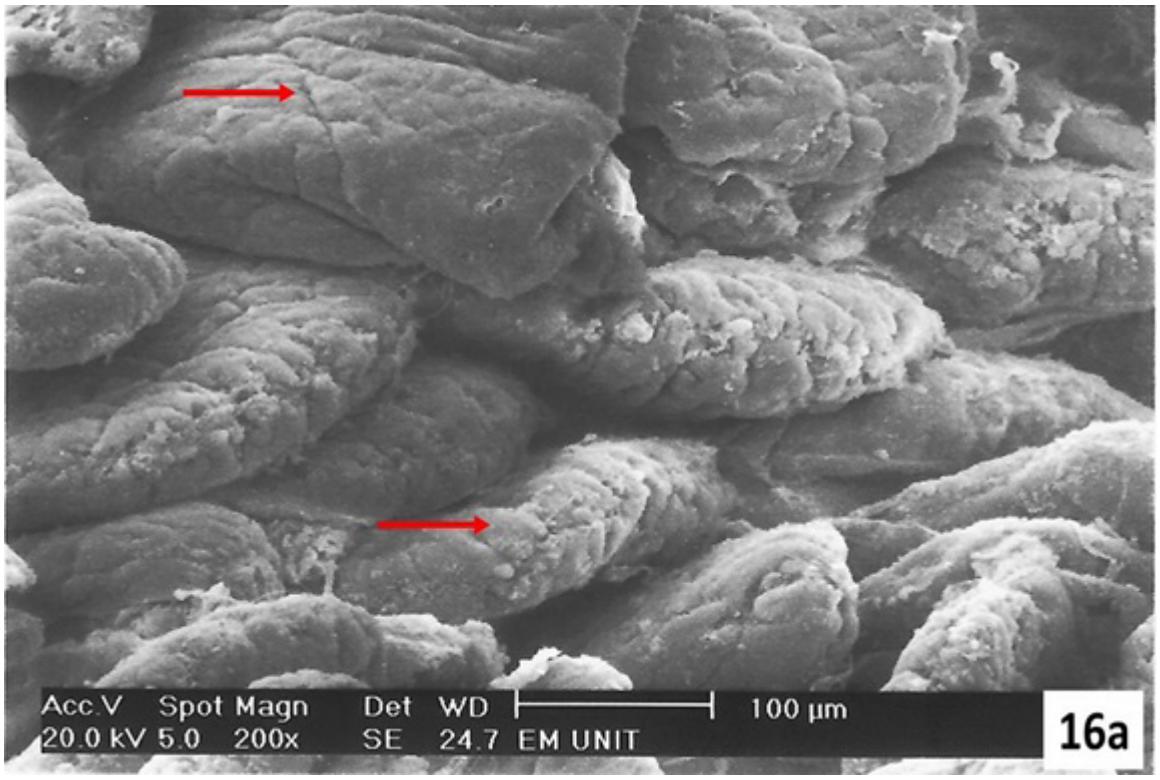

Fig. 16a: A scanning electron micrograph of a section in the jejunum from subgroup IIIb showing regular arrangement of villi which covered by enterocytes with regular hexagonal appearance (↑). SEM X 200

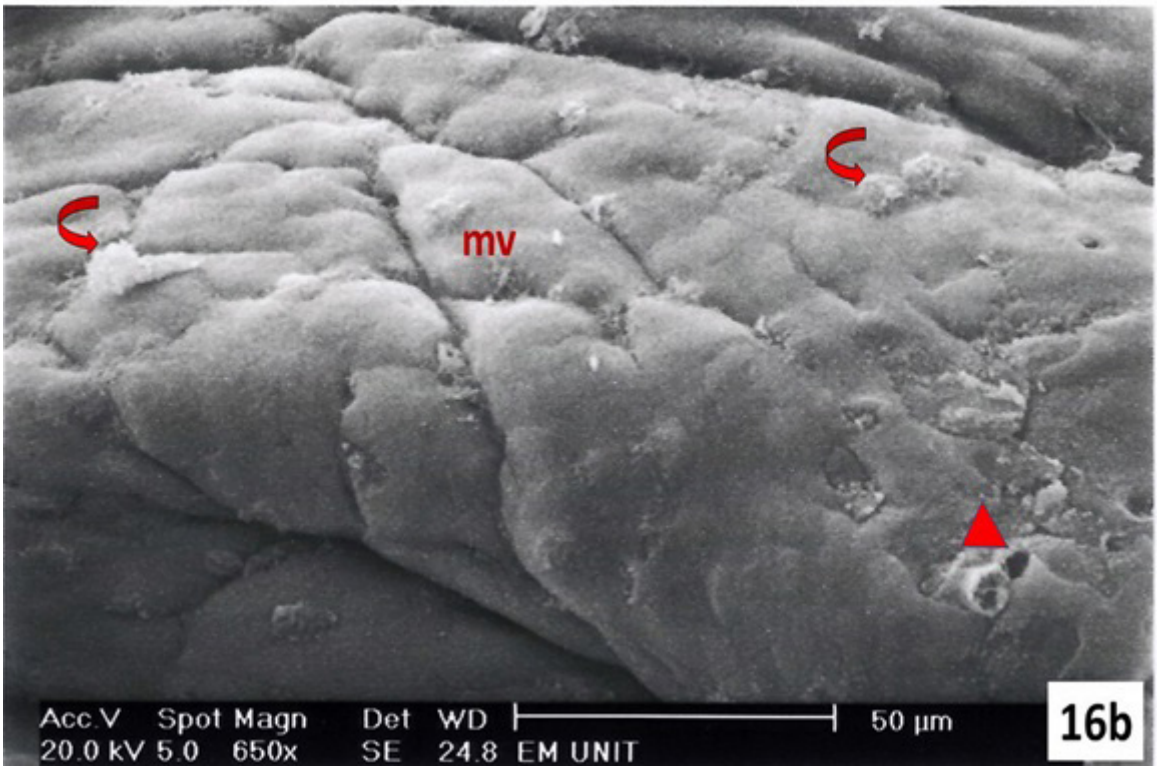

Fig. 16b: A scanning electron micrograph of a section in the jejunum from subgroup IIIb showing some goblet cells with depleted mucin content (▲) while others appear with their mucin secretion (curved arrow). Notice the apical part of the enterocytes is covered by microvilli (mv). SEM X 650

Table 1: showing mean ± SD of percentage area of collagen fibers and number of goblet cells in different groups:

Groups	Area % of Collagen fibers	Number of Goblet cells
Control group I	3.09±0.4	49±2.23
Group II	20.72±0.81▲	4.6±1.14Δ
Subgroup III a	11.1±0.69♦	22.2±2.28♠
Subgroup III b	5.08±0.59■	32.4±2.7○

Δ Significant decrease in comparison to all other groups.
▲Significant increase in comparison to all other groups.
♠ Significant increase in comparison to group II.
♦ Significant decrease in comparison to group II.
○ Significant increase in comparison to group II & subgroup IIIa.
■ Significant decrease in comparison to group II & subgroup IIIa.

DISCUSSION

The GIT is one of the most susceptible organs to the hazards of radiation. Acute structural damages of the intestine occurred within 24–48 hours of radiation exposure[1]. In the present study, after 7 days from exposure to gamma radiation in group II the jejunal mucosa showed changes in the form of distortion, fusion and even sloughing of some villi among with mononuclear cellular infiltration. Moreover, the brush border was disrupted in most of the enterocytes with significant decrease of the goblet cells and their mucin content as appeared in PAS stained sections. These findings were in accordance with Wang *et al*.[12]. Meanwhile, the crypts showed focal proliferation of its lining cells with many mitotic figures and its invasion with inflammatory cells. The lamina propria was also infiltrated with many mononuclear inflammatory cells.

The results of the current study coincided with other investigators[13] who reported erosions, necrosis, infiltration with inflammatory cell, and hemorrhage in the intestinal crypts. Infiltration of the villi and crypts with inflammatory cells might contribute to the pathogenesis of radiation enteropathy as emphasized by some investigators[14]. Meanwhile, Paris *et al*.[15] reported that apoptosis of the microvascular endothelial cells is the primary lesion in radiation damage and this might cause stem cell dysfunction. Subsequently, the microvascular endothelial cells, not epithelial cells, are the main target of radiation-induced gastrointestinal syndrome. In addition, other scientists[16] suggested that early apoptosis (4 hours after radiation) of endothelial cells is the primary lesion in radiation-induced damage to the gastrointestinal tract. However, another study concluded that endothelial injury was a consequence of radiation-induced damage of normal tissues and was not an initiating factor[17].

Meanwhile, Kim et. al.,[18] postulated that the intestinal epithelium regenerative response against gamma irradiation can be divided into three phases: apoptotic phase in the first 2 days, proliferation phase 2 to 4 days post-radiation, and stabilization phase 5 days post-radiation. They revealed that during the proliferative phase the crypts enlarge in size as the number of proliferating cells increased. Meanwhile, during the stabilization phase, the crypts and the villi are almost restored to the pre-radiation condition.

All the previous various studies showed that radiation can induce damage to both intestinal epithelial and endothelial cells. In addition, other researchers[19] postulated that the persistent intestinal damage might result from sensitivity of the intestine to radiation oxidative stress mechanisms with subsequent failure of the crypt–villus axis to regenerate. These outcomes are accompanied by continuous inflammatory response and vascular impairment with lack of regenerative cell enrollment. Moreover, Abdul-Hamid and Salah[20], stated that formation of reactive oxygen species (ROS) and the increase of neutrophil accumulation in the mucosa could disrupt the microcirculation and lead to the formation of ulcers. Moreover, accumulation of these hazard might cause structural damage by reacting with polyunsaturated fatty acids that present in DNA nucleotides and cellular membranes. Recently, other investigators[3] reported that the pathogenesis of radiation enteropathy might be due to the altered expression of long noncoding RNAs (lncRNAs) in the enterocytes as a result of accumulated

inflammatory mediators, mainly oxidative free radicals.

However, other researchers[21] declared that in severe oxidative stress, the intestinal Paneth cells release pro-inflammatory TNF-α and antimicrobial molecules which activate the caspases with subsequent intestinal epithelial cell injury and apoptosis[22].

Furthermore, other studies recorded that exposure to radiation resulted in damage to the intestinal epithelial barrier, decrease number and structural alteration of goblet cells, increased bacterial invasion, and increased inflammatory responses in intestinal tissue[23]. These results clarified the significant decrease in goblet cells numbers in (group II) gamma irradiated group stained by PAS in the current study.

Although the radiation exposure in the present experiment was limited to one radiation dose, yet it was enough to elicit a significant increase in the percentage area of collagen fibers in the submucosa and lamina propria in the groupII stained by Masson's trichrome. In view of this point, a researcher[24] postulated that a cytokine produced by Variety of immune cells limit the inflammatory reactions and induce fibrosis. It also stimulates fibroblast maturation and angiogenesis inducing matrix deposition and remodeling.

Many chemical compounds have been used as radioprotective agent; however, their high toxicity at optimum protective doses prohibited their clinical use. Moreover, Patients might tolerate dietary ingredients better than other drugs[25].

Ginger extract decreased mononuclear infiltration and improved the architecture of the colon[26]. It is also ranked one of the highest antioxidant value plants with anti-inflammatory properties through its ability to restore the redox form and inhibiting free-radical generation[27]. Furthermore, some studies stated that ginger treatment improved the antioxidant defense capacity of rats[28]. Ginger decreased lipid peroxidation of biological membranes as it cleared ROS, and hence improved intestinal blood flow[29].

In the present study and as far as we know, it was the first time to use ginger to decrease hazards of radiation enteropathy. Nevertheless, the present experiment tried ginger not only as a therapeutic agent but also, we tried to predict its possible prophylactic role if taken prior to radiation. The results of the present work showed that maximum benefit of ginger ingestion would be obtained if it was given before and after radiation as the jejunal mucosa almost restored its normal structure for both the villi and the crypts. The enterocytes retained its apical brush border and minimal intraepithelial lymphocytes and mononuclear cells in the lamina propria were detected. Moreover, significant increase in the number of PAS positive goblet cells in the villi with apparent increase in their mucin content with normal collagen deposition in the lamina propria and submucosa. In contrast, giving ginger before radiation only; showed partial improvement of the jejunal mucosa with patchy areas of sloughing and necrosis with evidence of collagen deposition. These was in accordance with some investigators who used ginger for treatment of ulcerative colitis[5], necrotizing enterocolitis[6], ischemia[30] and methotrexate toxicity[20]. Although the cause for enteropathy was different in all previous studies yet the pathogenesis is still the same and had responded exquisitely to ginger.

CONCLUSIONS

In view of our findings ginger was considered as potent antioxidant, anti-inflammatory natural herb. It appeared to be a potential promising agent for preventing damage of the intestinal tissues against radiation enteropathy. We also recommend careful planning of irradiation protocols of the small intestine with a protective dose of ginger to prevent radiation enteropathy.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الدور التحسيني المحتمل للزنجبيل في اعتلال الصائم المعوي الناجم عن اشعاع غاما في ذكور الجرذان البالغة. دراسة بالميكروسكوب الضوئي والميكروسكوب الاليكترونى الماسح

ملخص البحث

الخلفية: تعتبر الأمعاء واحدة من أكثر الأجهزة تضررا من سمية الإشعاع. لذلك، لا تزال معدلات الأمراض والوفيات الناجمة عن التهاب الأمعاء والقولون الإشعاعي مرتفعة. و ذكرت الدراسات الحديثة أن الزنجبيل قد يمتلك خصائص مضادة للالتهابات والأكسدة. لذلك كان الهدف من هذه الدراسة هو استكشاف الدور المحتمل للزنجبيل كعامل وقائي وكعامل علاجي محتمل في اعتلال الأمعاء الناجم عن اشعاع غاما.

المواد والطرق: تم استخدام ست وثلاثون جرذا أمهقا من الذكور البالغين في هذه الدراسة. تم تقسيم الحيوانات بشكل عشوائي إلى ثلاث مجموعات: المجموعة الأولى (I) استخدمت كمجموعة ضابطة، المجموعة الثانية(II) (أشعة جاما): حيث تعرض كل جرذ لجرعة واحدة إشعاعية و قدرها ٠,٧٣٩(١١جراي/ دقيقة) من إشعاع جاما ، المجموعة الثالثة (III) (أشعة جاما و زنجبيل): تم تقسيمها إلى مجموعتين فرعيتين تم تعرض كلاهما للإشعاع كما هو الحال في المجموعة الثانية وتم إعطاؤهم مستخلص الزنجبيل عن طريق الفم مرة واحدة يوميًا ١٢٠ ملغم / كغم. المجموعة الفرعية (-IIIأ): تلقت مستخلص الزنجبيل سبعة أيام قبل الإشعاع بينما المجموعة الفرعية (-IIIب): تلقت مستخلص الزنجبيل سبعة أيام قبل وبعد الإشعاع. في نهاية التجربة تم ذبح جميع الحيوانات، وتم فحص عينات الصائم المعوي بواسطة الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح. كما تم إجراء دراسة مورفومترية وتحليل إحصائي.

النتائج: أظهر الفحص الهستولوجى للمجموعة الثانية (II) انفتال واندماج لبعض الخملات. بينما أظهرت الخبايا تكاثر بؤري لخلاياها الطلائية وغزوها بالخلايا الالتهابية. علاوة على ذلك، أظهرت المجموعة الفرعية (-IIIأ) اختلال بؤري لجدار الخبايا. كما لوحظ انخفاض معتد في متوسط عدد خلايا الكأسية وزيادة معتدة في النسبة المئوية للمساحة المتوسطة لألياف الكولاجين، في المجموعات السابقة مقارنة بالمجموعة الضابطة. ومع ذلك، أظهرت المجموعة الفرعية (-IIIب) استعادة معظم هيكل الغشاء المخاطي للصائم ليصبح تقريبا مشابه للمجموعة الضابطة.

الخلاصة: يبدو أن الزنجبيل له تأثير واعد محتمل لتحسين إصابة الأنسجة المعوية الناجمة عن الإشعاع.