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## The Possible Impact of Synbiotics on Intestinal Mucosa and Immune Responses against Gamma Irradiation in Experimental Rats

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### ABSTRACT

Ionizing radiation has hazardous effects on humans, from cell damage to complete organ failure, depending on the radiation energy and the exposure time. A well-designed diet could treat and reduce or delay radiation hazards. This study aimed to enhance immunity and monitor the effect of four designed diets on the 6 Gy irradiated rats. 1010 lacteal forte, two groups of rats were fed 5 and 10% garlic, another two groups were fed 5 and 10% banana peels. Parameters related to nutrition and vitality were tracked. Comparing the effects of both kinds of treatment showed that the 5% garlic treatment had the best result on nutritional parameters, whereas the 10% garlic treatment had the best results from the side of medicine. Also, the 5 and 10% banana peel groups showed acceptable treatment results. Garlic as a food and banana peels as a food waste could be helpful in the presence of lacteal forte to treat and overcome the harmful hazard of radiation.

### INTRODUCTION

Ionizing radiation endangers living cells and has detrimental effects. Additionally, because of the quickening pace of technological development, human exposure to this radiation has significantly increased (Syaifudin *et al.* 2017)

Since the human gut is home to over 105 microbial species, which primarily regulate the organism's internal environment, it plays a significant role in human health. As a result, the gut plays a crucial role in both catabolism and anabolism, as well as a defense function. Due to their remarkable capacity to compete with pathogenic microbiota for sticking sites, immunological supplementation with probiotics, prebiotics, or a combination of both, known as synbiotics, has shown encouraging results against many enteric pathogens by triggering the activation of particular genes inside and outside the host's intestinal tract, one can stimulate, modulate, and regulate the immune response of the organism. Additionally, probiotics control fat storage and promote intestinal angiogenesis (Kerry *et al.* 2018).

The unique biological and functional properties of prebiotics derived from lactose make them safe. Additionally, doctors recommended them as therapeutics in their pure form or combined with dairy products (such as yogurt, milk, and infant formulas) or fruit juices. Prebiotics' bioactivity was demonstrated when intestinal bacteria, particularly probiotic ones (*Lactobacillus* spp. in the small intestine and *Bifidobacterium* spp. in the large intestine), were present. According to clinical studies, galacto-oligosaccharide lowers the risk of several types of diarrhea. Using lactulose, inflammatory bowel diseases may be lessened or even prevented. Hepatic encephalopathy was made less likely by lactitol and lactulose (Nath *et al.* 2018).

Through the gut-brain axis, probiotics control the secretion of gastrointestinal hormones and brain activity. Additionally, it is crucial for triggering intestinal angiogenesis signaling, which controls acute and chronic inflammation in the intestinal mucosal tissue brought on by the progression of inflammatory bowel disease via the vascular endothelial growth factor receptor (Paulina & Katarzyna 2017).

Garlic (*Allium sativum* L.), for its medicinal properties, has been used since ancient times as a food ingredient and therapeutic agent. Garlic contains different bioactive components include alliin, allicin-ajoene, diallyl sulphide, diallyl disulfide, diallyl trisulfide, and S-allyl-cysteine. Garlic could be used as antioxidant, anticancer, anti-inflammatory, anti-diabetic, anti-obesity, antibacterial, antifungal, immune-modulatory, cardiovascular protective, hepatoprotective, neuroprotective, renal protective, high blood pressure treatment, coronary artery disease (hardened arteries), stomach, colon, and rectal cancers, and to prevent tick bites (Tahir *et al.* 2022). The banana (*Musa* species), one of the most important and valuable plant species, has various pharmacological effects. One of the foods thought to be high in antioxidants is the banana. *Musa acuminata* contains antioxidant compounds like ascorbic acid, tocopherol, beta-carotene, phenolic groups, dopamine, and gallic catechin. The banana peel is more affluent in phytochemical compounds than its pulp (Kamal *et al.* 2019).

The purpose of this study was to determine whether specific concentrations of lacteal forte, a source of *Lactobacillus acidophilus* probiotics, and both garlic and banana peels as prebiotics could boost immunity and counteract the harmful effects of ionizing radiation in vivo.

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## MATERIALS AND METHODS

### Materials

Garlic and banana peel samples were obtained from the local market in Tanta, Egypt. Lacteal forte (The starter culture of *Lactobacillus acidophilus*) was purchased from Sigma Chemical and Drugs Company.

### Experimental Animals

Thirty-six male albino rats (Sprague Dawley strain), weighing approximately (100±10g), were obtained from the animal house of the national center for radiation research and Technology (Atomic Energy Authority, Egypt).

### Methods

#### Preparation of Garlic and Banana Peels Powders

To dry garlic and banana peel samples, it was placed in an aluminum tray and then dried in the vacuum oven at 65°C and a vacuum pressure of 67 KPa. The samples were dried until constant weight. Samples were weighed every two hours at first, followed by every one hour

and every 30 min using a digital balance with an error of 0.001g. Powder samples were obtained by grounding them in an electric blender and sieved to a mesh of size 0.5mm. The powder was stored in clean brown bottles at room temperature (30±5°C) until using (El Ashmawy & Ahmed 2016).

#### Determination; Determination of the Composition of Banana Peel and Garlic Powders

Different components of both powders, moisture, protein, fat, fiber, and ash% were determined according to the international methods (AOAC 2010). Total carbohydrates were calculated as difference:

#### Nutritional and Biological Assessment

Rats were fed pellets as a primary diet composed of the main components of the diet and mixed well with the suggested ratios of the additives needed for the experiment to meet all of the rat's nutritional needs (Table 1).

**Table 1:** Diets consumed by rat groups throughout the experiment time (12g/rat/day)

Diet composition Groups	Basal weight (g)	Garlic weight (g)	Lacteol ( <i>Lactobacillus</i> genus bacteria)	Total weight (g)
C(-ve).	2000	-	-	2000
C(+ve).	2000	-	-	2000
5% Gar.	1900	100	2x10 <sup>10</sup> CFU*	2000
10% Gar.	1800	200	2x10 <sup>10</sup> CFU	2000
5% Ban.	1900	100	2x10 <sup>10</sup> CFU	2000
10% Ban.	1800	200	2x10 <sup>10</sup> CFU	2000

\* CFU colony forming unit.

### Gamma Irradiation Facility

Irradiation was conducted in Nasr City, Cairo, Egypt, at the National Centre for Radiation Research and Technology (NCRRT). The radiation source was Gamma Cell-40 (Cesium 137), which allowed for a homogeneous irradiation distribution. The dose rate during the experimental phases was 0.61 Gy/minute. Rats were exposed to 6 Gy as one single dose of whole body (Nasr *et al.* 2021).

### Experimental Design

The adapted rats were divided into two main groups:

The first leading group (6 rats) was fed a basal diet and kept as C(-ve) control.

The second leading group (30 rats) was exposed to a single dose of 6.0 GY of gamma rays to the whole body (Abou-Zeid *et al.* 2018). After that, the second leading group was divided into five subgroups as follows:

The first subgroup: Irradiated rats, were fed with a basal diet and left as a positive control group (C(+ve))

The second subgroup (5% Gar.): Irradiated rats were fed a basal diet supplemented with garlic powder (50 g/kg diet) and Lacteal forte (the starter culture of *Lactobacillus acidophilus* (1010CFU/kg diet)) (Bahr *et al.* 2019).

The third subgroup (10%Gar.): Irradiated rats were fed a basal diet supplemented with garlic powder (100 g/kg diet) and Lacteol forte (The starter culture of *Lactobacillus acidophilus* (1010CFU/kg diet)).

The fourth subgroup (5%Ban.): Irradiated rats were fed a basal diet supplemented with garlic powder (100 g/kg diet) and Lacteol forte (The starter culture of *Lactobacillus acidophilus* (1010CFU/kg diet)).

The fifth subgroup (10%Ban.): Irradiated rats were fed a basal diet supplemented with garlic powder (100 g/kg diet) and Lacteol forte (The starter culture of *Lactobacillus acidophilus* (1010CFU/kg diet)).

The experiment lasted for 28 days.

### Biochemical Analysis

At the end of the experiment, rats were sacrificed, and blood samples were collected from hepatic portal veins in two tubes. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes and kept in plastic vials at -20 °C until analysis.

### Haematological Investigations

A complete blood count was performed with anticoagulants using a hematological analyzer (Exigo

Eos Vet, Sweden). The arthrogram consisted of red blood cell count (RBCs), hematocrit (HCT), hemoglobin (Hb) concentration, red cell index, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width absolute (RDW<sub>a</sub>), leukogram (which includes white blood cell count (WBCs), lymphocytes (%), monocytes (%), granulocytes (%), platelet count (PLT), and mean platelet volume (MPV) (Prakash *et al.* 2014).

**Malondialdehyde Levels and Antioxidant Enzyme Levels as Indicators of Lipid Peroxidation**

Serum malonaldehyde (MDA) was estimated spectrophotometrically as thiobarbituric acid reactive substances (TBARS). Also, superoxide dismutase (SOD) and Catalase (CAT) were determined according to (Sangeetha *et al.* 2020).

**Determination of Albumin and Total Protein**

Albumin and total protein were determined according to (Drupt 1974, Paulina & Rafa 2017 and Sonnenwirth, & Jaret 1980).

**Determination of Liver Function Parameters**

Liver function markers were assessed calorimetrically in serum samples of all groups using commercial kits (Bio-Diagnostic Co., Cairo, Egypt). Aspartate aminotransferase

(AST), and Alanine aminotransferase (ALT), were determined (Reitman & Frankel 1957).

**ELISA Assay for the Plasma Cytokine Profile**

Serum was tested in duplicate by ELISA for granulocyte-macrophage colony-stimulating factor IL-6 and tumor necrosis factor-alpha (TNF- $\alpha$ ) using an enzyme-linked immunosorbent assay (ELISA) (Pharmingen, USA) (Strachan *et al.* 2001).

**Histopathological Examination**

Ileum intestinal samples were taken (30 cm distal to the pylorus). The samples were washed with normal saline to remove gut contents. A 1cm long piece from each intestinal tissue was fixed overnight in 4% paraformaldehyde and embedded in paraffin for histological analysis (Perry *et al.* 2016).

**Results**

**Proximate Analysis of both garlic and banana peels powders**

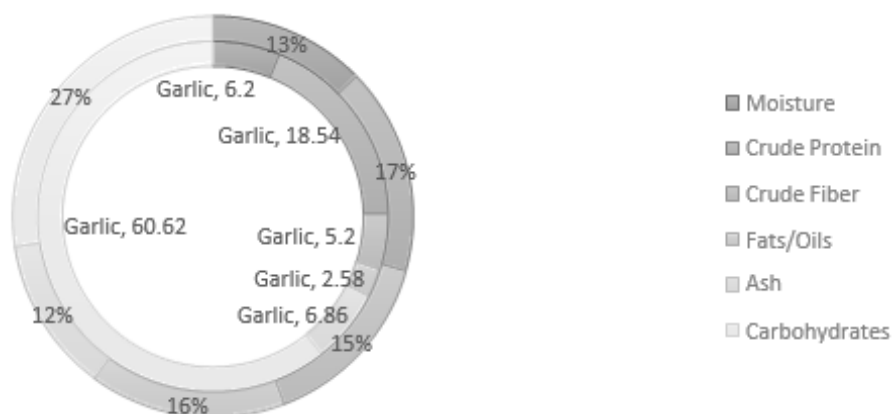
Proximate composition analyses of both banana peels and garlic powders were determined, and the results of these analyses are recorded in Table 2. Banana peel contain more moisture, ash, fiber, fats, and oils than garlic powder. However, garlic powder content from crude protein and carbohydrates were higher than that in banana peels (Anhwange *et al.* 2009, Kurhade *et al.* 2016).

**Table 2:** Proximate composition of both banana peel and garlic powders

Constituent	Banana peels powder (%)	Garlic powder (%)
Moisture	12.65+0.35	6.20+0.21
Crude Protein	16.72+0.12	18.54+0.10
Crude Fiber	14.86+0.31	5.20+0.2
Fats/Oils	15.86+0.24	2.58+0.15
Ash	12.52+0.14	6.86+0.14
Carbohydrates	27.39+0.13	60.62+0.01
pH	6.08+0.06	6.36+0.03
Food Energy g/calories	252.3+0.14	265.70+0.04

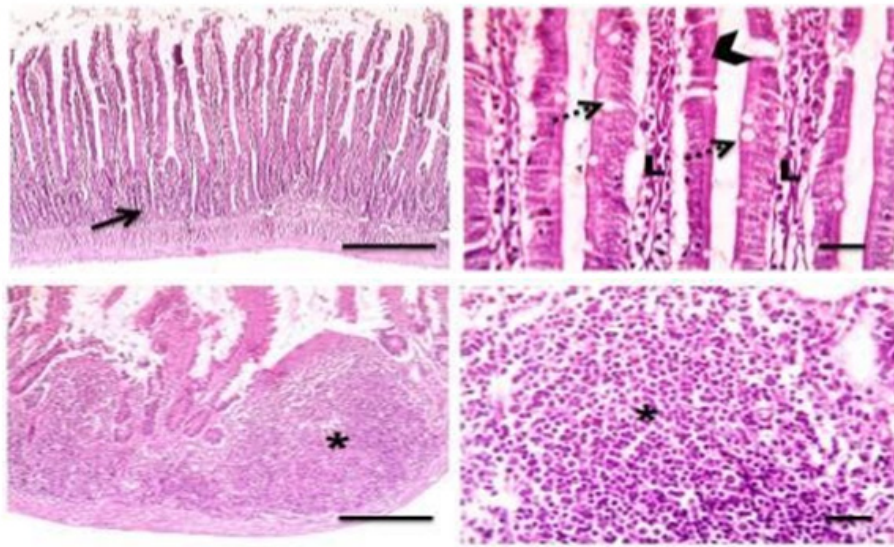
\*Values are means + standard deviation of three determinations (n=3)

\*Energy was calculated by summation of (fat x 9 kcal) + (carbohydrate x4 kcal).

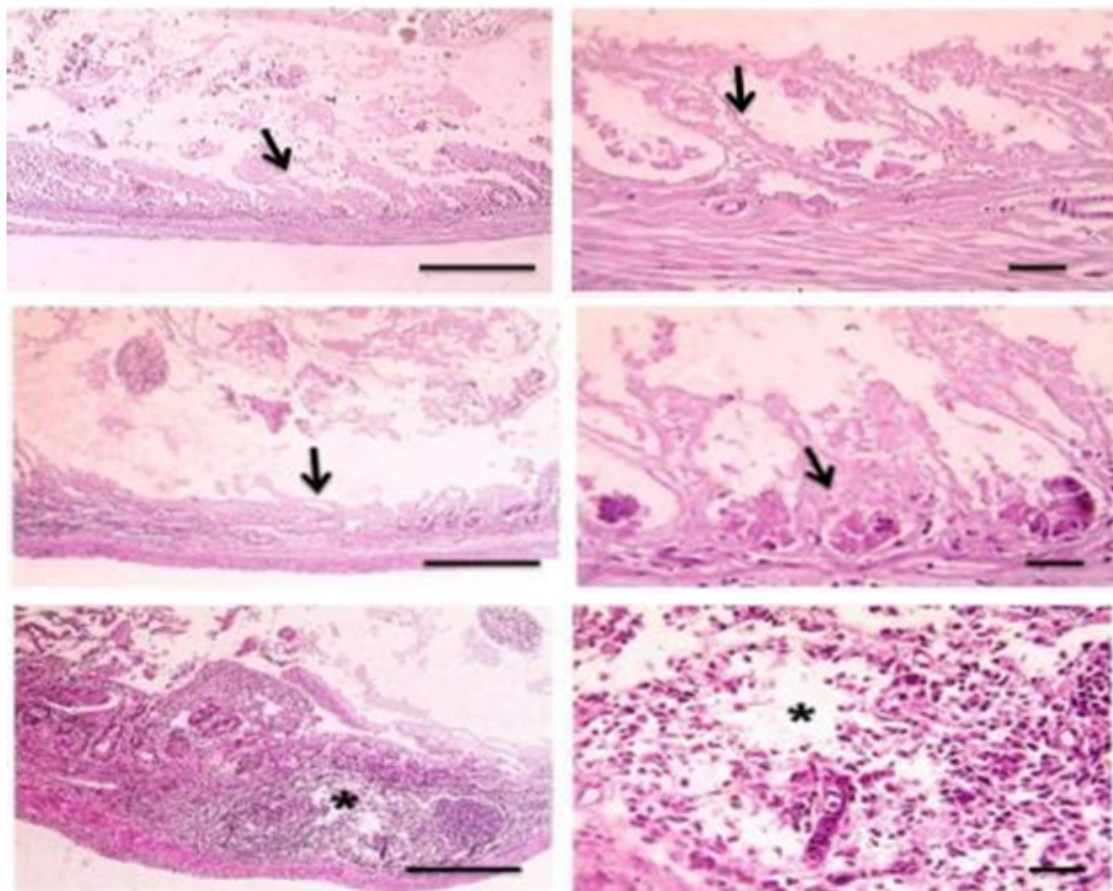


**Figure 1:** Banana Peels and Garlic Nutritional

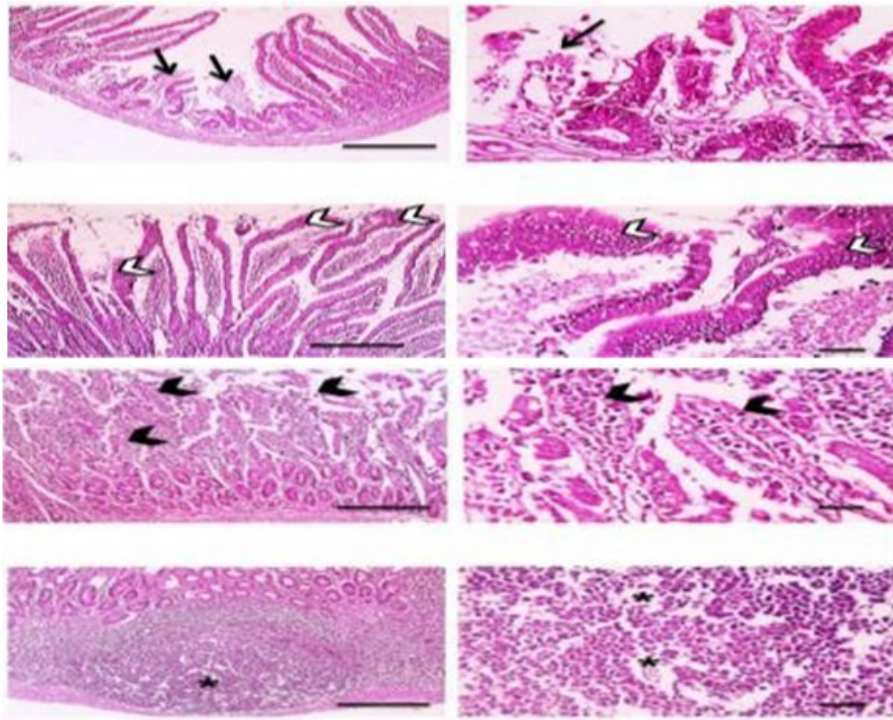
Intestine Pathological Studies



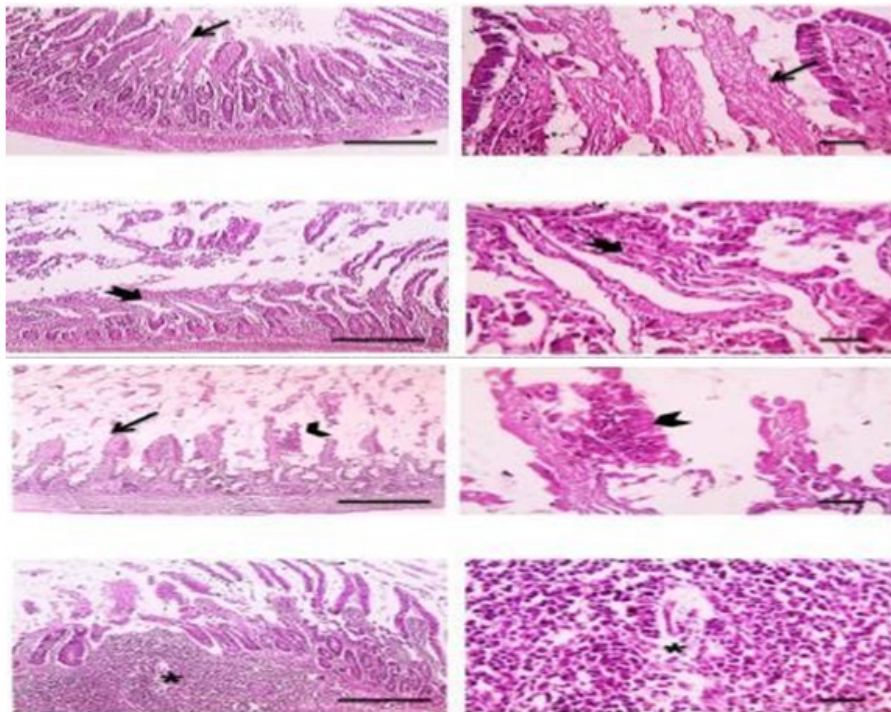
**Figure 2:** Microscopic pictures of H&E-stained ileal sections show normal long villi covered with columnar epithelium (arrowheads) and goblet cells (dashed arrows), normal lamina propria (L), normal crypts (arrows), and normal lymphocytic population in Payer's patches (\*) in control negative (C(-ve)) group. Low magnification is 100, and high magnification is 400.



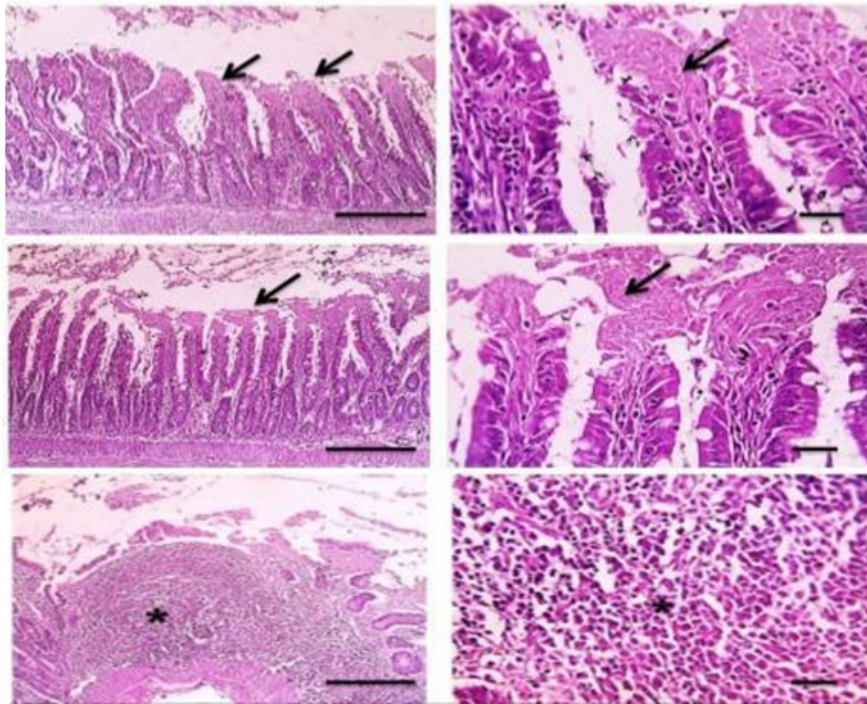
**Figure 3:** Microscopic pictures of H&E-stained ileal sections from the irradiated (C(+ve)) group show diffuse and deep mucosal necrosis leading to a severe loss of villi and crypts (arrows) with prominent necrosis and depletion of lymphocytes in Payer's patches (\*). Low magnification is 100, and high magnification is 400.



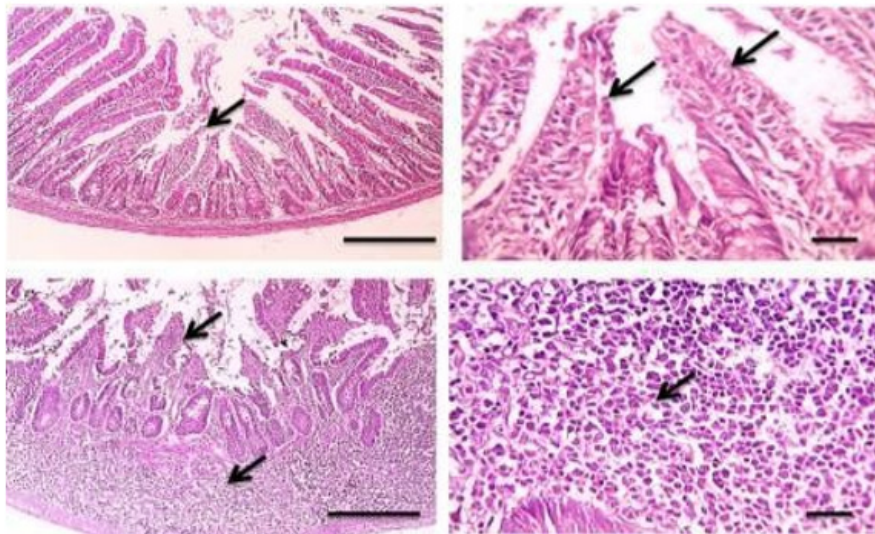
**Figure 4:** Microscopic pictures of H&E-stained ileal sections from (5% Gar.) group showed partially decreased severity of pathological lesions that varied from necrosis of a few villi (arrows) to desquamation of covering columnar epithelial cells (arrowheads). Signs of regeneration are observed in some sections, indicated by marked hyperplasia of columnar epithelial cells. Moderate depletion of lymphocytes in Payer's patches (\*) is seen. Low magnification is 100, and high magnification is 400.



**Figure 5:** Microscopic pictures of H&E-stained ileal sections from (10% Gar.) group showed slightly decreased severity of pathological lesions that varied from mild (thin arrows) to moderate (thick arrows) necrosis of villi. Signs of regeneration are very soft, as indicated by hyperplasia of columnar epithelial cells in very few villi. A mild depletion of lymphocytes in Payer's patches (\*) is seen. Low magnification is 100, and high magnification is 400.



**Figure 6:** Microscopic pictures of H&E-stained ileal sections from (5% Ban.) group show a decreased severity of pathological lesions than in previous groups, including necrosis in the tips of some villi (thin arrows) with restored lymphocyte density in Payer's patches (\*). Low magnification is 100, and high magnification is 400.



**Figure 7:** Microscopic pictures of H&E-stained ileal sections from (10% Ban.) group show mild pathological lesions, including loss of columnar epithelial cells covering a few villi (thin arrows), with restored lymphocyte density in Payer's patches (\*), are seen. Low magnification is 100, and high magnification is 400.

Several histopathologic changes were observed in the intestine tissue of the irradiated group (C(+ve)) compared to the control (C(-ve)) group. These changes included diffuse and deep mucosal necrosis leading to a severe loss of villi and crypts, prominent necrosis, and depletion of lymphocytes in Payer's patches.

The (5% Ban.) group showed partially decreased severity of pathological lesions ranging from necrosis of a few villi to desquamation of covering columnar epithelial

cells. Signs of regeneration are observed in some sections, indicated by marked hyperplasia of columnar epithelial cells. Moderate depletion of lymphocytes in Payer's patches is seen.

The (10%Ban.) group showed slightly decreased severity of pathological lesions that varied from mild to moderate necrosis of villi. Signs of regeneration are very mild, as indicated by hyperplasia of columnar epithelial cells in very few villi. A mild depletion of lymphocytes in Payer's

patches is seen.

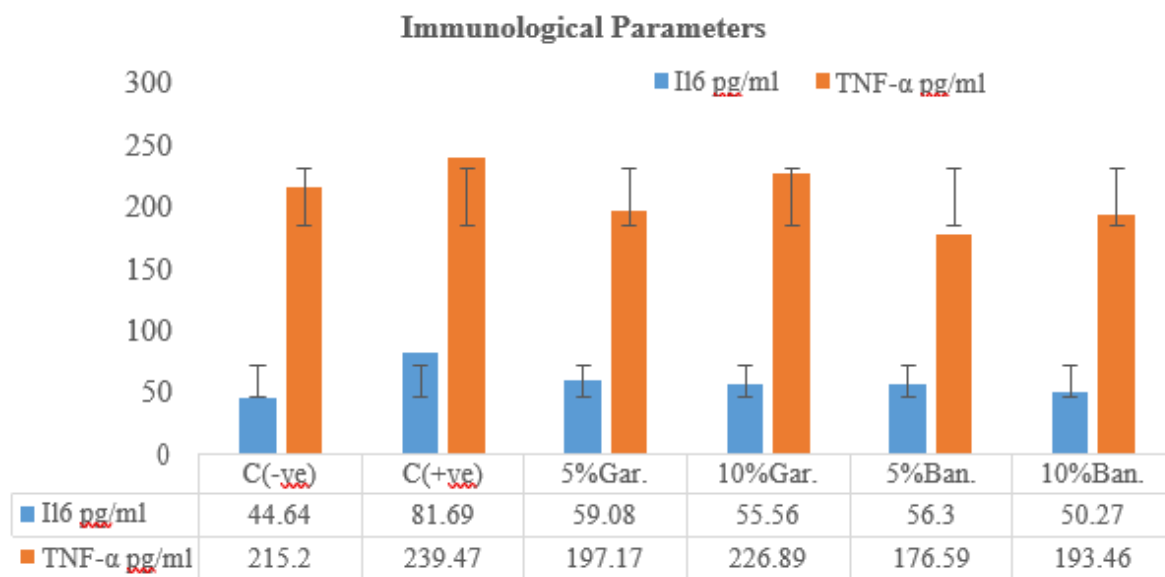
The (5% Gar.) group displayed partially less severe pathological lesions, ranging from desquamation of the covering columnar epithelial cells to necrosis of a few villi. In some sections, columnar epithelial cells exhibit pronounced hyperplasia, a sign of regeneration. Payer's patches indicate mild lymphocyte depletion.

The pathological lesions in the (10% Gar.) group, which ranged in severity from mild to moderate villi necrosis, were slightly less severe. Very few villi have hyperplasia of columnar epithelial cells, a very gentle sign of

regeneration. In Payer's patches, a slight lymphocyte depletion is observed.

The results were consistent with those observed, showing that stem cells in the intestinal crypts were responsible for inducing apoptosis in response to radiation symptoms (Becker 2018).

**The impact of adding a lacteal forte to the diet and supplementing it with banana peel and garlic powders on the immunological parameters of radiation-exposed rats**



**Figure 8:** The impact of the entire treatment on immunological parameters

Serum (IL-6) of the irradiated group (81.69+1.04) significantly increased compared to the C(-ve) (44.64+1.11) group, whereas the (5% Gar. and 10% Gar.) groups showed a significant decrease compared to the C(+ve) group. The (10% Gar.) group showed the best value relative to the C (-ve) group. Serum TNF- of the irradiated group (239.47+1.52) significantly increased compared with the C(-ve) (215.20+1.14) group), whereas the (5% Gar.) and (10% Gar.) groups showed a significant decrease compared with the C(+ve) group. On the other hand, in the (5%Ban.) and (10%Ban.) groups, their (IL-6) values were (56.30+1.05 and 50.27+2.04) and their (TNF-) values were (176.59+2.39 and 193.46+1.83), respectively, and showed a significant decrease compared with the irradiated (C(+ve)) group. 10%Ban. group showed the most related value compared with the (C(-ve)) group, respectively, and showed a significant decrease compared with the irradiated (C(+ve)) group. (10%Ban.) group showed the best value related to the (C(-ve)) group.

**The impact of adding a lacteal forte to the diet and supplementing it with banana peel and garlic powders on the Oxidative Stress Factors in gamma irradiated rats**

As we could see from these results, serum (SODs

and CAT) of the irradiated group (92.01+2.99 and 181.13+15.49), respectively, significantly decreased compared with C(-ve) (429.08+42.79 and 306.64+22.9) group, whereas serum (MDA) of the irradiated group (28.97+4.58) significantly increased compared with C(-ve) (20.82+2.77) group. The present treatment overcame these radiation hazards and equilibrated the oxidative stress indicators. That may be due to both banana peel and garlic powder's anti-oxidative effects, as pointed out in the literature [Bertrand *et al.* 2016 and Karabulutoglu *et al.* 2018).

The consequences of adding a lacteal forte to the diet and supplementing it with banana peel and garlic powders on irradiated rats' liver function parameters

ALT and AST present in the serum of the irradiated group (63.80 + 2.77 and 245.2 + 22.52), respectively, significantly increased compared with the C(-ve) (36.00 + 2.00 and 196.80 + 16.65) group. In contrast, all treated groups showed a significant decrease compared with the C(+ve) group. Also, In comparison to the C(-ve) group (6.02 + 0.56 and 3.42 + 0.11, respectively), the total protein and albumin of the C(+ve) group (6.22 + 0.45 and 3.32 + 0.29, respectively) did not differ significantly.

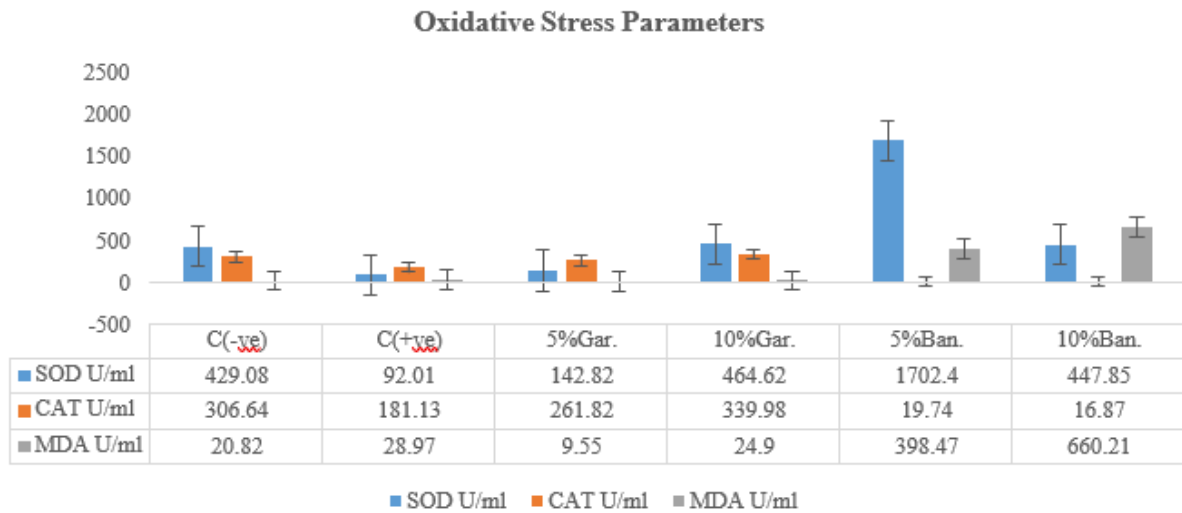


Figure 9: The impact of the entire treatment on oxidative stress parameters

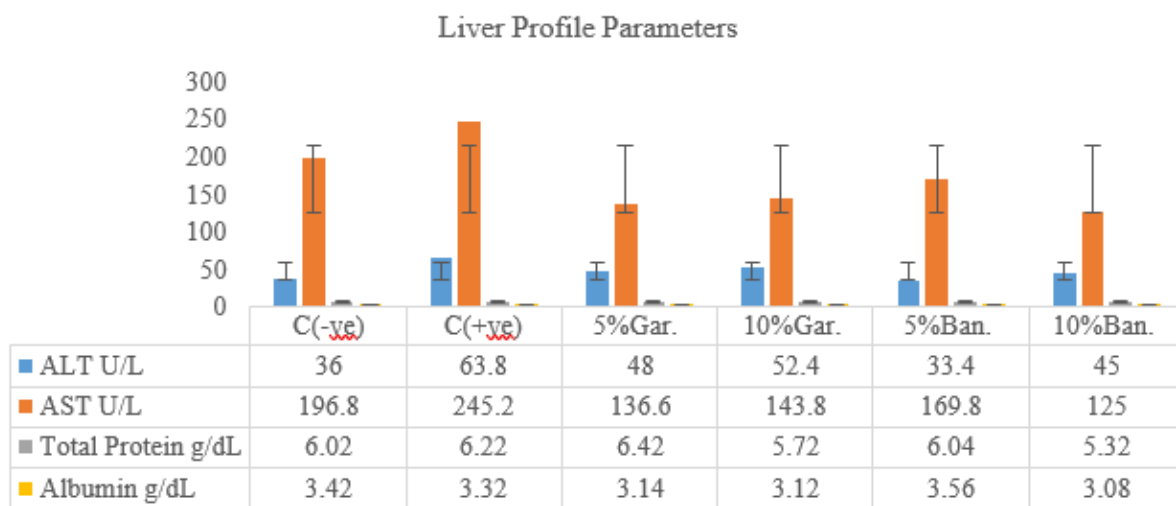


Figure 10: The impact of the entire treatment on live profile parameters

**Hematological assessment of the effect of banana peel and garlic powders and a lacteal forte mixture supplemented diet on irradiated rats**  
 Significant deviation in hematological parameters due to

ionizing radiation exposure, and the disturbance in the blood constituents due to radiation had been overcome with the entire treatment (Kouam *et al.* 2016 and Shahid *et al.* 2015).

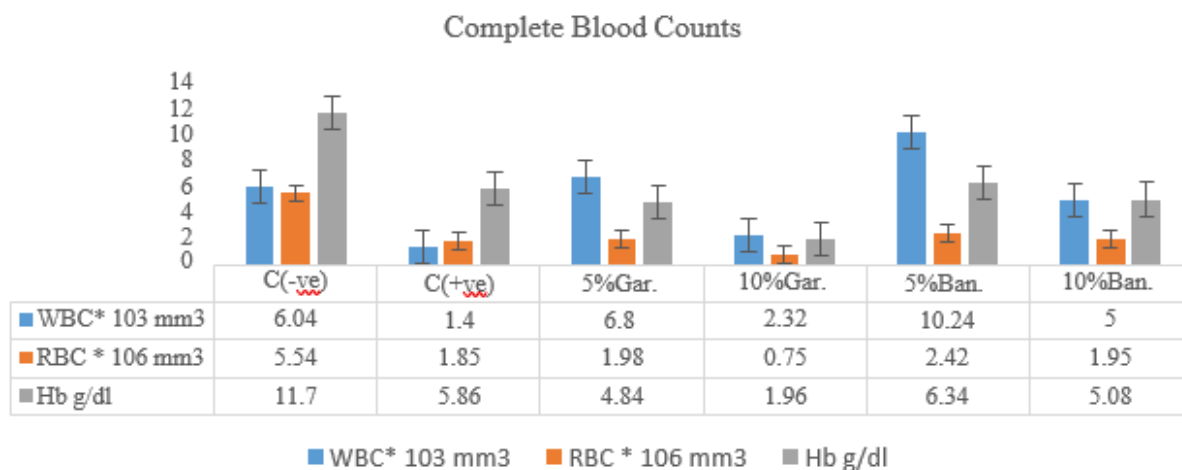


Figure 11: The impact of the entire treatment on complete blood counts

## Discussion

The main composition of garlic was carbohydrates (60.62±0.01), in addition to acceptable amounts of protein, fiber, fat/oil, minerals, and vitamins.

Exposure to 6Gy whole-body gamma radiation resulted in damage to the hematopoietic system characterized by the destruction of hematopoietic cells in the bone marrow, thus reducing the production of blood cells. The blood-forming hematopoietic stem cells were excited due to ionizing radiation exposure. (HSCs) were proliferated and migrated throughout medullary tissues to expand the number of HSCs and increase hematopoiesis. The entire treatment explained how dietary and metabolic factors could alter the risk of leukemia development following ionizing radiation (Nwachukwu *et al.* 2014).

The results of plasma ALT and AST activity indicated that exposure 6Gy radiation significantly ( $P < 0.05$ ) increased plasma ALT and AST activity relative to control group, while feeding with a diet enhanced with synbiotics reversed the radiation effect (Saad 2012).

Serum (SODs) and (CAT) of the irradiated group significantly decreased compared with C(-ve) whereas (MDA) significantly increased, which means that there is great stress in the cells due to radiation exposure. Literature points that garlic had anti-oxidative effect on oxidative stress. Hence, the results of treatment with synbiotics enhanced diet showed significant reduction in the levels of MDA while, significant elevations in SOD, and CAT activities. The best result was due to 10% garlic treatment (Elhadary *et al.* 2019).

These results agreed with the previous studies, the effect of oil from flaxseed (FSO) with (7 Gy) gamma- irradiation only or with carbon tetrachloride (CCl<sub>4</sub>), which induced brains acute neurotoxicity in rats. There were significant elevations in proinflammatory cytokines TNF- $\alpha$  and IL-6 levels (Arreola *et al.* 2015).

Also, radiation exposure caused many changes in several cytokines production. There were noticed elevations in IL6 and TNF- $\alpha$ . The elevations in IL6 and TNF- $\alpha$  could be explained due to LDR's increased capacity of T cells. Garlic affected pro-inflammatory cytokines and a dipocytokines IL-6 and TNF- $\alpha$ , as alliin through suppressing LPS inflammatory signals and generating an anti-inflammatory gene expression also, prevented the increase in expression of proinflammatory cytokines IL-6 and TNF- $\alpha$ . (Najman *et al.* 2022).

The obtained result showed that the stem cells located in the intestinal crypts symptoms due to radiation exposure were triggering apoptosis. The cells shed into the intestinal lumen can no longer be replaced as a result of the stem cells lost, which leads to damage to intestinal villi and weakened function. Also, degradation of the mucosa, and the length of the intestinal villi shortened, deterioration of the brush border, and thus morbidity of the intestinal absorption, all these effects were fulfilled by synbiotics enhanced diets (Najman *et al.* 2022).

## CONCLUSION

Proper food can be an effective treatment for many

diseases, especially in cases of prevention. From here, the aim was to fortify the meals for some patients exposed to ionizing radiation with some components necessary to enhance immunity and overcome some harmful effects. Many anatomical changes appeared in the intestinal tissues of the irradiated rats. These changes include fibrosis and deep damage in the cilia and bumps, as well as permanent damage in the lymphatic layer lining the intestine, which indicates that the immunological system of the irradiated rats urgently damaged. However, these apparent defects began to disappear, and the body started to heal itself anatomically in the treated groups, and the effects were less harmful and the reconstruction process more rewarding in the treated groups, where new cilia began to appear in the lining of the intestine. It could be indicated that the treatment with 5% or 10% garlic or 5% or 10% banana peels and lacteal forte mixtures enhanced the immunity of the irradiated rats, which appeared throughout the biological, hematological, liver profile, and oxidative stress parameters. Finally, we could conclude that the present treatment enhanced the immunity of the irradiated rats and began with intestine repair, immunological parameters, oxidative stress, and other vital parameters.

## List of Abbreviations

CFU Colony Forming Unit (RBCs) red blood cell count (HCT) haematocrit, (Hb) haemoglobin (MCV) mean corpuscular volume, (MCH) mean corpuscular hemoglobin, (MCHC) mean corpuscular hemoglobin concentration (RDW<sub>a</sub>) red blood cell distribution width absolute, (WBCs) white blood cell count, LYM# lymphocytes count MON# monocytes count GAR# granulocytes count (PLI) platelet count and (MPV) mean platelet volume ALT alanine transaminase AST aspartate transaminase TP total protein Alb albumin SODs super oxide dismutase CAT catalase MDA malonaldehyde IL-6 Inter Luckine 6 TNF- $\alpha$  Tumor Necrosis Factor

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