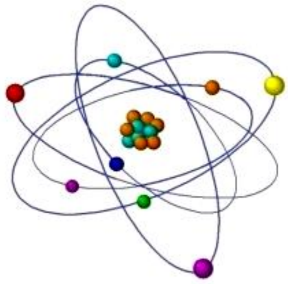
PHYTO THERAPEUTIC TREATMENT OF RADIATION-INDUCED TYPICAL MORPHOLOGICAL CHANGES OF SMALL INTESTINE



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ABSTRACT: *This study aims to identify the morphological changes in the small intestine after exposure to gamma irradiation (5Gy) and reduce the negative impact of irradiation with. Radiation-induced morphological changes of jejunum are thickening and shortening of intestinal microvilli and epithelial shedding. The villi are broken or dissolved to varying degrees, with a loose arrangement between the intestinal villus.The duckweed -Lemna minor has anti-inflammatory and antibacterial activity that reduces the negative impact of gamma irradiation on small intestines.Intake of Lemnan in the early post-irradiation period has a great significanceintheregenerative process of small intestine mucosa and increased survival rate of irradiated animals. Phytotherapeutic substance - Lemna minor. The active component of the duckweed is pectin polysaccharide - Lemnan, which increases the barrier-immune function of the small intestineThe results of the study indicate that Phytotherapeutic treatment on early post-radiation period reduces acutedelayed irradiation effects resulting in increased survival rate of irradiated animals.*

Key words: gamma- radiation, small intestine, pectin polysacarydes, *Lemna minor L.*

INTRODUCTION

Acute Radiation Syndrome is one of the most challenging aspects of human irradiation injury. Organs, tissues and cells are exposed to gamma irradiation causing three main acute radiation syndromes: Hematopoietic syndrome, Gastrointestinal syndrome, Cardiovascular central nervous system syndrome. The gastrointestinal tract is among the most radiosensitive organ systems in the body. According to radiosensitivity they are arranged in the following order: small intestine, salivary glands, stomach, rectum, colon, pancreas andliver [1].

Early radiation enteropathy is aresult of epithelial barrier dysfunction and mucosal inflammation. Delayed radiation enteropathy symptoms occur 3 months or more after irradiation and are characterized by mucosal atrophy, vascular sclerosis, and progressive intestinal wall fibrosis. These symptoms are progressive and mainly characterized by malabsorption of nutrients and abnormal intestinal peristalsis [2]. Cell damage can occur by direct or indirect action of ionizing radiation (IR). The direct effect of IR is the breaking of macromolecules. The indirect effect of IR is revealed in the production of highly reactive free radicals from the radiolysis of water and disfunctionof mitochondria. Under normal conditions, the mitochondria participate in the regulation of the physiological reactive oxygen species that are involved in the essential signaling pathways involved in cellular differentiation and proliferation process. Reactive oxygen and nitrogen species (ROS/RNS) produced after IR interact with biological targets causing damage of DNA, proteins, and lipids. Activation of the transcription factors results in the secretion of proinflammatory cytokines (tumor necrosis factor-α, interleukin-1β, and IL-6), chemokines (IL-8 and monocyte chemoattractant protein), cell adhesion molecules, stress response genes, and cell surface receptors. The target of the inflammatory factors is intestinal mucosa and submucosa; they not only induce tissue injury but also amplify the initial damage caused by gamma irradiation [8,9,10]. The outcome of this process is the development of fibrosis. Increased expression of Interleukin 1 alpha (IL-1α), Transforming growth factor-beta 1 (TGF-β1), and Platelet derived growth factor-AA (PDGF-AA) is in correlation with fibrosis and inflammatory cell infiltrates in the irradiated intestine. The sustained increase in expression of these cytokines from 24 h to 26 weeks after irradiation suggests an ongoing process that is initiated at the time of irradiation [3]. Ionizing Radiation promotes the induction of apoptosis and clonogenic cell death, which leads to mucosal breakdown. It is known that exposure to IR leads to inflammation and injury of the tissues [8,9]. The process of inflammation is amplified by the production of proinflammatory cytokines, chemokines, and growth factors decreasing the immune-barrier function of the intestinal epithelial cells. Translocation of the intestinal microflora in the basement membrane leads to bacterial infection, development of toxicosis, and sepsis [4,5,6,10].

Moreover, vascular injury is one of the most common effects of radiotherapy on normal tissues. Irradiated endothelial cells acquire a proinflammatory, procoagulant, and prothrombotic phenotype. It is suggested that the endothelial lesion occurs before crypt stem cell damage in the evolution of the GI syndrome [7].

MATERIALS AND METHODS

The experimental protocol was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU).

Animal care and maintenance

Three month old male mice (Mus musculus), were obtained from Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 20^0-220C, relative humidity of 50-70% and an airflow rate of 15 exchange/h. Also, a time-controlled system provided 08:00-20:00 h light and 20:00-08:00 h dark cycles. All mice were given standard rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes.

After acclimatization for a week to laboratory conditions, the mice were divided into three different groups. The first control group of three months old mice not irradiated, second group - experimental group of three months old irradiated mice, third group- irradiatedmice receiving lemnan with food. Mice whole-body irradiation with ^{137}Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a “Gamma-capsula-2” (group 2 and 3). The duckweed - Lemna minor L. was used asa herbal supplement.Cultivation of Lemna was performed onsynthetic leachate under controlled conditions using standard methods. After Cultivation biomass was dried at room temperatureand after mechanical fragmentation extracted powder was used in experiment [11,12,13].

Jejunum sections obtained from three groups of mice were fixed in 10% formalin for 48 h and embedded in paraffin blocks. Eight-micrometerthick slides were sectioned using a rotarymicrotome, andstained with hematoxylin and eosin (H&E). Images were captured with digital camera at 4x14 magnifications.

RESULTS AND DISCUSSION

In this study, we used acute gamma irradiation causing the gastrointestinal syndrome. Acute symptoms are developed after radiation exposure resulting in the morphological changes of the intestinal villus. Experimental analysis revealed that compared to bone marrow degenerative and regenerative phases are shorter in small intestines. In the case of damage, the integrity of the epithelial layer must be rapidly restored in order to prevent infections. In mice, this period varies from 24 to 55 hours. Consequently, the aim of our study was to determine morphological changes in the acute post-radiation period (48 hours) and reducing these changes using Lemnan.Morphological changes in jejunum were analyzed using H&E staining. The intestinal villi were arranged clearly and regularly in the control group (Fig.1)

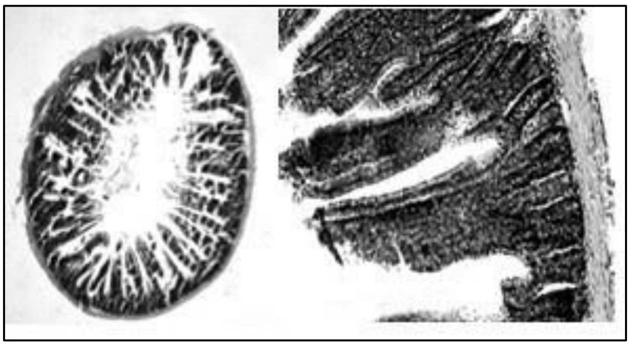

Fig.1 Histological sections of the small intestines of mice (H&E ×14x4)

The microvilli were arranged compactly, and the size of cells was approximately the same, forming an integral tight junction. Meanwhile, after irradiation (5 Gy) a thickening and shortening of intestinal microvilli and villi occurred, as well as a disruption of the tight junction structure. Furthermore, the villi were broken or dissolved to varying degrees, with a loose arrangement between the intestinal villus (Fig2). Epithelial shedding of the villi and disintegration was observed (Fig.3). The third experimental group eating lemnan showed regenerative process in small intestine mucosa after irradiation (Fig.4)

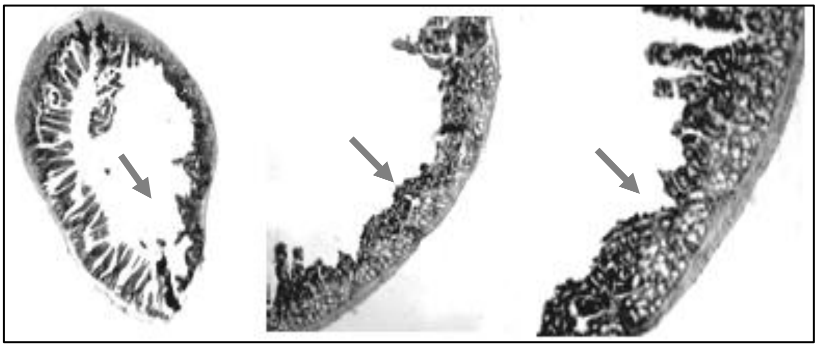

Fig.2 Shortened and irregular villi and breaking, dissolved epithelial cells (arrow**)** H&E-stained tissue sections of jejunum

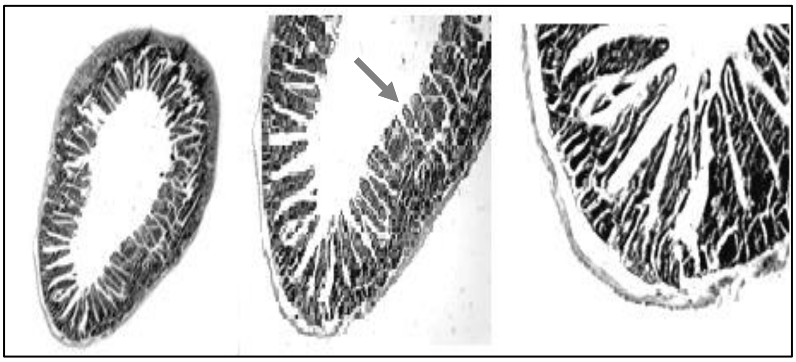

Fig.3 epithelial shedding of the villi and disintegration. H&E-stained tissue sections of jejunum.

Fig. 4 Phytotherapeutic impact on the postradiation regenerative process of tissue
H&E-stained tissue sections of jejunum. (*Arrow, indicates on the regenerative area of mucosa*)

Obtained result of the intestinal regenerative process is determined by the nature of Pectin. The physicochemical properties of pectins give them several advantages in the wound healing process, their hydrophilicity permits removal of exudates, maintenance of an acid pH, which is expected to act as a barrier against bacteria or fungi. Furthermore, pectins have the potential to bind active molecules and protect them from degradation [15,16]. Pectin reduces the secretion of pro-inflammatory factors and immunoglobulins during radiation enteritis [17].

Post-radiation recovery is a multi-component process. The universal parameter of the recovery process is reducing the mortality of irradiated mice. According to the specificity of gastro-intestinal radiation syndrome for evaluation of the positive impact of herbal supplements dose-dependent survival rate of mice was established [Fig.5].

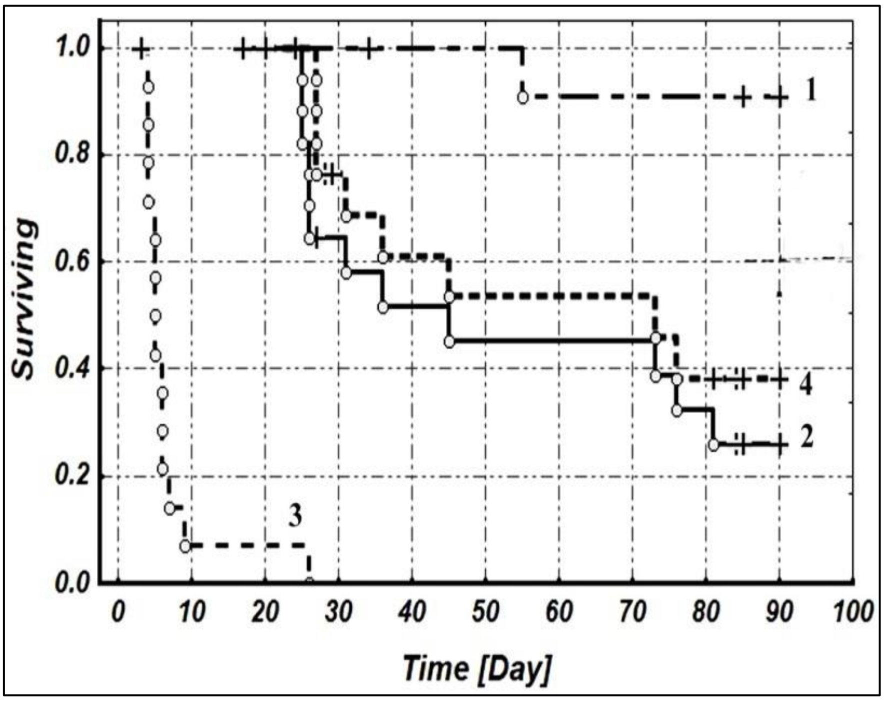

Fig. 5 Impact of Phytotherapeutic treatment on the survival rate of irradiated white mice.

5-Irradiation 3,5Gy, 2-5,0 Gy, 3-6,5, 4-Irradiation+Lemna,
0 -Complete, + - CensoredAcute ionizing radiation
X –Lifespan of the mice Y –Survival rate.Cumulative proportion surviving (Kaplan-Meier)

Threedifferent doses were used for mice irradiation: 3,5 Gy (minimal injury); 5 Gy (medium rate of injury); 6,5 Gy (acute injury). As Fig.5 shows high survival rate after 3,5 Gy irradiation (Fig.5-1). Opposite results are obtained after 6,5 Gy irradiation. Experimental animals mortality. All animals die in 2-3 weeks. (Fig.# 5-3). Consequently, for our using herbal supplement was selected 5 Gy irradiation causing medium rate of injury (Fig.#5-2). Survival rate of third experimental group (IR+Lemna) increased compared to second experimental group of irradiated mice. Survival rate increased with 15±5%(Fig.5-4).

CONCLUSION

Anti-inflammatory and antibacterial properties of Lemna minor reduces typical morphological injury developed after exposure of ionizing radiation. Radiation induced tissue-injury and post-radiation recovery is characterized by diversity of process, it can be considered that activation of recovery process and increasing survival rate of irradiated animals is related to recovery of small intestine barrier-immune function.The results of the study indicate that Phyto therapeutic treatment on early post-radiation period reduces acute delayed irradiation effects resulting in increased survival rate of irradiated animals.

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