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Resveratrol Modulates γ-rays-induced Acute Small Intestinal Injury in Rats

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RESVERATROL is a natural product that exhibits beneficial properties. Intestinal injury is a potential cause of death after high-dose radiation exposure. The study was assumed to determine whether resveratrol could modulate γ-rays-induced acute ileum injury in rats using biochemical and histopathological techniques. Rats were exposed to 8.5Gy-γ-rays and treated with or without resveratrol then, after 7-days post-irradiation, oxidative-stress; malondialdehyde (MDA), total nitrate/nitrite (NO(x)), and myeloperoxidase), antioxidant; superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd), anti-inflammatory markers; tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels, and histopathological study were evaluated in intestinal wall. Data has revealed a significant reduction in radiation-induced lipid-peroxidation (MDA), total NO(x), myeloperoxidase), inflammatory markers (TNF-α and IL-6) and escalation in antioxidant enzymes (SOD, GPx and GRd). In resveratrol and γ-rays treated rat group, obvious modulation in γ-rays-induced histopathological injury into villi and crypts of the ileum mucosa were distinguished. Resveratrol exhibits radio-protective effects against whole-body radiation-induced acute ileum injury in rats, for the first time and thus, could be of clinical value for patients undergoing radiotherapy.

Keywords: Resveratrol, ileum, radio-protector, γ-rays, rats

Introduction

Radiation-induced gastrointestinal injury results from the acute damage of intestinal stem cells reduced epithelial regeneration and consequent impairment of the mucosal barrier, resulting in electrolyte disturbance, diarrhea, weight loss, sepsis and death (Livanova et al., 2023). Intestinal injury is a limiting factor for ultimate chemo-radiation therapy of abdominal malignancies such as gastric, pancreatic and colorectal cancer (Kullenberg et al., 2023). The prime radiation-injury target is small intestine (Liu et al., 2022). Furthermore, radiation-induced gastrointestinal disorder limits the survival of injured persons in a mass casualty setting from nuclear accidents or threats (Yang et al., 2023).

Natural foodstuffs have been recognized for years to have active pharmacological reactions and have been a preliminary rule for several remedies which are presently on the marketplace. Resveratrol is a natural product generated in plants in response to environmental stress and growing conditions (Hibi, 2023). It is mainly produced in red grape and berries, exhibits anti-oxidative, anti-inflammatory, anti-proliferative, and anti-cancer properties. It is now being increasingly used in cosmetology and dermatology (Chen et al., 2023; Hai et al., 2023; Zhao et al., 2022). Owing to the strong antioxidant and anti-inflammatory properties, search on natural resources has become one of the most encouraging subjects to hostage the deleterious effects of radiation (Sun & Shahrajabian, 2023). Besides, several herbs valued to reduce radiation-induced intestinal

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injuries, the quercetin treatment was found to instruct significant benefits against radiation-induced intestinal injury in rats (Zhu et al., 2023). The protection of intestinal epithelial cells from the lethal effects induced by ionizing-radiation is an important topic in radiotherapy and in dealing with acute radiation pattern. However, the influences of middle- and low-dose radiation on intestinal epithelial cells remain unclear (Otsuka & Suzuki, 2016; Guo et al., 2023). Currently, there is no accepted protective treatment to mitigate radiation-induced intestine toxicity. The current study discusses if resveratrol as a polyphenol protective agent, could be modulate whole body γ-rays-induced acute ileum injury and promotes ileum epithelial repair in rats.

Materials and methods

Animals

40 adult male Swiss albino rats (220-250g) were obtained from the Egyptian organization for biological product and vaccines Giza, Egypt. The animals were fed normal nourishment and water ad-libitum and kept under regular conditions of humidity, temperature (20-24°C), 12-hours light, and 12-hours dark cycle. The animals were deprived of nutrition, overnight before samples collection. All experiments were conformed to the procedure and guides for the care and use of laboratory animals published earlier, National Institutes of Health, No. 85–23, revised 1996.

Radiation processing

It was performed by using gamma cell-40 (caesium-137) located at NCRRT, Cairo, Egypt. The animals were irradiated with a single dose level of 8.5Gy γ-rays, delivered at a dose rate of 0.38Gy/ minute at the time of experimentation.

Chemicals

Resveratrol was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals and solvents used were of the highest purity grade available.

Experimental design

Animal grouping

Forty rats were divided into four groups (n = 10). Control group, rats were administered normal saline (2ml/rat) orally by gastric tube for 7 days as a vehicle. Resveratrol group (each rat received 10mg/kg body weight orally, suspended in 2ml saline, once daily for 7 days) according to **Berbée** et al. (2011). **γ**-rays group; animals received the vehicle (2ml normal saline/rat) with the same dose and time; then subjected to a single dose of the whole body γ-rays (8.5Gy) and resveratrol & γ-rays group (each rat received resveratrol with the same dose and time, then one hour later after the last dose, rats were exposed to γ-rays (8.5Gy). The animals were decapitated 7 days after the end of the experiment.

The blood samples were collected from the rat heart's puncture. The serum samples were separated to determine TNF-α and IL-6. In an ice-cold 50mM phosphate buffer, pH 7.2, the ileum samples homogenate (10% w/v) was divided into 2 parts. One part was centrifuged at 3000 xg for 5 minutes. The supernatant was used for evaluating MDA, NO(x), myeloperoxidase, TNF-α and IL-6. The second part was centrifuged at 10000 xg for 10 minutes. The supernatant was used for evaluating SOD, GPx and GRd.

In ileum homogenates: Hillegas et al. (1990) method was used to measure myeloperoxidase. The glutathione reductase (GRd) activity was measured using Biodiagnostic (GR 25 11), Egypt, according to the manufacturer's instructions. Detection of total nitrate/nitrite (NO(x)) was performed by colourimetric technique according to Miranda et al. (2001) method. Estimation of malondialdehyde (MDA) level, and the relative enzyme activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured using Ohkawa et al. (1979), Kakkar et al. (1984) and Lawrence & Burk (2012) methods, respectively.

In serum: Detection of tumour necrosis factor-α (TNF-α, Catalogue No. CSB-E11987r) and interleukin-6 (IL-6, Catalogue No. CSB-E04640r) were performed by ELISA-technique commercially Kits-(CUSABIO Technology Biotech Co., China) according to the manufacturer's instructions.

Histopathological analysis

The ileum tissue samples were fixed in 10% normal formalin reagent. After 72 hours, tissues were dehydrated through a series of graded alcohol, embedded in paraffin, and cut into 4-micron sections and stained with haematoxylin and eosin (H&E) according to Suvarna et al. (2013) and examined microscopically.

Statistical analysis

The one-way analysis of variance (ANOVA) technique was used for analyses then, all data was tracked by LSD post hoc examination. The results obtained were expressed by mean ± standard deviation. Differences were considered significant at $p \leq 0.05$ (Snecdecor & Cochran, 1989).

Results

As presented in Table 1-3, the animal group treated with resveratrol showed non-significant changes in the levels of all estimated biochemical parameters of the current study.

In the γ-rays group, regarding the markers of ileum tissue injury, there were significant rises in MDA and NO(x) levels and in myeloperoxidase activity in irradiated rat groups as compared to controls and resveratrol groups.

In resveratrol & γ-rays group, the resveratrol treatment; 10 mg/kg before irradiation exerted statistical reduction on these markers (MDA, NO(x) and myeloperoxidase), when compared to the irradiated group, but their levels still significantly changed compared to the control and resveratrol rat groups, Table (1).

Legends as in Table (1).

In Table (2), In the γ-rays group; the markers of inflammation, TNF-α and IL-6 in serum, were elevated following γ-rays-irradiation experiment as compared to the control and resveratrol rat groups. In the resveratrol & γ-rays group, the pre-treatment with resveratrol produced significant reductions in their levels when compared to the irradiated group ($p \leq 0.05$), but their levels still significantly changed compared to the control and resveratrol rat groups.

TABLE 1. The markers of ileum-tissues oxidative stress levels of MDA, NO(x) and myeloperoxidase of different animal groups

Groups	Control	Resveratrol	γ-rays	Resveratrol & γ-rays
MDA (nmol/ g protein)	65.13± 6.31	64.14± 6.23	98.12± 9.02[a,b]	76.14± 7.31[a,b,c]
NO(x) (µmol/ mg protein)	10.06± 1.11	10.18± 1.18	19.50± 2.06[a,b]	14.28± 1.46[a,b,c]
Myelo-peroxidase (µU/ g tissue)	286± 26.14	277.87± 25.12	450.13± 46.16[a,b]	314.23± 29.33[a,b,c]

[a]Significant difference from the control group
bSignificant difference from the resveratrol group
cSignificant difference from the γ-rays group

TABLE 2. The inflammatory marker levels of TNF-α and IL-6 in the serum of different animal groups

Groups	Control	Resveratrol	γ-rays	Resveratrol & γ-rays
TNF-α (pg/ mL)	19.84± 2.42	19.54± 1.72	41.26± 5.01[a,b]	26.72± 2.34[a,b,c]
IL-6 (pg/ mL)	50.23± 4.32	48.95± 5.13	150.54± 16.16[a,b]	80.22± 8.24[a,b,c]

Legends as in Table (1).

TABLE 3. The anti-oxidant marker enzymes levels of SOD, GPx and GRd in the ileum-tissues of different animal groups

Groups	Control	Resveratrol	γ-rays	Resveratrol & γ-rays
SOD (U/ mg protein)	10.24± 4.13	10.34± 4.18	5.59± 3.26[a,b]	8.45± 5.01[a,b,c]
GPx (nmol/ mg protein)	140.47± 13.17	137.22± 13.15	77.16± 8.07[a,b]	112.14± 10.11[a,b,c]
GRd (nmol/ mg protein)	9.17± 3.17	10.01± 3.15	5.16± 2.07[a,b]	7.04± 2.31[a,b,c]

Legends as in Table 1.

Table (3) shows in the γ-rays group, a significant reduction in antioxidant intestine marker enzymes; SOD and GPx and DRd activity in ileum tissues in irradiated groups as compared to control and resveratrol values. In the resveratrol & γ-rays group, the pre-treatment with resveratrol developed a significant rise in their levels when compared to the irradiated group, but their levels still significantly changed compared to the control and resveratrol rat groups, Table (3).

Histopathological results

In the control group, ileum sections showed all layers; mucosa, submucosa, muscularis externa and serosa. The ileum villi appeared irregular. The Payer 's patches showed aggregation of the lymphatic nodules that is a characteristic feature of normal ileum structure (Fig. 1: A). Moreover, in the resveratrol group, a normal structure appears as in the control group (Fig. 1: B). In the γ-rays group, the ileum showed a marked enteritis represented by congested mucosal and sub mucosal blood vessels, villous tips loss (denudation), mucosa layer necrosis and inflammatory cell invasions in the submucosal layer with or without degenerative changes and expanded crypts in ileum mucosa (Fig. 2: A-D). In the resveratrol & γ-rays group, the ileum showed more significant stable tissue structures than in the γ-rays group. In some cases, showing moderate sloughing intestinal villi (Fig. 3: A, B).

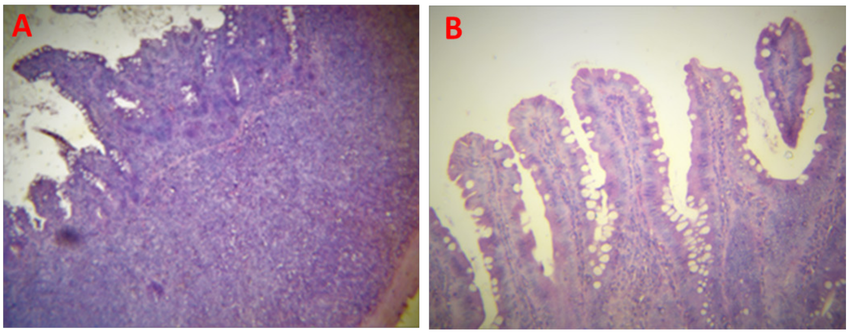

Fig (1. A). Control group section showing normal structure (H& E ×100), and B) Resveratrol group section showing normal ileum villi (H& E × 200).

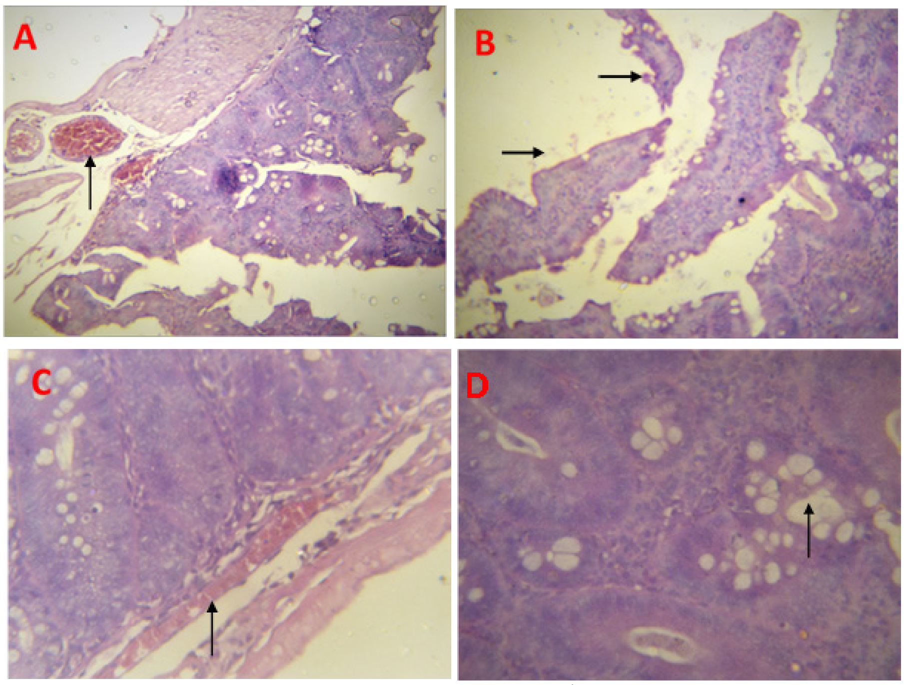

Fig. 2. In the γ-rays group, A) Congested sub mucosal blood vessels (↑) with villi denudation (H&E ×200), B) Sloughing mucosal villi and denudation (→) (H&E ×200), C) Congested sub mucosal blood vessels (↑), oedema increasing leukocytes around ileum crypts (H&E ×200), and D) Leukocytic infiltration around expanded and degenerated crypts (↑) (H&E ×400).

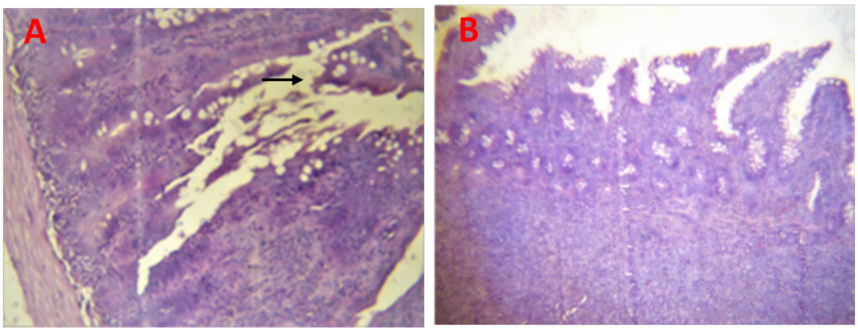

Fig. 3. In resveratrol & γ-rays group, A) Moderate denudation of villi (→), and B) Normal villi and normal crypts.

Discussion

Radiation induced gastrointestinal injury, resultes in impairing regeneration of epithelial wall (Meena et al., 2022). The injured epithelial cells activate the general invasion of bacterial pathogens causing death. These lethal gastrointestinal symptoms after radiation exposure are clinically known as radiation enteritis (Korany et al., 2022). So, there is a remarkable need for therapeutic measures after the radiation episode (Yang et al. 2022).

Oxidative-stress is an important aspect involved in the mechanism of radiation-induced tissue injury. The antioxidants are important concerns for the development of preventive therapeutics (Ahmed et al., 2023). Antioxidants have been used to suppress oxidative stress and protection of various organs from acute radiation injury in rats and mice (Ji et al., 2023; Motallebzadeh et al., 2023). Radiation damage is caused by the overproduction of ROS, which devastates the levels of antioxidants, resulting in oxidative stress that damages cellular membranes, leading to lipid peroxidation (Sun et al., 2022).

The current study revealed an increased level of MDA, NO(x) and myeloperoxidase in ileum tissues, and an increased level of TNF-α, and IL-6 in blood serum of the γ-rays group. Additionally, there was a decreased activity of SOD, GPx, and GRd in ileum tissues of the γ-rays group. All these indices were modulated in the resveratrol & γ-rays group.

A significant increase in lipid peroxidation, measured as MDA and NO(x) were detected post whole-body gamma-irradiation in the Paneth cells that are abundant in the tissue of the ileum region in rats (Eltahawy et al., 2017). Administration of resveratrol completely reversed this oxidative stress induced by radiation in ileum tissue (Qin et al., 2021), suggesting that it could protect its cellular membrane from radiation-induced lipid peroxidation and oxidative stress.

Myeloperoxidase activity is an indicator of quantitative inflammation and the infiltration of the mucosa of the ileum with leukocytes (Arciniega-Martínez et al., 2022). In a recent study, the intestinal injury was estimated by an uprise in myeloperoxidase enzyme activity in ileum tissue showing the intestinal injury in irradiated rat group (Harata-Lee et al., 2022). The current study, resveratrol pre-treatment significantly ameliorates ileum γ-rays injury, made evident by a decrease in myeloperoxidase activity. Also, Borges et al. (2018) found that resveratrol pre-treatment significantly combats ileum injury caused by ischemia and reperfusion injury in the rat model due to its effectiveness in increasing the durability and bioavailability in the rat's ileum-epithelium.

Gamma-rays-induced intestinal toxicity achieved a reduction in cellular antioxidant GPx, GRd and SOD enzymes activity (Fernández-Gil et al., 2017; Gagnaire et al., 2019). In a recent study, ileum tissues attained decreased in GPx, GRd and SOD activity, that is indicative of cellular oxidative stress and intestine dysfunction leads to enhanced production of ROS and reactive nitrogen species in rat's ileum (Banerjee et al., 2018). Resveratrol pre-treatment significantly reversed all these antioxidant indices as a pre-treatment for intestinal injury in irradiated rats and mice (Radwan & Karam, 2020; Farhood et al., 2020).

In rats, irradiation induced escalation of serum TNF-α and IL-6 levels contributes to DNA damage and cell death (Sayed et al., 2023). In the current study, this rise in serum TNF-α and IL-6 level was significantly obstructed by resveratrol pre-treatment when compared to the equivalent gamma-irradiated rats' group. Suppression of TNF-α and IL-6 levels clearly indicate the anti-inflammatory effect of resveratrol during the course of intestinal inflammation (Yu et al., 2023).

In the small intestine, whole-body irradiation of rats induced epithelial-cell destruction which contributes to the reduction of the lining epithelial cells regeneration, structural alterations of the villi, vascular injury and mucosal superficial damage (Carabajal et al., 2012). Radiation doses more than 8Gy γ-ray-induce cell cycle arrest and apoptosis of the crypt of intestinal epithelial cells within day 1 post-radiation, resulting in a decrease in regenerating crypt colonies by day 3 and finally villi denudation by day 7 post-radiation exposure (Bhanja et al., 2018).

In the present study, the animals were killed after 7 post-radiation exposures to 8.5 Gy γ-rays, and ileums were collected for examination. Histopathological analysis of rats' ileum clearly demonstrated a loss of crypts and leukocytes in the lamina propria with a significant denudation of villus length, indicating that γ-rays-induced ileum injury that is the primary cause of small intestinal toxicity. The results in the current study are in accordance with the literature, as the acute enteritis is observed in the ileum portions of the intestine of the irradiated mice (Jeena et al., 2016).

The small intestinal mucosal epithelium is one of the highest radio-sensitive tissues following contact to sub lethal doses of gamma radioactivity (Zhao et al., 2023). Irradiation pointedly disturbs the villi of the ileum in rats (Cromer and Zawieja, 2018).

Many drugs are being tested as having radio-protective effects against radiation induced acute ileum injury, such as melatonin, metformin, lycopene with promising results (Najafi et al., 2020; Sadic et al., 2017; Sezen et al., 2021). Rats receiving resveratrol pre-treatment demonstrated normal crypt-villus structure with an increase in the number of crypts and preserved villous length and the mucosal and submucosal layers were found relatively intact, compared to the irradiated group. Although, villi were seen desquamated, erosion or reduced in size (Zhang et al., 2017), rats treated with resveratrol only induce minute changes in crypt-villus morphology compared with the γ-rays group.

Conclusion

Based on the existing findings, the use of resveratrol is proposed as a safe and effective radio protective strategy to restore small-intestinal tract injuries induced by γ-rays exposure and induced epithelial repair.

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