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## The Protective Role of Red Beetroot (*Beta Vulgaris* L.) Peel Extract against Gamma Irradiation Induced Hepatic Apoptosis in Rats

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### ABSTRACT

Exposure to gamma radiation causes oxidative stress and generation of reactive oxygen species, which cause hepatocellular apoptosis, so it is necessary to keep safe the living organisms especially humans and animals. Red beetroot (*Beta vulgaris* L.) is thought to be crucial for enhancing hepatic health. Male Wistar albino rats were designated to evaluate the possible protective role of red beetroot peel extract (RBPE) to alleviate oxidative stress that associated with gamma-ray exposure. Biochemical, histopathology, and immunohistochemical analyses of rat livers exposed to acute radiation dose revealed different apoptotic patterns. According to the findings, RBPE has a DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity of  $1.22 \pm 0.23$  mg TE/g. The total phenolic and flavonoid contents of RBPE were  $126.21 \pm 0.39$  mg GE/g and  $67.18 \pm 0.78$  mg QE/g, respectively. Results showed that pre-administration of RBPE (50 mg/kg body weight for two weeks before and after exposure to 6 Gy gamma irradiation reduce elevations in liver enzymes and apoptotic markers [caspase-3, and B-cell lymphoma-2-associated protein X (Bax) and elevate the expression of the B-cell lymphoma-2 (Bcl-2). These findings were supported by the histological analyses, which demonstrated that RBPE shielded the rats' livers against gamma-irradiation-induced damage. The protective effects are most likely due to the potent antioxidant phenols in RBPE that working together synergistically. It is concluded that RBPE is a promising radioprotector for liver against oxidative stress induced by exposure to gamma ionizing radiation due to its antioxidant and anti-apoptotic power.

### INTRODUCTION

The human irradiation load has increased as a result of scientific and technological advancements, as exposure to low-dose radiation has become commonplace during medical diagnosis, use of some electronic devices, and cosmic radiation exposure from space and terrestrial radiation from earth. Ionizing irradiation (IR) can alter the liver's structures and functions [1]. Hepatic nonparenchymal cells are known to be radiosensitive including Kupffer cells (KCs), stellate cells (SCs), and sinusoidal endothelial cells (SECs). When these cells exposed to radiation, they discharge a variety of chemicals that cause liver fibrosis, which contributes to the distortion of structure and function of the liver [1-4]. Hepatocytes undergo apoptosis as a response to liver damage, according to compelling data [5, 6]. Caspases, a group of cysteine proteases, are activated during apoptosis, and a complex series of

processes link the initial stimulus with the cell's eventual death. The most crucial is Caspase-3 (Casp-3), which is in charge of DNA fragmentation and chromatin condensation [7]. Bcl-2 is an essential survival enhancer protein that regulates intrinsic apoptotic signaling, whereas BAX is known as the antithesis of Bcl-2 [8, 9].

Administration of synthetic chemical radio protective agents may aid in DNA repair, lessen inflammation long term of radiation-induced oxidative stress, and promote the death of damaged cells. Its value as a radioprotective substance can cause side effects like cephalalgia, nausea, sickness, vomiting, changes in blood pressure, and both local and generalized cutaneous reactions [10, 11]. As a result, there is a growing need for a natural radioprotective and active drug to safeguard not only the general public from the health risks of unintentional ionizing radiation exposures, but also high-risk groups

like those who handle harmful radioactive materials, and apparatus [12].

Hence, search for an ideal and natural radioprotector without side effects is a compelling urgency. Dietary phytochemicals have recently attracted interest due to their potential health benefits. Natural pigments, such as red beetroot (*Beta vulgaris* L.), have moreover gotten a lot of attention because of their antioxidant capabilities [13], which can help to prevent disease and improve human health [14]. The red beetroot (RB), known as shamandar in Egypt, is a vegetable plant which belongs to Amaranthaceae family. The RB is frequently consumed as food and employed as a natural food colorant and traditional Arab medicine, where it has been used for a long time to treat a wide range of illnesses. The therapeutic benefits of RB are said to include its anticancer, antimicrobial abilities [15], emmenagogue, carminative, hemostatic, renal protective and also has a potent herb properties used in cardiovascular conditions [16]. Further uses for RB juice include the natural treatment of sexual weakness and the removal of kidney and bladder stones. RB has grown in popularity as a "superfood" in recent years: a naturally occurring food item rich in nutrients that is used to increase athletes' energy [17, 18]. It is especially rich in sucrose but with moderate caloric value.

Based on information provided by the United States Department of Agriculture [19], 100 g of raw RB contains an average amount of energy of 43 kcal, 2.8 g of total dietary fiber, 1.61 g of total proteins, 6.76 g of total sugars, and 0.17 g of total lipids [20]. It also contains micronutrients as vitamins (C, B1, B2, B3, B6) [15, 21], and minerals as (calcium, potassium, magnesium, iron, selenium, and zinc). In addition, it contains phenolic and carotenoid compounds [15]. RB contains both yellow (betaxanthins) and red (betacyanins) pigments, which are collectively known as betalains [20] and are one of the most readily available antioxidants [22]. Betalains have the potential to be potent radical scavengers and therefore advantageous oxidative stress development inhibitors [23]. Analysis of 80% aqueous methanol of RB peel extract (RBPE) revealed the existence of glucoside, indicaxanthin, vulgaxanthin (I & II), betanin (isobetanin, neobetainin, prebetainin), p-coumaric acid, L-tryptophan, ferulic acid, and betalamic acid [24].

Based on the above literature, RBPE is considered as antioxidant and anti-inflammatory. The current study aimed to estimate the possible shielding effect of RBPE

against gamma ( $\gamma$ ) ray exposure-induced oxidative stress in the livers of male rats.

## MATERIALS AND METHODS

### Chemicals

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu's reagent and aluminum chloride ( $AlCl_3$ ) were bought from Fluka (Switzerland); trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), methanol, gallic acid (3,4,5-trihydroxybenzoic acid), and quercetin [2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one] were from Sigma Chemical Co. (USA).

### Plant Extraction

Commercial RB were obtained, washed, and cut their peels manually into small sliced, lyophilized and grinded in a mortar. 500 mg of lyophilized RB were homogenized for one minute in 10 ml of 80% aqueous methanol. According to the procedures outlined by Kujala *et al.* [24], the sample was then centrifuged (3000 rpm) for 10 min, and the clear supernatant was collected and dried using a rotary evaporator RE200 (BIBBY STERILIN LTD. U.K.).

### Determination of Antioxidant Capacity

To assess the scavenging potential of RBPE, the DPPH experiment was carried out [25]. The results of the DPPH assay were expressed as RSC% (radical scavenging activity). 4 ml of DPPH solution (0.1 mM DPPH in ethanol) was employed as a control sample. 0.2 ml of the test sample was added to 3.8 ml of the DPPH solution, the mixture were incubated at 25 °C for 30 minutes in the dark, the absorption of samples were measured using a UV spectrophotometer at  $\lambda$  517 nm. The following equation was used to calculate the RSC%

$$RSC\% = \frac{A_0 - A_1}{A_0} \times 100$$

Since A1 represents the absorbance of the sample, and A0 represents the absorbance of the control. The standard curve was created using trolox. The results were expressed in milligram of trolox equivalents (TE) per gram of beetroot extract.

### Measurement of Phenolic Content

The Folin-Ciocalteu reagent was used to determine the total phenolic and polyphenolic antioxidants content of RB extracts [26]. The absorbance was measured at  $\lambda$  750 nm with a "Umicum UV-300" Spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (GE) per gram of beetroot extract.

### Measurement of Total Flavonoid (TF) Content

A mixture of 1 ml of RBPE, 4 ml of distilled H<sub>2</sub>O, and 0.3 ml of 5 percent NaNO<sub>2</sub> was prepared then incubated at 25 °C for five min. 10% AlCl<sub>3</sub> solution was added carefully after the incubation. 2 ml of 1 M NaOH was added then formulated the mixture up to 10 ml with distilled water at the sixth minute of the incubation, and thoroughly mix it [27]. The absorbance was measured with a "Unicum UV-300" Spectrophotometer. Results were expressed in mg quercetin equivalents (QE) per gram of beetroot extract.

### γ Rays Irradiation

At the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), a cell-40 irradiation unit was supported by a  $\gamma$ -cesium-137 radiation (Atomic Energy of Canada Limited; Sheridan Science and Technology Park, Mississauga, Ontario, Canada). The male rats were exposed to an acute 6 Gray (Gy) dose  $\gamma$  ray at a rate of 0.41 Gy/min.

### Experimental Design

Healthy thirty-two adult Wistar albino male rats (weighing 150–160 g) were obtained from the animal facility of the Biological Applications Department, Nuclear Research Center (NRC), EAEA. Rats were housed in polypropylene cages at the animal house of the Radioisotopes Department, NRC, EAEA, for two weeks before starting the experiment. They acclimatized at a humidity of 60 ± 5%, a temperature of 25 ± 5°C, and a 12/12 hours cycle of light and darkness. Throughout the experiment, the rats were given unlimited access to fresh water and a regular pellet meal. The ethical standards for the care of research laboratory animals were followed in the handling and treatment of every rat. Rats were divided into four groups randomly (eight rats per group). G1 (control): normal rats were injected intraperitoneally (IP) with saline (1 ml/kg b wt) three days/week for four weeks. G2 (RBPE): normal rats were injected IP with RBPE at a dose of 50 mg/kg b.wt according to Al-Balawi *et al.* [28], three days/week for four weeks. G3 (IR): rats were whole body irradiated with acute dose of 6 Gy of  $\gamma$  rays. G4 (RBPE+IR): rats were injected IP with RBPE at a dose of 50 mg/kg b.wt for two weeks before irradiation with acute dose of 6 Gy of  $\gamma$  rays. 24 h post irradiation rats were continued to intake RBPE for more two weeks.

### Ethical Approval

All procedures involving animals were in compliance with the CIOMS and ICLAS International Guiding Principles for Biomedical Research Involving Animals 2012. The Egyptian Atomic Energy Authority's National Center for Radiation and Technology's Research Ethics Committee revised and approved the protocol (REC-NCRRT-26A/19), approval valid from 19/11/2019.

### Samples Preparation

Samples were collected from all groups twenty-four hours after the final dose of RBPE injection. Rats were anaesthetized and slaughtered to collect blood and organ samples. Blood samples were centrifuged to separate sera. Liver samples were dissected out and stored in 10% neutral buffered formalin for histological analysis. A series of (methyl, ethyl and absolute ethyl) alcohol dilutions were used to dehydrate the liver tissue after washing with tap water. Samples were cleaned in xylene and then heated to 56 °C in a hot air oven for 24 hours before being embedded in blocks of paraffin-beeswax. Wax blocks were sliced using sledge microtomes into a thickness of 4 to 6 microns. On glass slides, the tissue sections were assembled, deparaffinized, and stained with eosin and hematoxylin [29].

### Histopathological Alterations

The incidence and severity of liver lesions were graded semi-quantitatively, as described by Duman *et al.* [30], where grade 0: evident no injury, grade I: evident infiltration of pigmented hepatocyte cells, grade II: evident hepatocyte degeneration, and grade III: evident inflammatory cell infiltration.

### Liver Function

The commercial assay kits from Vitro Scientific Co. (Cairo, Egypt), were used to evaluate the serum activity of the alanine aminotransferase (ALT) [31], aspartate aminotransferase (AST) enzymes [32], as well as total protein [33] and albumin [34].

### Apoptotic Marker

#### Enzyme-Linked Immunosorbent Assay (ELISA)

Rat Casp-3 ELISA Kit was used to estimate the Casp-3 activity in serum (Cat. No. CSB-E08857r from CUSABIO, Wuhan, China).

### Immunohistochemistry

Bcl-2 (anti-apoptotic markers) and Bax (pro-apoptotic markers) were detected in liver sections. Sections were incubated with polyclonal antibody of Bcl-2 (catalog number, PA1-30411; Bcl-2, Thermo Fisher Scientific) and monoclonal antibody of Bax (catalog number, MA5-14003; Bax, Thermo Fisher Scientific), according to Petrosyan *et al.* [35].

### Statistical Analysis

Sera results are represented as mean values  $\pm$  standard error. A parametric test was used to determine whether the data had a normal distribution, and after that, various assessments were statistically analyzed using one-way ANOVA tests, followed by Tukey's HSD multiple comparisons as a post-hoc test, to identify the significant differences between the various groups.  $p < 0.05$  were chosen as statistically significant. The software SPSS statistical version 20 (SPSS® Inc., USA) was used for all statistical evaluates

## RESULTS

### In Vitro Finding

The current study investigated the protective effects of RBPE administration against the oxidative stress effects of IR exposure by using biochemical analyses, immunohistochemical assays, and histopathological

investigations. According to the findings, RBPE has a DPPH scavenging activity of  $1.22 \pm 0.23$  mg TE/g, total phenolic content  $126.21 \pm 0.39$  mg GE/g and flavonoids  $67.18 \pm 0.78$  mg QE/g.

### In Vivo Finding

Table (1) shows that irradiation elevates significantly the level of AST, ALT and Casp-3 and lowers significantly both total protein and albumin compared to control. The group that has been administered RBPE with irradiation confirmed a significant improvement in the levels of all parameters except albumin was nonsignificant.

There is a substantial difference between the four groups in terms of the pro- and anti-apoptotic markers expression in liver sections (Bax and Bcl-2). BAX is significantly expressed in the IR group, as demonstrated by the severe apoptotic changes in comparison to the lack of changes in the other groups. Bcl-2 is significantly expressed in the control and RBPE groups, as demonstrated by the moderate apoptotic changes in comparison to the IR group (Table 2). The degree of immunological activity is determined by the density and dispersion of the brown to black color. The immunoreactivity is localized intracellular in the damaged and apoptotic cells, as shown in figs. 1 and 2.

**Table (1): Shows  $\bar{x} \pm SE$  values of liver functions and Casp3 in different groups, ANOVA test and post hoc Tukey's HSD multiple comparisons**

Parameters	control (N=8)	RBPE (N=8)	IR (N=8)	RBPE+IR (N=8)	ANOVA
AST (U/L)	84.87 $\pm$ 6.70 <sup>a</sup>	89.25 $\pm$ 6.01 <sup>a</sup>	208.12 $\pm$ 14.12 <sup>c</sup>	160.0 $\pm$ 22.64 <sup>b</sup>	*
ALT (U/L)	42.0 $\pm$ 6.59 <sup>a</sup>	42.37 $\pm$ 5.70 <sup>a</sup>	96.0 $\pm$ 9.10 <sup>c</sup>	63.87 $\pm$ 5.35 <sup>b</sup>	*
Total protein (g/dL)	5.41 $\pm$ 0.22 <sup>c</sup>	5.30 $\pm$ 0.20 <sup>b,c</sup>	3.56 $\pm$ 0.18 <sup>a</sup>	5.11 $\pm$ 0.16 <sup>b</sup>	*
Albumin (g/dL)	3.90 $\pm$ 0.11 <sup>b</sup>	4.30 $\pm$ 0.98 <sup>b</sup>	2.57 $\pm$ 0.21 <sup>a</sup>	3.02 $\pm$ 0.13 <sup>a</sup>	*
Casp-3 (ng/ml)	1.26 $\pm$ 0.09 <sup>a</sup>	1.10 $\pm$ 0.04 <sup>a</sup>	5.73 $\pm$ 0.63 <sup>c</sup>	2.25 $\pm$ 0.15 <sup>b</sup>	*

Since ( $\bar{x} \pm SE$ ) are the mean and standard error, N is the number of samples; \* indicate significant ANOVA for the same parameter at  $P < 0.05$ , Different letters (a, b and c) indicate significant difference of post hoc Tukey's HSD multiple comparisons at  $P < 0.05$

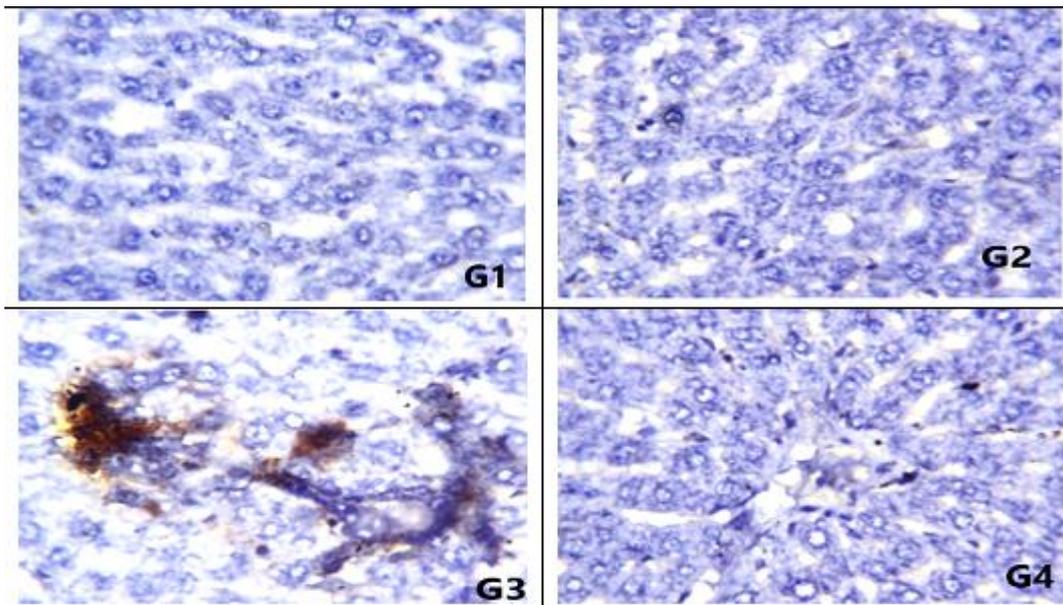
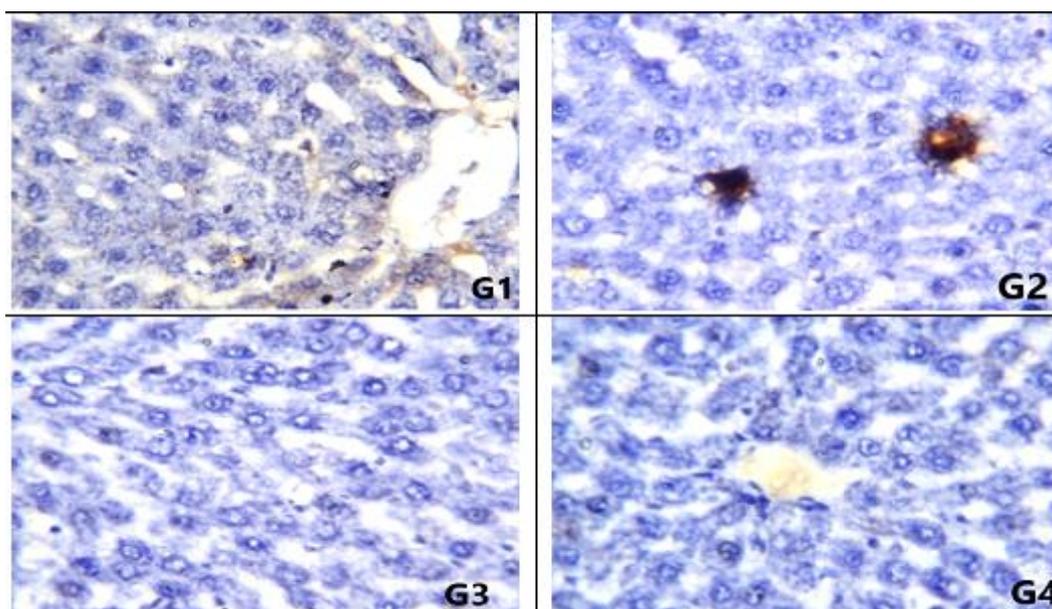
**Table (2): The severity of immunohistochemical alterations in the liver groups in terms of the pro- and anti-apoptotic markers (Bax and Bcl-2)**

marker	group			
	G1	G2	G3	G4
<b>Bax (Fig. 1)</b>	<b>0</b>	<b>0</b>	<b>III</b>	<b>0</b>
<b>Bcl-2 (Fig. 2)</b>	<b>II</b>	<b>II</b>	<b>0</b>	<b>I</b>

B-cell lymphoma-2-associated protein X (Bax)

B-cell lymphoma-2 (Bcl-2)

Nil (0) mild (I) moderate (II) sever (III)

**Fig. (1): The immunohistochemical alterations of Bax in the liver sections: (G1) control group, (G2) RBPE group, (G3) IR group and (G4) RBPE+IR group.****Fig. (2): The immunohistochemical alterations of Bcl-2 in the liver sections: (G1) control group, (G2) RBPE group, (G3) IR group and (G4) RBPE+IR group.**

### Histopathological Findings

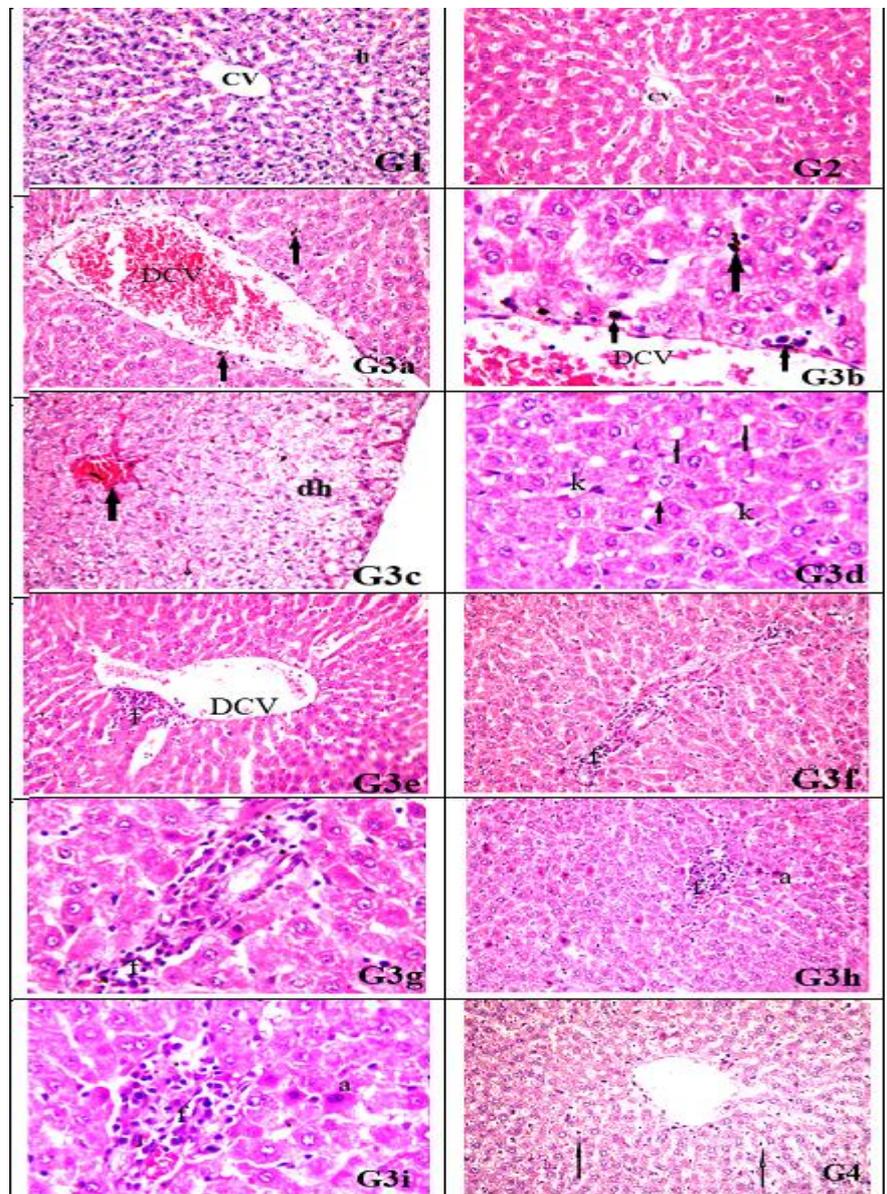
In the control and RBPE groups, the central vein's normal histological structure and the parenchymal hepatocytes around it were noted (fig. 3, G1 and G2). In irradiated rats, the central vein showed congestion and severe dilatation, but they also showed infiltration of some pigmented cells in the surrounding adjacent parenchyma between the hepatocytes (fig.3, G3a and b). Under the capsule, hepatocytes were seen to undergo degenerative alteration in association with congestion in the hepatic sinusoids (fig.3, G3c). In some individual hepatocytes, there was a fatty alteration accompanied by diffuse kupffer cell proliferation in the spaces between (fig.3, G3d). The parenchyma next to the dilated central

vein showed focal inflammatory cell aggregation (fig.3, G3e). There was a massive inflammatory cell infiltration in the portal area (fig.3, G3f and g). There was localized inflammatory cell infiltration in the hepatic parenchyma that was accompanied by apoptosis in some individual hepatocytes in the surrounding area (fig.3, G3h and i). G4 of rats which administrated RBPE and exposed to  $\gamma$  irradiation were shown to have diffuse kupffer cell proliferation in the spaces between their hepatocytes (fig.3, G4).

### The Histopathological Alterations

The incidence and severity of liver lesions were graded semi-quantitatively, as shown in table 3.

**Fig. (3): The histopathological findings in liver;** (G1) control group and (G2) rats administrated RBPE showing normal liver structure [central vein (cv) and surrounding hepatocytes (h) in the parenchyma] (H&E  $\times 40$ ). (G3a) irradiated rats (6 Gy), the central vein showed congestion and severe dilatation (DCV) with infiltration of pigmented cells (arrow) surrounding adjacent area (H&E,  $\times 40$ ). (G3b) magnification of (G3a) to identify the infiltrated pigment cells (arrow) surrounding the congested central vein (DCV) (H&E  $\times 80$ ). (G3c) showing degeneration in the hepatocytes (dh) underneath the capsule with congestion in hepatic sinusoids (arrow) (H&E  $\times 40$ ). (G3d) showing fatty change in some individual liver cells (arrow) with Kupffer cells proliferation (K) in between (H&E  $\times 80$ ). (G3e) showing focal inflammatory cells aggregation (f) adjacent the dilated central vein (DCV) (H&E  $\times 40$ ). (G3f) showing inflammatory cells infiltration (f) in the portal area (H&E  $\times 40$ ). (G3g) showing inflammatory cells infiltration (f) in the portal area (H&E  $\times 80$ ). (G3h) showing focal inflammatory cells infiltration (f) in the hepatic parenchyma with apoptosis (a) in some hepatocytes (H&E  $\times 40$ ). (G3i) showing focal inflammatory cells infiltration (f) in the hepatic parenchyma with apoptosis (a) in some liver cells (H&E  $\times 80$ ). (G4) showing diffuse kupffer cells proliferation (arrow) in the spaces between the hepatocytes (H&E  $\times 40$ ).



**Table (3): The histopathological alterations severity in the different groups of liver**

Histopathological alterations	G1	G2	G3	G4
Congestion in central vein	0	0	III	0
Infiltration of pigmented cells surrounding the central vein	0	0	I	0
Degeneration in hepatocytes	0	0	II	0
Fatty change in individual hepatocytes	0	0	II	0
Diffuse kupffer cells proliferation	0	0	II	II
Focal inflammatory cells infiltration in parenchyma	0	0	II	0
Inflammatory cells infiltration in the portal area	0	0	III	0
Apoptosis in some individual hepatocytes	0	0	II	0

Nill (0) mild (I) moderate (II) sever (III)

## DISCUSSION

This study aimed to demonstrate that RBPE had a protective impact on hepatocytes after  $\gamma$  irradiation. IR exposure causes oxidative stress in several tissues [36, 37]. This causes modifications to the detoxification system, an increase in Casp-3 activity, and other effects. Recently, there has been a greater emphasis on discovering and employing natural antioxidant molecules with pharmaceutical potency and have little to no side effects [38]. The results revealed the protective effect of RBPE (50 mg/kg bd. wt.) that could be normally supplied in the usual diet. The findings imply that the radio protective effect of RBPE on hepatocyte may lessen apoptosis in rats exposed to radiation. There was no significant increase in liver enzymes of the rats received RBPE and the control group indicating the safe and non-toxic nature of the RBPE. Moreover there was a significant reduction in the liver ALT and AST enzymes in the rats exposed to  $\gamma$  rays and pre-administrated by RBPE as compared to the IR group indicating its radio protective effect. A further evidence of the protective effect was provided by the difference in Casp-3 levels between the control group and those who received RBPE alone. The activities of the Casp-3 were also significantly lower in the group that received RBPE with IR exposure than the irradiated group. Shedid *et al.* [39] found that administering betaine to radiation-exposed rats (at a dose of 9 Gy administered in 3 portions of 3 Gy/wk) greatly reduced the liver enzymes and decreased the Casp-3 activities. Cho *et al.* [40] found that beetroot treatment increased the synthetic phase size (S-phase) cells in addition to reducing the radiation-induced apoptotic cell fraction size when the number of apoptotic cells was measured using a flow cytometric technique.

Regarding immunohistochemical changes in the liver, the results also revealed the stressful effect of IR on the liver cells and the protective effect of RBPE. Exposure to  $\gamma$  rays significantly elevate the expression of the Bax leading to severe apoptotic changes in the liver cells and reduced the expression of the Bcl-2 compared to the other groups. RBPE therapy clearly inhibited the increase in Bax expression levels as demonstrated by the no apoptotic changes in liver cells. Albasher *et al.* [41] reported that red beetroot methanolic extract prevented the casp-3 and Bax expression while increased Bcl-2 expression, in response to chlorpyrifos-induced brain injury in rats. El Gamal *et al.* [42] stated that the red beetroot ethanolic extract prevented the casp-3 and Bax expression while increased Bcl-2 expression, in response to gentamicin-induced nephrotoxicity.

Regarding the pathological changes in the liver, our results revealed the stressful effect of IR on the liver cells and the protective effect of RBPE. Exposure to  $\gamma$  irradiation significantly enhanced congestion in the central vein, and inflammatory cell infiltration in the portal region. There was localized inflammatory cell infiltration in the hepatic parenchyma that was accompanied by apoptosis in some individual hepatocytes in the surrounding area. Rats protected by RBPE and exposed to  $\gamma$  irradiation were shown to have diffuse kupffer cell proliferation in the spaces between their hepatocytes.

As we used the RBPE as a whole, we were unable to determine which part of it was responsible for the extract's radio protective effectiveness. An earlier study, however, suggests that betalain pigments might be a possible active component. Beetroot contains a high concentration of betalains, which have been linked to the

protection of oxidative stress and inflammation [43]. Betaine lessens oxidative stress, lowers the rise in apoptotic and fibrotic markers, and ameliorates radiation-induced liver damage [39]. Furthermore, beetroot has high levels of phenolic compounds and organic acids, which should be regarded as antioxidant defense adjuvants in the promotion of health and the prevention of chronic diseases [44]. Salamatullah *et al.* [45] reported that total flavonoids, total phenols, and reducing power activity were all high in RBPE. Beetroot and its nitrate content have lately gained popularity due to their high biological activity. It has a DPPH scavenging function, and prevents DNA damage. It has been demonstrated that betalains can reduce oxidative damage. It has also been shown to have anticancer properties by inhibiting cell growth, increasing angiogenesis, initiating cell death, and stimulating autophagy [46]. Finally, we found that RBPE against  $\gamma$  irradiation enhanced the animal shield after whole-body  $\gamma$  irradiation.

## CONCLUSIONS

It was concluded that administration of RBPE can induce a significant decrease in liver apoptosis post  $\gamma$  irradiation as demonstrated by a significant decrease in liver enzymes, caspase-3 and Bax, as well as preventing degeneration, inflammation and fatty change in hepatocytes, demonstrating that RBPE has protective properties against  $\gamma$  irradiation and is considered a nontoxic radio protective agent.

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## CONFLICT OF INTEREST

The authors have stated that they have no conflicts of interest with the content of this paper.

## CONSENT FOR PUBLICATION

It is hereby confirmed that all authors are aware of the contents of this manuscript and provide consent for its publication.

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