Biomedicine & Pharmacotherapy 94 (2017) 569–576

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Original article

Rescue effects of ginger extract on dose dependent radiation-induced histological and biochemical changes in the kidneys of male Wistar rats

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ARTICLE INFO

Article history:
Received 31 May 2017
Received in revised form 23 July 2017
Accepted 24 July 2017

Keywords:
γ-Ray
Kidney
8-OHdG
Proliferation
Cystatin C
Ginger

ABSTRACT

Radiation is an essential modality in the management of cancer therapy, but its acute and chronic side effects on the normal organs limit the helpfulness of radiotherapy. The deleterious effects of radiation begin with oxidative stress and inflammatory reaction to radiolytic hydrolysis and formation of free radicals. The aim of the current study was to investigate the effect of dose dependent whole body radiation exposure on histological and biochemical alterations in rat kidney. It was also planned to find out whether ginger extract mitigated the deleterious effects of different doses of radiation in rat kidney. Male Wistar rats were exposed to three doses (2, 4, and 8 Gy) of γ- ray with or without a 10 day pretreatment with ginger extract. After 10 days of whole body γ- ray exposure, the results revealed proliferation of glomerular and tubular cells, fibrosis in glomerular and peritubular and a significant increase in 8-OHdG, CRP, cystatin C (in 8 Gy), plasma urea and creatinine levels, as well as a significant decrease in total antioxidant capacity of radiation groups compared to those of the control group. Ginger extract administration once daily for 10 consecutive days before exposure to 2–4–8 Gy radiotherapy, which ameliorated histological and biochemical alterations in kidneys of the rats entirely or partially compared to those in the ethanol group rats. These findings indicate that whole body exposure to radiation induces kidney damage through oxidative DNA damage and inflammatory reactions, and that these effects can be alleviated using ginger pretreatment as an antioxidant and anti-inflammatory agent.

1. Introduction

Radiotherapy is an effective protocol in destroying cancer cells. Moreover, radiation is increasingly applied for diagnostic purposes in medical sciences. However, beside killing cancer cells, the radiotherapy-induced deleterious effects in normal tissues, especially in the marked radiosensitive organs such as the kidney, are limiting issues in treating cancers of the abdominal area [1–3]. A growing body of evidence indicated that hazardous effects of radiation on normal organs during radiotherapy are caused mainly by the generation of reactive oxygen species (ROS) and other toxic substances [3,4]. Radiation-induced ROS generation interacts with biological macromolecules, such as DNA, lipids, and proteins located in cell membrane leading to oxidative DNA damage, lipid peroxidation, and cell injury or death [5,6]. Moreover, radiation-induced ROS generation causes cell injury and death through alteration to the balance of endogenous antioxidant enzymes, such as super oxide dismutase, catalase, and glutathione [7]. Furthermore, previous studies have shown that antioxidant treatment during exposure to radiation were ameliorated or protected against damage induced by radiation in normal organs [8–10]. Oxidative nature of radiation-induced injury on the one hand, and the protective effect of antioxidant therapy, on the other, tempted us to design the current work with considering some precise underlying molecular mechanisms that may help radiation to exert its harmful effects on the kidney during whole abdomen exposure to different doses of γ- ray. In addition, due to the very well documented antioxidant and anti-inflammatory properties of ginger, a second aim of this work was to determine the possible protective effects of ginger extract pretreatment against dose

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http://dx.doi.org/10.1016/j.biopha.2017.07.128
0753-3322/

dependent radiation-induced histopathological alterations and biochemical changes in the kidneys of rats. In the current study, radiation were applied by gamma-ray. X-ray and gamma ray both are high energy electromagnetic radiation and could transfer energy to matter with analogous physical processes [11]. Denomination of gamma ray or x-ray only depends on the way of production. X-ray are produced by x-ray machines (or other systems such as linear accelerators), and gamma ray emitted by nucleus of atoms in radioactive isotopes. So, for example in X-ray absorptiometry, both x-ray and gamma ray can be used for measuring bone density in the same process [12].

2. Materials and methods

The animal care and handling herein were done according to the Principles of Laboratory Animal Care (NIH publication, no. 85–23, revised 1985) and were approved by the Urmia University of medical sciences Animal Care Committee. In the current study, 56 male Wistar rats weighing 220 ± 20 g were divided into the following seven groups of eight animals each. The group I served as the normal control. The groups II, III, and IV were exposed to the whole body single dose γ-radiation at the three doses of 2, 4, and 8 Gy respectively. The groups V, VI, and VII were pretreated for 10 days by hydro-alcoholic extract of ginger with a dose of 50 mg/kg body weight intragastrically by gavage and were then exposed to the whole body γ-radiation once at doses of 2, 4, and 8 Gy respectively. Wistar rats were exposed to single dose total of body of gamma rays 2, 4 and 8 Gy. According to the output of gamma ray machine (28.336 CGy/min), the exposure time for 2, 4 and 8 Gy radiation were 7.06, 14.12 and 28.24 min respectively.

Ginger extract was prepared according to our previous protocol. Briefly, a dried ginger rhizome (originally Chinese) was purchased from a local market. Sufficient quantity of rhizome was powdered in an electric grinder. Hydro-alcoholic extract of ginger was prepared by mixing three kg of powder with six liters of ethanol 70% in a suitable container. It was then left for 72 h in room temperature. Next, the extract was filtered through filter paper and was then concentrated using a rotary evaporator. The yield of the extract was kept in a refrigerator until the time of use [13]. After a post-radiation of 10 days, the rats were anesthetized by 10% chloral hydrate (0.5 mL/100 g body weight, IP). Next, after weighing the animals, the thoracic cavity was opened and the blood samples were collected directly from the heart and mixed with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood samples were then centrifuged at 4000 × *g* for 20 min within 30 min of collection. Furthermore, the yielded plasma was stored at −80 °C without repeated freeze-thaw cycles. Then, the abdominal cavities were opened and both kidneys were dissected. Excised kidneys were freed from adventitial tissues, fat, and blood clots and were subsequently weighed. The right kidney was divided into two parts. For the purpose of histopathological investigations, a part of the kidney was immediately fixed in 10% buffered formalin and then, after standard dehydration steps, it was embedded in paraffin. To perform biochemical analysis, other parts of the kidneys were washed with ice-cold physiological saline and then dried on filter papers. Subsequently, an ice-cold extraction buffer (10% wt/vol), containing a 50 mM phosphate buffer (pH 7.4) was added. It was then homogenized using Ultra Turrax (T10B, IKA, Germany). Next, the homogenates were centrifuged at 10,000 × *g* at 4 °C for 20 min. As the last step, the supernatant sample was collected and stored at −80 °C until the time of analysis [13].

2.1. Histopathological examinations

To evaluate general histological changes, after tissue processing steps, 5 μm sections from paraffin-embedded kidneys were cut and then stained with Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Proliferating cells were implemented, in accordance with our published protocol, by performing immunohistochemistry using an antibody against the proliferation cell nuclear antigen (PCNA). In brief, after taking tissue processing steps, such as deparaffinization, rehydration, and gradual ethanol passage, sections from the kidney tissue with a thickness of 5-μm were stained using the Monoclonal anti-PCNA antibody (Dako Denmark A/S, Denmark). Optimal results were achieved with the EnVision™ visualization system (Dako Denmark A/S, Denmark). Furthermore, Hematoxylin was used as a counterstain. The assessment included proper negative controls. Moreover, all the slides were inspected by two expert pathologists, independently. PCNA-positive indices were considered as indicators of kidney cell proliferation. In order to assess percentages of PCNA-positive indices, four non-overlapping fields of view per section from two to three sections per animal were analyzed. The number of positively stained cells and the total number of cells were counted for each field of view. In addition, for each animal, the number of positively stained cells was then presented as a percentage of the total number of counted cells. The criteria applied in scoring the quality of PCNA-positive indices were as follows: normal (i.e. PCNA-positive indices are present in less than 5% of the kidney cells), mild (i.e. PCNA-positive indices are present in less than 25% of the kidney cells), mild to moderate (i.e. PCNA-positive indices are present in 25–50% of the kidney cells), moderate to severe (i.e. PCNA-positive indices are present in 50–75% of the kidney cells), and severe (i.e. PCNA-positive indices are present in 75–100% of the kidney cells) [14]. In order to evaluate the kidney tissue fibrosis, 5 μm kidney tissue sections were stained using Masson Trichrome, in accordance with the manufacturer's instructions (Asiapajohesh, Amol, Iran). The severity of tissue fibrosis was estimated maintaining a semi-quantitative method explained by Ashcroft et al. and our published protocol [14,15]. A score ranging from zero (normal kidney) to eight (total fibrosis) was set. The criteria appointed in scoring kidney fibrosis were as follows: grade 0 = normal kidney; grade 1 = minimal fibrosis thickening of kidney tissue, grade 2 and 3 = moderate thickening of kidney tissue without obvious damage to the structure of kidney tissue; grade 4 and 5 = increased fibrosis with definite damage to architecture of the kidney and formation of fibrosis bands or small fibrosis masses; grade 6 and 7 = severe distortion of structure and large fibrosis areas; and finally grade 8 = total fibrotic obliteration [15].

2.2. Biochemical assays

Plasma creatinine and urea levels were estimated using urea and a creatinine commercial kit (Pars Azemoon, Karaj, IRAN). Levels of 8-OHdG in kidney tissue homogenates were measured by the quantitative sandwich enzyme immunoassay method using a commercial rat 8-hydroxy-deoxyguanosine Elisa kit (Cusabio, China), following the manufacture guidelines. The total antioxidant capacity (TAC) was measured in kidney homogenate using antioxidant assay kit (Cayman Chemical, USA), in accordance with the manufacture guidelines. The plasma cystatin C levels were determined employing the quantitative sandwich enzyme immunoassay method using a commercial rat cystatin C Elisa kit (Cusabio, China).

2.3. Statistical analysis

To verify normal distribution of data within each group, a Kolmogorov-Smirnov test was carried out. Furthermore, a one-way ANOVA and then the Tukey's post hoc test were conducted to test the statistical differences between the groups. The data obtained

from each test are presented as the mean ± S.E.; $p < 0.05$ is considered to be statistically significant.

3. Results

The effects of different doses of γ-Ray exposure and ginger extract pretreatment on biochemical parameters alteration are presented in Table 1. The creatinine amount in plasma obtained from 4 and 8 Gy γ-Ray groups showed significant increases compared to that obtained from the control group (0.05). There were no significant differences among the ginger pretreatment groups (V, VI, and VII) and the control group (0.05). Plasma urea amounts in all γ-Ray exposure groups showed significant increases compared to that in the control group (0.05). In the three groups with Ginger extract pretreatment, the amount of plasma urea was reduced significantly compared to that in the γ-Ray (II, III, and IV) groups (0.05).The cystatin C levels in radiation animals (4 Gy, and 8 Gy) exhibited a rise when compared with group I (0.05). However, a significant decrease in cystatin C levels was recorded in rats pre-treated with ginger extract (VI, and VII groups) compared to the 4 and 8 Gy radiation groups respectively ($p < 0.05$).

Irradiation in all applied doses (2, 4, and 8 Gy) decreased the total antioxidant capacity in kidney tissue of animals compared to those in the control group (0.05). Administration of ginger extract for 10 consecutive days before radiation elevated the total antioxidant capacity in kidney tissue of the animals compared to those of the II, III, and IV groups (0.05). The 8-OHdG levels in the kidney showed no significant change in gamma irradiated group (II) as compared to the control group ($p < 0.5$), but, in γ-Ray with 4 and 8 Gy doses, the 8-OHdG levels in the kidney were higher than those of the control group ($p < 0.05$). Ginger extract pretreatment with gamma irradiation exposure (groups VI, and VII) showed a significant reduction in 8-OHdG levels in the kidney as compared to those in the III and IV groups ($p < 0.05$), but it was still significantly higher than those in the control group. The CRP amount of plasma obtained from γ-Ray groups (II, and IV) was significantly higher than that obtained from the control group ($pp < 0.05$). Although, pretreatment with ginger extract reduced CRP amount in plasma obtained from VI and VII animals, but it was still higher than that obtained from the control group. The ratio of plasma cystatin C to plasma creatinine increased significantly on the IV group (γ-Ray-8 Gy dose) compared to that in the control group ($p < 0.03$). There were no significant differences found between the ginger extract pretreatment group and the control group in terms of cystatin C/creatinine ratio.

Histopathological examination results from the kidney tissue are presented in Figs. 1–3. The histology of the rats' kidneys exposed to gamma irradiation at a dose of 2, 4, and 8 Gy (groups II, III,and IV), compared to that of the control group, revealed several histopathological changes, such as scattered cytoplasmic vacuoles, proteinous cast, mesangial widening expansion, dilation of Bowman's space, interstitial plasma cell infiltration, focal PMN infiltration, as well as mesangial hypercellularity and tubular atrophy. The severity of all changes was more pronounced in those with 2 Gy–8 Gy doses respectively. Ginger extract pretreatment attenuated all histological changes in the kidney tissue of the pretreated groups compared to the irradiation groups (Fig. 2). The ratio of proliferated cells (PCNA-positive indices) in kidney tissue (regardless of cell types) of samples obtained from rats exposed to different doses of γ-Ray are given in Fig. 2. The average ratios of proliferated cells in the kidney tissue were 1.5, 20, 27, 38, 3, 3.7, and 4.2 in the control, II, III, IV, V, VI, and VII groups respectively. The PCNA-positive indices were dramatically increased in the rats of the 2GY, 4 and 8GY groups (mild in 2GY and mild to moderate in 4 and 8 Gy groups) compared to those of the control group ($p < 0.05$). There were no significant differences between the ginger extract pretreatment groups and the control group (Fig. 2).

Fig. 3 shows microscopic fibrosis scores in different parts of nephron tubules and peritubular vessels in different groups. There was no lesion score in the glomerulus, proximal tubules, distal tubules, as well as around peritubular vessels in the control and 2 Gy γ-Ray groups (grade 0). The microscopic lesion score in the glomerulus, proximal tubules, distal tubules, and peritubular vessels was 1 in the γ-Ray group with the 4 Gy dose, which is an indication of minimal fibrosis thickening of kidney tissue. There were no significant differences between the group receiving ginger extract pretreatment 10 days before 4 Gy γ-Ray exposure and the control group. The 8 Gy dose of γ-Ray exposure induced grade 2–3 fibrosis, which is an indication of moderate thickening of kidney tissue without obvious damage to its structure. In the VII group, the lesion score was 1(minimal fibrosis thickening) compared to the 8 Gy γ-Ray group. Although ginger extract pretreatment of 8 Gy γ-Ray exposure reduced fibrosis lesion compared to the VI group, there was still more fibrosis lesion in the VII group compared to the control group.

4. Discussion

The results of current study indicated that whole-body irradiation with different doses of γ-Ray leads to oxidative and inflammatory damage in the kidney of rats, as manifested by increased oxidative DNA damage and decreased total antioxidant capacity in the kidney tissue. In addition, γ-Ray exposure causes plasma levels of urea, creatinine, CRP,and cystatin C of the rats to significantly increase compared to those of the rats in the control group. Moreover, structural changes, such as scattered cytoplasmic vacuoles, proteinous cast, mesangial widening expansion, dilation of bowman's space, interstitial plasma cell infiltration, focal PMN infiltration, minimal fibrosis thickening of kidney tissue, and mild

Table 1
Effect of different doses of gamma ray with or without ginger extracts pretreatment serum and plasma biochemical parameters.

	control	2 Gy	4 Gy	8 Gy	2Gy + Gin	4Gy + Gin	8Gy + Gin
Creatinine (mg/dl)	0.526 ± 0.005	0.558 ± 0.022	0.633 ± 0.013*	0.595 ± 0.010*	0.515 ± 0.007	0.528 ± 0.009†	0.520 ± 0.008†
BUN (mg/dl)	15.53 ± 0.24	18.53 ± 0.29*	19.63 ± 0.13*	21.91 ± 0.49*	17.06 ± 0.41	15.13 ± 0.57†	14.15 ± 0.43†
Cys.C (ng/ml)	3.50 ± 0.096	3.56 ± 0.098	4.02 ± 0.060*	4.28 ± 0.113*	3.43 ± 0.084	3.22 ± 0.142†	3.13 ± 0.066†
TAC (ng/ml)	0.58 ± 0.0098	0.53 ± 0.0074*	0.48 ± 0.0025*	0.38 ± 0.0089*	0.58 ± 0.0134	0.59 ± 0.0098†	0.57 ± 0.0049†
8-OHdG (ng/ml)	0.94 ± 0.016c	1.18 ± 0.030	1.91 ± 0.087*	2.06 ± 0.061*	0.94 ± 0.037†	0.97 ± 0.027†	1.68 ± 0.107*,†
CRP (mg/l)	2.40 ± 0.093	2.80 ± 0.051*	3.36 ± 0.117*	3.86 ± 0.055*	2.73 ± 0.122	2.83 ± 0.091*,†	3.41 ± 0.130*,†
Cys.c/creatinine (ng/ml/mg/dl)	6.50 ± 0.14	6.45 ± 0.39	6.35 ± 0.09	7.58 ± 0.14*	6.68 ± 0.23	5.90 ± 0.24	6.04 ± 0.15

TAC: Total antioxidant capacity, Gin: Ginger.
Values are mean ± SE for 8 rats per group.
* Denotes significant difference ($p < 0.05$) compared to the control.
† Denotes significant difference ($p < 0.05$) compared to the gamma ray exposed groups.

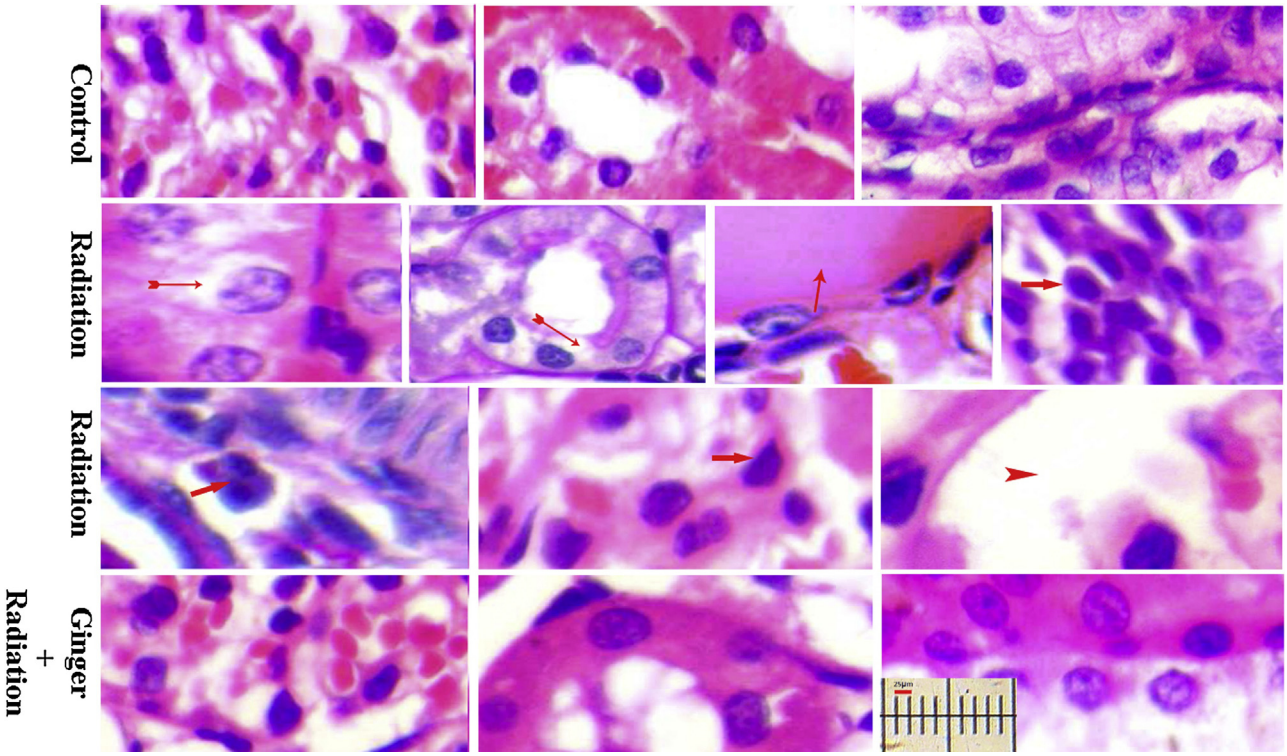

Fig. 1. Hematoxylin-eosin (H&E) and periodic acid – Schiff (PAS) staining show scattered cytoplasmic vacuoles, proteinous cast, mesangial widening expansion, dilation of bowman's space, interstitial plasma cell infiltration, were observed in all different parts of the kidney tissue in radiation groups (B and C) compared to control group animals (A). There were no significant differences in terms of kidneytissue structure between the ginger extract pretreatment and control groups. (Original magnification ×400). Vacuolization (➡), Proteinous cast (→), infiltrated plasma cell (➡), dilation of bowman's space (➤).

to moderate kidney tissue cells proliferation were also present in the kidney of rats in all γ-Ray exposure groups compared to the kidney of rats in the control group. The severity of all biochemical and structural alterations were more pronounced in2 Gy to 8 Gy of γ-Ray exposure doses respectively. Furthermore, Significant amelioration of urea, creatinine, cystatin C levels, 8-OHdG amount, and restoration of structural alterations, similar to those of the control group animals, were observed in the group with ginger extract pretreatment 10 days before 2, 4, and 8 Gy doses of γ-Ray exposure. Since the degree of ionizing radiation-induced cell damage depends on various factors including the radiation dose, levels of cellular antioxidant defense, time of administration, and radiation induced physiological and biochemical changes that limit the efficacy of the radiotherapy, the protective effect of ginger extract pretreatment may be a choice for adjuvant therapy to reduce irradiation-induced deleterious effects [16,17]. An increasing body of evidence has revealed that the deleterious effects of radiation are mediated by oxidative stress and inflammation [18–20]. It has been observed that increased free radical production leading to the formation of peroxides such as lipid–peroxidation, oxidation of DNA and proteins, as well as pro-inflammatory factors damage the cell membrane and also the cell [21]. As evidenced in the present study, γ-Ray exposure resulted in increased plasma CRP, kidney tissue DNA damage, fibrosis and kidney cell proliferation as an indicator of oxidative stress and inflammation. Previous studies have demonstrated that radiation exposure led to DNA damage due to insufficient antioxidant capacity in mammalian cells [22]. Interestingly, in the current study, DNA damage was observed along with a decline in the total antioxidant capacity in the kidney tissue of rats exposed to γ-Ray. Since, oxygen radicals such as $^{\bullet}OH$ are very reactive short-lived species, their direct measurement is very difficult. In general, measurement of secondary products of oxidation has been accepted as a trust worthy way for measuring the ongoing oxidative stress and damage [23]. Among dozens of oxidative stress resultant secondary products, the 8-OHdG is a very popular marker for evaluation of oxidative stress and oxidative DNA damage [24]. For the aforementioned reason, we used changes in the amount of 8-OHdG in the kidney tissue to examine the hypothesis that irradiation-induced structural and functional alterations in the kidney are in part associated with oxidative stress. In the current study, as assessed by light microscopy, it was found that rats in the γ-Ray groups had gone through alterations in the structure of their kidneys. The alteration included an increase in the fibrosis, an elevation in the number of PMNs, an increase in the kidney cells proliferation, and dilation of bowman's apace which are all indicators of inflammation reaction induced by γ-Ray. The mechanism through which γ-Ray exposure induced cell proliferation and fibrosis is not understood fully, but it may be due to oxidative stress and inflammatory reactions. According to the literature, infiltrated leukocytes in the tissue and their resultant PMN promote inflammation in several ways: PMN activates cytoplasmic granules and secretory vesicles which contain a variety of membrane-bound receptors for endothelial adhesion molecules, extracellular matrix proteins, soluble mediators, different interleukins, neutrophil elastase, and ROS [25,26]. In addition, signals from PMNs result in the release of secretory organelle contents and secreted effectors arouse inflammatory responses [25]. Moreover, PMNs generate lipid mediator of leukotrine B4, a substance that in addition to stimulating the generation of ROS constitutes a chemotactic factor for neutrophils and other leukocytes. PMNs also cause an increase in the endothelial adhesiveness and augment vascular permeability through promoting the release of HBP/CAP37/azurocidin [27].

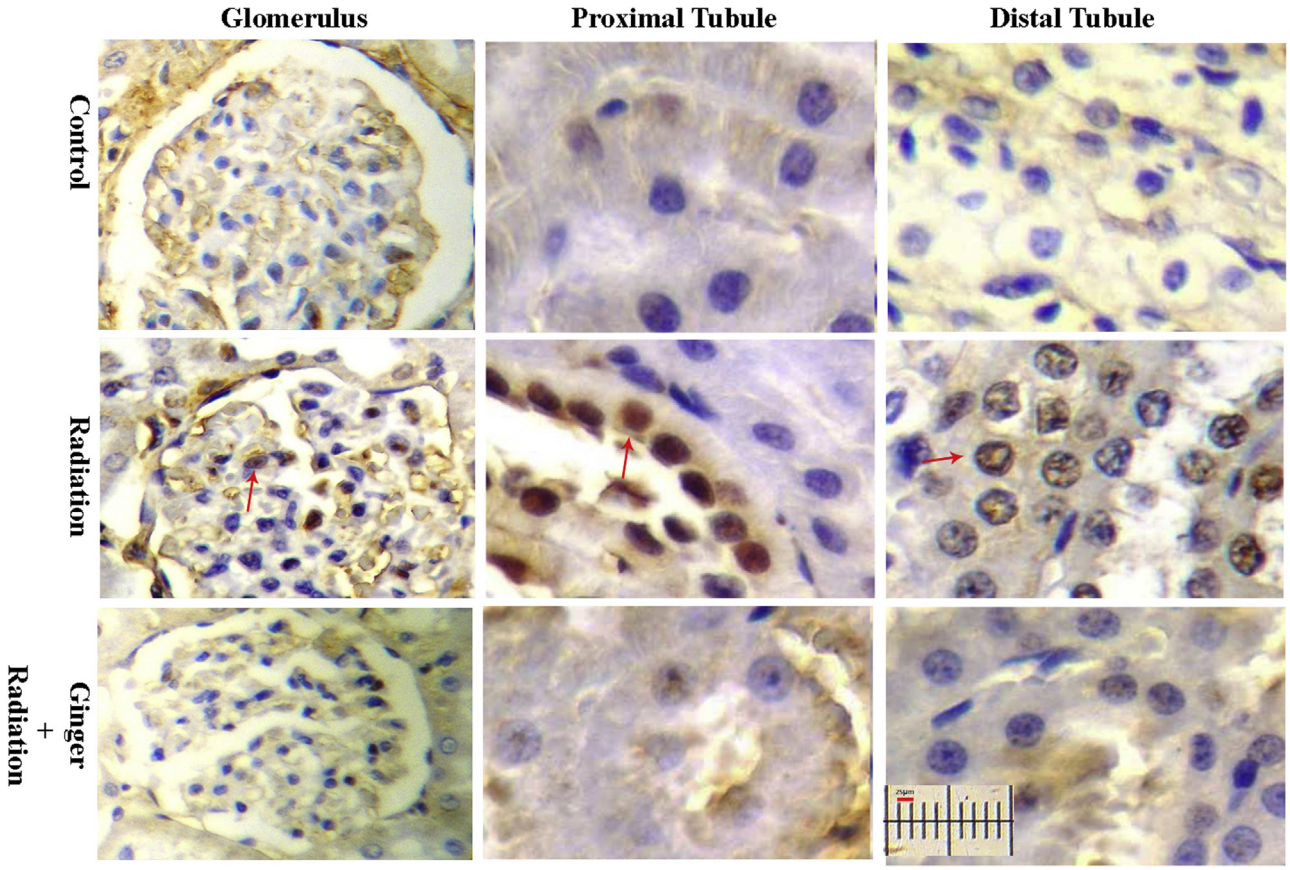

Fig. 2. Immunohistochemical staining of kidney tissue by proliferating cell nuclear antigen(PCNA) anti-body showed mild to moderate kidney cell proliferation(E), in γ- ray exposed groups compared to control group©. Ginger extract pretreatment prior to radiation reduced cell proliferation in kidney tissue. (Original magnification ×400). PCNA positive indices (⟶).

Furthermore, PMNs induce production of several cytokines that have a proinflammatory role in promoting systemic inflammatory responses and recruiting inflammatory cells locally [28,29]. Accordingly, leukocytes infiltration in kidney tissues may predispose development of kidney tissue fibrosis and proliferation via contribution of inflammatory responses in the kidney tissue. A recent study by Sasaki et al. demonstrated the functional role of infiltrated leukocytes and their resultant PMNs in the formation of fibrosis and proliferation in the skin tissue cultured cells [30].

Another important result of the current study was that γ-Ray exposure caused a significant rise in plasma creatinine and cystatin C levels in the 4 and 6 Gy doses. Plasma creatinine and cystatin C are two important predictor markers of kidney function. The elevation of plasma cystatin C and creatinine levels indicated that γ-Ray exposure affected renal function in rats exposed to γ-Ray with doses of 4 and 8 Gy. Because the levels of serum creatinine and cystatin C are affected by different non-renal factors, such as body weight, higher white blood cell count, and increased markers of inflammation [31], Grubb et al. proposed the ratio of cystatin C/creatinine to be used as an indicator of quality alterations in the glomerular filtration [32]. The enhancement of plasma cystatin C/creatinine ratio occurs in a condition called 'shrunken pore syndrome', due to shrinkage of glomerular pores [33]. Such pathophysiological phenomenon in glomerular barrier with shrinkage of glomerular pores affects the glomerular filtrate composition as follows: A small reduction in pore size is identified by increased serum concentrations of large molecules such as cystatin C, but further pore shrinkage leads to retention of smaller molecules such as urea and creatinine, which in turn results in their accumulation in the blood [33]. Cystatin C, compared to creatinine, is 100 times larger and is retained in a lower degree of pore shrinkage. Therefore, the ratio of cystatin C/creatinine can be an indicator of kidney disease at an earlier stage [34]. Interestingly, results of the current study showed a significant increase in the cystatin C/creatinine ratio concurrent with a high plasma creatinine and urea level in the group exposed to γ-Ray (only in 8 Gy dose) compared to those in the control group. To our knowledge, this is the first *in vivo* study to show γ-Ray exposure influences kidney function by shrinking glomerular pores and to document a rise in the cystatin C/creatinine ratio.

The second issue addressed in the current study was the rescue effect of a 10 day consecutive pretreatment with ginger extract prior to γ-Ray exposure against biochemical and structural alterations induced by irradiation in the plasma and kidney tissue. Ginger extract pretreatment mitigated all biochemical and structural changes induced by γ-Ray exposure compared to the γ-Ray treatment groups with different doses respectively. In agreement with the current study, several earlier studies demonstrated that pretreatment or co-treatment with irradiation exposure by medical plants such as *ocimumsanctum*,*menthe arvensis*,*curcumuin*, and *citrus* extract alleviated radiation induced deleterious effects in different organs [35–38].

Multiple lines of evidence have highlighted that the plant/natural products' radioprotective possession is due to their antioxidant and anti-inflammatory properties. As a well known medical plant, ginger has been reported to possess compounds, such as flovonoids, gingerol, shogaols, vitamin C, and dozens of polyphenolic compounds with antioxidant and anti-inflammatory

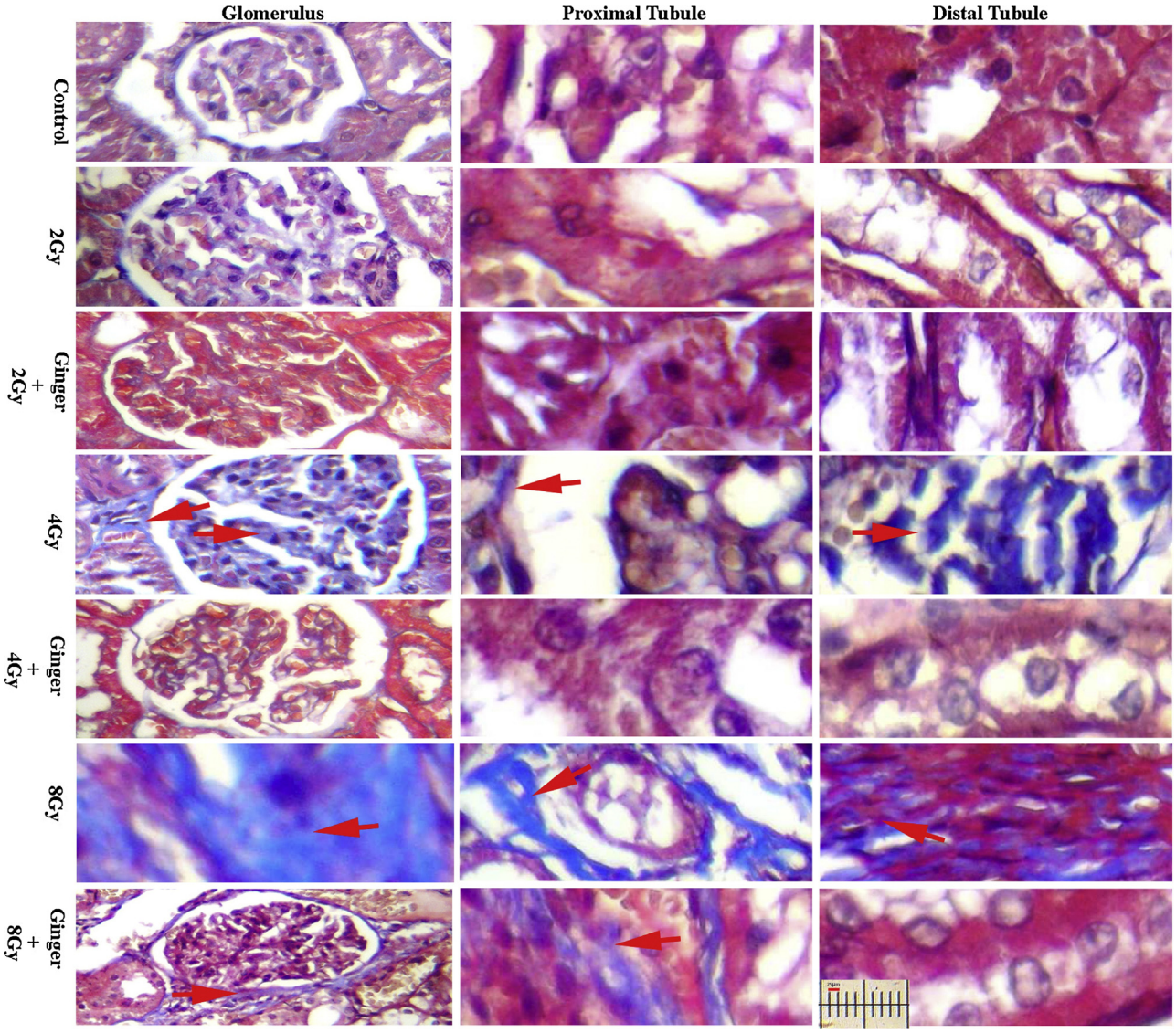

Fig. 3. Photomicrograph of kidney tissue of rats (Masson trichrome staining). In ©, sample obtained from the different groups. (Original magnification ×400). Fibrosis bond (➡).

properties [39–41]. Mainly, the antioxidant activity of ginger is due to two bioactive flovonoids of ginger including gingerol and shogaol that suppress the accumulation of reactive oxygen or nitrogen species by preventing the production of free radicals and scavenging free radicals produced in the body as well as chelate prooxidant transition metals as iron [42]. In addition, the study by El-sharaky et al. showed that ginger flavonoids, especially gingerol, increased the activity and levels of antioxidant enzymes such superoxide dismutase and glutathione peroxidase in liver of male rats [43]. Our recent work indicated that ginger administration to diabetic rats enhanced their total antioxidant capacity and reduced lipid peroxidation and protein oxidation "as two main free radical generation sources" in diabetic rats [13]. Results of the current study showed inflammatory reaction in rats exposed to γ-Ray, which were manifested by rising CRP levels and kidney cells proliferation. Our data also demonstrated pretreatment by ginger extract prior to γ-Ray exposure significantly reduced CRP levels and kidney cell proliferation. Based on previous studies, ionizing radiation exposure causes overproduction of proinflammatory enzymes such as ODO, iNOS, COX2, proinflammatory cytokines (TNFα and IL-β) that all have a role in the inflammatory disease pathogenesis like fibrosis, mutagenesis and cancer [44,45]. As an anti-inflammatory agent, ginger exerts its anti-inflammatory properties in several ways: ginger supplementation inhibits a number of proinflammatory gene expressions, such as TNF-α, arachidonic acid cascade, IL-1β, and macrophage chemoattractant protein-1(MCP-1) [46,47].

In addition, ginger also inhibits prostaglandin and leukotriene biosynthesis via suppression of 5-lipooxygenase synthetase activities [46,47]. Furthermore, Ginger supplementation suppresses iNOS enzymatic activity by justifying NF-κB mediated iNOS expression [45], and finally, at the molecular level, ginger demonstrates anti-inflammatory properties via inhibiting phosphorylation of MAPKs, ERK1/2, and the activation of NF-κB [48]. The above mentioned reasonson the one hand, and the results of the current study and some previous ones, on the other hand,

provided strong evidence that γ-Ray exposureinduces some functional and structural abnormalities in kidneys through oxidative stress and inflammation processes. Accordingly, the rescue effects of ginger supplementation pretreatment on these abnormalities are due to antioxidant and anti-inflammatory properties of ginger.

In conclusion, the results obtained from the current study indicate that ginger extract pretreatment protects against different doses of γ-Ray exposure-induced functional and structural alterations in kidney tissue, and more pronounced rescue effects of ginger extract are found in lower doses of γ-Ray (2and 4 Gy) than in the 8 Gy. To overcome the toxic effects of synthetic radioprotective compounds such as sulfhydryl compounds, our results also provide evidence that ginger extract may be a good alternative for synthetic radioprotective compounds, due to fewer side effects and highly antioxidant and anti-inflammatory properties of ginger supplementation compared to synthetic radioprotective compounds. However, further research is still required to elucidate the comprehensive details of subjects.

Funding source

This research didn't receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure statement

The authors report no conflict of interest.

Acknowledgment

This work derived from a Master of Science thesis in Medical physics by Urmia University of Medical Sciences, Urmia, IRAN

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