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Platelet-rich plasma ameliorates gamma radiation-induced nephrotoxicity *via* modulating oxidative stress and apoptosis

Ahmed F. Soliman[a,*], Mostafa Saif-Elnasr[b], Salma M. Abdel Fattah[c]

[a] Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt
[b] Health Radiation Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt
[c] Drug Radiation Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt

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ABSTRACT

Aims: As a source of growth factors and with its cytoprotective properties, platelet-rich plasma (PRP) received considerable attention in regenerative medicine. Thus, this study was designed to evaluate the protective efficacy of PRP against γ-radiation-induced nephrotoxicity.

Main methods: Forty male rats were distributed in four groups: 1) control, 2) PRP, 3) Radiation, and 4) PRP + radiation. Nephrotoxicity was examined in rats after a whole body γ-irradiation at a single dose of 8 Gy. Activated PRP (0.5 ml/kg BW) was injected subcutaneously twice weekly for three successive weeks prior to γ-irradiation. At the end of the experiment, creatinine, urea, albumin, and neutrophil gelatinase-associated lipocalin (NGAL) serum levels, as well as renal relative gene expression level of kidney injury molecule-1 (*KIM-1*) were estimated. Further, malondialdehyde level, nitric oxide content and reduced glutathione content in addition to superoxide dismutase and catalase activities were measured. Moreover, the expression levels of B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X (Bax), and caspase-3 proteins were assayed.

Key findings: PRP pre-treatment significantly reduced the radiation-induced abnormalities in kidney histology and attenuated the induced cell injury. Furthermore, PRP notably ameliorated the state of oxidative stress and appeared to inhibit the induced apoptosis.

Significance: This study lends a probable protective role of PRP against γ-radiation-induced nephrotoxicity which can highlight the possibilities of its application as a complementary procedure during radiotherapy.

1. Introduction

Radiotherapy is an important medical tool that contributes significantly to the cure or the alleviation of cancer patients [1]. Being radiosensitive, the kidneys are the dose-limiting organ for radiotherapy to pelvic malignancies including lymphomas and sarcomas of the upper abdomen, gastrointestinal and gynecologic cancers and during total body irradiation in preparation for bone marrow transplantation [2]. However, a significant number of patients received radiotherapy suffers from late radiation side effects, which are considered the main restrictive factor. One of these side effects is radiation-induced kidney injury, especially radiation nephropathy. The incidence of radiation-induced kidney injury is likely underreported because of the long latency and being likely often attributed to more common causes [3,4].

In radiation nephropathy, renal endothelial dysfunction and altered hemodynamics are known features [5–7]. Further, tubular cell loss and progressive interstitial scarring coincide with the progressive loss of renal function [8]. The radiation-induced injury is mainly due to the generation of reactive oxygen species (ROS) which results in a disproportion between pro-oxidant and antioxidant molecules inside the cell and in turn oxidation of proteins, lipids, and DNA, and may finally end by cell death [9]. Further, there is some evidence for apoptosis as the mechanism of renal tubular cell loss in radiation nephropathy [10].

Organ repair can be performed *ex vivo*, for example by injection of embryonic or adult stem cells, or *in situ*, for example by delivering factors that will promote or enhance the repair mechanisms of the organ itself. Within the last decade, many attempts have been made towards the development of a method for effective growth factors (GFs) delivery *in vivo* to initiate cellular repair and tissue regeneration [11]. Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma which contains considerable quantities of GFs such as platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-β1) and

* Corresponding author.
E-mail address: ahmed.fathi@sci.asu.edu.eg (A.F. Soliman).

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insulin-like growth factor-1 (IGF-1) [12]. The major advantage of PRP over other ways of administering GFs is that it is an inexpensive product, easy to obtain, and there is no risk of rejection or immune reaction because of its autologous preparation. Furthermore, PRP containing leukocytes was described to have an antimicrobial activity which suggests a low risk of infection [13,14].

Many GFs released by PRP are known to play a key role in angiogenesis and tissue regeneration by controlling cell migration, differentiation, proliferation and physiological functions [15,16]. For example, EGF is a potent promoter of growth in the renal tubular cells that attenuates tubular necrosis [17], IGF is a hormone that ameliorates acute tubular necrosis [18], TGF-β1 increases the anti-apoptotic B-cell lymphoma 2 (Bcl-2) expression, maintains epithelial homeostasis and protects renal cells from apoptosis [19,20] and VEGF protects peritubular endothelium, induces the proliferation of tubular epithelial cells, promotes angiogenesis and accelerates renal recovery [21–23].

In the light of the above findings, the present study was conducted to investigate a possible protective effect of PRP against γ-radiation-induced nephrotoxicity.

2. Material and methods

2.1. Chemicals

Trichloroacetic acid, thiobarbituric acid, reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene, glacial metaphosphoric acid, 5,5′-dithiobis-(2-nitrobenzoic acid) and Bradford reagent were purchased from Sigma-Aldrich Co (MO, USA). All other chemicals and solvents used were of the highest purity grade available.

2.2. Animals

Forty adult male and twenty adult female albino rats (200–250 g) were obtained from the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo, Egypt. Animals were housed in a well-ventilated room with 12/12 h light/dark cycle in polypropylene cages. Rats were kept on a standard diet and provided with food and water *ad libitum*. Animals were adapted to the laboratory conditions for one week before experimentation.

2.3. PRP preparation

A double-spin method was used to obtain PRP from the female albino rats. Briefly, rats were anesthetized with intraperitoneal injection of urethane at a dose of 1.5 g/kg BW and sacrificed by cervical dislocation then the whole blood was taken through cardiac puncture and pooled into test tubes containing 3.8% sodium citrate at a blood/citrate ratio of 9/1.

Hemocytometer was used to count platelets in 100 μl of the anticoagulated blood. The remaining part was centrifuged at 1000 rpm for 15 min at room temperature to separate plasma which was then centrifuged for additional 10 min at 3000 rpm to obtain platelet poor plasma (PPP) and a pellet containing the platelet concentrate. PPP was aspirated leaving 1 ml solution in which the pellet was resuspended and considered as PRP which was then incubated at room temperature for 30 min on a rotating platform to eliminate platelet agglomerates. The number of platelets in PRP was counted for each trial of PRP preparation. The final concentration of platelets obtained in PRP was approximately 3 times as great as that in the whole blood.

10% $CaCl_2$ activator solution was added to the PRP solution in a ratio of 50 μl $CaCl_2$ for every 1 ml of PRP to facilitate the release of growth factors from the α-granules of platelets. The mixture was incubated in glass tubes at 37 °C for 1 h, yielding activated PRP. The activated PRP was centrifuged at 4000 rpm and 4 °C for 10 min then the supernatant was collected and stored in aliquots at −20 °C for subsequent use.

2.4. Experimental design

Male albino rats were randomly divided into four groups, each of ten animals, as follows: Group I served as normal control group, Group II (PRP control group) received activated PRP subcutaneously (0.5 ml/kg BW) twice weekly for three successive weeks, Group III (γ-irradiated control group) was exposed to whole-body γ-irradiation at an acute single dose level of 8 Gray (Gy) delivered at a dose rate of 0.4319 Gy/min using Gamma Cell-40® biological irradiator with a ^{137}Cesium source (Atomic Energy of Canada Limited, Ontario, Canada) at the NCRRT. Group IV (PRP and radiation group) was injected with activated PRP subcutaneously twice weekly for three successive weeks then exposed to a single dose of γ-radiation in the next day of the last PRP dose.

48 h after irradiation, rats were anesthetized with urethane, and sacrificed by cervical dislocation after an overnight fasting period. The blood samples were collected by cardiac puncture and processed to separate sera which were then stored at −20 °C as aliquots for further analysis. Laparotomy was performed to expose the abdominal viscera then the gut was displaced to reveal the retroperitoneal and posteriorly situated kidneys which were excised, immediately washed with saline, dried with filter papers, and weighted. Kidneys were homogenized in ice-cold phosphate buffered saline (PBS) to form 10% homogenate for biochemical assays. A small part of each kidney was snap-frozen in liquid nitrogen and stored at −80 °C until used for assessment of renal kidney injury molecule-1 (*KIM-1*) relative gene expression.

The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH) and the study protocol was approved by the ethical committee of NCRRT.

2.5. Assessment of kidney functions and nephrotoxicity

After the determination of body weight at the beginning and at the end of the experiment in addition to kidneys weight, the body weight change and the ratio of kidneys to body weight were calculated as indices of kidney hypertrophy. Furthermore, kidney functions were assessed in rats by estimating serum creatinine, urea and albumin levels using available commercial kits provided by Spectrum diagnostics (Cairo, Egypt).

Moreover, the development of tubular injury was assessed by estimating serum level of neutrophil gelatinase-associated lipocalin (NGAL) using enzyme linked immunosorbent assay (ELISA) commercial kit (Cusabio Biotech, China) and by the determination of renal *KIM-1* relative gene expression level using quantitative real time RT-PCR (qRT-PCR). Briefly, total RNA was extracted from 30 mg kidney tissues using RNeasy Mini Kit (Qiagen, Hilden, Germany) as per the manufacturer's instruction. The concentration and purity of RNA were determined spectrophotometrically at 260 and 280 nm and its integrity was assessed by gel electrophoresis on 1% agarose gel stained with ethidium bromide. The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used to synthesize the first cDNA strand from 1 to 2 μg of the total RNA. The reactions of qRT-PCR were performed in a final volume of 25 μl with 5 μl of cDNA as a template, 1.5 μl (200 ng) of forward primer, 1.5 μl of reverse primer, 12.5 μl of 2× SYBR® Green PCR Master Mix (Eurogentec, Liege, Belgium) and the reaction's volume was then completed with nuclease-free water. Reactions were performed in duplicate in 7500 Real-Time PCR system (Applied Biosystems) using MicroAmp® fast optical 96-Well reaction plate with MicroAmp® optical adhesive film (Applied Biosystems, CA, USA) and the conditions were as follows: 10 min at 95 °C for initial denaturation, followed by 40 amplification cycles of denaturation at 95 °C for 15 s and 1 min at 60 °C for annealing and extension. Melt curve analyses of all products were performed and shown to produce a single DNA duplex. The $2^{-\Delta\Delta Ct}$ method [24] was used to determine the quantitative measurements with the usage of *β-actin* as the housekeeping gene. Specific

primers for *KIM-1* and *β-actin* were designed as follows

KIM-1	Forward primer, 5′-AACGCAGCGATTGTGCATCC-3′ Reverse primer, 5′-GTACACTCACCATGGTAACC-3′
β-actin	Forward primer, 5′-TCTGGCACCACACCTTCTACA ATG-3′ Reverse primer, 5′-AGCACAGCCTGGATAGCAACG-3′

2.6. Assessment of renal oxidative stress

Kidney homogenates were used to estimate different oxidative stress parameters. Lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) measured as malondialdehyde (MDA) according to the method of Yoshioka et al. [25], nitric oxide (NO) content was determined as nitrite concentration using a kit provided by Biodiagnostics (Giza, Egypt) according to the method described by Miranda et al. [26], GSH content was determined as described by Beutler et al. [27], superoxide dismutase (SOD) activity was assayed using SOD assay kit (Sigma-Aldrich Co.) as instructed by the manufacturer and catalase (CAT) activity was measured by the method of Sinha [28].

2.7. Western blot analysis

In order to determine the effect of PRP on renal apoptosis, immunoblotting was used to study the expression level of some specific apoptosis-related proteins, Bcl-2, Bcl-2-associated X protein (Bax), and caspase-3. Briefly, kidney tissues were homogenized in RIPA buffer (Sigma-Aldrich Co.). Protein concentration was determined using the Bradford method then equal amounts of protein samples (30 μg) were mixed with sample buffer (100 mM Tris·HCl, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, and 20% glycerol) and boiled for 5 min. Proteins were separated by 10% SDS-PAGE and subsequently transferred onto a nitrocellulose membrane (Sigma-Aldrich Co, MO, USA). Membranes were blocked with 5% non-fat milk at room temperature for 60 min then were washed three times for 5 min with Tris-buffered saline with Tween 20 (TBS-T) and incubated overnight with the appropriate primary antibodies against Bcl-2, Bax and caspase-3 (1:100; Santa Cruz Biotechnology, CA, USA) at 4 °C. Subsequently, the membranes were washed three times with TBS-T and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000, Cell Signaling Technology Inc., MA, USA) for 2 h at room temperature. Reactions were revealed with the pierce™ enhanced chemiluminescence (ECL) substrate (Thermo Fisher Scientific, MA, USA). Loading accuracy was evaluated by membrane rehybridization with monoclonal antibody against β-actin (1:500; BioVision, CA, USA) with expected molecular weight of 26, 23, 32, and 43 kDa for Bcl-2, Bax, caspase-3, and β-actin, respectively. Densitometric analysis of the bands was performed by a Diana 95.1 camera (Raytest Isotopenmebgeräte GmbH) and analyzed by the Aida 2.1 software (Raytest Isotopenmebgeräte GmbH, Straubenhardt, Germany).

2.8. Histopathology

Immediately after dissection, kidneys (n = 3/group) were excised and fixed in 10% neutral buffered formalin solution. Samples were dehydrated in graded concentrations of alcohol, cleared in xylene, and embedded in paraffin. Blocks were cut at 5 μm thicknesses on a rotary microtome, mounted on slides, deparaffinized and stained with hematoxylin and eosin. Slides were evaluated for histological changes under light microscopy.

Further, histological scoring for the severity of kidney injury was graded on scale of 0 (no damage), 1 (mild damage), 2 (moderate damage), and 3 (severe damage). Scoring was based on congestion in cortical blood vessels, focal inflammatory cells infiltration in between cortical tubules and focal extravasation of red blood cells in between tubules in the corticomedullary portion. A semi-quantitative assessment of histological lesion was performed by adding histological scores for each parameter analyzed.

2.9. Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (IBM Corp, NY, USA). All data were expressed as mean ± SEM. Statistical significance of differences among the different studied groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. All p values were 2-sided and statistical differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of PRP on nephrotoxicity indices

Compared to control group, rats exposed to γ-radiation showed a significant decrease in the body weight gain by 49.82% ($p < 0.001$) accompanied with a significant increase in the kidneys/body weight ratio by 19.70% ($p < 0.001$) confirming the effectiveness of γ-radiation in causing renal damage. However, PRP pre-treatment was able to ameliorate this effect by increasing the weight gain and decreasing the kidneys/body weight ratio compared to the irradiated group by 86.83% and 12.66%, respectively ($p < 0.001$). Further, non-significant effects on the body weight gain ($p = 0.854$) and the kidneys/body weight ratio ($p = 0.980$) were observed in normal animals treated with PRP (Table 1).

Significant increases in serum creatinine and urea levels were observed in γ-irradiated rats as compared to controls with percentage increases of 270.37% and 118.35%, respectively ($p < 0.001$). On the other hand, γ-radiation induced a significant decline in serum albumin level in comparison with normal control by 29.47% ($p < 0.001$). Administration of PRP prior to irradiation was able to diminish the increase in serum creatinine and urea levels by 42.00% ($p < 0.001$) and 36.30% ($p = 0.001$), respectively. Moreover, PRP produced a significant increase in serum albumin levels by 23.50 ($p < 0.001$) (Table 2).

Moreover, γ-radiation induced a significant elevation in serum

Table 1
Effect of PRP on γ-radiation-induced changes in some indices of renal hypertrophy.

	Initial body weight (g)	Final body weight (g)	Changes in body weight (%)	Kidneys weight (g)	Kidneys/body weight (%)
Control	245.33 ± 1.84	265.33 ± 0.92	8.15 ± 0.70	1.76 ± 0.05	0.66 ± 0.01
PRP	239.67 ± 1.52	257.83 ± 0.79	7.58 ± 0.52	1.69 ± 0.01	0.65 ± 0.01
Radiation	230.17 ± 6.93	239.50 ± 6.65	4.05 ± 0.33[a]	1.90 ± 0.04	0.79 ± 0.01[a]
PRP + radiation	232.85 ± 7.18	250.58 ± 7.05	7.61 ± 0.44[b]	1.72 ± 0.06	0.69 ± 0.01[b]

Data are expressed as mean ± SEM. n = 7 for each experimental group. PRP: platelet rich plasma. In multiple comparisons.
[a] $p < 0.05$ *vs* normal control group.
[b] $p < 0.05$ *vs* irradiated control group.

Table 2
Effect of PRP on γ-radiation-induced changes in some markers of renal functions and acute kidney injury.

	Creatinine (mg/dl)	Urea (mg/dl)	Albumin (g/dl)	NGAL (ng/ml)	Renal *KIM-1* relative gene expression
Control	0.27 ± 0.01	33.34 ± 1.98	5.43 ± 0.12	2.50 ± 0.13	1.06 ± 0.01
PRP	0.20 ± 0.01	32.83 ± 2.71	5.37 ± 0.09	2.35 ± 0.12	1.30 ± 0.09
Radiation	1.00 ± 0.09[a]	72.80 ± 6.94[a]	3.83 ± 0.13[a]	19.00 ± 1.93[a]	8.11 ± 0.29[a]
PRP + radiation	0.58 ± 0.01[a,b]	46.37 ± 1.35[b]	4.73 ± 0.06[a,b]	9.73 ± 0.16[a,b]	3.99 ± 0.54[a,b]

Data are expressed as mean ± SEM. n = 7 for each experimental group. PRP: platelet rich plasma, NGAL: neutrophil gelatinase-associated lipocalin and KIM-1: kidney injury molecule-1. In multiple comparisons.
[a] $p < 0.05$ *vs* normal control group.
[b] $p < 0.05$ *vs* irradiated control group.

NGAL level by 6.6 folds ($p < 0.001$) and up-regulation in renal *KIM-1* gene expression by 6.65 folds ($p < 0.001$) as compared to control rats. On the other hand, γ-irradiated animals pre-treated with PRP showed a significantly lower level of serum NGAL (by 48.79%, $p < 0.001$) and down-regulation of renal *KIM-1* gene expression (by 50.80%, $p < 0.001$) compared to their γ-irradiated control group (Table 2).

3.2. Effect of PRP on oxidative stress parameters

The pre-treatment of normal animals with PRP produced a non-significant decrease in the spontaneous lipid peroxidation level in kidney tissues by 10.12%. Despite the renal MDA level was significantly increased in γ-radiation exposed rats compared to normal control (54.77 ± 5.31 *vs* 8.20 ± 1.30 μmol/g tissue, $p < 0.001$), PRP pre-treatment was able to diminish this increase by 59.70% ($p < 0.001$) (Fig. 1A). Further, the exposure to γ-radiation induced a significant increase in the renal nitrite levels (10.60 ± 1.17 *vs* 1.60 ± 0.06 μmol/g tissue, $p < 0.001$) which indicates the extent of NO. However, the pre-treatment of the exposed group using PRP decreased the tissue nitrite levels by 54.62% ($p < 0.001$) (Fig. 1B).

Although rats exposed to γ-radiation showed a significant decrease in their renal GSH content compared to controls (48.08 ± 5.99 *vs* 91.10 ± 4.31 μmol/g tissue, $p < 0.001$), PRP pre-treatment reversed this decrease by 59.32% ($p = 0.001$). Moreover, PRP produced a non-significant elevation in the renal level of GSH content in normal rats by 11.42% (Fig. 1C). In the same context, renal SOD and CAT activities were significantly decreased in γ-irradiated group compared to normal controls (2.59 ± 0.32 *vs* 6.77 ± 0.49 U/mg protein; $p = 0.001$ and 62.80 ± 6.16 *vs* 120.37 ± 3.55 U/mg protein; $p < 0.001$, respectively). Surprisingly, PRP pre-treatment succeeded to bring up the enzymes' activities nearly to the normal levels. Further, PRP produced a non-significant increase in the renal SOD and CAT activities in normal rats by 5.32% and 3.54%, respectively (Fig. 1D and E).

3.3. Effect of PRP on renal apoptosis

As evident from immunoblotting, the exposure to γ-radiation significantly increased the expression level of the pro-apoptotic proteins Bax and caspase-3 (by 3.83 and 5.41 folds, respectively, $p < 0.001$) and significantly decreased the expression level of the anti-apoptotic protein Bcl-2 (by 59%, $p < 0.001$) compared to controls. However, in rats receiving PRP prior to γ-irradiation, such alterations were significantly ameliorated by 51.34%, 44.30%, and 92.68%, respectively ($p < 0.001$).

Further, the Bcl-2/Bax ratio was significantly reduced by 90.73% in γ-irradiated rats compared to the controls ($p < 0.001$), while the ratio was 2.88 times higher in PRP pre-treated group than this in the irradiated group ($p < 0.001$) (Fig. 2).

3.4. Histopathological examination

To further characterize the nephrotoxicity induced by γ-radiation, histopathological examination of kidney tissues was done. No histopathological alteration and normal histological structure of the glomeruli, tubules, and blood vessels at the renal cortex was observed in both normal and PRP control groups (Fig. 3A and B). In contrast, histological examination of kidneys from γ-irradiated animals revealed severe congestion in the cortical blood vessels (Fig. 3C), associated with focal inflammatory cells infiltration in between the renal tubules at the cortex (Fig. 3D), and focal extravasation of red blood cells in between tubules in the corticomedullary portion (Fig. 3E). Pre-treatment with PRP ameliorated the cortical blood vessels congestion and minimized the architecture distracter of glomeruli and tubules at the renal cortex (Fig. 3F). Cumulative histological score of the severity presented in Table 3 showed that the total histopathological score was significantly higher in γ-irradiated group compared to the control group ($p < 0.001$). However, this damage was significantly attenuated in PRP pre-treated animals compared to the irradiated ones ($p < 0.001$).

4. Discussion

The ability to mitigate the severity of radiation-induced kidney injury could have a significant clinical impact in cancer therapy because as many as 10–30% of patients undergo radiotherapy develop kidney disease in the form of acute radiation nephritis, chronic radiation nephritis, malignant hypertension, or benign hypertension [29,30]. Accordingly and because of its healing properties attributed to the autologous growth factors and secretory proteins [31], this study aimed to assess the protective effect of PRP against kidney injury induced by γ-radiation in rats.

In the present study, γ-irradiated rats exhibited a significant decrease in the body weight gain which could be attributed to the fact that the intestines are highly radiosensitive organs, and acute radiation exposure is known to cause death of rapidly proliferating crypt cells, disruption of the epithelial barrier, and mucosal inflammation resulting eventually to decrease the food consumption [32]. Further, it is established that tissue injury elicits acute inflammation whose features, among others, include swelling of the affected part due to the accumulation of exudates particularly fluids, proteins, and cells from local vessels unto the damaged part [33] which can provide an explanation for the increase in kidney/body weight ratio in the γ-radiation group. PRP pre-treatment was notably able to ameliorate these alterations possibly *via* its anti-inflammatory activity. Previous studies support this hypothesis where PRP was reported to increase the intracellular expression of the anti-inflammatory mediators (IL-4, IL-10, and IL-13) known to play a major role in inhibiting inflammation; the anti-inflammatory role of PRP had been determined due to the presence of HGF [34,35].

The exposure to γ-radiation induces oxidative deamination of the amino acids, protein catabolism, alterations of the membranes' permeability, and damage of the tubular epithelium and other tubulointerstitial components, which deteriorate the kidney function [36–38]. This is supported by the results of the present study which showed a significant elevation in serum creatinine and urea levels

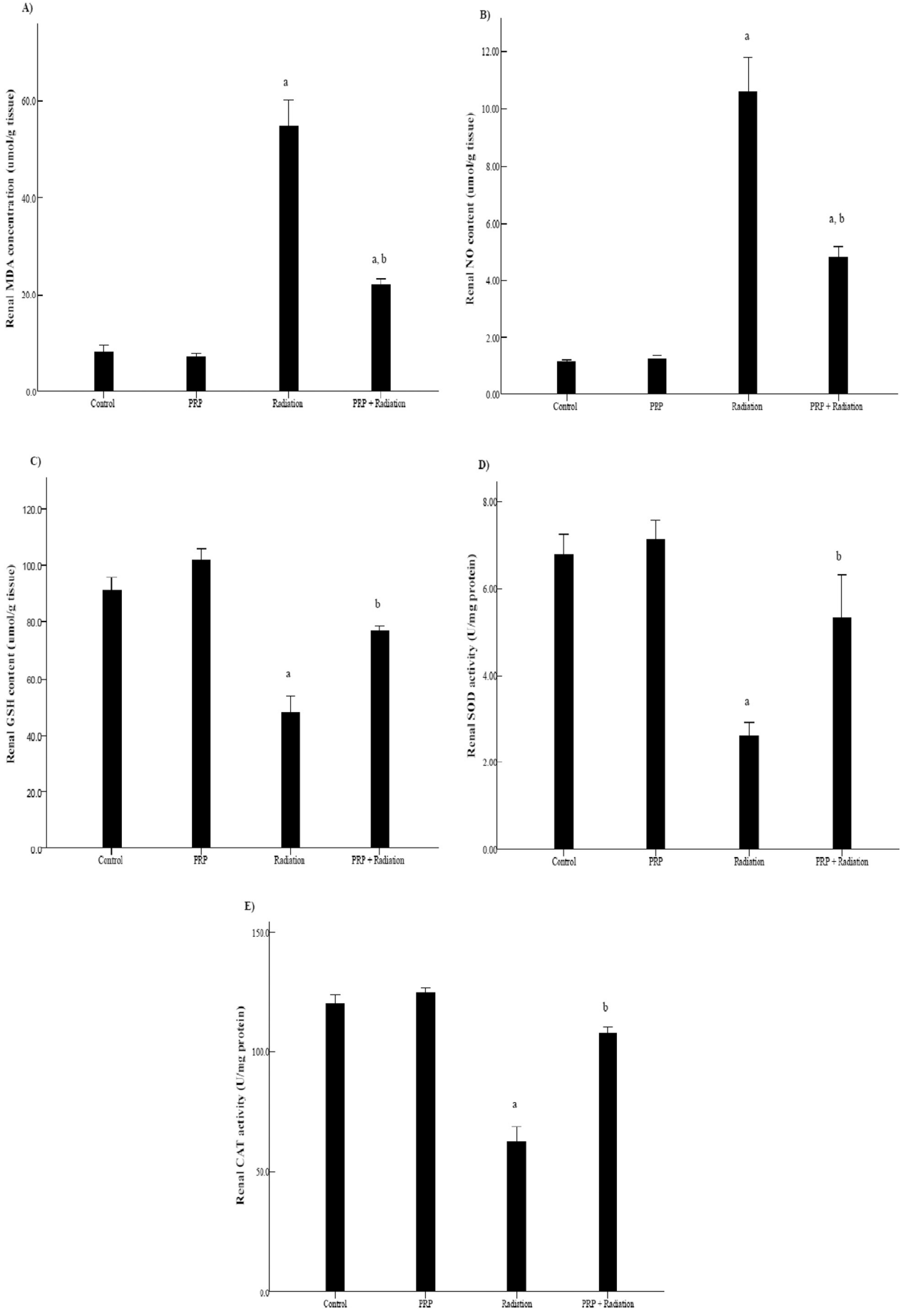

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Fig. 1. Effect of platelet rich plasma (PRP) on γ-radiation-induced changes in renal oxidative stress markers. A): Effect on malondialdehyde (MDA) level, B): Effect on nitric oxide (NO) content, C): Effect on reduced glutathione (GSH) content, D): Effect on superoxide dismutase (SOD) activity, and E): Effect on catalase (CAT) activity. In multiple comparisons, $^{a}p < 0.05$ *vs* normal control group, and $^{b}p < 0.05$ *vs* irradiated control group. Bar length represents mean value for each group with error bars depicting standard error of mean.

accompanied with a marked decrease in serum albumin level in γ-irradiated group designating a marked impairment of the kidney function. Furthermore, the current data revealed that the exposure to γ-radiation led to severe tubular injury as evidenced by the up-regulation of relative renal *KIM-1* gene expression and increased the serum NGAL level. KIM-1 is a type I transmembrane glycoprotein whose expression is undetectable in healthy kidneys but increases in proximal tubular cells following kidney injury which makes it a specific blood biomarker for acute and chronic kidney injuries [39]. NGAL is another marker expressed in several mammalian tissues at low levels, however; its level rapidly rises after the injury to renal tubular cells and is considered as one of the most promising biomarkers of severity in nephropathies [40].

The fact that PRP releases considerable quantities of GFs that enhance renal tubule cell regeneration, renal function restoration, and repair kidney structure and function after damage [17,20,22] in addition to the improved antioxidant status in the present study would provide a satisfactory explanation for the ability of PRP pre-treatment to attenuate the renal dysfunction and the tubular injury as evidenced by decreased the nephrotoxicity indices compared to irradiated control group. These results are in agreement with those of Salem et al. [41] who found that treatment with PRP significantly decreased the serum levels of creatinine, blood urea nitrogen, *N*-acetyl glucosaminidase (NAG) and KIM-1following cisplatin-induced nephrotoxicity. Nevertheless, other studies demonstrated the reverse; a non-significant difference of blood urea nitrogen and serum creatinine levels was observed between gentamicin-intoxicated rats treated with PRP and their gentamicin control group [42]. Similarly, Martín-Solé et al. [43] reported a non-statistical difference in serum creatinine and urea levels in PRP-treated rats seven days after ischemia compared to the ischemic control group.

The results of present work showed that the exposure to γ-radiation provoked an imbalance between oxidant and antioxidant species. This is in concordance with the results of Elkady and Ibrahim [36] who demonstrated that compared to the control group, adult male Swiss Albino rats showed a significant decrease in their renal CAT and glutathione peroxidase (GPx) activities and GSH content five days after whole body γ-irradiation at a single dose of 5 Gy. Further, Abozaid et al. [44] showed that the exposure of male Wistar rats to a single 6 Gy dose of whole-body γ-radiation resulted in a significant increase in renal content of MDA accompanied with an obvious depletion in GSH content and SOD activity in comparison with normal control group. Additionally, the exposure to whole body γ-radiation at a dose of 5 Gy resulted in a highly significant increase in the levels of lipid peroxides and a highly significant decline in the GSH content in kidneys of adult male Sprague-Dawley rats seven days post-irradiation compared with control rats [45].

The increased level of renal MDA might be probably due to the interaction between the hydroxyl group, formed as a by-product from water radiolysis, and the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes [46], Further, the elevated level of NO could be explained in the light of the fact that γ-irradiation results in direct DNA damage which activates poly ADP-ribose polymerase (PARP) that in turn induces the activation of the transcription factor nuclear factor kappa B (NF-κB) leading finally to increase the expression of inducible NO synthase (iNOS) and NO production [47]. Moreover, ROS can promote inflammation by stimulating NF-κB which in turn activates iNOS [48].

In an attempt to detoxify radiation generated free radicals, the utilization of the antioxidant system is enhanced which could explain the reduction of GSH content observed in γ-radiation group [49]. Furthermore, the significant decrease in the activities of the first line defense enzymatic antioxidants SOD and CAT can be explained by the interaction of the excess ROS with enzymes' molecules leading eventually to denaturation and partial inactivation of the enzymes which aggravates the free-radical mediated damage [50].

PRP pre-treatment markedly succeeded to reduce the levels of lipid peroxidation products alongside with NO content. Accordingly, it significantly increased the renal GSH content and the activities of SOD and CAT enzymes which indirectly indicates the significant reduction in lipid peroxidation. These findings are in accordance with those of Hesami et al. [51] who found that the treatment of rats with PRP five weeks after CCl_4-induced toxicity led to the reduction of hepatotoxicity probably due to lipid peroxidation inhibition and effective recovery of the antioxidant defense system. Further, Martins et al. [52] observed an improvement of the enzymatic and non-enzymatic antioxidant levels upon treatment of skeletal muscle injuries using PRP. In addition, a study by Bakacak et al. [53] reported that intraperitoneal PRP treatment decreased total oxidant status and oxidative stress index in ischemia and ischemia/reperfusion injury in rat ovary. It has been shown that PRP may prevent oxidative damage through the activation of transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant response element signaling [54]. Further, several GFs released from PRP can trigger cell activation and activate related signal path including the phosphatidylinositol-3 kinase (PI3K)/Akt pathway which can reduce ROS production and increase the level of resistance to oxidation [55–57].

Tissue injury both in tumors and normal tissues may occur by radiation *via* free radical-mediated DNA damage which is one of the most imperative deleterious effects of ionizing radiation that can trigger apoptosis [58,59]. Additionally, Flora et al. [60] demonstrated that increased ROS levels may cause an imbalance in Ca^{2+} regulation, which in turn induces an imbalance in anti- and pro-apoptotic proteins, such as Bcl-2 and Bax, leading to the release of cytochrome c from mitochondria for the activation of the terminal cascades of apoptosis. Moreover, Prise et al. [61] reported that radiation-induced recruitment of the intrinsic apoptotic pathway involves the release of cytochrome *c* from mitochondria leading to the sequential activation of caspase-9 and -3. Our study falls in line with the above findings, in which active apoptosis was induced by γ-radiation in renal cells as evidenced by the significant increase in the pro-apoptotic Bax protein level accompanied with the significant decrease in the anti-apoptotic Bcl-2 protein level which led accordingly to a significant decrease in Bcl-2/Bax ratio that is consistent with the increase in caspase-3 protein expression level.

Herein, PRP pre-treatment induced a significant decrease in the protein expression level of Bax and a significant increase in Bcl-2 expression level which subsequently caused a significant increase in Bcl-2/Bax ratio resulting in a significant decrease in the protein expression level of caspase 3. This concurs with the results of Moussa et al. [34] who observed that PRP significantly decreased the mRNA level of Bcl-2-associated death promoter (BAD) and caspase-3 and increased the mRNA level of Bcl-2 in human chondrocytes. Further, Salem et al. [62] demonstrated a significant elevation in the levels of the anti-apoptotic Bcl-2 marker following PRP infusion in the liver of dimethylnitrosurea-intoxicated rats compared to their corresponding controls.

The mechanism of action of PRP can be implied by the effect of its plentiful content of GFs. GFs and the adhesive glycoproteins secreted from the activated platelets interact with the cells by binding to their specific cellular membrane receptors. After that, they activate intracellular processes that stimulate cell proliferation, migration, and

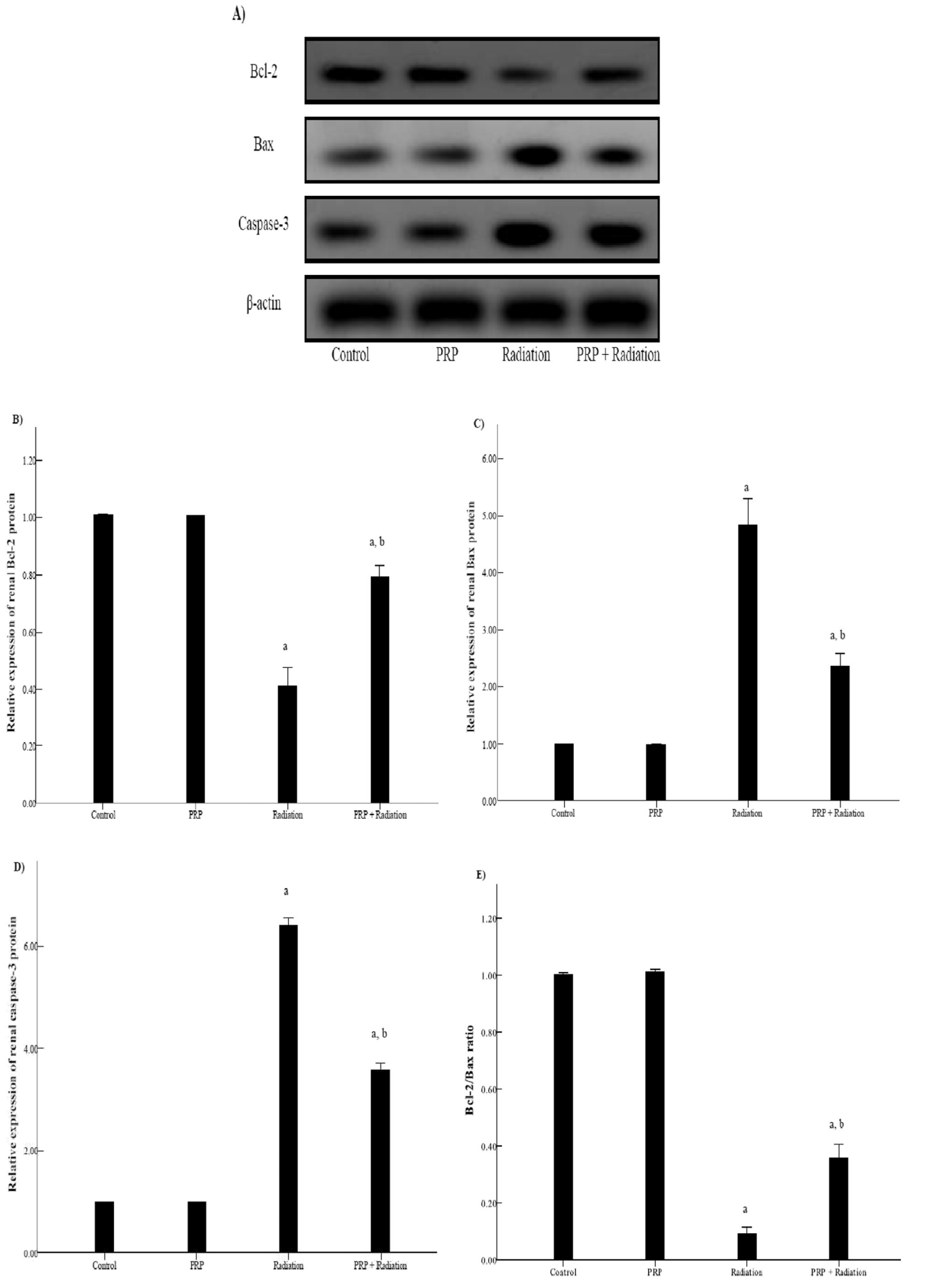

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Fig. 2. Effect of platelet rich plasma (PRP) on γ-radiation-induced changes in the renal expression level of apoptosis-related proteins, Bcl-2, Bax, and caspase-3. A): Western blots of Bcl-2, Bax, and caspase-3 in different experimental groups, B, C and D): Cumulative data obtained from densitometric analysis of Western blots expressed as ratios *vs* β-actin band intensities, E) Relative Bcl-2/Bax ratio. The relative protein level was expressed as 100% in control group. In multiple comparisons, [a]$p < 0.05$ *vs* normal control group, and [b]$p < 0.05$ *vs* irradiated control group. Bar length represents mean value for each group with error bars depicting standard error of mean.

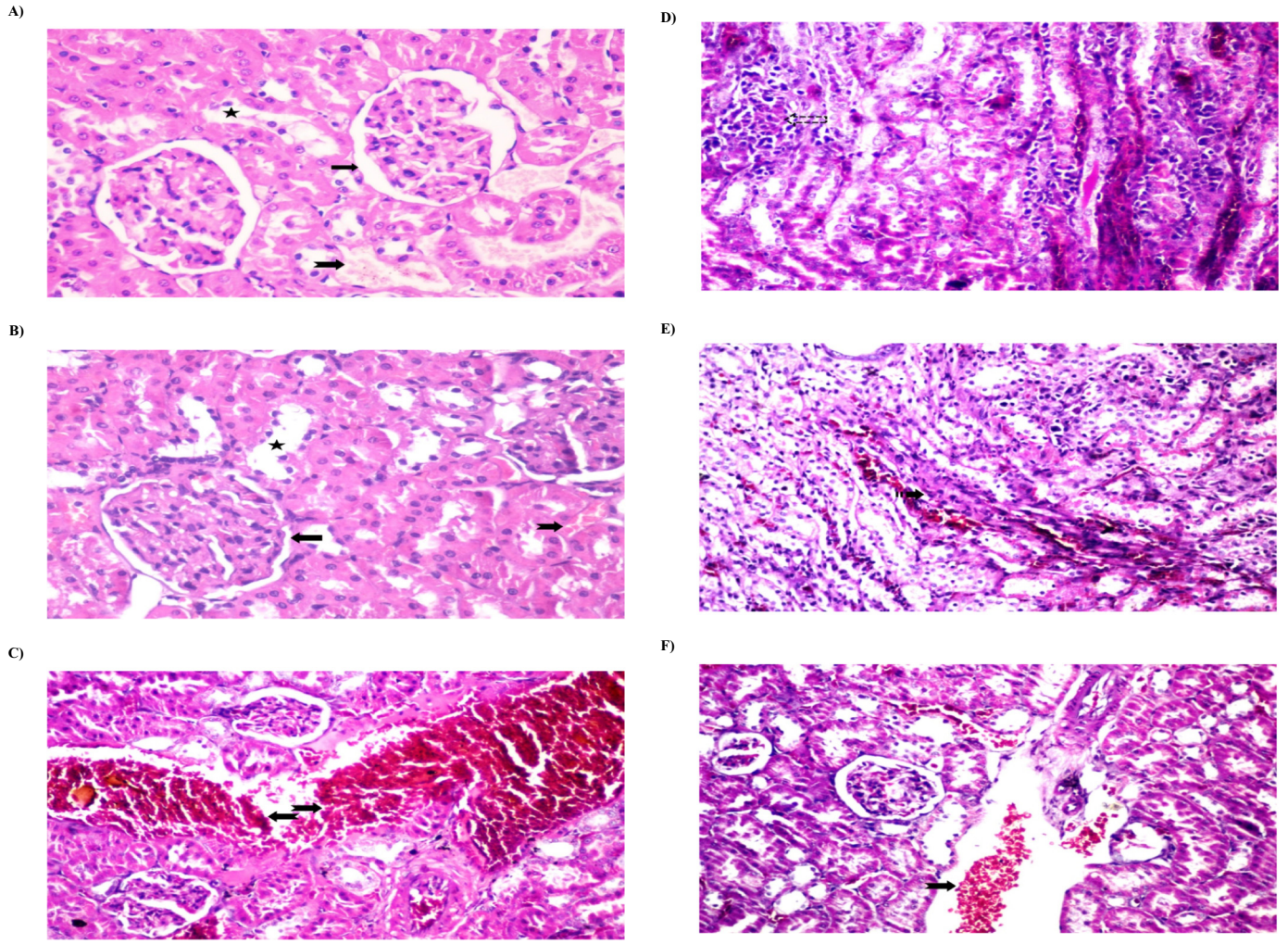

Fig. 3. Effect of platelet rich plasma (PRP) on γ-radiation-induced histological alterations of kidney tissue (40×). Photomicrographs of hematoxylin and eosin stained sections of kidney depicting no histopathological alteration and normal histological structure of the glomeruli (solid arrows), tubules (stars), and blood vessels (notched arrows) at the renal cortex in both normal and PRP control groups (A and B, respectively). γ-Radiation induced a severe congestion in the cortical blood vessels (notched arrows) (C), associated with focal inflammatory cells infiltration in between the renal tubules at the cortex (dashed arrow) (D), and focal extravasation of red blood cells in between tubules in the corticomedullary portion (striped arrow) (E). PRP pre-treatment ameliorated the cortical blood vessels congestion (notched arrow) and minimized the architecture distracter of glomeruli and tubules induced by γ-radiation at the renal cortex (F).

Table 3
Histological scores for the severity of renal injury in the different experimental groups.

	Control	PRP	Radiation	PRP + radiation
Congestion in cortical blood vessels	0.00	0.00	2.67	1.17
Focal inflammatory cells infiltration in between cortical tubules	0.00	0.00	2.00	0.00
Focal extravasation of red blood cells in between tubules in the corticomedullary portion	0.00	0.00	2.00	0.00
Total histological score	0.00	0.00	6.67 ± 0.21[a]	1.17 ± 0.17[a,b]

For each parameter and for each group, values represent the mean of scores obtained in three animals. Total histological score is expressed as mean ± SEM of cumulated histological scores obtained. PRP: platelet rich plasma. In multiple comparisons.

[a] $p < 0.05$ *vs* normal control group.

[b] $p < 0.05$ *vs* irradiated control group.

survival [63]. The downstream complex interactions of GFs present in PRP that has been reported as anti-apoptotic factors were confirmed. For example, the binding of IGF, EGF, and HGF to their cell-surface receptor seems to promote the survival of tubular cells by inhibiting apoptosis *via* activating the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) or PI3K/Akt signaling pathway and modulating Bcl-2 family protein expression [64–66].

Finally, our biochemical findings were confirmed by the histopathological examination of kidney tissues of γ-irradiated group which showed a severe congestion in the cortical blood vessels, associated with focal inflammatory cells infiltration in between the renal tubules at the cortex, and focal extravasation of red blood cells in between tubules in the corticomedullary portion. Meanwhile, these histopathological observations supported our hypothesis that PRP may protect the renal tissue from γ-radiation-induced nephrotoxicity as evidenced by the amelioration of the cortical blood vessels congestion and the minimized architecture distracter observed in the glomeruli and tubules at the renal cortex.

5. Conclusion

PRP exhibited effective protective effects against γ-radiation-induced renal injury on various levels which sheds the light on the possible applications of PRP during cancer radiotherapy.

Study limitation

Similar to other PRP studies, a critical limitation of this study is that the biologic effects of PRP depend on several factors such as the dose, application frequency and administration technique that should be verified with additional studies.

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Conflict of interest

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