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ORIGINAL ARTICLE

Differential effect of *Taraxacum officinale* L. (dandelion) root extract on hepatic and testicular tissues of rats exposed to ionizing radiation

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Abstract

Exposure to high doses of radiation negatively impacts on human organs. Dandelion (*Taraxacum officinale*) L. has been used as a traditional folk. This study was to investigate the effect of dandelion root extract (DRE) on radiation -induced hepatic and testicular tissues injury. Animals were exposed to 8.5 Gy of gamma radiation applied as a shot dose and DRE (200 mg/kg/day), was orally supplemented to rats 14 days before and after irradiation. The results showed that DRE administration attenuated oxidative stress in the liver and testis denoted by a significant reduction in the level of MDA and PCO with a marked elevation in GSH and the activity of SOD, CAT and Gpx. Moreover, DRE administration showed positive modulation in the activity of PNPase, GLDH and GSH-Ts. Additionally, these alterations were associated with a significant decrease in the activity of ALT, AST, ALP, and LDH with a marked increase of AL level. Further, elevated levels of testosterone, LH and inhibin B, as well as StAR and P450scc gene expression and Zn level with a decrease of FSH level were noticed. Also, DRE reduced the level of IL-1β, TNF-α, and caspase-3. Also administration of DRE significance diminished the histopathological changes in the hepatic and testicular tissues, denoted by a reduction in the necrotic and degenerative changes of hepatocytes or fibrinoid necrosis of congested central vein and improving the seminiferous tubules and interstitial tissue between the tubules of the testis. In conclusion, treatment with DRE pre-irradiation is effective on both liver and testicular tissues of rats. Meanwhile, in the case of post-radiation administration, DRE was more effective on testicular tissue than liver. So we suggest that it is better to use the dandelion before exposure to radiation rather than after it.

Keywords Ionizing radiation · Dandelion · Liver · Testes

Abbreviations

MDA	Malondialdehyde
PCO	Protein carbonyl
SOD	Superoxide dismutase
CAT	Catalase
Gpx	Glutathione peroxidase
GSH	Glutathione
PNPase	Purine nucleoside phosphorylase
GLDH	Glutamate dehydrogenase
GSH-Ts	Glutathione-S-transferases
AL	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
LDH	Lactate dehydrogenase
FSH	Follicular stimulating hormone
LH	Letulizing hormone
Zn	Zinc
StAR	Steroidogenic acute regulatory protein
P450scc	Cholesterol side-chain cleavage enzyme

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IL-1β	Interleukin-1beta
TNF-α	Tumor necrotic factor alpha
DRE	Dandelion root extract
SEL	Sesquiterpene
TS	Lactones taraxasterol
CGA	Taraxerol, chlorogenic acid
CRA	Chicoric acid

Introduction

Exposure to ionizing radiation (IR) negatively affects human health, elevating the hazards of tissue damage [1]. IR comes from the medical applications and various earthy sources and is considered to be one of the most risk factors that stimulate oxidative stress (OS), whereby reactive oxygen and nitrogen species (ROS and RNS) react with the intercellular molecules, promoting diverse changes such as DNA damage, genomic instability, leading to the beginning and development of tissue injury [2]. Additionally, the change in the cellular redox state stimulates several antioxidant enzymes redox, which are critial in signaling mechanisms inside the cell.

The liver is an important tissue because it is responsible for several vital processes, sensitive to many forms of injury and has the capability to repair itself. However, liver tissue toxicity following exposure to radiation causes various pathological changes like an increase in ROS, and an increase in the level of inflammatory markers, which further cause failure of hepatic cells to function [3].

Moreover, previous findings documented that male infertility is linked to exposure to toxic chemicals, radiation, and other environmental pollutions [4]. Testicular tissue is considered to be one of the most radiosuscepitable organs due to its highly active without cell regeneration system and antioxidant defense. It can be stimulated by radiation through the production of free radicals that cause disruptions in the normal metabolism, proliferation, and differentiation of the testis, which result in abnormalities in the spermatogenesis and infertility [5].

Testosterone related to the sex hormones that react with androgen receptors. In males, testosterone is naturally produced from the cholesterol through multiple enzymatic mechanisms, mostly in the Leydig cells of the testes and the adrenal glands [6]. In addtion, Increased biosynthesis of testosterone and high plasma levels depend on the regulation of steroidogenic acute regulatory protein (StAR) and the catalyzing Cholesterol side-chain cleavage enzyme (P450scc). StAR protein regulates the transferring of cholesterol from the external mitochondrial membrane to the inner membrane [7], where, P450scc increases the conversion of cholesterol to pregnenolone, and this process is considered to be the first step, whereby the steroidogenesis process begins to produce various steroid hormones [8]. Furthermore, previous finding by Sivakumar et al. [9] in which radiation exposure could induce steroidogensis impairment and hormonal changes in the culture testicular cells of human.

The radioprotectors and radiomitigators could reduce the hazards effects induced by exposure of the radiation. Application of traditional plants has demonstrated potential to alleviate symptoms of illness involving oxidative stress and improve health [10].

It is well documented that medicinal plants have the ability [11] to overcome the deleterious effects of oxidative stress due to their several antioxidant components.

Taraxacum officinale, commonly called dandelion, is a herbaceous perennial belonging to family *Asteraceae*. It was native to Eurasia, but it is now found in several areas around the world [12, 13]. It has been used as a traditional and modern herbal medicine system in many countries. Dandelion was included as a diuretic plant in the US pharmacopeia from 1831 to 1926 and the traditional Chinese, India medicine merges it with other herbs to enhance the immune system, to treat gallbladder disorders and hepatitis, and also to use as a topical compress to treat mastitis [14]. Dandelion is rich in flavonoids and ploypheno lic compounds [15] (such as sesquiterpene lactones, taraxasterol (TS), taraxerol, chlorogenic acid (CGA), and chicoric acid (CRA), vitamins (such as A, C, D, E, and B), inositol, lecithin, and minerals (such as iron, magnesium, sodium, calcium, silicon, copper, phosphorus, zinc, and manganese) [16]. These components are non-toxic and can be used as anti-inflammatory, diuretic, digestive stimulant, insulin stimulant, anti-neoplastic, anti-diabetic and antioxidative effects to protect against hepatic and testicular damage [17–21]. In contrast, other authors exhibited the side effects of dandelion on the testis [22].

Accordingly, in this study, we aims to evaluate the role of dandelion root extract on the hepatic and testicular injury induced by exposure to radiation.

Materials and methods

Experimental animals

Male Wistar rats (220 ± 10 g) obtained from the Atomic Energy Authority, National Centre for Radiation Research and Technology (NCRRT) were used in the current study. Animals were kept under standard conditions of ventilation, temperature, humidity, lighting (light/dark: 13 h/11 h) for 1 week before the beginning of the experiment. Rats were under standard diet with pellets including all alimentary elements. Food and water were available ad libitum.

Ethics approval

The study was approved by Research Ethics Committee (REC) for experimental studies for experimental studies (Human and Animal subjects) at the National Centre for Radiation Research and Technology-Egyptian atomic Energy Authority, Cairo, following the 3Rs principles for animal experimentation (Replace, Reduce and Refine) and is organized and operated according to CIOMS and ICLAS International Guiding Principles for Biomedical Research Involving Animals 2012. Serial number of the study 5A/19.

Gamma-irradiation

Gamma irradiation of rats was done at National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt via a Canadian Gamma Cell-40 (Cs^{137}). The rat's whole body was exposed to gamma rays at a dose of 8.5 Gy as an acute single dose and the dose rate was 0.66 Gy/s throughout the experimental periods. We obtained permission from the publishing committee (Atomic Energy Authority-Radiation Research and Technology (NCRRT)-Central Scientific Publishing Committee and was approved No.179.

Dandelion (*T. officinale* L.) preparation and treatment

Dandelion root was purchased from Alma Natural Herbs Co., Egypt. Common name: dandelion, Botanical name: *T. officinale* (L.) Weber ex F.H. Wigg. Plant Family: Asteraceae, Origin: USA. The plant was authenticated by a plant taxonomist and a voucher specimen was deposited at the herbarium of the Departmentof plant, Cairo University (N65724). 100 gm of root was mixed in 200 ml of distilled water and homogenized using a blender. The homogenate was filtered and centrifuged at 8000×*g* for 5 min at 25 °C. The supernatant was filtered using 0.45 µm filters, followed by lyophilisation. The dry powder was dissolved in water to get a stock solution of 100 mg/ml DRE [23] and the dose was administrated (200 mg/kg/day) for 14 consecutive days [24]. The treatment of DRE 14 days was selected according to the previous studies on the effect of antioxidants as radioprotectors or radiomitigators on radiation-induced tissue damage [25–27].

Experimental design

Rats were divided into five groups of six rats. Control group: rats received distilled water during 14 consecutive days via gavages; Dandelion group: rats received DRE (200 mg/kg b.wt) daily during 14 days via gavages; IR: rats exposed to 8.5 Gy of gamma rays; DRE + IR: rats received DRE (200 mg/kg) for 14 days, then were exposed to irradiation 8.5 Gy; IR + DRE: rat's whole body was irradiated, then received DRE (200 mg/kg) for 14 days, 1 h post- irradiation.

Sample preparation and biochemical analysis

Rats were sacrificed after a fasting period of 12 h the 15th day post exposure to irradiation. Blood samples were collected through the heart puncture by sterilized syringe, liver and testis tissues were rapidly excised. The blood was left to coagulate to obtain serum after centrifugation at 3000×*g* for 15 min. Liver and testis tissues (10% w/v) were homogenized in phosphate-buffered-saline (0.02 M sodium phosphate buffer with 0.15 M sodium chloride, pH 7.4) using Teflon homogenizer (Glass-Col, Terre Haute, Ind., USA) and after centrifugation at 10,000×*g* for 15 min using refrigerated centrifuge (K3 Centurion Scientific, Ltd, London, UK), the supernatant was stored at − 80 for further analysis. Measurement of absorbance was performed using a T60 UV/VIS spectrophotometer, PG instruments, London, UK.

Assessment of redox state in the hepatic and testicular tissues

Malondialdehyde (MDA) was assessed according to the method of Ohkawa et al. [28] using Assay Kit Cat. No. MAK085 from Sigma Aldrich, St Louis, MO, USA. The method is based on the reaction of MDA end product of lipid peroxidation with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen that can be measured spectrophotometrically at 532 nm. Protein carbonyl (PCO) was assayed according to the method of Levine et al. [29] using assay Kit Cat. No. MAK094 from Sigma Aldrich, St Louis, MO, USA. The principle of the method is depended on the formation of a Schiff base from the reaction of dinitrophenylhydrazine with protein carbonyls to form protein hydrazones which was measured spectrophotometrically at 375 nm. Liver total Glutathione-S-transferase activity (GSH-T) was determined using glutathione S-transferase assay Kit Cat. No. CS0410 from Sigma Aldrich Co., the assay is based on the conjugation of 1-Chloro-2,4-dinitrobenzene (CDNB) with the thiol group of glutathione in the presence of GST to form the CDNB substrate, which absorbs at 340 nm. The rate of increase in the absorption is directly proportional to the GSH-T activity in the liver. Glutathione content (GSH) was determined according to the method of Beutler et al. [30] and the method is based on the reaction of GSH with DTNB (5,5-dithiobis 2-nitrobenzoic acid) to give a yellow colored compound to absorb at 412 nm. The activity of Catalase (CAT) was determined according to Aebi [31], the method is based on the catalytic function of the enzyme where it catalyzes the decomposition of H_2O_2 into water and oxygen. Superoxide dismutase (SOD) activity was measured by Nishikimi et al.

[32] method and was modified by Kakkar et al. [33]. Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 min, under study conditions. Glutathione peroxidase (Gpx) was measured according to Paglia and Valentine [34] and the method is relied on the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione. Determination of antioxidant molecule and enzymes using commercial kits (Biodiagnostic, Egypt).

Assessment of liver injury markers

Serum purine nucleoside phosphorylase (PNPase) activity was estimated using Rat PNPase ELISA assay Kit MBS749080, from MyBioSource. Serum glutamate dehydrogenase (GLDH) was estimated using GLDH activity assay Kit Cat. No. MAK099 (Sigma Aldrich Co.). Alkaline phosphatase (ALP) was assayed according to the method of Belfield and Goldberg [35]. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), activities were measured according to Reitman and Frankel [36] and albumin (AL) was determined according to Doumas et al. [37], using assay Kits (Biodiagnostic, Egypt). Lactate dehydrogenase (LDH) was determined using LDH activity assay Kit Cat No. MAK066 (Sigma Aldrich Co.)

Assessment of testicular injury markers

Serum testosterone was determined using Rat Testosterone Enzyme-Linked Immunosorbent Assay (ELISA) Kit Cat. No. SE120089 from Sigma Aldrich, St Louis, MO, USA. Serum concentration of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined using Rat LH ELISA Kit Cat. No.CSB-E12654r and Rat FSH ELISA Kit Cat. No. CSB-E06869r, respectively from CUSABIO. These assays employ the competitive inhibition enzyme immunoassay technique.

Assessment of inflammatory and apoptosis markers in the hepatic and testicular tissue

Tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and caspase 3 were determined using Rat TNF-α ELISA Kit Cat. No. CSB-E08055r, Rat IL-1β ELISA Kit Cat.No. CSB-E08055r and Rat Casp-3 ELISA Kit Cat. No. CSB-E08857r, respectively from CUSABIO.

Detection of StAR and P450scc, gene expression using real time-polymerase chain reaction (RT-PCR)

RNA extraction

Total RNA was isolated from testicular tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. The purity (A260/A280 ratio) and the concentration of RNA were detected by using spectrophotometry (Gene Quant 1300, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis.

cDNA synthesis

5 µg RNA was reverse transcribed using oligonucleotide (dT) 18 primer [final concentration, 0.2 mM and was denatured at 70 °C for 2 min. Denatured RNA was kept on ice and reverse transcription mixture including 50 mM KCl, 50 mM Tris HCl (pH 8.3), 0.5 mM of deoxyribonucleotide triphosphate (dNTP), 3 mM MgCl2, 1U/ml RNase inhibitor, and 200 units of Moloney murine leukemia virus reverse transcriptase. The reaction tube was placed at 42 °C for 1 h, followed by heating to 92 °C to stop the reaction.

Real-time quantitative polymerase chain reaction

Real-time PCR (RT-PCR) amplification was carried out using 10 µL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), equivalent to 8 ng of reverse-transcribed RNA and 300 nM of each primer as shown in the Table 1. PCR reactions consisted of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles) were performed on step one plus real-time PCR system (Applied Biosystems). Data were analyzed with the ABI Prism sequence detection system software and quantified using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA, USA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to β-actin, which was used as the control

Table 1 The primer sequence of the studied genes

	Primer sequence
StAR	Forward primer: 5′-TCA GAG TAG CAG CTC CCT TGT TTG-3′ Reverse primer: 5′-CTC CAA ATC CTG AAA CGG GAA TGC-3′
P450scc	Forward primer: 5′-AGAAGCTGGGCAACATGGAGTCAG-3′ Reverse primer: 5′-TCACATCCCAGGCAGCTGCATGGT-3
Beta actin	Forward primer: 5′-CAA CCG TGA AAA GAT GAC CCA G-3′ Reverse primer: 5′-ATG GGC ACA GTG TGG GTG AC-3′

housekeeping gene and reported as fold change over background levels detected in the diseased groups.

Histopathological study

Tissue specimens from liver and testes were collected and fixed in 10% buffered formalin solution followed by dehydration, clearing and embedding in paraffin. Paraffin sections of 5 µm thickness were prepared and stained routinely with haematoxylin and eosin according to Bancroft and Stevens [38] and examined microscopically.

Statistical analysis

All the values are expressed as mean ± standard deviation (SD). Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test to determine the significant differences between means. The significance levels were set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

Effect DRE on hepatic and testicular tissues redox state

In the current study, exposure of male albino rats to γ-rays (a dose of 8.5 Gy) has triggered oxidative stress, demonstrated by a significant elevation ($p \leq 0.001$) in the level of MDA and PCO, with a significant reduction ($p \leq 0.001$) in the activity of SOD, CAT and Gpx and GSH content, compared to their respective values in the liver and testis of control rats (Tables 2, 3). DRE treatment has the ability to attenuate the oxidative damage via diminishing the elevation of MDA and PCO levels, additionally to DRE reversed the decrease of GSH content and SOD,Gpx and CAT activity ($p \leq 0.001$), compared to their respective values of hepatic and testicular tissues of irradiated rats.

Table 2 Effect of DRE (200 mg/kg) on the level of oxidant and antioxidant markers in the liver tissue of different animal groups

Parameters groups	MDA (nmol/g)	PCO (nmol/g)	GSH (mg/g)	Gpx (mg consumed GSH/min/g)	SOD (U/g)	CAT (U/g)
Control	33 ± 2	234 ± 31	1.60 ± 0.09	61 ± 5	75 ± 7	194 ± 20
DRE	31 ± 2 (−6)	230 ± 29 (−2)	1.62 ± 0.09 (+1)	62 ± 5 (+2)	72 ± 7 (−4)	199 ± 22 (+3)
IR	67 ± 5[a3] (+103)	390 ± 39[a3] (+67)	0.80 ± 0.04[a3] (−50)	35 ± 3[a3] (−43)	48 ± 3[a3] (−36)	92 ± 10[a3] (−53)
DRE + IR	39 ± 3[a1b3] (+18)	270 ± 30[a1b3] (+15)	1.39 ± 0.08[a1b3] (−13)	52 ± 4[a1b3] (−15)	65 ± 7[a1b3] (−13)	165 ± 14[a1b3] (−15)
IR + DRE	46 ± 5[a2b3] (+39)	300 ± 33[a2b3] (+28)	1.40 ± 0.07[a1b3] (−13)	45 ± 3[a2b3] (−26)	61 ± 6[a1b3] (−19)	136 ± 12[a2b3] (−30)

Values are expressed as Mean ± SD ($n = 6$). Values between brackets show percentage of change from control

[a]Significance versus control

[b]Significance versus irradiated group (IR). Differences between means were considered significant (a1,b1) at $p \leq 0.05$, highly significant (a2,b2) at $p \leq 0.01$ and very highly significant (a3,b3) at $p \leq 0.001$

Table 3 Effect of DRE (200 mg/kg) on the level of oxidant and antioxidant markers in the testicular tissue of different animal groups

Parameters groups	MDA (nmol/g)	PCO (nmol/g)	GSH (mg/g)	Gpx (mg consumed GSH/min/g)	SOD (U/g)	CAT (U/g)
Control	49 ± 4	267 ± 35	66 ± 6	55 ± 5	71 ± 7	144 ± 18
DRE	48 ± 4 (+2)	260 ± 34 (−3)	67 ± 6 (+2)	57 ± 5 (+4)	73 ± 7 (+3)	149 ± 22 (+3)
IR	90 ± 8[a3] (+84)	427 ± 49[a3] (+60)	31 ± 3[a3] (−53)	25 ± 2[a3] (−55)	41 ± 3[a3] (−42)	82 ± 10[a3] (−53)
DRE + IR	56 ± 5[a1b3] (+14)	315 ± 39[a1b3] (+18)	57 ± 5[a1b3] (−14)	50 ± 5[a1b3] (−9)	60 ± 5[a1b3] (−15)	125 ± 14[a1b3] (−13)
IR + DRE	58 ± 7[a1b3] (+18)	290 ± 47[a1b3] (+9)	56 ± 4[a1b3] (−15)	48 ± 3[a1b3] (−13)	64 ± 5[a1b3] (−10)	129 ± 12[a1b3] (−10)

Values are expressed as Mean ± standard deviation ($n = 6$). Values between brackets show percentage of change from control

[a]Significance versus control

[b]Significance versus irradiated group (IR). Differences between means were considered significant (a1,b1) at $p \leq 0.05$, highly significant (a2,b2) at $p \leq 0.01$ and very highly significant (a3,b3) at $p \leq 0.001$

Effect of DRE on hepatotoxicity marker enzymes

Rats exposed to γ -radiation had a significantly increased ($p \leq 0.001$) activity serum PNPase and GLDH, while a marked decrease ($p \leq 0.001$) in the level of hepatic GSH-T were noticed as markers of liver necrosis and injury, compared to their respective values in the liver. Meanwhile, DRE treatment was able to repair the live injury through reducing the enzymatic activity of PNPase and GLDH and restoring the activity of GSH-T ($p \leq 0.001$), compared to their respective values of irradiated rats (Fig. 1A–C).

Further, rats exposed to gamma irradiation (8.5 Gy) caused a significant elevation ($p \leq 0.001$) in the activity of AST, ALT, ALP and LDH, as well as a noticeable decrease ($p \leq 0.001$) of albumin level, compared to their respective values of control rats. Meanwhile, DRE treatment motivated a marked reduction ($p \leq 0.001$) in the serum activities of AST, ALT and ALP and LDH with a significant increase ($p \leq 0.001$) of albumin level, compared to their respective values of irradiated rats (Table 4).

Effect of DRE on the inflammatory markers

In the current study, rats exposed to irradiation showed a significant increase ($p \leq 0.001$) in the level of inflammatory and apoptotic markers (TNF-α, IL-1β, caspase-3) of hepatic and testicular tissue, compared to their respective values of control rats. DRE treatment significantly decreased ($p \leq 0.001$) the levels of TNF-α, IL-1β, caspase-3, confirming the anti-inflammatory and antiapoptotic properties of DRE (Figs. 2, 3A–C).

Effect of DRE on testicular tissue injury marker

As shown in Fig. 4A–E, a marked increase ($p \leq 0.001$) in the level of FSH, associated with a significant decrease ($p \leq 0.001$) in the level of inhibin B, testosterone, LH and Zn were observed in rats exposed to irradiation. As shown in Fig. 5A, a significant decrease in the level gene expression of StAR mRNA and P450scc as indicators of testicular damage, compared to their respective values of control rats. In contrast, the administration of DRE to irradiated rats has significantly attenuated the severity of oxidative

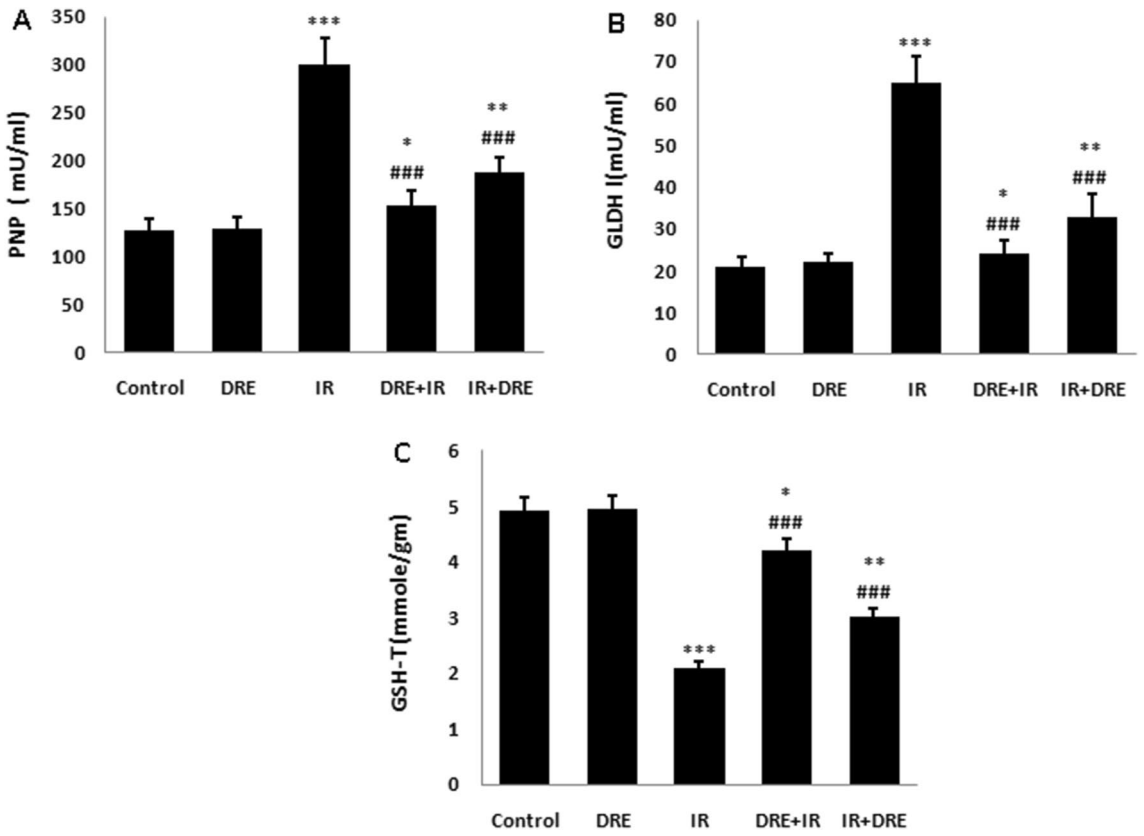

Fig. 1 Effect of DRE (200 mg/kg/day) on serum levels of PNP (**A**), GLDH (**B**) and liver GSH-T (**C**) in the animal groups. Values are expressed as Mean ± SD ($n=6$). *Significance versus control. $^{\#}$significance versus irradiated group (IR). Differences between means were considered significant ($^{*,\#}$) at $p \leq 0.05$, highly significant ($^{**,\#\#}$) at $p \leq 0.01$ and very highly significant ($^{***,\#\#\#}$) at $p \leq 0.001$

Table 4 Effect of DRE (200 mg/kg) on the liver biomarkers injury of different animal groups

Parameters groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Albumin (g/dL)	LDH (IU/L)
Control	18 ± 2	22 ± 2	117 ± 23	4.90 ± 1.0	1440 ± 200
DRE	19 ± 2 (+1)	20 ± 2 (−1)	118 ± 24 (+1)	5.00 ± 1.30 (+2)	1421 ± 213 (−1)
IR	35 ± 4[a3] (+94)	44 ± 4[a3] (+118)	299 ± 38[a3] (+156)	2.17 ± 0.8[a3] (−56)	2590 ± 320[a3] (+80)
DRE + IR	22 ± 2[a1b3] (+22)	25 ± 3.7[a1b3] (+14)	138 ± 41[a1b3] (+18)	4.20 ± 1.1[a1b3] (−14)	1595 ± 188[a1b3] (+11)
IR + DRE	23 ± 2[a2b3] (+28)	27 ± 3.8[a1b3] (+23)	155 ± 29[a2b3] (+32)	3.07 ± 0.9[a2b3] (−37)	1635 ± 180[a1b3] (+14)

Values are expressed as Mean ± SD (n = 6). Values between brackets show percentage of change from control

[a]Significance versus control

[b]Significance versus irradiated group (IR). Differences between means were considered significant (a1,b1) at $p \leq 0.05$, highly significant (a2,b2) at $p \leq 0.01$ and very highly significant (a3,b3) at $p \leq 0.001$

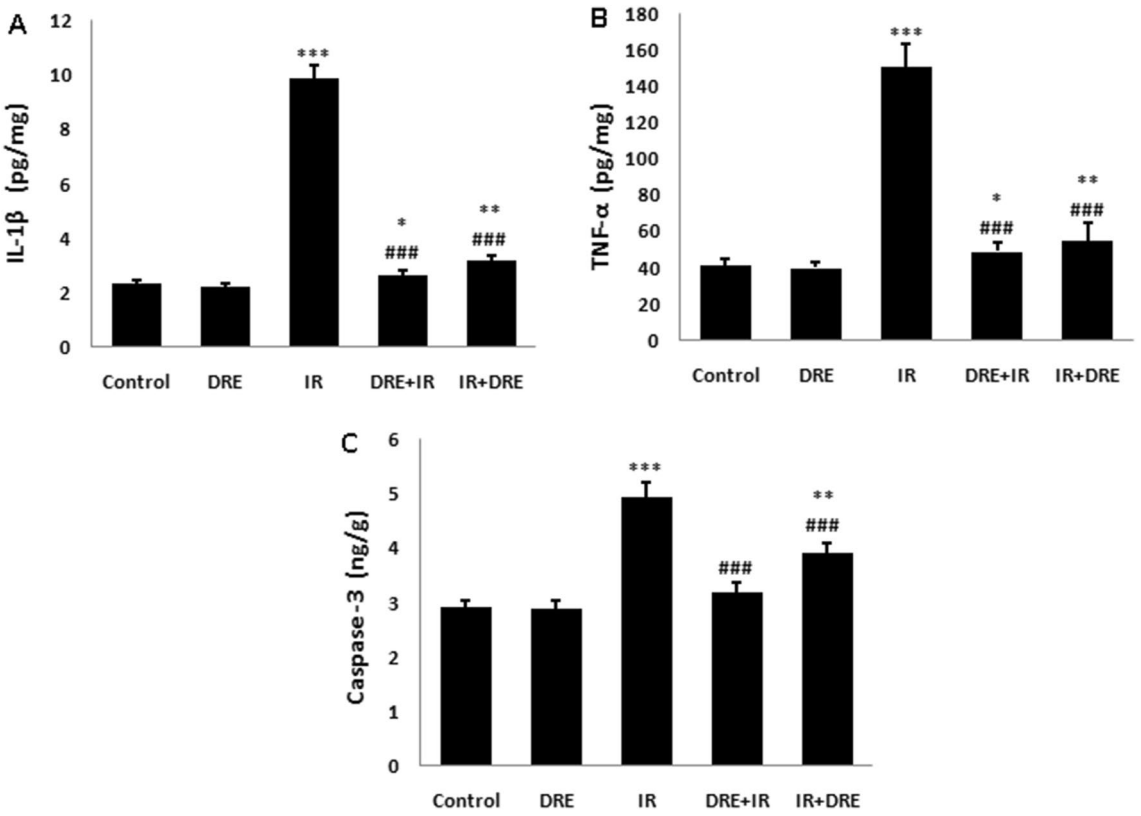

Fig. 2 Effect of DRE (200 mg/kg/day) on the levels of IL-1β (**A**), TNF-α (**B**) and caspase-3 (**C**) in the hepatic tissue of different animal groups. Values are expressed as Mean ± SD (n = 6). *Significance versus control. #Significance versus irradiated group (IR). Differences between means were considered significant (*,#) at $p \leq 0.05$, highly significant (**,##) at $p \leq 0.01$ and very highly significant (***,###) at $p \leq 0.001$

damage in the testis by a significant decrease ($p \leq 0.001$) of FSH and a marked increase ($p \leq 0.001$) of inhibin B, testosterone and LH and Zn (Fig. 4A–E). Additionally, DRE treatment significantly increased testicular mRNA gene expression of StAR and P450scc (Fig. 5A, B).

Histopathological results

The hepatic tissue of normal group showed anastomosing cords which separated by sinusoids. The liver cells showed polygonal, differ in size, contain large, round nucleus and might occasionally be binucleate. The cells have a granular acidophilic cytoplasm (Fig. 6A). In DRE group, the

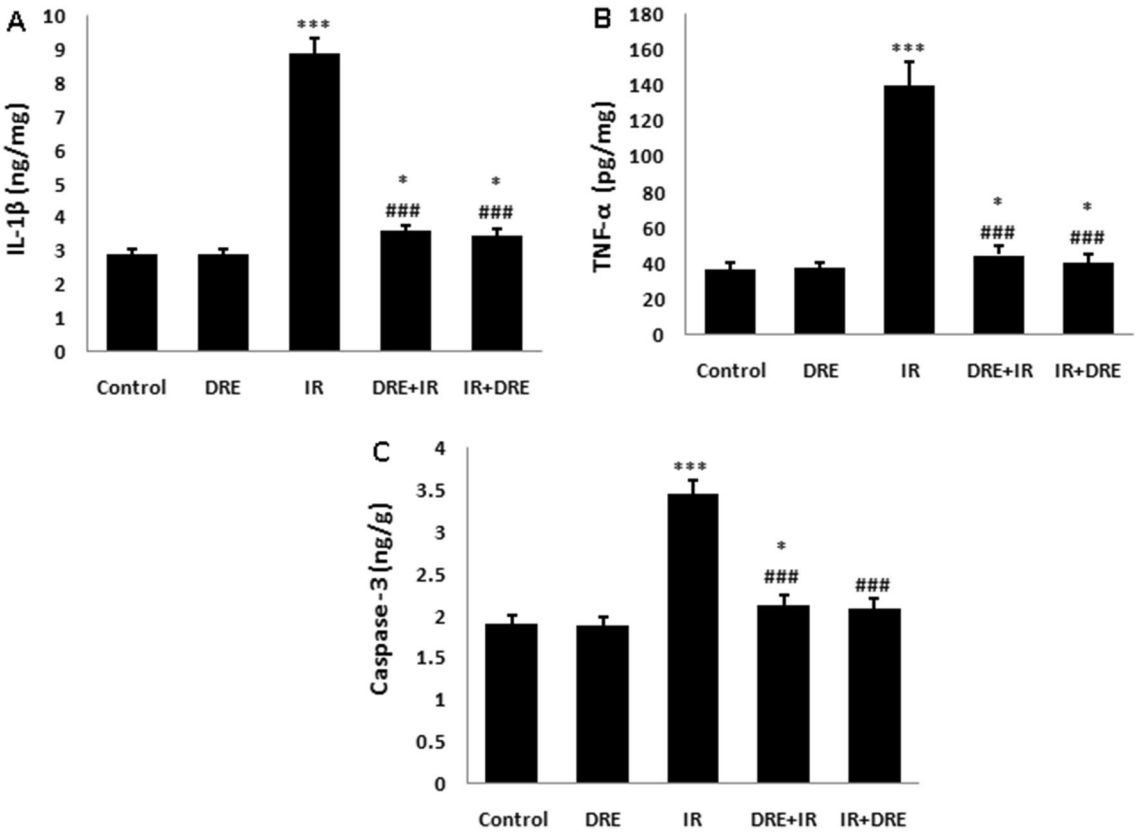

Fig. 3 Effect of DRE (200 mg/kg/day) on the levels of IL-1β (**A**), TNF-α (**B**) and caspase-3 (**C**) in the testicular tissue of different animal groups. Values are expressed as Mean ± SD ($n=6$). *Significance versus control. #Significance versus irradiated group (IR). Differences between means were considered significant ($^{*,\#}$) at $p \leq 0.05$, highly significant ($^{**,\#\#}$) at $p \leq 0.01$ and very highly significant ($^{***,\#\#\#}$) at $p \leq 0.001$

hepatic tissues displayed normal structure as control rats (Fig. 6B). While, the liver of irradiated rats showed an apparent widespread swelling and expanding of hepatocytes owing to various degeneration changes and might be progressive to necrosis changes within the lobules (Fig. 6C). Moreover, portal area showed congestion portal vein with fibrinoid necrosis, numerous bile ductulas, periductular fibrous beside degenerative and necrosis changes in hepatocytes (Fig. 6D, E). On other hand, most cases of rats received DRE before γ-rays exposure showed the histological structure of hepatic tissues relatively well conserved architecture without necrosis, degenerative changes and dilated blood vessels. Few cases in this group showed mild reversible degenerative changes in hepatocytes (Fig. 6F). While in rats received DRE after γ-rays exposure, the hepatic tissues showed mild reversible lesions as congested central vein, microstatosis (fatty changes) and hydropic degeneration of hepatocytes (Fig. 6G).

Testicular tissue in control group is surrounded from outside by a thick connective tissue capsule called tunica albuginea, inside it many circular or ovoid structures with a thick wall called seminiferous tubules, which lined with a specialized stratified epithelium called the germinal epithelium that consists of the spermatogenic and supporting or sertoli cells. These cells rest on a thin basement membrane. Between the tubules is the interstitial tissue consisting of fibroblasts, blood vessels, nerves, lymphatic and interstitial (Leyding) cells (Fig. 7A). In DRE group, testicular tissues appear normal histological structure as in case of control group (Fig. 7B). While, the testis of irradiated rats showed mildly lesions represented by degenerative changes in primary and secondary spermatocytes with serous exudate and edema in interstitial tissues (Fig. 7C, D). Moreover, in more severe cases, little or complete absence of spermatozoa with dissociation of spermatogonial cell layer and spermatocytes of tubules beside interstitial fibrosis, congested blood vessels were observed (Fig. 7E, F). Testicular tissue of rats received DRE before γ-rays exposure group showed normal structure and other few cases showed edema with slightly thickness of interstitial tissues (Fig. 7G), while in case of rats received DRE after γ-rays exposure displayed normal primary and secondary spermatocytes (Fig. 7H).

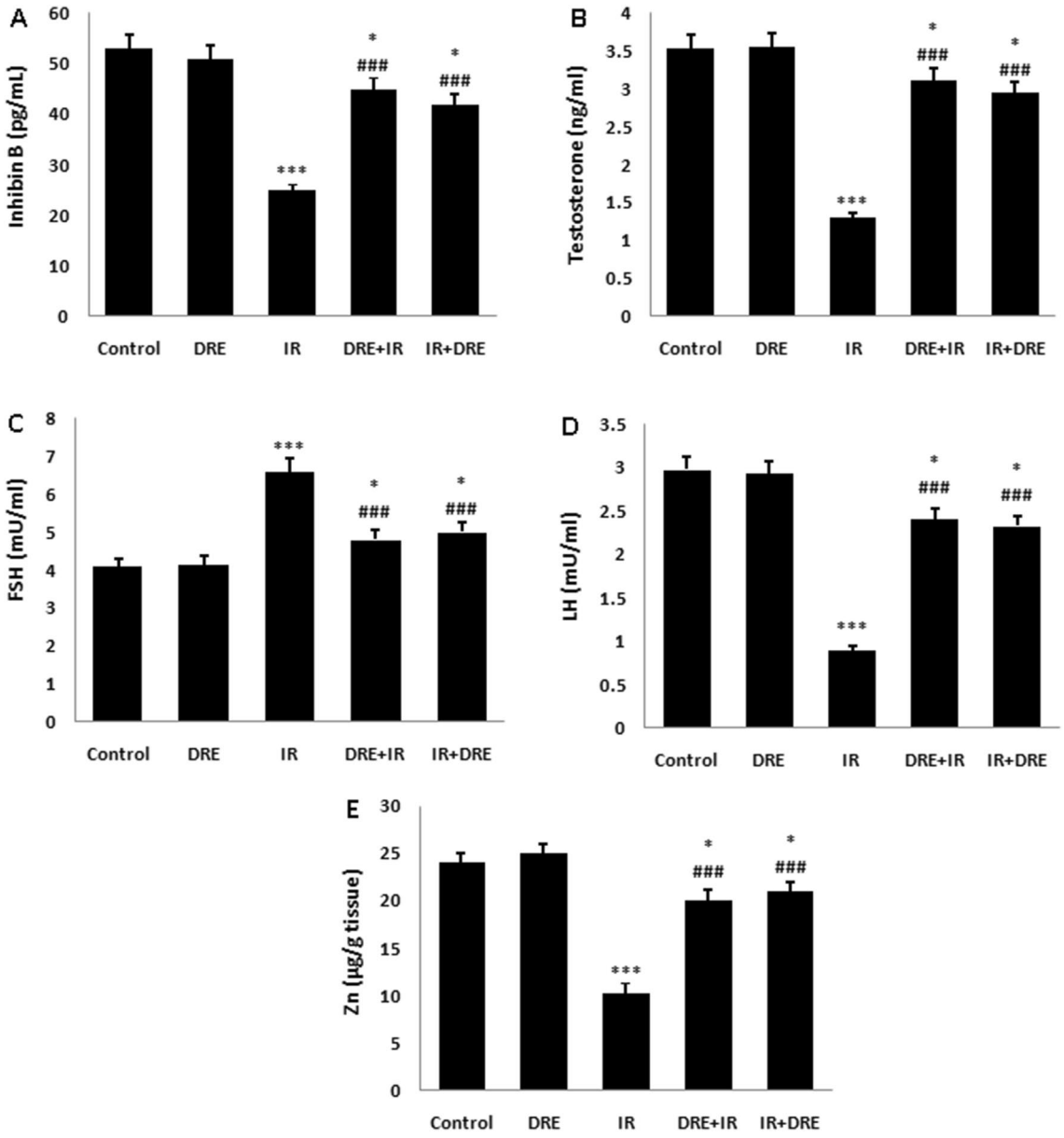

Fig. 4 Effect of DRE (200 mg/kg/day) on the serum levels of inhibin B (**A**), testosterone (**B**), FSH (**C**), LH (**D**) and testicular Zn (**E**) in the animal groups. Values are expressed as Mean ± SD ($n=6$). *Significance versus control. #Significance versus irradiated group (IR). Differences between means were considered significant ($^{*,\#}$) at $p \leq 0.05$, highly significant ($^{**,\#\#}$) at $p \leq 0.01$ and very highly significant ($^{***,\#\#\#}$) at $p \leq 0.001$

Discussion

In the current study, the whole body exposure of rats to gamma-radiation has triggered oxidative stress and induced alterations in the redox system of hepatic and testicular tissue evidenced as an apparent widespread swelling and expanding of hepatocytes owing to various degeneration changes and might be progressive to necrosis changes though the lobules as well as significantly elevated levels of oxidant markers. A significant elevation in the level of MDA and PCO might be attributed to the interaction of hydroxyl radical (·OH), with lipids, proteins, respectively accompanied with a significant reduction in the activity of the antioxidant enzymes Gpx and SOD and GSH content. The depletion of the antioxidants enzymes might result from their increased utilization to neutralize ROS. Additionally, the decrease in GSH post-irradiation might be attributed to the reduction of mRNA expression of glutathione reductase, an enzyme reduces glutathione disulfide to form GSH and is an important GSH-maintaining gene [39, 40].

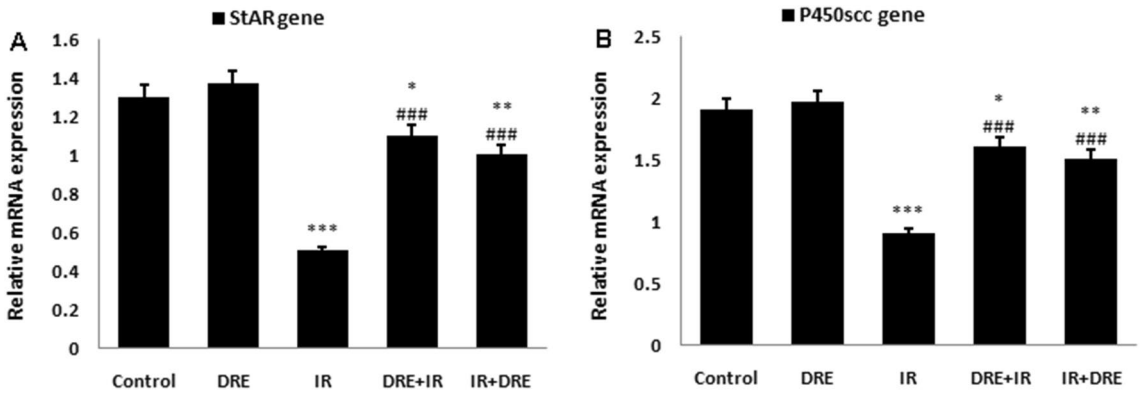

Fig. 5 Effect of DRE (200 mg/kg/day) on gene expression mRNA of StRA (**A**) and P450scc (**B**) in the testicular tissues of different animal groups. Results are expressed as mean ± SD (*n* = 6) *Significance versus control. #Significance versus irradiated group (IR). Differences between means were considered significant ($^{*,\#}$) at $p \leq 0.05$, highly significant ($^{**,\#\#}$) at $p \leq 0.01$ and very highly significant ($^{***,\#\#\#}$) at $p \leq 0.001$

Irradiated rats also showed a significant decrease of GSH-T activity, indicating that exposure to ionizing radiation motivated alteration in the detoxification system of the liver [41].

GLDH is an enzyme, localized in mitochondria and its physiological function is to catalyze the reversible conversion of glutamate into α-ketoglutarate, accompanied by a reduction of nicotinamide adenine dinucleotide [NAD $(P)^+$] to NAD(P)H [42]. High elevated level of GLDH is indicative of hepatocellular necrosis and liver damage. Thereby, we speculate that the elevation of GLDH might be attributed to the necrosis in the hepatic tissue, which also associated by an elevation of caspase-3 mediated apoptosis post-irradiation, the result was confirmed by histopathological examination (Fig. 6F, G). This is in concordance with Plaitakis et al. [43].

Moreover, irradiated rats showed an increase in the serum level of PNPase, an enzyme involved in purine metabolism containing a thiol (SH) group. PNPase cleaves a purine nucleoside by the ribose phosphorylation to create a nucleobase and ribose one phosphate. It occurs in many tissues but appears to be highest in the hepatocytes, Kupffer cells, and sinusoidal endothelial cells of liver. PNPase is a leakage marker and its activity could be explained as an important indicator for hepatocellular injury [44]. Hence, the elevation of this enzyme might result from the cells of the liver injury post-irradiation.

Additionally, oxidative stress elevates the levels of liver enzymes AST, ALT, ALP, LDH post- irradiation and might be attributed to the degeneration of hepatic cells and liberation these enzymes Kwo et al. [45]. The negative modulation of the studied parameters in the liver tissue was confirmed by the histopathological examination, which appeared the necrosis and hydropic degeneration of hepatocytes causing hepatic injury (Fig. 6C, D, F).

Several studies have established that IR influences testicular function, morphology and spermatogenesis [5, 46, 47]. IR causes defects in the normal metabolism, which might lead to mutagenesis, apoptosis, and necrosis of radiosensitive cells.

GSH has a critical role in sperm nucleus decondensation and spindle microtubule construction [48]. Besides, SOD and CAT protect spermatozoa against O_2 toxicity and lipid peroxidation [49]. SOD dismutases (O_2) anion to form O_2 and H_2O_2, while CAT coverts H_2O_2 to O_2 and H_2O. Gpx considers as an essential enzyme in the peroxyl scavenging mechanism and in keeping functional integration of the cell membranes, spermatogenesis, and sperm morphology and motility [50]. Thereby, in the current study, high levels of lipid peroxidation (MDA) and PCO with significant declines in the activities of antioxidant enzymes, including SOD, CAT, GSH and GPx suggesting a decrease in the testosterone biosynthesis and a disruption in the spermatogenesis post-irradiation. The disturbance in the antioxidant system is a critical factor in reducing the ability of Leydig cells to synthesize testosterone [51]. Previous studies demonstrated that Leydig cells consider as the main target for the negative effects of radiation exposure on male reproduction [52]. Further, StAR that is the rate-limiting process in the production of steroid hormones [7], acts as a crucial protein, present in Leydig cells involving in testosterone production. In the same context, cytochrome P450scc enzyme is limited to a small area on the matrix side of the mitochondrial inner membrane of Leydig cells, it catalyzes the change of cholesterol to pregnenolone, which transfers to testosterone by other steroid synthesis enzymes [53]. The results of the current study exhibited a significant decrease in the StAR and P450scc gene expression, and this might explain the decrease of testosterone level in rats subjected to radiation and this is in line with Lin et al. [54]. Moreover, the

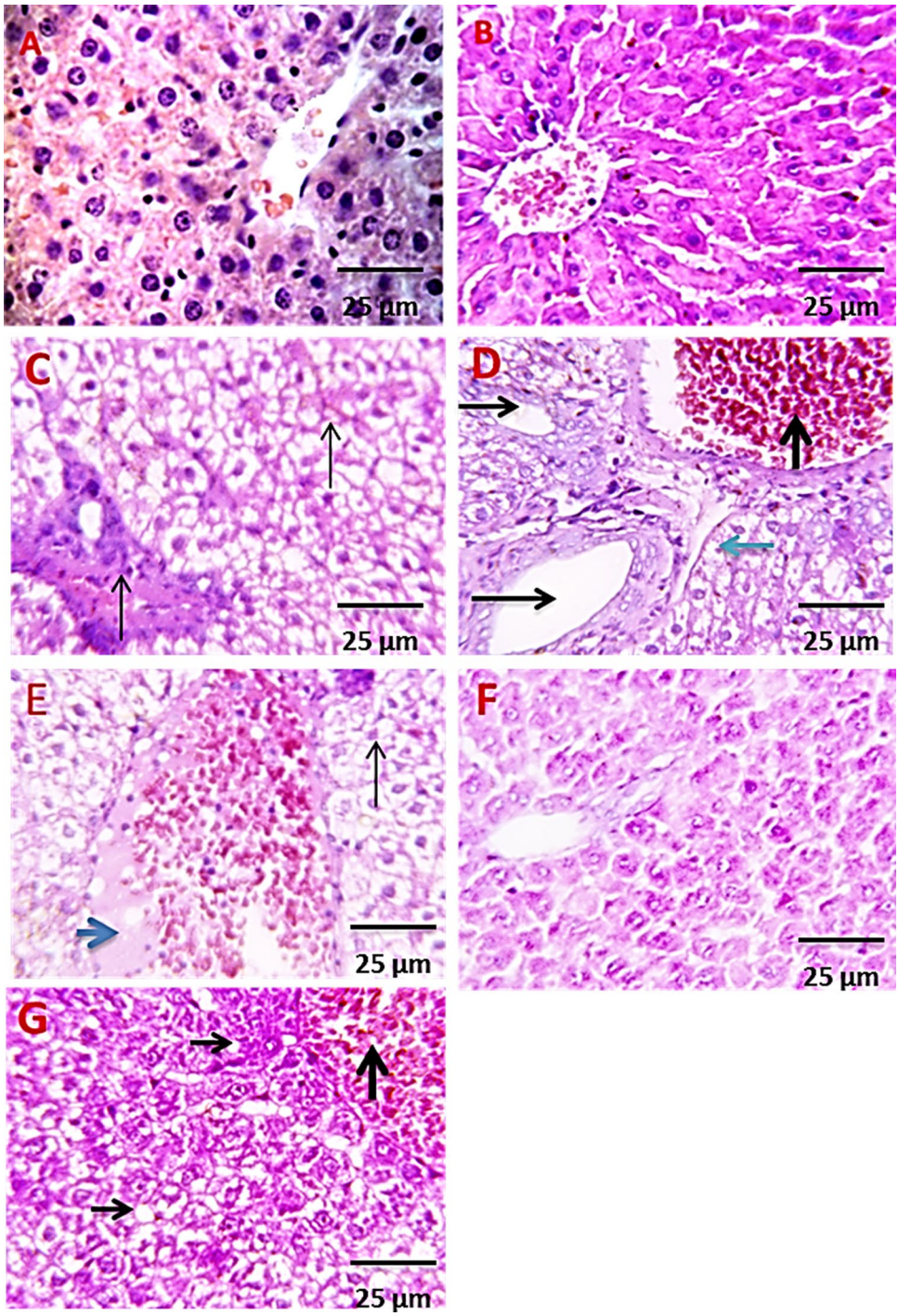

Fig. 6 Effect of dandelion root extract (DRE) on normal and γ-rays-induced histopathological alterations of liver tissues (H&E ×400). H&E: hematoxylin and eosin. **A**, **B** Rat liver of control and DRE groups showing normal structures. **C** Rat liver of irradiated group showing various degenerative or necrotic changes in hepatocytes (↑). **D** Rat liver of irradiated group shows congested portal vein (↑), numerous bile ductulas (→), periductular fibrous and necrosis in hepatocytes (←). **E** Rat liver of irradiated group showing fibrinoid necrosis of congested central vein (→) and hydropic degeneration of hepatocytes (↑). **F** liver of rat received DRE befor γ-rays exposure group showed mild reversible degenerative changes in hepatocytes. **G** liver of rat received DRE after γ-rays exposure group showing congested central vein (↑), microstatosis and hydropic degeneration of hepatocytes (→) (H&E ×400)

results are supported by the histopathological examination (Fig. 7C–F) that displayed Leydig cells impairment.

The decrease of LH level post-irradiation might result from the damage in Leydig cells observed in the current histopathological result, where luteinizing hormone-releasing hormone (LHRH) receptors are located [9], while Midzak et al. [55] attributed the reduction in LH to the disturbance in mitochondrial Leydig cells, where a complex III blocker inhibits LH-stimulated testosterone production at several sites along the steroidogenic mechanism. Another reason for the reduction of LH and testosterone production post-irradiation might be explained by inducing the release of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter of LHRH release [56].

FSH dysregulation may cause infertility and sexual dysfunctions. Herein, the significant increase in the serum FSH can be explained by the reduction in the inhibin B (a peptide regulator hormone) level due to the damage

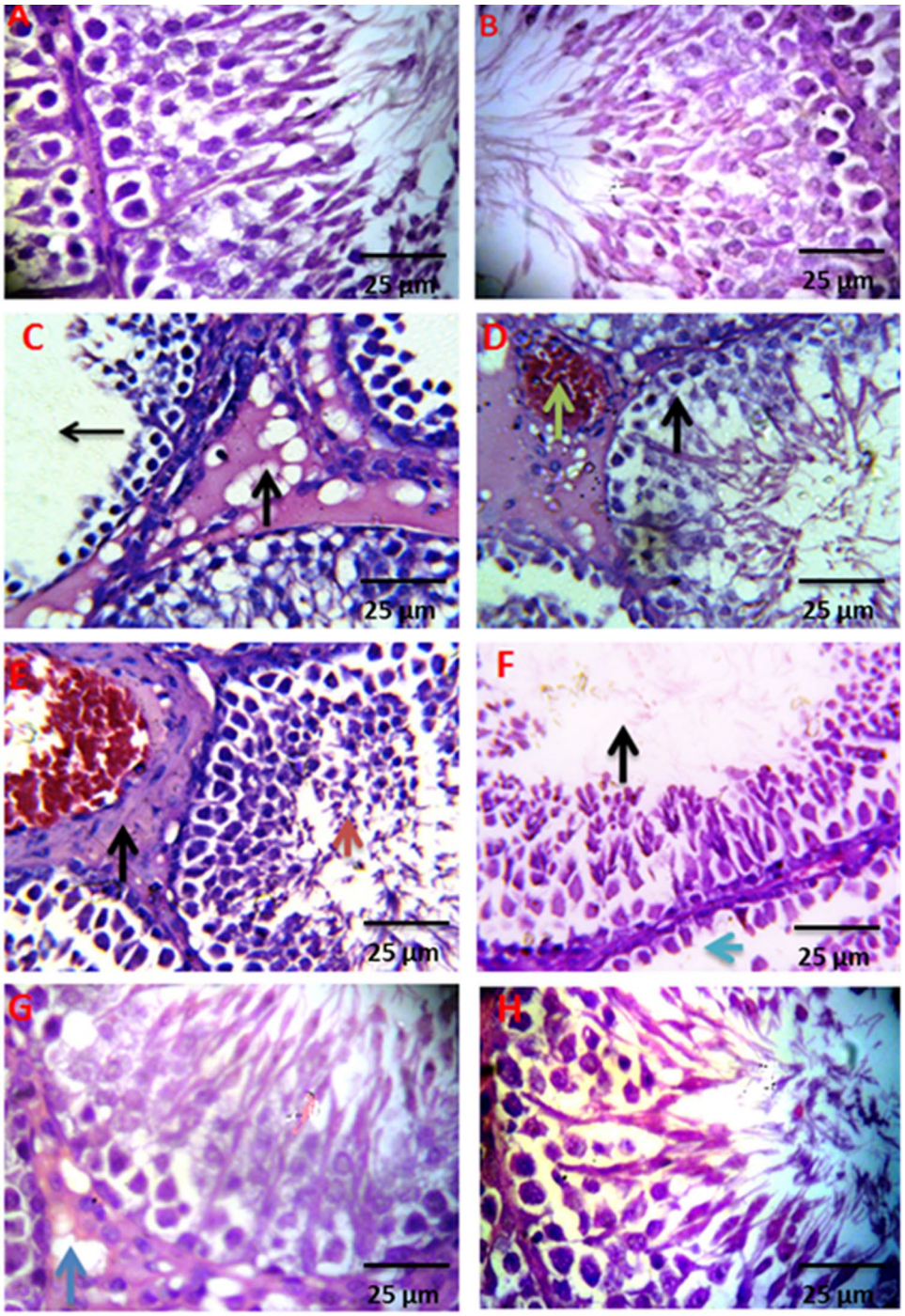

Fig. 7 Effect of dandelion root extract (DRE) on normal and γ-rays-induced histopathological alterations of testes tissues (H&E 400 x). H&E: hematoxylin and eosin. **A**, **B** Rat testis of control group and DRE group showing normal structure. **C** Rat testis of irradiated group showing absence of spermatogonial in the lumen (⟵), serous exudate and interstitial edema (↑). **D** Rat testis of irradiated group showing congested blood vessels (↑) beside degenerative changes in primary and secondary spermatocytes (↑). **E** Rat testis of irradiated group showing, Interstitial fibrosis (↑), congested blood vessels with little spermatozoa in lumen (↑). **F** Rat testis of irradiated group absence of spermatozoa (↑) with dissociation of spermatogonial cell layer and spermatocytes of tubules (⟵). **G** Testis of rat received DRE after irradiation shows edema (↑) with slightly thickness of interstitial tissues. **H** Testis of rat received DRE after irradiation showing normal histological structure

in Sertoli cells of the testicular tissue, in which inhibin B has been produced. Inhibin acts by the negative feedback mechanism to modulate the production of FSH. Suppressed inhibin-B levels in the testicular tissue might be a marker of oxidative stress [57]. The result is assured by histopathological examination (Fig. 7C–F).

Cytokines are glycoproteins, act as an immune defense response to infection or injury in the body through producing signaling molecules between immune cells. Cytokines may produce signals from the blood stream extending to the other tissues such as brain, leading to activation of other inflammatory mediators [58]. In this study, the elevation of pro-inflammatory cytokines (TNF-α and IL-1β) levels in the liver and testicular tissues post-γ-radiation could be interpreted by the correlation between oxidative stress and inflammation that are closely connected with

pathophysiological processes, one of which can be easily stimulated by another. ROS and RNS resulted from irradiation can begin the intracellular signaling cascades that elevate the pro-inflammatory gene expression. On the other side, inflammatory cells release a number of reactive species resulting in excessive oxidative stress [59]. Moreover, the increase of caspase 3 act as an indicator for apoptosis, where apoptosis-related genes (p53, and TNF-α).

On the other hand, we studied the differential antioxidant effects of DRE on the liver and testicular tissues pre and after-irradiation exposure. The results showed positive modulation of DRE on the studied biochemical parameters on the two tissues pre-irradiation, while DRE treatment post-irradiation exhibited a greater positive effect in the testicular tissue than in the liver tissue. The modulation might attribute to antioxidant, ant-inflammatory and anti-apoptotic effect of DRE due to its ingredients. Also administration of DRE significance diminished the histopathological changes in the hepatic and testicular tissues, denoted by a reduction in the necrotic and degenerative changes of hepatocytes or fibrinoid necrosis of congested central vein (Fig. 6F, G) and improving the seminiferous tubules and interstitial tissue between the tubules of the testis (Fig. 7G, H) The results are in similar with that of the previous studies [19, 21, 60–63].

The bioactive components in dandelion (*T. officinale*) extract have demonstrated progression of antioxidant effects, which are due to the pharmacological actions of components such as sesquiterpene lactones (SEL), taraxasterol (TS), taraxerol, chlorogenic acid (CGA) and chicoric acid (CRA) [64, 65]. Herein, the decrease in the levels of oxidative stress biomarkers in the liver and testes of rats treated with DRF before and after exposure to radiation might be attributed to CGA which is an ester of quinic acid with caffeic, ferulic, or coumaric acids. CGA considers as the richest phenolic compound of dandelion and binds to enzymes or multi-subunits, generating natural antioxidant property to change their biological activities [63]. Moreover, in the previous findings demonstrated that CGA reduced expression of cyclooxygenase-2 (COX-2) and TNF-α with blocking inflammatory pathways. Besides this, CGA exhibited the inhibiting impact on apoptosis and autophagy via inhibition of p53 and caspase-3 [66]. According to Zhang et al. [67], TS, pentacyclic-triterpene component in dandelion root extract possesses anti-inflammatory properties and has been considered as a therapeutic agent for the treatment of inflammatory diseases. In agreement with these studies, it has been shown that DRE possesses anti-inflammatory and antiapoptotic effect against γ-radiation through reduction of TNF-α, IL-1β and caspase 3.

Further, it has been documented that dandelion is a very rich source of vitamins and minerals such as Fe, Mg, Cu, and Zn and this leads to the positive modulation of zinc in the testicular tissue before and after radiation exposure [15, 68]. Zinc is an essential element for elevating the body immune response, decreasing inflammation, as an antioxidant [58].

Also, data from the current biochemical and histopathological results concluded that DRE treatment on testicular tissues of rats pre or post-irradiation is better than it in the liver, and this response might be due to the lacking sufficient vascularization of the testes means that oxygen tensions in this tissue, preventing an excess of oxidation of molecules. Another reason has been reported in previous finding in which the testes not only contain the conventional cytosolic (Cu/Zn) and mitochondrial (Mn) forms of SOD, but also includes a form of extracellular SOD (SOD-Ex) that is created by both Sertoli and germ cells [69].

Conclusion

The current findings suggested that DRE supplement pre-irradiation was associated with beneficial effects on the improvement of biochemical and histopathological changes produced by radiation in both the liver and testicular tissues of rats. While DRE administration to rats post-irradiation showed greater improvement in the testis compared with liver.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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