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Article

Protective effects of *Urtica dioica* L. seed extract on liver tissue injury and antioxidant capacity in irradiated rats

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Radiotherapy is often used for the treatment of cancer. However, it causes some side effects in patients. This study aimed to determine the hepatoprotective effects of *Urtica dioica* L. seed-extract (UDSE) in radiation-induced liver injury. Thirty-two male rats were randomly divided into 4 groups (n=8): control(C) group: no action was taken; radiation (R) group: irradiation was administrated at 5Gy single-fraction, radiation with UDSE(R+UDSE) group: irradiation was administrated at 5 Gy single-fraction and animals were fed pellets with 30 mL UDSE/kg; UDSE group: animals were fed pellets with 30 mL UDSE/kg. All of the experiments were performed in all of the groups over 10 days. Malondialdehyde (MDA) and reduced-glutathione (GSH) levels and superoxide-dismutase (SOD), catalase (CAT), glutathione-peroxidase (GSH-Px), aspartate-transaminase (AST), and alanine-aminotransferase (ALT) activities were determined. Histopathological findings were also evaluated in liver tissues. SOD, CAT and GSH-Px activities and GSH levels in the serum and liver were significantly increased, while MDA levels decreased in the R+UDSE group compared with the R group ($P<0.05$). Moreover, AST and ALT serum activities in the R+UDSE group were lower than those in the R group ($P<0.05$). In addition, radiation induced degenerative/necrotic changes in the R group were significantly compensated in the R+UDSE group. The results showed that radiation increased oxidative stress and decreased antioxidant capacity, as well as degeneration in the liver. However, UDSE attenuated these degenerative changes.

Keywords: Radiation. *Urtica dioica* L. seed extract. Oxidative stress. Antioxidant. Tissue injury.

INTRODUCTION

Every year, almost 14 million new cancer cases are diagnosed worldwide, and most of these patients utilize radiotherapy as a treatment or a strategy to relieve their pain (Jaffray, Gospodarowicz, 2015). Radiotherapy aims to destroy cancer cells by targeting the maximum radiation dose at the tumour tissue (Perez, Fields, 1987). The first aim of radiotherapy is to damage DNA in tumour cells to prevent uncontrolled replication and division mechanisms (Taysi *et al*., 2008). However, it is known that this also negatively affects non-target tissues. In addition, the dose increments and the localization of tumours in providing local tumour control are associated with complications in normal tissues. Therefore, radiation tolerance of non-target cells plays an important role in tumour control and cancer treatment (Perez, Fields, 1987).

Radiotherapy not only leads to DNA damage but also triggers the formation of free radicals through indirect mechanisms (Taysi *et al*., 2008). Radiation results in the breakdown of water molecules in the body to form free oxygen radicals such as hydroxyl radicals (OH•) and superoxide ($O_2^{\bullet -}$) during radiotherapy. It also leads to a distortion of the oxidant and antioxidant balance in the cells (Alizadeh *et al*., 2013). Reactive oxygen species (ROS) are responsible for the formation of adverse situations such as carcinogenesis, mutagenesis, ageing and atherosclerosis (Halliwel, 1989). Antioxidants have an important role in cancer treatment and complementary or preventive medicine, although they are still controversial in this field (Song, Yan, Chai, 2006).

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Nettle (*Urtica dioica L.*) (UD) is one of the most widely used plants in alternative medicine in Turkey, because of its therapeutic properties (Kav *et al*., 2008). Nettle seeds contain 26.4% nitrogen-free extract, 25% fat, 21.9% protein, 11.4% fibre 8.5% water, and 6.8% ash (Kavalali, Oztekin-Mat, 1996). Research has also reported that *Urtica dioica* L. seed extract (UDSE) has antioxidant properties, as well as immunomodulatory, antiinflammatory, antimicrobial, and pharmacological effects in *in vitro* and *in vivo* studies (Gulçin *et al*., 2004; Hajhashemi, Klooshani, 2013). Although the therapeutic effects of UDSE have been shown in some experimental studies, there are no reports about the protective role of UDSE against the formed tissue damage due to ionizing radiation (Yener *et al*., 2009; Körpe *et al*., 2013; Telo, Halifeoglu, Özercan, 2017). Determination of the inhibitory effects of UDSE on free radical generation will be important in patients receiving radiotherapy.

In this study, we aimed to investigate the protective effects of UDSE on liver tissue damage and selected antioxidant enzyme activities in irradiated rats. Therefore, we evaluated the serum activities of aspartate transaminase (AST), alanine transaminase (ALT) and intracellular antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the levels of reduced glutathione (GSH) and lipid peroxidation product malondialdehyde (MDA) in liver tissue and serum samples. In addition, the pathological effects of radiation in the liver tissue samples in all of the groups were also histomorphologically examined.

MATERIAL AND METHODS

Experimental design and study groups

Thirty-two male Wistar albino rats with a body weight of 180-200 grams and aged 8 weeks, were used. The present study was performed in accordance with the Van Yuzuncu Yıl University Experimental Animals Local Ethics Committee regulations and animal care and rights policies (28.07.2016, decision number: 07). The rats were quarantined for at least 7 days before irradiation and fed standard chow and water at a standard temperature (22 ± 1 °C) and light conditions (12 h light/12 h dark) in a windowless laboratory room. The rats were randomly divided into 4 groups (n = 8): the control (C) group was fed standard pellets for 10 days, the radiation (R) group was fed standard pellets for 10 days after irradiation with a 5 Gy single fraction, the radiation with UDSE (R+UDSE) group was exposed to 5 Gy radiation as a single fraction and fed pellets with 30 mL UDSE/kg for 10 days, and the UDSE group was fed pellets with 30 mL UDSE/kg for 10 days.

Plant materials and extraction procedure

The UDSE were purchased from a local herb store (Van, Turkey) and the species description was identified by biologist Kenan Yıldızhan (who has a master's degree in botany). Voucher specimens were preserved in herbarium of the Department of Biology, Faculty of Sciences, at Van Yuzuncu Yıl University (Herbarium code: VANF-16778).

The seed extract was prepared by partially modifying the method used by Yener *et al.* (2009). Nettle seed (*Urtica dioica* L. seed) was used as the plant material. For extraction, the plant seeds were ground in an electric mill. One kilogram of the milled seeds was placed in a glass beaker and 2 L ethanol (80%) was added. The beaker was covered with aluminium foil and the milled seeds were homogenized on a shaker for 24 h. The homogenized mixture was poured through the filter and the supernatant was pipetted into a tube (volume: 10 mL). Next, supernatant was centrifuged for 5 min at 3500 *xg*. The supernatant was poured through a 0.45 µm hydrophilic filter (millipore) with the aid of an injector. This process was repeated at least twice, and all of the supernatants were placed in the same container. Then, 400 mL of the filtrate was placed in the evaporator and purified from the solvent at 37 °C for aproximately 1 h and 40 min. At the end of the process, the condensed extract was placed in the falcon tubes and stored at - 20 °C in the freezer. At the beginning of the study, the UDSE was mixed with the milled pellet (30 mL/kg). The mix was compressed and dried, and the indicated groups were fed this mixed pellet food.

Irradiation process

First, a preliminary study was carried out for the application procedure. The optimal radiation dose distribution was set as 5 Gy on a rat that had the same physiological conditions. Radiation application to the rats was performed with a SiemensTM brand Sensation4 model CT-simulator device and total body CT images (2.5 mm section thickness) were obtained in the prone position after being anaesthetized with 50 mg/kg, IP (intraperitoneal) ketamine. These cross-sectional images were transferred to a ProwessTM brand three-dimensional (3-D) radiotherapy treatment planning system, allowing for contouring of all of the rat's tissues and organs. After the contouring process was finished, the rat's whole body 3-D dose plan was applied using 6-MV energized photons

from 2 areas of equal weight to obtain a single fraction of radiation of 5 Gy in the liver while the total body was irradiated. Irradiation was performed at gantry angles of 00 and 180 degrees with equal dose weights on the front and back sides. This dose schedule was designed to be the same for all of the rats and the planned dose procedure was sent to the SiemensTM Artiste (160 MLC) model linac for total body irradiation (Figure 1).

Preparation of the supernatant from the liver tissue samples and obtaining the serum samples

All of the rat groups were anaesthesied with 50 mg/kg IP ketamine at the end of day 10. Whole blood was collected via intracardiac injection, transferred into biochemistry tubes, and centrifuged at 3500 *x g* for 10 min. The serum was carefully transferred using a dropper. In addition, 1 g of liver tissue was homogenized using a homogenizer (Ultra Turrax-T25) in 9 mL 20 mM Tris-HCl (pH: 7.4, g/V, 1/10 ratio) as a previously described (Gumustekin *et al*., 2010). Next, homogenized liver tissue was centrifuged at 15 000 ×g and 4 °C for 30 min. The upper phase was transferred into tubes and stored at -80 °C until the biochemical analysis was performed.

Biochemical analysis

Measurement of the MDA levels from the serum and liver tissue samples was carried out using a fluorescence detector at 527 nm for excitation and 551 nm for emission wavelengths in a high performance liquid chromatography device (Agilent 1200 series from Germany), according to

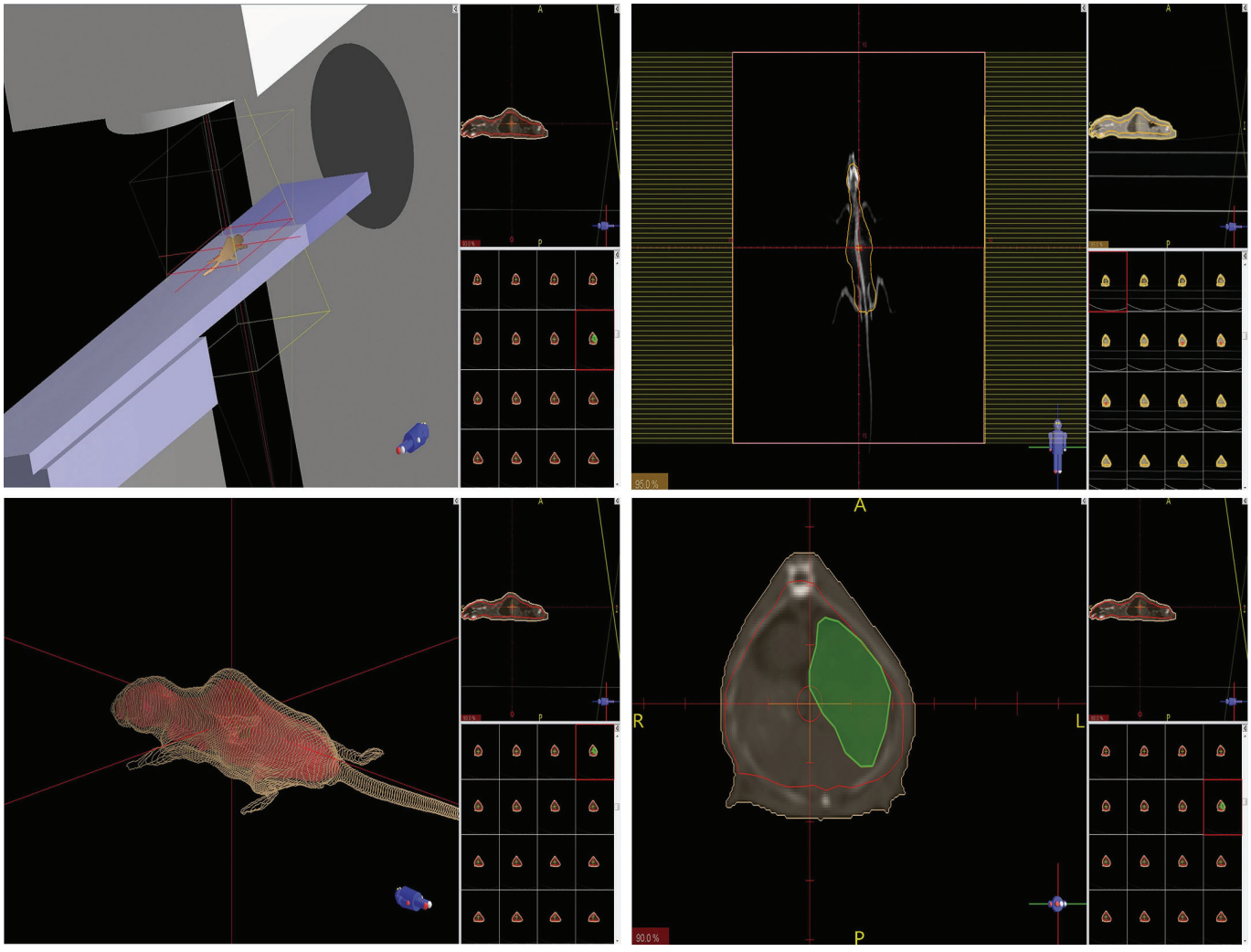

FIGURE 1 - Rat whole body three-dimensional (3-D) dose planning. Gantry 00 degrees beam angle and rat position. b) Irradiation field on the rat. c) 3-D radiation dose (5Gy) distribution on the rat. d) Contouring of the rat tissues and organs (green colored area: liver) in axial cross-sectional CT image (red line: field containing 5 Gy radiation dose). After the calculation of the radiation dose, the rats were placed and irradiated by a Linear Particle Accelerator SiemensTM Artiste (160 MLC) model LINAC using X-rays.

the previously described method by Khoschsorur *et al.* (2000). A RP18 column was used (150 × 4.6 mm length and 5 µm particle size) for the experiments. For the mobile phase, a mixture of 50 mM phosphate buffer (400 mL, pH: 6.8) and 600 mL pure methanol was used. GSH-Px activity was determined by the previous method of Valentine and Paglia (1983). Decreased absorbance values were recorded at intervals of 30 sec at 340 nm for 3 min. CAT activity was measured at 405 nm on a spectrophotometer (Shimadzu UV mini 1240, Japan) using the Goth colorimetric method (Goth, 1991). Cu and Zn-coupled SOD activities in the liver supernatant and serum were determined spectrophotometrically at 560 nm using the colorimetric method based on the rate of nitroblue tetrazolium inhibition (Sun, Oberley, Li, 1988). The activities were calculated by comparison with the standard curve. SOD activity in the tissue samples was expressed as IU/mg protein. In the serum samples, measurement was performed in accordance with the prospectus of the commercial kit (*Rel Assay Diagnostics™).* The AST and ALT activities were measured in an Architect c8000 Clinical Chemistry Analyzer. The obtained values were expressed as IU/mL.

Histopathological examination

At the end of the necropsy, the liver tissues for histopathological evaluation were fixed in a 10% formalin solution for 48 h. After conduction routine processes for tissue tracking, the liver tissues were embedded in paraffin blocks. Sections 4 µm thick were taken from each block and prepared on slides. The sections were stained with haematoxylin-eosin (HE) for histopathologic examination and visualized by light microscopy (Nikon Eclipse 80i-DS-Ri2). A scoring system was used in the obtained micrograph images to evaluate the radiation damage in the liver. According to this, all of the liver sections were examined for degeneration, necrosis and proliferation of the perisinusoidal cells by a board certified pathologist blinded to the treatment groups and then scored. Scoring was defined as slight and moderate, as shown in Table III.

Statistical analysis

All of the results were expressed as the mean ± standard deviation (SD). Following the Kruskal Wallis analysis, a post hoc (Tukey's) test was performed to identify the different groups. Data were analysed using SPSS (version 20, Inc., Chicago, Illinois). P values of 0.05 or less were considered statistically significant.

RESULTS AND DISCUSSION

Clinical studies have shown that radiation increases the level of oxidant molecules and reduces the activity of antioxidants such as SOD, CAT, and GSH, even if used in cancer diagnostics and treatment (Srinivasan *et al*., 2009). Therefore, to ensure tumour control, the side effects to healthy tissues after the applied dose increments must also be considered (Kushi *et al*., 2006). Plants containing natural antioxidants have been widely used in the past. Because of the harmful effects of synthetic antioxidants, natural antioxidants and plants have become the focus with increased interest. It was reported that UD extract possesses effective antioxidant properties and inhibits peroxidation (Lou *et al*., 1999). UD seeds include phytochemicals such as flavonoids, which have been shown to be effective against scavenger free radicals (Akbay *et al*., 2003). In this study, the protective effects of UDSE on serum and liver tissue were investigated in irradiated rats for the first time.

In this study, there were no significant differences between the weight averages of the rats in any of the groups.

Biochemical findings

Serum AST activities were significantly different in all of the groups ($p<0.05$). The lowest AST activities were observed in the UDSE group, whereas the highest activities were determined in the R group. In addition, the AST activities of the UDSE and R+UDSE groups were lower than those in the R group ($P < 0.001$).

When the serum ALT activities were examined, the R group values were higher than those in the other groups ($P < 0.05$). Additionally, the serum ALT activities of the R+UDSE and UDSE groups were lower than those of the R group ($P < 0.001$, Table I).

The MDA and GSH levels, and the SOD, CAT, and GSH-Px enzyme activities in the serum and liver tissue samples of the experimental groups are shown in Tables I and II. The MDA levels in the serum and liver tissue samples were higher in the R group ($P < 0.05$) than those in the other groups. However, there was no sitatistically significant differences among the MDA levels in the liver tissue samples of the C, R+UDSE, and UDSE groups ($P > 0.05$). In addition, the serum MDA levels of the C and UDSE groups were lower than those of the R and R+UDSE groups ($P < 0.05$).

Membrane lipids are more sensitive to the deleterious effects of free radicals. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and

an increased MDA content is an important indicator of lipid peroxidation (Huyut *et al*., 2016; Huyut *et al*., 2018; Sekeroglu *et al*., 2017). In other studies, it was shown that the supplementary UDSE helped to prevent the aflatoxin-induced increase in the MDA of the liver and increased a hepatoprotective effect in the pathologic examinations. (Gülçin *et al*., 2004; Uyar, Yener, Dogan, 2016). We observed that the MDA level and lipid peroxidation increased in only the irradiated groups. On the other hand, UDSE treatment decreased the MDA concentrations to the control level, which showed that UDSE may be successful in inhibiting lipid peroxidation, and thereby may protect the membrane lipids from oxidative damage, as was found similarly in previous study (Uyar, Yener, Dogan, 2016).

As an alternative radioprotector, herbal medicines are generally considered a well-known form of complementary therapeutic strategy. Researchers have focused on herbal medicines, which have gained increasing importance to eliminate side effects of irradiation (Jagetia, Baliga, 2002). UD or nettle has more powerful antioxidant properties than well-known antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), vitamin E and quercetin (Gülçin *et al*., 2004). UD presents scavenging activity against the iron-promoted oxidation of phospholipids, deoxyribose, and linoleic acid (Matsingou, Kopsakefalou, Salifoglou, 2001). When the electrogenerated bromine technique was used to estimate the antioxidant capacity of UD and plant-based medicinal preparations, it was shown that UD prevented damage from rat liver tissue.

There is a balance between ROS production and the antioxidant defence system in healthy individuals. The free radical scavenging activity of antioxidant defensive systems could be adversely affected by formed ROS with radiotherapy (Yu, 1994). GSH, GSH-Px, CAT, and glutathione S-transferase (GST) are among the major intracellular antioxidant defence systems that eliminate lipid peroxides and free oxygen radicals (Trakshel, Maines, 1988). In an previous study, it was reported that UD extract increases SOD, CAT, and GST activities at varying rates (Celik, Tuluce, 2007). Ekici et al. have shown that oxidative stress and the stress index were significantly increased and the SOD, CAT, and GSH levels or activities were decreased when compared to the control group in the kidney and testis tissues of male rats treated with 800 cGray radiation (Ekici *et al*., 2016).

In our study, the SOD activity of both serum and liver tissue samples in the R group was the lowest, while it was highest in the UDSE group ($P < 0.05$). In addition, the SOD activities of the R+UDSE group were similar to those in the C group ($P > 0.05$).

The CAT activity and GSH levels in the serum samples of the R group were lower than those in the other groups ($P < 0.05$). In addition, although the CAT activity and GSH values of the R group in the liver tissue samples were lower than those in the other groups, they were not significantly different ($P > 0.05$). Additionally, there were no differences between the CAT and GSH levels in the serum samples of the C and R+UDSE groups.

In the liver samples, the GSH-Px activities in the R group were lower than those in the other groups. The GSH-Px levels in the R+UDSE, UDSE and C groups were similar ($P < 0.05$). Moreover, the GSH-Px activities in the serum samples were higher in the UDSE group than that in all of the other groups, while they were lowest in the R group ($P < 0.05$).

The results of the current study show that antioxidant enzyme activities, such as CAT, and especially SOD and GSH-Px, were significantly decreased in the serum and liver tissues of the groups exposed to radiation, whereas

TABLE I - Protective effects of UDSE on selected antioxidant enzyme activities and AST, ALT, MDA levels in the serum of rats

Groups	AST (IU/L)	ALT (IU/L)	Serum (MDA) (µM/mL)	Serum (SOD) (ng/mL)	Serum (CAT) (IU/mL)	Serum (GSH) (mg/dL)	Serum (GSH-Px) (IU/mL)
C	76.3 ± 2.5*	57.5 ± 5.8	1.82 ± 0.1	5.25 ± 0.52	3.64 ± 0.1	1.25 ± 0.08	58.06 ± 2.9*
R	120.5 ± 6.2*	71.00 ± 11*	3.00 ± 0.52*	4.74 ± 0.19$^{\neq}$	2.50 ± 0.1*	0.84 ± 0.03*	44.07 ± 2.7*
R+UDSE	89.8 ± 7.2*	61.2 ±7.5$^{\theta}$	2.51 ± 0.2$^{\neq}$	4.97 ± 0.30	3.36 ± 0.2	1.06 ± 0.07$^{\theta}$	50.40 ± 1.6*
UDSE	64.5 ± 4.8*	51.5 ± 4.7$^{\phi}$	1.52 ± 0.2	6.06 ± 0.25*	3.43 ± 0.3	1.44 ± 0.06$^{\phi}$	66.05 ± 1.6*

The data with symbol are significantly different ($P < 0.05$), according to the Tukey T test. (*$p<0.05$): according to the other groups, ($^{\neq}p<0.05$): according to the C and UDSE groups, ($^{\phi}p<0.05$): according to the R and R+UDSE groups, ($^{\theta}p<0.05$): according to the R and UDSE groups, AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde: SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; Groups: C; control, R; radiation group, R+UDSE; radiation+ *Urtica dioica* seed extract group, UDSE; *Urtica dioica* seed extract group.

TABLE II - Actions of UDSE on selected antioxidant enzyme activities and MDA in the liver tissue of rats

Groups	Liver MDA (nmol/mg protein)	Liver (SOD) (IU/mg protein)	Liver (CAT) (IU/mg protein)	Liver (GSH) (mg/mg protein)	Liver (GSH-Px) (IU/mg protein)
C	3.58 ± 0.42	2.53 ± 0.15	0.73 ± 0.10	2.08 ± 0.25	4.18 ± 0.46
R	3.86 ± 0.21*	2.28 ± 0.20$^{\neq}$	0.68 ± 0.05	1.95 ± 0.12	3.85 ± 0.12*
R+UDSE	3.59 ± 0.19	2.37 ± 0.07	0.71 ± 0.07	2.01 ± 0.15	3.92 ± 0.19
UDSE	3.43 ± 0.16	2.67 ± 0.25	0.75 ± 0.05	2.13 ± 0.12	4.26 ± 0.21$^{\phi}$

The Values with sign are significantly different (P < 0.05), according to the Tukey T test. (*p<0.05): according to the other groups, ($^{\neq}$p<0.05): according to the C and UDSE groups, ($^{\phi}$p<0.05): according to the R and R+UDSE groups. AST: aspartate aminotransferase; ALT: alanine aminotransferase; MDA: malondialdehyde: SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; Groups: C; control, R; radiation group, R+UDSE; radiation+ *Urtica dioica* seed extract group, UDSE; *Urtica dioica* seed extract group.

TABLE III - Observational comparison of the effects of UDSE and radiation on the hepatic structure formation

Lesions (Liver)	C	R	R+UDSE	UDSE	p Value
Degeneration	–/8 ^{b}	8/8 ^{b}	2/8 ^{b}	–/8 ^{b}	*
Slight	–	7	2	–	
Moderate	–	1		–	
Proliferation in perisinusoidal cells	–/8 ^{b}	8/8 ^{a}	4/8 ^{ab}	–/8 ^{b}	**
Slight	–	7	4	–	
Moderate	–	1		–	
Necrosis	–/8 ^{b}	8/8 ^{a}	1/8 ^{b}	–/8 ^{b}	*
Slight	–	7	1	–	
Moderate	–	1	–	–	

^{a,b}: Values with different letters in same row are significantly different, *(P < 0.05), **(P < 0.01). According to the chi-square test range". Groups: C; control, R; radiation, R+UDSE; radiation+ *Urtica dioica* seed extract, UDSE; *Urtica dioica* seed extract.

there were no significant changes in the activities of these enzymes and GSH levels in the R+UDSE group when compared with the C group.

Histopathological findings

Normal histological findings were observed in the liver tissues samples in the C groups (Figure 2 A). In the R group, degenerative and necrotic changes were detected in the majority of the liver lobules, especially in the periacinar hepatocytes (stars). The integrity of these hepatocytes was impaired, the cytoplasm was pale in colour, and the nuclei had a pyknotic or fragmented appearance (arrows). Due to these degenerative-necrotic changes in the hepatocytes, the structure of the remark cords was corrupted (dissociated) (Figure 2 B). In addition, the hyperaemia in the venules and the activation of the perisinusoidal cells were noted. In the R+UDSE group, when compared with the control group, almost normal histological appearances were observed. In the UDSE group, normal histological findings were found in the liver tissue samples (Figure 2 D).

In recent years, significant hepatic lesions have been detected from the histopathological examinations related to irradiation in the abdominal region. The liver is a relatively more radiosensitive organ and radiation treatment causes many significant changes in the metabolic functions of the liver (Barshishat-Kupper *et al*., 2014). Some radio-protective agents have been used prior to irradiation to prevent the harmful effects of radiation on healthy tissues or cells, and many studies have reported that the use of these agents had a radio-protective effect on the kidney and liver (Jirtle, Anscher, Alati, 1990).

Recent histopathological studies have shown that radiation exposure to liver tissue can cause swelling in hepatocytes, deterioration of the membrane structure, and sinusoidal dilatation. However, it was proven that antioxidant treatment together with radiation protected the liver tissues and showed a healthy structure similar to that of the control group (Das *et al*., 2014). Our findings

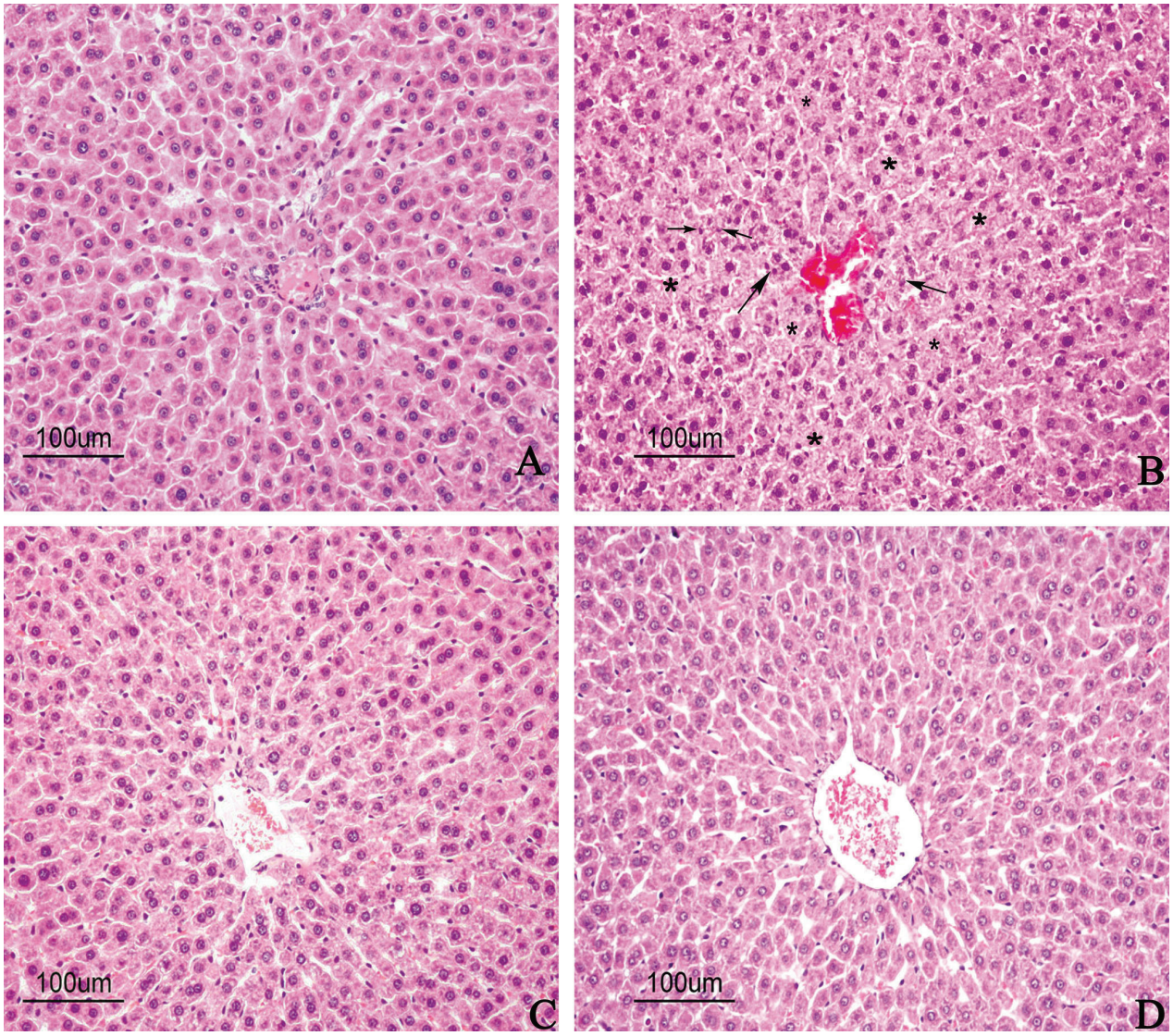

FIGURE 2 - (A) Liver of the rats shows normal architecture of lobules from the C group, (B) Hepatocytes in the periacinar and midzonal regions are swollen, their cytoplasm is stained in a pale color and appear as fine granular or homogeneous (*). In addition, the nuclei of some hepatocytes are dark color and picnotical (⟶) from the R group, (C) Parenchymal degeneration is observed in some hepatocytes in the periacinar (*) from the R+UDSE group and (D) liver of rats shows normal architecture of lobules from the UDSE group. Groups: C; control, R; radiation, R+UDSE; radiation+ *Urtica dioica* seed extract, UDSE; *Urtica dioica* seed extract. (haematoxylin-eosin, bar: 100 µm.).

have supported previous studies and showed that the use of UDSE together with radiotherapy decreased the harmful and destructive effects of irradiation in liver tissue.

CONCLUSION

These data show that UDSE prevents radiotherapy-induced liver damage, lipid peroxidation and oxidative stress, and protects antioxidant enzyme activities. For this reason, UDSE may be used as a radioprotector after irradiation. In addition, this study has indicated that local radiation therapy may be a more beneficial model than whole-body radiation therapy. However, future studies with different local or mimic radiation models are needed to test whether UDSE protects tumours or healthy cells from radiation.

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CONFLICTS OF INTEREST

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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