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*ORIGINAL ARTICLE*

# Can radiation-induced chronic oxidative stress in kidney and liver be prevented by dimethylsulfoxide? Biochemical determination by serum and tissue markers

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## Summary

***Purpose:*** *To investigate the protective effects of dimethylsulfoxide (DMSO) on chronic oxidative stress in the liver, kidney and serum with biochemical parameters such as malondialdehyde (MDA), advanced oxidation protein product (AOPP), catalase, glutathione (GSH), and free-thiols (F-SH).*

***Methods:*** *Thirty Wistar albino female rats were randomly divided into 3 groups: group I (control, n=10), group II (irradiation-alone group, n=10) and group III (DMSO and irradiation group, n=10). Rats in groups II and III were irradiated with a single dose of 6 Gy to the entire liver and right kidney. Group III received DMSO 4.5 g/kg by intraperitoneal injection 30 min before irradiation. At the end of the 24th week, the rats were sacrificed and their trunk blood, kidney and liver tissues were collected.*

***Results:*** *Group II rats showed increased levels of lipid peroxidation and protein oxidation, with decreased GSH, F-SH and catalase levels in all specimens when compared with group I. Serum and kidney MDA and AOPP levels were significantly lower in group III when compared with group II. However, serum and kidney GSH and F-SH levels were significantly higher in group III when compared with group II. The additive effect on catalase was seen only in the serum.*

***Conclusion:*** *DMSO is a protective agent on chronic oxidative stress in the serum and kidney tissue. No oxidant or antioxidant effect of DMSO in the liver was seen.*

**Key words:** antioxidative markers, dimethylsulfoxide, oxidative markers, radiation-induced oxidative stress

## Introduction

The number of long-term cancer survivors continues to grow with ongoing improvements in cancer therapy; 62% of adult cancer patients survive beyond 5 years. Cancer can be considered as a chronic disease for most of the patients. Late complications of cancer therapy are becoming an important concern for both physicians and patients with the increasing number of long-term cancer survivors [1,2]. Currently, physicians should try to prevent complications primarily by restricting the radiation dose and irradiated volume. Abdominal irradiation is an integral part of treatment in most of the frequent extracranial pediatric tumors like Wilms' tumor, neuroblastoma, hepatoma, non-Hodgkin's lymphoma, and Hodgkin's lymphoma and has been associated with renal and liver damage. Another important part of treating malignancies is bone marrow transplantation. The most common way of ablative therapy in preparing patients for allogeneic bone marrow transplantation is total body irradiation. Cumulative dosage, irradiated organ volume and additional treatments such as chemotherapy, are important risk factors for developing long-term side effects [1-4].

It is well known that irradiation of biological material leads to development of reactive oxygen species (ROS), and increase of ROS can persist for several days in irradiated cells. Radiation-induced increased ROS generation and/or oxidative stress has been observed *in vivo*. Generated ROS species have a transient nature, for this reason their direct measurement is extremely hard. The evidence has been derived from studies that showed increase in the formation of oxidized products [3,4].

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Administration of compounds that reduce radiation damage constitutes a different approach in the management of radiation-induced toxicity. DMSO is an antioxidant and free radical scavenger [5]. There are many data accumulated lately about the mechanisms of the radioprotective effect of DMSO. Free radical scavenging, interference hydrogen bonding, capture of H atoms, holes and/or electrons, inhibition of lipid peroxidation, decreasing hypoxia and polycythemia effects have been the subject of intense investigation [6-8]. The radical scavenger effect of DMSO can be based on the ability to prevent secondary induced oxidative stress [9].

In this study we investigated the protective effect of DMSO on chronic oxidative stress by biochemical parameters such as MDA, AOPP, catalase, GSH and F-SH. To the best of our knowledge, the protective effect of DMSO against radiation-induced chronic oxidative stress has not been studied before in an animal model *in vivo*.

## Methods

Thirty Wistar albino 3-4 month old female rats, weighing 200 ± 25 g, were selected from an inbred colony maintained under controlled conditions of temperature and humidity. The rats were maintained on a 12-h light/12-h dark cycle. The animals had free access to sterile water and food and were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. Ethical approval was obtained from the Ethical Committee of Trakya University.

Rats were randomly divided into 3 groups without differences in their body weight (BW) between the groups: group I (control, n = 10), group II (irradiation-alone group, n = 10), and group III (DMSO and irradiation group, n = 10).

Group I and II were given phosphate-buffered saline as vehicle, whereas group III received DMSO (4.5 g / kg, 40% in phosphate buffer in saline) by intraperitoneal injection 30 min before irradiation. Group I was treated with sham irradiation in the same volumes. The dose and time of treatments were defined on the basis of literature data. All experimental procedures were performed on anesthetized rats; anesthesia was maintained with ketamine (50 mg/kg BW) and xylazine (3.9 mg/kg BW) intraperitoneally. Then, all rats were subjected to veterinary care.

Group II and III were irradiated individually with a single dose of 6 Gy using a $Co^{60}$ treatment unit (Cirus, *cis*-Bio Int., Gif Sur Yvette, France) at a source-skin distance of 80 cm to the whole liver and right kidney using an anterior 4 × 5.5 cm single field. The dose rate was 0.89 Gy/min. The rats were anesthetized, and fixed on their blocks across a blue Styrofoam (Med-Tec, Orange City, IA) treatment couch in prone position. Correct positioning of the fields was controlled for each individual rat using a therapy simulator (Mecaserto-Simics, Paris, France). Special dosimetry was done for irregular fields.

At the end of the 24th week the rats were anesthetized by ketamine plus xylazine and their trunk blood and liver and kidney tissues were collected. Blood samples were centrifuged for 10 min at 1500 rpm in cold centrifuge and the serum was kept in deep-freeze at –20° C until analysis.

Liver and kidney specimens were washed with cold 0.9% NaCl solution and stored at –20° C until the biochemical studies. The frozen tissues were weighed separately and then homogenized in 10 volumes of cold phosphate buffer (pH 7.0) in a Potter-type homogenizer. Samples were centrifuged at 8,000 rpm for 10 min at 4° C.

The GSH levels of serum, and tissues were measured according to the method of Beutler et al. [10]. In this system, GSH is oxidized by Ellman reagent, which can be detected spectrophotometrically by a change of absorption at 412 nm. The results were expressed in mmol/L in the serum and nmol/mg protein in the tissues.

Determinations of free sulfhydryl group levels were performed according to Hu in plasma and to Sedlak and Lindsay in tissues [11,12]. The results were expressed in μmol/L in plasma and nmol/mg protein in tissues.

Serum and tissue levels of MDA, a marker of lipid peroxidation, were measured as thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. [13]. The results were expressed in nmol/ml in the serum and nmol/mg protein in the tissues.

Spectrophotometric determination of AOPP levels was performed according to Witko's method [14]. The results were expressed in μmol/L in the serum and nmol/mg protein in the tissues.

Catalase activity was measured according to the method of Aebi [15] by spectrophotometrically following up the decrease in the $H_2O_2$ concentration at the 240 nm. The results were expressed in U/L in the serum and U/mg protein in the tissues. The protein content of the tissues was determined by the method of Lowry et al. [16].

*Statistical considerations*

Statistical analyses were performed by STATISTICA AXA 7.1. Data fitting to normal distribution were expressed as mean ± SD, and data not fitting to normal distribution as median and range. One sample Kolmogorov-Smirnov test was used for the conformity of the data fitting to normal distribution. Intergroup comparisons were tested by One Way ANOVA, Post-hoc Bonferroni and Dunnett T3 tests. Kruskal-Wallis analysis of variance was used to compare the data which did not fit to normal distribution. Mann-Whitney U-test was used to compare statistically meaningful doublets. Descriptive statistics were expressed in mean ± SD. A p-value $<0.05$ was considered statistically significant for all differences.

## Results

None of the animals died during the experiment. All results for serum, kidney and liver tissues levels / activities are presented in Table 1. The effect of DMSO administration for all markers, compared to control and irradiation-only groups are shown in Figures 1-5.

*Serum*

Administration of DMSO decreased the MDA and AOPP levels when compared with the irradiation-alone group (p=0.018, p=0.001, respectively) (Figures 1a, 2a). The mean serum GSH level and catalase activity increased significantly following DMSO administration

**Table 1.** Serum, kidney, and liver tissue levels of oxidative and antioxidative parameters in each group

| *Antioxidative agents* | | *Control group (I)* | *Irradiation-only group (II)* | *DMSO + RT group (III)* | | *p-value* |
|---|---|---|---|---|---|---|
| Serum levels | MDA‡‡ | 7.8 (4.38-8.34) | 9.68 (8.1-10.4) | 6 (4.6-6.3) | I-II<br>II-III<br>I-III | 0.002**#<br>0.018**#<br>0.720** |
| | AOPP‡ | 257.7±17 | 356±26 | 290.2±40.2 | I-II<br>II-III<br>I-III | 0.000*#<br>0.001*#<br>0.482* |
| | GSH‡‡ | 2.3 (1.2-2.4) | 1.2 (1-1.5) | 2.9 (2.7-3.1) | I-II<br>II-III<br>I-III | 0.001**#<br>0.000**#<br>0.000**# |
| | F-SH‡ | 36.9±2.3 | 37.5±2.4 | 47.7±2.6 | I-II<br>II-III<br>I-III | 1.000*<br>0.000*#<br>0.000*# |
| | Catalase‡ | 7674±3250 | 1655±277 | 5927.7±1196 | I-II<br>II-III<br>I-III | 0.004*#<br>0.012*#<br>0.573* |
| Kidney tissue | MDA‡ | 3.5±0.25 | 4.1±0.62 | 3.1±0.5 | I-II<br>II-III<br>I-III | 0.013**#<br>0.007**#<br>0.637** |
| | AOPP‡ | 16±3.7 | 40.2±2.8 | 26.8±3.1 | I-II<br>II-III<br>I-III | 0.000*#<br>0.000*#<br>0.000*# |
| | GSH‡‡ | 12.9±0.6 | 8±1.2 | 10.8±2.5 | I-II<br>II-III<br>I-III | 0.000***#<br>0.006***#<br>0.000***# |
| | F-SH‡ | 23.3 (21.7-24.1) | 15.2 (13.9-19.5) | 18.3 (18.1-22.6) | I-II<br>II-III<br>I-III | 0.000**#<br>0.001**<br>0.101** |
| | Catalase‡ | 41.2±6.3 | 33.2±6 | 31±2.7 | I-II<br>II-III<br>I-III | 0.002*#<br>1.00*<br>0.000*# |
| Liver tissue | Catalase | 89±21.3 | 71±11.4 | 69.5±5.9 | I-II<br>II-III<br>I-III | 0.042<br>0.946<br>0.027 |
| | MDA | 1.8±0.31 | 2.5±0.6 | 2.9±0.5 | I-II<br>II-III<br>I-III | 0.002<br>0.086<br>0.000 |
| | AOPP | 21.6±7 | 30.7±6 | 27.4±7.1 | I-II<br>II-III<br>I-III | 0.053<br>0.444<br>1.00 |
| | GSH | 13.6±1.2 | 8.9±1.7 | 8.8±1.0 | I-II<br>II-III<br>I-III | 0.000<br>0.203<br>0.001 |
| | F-SH | 31±4.1 | 17.4±3.6 | 21.2±5.2 | I-II<br>II-III<br>I-III | 0.000<br>0.075<br>0.000 |

‡: Mean±SD since fits to normal distribution, ‡‡: Median (range) since does not fit to normal distribution, *: ANOVA, Bonferroni t-test, **: ANOVA, Dunnett T3, ***: Kruskal Wallis H analysis and Mann-Whitney U-test, #: $p < 0.05$, MDA: malondialdehyde, AOPP: advanced oxidation protein product, GSH: glutathione, F-SH: free thiols, RT: irradiation, DMSO: dimethylsulfoxide

(p=0.000, p=0.012, respectively) (Figures 3a, 4a). Contrary to other markers, serum F-SH levels did not show any differences in the irradiation-alone group when compared with the control group (p=1.000). However, administration of DMSO significantly increased the F-SH levels in serum (p=0.000) (Figure 5a).

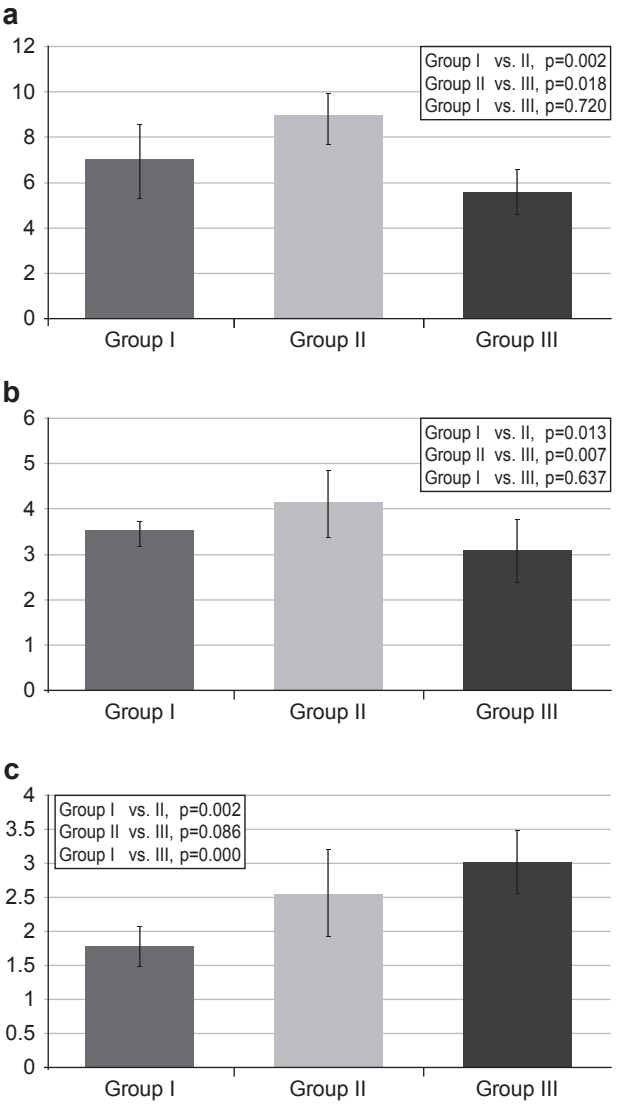


**Figure 1.** Bar graphics showing the effects of 4.5 g/kg DMSO applied 30 min prior to irradiation on MDA levels at 24 weeks in serum (**a**), in kidney tissue (**b**) and in liver tissue (**c**). In the DMSO + RT group, MDA levels in the serum and kidney tissue were decreased when compared to irradiation-alone group, and there was an increasing trend in the liver tissue.

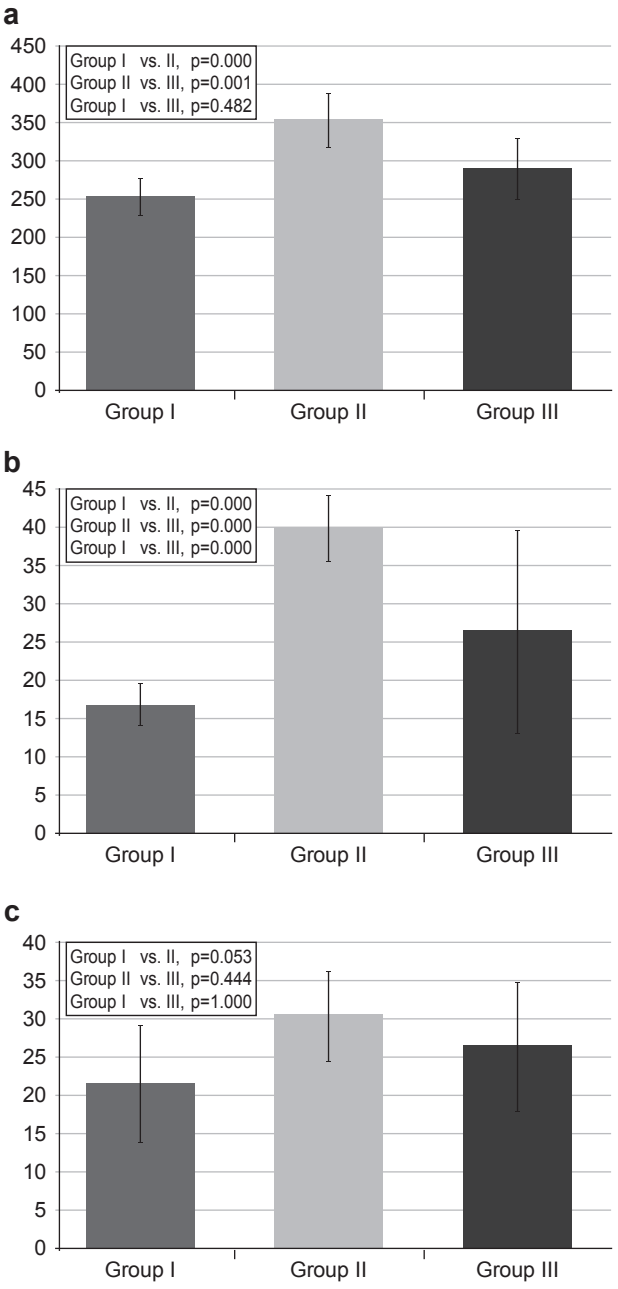


**Figure 2.** Bar graphics showing the effects of 4.5 g/kg DMSO applied 30 min prior to irradiation on AOPP levels at 24 weeks in serum (**a**), in kidney tissue (**b**) and in liver tissue (**c**). In the DMSO + RT group, AOPP levels in the serum and kidney tissue were decreased when compared to irradiation-alone group, but remained constant in the liver tissue.

*Kidney*

Administration of DMSO decreased the MDA and AOPP levels when compared with the irradiation-alone group (p=0.007, p=0.000, respectively) (Figures 1b, 2b). The mean kidney tissue levels of GSH and F-SH increased significantly following DMSO administration (p=0.006, p=0.001, respectively) (Figures 3b, 5b). The mean catalase activity was significantly decreased following irradiation (p=0.002). DMSO administration did not affect catalase activity when compared to other groups (p=1.000) (Figure 4b).

*Liver*

Administration of DMSO did not affect the MDA and AOPP levels when compared with the irradiation-alone group (p=0.086, p=0.444, respectively) (Figures 1c, 2c). DMSO administration did not affect GSH and F-SH levels and catalase activities when compared

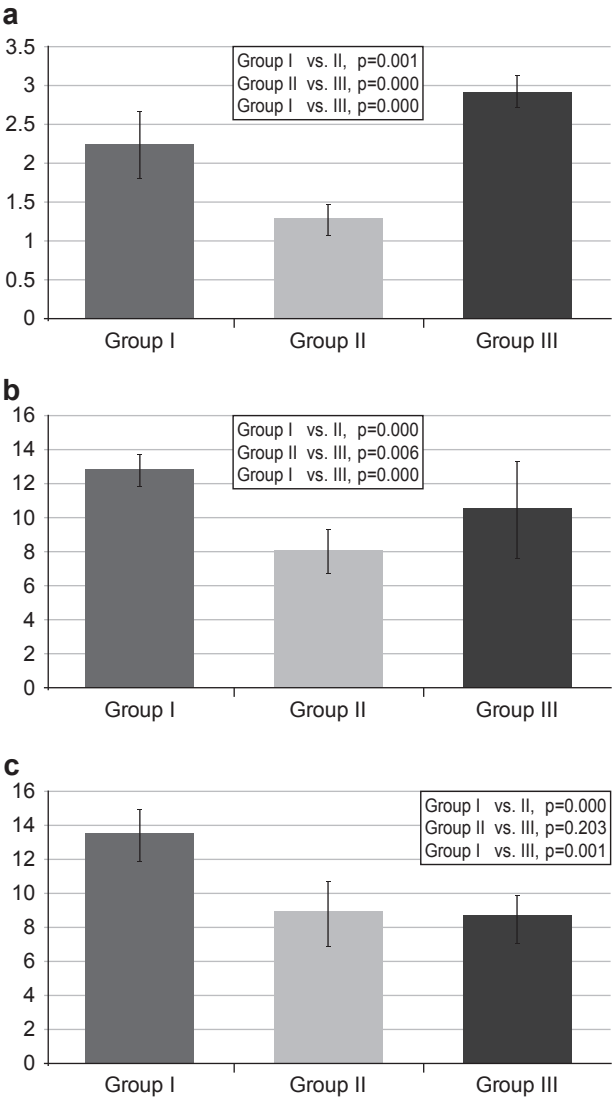


**Figure 3.** Bar graphics showing the effects of 4.5 g/kg DMSO applied 30 min prior to irradiation on GSH levels at 24 weeks in serum (**a**), in kidney tissue (**b**) and in liver tissue (**c**). In the DMSO + RT group, GSH levels in the serum and kidney tissue were increased when compared to irradiation-alone group, but remained constant in liver tissue.

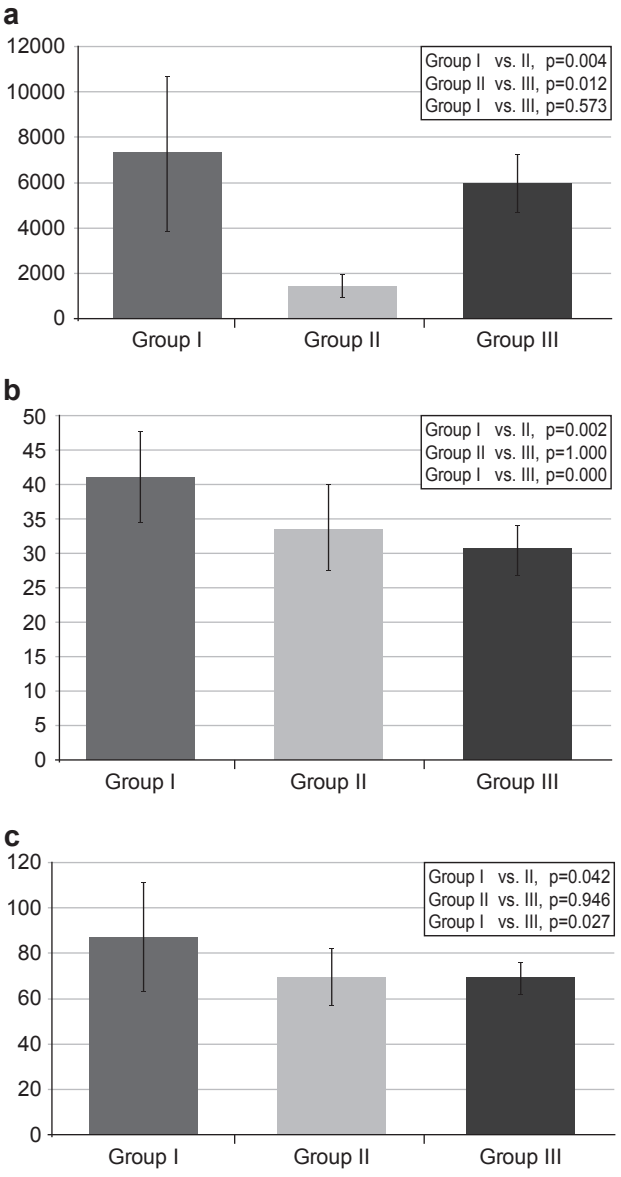


**Figure 4.** Bar graphics showing the effects of 4.5 g/kg DMSO applied 30 min prior to irradiation on catalase activity at 24 weeks in serum (**a**), in kidney tissue (**b**) and in liver tissue (**c**). In the DMSO + RT group, the serum catalase activity was increased when compared to irradiation-alone group, but remained constant in the kidney and liver tissue.

with the irradiation-alone group (p=0.203, p=0.075, p=0.946, respectively) (Figures 3c, 4c, 5c).

## Discussion

Organisms have developed a comprehensive array of antioxidant defenses to prevent free radical formation or limit their damaging effects. This protection involves complex pathways. The protective mechanisms are controlled *in vivo* by a wide spectrum of enzymatic and non-enzymatic systems. These defense systems have been thought to prevent free radicals which may cause irreparable damage by reaction with lipids, proteins, and nucleic acids. ROS can influence antioxidative defense mechanisms negatively as a result of decreased catalase activity, increased MDA levels and reduced GSH and F-SH intracellular concentration [17-21].

We used biochemical parameters such as MDA, AOPP, catalase, GSH and F-SH, for testing the hypothesis that irradiation might be associated with chronic oxidative stress. In our study, lipid peroxidation and protein oxidation were higher, but antioxidants (GSH, F-SH and catalase) were significantly lower at the end of the 24th week. Administration of DMSO exerted a protective effect in the serum and

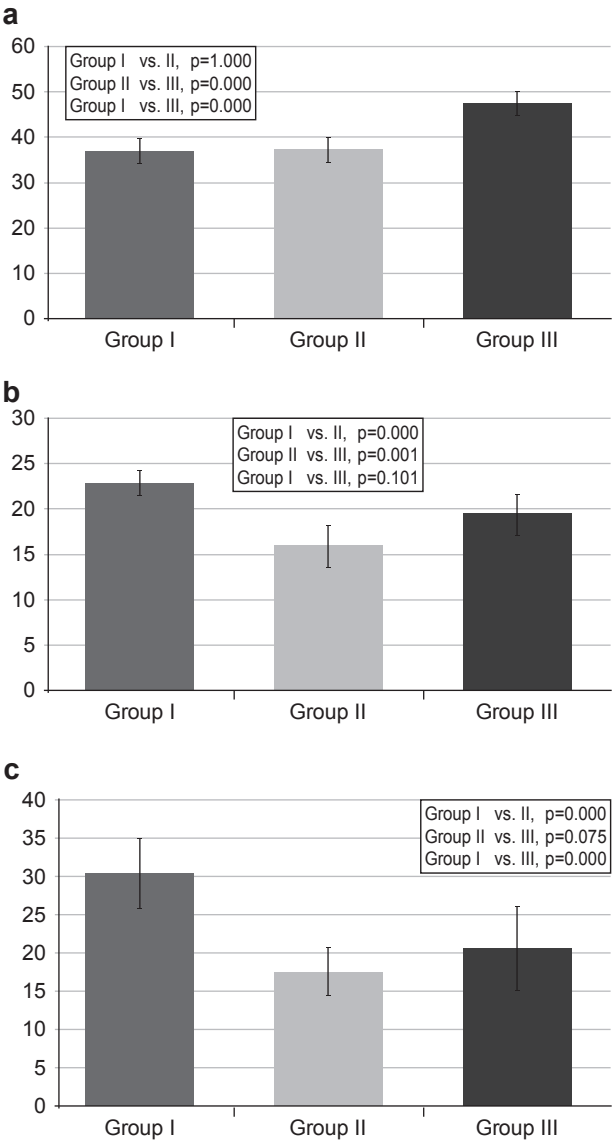


**Figure 5.** Bar graphics showing the effects of 4.5 g/kg DMSO applied 30 min prior to irradiation on F-SH levels at 24 weeks in serum (**a**), in kidney tissue (**b**) and in liver tissue (**c**). In the DMSO + RT group, F-SH levels in the serum and kidney tissue were increased when compared to irradiation-alone group, and there was an increasing trend in the liver tissue.

kidney tissue, while such an effect was not evident in the liver tissue.

The lipid peroxidation process is one of the oxidative conversions of polyunsaturated fatty acids known as MDA or lipid peroxides and it is the most commonly studied free radical reaction. MDA takes part at the end of lipid peroxidation, which serves as an index of oxidative damage [17,18]. In our study, irradiation caused elevation in the levels of lipid peroxidation in kidney and serum. DMSO-administered groups showed significantly lower lipid peroxidation in kidney and serum in comparison with the irradiated-alone group.

The oxidative modifications of proteins are good oxidative stress markers with much better stability when compared to lipids [17-19]. Several forms of protein oxidation can occur, including the formation of protein carbonyls or the formation of cross-linking molecules by oxidation of sulfhydryl groups or AOPP. AOPP are defined as dityrosine-containing cross-linked protein products [14,19,20]. We found significantly increased protein oxidation levels in the kidney and serum after irradiation. AOPP levels decreased after DMSO application and this differences was significant in the kidney and serum. This result may be related to the fact that DMSO exerts protective effects against irradiation-induced protein oxidation in the kidney.

The catalase activity represents one of the major enzymatic events in the antioxidant defense mechanisms. Catalase catalyses the decompensation of $H_2O_2$ to produce water and molecular oxygen, and it plays a major role in protecting cells against oxidative damage. Decreased catalase activity may compromise all the antioxidant enzyme defense system. It is known that the enzymatic activity of catalase diminishes after irradiation [17-21]. In this study, administration of DMSO was sufficient to enable a highly enhanced detoxification of $H_2O_2$, which is the substrate of catalase in the serum and kidney.

Among the non-enzymatic antioxidants, we evaluated GSH and F-SH levels. Administration of DMSO protects the endogenous GSH and F-SH depletion which results from irradiation in the kidney and serum. The increased GSH and F-SH levels suggest that protection of DMSO may be mediated through the modulation of cellular antioxidant levels. The delayed onset of lipid peroxidation and oxidative protein damage in the kidney can be prevented by treatment with DMSO.

DMSO has shown significant changes in the oxidative defense system in the kidney and serum except liver. DMSO did not restore oxidative or antioxidative markers in liver tissue. Irradiation caused chronic oxidative stress in the liver but DMSO did not provide a protective effect for radiation injury. Ashwood-Smith verified a protective effect of DMSO using 4.5 g/kg intraperitoneally 30 min before irradiation in rats [22]. Denko et al. studied the distribution of DMSO in organs 2 h after application of 35S-labeled DMSO to the skin of rats. The highest values occurred in decreased order in the following soft tissue: spleen, stomach, lung, vitreous humor, thymus, brain, kidney, sclera, colon, heart, skeletal muscle, skin, liver, aorta, adrenals, lens of the eye, and cartilage [23]. In animal models of radiation-induced hepatotoxicity by Ueda et al., DMSO has been shown to be able to protect the liver by inhibition of lipid peroxidation when administered on the 30th day following radiotherapy [7]. The trend which was ob-

served in our study (F-SH) might indicate that DMSO could confer a small degree of protection in liver tissue. The concentration of DMSO used in the previous two studies [7,23] was different from our study. Ueda et al. applied higher doses of DMSO in their study to obtain a protective effect in liver tissue [7]. We think that the dose of DMSO used in our study, despite the higher concentration in liver, might be low to provide a protective effect. It is likely that larger dose of this drug in higher concentration is required for liver protection.

Administration of DMSO resulted in a certain degree of polycythemia that helped restore peripheral red blood cell count after irradiation [8]. Hypoxia is known that generates ROS and promotes inflammation and vascular permeability, and it might be an important contributor to the maintenance of progressive oxidative stress. In addition, it might be a part of the driving force behind chronic radiation injury [24]. As noted above, recent evidence suggests that the polycythemic effect of DMSO protects from the hypoxia which leads to radioprotection through indirect activation of internal antioxidant systems such as GSH, F-SH and catalase.

Lenarczyk et al. have highlighted the necessity of minimally invasive laboratory tests in the determination of the degree of chronic oxidative stress in radiation-induced nephropathy, for which we also agree; they also have used urine samples in their study [4]. Within the same context, the results of our study showed that the levels of the serum markers might be an easy, fast, reproducible and cost-effective method for the determination of the chronic oxidative stress.

Besides those issues mentioned above, future trials are needed to assess the efficacy of DMSO administration for radiation-induced nephropathy prevention in groups that combine radiotherapy and chemotherapy. In addition, because of the low systemic toxicity of DMSO, it might be a more popular agent for studies concerning radiation-induced liver damage. The protective effect of DMSO should be studied in higher doses and higher concentrations [21,25].

## Conclusions

Liver and kidney irradiation stimulates chronic oxidative stress which is caused by the radiation-induced late toxicity. In the presented study, DMSO showed significant positive impact on the oxidative defense system in both kidney and serum except liver. Therefore, it might be considered as a promising radioprotective agent against chronic oxidative stress in radiation-induced nephropathy. It would be worth emphasizing the importance of studying the protective effect of DMSO in radiation-induced late tissue injury in different animal models. It seems that DMSO will open new perspectives in radioprotection in the near future.

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