ORIGINAL ARTICLES

The Radioprotective Effects of Caffeic Acid Phenethyl Ester and Thymoquinone on Oxidative and Nitrosative Stress in Liver Tissue of Rats Exposed to Total Head Irradiation

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ABSTRACT

Objective: *The aim of this study is to investigate the effects of addition of caffeic acid phenethyl ester (CAPE) and thymoquinone (TQ) on oxidative and nitrosative stress in the liver tissue of irradiated rats.*
Methods: *Forty Sprague-Dawley rats were divided into five groups to test the radioprotective effectiveness of TQ and CAPE administered by intraperitoneal injection. Appropriate control groups were also studied.*
Results: *Liver antioxidant capacity, as measured by levels of total superoxide scavenger activity (TSSA), non-enzymatic superoxide scavenger activity (NSSA) and glutathione-S-transferase (GST) activity except superoxide dismutase (SOD) activity, were statistically lower in the irradiation (IR) group compared to all other groups. Total superoxide scavenger activity and NSSA were statistically higher in the IR plus TQ and IR plus CAPE groups compared to all other groups. In contrast, glutathione peroxidase (GSH-Px) activity was significantly found to increase in the IR plus CAPE group compared to control groups. The xanthine oxidase (XO), nitric oxide synthase (NOS) activities, nitric oxide ($NO^{\bullet}$) and malondialdehyde (MDA) levels in the IR group were statistically higher than in the other groups. Moreover, XO activity in the IR plus TQ group was statistically lower than all other groups including the IR plus CAPE group. In addition, $NO^{\bullet}$ level was found to increase in all groups when compared to the normal control group.*
Conclusions: *Thymoquinone and CAPE decrease oxidative and nitrosative stress markers and have antioxidant effects, which also increase antioxidant capacity in the liver tissue of irradiated rats.*

Keywords: Antioxidant, caffeic acid phenethyl ester, free radicals, irradiation, thymoquinone

Los Efectos Radioprotectores del Éster Feniletílico del Ácido Cafeico y la Timoquinona sobre el Estrés Oxidativo y Nitrosativo en el Tejido del Hígado de Ratas Expuestas a Irradiación Total de Cabeza

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RESUMEN

Objetivo: *El objetivo de este estudio es investigar los efectos de la adición del éster feniletílico del ácido cafeico (CAPE) y la timoquinona (TQ) sobre el estrés oxidativo y nitrosativo en el tejido del hígado de ratas irradiadas.*
Métodos: *Cuarenta ratas Sprague-Dawley fueron divididas en cinco grupos para probar la efectividad radioprotectora de TQ y CAPE administrados por inyección intraperitoneal. También se estudiaron gru*pos de control apropiados.

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Resultados: *La capacidad antioxidante de hígado – medida por los niveles de actividad total de barrido de superóxido (ATBS) —, la actividad no enzimática de barrido de superóxido (ANBS), y la actividad de la glutatión-S-transferasa (GST), excepto la actividad de la superóxido dismutasa (SOD), fueron estadísticamente inferiores en el grupo de irradiación (IR) en comparación con todos los otros grupos. La actividad total de barrido de superóxido y la ANBS, fueron estadísticamente más altas en los grupos de IR más TQ e IR más CAPE que en todos los otros grupos. En contraste con ello, se halló que la actividad de la glutatión peroxidasa, aumentó significativamente en el grupo de IR más CAPE en comparación con los grupos control. Las actividades de la xantina oxidasa (XO), la óxido nítrico sintasa (NOS), y los niveles de óxido nítrico (NO) y de malondialdehído (MDA) en el grupo de IR, fueron estadísticamente superiores en otros grupos. Además, la actividad de la XO en el grupo IR más TQ fue estadísticamente inferior a todos los grupos, incluyendo el grupo IR más CAPE. A esto hay que añadir que se halló que el nivel de NO, aumentó en todos los grupos, en comparación con el grupo normal de control.*

Conclusiones: *La timoquinona y el CAPE disminuyen los marcadores de estrés oxidativo y nitrosativo, y tienen efectos antioxidantes, que también aumentan la capacidad antioxidante en el tejido del hígado de ratas irradiadas.*

Palabras claves: antioxidantes, ácido cafeico, radicales libres, irradiación, éster feniletílico, timoquinona

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INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive species generated by biochemical redox reactions as a part of normal cell metabolism. Oxygen is a critical element for living organisms and has some advantages. In view of the generation of ROS, certain adverse effects also occur. Basically, all the essential biomolecules can undergo oxidative reactions mediated by ROS. The ROS, RNS and their suggested effects on biological systems have become an important area of biomedical research in recent years (1–3). The increase in ROS and RNS production or the decrease in antioxidant mechanisms generates a condition called oxidative stress, defined as the imbalance between pro- and antioxidants in favour of the oxidants. These highly reactive compounds are dangerous for the cells when their cellular production exceeds the antioxidant capacity (4, 5). Reactive oxygen species, RNS and lipid peroxides have been implicated in the pathogenesis of a large number of diseases such as diabetes mellitus, cataract, cancer, Behçet's disease and rheumatoid arthritis (6–9).

To prevent the damage caused by ROS and RNS, multiple defence systems, collectively called antioxidants, are present in various organs and tissues as well as in serum and erythrocytes. Antioxidants prevent the organism from the harmful effects of free radicals by scavenging or inhibiting their formation. Cells maintain their vital functions against oxidative damage with the help of a system that involves glutathione (GSH) and GSH-dependent enzymes glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST), and superoxide dismutase (SOD) against oxidative damage. However, after an increase in the production of free radicals or a decrease in the defence against toxic species or both, oxidative stress can occur. Oxidative stress reflects the consequences of a mismatch between the rate of formation of free radicals and the ability of the cell to transform them to less toxic species (2, 3, 10).

Radiotherapy is an important treatment modality for a large variety of malignancies, and over 50% of cancer patients repeatedly receive radiotherapy during treatment (11–13). Ionizing radiation is known to generate free radicals in irradiated tissue. In order to obtain improved tumour control with a higher dose, normal tissues should be protected against radiation injury. Therefore, the role of radioprotective compounds is highly important in clinical radiotherapy (7, 14). Efforts to decrease toxicity of irradiation to normal tissue, organs and cells have led the search for cytoprotective agents. Unfortunately, most radioprotectors possess toxic side effects, which limit their role in medical treatment (15). For this reason, investigations for effective and non-toxic compounds with radioprotection capability led to increasing interest in antioxidants such as caffeic acid phenethyl ester (CAPE) and thymoquinone (TQ).

Thymoquinone is reported to possess strong antioxidant properties against oxidative damage induced by a variety of free radical generating agents. Many pharmacological and toxicological studies have reported it to possess diverse pharmacological properties such as antioxidant, hepatoprotective, neuroprotective, antidiabetic, anti-inflammatory, nephroprotective and anti-carcinogenic (16, 17).

Caffeic acid phenethyl ester, a phenolic compound, is found and isolated from propolis, has immunomodulatory, anti-tumoral, cytotoxic, anti-metastatic, anti-inflammatory and antioxidant properties and has been shown to inhibit lipoxygenase activities as well as suppress lipid peroxidation (18).

Although the antioxidant property of CAPE and TQ has been investigated through a number of studies, data on the radio-modifying ability of these are limited. Therefore, we aimed to investigate the effect of CAPE and TQ supplementa-

tion on oxidant, antioxidant and nitrosative parameters in the liver tissue of rats exposed to total head irradiation.

SUBJECTS AND METHODS

Forty Sprague-Dawley rats fed with standard laboratory chow and water were used. The rats were quarantined for at least one week before gamma irradiation. They were randomly divided into five groups (eight rats per group) and placed in separate cages during the study. The groups were as follows:

Group 1: [Irradiation (IR) plus TQ group] received both 5 Gy of gamma irradiation as a single dose to total cranium and TQ (50 mg $kg^{-1}day^{-1}$) daily by intraperitoneal (IP) injection starting 30 minutes before the radiation dose and subsequently daily for 10 days after irradiation. Thymoquinone was dissolved in dimethyl sulfoxide (DMSO) just before giving to the rats.

Group 2: (IR plus CAPE group) received both 5 Gy of gamma irradiation as a single dose to total cranium and CAPE (10 μmol $kg^{-1}day^{-1}$, IP) injection starting 30 minutes before the radiation dose and subsequently daily for 10 days after irradiation. Caffeic acid phenethyl ester was dissolved in DMSO just before giving to the rats.

Group 3: IR group received total head 5 Gy of gamma irradiation as a single dose.

Group 4: (the control group of groups 1 and 2) received DMSO injections intraperitoneally at an equal volume of that of TQ and CAPE dissolved for groups 1 and 2, respectively.

Group 5: (normal control group) did not receive TQ, CAPE and irradiation.

Animal experiments were carried out in an ethically proper way by following guidelines as set by the Ethical Committee of the Gaziantep University. Supplementation period was 10 days.

Prior to total head irradiation, all rats were anaesthetized with 80 mg/kg ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) and placed on a tray in the prone position. The rats in the IR and the IR plus TQ groups received irradiation *via* a Cobalt-60 teletherapy unit (Picker, C9, Maryland, NY, USA) from a source-to-surface distance of 80 cm by 5 x 5 cm anterior fields, with the total head gamma irradiation being a single dose of 5 Gy, while the rats in the control and sham control groups received sham irradiation. The dose rate was 0.49 Gy/min. The central axis dose was calculated at a depth of 0.5 cm.

Biochemical analysis

Ten days after total head irradiation, all rats were anaesthetized with 80 mg/kg ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) and then were killed by decapitation, and their livers were removed. The liver was homogenized in physiological saline solution (IKA-NERKE, GmBH & Co. KB D-79219, Staufen, Germany), and the homogenate was centrifuged at 10 000 g for one hour to remove debris. The clear upper supernatant was collected and all assays were conducted on this fraction. All of the procedures were performed at 4 ˚C.

Total superoxide scavenger activity (TSSA) and non-enzymatic superoxide scavenger activity (NSSA) were performed on the samples before and after adding trichloroacetic acid (TCA, 20%), as described (19, 20). First, TSSA is measured. In this method, xanthine-xanthine oxidase complex produces superoxide radicals that react with nitroblue tetrazolium (NBT) to form a farmazone compound. Total superoxide scavenger activity activity is measured at 560 nm by detecting inhibition of this reaction. By using a blank reaction in which all reagents are present except the supernatant sample and by determining the absorbance of the sample and blank, TSSA activity is calculated. Second, NSSA activity is measured in TCA-treated fractions prepared by treating part of the sample with a final concentration of 20% (w/v) TCA solution (to remove all enzymes and proteins), and centrifuging at 5000 g for 30 minutes. After the elimination of proteins by this procedure, NSSA activity assay is performed in the supernatant fraction. Superoxide dismutase activity is calculated as the difference between TSSA and NSSA. One unit of TSSA, NSSA and SOD is defined as the amount of enzyme protein causing 50% inhibition in NBT reduction rate. Results are expressed as U/mg protein.

Glutathione peroxidase activity was assayed according to Paglia and Valentine (21). In this method, GSH-Px catalyses the oxidation of glutathione in the presence of tert-Butyl hydroperoxide. Oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to NADP. The reduction in absorbance of NADPH at 340 nm is measured. The absorbance change per minute and the molar extinction coefficient of NADPH are used to calculate GSH-Px activity, which is expressed as U/mg protein.

Xanthine oxidase (XO) was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm (22). Xanthine oxidase activity is expressed as U/g protein. Malondialdehyde (MDA), the final product of lipid peroxidation, was determined spectrophotometrically according to a similar method described by Ohkawa *et al* (23). The concentration of MDA was calculated using 1.56 x 10^5 Mcm^{-1} as molar extinction coefficient. The total thiobarbituric acid-reactive substances were expressed as MDA. Results are expressed as nmol/mg protein.

Nitric oxide synthase (NOS) activity is based on the diazotization of sulfanilic acid by nitric oxide ($NO^{\bullet}$) at acid pH and subsequent coupling to N-(1-naphthyl) ethylenediamine. To 0.1 mL of sample, 0.2 mL of 0.2 M arginine was added and incubated at 37 ˚C for one hour. Then the combination of 0.2 mL of 10 mM hydrochloric acid (HCl), 100 mM sulfanilic acid and 60 mM N-(1-naphthyl) ethylenediamine was added. After 30 minutes, the absorbance of the sample tube was measured against a blank tube at 540 nm (24). Results are expressed as U/mg protein. Nitric oxide levels in tongue tissue were measured using the Griess reagent as previously described (25). Results are expressed as μmol/g wet weight. The protein content

was determined as described (26). Biochemical measurements were carried out using a spectrophotometer (Shimadu U 1601, Japan).

Statistical analyses

Statistical analyses were undertaken using a one-way variance analysis and Spearman's rank correlation test, respectively. Least significant difference (LSD) multiple range test was used to compare the mean values. Acceptable significance was recorded when *p*-values were < 0.05. Statistical analysis was performed with SPSS for Windows (SPSS, version 11.5, Chicago, IL, USA).

RESULTS

Antioxidative parameters

As shown in Table 1, there were statistically important differences between the IR group and other groups. Total superoxide scavenger activity, NSSA and GST activities except SOD activity were statistically lower in the IR group compared to all other groups ($p < 0.0001$). Total superoxide scavenger activity and NSSA activities were statistically higher in the IR plus TQ and IR plus CAPE groups compared to all other groups. In contrast, GSH-Px activity was found to significantly increase in CAPE-administered rat livers compared to control group.

Oxidative stress parameters

As for the comparison from the point of view of the XO activity and MDA level, the indicators of oxidant stress (Table 2), there were statistically significant differences between the IR group and other groups. The XO activity and MDA level in the IR group were statistically higher than other groups ($p < 0.0001$). Also, XO activity in the IR plus TQ group was statistically lower than all other groups including the IR plus CAPE group. Irradiation-induced increase in these oxidative parameters was prevented by TQ and CAPE.

Nitrosative stress parameters

As presented in Table 3, NOS activity and $NO^{\bullet}$ level in the IR group was statistically higher than other groups ($p < 0.05$ for $NO^{\bullet}$, $p < 0.0001$ for NOS). In addition, $NO^{\bullet}$ level was found to increase in all groups when compared to the normal control group.

Table 1: Antioxidative parameters measured in the liver tissue of the rats

	Normal control group	Control group of TQ and CAPE	IR group	IR plus TQ group	IR plus CAPE group
TSSA (U/mg protein)	2292.1 ± 264.9^{a}	2198.5 ± 218.0	2077.2 ± 65.9	$2717.0 \pm 426.2^{b,e,g}$	$2663.9 \pm 439.1^{b,e,g}$
NSSA (U/mg protein)	1646.2 ± 196.1	1525.8 ± 159.9	1380.5 ± 164.2	$1919.9 \pm 313.8^{c,e,g}$	$1914.0 \pm 385.2^{c,e,g}$
SOD (U/mg protein)	697.2 ± 101.7	672.6 ± 92.4	696.6 ± 134.2	809.7 ± 183.0	749.8 ± 150.6
GST (U/mg protein)	170.6 ± 20.5^{a}	142.9 ± 17.2^{g}	141.9 ± 20.7	145.7 ± 22.3^{g}	153.4 ± 33.5
GSH-Px (U/mg protein)	9.6 ± 1.3^{c}	$11.9 \pm 2.0^{b,g}$	17.8 ± 1.8	$10.4 \pm 1.2^{c,f}$	$25.7 \pm 6.4^{c,f,m}$

a: $p < 0.05$, b: $p < 0.001$, c: $p < 0.0001$ *vs* irradiation group, d: $p < 0.05$, e: $p < 0.01$, f: $p < 0.0001$ *vs* control group of TQ and CAPE, g: $p < 0.05$, m: $p < 0.0001$ *vs* normal control group

IR group: irradiation group; IR plus TQ group: irradiation plus thymoquinone group; IR plus CAPE group: irradiation plus caffeic acid phenethyl ester group; TSSA: total superoxide scavenger activity; NSSA: non-enzymatic superoxide scavenger activity; SOD: superoxide dismutase, GST: glutathione-S-transferase; GSH-Px: glutathione peroxidase

Table 2: Oxidative stress parameters measured in the liver tissue of the rats

	Normal control group	Control group of TQ and CAPE	IR group	IR plus TQ group	IR plus CAPE group
XO U/g protein	3.8 ± 0.82^{b}	3.5 ± 0.69^{c}	5.0 ± 0.96	$2.6 \pm 0.77^{c,d,k,n}$	4.1 ± 0.64^{a}
MDA nmol/mg protein	9.8 ± 1.6^{c}	10.2 ± 0.32^{c}	13.4 ± 1.92	10.5 ± 0.93^{c}	$10.9 \pm 1.66^{c,g}$

a: $p < 0.05$, b: $p < 0.001$, c: $p < 0.0001$ *vs* irradiation group, d: $p < 0.05$ *vs* control group of TQ and CAPE, g: $p < 0.05$, k: $p < 0.01$ *vs* normal control group, n: $p < 0.001$ *vs* normal control group.

IR group: irradiation group; IR plus TQ group: irradiation plus thymoquinone group; IR plus CAPE group: irradiation plus caffeic acid phenethyl ester group; XO: xanthine oxidase; MDA: malondialdehyde

Table 3: Nitrosative stress parameters measured in the liver tissue of the rats

	Normal control group	Control group of TQ and CAPE	IR group	IR plus TQ group	IR plus CAPE group
NOS U/mg protein	4.7 ± 0.93[c]	5.8 ± 1.55[b]	8.1 ± 1.27	5.2 ± 1.30[c]	5.1 ± 1.21[c]
NO• µmol/g wet weight	3.63 ± 0.80[c,e]	4.93 ± 096[a,g]	5.66 ± 0.77	4.82 ± 0.70[a,k]	4.68 ± 0.69[a,k]

a: $p < 0.05$, b: $p < 0.001$, c: $p < 0.0001$ *vs* irradiation group, g: $p < 0.05$, k: $p < 0.01$ *vs* normal control group.

IR group: irradiation group; IR plus TQ group: irradiation plus thymoquinone group; IR plus CAPE group: irradiation plus caffeic acid phenethyl ester group; NOS: nitric oxide synthase; NO•: nitric oxide

DISCUSSION

Organisms have two types of antioxidants, enzymatic (SOD, GSH-Px, GST) and non-enzymatic antioxidant mechanisms (NSSA, GSH, vitamin C, vitamin E) that act by neutralizing excessive ROS and RNS and prevent them from damaging the cellular structure (27). Normal cell functions and integrity of cell structures may be damaged *via* considerable reactivity of ROS and RNS. Free radical-scavenging antioxidants are consumed by the increased free radical activity associated with several conditions (28). The oxidative stress to an organism is mitigated by scavenging or neutralization of oxidative radicals with the help of endogenous antioxidants such as SOD, GSH-Px and GST, albumin, ceruloplasmin (3, 29, 30).

The present study characterizes the irradiation-mediated oxidative and nitrosative stress in liver tissue. Our results demonstrated that XO, NOS activities, MDA and NO• levels, oxidative and nitrosative stress parameters in the liver tissue of irradiated rats were significantly higher than in the control rats. This is consistent with the hypothesis that irradiation may generate oxidative stress. The levels of parameters measured in this tissue were also lower in irradiated rats that were administered TQ and CAPE than in those that did not receive TQ and CAPE. Our results support the research hypothesis that the systemic administration of TQ and CAPE would reduce the oxidative and nitrosative damage in irradiated liver tissue in a rat model.

Most individuals are exposed to ionizing radiation from radiotherapy or from diagnostic or therapeutic procedures that involve radionuclides in nuclear medicine. It is accepted that ionizing radiation is an important modality for the treatment of human malignancies. However, acute and late toxicity of the radiation on normal tissues limits its role in cancer treatment. The aim of radiotherapy is to kill cancer cells while causing as little damage as possible to normal cells. Ionizing radiation generates free radicals when it passes through living tissues. Interactions of free radicals with DNA can induce genetic damage and cause mutagenesis and carcinogenesis. However, while it is important to protect normal tissues, it is not always possible to keep these tissues outside of the treatment field and avoid the side effects and complications of irradiation. Furthermore, free radicals produced by local radiation applications may be carried to distant organs *via* the systemic circulation (31, 32).

Exposure to high amounts of ionizing radiation results in damage to the haematopoietic, gastrointestinal and central nervous systems, depending on the radiation dose. Therefore, it is important to protect normal tissues in the treatment field. The nature and degree of such side effects depend on the dose of radiation and the sensitivity of the organs irradiated. The development of effective radiomodifiers is of great medical importance. Radioprotective agents are synthetic compounds or natural products immediately administered before irradiation to reduce radiation-induced injuries. Naturally occurring compounds that function as antioxidants and immunostimulants are important for the development of radioprotective agents. Approved radioprotective agents are expensive and have some severe side effects (31, 32). For this reason, to prevent injury caused by radiation on healthy tissue, many investigations related to natural products that have antiviral, anticancer, immunostimulant and antioxidant effects have been constructed. Several studies on radioprotective agents are ongoing. To our knowledge, the present study on TQ and CAPE on oxidative and nitrosative stress in liver tissue of rats exposed to total head irradiation is the first, and results obtained in the study are important.

Reactive oxygen species and RNS are exceedingly reactive compounds that can be formed in the body during metabolic activities as part of regular metabolic reactions or from environmental causes such as ionizing radiation *eg* X- and γ-rays and ultraviolet light (5, 7). Even though free radicals are necessary for some reactions, they can generate oxidative stress if they exceed the antioxidant capacity of the cells. For this reason, their concentrations are strictly regulated with a variety of enzymatic and non-enzymatic systems in the body during metabolic activities. The free radicals can react with many biological molecules like DNA, proteins and membranes and consequently damage the cells in a number of ways (5).

Nitric oxide is a multifunctional molecule that is implicated in a wide variety of physiological and pathological processes. We found that NO• level and NOS activity in the IR group were significantly increased compared to those of all other groups. This confirms the presence of increased nitrosative stress in the liver tissue of irradiated rats. The concentrations of NO• under nonpathological conditions are in the nanomolar, and under conditions of oxidant injury in the micromolar range. Nitric oxide reacts rapidly with superoxide

radical ($O_2^{\bullet-}$) to form peroxynitrite, which is itself cytotoxic and readily decomposes into the highly reactive and toxic hydroxyl radical and nitrogen dioxide. Peroxynitrite is much more reactive than $NO^{\bullet}$ or $O_2^{\bullet-}$, which causes diverse chemical reactions in biological systems including nitration of tyrosine residues of proteins, triggering of lipid peroxidation, inactivation of aconitases, inhibition of the mitochondrial electron transport and oxidation of biological thiol compounds (3, 33). Total superoxide scavenger activity, NSSA, SOD, GSH-Px and GST are very important antioxidants that play pivotal roles in the elimination of $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2), which prevent the propagation of lipid peroxidation reactions to remove ROS and RNS. In this period, SOD, the first line of defence against oxygen-derived free radicals, catalyses the dismutation of the $O_2^{\bullet-}$ into H_2O_2. Hydrogen peroxide can be transformed into H_2O and O_2 by GSH-Px (10, 34, 35). These enzymes can act as protectors that preserve against the oxidative damage induced by ROS and RNS in different tissues (34–36). In the present study, we found a significant reduction in unchanged SOD activity, a decrease in TSSA, NSSA and an increase in GSH-Px and XO activities in liver tissues of irradiated rats when compared to other groups. Lipid peroxidation is the primary cellular damage resulting from free radical reactions. Elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides. Malondialdehyde level, a marker of lipid peroxidation, was found to be similar to that reported by previous studies (34, 35, 37–39). Interestingly, when supplemented with TQ and CAPE, XO activity and MDA levels in these groups were significantly lower than those in liver tissues of irradiated rats. Thus, in the liver tissues of rats exposed to irradiation only (IR group), there was a significant reduction of TSSA and NSSA, a significant increase of XO activity, oxidant enzyme, and increased membrane damage due to MDA. The findings of the current study are in line with previous studies, which have reported the antioxidant effects of CAPE (18). In addition, the finding that these antioxidant substances provide protective effects against radiation-induced oxidative stress is consistent with previous reports on the radioprotective effects of CAPE (40). Furthermore, it has been reported that TQ could act as a free radical and superoxide radical scavenger (16). The finding that TQ and CAPE prevent oxidative and nitrosative stress by inhibiting lipid peroxidation is also very important.

In conclusion, to our knowledge, this is the first study that simultaneously investigates the radioprotective effects of TQ and CAPE on oxidative and nitrosative damage in the liver tissue of irradiated rats. These results suggest that TQ and CAPE exhibit radioprotective effects against oxidative and nitrosative damage in the liver tissue of irradiated rats.

Thymoquinone and CAPE, having multi-pharmacological activities, can be considered in treating irradiation-associated complications. Since free radicals are the major mediators for radiation-induced damage, a treatment combining radiation with an antioxidant might provide a strategy for preventing radiation injury to normal tissues.

AUTHORS' NOTE

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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