European Journal of Therapeutics

DOI: 10.5152/EurJTher.2019.18052

Original Research

Radioprotective Effect of Caffeic Acid Phenethyl Ester on the Brain Tissue in Rats Who Underwent Total-Head Irradiation

Nesrin Khayyo[1], Muhammed Enes Taysı[2], Elif Demir[3], Hasan Ulusal[1], Kadir Çınar[4], Mehmet Tarakçıoğlu[1], Seyithan Taysı[1]

[1]Department of Medical Biochemistry, Gaziantep University School of Medicine, Gaziantep, Turkey

[2]Gaziantep University School of Medicine, Gaziantep, Turkey

[3]Division of Biochemistry, Harran University Faculty of Health, Şanlıurfa, Turkey

[4]Department of Neurosurgery, Sanko University School of Medicine, Gaziantep, Turkey

ABSTRACT

Objective: In this study, we evaluated whether caffeic acid phenethyl ester (CAPE) has a radioprotective effect on the damage in the rat brain tissue induced by gamma radiation, considering that it may inhibit the ionizing radiation damage.

Methods: A total of 36 Sprague–Dawley rats were divided into four groups to test the radioprotective effect of CAPE administered by intraperitoneal injection. An appropriate control group was also studied. On day 11, the brain tissue of all rats was removed and homogenized in phosphate buffer, and the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), paraoxonase (PON), arylesterase (ARE), ceruloplasmin (CER), lipid hydroperoxide (LOOH), and total-SH parameters were measured to determine if CAPE had a protective effect.

Results: The ARE and PON activity and the total-SH level were statistically increased compared to the IR group, whereas the LOOH, TOS, and OSI levels were significantly decreased.

Conclusion: The data obtained in the study suggest that the CAPE administration prior to irradiation may prevent the irradiation brain damage.

Keywords: Antioxidant enzymes, brain, caffeic acid phenethyl ester, irradiation, oxidative stress

INTRODUCTION

Brain tumors are recognized to be among the most malign tumors. According to the World Health Organization's (WHO) report, being the fourth-degree diffuse-type tumors, they have a high rate of mortality (1, 2). However, current treatment approaches are very limited, consisting of radical surgery, radiotherapy, and chemotherapy. Tumoral gliomas can diffuse throughout the brain by infiltrating into the lymphatic drainage system. Being effective in the brain, radiotherapy is preferred for diffuse tumors such gliomas. Nevertheless, the selective permeability of the blood–brain barrier prevents chemotherapeutic agents from substantially accessing tumoral cells that are diffuse throughout the brain (3, 4).

Radiotherapy, as one of the most frequent treatment methods, can be used in all types of cancer. Radiotherapy is preferred in 2 out of 3 patients who apply to clinics. In addition, the radiotherapy dose required to establish control over cancer cells is higher than usual, and that is why when applied, it causes damage in normal tissues (5). As the degree of damage in normal tissues caused by radiotherapy changes depending on the tissue radio-sensitiveness, it was shown in studies that there is a risk up to 8 times higher when the whole-body irradiation is used. When radiotherapy is applied to tumors located within the head and neck, brain, or eye, it can be observed that there are destructive effects on other nearby tissues, which depend on radiotherapy. It is known that reactive oxygen species (ROS) are accumulated and that DNA fragments are generated which cause these destructive effects following radiotherapy. Leading to the endoplasmic reticulum and mitochondrial membrane damage, accumulated ROS increase the damage to surrounding tissues, which depends on radiotherapy. It is pointed out in the literature that ROS and reactive nitrogen species (RNS) play a role in the pathogenesis of many diseases (6-9). Furthermore, it has been

How to cite: Khayyo N, Taysı ME, Demir E, Ulusal H, Çınar K, Tarakçıoğlu M, et al. Radioprotective Effect of Caffeic Acid Phenethyl Ester on the Brain Tissue in Rats Who Underwent Total-Head Irradiation. Eur J Ther 2019; 25(4): 265–72.

ORCID IDs of the authors: N.K. 0000-0003-4583-4538; M.E.T. 0000-0001-7419-5988; E.D. 0000-0003-4545-5175; H.U. 0000-0003-3890-2088; K.Ç. 0000-0002-4517-3808; M.T. 0000-0002-2398-8264; S.T. 0000-0003-1251-3148

Corresponding Author: Seyithan Taysı **E-mail:** seytaysi@hotmail.com

Received: 17.10.2018 • **Accepted:** 20.03.2019

demonstrated in many studies that free radicals formed due to radiation infest other tissues and organs through systemic circulation (10, 11). In addition to the known antioxidant properties of caffeic acid phenethyl ester (CAPE), data on the radiation-protective ability of this agent are limited (6, 12). We hypothesized that CAPE, which antioxidant effects have been proven in many studies, could protect the brain tissue from radiation-induced oxidative damage. For this reason, we measured the oxidative biomarkers, total oxidant status (TOS), oxidative stress index (OSI), lipid hydroperoxide (LOOH), and antioxidant biomarkers, total antioxidant status (TAS), paraoxonase (PON), arylesterase (ARE), ceruloplasmin (CER), and total-SH in the brain tissue of rats with or without gamma radiation exposure to total cranium with a single dose of 5 Gray (Gy).

METHODS

This study is conducted using 36 male Wistar Albino rats weighing 200±20 gr. The rats were divided into four groups as the control (n=8), sham control (n=8), irradiation (IR) (n=10), and IR+CAPE group (n=10). Prior to total cranium irradiation, all rats except the sham control group were anesthetized with 80 mg/kg ketamine hydrochloride (Pfizer İlaç, İstanbul, Turkey) and placed on a tray in the prone position. The rats in the IR and the IR+CAPE groups received irradiation via a cobalt-60 teletherapy unit (Picker, C9, Maryland, NY, USA) from a source-to-surface distance of 80 cm by 5×5 cm anterior fields, with the total cranium gamma irradiation being a single dose of 5 Gy, while the rats in the control and sham control groups received sham irradiation. Ten days after irradiation, all animals were killed by decapitation, and their brain tissues were removed and homogenized within a phosphate buffer (a single volume of the tissue sample and nine volumes of phosphate buffer as cold as ice). In the aftermath of this homogenization procedure, the obtained supernatant was put in five eppendorf tubes and kept at −80°C until biochemical assessment for protection from deformations. This study spectrophotometrically analyzed biochemical parameters like TAS, TOS, OSI, LOOH, PON, ARE, CER, and total SH, which have been chosen for assessing CAPE's antioxidant effectiveness.

The information regarding the groups can be found as following: Group 1: Sham control group (SCG): No drug application and/or surgical intervention was conducted in this control group.

Group 2: Control group (CG): As this group is the positive control of the fourth group, rats in this group were injected intraperitoneally (IP) with 0.25 ml dimethyl sulphoxide (DMSO) for 10 days.

Group 3: IR group: The head areas of the rats from this group were applied a single dose of 5 Gy on the 1st day. Thirty minutes prior to and throughout 10 days following this application, rats were given physiological saline solution IP.

Group 4: IR+CAPE group: The rats within this group were given a single dose of 5 Gy on the 1st day. Thirty minutes prior to and throughout 10 days following this application, subjects were applied IP 10 µmol/kg/day CAPE, which was thawed in DMSO. Rats in all groups were fed with regular food and water. At the end of the experiment, animals were decapitated being, which was identical to the protocol carried out in the first group, and advanced biochemical analyses were made following the removal of the brain tissue. CAPE was dissolved in DSMO immediately before the application. The final concentration of DMSO was 0.1%.

This study was conducted at the Department of Medical Biochemistry after obtaining ethical approval from the Animal Ethics Committee of Gaziantep University School of Medicine (2017/2).

Measurement of Antioxidant Parameters

Measurement of total antioxidant status

The method of measuring the TAS levels was as follows: This molecule gets decolorized as all antioxidant molecules reduce the ABTS cationic radical. The degree of decolorization is proportional to the total concentration of antioxidant molecules (13). During this procedure, Trolox, that is a water-soluble analogue of Vitamin E, was used as a calibrator. Results were presented as mmol Trolox equivalent/gr protein.

Measurement of Total SH

The level of total-SH groups in samples was measured according to Ellman's method (14). Results were presented as mmol/gr protein.

Measurement of Paraoxonase Enzyme Activity

Attached to paraoxonase HDL-cholesterol, lipophilic is a hydrophobic antioxidant enzyme. This enzyme's activity was measured by using a kit of Real Assay. In short, in this method, the PON enzyme hydrolyses the paraoxon substrate (O, O-diethyl-O-pnitrophenylphosphate) by reacting with it. Colored p-nitrophenol is a product of this hydrolyzing procedure. Monitored in the kinetic mode at 412 nm, this product's absorbance is expressed as U/g protein (15).

Measurement of Arylesterase Activity

The ARE activity e is measured with the Real Assay commercial kit. In this test, the enzyme contained by the sample that is to be measured triggers an enzymatic reaction with phenylasetat substrate, and hence creates phenol. The obtained phenol is measured colorimetrically, and the activity is thus determined (16). Results are presented as U/g protein.

Measurement of Ceruloplasmin Level

The ceruloplasmin level is determined according to the method suggested by Erel (17). Being a colorimetric method, it measures enzymatic oxidation of the ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Results are presented as U/gr protein.

Measurement of Oxidant Parameters

Measurement of lipid hydroperoxide level

The level of lipid hydroperoxide was measured by using the modified FOX2 assay method (18). In this method, ferrous ions that are in the reaction medium are oxidized to ferric ions by lipid hydroperoxides. The generated ferric ion chromogens form a complex molecule with xylenol orange and ferric ion and the absorbance of this formed colored molecule is measured at 560

Table 1. TAS, TOS, OSI, LOOH, PON, ARE, SER, and Total SH values

	TAS	TOS	OSI	LOOH	PON	ARE	CER	Total SH
Sham control group	0.36±0.078	25.33±2.76[e]	7.92±2.47[a]	1.31±0.076[b]	0.571±0.098[b]	10.30±0.468	56.91±3.32	0.0669±0.003
Control group	0.36±.078	28.16±3.69[e]	7.42±2.43[a]	1.27±0.043[c]	0.921±0.285[e]	10.59±0.697[a]	57.28±3.99	0.0666±0.002
IR group	0.32±0.103	35.16±3.84	11.97±3.54	1.46±0.093	0.351±0.060	9.48±0.745	53.37±7.48	0.0606±0.008
IR+CAPE group	0.38±0.043	27.39±2.35[e]	7.24±1.14[a]	1.17±0.169[e]	0.551±0.061[d]	10.68±0.657[a]	58.61±5.77	0.0710±0.005[a]

[a]: p<0.05; [b]: p<0.01; [c]: p<0.001; [d]: p<0.005; [e]: p<0.0001 vs. IR group

TAS: mmol Trolox equivalent/ gr protein; TOS: mmol/gr protein; OSI: arbitrary unit; LOOH: μmol/gr protein; PON: U/g protein; ARE: U/g protein; CER: U/ gr protein; Total SH: mmol/gr protein

nm in the endpoint mode. It is t-butyl hydroperoxide standard, which is freshly prepared as a calibrator used for this measurement. Results are presented as μmol/gr protein.

Measurement of total oxidant status level

While measuring TOS levels of samples, an assessment was made on the color change due to the oxidization of ferrous ion to ferric ions by oxidant molecules that samples contain. This is a method of colorimetric TOS measurement, which has been previously acknowledged within the scholarly literature (19). Results were expressed as μmol H_2O_2 equivalent/gr protein.

Calculation of Oxidative Stress Index

First, TOS and TAS units were calculated as μmol for the OSI calculation of samples. Then, OSI was calculated according to OSI (AU)=[(TOS μmol/L)/(TAS μmol/L)]x100 formula.

Measurement of Protein

It was the Bradford method that has been used during the protein designation conducted within this study (20). For standard curve, 25–300 μg series solutions that contain cattle serum albumin were prepared. Taken 0.1 mL from the prepared solution was added to 5 mL Coomassie Blue reactive dye. Five minutes after the mixture was made, its absorbance was measured at 595 nm. Calculations were made in accordance with the standard curve.

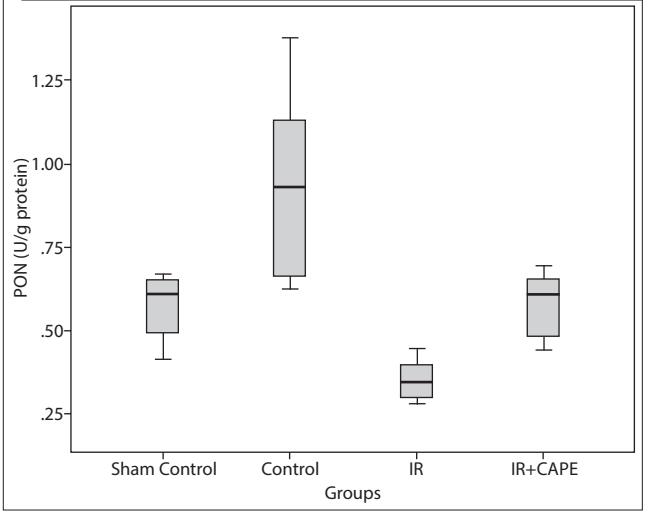
Statistical Analysis

The Kolmogorox–Smirnov test was used to check compliance with normal distribution. For comparing three independent group variables that have a normal distribution, analysis of variance and LSD multi-comparison tests were used. The interrelations between variables were tested using the Pearson correlation analysis. The frequency, percentage, and average of standard deviation values were given as introductory statistics. For statistical analyses, the SPSS for Windows version 22 (IBM Corp.; Armonk, NY, USA) package program was used, and a p-value ≤0.05 was accepted as statistically significant.

RESULTS

The TAS, TOS, OSI, LOOH, SER, total SH values, PON, and ARE activities of four groups that have been taken into account are summarized in Table 1 and Figures 1–6. When groups were assessed in terms of TAS levels, no significant change was observed in relation to control groups (p>0.05). However, when assessed in terms of TOS levels, there were statistically significant changes. A significant increase was observed when the TOS level of Group 3 (IR group) was compared with other groups (p<0.0001). It was detected that there were statistical differences in terms of OSI levels. The OSI level in Group 3 was found to be significantly increased in relation to Groups 1, 2, and 4 (p<0.05). Furthermore, there was no statistically significant difference observed between Groups 1, 2, and 4 (p>0.05).

Figure 1. Demonstration of the PON level distribution among groups by Box PLOT graph



It was observed that there were statistically significant differences when groups were assessed in terms of LOOH levels. It was noted that there was a significant increase in the LOOH level of Group 3 in comparison to Groups 1, 2, and 4 (p<0.01, p<0.001, p<0.0001, respectively). In a similar vein, it was noticed that there were significant differences between groups in terms of the PON activity. As there was a significant difference between the PON activity in Group 2 and the PON activity

Figure 2. Demonstration of the ARE level distribution among groups by Box PLOT graph

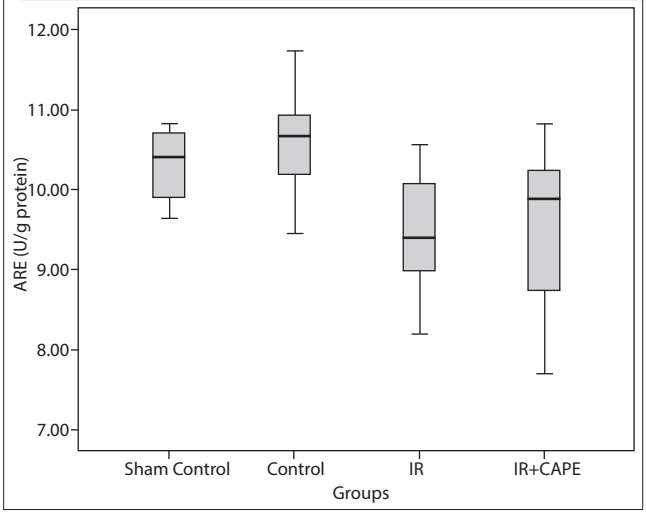


Figure 3. Demonstration of the TOS level distribution among groups by Box PLOT graph

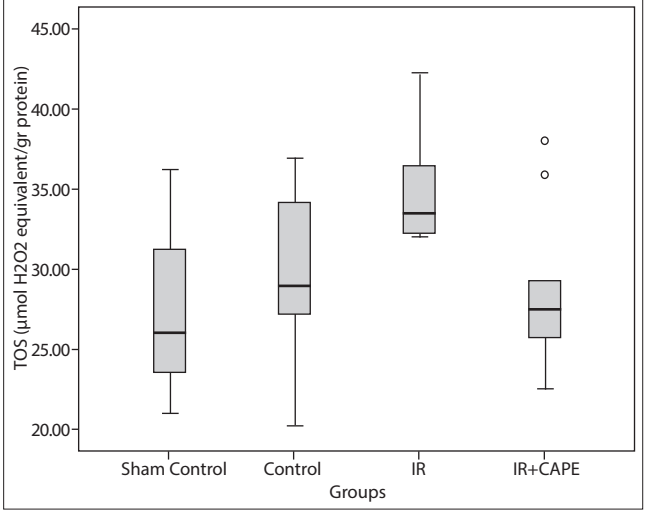


Figure 4. Demonstration of the LOOH level distribution among groups by Box PLOT graph

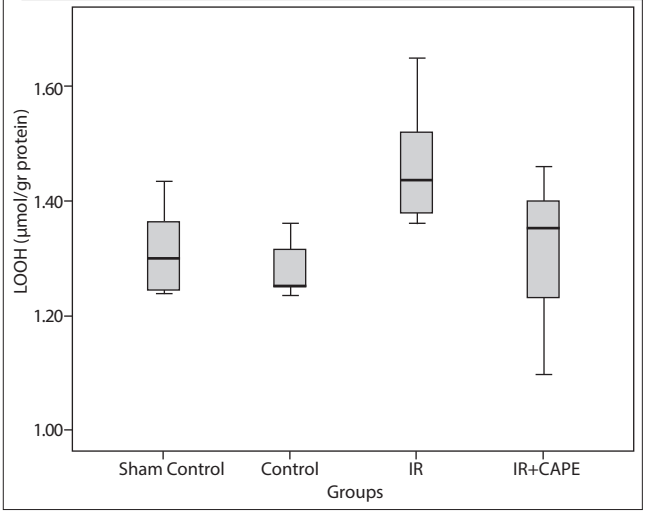
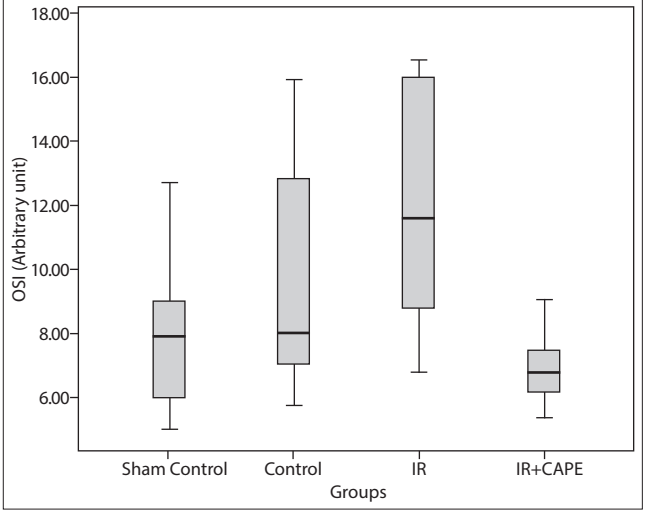


Figure 5. Demonstration of the OSI level distribution among groups by Box PLOT graph

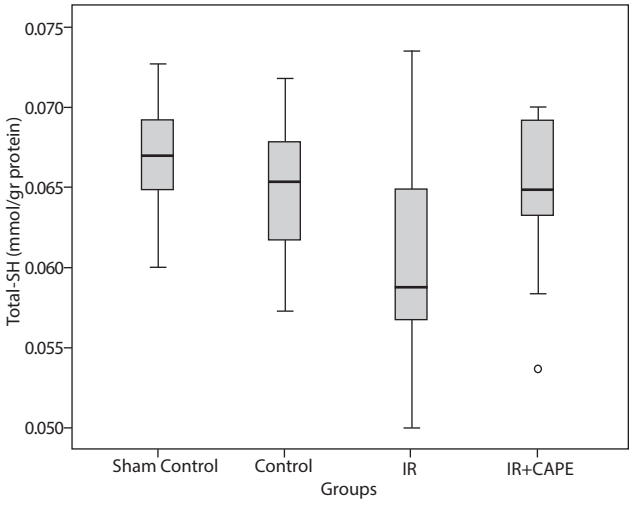


in Group 1, the value was higher in Group 2 ($p<0.05$). This increase in Group 2 was not only to be considered as a significant change in relation to Group 1, but also the others ($p<0.001$). Moreover, the PON activity in Group 3 decreased significantly in comparison with Groups 1, 2, and 4 ($p<0.01$, $p<0.0001$, $p<0.005$, respectively). By the time the total-SH assessment was made, it was observed that there were statistically significant differences between groups ($p<0.05$). The level of total SH in Group 4 was found to be significantly higher than in Group 3. Finally, when groups were compared in terms of the ARE activity and CER level, it was observed that there were statistically significant differences in the ARE activity, whereas there was no such difference in terms of the CER level. The ARE activity in Group 3 was found to be significantly low in comparison with that of Groups 2 and 4 ($p<0.05$). Yet, there were no significant changes detected between Groups 3 and 1 ($p>0.05$).

DISCUSSION

Ionized radiation, radiology in particular, is a risk factor for the personnel working in the radiotherapy and nuclear medicine departments. Such a risk originates from used radioactive materials such as the isotopes of radium, uranium, and thorium (21). Despite its detrimental effects, radiotherapy is one of the most significantly effective treatment modalities. Hence, approximately more than a half of patients with cancer is treated via radiotherapy. During the radiation treatment, an effective dose is to be determined to maximize toxicity upon cancer cells; however, this dose may also show toxic effects on healthy tissues that remain within the application area of radiotherapeutic treatment. Each tissue's sensitivity level is different. Therefore, the extent of damage on a tissue that has been subject to radiation depends on that tissue's sensitivity. These issues reflect the increasing importance of the need for research on the acute effects and af-

Figure 6. Demonstration of the total-SH level distribution among groups by Box PLOT graph



ter-effects of ionized radiation on tissues and cells (6, 12, 22, 23). In accordance, among the scholarly work focusing on relevant radiotherapy studies, the ones researching the oxidative stress occurrence, the consequent free radical generation, and furthermore the interaction of antioxidants with this oxidative mechanism bear vital importance.

It has been reported that accumulated within the cell, ROS damages its components and therefore pave the way for diseases (24, 25). One kind of radiotherapy cell damage is lipid peroxidation, thus the deformation of the epicyte. The failure of its structure and function causes an uncontrolled flow of free radicals coming in and going out of the cell, resulting in damage.

When the cell and the tissue are radiated, the oxidative damage begins within the cell. The degree of this damage changes depending on the balance between the cell's antioxidant defense system and the ROS levels. When this balance is altered in favor of ROS, the degree of damage within the cell increases (26, 27). Categorized by effect types, basic antioxidants within cells are divided into two main groups as enzymatic and non-enzymatic. Enzymatic antioxidants operate by activating enzymes within the cell. These, in short, can be outlined as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and glutathione reductase (GR). Non-enzymatic ones, on the other hand, take effect through inhibiting the ROS production. These can be exemplified as GSH, Vitamins C and E, melatonin, zinc, ginkgo biloba and carotenes (28-31).

In the central nervous system, the amount of endogenic antioxidants is relatively low compared to other tissues, and for this reason, nerve cells are more sensitive to oxidative damages that can potentially increase (32). As in all tissue types, there are ROS cleaner enzymes within the ones in brain. Most important among these are SOD and GSH-Px. SOD converts the superoxide radical ($O_2^{\bullet-}$) that is accumulated within the cell into hydrogen peroxide (H_2O_2). Being different enzymatic antioxidants, GSH-Px and CAT prevent the harm of H_2O_2 by converting it into H_2O and molecular oxygen (O_2).

Scholarly work within the literature reports that SOD may protect the cell from the damage caused by ROS accumulated within (33, 34). In addition, thanks to the available cell culture studies, it is known that SOD shows a protective effect against ROS caused by tumor necrosis factor, interleukin1, and ionized radiation within cells (35). Many studies examined the parameters that display the damage on the brain tissue caused by radiation (11, 36, 37). Among these, the research conducted by Kojima et al. (37) can be pointed out as one of the milestones within this field. In this study, mice of different age (1, 4, and 12 week old, and 1 year old) were subjected to whole-body radiotherapy, and the effect of this application on the lipid peroxidation within the mouse brain tissue was examined. In this approach, 1-week-old mice were considered to be equivalent to newborn humans, 4 week old to adolescents, 12 week old to adults, and 1 year old to the elderly. As a result, when compared to control groups, it was reported that there were no statistically significant changes in SOD, GSH-Px, and CAT activities and MDA levels in the brain tissue of adult (12-weeks-old) mice (37, 38).

Another important study is the one by Collins-Underwood et al. (39), where rats were subjected to cranial radiation therapy, and primary neuronal culture was shown. When cells within the culture reach a sufficient number, the NADPH oxidase activity that converts O_2 to $O_2^{\bullet-}$, an oxidative type, was studied, and as a result, a decrease was observed. When rats were given a NADPH oxidase inhibitor IP before irradiation to verify this finding, it was demonstrated that a ROS increase was substantially prevented within the cell induced with radiation. Moreover, Kojima et al. (37) show that irradiating the mouse brain with a low-dose (50 cGy) gamma ray induces endogenic antioxidant potential. In line with this finding, it is thought that low-dose irradiation can be used in the treatment of neurodegenerative diseases that are induced with ROS accumulated within nerve cells. This issue remains as a new research question.

There are some parameters found in the literature used to assess the oxidative level and lipid peroxidation in the brain tissue. Whereas the MDA level is used to assess lipid peroxidation, SOD, GSH-Px, CAT, and XO activities are acknowledged as valid parameters for the evaluation of oxidative damage. Accordingly, in our study, TAS, TOS, OSI, PON, ARE, CER, and SH parameters in the brain tissue are measured and assessed to detect whether there are any protective effects of CAPE, which has antitumoral and antiinflammatory and antioxidant effects, in averting the impact of locally applied radiotherapy on the oxidant–antioxidant system.

In their study with rabbits, Ilhan et al. (40) researched the protective effect of CAPE and methyl prednisolone on the irradiated spinal cord. The study assessed the spinal cord's post-irradiation MDA level, SOD and CAT activities, and histopathological changes. It is reported that MDA levels are significantly low in the group that was applied CAPE in comparison with the methyl prednisolone group, and compared with the control group, there was no observed tissue damage in the CAPE group. Con-

ducted by Yilmaz et al. (41), in another study, the MDA levels and SOD and CAT activities in the liver of rats with diabetes induced with streptozotocin were researched. As a result, it was reported that MDA levels increased in diabetic rats compared to the control group, and in the group injected with CAPE, it remained on the same level with the control group. Hence there was no significant change observed. Moreover, it is detected that CAPE reduces the SOD and CAT activities in these rats. This is because of CAPE's cleansing of ROS and pressure on the SOD and CAT activities.

To the best of our knowledge, there are no existing studies on TAS, TOS, OSI, PON, ARE, CER, LOOH, and SH parameters in the brain tissue of rats subjected to ionized radiation. In our study where these parameters were assessed in the case of rats subjected to total-head irradiation for the first time, it was observed that there was no significant difference on TAS levels among rat groups. In line with the existing literature, it has been verified that radiotherapy does not affect the anti-oxidative mechanism in the brain tissue. In addition, it has also been verified with a significant increase in the TOS level that radiotherapy generated oxidative damage in rats.

One of the most important outcomes of the ROS damage on tissues is lipid peroxidation. In the recent years, lipid peroxidation has been emphasized as an important topic. LOOHs that emerge at the chain stage of lipid peroxidation are weak outputs, and they form aldehydes, ketones, carboxylic acids, alkanes, alkenes, and various polymerization products as breaks and dissolutions occur on the chain. As a result of this reaction, products such as MDA emerge and determine the degree of peroxidation (42). When assessing our working groups in terms of LOOH levels, a significant difference was detected between Group 4 injected with CAPE and Group 3 subjected to radiotherapy ($p<0.05$). The LOOH level in Group 4 was lower. This finding indicates that CAPE plays a protective role against oxidative damage on rats induced by radiotherapy. The MDA levels increase in Group 3 and a significant decrease in the CAPE+R group supports the claim of CAPE's antioxidant properties.

Being a serum enzyme, PON is related to HDL, and in addition, it was reported that it has an antioxidant function (43). Although PON and ARE activities are considered to be two distinct enzymes within the scholarly literature, advanced molecular studies suggest that the PON enzyme in the human serum shows both the ARE and PON activity (44). These two enzymes are components of the antioxidant enzymatic system that plays a role against oxidant accumulation. Therefore, when oxidative damage increases, to put another way, the balance is altered toward ROS, and it is expected that there will be a decrease in PON and ARE activities. In our study, we aimed to determine whether CAPE had a protective effect by assessing whether there was any significant increase in the IR+CAPE group in relation to IR group. In this sense, examining Figure 4, it can be observed that the PON activity in Group 3 decreased significantly in comparison with its control group (Group 1). Although a dramatic increase was observed in the IR+CAPE group contrary to expectations, the difference between the IR+CAPE and IR groups is statistically significant. Moreover, when groups are evaluated in terms of the ARE activity, whereas the ARE activity in the IR group decreased significantly in relation to control groups, the ARE activity in the IR+CAPE group is found to be significantly high in comparison with the IR group. The ARE finding indicates that CAPE is protective against ROS induced by radiotherapy.

Finally, this study assessed CER and total SH levels for examining whether CAPE is protective against ROS accumulation induced by total-head irradiation. Being an important antioxidant, CER resembles SOD in terms of its mechanism of action. In sum, its most important physiological task within the organism is to transform the ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}). In doing so, it prevents the hydroxyl radical ($OH^{\cdot}$) generation through halting the Fenton reaction within the cell (45, 46). Hence, the decrease in the CER level increases the free $OH^{\cdot}$ oscillation. In this study, whereas there is no significant difference between groups in terms of CER, the total SH level in the IR+CAPE group was found to be significantly higher than in the IR group ($p<0.05$).

Many agents that decrease the cellular toxicity of ionized radiation within the normal tissue have been used before the application in organs such as the brain, heart, bladder, kidney, etc. to prevent early and late complications caused by ionized radiation (47). In these studies, it was reported that enzymatic and non-enzymatic antioxidants decreased as a result of overproduction of free radicals within the brain tissue, linked to ionized radiation. For this reason, studies suggest either a treatment aiming to raise the antioxidant enzyme activity of the tissues subjected to ionized radiation or using agents that increase the antioxidant enzyme activity to avert the rise of oxidative stress seen in patients receiving radiotherapy (48).

There are many studies conducted with a variety of antioxidant materials believed to have preventive or reducing effects regarding the tissue or organ damage in radiotherapy. The preventive role of CAPE on the development of tissue and organ damage might be inferred because of the antioxidant effect.

One of the major limitations of this study is the lack of histological evaluation. Although biochemical analyses suggest that CAPE exhibits radioprotective effects against oxidative damage in the brain tissue of irradiated rats, it may be reasonable to support these data with histological evaluations. Moreover, radioprotectors are ideally expected to have selectivity for normal tissues, but not for tumor tissues from the effect of radiotherapy. However, the study does not provide any data for such comparison with CAPE. This is another limitation of our study.

CONCLUSION

By reducing the formation of TOS, LOOH, and OSI, oxidant stress parameters, and increasing the ARE and PON activity, total-SH levels, and antioxidant parameters, CAPE reduces irradiation-induced oxidative stress in the rat brain tissue. 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.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University School of Medicine (2017/2).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept -S.T.; Design - S.T., N.K.; Supervision - S.T.; Materials - K.Ç., N.K., E.D.; Data Collection and/or Processing - N.K., E.D., S.T.; Analysis and/or Interpretation - S.T., H.U., N.K.; Literature Search - M.E.T., S.T.; Writing Manuscript - S.T.; Critical Review - S.T., M.T.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study was supported by Gaziantep University Scientific Research Projects Unit with a project number of TF.YLT.17.20.

REFERENCES

1. Joseph JV, Balasubramaniyan V, Walenkamp A, Kruyt FA. TGF-beta as a therapeutic target in high grade gliomas - promises and challenges. Biochem Pharmacol 2013; 85: 478-85. [CrossRef]
2. Yan YR, Xie Q, Li F, Zhang Y, Ma JW, Xie SM, et al. Epithelial-to-mesenchymal transition is involved in BCNU resistance in human glioma cells. Neuropathology 2014; 34: 128-34. [CrossRef]
3. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol 2012; 14: 1-49. [CrossRef]
4. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109. [CrossRef]
5. Akyuz M, Taysi S, Baysal E, Demir E, Alkis H, Akan M, et al. Radioprotective effect of thymoquinone on salivary gland of rats exposed to total cranial irradiation. Head Neck 2017; 39: 2027-35. [CrossRef]
6. Demir E, Taysi S, Al B, Demir T, Okumus S, Saygili O, et al. The effects of Nigella sativa oil, thymoquinone, propolis, and caffeic acid phenethyl ester on radiation-induced cataract. Wien Klin Wochenschr 2016; 128: 587-95. [CrossRef]
7. Ustun K, Taysi S, Sezer U, Demir E, Baysal E, Demir T, et al. Radio-protective effects of Nigella sativa oil on oxidative stress in tongue tissue of rats. Oral Dis 2014; 20: 109-13. [CrossRef]
8. Buyukokuroglu ME, Taysi S, Polat F, Gocer F. Mechanism of the beneficial effects of dantrolene sodium on ethanol-induced acute gastric mucosal injury in rats. Pharmacol Res 2002; 45: 421-5. [CrossRef]
9. Baysal E, Gulsen S, Aytac I, Celenk F, Ensari N, Taysi S, et al. Oxidative stress in otosclerosis. Redox Rep 2017; 22: 235-9. [CrossRef]
10. Taysi S, Abdulrahman ZK, Okumus S, Demir E, Demir T, Akan M, et al. The radioprotective effect of Nigella sativa on nitrosative stress in lens tissue in radiation-induced cataract in rat. Cutan Ocul Toxicol 2015; 34: 101-6. [CrossRef]
11. Ahlatci A, Kuzhan A, Taysi S, Demirtas OC, Alkis HE, Tarakcioglu M, et al. Radiation-modifying abilities of Nigella sativa and thymoquinone on radiation-induced nitrosative stress in the brain tissue. Phytomedicine 2014; 21: 740-4. [CrossRef]
12. Cikman O, Taysi S, Gulsen MT, Demir E, Akan M, Diril H, et al. 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. W Indian Med J 2016; 65: 1-7. [CrossRef]
13. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-9. [CrossRef]
14. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-7. [CrossRef]
15. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983; 35: 1126-38.
16. Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. Eur J Clin Chem Clin Biochem 1992; 30: 391-5. [CrossRef]
17. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37: 277-85. [CrossRef]
18. Arab K, Steghens JP. Plasma lipid hydroperoxides measurement by an automated xylenol orange method. Anal Biochem 2004; 325: 158-63. [CrossRef]
19. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-11. [CrossRef]
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-54. [CrossRef]
21. Cardozo-Pelaez F, Brooks PJ, Stedeford T, Song S, Sanchez-Ramos J. DNA damage, repair, and antioxidant systems in brain regions: a correlative study. Free Radic Biol Med 2000; 28: 779-85. [CrossRef]
22. Cikman O, Taysi S, Gulsen MT, Demir E, Akan M, Diril H, et al. The Radio-protective effects of Caffeic Acid Phenethyl Ester and Thymoquinone in rats exposed to total head irradiation. Wiener Klinische Wochenschrift 2015; 127: 103-8. [CrossRef]
23. Cikman O, Ozkan A, Aras AB, Soylemez O, Alkis H, Taysi S, et al. Radioprotective Effects of Nigella Sativa Oil Against Oxidative Stress in Liver Tissue of Rats Exposed to Total Head Irradiation. J Invest Surg 2014; 27: 262-6. [CrossRef]
24. Sies H. Oxidative stress: from basic research to clinical application. Am J Med 1991; 91: 31-8. [CrossRef]
25. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J 1995; 9: 1173-82. [CrossRef]
26. Ertekin MV, Kocer I, Karslioglu I, Taysi S, Gepdiremen A, Sezen O, et al. Effects of oral Ginkgo biloba supplementation on cataract formation and oxidative stress occurring in lenses of rats exposed to total cranium radiotherapy. Jpn J Ophthalmol 2004; 48: 499-502. [CrossRef]
27. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-47. [CrossRef]
28. Taysi S, Cikman O, Kaya A, Demircan B, Gumustekin K, Yilmaz A, et al. Increased oxidant stress and decreased antioxidant status in erythrocytes of rats fed with zinc-deficient diet. Biol Trace Elem Res 2008; 123: 161-7. [CrossRef]
29. Taysi S, Memisogullari R, Koc M, Yazici AT, Aslankurt M, Gumustekin K, et al. Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. Int J Radiat Biol 2008; 84: 803-8. [CrossRef]
30. Taysi S, Okumus S, Akyuz M, Uzun N, Aksor A, Demir E, et al. Zinc administration modulates radiation-induced oxidative injury in lens of rat. Pharmacogn Mag 2012; 8: 245-9. [CrossRef]
31. Baysal E, Taysi S, Aksoy N, Uyar M, Celenk F, Karatas ZA, et al. Serum paraoxonase, arylesterase activity and oxidative status in patients with obstructive sleep apnea syndrome (OSAS). Eur Rev Med Pharmacol Sci 2012; 16: 770-4.
32. Peuchen S, Bolanos JP, Heales SJ, Almeida A, Duchen MR, Clark JB. Interrelationships between astrocyte function, oxidative stress and antioxidant status within the central nervous system. Progress Neurobiol 1997; 52: 261-81. [CrossRef]
33. Brennan LA, Kantorow M. Mitochondrial function and redox control in the aging eye: role of MsrA and other repair systems in cataract and macular degenerations. Exp Eye Res 2009; 88: 195-203. [CrossRef]
34. Panz T, Wojcik R, Krukar-Baster K. Activity of superoxide dismutase obtained from senile cataract lens - effect of diabetes mellitus. Acta Biochim Pol 2008; 55: 821-3. [CrossRef]
35. Hirose K, Longo DL, Oppenheim JJ, Matsushima K. Overexpression of mitochondrial manganese superoxide dismutase promotes the survival of

tumor cells exposed to interleukin-1, tumor necrosis factor, selected anticancer drugs, and ionizing radiation. FASEB J 1993; 7: 361-8. [CrossRef]
36. Gisone P, Robello E, Sanjurjo J, Dubner D, Perez Mdel R, Michelin S, et al. Reactive species and apoptosis of neural precursor cells after gamma-irradiation. Neurotoxicology 2006; 27: 253-9. [CrossRef]
37. Kojima S, Matsuki O, Nomura T, Yamaoka K, Takahashi M, Niki E. Elevation of antioxidant potency in the brain of mice by low-dose gamma-ray irradiation and its effect on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced brain damage. Free Radic Biol Med 1999; 26: 388-95. [CrossRef]
38. Sivonova M, Tatarkova Z, Durackova Z, Dobrota D, Lehotsky J, Matakova T, et al. Relationship between antioxidant potential and oxidative damage to lipids, proteins and DNA in aged rats. Physiol Res 2007; 56: 757-64.
39. Collins-Underwood JR, Zhao WL, Sharpe JG, Robbins ME. NADPH oxidase mediates radiation-induced oxidative stress in rat brain microvascular endothelial cells. Free Radical Bio Med 2008; 45: 929-38. [CrossRef]
40. Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. Eur J Cardiothorac Surg 1999; 16: 458-63. [CrossRef]
41. Yilmaz HR, Uz E, Yucel N, Altuntas I, Ozcelik N. Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. J Biochem Mol Toxicol 2004; 18: 234-8. [CrossRef]
42. Taysi S, Tascan AS, Ugur MG, Demir M. Radicals, Oxidative/Nitrosative Stress and Preeclampsia. Mini-Rev Med Chem 2019; 19: 178-93. [CrossRef]
43. Suchocka Z, Swatowska J, Pachecka J, Suchocki P. RP-HPLC determination of paraoxonase 3 activity in human blood serum. J Pharm Biomed Anal 2006; 42: 113-9. [CrossRef]
44. Aslan M, Kosecik M, Horoz M, Selek S, Celik H, Erel O. Assessment of paraoxonase and arylesterase activities in patients with iron deficiency anemia. Atherosclerosis 2007; 191: 397-402. [CrossRef]
45. Taysi S, Kocer I, Memisogullari R, Kiziltunc A. Serum oxidant/antioxidant status in patients with Behcet's disease. Ann Clin Lab Sci 2002; 32: 377-82.
46. Taysi S, Polat F, Gul M, Sari RA, Bakan E. Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol Int 2002; 21: 200-4. [CrossRef]
47. Links M, Lewis C. Chemoprotectants: a review of their clinical pharmacology and therapeutic efficacy. Drugs 1999; 57: 293-308. [CrossRef]
48. Belkacemi Y, Rat P, Piel G, Christen MO, Touboul E, Warnet JM. Lens epithelial cell protection by aminothiol WR-1065 and anetholedithiolethione from ionizing radiation. Int J Cancer 2001; 96: 15-26. [CrossRef]