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Original Research

Radioprotective Effects of Propolis and Caffeic acid Phenethyl Ester on the Tongue-Tissues of Total-Head Irradiated Rats

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ABSTRACT

Objective: This study aimed to investigate the radioprotective effects of propolis and caffeic acid phenethyl ester (CAPE) against radiation-induced damage in the tongue-tissues of rats exposed to total cranial gamma irradiation.

Methods: Fifty-four Sprague-Dawley rats were randomly divided into six groups. An appropriate control group was also studied. The animals were euthanized on day 10, and tongue-tissues were collected for evaluating biochemical oxidative parameters.

Results: Lipid hydroperoxides (LOOH), total oxidant status (TOS), oxidative stress index (OSI) levels and xanthine oxidase (XO) activity, and markers of oxidative stress were significantly higher, while paraoxonase (PON) activity was significantly lower for irradiated rats (IR) group compared to the other groups. When LOOH, TOS, OSI values, and XO activity in the propolis+IR and CAPE+IR groups were evaluated, there was no statistically significant difference between those ones and all control groups. In terms of the total -SH levels, the propolis+IR group was found to be significantly higher than all other groups. There was no significant difference in arylesterase (ARE) activity, ceruloplasmin (Cp) levels.

Conclusion: Propolis and CAPE reduce oxidative stress and have antioxidant effects that may be useful agents of ionizing radiation-induced tissue damage

Keywords: Antioxidants, caffeic acid phenethyl ester, irradiation, oxidative stress, propolis

INTRODUCTION

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals, which induce oxidative/nitrosative stress, play a role in the pathogenesis of many diseases (1-4). Studies have shown that head and neck cancer (HNC) is the sixth most common malignancy in the world, with an annual worldwide incidence of over 600,000 cases and 350,000 deaths per year (5, 6). HNCs include cancers of the buccal cavity, head and neck subset, larynx, pharynx, thyroid, salivary glands, and nose/nasal passages (6).

Radiotherapy is an indispensable modality method in cancer therapy, and approximately 2/3 of the patients are on radiotherapy. When the total dose required for effective local control with radiotherapy is exceeded, damage occurs in normal tissues in the field of irradiation. The resulting damage is also closely associated with the sensitivity to radiation. It is known that ionizing radiation forms free radicals (1, 6).

Radiotherapy-induced free radicals cause DNA breaks, and hence cell death, as well as inviting many diseases. An approach to reduce radiation-induced side effects is the systematic use of protective substances that protect normal tissues against radiation, but do not adversely affect the tumor such as amifostine and N-acetyl cysteine. Thus, early and late complications that could occur when the tumor is given a higher dose of radiation may be prevented (1, 7).

Propolis and an active component of its extract, caffeic acid phenethyl ester (CAPE), have been shown to have immunomodulatory, anti-humoral, cytotoxic, anti-metastatic, anti-inflammatory, and antioxidant properties. They inhibit lipoxygenase activities and suppress lipid peroxidation (7, 8).

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Efforts to diminish the toxicity of irradiation to normal cells, tissues, and organs have caused investigations to identify cytoprotective agents. Unfortunately, most of these radioprotectors possess toxic side effects limiting their role in medical treatment. Therefore, investigations for effective and non-toxic compounds with radioprotective capabilities caused increasing interest in naturally-occurring antioxidants such as *Nigella sativa*, CAPE, and propolis.

In this study, we focus on the protective effects of propolis and CAPE on oxidative stress due to radiation in rat lingual tissue by examining total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

It also investigates the radical scavenger activities involved in the radioprotective activities of these naturally-occurring substances and their possible mechanisms for the effects of lipid hydroperoxides (LOOH), xanthine oxidase (XO) activity, oxidant enzyme, and antioxidant systems, which contain enzymes such as paraoxonase (PON) and arylesterase (ARE), including low molecular weight free radical scavengers such as total sulfhydryl (-SH) groups and proteins such as ceruloplasmin (Cp) having antioxidant properties.

METHODS

Study Protocol, Rats, and Experimental Groups
In this study, 54 male Sprague-Dawley rats weighing 200±20 grams were used. A total of 6 groups were formed in the study, and there were 8 rats in the control groups and 10 rats in the other groups.

Group information was as follows
Sham control group (n=8): no propolis, no CAPE but sham irradiation.

The control group of propolis+irradiation (IR) (n=8): Only 1 ml of saline was given by an orogastric tube.

The control group of CAPE+IR (n=8): The amount of dimethyl sulfoxide (DMSO) used to dissolve CAPE was given intraperitoneally (IP) to this group.

IR group (n=10): received only IR of 5 Gy.

Propolis+IR group (n=10): received IR (5 Gy) plus propolis (80 mg $kg^{-1}day^{-1}$, by an orogastric tube, one hour before IR).

CAPE+IR group (n=10): received IR (5 Gy) plus CAPE (10 µmol $kg^{-1}day^{-1}$, IP, 30 min before IR). CAPE was dissolved in DSMO just before giving it to the rats.

Main Points:

- Ionizing radiation causes oxidative stress.
- Oxidative stress plays a role in the pathogenesis of many diseases.
- In the tongue-tissue, Propolis and Caffeic acid phenethyl ester significantly prevented oxidative stress caused by ionizing radiation.

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 (ethical committee number: 2017/2).

Biochemical Analysis
Except for Wistar-Albino male rats in the control groups, those in the other group were anesthetized with 50 mg/kg/ip ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) and the rats were placed in the prone position on the radiotherapy device. Irradiation of these rats was performed with a single dose of 5 Gy to the head area with a Co 60 teletherapy device and an area of 25 cm^2 with an SSD of 80 cm. The rats in the control groups were given a certain volume of isotonic serum. At the end of the experiment, the rats were first anesthetized with ketamine 50 mg/kg/ip. The rats were killed by decapitation. The brain tissue was homogenized with a phosphate buffer, and the supernatant was collected in 5 ependorf tubes and stored at –80°C until biochemical tests were performed. Biochemical analyses were performed by spectrophotometric methods. Group information was as follows.

All rats that received 80 mg/kg ketamine HCl (Pfizer Pharmaceuticals, Istanbul, Turkey) were anesthetized and placed in a tray in the prone position. The rats in the IR, propolis plus IR, and the IR plus TQ groups underwent a single dose of 5Gy irradiation using 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 covering the total, while the rats in the control and sham control groups received sham irradiation. The irradiation rate was 0.49 Gy/min. The central axis dose was calculated at a depth of 0.5 cm.

Biochemical Analysis
All rats were anesthetized with ketamine hydrochloride 50 mg/kg ip on day 11. Then, all animals were killed by decapitation, and their tongue-tissues were taken. The tongue-tissues were homogenized in physiological saline solution (IKA-NERKE, GmBH & CO. KB D-79219, Staufen, Germany). The supernatant obtained after homogenization was collected using eppendorf tubes and stored at –80°C until biochemical analysis.

TAS, TOS, OSI, Cp, thiol-disulfide, and total SH levels were measured as previously described (9, 10). PON and ARE activities were measured with commercially-available kits (Rel Assay Diagnostics, Gaziantep, Turkey). LOOH levels were measured with the ferrous ion oxidation-xylenol orange method, as previously described (10). XO activity was measured spectrophotometrically with uric acid formation from xanthine with an increase in absorbance at 293 nm as previously described (1), which expressed as U/mg protein. The protein content was measured as described (11). All parameters were performed using a spectrophotometer (Shimadu U 1601, Japan).

Statistical Analysis
Analysis of the data obtained from the study was performed with Statistical Package for the Social Sciences for Windows version 11.5 (SPSS Inc.; Chicago, IL, USA). The Kolmogorov Smirnov test was used to test whether or not the data were normally distributed. The significance of the differences between the groups

Table 1. Antioxidant parameters assigned in the tongue–tissues of the rats

	Sham control group	The control group of propolis+IR	The control group of CAPE+IR	IR group	Propolis+IR group	CAPE+IR group
TAS (mmol Trolox Eq./g protein)	0.23±0.027	0.21±0.028[a]	0.22±.012[a]	0.21±0.013[a]	0.25±0.03	0.22±0.018[a]
Total–SH (µmol/g protein)	0.436±0.002[a]	0.445±0.0047[a]	0.455±0.003[a]	0.488±0.006[a]	0.560±0.009	0.460±0.007[a]
ARE (U/g protein)	8.76 1.0	8.31±0.99	8.6±0.45	8.14±0.74	8.49±0.84	8.77±0.69
Cp (mg/g protein)	106.±9.84	96.6±8.45	94.4±9.75	92.94±30.94	96.51±10.1	100.2±7.52

[a]: p<0.01vs. Propolis+IR group,

Figure 1. Mean± Standard deviation (SD) of xanthine oxidase (XO) activity assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR

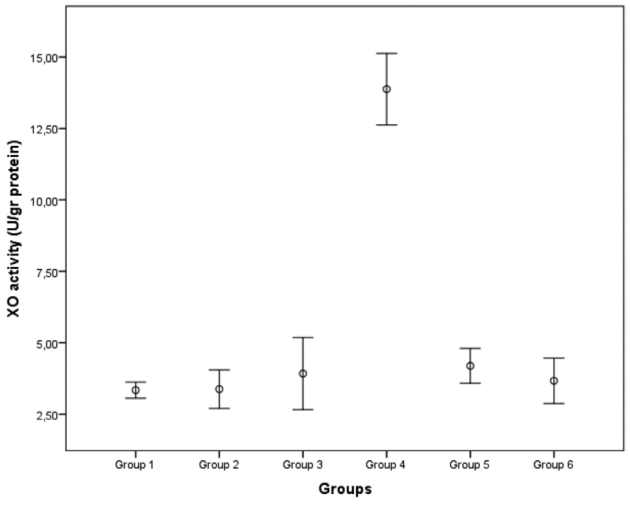
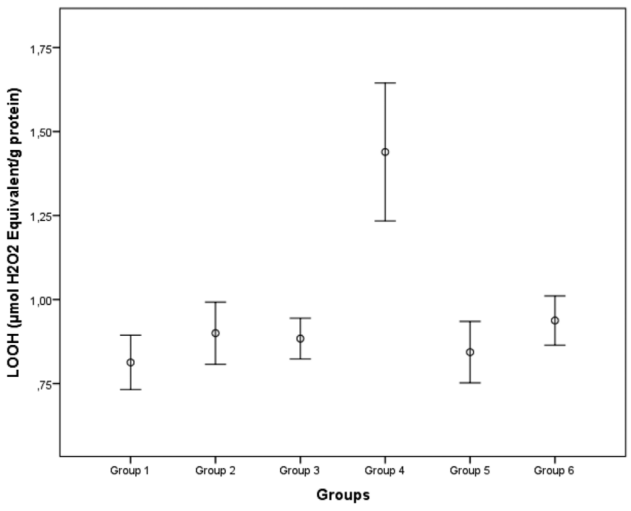


Figure 2. Mean±SD of lipid hydroperoxides (LOOH) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR



was analyzed using post-hoc, the lowest significant difference test procedure. Pearson correlation analysis was used to test the correlation between variables. Quantitative variables were expressed as mean±standard deviation, while qualitative variables were expressed as frequencies and percentages. P values <0.05 were considered statistically significant.

RESULTS

Antioxidant Parameters

As seen in Table 1, TAS values in the propolis+R group were significantly higher than those of all other groups, except for the sham control group. The total -SH levels of the propolis+IR group were found to be significantly higher than those of all other groups (p< 0.01). On the other hand, no statistically significant difference in Cp levels and ARE activity was found between the propolis+IR group and all groups.

Oxidant Parameters

As seen in Figures 1–4, there were statistically significant differences in certain parameters between the IR group and other groups. XO activity, LOOH, TOS, and OSI levels (p< 0.0001 for all parameters) were significantly higher, while PON activity was significantly lower for the IR group than in the other groups. When LOOH, TOS, OSI levels, and XO activity in the propolis+IR and CAPE+IR groups were evaluated, there were no statistically significant differences between those ones and the values for all control groups. This demonstrates that it reverses oxidative stress in irradiated rats given propolis and CAPE, and that these natural substances protect rats from ionizing radiation. In terms of XO activity, this protection appears to be even more evident.

DISCUSSION

Radiotherapy (RT) is a commonly-used therapeutic method in the precise and palliative treatment of cancer. The effects of RT are mediated by the production of free radicals, which react with the unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids. ROS, RNS, and other free radicals produced in biological systems are the major causes of oxidative/nitrosative stress (12, 13).

They can also mediate the activation of carcinogens to electrophilic, DNA-damaging components. For example, peroxyl

Figure 3. Mean±SD of total oxidant status (TOS) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR

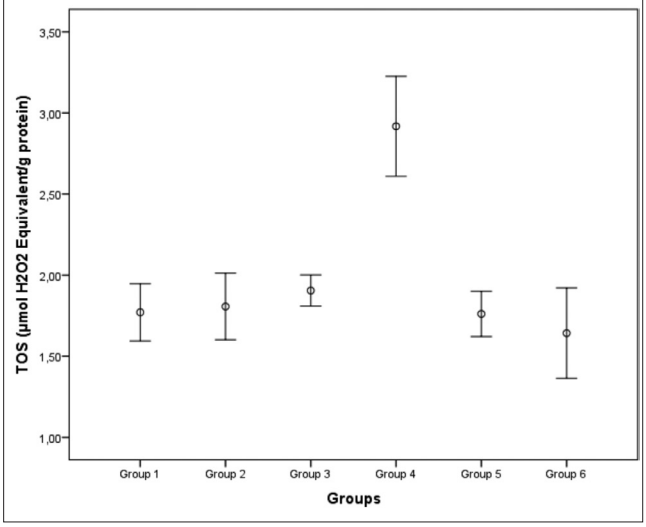
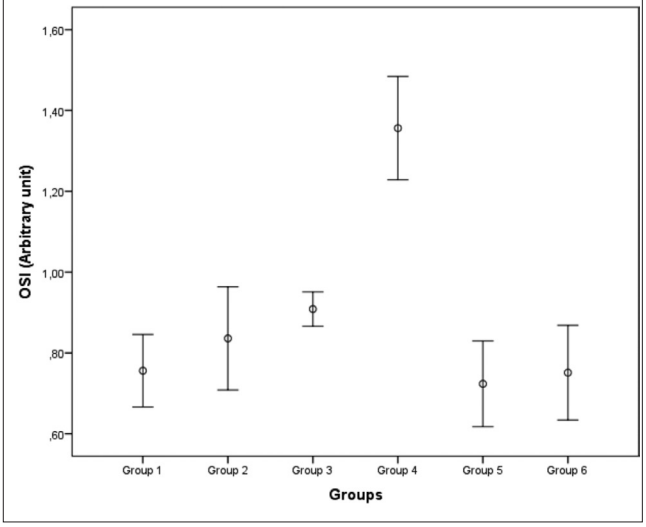


Figure 4. Mean±SD of oxidative stress index (OSI) levels assigned in the tongue-tissue of rats.
IR: Irradiated, Group 1: Sham control group, Group 2: The control group of propolis+IR, Group 3: The control group of CAPE+IR, Group 4: IR, Group 5: Propolis+IR, Group 6: CAPE+IR



radicals may have an indirect role in carcinogens as mediators of oxidation related to hydroperoxide in carcinogens. Patients undergoing radiotherapy suffer serious side effects during and after treatment. When the total dose required for effective local control with radiotherapy is exceeded, the normal tissues in the irradiated area may be damaged. The damage that occurs in normal healthy tissues in this way varies with the sensitivity of the affected tissue to radiation. For this reason, it is essential to know the effects of radiation on many biological systems and organs. Investigation of the acute and long-term effects of ionizing radiation on tissues and cells are among the important topics in radiotherapy (7, 14). Many experimental studies have stated that the radicals formed caused by ionizing radiation prepare the ground for the formation of other ROS and RNS, which play a role in the pathophysiology of many diseases and cause cell death due to DNA breaks. Numerous studies have been carried out to prevent early and late complications, which may occur due to ionizing radiation. For this objective, the researchers have focused on bio-protectors that decrease or inhibit the level of the damage caused by ionizing radiation (1, 6, 7, 13).

In our previous study, we found a significant increase in XO activity in different tissues of irradiated rats when compared to other groups. In the current study, when the comparison is made in terms of oxidative stress markers, XO activity, LOOH, TOS, and OSI levels were significantly higher in the IR group compared to the other groups.

Lipid peroxidation is the primary cellular damage caused by free radical reactions. High lipid peroxidation is responsible for the formation of lipid hydroperoxides. Levels of LOOH, TOS, and OSI, markers of oxidative stress, were similar to those reported in previous studies (15, 16).

Interestingly, XO activity in these groups, supplemented with propolis and CAPE, was significantly lower than in these tissues of irradiated rats. Therefore, in rats exposed to irradiated rats only (IR group), there was a significant increase in XO activity, the oxidant enzyme. The findings of the present study are consistent with previous studies reporting the antioxidant effects of these natural substances. Also, the finding that these antioxidants provide protective effects against radiation-induced oxidative stress is consistent with previous reports of their radioprotective effects (7).

The paraoxonases (PON1, PON2, and PON3) are the protein products of a gene family that evolved via the duplication of a common precursor and have high structural homology with each other. PON1 and PON3 synthesized in the liver are both associated with high-density lipoprotein (HDL) particles and exhibit antioxidant and anti-inflammatory properties that hydrolyze lipid peroxides in low-density lipoproteins and HDL. PON2 and PON3 are intracellular enzymes modulating mitochondrial superoxide radical production and endoplasmic reticulum stress-induced apoptosis (17, 18). However, there has been an increasing interest in the biological function of PONs in human cancers. Changes of PON status encompassing genotype, activity and/or expression have been reported in patients affected by cancer, as well as in various cancer cells in vitro. Cells undergo neoplastic transformation through a number of different events in which the cell death program is regulated, and apoptosis resistance is impaired. There is evidence supporting the role of PON2 and PON3 enzymes in cancer cell survival, which can be ascribed to the antioxidant and anti-apoptotic activities of these cells. A role in cancer cell chemotherapeutic resistance and survival has also been ascribed to PON2 and PON3 (17). Previous reductions in PON activity have been reported in experimental and human studies (19, 20). The current study found that the PON activity in the tongue-tissue

of irradiated rats was statistically significantly lower than those when compared to all the other groups. Our results showed that PON activity was reduced only in irradiated rats and that propolis and CAPE administration reversed the decrease in PON activity in irradiated rats.

LOOH is a well-known marker of oxidative stress caused by glycolipid, cholesterol, and unsaturated phospholipids by peroxidative reactions under oxidative stress (21). The levels of LOOH have also been found to be increased in irradiated rats, and propolis and CAPE applications reversed an increase in LOOH levels in irradiated rats. Lipid hydroperoxides in studies done have been reported to inhibit PON activity, and researches with pomegranate juice have shown that it can maintain PON activity during lipoprotein oxidation. Also, for preservation, these studies have shown that pomegranate juice can increase PON activity (22). In this study, the reduction of PON activity and the consideration of high LOOH levels in irradiated rats can help to understand the underlying mechanisms of oxidative stress elevation caused by irradiation. The current study correlates prevention of the decrease in PON activity with protection of lipid peroxidation, in terms of reduced LOOH levels, by the administration of propolis and CAPE. The healing found in irradiated rats treated with these natural substances may provide an explanation of how these inhibitory effects occur.

One of the major limitations of this study is the lack of histological evaluation. Although biochemical analyzes suggest that propolis and CAPE exhibit radioprotective effects against oxidative damage in the tongue-tissue of irradiated rats, it may be reasonable to support these data with histological evaluations. Also, radio protectants are ideally expected to have selectivity for normal tissues, but not for tumor tissues from the effect of radiotherapy. However, this study does not provide any data for such a comparison with propolis and CAPE. This is another limitation of our work.

To our knowledge, this is the first study that simultaneously investigates the radioprotective effects of propolis and CAPE on the oxidative stress in the tongue-tissues of irradiated rats. The results obtained in the study suggest that propolis and CAPE exhibit radioprotective effects against oxidative stress in the tongue-tissues of irradiated rats. It is now apparent that the future approach to treating irradiation-associated complications can consider the use of propolis and CAPE having multi-pharmacological activities. ROS have been implicated in many disease processes, including aging and carcinogenesis, and have been associated with a variety of complications resulting from the treatment of cancer.

CONCLUSION

The treatment of free radical-induced diseases with antioxidants or free radical scavengers has become an important therapeutic method. Since free radicals are the main mediators of radiation-induced damage, a treatment that combines radiation with an antioxidant may provide a strategy to prevent radiation damage to normal tissues.

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

Informed Consent: Informed consent is not necessary due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

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

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