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Nephroprotective effects of propolis and caffeic acid phenethyl ester against radiation-induced oxidative stress

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ABSTRACT
BACKGROUND: *Propolis and its active component, caffeic acid phenethyl ester (CAPE), have been shown to have immunomodulatory, anti-tumoral, cytotoxic, anti-metastatic, anti-inflammatory, and antioxidant properties. Ionizing radiation induces radiolysis of water and generates free radicals that result in oxidative stress. In this study, we aimed to investigate protective effects of propolis and CAPE on the kidney tissue of rats against ionizing radiation-induced oxidative stress.*
MATERIALS AND METHODS: *Forty-eight rats were divided into six groups; sham group, irradiation (IR), CAPE plus IR, propolis plus IR groups, and control groups of propolis and CAPE. Lipid hydroperoxide (LOOH) levels, total oxidant status (TOS), and oxidative stress index (OSI) were assayed to determine oxidative status. Total antioxidant status (TAS), total sulfhydryl (–SH) levels, paraoxonase, ceruloplasmin, and arylesterase activities were determined as antioxidant parameters.*
RESULTS: *Kidney TOS, OSI, and LOOH levels were significantly higher in the IR group (P<0.001). TAS and –SH levels were significantly lower in the IR group compared to propolis plus IR, CAPE plus IR, and sham groups (P<0.001). Total –SH levels in the CAPE plus IR group were significantly higher than sham group, but were significantly decreased compared to propolis plus IR group (P<0.001).*
CONCLUSION: *Ionizing radiation exposure results in oxidative stress in rat kidneys, and propolis and CAPE enhance antioxidant capacity and prevent kidney tissue from radiation-induced oxidative damage by improving antioxidant status.*
KEYWORDS: *Caffeic acid phenethyl ester, propolis, oxidative stress, kidney injury, radiation*

INTRODUCTION

Oxidative stress is known to be responsible for the mechanism of several disorders or diseases. In living organisms, cellular oxidative status is mainly influenced by the balance between the formation and scavenging of free radicals (1, 2). Formation of free radicals is induced by several factors, however, a potent one of these, ionizing radiation, has a strong effect on oxidative damage by inducing radiolysis of water and generating free radicals. Ionizing radiation is used both as diagnostic in imaging procedures and therapeutic in malignancies (3-5).

Ionizing radiation generates free radicals via radiolysis of water and these free radicals may leave from the irradiated region and reach into distant tissues/organs by systemic circulation (6). It is well established that free radicals influence the oxidative status of various systems in the body and finally lead to oxidative damage.

Protection of vital organs from oxidative damage by natural products became more important in the scope of preventive medicine (7-10). Various protective agents are reported to be effective in improving oxidative status. These agents' mechanism of action is explained by the decrease in free radical formation, improvement in radiation-related inflammation, and repair of DNA damage (3, 11, 12).

Propolis is a bee glue composed of pollen, beeswax, plant resins, and essential oils (13). Caffeic acid phenethyl ester (CAPE) is an active component of propolis, and both of these agents have been shown to have immunomodulatory, anti-tumoral, cytotoxic, anti-metastatic, anti-inflammatory, and antioxidant properties (7, 13-17)

In our study, we investigated the detrimental effects of free radicals on kidneys formed after irradiation of distant regions and carried by the blood circulation, and nephroprotective effects of propolis and CAPE against oxidative stress.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents except CAPE and DMSO (Sigma Chemical Co. St Louis, MO, USA) were supplied from the Department of Medical Biochemistry store.

Animals and experiments

A total of forty-eight male and albino Sprague-Dawley rats were used for the experiment. Rats were bred at the department of the animal laboratory and were 12-16 weeks old and weighing 220±25 g at the time of irradiation. At least seven days before irradiation, animals were quarantined. Rats were divided into six groups including eight for each, housed in the cages in a windowless laboratory room containing automatic temperature (22±1 $^{\square}$C) and lighting controls (12 h light/12 h dark). Standard laboratory chow and water were used for feeding rats. All stages of the experiment were performed according to the ethical procedure.

Experimental groups

The experiment design was made by dividing rats into six groups. Each group consisted of eight rats. The groups were;

Irradiation (IR) group: The rats in this group received total cranial IR with a single dose of 5 Gray (Gy) gamma radiation.

Propolis group: The rats in this group received total cranial IR with a single dose of 5 Gy and propolis (80 mg $kg^{-1}day^{-1}$) via an orogastric tube one hour before IR. This procedure continued for 10 days following IR.

CAPE group: The rats in this group received total cranial IR with a single dose of 5 Gy and intraperitoneal (IP) injection of CAPE (10 µmol $kg^{-1}day^{-1}$) 30 minutes before the IR. This procedure continued daily for 10 days following IR. CAPE was dissolved in DMSO

with 0.1% final concentration just before administration.

Control group of propolis: Rats in this group received 1-ml of saline via an orogastric tube. IR, propolis, or CAPE were not administered.

Control group of CAPE: The rats in this group received only DMSO by IP injections equal to the volume of CAPE in the CAPE group. This procedure continued for 10 days. IR, propolis, or CAPE were not administered.

Sham group: The rats in this group did not receive IR, propolis, or CAPE.

An ethically proper way according to the guidelines of the local Ethical Committee was pursued at all stages of the experiment. A dose of 80 mg/kg ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) was administered to the rats for anesthesia before IR. Thereafter rats were put on a tray and irradiated in the prone position. IR was administered by Cobalt-60 teletherapy unit (Theratron Equinox, MDS Nordion, Kanata, Ontario, Canada). Only an anterior field with 5x5 cm was arranged. The source-to-surface distance was 80 cm. The central axis dose was calculated at a depth of 0.5 cm. Dose rate was 0.49 Gy/min.

Biochemical analyses

All rats were sacrificed following anesthetization with 80 mg/kg ketamine hydrochloride on the eleventh day and the kidneys were removed. Kidneys were homogenized in isotonic saline. The homogenate was centrifuged at 10,000 *g* for 1 hour and debris was removed. Clear upper supernatant was picked to use in the assessments. Examinations were made at 4°C.

Lipid hydroperoxide (LOOH), total oxidant status (TOS), and oxidative stress index (OSI) were assessed as oxidative status parameters in the study. Total antioxidant status (TAS), total sulfhydryl (–SH) groups, and activities of ceruloplasmin (Cp), arylesterase (ARYL), and paraoxonase (PON) were determined as antioxidant status parameters.

TOS and TAS levels were measured by using Erel's method (18). TOS was expressed as micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/g protein). TAS was expressed as millimolar Trolox equivalent per liter (mmol Trolox equivalent/g protein). The resulting unit of TAS was translated into μmol/g protein. OSI was defined as the TOS-to-TAS ratio and calculated by using the formula (19);

OSI (arbitrary unit) = [TOS (μmol H_2O_2 equivalent/g protein)/ TAS (μmol Trolox equivalent/g protein)] × 100.

Paraoxonase activity was analyzed in the basal activity. An elevation in the absorbance at 412 nm at 37°C was monitored to measure the paraoxon hydrolysis rate. Molar absorptivity coefficient at pH 8 was used to calculate the amount of p-nitrophenol generation which was 17,000 M/cm and expressed as U/mg protein (20).

Phenylacetate was used as a substrate to determine ARYL activity, and the increase in absorbance at 270 nm at 37°C was monitored. The activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M/cm (21). Under these conditions, one unit of ARYL activity was defined as 1 μmol phenol generated/min. ARYL activity was expressed as U/g protein.

Erel's method was used to measure the enzymatic activity of Cp (22). Ferrous ion was oxidized to ferric ion by the activity of ceruloplasmin ferroxidase. Cp activity was expressed as mg/dl.

Total –SH levels were analyzed according to Ellman's method (modified by Hu et al.) and expressed as mmol/g protein (23).

LOOH levels were measured by the ferrous ion oxidation-xylenol orange method (24) and expressed as μmol/g protein.

Oxidative and antioxidant parameters analyzed in IR, propolis plus IR, and CAPE plus IR groups were compared with control groups. In addition, IR, propolis plus IR, and CAPE plus IR groups were compared with each other. Finally, all of the groups were compared with each other.

Statistical analyses

Data were analyzed using statistical analysis with the Statistical Package for the Social Sciences for Windows (SPSS, version 23.0, Chicago, IL). Kolmogorov Smirnov test was used in determining the normally distributed continuous variables. Data were expressed as mean ± SD. ANOVA test was used to analyze the differences between groups in normally distributed variables. Kruskal Wallis H test was used for abnormal distributed data. *P* value less than 0.05 was accepted statistically significant.

RESULTS

Antioxidant status parameters

Total –SH levels and TAS o the IR group were found significantly different compared to propolis, CAPE, and sham groups (Table 1). Total –SH levels were significantly lower in the IR group compared to sham, propolis, and CAPE groups (P<0.001). Furthermore, total –SH levels were significantly higher in the propolis group compared to the CAPE group (P<0.001). TAS was significantly elevated in the CAPE and sham groups compared to the IR group (P<0.001). PON, Cp, and ARYL activities were not significantly altered when all groups were compared with each other (P >0.05).

Oxidative status parameters

TOS, OSI, and LOOH levels in the IR group were significantly different when compared to all of the other groups (Table 2). TOS, OSI, and LOOH levels were significantly lower in propolis, CAPE, and sham groups than the IR group (P<0.001). Moreover, a statistically significant difference was found between the IR group and the control groups of propolis and CAPE (P<0.001).

Table 1. Antioxidant parameters in kidney tissue of rats

	ARYL (U/g protein)	PON (U/mg protein)	-SH* (mmol/g protein)	TAS* (mmol Trolox equivalent/g protein)	Cp (mg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Propolis group	10.7±0.413	1.24±0.433	0.07±0.005[a,b]	0.06±0.007	43.2±1.691
CAPE group	9.8±0.769	1.03±0.365	0.06±0.006[a,c]	0.07±0.014[e]	44.4±2.916
Control group of CAPE	10.1±0.850	1.27±0.326	0.07±0.007	0.06±0.005	44.3±1.649
Control group of Propolis	10.9±0.909	1.30±0.143	0.07±0.001	0.06±0.008	45.2±2.519
Sham group	10.2±0.941	1.29±0.211	0.07±0.009[c,d]	0.07±0.005[d]	43.7±2.517
IR group	10.0±0.829	1.04±0.194	0.06±0.004[b,d]	0.05±0.011[d,e]	43.7±2.074

* P<0.001; a: Propolis vs. CAPE, b: Propolis vs. IR group, c: CAPE vs. Sham group, d: Sham vs. IR group, e: CAPE vs. IR group.

CAPE; caffeic acid phenethyl ester, ARYL; arylesterase, PON; paraoxonase, SH; sulfhydryl, TAS; total antioxidant status, Cp; ceruloplasmin

Table 2. Oxidant parameters in kidney tissue of rats

	LOOH* (μmol/g protein)	TOS* (μmol H2O2 equivalent/g protein)	OSI * (Arbitrary unit)
	Mean ± SD	Mean ± SD	Mean ± SD
Propolis group	0.9±0.079a	4.2±0.645a	6.6±1.497a
CAPE group	0.9±0.151b	3.8±0.807b	6.1±1.428b
Control group of CAPE	0.9±0.052c	4.0±0.336c	7.2±0.788c
Control group of Propolis	0.8±0.061d	4.2±0.302d	7.4±1.236d
Sham group	0.8±0.070e	4.0±0.548e	6.3±1.144e
IR group	1.2±0.105a,b,c,d,e	5.9±0.533a,b,c,d,e	11.9±2.429a,b,c,d,e

*P<0.001; a: Propolis vs. IR group, b: CAPE vs. IR group, c: Control group of CAPE vs. IR group, d: Control group of Propolis vs. IR group, e: Sham vs. IR group.

LOOH; lipid hydroperoxide, TOS; total oxidant status, OSI; oxidative stress index

DISCUSSION

Organisms may be exposed to radiation by several conditions in daily life, however, harmful effects of radiation may occur either with imaging processes which use ionizing radiation (such as computed tomography) or when undergoing radiotherapy after a cancer diagnosis. Ionizing radiation is a potent agent which generates oxidative stress as a result of free radical formation via radiolysis of water. By therapeutic use of ionizing radiation in a region of the body, oxidative stress may occur in distant organs as well as in local irradiated tissues by joining of free radicals into the blood circulation (3).

Oxidative stress in the tissues may be evaluated by increase in various oxidative parameters (5). In the current study, we analyzed LOOH, OSI, and TOS to determine oxidative stress in the renal tissue and the increase in these parameters was accepted as an indicator of ionizing radiation-induced oxidative damage. LOOH generates from unsaturated phospholipids, glycolipids, and cholesterol as a result of peroxidative reactions (25). In our study, we found a significant elevation in LOOH levels in the IR group when compared to all other groups. TOS and OSI were also significantly increased in the IR group compared to the other groups. These results suggest that ionizing radiation-induced free radicals were carried through blood circulation and generated oxidative stress in the kidneys.

Oxidative damage is known to be responsible for the genesis of a wide scale of disorders from aging to malignancies. Therefore, natural products became important for effective prevention against oxidative stress and have been studied by several investigators (3, 6, 8-10, 26). In the study, we investigated propolis and CAPE for their antioxidant and protective effects on the renal tissue against radiation-induced oxidative stress. Propolis is a bee glue that has several composites including resin, pollen and

beeswax. Organic compounds such as polyphenols, esters, amino acids, and vitamins are also constituents of propolis (13).

CAPE is an active phenolic compound of propolis. Also, it is found in the nature as a component of the resinous exudates of the buds and leaves of plants (27). Effects of these products on various tissues and organs have been studied and shown to have immunomodulatory, anti-tumoral, cytotoxic, anti-metastatic, anti-inflammatory, and antioxidant properties (7, 11, 14, 15). Nephrotoxic drugs, sepsis, toxic agents, diseases, or ischemia may induce oxidative damage in kidneys and in recent studies, propolis and CAPE have been found to reverse this damage by improving antioxidant status (7, 27-31). In our study, we investigated ionizing radiation triggered oxidative damage in the kidney and found that supplementation with propolis and CAPE reduced renal oxidative parameters. Decrease in TOS, OSI, and LOOH levels support the hypothesis that propolis and CAPE prevent renal tissue from oxidative injury.

After ionizing radiation exposure, cellular antioxidants increase to minimize or eliminate oxidative damage (3). PON, ARYL, and Cp activities, total –SH groups and TAS were determined in the study as antioxidant parameters which were previously reported to protect cells against oxidative stress and improve antioxidant status (32). Cp exhibits extracellular antioxidant activity and is responsible for Fe^{+2} oxidation (33). PON is an antioxidant enzyme that hydrolyzes lipid peroxides into oxidized lipoproteins (34-36). Sulfhydryl groups are reactive constituents of non-protein and protein molecules that take role in important processes such as detoxification, protein metabolism, and activation of antioxidant enzymes (8). In the study, although an important difference was not found between all of the groups in terms of ARYL, PON, and Cp activities, significant changes in total –SH levels and TAS were determined. There was a statistically significant reduction in –SH levels in the kidney tissue in the IR group. However, propolis or CAPE administration has ameliorated this decrease. Furthermore, total –SH levels were significantly higher in the propolis group than the CAPE group. This may be explained by the superiority of propolis over CAPE, however, further research is needed to support these findings. TAS was found significantly decreased in the kidney tissue of rats in the IR group. Supplementation with CAPE reversed this reduction that support the protective effect of CAPE.

CONCLUSION

Ionizing radiation-induced free radical formation may cause oxidative stress in the kidneys via systemic circulation. However, systemic administration of propolis and/or CAPE may prevent renal tissue from oxidative damage and ameliorate renal injury by improving antioxidant status. Natural products may be useful in protecting normal tissues from harmful effects of radiation in case of exposing ionizing radiation either with imaging or irradiation.

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