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Neuroprotective effects of propolis and caffeic acid phenethyl ester (CAPE) on the radiation-injured brain tissue (Neuroprotective effects of propolis and CAPE)

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► ***Original article***

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ABSTRACT

Background: Our purpose was to investigate propolis and its component caffeic acid phenethyl ester (CAPE) for their antioxidant effects on the brain tissue of rats exposed to ionizing radiation (IR). ***Materials and Methods***: Fifty-four male albino Sprague-Dawley rats, divided into six groups, were designed as normal control group, cranial irradiation of 5 Gray alone, irradiation plus CAPE, irradiation plus propolis, control groups of propolis and CAPE. Oxidative/antioxidative status indicators, lipid peroxidation and antioxidant enzymes, were determined by biochemical methods in homogenized brain tissue of rats. ***Results***: Malondialdehyde level, the lipid peroxidation index, in the irradiation alone group was found to be significantly increased compared to all of the other groups ($p<0.001$). Enzyme activities of superoxide dismutase (SOD) were 504.93, 720.70 and 659.98 for irradiation alone group, irradiation plus CAPE group and irradiation plus propolis group, respectively. Enzyme activity of SOD in the irradiation alone group was found to be significantly decreased compared to the groups received propolis or CAPE ($p<0.003$). Enzyme activity of glutathione peroxidase was not found statistically different among all of the groups. ***Conclusion:*** Propolis and CAPE were found to be beneficial agents in protecting brain tissue against IR-induced oxidative damage.

Keywords: *Brain, caffeic acid phenethyl ester, ionizing radiation, oxidative stress, propolis.*

INTRODUCTION

Ionizing radiation (IR) is an important source in the generation of free radicals among the various physical/chemical agents; interacts with cells and produces cytotoxic effects. Many effects of IR are mediated through the production of free radicals such as superoxide radical and hydroxyl radical [(1)]. Recent studies emphasize that free radicals play an important role in the cellular damage [(2-5)].

Cells normally have various mechanisms acting to defend against free radical induced damage. Overproduction of free radicals is mainly eliminated by antioxidant defense system including superoxide dismutase (SOD), glutathione and catalase [(6, 7)]. Deficiency in SOD and Glutathione peroxidase (GSH-Px) results in relatively higher levels of free radicals and altered redox state which will induce a state of persistent oxidative stress [(8)]. Free radicals and lipid peroxidation are reported to play important role in various human diseases such as ischaemia-reperfusion injury, atherosclerosis, diabetes, neurodegenerative diseases, cancer and allergy [(9,10)].

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The most important organ of the central nervous system (CNS), the brain is more sensitive to free radical induced damage because of its high use of oxygen, its high concentration of polyunsaturated fatty acids, and its low concentration of antioxidant molecules compared to other tissues [11]. In CNS, oxidative stress results in acute and chronic injury and plays an important role in the pathogenesis of neuronal damage [12]. Therefore, herbal remedies which can protect cellular membranes against IR and free radicals will have potential benefits as radiation-protectors, antioxidant and antimutagens [13,14].

Propolis is a resinous material collected by honey bees from plants, and its flavonoid component, caffeic acid phenetyl ester (CAPE), possesses a number of important biological and pharmacological properties including antitumor, immunomodulatory, anti-inflammatory, anti-oxidant, anticarcinogenic, antiviral, anti-microbial, antiparasitic, and anti-diabetic activities [15,16].

Beside the known antioxidant and neuroprotective properties of propolis and CAPE, data on the radiation-protective ability of these agents in radiation-injured brain tissue have not been reported to date. In the current study, we hypothesized that propolis and CAPE could protect brain tissue from radiation-induced oxidative damage. For this reason, we measured the antioxidant defense system parameters, SOD and GSH-Px, and the marker of lipid peroxidation, malondialdehyde (MDA), in the brain tissue of rats with or without exposing to gamma radiation to total cranium with a single dose of 5 Gray (Gy).

MATERIALS AND METHODS

Rats and experiments

Fifty-four male albino Sprague-Dawley rats, 12-16 weeks old, weighing 220±25 g at the time of irradiation, bred at Gaziantep University Medical School, department of animal laboratory, were used for the experiment. All procedures involving the Sprague-Dawley rats adhered to the ARVO Resolution on the Use of Animals in Research. Animal experimentations were carried out in an ethically proper way by following guidelines as set by the Ethical Committee of the Gaziantep University. The rats were quarantined for at least seven days before irradiation, housed ten to a cage in a windowless laboratory room with automatic temperature (22±1˚C) and lighting controls (12 h light/12 h dark) and fed with standard laboratory chow and water. The rats were randomly divided into six groups. Control groups included 8 rats and the other groups included 10 rats for each. Group A (normal control group) did not receive CAPE, propolis or irradiation. Group B (irradiation plus CAPE group) received 5 Gy of gamma irradiation as a single dose to total cranium and CAPE [10 μmol $kg^{-1}day^{-1}$, intraperitoneally (i.p.)] injection starting 30 minutes before the irradiation and continuing daily for 10 days after irradiation. CAPE was dissolved in dimethyl sulfoxide (DSMO) just before giving to the rats. The final concentration of DMSO was 0.1%. Group C (control group of CAPE) received DMSO (i.p.) injections and sham irradiation. Group D (irradiation plus propolis group) received both 5 Gy of gamma irradiation as a single dose to total cranium and propolis (80 mg $kg^{-1}day^{-1}$) starting one hour before irradiation and continuing for 10 days through an orogastric tube. Group E (control group of propolis): received 1-ml saline through an orogastric tube and sham irradiation. Group F (Irradiation alone group) received 5 Gy of gamma irradiation as a single dose to total cranium plus 1-ml saline through an orogastric tube. Prior to total cranium irradiation, the rats were anesthetized with 50 mg/kg ketamine HCl (Pfizer Inc, Istanbul, Turkey) and placed on a plexiglas tray in the prone position. While the rats in the control group of CAPE or propolis received sham irradiation, the rats in the groups of B, D, F were irradiated using cobalt 60 teletherapy unit (Theratron Equinox, MDS Nordion, Kanata, Ontario, Canada) from a source-to-surface distance of 100 cm by 10×20 cm anterior fields with 5 Gy to the total cranium as a single fraction. Irradiation dose of 5 Gy was adjusted as previously described [17]. The central axis was calculated at a depth of 1 cm. The dose rate was 0.49 Gy/min.

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Fractionation of brain samples

At the 11th day of the experiment, the rats were anesthetized with 50 mg/kg ketamine i.p. Then an intracardiac withdrawal of blood was performed. Following this process, the rats were sacrificed and their brains were removed. Brain tissues were homogenized by a homogenizer (IKA -NERKE, GmBH & CO. KB D-79219, Staufen, Germany) in isotonic saline (1/10 weight/ volume) on ice for one minute. The supernatant was stored at -80°C in aliquots for biochemical measurements. Activities of the antioxidant enzymes, SOD and GSH-Px, and MDA levels were determined from these supernatants spectrophotometrically for one time.

Determination of MDA levels

Malondialdehyde was determined by spectrophotometry of the pink-colored product of the thiobarbituric acid-reactive substances complex. Total thiobarbituric acid-reactive substances were expressed as MDA, using a molar extinction coefficient for MDA of 1.56×10^5 cm^{-1} M^{-1} [18]. The MDA level was expressed as nmol/g wet weight.

Determination of SOD activity

Superoxide dismutase activity was determined by the method in which xanthine – xantine oxidase complex produces superoxide radicals and that react with nitroblue tetrazolium (NBT) to form the formazan compound [19]. SOD activity is measured at 560 nm by detecting the inhibition of this reaction. Activity was calculated by using a blank study in which all reagents except a supernatant sample are present and by determining the sample and blank absorbance. One SOD unit was defined as the enzyme amount causing 50% inhibition in the NBTH2 reduction rate. Superoxide dismutase activity was also expressed as U/mg protein of brain tissue sediment.

Determination of GSH-Px activity

Glutathione peroxidase activity was measured by the method in which GSH-Px catalyzes the oxidation of glutathione in the presence of H_2O_2 [20]. Oxidized glutathione is converted into the reduced form in the presence of glutathione reductase and NADPH, while NADPH is oxidized to NADP. Decrease in the absorbance of NADPH at 340 nm is measured. GSH-Px activity of the brain tissues were calculated by measuring the absorbance change per minute and by using the molar extinction coefficient of NADPH. GSH-Px activity was expressed as U/mg protein of brain tissue sediment. The protein content was determined using Bradford method [21]. Biochemical measurements were carried out at room temperature using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistical analyses

Analyses were conducted using Statistical Package for the Social Sciences (SPSS, version 18) software. Data were analyzed with one-way analysis of variance (ANOVA) followed by a post hoc test (LSD alpha) for multiple comparisons. Data were expressed as mean± standard deviation (SD) and p values <0.05 were considered to be statistically significant.

RESULTS

Enzyme activity of SOD and GSH-Px and MDA levels of the six groups are presented in table 1. Compared to the other groups, enzyme activity of SOD of the rats in the irradiation alone group was found lower ($p<0.006$). There was a statistically significant difference between irradiation alone group and the groups treated with propolis or CAPE ($p<0.003$). However, enzyme activity of SOD was not found different between irradiation plus propolis and irradiation plus CAPE groups.

As expected, lipid peroxidation as indicated by MDA levels in brain tissue of the rats in the

Table 1. Mean SOD, GSH-Px and MDA values of the groups.

Groups	SOD mean±SD (U/mg protein)	GSH-Px mean±SD (U/mg protein)	MDA mean±SD (µMol/mg protein)
A	691.26±149.78	990.05±85.96	9.13±1.11
B	720.70±142.01	956.51±149.31	8.24±2.28
C	579.12±105.69	1099.05±335.85	10.49±1.78
D	659.98±137.27	871.47±130.56	8.61±1.80
E	705.54±147.44	910.86±94.55	11.03±0.60
F	504.93±115.76 [a]	929.69±192.68	12.60±1.25 [b]

Group A: normal control group, B: irradiation plus CAPE, C: control group of CAPE, D: irradiation plus propolis, E: control group of propolis, F: irradiation alone group, SD: standard deviation

[a] $p<0.006$ as compared to other groups.

[b] $p<0.001$ as compared to other groups.

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irradiation alone group was found to be higher compared to the other groups ($p<0.001$). However, MDA level was not found different between irradiation plus propolis and irradiation plus CAPE groups ($p>0.2$).

Enzyme activity of GSH-Px was not found statistically different among all of the groups ($p>0.1$).

DISCUSSION

Herbal remedies which are effective as antioxidants and radiation-protectors due to their ability of scavenging free radicals or neutralizing their reactions are of great interest to health management due to their potential applications during radiotherapy (RT) in cancer care, etc. In the present study investigating antioxidant effects of propolis and CAPE on brain tissue of rats exposed to IR revealed that the MDA levels, indicator of lipid peroxidation, in the groups treated with propolis or CAPE was lower than in irradiation alone group whereas the antioxidant parameter, SOD activity, was found to be higher. The results of the current study support the research hypothesis that the systemic administration of propolis and CAPE would reduce the oxidative damage in radiation-injured brain tissue.

Ionizing radiation (IR) is known to be a common and a mandatory method for brain cancer care; beside its harmful effects. The effects of IR may change with dose, frequency, size of exposed area and time of irradiation [22]. IR damages tissues by producing free radicals which cause oxidative damage in biological molecules such as nucleic acids, proteins, and lipids, resulting in cellular injury. IR initiates lipid peroxidation that is believed to be an important cause of damage in cell membranes. In addition, lipid peroxidation is a contributing factor to the development of free radicals-mediated tissue damage [23]. The most susceptible substrates for autoxidation in oxidative stress are polyunsaturated fatty acids of the cell membrane among most components of cellular structure and function which are likely to be potential targets of oxidative damage. This may lead to impairment of the nervous system, the general deterioration of cellular metabolism, and finally cell death. Previous studies performed on various tissues suggested that the formation of MDA, a marker of lipid peroxidation, was increased by IR [24, 25]. In our study, MDA level, was found to be increased in the irradiation alone group, whereas the MDA levels in the groups treated with propolis or CAPE was significantly decreased compared to the irradiation alone group.

Increased cellular production of free radicals has been observed after exposure to IR and radiation-induced changes occur related to oxidative stress. Antioxidant enzymes SOD and GSH-Px, protect cells against oxidative stress by scavenging free radicals [5,26]. SOD is the first and most important line of antioxidant enzyme defense systems against free radicals, particularly superoxide radicals, and activity of SOD could be increased in oxidative stress, but also can be upregulated through numerous signaling pathways [27,28]. A decrease in the antioxidant enzyme capacity of the brain tissue could result in free radicals accumulation during IR. Certain pathological processes in CNS injury involve the generation of oxygen free radicals either as a cause or a result of disease progression [29]. Therefore, natural products which can protect healthy cells against oxidative damage by enhancing the antioxidant capacity and scavenging or inhibiting free radicals are becoming increasingly important in clinical RT.

Currently herbal remedies such as propolis or CAPE are becoming popular because of their beneficial effects with fewer side effects compared to synthetic/semi-synthetic drugs [30]. All flavonoids within propolis, except CAPE, are reported to have a low order of acute oral toxicity with a reported ED_{50} of 8–40 g/kg. Similarly, a safe dose in humans is estimated as 1.4 mg/kg body weight/day or approximately 70 mg/day [31]. Propolis can increase antioxidant capacity in animals [32] and humans [33] and the antioxidant capacity of it may be related to its chemoprevention effects. The flavonoids in propolis are capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation [34]. It is well known that propolis has various components and one of its major components, CAPE, inhibits free radical production in many systems [35].

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Antioxidant and neuroprotective effects of propolis and its active component CAPE have been reported in many studies previously. Izuta *et al.* [36] reported that inhibitory effects of propolis against neuronal cell death induced by endoplasmic reticulum stress or staurosporine could be exerted primarily by chrysin in the SH-SY5Y cells. Cardosa *et al.* [37] also showed neuroprotective effects of propolis on primary cerebral cortical neurons against staurosporine and H_2O_2 induced cytotoxicity. In addition, Jasprica *et al.* [33] demonstrated that propolis treatment decreased MDA levels and increased the SOD activity in human red blood cells.

Caffeic acid phenetyl ester has been reported to provide neuroprotection by reducing infarctions and decreasing free radicals in animal model of transient focal cerebral ischemia and reperfusion [38-40] and also has been reported to be an antioxidant in spinal cord [41]. Hosnuter *et al.* [35] demonstrated that CAPE possesses antioxidant activity by scavenging free radicals, saving SOD activity, preventing xanthine oxidase activity, and decreasing levels of MDA and nitric oxide in the rat models.

Furthermore, herbal remedies also can protect against late radiation toxicity to organs without interfering with the beneficial effect of RT and decrease the radiation-induced toxicity. For example, the administration of CAPE for radiosensitization of tumor cells has been studied. By IR, the increased death of the cells treated with CAPE has been reported. Since CAPE is an effective inhibitor of NF-κB and a stimulator of the functions of glutathione S-transferase, it drains GSH levels. Subsequently, tumor cells are radiosensitized due to this drainage [42].

In the present study, enzyme activity of SOD was found decreased in the irradiation alone group compared to the groups treated with propolis or CAPE. The significant decrease in the activity of the SOD in the irradiation alone group indicates the generation of oxidative stress and an early protective response to oxidative damage. Since the SOD acts as a free radical scavenger, the reduction in its concentration leads accumulation of free radicals which are blamed for cellular damage in brain tissue. In other words, the higher activities of SOD in the groups treated with propolis or CAPE than in irradiation alone group demonstrated that these agents may protect brain tissue against harmful effects of free radicals.

Different results were reported in various studies about the enzyme activity of GSH-Px in the oxidative stress. In the current study, statistically significant difference was not found among the groups according to the enzyme activity of GSH-Px. In this state, injured proteins of the GSH-Px enzyme may be repaired or loss of activity may be compensated by stimulating synthesis of the enzyme [43].

Although biochemical analyses suggested that propolis and CAPE exhibits radiation-protective effects against oxidative damage in the brain tissue of irradiated rats, limitation of this study is lack of histological evaluation which may support this data.

As a result, we found increased oxidative stress and impaired antioxidant defense system in brain tissue of irradiated rats in comparison to the other groups. This is the first study that concurrently investigates the effects of propolis and CAPE on the oxidant/antioxidant system in the brain tissue of the irradiated rats. We showed that these natural substances clearly appeared to prevent oxidative stress in radiation-injured brain tissue by decreasing the formation of lipid peroxidation and increasing the antioxidant enzyme activities, and also by inhibiting free radical generation. These results suggest an important role of naturally occurring compounds (propolis and CAPE) as an antioxidant and free radical scavenger on the oxidative stress in the radiation-injured brain tissue. These agents are likely to be valuable drugs for protection against IR and/or be used as an antioxidant against oxidative stress and other severe side effects occurred in head and neck cancer patients treated with radiation therapy. However, additional pharmacological and toxicological studies are required to support these findings.

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Conflicts of interest: *none to declare.*

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