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The Effect of Propolis on Oxidative Damage Induced by Whole Body Irradiation in Rats

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The antioxidant and radioprotective effects of propolis against ionizing radiation induced oxidative damage were investigated. Twenty one rats were divided in to 3 groups; 1st group was the control group; 2nd group was the irradiation group, physiological saline (i.p.)/ 3 days + 9 Gy gamma irradiation; 3rd group was irradiation + Propolis group, propolis (i.p. 100 mg / kg BW) / 3 days + 9 Gy gamma irradiation. As a result of irradiation, a significant increase was determined in malondialdehyde (MDA) levels of liver, kidney, and brain tissues of the rats (P<0.001). There was a significant decrease in glutathione peroxidase (GSH-Px) activity of all the tissues (P<0.001) and superoxide dismutase (SOD) activity and glutathione (GSH) level in the liver (P<0.01). Also, GSH level increased significantly in the kidney (P<0.001) and ovarium tissues (P<0.05). The MDA levels that increased in the liver as a result of irradiation, decreased significantly due the addition of propolis (P<0.001); whereas, the MDA levels that increased in kidney and brain did not change. In the spleen and ovarium tissues, MDA levels decreased significantly as a result of addition of propolis compared to the irradiation group (P<0.05). It was determined that GSH levels, that decreased in the liver (P<0.01), and increased in the kidney due to addition of propolis, and GSH levels, that decreased in the liver as a result of irradiation (P<0.01) and increased in the kidney (P<0.001), approached to the normal level (P<0.001) and the GSH levels in the spleen and brain increased compared to both the control and irradiation groups (P<0.05). The addition of propolis caused a decrease in the GSH-Px activities of heart, spleen, brain, and ovarium tissues compared to both the control and irradiation groups (P<0.001). Also, a decrease in the SOD activity of heart tissue compared to the control group was found (P<0.05).

Key Words: *İrradiation, oxidative stress, propolis*

Tüm Vücut Radyasyonu Uygulanan Ratlarda Oksidatif Hasar Üzerine Propolisin Etkisi

Radyasyona bağlı oksidatif hasara karşı propolisin antioksidan ve radyoprotektif etkileri araştırıldı. Yirmi bir rat 3 gruba bölündü; 1. grup kontrol grubu; 2. grup radyasyon grubu; serum fizyolojik (i.p.)/3 gün + 9 Gy gamma radyasyon; 3. grup radyasyon + propolis grubu; propolis (i.p. 100 mg/kg CA)/3 gün + 9 Gy gamma radyasyon uygulandı. Radyasyon sonucu, ratların karaciğer, böbrek ve beyin dokularının malondialdehit (MDA) düzeylerinde anlamlı bir artış tespit edildi (P<0.001). Tüm dokuların glutatyon peroksidaz (GSH-Px) aktivitesinde (P<0.001) ve karaciğerde süperoksit dismutaz (SOD) aktivitesi ile glutatyon (GSH) seviyesinde (P<0.01) önemli azalma vardı. Ayrıca, böbrek (P<0.001) ve yumurtalık (P<0.05) dokularında GSH düzeyi anlamlı olarak arttı. Propolis ilavesiyle radyasyon sonucu karaciğerde artan MDA düzeyleri önemli azalırken (P<0.001), böbrek ve beyinde artan MDA düzeyleri değişmedi. Dalak ve yumurtalık dokularında ise propolis ilavesiyle MDA değerleri radyasyon grubuna göre önemli azalma gösterdi (P<0.05). Propolis ilavesiyle radyasyon sonucu karaciğerde azalan (P<0.01), böbrekte artan GSH düzeyleri kontrol seviyelerine yaklaşırken (P<0.001), dalak ve beyinde hem kontrol hem de radyasyon uygulanan gruba göre GSH düzeylerinde artış saptandı (P<0.05). Propolis ilavesi kalp, dalak, beyin, yumurtalık GSH-Px aktivitesinde hem kontrol hemde radyasyon uygulanan gruba göre düşüşe neden oldu (P<0.001). Ayrıca, kalp SOD aktivitesinde kontrol grubuna göre önemli azalma bulundu (P<0.05).

Anahtar Kelimeler*: Radyasyon, oksidatif stres, propolis*

Introduction

Ionizing radiation (X, γ-ray) is widely used for diagnosis and therapy in medicine as well as in industrial applications. Ionizing radiation has attracted considerable attention due to its benefits as well as possible harmful effects to human population (1). Ionizing radiation in interaction with living cells causes a variety of changes depending on exposed and absorbed dose, duration of exposure and interval after exposure, and susceptibility of tissues (2). A detrimental effect of irradiation is the production of reactive oxygen species (ROS), which includes superoxide anion radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) (3). ROS are highly reactive and could diffuse to vital cellular targets like DNA, proteins and membrane, ultimately leading to cell death (4).

Cells develop different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase (SOD) catalyzes the dismutation of the $^{\bullet}O_2^-$ into H_2O_2 (5, 6). The major systems degrading H_2O_2 are

glutathione peroxidase (GSH-Px), glutathione reductase (GRD), and glutathione (GSH) (7, 8). Oxidative stress occurs when there is excessive free radical production and/or low antioxidant defense, and the results in chemical alterations of biomolecules cause structural and functional modifications (9). One of the oxidative damage indices is the malondialdehyde (MDA) formation as an end product of lipid peroxidation, which is a result of reaction of ROS with the unsaturated free fatty acids of membrane lipids (10).

Efforts to decrease toxicity of irradiation to normal tissues, organs, and cells have led to researches to identify cytoprotective agents. Propolis is a resinous product of the honey bee that has been employed in traditional medicine throughout history as it possesses a broad spectrum of biological activities, including antibacterial, antifungal, antiviral, antiinflammatory, antioxidant, hepatoprotective, and more recently, anticancer properties (11, 12). Antioxidant capacity is among its most important properties (13). More than 180 constituent compounds have been identified in propolis to date, including amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, terpenes and caffeic acid, as well as volatile oils, aromatic acids, waxes, resins, balms, and pollen grains (14). The active components of propolis that have been identified so far include polyphenols and flavonoids (13).

ROS is the major mediators for radiation-induced damage (15). Being a radiosensitive organ, liver has higher susceptibility against radiation damage (16). The polyphenolic molecular structure of the flavonoids that are universally present, with their innate antioxidant activity, suggests that propolis might also have useful radioprotective properties against irradiation. Therefore, the effect of propolis on the oxidant (MDA) and antioxidant parameters (SOD, GSH-Px, GSH) in the liver tissues and the other tissues of the rats exposed to whole-body irradiation was investigated.

Materials and Methods

Animals: Female fertile Wistar albino rats (200±10 g) aged between 4–5 months were supplied from the Animal Care Unit of Fırat University and were kept in plastic cages with stainless-steel grid tops. The experimental conditions were environmentally controlled in terms of temperature (23±2 °C), humidity (50±5%), and light (12 h of light and dark cycle). The animals were fed with pellet diet and water ad libitum. Three rats were kept together in polypropylene cages containing sterile husk bedding during the experiment. The experiments were carried out after obtaining the approval of the Local Ethics Committee of the Veterinary Research Institute (Official form date and number: 18.04.2013 and 2013/4-1) in Elazığ.

Irradiation of the Rats: A γ-ray source was used to perform the whole-body irradiation. The animals were placed in Plexiglass^{R} cages and irradiated in each group including seven rats, simultaneously. The source-to-skin distance was 291 cm with a dose of 0.0233 Gy/s (17) and an absorbed dose of 9 Gy. They were irradiated by a 160 MLC LINAC (Siemens Artiste linear accelerator, using 6 MV photons). The rats were irradiated under continuous isoflurane anesthesia in a specially fabricated plexiglass chamber radiating out from the center.

Experimental Design: The rats were assigned to three groups including 7 rats in each.

Control Group: The rats did not receive any treatment.

Irradiation: The rats were treated with intraperitoneal injection (i.p.) containing a physiological saline solution for three days. All rats in this group were irradiated with gamma-rays at dose of 9 Gy.

Irradiation + Propolis Group*:* The rats were treated with i.p. injection containing ethanolic propolis extract (EEP) at dose of 100 mg/ kg body weight (2, 17) for 3 consecutive days. Then, all the rats were irradiated with gama-rays at dose of 9 Gy.

Ketamine (ketamine hydrochloride, 50 mg/kg [Ketalar® 5%, Parke-Davis]) and xylazine (8 mg/kg [Rompun® 2%, Bayer]) mixture was used intraperitoneally in order to anesthetize the rats. All rats were sacrificed 24 hours after irradiation exposure. After decapitation, whole liver, spleen, kidney, brain, heart and ovarium tissues were rapidly resected. The tissues were stored at -80°C.

Biochemical Analysis in Tissues: The tissues were homogenized by using a Teflon-glass homogenizer with 1.15% KCl in order to obtain 1:10 (w/v) homogenate. MDA concentration of tissue homogenates expressed as the thiobarbituric acid reactive substances (TBARS) was determined spectrophotometrically in accordance with the method described by Placer et al. (18). MDA concentrations were expressed as nmol/g protein. GSH concentration of tissue homogenates was measured by an assay based on the dithionitrobenzoic acid recycling method of Sedlak and Lindsay (19). GSH-Px activity was determined according to the method of Lawrance and Burk (20), recording the decrease of NADPH at 340 nm. SOD activity was performed based on the method of Sun et al. (21). Tissue protein contents were determined in accordance with the method of Lowry et al. (22).

Ethanolic Propolis Extract (EEP): The propolis samples were kept desiccated at room temperature in the dark before the analysis. EEP was prepared based on the method described by Kosalec et al. (23). Briefly, propolis (10 g) was crushed into small pieces in a mortar and mixed vigorously with 50 ml 80% (V/V) ethanol p.a. (Merck, Sigma) for 48 h at 41±1 °C and filtered through Whatman No. 4 paper. At the end of procedure, it was evaporated in an oven at 45 °C and kept in dark before it was used. EEP used in the experiment was dissolved in ethanol, which was used as solvent of lipophilic compounds from EEP. Further dilutions were made in water. The final concentration of ethanol was less than or equal to 1% (V/V).

Statistical Analysis: The SPSS statistical software (SPSS for windows, version 21.0) was used for all statistical analyses. All the data were presented in mean (±) and standard error (SE). Analysis of variance (ANOVA) followed by Duncan test was used to determine whether there were significant differences among the groups. The 5% level of significance was used to establish differences (24).

Results

Lipid peroxidation (MDA), nonenzymatic antioxidants (GSH) and enzymatic (SOD, GSH-Px) activities were presented in Table 1.

As a result of irradiation, a significant increase was determined in MDA levels of liver, brain, ($P<0.001$) and kidney tissues ($P<0.01$) of the rats. Also, there was a significant decrease in GSH-Px activity of all the tissues ($P<0.001$) and SOD activity and GSH level in the liver ($P<0.01$). On the other hand, GSH level increased significantly in the kidney ($P<0.001$) and ovarium tissues ($P<0.05$).

The MDA levels that increased in the liver as a result of irradiation, decreased significantly with the addition of propolis ($P<0.001$) and the MDA levels that increased in kidney and brain were not affected. In the spleen and ovarium tissues, MDA levels decreased significantly by addition of propolis compared to the irradiation group ($P<0.05$).

Due to addition of propolis, GSH levels, that decreased in the liver as a result of irradiation ($P<0.01$) and increased in the kidney ($P<0.001$), approached to the normal level and it was determined that the GSH levels in the spleen and brain increased compared to both the control and irradiation groups ($P<0.05$). The addition of propolis caused a decrease in the GSH-Px activities of heart, spleen, brain, and ovarium tissues compared to both the control and irradiation groups ($P<0.001$). Also, a decrease in the SOD activity of heart tissue compared to the control group was found ($P<0.05$).

Table 1. The effect of propolis on oxidant/antioxidant status in the tissues of the irradiated rats

	Control	Irradiation	Irradiation + Propolis	P
		LIVER		
MDA (nmol/g prot)	4.56 ± 0.38^{c}	12.57 ± 0.56^{a}	7.12 ± 0.83^{b}	***
GSH-Px (U/g prot)	2.12 ± 0.33^{a}	0.75 ± 0.16^{b}	0.61 ± 0.13^{b}	***
GSH (nmol/g prot)	0.59 ± 0.05^{a}	0.35 ± 0.04^{b}	0.55 ± 0.05^{a}	**
SOD (U/g prot)	1.80 ± 0.19^{a}	1.13 ± 0.04^{b}	1.36 ± 0.04^{b}	**
		KIDNEY		
MDA (nmol/g prot)	9.10 ± 0.42^{a}	22.57 ± 2.86^{b}	19.92 ± 3.00^{b}	**
GSH-Px (U/g prot)	2.54 ± 0.27^{a}	1.10 ± 0.17^{b}	0.91 ± 0.17^{b}	***
GSH (nmol/g prot)	0.41 ± 0.02^{b}	1.92 ± 0.43^{a}	0.62 ± 0.04^{b}	***
SOD (U/g prot)	1.56 ± 0.05	1.64 ± 0.09	1.59 ± 0.09	NS
		HEART		
MDA (nmol/g prot)	12.24 ± 0.99	15.87 ± 1.37	9.25 ± 1.45	NS
GSH-Px (U/g prot)	45.15 ± 3.79^{a}	22.16 ± 1.56^{b}	3.91 ± 0.21^{c}	***
GSH (nmol/g prot)	0.72 ± 0.08	0.82 ± 0.10	0.66 ± 0.06	NS
SOD (U/g prot)	3.36 ± 0.33^{a}	2.76 ± 0.24^{ab}	2.19 ± 0.18^{b}	*
		SPLEEN		
MDA (nmol/g prot)	14.09 ± 1.47^{ab}	16.76 ± 0.80^{a}	10.85 ± 1.44^{b}	*
GSH-Px (U/g prot)	18.35 ± 1.12^{a}	11.23 ± 1.11^{b}	3.05 ± 0.64^{c}	***
GSH (nmol/g prot)	0.42 ± 0.01^{b}	0.55 ± 0.05^{ab}	0.6 ± 0.05^{a}	*
SOD (U/g prot)	1.43 ± 0.07	1.59 ± 0.12	1.62 ± 0.07	NS
		BRAIN		
MDA (nmol/g prot)	13.07 ± 0.86^{b}	21.65 ± 2.02^{a}	20.17 ± 1.99^{a}	***
GSH-Px (U/g prot)	38.41 ± 1.99^{a}	21.36 ± 1.77^{b}	3.02 ± 0.90^{c}	***
GSH (nmol/g prot)	0.52 ± 0.05^{b}	0.67 ± 0.04^{ab}	0.75 ± 0.06^{a}	*
SOD (U/g prot)	1.97 ± 0.14	1.97 ± 0.12	2.06 ± 0.20	NS
		OVARIUM		
MDA (nmol/g prot)	12.94 ± 0.82^{ab}	14.64 ± 1.40^{a}	6.61 ± 2.80^{b}	*
GSH-Px (U/g prot)	57.76 ± 2.99 ^{a}	38.74± 2.24^{b}	4.68 ± 1.32^{c}	***
GSH (nmol/g prot)	0.82 ± 0.03^{b}	1.34 ± 0.18^{a}	1.2 ± 0.50^{ab}	*
SOD (U/g prot)	3.78 ± 0.15	4.01 ± 0.24	3.70 ± 0.36	NS

*****P<0.05, ******P<0.01, *******P<0.001, **NS:** No Significant

^{abc}Mean values with different superscripts within a row differ significantly.

Discussion

Irradiating biological material leads to a rapid increase in ROS, generated primarily due to the ionizing of water molecules. ROS are highly reactive and could diffuse to vital cellular targets like DNA, proteins and membrane, thus leading to cell death (4, 25). Cellular and tissue resistance against ionizing radiation depends on many endogenous parameters, including antioxidant systems and their capacity for adaptive response (26). A number of studies have revealed that irradiation caused oxidative damage by causing an increase in MDA levels and a decrease in the levels of the antioxidants such as SOD, GSH, and GSH-Px depending on time and dosage (10, 26-31). In this study, MDA levels in the liver, kidney, and brain increased significantly as a result of γ-radiation; whereas, a significant decrease was detected in the GSH-Px activity in these tissues and also in the SOD activity and GSH levels in the liver. Additionally, as a result of γ-radiation, significant decreases in GSH-Px activity were determined in the heart, spleen and ovarium tissues; whereas, MDA had an insignificant increase. The reduction in the antioxidant enzymes is associated with the depletion of enzymes during oxidative stress that occurred as a result of irradiation (10, 26, 28). Differently from studies indicating a decrease in GSH in the kidney and ovarium tissues (29, 32, 33), a significant increase was observed in GSH levels in these tissues as a result of γ-radiation in the present study. Also in a study conducted on the acute ovarian toxicity induced by the whole body irradiation, it has been reported that irradiation does not cause a change in the MDA level in the ovarium tissue and causes an increase in GSH-Px and CAT activities (34). Kandemir et al. (35), suggested that the activation of antioxidant defenses, in which the actual production of oxyradicals should decrease, is a preparative mechanism against oxidative stress caused by stress situations.

Irradiation causes oxidative damage in more than one tissue and although the cells have both enzymatic and non-enzymatic antioxidant systems to scavenge ROS formed as a result of irradiation, the antioxidant systems fall behind as mentioned above. In order to prevent the oxidative stress caused by irradiation, natural exogen antioxidants may be used. Propolis is the generic name for a complex resinous mixture collected by honey bees from the buds and exudates of various plants. The flavonoids in propolis are powerful antioxidants and capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation (13, 31, 36). Antioxidant activity of flavonoids is based on ability of direct free radicals scavenging or stabilizing the reactive oxygen species (ROS) by interacting with the reactive compound of the radical. Due to the high reactivity of the phenolic hydroxyl substituents of the flavonoids, radicals are made inactive (2). In this study, the MDA levels increased in the liver as a result of irradiation decreased significantly with the propolis addition; however, the MDA levels in the kidney and brain tissues were not affected by the addition of propolis. The decreases in the MDA levels by the propolis addition in heart, spleen, and ovarium tissues together with the liver tissues compared to the irradiation group may be associated with the antioxidant and free radical scavenging capability of propolis. In numerous studies, it has been determined that the addition of propolis decreases the MDA levels increasing in the plasma (31), lens (36), skin (14), small intestine (37) occurring as a result of irradiation and increased the decreased antioxidant levels such as SOD (36), and GSH (31, 37). Being an active phenolic compound of propolis extract, caffeic acid phenethyl ester (CAPE) blocks ROS production (13, 36). Also in various studies investigating the effect of CAPE on radiation, similar results have been obtained about the oxidant and antioxidant parameters in the tissues such as liver (4), intestine (38), heart (26), and lens (36). Moreover, it has been reported that propolis is more effective than CAPE in preventing the oxidative damage occurring as a result of irradiation (36). In contrast with other studies, the fact that the addition of propolis decreased the GSH-Px activity from antioxidant enzymes in all the tissues, SOD activity in the liver and heart tissue, GSH level in the kidney compared to the control and/or irradiation groups may be associated with the fact that propolis decreased the need for the antioxidants due to its antioxidant and free radical scavenging properties.

GSH is one of the most important anti-oxidant molecules. Propolis has been determined to reverse the consumption of glutathione, which is synthesized in the liver and has radical scavenging activity (39). Also in this study, it was determined that while due to the addition of propolis, GSH levels, which decreased in liver as a result of irradiation, returned to the normal level, the GSH levels in the spleen and brain increased compared to the control group.

Radiation causes oxidative stress in more than one tissue and often the antioxidants run out. When the MDA levels were examined, it was observed that propolis decreased the oxidative stress in the tissues other than kidney and brain in the irradiated rats due to its antioxidant and free radical scavenging property. As propolis decreases the formation of lipid peroxidation in the irradiated rats, there is a need for conducting further studies on high doses of propolis by which the oxidative damage in the kidney and brain is prevented.

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