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Original Article

Propolis Attenuates Nitrosative Stress in the Brain Tissue of Rats Exposed to Total Head Irradiation

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ABSTRACT

Objective: This study aimed to research the radioprotective aspect of propolis on the brain tissue of rats exposed to ionizing radiation and its ability in reducing nitrosative stress resulting from ionizing radiation.

Methods: In this study, 32 rats were used. They were randomly divided into four equal groups: two control groups, one irradiation only group, and the last group is both IR exposed and propolis administered. A single dose of 5 Gy was given to the other two groups except the control groups. Biochemical parameters were measured by spectrophotometry to determine whether protective effects of propolis were present.

Results: In this study, it was determined that nitric oxide, peroxynitrite values, and nitric oxide synthase activity were significantly higher in the radiotherapy only group in comparison to both propolis and irradiation-treated groups.

Conclusion: These findings suggest that the use of propolis has a protective effect against the adverse effects of nitrosative stress caused by ionizing radiation. However, to be assured of these beneficiary effects of propolis should be supported by further pharmacological and toxicological research.

Keywords: Irradiation, propolis, nitric oxide, nitric oxide synthase, peroxynitrite

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly produced in living organisms and are rendered harmless.[1–3] Reduced antioxidative capacity and increase in free radical (FR) production cause significant damage to the components of the cell by causing oxidative/nitrosative stress.[4–7]

Cancer, which was accepted as the second cause of death in 2015, caused the death of 8.8 million people in the world and is an expensive and important health problem. Although many strategies have been found to treat cancer such as surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, great care has been taken to investigate additional strategies to combat cancer using natural plants and their bioactive components.[8]

Radiotherapy is one of the indispensable treatment methods in cancer treatment, and approximately two-third of the cancer patients are receiving radiotherapy. While the total dose required for effective local control with radiotherapy is exited, damage occurs in normal tissues in the field of irradiation. The resulting damage is also closely related to the sensitivity to radiation. It is known that ionizing radiation forms FRs.[4,9,10]

Radiation effects can be grouped into two types: direct and indirect effects. The direct effect occurs with ionization in DNA, whereas the indirect effect occurs with water ionization in the organism. Since about two-thirds of the human body weight is water, most of the radiation damage is caused by FR, which is caused by the effect of radiation on water.[11] The aim of the radiotherapy is to preserve as much as of the normal tissue possible and to give maximum ionizing radiation to the tumor tissue. Because radiation is toxic to both tumor tissues and healthy tissues, normal tissue damage can also increase as the radiation dose increases as a result of the near healthy tissues exposure to radiation. Unfortunately, most of the radioprotectors used today have significant toxic side effects that limit their role in medical treatment.[12] Therefore, the search for effective and nontoxic compounds with radioprotective ability has led to increased interest in antioxidants such as thymoquinone and propolis.[13]

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Table 1. Parameters Measured in Brain Tissue of Rats

	Nitric Oxide (μmol g^{-1} Weight)	Nitric Oxide Synthase (U mg^{-1} Protein)	Peroxynitrite (μmol g^{-1} Weight)
Sham control group	4.58 ± 0.3[b]	3.48 ± 0.7[b]	74.41 ± 23.7[b]
The control of IR + propolis	5.52 ± 1.9[a]	3.28 ± 0.9[b]	62.96 ± 23.2[b]
IR group	7.21 ± 0.8	5.12 ± 1.0	189.34 ± 60.4
IR + propolis group	5.65 ± 1.2[a]	2.99 ± 0.5[b]	63.06 ± 20.9[b]

IR, Irradiated.
[a] $P < .01$.
[b] $P < .0001$ vs IR group.

Propolis has immunomodulatory, antitumoral, cancer-preventative, antimetastatic, anti-inflammatory, cytotoxic, and antioxidant properties. Our main focus here is the antioxidant activity and radioprotective effects of propolis.[13,14]

To our knowledge, we did not find an experimental study investigating the effect of propolis on nitrosative stress parameters in the brain tissues of rats given ionizing radiation. Therefore, in this study, we aimed to investigate the possible effects of propolis from total cranial irradiation on nitrosative stress.

METHODS

Study Protocol and Rats and Experimental Groups

This research was conducted at the Departments of Medical Biochemistry and Radiation Oncology after obtaining ethical approval from Animal Ethics Committee of Gaziantep University (ethical committee number: 2017/2). Thirty-six Sprague–Dawley rats (200 ± 20 g) fed with standard laboratory chow and water were used. Experimental animals were quarantined for at least 1 week. Later, they were randomly divided into four groups, including eight rats in each group. Information on groups is as follows:

Sham control group: no propolis but sham irradiation.

The control group of irradiation (IR) plus propolis group: saline only (1 mL) through an orogastric tube, no IR, no propolis.

The IR group received 5 gray (Gy) gamma irradiation and 1 mL saline as a single dose. IR 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 1 hour before irradiation and continuing for 10 days through an orogastric tube. Supplementation period was 10 days.

All rats that received 80 mg kg^{-1} ketamine hydrochloride (Pfizer Pharmaceuticals, Istanbul, Turkey) were anesthetized. Then, the rats in the IR and IR plus propolis groups were given a single dose of ionizing radiation with the aid of a Cobalt-60 teleotherapy unit (Picker, C9, Maryland, NY, USA).

Biochemical Analysis

Determination of nitric oxide synthase activity, nitric oxide, and peroxynitrite levels

All rats were anesthetized with ketamine hydrochloride 50 mg kg^{-1} i.p. on Day 11. Approximately equal amounts of tissue were obtained from the frontal lobes of each rat brain. The homogenization of tissues was carried out in a Teflon glass homogenizer. The supernatant obtained after homogenization was taken into eppendorf tubes and kept at –80°C until biochemical analyzes.

Nitric oxide synthase (NOS) was studied according to the method described by Durak et al.[15] NOS activity was determined by the diazotization of sulfanilic acid by NO at acid pH and subsequent coupling to *N*-(1-napthyl) ethylenediamine. To 0.1 mL of sample, 0.2 mL of 0.2 M arginine was added, and the mixture was incubated at 37°C for 1 hour, after which 0.2 mL of 10 mM HCl, 100 mM sulfanilic acid, and 60 mM N-(1-napthyl) ethylenediamine were added. Absorbance at 540 nm was measured after 30 minutes. Sodium nitroprusside standard solution (25 mM) was used as standard. Results were expressed as U mg^{-1} protein. Nitric oxide ($NO^{\bullet}$) levels were measured as previously described,[16] which express as μmol g^{-1} weight. Peroxynitrite ($ONOO^{-}$) determination was performed according to the method proposed by Vanuffelen et al.[17] and modified by Al-Nimer et al.,[18] which expresses as μmol g^{-1} wet weight. The protein content was determined as described.[19]

Statistical Analysis

The data obtained from the study were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). The Kolmogorov–Smirnov test

Main Points

- Ionizing radiation produces free radicals.
- Excessive free radicals create nitrosative stress.
- Nitrosative stress plays an important role in the pathogenesis of many diseases.
- Propolis prevented nitrosative stress caused by ionizing radiation.

Figure 1. Mean ± SD of nitric oxide ($NO^{\bullet}$) assigned in the brain tissue of rats. IR: irradiated.

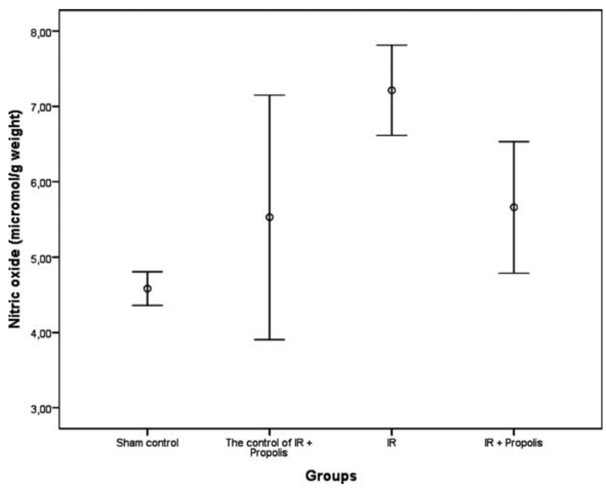
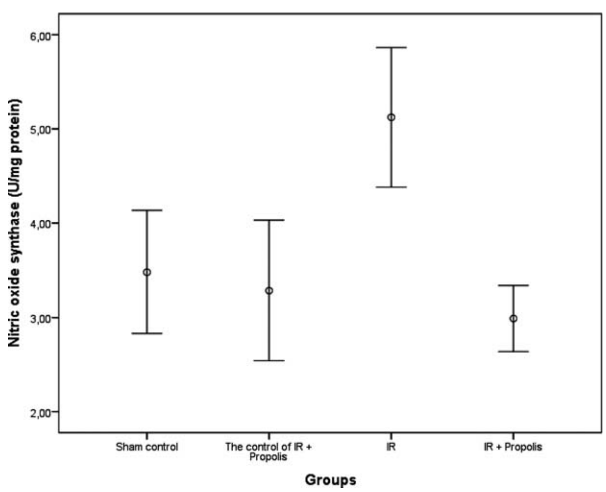


Figure 2. Mean ± SD of nitric oxide synthase (NOS) assigned in the brain tissue of rats. IR: irradiated.

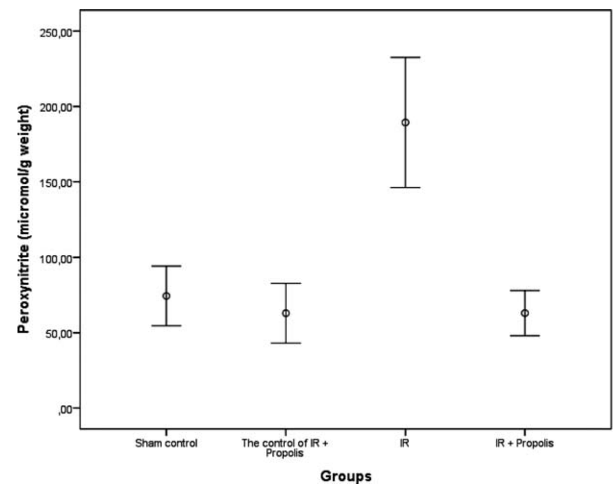


was used to test whether the data are parametrical or not. ANOVA and LSD multiple comparison tests were used to compare three independent groups of variables with normal distribution. Mean ± standard deviation was given as descriptive statistics. $P \leq .05$ was considered statistically significant.

RESULTS

Results are shown in Table 1 and Figures 1-3. In statistical analysis, the difference between the groups in terms of $ONOO^{-}$ ($P <$ .0001) and $NO^{\bullet}$ ($P < .0001$) values, and NOS activity ($P < .01$) was found to be statistically significant. $ONOO^{-}$, $NO^{\bullet}$ values, and NOS activities were significantly higher in rats in the irradiation-only group than sham control group, irradiation plus propolis, and control group of group given propolis.

Figure 3. Mean ± SD of peroxynitrite ($ONOO^{-}$) assigned in the brain tissue of rats. IR: irradiated.



DISCUSSION

The physiological processes of all aerobic organisms generate FRs necessary for the maintenance of normal life activities. There are many endogenous FR scavenging pathways in living things, and the balance between FRs and antioxidant systems is crucial to maintain the health of the organism. However, excessive accumulation of FRs damages normal cells and tissues, damages human tissues, and organs and cells and plays a role in the pathogenesis of many diseases.[3,9,20]

Although there are some possible mechanisms by which oxidative/nitrosative stress plays a regulatory role in tumor growth and progression, some important questions remain unanswered. Whether oxidative/nitrosative stress is caused by the increased oxidant production of the tumor, or the deficiency of the antioxidant defense system is not completely explained. Although significant changes in cellular redox homeostasis during tumor growth have been documented in experimental models, such changes have not been demonstrated in humans. Most of the difficulties encountered in these studies are related to the complexity of the biochemical pathways that regulate the cellular redox balance.[21,22]

The purpose of radiation therapy is to carefully give ionized radiation doses to a defined tumor volume to protect the cells and tissues around the tumor with minimal damage, destroy tumor cells, provide the patient with a high quality of life, and prolong survival. The purpose of radiation therapy is to carefully give ionized radiation doses to a defined tumor volume to protect tissues around the tumor with minimal damage, destroy tumor cells, provide the patient with a high quality of life, and prolong survival.[9,11,23]

In some studies, investigating the parameters affected by ionizing radiation as a result of oxidation, it has been reported that ionizing radiation increases the levels of NO in cells and tissues, leading to an increase in RNS and consequently cause oxidative and nitrosative stress.[9,23] Even though antioxidant properties of propolis are proven in a large number of studies, to our knowledge, this is the first investigation that studied the effects of propolis on radiation-induced nitrosative stress in brain tissue. In the present study, we found that NO• and ONOO− levels and NOS activity in the rats given propolis were significantly lower compared to the rats received irradiation-only group. The results obtained in the study support the research hypothesis that the systemic administration of propolis would reduce the nitrosative damage in irradiated brain tissue.

The brain's antioxidant defense system is vulnerable to brain FR damage, as its ability to cleanse FRs is weaker compared to other tissues. Therefore, a decrease in the antioxidant defense system of brain tissue can cause ROS/RNS deposition during IR.[23–25]

In the present study, we found a significant increase in $NO^{\bullet}$ and $ONOO^{-}$ levels and NOS activity in irradiated rats when compared to other groups, which is similar to that reported by previous studies.[9,23,26] The results support the research hypothesis that the systemic administration of propolis would reduce the nitrosative damage in irradiated brain tissue in the experimental rat model. Our findings support the antioxidant properties of propolis in radiation-induced brain tissue damage.

Although biochemical analyzes suggest that propolis has a radioprotective effect against nitrosative damage in the brain tissue of irradiated rats, the absence of histopathological evaluation supporting this study was one of the limitations of the study.

CONCLUSION

Our findings demonstrated increased nitrosative stress in brain tissue of irradiated rats. This is the first study that investigates the effects of propolis on the nitrosative stress in the brain tissue of the irradiated rats. Oxidative/nitrosative stress is mainly a result of increased ROS and RNS productions and may be one of the main causes of cardiovascular, inflammatory, neurodegenerative, and autoimmune diseases, as well as important in cancer. The current results have shown that propolis clearly prevents from nitrosative stress in radiation-induced brain tissue damage by inhibiting FR generation or scavenging ROS/RNS. The use of propolis as an antioxidant against serious side effects in patients with head and neck cancer exposed to ionizing radiation may be beneficial in obtaining a more beneficial treatment. However, further research is needed to support these results.

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Informed Consent: N/A.

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