The Effects of Royal Jelly Against Radiation-Induced Acute Oral Mucositis

Yasemin B. CIHAN[1], Kemal DENIZ[2]

[1] Kayseri Education and Research Hospital, Department of Radiation Oncology
[2] Erciyes University Medical School, Department of Pathology, Kayseri, TURKEY

ABSTRACT

The purpose of this study was to investigate the effects of royal jelly against radiation-induced oral mucositis in rats. This study was comprised of 48 adult male Sprague-Dawley rats, each between eight and 12 weeks old and weighing 275±35 g. These were divided randomly into six groups; Group C (control), Group IR (irradiation), Group IR+RJ50 (irradiation plus 50 mg/kg royal jelly), Group IR+RJ100 (irradiation plus 100 mg/kg royal jelly), Group RJ50 (50 mg/kg royal jelly), and Group RJ100 (100 mg/kg royal jelly). The degree of mucositis, the animal's body mass, and food intake were evaluated. Biochemical and histopathological methods were utilized for the evaluation of mucositis, and a hemogram, oxidative stress markers, and biochemical parameters were analyzed. Tongue samples were also processed for histopathological examination. Irradiation significantly increased oral mucositis, decreased the thrombocyte and neutrophil counts, and resulted in a reduction of food intake accompanied by weight loss. In addition, a significant increase in malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) and catalase (CAT) activities were found (p< 0.001). Irradiation plus 50 mg/kg royal jelly caused normalization in the quantitative, biochemical, and histopathological parameters compared with the group that underwent irradiation alone. Irradiation plus 100 mg/kg royal jelly did not provide superior radioprotection against radiation-induced toxicities. Royal jelly was administered orally to reduce oral mucositis as it may be more suitably applied.

Keywords: Radiotherapy, Oral mucositis, Royal jelly, Rat, Antioxidant

ÖZET

Ratlarda Radyasyonun İndüklediği Akut Oral Mukozite Karşı Arı Sütünün Etkisi

Bu araştırmanın amacı, ratlarda baş-boyun bölgesine uygulanan radyoterapiye bağlı oluşan oral mukozite karşı arı sütünün etkisini belirlemektir. Bu çalışmaya 48 adet erişkin, 8-12 haftalık yaşta ve 213±27 gram ağırlığında Sprague-Dawley erkek rat alındı. Hayvanlar 6 gruba ayrıldı; Grup K (kontrol), Grup RT (sadece ışınlama yapılan), Grup RT+AS50 (ışınlama ile birlikte 50 mg/kg arı sütü verilen); Grup RT+AS100 (ışınlama ile birlikte 100 mg/kg arı sütü), Grup AS50 (sadece 50 mg/kg arı sütü) ve Grup AS100 (sadece 100 mg/kg arı sütü) verilen grup olarak belirlendi. Deney boyunca hayvanların mukozit skorları, ağırlık ölçümleri ve yiyecek tüketimleri ölçüldü. Biyokimyasal ve histopatolojik yöntemlerle mukozit, hemogram, antioksidan aktiviteleri ölçüldü ve biyokimyasal paremetrelerin analizi yapıldı. Ayrıca dil dokusunda da histopatolojik inceleme yapıldı. Işınlamadan sonra oral mukozitte artış, trombosit ve nötrofil sayısında azalma ve oral alımın azalmasına bağlı olarak kilo kaybında önemli düşme gözlendi. Ayrıca serumda malondialdehit (MDA) seviyesinde önemli bir artış, süperoksit dismutaz (SOD) ve katalaz (CAT) aktivisinde düşme görüldü (p< 0.001). Işınlama ile birlikte 50 mg/kg arı sütü verilen grup, RT grubu ile kıyaslandığında kantitatif, biyokimyasal ve histopatolojik paremetrelerde normalizasyon sağladığı görüldü. Işınlama ile birlikte 100 mg/kg arı sütü verilen grupta radioprotektif etkinin doza bağlı olarak artmadığı görüldü. Arı sütünün uygun dozlarda verildiğinde oral mukoziti azaltmada etkili olduğu görüldü.

Anahtar Kelimeler: Radyoterapi, Oral mukozit, Arı sütü, Sıçan, Antioksidan

INTRODUCTION

Radiotherapy (RT) is an essential therapeutic modality in the management of head and neck cancers. Despite advances in RT technology, oral mucositis has not been ameliorated in head and neck cancer patients treated with radiotherapy. The current head and neck radiotherapy protocols have a mucositis incidence of 85-100%. The severity of mucositis depends on many variables, such as anti-cancer treatment protocol, age, diagnosis of the patient, level of oral hygiene during therapy, and genetic factors.[1,2,3,4]

Oral mucositis begins five to seven days after the initiation of radiotherapy with cumulative doses of about 10-15 Gy of standard fractionated radiation. Acute mucositis is defined as a reactive inflammation of the mucosa and atrophy of the squamous epithelial tissue and with an absence of vascular damage. Early evidence of mucositis is characterized by erythema and pain causing discomfort.

At 20-30 Gy of standard fractionated radiation, the lesions often start off as discrete breaks in the mucosa. A typical lesion of advanced mucositis is seen as an ulceration, either with or without a fibrinous pseudomembrane, on a bed of erythema. This treatment-induced complication causes discomfort as well as difficulty in drinking, eating and swallowing and is accompanied by speech and nutritional problems. It can also lead to other complications, such as poor nutrition due to pain along with delays in drug administration and can have a significant impact on quality of life, the risk of infection and cost of care.[1,2,5-7]

A variety of approaches for the management of early oral mucosal radiation effects have been tested preclinically as well as clinically. However, there is no universally accepted treatment or prevention protocol. Diagnosis is clinical and based on the use of known stomatotoxic therapy along with the timing and location of oral lesions.[6,8-14]

For several decades, there have been publications which have pointed to the potential of antioxidants to reduce mucosa damage induced by ionizing radiation. Some naturally occurring antioxidants have the advantage of offering an extended window of protection against low-dose and low-dose rate irradiation, including therapeutic potential when administered after irradiation.[9,12,13] In addition, further studies are required to determine the potential value of specific antioxidant nutrients during radiotherapy for cancer. The use of antioxidants, such as vitamin E, and zinc sulfate, for the prevention of oral mucositis in RT or chemoradiation is promising but more research is needed.[6,13]

In recent years, there have been reports on the antioxidant activities of royal jelly (RJ) that attracted attention from various scientists. It has been studied in vitro and in vivo by many researchers. Royal jelly is consumed as a dietary supplement and is widely used in commercial medical products, health foods and cosmetics in many countries. It is produced by the hypopharyngeal and mandibular gland of worker honeybees and is known to be necessary food for the growth of the honeybee queen since it contains major nutrients including proteins, sugars, lipids, vitamins, minerals and free amino acids. One of the important physiological functions of RJ is its antioxidative action because it has the ability to help prevent various chronic diseases, such as cancer and cardiovascular disease caused by the attack of free radicals.[15-21] Recent reports suggest that RJ offers protection against oxidative, stress-induced biological dysfunction. Therefore, it is possible that RJ may offer some protection against radiation side effects. However, no previous study exists on the ability of RJ to eliminate or alleviate oral mucositis generated by head and neck irradiation in rats.

The aim of the present research was to evaluate the level of radiation-induced mucositis, myelosuppression, and oxidative stress in rats and to investigate the possible protective effects of RJ on mucositis using quantitative, biochemical, and histopathological approaches.

MATERIAL and METHODS

Appropriate permission for the study was obtained from the ethics committee of Erciyes University (Kayseri, Turkey) and this experimental study was performed in accordance with national guidelines for the use and care of laboratory animals.

2.1. Animals:

The study was carried out in 48 adult male Sprague-Dawley rats that between eight and 12 weeks old and weighed 275±35 g. The rats were quarantined for at least three days before irradiation and housed in groups of four in polycarbonate cages with free access to standard rat food and water under hygienic conditions. All rats were kept in a room with standardized air conditioning (23⁰C; 50-60% humidity) in 12-hour light/dark cycles.

Study groups:

The rats were divided into six groups with eight animals in each group, one group for control and five for experimental purposes, and were treated as follows:

Group 1 (C): Received neither RJ nor IR (control group).

Group 2 (IR): Received total cranium irradiation of 22 Gy as a single dose and received distilled water 0.2 mL sham per day topical administration for seven days beginning 30 minutes before irradiation; the rats were given an equal volume of distilled water instead of RJ.

Group 3 (IR+RJ50): Received total cranium irradiation plus an RJ dosage of 50 mg/kg/day topical administration for seven days which began 30 minutes before irradiation and continued for a week.

Group 4 (IR+RJ100): Received total cranium irradiation plus an RJ dosage of 100 mg/kg/day; otherwise, the procedure was the same as for Group 3.

Group 5 and 6 (RJ50 and RJ100): Received RJ alone at a dose of 50 and 100 mg/kg/day, respectively.

Irradiation procedure

Prior to irradiation, each of the rats were weighed after they were anesthetized by a subcutaneuos injection of 30 mg/kg ketamine hydrochloride (Ketalar®, Parke Davis, İstanbul, Turkey) and 5 mg/kg xylasine (Rompun®, Bayer, İstanbul, Turkey). They were then placed on a Plexiglas tray in a prone position. The rats in Groups 2, 3 and 4 were irradiated using a Cobalt-60 teletherapy unit (Theratron 780 C) from a source-to-surface distance of 80 cm by a 5x15 cm anterior field with 22 Gy to the total cranium as a single fraction. Two rats were irradiated simultaneously. The dosage was calculated for the central axis at a depth of 1 cm and the dose rate was 3.00 cGy/MU. Groups 1, 5 and 6 were subjected to the same procedure but were not irradiated. The irradiation of a 22 Gy in a single dose was selected from previous studies.[22]

Preparation of water extract RJ

Royal jelly was acquired from the Civan Bee Farm in Bursa, Turkey, and kept at -20oC until used. It was diluted in sterilized distilled water (100 mg/Ml, wt/vol) and ultrasonicated for at least 60 min and shaken slowly overnight at 4^0C. The mixture was centrifuged at 15000 g for 10 min at 4^0C, and the supernatant was further diluted with the culture medium and used as the sample solution for the upcoming tests. The royal jelly was per day topical administration for seven days to Groups of 3, 4, 5 and 6 (0.2 ml for a day per rat) and was well-tolerated without any toxic effects. The dosage was selected from previous studies.[15-21]

Determination of the clinical findings of radiation-induced mucositis, body weight, and food intake:

Body weight was measured in each rat every two days, and the amount of oral intake was evaluated in each rat per group. After irradiation, the mouths of the rats were examined of daily for signs of mucositis. The extent of mucositis was scored based on the scale proposed by Parkins et al.[5] as follows: *Score 0:* Normal; *Score 0.5:* Slightly pink; *Score 1:* Slightly red; *Score 2:* Severe reddening; *Score 3:* Focal desquamation; *Score 4:* Exudation or crusting covering less than one-half of the lip; and *Score 5:* Exudation or crusting covering more than one-half of the lip.

Collection and processing of blood and tissues:

At the end of the study, the rats were anesthetized by a subcutaneous injection of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine 12 hours after the last dose of RJ. Blood samples from the hearts of all the rats were placed into dry, and heparin-coated tubes. After the blood samples were collected, the tongue tissue of each animal was dissected.

Measurement of the hemogram, biochemical parameters and oxidative stress markers:

The leucocytes, thrombocytes, and hemoglobin were removed, formed in heparinized tubes, and measured using Johnson & Johnson label kits. They were then evaluated spectrophotometrically using an automated analyzer.

The blood samples collected in the heparinized tubes were centrifuged at 2000x g for the separation of plasma and serum which, had been stored at -2^0C until the measurements. The erytrocytes were washed and diluted as described by Winterbourn et al.[23] A total of 0.8 ml of washed erythrocytes were hemolyzed by using 3.2 ml of ice-cold distilled water. This hemolysate was used for for the measuring catalase (CAT) activities. For measuring of superoxide dismutase (SOD) activity, 1 ml of the hemolysate was added to 1 ml of a 3/5 chloroform/ethanol mixture and was vortexed. Following centrifugation at 2000x g for 10 min, the clear supernatant was used.

Serum was used for measuring blood urea nitrogen (BUN), glucose, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline

phosphatase (ALP), gamma glutamyl transpeptidase (GGT), creatinine, lactate dehydrogenase (LDH), sodium, potassium, calcium and chloride activities. Spectrophotometric diagnostic kits (Konelab Thermo Clinical Labsystems, Finland) were used for biochemical parameters.

The enzyme activities in the serum were evaluated by an autoanalyzer (Konelab–60 Thermo Clinical Labsystems, Finland). The measurements of malondialdehyde (MDA), SOD and CAT activity were performed in accordance with the methods described by Yoshiaka et al.[24], Luck[25] and Sun et al.[26] The MDA results were expressed as nmol/mL. Activities for SOD and CAT activity were expressed as U/mg Hb.

Histolopathological analysis:

The tongues of the rats were excised, washed in ice-cold isotonic saline, and fixed in 10% formalin. They were then hydrated in ascending grades of ethanol, cleaned in xylene, and embedded in paraffin wax. Sections (5 μm thick) were cut and stained with hematoxylin and eosin (H&E) stain. Histopathological findings in all groups were evaluated based on the severity of inflammation and ulceration in the tongue. Inflammation was scored on four-point ordinal systems as follows; *grade 0:* normal; *grade 1:* focal infiltration of inflammatory cells; *grade 2:* slight inflammation of only the lamina propria; *grade 3:* severe inflammation; and *grade 4:* severe inflammation extended to the muscle layer. Ulceration was scored as follows; 0: normal; 1: epithelial desquamation; 2: total loss of epithelium; and 3: destruction of the muscle layer. Histopathological examination times of the oral mucosa were based on the literature.[6,11]

Statistical analysis:

All results were analyzed using the general linear model procedure available from Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) 15.0 version statistical software program. All values were expressed as arithmetical mean and ± standard error. Analysis of data among all groups was done by one-way analysis of variance (ANOVA). Statistical significance was assigned when p< 0.05. Differences between groups were determined by Duncan's multiple range test.

RESULTS

No animal mortality was observed in any of groups. Figure 1 shows the body weight of the rats in the groups. There appeared to be a significant (p< 0.05) decrease in body weight in the IR group of animals, but there was an increase in the IR+RJ50 group. All groups showed weight gains during day one, but the irradiated rats began to show sudden decreases in weight beginning on day two with significant differences occuring between the IR, IR+RJ100, and the control groups (p< 0.05). From days three to seven, the IR+RJ50 group showed a significant (p< 0.05) increase in body weight. The IR+RJ100 group showed a tendency to gain weight beginning on day two but these rats were losing weight at the end of the experiment period.

Figure 2 shows that the IR group had a decrease in oral intake, and significant differences were noticed in the control group (p< 0.05). Beginning on day three, all irradiated rats showed a sudden decrease in oral intake compared with the non-irradiated rats. The IR+RJ50 group showed a slow increase in oral uptake beginning on day four and accelerating on day seven. In the irradiated rats treated with 100 mg/kg RJ, oral intake declined until the end of the experiment period. Figure 3 displays the course of mucositis during the experimental period. Irradiation caused statistically significant increases in the severity of mucositis in the beginning days of the study (p< 0.05). However, the administration of RJ50 to IR-treated rats significantly prevented the radiation-induced negative effects of mucositis (p< 0.05). The mucositis scores were higher in the IR+RJ100 group during the entire experimental period.

As seen in Table 1, animals treated with IR showed a significant decrease in leucocyte and thrombocyte counts (p< 0.001), but there was no significant difference in the hemoglobin (Hb) levels when compared with the other groups. Moreover, the leucocyte and thrombocyte counts were significantly high in the IR+RJ50 group when compared to the IR group. In the IR+RJ100 group, the leucocyte and thrombocyte counts were significantly lower than in the IR group (p< 0.001).

The effects of RJ and IR on serum biochemical parameters are depicted in Table 2. Radiation caused a significant changes in almost all biochemical paremeters. Compared with the controls, LDH and BUN levels were determined to have increased, but ALP, AST, glucose, calcium, and sodium levels showed
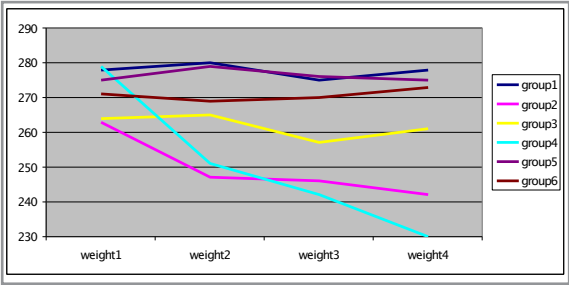
Figure 1. Rats' body mass in groups during the seven days of the experiment. The groups were considered statistically different (p< 0.05).
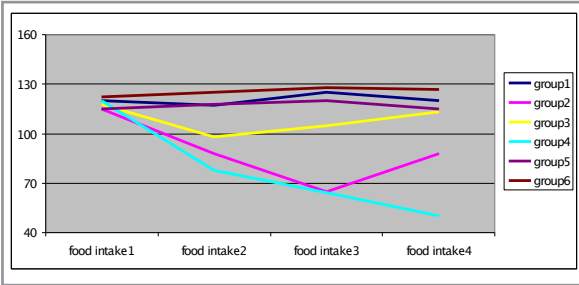

Figure 2. Rats' food intake in groups during the seven days of the experiment. The groups were considered statistically different (p< 0.05).
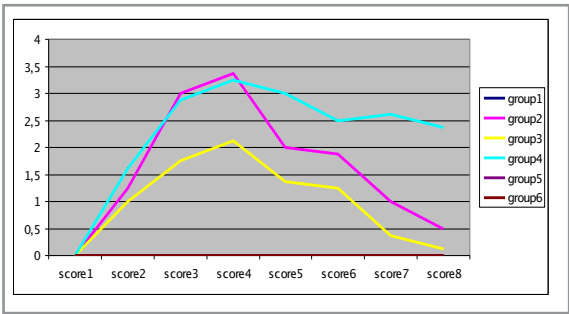

Figure 3. Mucositis development after irradiation during the experimental period. The groups were considered statistically different (p< 0.05).

significant decreases. The IR+RJ50 group showed significant improvement in all tested parameters related to the control values (p< 0.001). Compared with the IR group, the BUN and LDH levels were determined to have increased in the IR+RJ100 (p< 0.001). In the biochemical findings, no statistically significant differences were determined in serum ALT, GGT, or potassium levels. When compared with the control group, no statistically significant difference was determined to exist in the group which was administered RJ alone with respect to all biochemi-

cal parameters. Values pertaining to the parameters investigated were determined to be closer to those of the control group   (p> 0.05).

The results in Table 3 show that IR induced an oxidative stress as indicated by the increase in MDA level accompanied with decreases in CAT and SOD (p< 0.001). Treatment with IR+RJ50 resulted in a significant improvement in CAT and SOD and a decrease in MDA levels. The combined treatment of IR with high doses of RJ resulted in no significant improvement in the antioxidant status.

In all the tongue tissue sections from the control group, the RJ50 and RJ100 groups were found to have normal epitheliums (Figure 4a). In the IR group, the histology was consistent with epithelial atrophy, oral ulcerations, mild to moderate nonspecific acute inflammatory infiltrate and partially necrotic alterations (Figure 4b). However, the IR+RJ50 group presented less inflammatory infiltrate; moreover, this group presented a more pronounced granulation tissue, and the general characteristics indicated a more advanced healing process (Figure 4c). The IR+RJ100 group had developed ulcers, areas of necrosis, and massive infiltrate neutrophils (Figure 4d).

Table 1. Hemoglobin level along with the neutrophil, and thrombocyte counts in all groups. Results are presented as mean±Standard deviation(Mean±SD).

Parameters	Control Mean±SD	IR Mean±SD	IR+RJ50 Mean±SD	IR+RJ100 Mean±SD	RJ50 Mean±SD	RJ100 Mean±SD	p (<0.001)
Hemoglobin (g/dL)	15.03±1.08[4]	15.78±1.1[4]	15.63±2.41[4]	9.06±0.9[12356]	14.80±1.37[4]	15.67±1.08[4]	***
Neutrophile (10³/ml)	1.33±0.26[24]	0.35±0.06[1356]	0.95±0.09[24]	0.28±0.09[1356]	1.31±0.5[24]	1.44±0.55[24]	***
Thrombocyte	619.37±76.66[234]	350.12±69.18[1456]	417.75±102.7[1456]	103.37±78.81[12356]	564.87±73.74[234]	553.12±48.12[234]	***

Table 2. Biochemical paremeters in all groups. Results have been represent as Mean±SD

Parameters	Control Mean±SD	IR Mean±SD	IR+RJ50 Mean±SD	IR+RJ100 Mean±SD	RJ50 Mean±SD	RJ100 Mean±SD	p (<0.001)
ALP(IU/L)	266.37±6.54[234]	234.00±22[145]	239.37±4.53[145]	132.62±14.52[12356]	258.75±12.32[234]	252.00±4.07[4]	***
ALT(IU/L)	63.75±9.14	63.62±24.41	63.50±17.37	60.87±22.40	69.00±4.14	76.25±2.81	ns
AST(IU/L)	179.12±65.18[2]	109.62±52.50[1]	127.12±53.21	120.00±17.79	156.12±4.85	164.62±3.11	***
GGT(IU/L)	0.12±0.04	0.12±0.46	0.13±0.05	0.11±0.03	0.1±0.07	0.1±0.05	ns
Glucose (mg/dL)	247.50±12.30[234]	181.52±16.90[1456]	184.87±14.78[1456]	144.37±20.66[12356]	250.62±13.24[234]	257.12±9.21[234]	***
Bilirubin (mg/dL)	0.05±0.05	0.09±0.07[6]	0.1±0.0[56]	0.11±0.05[56]	0.03±0.0[34]	0.02±0.0[234]	***
BUN (mg/dL)	16.87±1.8[24]	21.25±1.28[1345]	15.37±1.40[246]	25.0±2.13[12356]	18.0±0.75[24]	19.12±2.9[34]	***
Creatinine (mg/dL)	0.43±0.02	0.47±0.01[3]	0.38±0.03[24]	0.49±0.0[36]	0.43±0.0	0.42±0.0[4]	***
Calcium (nmol/L)	9.41±0.42[24]	8.75± 0.3[135]	9.58±0.54[24]	8.41±0.22[1356]	9.28±0.13[24]	9.23±0.16[4]	***
Chloride (nmol/L)	103.0±3.0[4]	101.5±3.0[4]	103.12±2.23[4]	94.37±5.62[12356]	102.25±1.28[4]	102.62±1.5[4]	***
Potassium (nmol/L)	4.4±0.13	4.13± 0.19	4.22±0.19	4.26±0.31	4.26±0.09	4.28±0.06	ns
Sodium(nmol/L)	139.2±1.66[26]	134.3±2.6[13]	141.0±0.92[2456]	136.6±2.9[3]	137.0±2.0[3]	135.8±1.8[3]	***
LDH (IU/L)	1029.0±28.25[234]	1363.87.5±48.8[13456]	1130.87±99.3[12456]	1443.75±51.1[12356]	1028.62±33.9[234]	1007.37.62±17[4]	***

The groups in the same column with different letters number are statistically significant (p<0.05).

DISCUSSION

In this study, we looked for so-called irradiation side effects, which are assumed to be transient, on the mucosa of the rat during the first seven days of mucositis. Previous studies have shown that a dose-effect curve has been obtained for the incidence and severity of mucositis.[1-3] It is important to remember that the rats were subjected to a single, relatively high dose of radiation whereas human patients receive radiotherapy as a series of fractionated or hyperfractionated doses. There were noticeable changes observed in the irradiated animals with respect to mucositis, body weight gain, and decrease in food intake, and these are shown in Figures 1, 2, and 3. The tested animals were given a low-dose RJ challenge to ensure the induction of a severe response. The rats who received irradiaton indicated the presence of oral mucositis in the head and neck.[1,2,6-8] Similar results were reported by Nagler et al.[27] who delineated systemic side effects in post-IR with 15 Gy per single dose in animal models. They reported that a severe cachectic and immunocompromised status occurred in the head and neck with irradiation and recommended that the animals needed nutritional and immunological support during the irradiation. Kohno et al.[17] reported that oral administration of RJ neither promoted cytotoxic ef-

Table 3. MDA, SOD and CAT activity/levels in all groups. Results are representing as Mean±SD.

Parameters	Control Mean±SD	IR Mean±SD	IR+RJ50 Mean±SD	IR+RJ100 Mean±SD	RJ50 Mean±SD	RJ100 Mean±SD	p (<0.001)
MDA (nmol/ml)	5.08±0.18[2346]	9.13±0.15[13456]	7.26±0.09[12456]	9.53±0.13[1235]	4.89±0.06[234]	4.85±0.07[1234]	***
CAT (U/mg-protein)	0.92±0.04[2346]	0.68±0.02[13456]	0.76±0.05[12456]	0.59±0.04[1235]	0.92±0.02[234]	0.93±0.01[1234]	***
SOD (U/mg-protein)	0.60±0.07[2346]	0.37±0.01[13456]	0.55±0.02[12456]	0.30±0.04[1235]	0.64±0.03[234]	0.67±0.04[1234]	***

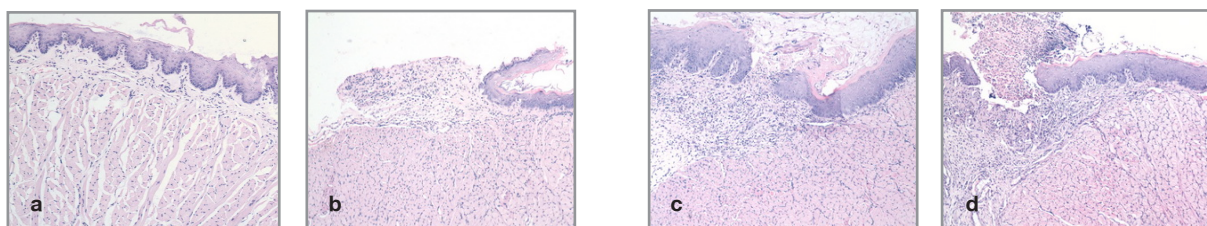The groups in the same column with different letters number are statistically significant (p< 0.05).

Figure 4. The histopathological images in all groups in the present study.
Figure 4a: Healthy mucosa with normal epithelium and no inflammatory infiltrate in the control, RJ50, and RJ100 groups.
Figure 4b: The IR group clearly reflected degeneration, congestion, and moderate inflammatory infiltrate in the submucosa.
Figure 4c: The IR+ RJ50 group showed mucosa repair and little inflammatory infiltrate and granulation tissue
Figure 4d: The IR+RJ100 group showed intense inflammatory infiltrate

fects on macrophage nor caused of weight loss. A similar increase in body weight was reported in rats that were fed RJ. Biswall et al.[9] were the first to use topical honey to successfully manage mucositis due to radiation. Rashad et al.[12] reported that prophylactic natural honey is effective in reducing mucositis resulting from radiochemotherapy in patients with head and neck cancer.

Our study demonstrated that RJ50 protects against hematological indices. The cells of leucocytes, lymphocytes, and platelets are highly radiosensitive to irradiation.[6] Irradiation produced severe leukopenia, and the severity of oral mucositis coincided with the suppressed immune state in rats in our study.[4,6] In vivo studies in experimental animals have included protection against radiation-induced hematopoietic or oral mucosa injury. To our knowledge, the anti-inflammatory and antioxidant actions of RJ have appeared in the literature. Several reports in the literature have demonstrated the immunomodulatory properties of RJ.[16,17,21] Cheng et al.[29]) reported that RJ is effective against the hematopoietic dysfunction observed in X-irradiated mice and that is also promotes macrophage activity and hematopoietic stem cell proliferation. According to Sver et al.[30] RJ stimulates antibody production and the proliferation of immunocompetent cells in mice and depresses humoral immune functions in rats. In addition, Fujiwara et al.[18] reported that the anti- inflammatory properties of RJ are expressed both by the inhibitory effects produced by this substance on capillary permeability during the acute phase of inflammation and by the reduced granulation tissue on the inflammatory chronic phase in streptozotocin-diabetic rats.

In the present study, in the group which received IR, the increase determined in BUN and LDH is related

to increased protein catabolism or necrosis. The reduction of food intake may also indicate protein catabolism, thereby contributing to the observed kidney injury and causing impaired glomerular filtration. In addition, changes in serum creatinine and electrolyte levels have been observed primarily in cases of functional disorders of the kidney tissue and tubule damage.[3,19] The decrease observed in blood glucose levels in the IR group may be related to reduction in food intake. Due to the scarcity research on the investigation of the effects of irradiation on other biochemical parameters, it was not possible to include information about this in our study. However, we can say that the cotreatment of IR and RJ50 resulted in a significant improvement in all the tested parameters regarding the normal values of the control.

In our study, parallel with the literature, the decrease in the MDA level in group of rats that received a lower dosage of RJ indicates that RJ prevents mucosa injury by decreasing lipid peroxidation. Sver et al.[30] demonstrated that the reduced antioxidant capability of blood plasma after radiaton therapy was significantly related to the severity of the mucositis. According to the authors, an improvement in the antioxidant status may counteract the damage caused by the reactive oxygen species formed as a result of chemo/radiotherapy. França et al.[10] cited the decreased antioxidant activities of SOD and CAT in mucositis severity. In the present study, we also observed a decline in these enzymes. This may have been due to an increase in circulating lipid peroxidation. The activities of SOD and CAT were increased considerably after treatment with RJ50. These results show that RJ has proven oxygen radical scavenging effects along with antioxidant properties. To our knowledge, the anti-inflammatory and antioxidant actions of RJ have ap-

peared in the literature to date.[16-21] In our study, the absence of any difference between the control group and the groups using only RJ50 and RJ100 alone groups demonstrates that RJ did not cause oxidative stress individually and did not involve a direct change in antioxidant enzyme activity. Its antioxidant effects mentioned above further confirm this result. Among relevant researches, Kobayashi et al.[31] reported that RJ has a cytotoxic and bactericidal effect in vitro; thus, they suggested that it may inhibit both prooxidant and antioxidant activities.

Regarding the therapeutic analysis, it is clear noticed that RJ50 was effective in protecting the tongue tissue from the irradiated head and neck region as indicated by the improvement of the histological picture in those tissues. In irradiation, the epithelial layer was destroyed, and there was an increase in inflammatory cells. In contrast, the IR+RJ50 group presented higher organized tissue. These findings are in agreement with other reports which observed differences in the healing process between non-irradiated and irradiated wounds. It was correlated with the increased intake of food and reduction of oral mucositis. We also concluded that the rats which received a hig dose of irradiated RJ did not have superior radioprotection. The reason for the side effects may be immunological and oxidative. Reactive oxygen species mobilized by IR cause the activation of leukocytes which secrete free radicals and enzymes that contribute to tissue damage. In addition, free radicals contribute to an increase in transepithelial permeability and enable bacteria and endotoxins to permeate the mucosal barrier. The increase of neutrophile accumulation and the formation of reactive oxygen species in the mucosa disrupt microcirculation and play a role in the formation of ulceration. Consequently, ulceration of the oral mucosa results in an increased risk of infection, particularly when there is immunosuppression.[8,13,14,28,32] Within the scope of our research, RJ administration decreased the mucosal damage and oxidant properties formed by irradiation. The rats treated with RJ50 plus RT treated was not as severely affected by inflammation and ulcers as those to which received only IR or IR+RJ100. It was shown that RJ decreases leukocyte infiltration. The decrease in the mucosal adhesion of neutrophils caused indirectly by RJ has also been demonstrated recently.[6,7,11] In the present study, we examined the protection of RJ50 against radiation-induced mucositis. We also demonstrated a dose re-

sponse, showing full protection at 50 mg/kg RJ but little or no protection at 100 mg/kg.

The results of the current study suggest the healing effect of 50 mg/kg RJ regarding severe oral mucositis induced by RT. Royal jelly high antioxidant activity in 50 mg/kg has been found to protect against reactive oxygen species, and its antioxidant activity may be attributed, in part, to the vitamins, polyphenolic compounds, or protein fractions. Royal jelly has the advantage of therapy, although it is generally protective when administered at pharmacological doses. However, further clinical studies are required to determine the potential value of its antioxidant effects and to identify the exact biological mechanism and antioxidant effects of RJ in the human body.

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Correspondence

Dr. Yasemin Benderli CİHAN

Kayseri Eğitim ve Araştırma Hastanesi

Radyasyon Onkolojisi Bölümü

38010, Kocasinan

KAYSERİ / TURKEY

Tel: (+90.352) 336 8884 / Ext 1800

Fax: (+ 90.352) 320 73 13

e-mail: cihany@erciyes.edu.tr