Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(4):1516-1522
DOI: 10.17826/cumj.1139113

ARAŞTIRMA / RESEARCH

Effects of different doses of royal jelly on oxidative stress and telomerase enzyme in rats with Cadmium toxicity

Kadmiyum toksisitesi oluşturulan ratlarda arı sütünün farklı dozlarının oksidatif stres ve telomeraz enzimi üzerine etkileri

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Cukurova Medical Journal 2022;47(4):1516-1522

Abstract

Purpose: Cadmium (Cd) is a toxic metal that seriously threatens human health due to environmental pollution, is widely used in industry and agriculture, and causes oxidative stress and tissue damage. This study aims to examine the effect of royal jelly (RJ) on oxidative status and telomerase enzyme activity in tissue damage induced by Cd.

Materials and Methods: The experimental design was made with 6 rats in each group. A total of 6 groups were created: control group, Cd group, 250 mg/kg RJ group, Cd + 250 mg/kg RJ group, 400 mg/kg RJ group, Cd + 400 mg/kg RJ group. In the study, total oxidant status and total antioxidant status in blood serum were investigated by colorimetric method, and telomerase enzyme activity in ovarian tissue was investigated by ELISA method.

Results: Cd caused an increase in oxidative capacity (23.80 ± 2.4) and a significant decrease was determined after RJ applications compared to the control group. After RJ application, the best total antioxidant response was observed in the 250 mg/kg RJ and Cd + 250 mg/kg RJ groups. Cd significantly reduced telomerase enzyme activity (0.90 ± 0.13). RJ administered for treatment after Cd application increased telomerase levels up to the control level (1.40 ± 0.05). The best treatment response was observed in the Cd + 250 mg/kg RJ group (1.42 ± 0.05).

Conclusion: Cd causes oxidative stress and that RJ may have curative effects by increasing the antioxidant capacity and telomerase enzyme activity RJ is a promising natural product and can contribute to recovery.

Keywords:. Cadmium, royal jelly, telomerase, ovary, antioxidant

Öz

Amaç: Kadmiyum (Cd), çevre kirlenmesi nedeni ile insan sağlığını ciddi oranda tehdit eden endüstride ve tarımda yaygın şekilde kullanılan oksidatif strese ve doku hasarlarına sebep olan toksik bir metaldir. Bu çalışma Cd ile oluşturulmuş doku hasarında arı sütünün (RJ) oksidatif statü ve telomeraz enzim aktivitesine etkisini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Deneysel tasarım her grupta 6 rat olacak şekilde; Kontrol grubu, Cd grubu, 250 mg/kg RJ, Cd + 250 mg/kg RJ, 400 mg/kg RJ, Cd + 400 mg/kg RJ grubu olacak şekilde 6 guruba ayrılarak oluşturuldu. Çalışmada kan serumundan total oksidan düzeyi, total antioksidan düzeyi kolorimetrik yöntemle ve ovaryum dokusundan telomeraz enzim aktivitesi ELISA yöntemiyle araştırıldı.

Bulgular: Cd oksidatif kapasitede artışa sebep oldu (23.80 ± 2.4) ve RJ uygulamaları ardından kontrol grubuna göre anlamlı azalma izlendi. RJ uygulaması ardından en iyi total antioksidan yanıtı 250 mg/kg RJ ve Cd + 250 mg/kg RJ gruplarında görüldü. Cd telomeraz enzim aktivitesini önemli düzeyde düşürmüştür. (0.90 ± 0.13) Cd uygulaması ardından tedavi amaçlı verilen RJ nin telomeraz düzeylerini yeniden kontrol seviyesine (1.40 ± 0.05) yükselttiği görüldü. Tedaviye en iyi cevap Cd + 250 mg/kg RJ grubunda izlendi (1.42 ± 0.05).

Sonuç: Cd un oksitatif strese sebep olduğu ve RJ uygulamaları ardından antioksidan kapasitede ve telomeraz enzim aktivitesinde görülen artışla RJ nin iyileştirici etkilerinin olabileceği sonucuna ulaşıldı.

Anahtar kelimeler: Kadmium, arı sütü, telomeraz, ovaryum, antioksidan

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Geliş tarihi/Received: 01.07.2022 Kabul tarihi/Accepted: 28.09.2022

INTRODUCTION

Today, the rate of exposure to toxic metals is increasing day by day, and as a result, dysfunctions are seen in different tissues of humans and animals[1]. Cadmium (Cd) has a high rate of spreading in nature and is not one of the basic elements that are necessary for human life. Cd can easily affect humans and other living things by polluting the soil, air, and water due to industrial wastes, pesticides, phosphate fertilizers used in agriculture, mines, refineries, and cigarette smoke[2].

Previous studies have discussed the negative effects of Cd on different tissues, including the ovary. It was stated that Cd causes damage to the reproductive system and deteriorates the endocrine system[3,4]. Long-term exposure to Cd increases lipid peroxidation, causing oxidative stress. The increase of reactive oxygen species in the cell causes damage to the tissues and dysfunctions in the organs where this damage occurs[5]. Cd was associated with the damage seen in the liver, kidney, testes, and ovaries and with changes in superoxide dismutase activity and enzymatic and non-enzymatic antioxidant defense systems including enzymes such as glutathione peroxidase, glutathione-S-transferase, and catalase[4-6]. Cd does not directly produce free oxygen radicals, but indirectly leads to the formation of free radicals by affecting the mitochondrial electron transfer chain or increasing glutathione consumption. This leads to the disruption of endocrine secretions in related tissues due to apoptosis and tissue damage[4,5,7].

Telomeres are the DNA sequences containing guanine-rich base repeats and special proteins at the end of linear chromosomes in eukaryotic cells and protect the end of chromosomes from degradation. The change in telomere length is controlled by telomere proteins and the telomerase enzyme complex[8]. Telomerase activity can be observed mostly in cancer tissues and in fetal and adult testes, fetal ovary, and proliferating cells of repaired tissues. Moreover, telomerase enzyme activity was also reported in hematopoietic stem cells, lymphocytes, hair follicles, and intestinal crypt cells[9,10]. Apart from these, it is very difficult to monitor the telomerase enzyme in tissues. However, it is thought to be an important diagnostic indicator of cancer due to its very prominent activity in cancer tissues[11]. Telomerase is a large reverse transcriptase enzyme complex in ribonucleoprotein structure and is synthesized from a strand of telomeric DNA. It has two subunits that are crucial for its activity, telomerase RNA and telomerase reverse transcriptase. It plays a role in completing the end of the linear chromosomal DNA molecule during replication. It protects the ends of chromosomes against abnormal conditions such as recombination, degradation, and fusion and ensures the integrity and stability of chromosomes[8,9,12,13].

Royal jelly (RJ) is a beige-colored, viscous, acidic, and sour-tasted substance that is secreted from the mandibular and hypopharyngeal glands of young worker bees of *Apis mellifera* species. Although all bee larvae are fed with RJ for the first three days after hatching, the queen bees continue to be fed with royal jelly throughout their development[14,15]. This nutrient allows the queen bee to synthesize more juvenile hormones. The rich biological composition of RJ leads to changes in gene expression, allowing a complete ovarian development in the queen bee. The queen bee can live up to five years thanks to RJ. However, worker bees have a shorter lifespan and are not reproductive. Royal jelly contains a complex composition of water, proteins, carbohydrates, fatty acids and lipids, minerals, and small amounts of vitamins, free amino acids, and essential compounds. The most characteristic compound of RJ is a unique active ingredient, trans-10-hydroxy-2-decenoic acid (10-HDA), and major royal jelly proteins (MRJPs). MRJP1, a weak acidic glycoprotein, is the most abundant protein in RJ and has monomeric or oligomeric forms[16-18]. In recent years, royal jelly has been preferred in studies conducted in the field of health sciences since it is a natural bee product. Its use has increased especially in burns, gastrointestinal diseases, ulcers, and cancer treatments[19,20].

Recently, the interest in natural products has increased as they can be both nutritional supplements and can support the treatment of diseases. There has been a great interest in bee products in recent years and many studies are carried out about them. Royal jelly is the product with the most interesting biological content. Although it is stated that RJ regulates mechanisms such as longevity and fertility, there is not enough information about this product and the telomerase enzyme, which is also a concept related to life expectancy. This study aimed to investigate the effects of RJ on antioxidant protection and telomerase activity against the harmful effects of Cd.

MATERIALS AND METHODS

Animals

The animal experiments of this study were carried out with the approval numbered 2020/10-01 taken from Çanakkale Onsekiz Mart University Local Ethics Committee.

G*Power-3.1 program was used in the experimental design. According to the principle of Cohen[21], the sample size was determined as 36 with an effect size of 0.70, an α error of 0.05, and a power of (1–β) 80%. The groups were formed to include 6 rats in each group.

All laboratory animals were kept at a temperature of 22±2 °C, a humidity of 50±5%, and in a 12/12 light-dark cycle in the laboratories of Çanakkale Onsekiz Mart University. Each cage contained 3 experimental animals. A total of 6 experimental groups were created. All rats were fed ad libitum with standard pellet feed and tap water placed in easily accessible compartments on the cage.

In this study, 36 Wistar albino female rats weighing 200-250 g were kept on a standard laboratory diet under optimum conditions. Preliminary trials were performed for 1 week. The study lasted 4 weeks, excluding preliminary trials. Two doses (250 mg/kg, 400 mg/kg) of RJ were administered to the rats 5 times a week by the gavage method. Cd was administered at the first and third weeks (1 ml/kg) by intraperitoneal injection.

Experimental design

36 rats were randomly divided into 6 groups and the names of the groups were determined as follows: control group, 250 mg/kg Rj group, 400 mg/kg RJ group, Cd group, Cd + 250 mg/kg +RJ group Cd + 400 mg/kg +RJ group.

Collection of blood and tissue samples

After the administration, the food of the rats was withdrawn the night before. The rats were anesthetized with 50mg/kg ketamine and 10mg/kg xylazine injection. Blood samples were collected from the heart and then tissue collection procedures were performed. Blood samples were taken into tubes and rested for 1 hour. The tubes were then centrifuged at 4000 rpm for 10 minutes. After centrifuge, blood serum was taken, and the samples were stored at -80 °C. At the end of the experiment, an abdominal skin incision was made with a scalpel in the midline of the abdomen in the dorso-ventral position under anesthesia. The peritoneum was passed through, and the ovaries were removed. Then, the fat layer around the ovaries was cleaned in a way that would not damage the ovarian tissue. Collected ovarian tissues were homogenized in 1.5 mL of phosphate buffer (PBS, pH: 7.4). Homogenates were centrifuged at 4000 rpm for 10 minutes.

Analysis of samples

Serum TOS levels were determined using a colorimetric kit (Rel Assay Diagnostics, Cat No: RL0024, Gaziantep, Turkey) and the results are given in μmol H_2O_2 equivalent/L. Serum TAS levels were determined using a colorimetric kit (Rel Assay Diagnostics, Cat No: RL0017, Gaziantep, Turkey) and the results are given in mmol Trolox equivalents/L. Oxidative stress indices are given as a proportional expression. OSI was calculated according to the following formula: OSI= [μmol/ H_2O_2 equivalent/L) / mmol Trolox equivalents/L) x 100]. Telomerase enzyme was determined with homogenates obtained from ovarian tissues using rat Telomerase Sandwich-ELISA principle (Elabscience Biotech Co. Ltd, USA, Cat No: E-EL-R0947) commercial kit.

Statistical analysis

The Microsoft SPSS 23.0 software package was used for statistical analysis of the data. The homogeneity of the data was examined using the Levene test. Any difference(s) between the groups were determined using the one-way analysis of variance (ANOVA). For a significant p-value ($p<0.05$), Tukey's multiple range test was used to determine the origin of the difference. Data are presented as mean and standard deviation (M ± SD).

RESULTS

There was a statistically significant difference in Cd, Cd+250 mg/kg RJ, and Cd+ 400 mg/kg RJ groups compared to the control group in terms of TOS levels after Cd application ($p<0.05$). Although this difference numerically approached the control group in the 250 mg/kg RJ group, no statistically significant improvement was observed. There was no statistical difference in serum TOS levels between the control, 250 mg/kg RJ and 400 mg/kg RJ groups ($p>0.05$).

There was a statistical difference between the control group and the Cd, Cd+250 mg/kg RJ, Cd+400 mg/kg RJ groups in terms of serum TAS levels after Cd applications and RJ applications ($p<0.05$). There was no statistical difference between the control, 250 mg/kg RJ and 400 mg/kg RJ groups ($p>0.05$). While there was no statistical difference ($p<0.05$) between the control and 250 mg/kg RJ group in terms of OSI, there was a statistical difference between the 400 mg/kg RJ, Cd, Cd+250 mg/kg RJ, Cd+400 mg/kg RJ groups and the control group (Table 1).

Table 1. Blood serum TAS, TOS and OSI Levels.

Groups	TAS (mmol Trolox equivalents/L)	TOS (μmol/ H_2O_2 equivalent/L)	OSI (μmol/ H_2O_2 equivalent/L) / mmol Trolox equivalents/L) x 100
1. Control	2.13 ± 0.14	16.75 ± 2.52	0.79 ± 0.13
2. 250 mg/kg RJ	2.17 ± 0.02	18.71 ± 1.59	0.86 ± 0.07
3. 400 mg/kg RJ	1.95 ± 0.13	20.59 ± 3.44	1.07 ± 0.23*
4. Cd	1.61 ± 0.11*	23.80 ± 2.4*	1.52 ± 0.16*
5. Cd + 250 mg/kg RJ	1.74 ± 0.10*	23.33 ± 3.65*	1.33 ± 0.15*
6. Cd + 400 mg/kg RJ	1.73 ± 0.13*	23.05 ± 3.29*	1.19 ± 0.14*

"*" indicates a statistical difference compared to the control group ($p<0.05$, M ± SD).

According to ovarian telomerase analysis, there was a statistically significant difference in the Cd group ($p<0.05$), but there was no significant difference between the other groups. It was observed that RJ had a significant effect ($p<0.05$) on the telomerase enzyme in the Cd applied groups by bringing it closer to the control level. The highest increase in the Cd +250 mg/kg RJ group was found to be close to the control group (Table 2).

Table 2. Ovarian telomerase levels

Groups	Telomerase (ng/ml)
1. Control	1.40 ± 0.05
2. 250 mg/kg RJ	1.42 ± 0.11
3. 400 mg/kg RJ	1.29 ± 0.09
4. Cd	0.90 ± 0.13*
5. Cd + 250 mg/kg RJ	1.42 ± 0.05
6. Cd + 400 mg/kg RJ	1.34 ± 0.07

"*" indicates a statistical difference compared to the control group ($p<0.05$, M ± SD).

DISCUSSION

Oxidative stress directly damages the ovaries and can indirectly lead to ovarian aging by causing telomere shortening,mitochondrial dysfunction, inflammation, and apoptosis[22]. The ovary functions like a biological clock and controls the aging process[23]. Telomeres are repetitive sequences found at the end of eukaryotic chromosomes and are associated with aging since they shorten at each cell division[8]. Previous studies showed that telomere length plays an essential role in the mitotic index of ovarian cells[22,24]. Telomerase is an enzyme that catalyzes telomere lengths but its expression cannot always be monitored. Telomerase enzyme is usually found in cancer tissues. However, telomerase enzyme activity can be observed in tissues such as the ovary and testis, where cellular activities such as spermatogenesis, oogenesis, and folliculogenesis are intense[9,10].

According to the study results, it was observed that Cd reduced telomerase enzyme activity, and this result was consistent with the oxidant status. Previous studies reported that oxidative stress accelerates cellular aging by referring to the relationship between telomerase enzyme and oxidative stress[24]. In this case, Cd administration caused oxidative stress in ovarian tissues. Today, many natural products are investigated in order to improve the effects of oxidative stress. Among these, one of the most popular is RJ, thanks to its rich biological content[25,26]. Research on RJ indicates that it has potential estrogenic activity. RJ competes with 17β-estradiol for binding estrogen receptors α and β[12]. RJ also has antioxidant and radical scavenging activities thanks to

its rich content[14,15,26,27]. In rats, inhibition of telomerase is prevented by estrogen administration[28]. In a study conducted on mice, it was stated that RJ activates estrogen receptors in reporter gene expression experiments and that this increases the transcription of a reporter gene via an estrogen-sensitive element[29]. It was stated that treatment with royal jelly protects against the harmful effects of cisplatin. This protection was attributed to the antioxidant and radical scavenging activity of royal jelly[15]. Moreover, it was reported that dietary RJ increases the mean lifespan of C3H/HeJ mice, possibly through reduced oxidative damage[16].

In a healthy physiological process, ovarian function is expected to decrease over time. However, various reasons such as heavy metal pollution, smoking, stress, chemotherapy, and industrial pollution can cause pathological ovarian aging, which causes early reduction of ovarian function[24,30]. Although previous studies reported that free radicals and reactive oxygen species (ROS) cause various adverse conditions in tissues and organs, ROS induces apoptosis in granulosa cells, leads to the rupture of the follicular wall and ovulation, and is involved in the regulation of oocyte growth, meiosis, ovulation, and other physiological processes in the ovary[24]. For this reason, a certain level of ROS must be maintained in the ovaries. Cells have a natural antioxidant defense mechanism in order to maintain this balance. Studies conducted in recent years have been investigating the functioning of this mechanism in the body or with external supplements. Studies showed that estrogen is involved in the antioxidant defense mechanism as well[20]. Increased ROS and increased secretion of estradiol during the growth of follicles trigger the expression of antioxidant enzymes, resulting in a dynamic balance between ROS and antioxidants[31]. Cd disrupts the integrity of the cell membrane, causing cellular damage, accumulation of reactive oxygen species, tissue damage, and endocrine disorders[6,32]. It was reported that Cd leads to hormonal disorders and a decrease in estrogen levels[3,7]. Cd toxicity causes tissue damage and negatively affects the dynamics of the ROS-antioxidant balance that should be maintained in the ovary[24].

In addition, toxicity caused by cadmium or other harmful agents in the ovaries results in decreased follicular growth[6,32]. According to these data, telomerase enzyme activity may have decreased due to intense damage to the tissues in Cd-administered groups. However, after RJ applications, telomerase activity increased again similar to the level of the control group.

According to the results of this study, it was observed that Cd reduced telomerase enzyme activity and that this result was consistent with the oxidant status. However, this consistency was not observed at the same rate between antioxidant capacity and telomerase. There was a numerical increase in antioxidant status but telomerase significantly approached the control level. The reason for this may be the intense activity of telomerase enzyme in ovarian cells[28,30]. Previous studies reported that ovarian tissues show extremely high telomerase activity compared to other tissues. This information was also supported in this study.

This study has some limitations. The study contributes to the literature regarding the effect of RJ on telomerase activity and antioxidant activity; however, these data should be supported with further immunohistochemical and RT-PCR studies in order to provide more comprehensive results. For this reason, it is aimed to conduct future studies by including these methods.

In conclusion, Cd causes an increase in oxidative stress and a decrease in total antioxidant capacity. Although RJ quantitatively increased the antioxidant level, the selected doses of RJ did not show a statistically significant change. Therefore, different doses of RJ need to be evaluated. The decreased telomerase enzyme activity after Cd toxicity approached the control group level with RJ, indicating cellular activity and healing in these tissues. Therefore, the selected doses proposed important results for telomerase activity. The fact that the RJ doses selected in the study support the cellular potential in the ovary indicates the importance of this product in terms of the biological clock. It was also shown that this product can be used therapeutically due to its antioxidant properties. More studies are required to get the most out of this natural nutrient.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SÇ; Veri toplama: SÇ; Veri analizi ve yorumlama: SÇ; Yazı taslağı: SÇ; İçeriğin eleştirel incelenmesi: SÇ; Son onay ve sorumluluk: SÇ; Teknik ve malzeme desteği: SÇ; Süpervizyon: SÇ; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Çanakkale Onsekiz Mart Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan 27.11.2020 tarih ve 2020/10-01 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.
Author Contributions: Concept/Design : SÇ; Data acquisition: SÇ; Data analysis and interpretation: SÇ; Drafting manuscript: SÇ;; Critical revision of manuscript: SÇ; Final approval and accountability: SÇ;;

Technical or material support: SÇ; Supervision: SÇ; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained for this study from the Local Ethics Committee of Çanakkale Onsekiz March University Animal Experiments with the decision dated 27.11.2020 and numbered 2020/10-01.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

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