Cilt 12, Sayı 2, Sayfa 1-7, 2023 Volume 12, Issue 2, Page 1-7, 2023
Araştırma Makalesi https://doi.org/10.46810/tdfd.1205005 Research Article
BİNGÖL ÜNİVERSİTESİ
Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

The Role of Royal Jelly on Exhaustive Exercise-Induced Oxidative Stress

Murat BAKIR[1*], Tülay ÖZHAN BAKIR[2]

[1] Bingöl University, The School of Physical Education and Sports Faculty, Sports Management Department, Bingöl, Türkiye
[2] Bingöl University, The School of Physical Education and Sports Faculty, Coaching Education Department, Bingöl, Türkiye
Murat BAKIR ORCID No: 0000-0003-0149-7162
Tülay ÖZHAN BAKIR ORCID No: 0000-0003-3526-0446

**Corresponding author: muratbakir6@gmail.com*

(Received: 15.11.2022, Accepted: 07.04.2023, Online Publication: 22.06.2023)

Keywords
Royal Jelly, Liver, Kidney, Skeletal muscle, Exhaustive exercise, Antioxidant

Abstract: In this study, the effects of Royal Jelly (RJ) on oxidative stress caused by exhaustive swimming exercise in rat tissues were evaluated. Methods: Twenty four male Wistar albino rats were indiscriminately distributed into four experimental groups: Sedentary control (SC); SC with the administration of RJ (100 mg kg-1) (SC + RJ); Exhaustive swimming exercise (E); Exhaustive swimming exercise with the administration of RJ (100 mg kg-1) (E + RJ). 100 mg kg-1 of RJ was dissolved in drinking water. Rats in the SC+RJ and E+RJ groups were supplemented with RJ (100 mg kg-1) orally once a day for two weeks. Rats in groups E and E+RJ were subjected to acute exhaustive swimming exercise on the 14th day of the study, then some biochemical parameters related to oxidative stress of all groups were measured. Results: The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (CRE) significantly increased in the exercised rats compared with the sedentary rats ($P < 0.05$). The decreased superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT) activities of muscular and hepatic tissues significantly increased and the high malondialdehyde (MDA) levels of muscular, hepatic, and kidney tissues significantly reduced in exercised rats treated with RJ ($P < 0.05$). Conclusion: In this study, protective effects of RJ against oxidative damage on tissues after exhaustive exercise were observed.

Arı Sütünün Tüketici Egzersize Bağlı Oksidatif Stres Üzerindeki Rolü

Anahtar Kelimeler
Arı sütü, Karaciğer, Böbrek, İskelet kası, Tüketici egzersiz, Antioksidan

Öz: Bu çalışmada, Arı Sütü (RJ)'nin sıçan dokularındaki tüketici yüzme egzersizinin neden olduğu oksidatif stres üzerindeki etkileri değerlendirildi. Metodlar: Yirmi dört adet erkek Wistar albino sıçan rastgele dört deney grubuna ayrıldı: Sedanter kontrol (SC); 100 mg kg-1 RJ uygulanmış SC (SC + RJ); tüketici yüzme egzersiz grubu (E); 100 mg kg-1 RJ uygulanmış tüketici yüzme egzersiz grubu (E + RJ). 100 mg kg-1 RJ içme suyunda çözüldü. SC + RJ ve E + RJ gruplarındaki sıçanlara, iki hafta boyunca günde bir kez ağızdan RJ (100 mg kg-1) verildi. E ve E + RJ gruplarındaki sıçanlar çalışmanın 14. gününde akut tüketici yüzme egzersizine tabi tutulduktan sonra tüm grupların oksidatif stres ile ilgili bazı biyokimyasal parametreleri ölçüldü. Bulgular: Alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), alkalin fosfataz (ALP), kan üre azotu (BUN) ve kreatinin (CRE) seviyeleri, egzersiz yapılan sıçanlarda, sedanter sıçanlara kıyasla önemli ölçüde artmıştır ($P < 0.05$). RJ ile beslenen sıçanlarda kas ve hepatik dokuların azalmış olan süperoksit dismutaz (SOD), glutatyon peroksidaz (GSHPx) ve katalaz (CAT) aktiviteleri önemli ölçüde artmış ve kas, hepatik ve böbrek dokularının yüksek malondialdehit (MDA) seviyeleri önemli ölçüde azalmıştır ($P < 0.05$). Sonuç: Sonuç olarak, bu çalışmada, tüketici egzersiz sonrası oluşan dokular üzerindeki oksidatif hasara karşı RJ'nin koruyucu etkileri gözlenmiştir.

1. INTRODUCTION

As it is known, the effects of regular physical exercise on health have been investigated for years. Studies have shown that regular physical exercise has preventive effects on cardiovascular diseases, obesity, type 2 diabetes, and cancer [1]. During exercise, the amount of oxygen needed by the body increases 10-20 times compared to resting conditions. This rate is even higher in actively working muscles (200 times). Molecular oxygen is reduced in the mitochondrial electron transport chain as a result of the large flow of oxygen to the mitochondria. As a result of this process, the amount of superoxide ($O_2^{\cdot}$) and the formation of hydroxyl radicals increase [2]. If the intensity of exercise is high, it causes oxidative stress and cell damage. It has been previously shown that acute exhaustive exercise increases the production of reactive oxygen species (ROS) that causes glutathione oxidation and lipid peroxidation. In addition, it increases the amount of oxidative stress markers [3, 4]. Aerobic organisms produce free radicals in certain amounts within their normal metabolic processes. Free radicals are produced in cells from oxygen or nitrogen sources. Free radicals generated from oxygen sources are called ROS, and produced from nitrogen sources are named reactive nitrogen types (RNS) [5]. Studies have shown that excessive and continuous production of ROS causes adverse effects on nucleic acids, proteins, and lipids, resulting in cellular death [6]. All aerobic organisms, including humans, have developed a system called antioxidant defense systems to keep free radical production within certain limits and to prevent the harmful effects of these molecules [4]. In cell metabolism, ROS production is kept in balance by antioxidant enzyme system [7]. Antioxidants, which have an important role in ending oxidative chain reactions by preventing the emergence of free radical intermediates, are called free radical scavengers [8]. These substances strengthen the immune mechanism and also try to minimize the risk of cancer and degenerative diseases by preventing and repairing the destruction caused by ROS and RNS [9]. These enzymatic antioxidants are SOD, CAT, GSHPx, and glutathione reductase (GR) [4].

Antioxidants can be produced from the body by endogenous sources and can be taken exogenously from food [9]. In some cases (such as radiation, exercise, smoking and alcohol use), the endogenous antioxidant defense system is inadequate in regulating the oxidation-reduction (redox) balance and so oxidative stress occurs. Oxidative stress can be defined as disruption of redox signaling and oxidation-reduction balance in favor of oxidants [10]. Oxidative stress is caused by an imbalance among the generation and accumulation of ROS in the organism and the ability of the antioxidant defense system to eliminate the harmful effects of these reactive products [11]. The imbalance between pro- and antioxidant species (oxidative stress) is responsible for the development of many diseases such as diabetes, cancer, atherosclerosis, obesity, and osteoporosis [12].

As mentioned above, antioxidants can be produced endogenously in the biological system or can be taken exogenously from natural compounds. However, the endogenous antioxidant defense mechanism may be insufficient during intense exercise, therefore, oxidative stress biomarkers can be eliminated with external antioxidant supplements [13].

RJ is made and secreted in the hypopharyngeal (mandibular) and mandibular glands of young worker bees. The queen bee fed with RJ has different physical and structural featuress than the working bees, and the longer life span has led to the idea that this product can also be beneficial for humans and it has become attractive to consume this product. RJ contains high amounts of sugars, lipids, proteins, vitamins, minerals and water. In addition phenolic compounds (PC) and flavonoids play an important role in the chemical structure of RJ [14]. RJ has been found to have biological activities such as antioxidant and antiinflammatory [15].

In a study, it was observed that Bingöl Royal Jelly (BRJ) contains some medium and short-chain fatty acids such as essential fatty acids linoleic acid and propionic acid, in addition to major flavonoids and phenolic compounds such as apigenin, quercetin, naringenin, gallic acid, caffeic acid, which increase antioxidant capacity in metabolism. In the same study, the antimicrobial activity of BRJ against pathogens was also demonstrated [16].

In an oxidative stress model by Asadi *et al*. [17] RJ has been shown to reduce oxidative stress by increasing antioxidant capacity in the experimental varicocele model in rats. They observed that SOD, CAT, and GPx values of RJ treated experimental varicocele group increased significantly ($P < 0.05$) and MDA showed a significant ($P < 0.05$) decrease compared to the experimental varicocele group. The antioxidant activity of RJ has been shown in a rat model by reducing liver and pancreas MDA levels and increasing both ferric-reducing antioxidant power (FRAP) and CAT levels in the pancreas and liver ($P < 0.05$) [18]. These experimental findings appear to support the antioxidative effects of RJ on oxidative stress induced tissue damage.

It was previously stated that acute exhaustive exercise increases ROS production, which induces lipid peroxidation, glutathione oxidation, and increases oxidative stress markers. These effects of acute exhaustive exercise allow it to be used as a model that generates oxidative stress [3]. Therefore, many studies have emphasized on the recognition of natural compounds that inhibit high production of ROS and reduce oxidative stress through ROS capture properties. For this reason, the present study aimed to reveal oxidative stress biomarkers by applying the exhaustive swimming exercise model and to demonstrate the protective effects of RJ through biochemical methods. Thus, it was aimed to test whether RJ would be a protective supplement product against oxidative damage occurring in tissues.

2. MATERIAL AND METHOD

2.1. Animal Models and Royal Jelly Administration

The study was approved by the Animal Ethics Committee of Bingöl University (approval date and no: 26/06/2018, BÜHADYEK-2018-02). The authors confirm that all the experiments were performed under approved guidelines and regulations. The study was carried out at Bingöl University's Experimental Researches Center on the rats supplied by the same Center. A total of twenty four male Wistar-Albino-type rats (9 weeks old, weight 230 ± 20 g) were used for all experiments. Rats were provided standard pelleted rat food and water than housed in a controlled room with a 12-h light-dark cycle at 22 ℃. Fresh Royal Jelly (RJ) was acquired from a commercial firm in Turkiye (Bingöl, Turkiye) and kept at -20 ℃ until use. 100 mg kg^{-1} of RJ was dissolved in drinking water and subjected orally for 14 consecutive days to RJ groups [19]. In addition, the last RJ was administered on the 14th day of study, 1 h prior to the induction of exhaustive exercise.

2.2 Experimental Desing and Exhaustive Exercise

The study registered 24 Wistar-Albino-type (9 weeks old, weight 230 ± 20 g) male rats which were divided into four groups in equal numbers: a sedentary control group fed on a standard diet (SC, n = 6), a sedentary control group fed on standard diet containing RJ (100 mg kg^{-1}) for 14 days (SC + RJ, n = 6), an exhaustive exercise group fed on standart diet and subjected to acute exhaustive swimming exercise on the 14th of the study (E, n = 6) and an exhaustive exercise group fed on standart diet containing RJ (100 mg kg^{-1} body $weight^{-1}$) for 14 days and subjected to acute exhaustive swimming exercise on the 14th of the study (E + RJ, n = 6). Exercised rats were exposed a single exhaustive swimming test in barrels (60 cm x 90 cm x 50 cm) at a temperature of 35 ℃ ± 0.5 ℃. The rats exposed to exhaustive exercise were primarily adapted to a swimming training 20 min day^{-1} for 3 days. The rats were kept in shallow water for 20 min on the first day. On the second day, the rats started to swim at the level of water that would exceed the height for 20 min. On the third day, they started swimming in deep enough water for 20 min. Then the rats were forced to swim with a weight (3% of their body weight) bonded to the tail till exhaustion [20]. Exhaustion was described by the following situation: more than 10 s drowning down the surface and loss of a righting reflex when put on a flat surface [21]. After 2 weeks all rats were anesthetized with intraperitoneal ketamine/xylazine (60 mg kg^{-1} and 6 mg kg^{-1}, respectively) and euthanized by cervical dislocation immediately after the exhaustive swimming exercise, and samples were collected for analysis.

2.3. Biochemical Analyses

Blood was taken from the heart and the serums were obtained by centrifugation (4500 rpm, 4 ℃, 10 min) and preserved at -80 ℃. The ALT (Sigma -MAK052A), AST (Sigma-MAK055A), and ALP (Sigma-MAK447A) enzyme activities, as a measure of hepatic tissue damage, were measured using the commercial kits by an automated biochemical analyzer (Olympus AU 2700) based on instructions provided from Sigma–Aldrich Chemicals Co., St. Louis, USA. The intensities of BUN (Cayman-700623) and CRE (Cayman-10005314) were analyzed in triplicates with a commercially available assay kit (Cayman Chemical, Michigan, USA) in accordance with the instructions. Liver, kidney and muscle tissues were removed and kept at −80 ℃ until use. Assay of SOD (Cayman-706005), CAT (Cayman-707011), GSHPx (Cayman-703114) activities and MDA (Cayman-10009202) level were analyzed in triplicates with a commercially available assay kit (Cayman chemical, Michigan, USA) by the instructions.

2.4. Statistical Analysis

SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical calculations. Results were expressed as means ± SD. One-way ANOVA test was used to compare multiple groups. Distinctions were considered significant when $P < 0.05$.

3. RESULTS

Plasma levels of AST, ALT, ALP, BUN, and CRE levels in group E were all significantly elevated by 87.6%, 116%, 33.8%, 68.7%, and 75% respectively, compared to group SC ($P < 0.05$) (Table 1). However, there were no differences between groups E and E + RJ in plasma levels of AST, ALT, ALP, BUN, and CRE. Rices in cytosolic enzymes such as AST, ALT, and ALP in the plasma are replies to exhaustive exercise and are frequently used as signs of hepatic injury. Furthermore, alters in the levels of CRE and BUN have previously been used as signs of renal damage.

Table 1. Effects of RJ supplementation on plasma parameters of rats after exhaustive exercise.

Parameter	Units	SC	SC + RJ	E	E + RJ
AST	(U L^{-1})	81±6^{a}	107±12^{a}	152±13^{b}	134±9^{a,b}
ALT	(U L^{-1})	25±3^{a}	27 ± 5^{a}	54±10^{b}	52±7^{b}
ALP	(U L^{-1})	177±21^{a}	181±19^{a}	237±28^{b}	203±16^{a,b}
BUN	(mg dL^{-1})	16±1^{a}	17±1^{a}	27±2^{b}	25±2^{b}
CRE	(mg dL^{-1})	0.44±0.01^{a}	0.46±0.02^{a}	0.77±0.06^{b}	0.66±0.03^{b}

Data are the means ± SEM (n = 6). Values in the same row with different superscript letters are significantly different at $P < 0.05$. SC, sedentary control; RJ, royal jelly; E, exhaustive swimming exercise; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRE, creatinine.

Table 2 shows the antioxidant enzyme activities. In comparison with the control group, the antioxidant enzyme activities of SOD, CAT, and GSHPx in the liver and skeletal muscles and the SOD, and GSHPx activity

in the kidney of the E group were all significantly lower ($P < 0.05$). Compared to the exercise group, these antioxidant enzymes activities were all significantly higher in E + RJ group in the liver and skeletal muscles ($P < 0.05$). But, there were no distinctions between the exercise group and E + RJ group in antioxidant enzyme activities in the kidney.

Table 2. Effects of RJ supplementation on antioxidant enzymes, SOD, CAT and GSHPx activities in tissues of rats after exhaustive exercise.

Measurements	Units	SC	SC + RJ	E	E + RJ
Skeletal muscle					
SOD	(U mg^{-1} protein)	2.08 ± 0.15^{a}	2.03 ± 0.19^{a}	0.56 ± 0.13^{b}	2.20 ± 0.18^{a}
CAT	(U mg^{-1} protein)	6.01 ± 1.24^{a}	6.22 ± 1.16^{a}	1.87 ± 0.12^{b}	6.10 ± 1.23^{a}
GSHPx	(U mg^{-1} protein)	72.33 ± 5^{a}	68.62 ± 4^{a}	21.36 ± 2^{b}	53.19 ± 6^{a}
Liver					
SOD	(U mg^{-1} protein)	2.80 ± 0.46^{a}	2.85 ± 0.66^{a}	1.05 ± 0.31^{b}	2.77 ± 0.62^{a}
CAT	(U mg^{-1} protein)	17.72 ± 1.6^{a}	18.64 ± 1.9^{a}	9.15 ± 0.6^{b}	18.71 ± 1.4^{a}
GSHPx	(U mg^{-1} protein)	75.46 ± 7^{a}	77.52 ± 4^{a}	38.63 ± 19^{b}	72.44 ± 31^{a}
Kidney					
SOD	(U mg^{-1} protein)	1.43 ± 0.11^{a}	1.36 ± 0.25^{a}	0.40 ± 0.13^{b}	$0.93 \pm 0.16^{a,b}$
CAT	(U mg^{-1} protein)	13.69 ± 2.8^{a}	14.11 ± 2.7^{a}	12.46 ± 1.9^{a}	12.73 ± 1.5^{a}
GSHPx	(U mg^{-1} protein)	21.64 ± 3.1^{a}	22.13 ± 2.7^{a}	13.49 ± 2.4^{b}	$16.87 \pm 1.9^{a,b}$

Data are the means ± SEM (n = 6). Values in the same row with different superscript letters are significantly different at $P < 0.05$. SC, sedentary control; RJ, royal jelly; E, exhaustive swimming exercise.

MDA was measured in the liver, kidney, and skeletal muscles (Figure 1). MDA levels of the liver, skeletal muscles, and kidneys were all significantly higher in group E compared to group SC ($P < 0.05$). However compared to group E, the MDA levels were significantly lower in group E + RJ ($P < 0.05$).

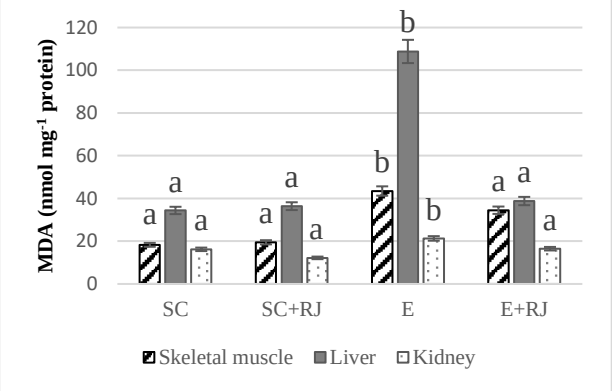

Figure1. The effects of RJ on skeletal muscle, liver, and kidney MDA levels. The values are the means ± S.D. for groups of six rats, different letters are significantly different at $P < 0.05$. SC, sedentary control; RJ, royal jelly; E, exhaustive swimming

4. DISCUSSION AND CONCLUSION

Exercise is a process that causes many physiological and biochemical changes that can alter the redox state. During exercise, oxygen consumption in the whole body and especially in skeletal muscle increases significantly. This process results in a drastic increase in ROS production. Whether exercise-induced ROS production is detrimental or beneficial probably depends on the balance between the levels of ROS produced during exercise and the competence of cellular antioxidant systems to protect cells against an oxidant challenge. Some recent studies have concluded that regular exercise does not lead to chronic oxidative stress in active muscles [22-26].

Low and moderate levels of exercise-induced ROS production play an important role in the exercise-induced adaptation of skeletal muscle. In contrast, high levels of ROS production cause muscle damage and a decrease in physiological benefits associated with low and moderate ROS production [27]. This result is explained by the concept of exercise-induced hormesis. Hormesis is used to describe a biphasic dose-response curve in which a transient increase in low levels of a stressor produces a beneficial adaptive effect on cells, whereas a chronic high dose of a stressor causes damage to cells. Exercise-induced increases in ROS production in skeletal muscle have been found to play a necessary role in the adaptation of skeletal muscle to training. The bell-shaped hormesis curve predicts that exercise-induced increases in ROS production promote significant physiological benefits until an optimum level of ROS production is reached. However, if exercise leads to a true hormetic effect on the body, after this peak of physiological benefit is reached, any increase in exercise-induced ROS production will result in tissue damage and reduced exercise-induced adaptations [27]. Although short-term, low-intensity exercise does not appear to increase oxidative stress, it is well known that long-term, high-intensity endurance exercise increases oxidative stress. Prolonged and high-intensity endurance exercise causes increases in biomarkers of oxidative stress (eg, increased protein oxidation and lipid peroxidation) in both blood and active skeletal muscles in untrained humans and animals. The effect of ROS on muscle power production is biphasic and dependent on the level of ROS within the muscle fiber. Likewise, the main molecule in the ROS cascade is the superoxide radical dismutating to H_2O_2, and it seems likely that both $O_2^{\cdot -}$ and H_2O_2 affect muscle contraction function. At rest, superoxide radicals are produced at low rates in skeletal muscle fibers. During exercise, the rate of $O_2^{\cdot -}$ production in the muscle increases markedly; the total amount of $O_2^{\cdot -}$ production in the muscle fiber depends on both the intensity and duration of the exercise and the temperature of the contracted muscle. In general, relatively high-intensity, prolonged aerobic exercise (ie 65%-75% VO_2max) results in greater ROS production compared to low-intensity (ie <40% VO_2max) short-term exercise. Also, increased muscle temperature results in higher ROS levels during contractions [28].

Increased ROS causes oxidative stress and lipid peroxidation, which have detrimental effects on the organism [29].

The current study focuses on determining the inhibitory effects of RJ against exhaustive swimming-induced oxidative stress. In this study, the antioxidant activity of royal jelly, which has different types of antioxidants, was tested. Exhaustive exercise causes oxidative stress, which is defined as the disruption of redox signaling and control [10]. Pending the performance of exhaustive exercise, different substances are made by the metabolic process, such as ROS [30]. Oxidative stress caused by increased ROS causes oxidative damage to tissues [4, 27]. When tissues such as muscle, kidney, and liver are damaged, various enzymes are released from these tissues into the bloodstream. Indicators of cellular damage can be observed in the blood after exhaustive exercise [31].

The previous studies demonstrated that exhaustive exercise raises AST, ALT CRE, and BUN levels in plasma and causes important skeletal muscle, kidney and, liver damage [32]. In this study compared to group SC, the CRE, AST, ALT ALP and BUN levels of group E were significantly higher ($P < 0.05$). It is a remarkable protocol to try antioxidant food supplements to prevent the damage caused by exhausting exercise to the organism [32]. External antioxidant applications are frequently used to bring the increased enzyme levels to normal values. In the study of Ghanbari *et al*. [18], it has been reported that RJ treatment reduces liver injury by decreasing serum AST, ALT and, ALP levels. In another study, Kanbur, Eraslan [33] showed that RJ administration had protective effects against paracetamol-induced liver tissue damage. But Huang *et al*. [34] observed that the administration of L-arginine did not cause any changes in AST and ALT levels in rats exposed to exhaustive exercise. In the same vein, in the current study, rats in the RJ-supplemented group displayed no differences in AST, ALT, ALP, BUN, or CRE levels compared to group E (Table 1). No significant changes in AST, ALT, ALP, BUN, and CRE levels in RJ exercise groups show that RJ does not have beneficial effects on hepatocyte cells and kidney function.

Regarding lipid peroxidation, the traditional oxidative stress marker is MDA produced during fatty acid oxidation. This product is measured by the reaction of thiobarbituric acid, which generates thiobarbituric acid reactive substances (TBARS) in blood samples [27]. MDA levels were measured in the liver, kidney, and skeletal muscles (Figure 1). MDA is indicated as a substantial oxidative stress marker that can cause direct detriment to membrane structure, or indirect damage to other cellular ingredients via the production of reactive aldehydes [35]. In addition, MDA has been used as an indication of lipid peroxidation in exercise studies [36]. Previous studies have shown significant increases in lipid peroxidation products in the liver, kidney, and skeletal muscle as a result of exhaustive exercise ($P < 0.05$) [36, 37]. MDA levels in our study are consistent with the above-mentioned studies. In this study, the MDA levels in muscular, renal and hepatic tissues were significantly higher in group E compared to group SC ($P < 0.05$). But, RJ supplementation substantially preserved the liver, skeletal muscles, and kidneys from ROS-mediated oxidative damage after exhaustive exercise. Oxygen consumption increases during exhaustive exercise, which can lead to an increase in ROS [37]. High amounts of ROS are potentially detrimental to the body if they are not rapidly deactivated [30]. It was previously showed that antioxidant enzymes are important in protecting the body against ROS. Antioxidant enzymes such as SOD, CAT and GSHPx inhibit ROS accumulation in the body [4, 36]. SOD catalyses the reduction of the superoxide to H_2O_2 and H_2O; GSHPx reduces H_2O_2 to H_2O. Also, GSHPx can reduce lipid peroxides stright. CAT catalyzes the conversion of H_2O_2 to H_2O [38]. The substantial reduction in the activities of SOD, GSHPx, and CAT in the skeletal muscle and liver tissues may be a sign of oxidative stress [4]. In the oxidative stress model developed by Asadi *et al*., it has been shown that royal jelly reduces oxidative stress by increasing antioxidant capacity. They observed that SOD, CAT, and GPx values of the experimental varicocele group treated with RJ were significantly increased ($P < 0.05$) and MDA was significantly decreased ($P < 0.05$) compared to the experimental varicocele group [17]. Table 2 shows the antioxidant enzyme activities. In this study, a significant decrease in SOD, GSHPx, and CAT activities was observed after exhaustive exercise ($P < 0.05$). The findings of this study are in accordance with some previous observations in the literature [37, 38]. In our study, the data also demonstrated that the GSHPx, SOD, and CAT activity values in the liver and skeletal muscles of RJ + E group were significantly higher than group E ($P < 0.05$).

PCs in royal jelly are effective scavengers of free radicals and other non-radical ROS/RNS in vitro. The physiological ability of PCs to induce endogenous antioxidant gene expression, regulate ROS production by enzymes and redox-related transcription factors, causes PCs to be key reducers of OS and inflammation and protect lipids, proteins, and nucleic acid damage [39]. In this study, it is assumed that the functional hydroxyl group (OH) of PCs plays a key role in antioxidant defense. The results showed that RJ was able to restore antioxidant enzyme activities to control group levels, thereby protecting the tissues from oxidative stress caused by exhaustive swimming exercise.

In conclusion, the data demonstrated that RJ can increase SOD, GSHPx and CAT activities, but decrease the MDA levels. These effects might be due to its antioxidant feature. RJ treated as a good scavenger against free radical generation and thereby obstructs lipid per-oxidation. These findings showed that RJ had protective effect against the oxidative stress caused by exhaustive exercise. The study shows that RJ can be used as a protective product against damages caused by oxidative stress that may occur for various reasons. The present study will provide a basis for subsequent similar studies.

REFERENCES

[1] Moore SC, Lee I-M, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, et al. Association of leisure-time physical activity with risk of 26 types of cancer in 1.44 million adults. JAMA internal medicine. 2016;176(6):816-25.

[2] Morillas-Ruiz JM, Hernández-Sánchez P. Oxidative stress and antioxidant defenses induced by physical exercise. Basic principles and clinical significance of oxidative stress Rijeka: InTechOpen. 2015:221-41.

[3] Malaguti M, Angeloni C, Garatachea N, Baldini M, Leoncini E, Collado PS, et al. Sulforaphane treatment protects skeletal muscle against damage induced by exhaustive exercise in rats. Journal of Applied Physiology. 2009;107(4):1028-36.

[4] Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiological reviews. 2008;88(4):1243-76.

[5] Halliwell B, Gutteridge JM. Free radicals in biology and medicine: Oxford university press, USA; 2015.

[6] Liu Z, Ren Z, Zhang J, Chuang C-C, Kandaswamy E, Zhou T, et al. Role of ROS and nutritional antioxidants in human diseases. Frontiers in physiology. 2018;9:477.

[7] Estruel-Amades S, Massot-Cladera M, Garcia-Cerdà P, Pérez-Cano FJ, Franch À, Castell M, et al. Protective effect of hesperidin on the oxidative stress induced by an exhausting exercise in intensively trained rats. Nutrients. 2019;11(4):783.

[8] Gholamian-Dehkordi N, Luther T, Asadi-Samani M, Mahmoudian-Sani MR. An overview on natural antioxidants for oxidative stress reduction in cancers; a systematic review. Immunopathologia Persa. 2017;3(2):e12.

[9] Chatterjee M, Saluja R, Kanneganti S, Chinta S, Dikshit M. Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. Cellular and Molecular Biology. 2007;53(1):84-93.

[10] Sies H. Oxidative stress: a concept in redox biology and medicine. Redox biology. 2015;4:180-3.

[11] Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity. 2017;2017.

[12] Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. Oxidative medicine and cellular longevity. 2017;2017.

[13] Atashak S. The antioxidant role of artichoke (Cynara scolymus L.) extract against exhaustive exercise-induced oxidative stress in young athletes. Journal of Medicinal Plants. 2019;18(71):37-48.

[14] Bărnuțiu LI, Mărghitaș LA, Dezmirean DS, Mihai CM, Bobiș O. Chemical composition and antimicrobial activity of Royal Jelly-REVIEW. Scientific Papers Animal Science and Biotechnologies. 2011;44(2):67-72.

[15] Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, et al. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. Bioscience, biotechnology, and biochemistry. 2004;68(1):138-45.

[16] Bengü AŞ, Adnan A, Özbolat S, Abdullah T, Aykutoğlu G, ÇiftçİM, et al. Content and antimicrobial activities of bingol royal jelly. Türk Tarım ve Doğa Bilimleri Dergisi. 2020;7(2):480-6.

[17] Asadi N, Kheradmand A, Gholami M, Saidi SH, Mirhadi SA. Effect of royal jelly on testicular antioxidant enzymes activity, MDA level and spermatogenesis in rat experimental Varicocele model. Tissue and Cell. 2019;57:70-7.

[18] Ghanbari E, Nejati V, Khazaei M. Improvement in serum biochemical alterations and oxidative stress of liver and pancreas following use of royal jelly in streptozotocin-induced diabetic rats. Cell Journal (Yakhteh). 2016;18(3):362.

[19] Silici S, Ekmekcioglu O, Eraslan G, Demirtas A. Antioxidative effect of royal jelly in cisplatin-induced testes damage. Urology. 2009;74(3):545-51.

[20] Zhang H, Liu M, Zhang Y, Li X. Trimetazidine attenuates exhaustive exercise-induced myocardial injury in rats via regulation of the Nrf2/NF-κB signaling pathway. Frontiers in pharmacology. 2019;10:175.

[21] Thomas D, Marshall K. Effects of repeated exhaustive exercise on myocardial subcellular membrane structures. International journal of sports medicine. 1988;9(04):257-60.

[22] Ji LL, Kang C, Zhang Y. Exercise-induced hormesis and skeletal muscle health. Free Radical Biology and Medicine. 2016;98:113-22.

[23] de Sousa CV, Sales MM, Rosa TS, Lewis JE, de Andrade RV, Simões HG. The antioxidant effect of exercise: a systematic review and meta-analysis. Sports medicine. 2017;47:277-93.

[24] Radak Z, Ishihara K, Tekus E, Varga C, Posa A, Balogh L, et al. Exercise, oxidants, and antioxidants change the shape of the bell-shaped hormesis curve. Redox biology. 2017;12:285-90.

[25] Di Meo S, Napolitano G, Venditti P. Mediators of physical activity protection against ROS-linked skeletal muscle damage. International journal of molecular sciences. 2019;20(12):3024.

[26] Nocella C, Cammisotto V, Pigozzi F, Borrione P, Fossati C, D'Amico A, et al. Impairment between oxidant and antioxidant systems: short-and long-term implications for athletes' health. Nutrients. 2019;11(6):1353.

[27] Elejalde E, Villarán MC, Alonso RM. Grape polyphenols supplementation for exercise-induced oxidative stress. Journal of the International Society of Sports Nutrition. 2021;18(1):3.

[28] Powers SK, Deminice R, Ozdemir M, Yoshihara T, Bomkamp MP, Hyatt H. Exercise-induced oxidative stress: Friend or foe? Journal of sport and health science. 2020;9(5):415-25.

[29] Popovic LM, Mitic NR, Radic I, Miric D, Kisic B, Krdzic B, et al. The effect of exhaustive exercise on oxidative stress generation and antioxidant defense

in guinea pigs. Advances in Clinical and Experimental Medicine. 2012;21(3):313-20.
[30] Vale A, Ferreira H, Benetti E, Rebelo A, Figueiredo A, Barbosa E, et al. Antioxidant effect of the pequi oil (Caryocar brasiliense) on the hepatic tissue of rats trained by exhaustive swimming exercises. Brazilian Journal of Biology. 2018;79:257-62.
[31] Huang Q, Ma S, Tominaga T, Suzuki K, Liu C. An 8-week, low carbohydrate, high fat, ketogenic diet enhanced exhaustive exercise capacity in mice part 2: effect on fatigue recovery, post-exercise biomarkers and anti-oxidation capacity. Nutrients. 2018;10(10):1339.
[32] Huang K-C, Wu W-T, Yang F-L, Chiu Y-H, Peng T-C, Hsu B-G, et al. Effects of freshwater clam extract supplementation on time to exhaustion, muscle damage, pro/anti-inflammatory cytokines, and liver injury in rats after exhaustive exercise. Molecules. 2013;18(4):3825-38.
[33] Kanbur M, Eraslan G, Beyaz L, Silici S, Liman BC, Altınordulu Ş, et al. The effects of royal jelly on liver damage induced by paracetamol in mice. Experimental and Toxicologic Pathology. 2009;61(2):123-32.
[34] Huang C-C, Lin T-J, Lu Y-F, Chen C-C, Huang C-Y, Lin W-T. Protective effects of L-arginine supplementation against exhaustive exercise-induced oxidative stress in young rat tissues. Chin J Physiol. 2009;52(5):306-15.
[35] Aksoy L, Aslan Z. Nephroprotective and antioxidative effects of royal jelly on ethylene glycol induced nephropathy in rats. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2017;64(4):241-8.
[36] Yan F, Hao H. Effects of Laminaria japonica polysaccharides on exercise endurance and oxidative stress in forced swimming mouse model. Journal of Biological Research-Thessaloniki. 2016;23(1):1-7.
[37] Yang Q, Jin W, Lv X, Dai P, Ao Y, Wu M, et al. Effects of macamides on endurance capacity and anti-fatigue property in prolonged swimming mice. Pharmaceutical biology. 2016;54(5):827-34.
[38] Yan F, Wang B, Zhang Y. Polysaccharides from Cordyceps sinensis mycelium ameliorate exhaustive swimming exercise-induced oxidative stress. Pharmaceutical Biology. 2014;52(2):157-61.
[39] Kruk J, Aboul-Enein BH, Duchnik E, Marchlewicz M. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. The Journal of Physiological Sciences. 2022;72(1):1-24.