The inducing of caspase and bcl-2 pathway with royal jelly decreases the muscle tissue damage exposed with fluoride in rats

Abdullah Aslan (aaslan@fирat.edu.tr)  
Firat University: Firat Universitiesi  https://orcid.org/0000-0002-6243-4221

Muhammed Ismail Can  
Inonu University: Inonu Universitiesi

Ozlem Gok  
Firat University: Firat Universitiesi

Seda Beyaz  
Firat University: Firat Universitiesi

Gozde Parlak  
Firat University: Firat Universitiesi

Ibrahim Hanifi Ozercan  
Firat University: Firat Universitiesi

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Abstract

In this study, 42 Wistar albino female rats (n = 42, 8 weeks old) were used. Rats were divided into 6 groups and 7 rats included each group. Groups: (i) Control Group: Standard diet; (ii) RJ (royal jelly) Group: Standard diet + royal jelly; (iii) F50 Group: Standard diet + 50 mg/kg fluoride; (iv): F100 Group: Standard diet + 100 mg/kg fluoride; (v) F50 + RJ Group: Standard diet + 50 mg/kg fluoride + royal jelly; (iv): F100 + RJ Group: Standard diet + 100 mg/kg fluoride + royal jelly. After the 8-week study period, the rats were decapitated and their muscle tissues were removed. Expression levels of Caspase-3, Caspase-6, Bax, Tnf-α, IL1-α and Bcl-2 proteins in muscle tissue were determined by Western Blotting method. Histopathological analyzes were also performed on the muscle tissue. MDA, GSH, and CAT analyzes were determined by spectrophotometric analysis. According to our findings, Bcl-2, Tnf-α and IL1-α protein expression were increased in damage groups compared to control and royal jelly groups, Caspase-3, Caspase-6 and Bax protein expression levels decreased in damage groups. There was an increase in MDA level in damage groups compared to the control and royal jelly groups, CAT and GSH levels decreased in damage groups. According to histopathological analysis results, edema and inflammatory cell formations were found in the injury groups, a tendency to decrease in these injuries was observed in the treatment groups. Based on these results, we can say that royal jelly has protective effects against fluoride damage.

Introduction

In recent years, especially nutritional based measures have begun to be taken for reduce or eliminate the effects of free radicals that play a leading role in the origin of many diseases, including cancer. One of these foods is royal jelly. Royal jelly is secreted from the hypopharyngeal glands of worker bees. It is used to improve human health due to its remarkable antimicrobial and antioxidant properties. It is made up of various bioactive substances such as water (50–60%), proteins (18%), carbohydrates (15%), lipids (3–6%), vitamins, free amino acids and mineral salts (1,5%) (Aslan 2015; Ozsahin et al. 2019; Sharif and Darsareh 2019; Park et al. 2020; Aslan et al. 2020a; Demir et al. 2020). Royal jelly is known to have antitumor, antioxidative, antidiabetic, antiallergic, antibacterial, antiinflammatory and antihypertensive effects. It is also used as a semen diluent due to its sperm quality and fertility enhancing properties (Ghanbari et al. 2015). In a study conducted by creating a sporadic Alzheimer's disease model in 2020, it was stated that royal jelly has positive effects on memory and neuroprotective effects in rats (Silva et al. 2020).

Fluoride is one of the most common chemical pollutants used especially in coal power plants and industrial areas (Wang et al. 2020). Halogenated fluoride is highly reactive and shows high electronegative properties. Excessive fluoride consumption causes fluorosis (fluorine poisoning) in drinking water. Symptomatic fluorosis causes more damage to hard tissues such as teeth and skeletal structures. However, fluoride also damages other organs (heart, brain, liver, kidney) (Orta 2012; Wang et al. 2018; Xie et al. 2020). Fluoride toxicity creates widespread damage on various tissues through oxidative stress and apoptosis (Liu et al. 2019).
Bax, Bcl-2, Caspase-3 and Caspase-6 proteins play an important role in the mechanism of apoptosis. Caspases are localized in the cell cytosol. They are divided into two groups as initiator and effector caspases. They inhibit DNA polymerase enzyme activity and thus allow the cell to undergo apoptosis and disappear. Caspase-3 and Caspase-6 are effector caspases. Bcl-2 protein is localized in the cytosol. Some of the members of the Bcl-2 protein family are proapoptotic and some are antiapoptotic. They control the permeability of the mitochondrial pores. Bax protein is located on the mitochondria. It is a proapoptotic protein and has an important role in the occurrence of apoptosis. Tumor necrosis factor-α (TNF-α) protein is a cytokine that regulates immune functions. TNF-α is a 26 kDa transmembrane protein and the most important place where it is secreted is subcutaneous adipose tissue. TNF-α increases leptin expression in adipocytes. Interleukin 1 alpha (IL-1α) is a cytokine of the interleukin 1 family encoded by the IL1A gene in humans. Usually Interleukin 1 is responsible for producing inflammation as well as promoting fever and sepsis. IL-1α is produced by activated macrophages as well as neutrophils, epithelial cells and endothelial cells (Tilg and Moschen 2006; Feng et al. 2011; Aslan and Can 2014; Pekmezsi et al. 2018; Aslan et al. 2020b; Aslan et al. 2020c).

Materials And Methods

In vivo analyses

The rats were revealed to 12 h of a light period and 12 h of darkness per day. The rats were separated into four groups. The animals housed in standard laboratory conditions and cages and maintained at 20–25 C, 50–60% relative moisture. The rats were divided into 6 groups, our experimental groups were;

Group 1 → Control (C) group: The group fed with standard diet

Group 2 → Royal Jelly (RJ) group: Fed with standard diet + RJ (100 mg/kg bw, oral gavage)

Group 3 → F50 group: Fed with standard diet + fluoride (50 mg/kg bw, drinking water)

Group 4 → F100 group: Fed with standard diet + fluoride (100 mg/kg bw, drinking water)

Group 5 → F50 + RJ group: Fed with standard diet + fluoride (50 mg/kg bw, drinking water) and RJ (100 mg/kg bw, oral gavage)

Group 6 → F100 + RJ group: Fed with standard diet + fluoride (100 mg/kg bw, drinking water) and RJ (100 mg/kg bw, oral gavage)

The study continued for 16 weeks. Muscle tissues of rats were removed and stored at -80°C until the analysis was done.

Application of royal jelly
Royal jelly was given to rats by oral gavage (100 mg/kg bw) five times a week for eight weeks (Ghanbari et al. 2015).

**Application of fluoride**

Fluoride was given to rats five times a week by adding drinking water (50 mg/kg bw and 100 mg/kg bw) (Wang et al. 2018). The animal experiment phase of the study continued for eight weeks.

**Analysis of proteins via SDS-PAGE and western blotting method**

Protein samples belonging to tissues are run in 12% gel. Then, these proteins are transferred to nitrocellulose membrane by Western blotting method (Laemmli 1970) and Protein levels are measured with a density detecting analysis system (Image J, National Institute of Health, Bethesda, USA). Proteins are separated from each other in the gel by SDS-PAGE before blotting in the protein transfer stage. Proteins are transferred from the gel to the nitrocellulose membrane by blotting. After blotting, the membrane is washed in PBS Tween-20 solution for 15 minutes. It is kept in milk powder for one night. After blocking, it is kept at room temperature for 90 minutes. Then the primary antibody (1/1000) is added and left at room temperature for 90 minutes. Wash with PBS solution for 15 minutes. Then, by adding secondary antibody (1/5000), it is kept at room temperature for 90 minutes. Likewise, it is washed with PBS solution for 15 minutes. Then it is kept in Diaminobenzidine (DAB, Amresco) solution until the bands become apparent. Finally, the reaction is stopped by taking it into distilled water (Aslan et al. 2016a; Aslan et al. 2020b; Aslan 2018).

**GSH (Glutathione) activity measurement in muscle tissue**

GSH (Glutathione) concentrations in muscle tissue samples will be measured spectrophotometrically by means of glutathione reductase and NADPH (nicotinamide adenine dinucleotide phosphate) based on enzymatic reaction. This method is based on measuring the colored compound (2-nitro-5-thiobenzoic acid), which is formed as a result of the reaction of 5,5'-dithiobis-2 nitrobenzoic acid and GSH. By recording the absorbance changes at 412 nm in the UV (ultraviolet) spectrophotometer, using the molar absorption coefficient of 2-nitro-5-thiobenzoic acid, GSH concentrations in seminal plasma will be calculated and the results will be expressed in pmol/ml. In this method, a standard graphic will be created using bovine serum albumin solution. Protein concentrations of the samples will be calculated in mg/ml based on the standard chart (Aydemir et al. 2008).

**Malondialdehyde (MDA) activity measurement in muscle tissue**

Muscle tissue samples are divided into small sizes, 4.5 ml 1.15% KCl is added to 0.5 gram tissue sample and broken down in a mechanical homogenizer. From this homogenate, some changes were made based on the Ohkawa (1979) method to determine MDA, which is the final product of lipid peroxidation. This method is based on the reaction of the aldehyde products of lipid peroxidation, MDA and TBA.
(thiobarbutyric acid). In the measurement method, 8.1% SDS, 750 µl 20% (pH: 3.5) acetic acid is added to 0.1 ml tissue homogenate. Then 750 µl of 0.8% (pH: 3.5) TBA and distilled water is added until the final volume is 4 milliliter. It's left in a hot water bath (95°C) for 45 minutes. After cooling, add 1 ml of distilled water and 5 ml of n-butanol-pyridine at a level of 15:1 (v/v) and mix with a vortex device. After centrifugation at 5000 rpm for 10 minutes, organic layer in upper part is removed. It is measured in a spectrophotometer (532 nm) and values are saved in nmol/g (Aslan 2018).

**Catalase (CAT) activity measurement in muscle tissue**

To be used in catalase measurement, concentrated (35%) H₂O₂ is added to 1/15 M Na-K-phosphate buffer (Na₂HPO₄ - KH₂PO₄, pH: 7) solution until the absorbance at 240 nm in the spectrophotometer becomes 0.7–0.9. In order to determine the catalase content of the samples, 1ml of this mixture is taken and placed in the bathtub. Depending on the working range, starting from 30 µl, gradually increasing concentrations of supernatant are added and mixed once. In the spectrophotometer, the absorbance change of H₂O₂ is read at 240 nm wavelength for 30 seconds. The number of enzyme units per milliliter is calculated from this optical density difference (Luck 1963; Yigitcan 2012; Aslan et al. 2020a).

**Histopathological analysis**

At the end of the research, the muscle tissues of the rats were decapitated under anesthesia. Then, sections and blocks were prepared from the tissues, and the development and spread of the damage was examined histopathologically. Macroscopic samples were taken, fixed in 4% paraformaldehyde solution and examined under a light microscope and histopathological analyzes were performed.

**Statistical analysis**

All data were evaluated with analysis of variance in Prism 5.0 (GraphPad Inc., San Diego, CA, USA). One Way ANOVA test was applied to determine the differences within the groups.

**Results**

**GSH analysis results**

When Table 1 and Fig. 1 are examined, it is seen that the GSH levels of the F50 and F100 groups are lower than the F50 + royal jelly and F100 + royal jelly groups (p < 0.05).
Table 1
Muscle tissue GSH levels (µmol/mg protein)

| Groups               | Muscle tissue GSH levels |
|----------------------|--------------------------|
|                      |                          |
| Control              | 850.58 ± 1.00<sup>a</sup>|
| RJ                   | 855.29 ± 1.00<sup>a</sup>|
| 50 mg/kg F           | 500.58 ± 1.00<sup>d</sup>|
| 100 mg/kg F          | 400.94 ± 1.00<sup>e</sup>|
| RJ + 50 mg/kg F      | 638.82 ± 1.00<sup>b</sup>|
| RJ + 100 mg/kg F     | 560.94 ± 1.00<sup>c</sup>|

<sup>a–e</sup> Differences between groups with different letters are statistically significant (p < 0.05). One-way ANOVA Post Hoc LSD Test

MDA analysis results

When the results of MDA analysis are examined (Table 2 and Fig. 2), it is seen that the highest MDA levels are in the F100 and F50 groups, respectively. When the F50 + RJ and F100 + RJ groups are compared with the F50 and F100 groups, a statistically significant observed (p < 0.05).

Table 2
Muscle tissue MDA levels (nmol/g)

| Groups           | Muscle tissue MDA levels (nmol/g) |
|------------------|-----------------------------------|
| Control          | 1.02 ± 0.02<sup>e</sup>           |
| RJ               | 0.95 ± 0.02<sup>e</sup>           |
| 50 mg/kg F       | 1.77 ± 0.02<sup>b</sup>           |
| 100 mg/kg F      | 2.05 ± 0.02<sup>a</sup>           |
| RJ + 50 mg/kg F  | 1.55 ± 0.02<sup>d</sup>           |
| RJ + 100 mg/kg F | 1.72 ± 0.02<sup>c</sup>           |

<sup>a–e</sup> Differences between groups with different letters are statistically significant (p < 0.05). One-way ANOVA Post Hoc LSD Test

CAT activity results
When CAT analysis results are examined (Table 3 and Fig. 3), it is seen that the lowest CAT levels are in the F100 and F50 groups, respectively. When the F50 + RJ and F100 + RJ groups are compared with the F50 and F100 groups, a statistically significant observed (p < 0.05).

| Groups             | Muscle tissue catalase activity (U/mg protein) |
|--------------------|-----------------------------------------------|
| Control            | 42.26 ± 2.00<sup>a</sup>                      |
| RJ                 | 42.02 ± 2.00<sup>a</sup>                      |
| 50 mg/kg F         | 21.08 ± 2.00<sup>d</sup>                      |
| 100 mg/kg F        | 15.17 ± 2.00<sup>e</sup>                      |
| RJ + 50 mg/kg F    | 32.75 ± 2.00<sup>b</sup>                      |
| RJ + 100 mg/kg F   | 25.52 ± 2.00<sup>c</sup>                      |

a–e Differences between groups with different letters are statistically significant (p < 0.05). One-way ANOVA Post Hoc LSD Test

Expression levels of bax, caspase-3, caspase-6, bcl-2, TNF-α, and IL-1α proteins

When Bax protein expression rates in muscle tissue are examined (Fig. 4A,E), it is seen that there is a statistically significant decrease in the fluoride damage groups compared to the control and royal jelly groups. When these groups are compared with treatment groups, a statistically significant observed (p < 0.05).

When the Bcl-2 protein expression rates are examined in Fig. 4B,E it is observed that there is a statistically significant increase in the fluoride damage groups compared to the control and RJ groups. When these groups are compared with the treatment groups, a statistically significant decrease was observed (p < 0.05).

When the Caspase-3 protein expression rates in muscle tissue are examined (Fig. 4C,E), a statistically significant decrease is sighted in fluoride damage groups compared to control and RJ groups. The expression rates of the F50 and F100 groups are close to each other. When the F50 + RJ and F100 + RJ treatment groups are compared with the fluoride damage groups, a statistically significant increase are observed (p < 0.05).
When the Caspase-6 protein expression rates are examined in Fig. 4D,E, it is observed that there is a statistically significant decrease in the F50 and F100 damage groups compared to the control and RJ groups. When the damage groups are compared with the treatment groups, it is seen that there is a statistically significant increase in the treatment groups (p < 0.05). Among the groups, it is seen that the lowest expression rate is in the F100 group.

When Bax, Bcl-2, Caspase-3 and Caspase-6 protein expressions are examined in muscle tissue, no statistically significant difference is found between control and RJ groups.

When the TNF-α and IL-1α proteins expression rates are examined in Fig. 4E.E and 4F.E it is observed that there is a statistically significant increase in the fluoride damage groups compared to the control and RJ groups. When these groups are compared with the treatment groups, a statistically significant decrease was observed (p < 0.05).

**Histopathological analysis results**

When the histopathological analyzes of muscle tissue examined (Fig. 5A,B,D), it is seen that there is no significant difference between the groups in terms of streak loss. According to this results, signs of inflammatory cell infiltration and edema formation are more common in the F100 damage group compared to the other groups. It is understood that the damages seen in the F100 and F50 groups decreased in the treatment groups (Fig. 5C,E,F).

**Discussion**

In recent years, the use of medicinal and aromatic herbs and many foods for therapeutic purposes has been increasing against many diseases including cancer. Thanks to the developments in the science of biology, detailed information about the therapeutic effect of many foods is obtained and many researches are carried out on these foods. One of these foods is royal jelly (Ghanbari et al. 2015; Aslan et al. 2018; Tekeli et al. 2019). There are many studies in the literature on royal jelly, especially its positive effects on testicular tissue and sperm quality. However, there are not many studies about the effect of royal jelly on some damages on muscle tissue. For this purpose, we examined the possible therapeutic effects of royal jelly against muscle damage caused by fluoride in our study. We think that the molecular biological, biochemical and histopathological findings which we obtained will contribute to the literature.

Quadri et al. (2018) created 50 and 100 ppm fluoride-induced tissue calcemia in rats in their study. Then, they evaluated the results on some apoptotic pathways and biochemical findings. They also performed histopathological analyzes using the TUNEL method. The study team stated that there was a significant increase in oxidative stress in groups exposed to fluoride (p < 0.05). They stated that after chronic fluoride exposure, there was a significant increase in the level of Caspase-3 expression and a decrease in the expression level of Bcl-2 in the heart muscle. They emphasized that swelling, tissue edema and cell loss occur in damage groups. They stated that catalase activity was lower in the groups that were damaged by giving 50 ppm and 100 ppm fluoride with drinking water compared to the control group given normal
drinking water. The main functions of caspases are to prevent the DNA polymerase enzyme activity and to destroy the cell by apoptosis. In our study, when Caspase-3 expression levels examined in Fig. 4C, it is seen that there was no statistically significant difference between the control group and royal jelly group. It is seen that there was a statistically significant decrease in the fluoride damage groups compared to the control group, and an increase in the expression levels of Caspase-3 in the treatment groups when compared to the treatment groups (p < 0.05).

When Caspase-6 expression levels examined in Fig. 4D, it is seen that the lowest rate is in the F100 damage group. It was observed that there was a statistically lower level of Caspase-6 expression in the damage groups compared to the control and royal jelly groups. Caspase-6 expression levels increased in F50 + royal jelly and F100 + royal jelly groups compared to the damage groups (p < 0.05). Almeer et al. (2018) examined the possible protective effect of royal jelly in a study that they induced cadmium-induced hepatotoxicity in mice. When the study team evaluated the results, they stated that royal jelly showed a hepatoprotective effect by preventing liver damage, oxidative stress and inflammation caused by the increase in expression of Nrf-2 and the antiapoptotic protein Bcl-2.

In our study, it is seen that the lowest Bax protein level in muscle tissue is in the F100 and F50 groups, respectively, and these groups show a statistically significant difference compared to the control and royal jelly groups. A statistically significant increase is seen when injury groups compared with treatment groups (p < 0.05). It was observed that there was no significant difference between the control and royal jelly groups (Fig. 4A). The expression rates of Bax protein, which is a proapoptotic protein, are low in the damage groups and high in the treatment groups. In this respect, we can emphasize that royal jelly has a protective effect against fluoride damage. When the expression levels of Bcl-2 protein, an antiapoptotic protein, are examined (Fig. 4B), the damage groups were statistically significantly higher compared to the control group (p < 0.05). The highest Bcl-2 level observed in the F100 group. There was a statistically significant decrease in treatment groups compared to the injury groups (p < 0.05). Considering Bcl-2 expression levels, we can say that royal jelly has a protective effect against fluoride damage. Habashy and Abu-Serie (2020) investigated the potential antiviral effect of royal jelly protein 2 (MRJP2) and its isoform X1 against SARS-CoV-2 in their study. Molecular docking method was also used in this study in which the sialydase activity of royal jelly proteins was determined. The team examined the interactions of MRJP2 and X1 proteins with SARS-CoV-2 vital proteins. According to docking analysis, they stated that royal jelly proteins can bind to the active site or cofactor binding site on the viral nsp3, nsp5, nsp9, nsp12, and nsp16, thus inhibiting their activity. The team found that the MRJP2 and X1 proteins could be a hope against this deadly virus; However, they stated that they should be supported in new studies.

When the expression levels of TNF-α and IL-1α proteins are examined (Fig. 4E, 4F), the damage groups were statistically significantly higher compared to the control group (p < 0.05). There was a statistically significant decrease in treatment groups compared to the injury groups (p < 0.05). Kanbur et al. (2009) examined the protective effects of royal jelly in their study of fluoride damage in mice. When MDA levels were examined, they stated that there was a statistically significant difference in liver tissue between the control group and the experimental group. They stated that this difference was not significant only in the
royal jelly group compared to the control group, but statistically significant in the fluoride and fluoride + royal jelly groups. They stated that the MDA levels of the control group and royal jelly group were similar. When examining CAT activity, they observed a significant decrease in fluoride group compared to control group. They stated that CAT activity increased in royal jelly + fluoride group, but no significant difference was found in only royal jelly group. When the GSH-Px activity was examined, they stated that it increased significantly in the fluoride group compared to the control group, while it decreased in the fluoride + royal jelly group.

When we look at the therapeutic effects of royal jelly in the literature are examined, it is seen that many studies have not been done on muscle tissue. Our study investigates the possible healing effects of royal jelly against to muscle tissue damage in fluoride-induced rats. In this respect, we think that our study will make an important contribution to the literature. In our study, when the analysis of GSH activity examined (Table 1, Fig. 1), it was seen that there was a statistically significant decrease in the F50 and F100 groups compared to the control and royal jelly groups (p < 0.05). There was no statistically significant difference between the control and royal jelly groups. A statistically significant increase was observed in treatment groups compared to the damage groups (p < 0.05). Fluoride damage caused a decrease in GSH activity in rats, and an increase in GSH activity was observed in rats treated with royal jelly. When MDA levels, which is the final product of lipid peroxidation, examined (Table 2, Fig. 2), it was observed that it was low in the control and royal jelly groups, and increased in the damage groups. In the treatment groups, a decrease was observed in MDA levels. There was a statistically significant difference between treatment groups and damage groups (p < 0.05). Among all groups, it’s seen that the lowest MDA level is in the only royal jelly given group. The highest MDA level is seen in the F100 group. We can say that royal jelly provides a decrease in MDA levels by being effective against fluoride damage.

When the CAT activity results examined among the groups (Table 3, Fig. 3), it was observed that there is a statistically significant decrease in damage groups compared to control group (p < 0.05). When the injury groups are compared with the treatment groups, a statistically significant increase observed in treatment groups. There was no statistically significant difference between the control group and royal jelly group. We can say that fluoride damage causes a decrease in catalase activity, while royal jelly resists this damage and creates an increase in this activity. In our study, histopathological analyzes were also performed to support molecular biological analyzes and to show stronger evidence. When histopathological analyzes of muscle tissue examined (Fig. 5A,B,D), inflammatory cell infiltration and edema formation are observed in the F100 injury group compared to the other groups. Edema formation and inflammatory cell infiltration are relatively less in the treatment groups. There was no significant difference between the groups in terms of striation loss in muscle tissue. When the groups examined in terms of both edema and inflammatory cell infiltration, it was understood that the damages seen in the F100 and F50 groups tend to decrease in the treatment groups (Fig. 5C,E,F).

Conclusions
The results of present study illustrate that royal jelly has a protective effect on muscle tissue against fluoride damage. It was observed that fluoride-induced damage occurred mostly in the F100 group and the secondly in the F50 group. When MDA, CAT and GSH activities and Bax, Bcl-2, Caspase-3 and Caspase-6 protein expression levels and muscle tissue histopathological analyzes are examined, we can state that royal jelly plays a protective role against fluoride damage (Fig. 6)

**Declarations**

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**Authors’ contributions**

OG, SB, MIC, GP performed laboratory analysis, AA wrote the article, review-editing, investigation, methodology, formal analysis, IHO performed pathological analysis, all authors read the article and approved the manuscript.

**Compliance with ethical standards**

**Competing interests**

The authors have declared no conflicts of interest.

**Ethical approval and consent to participate**

All applicable international, national and institutional guidelines for the care and use of experimental animals were followed. The animals of this research were provided by Firat University Experimental Animals Research Center (FUDAM, ethical committee approval number: 02/09/2020, 2020/12, Elazig, Turkey). The authors declare that they consent to participate to this study.

**Consent to publish**

The authors declare that they consent for publication of this study

**Availability of data and materials**

Not applicable
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**Figures**
Figure 1

Muscle tissue GSH levels in rats. a-e: different letters indicate statistically significant difference between groups. Values are statistically significant at p<0.05 One-way analysis of variance (ANOVA) Post Hoc LSD Test.
Muscle tissue MDA results in rats. a-e: different letters indicate statistically significant difference between groups. Values are statistically significant at p<0.05 One-way analysis of variance (ANOVA) Post Hoc LSD Test.

Figure 2
Figure 3

Muscle tissue catalase enzyme expression levels in rats. a-e: different letters indicate statistically significant difference between groups. Values are statistically significant at p <0.05 One-way analysis of variance (ANOVA) Post Hoc LSD Test.
Figure 4

Muscle tissue western blotting mean protein expression results; A: Bax, B: Bcl-2, C: Caspase-3, D: Caspase-6, E: IL-1α, F: TNF-α, G: western blotting protein bands. a-e: different letters indicate statistically significant difference between groups. Values are statistically significant at p<0.05 One-way analysis of variance (ANOVA).
Figure 5

Muscle tissue histopathological results
Figure 6

The protective effect of royal jelly on muscle tissue apoptotic pathway.