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

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Abstract

In this study, 42 Wistar albino male 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; (vi): F100+RJ group: Standard diet + 100 mg/kg fluoride + royal jelly. After 8 weeks, the rats were decapitated, and their muscle tissues were removed. Expression levels of Caspase-3, Caspase-6, Bax, tumor necrosis factor- α (TNF- α), interleukin 1 alpha (IL1- α) and Bcl-2 proteins in muscle tissue were determined by western blotting method. Histopathological analyses were also performed on the muscle tissue. Malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) analyses were determined by a spectrophotometer. According to the obtained results, Bcl-2, TNF- α and IL1- α protein expression was increased in damage groups compared to the control and royal jelly groups, while Caspase-3, Caspase-6 and Bax protein expression levels decreased in damage groups. MDA level increased in damage groups compared to the control and royal jelly groups, while CAT and GSH levels increased with royal jelly application in royal jelly-given group in comparison to the fluoride-exposed group. 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 on muscle tissue against fluoride damage.

Keywords Bax · Bcl-2 · Fluoride · IL1- α · Muscle damage · Royal jelly · TNF- α

Introduction

In recent years, especially nutritional-based measures have begun to be taken to 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 and Aslan 2020). Royal jelly contains many active constituents and antioxidants such as polyphenols (Hossen et al. 2017). Royal jelly is known to have antitumor, antioxidative, antidiabetic, antiallergic, antibacterial, antiinflammatory and antihypertensive effects. There are many vitamins in royal jelly such as folate, inositol, riboflavin, vitamin B6 (Guo et al. 2021). 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). Latterly, honeybee and royal jelly products have been recommended as a potential coherent antiseptic to help preserve against the Covid-19 based on the biocidal effect of H₂O₂ and other phytochemicals existing in bee and royal jelly products (Shaldam et al. 2021).

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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-1 α) 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-1 α 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; Pekmezci et al. 2018; Aslan et al. 2020b, c).

Materials and methods

In vivo analyses

The rats were revealed to 12 h of a light period and 12 h of darkness per day, and they were separated into six groups. The animals were housed in standard laboratory conditions and cages and maintained at 20–25 °C, 50–60% relative moisture. A total of 42 Wistar albino (8 weeks old) male rats, 7 in each group, were used in the study. The rats were divided into 6 groups. Our experimental groups were as follows:

- Group 1 → Control (C) 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. At the end of the study, the rats were decapitated under anesthesia, and their leg muscles were removed. These muscle tissues were stored at −80 °C until analysis.

Application of royal jelly

Royal jelly was given to rats by oral gavage (100 mg/kg bw) five times a week for 8 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). Excess fluoride in drinking water is known to cause fluoride poisoning. In addition, fluoride was added to the drinking water of the rats in order not to create any other stress in addition to the oral gavage stress in the rats (Xie et al. 2020). The animal experiment phase of the study continued for 8 weeks.

Analysis of proteins via SDS-PAGE and western blotting method

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

GSH (glutathione) activity measurement in muscle tissue

GSH (glutathione) concentrations in muscle tissue samples were 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 were calculated, and the results were expressed in pmol/ml. In this method, a standard graphic was created using bovine serum albumin solution. Protein concentrations of the samples were calculated in mg/ml based on the standard chart (Aydemir et al. 2008).

Malondialdehyde (MDA) activity measurement in muscle tissue

Muscle tissue samples were divided into small sizes, 4.5 ml 1.15% KCl was added to 0.5 g 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 was added to 0.1 ml tissue homogenate. Then, 750 µl of 0.8% (pH: 3.5) TBA and distilled water was added until the final volume is 4 ml. It is left in a hot water bath (95 °C) for 45 min. After cooling, 1 ml of distilled water was added, followed by 5 ml of n-butanol-pyridine at a level of 15:1 (v/v) and mixed with a vortex device. After centrifugation at 5000 rpm for 10 min, the organic layer in the upper part was removed. It was measured in a spectrophotometer (532 nm), and values were saved in nmol/g (Aslan 2018).

Catalase (CAT) activity measurement in muscle tissue

To be used in catalase measurement, concentrated (35%) H₂O₂ was 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, 1 ml of this mixture was taken and placed in the bathtub. Depending on the working range, starting from 30 µl, gradually increasing concentrations of supernatant were added and mixed once. In the spectrophotometer, the absorbance change of H₂O₂ was read at 240-nm wavelength for 30 s. The number of enzyme

units per milliliter was 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 were examined histopathologically. Macroscopic samples were taken, fixed in 4% paraformaldehyde solution and examined under a light microscope, and histopathological analyses were performed.

Statistical analysis

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

Results

GSH analysis results

When Table 1 and Fig. 1 were examined, it was seen that the GSH levels of the F50 and F100 groups were lower than the F50 + royal jelly and F100 + royal jelly groups ($p < 0.05$).

MDA analysis results

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

Table 1 Muscle tissue GSH levels

Groups	Muscle tissue GSH levels (µmol/mg protein)
Control	850.58 ± 1.00 ^a
RJ	855.29 ± 1.00 ^a
50 mg/kg F	500.58 ± 1.00 ^d
100 mg/kg F	400.94 ± 1.00 ^e
RJ + 50 mg/kg F	638.82 ± 1.00 ^b
RJ + 100 mg/kg F	560.94 ± 1.00 ^c

^{a–e} Differences between groups with different letters are statistically significant ($p < 0.05$). One-way ANOVA post hoc LSD test

Fig. 1 Muscle tissue GSH levels in rats. The letters a,b,c,d,e indicate that the groups differ from each other statistically ($p < 0.05$). Values are statistically significant at $p < 0.05$ One-way analysis of variance (ANOVA) post hoc LSD test

CAT activity results

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

Expression levels of Bax, Caspase-3, Caspase-6, Bcl-2, TNF-α and IL-1α proteins

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

Table 2 Muscle tissue MDA levels (nmol/g)

Groups	Muscle tissue MDA levels (nmol/g)
Control	1.02 ± 0.02 ^c
RJ	0.95 ± 0.02 ^c
50 mg/kg F	1.77 ± 0.02 ^b
100 mg/kg F	2.05 ± 0.02 ^a
RJ + 50 mg/kg F	1.35 ± 0.02 ^d
RJ + 100 mg/kg F	1.62 ± 0.02 ^c

^{a-c} Differences between groups with different letters are statistically significant ($p < 0.05$). One-way ANOVA post hoc LSD test

Fig. 2 Muscle tissue MDA results in rats. The letters a,b,c,d,e indicate that the groups differ from each other statistically ($p < 0.05$). Values are statistically significant at $p < 0.05$ One-way analysis of variance (ANOVA) post hoc LSD test

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

When the Caspase-3 protein expression rates in muscle tissue were examined (Fig. 4C,G), a statistically significant decrease was sighted in fluoride damage groups compared to the control and RJ groups. The expression rates of the F50 and F100 groups were close to each other. When the F50 + RJ and F100 + RJ treatment groups were compared with the fluoride damage groups, a statistically significant increase was observed ($p < 0.05$).

When the Caspase-6 protein expression rates were examined in Fig. 4D,G, it was observed that there was a statistically significant decrease in the F50 and F100 damage groups compared to the control and RJ groups. When the damage groups were compared with the treatment groups, it was seen that

Table 3 Muscle tissue catalase activity

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

^{a-c} Differences between groups with different letters are statistically significant ($p < 0.05$). One-way ANOVA post hoc LSD test

Fig. 3 Muscle tissue catalase enzyme expression levels in rats. The letters a,b,c,d,e indicate that the groups differ from each other statistically ($p < 0.05$). Values are statistically significant at $p < 0.05$ One-way analysis of variance (ANOVA) post hoc LSD test

there was a statistically significant increase in the treatment groups ($p < 0.05$). Among the groups, it was seen that the lowest expression rate was in the F100 group.

When Bax, Bcl-2, Caspase-3 and Caspase-6 protein expressions were examined in muscle tissue, statistically significant difference was not found between control and RJ groups.

In our study, when the Bax/Bcl-2 ratio was considered, it was seen that there were statistical differences between the injury groups and the treatment groups. The highest Bcl-2 expression and the lowest Bax expression rates were found in the F100 group.

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

Histopathological analysis results

When the Fig. 5 histopathological analyses of muscle tissue were examined. Since there was no damage in the control group (Fig. 5A), inflammatory cells and edema were not observed (HE $\times$ 200). Since there was no damage in the royal jelly group (Fig. 5B), inflammatory cells and edema were not observed (HE $\times$ 200). The parts indicated by arrows in F50 (Fig. 5C) show the formation of inflammatory cells and edema (HE $\times$ 200). The parts indicated by arrows in F100 (Fig. 5D) show loss of inflammatory cells, edema formation and streaking (HE $\times$ 200). The arrowed portions in F50 + RJ (Fig. 5E)

show mild edema formation and fewer inflammatory cells (HE $\times$ 200). The arrowed portions in F100 + RJ (Fig. 5F) show sparse streak loss, mild edema formation and few inflammatory cells (HE $\times$ 200) (Table 4).

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.

Ibrahim et al. (2015) investigated the protective effects of royal jelly against cisplatin toxicity in rats. They stated that royal jelly has a nephroprotective role against cisplatin-induced damage in rats.

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 analyses using the TUNEL method. They stated that there was a significant increase in oxidative stress in groups exposed to fluoride ($p < 0.05$), and they stated that after chronic fluoride exposure, there was a significant increase in the Caspase-3 expression level and a decrease the Bcl-2 expression level in the heart muscle. In addition, they emphasized that swelling, tissue edema and cell loss occur in damage groups. 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. 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 were examined in Fig. 4C, it was seen that there was no statistically significant difference between the control group and royal jelly group. It was 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 fluoride damage groups ($p < 0.05$).

Shaldam et al. (2021) stated that active compounds in honey bee products (P-coumaric acid, ellagic acid, kaempferol

Fig. 4 Muscle tissue western blotting mean protein expression results: **A** Bax, **B** Bcl-2, **C** Caspase-3, **D** Caspase-6, **E** IL-1 α , **F** TNF- α , **G** western blotting protein bands. The letters a,b,c,d,e indicate that the groups differ

from each other statistically ($p < 0.05$). Values are statistically significant at $p < 0.05$ One-way analysis of variance (ANOVA)

Table 4 Muscle tissue histopathological results

Groups	Streaking loss	Edema	Inflammatory cell infiltration
Control	-	-	-
RJ	-	-	-
50 mg/kg F	+	++	++
100 mg/kg F	+++	+++	+++
RJ + 50 mg/kg F	-	+	+
RJ + 100 mg/kg F	+	+	++

Fig. 5 Muscle tissue histopathological results: **A** Control; **B** RJ; **C** F50; **D** F100; **E** F50 + RJ; **F** F100 + RJ

and quercetin) may have a protective role against Covid-19 by showing potential antiviral activity. The research team supported the findings in their studies with the molecular docking method, and they emphasized that it would be more efficient to support these findings with in vivo studies.

When Caspase-6 expression levels were examined in Fig. 4D, it was seen that the lowest rate was 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 the expression of Nrf-2 and the antiapoptotic protein Bcl-2.

In our study, it was 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 was seen when injury groups were 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, were 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, were examined (Fig. 4B), the expression of Bcl-2 protein was statistically significantly higher in the damage groups compared to the control group ($p < 0.05$). The highest Bcl-2 level was 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 analysis was also used in this study in which the sialidase 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. They 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-1 α proteins were examined (Fig. 4E,F), the expression levels of these two proteins were statistically higher in the damage groups 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 on 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. In addition, they stated that the MDA levels of the control group and royal jelly group were similar. They indicated that CAT activity decreased in the fluoride group compared to the control group. They stated that CAT activity increased in the 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 examined literature, it is seen that many studies have not been done on muscle tissue. Our study investigates the possible healing effects of royal jelly against 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 was 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, were examined (Table 2, Fig. 2), it was observed that they were 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 was seen that the lowest MDA level was in the only royal jelly given group. The highest MDA level was 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 were examined among the groups (Table 3, Fig. 3), it was observed that there was a statistically significant decrease in damage groups compared to the control group ($p < 0.05$). When the injury groups were compared with the treatment groups, a statistically significant increase was observed in the treatment groups. There was no statistically significant difference between the control group and royal jelly groups. 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 analyses were also performed to support molecular biological analyses and to show stronger evidence. When histopathological analyses of muscle tissue were examined (Fig. 5A,B), inflammatory cell infiltration and edema formation were not observed in the control and RJ groups. compared to the other groups. The parts indicated by arrows in F50 (Fig. 5C) show the formation of inflammatory cells and edema (HE $\times$ 200). The parts indicated by arrows in F100 (Fig. 5D) show loss of inflammatory cells, edema formation and streaking (HE $\times$ 200). Edema formation and inflammatory cell infiltration were relatively less in the treatment groups (Fig E,F); it was understood that the royal jelly protects muscle tissue against fluoride injury.

Fig. 6 The protective effect of royal jelly on muscle tissue apoptotic pathway

Conclusions

The results of the 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 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 analyses are examined, we can state that royal jelly plays a protective role against fluoride damage (Fig. 6).

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

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Data availability Not applicable.

Declarations

Ethics 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 for publication The authors declare that they consent for the publication of this study.

Competing interests The authors declare no competing interests.

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