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Original Article

Potential Anti-Apoptotic Impacts and Telomerase Activity of Royal Jelly on Different Tissues of Rats

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Abstract

Background and Aim: Royal jelly (RJ) has a broad range of pharmaceutical activities, including antioxidant, anti-aging, anti-tumor, and anti-apoptotic. The current study aimed to investigate RJ impacts on cell survival by measuring the amount of telomerase enzyme, protein BCL2, and BAX in different tissues of rats.

Methods: In this study, male Wistar rats (n=21) were randomly divided into 3 groups; Group 1 was the control group. Group 2 and group 3 were treated with royal jelly at a concentration of 150 mg/kg and 300 mg/kg for 30 days, respectively. The contents of Bax, BCL-2, and telomerase in the tissues Brain, Liver, Kidney, and lymphocytes were measured using the ELISA method.

Results: Telomerase increased in all the tissues involved in both treatment groups compared to the control group; however, the changes were not statistically significant. Although BAX and BCL-2 proteins showed irregular patterns, the ratio of BAX/BCL-2 declined in almost all the studied tissues with a significant decline in the rats' liver and kidney treated with RJ at the dose of 300 mg/kg and in the lymphocytes of the group administered 150 mg/kg of RJ.

Conclusion: RJ appears to have potential anti-apoptotic effects on the rats' tissues studied via regulating the levels of BAX, BCL-2, and telomerase proteins. Regarding telomerase, its levels increased in a dose-dependent manner in all involved tissues. Concerning the ratio of BAX/BCL-2, it is sensible to conclude that RJ tends to positively impact the cell survival rate at the dose of 300 mg/kg in the brain, Liver, and Kidney. Nonetheless, this ratio decreased more significantly at the dose of 150 mg/kg in lymphocytes, showing more potential to survive brain cells in this concentration.

Keywords: Apoptosis; BAX; BCL2; Royal Jelly; Telomerase.

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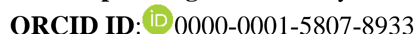
ORCID ID:0000-0001-5807-8933

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Introduction

Natural products are increasingly used around the world because of their therapeutic effects on human health. They have shown remarkable physiological benefits, reduced risk of chronic diseases, and no side effects. Apoptosis, also called programmed cell death, occurs in multicellular organisms and is essential in

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tissue homeostasis, removing unwanted cells (1, 2). Mitochondrial proteins particularly the Bcl-2 family proteins have a major role in controlling this process. The Bcl-2 family proteins are generally found in two types: anti-apoptotic ones, including Bcl-2 and Bcl-xL and Mcl-1, and pro-apoptotic ones such as Bak, Bax, and Bad (3, 4). While anti-apoptotic proteins hinder apoptosis by preventing the release of cytochrome C from the mitochondria, pro-apoptotic proteins accelerate its release, preventing apoptotic cell death. The Bcl-2 family proteins maintain a balance between new and old cells (5).

Royal jelly (RJ), secreted from the mandible and pharyngeal glands of worker bees, has been widely used as a dietary supplement. The main and active component of RJ is proteins, making up about 50% of its dry matter. RJ proteins include water-soluble proteins known as royal jelly core proteins (MRJPs) (6, 7). It also contains water-soluble B vitamins such as thiamine, riboflavin, pyridoxine, niacin, biotin, folic acid, inositol, minerals, essential amino acids, sugar, sterols, phosphorous compounds, acetylcholine, and other constituents beneficial to human health (8, 9). 10-hydroxy-2-decanoic acid is another significant component modulating the immune system by affecting peripheral blood mononuclear cells (PBMC) (10, 11). The effects of RJ on collagen tissue repair, atherosclerosis, arthritis, diabetic foot ulcers, and wart treatment have been proven (12, 13). Various studies have shown that RJ has a broad range of pharmaceutical activities, including antioxidant, anti-aging, anti-inflammatory, anti-tumor, anti-bacterial, and anti-apoptotic (14-17).

Bcl-2 (B cell lymphoma 2) protein is deemed crucial in cell survival and can prevent in vitro cell death induced by different stimuli including chemotherapeutic agents. Its anti-apoptotic activity has been shown in vivo in a generation of rats lacking the BCL-2 gene, representing a range of anomalies including excessive cell death (18). In contrast, Bax protein with a weight of 24 Kilo Dalton is considered one of the most well-known proteins involved in the cell death process. The promoter of this gene contains the p53 gene sequence, which causes this gene to be expressed following DNA damage and P53 activation (5). Following the accumulation of Bax in the mitochondria and creating a channel in the membrane by weakening it, cytochrome C eventually activates the caspase cascade and causes its apoptosis, and its release (18).

Based on the available literature, it has been shown that the telomerase enzyme can also play a role in the process of apoptosis via adding a replication of the TTAGGG sequence to the telomeres, the protective caps on the ends of the chromosomes, preventing telomere shortening and thus cell death. In each cell division, a part of the telomere is continuously shortened. Continuous telomere shortening leads to the separation of several proteins from the telomere structure and changes in the expression patterns of different genes. As a result, genes that have already been silenced are expressed. Eventually, continuous telomere shortening leads to cell cycle arrest and cell death (19).

The anti-apoptotic impacts of RJ have been a focus in various studies to date, as Jenkhetkan et al showed potential advantages for RJ in increasing life span and anti-oxidant activation by measuring some BCL-2 family proteins in human lymphocytes (20). Owing to the anti-apoptotic effects of this natural product, the current study aimed to investigate its impacts on cell survival using different tissues of rats.

Methods

Animals and treatment groups

In this experimental study, 21 male Wistar rats were experimented with. Each rat weighed between 200 to 300 grams. The animals were randomly divided into 3 groups consisting of 7 rats as follows: Group 1 was the control group who were administered distilled water for 30 days. Group 2 was treated with royal jelly at a concentration of 150 mg/kg for 30 days and group 3 was treated with royal jelly at a concentration of 300 mg/kg for the same duration (21-24). The rats were preserved in a standard condition in terms of food and water with a regular 12-hour dark-light period. The ambient temperature and humidity were defined as standard. In this study, except for the control group, royal jelly at concentrations of 150 mg/kg and 300 mg/kg was administered as oral gavage to the rats daily for 30 days until the end of the investigation according to previous studies. Royal Jelly was obtained from Ponik Pharmaceutical co (Iran). Laboratory research was approved by the Ethics Committee of Gonabad University of Medical Sciences with the project ethics code IR.GMU.REC.1396.54.

Sample collection

After the course of treatment of rats with RJ, the animals were sacrificed according to the guidelines of the Ethics Committee of Gonabad University of

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Medical Sciences for research on laboratory animals. Firstly, the rats were anesthetized using ketamine and xylazine (100 mg/kg and 10 mg/kg respectively). Then the tissues of the kidney, brain, and liver of the rats were removed. After grinding in liquid nitrogen using a mortar and pestle, the tissues were transferred to certain micro-tubes and stored at -70°C.

Isolation of lymphocytes using Ficoll

Briefly, after taking blood samples using EDTA tubes, it was diluted with Hanks' Balanced Salt Solution (HBSS) at a ratio of 1: 2. Then 15 mL of ficoll was covered with a layer of diluted blood (30 mL). Afterward, it was centrifuged at 2800 RPM for 20 minutes. The lymphocyte-containing layer, which turned white just above the erythrocyte deposition, was removed using a Pasteur pipette. It was later washed twice with normal saline and the precipitate was stored at -70°C for subsequent experiments.

Measuring the total protein content

The Broadford method, a time-tested colorimetric assay, was used to measure the concentration of total protein in the tissues based on the standard protocols (25). Bovine Serum Albumin (BSA) was used as a standard for this test.

Measurement of BAX, Bcl2, and telomerase

The contents of Bax, BCL-2, and Telomerase in the tissues were measured using Rat B-Cell Leukemia/Lymphoma 2 ELISA Kit (Bcl2), Rat Bcl2 Associated X Protein ELISA Kit (Bax), and Rat Telomerase ELISA kit made by ZellBio GmbH (Germany) Company according to the manufacturer's instructions. The concentrations were expressed as ng/mg of tissue according to the total concentration of tissue protein. Briefly, reagents, samples, and the standards were prepared. In the next step added 40 microliter sample (s) + 10 microliter BCL-2-Ab, (for Bax protein added Bax-Ab) and for telomerase enzyme added Then, 50 microliter standards and 50 microliters Streptavidin-HRP were added and incubated for 60 minutes at 37. After washing plates Chromogen solution A and 50 microliters B were added and incubated for 10 minutes at 37 for color development. Finally, absorbance values were read within 10 minutes at 450 nm.

Statistical analysis

Data were analyzed using SPSS software (version 18) and presented as mean ± standard error of the mean (SEM). Comparison of data between the groups was performed by one-way ANOVA and Tukey test for comparisons of the means, using the Graph Pad Prism 7 program. P value < 0.05 was considered significant.

Results

Levels of BAX, BCL-2, and Telomerase in blood lymphocytes

As is shown in Figure1, the levels of BAX and telomerase are increased in the rats treated with 150 and 300 (mg/kg) of RJ compared to the control group. Concerning the BCL-2, the levels of this protein did not change (P>0.5).

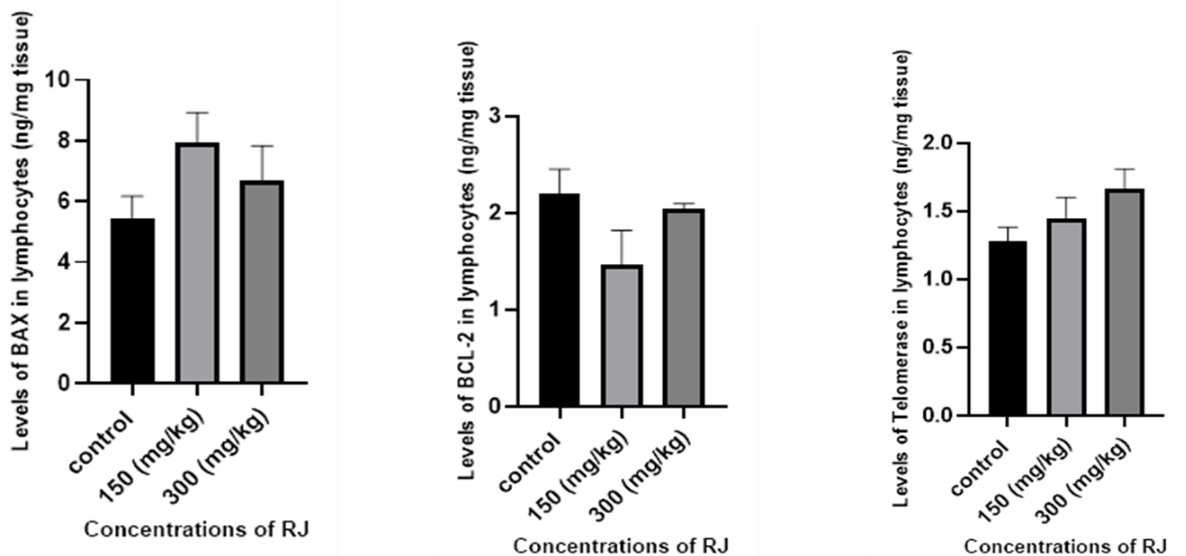

Figure 1. Levels of BAX, BCL-2, and telomerase (ng/mg tissue) in lymphocytes of rats received different doses of RJ (150 and 300 mg/kg).

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Levels of BAX, BCL-2, and Telomerase in brain

It is shown in Figure 2 that while the BAX levels decreased slightly in both two treated groups of the rats, levels of telomerase rose considerably. However, none of these alterations were statistically significant (P>0.5). Furthermore, there were minor changes in the BCL-2 levels in the brain.

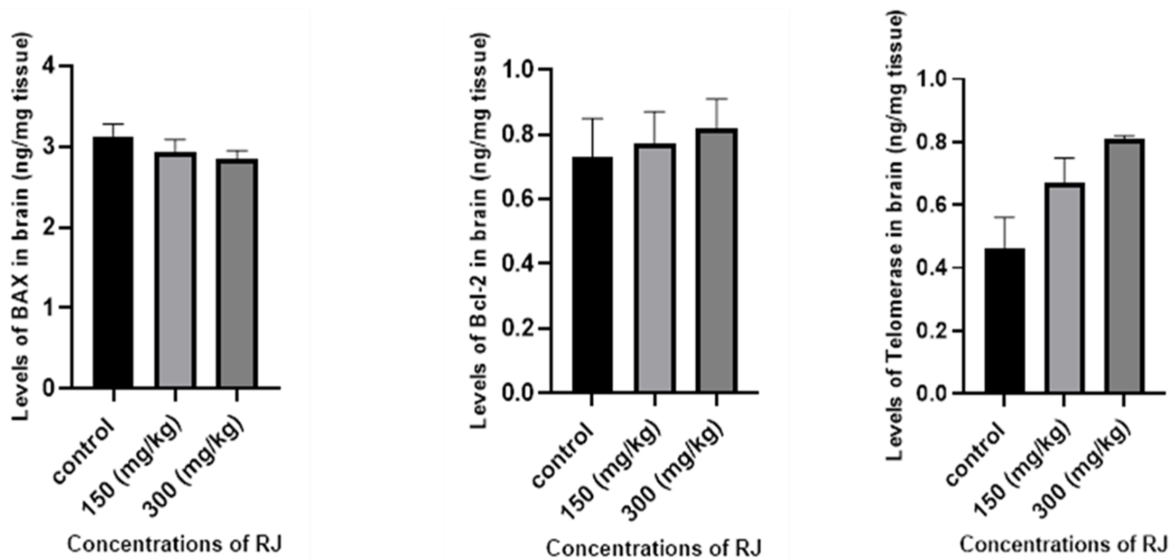

Figure 2. Levels of BAX, BCL-2, and telomerase (ng/mg tissue) in the brain of rats received different doses of RJ (150 and 300 mg/kg).

Levels of BAX, BCL-2, and Telomerase in liver

It is clear from Figure 3 that changing patterns of the BAX and BCL-2 levels in the liver are analogous. After treatment with 150 mg/kg of RJ, the levels of BAX and BCL-2 increased slightly (from 1.58 to 0.38 and from 0.38 to 0.1 ng/mg tissue, respectively). Nevertheless, both the BAX and BCL-2 levels fell significantly in rats treated with 300 (mg/kg) of RJ (P<0.001). Regarding telomerase, there was a steady rise in a dose-dependent manner, though not statistically significant.

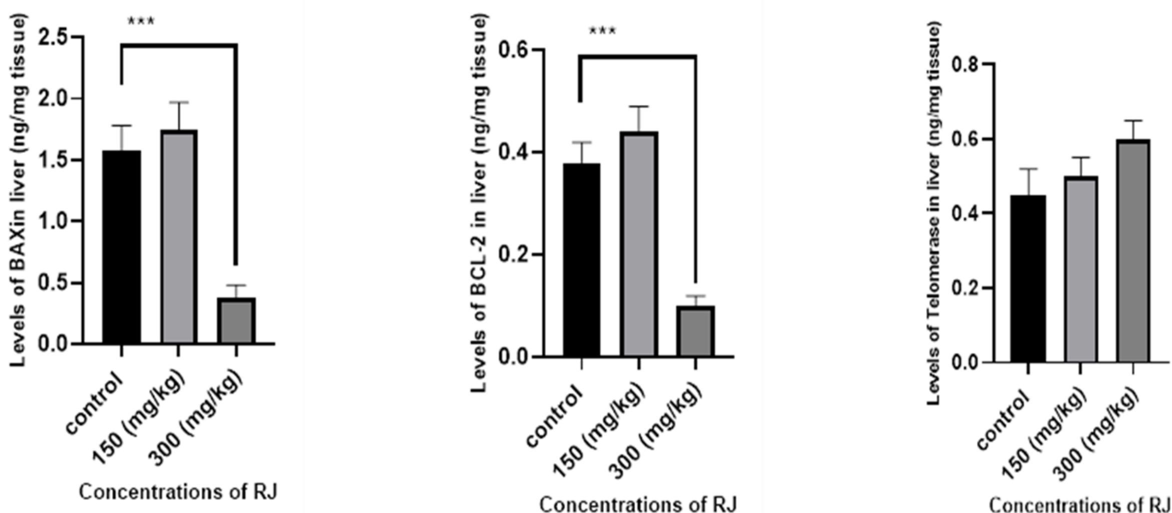

Figure 3. Levels of BAX, BCL-2, and telomerase (ng/mg tissue) in the liver of rats received different doses of RJ (150 and 300 mg/kg).

Levels of BAX, BCL-2, and Telomerase in kidney

The levels of BAX and BCL-2 dropped remarkably compared to the control group (P<0.001) _from 1.63 to 0.29, and from 0.41 to 0.09 ng/mg tissue, respectively (Figure 4). In addition, the levels of telomerase increased, particularly at the dose of 300 mg/kg of RJ. The level changes of these proteins in the rats treated with 150 mg/kg of RJ were not significant.

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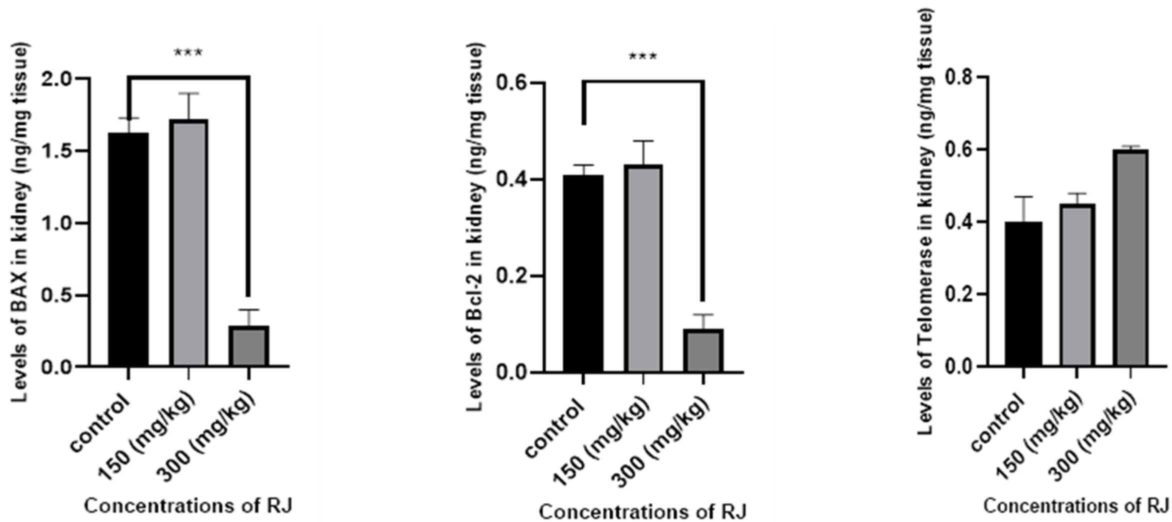

Figure 4. Levels of BAX, BCL-2, and telomerase (ng/mg tissue) in the Kidney of rats received different doses of RJ (150 and 300 mg/kg).

The ratio of BAX/BCL-2 in the tissues

To gain a more intelligible interpretation, the ratio of BAX/BCL-2 was calculated for all the tissues (Figure 5). A statically significant decline was observed in this ratio at the dose of 150 mg/kg in the lymphocytes. In the Brain, this ratio was decreased at both doses; however, these changes were not statically significant.

With regard to the remaining tissues, in the Liver, while the ratio of BAX/BCL-2 saw a slightly rose at the dose of 150 mg/kg, it declined significantly in the rats treated with the RJ at 300 mg/kg dose. Likewise, the kidney witnessed somewhat similar changes, with a significantly decreased level of BAX/BCL-2 in the higher dose of RJ.

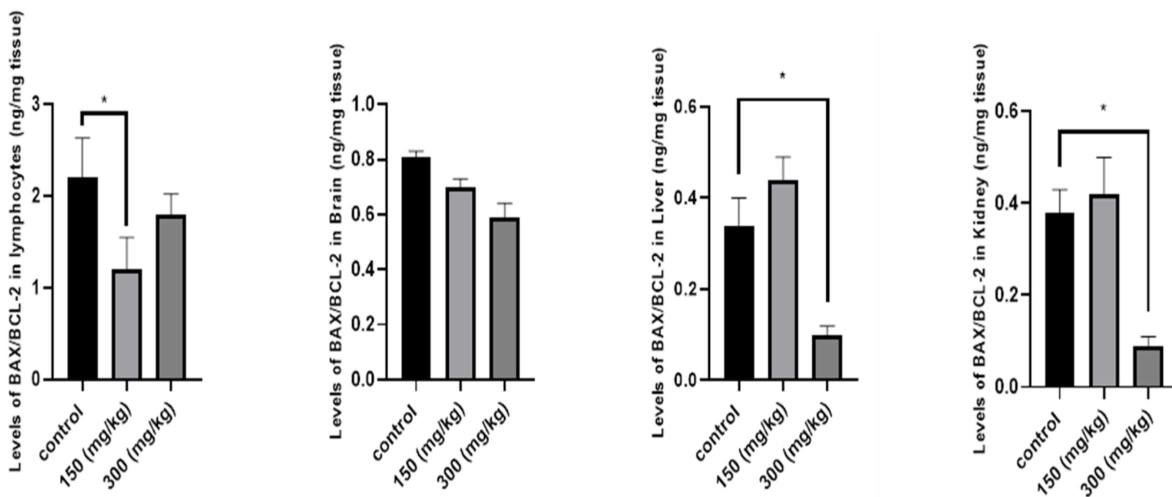

Figure 5. Levels of BAX/BCL-2 (ng/mg tissue) in the different tissues (blood lymphocytes, the brain, liver, and kidney) of the rats received different doses of RJ (150 and 300 mg/kg).

Discussion

The current study was performed to measure the levels of Telomerase, BAX, and BCL-2 proteins in different tissues of male rats treated with different doses of RJ, reflecting insight into the survival rates of these cells as a result of treatment with RJ.

The results showed that the levels of Telomerase in all the studied tissues (Brain, Liver, Kidney, and lymphocytes) in both treatment groups were elevated compared to the control group. Although this rise was not statistically significant, it appears that this herb may possess anti-senescence activities. In a study conducted by Jiang et al, it was found that the

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treatment of human embryonic lung fibroblast cell line (HFL-I) with different concentrations of the major royal jelly proteins (0.1–0.3 mg/mL) had beneficial impacts on the proliferation activity, the senescence process, and the length of telomeres (26). Likewise, in a recent investigation, the advantageous effects of RJ against Doxorubicin (DXR), a potent genotoxic chemotherapeutic compound, in human lymphocytes were illustrated through an increased hTERT/BAX which indicates a greater longevity (20).

The levels of proteins BAX and BCL-2 altered in quite erratic patterns. Nevertheless, the Bax and BCL-2 levels declined and rose in the brain tissue, respectively. Moreover, the levels of these proteins fell significantly in both the liver and kidney. A study was performed on male Balb/c-rats with kidney ischemia/reperfusion (I/R) injury and the levels of BAD, BAX, and FADD gene expression profiles showed a significant reduction in these genes in the groups receiving royal jelly/mesenchymal stem cells. Although the expression of the BCL2 gene increased in the I/R + MSCs and ischemic preconditioning groups, it was not statistically significant (27). In a study of cadmium induced-nephrotoxicity rats that were exposed to royal jelly and CdCl2, RJ pretreatment significantly reduced the expression of Bax and caspase-3 and significantly increased Bcl-2 expression compared with the Cd-treated group (28).

During research conducted by Aslan et al, the protective effects of RJ against heart damage were investigated. In this study, 42 rats were treated with 100 mg/kg RJ. Protein expression levels of caspase-3, caspase-6, caspase-9, Bcl-2, Bax, BDNF, Gsk-3, Nrf-2, and NF-κB proteins in heart tissues were assessed by the western blots technique and hearth tissues were examined histopathologically. The study showed a protective impact for RJ against fluoride-induced oxidative damage by decreased and increased levels of Bcl2 and Bax, respectively (29). In another study, the protecting effects of RJ against lung damage were evaluated. The levels of COX-2, Bcl-2, GSK3, TNF-α, Bax, BDNF, caspase-3, caspase-6, and caspase-9 proteins were determined. Similarly, RJ showed a healing impact on fluoride-exposed lung damage in rats (30).

As it appears, most studies on RJ have been focused on investigating the anti-damaging impacts of this product on different tissues, while in the current study the aim was to assess the anti-aging impacts of RJ on different tissues of healthy rats. Furthermore, the ratio of BAX/BCL-2 has not much been used as an indication of apoptosis. To illuminate a more intelligible interpretation of BAX and BCL-2 changes, the current study made use of this ratio too. As a result, it is evident that in almost all measurements, this ratio declined, indicating an anti- apoptotic effect of RJ. In a similar study, it was illustrated that RJ could ameliorate mitochondrial-induced ovarian apoptosis caused by cisplatin by regulating anti-apoptotic Bcl2, and pro-apoptotic Bax mRNAs, showing a decreased ratio of BAX/BCL-2 (31).

In a study that examined the protective effects of honey on cisplatin-induced hepatic and renal toxicity in male rats, BAX mRNA expression in the liver and kidney was augmented after cisplatin treatment and showed significantly higher expression levels than that in the control animals. Treatment of the rats with honey significantly lowered the liver and kidney NF-kB mRNA and BAX mRNA expression compared to the cisplatin-treated rats. Conversely, the Bcl-2 levels in the hepatic and renal tissues were significantly lower in the cisplatin-treated animals than in the control animals. Pre-treatment with honey resulted in the restoration of Bcl-2 levels in both the liver and kidney compared to the cisplatin-treated group (32). Furthermore, Almeer et al found that RJ had potential hepatoprotective effects against cadmium chloride-induced hepatotoxicity in rats by upregulating Nrf2 and BCL-2 (33). The results of these studies were almost congruous with ours.

Concerning lymphocytes, research conducted by Hosseini et al showed that RJ had no apoptotic impacts on Peripheral Blood Mononuclear Cells (PBMC). The survival rate of these cells did not change considerably in vitro (34).

However, the results of our study illustrated a significantly decreased ratio for BAX/BCL-2 in the group of rats treated with 150 mg/kg RJ. This inconsistency may be attributed to the fact that this study was performed on human cell culture with different doses of RJ. In another study by Jenkhetkan et al on rat lymphocytes, anti-apoptotic effects of RJ was shown to be important, increasing the ratio of BCL-2/BAX which was consistent with our results (20).

The potential neuroprotective effects of RJ against Cd-induced neuronal damage was assessed in an investigation and the levels of BAX and BCL-2 fell and rose respectively in cerebral cortices of the treated rats. Similarly, the same patterns for these proteins occurred in our study in the Brain of the rats (35).

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Conclusion

In conclusion, Royal jelly appears to have potential anti-apoptotic impacts on the rats' tissues studied via regulating the levels of BAX, BCL-2, and Telomerase proteins. Regarding Telomerase, its levels increased in a dose-dependent manner in all tissues involved. With regard to the ratio of BAX/BCL-2, it is sensible to conclude that RJ tends to positively impact the cell survival rate at the dose of 300 mg/kg in the brain, liver, and kidney. Nonetheless, this ratio declined more remarkably at the dose of 150 mg/kg in lymphocytes, showing more potential to protect cells in this concentration.

Acknowledgments

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Conflict of Interest

All authors have no conflict of interest to declare.

Funding/Support

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Ethics

Laboratory research was approved by the Ethics Committee of Gonabad University of Medical Sciences with the project ethics code IR.GMU.REC.1396.54.

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