Cerrahpaşa Med J 2026; 50: 1-8 ORIGINAL ARTICLE

Protective Effects of Royal Jelly in Diabetic Rat Testis Tissue Through Apoptotic and Antiapoptotic Pathways

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What is already known on this topic?

- *The protective role of royal jelly (RJ) in diabetes-induced testicular damage has been demonstrated in some studies.*

What this study adds on this topic?

- *This article investigates and identifies some of the molecular factors—mitogen-activated protein kinases, Fas/FasL, and insulin-like peptide-3—that may be responsible for the protective effect of RJ in diabetic testicular tissue.*

Abstract

Objective: Diabetic male subfertility and/or infertility represents an area of research that has attracted attention and therefore become a subject of investigation at the molecular level. The insulin-like effects, antihypertensive, antitumor, antioxidative, anti-inflammatory, and immunomodulatory roles of royal jelly (RJ) have been demonstrated by scientific research. This study was designed based on the hypothesis that RJ may have the potential to prevent apoptosis in testicular tissue and, consequently, exert a beneficial impact on spermatogenesis through its ability to mitigate the adverse effects of diabetes on testicular tissue. The aim is to identify some of the molecular factors that may be responsible for the therapeutic effects of RJ on diabetic testicular tissue.

Methods: Apoptosis, proliferation, and Leydig cell functions were determined using the following markers: transferase dUTP nick end labeling analysis, c-Jun N-terminal kinase (JNK), p38, Fas, and FasL for apoptosis; proliferating cell nuclear antigen (PCNA) and extracellular signal-regulated kinase (ERK) for proliferation; and insulin-like peptide-3 (INSL3) for Leydig cell function. A total of 20 Wistar albino male rats were randomly assigned to 3 experimental groups. The control group (C) and the diabetic group (D) were administered a single dose of 50 mg/kg STZ (intraperitoneally), and the RJ group was given 400 mg/kg/day (ig.) RJ after a single dose of 50 mg/kg STZ.

Results: While PCNA, ERK, and INSL3 immunoreactivities were significantly increased, the apoptotic cell and tubule indices, JNK, p38, Fas, and FasL immunoreactivities were significantly decreased in the RJ group compared to the D group.

Conclusion: Royal jelly may have a protective effect in preventing diabetes-related male infertility through apoptosis and anti-apoptotic pathways.

Keywords: Diabetic testes, Fas, FasL, Insulin-Like 3, mitogen-activated protein kinases, proliferating cell nuclear antigen

Introduction

Diabetes mellitus (DM) is 1 of the fastest-growing global health problems of the 21st century.[1] Diabetes mellitus is characterized by hyperglycemia and it has crucial complications such as angiopathy, neuropathy, and retinopathy. Additionally, male infertility associated with diabetes has been 1 of the frequently studied topics in recent years.[2-6] Due to the hyperglycemia caused by diabetes, the rate of free radical formation increases and oxidative stress occurs. Oxidative stress damages antioxidant defense systems, and diabetic mammalian spermatozoa are at increased risk of oxidative damage. Studies have shown that elevated reactive oxygen species (ROS) and free radical levels have a devastating effect on sperm motility in diabetic patients and reduce fertility. There are studies showing that oxidative stress and related germ cell apoptosis reduce fertility in male individuals with diabetes.[7-11]

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Royal jelly (RJ) and other apitherapy-derived products have been proposed as complementary agents for diabetes management due to their potential to alleviate disease-related symptoms. Royal jelly is a valuable bee product produced by the hypopharyngeal and mandibular glands of worker bees. It possesses strong antioxidant properties and has been extensively investigated for its potential therapeutic effects in the treatment of various diseases.[12-16]

The protective role of RJ in diabetes-induced testicular damage has been demonstrated in some studies.[14,15] However, the molecular factors responsible for the effect of RJ have not been fully elucidated.

For these reasons, this study investigated the RJ-induced changes in c-Jun N-terminal kinase (JNK), p38, Fas, and Fas ligand (FasL) proteins, which are known to have regulatory effects on apoptosis, in testicular tissue. In addition, the possible therapeutic effect was to be elucidated at the molecular level by determining the expression of insulin-like peptide-3 (INSL3), proliferating cell nuclear antigen (PCNA) and extracellular signal-regulated kinase (ERK) proteins to observe the effect of RJ on Leydig cells and spermatogonia.

Methods

This study did not use AI and/or LLM tools during the preparation of this manuscript.

Animals and Experimental Design

The study was approved by the local ethics committee of Trakya University (Approval No.: TUHADYEK-2016/38; Date: June 29, 2016) and 2017/155 (Decision date: March 31, 2017). A total of 20 male Wistar albino rats (weight: 260-340 g, age: 3 months) were obtained from the Experimental Animal Research Unit of Trakya University. The animals were housed under stable laboratory conditions at 22 ± 1°C with a 12 hour light: 12 hour dark cycle. The animal experimental procedures and data collection were conducted between October 2016 and June 2017.

Streptozotocin (STZ, Sigma–Aldrich, Taufkirchen, Germany) was used to establish a type I diabetes model. Streptozotocin is soluble and stable in 0.1 M citrate buffer (pH = 4.5, Thermo Scientific, Fremont, CA). Streptozotocin was administered as a single dose of 50 mg/kg. Blood glucose levels of all animals were measured using a commercial glucometer (IME-DC, Oberkotzau, Germany) before STZ treatment, 2 days after STZ administration, and every 7 days until the end of the experiment, and diabetes was confirmed by measuring blood glucose concentrations ≥250 mg/dL. At the end of the experiment, both testes were removed under anesthesia with Ketasol (Ricterpharma, Vienna, Austria) and Basilazin (Rompun, Istanbul, Türkiye), and the testes were weighed.

Twenty rats were divided into 3 groups. The control group (C) was treated only with citrate buffer (0.1 M, pH: 4.5) intraperitoneally (ip.); the diabetes group (D) was injected with a single dose of 50 mg/kg STZ dissolved in citrate buffer (0.1 M, pH: 4.5) intraperitoneally (ip).; the RJ was treated with 50 mg/kg STZ i.p.+400 mg/kg/day RJ intragastric (ig.) (diluted in sterile water after diabetes induction) for 4 weeks.[15]

Biochemical Analysis

The Advia Centaur XP device was used to measure testosterone levels from the cardiac blood samples.

Light Microscopy and Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling

The testes were fixed in 10% formalin (Sigma-Aldrich) for 24 hour. The testes were then washed, dehydrated, and cleared. The samples were embedded in paraffin. Sections were cut at 5 µm and stained with hematoxylin–eosin (H–E) to assess histological changes, mean seminiferous tubule diameter (MSTD). Transferase dUTP nick end labeling (TUNEL) was measured to determine apoptosis between groups.[17]

For TUNEL, 5 µm thick testis sections were incubated overnight at 37°C. The sections were passed through toluene, graded alcohol series and then washed with phosphate-buffered saline (PBS) (pH: 7.4, Invitrogen, California, USA). Sections were treated with proteinase K and then rinsed with distilled water. Subsequent steps were performed using the ApopTaq Plus Peroxidase in Situ Apoptosis Detection Kit (Chemicon SA, Merck Millipore) according to the manufacturer's instructions. Finally, the sections were passed through an ascending series of alcohols and toluene and mounted with Entellan (Invitrogen).

Immunohistochemistry Procedure

The expression levels of phosphorylated (p-) and total (t-) JNK, phosphorylated and total p38 MAPK, Fas/FasL, proliferating cell nuclear antigen (PCNA), phosphorylated and total extracellular signal-regulated kinase (ERK), and insulin-like peptide-3 (INSL3) were evaluated using immunohistochemical methods.

Paraffin-embedded sections with a thickness of 5 µm were kept in an oven at 56°C overnight. Following deparaffinization and rehydration, antigen retrieval was performed by boiling the sections in citrate buffer (pH 6; Invitrogen). The sections were subsequently treated with 3% H_2O_2 and then incubated with a blocking solution (Invitrogen). The sections were then treated with rabbit monoclonal p-JNK antibody (overnight, 1/50 dilution, cell signaling technology (CST-4668S), Massachusetts, USA), t-JNK antibody (overnight, 1/50 dilution, CST-9252), p-p38 MAPK (overnight, 1/200 dilution, CST-4511S), t-p38 MAPK (overnight, 1/50 dilution, CST-8690S), Fas antibody (1 hour room temperature, 1/100 dilution, Novus Biologicals (NB120-13550), Colorado, USA), FasL antibody (1 hour room temperature, 1/300 dilution, Abcam (ab15285), Cambridge, UK), rabbit monoclonal PCNA antibody (1 hour room temperature, 1/16000 dilution, CST-13110S), rabbit monoclonal pERK antibody (1/200 dilution, CST-4370S), rabbit monoclonal tERK antibody (overnight, 1/50 dilution, CST-4695S) and INSL3 antibody (1 hour room temperature, 1/500 dilution, Lifespan Biosciences (LSBio-C171758), Washington, USA). Diluent solution (Invitrogen) was applied to just negative control sections instead of the primary antibody. Following incubation with the appropriate secondary antibodies (Invitrogen) for 10 minutes, the sections were washed with PBS and subsequently exposed to horseradish peroxidase (HRP)-conjugated streptavidin (Invitrogen) for an additional 10 minutes. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB; Invitrogen) as the chromogen, followed by hematoxylin counterstaining.

Cell Counting and Statistical Analyses

Histological sections were evaluated using a light microscope (Olympus BX-51, Tokyo, Japan). The MSTD was determined on hematoxylin and eosin (H & E)-stained sections at ×200 magnification with the aid of an ocular micrometer. Ten randomly selected cross-sections of seminiferous tubules were evaluated for each specimen, and the mean value was calculated. The TUNEL technique was applied to testis sections to evaluate apoptotic cells and apoptotic tubule indices.[17,18] c-Jun N-terminal kinase, p38, and ERK immunoreactivities were assessed using a histological score (HSCORE).[19] Proliferating cell nuclear antigen, Fas, and FasL immunoreactivities were evaluated by counting the number of immunopositive cells per 1000 cells on each slide. Also, INSL3

immunoreactivities were scored as the number of immunopositive cells/1 mm²/10 different areas/slide.[18]

Statistical analyses were conducted using Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) at the Department of Biostatistics and Medical Informatics, Faculty of Medicine, Trakya University. Data were expressed as mean ± SD, and statistical significance was set at $P \leq .05$. The normality of quantitative variables was evaluated using the 1-sample Kolmogorov–Smirnov test. Non-normally distributed variables, including testosterone, PCNA, JNK, and MSTD, were compared between groups using the Mann–Whitney *U*-test. Normally distributed variables were analyzed by 1-way analysis of variance (ANOVA). For post hoc analyses, Tukey's test was applied to variables with homogeneous variances (p38 and TUNEL), whereas Tamhane's *T2*-test was used for variables with heterogeneous variances (INSL3, ERK, Fas, and FasL). Differences in body weight and blood glucose levels between groups were assessed using the independent samples *t*-test.

Results

Blood Glucose Levels, Body and Testis Weights, and Testosterone Levels

Blood glucose concentrations were markedly elevated in both the D and RJ groups compared with the C group ($P < .001$). Moreover, glucose levels were significantly higher in the RJ group than in the D group ($P = .002$; Table 1).

Final body weight was significantly reduced in the D and RJ groups relative to the C group ($P < .001$and $P = .002$, respectively; Table 1). Testicular weight was significantly lower in the D group than in the C group ($P = .018$; Table 1), whereas a significant increase was observed in the RJ group compared with the D group ($P = .044$; Table 1).

Serum testosterone levels were significantly lower in both the D and RJ groups than in the C group ($P = .002$ for both comparisons; Table 1). However, no significant difference was observed between the D and RJ groups ($P = .686$; Table 1).

Morphological and Morphometric Findings

In the H–E slide, the D group slides showed basement membrane undulation, disorganised seminiferous tubules, and spermatogenesis. In the RJ group, although some characteristics were similar to the D group, the histological appearance of the seminiferous tubules was similar to that of the control group (Figures 1a-c). When the MSTD values were examined among the groups, the values of the D and RJ groups were significantly decreased compared to the C group ($P < .001$, $P < .001$, respectively; Figure 1g). In addition, MSTD values increased in the RJ group compared to the D group ($P < .001$; Figure 1g).

The TUNEL method used to identify apoptotic cells, the apoptotic tubule index and apoptotic cell index were evaluated and no significant difference was observed in the RJ group compared to the C group (respectively $P = .093$ $P = .402$; Figures 1d,1f,1h, and1i), but a statistically significant decrease was observed compared to the D group (both $P < .001$; Figures 1e, 1f, 1h, and1i).

Immunohistochemical Findings

Both phosphorylated JNK (pJNK) and total JNK (tJNK) proteins were localized in the nuclei of cells in all experimental groups (Figures 2a-g). p-Jun N-terminal kinase immunoreactivity was significantly increased in the D group compared with the C group ($P < .001$; Figure 2d). In contrast, no significant difference was observed between the RJ and C groups ($P = .059$; Figure 2d). Weak pJNK immunoreactivity was detected in the C group (Figure 2a), whereas strong immunoreactivity was observed in the D group. The RJ group exhibited weak to moderate pJNK immunoreactivity (Figures 2b-d). The distribution pattern of tJNK immunoreactivity was similar to that of pJNK (Figures 2e-h). However, tJNK immunoreactivity was significantly higher in the RJ group than in the C group, while it was significantly lower than that observed in the D group ($P < .001$; Figure 2h). The tJNK immunoreactivity of the C group was weak in other cells of the spermatogenic series except primary spermatocytes (Figure 2e). In group D, moderate immunoreactivity was observed in a small number of spermatogonium and strong immunoreactivity in Sertoli cells and spermatocytes (Figure 2f). In the RJ group, there was usually weak and moderate immunoreactivity in primary spermatocytes, Sertoli cells and spermatogonium (Figure 2g).

The p-p38 immunoreactivity was generally detected in the cytoplasm and rarely in the nucleus (Figures 2i-k). The immunoreactivities in the D and RJ groups were higher than those in the C group ($P < .001$, $P < .001$; Figures 2i-l). In the RJ group, p-p38 immunoreactivity was found to be decreased compared to the D group ($P = .001$; Figures 2j-l). The t-p38 immunoreactivity was located in the nucleus in all sections (Figures 2m-o). This was significantly increased in the D and RJ groups compared to the C group section ($P < .001$, $P = .006$; Figures 2m-p), while the RJ values were significantly decreased compared to the D group ($P = .001$; Figures 2n-p).

Table 1. Blood Glucose Levels, Body and Testis Weights, and Testosterone Levels

	C	D	RJ
Initial blood glucose levels (mg/dL)	105.83 ± 8.280	94.00 ± 3.464	97.33 ± 4.131
End of experiment blood glucose levels (mg/dL)	108.67 ± 6.154	501.00 ± 22.984*	544.67 ± 23.712*,†
Initial body weights (g)	314.50 ± 20.560	305.63 ± 15.547	307.83 ± 13.615
Final body weights (g)	328.50 ± 18.163	214.88 ± 4.518*	243.83 ± 34.284*
Testis weights (g)	1.35 ± 0.120	1.16 ± 0.084*	1.33 ± 0.151†
Testosterone levels (mg/mL)	143.13 ± 38.277	34.08 ± 7.815*	39.46 ± 10.540*

RJ, royal jelly.
*Significant compared to the C.
†Significant compared to the D.
$P < .05$ was considered statistically significant.

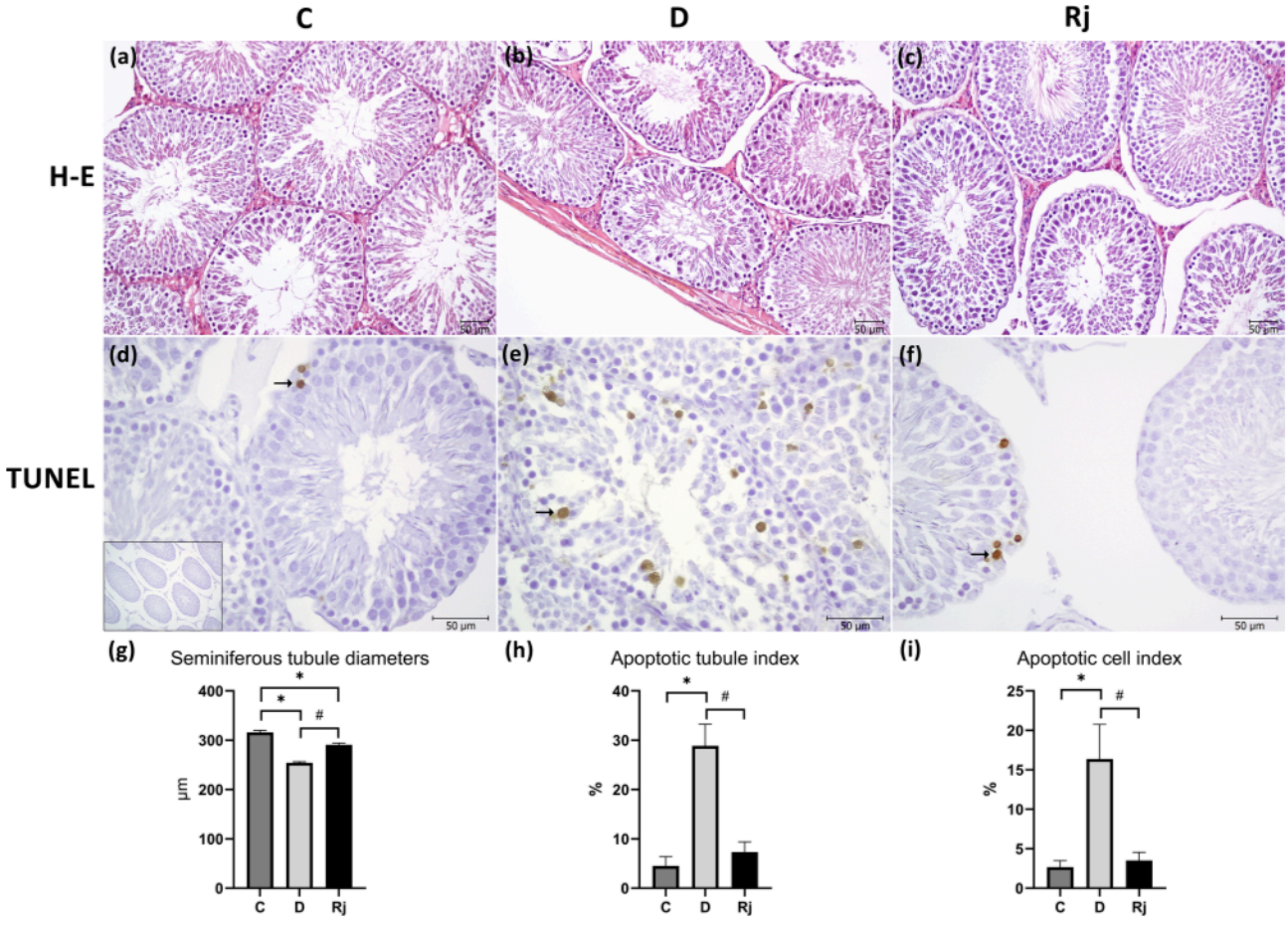


Figure 1. Sections with H–E staining (a-c), sections with TUNEL micrographs (d-f), graph of mean seminiferous tubule diameters (MSTD) (g), graphs of apoptotic tubule and apoptotic cell index (h-i). H–E, hematoxylin–eosin; MSTD, mean seminiferous tubule diameter; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

In all experimental groups, Fas immunoreactivity was generally detected in a small number of spermatogonia and some spermatocytes (Figures 2q-s). In the D and RJ groups, Fas immunoreactivity was significantly increased compared to the C group ($P < .001$, $P < .001$; Figures 2q-t), but decreased between RJ and D ($P < .001$; Figures 2r-t). FasL immunoreactivity was generally observed in Sertoli and Leydig cells of the C group sections (Figure 2u). In D and RJ groups, FasL immunopositivity was found not only in Sertoli and Leydig cells, but also in spermatogenic cells (Figures 2v-w). The number of FasL-immunopositive cells was higher in the D group than in the C group ($P < .001$; Figures 2u, 2v, and 2x), but it was observed that this immunoreactivity in the D group showed a significant decrease in the RJ group ($P < .001$; Figure 2v, 2w, and 2x).

A large number of immunopositive cells with nuclear localization were observed in PCNA immunostaining sections (Figures 3a-c). The proliferation indices of the D group were lower than those of the C group ($P < .001$; Figure 3d). In the RJ group, proliferation indices were found to be lower than in the C group ($P = .004$; Figure 3d), but increased compared to the D group ($P < .001$; Figure 3d).

Extracellular signal-regulated kinase immunoreactivity was observed at both cytoplasmic and nuclear levels (Figures 3e-k). The pERK immunoreactivity was significantly higher in the C group than in the D and RJ groups ($P < .001$, $P = .003$; Figures 3e-h). In the RJ group, pERK immunoreactivity is higher than in the D group ($P < .001$; Figures 3f-h). In the D and RJ groups, tERK immunoreactivities were found to be decreased compared to the C group ($P < .001$, $P = .001$; Figures 3i-k). When compared with the D group, tERK immunoreactivity was found to be increased in the RJ group ($P = .002$; Figures 3j-l).

The number of INSL3-positive cells was significantly decreased in the D group compared to the C group (Figures 3m,3n,3p). Although the number of INSL3-positive Leydig cells in the RJ group was decreased compared to the C group ($P < .001$), it was significantly increased compared to the D group ($P = .008$; Figures 3m-p).

Discussion

Diabetic male infertility is a topic with many unexplained research questions regarding its molecular mechanisms. The histopathologic, biochemical, and endocrinologic changes caused by diabetes in the male reproductive system have been revealed in many experimental studies.[7,18] Among the histopathologic changes, testicular apoptosis, abnormal spermatogenesis, and decreased seminiferous tubule diameter are the most obvious findings.[7,20,21]

In many studies with experimental diabetic rats, body weight and testicular weight were decreased, similar to the findings.[15,19,22] The positive change in the testicular histopathology in the RJ group was statistically verified by the decrease in the apoptotic tubule and cell indices through the increase in the MSTD and proliferation indices. As in many previous studies, the study also showed a significant decrease in the MSTD values of the D group and an

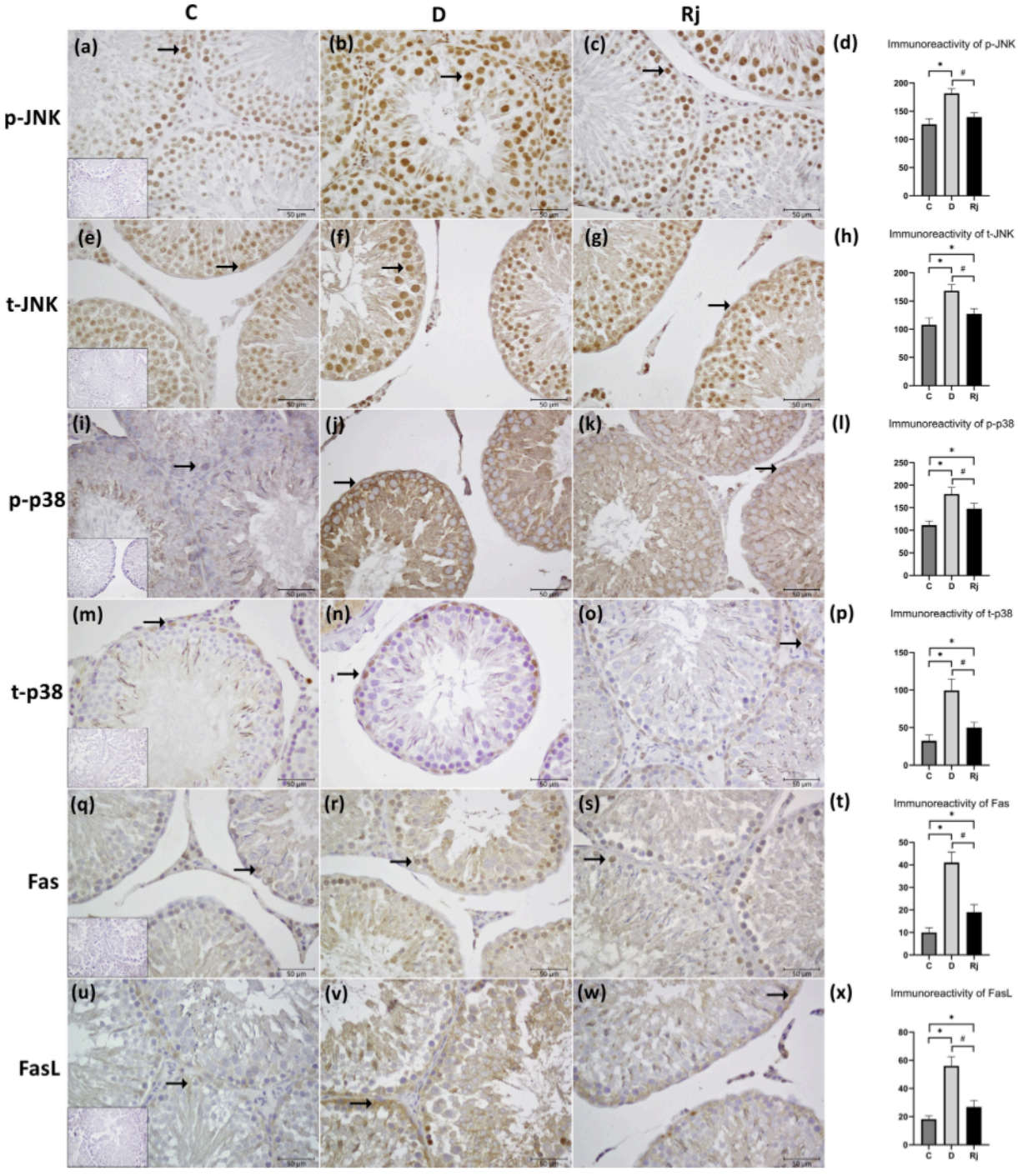


Figure 2. pJNK (a-d) and tJNK (e-h) micrographs and graphs, p-p38 (i-l) and t-p38 (m-p) micrographs and graphs, Fas (q-t) and FasL (u-x) micrographs, and graphs of testis tissue. *Compared with group C. ǂCompared with group D; inset: negative control, hematoxylin counterstaining. pJNK, p-Jun N-terminal kinase; tJNK, t-Jun N-terminal kinase.

increase in the RJ group compared to the D group, suggesting the antioxidant effect of RJ.[23-25]

Karaca et al suggested that testosterone levels were increased in the RJ group compared to the D group. In the study, it was observed that the increase in testosterone levels was not significant between the D and the RJ groups. The decrease in serum testosterone levels is parallel to both clinical and experimental findings.[15,22,23]

Testicular dysfunction in diabetic patients has been reported to be closely related to apoptosis.[19,24] Increased ROS caused by hyperglycemia leads to oxidative stress. Cytochrome C release from mitochondria leads to activation of caspases and induction of apoptosis.[15] In the study, analysis of the diabetic group revealed a significant increase in apoptotic tubule and cell indices, consistent with findings reported in previous studies.[26] The average values obtained in the apoptotic tubular and cell indices in the RJ group were almost equivalent to those in the C group. These results contribute to the anti-apoptotic activity of RJ. Other studies related to apoptosis in testicular tissues were conducted by Karaca et al and Ghanbari et al. Karaca et al[15,25] administered 400 mg/kg/day of RJ for 4 weeks and reported that caspase-3

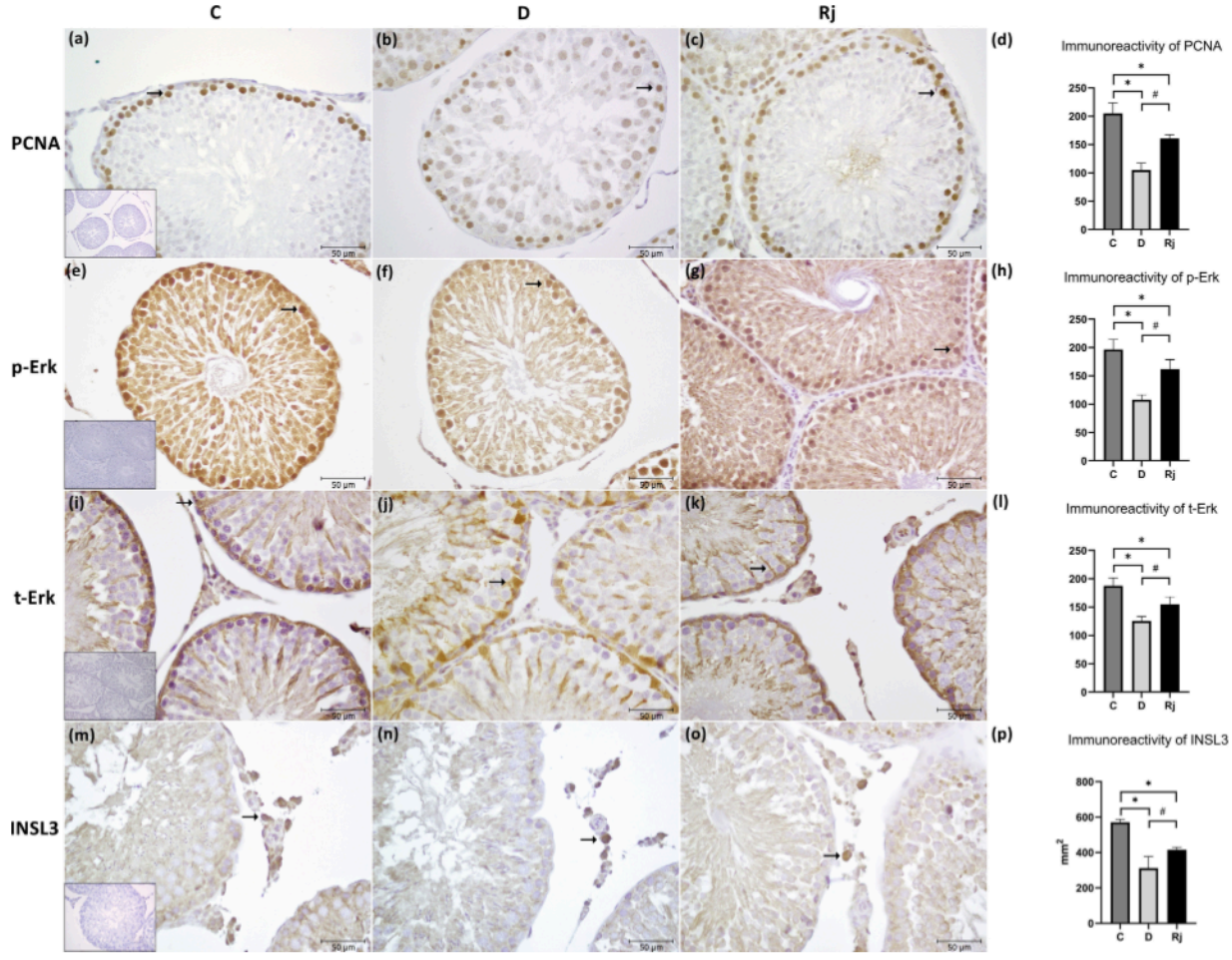


Figure 3. PCNA micrographs and proliferation indices graph (a-d), pERK (e-h) and tERK (i-l) micrographs and graphs, INSL3 (m-p) micrographs, and graph of testis tissue. *Compared with group C. ǂCompared with group D; inset: negative control, hematoxylin counterstaining. INSL3, insulin-like peptide-3; PCNA, proliferating cell nuclear antigen; pERK, extracellular signal-regulated kinase.

immunoreactivity was significantly decreased in diabetic subjects. Ghanbari et al administered 100 mg/kg/day RJ for 6 weeks and many reproductive parameters improved compared to diabetic subjects.

c-Jun N-terminal kinases, members of the MAPK family, not only regulate cell life and death but also control cell migration. c-Jun N-terminal kinase activity also includes cell differentiation, development, and apoptosis.[19,27] Another member of the MAPK family is p38 MAPK, which is activated by inflammatory cytokines and environmental stress. Phosphorylation of p38 MAPKs leads to pro-apoptotic signaling and cell apoptosis.[27,28] The ERK pathway is activated by growth factors. Activation of ERK is more important in cell differentiation, proliferation, and migration.[27] Koh[26] showed that apoptosis was increased as a result of activation of JNK and Bax signaling pathways in diabetic testis tissue. Donmez et al[19] evaluated MAPK immunoreactivity in STZ-induced diabetes. They found an increase in pJNK and a decrease in pERK expression in the diabetic group. These results are consistent with the findings. Kushawa and Jena[29] reported an increase in oxidative stress via activation of p53 and p38 MAPK signaling pathways, triggering apoptosis. Zhao et al suggested that diabetic testis was further increased by zinc deficiency and correlated with oxidative stress, p38 MAPK, and p53 activation.[28] In the study, while JNK and p38 protein expressions were increased in diabetic testis tissue, these expressions were significantly decreased in the RJ group. This outcome is likely attributable to the potent antioxidant properties of RJ.

Testicular germ cell apoptosis plays an important role in spermatogenesis. In diabetic men, the apoptotic index is known to increase in the testicular tissue.[15,17,25] Demirtas et al[30] similarly found an increase in TUNEL findings in the diabetic group. In the intrinsic pathway of apoptosis, Bcl-2 family proteins alter the permeability of the mitochondrial membrane. In the extrinsic pathway, apoptosis is initiated by transmembrane receptors. The association of the ligand and the counteracting lethal receptors (Fas/FasL interaction) causes the Fas receptor to bind to Fas-associated death domain (FADD).[17] Activation of the caspase pathway results in apoptosis. Bayram et al[17] investigated the Fas/FasL pathway in diabetic rat testis tissues and reported increased expression of caspase-3, caspase-8, and Fas and FasL. Furthermore, elevated Fas/FasL expression was previously identified in diabetic testicular tissues.[4] In the study, the expression of Fas and FasL was increased in the diabetic group, whereas the expression of Fas/FasL was decreased in the RJ group. This result may contribute to the reduction of ROS production by RJ.

Proliferating cell nuclear antigen immunoreactivity is often used to evaluate cell proliferation in a tissue (proliferation index). In the study, the proliferation indices of the diabetic subjects were significantly decreased, similar to the studies of Donmez et al. Ghanbari et al evaluated the spermiogenesis index (SPI), Sertoli cell index (SCI), and the meiotic index (MI) in the testicular tissues of the subjects.+ These parameters were increased with the administration of RJ. They concluded that the testicular damage

caused by diabetes was probably ameliorated by the antioxidant effect of RJ.

The other member of the MAPK family is ERK, which affects substrate phosphorylation and gene transcription, thereby regulating cell proliferation.[31] In the present study, both t-ERK and p-ERK immunoreactivities were significantly reduced in the D group, suggesting an association with decreased proliferation of spermatogenic cells. These findings are consistent with the reduced PCNA immunoreactivity observed in diabetic testicular tissue, supporting the role of ERK signaling in the regulation of spermatogenic cell proliferation.

In addition to testosterone levels, INSL3 is an important biomarker defining Leydig cell function.[32,33] Evaluation of INSL3 immunoreactivity revealed a significant reduction in diabetic testicular tissue, consistent with previous studies reporting decreased serum INSL3 levels under diabetic conditions. In a study of patients with type 2 diabetes, Ermetici et al[32] reported that serum INSL3 levels remained unchanged and were not significantly correlated with serum testosterone concentrations. In the present study, serum testosterone levels showed a non-significant increase in the RJ group compared with the D group, whereas INSL3 levels were significantly elevated in the RJ group. Ivell and Anand-Ivell[33] reported in their review that serum testosterone levels affect the feedback mechanisms in the hypothalamic-pituitary-testicular axis. Based on the findings, the accumulated data suggest an active involvement of Leydig cells and INSL3 in this interaction.

The findings demonstrated that RJ attenuated diabetes-induced testicular injury by suppressing the expression of the apoptotic proteins JNK, p38, Fas, and FasL. In contrast, the expression levels of PCNA, ERK, and INSL3 were markedly enhanced following RJ treatment. Collectively, these results indicate that RJ exerts a protective effect against diabetes-associated testicular damage by modulating molecular pathways involved in apoptosis, cell proliferation, and testicular function.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Artificial Intelligence Usage Statement: The authors declared that no artificial intelligence tool was used in the preparation of the manuscript.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of University of Trakya (Approval No.: 2016/38, 2017/155; Date: June 29, 2016).

Informed Consent: Informed consent was not applicable as this was an experimental animal study and did not involve human participants.

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Author Contributions: Concept – O.E., G.K.; Design – Ş.B., Ş.Y.; Supervision – G.K., Ö.D.; Resources – O.E., Ş.Y.; Materials – O.E., Ş.Y.; Data Collection and/or Processing – Ş.Y., Ş.B.; Analysis and/or Interpretation – O.E., G.K.; Literature Search – O.E., Ş.Y.; Writing – O.E., Ş.Y.; Critical Review – G.K., Ö.D.

Declaration of Interests: The authors have no conflicts of interest to declare.

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