Original Research

The effect of rhodiola rosea root extract on stress-induced ovarian damage, infertility and reproductive disorders in female rats

B.G. Kadioglu[1], B. Gundogdu[2], N. Kurt[3], A. Ozbek Bilgin[4], H. Suleyman[4,*], Z. Suleyman[5]

[1]*Department of Obstetrics and Gynecology, Regional Training Research Hospital, Health Sciences University, Erzurum (Turkey)*
[2]*Department of Pathology, Faculty of Medicine, Ataturk University, Erzurum (Turkey)*
[3]*Department of Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan (Turkey)*
[4]*Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan (Turkey)*
[5]*Department of Nursing, Faculty of Health Sciences, Erzincan Binali Yildirim University, Erzincan (Turkey)*

Summary

Purpose: The aim of the study was to examine the possible effect of Rhodiola rosea root extract (RRE) on ovarian damage, infertility (IF), delay in conception (DC), decrease in the number of offspring (NO) in female rats that have been stressed with compulsory immobilization method (CIM) and on retardation of intrauterine physical development (IPD) in offspring. *Materials and methods*: Female Albino Wistar rats were divided like: healthy (HG), stress-induced control (SC), and stress + 50 mg/kg RRE (S + REG) groups including sixteen rats in each group. Six rats from each group were killed for conducting biochemical and histopathological examinations in ovarian tissues and others were observed for IF, DC, NO, and IPD. *Results*: It was found that RRE significantly prevented the increase in oxidative parameters and proinflammatory cytokine levels in ovarian tissue of animals stressed with CIM. RRE suppressed the increase in IF rate and DC whereas caused a decrease in NO and in IPD retardation. *Conclusion*: These results suggested RRE may have beneficial effects in the treatment of stress induced reproductive dysfunction. *Content:* The effect of RRE on oxidative and inflammatory changes in ovarian tissue and reproductive changes of animals exposed to stress with compulsory immobilization method.

Key words: Ovarian damage; Stress; Infertility; Rhodiola rosea root.

Introduction

Infertility is known as the failure to achieve pregnancy after 12 months of unprotected intercourse in the literature [1]. Abnormalities such as tube damage, ovulation and implantation disorders are responsible for the infertility in women. These abnormalities leading to infertility are related with psychological stress [2]. Research has shown that chronic stress results in permanent and marked reproductive dysfunction, an increase in embryo resorption, and a decrease in conception rate [3, 4]. In fact, it is expressed in the literature that long-term or uncontrolled stress can adversely affect human health, including fertility [1]. It is also reported that stress-induced depression and anxiety can lead to infertility [5, 6]. Epidemiological studies have shown that depression and anxiety disorders occur simultaneously [7, 8]. Some of the studies show the increase of oxidative stress in depression and anxiety [9, 10]. In depressive disorders, an increase in the inflammatory response parallel to the increase in the production of proinflammatory cytokines is seen [11, 12]. Experimental studies have also shown that proinflammatory cytokines increase in anxiety disorders [13]. Therefore, oxidative stress and inflammation have been recognized among the basic reasons of reproductive dysfunction in many studies [14]. The act of oxidative stress and inflammation in pathologies like spontaneous abortions, embryopathies, preeclampsia, fetal growth restriction, preterm, and miscarriage affecting female reproductive processes is well known [15, 16]. This information suggests that an antioxidant, anti-inflammatory, antidepressant and anxiolytic agent may be effective in the treatment of stress-induced infertility and pathologies affecting reproductive processes. Rhodiola rosea root extract (RRE) used in the study is obtained from the root of Rhodiola rosea L. plant [17], which grows in the northern parts of Europe, Asia and the US. Preclinical studies have reported that RRE has anti-stress, neuropsychotropic, anxiolytic, antidepressant and neuroprotective effects [17, 18]. In addition, Rhodiola rosea extract has antioxidant and anti-inflammatory effects [19, 20]. No information was found in the literature about the effects of RRE in the treatment of stress-induced infertility and pathologies affecting reproductive processes. The present study aims to investigate the possible effect of RRE on ovarian damage, infertility (IF), delay in conception (DC), decrease in number of offspring (NO) and intrauterine physical development (IPD) retardation in experimental stress-induced rats.

Material and Methods

Animals

Forty-eight female albino Wistar rats weighing 265-272 grams were used in this study. The rats were obtained from Ataturk University Experimental Application and Research Center. The rats were housed and fed at standard room temper-

Table 1. — *Reproductive process and infertility of rats in experimental groups.*

Groups	Non-infertile rats n (%)	Infertile rats n (%)	p*	Delay in maternity period (days)	p**
HG (n = 6)	10 (100)	0 (0.0)		24 - 21 = 3	
SC (n = 6)	2 (20)	8 (80)	0.001	42 - 21 = 21	0.000
S + REG (n = 6)	7 (70)	3 (30)		27 - 21 = 6	

HG: healthy group, SC: stress control group, S + REG: stress + RRE group, * Pearson Chi-Square test.
** One-way analysis of variance (ANOVA) test followed by post-hoc Tukey test.

Table 2. — *Weight and gender differences of pups in experimental groups of rats*

Groups	Male pups				Female pups					Total weight
	n	Mean ± SD	p*	p**	n	Mean ± SD	p*	p**	n	Mean ± SD
HG	31	5.20 ± 0.47	-	<0.001	54	4.90 ± 0.66	-	<0.001	85	5.01 ± 0.62
SC	10	3.90 ± 0.42	< 0.001	-	5	3.70 ± 0.61	< 0.001	-	15	3.83 ± 0.48
S + REG	30	5.00 ± 0.79	0.629	<0.001	26	4.70 ± 0.44	0.605	0.001	56	4.91 ± 0.66

HG: healthy group, SC: stress control group, S + REG: stress + RRE group, SD: standard deviation.
*according to HG group, ** according to SC group.

ature (22 °C) before the experiment. The protocols and procedures were approved by the local Animal Experimentation Ethics Committee (Date: 27/06/2019, meeting no.: 7/120).

Chemicals

The ketamine was supplied from Pfizer (Turkey), and Rhodolia rosea root extract was supplied from Solgar (United States).

Experimental groups

Experimental animals were grouped as: healthy (HG), stress control (SC), and stress + RRE (S + REG) groups.

Experimental procedure

Widely known compulsory immobilization method (CIM) was used in order to create a stress model for animals [21]. All animals (except for the HG group) were brought to the supine position, feet and arms were tied and held in the same position for 24 hours to implement CIM. 50 mg/kg of RRE was orally applied to S + REG (n-16) rats at the end of this period. 50 mg/kg of RRE has also showed biological activity in previous studies [20]. Distilled water was administered to the SC (n-16) and HG (n-16) group animals in the same way and in equal doses as in S + REG group. CIM procedure was applied for three times with 10-day intervals. RRE was given once a day for 30 days. Then, six rats (n-6) from each group were killed with high dose anesthesia (50 mg/kg thiopental sodium) and ovarian tissues were taken for biochemical and histopathological examinations. The remaining animals were kept with mature male rats for two months in appropriate laboratory conditions for reproduction. The rats being pregnant during experimental process were taken to separate cages and kept in a suitable environment. Rats that were not conceived and gave birth within two months were accepted as infertile. DC, NO and IPD were also investigated. Furthermore, delay in maternity period was calculated by deducting the pregnancy period (21 days) from the period as of the coitus of the female and male rats until the delivery (A) (A-21 = delay in maternity period). The decrease in the number of offspring (NO) obtained

from the S+REG group was compared with HG. Weights of puppies born in SC and S + REG groups were compared with HG to assess the intrauterine physical development of puppies [21]. Experimental results were compared between groups and evaluated accordingly

Biochemical analyses

All tissues were rinsed with phosphate buffered saline solution prior to dissection. The liver tissues were homogenized in ice-cold phosphate buffers (50 mM, pH 7.4) appropriate for the variable to be measured [22]. The tissue homogenates were centrifuged at 4,000 rpm for 30 min at 4 °C, and the supernatants were extracted to analyze, malondialdehyde (MDA), Total Glutathione (tGSH), Total Anti-oxidant Status (TAS) and Total Oxidant Status (TOS), Nuclear Factor-Kappa B (NF-κB) and protein concentration. The protein concentration of the supernatant was measured using the method described by Bradford MM. The results were expressed by dividing to g protein [23]. All spectrophotometric measurements were performed via microplate reader (Bio-Tek, USA).

MDA assessment

The method used by Ohkawa *et al.* was considered for MDA assessment [24]. This method is based on the spectrophotometric assessment of the absorption of the pink-colored complex created by thiobarbituric acid (TBA) and MDA at high temperature (95 °C) at 532 nm wavelength. The amounts of MDA of the samples were determined by using the standard graphics formed with the MDA stock solution previously prepared considering the dilution coefficients.

tGSH assessment

The DTNB (5.5'-Dithiobis (2-nitrobenzoic acid)) in the assessment environment is a disulphide chromogen and is easily reduced by sulfhydryl group compounds. The resultant yellow color was spectrophotometrically assessed at 412 nm [25]. The GSH amounts of the samples were determined by using the standard graphics formed with the GSH stock solution previously prepared considering the dilution coefficients.
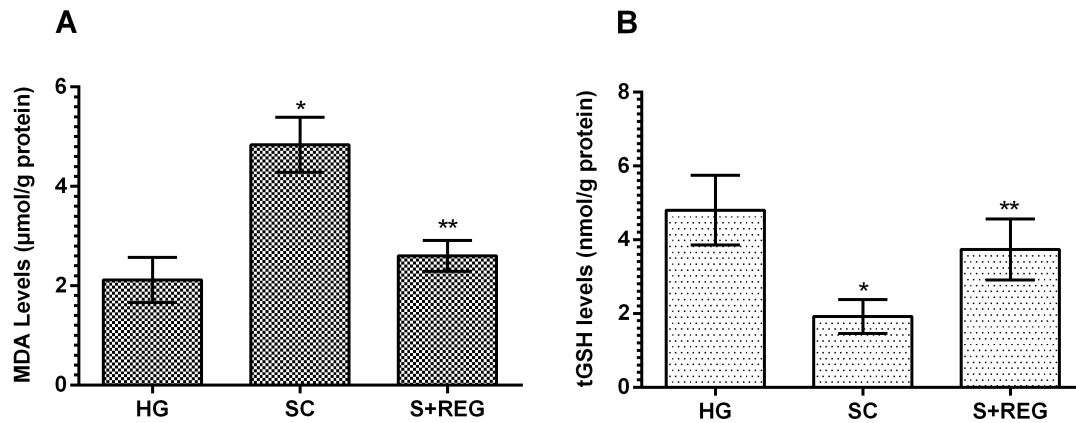
Figure 1. — MDA and tGSH levels in ovarian tissues of experimental groups. * $p < 0.001$, according to HG and S + REG group, ** $p > 0.05$, according to HG group.
HG: healthy group, SC: stress control group, and S+REG: stress + 50 mg/kg Rhodiola rosea root extract group. MDA: Malondialdehyde, tGSH: Total Glutathione, p according to one-way analysis of variance test followed by post-hoc Tukey test.
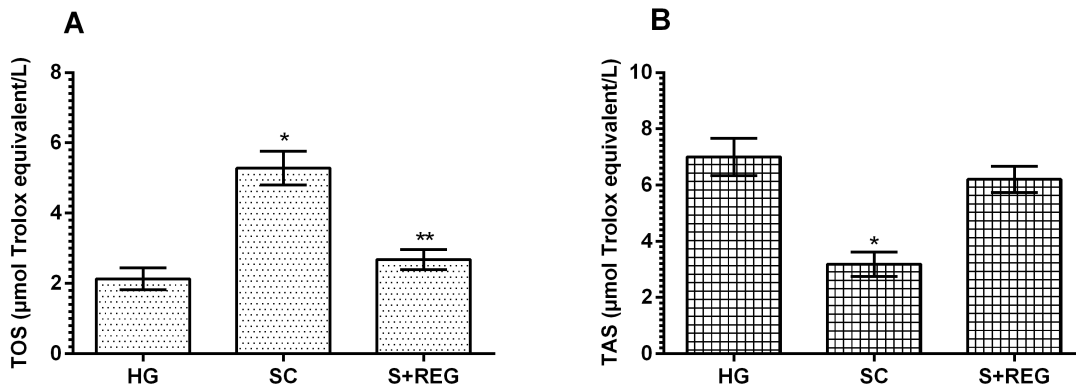

Figure 2. — Oxidant/antioxidant balance in the ovarian tissues of experimental groups. * $p < 0.001$, according to HG and S + REG group, ** $p > 0.05$, according to HG group.
HG: healthy group, SC: stress control group, and S + REG: stress + 50 mg/kg Rhodiola rosea root extract group. TAS: Total Anti-oxidant Status and TOS: Total Oxidant Status, p according to one-way analysis of variance test followed by post-hoc Tukey test.
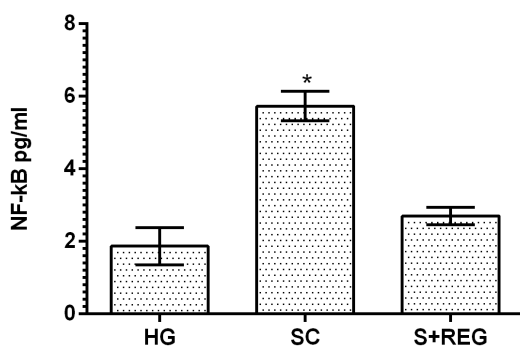

Figure 3. — NF-κB levels in in the ovarian tissues of experimental groups. * $p < 0.001$, according to HG and S + REG group.
HG: healthy group, SC: stress control group, and S + REG: stress + 50 mg/kg Rhodiola rosea root extract group. NF-κB: Nuclear Factor-Kappa B, p according to one-way analysis of variance test followed by post-hoc Tukey test.

Measurement of TOS and TAS

TOS and TAS levels of tissue homogenates were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Turkey) developed by Erel [26, 27]. The results were expressed as μmol Trolox equivalent/L. The percentage ratio of TOS to TAS was used as the oxidative stress index (OSI). OSI was calculated as TOS divided by $100 \times$ TAS.

NF-κB analysis in tissue

Tissue-homogenate NF-κB concentrations were measured using rat-specific sandwich enzyme-linked immunosorbent assay Rat Nuclear Factor- Kappa B ELISA immunoassay kits (Cat. No.: 201-11-0288, SunRed). Analyses were performed according to the instructions of the manufacturer. Briefly, monoclonal antibody specific rat NF-κB was coated onto the wells of the micro plates. The tissue homogenate, standards and biotinylated monoclonal antibody specific and streptavidin-HRP were pipetted into these wells and then incu-
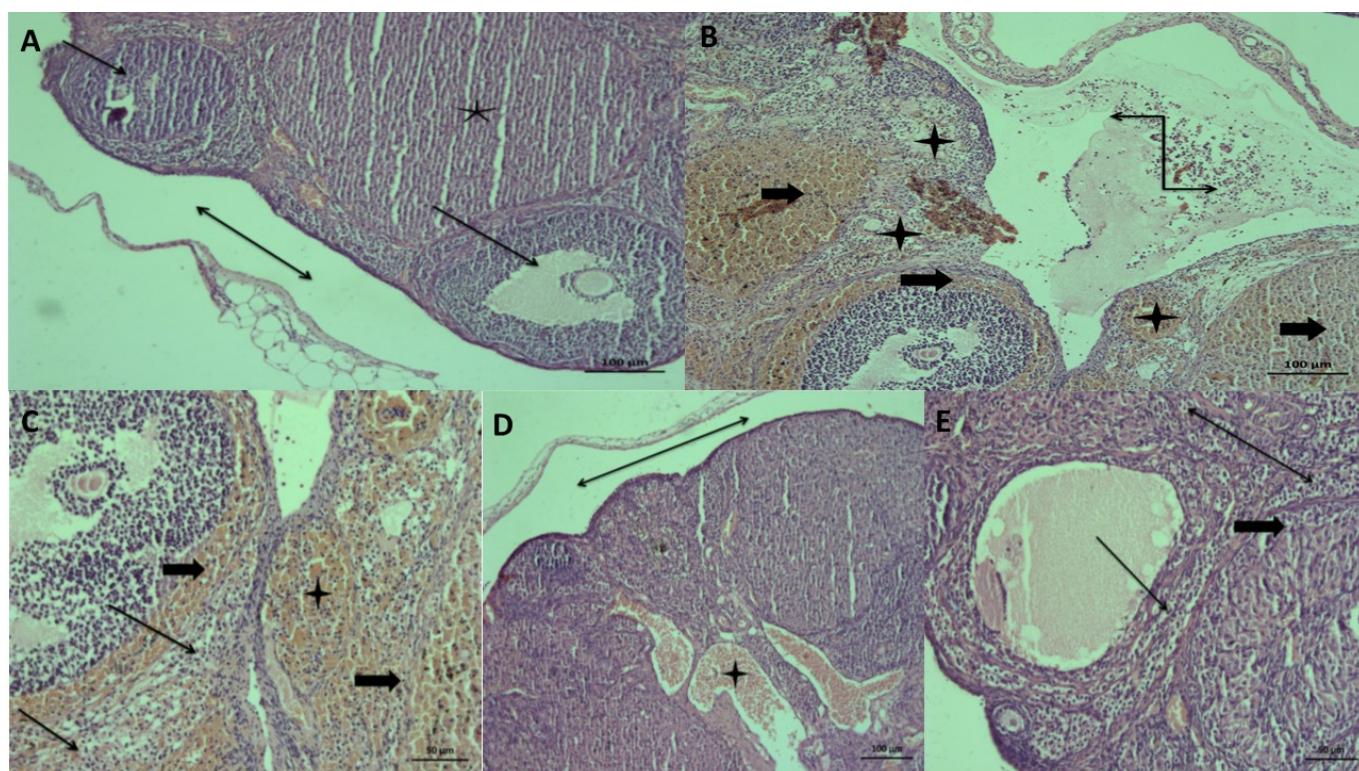
Figure 4. — Histopathological changes of the experimental groups. (A) The ovarian tissue section of healthy group: the corpus luteum (star), developing follicular structures (arrows), subcapsular area (bidirectional arrow) (H&E, × 100). (B) Ovarian tissue of the stressed group section: diffuse congestion (stars) and hemorrhage (block arrows), fluid accumulation and inflammatory cell infiltrate in subcapsular area (black zigzag arrows), (H&E, × 100). (C) Ovarian tissue of the stressed group section: necrotic changes (arrow), congestion (star) and hemorrhage (block arrow) (H&E, × 200) (D) The ovarian tissue of the RRE-treated group: mild congestion (asterisk), subcapsular area without fluid accumulation and inflammatory cell infiltrate (bidirectional arrow) (H&E, × 100). (E) Ovarian tissue section of the RRE-treated group showing mild necrosis in the parenchymal stroma (bidirectional arrow), follicular structure (arrows) and corpus luteum (block arrow) (H&E, × 200).

bated at 37 °C for 60 min. After washing, chromogen reagent A and chromogen reagent B were added acting by the bound enzyme to produce a blue color. It was incubated at 37 °C for 10 min. Then H_2SO_4 was added. The intensity of yellow color formed was directly proportional to the concentration of rat NF-κB present in the original specimen. The well plates were read at 450 nm and the absorbance of the samples was calculated with formulations using standard graphics at the end of the course.

Histopathological examination

All of the tissue samples were first fixed in a 10% formaldehyde solution for light microscope assessment. Following the identification process, tissue samples were washed under tap water in cassettes for 24 hours. Samples were then treated with conventional grades of alcohol (70%, 80%, 90%, and 100%) to remove the water within tissues. Tissues were then passed through xylol and embedded in paraffin. Four-to-five micron sections were cut from the paraffin blocks and hematoxylin-eosin staining was administered. Their photos were taken following the Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan) assessment. Histopathologial assessment was carried out by the pathologist blind for the study groups.

Statistical analysis

All statistical analyses were performed using "SPSS statistical software" (Version 18) and p values < 0.05 were considered significant. The results were expressed as "mean ± standard error of mean" (x ± SEM). The significance of the difference between the groups was determined using one-way analysis of variance (ANOVA) test followed by post-hoc Tukey test.

Results

Biochemical findings

1) The results of MDA and tGSH

As can be seen in Figure 1, the amount of MDA significantly increased in the ovarian tissue of the animals treated with immobilization method compared to healthy group and REG group ($p < 0.001$). The amount of MDA in REG group was not statistically significant according to healthy controls ($p > 0.05$). Stress significantly decreased the amount of tGSH in the ovarian tissue of the animals compared to healthy and REG group ($p < 0.001$). The amount of tGSH in REG group was statistically insignificant compared to healthy controls ($p > 0.05$).

2) The results of TOS and TAS analysis

It was found that the balance between TOS and TAS levels in the ovarian tissue of stress-treated animals changed in favor of TOS. The TOS level in the stress-treated group increased significantly compared to the healthy group and REG group ($p < 0.001$), whereas the TAS level decreased significantly ($p < 0.001$). As seen in Figure 2, the TOS levels in the ovarian tissue of RRE administered animals were not statistically significant compared to healthy group ($p > 0.05$). Moreover, the TAS levels in the ovarian tissue of RRE administered animals were close to healthy group ($p = 0.048$).

3) NF-κB analysis results

Stress caused an increase in NF-κB levels in the ovarian tissue of the animals. NF-κB levels significantly increased in the ovarian tissue of the stressed group compared to healthy and REG group ($p < 0.001$) as can be seen in Figure 3.

Reproductive test results

As shown in Table 1, two of the ten reproductive female rats in SC group gave birth whereas the remaining 8 female rats did not give birth in two months. The mean duration of maternity of the animals in the SC group was 42 days in SC group and 27 days in S + REG group. 7 of 10 female rats gave birth and 3 of them were infertile. All female rats in HG group gave birth within 24 days.

As can be seen in Table 2, two female rats gave birth to 10 males, with the average weight of 3.9 g, and 5 female offspring weighing 3.7 g in the SC group. 7 female rats who were pregnant gave birth to 3 males with the average weight of 5.0 g and 26 female pups with the average weight of 4.7 grams in the REG group. In the SG animal group, 10 pregnant rats gave birth to an average of 5.2 grams, 31 male and 54 female pups weighing 4.9 grams.

Histopathological findings

Figure 4A shows normal corpus luteum, developing follicular structures, and subcapsular area in ovarian tissue of the healthy group. In the ovarian tissue of the stressed animals, diffuse congestion, hemorrhage, sporadic accumulation of fluid and inflammatory cell clusters/infiltrates in the subcapsular area, and necrotic areas of the stroma in the parenchyma were detected (Figure 4B). Mild congestion was observed in the ovarian tissue of the RRE treated group. Fluid accumulation and inflammatory cell infiltrates in the subcapsular area were not observed. In addition, mild necrotic areas were found in the stroma in the parenchyma, follicle structure, and corpus luteum (Figure 4C).

Discussion

The possible effects of RRE on ovarian damage and reproductive process disorders were experimentally analyzed in female rats in the study. Our biochemical results showed that MDA, TOS and NF-κB levels in ovarian tissue of animals stressed with CIM increased whereas tGSH and TAS levels decreased compared to healthy and RRE groups. These results indicate that experimental stress changes the oxidant/antioxidant balance in the ovarian tissue in favor of oxidants. This situation is expressed as oxidative stress in the

literature [28, 29]. MDA being high in ovarian tissue of the stressed group is a toxic product of lipid peroxidation initiated by reactive oxygen species (ROS) causing cellular damage [30, 31]. It has been reported that serum MDA levels of patients with anxiety and various psychological disorders are higher than that of healthy controls [32, 33] and they return to normal with citalopram treatment [10]. The amount of tGSH in the ovarian tissue of the stressed group with elevated MDA was found to be lower compared to healthy and RRE groups in the present study. GSH which is an endogenous antioxidant molecule in tripeptide form detoxifies hydrogen peroxide and organic peroxides and protects cells from ROS damage [34]. A decrease in plasma antioxidant system has been reported in rats exposed to stress [35]. The oxidant/antioxidant balance in the ovarian tissue of the stressed group was also shown to be impaired in favor of oxidants by measuring TOS and TAS levels. TOS is used to determine the cumulative oxidative effects of various oxidants in biological systems [27], while TAS is used to determine the cumulative antioxidative effects of antioxidants [26]. In the present study, it was also observed that NF-κB levels significantly increased in ovarian tissue of stressed animals compared to healthy and RRE groups. As is known, NF-κB is an inducible transcription factor that plays a central role in immune and inflammatory responses [36]. Activated by excessive mechanical stress, NF-κB stimulates the release of inflammatory cytokines such as IL-1β, TNF- and IL-6 in cells [36, 37]. These cytokines increase in stress, anxiety and depressive disorders, and also increase the inflammatory response in the ovarian tissue [11-13]. Oral administration of RRE antagonized the stress-induced increase of MDA, TOS and NF-κB in ovarian tissue and decrease of tGSH and TAS. This effect of RRE may be due to its anti-stress, antidepressant and anxiolytic properties. In a clinical study, it was observed that the stress response decreased in patients treated with RRE [38]. In another clinical study, it was reported that RRE had an antidepressant effect [39]. It has also been found in animal models that RRE has an anxiolytic effect [17]. In the literature, it has been reported that the antioxidant effect of RRE is due to the active ingredient of oligomeric proanthocyanide in the content of Rhodiola rosea [40]. The fact that RRE inhibited stress-induced NF-κB increase in ovarian tissue indicates that it has both antioxidant and anti-inflammatory effect. It is known that inflammatory response increases parallel to the increase in production of cytokines in stress, depression and anxiety disorders [11-13].

It has been shown that stress results in reproductive dysfunction, increased embryo resorption, and decreased pregnancy rate in many studies [3, 4]. In our study, infertility developed in 80% of the stress group animals with high oxidant and NF-κB levels. Administration of RRE reduced stress-induced infertility in animals by 70%. These experimental results are consistent with the following literature data. Previous studies have shown that psychological disorders, oxidative stress and inflammation are associated with infertility [1, 5, 6, 14]. Aksoy *et al.* have showed that oxidative stress is one of the main causes of infertility due to ovarian I/R injury [41]. Unlubilgin

et al. have stated that proinflammatory cytokines play a role in the development of infertility in rats [42]. The benefit of Rhodiola rosea extract in the treatment of various psychological disorders is well known [17, 18]. In addition, RRE has been shown to have antioxidant and anti-inflammatory effect, and inhibitory effect on IL-1β, TNF-α and IL-6 production [19, 20, 42]. Data showing that psychological disorders negatively affect duration of conception are available in the literature [43]. In our study, duration of conception was prolonged in the stress group where oxidative and proinflammatory cytokine levels elevated compared to the healthy and RRE treated groups. This shows that oxidative stress and inflammation associated with psychological stress delay conception time. Aksoy *et al.* also showed that oxidative stress adversely affected the duration of conception [41]. Unlubilgin *et al.* have showed that the increase in proinflammatory cytokine levels in ovarian tissue prolongs duration of conception [42]. In the present study, it was found that the decrease in the number of offspring in the stress group was directly proportional to the increase in oxidant and proinflammatory parameters. The fact that the decrease in the number of offspring is related to oxidative stress is supported by previous studies [44]. Moreover, significant IPD retardation was found in animals exposed to stress with CIM in the present study. Weights of the pups born in the stressed group were found to be significantly lower compared to that of healthy and RRE-treated groups. Birth weight is considered to be an indicator of IPD [45, 46]. It has been reported that high oxidant parameters and low antioxidant parameters in ovarian tissue negatively affect IPD [40]. Furthermore, the role of oxidative stress and inflammation in pathologies such as embryopathies and fetal growth restriction is well known [15, 16]. The fact that oxidant and proinflammatory cytokine levels in ovarian tissue of animals treated with RRE is close to that of the healthy group and that the birth weight of the offspring is less affected by stress coincides with the literature. Diffuse congestion, hemorrhage, subcapsular fluid accumulation and inflammatory cell infiltrates, and necrotic areas in the parenchymal stroma were detected in the ovarian tissue of the stress group where oxidative stress and inflammation markers showed a significant increase. However, ovarian tissue treated with RRE was found to be normal except for mild congestion and mild necrotic areas. Oxidative stress and proinflammatory cytokine elevation have been reported to cause hemorrhage, congestion, follicle degeneration, inflammatory cell infiltration and necrosis in ovarian tissue. In addition, it has been noted that this histopathological damage causes infertility and delayed conception in animals [41].

Conclusion

In conclusion, we have demonstrated that oxidative and inflammatory damage develops in ovarian tissue of animals exposed to stress with CIM by using biochemical and histopathological methods. CIM-related stress negatively affected fertility, duration of conception, number of offspring and IPD in female rats. RRE reduced the severity of stress-related oxidative and inflammatory damage in the ovarian tissue. In addition, RRE antagonized the negative effect of stress on reproductive function and IPD. These results suggest that RRE may be useful in the treatment of ovarian damage and reproductive dysfunction due to psychological stress.

Abbreviations

CIM, Compulsory immobilization method; DC, Delay in conception; IF, Infertility; IPD, Intrauterine physical development; MDA, Malondialdehyde; NF-κB, Nuclear Factor-Kappa B; NO, decrease in the number of offspring; tGSH, Total glutathione; GSHRd, Glutathione reductase; RRE, Rhodiola rosea root extract; TAS, Total anti-oxidant status; TOS, Total oxidant status.

References

[1] Mascarenhas M.N., Flaxman S.R., Boerma T., Vanderpoel S., Stevens G.A.: "National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys". *PLoS Med.*, 2012, *9*, 1001356.

[2] Sominsky L., Hodgson D.M., McLaughlin E.A., Smith R., Wall H.M., Spencer S.J.: "Linking stress and infertility: a novel role for ghrelin". *Endocr. Rev.*, 2017, *38*, 432-467.

[3] Geraghty A.C., Muroy S.E., Zhao S., Bentley G.E., Kriegsfeld L.J., Kaufer D.: "Knockdown of hypothalamic RFRP3 prevents chronic stress-induced infertility and embryo resorption". *eLife*, 2015, *4*, 04316.

[4] Young A.J., Carlson A.A., Monfort S.L., Russell A.F., Bennett N.C., Clutton-Brock T.: "Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats". *Proc. Natl. Acad. Sci.*, 2006, *103*, 12005-120010.

[5] Damone A.L., Joham A.E., Loxton D., Earnest A., Teede H.J., Moran LJ.: "Depression, anxiety and perceived stress in women with and without PCOS: a community-based study". *Psychol. Med.*, 2019, *49*, 1510-1520.

[6] LakatosE., Szigeti J.F., Ujma P.P., Sexty R., Balog P.: "Anxiety and depression among infertile women: a cross-sectional survey from Hungary". *BMC Women's Health*, 2017, *17*, 48.

[7] Kessler R.C., Gruber M., Hettema J.M., Hwang I., Sampson N., Yonkers K.A.: "Co-morbid major depression and generalized anxiety disorders in the National Comorbidity Survey follow-up". *Psychol. Med.*, 2008, *38*, 365-374.

[8] Pirkola S.P., Isometsä E., Suvisaari J., Aro H., Joukamaa M., Poikolainen K., *et al.*: "DSM-IV mood-, anxiety-and alcohol use disorders and their comorbidity in the Finnish general population". *Soc. Psych. Psychiatr. Epidemiol.*, 2005, *40*, 1.

[9] Rigucci S., Serafini G., Pompili M., Kotzalidis G.D., Tatarelli R.: "Anatomical and functional correlates in major depressive disorder: the contribution of neuroimaging studies". *World J. Biol. Psychiatry*, 2010, *11*, 165-180.

[10] Atmaca M., Tezcan E., Kuloglu M., Ustundag B., Tunckol H.: "Antioxidant enzyme and malondialdehyde values in social phobia before and after citalopram treatment". *Eur. Arch. Psychiatry. Clin. Neurosci.*, 2004, *254*, 231-235.

[11] Irwin M.: "Immune correlates of depression". *Adv. Exp. Med. Biol.*, 1999, *461*, 1-24.

[12] Maddock C., Pariante C.M.: "How does stress affect you? An overview of stress, immunity, depression and disease". *Epidemiol Psychiatr Sci.*, 2001, *10*, 153-162.

[13] Alzarea S., Rahman S.: "Alpha-7 nicotinic receptor allosteric modulator PNU120596 prevents lipopolysaccharide-induced anxiety, cognitive deficit and depression-like behaviors in mice". *Behav. Brain. Res.*, 2019, *366*, 19-28.

[14] Abolaji A.O., Adedara I.A., Abajingin A.O., Fatunmibi O.J., Ladipo E.O., Farombi E.O.: "Evidence of oxidative damage and reproductive dysfunction accompanying 4-vinylcyclohexene diepoxide exposure in female Wistar rats". *Reproductive Toxicol.*, 2016, *66*, 10-19.

[15] Al-Gubory K.H., Fowler P.A., Garrel C.: "The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes". *Int. J. Biochem. Cell Biol.*, 2010, *42*, 1634-1650.

[16] Kalagiri R.R., Carder T., Choudhury S., Vora N., Ballard A.R., Govande V., *et al*.: "Inflammation in complicated pregnancy and its outcome". *Am. J. Perinatol.*, 2016, *33*, 1337-1356.

[17] Coors A., Brosch M., Kahl E., Khalil R., Michels B., Laub A., *et al*.: "Rhodiola rosea root extract has antipsychotic-like effects in rodent models of sensorimotor gating". *J. Ethnopharmacol.*, 2019, *235*, 320-328.

[18] Juřica J., Koupá T.: "Rhodiola rosea and its neuropsychotropic effects". *Ceska. Slov. Farm.*, 2016, *65*, 87-93. [In Czech]

[19] Palumbo D.R., Occhiuto F., Spadaro F., Circosta C.: "Rhodiola rosea extract protects human cortical neurons against glutamate and hydrogen peroxide-induced cell death through reduction in the accumulation of intracellular calcium". *Phytother. Res.*, 2012, *26*, 878-883.

[20] Doncheva N.D., Mihaylova A.S., Getova D.P.: "Antinociceptive and anti-inflammatory effects of Rhodiola rosea L. Extract in rats". *Fol. Med.*, 2013, *55*, 70-75.

[21] Aynaoglu Yildiz G., Yildiz D., Yapca O.E., Suleyman B., Arslan Y.K., Kurt N, *et al*.: "Effect of diazepam, sertraline and melatonin on the stress-induced reproductive disorders and intrauterine growth restriction in female rats". *J. Matern. Fetal Neonatal. Med.*, 2019, 1-7.

[22] Weydert C.J., Cullen J.J.: "Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue". *Nat. Protoc.*, 2010, *5*, 51-66.

[23] Bradford M.M.: "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". *Anal. Biochem.*, 1976, *72*, 248-254.

[24] Ohkawa H., Ohishi N., Yagi K.: "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". *Anal. Biochem.*, 1979, *95*, 351-358.

[25] Sedlak J., Lindsay R.H.: "Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent". *Anal. Biochem.*, 1968, *25*,192-205.

[26] Erel O.: "A novel automated method to measure total antioxidant response against potent free radical reactions". *Clin. Biochem.*, 2004, *37*, 112-119.

[27] Erel O.: "A new automated colorimetric method for measuring total oxidant status". *Clin. Biochem.*, 2005, *38*, 1103-1111.

[28] Yeum K.J., Russell R.M., Krinsky N.I., Aldini G.: "Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma". *Arch. Biochem. Biophys.*, 2004, *430*, 97-103.

[29] Kisaoglu A., Borekci B., Yapca O.E., Bilen H., Suleyman H.: "Tissue damage and oxidant/antioxidant balance". *Eurasian J. Med.*, 2013, *45*, 47-49.

[30] Gutteridge J.: "Lipid peroxidation and antioxidants as biomarkers of tissue damage". *Clin. Chem.*, 1995, *41*, 1819-1828.

[31] Davies K., Goldberg A.: "Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes". *J. Biol. Chem.*, 1987, *262*, 8220-8226.

[32] Kuloglu M., Atmaca M., Tezcan E., Gecici Ö., Tunckol H., Ustundag B.: "Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder". *Neuropsychobiology*, 2002, *46*, 27-32.

[33] Kuloglu M., Atmaca M., Tezcan E., Ustundag B., Bulut S.: "Antioxidant enzyme and malondialdehyde levels in patients with panic disorder". *Neuropsychobiology*, 2002, *46*, 186-189.

[34] Süleyman H., Gül V., Erhan E.: "Oksidatif stres ve doku hasarı". *Erzincan Tıp Dergisi.*, 2018, 1. [In Turkish]

[35] Liu J., Wang X., Mori A.: "Immobilization stress-induced antioxidant defense changes in rat plasma: effect of treatment with reduced glutathione". *Inter. J Biochem.*, 1994, *26*, 511-517.

[36] Hayden M.S., Ghosh S.: "NF-κB, the first quarter-century: remarkable progress and outstanding questions". *Genes Dev.;* 2012, *26*, 203-234.

[37] Choi M.C., Jo J., Park J., Kang H.K., Park Y.: "NF-κB Signaling Pathways in Osteoarthritic Cartilage Destruction". *Cells*, 2019, *8*, 734.

[38] Olsson E.M., von Schéele B., Panossian A.G.: "A randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract shr-5 of the roots of Rhodiola rosea in the treatment of subjects with stress-related fatigue". *Planta Med.*, 2009, *75*, 105-112.

[39] Darbinyan V., Aslanyan G., Amroyan E., Gabrielyan E., Malmström C., Panossian A.: "Clinical trial of Rhodiola rosea L. extract SHR-5 in the treatment of mild to moderate depression". *Nord. J. Psychiatry*, 2007, *61*, 343-348.

[40] Zhou Q., Yin Z.P., Ma L., Zhao W., Hao H.W., Li H.L.: "Free radical-scavenging activities of oligomeric proanthocyanidin from Rhodiola rosea L. and its antioxidant effects in vivo". *Nat. Prod. Res.*, 2014, *28*, 2301-2303.

[41] Aksoy A.N., Aydın F., Yılmaz E.P.T., Batmaz G., Suleyman B.: "The Effect of Controlled Reperfusion in the Prevention of Infertility Caused by Ischemia Induced in the Contralateral Ovary in Rats with Unilateral Ovariectomy". *Gynecol. Obstet Invest.*, 2015, *80*, 199-205.

[42] Unlubilgin E., Suleyman B., Balci G., Al R.A., Cankaya M., Nayki U.A., *et al*.: "Prevention of infertility induced by ovarian ischemia reperfusion injury by benidipine in rats: Biochemical, gene expression, histopathological and immunohistochemical evaluation". *J. Gynecol. Obstet. Hum. Reprod.*, 2017, *46*, 267-273.

[43] Zhou Q., Han X., Li R., Zhao W., Bai B., Yan C., *et al*.: "Anti-atherosclerosis of oligomeric proanthocyanidins from Rhodiola rosea on rat model via hypolipemic, antioxidant, anti-inflammatory activities together with regulation of endothelial function". *Phytomedicine*, 2018, *51*, 171-180.

[44] Smeenk J., Verhaak C., Eugster A., Van Minnen A., Zielhuis G., Braat D.: "The effect of anxiety and depression on the outcome of in-vitro fertilization". *Hum. Reprod.*, 2001, *16*, 1420-1423.

[45] Yapca O.E., Kumbasar S., Salman S., Yarali O., Sener E., Mammadov R., *et al*.: "Controlled reperfusion for different durations in the treatment of ischemia–reperfusion injury of the rat ovary: evaluation of biochemical features, molecular gene expression, and histopathology". *Can J Physiol Pharmacol.*, 2015, *93*, 269-274.

[46] Rödel H.G., Prager G., Stefanski V., von Holst D., Hudson R.: "Separating maternal and litter-size effects on early postnatal growth in two species of altricial small mammals". *Physiol. Behav.*, 2008, *93*, 826-834.

Corresponding Author:
HALIS SULEYMAN, M.D., Ph.D.
Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University,
24100, Erzincan (Turkey)
e-mail: halis.suleyman@gmail.com