EXPERIMENTAL STUDY

Protective role of melatonin against testicular damage caused by polymicrobial sepsis in adult rats

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ABSTRACT

BACKGROUND: This study aimed to investigate the possible protective effects of melatonin (MEL) against the damage to testicular tissue in rats caused by polymicrobial sepsis as a result of cecal ligation and perforation (CLP).

METHODS: In this study, 21 male Wistar albino rats were used. The rats were randomly divided into three groups (n=7): Sham Control (Group 1), CLP (Group 2), and CLP + MEL (Group 3). Sepsis was created using the CLP method. MEL was administered intraperitoneally in two equal doses of 10 mg/kg at 30 min before and 6 h after perforation. Tissue sections taken from paraffin blocks were stained with hematoxylin and eosin (H and E) and examined histopathologically under a light microscope. Intracellular H_2O_2 and apoptosis evaluations were carried out using the flow cytometric method.

RESULTS: Sepsis caused a significant reduction in all sperm parameters. There was a significant decrease in sperm density, motility and cell numbers with normal morphology ($p<0.05$). Intracellular H_2O_2 level and apoptotic cell percentages increased in sperm cells in the CLP group. MEL treatment was found to significantly reduce sperm abnormalities, testicular damage, intracellular H_2O_2 levels, and apoptosis.

CONCLUSION: This study showed that melatonin administration could be a potential treatment option to reduce acute testicular tissue damage due to sepsis.

Keywords: Melatonin; polymicrobial sepsis; sperm; testicular damage.

INTRODUCTION

Systemic or local infection may disrupt testicular steroidogenesis and spermatogenesis, causing temporary infertility and sexual dysfunction and even more permanent damage. Inflammation and infection lead to the formation of highly toxic reactive oxygen derivatives,[1] bioactive lipids, and pro-inflammatory and inflammatory cytokines.[2] Inflammation can show its effect on the testes by making a double effect on spermatogenesis and steroidogenesis, and decreasing spermatocytic arrest, serum testosterone, and luteinizing hormone serum levels.[3] There is a great deal of evidence that inflammation is the source of oxidative stress. Reactive oxygen derivatives reactive oxygen species (ROS) increase in oxidative stress, and these are important causes of male infertility. The released ROS react with the polyunsaturated fatty acids found in the sperm cell membrane, causing a decrease in cell movement and regression in fertility potential.[4]

The testicle is an organ with a unique immune system that helps maintain spermatogenesis. Thanks to the blood testicular barrier created by the Sertoli cells, it protects the sperm

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cells from the attacks of the body's own immune system. When infection occurs, inflammatory factors and free radicals are produced by testicular macrophages that inhibit gonadal steroidogenesis, including suppression of testosterone, a key molecule in maintaining normal spermatogenesis.[5] ROS such as hydrogen peroxide (H_2O_2), superoxide anion, and/or hydroxyl radical (OH) are causative agents in a range of pathologies, including inflammation, infection, alcohol toxicity, and cryptorchidism, and are known to mediate testicular damage in a variety of situations.[6] The previous studies in rat testes Sertoli cells have shown an increased production of H_2O_2 after the administration of lipopolysaccharide (LPS).[2,4]

Biological compounds with antioxidant properties can contribute to the protection of cells and tissues against the harmful effects of ROS. In experimental studies, it has been shown that the use of antioxidants protects spermatozoa from ROS-producing abnormal spermatozoa, gets rid of ROS produced by leukocytes, prevents DNA breaks, improves semen quality, prevents early sperm maturation, and increases the success of assisted reproductive techniques by supporting spermatozoa.[7] Melatonin (MEL) secreted from the pineal gland has various physiological effects such as regulating reproductive hormone secretion and affecting the immune system by crossing the blood testicular barrier and acting as an antioxidant.[8] Leydig cells promotes testosterone secretion. MEL in physiological concentrations increases the phagocytic power of testicular macrophage cells. MEL also cleans free radicals produced by macrophages.[9] Testicular macrophage cells are essential for maintaining the testicular immune response and exhibit reduced pro-inflammatory capacity. In patients with orchitis-related infertility, the number of macrophages with strong phagocytic activity in the interstitial tissue of the testes decreases; conversely, the number of macrophages that secrete large amounts of inflammatory cytokines increases. It has been reported that administration of MEL to septic mice induced by LPS exerts anti-inflammatory effects on mouse macrophages and regulates downwards the inflammatory response of macrophages.[10] It removes H_2O_2, increases endogenous antioxidant pathways.[11]

Studies examining the effects of sepsis on the testes in the literature are models of sepsis induced by LPS. In our study, we created sepsis with the method of cecal ligation and puncture (cecal ligation and perforation [CLP] creates a polymicrobial sepsis), which is a model that is very similar to surgical sepsis in the clinic. This study predominantly aimed to investigate the intracellular H_2O_2 levels (for ROS evaluation), apoptosis and the histomorphologically protective effects of MEL on sperm cells to prevent testicular damage under sepsis conditions.

MATERIALS AND METHODS

The experimental protocols were carried out in the Sakarya University Animal Laboratory in accordance with international guidelines after approval from the Animal Care and Use Ethics Committee of Sakarya University (01.07.2020/36). Rats were kept in wire cages under standard laboratory conditions (12-h light/12-h dark cycle, at a temperature of 22°C and 50–60% humidity). All rats were fed with standard pellet feed and tap water before the study. However, they were only allowed to take water for 12 h before and after the operation. Ketamine HCL (Ketalar®, Pfizer, Istanbul) and Xylazine HCL (fRompun®, Bayer, Istanbul) were used for anesthesia, and MEL was used as an antioxidant agent (Cayman Chemical East Ellsworth rd. Item no; 14427, USA).

Experimental Design and Animals

In this study, 21 adult male Wistar rats with an average weight of 250–300 g were used. The rats were randomly divided into three groups with seven animals in each group.

Group 1 (Sham-Control)
The rats in this group were operated on under general anesthesia, but CLP was not applied.

Group 2 (CLP)
Sepsis was created using the CLP method under general anesthesia.

Group 3 (CLP+MEL)
As in Group 2, sepsis was created using the CLP method under general anesthesia. Furthermore, 10 mg/kg of MEL was administered intraperitoneally to the rats in this group 30 min before the CLP procedure and 6 h after the operation. The MEL was dissolved in ethanol and applied after diluting with 0.09% NaCl (for an alcohol concentration of 1%).

The Cecal Ligation and Puncture-induced Sepsis Model

Sepsis was induced using the CLP-induced sepsis model in rats according to Rittirsch et al.[12]

All rats were anesthetized with Ketamine HCL (50 mg/kg) and Xylazine HCL (15 mg/kg) intraperitoneally. Anesthetized subjects were placed on the operating table in a supine position. After shaving the abdominal area, it was washed with Povidone-iodine. The cecum was isolated by reaching the peritoneum with a 2 cm incision from the abdominal region of the rats. The ascending colon contents were pushed down to fill the cecum. The cecum was tied under the ileocecal valve using 3/0 silk thread and then pierced with an 18 gauge needle (2 holes). Then the cecum was placed in the abdomen and the abdomen was closed with a double layer of 3/0 silk thread. After the surgical procedure, a 1% lidocaine solution was applied as an analgesic to the sutured area of the rats to reduce pain stress. The animals were not fed after surgery but were allowed to take water 6 h after the operation.

The rats were euthanized with a high dose blood collection while they were under general anesthesia 18 h after the oper-

ation, and the experiment was ended. Tissue and semen samples were taken. The samples taken were stored at −20°C until analyzed in the laboratory.

Epididymal Sperm Collection, Analysis and Storage

Mature sperm cells were obtained by scraping them from the epididymis. Epididymal sperm counts were determined using the method described by Yokoi et al.[13] At the end of the experiment, 10 µl of the epididymal sperm suspension in PBS was taken from the three groups, the sperm count was carried out in a Makler chamber and the concentrations were recorded by multiplying by the dilution ratio. The samples were evaluated in terms of motile sperm parameters, non-progressively motile and immotile sperm in 10 frames of the Makler counting chamber. Normal morphology and abnormal morphology of 200 sperm were evaluated and their percentages were calculated from the staining of the samples for each rat in the three groups by examination at ×40 magnification under a light microscope. The sperm morphology was assessed using the WHO classification and Kruger's strict criteria.[14]

Measurement of H_2O_2 by Flow Cytometry

Sperm cells obtained by stripping them from the epididymis were diluted at a concentration of 5×106 sperm/ml. Intracellular ROS concentrations were measured using 2'-7'dichlorofluorescin diacetate (DCFH-DA) (25 mg/ml) (AAT Bioquest, Inc. Sunnyvale, CA, USA) in dimethyl sulfoxide and were counterstained with propidium iodide (PI) (AAT Bioquest, Inc. Sunnyvale, CA, USA). A 5 µl volume of DCFH-DA (10 mM) and 3 µl of PI were added to 492 µl of sperm samples. The samples were gently mixed and incubated at 37°C for 30 min. The colorless DCFH-DA was converted into fluorescent DCFH by interacting with intracellular hydrogen peroxide (H_2O_2) and the green fluorescence intensity was assessed at a wavelength of 515–545 nm in the FI1 channel. PI red fluorescence was analyzed in the FL-2 channel at a wavelength of 564–606 nm. All fluorescence signals from labeled spermatozoa were analyzed by a flow cytometer (FACSCanto II; Becton Dickinson) equipped with a 488 nm argon–ion laser. Each sample of 10,000 sperm cell events were recorded at a flow rate of <200 events/s and analyzed using the FACSDiva software.

Testicular Histopathology

Testicular tissue samples of rats were fixed with Bouin's fixative and embedded in paraffin wax for evaluation under a light microscope. Samples were cooled by keeping at −20°C before sectioning. Sections between 3 and 5 µm thick embedded in gelatine (Gelatine, Foodland, Ewald-Gelatine GmbH, Meddersheimer Str. 50, 55566 Bad Sobernheim, Germany) were produced by a Thermo Scientific Microm HM40E (Otto-Hahn-Strasse1a 69190 Walldorf, Germany) microtome and floated in a hot water bath before placing on slides. To examine the histological structure of the sections, the samples were stained with Hematoxylin-Eosin (Merck KGaA 64271 Darmstadt, Germany). Evaluations were carried out using Johnson scoring.[15]

Statistical Evaluation

Statistical analysis was carried out using the SPSS 22.0 statistical software program (SPSS Inc. and Lead Tech. Inc. Chicago. the USA). The Shapiro–Wilk test was used for the normal distribution of data. In a comparison of more than two variables, the Kruskal–Wallis test was used, while the significance of the difference between groups was evaluated using the Mann–Whitney U test. All data are presented as a mean±SD. Statistical analysis at $p<0.05$ was considered significant.

RESULTS

Light Microscope Results for Sperm Parameters

The light microscope evaluation results for sperm parameters are presented in Table 1. Sepsis caused a significant decrease in all sperm parameters. Sperm density, percentage of sperm motility, percentage of sperm motility/immotility in place, and percentage of cells with normal morphology were all found to be significantly reduced ($p<0.05$). Comparing these parameters with the Sham Control group, it was determined that, statistically, the CLP group decreased significantly, and the mean values of the CLP + MEL group were close to the Sham Control group ($p<0.05$). It was observed that there was a high level of statistically significant differences, especially between the Sham Control group and the CLP and CLP + MEL groups ($p=0.000$ for comparisons of all parameters). Furthermore, in the results of this study, tail anomalies – one of the most common sperm morphological disorders – were more noticeable than other types of anomaly.

Flow Cytometric Evaluation

The basal intracellular H_2O_2 level of each group was determined by DCFDA staining, followed by flow cytometry. According to the DCFH (H_2O_2) staining results, there was a significant difference between the groups ($p<0.05$). The percentage of sperm stained with DCFH was calculated to be highest (31.414±9.064%) in the sepsis group and lowest (0.957±0.946%) in the Sham group. In the MEL -treated group, the ratio of intracellular H_2O_2 decreased from 31.414±9.064% to 4.271±1.703%. The differences between groups of dead, alive, and apoptotic cells, including H_2O_2, are given in Table 2 and Figure 1.

Histopathological Findings

A light microscope was used to evaluate hematoxylin-eosin stained sections taken from testicular tissue samples using Bouin's fixative on the tissue embedded in paraffin wax (Fig. 2). In the testicular tissue biopsies of the Sham Control group showing a normal seminiferous tubule and spermatogenetic structure (Fig. 2a), the tunica albuginea featuring tight connective tissue, the seminiferous tubules located within the testicular lobules separated by septa extending from the capsule, and the loose connective tissue structure rich in blood, lymph vessels and Leydig cells in the interstitial areas, were all

Table 1. Microscopic sperm parameter evaluation results for all groups

Parameters	Sham-Control (1)	CLP (2)	CLP+MEL (3)	p-value
	Mean±SD	Mean±SD	Mean±SD	
Sperm count (millions)	29.142±3.891	16.428±1.272	26.571±1.511	=0.000 (2–1.3)
Motility (%)	32.857±4.879	17.571±2.507	27.571±2.070	=0.000 (2–1.3)
				=0.024 (1–3)
Non-progressive mortility (%)	17.142±4.879	11.428±2.439	13.571±2.439	=0.016 (1–2)
Immotility (%)	50.000±7.071	71.000±4.509	58.857±2.340	=0.000 (1–2)
				=0.011 (1–3)
				=0.001 (2–3)
Morphology (%)	25.000±2.943	14.428±3.101	22.857±2.340	=0.000 (2–1.3)
Johnson_Score	8.857±0.690	6.000±0.816	8.428±0.534	=0.000 (2–1.3)

Sham-Control: Control group; CLP: Untreated sepsis group; CLP + MEL: Sepsis and melatonin treatment group; SD: Standard deviation. The mean difference is significant at the $p<0.05$ level.

Table 2. Flow cytometry evaluation results for all groups

Parameters %	Sham-Control (1)	CLP (2)	CLP+MEL (3)	p-value
	Mean±SD	Mean±SD	Mean±SD	
Propidium iodide	8.085±0.514%	3.800±3.074%	2.314±1.593%	=0.004 (1–2)
				=0.002 (1–3)
Apoptosis	0.0143±0.037%	4.928±4.370%	1.528±3.434%	=0.001(1–2)
				=0.006 (1–3)
				=0.018 (2–3)
Cell viability	90.928±0.0953%	58.028±6.587%	91.900±2.671%	=0.002 (1–2)
				=0.029 (1–3)
				=0.002 (2–3)
H_2O_2	0.957±0.946%	31.414±9.064%	4.271±1.703%	=0.002 (1–2)
				=0.006 (1–3)
				=0.002 (2–3)

Sham-Control: Control group, CLP: Untreated sepsis group; CLP + MEL: Sepsis and melatonin treatment group; SD: Standard deviation. The mean difference is significant at the $p<0.05$ level.

normal. For the testes of the CLP group (Fig. 2b), very severe atrophy, reduction in the number and layer of spermatogenic cells, spermatogenic arrest and separation in the intercellular junctions were observed in most of the seminiferous tubules. Tissue sections of the tests taken from the CLP + MEL treatment group were found to have areas similar to the healthy histological seminiferous tubule organizational structure of the Sham-Control group (Fig. 2c).

In the Sham-Control group showed a normal seminiferous tubule and spermatogenetic structure (Fig. 3a). In the CLP group, vacuolization and fragmentation of spermatogenetic layers were observed in some areas of the seminiferous tubules (Fig. 3b black arrows). In the CLP + MEL group it was observed that there were fewer separations in the intercellular junctions in the seminiferous tubules compared to the CLP group. Furthermore, spermatogenetic layers and spermatozoa were observed to have accumulated in the lumen of the seminiferous tubule (Fig. 3c).

DISCUSSION

The results of this study clearly showed that sepsis induced by CLP caused significant damage to the morphology of sperm cells and testicular tissue. The study also proved that MEL treatment had a strong protective effect in preventing the damage caused by oxidative effects in the testicular tissue by suppressing or eliminating ROS production in sperm cells.

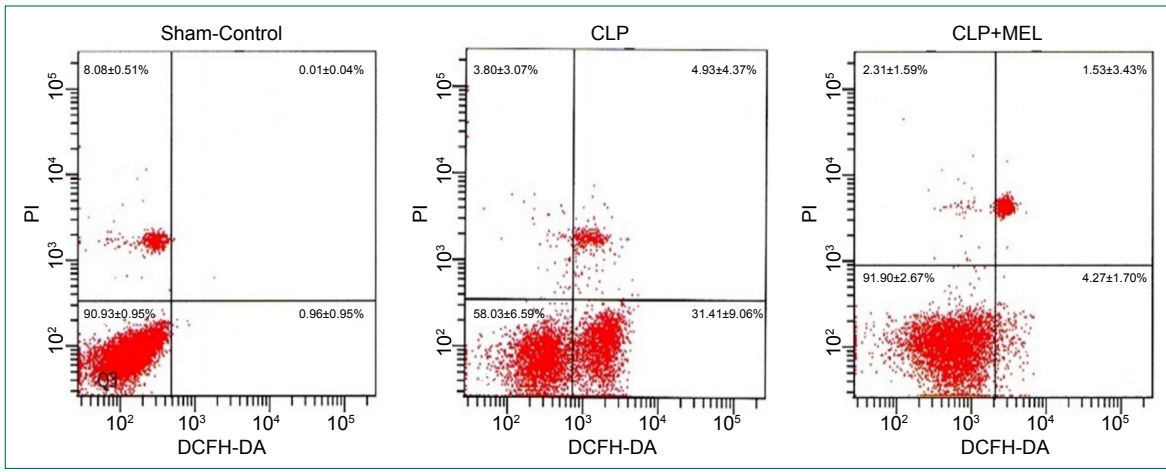

Figure 1. The flow cytometric analysis of the intracellular H_2O_2 measurements of sperm stained with 2', 7'-dichlorofluorescin propidium iodide (DCFH/PI). Each dot plot shows PI-positive dead cells on the upper left, apoptotic cells on the upper right, unstained viable cells on the lower left and the lower right shows the proportion of DCFH positive reactive oxygen species. Sham-Control: Control group CLP: Cecal ligation and perforation, Untreated sepsis group. CLP+MEL: Sepsis and melatonin treatment group.

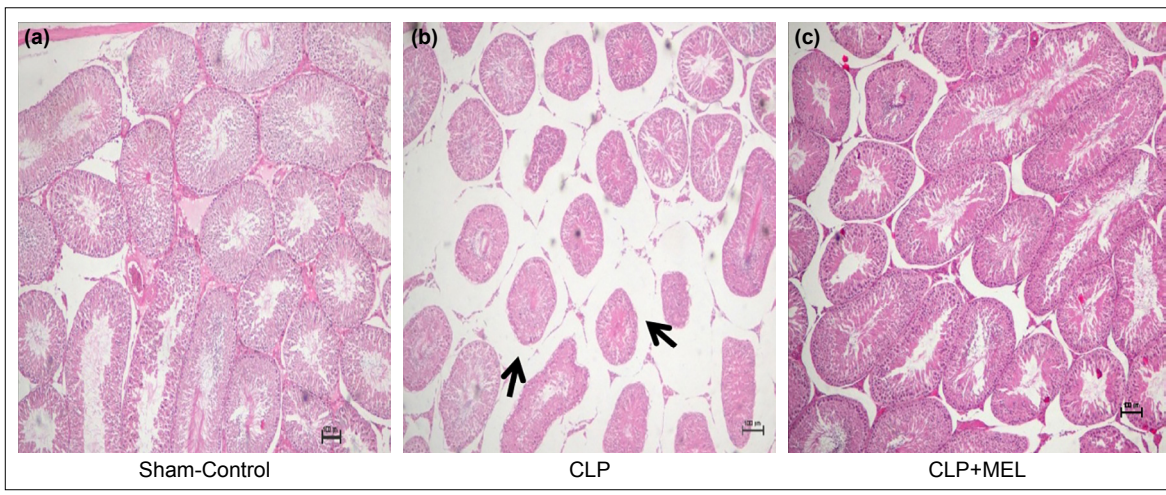

Figure 2. Histological testicular results. Sham-Control: Control group **(a)**. CLP: Cecal ligation and perforation, Untreated sepsis group **(b)**; Arrow: Disorganization in the membrane of the seminiferous tubules. CLP+MEL: Sepsis and melatonin treatment group **(c)**. Hematoxylin-Eosin ×40, 100 scale-bar.

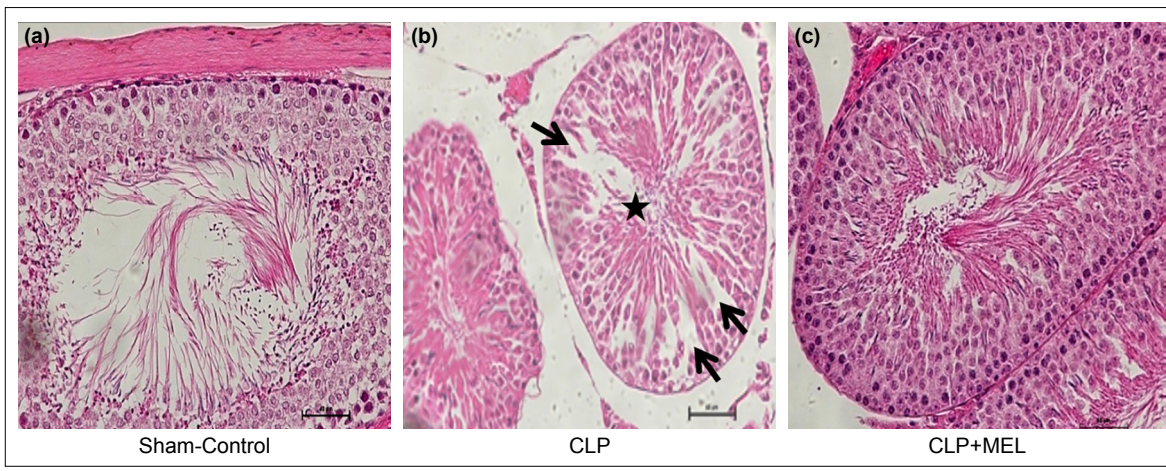

Figure 3. Histological structure of the seminiferous tubules. Sham-Control: Control group **(a)**. CLP: Cecal ligation and perforation, Untreated sepsis group **(b)**; Arrow: Vacuolization and fragmentation of spermatogenetic layers inseminiferous tubules. Star: decrease in the number of the spermatozoid. CLP+MEL: Sepsis and melatonin treatment group **(c)**. Hematoxylin-Eosin ×200, 50 scale bar.

Sepsis is a complex syndrome that occurs under the progression of a systemic inflammatory response mediated by endogenous factors affecting all organs and systems.[16] As the sepsis progresses, it may cause septic shock and eventually the loss of the organ at the initial injury site and then failure in organs far from the initial injury site.[17] The release of pro-inflammatory cytokines initiated by lipid peroxidation produced during sepsis triggers morphological and mitochondrial changes in many organs.[18]

Mitochondria are the major source of intracellular ROS, and recent studies have supported the importance of mitochondrial ROS.[19] Testicular tissue and spermatozoa are known to be extremely susceptible to ROS-induced damage. H_2O_2 is known to be key agents causing cytotoxicity in spermatozoa to produce oxidative stress by decreasing the enzymatic defenses. It was found that the excessive presence of ROS increased the apoptosis of germ cells and inhibited the activity of spermatozoa.[20] Sheweita et al.[21] reported that almost 40% of infertile men showed abnormally increased ROS levels.

There are only a few studies investigated the testicular injury induced by CLP sepsis on the laboratory animals. The results of this study clearly showed that sepsis induced by CLP caused a decrease in sperm count and motility in rat testes and a statistically significant increase in the morphologically abnormal sperm count, while MEL treatment reversed this situation. There is ample evidence that supports the fact that infection and inflammatory disease cause testicular dysfunctions. Inflammation triggered by in vivo bacterial lipopolysaccharide administration has been shown to inhibit testicular steroidogenesis and impair spermatogenesis.[1] It has been reported that acute inflammation induces inflammatory mediators in adult male albino rats and oxidative stress observed during acute endotoxemia are the main factors contributing to the elimination of steroidogenesis and spermatogenesis.[22] Kurcer et al.,[23] in their study on the protective effects of MEL in rat testicular ischemia/reperfusion (I/R), reported that MEL treatment did not prevent a decrease in sperm concentration caused by I/R, but it prevented sperm anomalies.

An examination of the flow cytometric results in the CLP group of this study showed that the intracellular H_2O_2 concentration and apoptosis in sperm cells increased at a statistically significant level compared to the Sham Control and CLP + MEL groups, that the number of living cells decreased, and that MEL treatment prevented apoptosis in cells and increased cellular viability by decreasing the intracellular H_2O_2 concentration. As shown in the histomorphological evaluation results, sepsis induced by CLP caused morphological changes in testicular tissue. Serious atrophy was observed in the seminiferous tubules in testicular samples of rats in the CLP group. In addition, it was noted that binding occurred in the number and layer of spermatogenic cells and separation at the intercellular junctions. The situation was reversed in the CLP + MEL group. MEL significantly preserved the testicular structure, sperm count, and morphology. This protective effect was thought to be a result of MEL's ROS scavenging properties. It has become clear in recent studies that MEL, which is the main secretion of the pineal gland, has a protective effect in the initial and progressive stages of diseases caused by reactive oxygen metabolites, and has become the focus of attention for researchers.[24,25] However, examining the studies on the effects of MEL on the reproductive system, it has been the tissue-level effects that have mainly been shown. This study is distinctive in that the effect of MEL on both testicular tissue and sperm cells has been demonstrated.

Conclusion

Severe tissue damage in testicular tissue due to sepsis induced by CLP. We thought that the damage could be suppressed by the antioxidant and anti-apoptotic effects of MEL. The determination of oxidative stress and antioxidant therapy has not yet been adopted in clinical use. ROS and oxidative stress determination should be applied in the near future so that it can be used routinely without the need for advanced techniques. Antioxidants to be used for treatment, their dosage and durations should also be determined. Today, when the use of assisted reproductive techniques is increasing, the sperm preparation environment should be supported with antioxidants. However, we think that more studies should be conducted to explain oxidative stress and antioxidants in male infertility.

One of the limitations of this study was that the efficiency of a single dose of MEL was investigated. Furthermore, the present research was an acute study aimed to clarify ways to work in future research at the cellular level and needs to be supported by further studies.

Ethics Committee Approval: This study was approved by the Sakarya University Animal Experiments Local Ethics Committee (Date: 01.07.2020, Decision No: 36).

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Authorship Contributions: Concept: S.D.; Design: S.D.; Supervision: Resource: S.D., O.B., G.E.; Data: S.D., O.B., G.E.; Analysis: S.D., O.B., G.E.; Literature search: S.D., O.B., G.E.; Writing: S.D., O.B., G.E., V.T., A.S.; Critical revision: S.D., O.B., G.E., V.T., A.S.

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REFERENCES

1. Hedger MP. Toll-like receptors and signalling in spermatogenesis and testicular responses to inflammation – A perspective. J Reprod Immunol 2011;88:130–41. [CrossRef]
2. Liew SH, Meachem SJ, Hedger MP. A stereological analysis of the response of spermatogenesis to an acute inflammatory episode in adult rats. J Androl 2007;28:176–85 [CrossRef]
3. Celino FT, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T, et al. Toler-

ance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. PLoS One 2011;6:e16938. [CrossRef]
4. Sarkar O, Bahrainwala J, Chandrasekaran S, Kothari S, Mathur PP, Agarwal A. Impact of inflammation on male fertility. Front Biosci (Elite Ed) 2011;3:89–95. [CrossRef]
5. Reddy MM, Suraneni Mahipal VK, Subhashinia J, Reddy CM, Roya RK, Reddy GV, et al. Bacterial lipopolysaccharide-induced oxidative stress in the impairment of steroidogenesis and spermatogenesis in rats. Reprod Toxicol 2006;22:493–500. [CrossRef]
6. Henkel R. The impact of oxidants on sperm function. Andrologia 2005;37:205–6. [CrossRef]
7. Ozyurt B, Parlaktas BS, Ozyurt H, Aslan H, Ekici F, Atis ÖJ. A preliminary study of the levels of testes oxidative stress parameters after MK-801-induced experimental psychosis model: Protective effects of CAPE. Toxicology 2007;230:83–9. [CrossRef]
8. Yu K, Deng SL, Sun TC, Jiang W, Liu YX. Melatonin regulates the synthesis of steroid hormones on male reproduction: A review. Molecules 2018;23:447. [CrossRef]
9. Deng SL, Zhang BL, Reiter RJ, Liu YX. Melatonin ameliorates ınflammation and oxidative stress by suppressing the p38MAPK signaling pathway in LPS ınduced sheep orchitis. Antioxidants 2020;9:1277.
10. Aparicio-Soto M, Alarcón-De-La-Lastra C, Cárdeno A, Sánchez-Fidalgo S, Sánchez Hidalgo M. Melatonin modulates microsomal PGE synthase 1 and NF-E2 related factor-2-regulated antioxidant enzyme expression in LPS-induced murine peritoneal macrophages. Br J Pharmacol 2014;171:134–44. [CrossRef]
11. Lowes D, Almawash A, Webster N, Galley H. Role of melatonin and indole derivatives on endothelial cells in an in vitro model of sepsis. Br J Anaesth 2010;104:525.
12. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009;4:31–6. [CrossRef]
13. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res 2003;93:141–54.
14. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe M, Jonas A, et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 1986;46:1118–23. [CrossRef]
15. Agger P, Johnsen SG. Quantitative evaluation of testicular biopsies in varicocele. Fertil Steril 1978;29:52–7. [CrossRef]
16. Lever A, Mackenzie I. Sepsis: Definition, epidemiology, and diagnosis. BMJ 2007;335:879–83. [CrossRef]
17. Exline MC, Crouser ED. Mitochondrial mechanisms of sepsis-induced organ failure. Front Biosci 2014;13:5030–41.
18. Galley HF. Oxidative stress and mitochondrial dysfunction in sepsis. Br J Anaesth 2011;107:57–64. [CrossRef]
19. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 2013;38:225–36. [CrossRef]
20. Zhou DX, Qiu SD, Zhang J, Tian H, Wang HX. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J Androl 2006;8:584–8. [CrossRef]
21. Sheweita SA, Tilmisany AM, Al-Sawaf H. Mechanisms of male infertility: Role of antioxidants. Curr Drug Metab 2005;6:495–501. [CrossRef]
22. Metukuri MR, Reddy CM, Reddy PR, Reddanna P. Bacterial LPS-mediated acute inflammation-induced spermatogenic failure in rats: Role of stress response proteins and mitochondrial dysfunction. Inflammation 2010;33:235–43. [CrossRef]
23. Kurcer Z, Hekimoglu A, Aral F, Baba F, Sahna E. Effect of melatonin on epididymal sperm quality after testicular ischemia/reperfusion in rats. Fertil Steril 2010;93:1545–9. [CrossRef]
24. Zhang WX, He BM, Wu Y, Qiao JF, Peng ZY. Melatonin protects against sepsis-induced cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1 in mice. Life Sci 2019;217:8–15.
25. Wang N, Li H, Zhu Y, Li N, Chen ZJ, Zhang C. Melatonin protects against Epirubicin-induced ovarian damage. J Reprod Dev 2020;66:19–27. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Yetişkin sıçanlarda polimikrobiyal sepsisin neden olduğu testis hasarına karşı melatoninin koruyucu rolü

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AMAÇ: Bu çalışmada, çekal ligasyon ve perforasyon (CLP) ile indüklenen polimikrobiyal sepsisin sıçan testis dokusunda oluşturduğu hasara karşı melatoninin olası koruyucu etkilerini araştırmak amaçladı.
GEREÇ VE YÖNTEM: Çalışmamızda 21 adet erkek Wistar-albino sıçan kullanıldı. Sıçanlar, Sham-Kontrol (Grup I), CLP (Grup II), CLP + MEL (Grup III) olmak üzere rastgele üç gruba (n=7) ayrıldı. Sepsis, çekal ligasyon ve perforasyon (CLP) yöntemi kullanılarak oluşturuldu. Melatonin perforasyondan 30 dakika önce ve altı saat sonra iki eşit dozda intraperitoneal olarak (10 mg/kg) uygulandı. Parafin bloklarından alınan doku kesitleri hematoksilen ve eozin ile boyandı ve ışık mikroskobu altında histopatolojik olarak incelendi. Hücre içi H_2O_2 ve apoptoz değerlendirmeleri flow stometrik yöntemle yapıldı.
BULGULAR: Sepsis, tüm sperm parametrelerinde önemli bir azalmaya neden oldu. Sperm yoğunluğunda, hareketliliğinde ve normal morfolojiye sahip hücre sayılarında önemli ölçüde azalma oldu ($p<0.05$). CLP grubunda sperm hücrelerinde hücre içi H_2O_2 seviyesi ve apopitotik hücre yüzdeliği arttı. Melatonin tedavisinin sperm anormalliklerini, testis hasarını, hücre içi ROS seviyelerini ve apopitozu anlamlı oranda azalttığı bulundu.
TARTIŞMA: Sunulan bu çalışma, sepsise bağlı gelişen akut testis doku hasarını azaltmak için melatonin uygulanmasının potansiyel bir tedavi seçeneği olabileceğini gösterdi.
Anahtar sözcükler: Melatonin; polimikrobiyal sepsis; sperm; testis hasarı.

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