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Relationship Between Antioxidant Enzyme Chains and Trace Elements and Electrolytes Levels and Potency of Melatonin on Sepsis-Induced Small Intestine Injury

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Abstract

BACKGROUND/AIMS: It was intended to determine the impacts of melatonin (Mel) on chitinase-3-like-1 protein, an early sepsis marker, and antioxidant enzymes, trace elements, and electrolytes in the small intestine tissue treated with sepsis with lipopolysaccharide (LPS) in Sprague Dawley.

MATERIALS AND METHODS: Control, LPS (20 mg/kg i.p.), Mel (10 mg/kg i.p.x3) and LPS + Mel groups were created from Sprague Dawley rats, with 8 individuals in each group. For the LPS group to be used as a sepsis model, the rats were decapitated at the 6th hour following LPS administration, and blood samples and small intestines were taken. In serum samples, antioxidant enzymatic defense molecules such as glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD), early sepsis marker YKL-40 were evaluated by enzyme-linked immunosorbent method. For statistical analysis, ANOVA and post hoc Tukey's tests were used.

RESULTS: The concentrations of GR, GSH-Px, SOD, and YKL-40 were decreased in LPS group. Antioxidant enzyme levels in the LPS + Mel groups were found to be close to the control. Also, trace element and electrolyte levels were significantly affected by LPS induced sepsis and Mel treatment returned them to control values. In the sepsis group, small intestine's images were shown damaged tissue sign and with decreased crypt depths-villus lengths in histological examinations. The images of the other groups are similar to the control group.

CONCLUSION: We suggest that Mel application improves serum antioxidant enzyme activity and trace element-electrolyte levels in small intestine tissue with sepsis, and may protect intestinal permeability and absorption by having a preventive effect on the intestinal barrier.

Keywords: Sepsis, trace elements, anti-oxidants, YKL-40, melatonin

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INTRODUCTION

Sepsis is a syndrome characterized by physiopathological disorders resulting from an increased and irregular inflammatory response. Severe sepsis can lead to multiple organ dysfunction syndrome (MODS), which arises from tissue and organ damage.[1] The pathogenesis of severe sepsis involves a cytokine storm, which is characterized by the release of inflammatory molecules, leading to oxidative stress due to an increase in prooxidant molecules and a failure to reduce or eliminate damage to the cell microenvironment.[2]

The pathogenesis of the disease is believed to be caused by a cytokine storm, which is characterized by the release of inflammatory mediators, an increase in reactive oxygen species radicals (ROS), and the creation of pro-oxidant molecules.[2] It has been established that the small intestines may serve as a source of MODS due to their role in facilitating bacterial translocation across the intestinal barrier. This process is compromised due to tissue damage resulting from inflammation and elevated oxidative stress.[3,4]

Melatonin (Mel), previously recognized as a neurohormone exclusively produced by the pineal gland during nocturnal hours, has been demonstrated to influence the regulation of circadian rhythm. However, recent studies have revealed that Mel possesses significant antioxidant properties.[5] It has been demonstrated that Mel reduces the occurrence of oxidative damage via lipopolysaccharide (LPS) by inducing antioxidant-enzyme expression and has anti-inflammatory effects as well as mitochondrial protection effects.[6, 7] In experimental studies, the exogenous application of Mel has been observed to increase the survival rate in sepsis induced by LPS.[7] However, the precise protective mechanism of sepsis remains to be fully elucidated. LPS is a glycolipid micromolecule derived from the cell wall of gram-negative bacteria. It has been demonstrated that LPS instigates the secretion of inflammatory molecules and reactive oxygen and nitrogen derivatives, which bind to Toll-like receptor-4. Consequently, it has been employed in experimental sepsis models.[8] Increased oxidative stress has been identified as a significant contributor to bacterial transmission observed in sepsis, resulting in enhanced intestinal permeability and impaired intestinal barrier function.[9,10] Consequently, Mel, a well-known antioxidant, has been identified as a potential protective agent against oxidative damage to the intestinal barrier and the subsequent increase in permeability.[3]

It has been established that trace elements play a pivotal role in maintaining metabolic continuity, a process that encompasses vital functions such as digestion and absorption, a wide range of essential bodily functions, and homeostasis. They act as cofactors for various metabolic enzymes, regulate gene transcription, and bolster the body's defense against oxidative stress and inflammation.[11] It has been proposed that trace elements play an essential role in the prognostication and therapeutic management of sepsis, given their function as cofactors in numerous enzymatic reactions involved in the oxidant/antioxidant system and pro/anti-inflammatory balance. These elements modulate immune responses through their antioxidant properties and the regulation of cytokine production, which is crucial in the context of sepsis. Antioxidant enzymes, such as superoxide dismutase (SOD), are contingent upon trace elements for their biological functions.[11,12] Alterations in trace element and electrolyte levels have been documented in numerous tissues and organs, particularly in serum, in the context of sepsis.[12,13] However, hypotension resulting from electrolyte imbalance in sepsis can lead to shock and signifies a poor prognosis. The primary treatment objective in shock is to regulate the disrupted fluid-electrolyte balance. This is essential for ensuring adequate perfusion of tissues and organs.

YKL-40, with its glycoprotein structure, chitinase 3-like protein 1, is released from many tissues such as macrophages and neutrophils and is a protein with a glycoprotein structure that is activated in many processes, including cancer, as well as in acute and chronic inflammatory events. Their levels are important in determining the prognosis of the disease. Therefore, it is recommended to be used as a marker in inflammatory diseases.[14,15]

The aim of this study was to determine how Mel due to its antioxidant and anti-inflammatory effects- affects antioxidant enzymes and YKL-40 in serum, small intestinal morphology and tissue trace element-electrolyte levels in LPS-induced sepsis in rats.

MATERIALS AND METHODS

Experimental Groups

Ethical approval for the research was received from İstanbul Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (approval number: 2017/63, date: 30.05.2017).

Adult male rats Sprague Dawley weighing around 200-250g were commercially supplied with a diet and tap water ad libitum. Rats were kept in an experimental environment where 12h light/12h dark, humidity (55-60%), and temperature constant at 22±2 °C. Our study consisted of control, LPS, Mel, LPS + Mel groups, with 8 rats in each group. Sample size was calculated with the power analysis method.

Experimental Procedures

We did not apply any treatment to the rats that constitute the controls. In the LPS, the rats were applied 20 mg/kg of LPS i.p. as a single dose, prepared in 1 mL sterile saline (*Escherichia coli* O127:B8, Sigma-Aldrich, Product No: L5668). Mel group was administered intraperitoneally 10 mg/kg at 2-hour at intervals, by dissolving total 30 mg/kg (Mel, Sigma Aldrich, Product No: M5250) in 2.5% mL ethyl alcohol. In the LPS + Mel group, other doses of Mel (10 mg/kg, i.p.) were executed 30 minutes before LPS application and at the 2nd and 4th hours thereafter.

Six hours after the first injection, the rats were cut off under ketamine-xylazine anesthetic conditions. (i.p. route) and blood samples were removed into yellow-dry tubes from the heart. Serum was taken at 3000 rpm for 5 minutes by centrifugation. The small intestinal tissue samples were cleaned with physiological saline, and some of them were frozen in liquid nitrogen for analyses of trace element and electrolyte levels such as sodium (Na^{+}), potassium (K^{+}), magnesium (Mg^{++}), calcium (Ca^{++}), iron (Fe^{++}), copper (Cu^{++}), zinc (Zn^{++}), selenium (Se^{++}). Also, other parts of intestinal tissues were taken in 10% formaldehyde for histological examinations, and stored at -80 °C with the serum samples to determine YKL-40, and antioxidant defense enzymes such as glutathione reductase (GR), glutathione peroxidase (GSH-Px), and SOD levels.

Analyzing YKL-40

Serum YKL-40 was measured by double antibody sandwich technique using antibody-coated plates and enzyme-linked immunosorbent (ELISA) technique (China, Lot: YHB20170315843) at 450 nm.

Analyzing Antioxidant Enzyme Chains (GSH-Px, GR, SOD enzymes)

The levels of serum GR (China, Lot: YHB20171106220), GSH-Px (China, Lot: YHB20171106219), and SOD (China, Lot: YHB20171106218) were assayed with ELISA test at 450 nm.

Analysis of the Levels of Trace Element and Electrolyte

The inductively coupled plasma-optical emission spectroscopy (ICP-OES) (PerkinElmer-Optima 7000 DV) was used the levels of Fe, Se, Zn, Cu, Na, Ca, K, Mg (PerkinElmer-Optima 7000 DV) in small intestine tissue samples. Approximately 0.200/0.250 g of small intestinal tissue samples were weighed; it was treated in 10 mL of 20% HNO3 for 5 minutes at 145 °C, 5 minutes at 165 °C and 20 minutes at 175 °C.

The test tubes warmed at ambient temperature were filtered through Whatman; it was filled to 50 mL in bottles using ultrapure water. The samples were determined at ICP-OES and had a standard concentration of 50 ppb-1 ppm. Results were calculated as mg/kg.

Quality Control, Assurance

To quantitatively calculate the levels of each element in small intestine tissue, calibration standard vials were done to diluted 1000 mg/L ICP standard solution of multi-element (Merck). Calibration charts belonging each element were prepared changing from low to high calibration standard concentrations; a robust R2 level excessive 0.999 was obtained.

The accuracy and precision of the assays were verified with repeated calculations of calibration solutions of known concentrations. Limit of detection and limit of quantification values for each element were calculated by analyzing blank solutions (Table 1).[12,15]

Statistical Analysis

A power analysis using G*Power for this study on Serum GR levels, based on a similar design, indicated a required sample size of 32, consisting of 4 groups with 8 animals each. The effect size was 1.02, α=0.05, and power=0.95. The critical F-value was 3.09 (df=20.3), resulting in an actual power of 0.978. Given the sufficiency of serum and homogenate for all parameters, the samples were subjected to two replicates.

The Statistical Package for the Social Sciences 22.0 Software Programme (SPSS, Inc., Chicago, IL, USA) was performed as blindly. The conformity of the data to normal distribution was evaluated with the Shapiro-Wilk test. For the Shapiro-Wilk test, if the significance value was p>0.5, it was accepted that the distribution was normal. The significancy was assessed using ANOVA and following post hoc Tukey's between groups. The obtained results were shown as the mean±standard deviation. The statistical significance limit was accepted as p<0.05.

RESULTS

Serum Antioxidant Enzyme Chain Findings

Serum GR was significantly lower in the LPS group than control, LPS + Mel (p<0.05, both) and Mel (p<0.001) groups. Moreover, GR was found significantly increased in Mel compared to control, LPS + Mel (p<0.01, both), and LPS (p<0.001) groups. There are no differences between control and LPS + Mel (Figure 1-a).

Table 1. Summary of instrument settings

Element	Spectral line, (nm)	LoD, (µg kg^{-1})	LoQ, (µg kg^{-1})	RSD, (%)	R^2
Ca	317,933	47.52	158.40	0.96	0.999983
Cu	327,393	1.22	4.07	0.70	0.999905
Fe	238,204	12.79	42.63	0.86	0.999894
K	766,490	76.94	256.47	0.92	0.999916
Mg	285,213	21.64	72.13	0.83	0.999932
Na	589,592	63.81	212.70	0.67	0.999897
Se	196,026	0.88	2.93	0.94	0.999879
Zn	213,857	6.79	22.63	0.90	0.999928

Ca: Calcium, Cu: Copper, Fe: Iron, K: Potassium, Mg: Magnesium, Na: Sodium, Se: Selenium, Zn: Zinc, LoD: Limit of detection, LoQ: Limit of quantification, RSD: Relative standard deviation, R^2: Determination coefficient.

Table 2.1. Comparison of GR (ng/mL) values according to groups

Groups	n	Mean	F	p
Control	8	26.9148±0.51		
LPS	8	17.7015±0.13		
Mel	8	39.3503±0.91	16.95	0.00
LPS + Mel	8	26.8193±0.69		
Total	32	27.6964±0.57		

GR: Glutathione reductase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.2. Comparison of GSH-Px (mU/mL) values according to groups

Groups	n	Mean	F	p
Control	8	165,797±18.24		
LPS	8	151,575±12.11		
Mel	8	287,143±11.92	6,808	0.0026
LPS + Mel	8	199,042±16.23		
Total	32	200,889±14.45		

GSH-Px: Glutathione peroxidase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.3. Comparison of SOD (ng/mL) values according to groups

Groups	n	Mean	F	p
Control	8	32.6342±0.76		
LPS	8	38.7994±1.13		
Mel	8	34.7321±1.91	8,274	0.0025
LPS + Mel	8	38.7150±1.95		
Total	32	35.9592±2.97		

SOD: Superoxide dismutase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.4. Comparison of YKL-40 (pg/mL) values according to groups

Groups	n	Mean	F	p
Control	8	19.012±0.32		
LPS	8	13.9289±1.10		
Mel	8	16.3200±0.80	11,934	0.0175
LPS + Mel	8	22.2171±1.93		
Total	32	17.8695±.077		

LPS: Lipopolysaccharide, Mel: Melatonin.

Serum GSH-Px was higher in the Mel compared to the results obtained in the control and LPS significant ($p<0.01$). There are no significancies among the other groups ($p>0.05$) (Figure 1-b).

Serum SOD was lower in the LPS than controls ($p<0.05$), and Mel ($p<0.01$) groups. While in the Mel, serum SOD was increased compared to LPS ($p<0.01$) and LPS+Mel ($p<0.05$) (Figure 1.c).

Table 3.1. Comparison of Fe^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	74.1989±0.76		
LPS	8	79.7941±0.13		
Mel	8	72.7111±0.19	70.47	<0.0001
LPS + Mel	8	75.7933±0.19		
Total	32	75.6241±0.29		

Fe^{++}: Iron, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.2. Comparison of Cu^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	6.1855±0.15		
LPS	8	6.6643±0.10		
Mel	8	6.0727±0.18	1,064	<0.0001
LPS + Mel	8	6.4699±0.11		
Total	32	6.3481±0.11		

Cu^{++}: Copper, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.3. Comparison of Zn^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	33.1812±0.1		
LPS	8	35.6833±0.3		
Mel	8	32.5158±1.1	65.02	<0.0001
LPS + Mel	8	34.1238±0.8		
Total	32	32.8760±0.5		

Zn^{++}: Zinc, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.4. Comparison of Se^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	0.7168±0.06		
LPS	8	0.7020±0.01		
Mel	8	0.7710±0.09	30.36	<0.0001
LPS + Mel	8	0.7363±0.05		
Total	32	0.7315±0.06		

Se^{++}: Selenium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.1. Comparison of Na^{+} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	649,373±18.2		
LPS	8	698,340±12.1		
Mel	8	636,352±10.1	137.5	<0.0001
LPS + Mel	8	673,147±11.8		
Total	32	664,305±10.3		

Na^{++}: Sodium, LPS: Lipopolysaccharide, Mel: Melatonin.

As an Early Biomarkers Serum YKL-40 Findings

YKL-40, an early marker of sepsis, was found decrease in the LPS compared to the results observed in other groups significantly ($p<0.05$). There is no observed significant change in the other groups ($p>0.05$) (Figure 1-d).

Electrolytes and Trace Elements Levels Analyzing

Intestinal tissue Na^{+}, K^{+}, Mg^{++}, Ca^{++}, Fe^{++}, Cu^{++}, Zn^{++}, Se^{++} levels were analyzed in all experimental groups and also Cu^{++}/Zn^{++} ratio was calculated. Except for Mg^{++} and Se^{++}, all electrolyte and trace element levels in the LPS group significantly increased compared to all experimental groups ($p<0.001$). Also, Mg^{++} and Se^{++} levels were lower in the LPS group than in other groups significant; for Se^{++}, and control and Mel groups for Mg^{++} ($p<0.001$). There were significantly changing between control and LPS + Mel group compared to control for Na^{+}, Mg^{++}, Ca^{++}, Fe^{++}, Cu^{++} ($p<0.05$) (Figures 2-3).

Histological Findings

It is observed that crypt depths and villus lengths decrease in the LPS group. It is determined that these lengths are relatively longer in the control, Mel and LPS + Mel groups compared to the LPS group. Additionally, it is observed that cell structures are disrupted in some places in the LPS (Figure 4).

DISCUSSION

The small intestine is the primary site of digestion and absorption within the gastrointestinal system. In the context of sepsis, the disruption

Table 4.2. Comparison of K^{+} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	772,917±17.6		
LPS	8	831,201±13.4		
Melatonin	8	757,419±21.5	71.07	<0.0001
LPS+Mel	8	778,321±12.9		
Total	32	784,965±17.7		

K^{++}: Potassium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.3. Comparison of Mg^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	43.7777±0.50		
LPS	8	42.1159±0.25		
Mel	8	46.2185±0.36	100.1	<0.0001
LPS + Mel	8	44.8372±0.91		
Total	32	44.2373±0.62		

Mg^{++}: Magnesium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.4. Comparison of Ca^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	895,880±7.6		
LPS	8	983,150±9.1		
Mel	8	914,245±3.2	757.6	<0.0001
LPS + Mel	8	954,479±5.9		
Total	32	936,938±8.1		

Ca^{++}: Calcium, LPS: Lipopolysaccharide, Mel: Melatonin.

of its microbial ecosystem, coupled with tissue injury resulting from inflammatory and oxidative damage, contributes to a marked deterioration of the intestinal barrier. This heightened significance of the small intestine in the prognosis of sepsis underscores its critical role in maintaining bodily functions.[16]

Oxidative stress is the result of an imbalance between oxidants and antioxidants, caused by an excess of ROS formation and a decrease in antioxidant levels. A multitude of studies have observed an increase in ROS levels and a decrease in antioxidant levels in septic patients. It has been proposed that oxidative stress may trigger the release of inflammatory mediators, thereby inducing inflammation through redox reactions that facilitate transcriptional activation, such as NF-κß.[17] Despite the development of numerous antioxidant therapeutic agents, treatment outcomes have been unsatisfactory. This failure has been attributed to the absence of effective antioxidant therapy at the mitochondrial level. The basis of the etiopathophysiology of organ dysfunction in septic conditions is suggested to be damage mediated by oxidative stress in mitochondria.[18]

Mel has been reported to have profound antioxidant activity by reacting with reactive species derived from both oxygen and nitrogen, and its metabolites also have antioxidant function. Mel exhibits both lipophilic and hydrophilic characteristics, with its highest concentrations being found in mitochondria. It has been demonstrated to possess both anti-inflammatory and antioxidant properties, capable of removing hydrogen peroxide, strengthening antioxidant pathways, and reducing nitric oxide formation. Mel has been documented to impede mitochondrial dysfunction, energy failure, and cell death. In animal models of oxidative damage, Mel has been shown to improve the release of inflammatory cytokines and increase GSH-Px, GR, and superoxide dismutase enzymes in the antioxidant enzyme chain.[19-21] Furthermore, Mel has been demonstrated to function as a mucosal protective agent, although its precise physiological role within the gastrointestinal tract remains to be fully elucidated. Studies have demonstrated that it reduces intestinal permeability, which increases due to damage caused by various reasons.[22, 23] The mechanism through which Mel exerts this effect remains to be fully elucidated, although studies have suggested a role for structural changes in the tight junctions of the intestinal epithelium.[24]

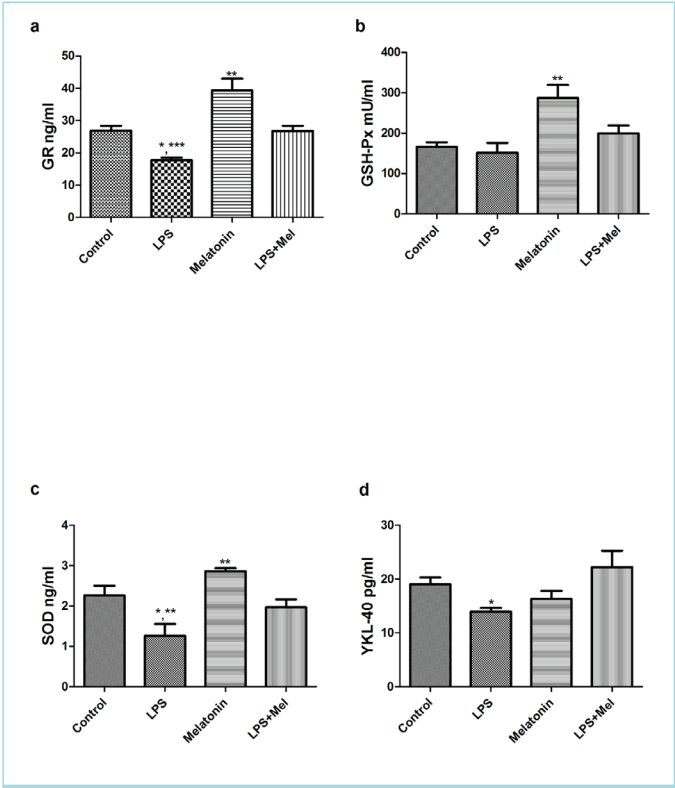

Figure 1. Serum antioxidant enzymes chain (GR, GSH-Px, SOD) and YKL-40 levels in all experimental groups; control, LPS, Mel, LPS + Mel. (a) *p<0.05 LPS vs. control and LPS + Mel; **p<0.01 Melvs. control and LPS + Mel; ***p<0.001 Melvs. LPS. (b) **p<0.01 Mel vs. control and LPS; (c) *p<0.05 LPS vs. control and Mel vs. LPS + Mel; **p<0.01 Mel compared to LPS; (d) *p<0.05 LPS vs. control.

GR: Glutathione reductase, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, LPS: Lipopolysaccharide, Mel: Melatonin.

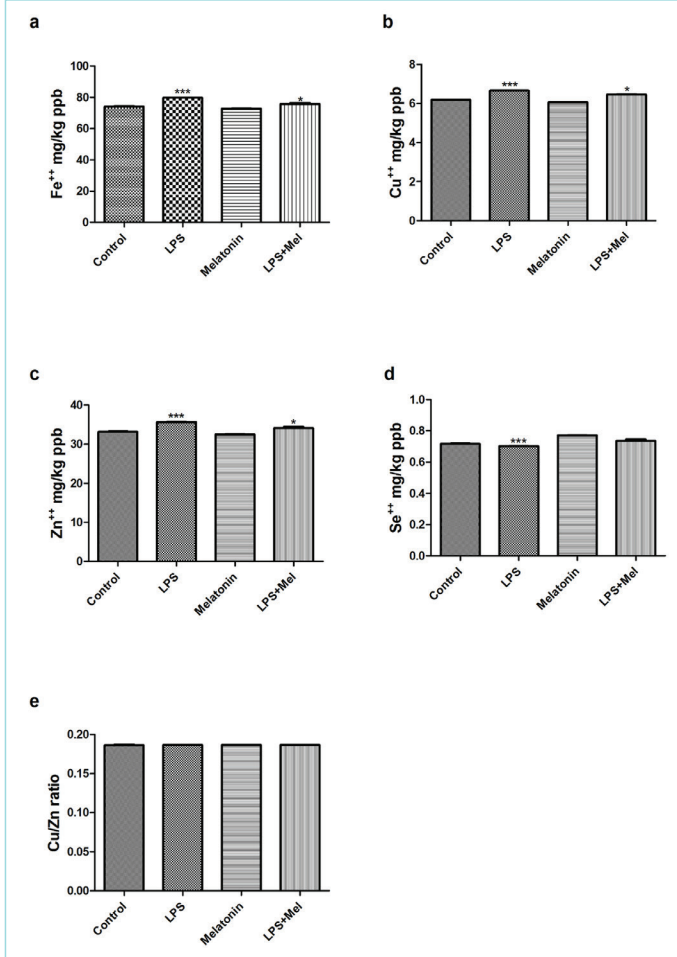

Figure 2. Trace elements levels for experimental groups (a-e). (a) Fe^{++} levels of intestinal tissue. (b) Cu^{++} levels of intestinal tissue. (c) Zn^{++} levels. (d) Se^{++} levels of intestinal tissue. (e) Cu^{++}/Zn^{++} ratio of intestinal tissue; *p<0.05 LPS + Mel vs. control and Mel; ***p<0.001 LPS vs. all experimental groups.

Fe^{++}: Iron, Cu^{++}: Copper, Zn^{++}: Zinc, Se^{++}: Selenium, LPS: Lipopolysaccharide, Mel: Melatonin.

Superoxide, an oxidant molecule, is released by mitochondria, primarily through the process of oxidative phosphorylation. It has been established that SOD represents the primary biochemical response to superoxide, the predominant harmful oxidant radical produced by mitochondria. SOD functions by dismutating superoxide radicals into hydrogen peroxide and molecular oxygen. An imbalance, whether it be an excess of superoxide anion or a deficiency in SOD, can lead to the accumulation of highly reactive superoxide radicals within the mitochondria. It has been reported that these radicals, when formed in excessive amounts, can interact with mitochondrial nitric oxide and form peroxynitrite, a reactive nitrogen species and a strong oxidant.[25]

The concentration of glutathione (GSH) is established by the catalysis process of GSH-related antioxidant enzymes called GSH-Px, which consumes reduced GSH, and GR, which regenerates reduced GSH from oxidized matter in mitochondria. Research has indicated that a decline in the activity of this enzyme may result in an accumulation of hydrogen peroxide, consequently leading to elevated oxidative stress and tissue damage.[20,26] It has been proposed that deficiencies in these enzymes may render cells more susceptible to oxidative damage.[27]

In our study, it was observed that in the group in which we induced sepsis with LPS, the levels of GSH-Px, GR, SOD, which are the main enzymes in the anti-oxidant chain, decreased significantly compared to other experimental groups, and enzyme levels increased significantly in septic rats administered Mel. In the group where Mel was administered alone, the levels of this internal enzyme were found to be higher compared to the control group. These outcomes align with existing literature, demonstrating that decreased mitochondrial antioxidant enzyme levels, which play a role in mitochondrial dysfunction due to oxidative damage in sepsis, can be mitigated by Mel. This finding further substantiates the substantial antioxidant activity of Mel.

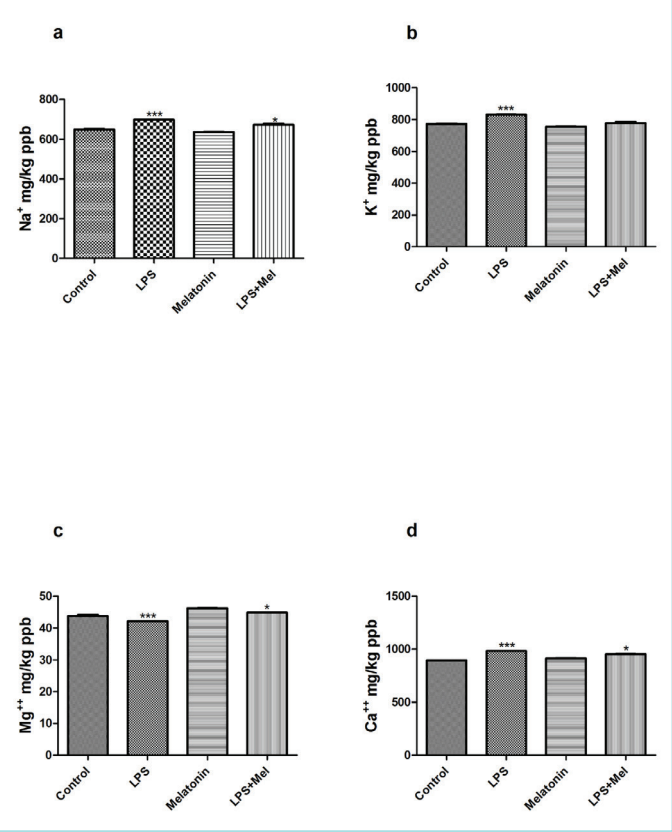

Figure 3. Electrolytes levels for all experimental groups (a-e). (a) Na^+ levels of intestinal tissue. (b) K^+ levels of intestinal tissue. (c) Mg^{++} levels of intestinal tissue**.** (d) Ca^{++} levels of intestinal tissue; *p<0.05 LPS + Mel vs. control and Mel; ***p<0.001 LPS vs. all experimental groups.

Na^{++}: Sodium, K^{++}: Potassium, Mg^{++}: Magnesium, Ca^{++}: Calcium, LPS: Lipopolysaccharide, Mel: Melatonin.

Disturbances in mineral and electrolyte homeostasis are common in many serious diseases, including sepsis. Moreover, the frequency of these changes in clinically critical is unclear.[28] It is reported that more than 66% of patients in intensive care have multiple electrolyte and acid-base abnormalities.[29] Since the functions of electrolytes and minerals in the protection and maintenance of cellular action, tissue perfusion and acid-base balance are vital in preventing complications and adverse outcomes. It has been reported that it is critical to keep aforementioned parameters at physiological concentrations.

It was detected that the levels of Na^+ and K^+ increased significantly in the LPS. In the LPS receiving Mel treatment, a significant decrease appeared compared to the LPS. It has been suggested that changes in electrolyte levels in the small intestine tissue in the sepsis group may occur due to impaired absorption levels due to sepsis, especially in studies that glucose transport decreases in sepsis and accordingly sodium accumulation may occur in the small intestine tissue.[30] It has also been stated that the effectiveness of the sodium potassium pump may change due to sepsis.[31,32]

Calcium is an ion that plays a role in triggering the apoptotic pathway, functions as a clotting factor (factor-IV), and has a role in the second messenger system. It has been reported that calcium can modify blood viscosity. Its potential involvement in coagulation, observed inflammation, and cell and tissue apoptosis in small intestinal injury has been suggested. In this study, calcium levels in the LPS group were found to be significantly lower compared to the other groups. The administration of Mel led to a reduction in this increase, approaching the level observed in the control group.

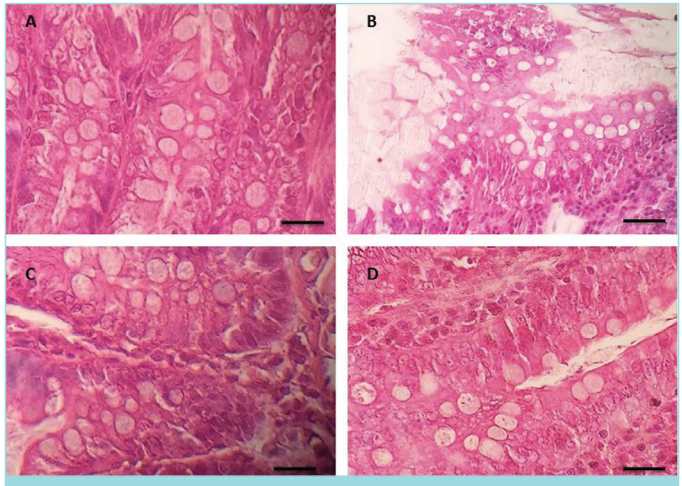

Figure 4. Image of histological section for all experimental groups (A-D). (A) control, (B) LPS, (C) Mel and (D) LPS + Mel groups.

LPS: Lipopolysaccharide, Mel: Melatonin.

Magnesium is a trace element that behaves as a cofactor of more than 300 biochemical catalyzer. In addition to its crucial for immunity, also magnesium has a function to induce of many enzymes such as phospho kinase and ATP.[33] In this study, we observed that magnesium levels in septic conditions intestinal tissue were significantly lower than the control group. We found that magnesium levels increased in the group to which we applied Mel. Low magnesium levels in sepsis are considered a sign of poor prognosis in terms of mortality.[34]

Selenium has antioxidant functions such as participating in the structure of selenoenzymes such as GSH-Px and being a component of other selenoproteins.[35] Many studies have shown that there is a insistent decrease in plasma selenium concentration in systemic inflammatory response syndrome and septic condition. It has been observed that the decrease in GSH-Px activity shows a parallel tendency with selenium levels.[12,36]

Copper is one of the trace elements required for many cellular enzymes to perform physiological function and also as a cofactor in the catalytic reactions that can lead to ROS formation when present in high intracellular concentrations.[37]

Research has demonstrated that Mel's primary function is not to serve as an effective radical-scavenging antioxidant by reacting with different pro-oxidant molecules, but rather to protect against the toxicity caused by copper by binding to copper via chelation within the cell.[38] In vitro and in vivo experiments have demonstrated that Mel, administered at concentrations of 1 mM and 50 mg/kg (i.p.), effectively mitigates the generation of hydroxyl radicals by high copper concentrations and polyphenols, thereby preventing DNA damage through copper chelation.[39] It has been reported that Mel exerts a bacteriocytic effect against pathological bacteria. However, the precise mechanism underlying this effect remains to be fully elucidated. Mel has been reported to exhibit high metal binding properties, including those of iron, copper, and zinc. It is imperative to note that bacteria require metals, particularly free iron, for their proliferation. One postulation suggests that Mel's metal-binding property may exert a bacteriocytic effect by diminishing the metal utilization of bacteria.[40]

In the present study, we observed that trace elements of Fe^{++}, Cu^{++}, and Zn^{++} exhibited a significant increase in the LPS group, while Se^{++} levels demonstrated a decrease. The administration of Mel resulted in a reversion of these trace element levels to levels more consistent with the control group.

It has been established that minerals are predominantly absorbed by the duodenum and subsequently transported into the circulation via the portal vein. It has been proposed that the assessment of villus height and crypt depth can serve as a metric for evaluating intestinal development, digestive processes, and absorptive capacity. While villus height and crypt depth are indicators of the digestive and absorptive function of the small intestine and the maturity of epithelial cells, the ratio of villus to crypt depth has been shown to be a more sensitive indicator of digestive and absorptive function.[41]

In the intestinal tissue sections of the LPS group, a decrease in crypt depths and villus lengths was observed. This phenomenon has been documented as a prevalent finding, suggesting a potential reduction in absorption. Acute excessive intestinal motility and especially glucose and amino acid absorption are impaired in sepsis. In the future, the intestinal barrier, which is disrupted as a result of tissue damage, may become excessively permeable and thus allow the invasion of bacteria.[3,40,42] It is noteworthy that the crypt depths and villus lengths in the sepsis group induced with LPS and in the group treated with Mel were greater than those observed in the LPS group.

Plasma levels of YKL-40, a promising prognostic marker for sepsis, have been found to be significantly elevated in patients with a poor prognosis. In this study, YKL-40 levels in the LPS group were found to be significantly lower compared to the other experimental groups. A previous study observed that the YKL-40 level exhibited an average increase by the 14th hour in patients with sepsis and poor prognosis. The present study is more acute than the aforementioned study. It is hypothesized that the results at the protein level may be influenced at a subsequent stage.[14,15]

Study Limitations

The limitations of our study include the inability to examine the acute model of sepsis as well as the chronic model and the lack of genetic or molecular pathway data to provide a balanced view of antioxidant mechanisms.

CONCLUSION

In this study, we evaluated the impacts of Mel on serum antioxidant enzymes, the trace element and mineral levels of small intestine tissue with the biomarker YKL-40. Our findings indicated that Mel ameliorated the injury caused by sepsis as a result of LPS stimulation. The observed activity of Mel can be attributed to its antioxidant, anti-inflammatory, and immunomodulatory.

MAIN POINTS

- Sepsis is one of the cases with the highest incidence. Small intestinal damage and impaired intestinal permeability due to sepsis are among the most frequently observed and fatal symptoms.

- Melatonin (Mel) is a powerful endogenous antioxidant. With its anti-oxidant and anti-inflammatory effects, it is very effective on the oxidative damage caused by sepsis and the resulting fetal damage.

- It is important to examine tissue trace element and electrolyte levels and the effects of Mel on these, as they are present in the structures of important enzyme systems, such as anti-oxidant enzymes, whose levels are affected in sepsis.

ETHICS

Ethics Committee Approval: Ethical approval for the research was received from İstanbul Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (approval number: 2017/63, date: 30.05.2017).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.A., H.Y., E.Ö., Concept: G.A., H.Y., E.Ö., Ş.T., Design: G.A., H.Y., E.Ö., Ş.T., Data Collection and/or Processing: G.A., H.Y., Ş.T., İ.E.Y., V.O., Analysis and/or Interpretation: G.A., H.Y., E.Ö., İ.E.Y., V.O., Literature Search: G.A., Ş.T., Writing: G.A., E.Ö.

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