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The Effect Of Melatonin On Blood and Tissue (Liver and Kidney) Lipid Peroxidation and Tissue NF-κB Levels In LPS Induced Obstructive Jaundice

LPS ile Uyarılmış Tıkanma Sarılığı Modelinde Melatonin Uygulanmasının Kan ve Doku (Karaciğer/Böbrek) Lipid Peroksidasyonu ile Doku NF-κB Düzeylerine Etkisi

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ABSTRACT

Objectives

In obstructive jaundice, free radical production is increased and antioxidative activity is reduced. Melatonin has a beneficial effect with antiinflammatory and antioxidant activity acting as a free radical scavenger. Melatonin inhibits the NF-κB expressions, suppresses cytokine expression/release and inhibits the adhesion molecule expressions. The aim of this study was to investigate the effects of Melatonin on liver and renal tissue NF-κB expressions, serum and tissue lipid peroxidation in lipopolysaccharide (LPS) induced obstructive jaundice.

Methods

We randomized 60 rats into 6 groups. Group A: Sham, Group B: Obstructive jaundice, Group C: Obstructive jaundice + Melatonin (20 mg kg-1 intraperitoneal), Group D: Obstructive jaundice+ LPS (10 mg kg-1), Group E: Obstructive jaundice + LPS + Melatonin. Group F: Obstructive jaundice + Melatonin +LPS. Biochemical markers of lipid peroxidation as Malondialdehyde(MDA) and Myeloperoxidase(MPO) were determined after sacrifice in each groups.

Results

Serum and Liver/renal tissue MDA, MPO levels and tissues NF-κB increased in group 2, 4 and 6 compared to group 1. After the administration of Melatonin (group 3 and 5), liver/renal tissue MDA and MPO levels decreased; and tissues NF-κB levels decreased as compared to other groups.

Conclusions
In this model of OJ stimulated by LPS, Melatonin suppressed the adverse effects of OJ in the absence of LPS. It is clearly shown that melatonin acts as a hepatic and renal protective effect against oxidative stress in OJ. However, it failed to prevent lipid peroxidation in case of LPS-induced OJ when it is administrated before or after LPS.

Key words: *Lipid peroxidation, Lipopolysaccharide, LPS, Melatonin, NF-κB expressions Obstructive jaundice, Liver, Kidney, Animal.*

Abbreviations
Mel: Melatonin
LPS: Lipopolysaccharide
NF-κB: Nuclear factor kappa B
OJ: Obstructive jaundice
MDA: Malondialdehyde
MPO: Myeloperoxidase

ÖZET

Amaç
Tıkanma sarılığında serbest oksijen radikal üretimi artmakta ve antioksidatif kapasite azalmaktadır. Melatonin serbest oksijen radikallerini azaltmakta, antioksidan etki göstermektedir. Melatonin, NF-κB ekspresyonunu azaltmakta, sitokin salınımı ve adhezyon molekül oluşumunu inhibe etmektedir. Bu çalışmanın amacı; LPS ile uyarılmış Tıkanma sarılığı modelinde Melatonin kullanımının karaciğer ve böbrek lipid peroksidasyonu ve NF-κB ekspresyonuna etkisini irdelemektir.

Materyal Metod
60 rattan oluşan 6 grup oluşturuldu. Grup A: Sham, Grup B: Tıkanma Sarılığı, Grup C: Tıkanma Sarılığı + Melatonin (20 mg kg-1 intraperitoneal), Grup D: Tıkanma sarılığı+ LPS (10 mg kg-1), Grup E: Tıkanma sarılığı+ LPS + Melatonin. Grup F: Tıkanma sarılığı + Melatonin +LPS.

Sonuçlar
Serum ve doku (karaciğer/böbrek) MDA, MPO düzeyleri ve doku NF-κB seviyeleri grup 2, 4 ve 6' da artmış olarak bulundu. Melatonin uygulanmasını takiben (grup 3 ve 5), karaciğer/böbrek doku MDA ve MPO seviyelerinde azalma saptandı Ayrıca bu gruplarda; doku NF-κB seviyeleri diğer gruplara göre düşük bulundu.

Yorum
Bu deneysel çalışma modelinde LPS ile uyarılan tıkanma sarılığında melatonin etkisi araştırılmıştır. Melatonin kullanımı ile tıkanma sarılığı oluşturulan deneklerde karaciğer ve böbrek dokusunda koruyucu etki sağlanmıştır. Ancak LPS ile uyarılan deneklerde melatonin verilmesinin endotoksemiyi önlemede etkili olmadığı görülmüştür.

Anahtar Kelimeler: *Lipid peroksidasyonu, Lipopolisakharid, LPS, Melatonin, NF-κB Düzeyleri Engellenmiş Sarılık,Karaciğer,Böbrek, Rat.*

INTRODUCTION

Patients with obstructive jaundice (OJ) have increased peroperative complications, such as sepsis, bleeding, wound problems, and renal and liver malfunction (1, 2). Endotoxemia is one of the major complications that can lead to complicated pathophysiological changes in the process of OJ (3).

Lipid peroxidation is associated with the pathogenesis of tissue damage in animals with OJ. Free oxygen radicals (FOR) seem to play a role in the pathogenesis. FOR scavengers reduce in the bile duct-ligated rats, thereby increasing the susceptibility of the liver to injury by oxygen derived free radicals (4). One of the important problems in OJ is the increased incidence of endotoxemia that results from defective host immune response. Systemic endotoxemia developed in OJ. There is a depression in the clearance of

lipopolysaccaride (LPS) and other endotoxins in OJ.

Melatonin (Mel) acts as a free radical scavengers. It acts as an antioxidant. It reduces oxidative stress, decreases MDA and MPO activities. Mel decreases NF-κB activities, reduces Superoxide dismutase, glutathione peroxidase, glutathione reductase enzyme activities. It decreases peroxinitrite and NO levels. (5).

The effects of Melatonin in OJ induced by LPS (OJ+LPS) and NF-κB activity in OJ treated by melatonin have not been investigated until this time. Therefore in this experimental study; we evaluated the effect of Melatonin in OJ and OJ+LPS by analyzing serum and tissue lipid peroxidation, liver and renal tissue histopathological changes. And finally we analyzed the effects of Melatonin on tissue NF- κB expression in OJ and OJ+LPS groups.

MATERIAL AND METHODS

Animals

This experimental study was conducted in adherence to National Institutes of Health guidelines on the use of experimental animals and was carried out in accordance with institutional policies and guidelines for the care and use of laboratory animals. Approval of the Institutional ethics committee was taken. (date and number of approval: November 20, 2006, B.30.2.MEU.0.01.00.00./4920–12/3). The experiments were performed in the Animal Experimental Laboratory of the Institution.

60 male, Wistar Albino rats, weighing 150–220 g, were housed at constant temperature with 14/10 h periods of light and dark exposure, respectively. Animals were given diet and tap water prior to the experiments. All rats were anaesthetized with intramuscular ketamine HCl (50 mg kg-1) (KetalarREczacıbası, Warner Lambert Ilac AS, Istanbul, Turkey) and xylazine (5 mg kg-1) (RompunR Bayer AG, Leverkusen, Germany). The animals were kept on a warm water mattress during the procedure and kept warm postoperatively until regaining consciousness. We randomized 60 rats into 6 groups. Group A: Sham; Group B: Obstructive jaundice (OJ); Group C: OJ + Melatonin (20 mg kg-1); Group D: OJ + LPS (E. Coli LPS serotype L-2630, 100 mgr, Sigma); Group E: OJ+ LPS+ Melatonin; Group F: OJ+ Melatonin+ LPS.

SURGICAL PROCEDURES

Bile duct ligation

The chest and abdomen were shaved and each animal was fixed in supine position on the operating table. The abdomen was cleaned with 1 percent polyvinyl iodine and the operating field was covered with a sterile drape. The abdomen was opened through a midline incision and experimental jaundice was created by ligation of the common bile duct according to the technique described in details by Lee (6).

The duodenum was retracted, the common bile duct was identified then ligated with 5/0 sutures and cut to prevent recanalisation. The abdominal wall was closed with interrupted silk sutures and the skin was approximated with a subcuticular stitch.

Jaundice was observed at the end of the fifth postoperative day. In Group C, Melatonin (20 mg kg-1) was administered intraperitoneally daily for 5 days, starting on day 5. In Group D, we first injected LPS (10 mg kg-1) intraperitoneally on day 5 and rats were sacrificed after 6 hours. In Group E, LPS was injected at the end of day 5, Melatonin was administrated after 6 hours and daily for 5 days. In Group F, Melatonin was administrated at the end of day 5 and repeated daily for 5 days. LPS was injected at the end of day 10 and rats were sacrificed 6 hours later. Rats were sacrificed on postoperative day 5 for Group B and D and on postoperative day 10 for Group C, E and F. The sham operation consisted of mobilization of the

bile duct only. The rats in this group were sacrified immediately after the procedure. In Group B, rats were sacrificed on the fifth day in order to reveal the prime changes on lipid peroxidation in the jaundiced rats. The rats in group C, E and F were sacrificed on the tenth day in order to find out and demonstrate clearly the biochemical changes developed when Melatonin was administrated in the jaundiced rats after or before the establishment of endotoxemia. These groups were designed to evaluate the therapeutic and protective effect of the drug in conditions like OJ and LPS-induced OJ.

The blood was taken by cardiac puncture for serum malondialdehyde (MDA), serum myeloperoxidase (MPO) activity. The liver and renal tissue samples were harvested. Liver and renal tissue MDA, MPO levels were determined. The liver and renal tissue histopathological changes and NF-κB levels were determined. The liver and renal tissue NF-κB expression was illustrated immunohistopathologically.

BIOCHEMICAL MEASURES

Determination of MDA

The levels of serum and tissue lipid peroxidation products as thiobarbituric acid (TBA)–MDA.
Adducts were measured spectrophotometrically by the method described by Yagi (7).

Determination of MPO

The determination of serum and tissue MPO activity depends on the fact that it reduces odianozidine. Reduced o-diazidine was measured at 410 nm by spectrophotometer (8). The MPO activity was expressed as units per liter for serum and units per gram for tissue.

HISTOPATHOLOGICAL EVALUATION

The tissues of each group were sectioned, fixed with 10 % formalin, dehydrated and embedded in paraffin for the histopathological examination. Histopathological scoring of hepatic and renal injury was performed semiquantitatively.

The hepatic injury scored as follows

0, no hepatocyte injury; 1, minimal cellular changes; 2, only minimal centrolobular injury; 3, severe centrolobular injury; 4, minimal centrolobular and midzonal injury; 5,severe centrolobular and midzonal injury; 6,totally destruction of hepatocytes.

The kidney sections were analysed

The changes were limited to the tubulointertisial areas and graded as: 0, normal; 1, areas of focal granulo-vacuolar epitelial cell degeneration and granular debris in the tubular lumen, with or with no evidence of tubular epitelial cell desquamation in small foci (< 1% of the tubule populaton involved by desquamation) 2, tubular epitelial necrosis and desquamation easily visible, but involving less than half of the cortical tubules; 3, more than half of the proximal tubules showing necrosis and desquamation but involved tubules easily visible; 4, complete or almost complete proximal tubular necrosis.

MORPHOMETRIC DETERMINATION

NF-κB Expression

Immunohistochemical evaluation of NF kappa B/p65 (Rel A) Ab–1 (RB-1638dilusyon 1:75, Neomarkers Labvision, Fremont, USA) were performed using a combination of streptavidinbiotin peroxidase method and microwave antigen retrieval on formalin fixed paraffin embedded tissues according to manufacturers methods. The sections were evaluated for diffuseness and intensity of staining in tubulointerstitial cells and hepatocytes.

For diffuseness sectios were graded as
0, no staining; 1, staining <25%; 2, staining 25–50%; 3, staining 50–75%; 4, staining >75%.

For staining intensity, sectons were graded as
0, no staining; 1, weak but detectable above control; 2, distinct; 3, intense.

Statistical Analysis
Descriptive statistics (median and % 25 and % 75 quartiles) is calculated in each group for HE and NF-Kappa Bscores parameters. Kruskall-Wallis is used to test differences between groups for HE and NF iNOS scores. Multiple comparisons groups were analyzed Dunn test. Box-plot graphs were used to show medians of liver and renal NF-kappa B scores.

Biochemical values were as mean ± standard deviation (SD) values. These parameters are assigned of homogeneity of variances. One- Way analysis of Variance (ANOVA) were used for parameters provide homogeneity of variances and Welch test were used for parameters provide heterogeneity of variance. Tukey test were used for multiple comparisons in One-Way analysis of Variance and Games- Howell test for multiple comparisons in Welch test statistics.

RESULTS

Biochemical Examination
Biochemical values of each group are showed in Table–1.The descriptive statistics of serum, liver, and renal MDA; and serum, liver, and renal MPO are presented in Figures 1 and 2 respectively. The significance levels of pairwise comparisons of groups according to Tukey's post hoc test are presented in each figure.

Serum, liver and renal MDA;
When the serum, liver, and renal MDA of the groups were compared, there was a significant difference between groups A and B, A and D, A and E, C and D, B and F ($p < .05$) (Figure 1). In Group C serum, liver and renal MDA levels were decreased compared with group B and D; but the difference were not found statistically significant. There were no significant difference between, Groups A (Sham group) and C (OJ+M) (Figure–1)

Serum, liver and renal tissue MPO;
There was a significant difference between Groups A(Sham group) and B(OJ), Groups A(Sham group) and D(OJ+LPS), Groups A(Sham group) and E(OJ+LPS+M), Groups B(OJ) and D(OJ+LPS), B(OJ) and E(OJ+LPS+Mel), B(OJ) and F(OJ+Mel+LPS), Group C(OJ+Mel) and Groups D(OJ+LPS) Group C(OJ+Mel) and Groups E(OJ+LPS+Mel) Group C(OJ+Mel) and Groups F(OJ+Mel+LPS). There was no significant difference between, Groups A (Sham group) and C (OJ+Mel), and Groups B and C (Figure–2)

HISTOPATHOLOGICAL RESULTS

Liver and renal HE scores values of each group described as median, with interquartile range in brackets were shown in Table-2.

Liver
In the histopathological examination of the liver of the rats (Figure 5) in the sham group (group A), normal histologic findings were detected. In group B (OJ group), severe focal inflammation and coagulation necrosis and bile duct proliferation was observed in the portal or ductal space. In group C (OJ+Mel) minimal focal inflammation or necrosis was detected. There was a significant difference between groups A and B, groups A and D, groups C and D, groups A and E, groups A and F.($p < .05$)(Figure-3) In groups D (OJ + LPS group) and E (OJ + LPS + Mel group), there was a severe focal inflammation, bile duct proliferation and hepatocyte necrosis was observed in the portal or ductal space. However, in groups C (OJ + Mel group), and F (OJ +

Mel + LPS group), these changes were minimal. The liver HE scores of group B and D was significantly higher than the control group. The median scores of group C (OJ + Mel group), and F (OJ + Mel + LPS group), were lower than the other groups.

Renal

In the histopathological examination of the kidney of the rats (Figure 6) in the sham group (group A), normal histologic findings were detected. In group B (OJ group), renal tubuler desquamation and granular debris was observed in the glomerular space. In group C (OJ+Mel), minimal renal tubuler desquamation and granular debris was observed in the glomerular space. There was a significant difference between groups A and B, groups A and D, groups A and E, groups A and F, groups C and D.($p < .05$)(Figure-3)

In groups D (OJ + LPS group) and E (OJ + LPS + Mel group), there was a severe tubuler desquamation, granular debris, tubuler cytoplasm acuolization and congestion was observed. However, in roups C (OJ + Mel group), and F (OJ + Mel + LPS group), these changes were minimal. The renal HE scores of group B and D was significantly higher than the control group. The median scores of group C (OJ + Mel group), and F (OJ + Mel + LPS group), were lower than the other groups.

IMMUNOHISTOCHEMICAL NF-κB EXPRESSION

Liver and renal Immunohistochemical expression values of each group described as median, with interquartile range in brackets were shown in Table-2.

Liver

In the immunohistochemical examination of expression in the liver of rats (Figure 7) in the sham group (group A), very weak NF-κB staining was detected in hepatocytes around the central venules of the lobules. In group B (OJ group), the number of NF-κB stained cells were increased. There was a significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups A and F.($p < .05$)(Figure-4)

In groups D (OJ + LPS group) and E (OJ + LPS + Mel group), there was intense NF-κB staining. However, in groups C (OJ + Mel group), and F (OJ + Mel + LPS group), NF-κB staining was sparse.

Renal

In the immunohistochemical examination of renal NF-κB expression (Figure 8), it was noted in the tubular epithelium. There was severe immunostaining in the tubular epithelium, in both the cortical and medullar region, and rominent in the outer medulla.

In sham group (group A), very weak NF-κB staining was detected in a few tubular epithelium samples. In groups B (OJ group), D(OJ + LPS group), and E (OJ+ LPS + Mel group) the numbers of NF-κB stained cells were increased. In groups C (OJ + Mel group) and F (OJ + Mel + LPS group), NF-κB staining was sparse. There was a significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups A and F, ($p < .05$)(Figure–4)

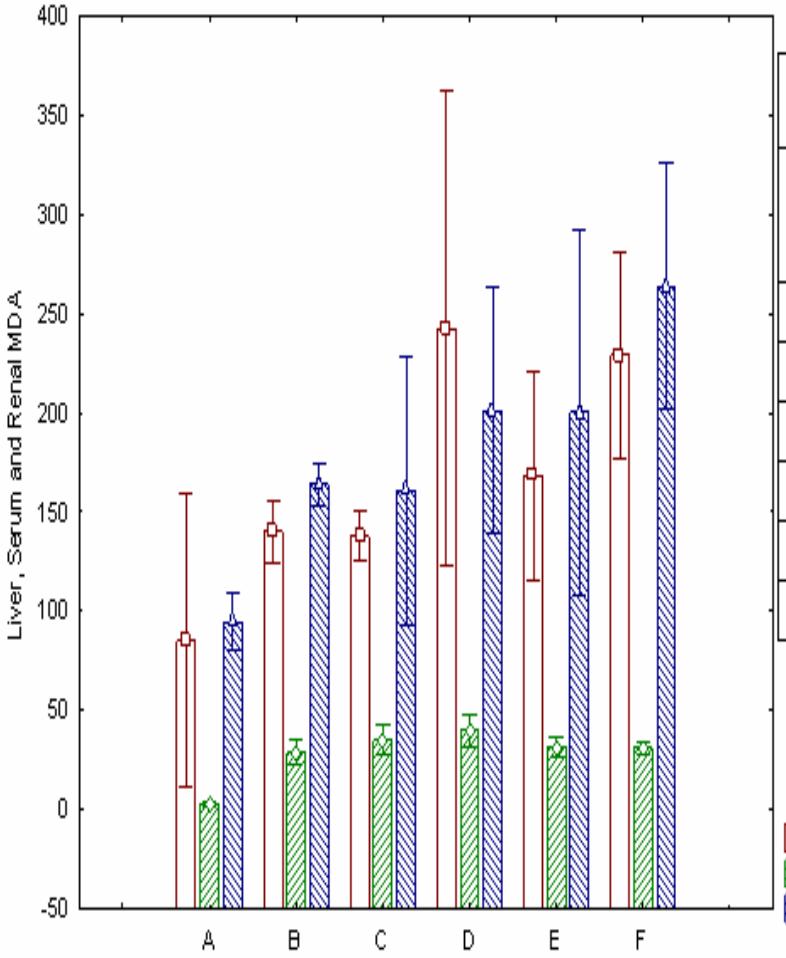
The tubular epithelium showed more intense staining in the cases of group D(OJ + LPS) and group E (OJ + LPS + Mel) when compared to group C (OJ +Mel) and group F (OJ + Mel + LPS group).But the difference were not found statistically significant.

Table-1: *Biochemical values of each group described as mean ± standard error of mean (SEM)*

	Sham	OJ	OJ+ Mel	OJ+LPS	OJ+LPS+Mel	OJ+Mel +LPS
Serum MDA	2.59 ± 0.53	28.45 ± 5.95	35.13 ± 7.38	40.02 ± 9.66	31.83 ± 5.82	30.94 ± 3.07
Serum MPO	265.20 ± 42.63	602.5 ± 180.9	513.79 ± 265.33	2724.53 ± 1361.65	2456.29 ± 508.83	2095.76 ± 769.61
Liver MDA	85.0 ± 46.45	140.04 ± 19.02	137.74 ± 14.87	242.69 ± 143.13	168.36 ± 63.43	229.13 ± 62.30
Liver MPO	0.21 ± 0.02	1.55 ± 0.68	1.21 ± 0.31	1.71 ± 0.36	1.96 ± 0.96	1.57 ± 0.41
Renal MDA	94.44 ± 13.69	163.91 ± 12.52	160.71 ± 81.08	201.07 ± 74.46	200.38 ± 110.12	263.84 ± 74.09
Renal MPO	0.45 ± 0.24	1.14 ± 0.46	0.74 ± 0.18	1.49 ± 0.39	1.53 ± 0.34	1.51 ± 0.41

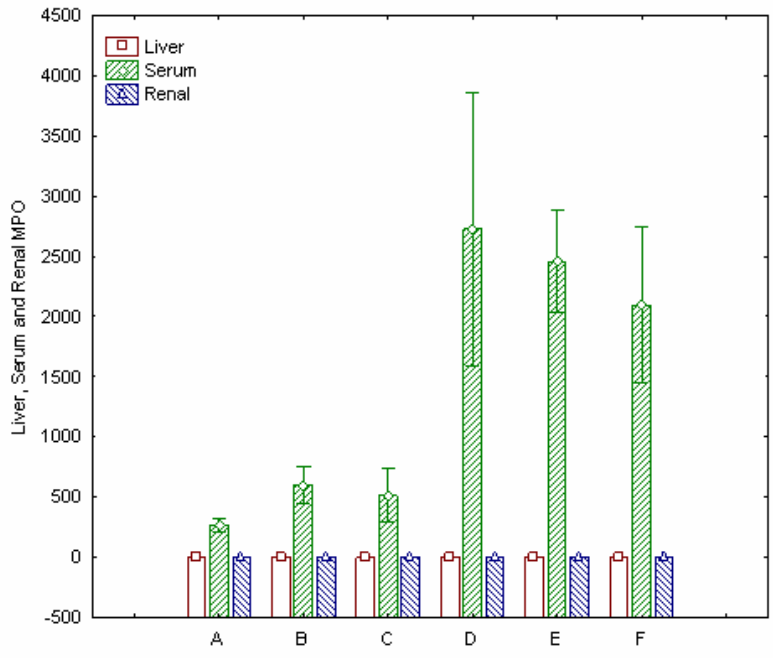
Table-2: *HE scores and immunohistochemical NF-κB expression values of each group described as median, with interquartile range in brackets*

	Group A (Sham)	Group B (TS)	Group C (TS+M)	Group D (TS+LPS)	Group E (TS+LPS+M)	Group F (TS+M+LPS)
Liver HE	0 [0 to 0]	5 [2.25 to 5]	4 [1.25 to 4]	6 [3.75 to 6]	6 [2.25 to 6]	5.5 [4.5 to 6]
Renal HE	0 [0 to 0]	1.5 [1 to 2]	1.0 [1 to 1.5]	2 [1 to 2]	2 [1 to 2]	1 [1 to 2]
Liver NF	0 [0 to 0]	4 [2 to 4]	3 [3 to 4]	5 [3 to 5]	4 [2 to 4]	3 [2 to 4]
Renal NF	0 [0 to 0]	4 [2 to 5]	3.5 [2.75 to 4]	5 [3 to 5]	3 [2 to 4]	3 [2 to 4]



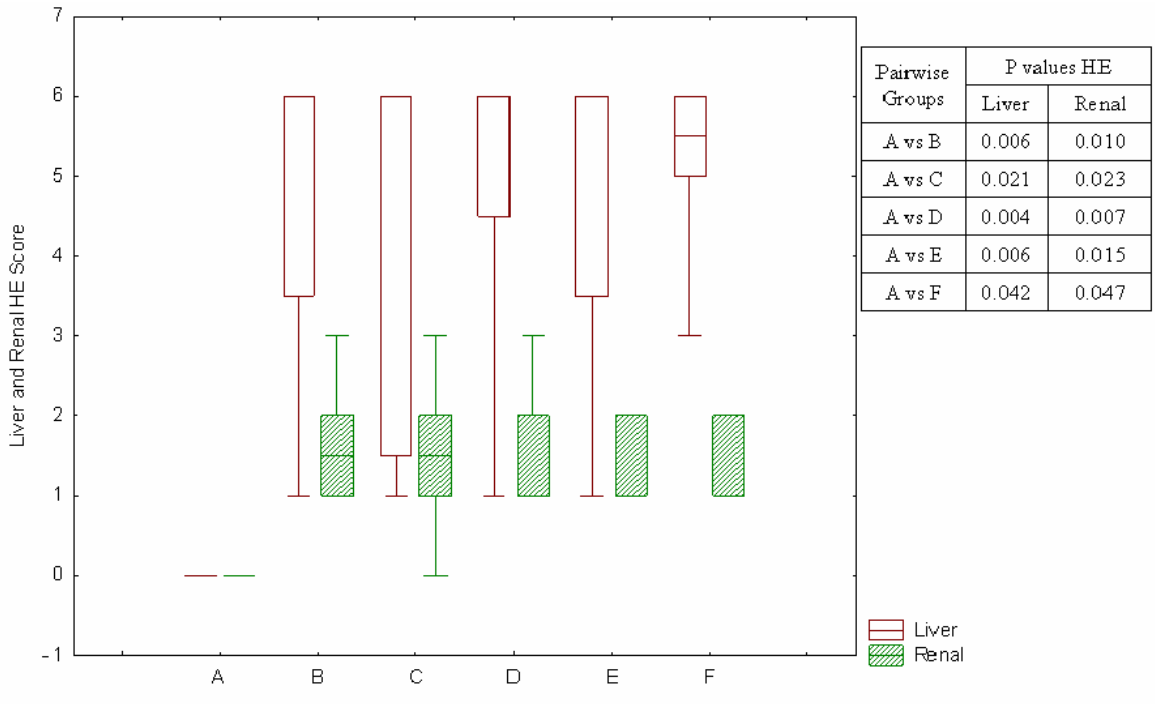
Pairwise Groups	P values MDA		
	Liver	Serum	Renal
A vs B	-	0.001	0.0001
A vs C	-	0.001	-
A vs D	0.015	0.0001	0.036
A vs E	0.033	0.0001	-
A vs F	-	0.0001	0.002
B vs F	-	-	0.048

Figure-1: *Liver, renal tissue and seru MDA levels*



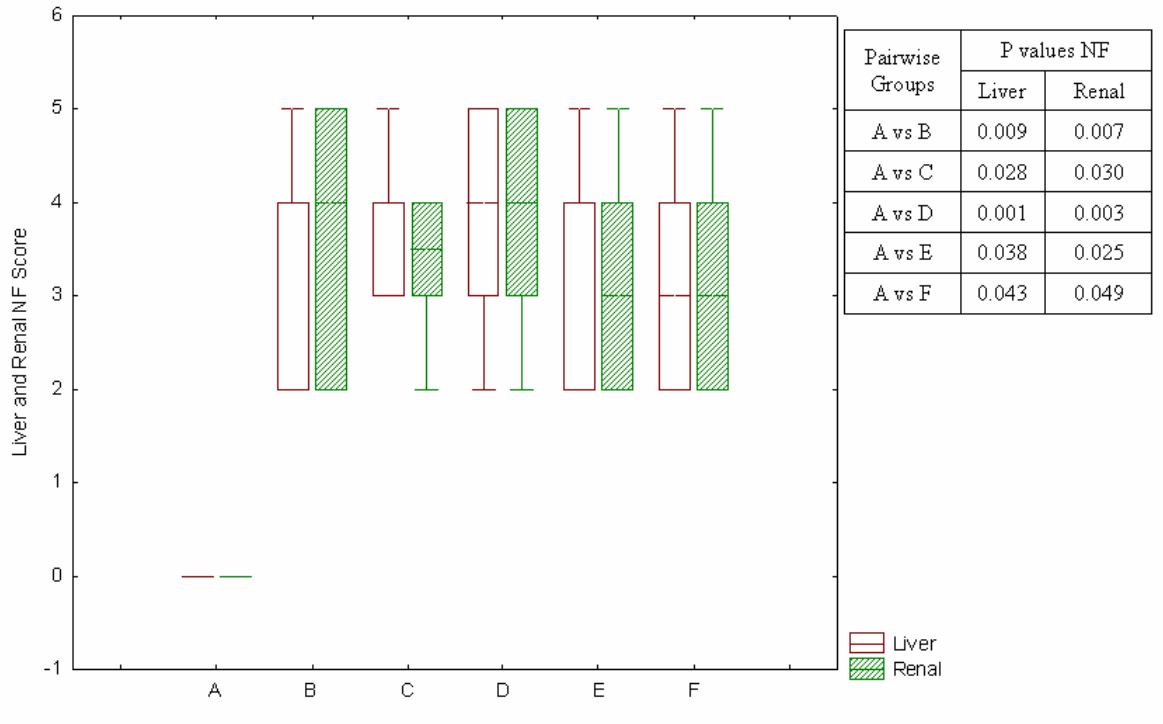
Pairwise Groups	P values MPO		
	Liver	Serum	Renal
A vs B	0.002	0.008	0.010
A vs C	0.036	-	-
A vs D	0.0001	0.011	0.0001
A vs E	0.0001	0.0001	0.0001
A vs F	0.002	0.002	0.0001
B vs D	-	0.024	-
B vs E	-	0.0001	-
B vs F	-	0.007	-
C vs D	-	0.019	0.002
C vs E	-	0.0001	0.002
C vs F	-	0.004	0.002

Figure-2: *Liver, renal tissue and serum MPO levels*



Pairwise Groups	P values HE	
	Liver	Renal
A vs B	0.006	0.010
A vs C	0.021	0.023
A vs D	0.004	0.007
A vs E	0.006	0.015
A vs F	0.042	0.047

Figure-3: *Liver and renal tissue HE scores*



Pairwise Groups	P values NF	
	Liver	Renal
A vs B	0.009	0.007
A vs C	0.028	0.030
A vs D	0.001	0.003
A vs E	0.038	0.025
A vs F	0.043	0.049

Figure-4: *Liver and renal tissue NF-κB scores*

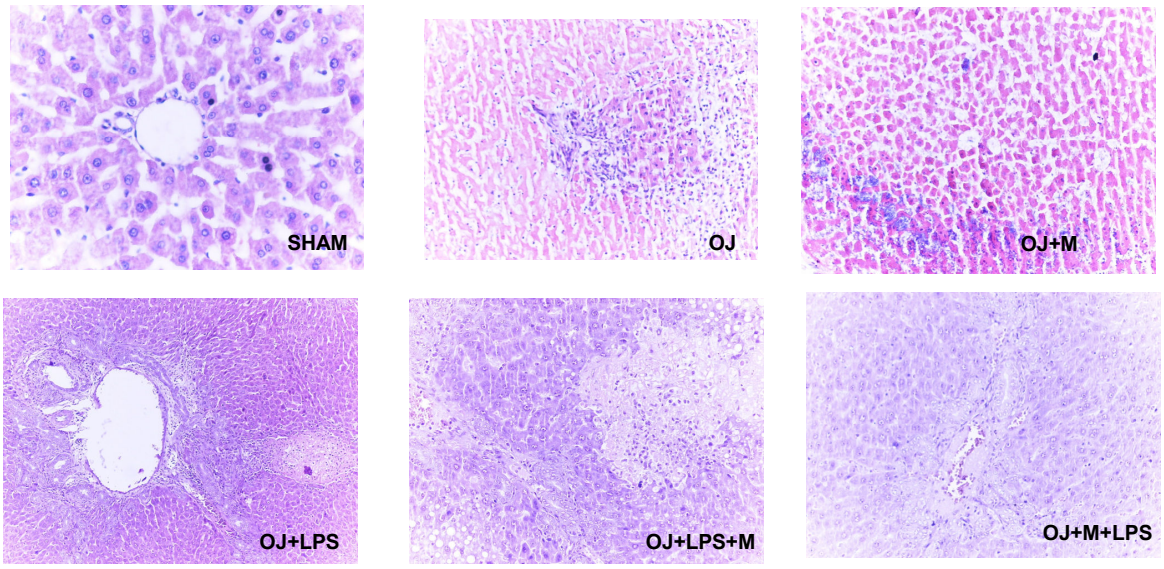


Figure-5: *Hematoxilene-Eosine (HE) Staining in Liver Tissue*

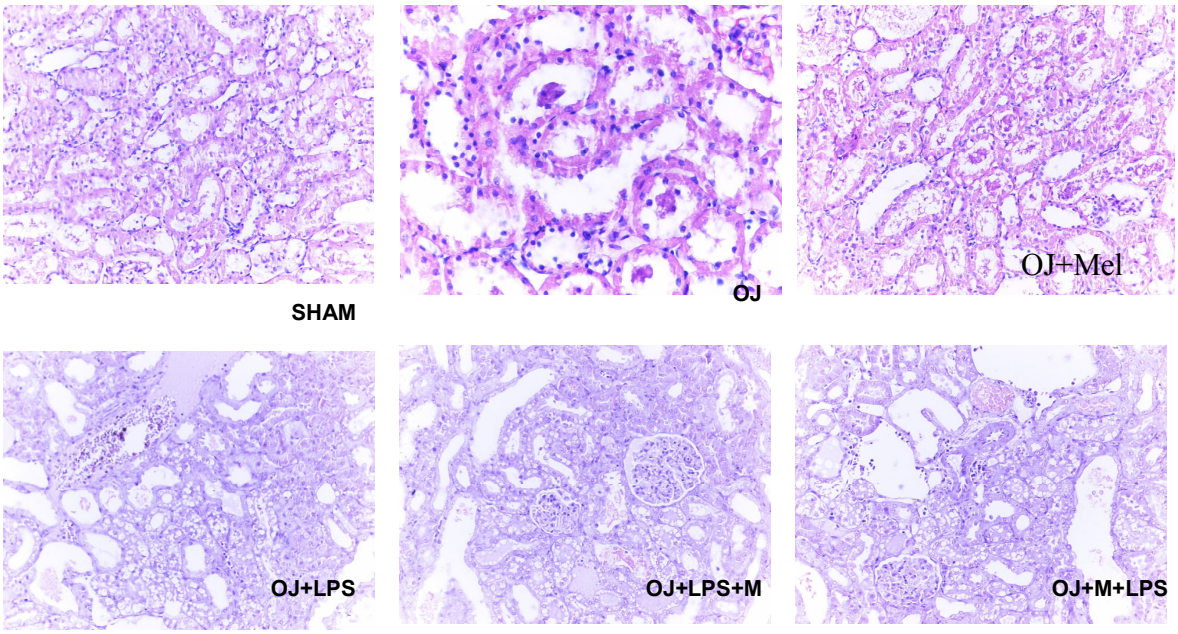


Figure-6: *Hematoxilene-Eosine (HE) Staining in Renal Tissue*

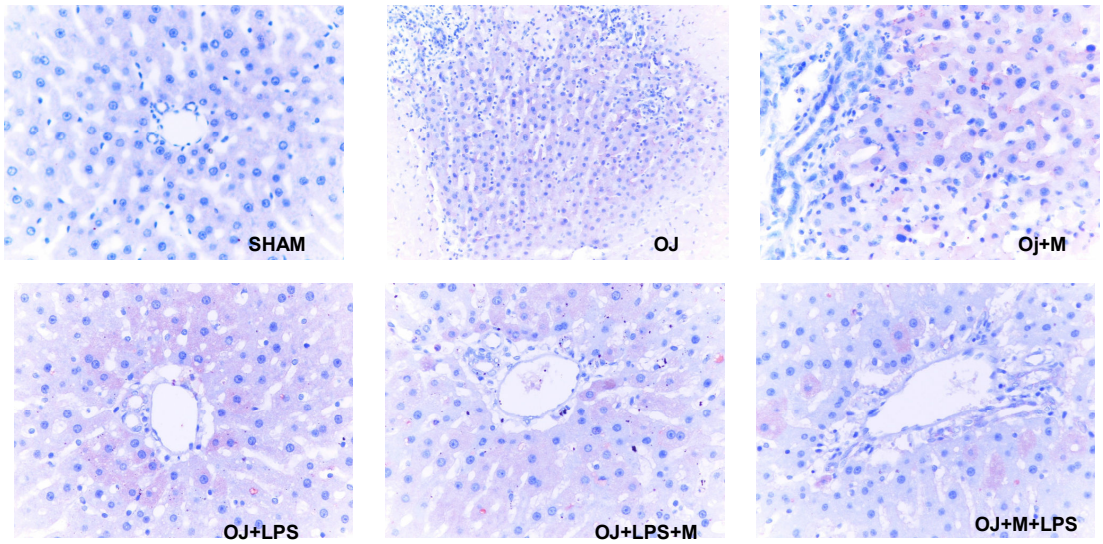


Figure-7: *Representative sparse and intense NF-κB staining in the immunohistochemical examination of liver tissue. ×400*

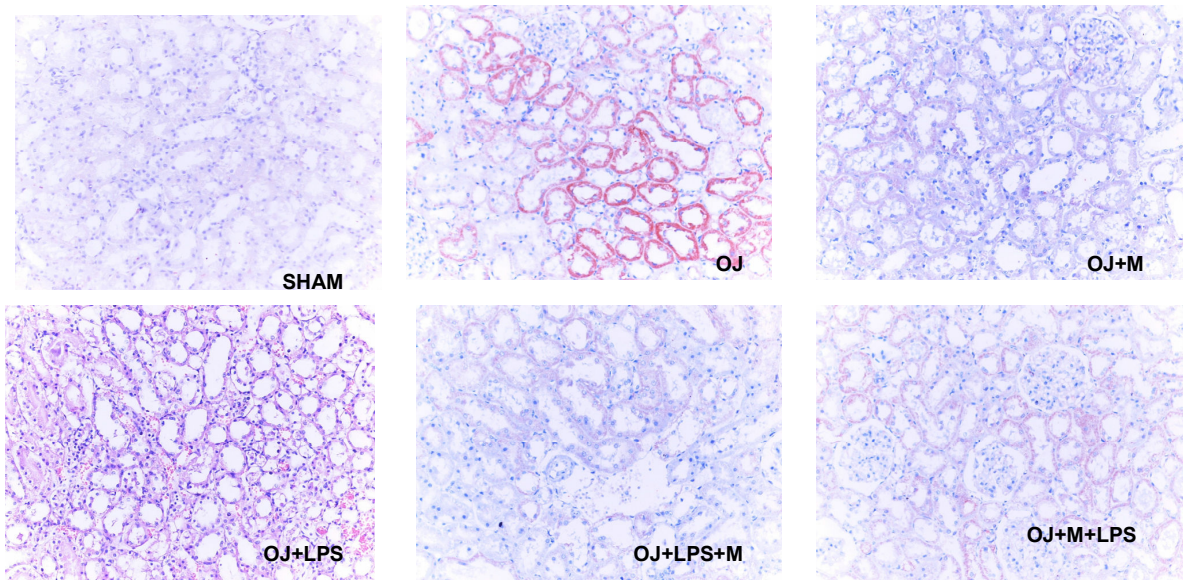


Figure-8: *Representative sparse and intense NF-κB staining in the immunohistochemical examination of renal tissue. ×400.*

DISCUSSION

Oxidative stress results from antioxidants depletion and oxidants overproduction. Free oxygen radicals have been implicated as mediators of tissue injury in a variety of diseases. Most free radical reactions involve the reduction of molecular oxygen leading to the formation of highly reactive oxygen species such as super oxide anion (O2-), hydroxyl radical(-OH), hydrogen peroxide(H2O2) and single oxygen(1O2). In OJ, the free radicals production is increased and the antioxidative activity is reduced. This phenomenon combined with lipid peroxidation at the site of inflammation leads to tissue damage. Combined portal endotoxemia resulting from impaired intestinal barrier and systemic endotoxemia resulting from dysfunctional Kupffer cells lead to sepsis (1,2,3).

Sepsis is a severe and major complication related to OJ, and it is also the reason of high morbidity and mortality. The neutralization and elimination of the intestinal endotoxin related to the lack of bile in the intestinal lumen are reduced in OJ (4). At the same time, impaired gut barrier function leads to bacterial translocation. The activation of polynuclear leukocytes, monocytes, and macrophages in OJ leads to release of many mediators, which contribute to the pathophysiology of the systemic inflammatory response syndrome, sepsis, and multiple organ failure (9,10,11). On the other hand, these mediators bind to the respective cell surface receptors and activate tyrosine kinase and nuclear factor kappa B (NFκB). This leads to transcription of iNOS protein in different cells and organs and to overproduction of NO (12,13,14).

NF-κB is an inducible nuclear transcription factor regulating the expression of many genes. NF-κB activation may function as a master switch in a variety of immune and inflammatory processes, including sepsis and transplant tolerance. NF-κB exists in the cytoplasm in an inactive form associaced with inhibitory proteins termed I B. When the cell is exposed to activation signals such as LPS or TNF-alfa binding to

cell surface receptors, the I B protein is phosphorylated and ubiquinated and broken down in proteosomes. After being freed NF-κB moves to the nucleus and promoter/enhancer regions of genes (15,16,17,18,19).

Melatonin(N-acetyl-5-methoxytryptamine) is a lipophilic indole amine derived from tryptophan. Melatonin is a potent antioxidant, well tolerate and without toxicity upon its administration. Melatonin has a powerful capacity to scavenge free radicals and prevents tissue damage (20,21,22,23,24). In particular, melatonin prevents oxidative stress induced by I/R in liver (25,26), brain (27,28), myocardium (29), intestine (30,31) and kidney (32). Melatonin acts as a free radical scavengers. It acts as an antioxidant. It reduces oxidative stress and MDA and MPO activities. It decreases NF-κB activities and reduces superoxide dismutase, glutathione peroxidase, glutathione reductase enzyme activities. It decreased peroxinitrite and NO levels. (33,34). As it is known; there is a significant decrease in hepatic and plasma reduced glutathione in rats with obstructive jaundice. The renal oxidative enzyme status has also been damaged in OJ. The literature review about the effect of Melatonin in OJ revealed that hepatic and renal oxidative stress was attenuated with melatonin treatment (35,36,37).

The present study is the first in the literature investigating the effect of Melatonin on liver and renal tissue NF-κB expression in LPS induced OJ. The results of our study showed that; the hepatic and renal tissue lipid peroxidation is increased in OJ. We observed an increase in serum, liver and renal tissue MDA and MPO levels in OJ, OJ+LPS, OJ+LPS+Mel groups. Lipid peroxidation and oxidative stress were ameliorated when LPS was administrated to the jaundiced rats. Melatonin decreased serum, liver and renal MDA and MPO levels in OJ, but the difference was not found statistically significant. In order to evaluate the protective and therapeutic effect of Melatonin, we administered it to the jaundiced rats in which endotoxemia is induced with LPS before (OJ+LPS+Mel) or after administration of Melatonin (OJ + Melatonin+ LPS), to see whether it exerts any beneficial effect on LP and NF-κB expression.

In the comparison of OJ+LPS group with OJ+M+LPS group (when melatonin given before LPS); serum, liver and renal tissue MDA and MPO levels were detected lower in OJ+M+LPS group. These results show that lipid peroxidation and oxidative stress are prevented with the protective effect of Melatonin.

In the comparison of OJ+LPS group with OJ+LPS+M group (when melatonin given after LPS); serum MDA and MPO, liver MDA and MPO levels were detected lower in OJ+LPS+M group.
In the histopathological examination of liver and renal tissue; groups were compared according to damage scores. Damage scores in OJ and OJ+LPS groups were higher than the damage scores of all groups. Damage scores in the groups in which Melatonin applied before and after LPS were lower than the damage score of group OJ+LPS.

NF-κB expression in OJ and OJ+LPS groups were higher than NF-κB expression in other groups. NF-κB expression in the groups (OJ+LPS+M and OJ+M+LPS) in which Melatonin applied before and after LPS were lower than the NF-κB expression group OJ+LPS.

Melatonin in OJ attenuated liver and renal tissue histopathological and NF-κB scores. In the immunohistochemical examination of NF-κB expression, we found that liver and renal NF-κB expression were increased significantly in group D as compared to sham and OJ groups This can be explained by the fact that the increased sensitivity to LPS and endotoxin in OJ can lead to exaggerated NF-κB expression, LP, and oxidative stress, which may lead to organ dysfunction. In

conclusion; in this model of OJ stimulated by LPS, Melatonin suppressed the adverse effects of OJ in the absence of LPS. It is clearly shown that melatonin acts as a hepatic and renal protective effect against oxidative stress in OJ. However, it failed to prevent lipid peroxidation in case of LPS-induced OJ when it is administrated before or after LPS.

For this reason, we think that Melatonin can be used as a protective agent in OJ; but it seems not to be a useful agent in endotoxemia established OJ. In our opinion; clinical applications for jaundiced patients need further investigation.

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