The protective effect of melatonin on remote organ liver ischemia and reperfusion injury following aortic clamping

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BACKGROUND: *Severe local and systemic tissue injuries can occur after restoration of tissue oxygenation which is also known as reperfusion injury. Our objective was to investigate the possible protective effects of melatonin against IR damage in hepatic tissue following infrarenal aortic occlusion.*
METHODS: *A total of twenty-one male Wistar-albino rats separated into three groups as follows: Group I: Laparotomy and dissection of the infrarenal abdominal aorta (AA) were concurrently performed. Group II: About 1 ml of 0.9% saline was intraperitoenally administered 30 min before and after the occlusion operation. After laparotomy and dissection, infrarenal AA was clamped for 30 minutes and then was exposed to two hours of reperfusion. Group III: The melatonin was administered 30 min before clamping of the infrarenal AA then 30 min of ischemia and two hours of reperfusion was applied.*
RESULTS: *Serum aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels were remarkably higher in IR group, when compared with the sham group, and the laboratory tests returned to normal levels in IR+MEL group after treatment. Although serum IL-1β, IL-6, IL-18, TNF-α, and IFN- γ levels have decreased in treatment group following melatonin administration, this decrement was statistically significant for serum IL-18, TNF-α, and IFN-γ parameters compared with the IR group. Serum levels of TOC and OSI were decreased and tissue levels of TAC were increased by melatonin.*
CONCLUSION: *As a result of this study, it can be suggested that melatonin has antioxidant, anti-inflammatory and hepatoprotective effects in case of IR.*

KEY WORDS: Aortic occlusion, Injury, Ischemia/Reperfusion, Liver, Melatonin

Introduction

Total aortic clamping is commonly used to control bleeding and this method is an important part of the surgical interventions including transplantation, cadaveric organ removal, and major abdominal surgery, post-traumatic and reconstructive treatments. Ischemia, which is characterized by poor organ perfusion, occurs during this period described above, when arterial or venous blood flow is interrupted [1]. Restoration of arterial blood flow, which is also known as the reperfusion period, constitutes the main factor to maintain the viability of ischemic organs. Severe local and systemic tissue injuries can occur following oxygenation of the tissues with reperfusion,

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which was firstly described by Haimovici as "reperfusion injury" in 1960 [2]. There is a balance between free radicals and antioxidant defense mechanisms under normal conditions. The disruption of this balance in favor of oxidants is called oxidative stress [3,4].
Melatonin, which is the main product released from the pineal gland, exerts well-known antioxidant, immune and reproductive functions 5and free radical scavenger [6]. Due to its low molecular weight and high lipophilicity, it easily penetrates the cellular membranes and fights against oxidative stress by adjusting intracellular calcium and malondialdehyde levels and suppressing tumor necrosis factor (TNF)-α, interleukin 1β and IL-6 levels [7,8]. In this study, we aimed to investigate the possible anti-inflammatory, antioxidant and protective effects of melatonin in hepatic damage following infrarenal aortic occlusion–reperfusion in rat models and in preventing livers from this kind of oxidative damage study.

Materials and Methods

Current study protocol and experimental methods were approved by Afyon Kocatepe University Ethical Committee of Experimental Animals (References No: Akuhadyek-329-14). Care of all rats was done according to the Experimental Animal Usage and Principles regulated by the National Health and Medical Research Council and according to the Guide for Experimental Animal Care and Usage prepared and issued by the National Institution of Health.

SUBSTANCE PREPARATION

Melatonin was initially dissolved in a very low volume of ethanol (96 %) and diluted in 0.9 % saline (a final concentration of 1% ethanol) and was intraperitoneally (i.p) administered 30 min before and after infrarenal abdominal aorta (AA) dissection and clamping.

ANIMALS

A total of twenty one Wistar-Albino rats, weighing between 250-300 grams (mean 270 g) were included in this study. Rats were inhabited in Eurotype-4 cages under animal laboratory conditions including diurnal rhythm of 12 h night and 12 h day, ambient temperature between 24-26°C and 50-60% humidity before the initiation of the experiment.

EXPERIMENTAL DESIGN

Animals were equally and randomly divided into three groups as follows:
Group I (SHAM, n=7):Laparotomy and infrarenal AA dissection were done during the same surgical time period and stress as in other groups but no clamping was done to infrarenal AA.
Group II (I/R, n=7):About 1 ml 0.9% saline was administered intraperitoenally (i.p.) 30 min before and after occlusion. After laparotomy and dissection of infrarenal AA, infrarenal AA was clamped by atraumatic microvascular clamp for 30 min and then was exposed to 2 hours of reperfusion.
Group III (IR+MEL, n=7): (2x10mg/kg body weight dissolved with ethanolin~1ml 0.9% saline solution) melatonin (Sigma-Aldrich, M5250, St. Louis, MO, USA) was administered i.p. 30min before infrarenal AA dissection and clamping, and then 30 min of ischemia was applied. Additionally, the same amount of Melatonin was administered in the same manner at the end of the occlusion period and then 2 hours of reperfusion was applied.

ANESTHESIA AND SURGICAL PROCEDURE

The rats were anesthetized with an intramuscular (IM) injection of 40 mg/kg ketamine (Ketalar, Parke-Davis, Eczacibasi, Turkey) following 5 mg/kg xylazine (IM) injection (Rompun, Bayer, Turkey) for premedication. The median laparotomy was applied to rats under sterile conditions. Following 10 mL of saline solution was administered i.p. to preserve fluid balance. After bowels were moved away by wet surgical gauze, infrarenal AA were carefully explored and clamped by atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, MN, USA) and abdominal incision was temporarily covered up by a plastic clothing to minimize loss of heat and fluid. Aortic ischemia was confirmed by loss of pulsation on distal aorta and aortic reperfusion was confirmed by return of pulsation on distal aorta after removal of clamp.
Biochemical Analyses: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities, indicators of liver ischemia, were analyzed in an autoanalyzer (Cobas 6000, Roche, Switzerland).

MEASUREMENT OF SERUM TOTAL OXIDANT STATUS (TOS)

The TOS of the serum was measured using an automated colorimetric measurement method for TOS [9,10]. In this method, oxidants presented in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which are abundantly presented in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules presented in the sample. The assay was calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2Eq/L).

MEASUREMENT OF SERUM TOTAL ANTIOXIDANT STATUS (TAS)

The TAS of the serum was measured using a novel automated colorimetric measurement method for TAS 10-12. This method is based on the bleaching of color char-

acteristics of a more stable ABTS (2,2'-azino-bis[3-ethylbenzothiazo-line-6-sulfonic acid]) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmolTrolox equivalent/L.

DETERMINATION OF SERUM OXIDATIVE STRESS INDEX (OSI)

The ratio of TOS to TAS represents the OSI, an indicator of the degree of oxidative stress (OS). The OSI value is calculated according to the formula:
OSI (arbitrary unit) = TOS (μmol H_2O_2Eq/l)/TAS (mmolTroloxEq/l) ×100 [12].

DETERMINATION OF BLOOD IL-1B, IL-6, IL-18, TNF-A AND IFN–Γ LEVELS

Serum samples were stored at -80°C until analysis. Serum IL-1β, IL-6, TNF-α, IFN-γ (E-Bioscience, Vienna, Austria) and IL-18 (Booster, Fremont, USA) levels were determined by ELISA technique using specific kits and the results were expressed as pg/mL [13].

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EXAMINATION

Liver tissue samples removed from sacrificed animals were fixed with 10 % neutral buffered formalin solution, have undergone routine histological tissue processing steps and embedded in paraffin blocks. Sections at five micron thickness were taken from paraffin blocks. Tissue slides were stained with hematoxylin-eosin stain for general morphological evaluation of tissues and poly-l-lyzin coated slides were stained with primary iNOS antibody via using indirect immunohistochemistry method. Stained slides were evaluated under light microscope (Eclipse E-600 Nikon, Japan).In general morphological evaluation of tissue slides, inflammatory cell migration, edema and sinusoidal enlargement were semi-quantitatively evaluated and scored between 0 and 4 according to their intensity degree. H-SCORE was used for immunohistochemical stain evaluation. The immunoreactivity of iNOS positive cells in six different areas under 40X objective magnification were counted and calculated for each slides and established data were analyzed.

Statistical Analyses: The data were analyzed by using SOFA statistics open source software. Results for descriptive statistics were expressed as mean±standard deviation (SD) or median [range (minimum–maximum)]. Statistical comparisons of continuous variables among the groups were performed using one-way analysis of variance (ANOVA) or Kruskal–Wallis test based on their distribution. Tukey test was performed for post hoc analysis after performing analysis of variance test. In cases where Kruskal–Wallis test yielded statistical significance, Bonferroni-corrected Mann–Whitney U-test was used to identify the groups which showed differences. P value <0.05 was considered statistically significant.

RESULTS EVALUATION OF BIOCHEMICAL DATA SUPPORTING LIVER DAMAGE

Blood AST, ALT, and LDH activities (Figs 1-3) were markedly higher in the I/R group compared to the sham group, the values were lower postoperatively in the IR+MEL treatment group (P=0,006). The statistical analysis of the data among the groups showed that the reduction in AST, ALT, and LDH activities were significant. The blood and statistical analysis results of the groups are summarized in Table I.

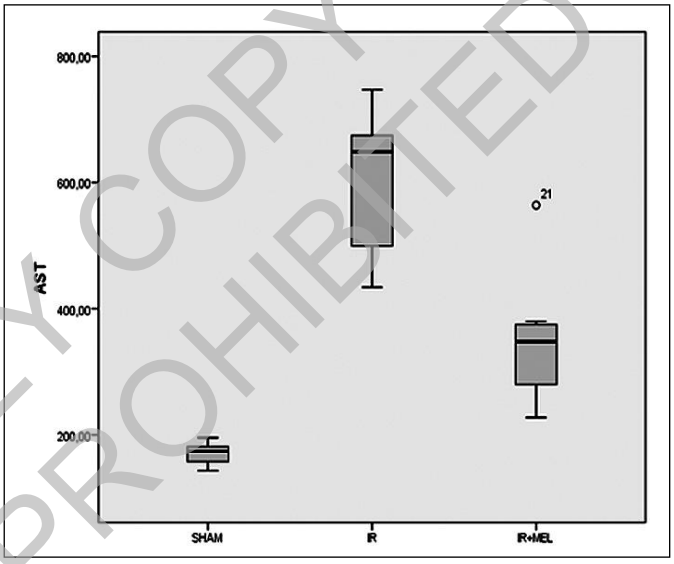

Fig. 1: Graphical analysis of the blood AST activities showing liver injury between the groups.

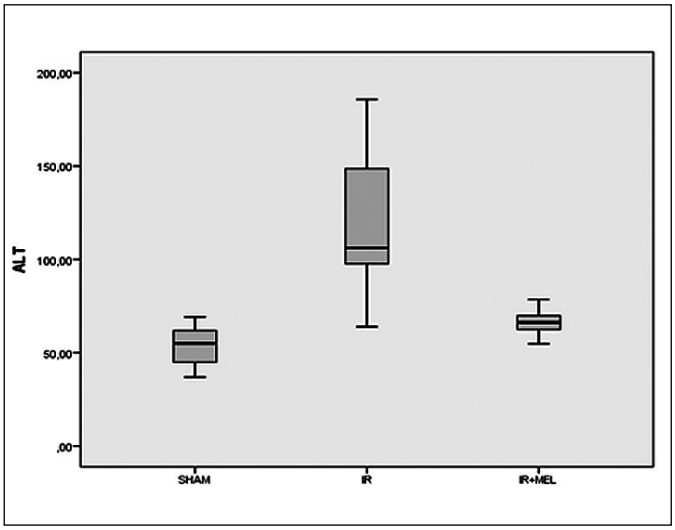

Fig. 2: Graphical analysis of the blood ALT activities showing liver injury between the groups.

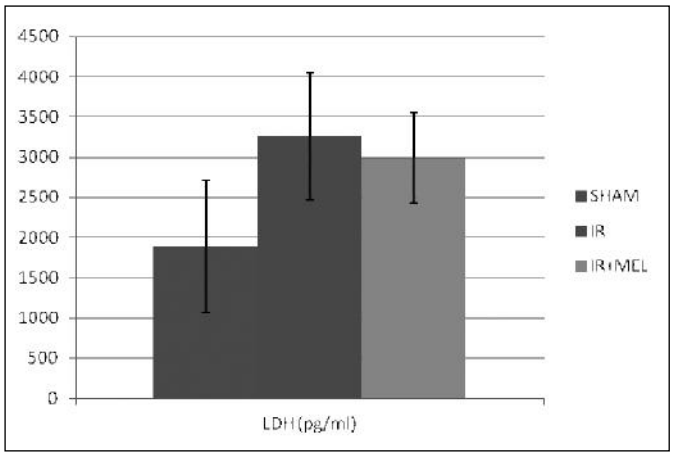

Fig. 3: Graphical analysis of the blood LDH activities showing liver injury between the groups.

EVALUATION OF BLOOD IL-1β, IL-6, IL-18, TNF-α, IFN- γ LEVELS

Blood IL-1β levels were median 39.5 (min:36.5-max:51.2) pg/ml in the sham group, median 45.7(min:37.1- max:94.1) pg/ml in the I/R group, and median 46.3 (min: 29.4- max: 78.8) pg/ml in the IR+MEL treatment group. A comparison of data among the groups revealed no significant findings (p>0.05).

Blood IL-6 levels were 94.9±9.4 pg/ml in the sham group, 105.5±10.7 pg/ml in the I/R group, and 99.2±7.6 pg/ml in the IR+MEL group. Although the treatment showed a decrease in IL-6 levels, the comparison of IL-6 levels among the groups revealed no significant findings (p>0,05).Blood IL-18 levels were median 174.7 (min: 162.7- max: 188.3) pg/ml in the sham group, median 195.2 (min: 171.2 – max. 270.5) pg/ml in the I/R group, and median 190.1 (min: 178.1-max: 195.2) pg/ml in the IR+MEL group. There was a statistically significant (p=0.047) reduction in IL-18 levels after the treatment in the IR+MEL group.

Blood TNF-α levels were 132.9±8,45 pg/ml in the sham group, 148,3±6,74 pg/ml in the I/R group, and 138,6±4,56 pg/ml in the IR+MEL group. There was a statistically significant (p=0.002) reduction in TNF-α levels after the treatment in the IR+MEL group.Blood IFN-γ levels were median 55.1±7.14 pg/ml in the sham group, median 75,7±12,14 pg/ml in the I/R group, and median 58,9±10,14 pg/ml in the IR+MEL group. There was a statistically significant (p=0.003) reduction in IFN-γ levels after treatment of the IR+MEL group. The relationships of blood IL-1β, IL-6, IL-18, TNF-α, and IFN-γ levels among the groups are summarized in Table II and Fig. 4.

TABLE I - *Enzyme results and statistical analyses showing liver injury between the groups*

	GROUPS			
	SHAM	**IR**	**IR+MEL**	**P**
AST				
Median (min.-max.) (U/L)	174,2(143,2- 195,6)[a,b]	648,6(434-747)[a,c]	347,7(227,3-564)[b,c]	**<0,001**‡
ALT Median (min.-max.) (U/L)	54,9(36,9-69,2)[a]	106,2(63,9-185,7)[a,b]	66,2(54,8-78,5)[b]	**0,003**‡
LDH (U/L)	1893,8±817,9[a,b]	3263,7±792,7[a]	2990,1±566,1[b]	**0,006**†

† One-way analysis of variance (ANOVA) and post hoc analysis with Tukey test,
‡ Kruskal-Wallis test and post hoc analysis with Bonferroni-corrected Mann–Whitney U-test,
Data are mean ± standard deviation (SD) unless otherwise indicated.
Bold P-value defines the significant difference (P < 0.05).

TABLE II - *Blood IL-1β, IL-6, IL-18, TNF-α, and IFN-γ results and their statistical analyses*

	GROUPS			
	SHAM	**IR**	**IR+MEL**	**P**
IL-1β Median (min.-max.) (pg/mL)	39,5 (36,5-51,2)	45,7 (37,1-94,1)	46,3 (29,4-78,8)	0,534‡
IL-6 (pg/mL)	94,9±9,4	105,5±10,7	99,2±7,6 0,132†	
IL-18 Median (min.-max.) (pg/mL)	174,7 (162,7-188,3)[a]	195,2 (171,2-270,5)	190,1 (178,1-195,2)[a]	**0,047**‡
TNF-α (pg/mL)	132,99±8,45[a]	148,37±6,74[a,b]	138,61±4,56[b]	**0,002**†
IFN- γ (pg/mL)	55,1± 7,14[a]	75,7± 12,14[a,b]	58,9±10,14[b]	**0,003**†

† One-way analysis of variance (ANOVA) and post hoc analysis with Tukey test,
‡ Kruskal-Wallis test and post hoc analysis with Bonferroni-corrected Mann–Whitney U-test,
Data are mean ± standard deviation (SD) unless otherwise indicated.
Bold P-value defines the significant difference (P < 0.05).

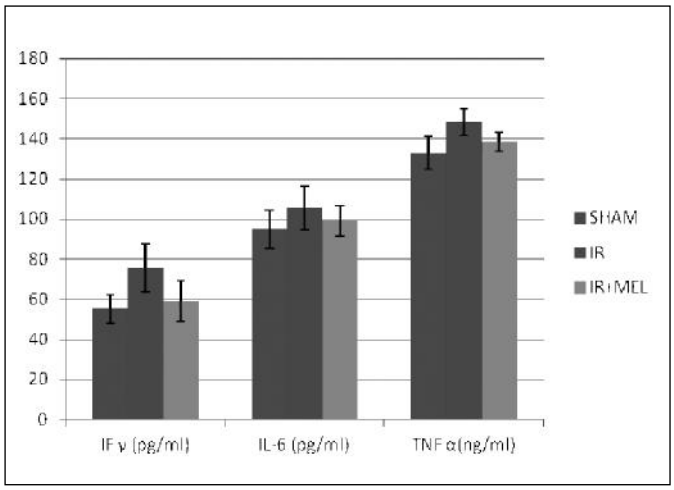

Fig. 4: Graphical analysis of the relationship of blood IL-1?, IL-6, IL-18, TNF-?, and IFN-? levels between the groups.

EVALUATION OF SERUM TOTAL ANTIOXIDANT STATUS(TAS), TOTAL OXIDANT STATUS (TOS), AND OXIDATIVE STRESS INDEX (OSI) LEVELS

Although total oxidant Status (TOS) data (12.4±1.51) were significantly higher in the I/R group compared to other groups, they were markedly lower (8,3±1.4) in the IR+MEL group (p<0,001) (Fig. 5).

TAS level were lower in the I/R group compared to other groups. The TAS value was higher in the IR+MEL group (1.31±0.45) compared to the I/R group (1,23±0,19). However, The comparison of TAS Levels among the groups revealed no significant findings (p>0.05).

The OSI value was significantly lower (p<0.001) in the IR+MEL group (682,5 ± 195.2) compared to the I/R group (1027.9±210.4). The OSI value was significantly higher (p<0.001) in the IR group compared to the Sham group (452,6±128,6) (Fig. 6).The TOS, TAS, and OSI data and results of statistical analysis are summarized in Table III.

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATIONS

In morphological evaluation, there was no significant difference among groups by means of mononuclear cell infiltration and edema (p>0.05) but, sinusoidal enlargement was prominent in IR and treatment groups, when compared with the shams (p<0,05). There was a prominent difference between the IR group and the other two groups by means of inflammatory cell infiltration (p<0,001). Also, there was significant difference by means of sinusoidal enlargement and necrotic cell density mass

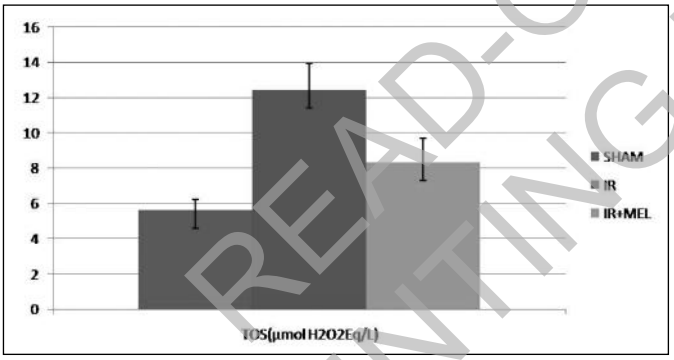

Fig. 5: Graphical analysis of TOS data.

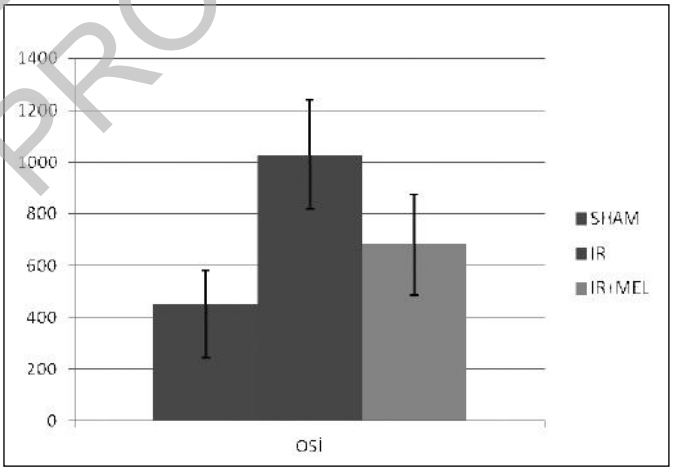

Fig. 6: Graphical analysis of OSI data

TABLE III - *TOS, TAS, and OSI data and their statistical analysis results*

	GROUPS			
	SHAM	**IR**	**IR+MEL**	**P**
TOC (µmol H_2O_2 Equiv./L)	5,6±0,6[a,b]	12,4±1,51[a,c]	8,3±1,4[b,c]	**<0,001†**
TAC (mmol Trolox equivalent/L)	1,3±0,31	1,23±0,19	1,31±0,45	**0,888†**
OSI	452,6±128,6[a]	1027,9±210,4[a,b]	682,5±195,2[b]	**<0,001†**

† One-way analysis of variance (ANOVA) and post hoc analysis with Tukey test,
‡ Kruskal-Wallis test and post hoc analysis with Bonferroni-corrected Mann–Whitney U-test,
Data are mean ± standard deviation (SD) unless otherwise indicated.
Bold P-value defines the significant difference (P < 0.05).

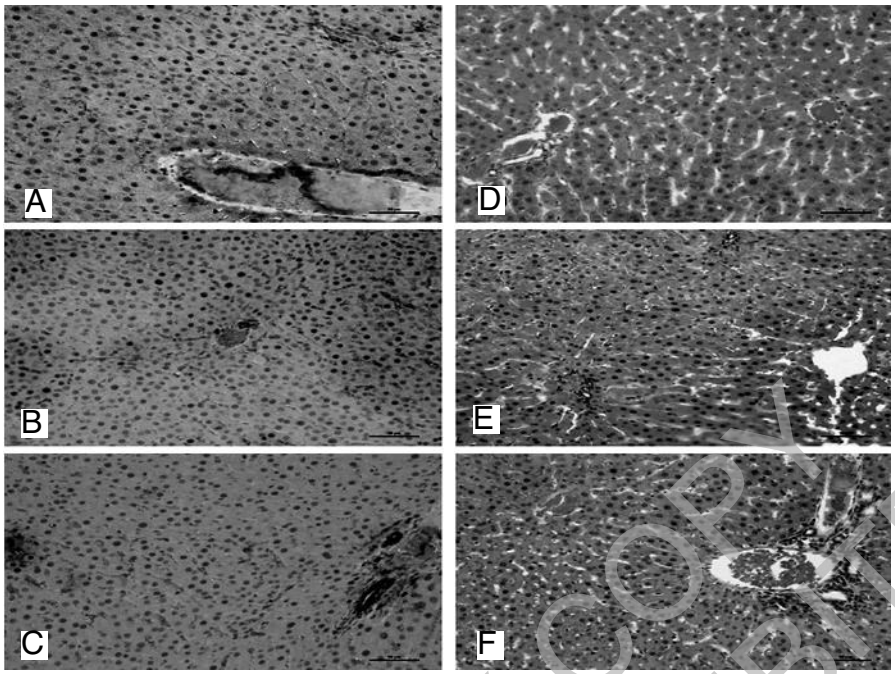

Fig. 7 (A): iNOS staining for control group. A few slight and mild immunopositive cells are seen around central veins which is normal because liver has massive metabolism and oxygen saturation is considerably low especially around central veins (iNOS Primary Antibody, 40X). (B): iNOS staining for IR group. It is obviously seen that virtually whole hepatocyte cells were stained with iNOS from mild to heavy degrees (iNOS Primary Antibody, 40X). (C): Liver tissue taken from IR / MLT group stained with iNOS. Immunopositivity and number of the stained hepatocyteds are decreased and a few immunopositive cell clusters are seen in paranchyme (circles) (iNOS Primary Antibody, 40X). (D): General view of the liver tissue of control group. Very slight edema is seen in parenchyma. But there is no inflammatory cell migration and sinusoidal enlargement (Hematoxylin-Eosin, 40X). (E): Liver tissue taken from IR group. Slight edema, moderate sinusoidal enlargements, diffused necrotic cell groups (arrows) and mononuclear cell infiltrations (asterix) are seen in parenchyma (Hematoxylin- Eosin, 40X). (F): General view of the liver tissue of IR / MLT group. Very slight edema and slight sinusoidal enlargements are seen in parenchyma. But there is no inflammatory cell migration and there are a few necrotic cells (arrow) (Hematoxylin-Eosin, 40X). A- SHAM, B- IR and C- IR+MEL (iNOS stain) D- SHAM, E- IR and F- IR+MEL (H&E stain).

TABLE IV - *Morphological evaluation and scoring of the groups.*

Groups	Inflammatory Cell Migration	Edema	Sinusoidal Enlargement	Total
SHAM	0	1	0	1
IR	0	1	1	2
IR+MEL	0	1	1	2

TABLE V - *Evaluation of iNOS expression (H-SCORE)*

RAT	SHAM	IR*	IR + MEL#
1	14	25	24
2	8	46	15
3	5	53	26
4	12	40	20
5	8	68	12
6	14	56	27
7	8	48	20
Mean ± SD	9,85 ± 3,48	48 ± 13,45	20,57 ± 5,59

*; p< 0.001 vs SHAM, #; p< 0.001 vs IR.

between the IR group and shams and between the IR and IR + MEL groups ($p< 0.001$).
In immunohistochemical evaluation, it was observed that hepatocytes, sinusoids and portal areas were stained with iNOS antibody at various degrees (Fig. 7). The intensity degrees and morphological change grades were given in Tables IV and V. There was a very significant difference between the IR and sham group and between IR and IR+Mel group by means of iNOS signaling intensity ($p<0.001$).

Discussion

In the present study, we showed that the anti-inflammatory, antioxidant and protective effects of melatonin in hepatic oxidative damage following infrarenal AA in rat models in preventing livers from this kind of damage. Energy deficiency in liver ischemia and reperfusion injury result in increased oxidative stress, and stimulated local and systemic inflammatory response. Increasing in oxidative stress causes to decrease in ATPase activity, and to increase in OFRs and peroxidation at the cellular level. Endothelial cellular swelling, vasoconstriction, and thrombocyte aggregation develop in the sinusoids, and hepatic micro circulation is impaired during the early stage of reperfusion. Inflammatory mediators released as a consequence of reperfusion activate endothelial cells and circulating neutrophils in remote organs that are not exposed to the initial ischemic insult. This distant response to I/R can result in leukocyte-dependent microvascular injury that is characteristic of multiple organ dysfunction syndrome.
Experimental studies found that melatonin is anti-inflammatory in various ischemia-reperfusion models[14]. Melatonin has been reported to have free radical sweeping properties on tumor necrosis factor (TNF)-α, IL-1β, and IL-6 levels in many different tissue ischemia/reperfusion (I/R) models due to its suppressive properties [15]. The contributions of the application of melatonin on pancreatic tissue lipid peroxidation and reduced cellular death in pancreatic ischemia and reperfusion injury have been studied in detail by Munoz et al. [16]. Yuji et al. [17] demonstrated that the application of melatonin reduced mitochondrial oxidative stress in HI/R injury. The suppressive effect of melatonin on pro-inflammatory cytokines has been clearly shown in the study by Raghavendra et al. [18]. Wang et al. [19] found that melatonin suppressed the production of TNF-α and IL-1β cytokines in carbon tetrachloride-induced hepatic fibrogenesis. In the study of Lahiri et al. [20], they demonstrated that experimental reflux esophagitis can be prevented by suppressing TNF-α, IL-1β, and IL-6 levels. Despite studies proving the protective efficiency of melatonin, Kurcer et al. [21] found that in the application of melatonin after unilaterally nephrectomized and subjected to 1 hr of renal pedicle occlusion followed by 2 hr reperfusion and resulted in no changes in the kidney TNF-α, IL-1β, and IL-6 levels of pro-inflammatory cytokines.
The current study found that blood AST, ALT, and LDH activities were significantly higher in the I/R group compared to the sham group, but that the values were lower in the IR+MEL treatment group after the treatment. Kaçmaz et al. [6] carry outed rats subjected to 1h of infrarenal aortic occlusion followed by 1h of reperfusion to induce I/R damage, evidenced by increases in the MDA and MPO activity, and a decrease in GSH. Furthermore the AST, ALT activities, which all increased due to I/R, were all observed to decrease after melatonin treatment.
Although blood IL-1β, IL-6, IL-18, TNF-α, and IFN- γ levels were lower than the I/R group after melatonin administration, there was a statistically meaningful reduction only in the IL-18, TNF-α, and IFN- γ groups. The increased inflammatory response in ischemia and reperfusion injury results in tissue oxidative stress. Tissue oxidative stress varies depending on the balance between oxidative and antioxidant substances. The balance is frequently against antioxidant substances in inflammatory processes [22]. Melatonin can improve TAS and TOS results and command ischemia and reperfusion injury, which has been proven both biochemically and histopathologically. Reiter and Pieri [23,24] have also found similar results in their study. Chen et al. [25] also studied neutrophil apoptosis in blood samples and the effects of melatonin application in their seven-case clinical study where they caused anhepatic IR injury and performed hepatectomy. They reported that there was a reduced delay in neutrophil apoptosis after melatonin administration, which in turn reduced hepatic IR injury. Sener et al. [26], in their study, they examined the antioxidant efficiency of melatonin, reported significant improvement after melatonin application in the levels of malonyldialdehyde (MDA), which is the final product of lipid peroxidation. They found increased MDA levels in ischemia and reperfusion despite reduced levels of glutathione, which is naturally antioxidative. Tuncdemir et al. [27] found reduced MDA levels with no reduction in glutathione levels after melatonin application. The current study, we also found reduced TOS and OSI values despite increased TAS levels. The researchers consider that this may be due to the natural antioxidative properties of melatonin.
Literature scans also showed the use of different agents with known anti-inflammatory properties in liver I/R models [28]. The present study found extensive histopathological changes including inflammatory cellular infiltration in remote organ liver following aortic clamping, necrotic cellular densities, and sinusoidal widening in the I/R group. Similar to Sener [29] et al., the present study found reduced histopathological changes in the melatonin treatment group and also Atik et al. [30] have indicated that there was a positive correlation between the severity of the disease and iNOS reactivity in liver biop-

sies taken from patients with acute or chronic hepatic diseases, which lead to damage in hepatocytes. Miriam Romero et al. [31] have reported that there was a relationship between the degrees of cellular damage occurred during transplant rejection and iNOS staining. This improvement may be due to the potential anti-inflammatory and antioxidative effects of melatonin.

In conclusion, melatonin ameliorated the disorders of liver functions and decreased serum levels of inflammatory cytokines like TNF-α, IL-6 and IL-18 related to infra-aortic IR injury and also we found that, melatonin reduced the serum levels of OSI in the infra-aortic occlusive rats. On the other hand, melatonin ameliorated histopathological disorders induced by IR injury compared to sham. Since the administration of melatonin inhibited the generation of free radicals and the accumulation of neutrophils in the damaged hepatic, ileal, and lung tissue, these agents appear to play a cytoprotective role in the liver, ileum, and lung insulted by I/R. In the current study, melatonin could be given at 30 min. prior to and after aortic clamping. The aim of this procedure is relevant in the clinical setting of ruptured abdominal aortic aneurysm where there is a higher incidence of remote organ injury compared to elective aortic surgery. In this rat model, the results demonstrated that melatonin protected the liver against aortic ischemia-reperfusion injury, which may be due to free radical scavenging activity of melatonin and its ability to reduce neutrophil infiltration. This is the first study evaluating the favorable effect of melatonin on IR-exposed liver injury after infrarenal occlusion of the aorta with our I-R (30-120 min) period. Although further studies using different dose regimens and time intervals are required, According to our results, we have shown that melatonin has anti-inflammatory, antioxidant activity and protective effect on damaged liver functions and histopathological findings in infra-renal AA IR exposed rats. We supposed that our results will put forward a new point of view to the literature about protective, antioxidant and anti-inflammatory effect of melatonin on remote organ liver IR injury following infra-renal aortic occlusions.

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Riassunto

Dopo il ripristino della circolazione sanguigna e dell'ossigenazione possono verificarsi gravi danni locali e sistemici ai tessuti temporaneamente ischemici, noti come danni da riperfusione. Lo scopo di questo studio è stato quello di indagare sui possibili effetti protettivi della melatonina nei confronti dei danni sistemici da riperfuzione nel tessuto epatico a seguito dell'occlusione dell'aorta sottorenale.

Per questo studio sono stati impiegati un totale di 21 ratti Wistar-albini di sesso maschile, suddivisi in tre gruppi:

I gruppo – laparotomia e contemporanea dissezione dell'aorta infrarenale;

II gruppo – somministrazione intraperitoneale di circa 1 ml di fisiologica al 0,9% di NaCl 30' prima e dopo l'operazione di occlusione. Dopo la laparotomia e sua dissezione, l'aorta sottorenale è clampata per 30' e quindi riabitata al circolo di riperfusione per 2 ore;

III gruppo – 30' prima del clampaggio dell'aorta sottorenale è stata somministrata la malatonina, seguita dal clampaggio aortico per 30' e un periodo di riperfusione di 2 ore.

Sono stati quindi dosati i tassi sierici di aspartate aminotransferasi, alanine aminotransferasi, and lattato dehydrogenase, risultati significativamente più elevate nei gruppi II e III rispetto al gruppo I di controllo. Gli esami di laboratorio sono tornati ai livelli normali nel III gruppo dopo il trattamento.

Sebbene si sia avuto un decremento del tasso sierico di IL-1β, IL-6, IL-18, TNF-α, e IFN- γ nel gruppo trattato con melatonina, questo decremento ha assunto valore statisticamente significativo per i livelli sierici di IL-18, TNF-α, e IFN- γ in paragone con quanto osservato nel II gruppo.

I tassi sierici dello stato totale di antiossidanti tissutali (TOC) e dell'indice di stress ossidativo dei tessuti (OSI) sono risultati diminuiti e quelli della capacità antiossidante (TAC) risultano accresciuti dalla melatonina.

Il risultato di questo studio suggerisce effetti antiossidanti della melatonina ed effetti epatoprotettivi nei confronti dei danni da riperfusione.

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