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ARAŞTIRMA / RESEARCH

Protective effect of melatonin on cisplatin-induced liver injury in rats

Melatoninin sıçanlarda cisplatin kaynaklı karaciğer hasarı üzerindeki koruyucu etkisi

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Abstract

Purpose: We aimed to evaluate the protective effect of melatonin (Mel) on experimental hepatatoxicity induced by cisplatin (Cis).
Materials and Methods: A total of 40 Wistar Albino rats were used. Control group (n = 10) daily 0.1 mg / kg isotonic solution (intraperitoneal) i.p. was administered. Cis group (n = 10) was given a single dose of i.p. Cis (7 mg/kg) was administered. Melatonin (Mel) group (n = 10) daily i.p. Mel (10 mg / kg) was administered. Cis + Mel group (n = 10) daily i.p. Mel (10 mg / kg) + single dose i.p. Cis (7mg / kg) was administered. On the 8th day of the experiment, liver tissues of rats were collected histologically and immunohistochemically for tumor necrosis factor-α (TNF-α) analysis. Analyzes were performed for the levels of Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), and albumin for liver function from the serum obtained from the blood.
Results: It was observed that liver tissue histopathological score and TNF-α immunoreactivity increased significantly with cisplatin administration compared to the control group. We found that the histopathological score and TNF-α immunoreactivity were decreased in the group treated with melatonin, and the liver function enzymes ALT, AST, and LDH were significantly decreased compared to the cisplatin group. Albumin level, on the other hand, showed a significant improvement in the group treated with melatonin.
Conclusion: Melatonin may play a protective role in hepatotoxicity caused by cisplatin by reducing inflammation and preventing the increase in liver enzymes.

Keywords:. Cisplatin, inflammation, melatonin

Öz

Amaç: Cisplatin ile oluşturulan deneysel hepatatoksisite üzerine melatoninin koruyucu etkisini değerlendirmeyi amaçladık.
Gereç ve Yöntem: Toplam 40 adet Wistar Albino türü rat kullanıldı. Kontrol grubuna (n=10) günlük 0.1 mg / kg izotonik solüsyon (intraperitoneal) i.p. uygulandı. Cisplatin grubuna (n = 10) 5. gün tek doz i.p. Sisplatin (7 mg / kg) uygulandı. Melatonin grubuna (n = 10) günlük i.p. Melatonin (10 mg / kg) uygulandı. Cisplatin + Melatonin grubuna (n = 10) günlük i.p. Melatonin (10 mg / kg) + 5. günde tek doz i.p. Cisplatin (7mg / kg) uygulandı. Deneyin 8. günü ratlardan karaciğer dokuları histolojik ve immünohistokimyasal olarak Tümör nekrozis faktör-α (TNF-α) inceleme için alındı. Kanlardan elde edilen serumlardan da karaciğer fonksiyonu için Alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), Laktat dehidrogenaz (LDH) ve albümin seviyeleri için analizler yapıldı.
Bulgular: Cisplatin uygulamasıyla birlikte karaciğer dokusu histopatolojik skorunun ve TNF-α immünoreaktivitesinin kontrol grubuna göre anlamlı olarak artış gösterdiği görülmüştür. Melatonin ile tedavi edilen grupta ise histopatolojik skorunun ve TNF-α immünoreaktivitesinin azaldığını ayrıca karaciğer fonksiyonu enzimleri olan ALT, AST ve LDH'ın cisplatin grubuna göre anlamlı şekilde azaldığını bulduk. Albümin düzeyi ise melatonin ile tedavi edilen grupta önemli bir iyileşme gösterdi.
Sonuç: Melatoninin, sisplatinin neden olduğu hepatotoksisitede inflamasyonu azaltarak ve karaciğer enzimlerindeki artışı önleyerek koruyucu rol oynayabilir.

Anahtar kelimeler: Cisplatin, melatonin, inflamasyon

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INTRODUCTION

Cisplatin (Cis) is known as an important drug used in the treatment of cancer patients. Cis is an inorganic, water-soluble substance containing platinum. Cis is found in high levels in the kidney, liver, intestine, and testicular tissues. Although the drug is used as chemotherapeutic, it has side effects on many tissues[1]. Studies show that one of the important side effects of Cis is hepatotoxicity[2]. Acutely administered Cis has been shown to cause liver damage. It causes damage, especially in the liver parenchyma. It causes degeneration in hepatocytes[3]. Cis causes oxidative stress. Reactive oxygen species occurring as a result of oxidative stress increase the release of pre-inflammatory cytokines. Among these increasing cytokines, TNF-α plays an important role. TNF- α is a proinflammatory cytokine. Its levels rise in acute and chronic disease conditions[4]. With the increase of this cytokine, the cytotoxic effect of Cis also increases[5].

There are important enzymes to show hepatocyte damage. High levels of liver enzymes such as ALT, AST, and LDH in serum are important markers to show liver damage[6]. Likewise, the amount of albumin is an important marker in determining liver function[4]. Cis has been shown in studies to increase ALT, AST, and LDH levels, as well as decreased serum albumin levels[4].

Melatonin (Mel) is known as N-acetyl-5-methoxytryptamine. It is a molecule found in more or less all living organisms. It is an indolamine found in any part of the organism. Although Mel, which is found as a hormone, is synthesized locally in many tissues, it is also produced in the pineal gland. It is then given to blood[7]. Mel has various physiological effects. It has a regulating effect on detoxification of free radicals and antioxidant activity, bone formation, reproduction, and cardiovascular disorders[8]. Previous studies have revealed additional various and multifunctional properties of Mel, including antioxidant and anti-inflammatory[9]. Studies have shown that Mel reduces hepatocellular damage and reduces the level of enzymes such as ALT and AST, which are important markers of liver damage[10]. These results showed that Mel has a hepatoprotective effect. In the light of this information, it is suggested that Mel may have a protective effect in Cis-induced liver damage. However, the effect of Mel on liver function and inflammation on Cis-induced liver injury has not been explained.

In the present study, we aimed to explain the protective effect of Mel in liver injury induced by Cis histopathologically, immunohistochemically, and biochemically.

MATERIALS AND METHODS

This study was planned and implemented in Erciyes University Faculty of Medicine Department of Histology-Embryology in accordance with the approval of Erciyes University Experimental Animals Local Ethics Committee. All procedures were carried out in accordance with the Universal Declaration of Animal Rights with the approval of Erciyes University Experimental Animals Ethics Committee (Date: 05.05.2021, Decision number: 21/126). The experimental phase of the study was carried out in Erciyes University Experimental Research Application and Research Center (DEKAM). Rats were used in our study. Because there is a need to study tissue samples for histopathology and immunohistochemistry methods. In addition, serum samples to be obtained from blood are needed for liver enzymes. Therefore, rats were included in our study with the necessary ethics committee decision.

In this study, 40 Wistar albino-type male rats produced in DEKAM, 8-12 weeks of age, 200-300 g, were used. Rats born on the same date were randomly divided into 4 groups. These processes were done by labeling the cages with group names. The rats to be treated were removed from their cages and injected with the determined dose. The rats kept in the cages were kept at 21 ° C in the normal order of the day in a light / dark environment for 12 hours to provide water and nutrient needs.

Experimental procedure

Group 1 (Control) (n = 10) daily 0.1 mg / kg isotonic solution (intraperitoneal) i.p. was administered.

Group 2 (Cisplatin) (n = 10) on the 5th day single dose i.p. Cis (7 mg / kg) was administered [11].

Group 3 (Melatonin) (n = 10) daily i.p. Mel (10mg / kg) was administered [12].

Group 4 (Cis+ Mel) (n = 10) daily i.p. Mel (10 mg / kg) + on the 5th day single dose i.p. Cis (7mg / kg) was administered.

On day 8, animals were sacrificed under general anesthesia, and liver tissue was harvested for histological examination and biochemically in serums.

Histopathological evaluation

Liver sections of 5-6 μm were taken from the paraffin blocks. The sections were kept in the oven for a certain period of time, then paraffin was removed with xylene and diluted by passing through graded alcohol series. Sections were stained with hematoxylin-eosin (H + E). The liver was then evaluated under the Olympus BX53-EP50 microscope[13]. While applying histopathological score, the following criteria were used; hemorrhage, necrotic hepatocytes, vacuolized hepatocytes, and the appearance of hepatocyte cords. Scoring was conducted as follows: 0 = not at all, 1 = 0–25%, 2 = 26–45%, 3 = 46–75%, and 4 = 76–100%.

Immunohistochemistry

Avidin biotin peroxidase method was used to determine the difference in tumor necrosis factor-α (TNF-α) (bs-2081R, Bioss, Boston, MA, USA) expression in liver sections. After the paraffin sections were incubated in the oven, they were cleared of paraffin in xylene. For antigen recovery, 10% citrate buffer was applied in the microwave at 600w for 5 minutes and then allowed to cool for 10 minutes at room temperature. Sections washed with phosphate buffer (PBS) were treated with 3% hydrogen peroxide (H_2O_2) for 12 minutes to prevent endogenous peroxidase activity. It was washed again with PBS for 3X5 minutes. The staining kit (Lab Vision, Ultra Vision Detection System Large Volume, Anti - Polyvalent Thermo Scientific HRP) was used for the next steps. The serum block was dropped and kept at room temperature for 10 minutes. TNF-α antibody was prepared and kept at +4 0C for 1 night. Sections from antibodies were washed 2x5 minutes with PBS. It was kept at room temperature in secondary antibody for 10 minutes and then washed again 2X5 minutes with PBS. It was treated with HRP streptavidin at room temperature for 10 minutes. After rewashing, it was treated with diaminobenzidine (DAB) in the kit for 1.5 minutes to make its immunoreactivity visible. It was sealed with entellan after being passed through the incremental alcohol series and xylene[14]. The immunoreactivity of the antibody was measured with the image j program. The staining intensity of the areas was measured. Measurements were made in 4 fields from each slide. Measurements were made from 40 different areas in total for each group.

Biochemical analysis

After the blood taken from the subjects was centrifuged, their serum was taken. Serums were removed to -80 ° C. ALT, AST, LDH, and albumin values of blood serum samples taken at the end of the experiment were then measured. These parameters were evaluated in Erciyes University Central Biochemistry Laboratory.

Statistical analysis

Power analysis was performed for the sample size of the study. Sample sizes of 10, 10, 10, and 10 are obtained from the 4 groups whose means are to be compared. The total sample of 40 subjects achieves 99% power to detect differences among the means versus the alternative of equal means using an F test with a 0,05 significance level (PASS 11 software). The Kolmogorov-Smirnov test was used to determine whether the data for all variants (histopathology, immunohistochemistry, and biochemistry) were normally distributed. The data has a normal distribution. One-way ANOVA analysis of variance was used to compare the differences of histopathology, immunohistochemistry, and biochemistry between Groups. Then, pairwise comparisons were made with the post hoc Tukey test. Data were expressed as mean ± standard deviation (SD). GraphPad Prism 8.0 software was used for statistical analysis. $P \leq 0.05$ was considered statistically significant.

RESULTS

At the end of the experiment, the histopathological score level in the liver was determined (Table 1). Multiple hemorrhagic areas and necrotic cells were observed in the Cis group. The histopathological score was significantly increased in the Cis group compared with the control group ($P < 0.0001$). The histopathological score was found to be decreased in the Cis+Mel group compared with the Cis group ($P < 0.0001$). (Figure 1).

Table 1. Histopathological score results

Groups	Control	Cis	Mel	Cis+Mel	*p*
Histopathological score	0.18±0.40[a]	2.18±0.40[b]	0.09±0.30[a]	1.09±0.53[c]	0.0001

Values are given as mean ± standard error. $p < 0.05$ was considered significant. There is no significant difference between groups containing the same letter (a, b, c).; Cis; Cisplatin, Mel; Melatonin.

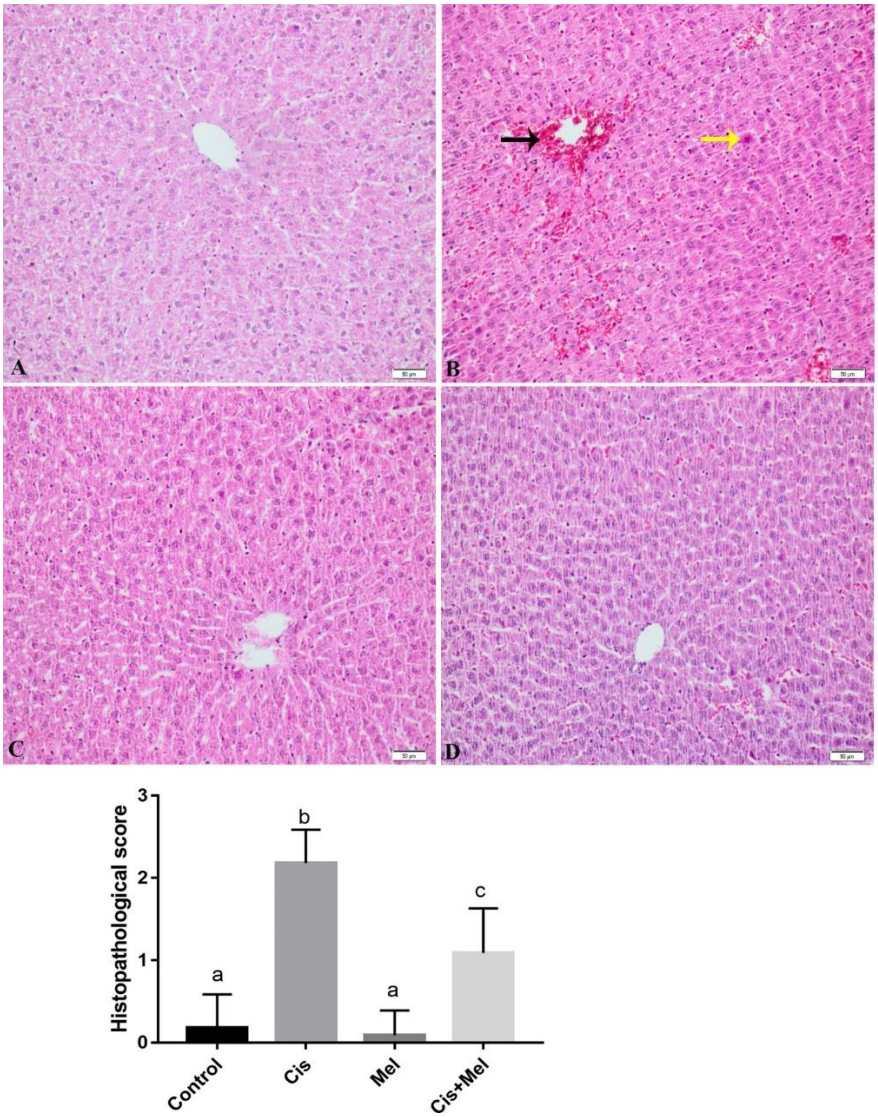

Figure 1. Liver tissues of all experimental groups. A-D, H&E staining of liver tissues.

A. Control group, B. Cis group, C. Mel group, D. Cis+Mel group. Black arrow: hemorrhage, yellow arrow: necrotic cell. Scale: 50 µm
Values are given as mean ± standard error. $p < 0.05$ was considered significant. There is no significant difference between groups containing the same letter (a, b, c).
Abbreviations: Cis; Cisplatin, Mel; Melatonin, H&E; hematoxylin-eosin

TNF-α level in the liver was determined at the end of the experiment. The TNF-α level was significantly increased in the Cis group compared with the control group ($P < 0.0001$). The TNF-α level was found to be decreased in the Cis+Mel group compared with the Cis group ($P < 0.0001$). (Table 2, Figure 2).

Table 2. Immunohistochemistry results of TNF-a.

Groups	Control	Cis	Mel	Cis+Mel	p
TNF-a immunoreactivity	77.96±3.99a	83.36±1.21b	78.36±1.31a	80.25±1.08a	0.0001

Values are given as mean ± standard error. $p < 0.05$ was considered significant. There is no significant difference between groups containing the same letter (a, b).; Abbreviations: Cis; Cisplatin, Mel; Melatonin, TNF-a; tumor necrosis factor-α.

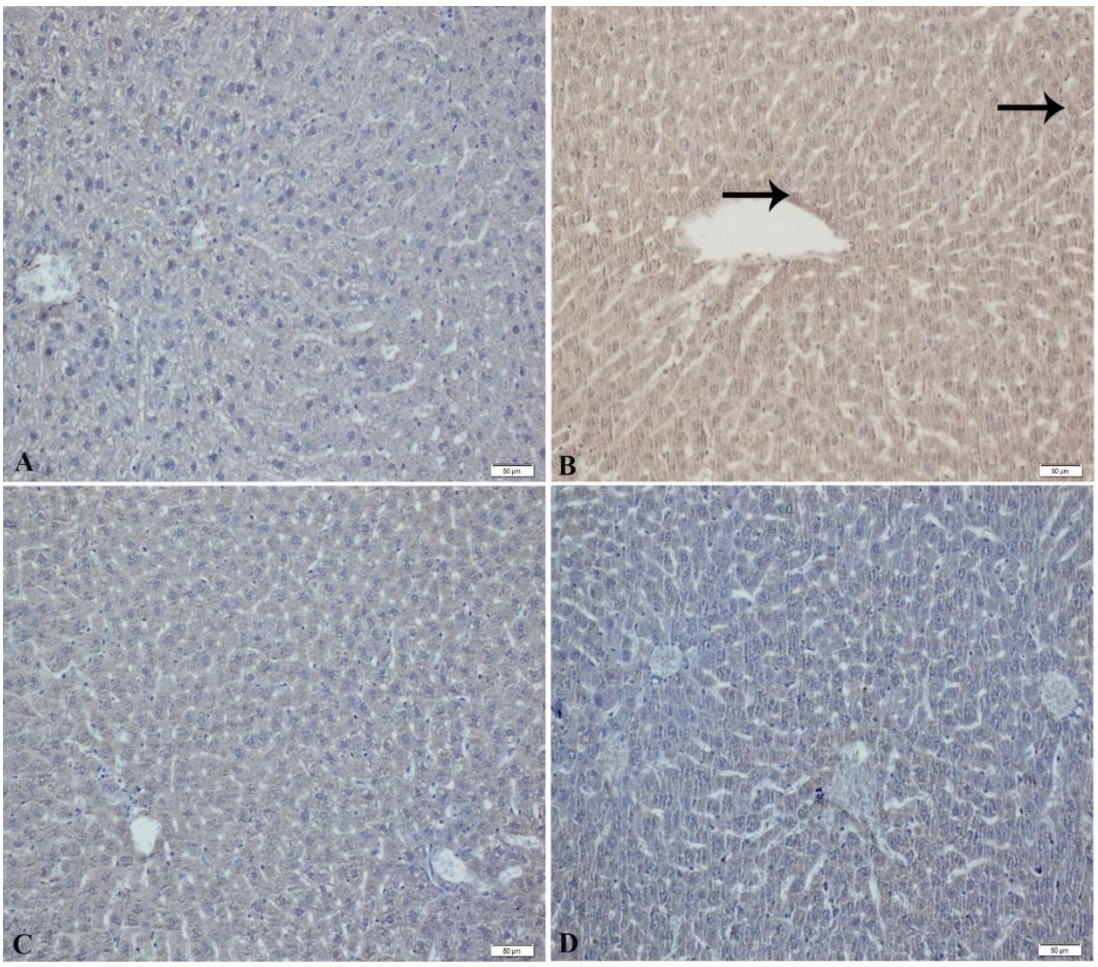

Figure 2. Immunohistochemistry staining of all experimental groups. A-D, TNF-a immunohistochemistry of liver tissues. A. Control group, B. Cis group, C. Mel group, D. Cis+Mel group. Immuunoreactive cells (arrow). Scale: 50 µm.

Cis; Cisplatin, Mel; Melatonin, TNF-a; tumor necrosis factor-α.

ALT, AST, LDH, and albumin levels in the serum were determined at the end of the experiment (Table 3). ALT, AST, and LDH levels were significantly increased in the Cis group compared with the control group (P < 0.0005). ALT, AST, and LDH levels were significantly decreased in the Cis+Mel group compared with the Cis group (P < 0.0005). Albumin level was significantly decreased in the Cis group compared with the control group (P < 0.0005). Albumin level was significantly increased in the Cis+Mel group compared with the Cis group (P < 0.0005). (Figure 3).

Table 3. Biochemistry results

Groups	Control	Cis	Mel	Cis+Mel	*p*
ALT	39±3.16[a]	50±5.09[b]	40.5±3.83[a]	41.6±6.18[a]	0.0021
AST	75.33±5.61[a]	83.33±3.38[b]	72.88±2.99[a]	75.52±2.78[a]	0.0004
LDH	567.8±84.6[a]	749.4±69.55[b]	564.2±75.46[a]	607.3±53.44[a]	0.0023
Albumin	3.65±0.18[a]	3.24±0.13[b]	3.73±0.27[a]	3.57±0.17[a]	0.0007

Values are given as mean ± standard error. p < 0.05 was considered significant. There is no significant difference between groups containing the same letter (a, b); ALP; alkaline phosphatase, ALT; alanine aminotransferase, Cis; Cisplatin, LDH; Lactate dehydrogenase, Mel; Melatonin.

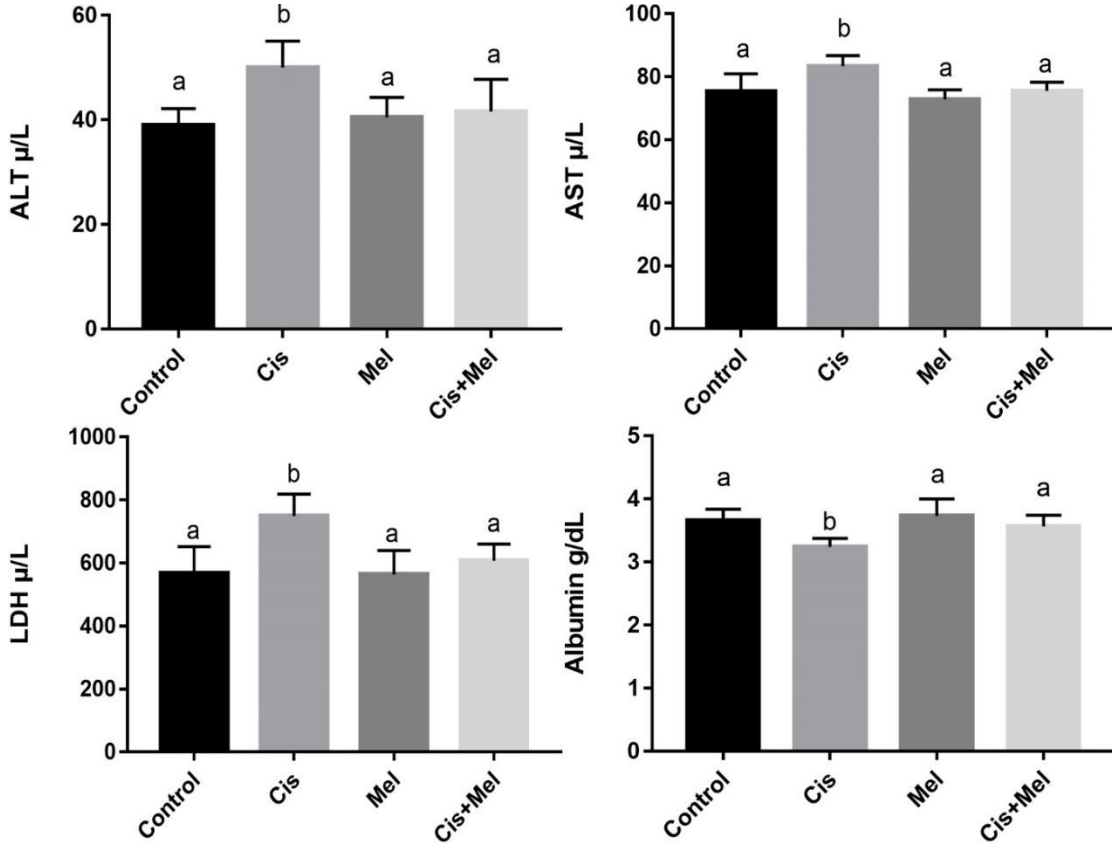

Figure 3. Biochemistry results. Values are given as mean ± standard error. p < 0.05 was considered significant. There is no significant difference between groups containing the same letter (a, b).

ALP; alkaline phosphatase, ALT; alanine aminotransferase, Cis; Cisplatin, LDH; Lactate dehydrogenase, Mel; Melatonin.

DISCUSSION

The liver is an important organ in drug metabolism. The liver has an important role in protein and lipid metabolism. It also has roles such as toxic substance removal and drug detoxification. For these reasons, the liver can be exposed to many substances[15]. One of the side effects of Cis, which is an important chemotherapeutic, is hepatotoxicity. It is known that the liver accumulates significant amounts of Cis. Therefore, Cis causes hepatotoxicity[2]. In the present study, we evaluated the protective effect of Mel on Cis-induced hepatotoxicity. We examined the Cis-induced liver injury histopathologically, immunohistochemically, and biochemically. We observed that Mel reduced Cis-induced liver damage.

Cis has been shown to have detrimental effects on the liver such as cell degeneration, cell loss, vacuolization, and inflammatory cell accumulation[3,5]. In another study, hepatic cord irregularity, necrotic cells, and sinusoidal occlusion were observed[2]. In the present study, we observed that there were necrotic cells, sinusoidal occlusion and hemorrhagic areas in the Cis applied group. We found that this damage was significantly reduced in the group treated with Mel. In a study, it was shown that melatonin inhibited liver necrosis caused by dextran sulphate sodium[16]. It has also been observed that melatonin reduces hepatocyte damage in the periphery of the lobule, which occurs in liver damage caused by carbon tetrachloride[10]. This result shows that Mel restores and reduces liver damage when evaluated histopathologically in accordance with other studies[9,10,12].

Cis induced toxicity leads to ROS production. This causes complications such as oxidative stress and inflammation. As a result of inflammation, cytokines that play a role in the inflammatory pathway such as TNF-α are released. TNF-α plays an important role in liver pathogenesis[17]. Studies have shown that Cis significantly increases the level of TNF-α in the liver[4,5]. In our study, TNF-α immunoreactivity increased significantly in the Cis applied group. It has been shown that melatonin decreases TNF-α level in liver ischemia-reperfusion injury[18]. It has been shown that melatonin significantly reduces TNF-α levels in carbon tetrachloride-induced liver injury[19]. In liver damage induced by the administration of Bacillus Calmette Guerin (BCG) and lipopolysaccharide, increased TNF-α activity was observed to be decreased by melatonin[20]. When Mel, an antioxidant and anti-inflammatory, was administered, TNF-α immunoreactivity was significantly reduced. This result shows that Mel reduces inflammation in accordance with other studies[9,15]. With this study, we showed that Mel inhibited the increase in TNF-α level caused by the inflammation in the liver induced by Cis and it protects the liver.

ALT is the enzyme formed in hepatocytes and is an important indicator of liver cell damage. AST is a mitochondrial and cytoplasmic enzyme in the liver. AST reflects liver damage. When liver cells are damaged, these enzyme levels increase significantly and pass into serum[1,21]. Another indicator of liver function is LDH, and its amount in serum increases significantly in liver degeneration[22]. Studies have shown that the levels of enzymes such as ALT, AST, and LDH are increased with Cis administration[1,2,23]. Similar to these studies, we found that enzymes such as ALT, AST, and LDH increased significantly with Cis administration in the present study. These results also confirm our histopathological findings. It was observed that melatonin decreased the liver enzymes ALT and AST, which are increased in cecal ligation and puncture (CLP)-induced sepsis injury, which is another liver injury model[9]. In addition, it has been shown by a study that melatonin reduces enzymes such as ALT and AST in liver damage caused by carbon tetrachloride[10]. In arsenic trioxide-induced liver injury, melatonin has been shown to improve important liver enzyme levels such as ALT and AST[24]. We observed that these enzymes decreased significantly in the Cis+Mel group. In line with previous studies, Mel reduced the amount of these enzymes, which are an important marker of liver damage[9,10].

Serum albumin is produced by the liver and is an important marker in acute-chronic liver diseases. As a result of liver damage, there is a significant decrease in serum albumin[25]. Serum albumin level decreases in liver damage caused by Cis administration[4]. As a result of Cis administered with the present study, it was observed that the serum albumin level produced by the liver decreased significantly. It was found that this amount of albumin increased significantly in the Mel applied group. Mel significantly increased the amount of albumin decreased in liver damage, showing that it is consistent with previous studies[26,27].

Cis-induced hepatoxicity has been the focus of many researchers. Although different treatment and protection options are tried to be applied, these treatment methods are still limited. Therefore, new

research is needed to reduce chemotherapy-induced damage. This study provides new information in terms of the protective effect of Mel on the inflammatory cytokine TNF-α, which is important for liver function. It also provides new insights into how enzymes important in liver function mediate the hepatoprotective effects of Mel.

In our study, histopathology, immunohistochemistry, and biochemistry analyzes were performed. As a result of these analyzes, important information was obtained for future studies. The inability to apply different pathways and methods due to the lack of sufficient budget limiting the scope of our study is shown as the limitation of the current study. The application of immunohistochemistry with more antibodies and also the application of methods such as western blot and PCR will enrich our study with new studies we will do.

This study shows that hepatotoxicity induced by Cis, which is an important chemotherapeutic, is restored by the administration of Mel. This has been achieved by reducing both inflammation and the amount of important enzymes in liver damage. Mel may be an important agent in preventing liver injury in patients receiving Cis therapy. However, more research is needed to determine the precise mechanism underlying the hepatoprotective effect of Mel. We think that the present findings and future studies will be important in developing therapeutic strategies.

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