**Research Article**

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# The Effects of Melatonin and N-Acetylcysteine on Obstructive Jaundiced Rats

## Sıçanlarda Tıkanma Sarılığında Melatonin ve N-Asetilsisteinin Etkileri

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**ABSTRACT**

Aim: The aim of this experimental study is to investigate the effects of N-acetylcysteine and melatonin on cholestasis and their protective effects on liver and renal injury.

Materials and Methods: Forty-eight rats were used in the study. Rats were divided into three main groups as sham, main control, and study groups. The main control group is further divided into early sacrification group and control group. The study group is divided into melatonin, N-acetylcysteine, and melatonin & N- acetylcysteine groups. A laparotomy was performed in study and control groups,and the common bile duct was ligated and divided. Five days after the first operation, blood samples liver and renal tissues were collected from early sacrification group. Between postoperative days five and ten, melatonin, N-acetylcysteine, melatonin & N-acetylcysteine solutions were applied to the rats in the study group subcutaneously and saline to the sham and control group. Blood samples, and liver tissues, and renal tissues of the rest of the rats were collected. Results: AST, ALT, BUN, creatinine, total bilirubin levels were significantly higher in rats with jaundice than in sham group . AST, ALT, total bilirubin, BUN, creatinine levels were significantly higher in the control group at the end of day ten. Among the melatonin group, N- acetylcysteine group, and melatonin & N-acetylcysteine group, all biochemical parameters were not different. Also, the MDA and NO levels were higher in control group in comparison with the study groups. On the other hand, there was no significant difference between the melatonin group, N-acetylcysteine group, and melatonin & N- acetylcysteine group on behalf of MDA and NO levels, and histopathologic findings.

Conclusion: Use of melatonin and N-acetylcysteine in rats with obstructive jaundice prevents damages to free oxygen radicals on the liver and renal tissue.

**Keywords:** Melatonin, N-acetylcysteine obstructive jaundice, free oxygen radicals

**ÖZET**

Amaç: Tıkanma sarılıklı sıçanlarda, koletastazda N–asetilsistein ve melatoninin etkilerini ve karaciğer ve böbrek hasarında koruyucu rollerini araştırmak ve karşılaştırmak amaçlandı.

Materyal ve Metod: 48 sıçan çalışma için kullanıldı. Sıçanlar sham, ana kontrol ve çalışma olarak 3 ana gruba ayrıldı. Daha sonra ana kontrol grubu erken sak-

rifikasyon grubu ve kontrol grubuna ayrıldı. Çalışma grupları kendi içinde melatonin, N–asetilsistein ve melatonin & N– asetilsistein olmak üzere üç ayrı alt gruba ayrıldı. Çalışma ve kontrol gruplarında laparotomi yapıldı, koledok dönüldü, bağlandı ve kesildi. İşlemden 5 gün sonra erken sakrifikasyon grubunda; kan örnekleri, karaciğer ve böbrek dokuları alındı. Çalışma gruplarında beşinci günden başlayarak 10. güne kadar subkutan olarak melatonin, N–asetilsistein ve melatonin & N–asetilsistein uygulandı, sham ve kontrol gruplarına serum fizyolojik verildi. 10. Günde tüm gruplardan kan örnekleri, karaciğer ve böbrek doku örnekleri alındı.

Bulgular: Total bilirübin, AST, ALT, kreatinin seviyeleri sarılıklı sıçanlarda anlamlı olarak yüksekti. 10. günde kontrol gruplarında AST, ALT, total bilirubin, BUN, kreatinin seviyeleri anlamlı olarak yüksekti. Melatonin, N–asetilsistein ve melatonin & N–asetilsistein grupları karşılaştırıldığında tüm biyokimyasal parametrelerin seviyelerinde anlamlı fark saptanmadı. Kontrol grubunda MDA ve NO seviyeleri çalışma grubundan daha yüksek saptandı. Melatonin, N–asetilsistein ve melatonin & N–asetilsistein grupları karşılaştırıldığında MDA ve NO seviyeleri açısından farklılık yoktu. Histopatolojik bulgularda MDA ve NO değerleri ile benzerlik gösteriyordu.

Sonuç: Tıkanma sarılıklı sıçanlarda melatonin ve NAC kullanılması karaciğer ve böbrek dokusunda serbest oksijen radikallerinin yarattığı hasardan korur.

**Anahtar kelimeler:** Melatonin, N–asetilsistein, tıkanma sarılığı, serbest oksijen radikalleri

## 1. INTRODUCTION

Obstructive jaundice is characterized by obstruction of bile flow and accumulation of bile in the liver. Various pathologies such as obstructive, inflammatory, and genetic processes result in cholestasis in the liver. The cause of hepatocyte damage is the accumulation of bile acids during cholestasis. In addition, cytotoxic bile acids cause necrosis and fibrosis in the liver and kidney(1).

In the etiopathogenesis of liver fibrosis, infarcts developing in the biliary tract, oxidative stress created by free radicals, and an increase in lipid peroxidation were found to be effective (2,3). It is previously known that bile acids accumulating in the tubules in the kidney tissue cause tubular necrosis and granulovacuolar degeneration with the damage caused by free oxygen radicals, similar to those in the liver. Oxidative stress and lipid peroxidation products can increase collagen synthesis by stimulating stellate cells in the liver, causing bile infarcts and tubular degeneration and necrosis in the kidney (4).

Melatonin or N-acetyl-5-methoxytryptamine (MEL) is the main substance secreted by the pineal gland (5). Melatonin has been shown to have a scavenging effect on highly toxic hydroxyl radicals and other oxygen-derived radicals. The receptors mediating this effect are unknown. In a study, it was shown that melatonin is more effective than other known antioxidants (such as mannitol, glutathione, Vit E, Vit C) (6,7). Yet, the antioxidant effect is probably only seen at pharmacological doses (8,9). N-Acetylcysteine (NAC) is the name given to the N-acetylated derivative of a thiol molecule, L-Cysteine, which is a natural amino acid(9). NAC exerts a direct antioxidant effect by interacting with the electrophilic group of oxidant radicals through the nucleophilic free thiol group(11).

Liver and kidney injury induced by common bile duct ligation has become a widely used experimental animal model in recent years. Melatonin and NAC were used in separate studies in the liver of rats developing jaundice after common bile duct ligation. However, to our knowledge, this study is the first one in which the effects of melatonin and NAC both in the liver and kidneys are examined (3,12,13). The aim of this study is to compare the seperate and possible synergistic antioxidant effects of melatonin and N-acetylcysteine on the liver and kidneys.

## 2. MATERIAL and METHOD

This experimental study was carried out at Erciyes University Faculty of Medicine Hakan Çetinsaya Experimental and Clinical Research Center (DEKAM). Prior to the study, Erciyes University Faculty of Medicine Ethics Committee approval was obtained.

### *2.1. Animals and experimental procedure*

Forty-eight Wistar–Albino rats (200–250 g) were housed under standart conditions with a 12/12 h light/dark cycle, with free access to food and wa-


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ter. Rats were randomly divided into eight groups. After fastin over night, Ketamine-HCL (10mg/kg) is administered intraperitoneally as an anesthetic agent to rats. Cefazolin (17 mg/kg im) prophylactic antibiotic is administered. After the administration of the anesthetic, the abdomen is shaved with a razor and cleaned with batticon solution. A laparotomy is performed through a midline incision under sterile conditions. The liver hilum is explored, the common bile duct is ligated with 5/0 silk and sectioned close to the liver hilum. The fascia is closed with 3/0 silk, the skin is closed with 4/0 silk, and the skin was cleaned with batticon.

Group I. Sham group (n=8): The common bile duct is mobilized without ligation. Group II. Control-1 (jaundice-early sacrification) group (n=8): The common bile ducts are surgically ligated and excised. After five days, 5 ml of blood was taken via relaparotomy and the liver and kidneys of the rats are harvested. Group III. Control–2 (jaundice) group (n=8): The common bile ducts of the rats in this group are surgically ligated and excised. Starting on the 6th day after laparotomy, 2 cc of saline was given subcutaneously for 5 days. Group IV. Study group (jaundice–melatonin) (n=8): The common bile ducts of the rats in this group were surgically ligated and excised. Starting on the 6th day after laparotomy, 10 mg/kg of melatonin is administered subcutaneously to each rat daily until the postoperative 10th day, approximately 2 mg/2cc of melatonin. Group V. Study group (jaundice–NAC) (n=8): The common bile ducts of the rats in this group are surgically ligated and excised. Starting on the 6th day after laparotomy, 150 mg/kg NAC is administered subcutaneously to each rat daily until the postoperative 10th day.

Group VI. Study group (jaundice–Melatonin & NAC) (n=8): The common bile ducts of the rats in this group are surgically ligated and excised. Starting on the 6th day after laparotomy, 10 mg/kg of melatonin and 150 mg/kg of NAC are administered subcutaneously to each rat daily until the postoperative day 10.

Relaparotomy is performed on the postoperative day 10, and 5 ml of blood is collected and the liver and kidneys are harvested in groups III, IV, V, VI. Alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities are determined, total bilirubin, blood urea nitrogen (BUN), and creatinine levels, and as indicators of lipid peroxidation in the liver, malondialdehyde (MDA) and Nitric oxide (NO) levels, are measured.

### *2.2. Histopathological Evaluation*

Liver: Micro-abscesses formed by cholestasis in the liver, enlarged bile canaliculi, and necrosis in the portal area were noted via light microscopy. In the evaluation of biliary infarcts and portal area necrosis, the decision was made according to the size of the largest necrosis and biliary infarct area. It was evaluated as 0 points if there was no biliary infarction and necrosis, 1 point if the size of the bile infarct and necrosis was smaller than 1 high magnification field, 2 points if it was equal to 1 high magnification field, and 3 points if it was larger than 1 high magnification field(14). Kidney: Granulovocouolar degeneration and width in renal tubules were examined semiquantitatively as stated by Chen et al. (11).

No renal tubule damage = 0
Renal tubule damage >25% = 1
Renal tubule damage 25–50% =2
Renal tubule damage 50–75% = 3
Renal tubule damage 75–100% = 4

## STATISTICAL ANALYSIS

Data are presented as mean±standard deviation (X±SD) and/or median (min.–max.). The distribution of the data was analyzed with the Kolmogorov-Smirnov test. Variable distributions of AST, ALT, creatinine, MDA, and NO were defined as mean±standard deviation (X±SD). The difference of these variables between the groups was examined using the Tukey test. Since the data did not show normal distribution according to the groups, the comparison of all groups was made with Kruskal Wallis analysis of variance. Statistics; It was done in Statistical Package for the Social Sciences (SPSS) for Windows (15.0 version). $p<0.05$ values were considered statistically significant.

## RESULTS

### *1. BIOCHEMICAL TESTS*

*1.1. Total Bilirubin (mg/dl):*

Total bilirubin values measured in early sacrification (P=0.001), control (P=0.001), and melatonin

(P=0.001), NAC (P=0.002) groups were significantly higher than in the sham group. There was no statistically significant difference between the Sham group and the melatonin & NAC group (P=0.166). Total bilirubin values were higher in the control group compared to melatonin group (P=0.001). Considering the time, no significant difference was found between the early sacrification and control groups on the 5th and 10th days in terms of total bilirubin values (P=0.614). There was no statistical difference between the melatonin and NAC groups (P=0.732), there was no statistical difference between the melatonin and melatonin & NAC groups (P=0.134), and there was no statistical difference between the NAC and melatonin & NAC groups (P=0.507) (Table 1).

*1.2. AST (U/L):*

Considering all groups, AST values were statistically significantly lower in the sham group than in the control (P=0.001) and early sacrification (P=0.001) groups. AST values in the

control group were higher than the AST values in the melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.001) groups. There was no statistical difference between the control group and the early sacrification group (P=0.665). There was no difference between the melatonin, NAC (P=0.961), melatonin & NAC (P=0.976) groups. There was no difference between the NAC group and the melatonin & NAC group (P=0.661) (Table 1).

*1.3. ALT (U/L)*:

When the ALT values of the sham group were compared with all other groups, there was a statistically significant difference in the early sacrification (P=0.001) and control (P=0.001) groups. Lower ALT levels were found in the Sham group. There was no statistically significant difference in ALT values in the early sacrification group compared to the control group, but they were relatively lower (P=0.665). ALT values in the control group were higher than the ALT values in the melatonin (P=0.007), NAC (P=0.002), melatonin & NAC (P=0.017) groups. There was no statistical difference between the control group and the early sacrification group (P=0.628). There was no difference between the melatonin, NAC (P=0.997), melatonin & NAC (P=0.954) groups. There was no difference between the NAC group and the melatonin & NAC group (P=0.970) (Table 1).

*1.4. BUN (mg/dl):*

There was no statistically significant difference between the Sham group and the early sacrification (P=0.356), melatonin (P=0.134), NAC (P=0.475) and melatonin & NAC (P=0.569) groups. BUN levels in the control group were significantly higher than the BUN levels in the melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.001) groups (Table 1). There was no difference between the melatonin group and the NAC (P=0.975) and melatonin & NAC (P=0.947) groups (Table 1).

*1.5. Creatinine (mg/dl):*

There was a statistically significant difference between sham group and early sacrification (P=0.001) and control group (P=0.001) in terms of creatinin levels. Higher creatinine levels were detected in the early sacrification and control groups. There was no statistically significant difference between the early sacrification group and the melatonin (P=0.684), NAC (P=0.217) and melatonin & NAC (P=0.126) groups.

A statistically significant difference was found between the control group and the melatonin group (P=0.001), the NAC group (P=0.006), and the melatonin & NAC (P=0.003) groups. There was no difference between the melatonin group and the NAC group (P=0.991) and the melatonin & NAC group (P=0.078) (Table 1).

## *2. LIVER AND KIDNEY MDA AND NO RESULTS*

*2.1. Liver MDA Results (nmol/g protein):*

The MDA level, which was measured in the study groups (melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.001)), was found to be significantly lower than the control group (Table 4). There was no statistical difference between the sham group and the study groups [melatonin (P=0.067), NAC (P=0.104), melatonin & NAC (P=0.381)]. MDA levels were not statistically significant in the comparison between the early sacrification group and the control group (P=0.765) (Table 2). 2. Kidney MDA Results (nmol/g protein):

MDA values , which were measured in kidney tissue on day 10, of the study groups were significantly lower than the control group [melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.001)] (Table 2).


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There was a statistically significant difference between the study groups in the sham group [melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.000)]. There was no statistical difference between the study groups (p>0.05). MDA levels were significantly different in the comparison between the early sacrification group and the control group ((P=0.001) (Table 2)

*2.3. Liver NO Results (nmol/g protein):*

The NO values measured in the liver on the 10th day in the study groups were significantly lower than in the control group [melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.001)] (Table 2). There was no statistical difference between the sham group and the study groups [melatonin (P=0.995), NAC (P=0.580), melatonin & NAC (P=0.480)]. There was no statistical difference between the study groups (p>0.05). In the comparison made between the early sacrification group and the control group (P=0.697), there was no significant difference between NO levels (Table 2).

*2.4. Kidney NO Results (nmol/g protein):*

When the NO levels measured on the 10th day were compared, NO level of study groups [melatonin (P=0.001), NAC (P=0.001), melatonin & NAC (P=0.000)] was significantly lower than the control group. There was no statistical difference between the sham group and the study groups [melatonin (P=0.989), NAC (P=0.384), melatonin & NAC (P=0.287)]. There was no statistical difference between the study groups (p>0.05). Similarly, there was no significant difference in NO levels between the early sacrification group and the control group (P=0.516) (Table 3).

### *3. HISTOPATHOLOGICAL FINDINGS*

Micro-abscesses formed by cholestasis in the liver, enlarged bile canaliculi, and lymphocyte infiltration were evaluated histopathologically. Granuvocouolar degeneration in the kidney and width of the renal tubules were evaluated semiquantitatively. Liver and kidney samples were stained with H&E and evaluated by beam microscopy. Bile infarcts, necrosis and fibrosis in the liver tissue were evaluated. There was a statistically significant difference between the Sham group and all other groups (p<0.001). There was no statistically significant difference between the early sacrification group and the control group (p>0.05) (Table 3). A statistically significant difference was found between the control group and the study group (p<0.001). There was no statistically significant difference between the melatonin, NAC or melatonin & NAC groups (p>0.05) (Table 3) (Picture 1). Granuvocouolar degeneration in kidney tissue and width of the renal tubules were examined semiquantitatively. A statistically significant difference was observed between the sham group and the groups other than the NAC group in terms of " Granuvocouolar degeneration in kidney tissue and width of the renal tubules (p<0.05). There was no significant difference between the early sacrification group and the control group (p>0.05). It was statistically significant (p<0.001) in the evaluation made between the control group (Picture 2) and the study group. There was no statistically significant difference between the melatonin, NAC and melatonin & NAC groups in the study group (p>0.05) (Table 3).

## DISCUSSION

Although hypovolemia at the cellular level and in the extracellular environment, increase in free oxygen radicals, endotoxemia and decrease in antioxidant functions play an important role in the deterioration in renal functions in obstructive jaundice, the etiology is still unclear (15).

In addition, the balance between oxidative andantioxidative systems and an increase in lipid peroxidation in the liver detoriorates during obstructive jaundice (1,4). This may lead to severe bacterial translocation as a result of intestinal barrier dysfunciton along with increased bacterial overgrowth int he course of obstructive jaundice (16,17).

Serious clinical problems such as acute respiratory distress syndrome, renal failure, hepatorenal syndrome, and cardiovascular failure may accompany obstructive jaundice (18, 19). Previous studies showed some pathological changes in tubular epithelial cells in both the control and treatment groups in the experimental model of obstructive jaundice, as well as, congestion in the vascular bed and venous dilatation (3). In our study, bile pigments were observed in the proximal tubules both in the study and control groups. Kidney damage was diminished in treated rats. The early increase in kidney function tests was found to be limited after antioxidant therapy. These results can be interpreted as early initiation of during jaundice will reduce kidney damage. It has been also determined that it is possible to detect the kidney damage due to the precipitation of bile pigment in the

early period of jaundice by kidney function tests, and oxidant activity parameters such as MDA and NO. Besides its antioxidant effect, melatonin also stimulates many antioxidant enzymes and inhibits the synthesis of prooxidant enzymes and nitric oxide (8,9). In our study, the kidney damage in rats given melatonin was found tobe diminished when compared to rats with jaundice, in line with the article mentioned above. The creatinine levels were statistically higher in the early sacrification group in comparison with the sham group, and jaundice in the earlier stage seemed to cause tissue damage in the kidney. We observed that on the day 10, there were irreversible foci of necrosis that developed in the kidney, and the degeneration of the tubules which were became widespread foci of necrosis in the kidney.

In our study, MDA levels were found to be significantly lower in the group given melatonin. The MDA levels started to decrease even on the first day of the the administration of melatonin. Eventhough an antioxidant treatment was given, it was thought that the damage to the tissue could not be completely prevented, but it was thought that permanent damage could be prevented via this antioxidant treatment by reducing apoptosis and minimizing tissue damage.

Hepatic damage and oxidative stress in jaundice develops through a nitric oxide-mediated system. The reason why we used NO measurement to determine oxidative stress in our study was to detect instant oxidative stress. A decrease in oxidative stress parameters, decline in MDA levels with diminished bile duct necrosis and hepatocyte thinning were detected with low-dose melatonin administration. However, it has been suggested in a previous study that new studies are needed to determine whether this effect of melatonin is dose-dependent or not (18). Contrary to its antioxidant effects against ischemia/reperfusion injuryin *in vivo* studies, melatonin has been reported to have no effect on reducing the extent of the infarct area as melatonin showed a decrease in MDA, which is an indicator of lipid peroxidation, the prevalence of the infarct area continued (19).

Chen et al. previously reported the tissue damage in kidneys during obstructive jaundice along with the increase in plasma and renal tissue levels of MDA. They found a correlation between MDA levels and histopathological damage in the kidneys. Lower levels of MDA, as well as, histopathological damage stage of kidneys with the melatonin treatment. The authors found kidney damage, even in the sham group, which they attributed to laparotomy and repeated injections (12). Yet, we did not encounter any pathological laboratory findings detected in the sham group in terms of MDA and renal tissue damage in our study. In fact, we detected lower MDA levels in the sham group than both the control and treatment groups and did not see any oxidative stress , or a damage caused by subcutaneous administration and laparotomy in rats. Oxidative stress plays a crucial role in complications associated with cholestasis. Tissue antioxidant capacity is inhibited in different tissues of cholestatic animals. Antioxidant therapy may play a role in the management of organ damage caused by cholestasis. NAC is known to reduce biomarkers of oxidative stress in cholestatic animals. NAC has been found to significantly improve tissue histopathological changes in cholestatic rats (20).

In the study examining the free radical scavenging effect of NAC in renal ischemia- reperfusion injury, they reported that free oxygen radicals play an important role in renal ischemia-reperfusion injury, and lipid peroxidation increases rapidly after reperfusion. They found that glutathione stores were reduced in ischemic tissue. It is known that glutathione protects the cell by interacting directly with hydrogen peroxide, superoxide, alkoxyl radicals (21). It is known that the rate of oxidized glutathione increases and the reduced form decreases in renal failure. It was determined that the amount of reduced glutathione increased with the administration of NAC. It has a protective effect against glutathione reperfusion damage with its direct free radical scavenging effect and indirect effects. NAC reduced MDA levels. Histopathologically, tubular necrosis was reduced by giving NAC in the current study. NAC reduced the level of MDA, a marker of lipid peroxidation, and kidney damage, and reversed the decrease in glutathione stores. In jaundice, an ischemia-reperfusion injury occurs after bilirubin precipitation into the tubules in the kidney (21). In our study, MDA and NO levels detected in kidney tissue of rats given NAC were lower than control group rats. Areas of granulovocuolar degeneration were lower in NAC-treated rats. It was thought that the regression of the damage developing in the kidney and the persistence of this damage can be prevented when treatment is started in the early period such as NAC and melatonin.

No significant difference was observed in terms of antioxidant effect in comparison of MDA and NO


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levels of the melatonin or NAC groups with the levels of melatonin & NAC group. This may be due to competition of the antioxidant systems of the rats treated with both the substances together with the antioxidant effects of mealtonin & NAC itself which may have caused a slowdown in antioxidant activity. Melatonin enters the cell more rapidly as it has a lipophilic structure. The relationship of melatonin specific receptors on the cell membrane with NAC is unclear. There may be a competition between melatonin & NAC in terms of binding to these receptors. NAC is the precursor of glutathione, and both substances show their antioxidant effects through NO synthetase. Therefore, there may be a decrease in activity because of the competition between these two of both antioxidants.

Bile infarction occurs as a result of duct damage due to increased biliary pressure, direct effects of bile components on hepatocytes, and indirect effects of bilirubin and bile acids in the blood. It has been shown that there is an increase in Kupffer cell activity, prominent phagocytic vacuoles, and moderate congestion in the sinusoids at the borders close to these areas of necrosis. In the first week of obstruction, edema, neutrophils and lymphocytes can be seen in the portal area (22). Bile duct proliferation in the periportal region is observed with portal inflammation. If the obstruction persists, fibrosis and fibrous septa bridging across the periportal and periseptal areas are observed, which are indicators of chronic cholestasis. Bile plugs are scattered or absent. Continuing morphological changes in the liver result in the formation of secondary biliary cirrhosis and regeneration nodules after months in living experimental animals (22). Developing infarct, fibrosis at the end of the first week; areas of necrosis increase, tissue damage becomes permanent. When antioxidant therapy is started in the early period, fibrosis can be slowed down, and bilirubin levels can decrease even though obstructive jaundice continues. It has been seen in our study that permanent damage to the tissue can be prevented. Less tissue damage was detected in the treated groups compared to the jaundiced rats (Picture 1).

In the comparison of total bilirubin, AST and ALT levels (Table 1), lower bilirubin values were obtained in treated rats when both control and treatment groups were taken into account in our study. In our study, a statistically significant decrease was found in the MDA and NO levels (Table 2) in both heaptic and renal tissues. We thought that co-administration of both antioxidant agents would create a synergistic effect. However, when we considered the AST, ALT and total bilirubin values (Table 1), we could not find any findings to prove our claim. The antioxidant activities of both melatonin and NAC were noted in many steps, and these activities were similar in action, such as the formation of peroxynitrite with NO synthase. With these properties, a relative decrease in antioxidant effect was detected in the groups given both drugs together, which suggests that both agents compete to interact chemically. No significant difference was observed between the melatonin and NAC groups. The superiority of melatonin over NAC in terms of antioxidant properties was not found in our study.

By extending the study period, including antioxidant parameters (measurement of superoxide dismutase, glutathione, etc.), oxidative parameters such as MDA and NO, it is possible to compare the efficacy of both substances against reactive oxygen species and to determine the differences between melatonin and NAC. Besides, intracellular cholestasis, biliary infarcts, and ductal proliferation were observed in the study and control groups. When both NO and MDA levels, and histopathological findings were evaluated, no significant difference was found between treatment . Yet, the comparison between sham group, early sacrification and the treatment groups, it was determined that especially the antioxidant activity and tissue damage were achieved only in the early sacrification group, and there was a significant difference between this group and sham group. It was concluded that when antioxidant treatment is started during or before the first surgical stress, more successful results will be obtained in the light of both biochemical parameters and oxidative and histopathological parameters.

The lower levels of total bilirubin levels, one of the important indicators of obstructive jaundice, were noted in the melatonin group and NAC group compared to control group. There was no significant difference between the melatonin group and NAC group, and there was no statistically superiority to the other study groups when both drugs were given together.

Melatonin and NAC have shown positive effects on obstructive jaundice when serum AST, ALT, BUN, and creatinine levels are compared between control,

csham and study groups. However, no significant difference was found in comparison between the melatonin group and NAC group, and co-administration of both drugs did not create synergistic effect either. Hepatic and renal MDA levels were found to be reduced after melatonin and NAC administration which may be interpreted as reduction in lipid peroxidation due to ischemia- reperfusion injury develops after obstructive jaundice. When the nitric oxide levels due to ischemia/reperfusion injury developed after obstructive jaundice were compared, it was thought that melatonin and NAC reduced the damage, most likely by causing a decrease in hepatic and renal NO levels.

A scoring system that examines biliary infarcts and necrosis in the portal area in terms of hepatic damage due to obstructive jaundice was prepared and a comparison between the groups was made. The histopathological changes in the liver due to the effect of obstructive jaundice are reduced by the effects of melatonin and NAC, suggesting that both agents play a role in the faster recovery of cellular damage on the liver.

In conclusion, the use of melatonin and N-acetylcysteine reduced tissue damage in both liver and kidneys in an experimental jaundice model in rats. It is suitable for clinical use in obstructive jaundice with no side effects, ease of administration.

## REFERENCES


1. Parola M, Leonarduzzi G, Robino G, et al. On the role of lipid peroxidation in the pathogenesis of liver demage induced by long-standing cholestasis. Free Radical Biol Med. 1996; 20:351–359.
2. Montilla P, Cruz A, Padillo FJ, et al. Melatonin versus vitamin E as protective treatment against oxidative stres after extrahepatic bile duct ligation in rats. J. Pineal Research. 2001; 31:138–144.
3. Küçük C, Sözüer EM, Ikizceli I. Role of oxygen free radical scavengers in acute renal failure complicating obstructive jaundice. Eur. Surg. Res. 2003; 35:143–47.
4. Halim AB. Biochemical effect of antioxidants on lipid and liver function in experimentally-induced liver demage. Ann Clin Biochem. 1997; 34: 656–663.
5. Hu C, Zhao L, Tao J, Li L. Protective role of melatonin in early-stage and end- stage liver cirrhosis. J Cell Mol Med. 2019 Nov;23(11):7151-7162.
6. Ballı E. Melatoninin Fonksiyonları. Mersin Üniversitesi Tıp Fak. Dergisi. 2003; 4:380–385.
7. Pazar A, Kolgazi M, Memisoglu A, Bahadir E, Sirvanci S, Yaman A, Yeğen BÇ, Ozek E. The neuroprotective and anti-apoptotic effects of melatonin on hemolytic hyperbilirubinemia-induced oxidative brain damage. J Pineal Res. 2016 Jan;60(1):74-83.
8. Tan DX, Chen LD, Poeggeler B, et al. Melatonin: a potent endogenous hydroxyl radical scavenger. Endocr J. 1993; 1:57–60.
9. Tan DX, Poeggeler B, Reiter RJ, et al. The pineal hormone melatonin inhibits DNA adduct formation induced by the chemical carcinogen role in vivo. Cancer Lett 1993; 70:63–71.
10. Zafarullah M, Li WQ, Sylvester J, et al. Molecular mechanisms of N–actylysteine actions. Cell Mol. Life Science 2003; 60:6–20.
11. Dülger H, Durmuş A, Sezgi C. KOAH akut atak tedavisinde antioksidan olarak N–asetilsistein'in etkinliği Van Tıp Dergisi. 2002; 9(3): 91–94.
12. Chen C, Shiesh S, Tsao HC, et al. Protective effect of melatonin on renal injury of rats induced by bile ligation. Digestive Diseases and Science. 2001; 46: 927– 931.
13. Bülbüller N, Akkuş MA, Çetinkaya Z. Effects of melatonin and lactulose on the liver kidneys in rats with obstructive jaundice. Pediatr Surg Int. 2002; 18: 678– 680.
14. Tsai LY, Lee KT, Lu FJ. Biochemical events associated with ligation of the common bile duct in Wistar rats. J Formos Med Assoc 1997; 96:17–22.
15. Deitch EA, Sitting K, Li M, et al. Obtructive jaundive promotes bacterial translocation from gut. Am J Surg 1990; 159:79–84.
16. Gümüs M, Tekin R, Firat U, Onder A, Kapan M, Böyük A, Aldemir M, Kilinç C. The effects of pomegranate on bacterial translocation in rats with obstructive jaundice. Eur Rev Med Pharmacol Sci. 2013 Jun;17(11):1488-94.
17. Bomzon A. Bile acids, oxidative stress and renal function in biliary obstruction Semin Nephro. 1997; 17: 549–562 (Review).
18. Eşrefoğlu M, Gül M, Emre MH. Protective effect of low dose of melatonin against cholestatic oxidative stress after common bile duct ligation in rats. World J Gastroenterol. 2005; 11:1951–1956.
19. Andreadau I, Iliodromitis EK, Mikros E, et al. Melatonin does not prevent the protection of ischemic preconditioning in vivo despite its antioxidant effect against oxidative stres. Free Radic Biol Med. 2004; 37:500–510.
20. Aydoğdu N, Kaymak K,Yalçın Ö. Sıçanlarda Böbrek iskemi/reperfüzyon hasarında N–asetilsisteinin etkileri. Fırat Tıp Dergisi 2005; 10: 151–155.
21. Ommati MM, Amjadinia A, Mousavi K, Azarpira N, Jamshidzadeh A, Heidari R. N-acetyl cysteine treatment mitigates



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biomarkers of oxidative stress in different tissues of bile duct ligated rats. Stress. 2021 Mar;24(2):213-228.

22. Cotran RS, Kumar V, Collis T ( Eds). Robbins Pathologic Basis Disease (6th ed). WB Saunders Company, Philadelphia 1999; pp 100–101.