RESEARCH ARTICLE

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Protective Effects of Melatonin against Chronic Sodium Nitrite Exposure in Rats

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In this study, anti-inflammatory effects melatonin (Mel) on liver and kidney damage induced by sodium nitrite ($NaNO_2$) used as a food additive were investigated. The study groups were control group (C), $NaNO_2$ group ($NaNO_2$) and melatonin + $NaNO_2$ group (Mel + $NaNO_2$). The first group received dimethyl sulfoxide (DMSO) and the second and third groups received $NaNO_2$ orally for twelve weeks. The third group received melatonin 2 hours before the administration of $NaNO_2$. Administration of $NaNO_2$ (80 mg/kg/day) for 12 weeks orally to rats increased serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) ($P<0.001$) and urea ($P<0.01$) levels. Interleukin 1-alpha (IL-1α) ($P<0.05$) and tumor necrosis factor alpha (TNF-α) ($P<0.01$, $P<0.001$ respectively) levels were found to be increased in $NaNO_2$ group in liver and kidney homogenates. It was also determined that IL-6 ($P<0.001$) levels were increased in kidney tissue. On the other hand, it was also found that there was a decrease at the levels of serum AST ($P<0.001$), ALT, urea ($P<0.05$), liver IL-1α, TNF-α ($P<0.01$), and kidney TNF-α, IL-6 ($P<0.05$) in group given melatonin (500 µg/kg/day) 2 hours before $NaNO_2$. In addition, it was observed that there was less liver and kidney damage than $NaNO_2$ group in the pathological examinations Mel + $NaNO_2$ applied group. The present data demonstrate that melatonin administration has visible modulatory effects and can eliminate inflammation; moreover, it can prevent the increase in biochemical markers caused by chronic sodium nitrite administration.

Key Words: *Melatonin, sodium nitrite, food additive, inflammation*

Sıçanlarda Melatoninin Kronik Sodyum Nitrit Maruziyetine Karşı Koruyucu Etkileri

Bu çalışmada, gıda katkı maddesi olarak kullanılan sodyum nitritin ($NaNO_2$) neden olduğu karaciğer ve böbrek hasarı üzerine melatoninin (Mel) anti-inflamatuvar etkileri araştırıldı. Çalışma grupları kontrol grubu (C), $NaN0_2$ grubu ($NaN0_2$) ve melatonin + $NaN0_2$ grubu (Mel + $NaN0_2$) olarak belirlendi. İlk gruba dimetil sülfoksit (DMSO), iki ve üçüncü gruplara $NaNO_2$ oniki hafta boyunca oral olarak uygulandı. Üçüncü gruba $NaNO_2$ uygulamasından 2 saat önce melatonin uygulandı. Sıçanlara oral yoldan 12 hafta süreyle $NaNO_2$ verilmesi (80 mg/kg/gün) serum aspartate aminotransferaz (AST), alanin aminotransferaz (ALT) ($P<0.001$) ve üre ($P<0.01$) seviyelerini arttırdı. Karaciğer ve böbrek homojenatlarında $NaNO_2$ grubunda interlökin 1-alfa (IL-1α) ($P<0.05$) ve tümör nekroz faktör alfa (TNF-α) (sırasıyla $P<0.01$, $P<0.001$) düzeylerinde artış olduğu bulundu. Ayrıca böbrek dokusunda IL-6 ($P<0.001$) seviyelerinin arttığı tespit edildi. Öte yandan, $NaNO_2$'den 2 saat önce melatoninin (500 µg/kg/day) verilen grupta serum AST ($P<0.001$), ALT, üre ($P<0.05$), karaciğer IL-1α, TNF-α ($P<0.01$) ve böbrek TNF-α, IL-6 ($P<0.05$) düzeylerinde azalma olduğu tespit edildi. Ayrıca, patolojik incelemelerde Mel + $NaNO_2$ uygulanan grupta $NaNO_2$ grubundan daha az karaciğer ve böbrek hasarı olduğu gözlendi. Mevcut veriler, melatonin uygulamasının görünür modülatör etkilere sahip olduğunu ve inflamasyonu ortadan kaldırılabileceğini, bununla birlikte kronik sodyum nitrit uygulamasının neden olduğu biyokimyasal belirteçlerin artmasını önleyebileceğini göstermektedir.

Anahtar Kelimeler*: Melatonin, sodyum nitrit, gıda katkı maddesi, inflamasyon*

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Introduction

The people are exposed to different chemicals and food additives cause of the changing consumption habits of the society, the sedentary lifestyle, the increase in mass food and food production, the low level of education and income, the increase in workload and the inadequacy of time, turn to ready food consumption, reasons such as uncontrolled food production, the extension of the shelf life of food. Many of these additives are increasingly recognized as a potential hazard for human health. One of the important food additives is sodium nitrite. Sodium nitrite is widely used for purposes as antibacterial, flavor-protecting and enhancer, color stabilizer and shelf-life extension (1, 2). Sodium nitrite is generally considered as a poor carcinogenic substance, but it can cause cancer, hepatotoxicity, nephrotoxicity, tissue damage, inflammation and functional impairment when exposed to high doses (3, 4). Also in toxicity cases the level of free radicals is increasing especially superoxide radical and nitric oxide interact easily and it causes the formation of another reactive free radical, peroxynitrite (5). Peroxynitrite a strong cytotoxic oxidant that plays an active role in free radical-induced, tissue injury, in the increase of reactive oxygen species (ROS) production and in the inhibition of the mitochondrial electron transport chain (5, 6). The preservation mechanisms of antioxidants that can be taken endogenously and / or exogenously

against nitrate and nitrite oxidation has not yet been defined. Today, research is very limited on the role of hormones such as growth hormone, estrogen, dehydroepiandrosterone and melatonin (7), which are found to be important contributors to the immune system against the adverse effects of nitrates and nitrites. The melatonin tryptophanamino acid derivative is a hormone and is produced especially in the pineal gland. However, it is also known that the melatonin receptors have been identified in the retina, ovaries, and the gastrointestinal system (7-9). Before the researches made in recent years melatonin has only been implicated in the regulation of biological rhythms but today it is known to be one of the strong endogenous antioxidants (9, 10). The reduction of immunological functions and the ability of the immune system to respond to antigenic stimuli may occur, due to aging, exposure to chemicals, oxidative stress, radiation and food additives. Therefore, the risk of infectious disease, cancer and prevalence may increase (1, 11). For this reason it was aimed to determine the effects of the melatonin (known as the neuro endocrine hormone which is expressed that antioxidant, antiinflammatory and have a protective effect against ischemia-reperfusion injury in various organs) on the pro-inflammatory cytokine markers that is important in the pathogenesis of these degenerative changes with liver and kidney damage induced with sodium nitrite.

Materials and Methods

In the study, 30 Sprague-Dawley rats of 2-3 months old were obtained from the Erzurum Veterinary Control Institute Experimental Animal Unit. Kafkas University Experimental Animals Local Ethics Committee (KAU-HADYEK) was held with the resolution of 2017-044 at the Kafkas University Experimental Animal Application and Research Center. Rats were fed *ad-libitum* under standard conditions (in constant temperature and ventilated rooms, 12 hours of daylight and 12 hours of darkness).

1. Control Group (C): 500 µL dimethyl sulfoxide (DMSO) was administered orally daily

2. Sodium Nitrite Group ($NaNO_2$): Sodium nitrite (80 mg/kg/day) administered was dissolved in drinking water

3. Melatonin + Sodium Nitrite Group (Mel + $NaNO_2$): 80 mg/kg/day sodium nitrite (dissolved in drinking water) + 500 µg/kg/day melatonin (dissolved in DMSO) was administered orally.

After the 7 day adaptation period, the study groups were administered with the treatment substances for 12 weeks according to the above procedure. Melatonin was given orally 2 hours before sodium nitrite administration. DMSO was administered orally to the control group for 12 weeks in order to minimize stress caused by oral gavages administration between control and study groups and to dissolve melatonin in DMSO. At the end of the study, liver and kidney tissue samples were taken following cervical dislocation after taking blood from the animals into the tubes without anticoagulant via the intracardiac route.

Tissue and serum samples from animals were stored at –20 °C until biochemical analyzes were performed. Some of the tissues were taken for 10% formaldehyde solution for histopathological examinations.

Biochemical Analyzes: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine levels were measured in the auto analyzer to determine liver and kidney damage induced by sodium nitrite in serum samples (Mindray BS 120). TNF-α, IL-1α and IL-6 levels of proinflammatory cytokines in liver and kidney tissue homogenates were determined using commercial ELISA kit (Elabscience-USA).

Histopathological Analyzes: Tissue samples of liver and kidney were collected at necropsy, and fixed in 10% phosphate buffered formaldehyde solution, and then embedded in paraffin. Tissue sections at 5 µ thickness were cut and stained routinely with hematoxylin and eosin (HE) for microscopic examination.

Statistical Analyzes: SPSS 18 package program was used for biostatistical evaluation of the data obtained in the study. As the significance level was accepted as $P<0.05$ a total of 10 subjects in each group corresponds to a power of about 90%. We conducted a normality test with the data and subsequently conducted ANOVA analyses when no evidence of deviation from the normality. One-way analysis of variance (ANOVA) and Tukey test were used to evaluate the variables between the groups. The values of the research results are given as mean ± standard deviation (12-14).

Results

Serum AST, ALT ($P<0.001$) and urea ($P<0.01$) levels were significantly increased when the $NaNO_2$ group is compared with the C group. When Mel+$NaNO_2$ group and $NaNO_2$ group were compared serum AST ($P<0.001$), ALT ($P<0.05$) and urea ($P<0.05$) levels decreased significantly. In addition, the serum AST, ALT and urea levels were determined to approach of the control group in the Mel + $NaNO_2$ groups. When serum creatinine levels were evaluated, it was found that there was no statistical significance between groups (Figure 1). When the levels of liver TNF-α ($P<0.01$) and IL-1α ($P<0.05$) were evaluated, there was a significant increase in the $NaNO_2$ group compared to the C group. Figure 2 shows that there is a significant decrease in the indicated parameters when the Mel + $NaNO_2$ group is compared with the $NaNO_2$ group ($P<0.01$). There was no statistically significant difference between the groups in terms of liver IL-6 levels.

When the levels of proinflammatory cytokines (TNF-α, IL-1α and IL-6) in renal tissue were evaluated. It was determined that the $NaNO_2$ group increased significantly in comparison with the C group ($P<0.001$, $P<0.05$ and $P<0.001$ respectively). When the Mel +

$NaNO_2$ group is compared with the $NaNO_2$ group it was found that there was a statistically significant decrease in kidney TNF-α and IL-6 levels ($P<0.05$). When both the C group and the $NaNO_2$ group with kidney IL-1α levels were compared with the Mel + $NaNO_2$ group it was found that there was no statistical significance (Figure 3).

In group C, a mild vascular congestion was observed in only one case in the liver. In the $NaNO_2$ group, moderate vascular congestion was observed in all cases and mild vascular congestion in one case. However, no necrosis and fibrosis were found in any of the cases. Moderate focal in the majority of events, moderate in a case and light common hepatocyte damage in one case were determined. Mild focal periportal inflammation was detected in all cases. Mild interhepatocytic bleeding was noted in all cases. In the Mel + $NaNO_2$ group, mild vascular congestion was observed in half of the cases and moderate vascular congestion in the other half. But no case of necrosis and fibrosis were observed in any animal. In cases with moderate congestion, moderate focal and in one case with common hepatocyte injury were observed. At the same time, focal mild periportal inflammation was detected in the case with moderate congestion. Cell damage was detected in two cases and there was mildly intra hepatic bleeding. When these histopathological findings are taken into account less liver damage was observed in the Mel + $NaNO_2$ group than in the $NaNO_2$ group (Figure 4). In group C, mild vascular congestion was observed in only one case in the kidney. These findings were found in only one case in Mel + $NaNO_2$ group. According to the histopathological results described, there was less kidney damage in the Mel + $NaNO_2$ group compared to the $NaNO_2$ group (Figure 5).

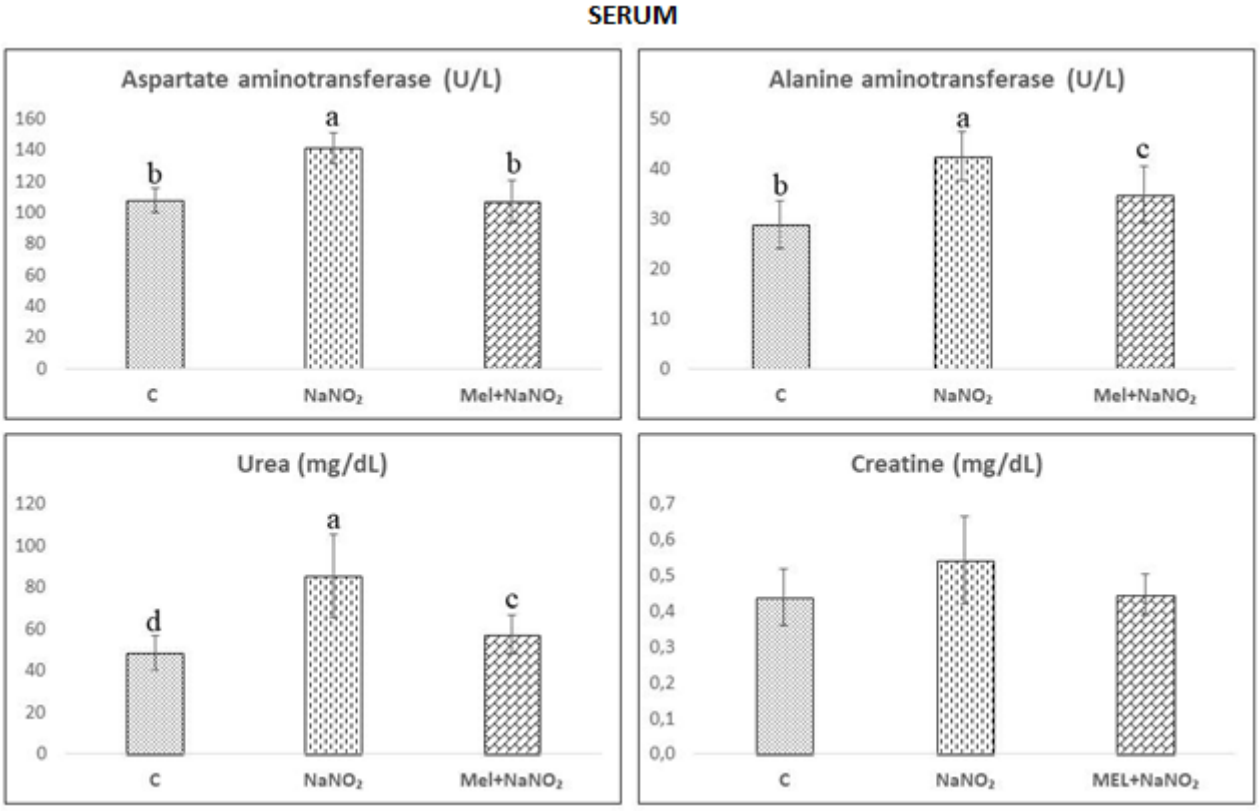

Figure 1. Aspartate aminotransferase, alanine aminotransferase, urea and creatine levels in serum samples, mean±SD, (n:10), a-b: $P<0.001$, a-c: $P<0.05$, a-d: $P<0.01$, b-c, c-d: $P>0.05$

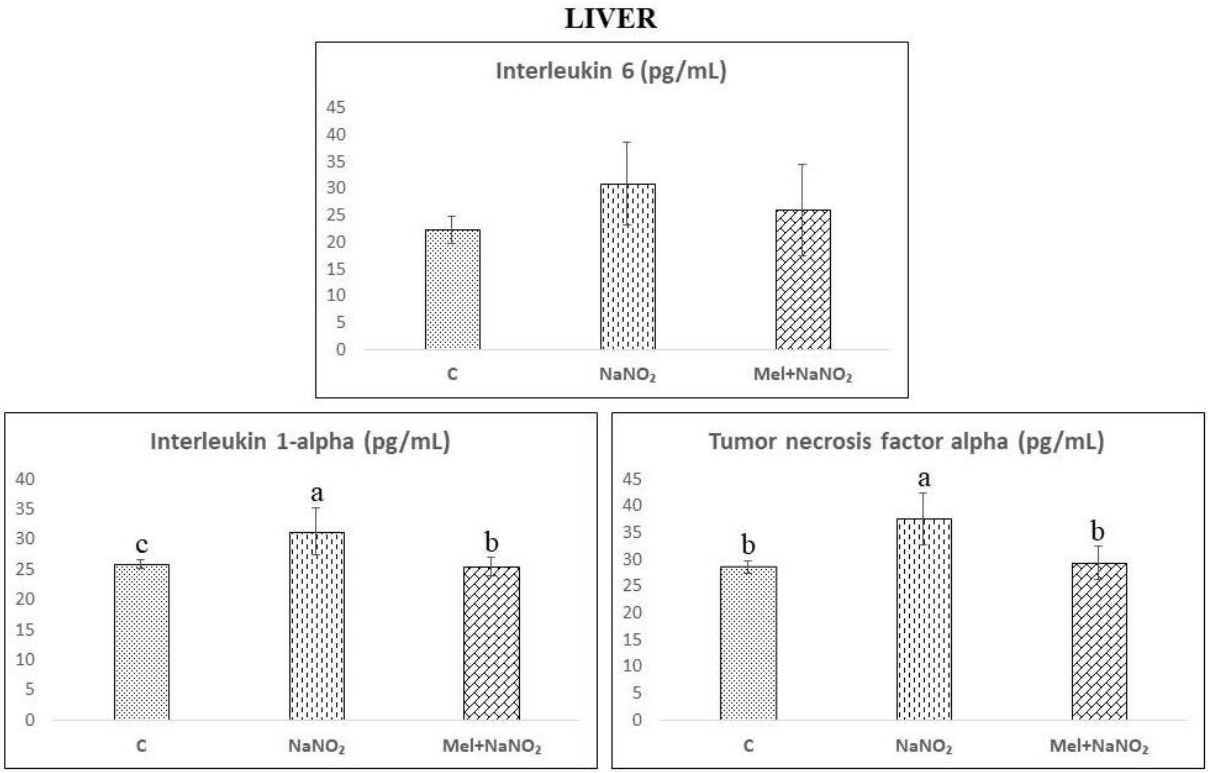

Figure 2. Interleukin 6, interleukin 1-alpha and tumor necrosis factor alpha levels in liver tissues, mean±SD, (n:10), a-b: P<0.01, a-c: P<0.05, b-c: P>0.05

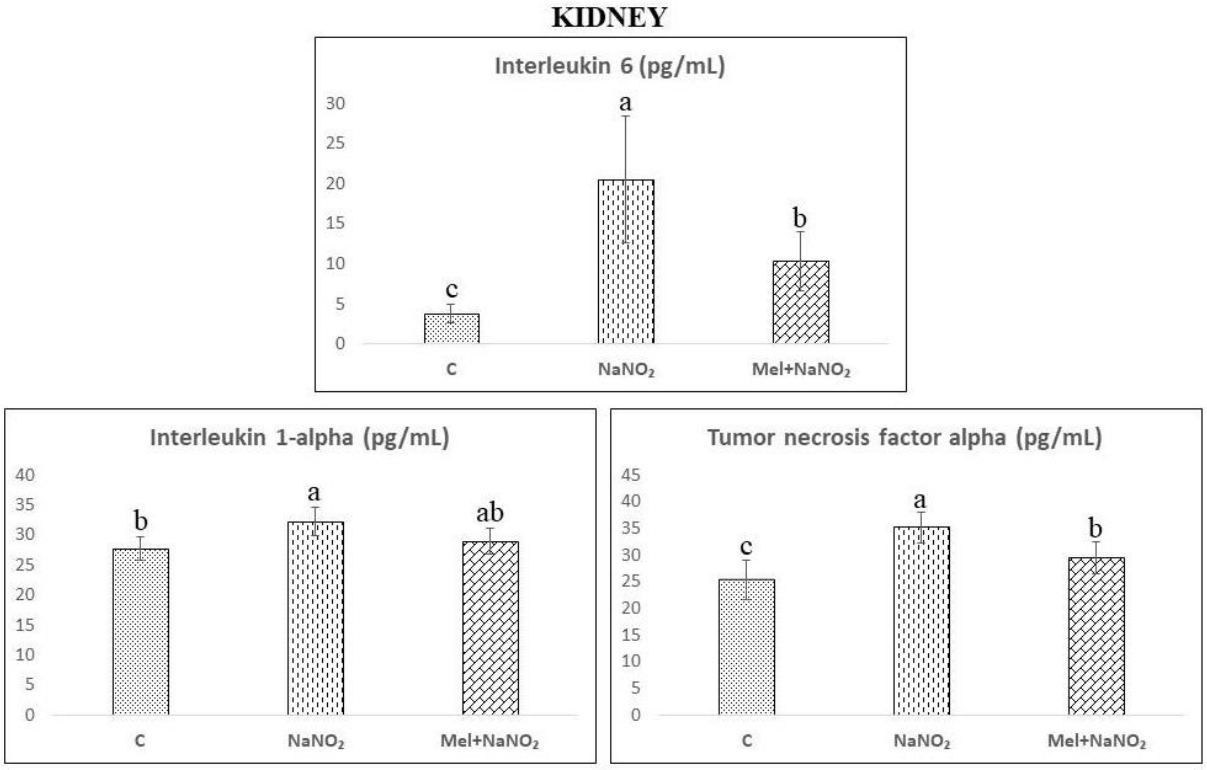

Figure 3. Interleukin 6, interleukin 1-alpha and tumor necrosis factor alpha levels in kidney tissues, mean±SD, (n:10), a-b: P<0.05, a-c: P<0.001, b-c: P>0.05

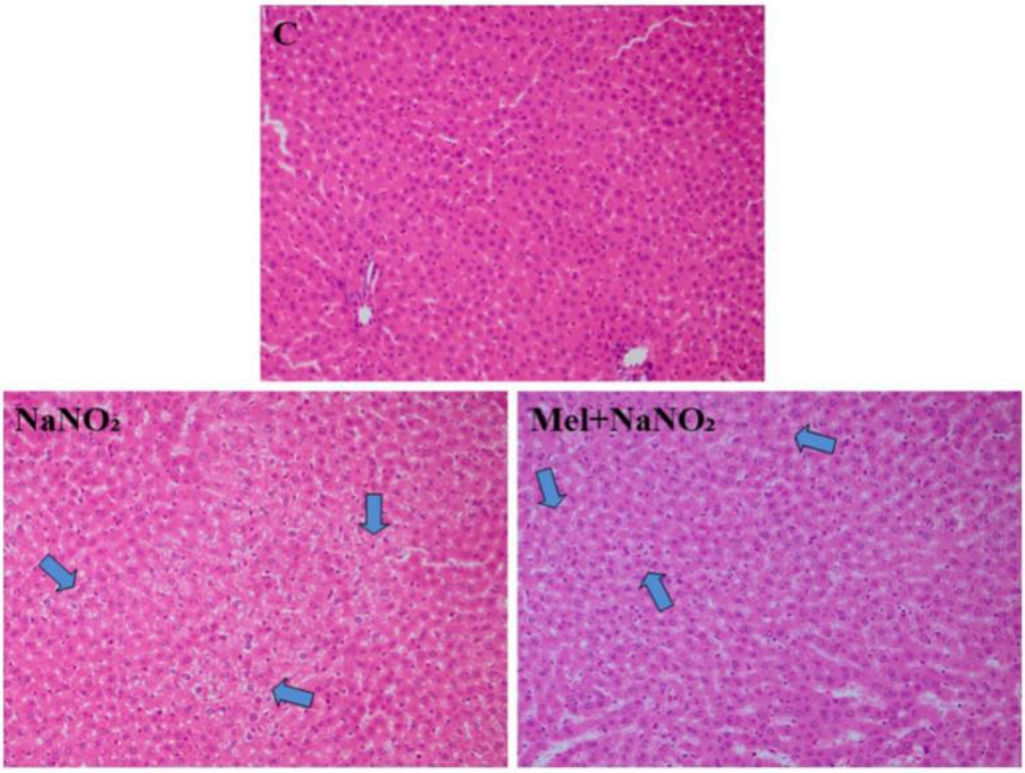

Figure 4. Group C; Liver tissue at morphological limits, $NaNO_2$ group; Moderate focal hepatic damage (arrow), Mel + $NaNO_2$ group; Mild common hepatocyte damage (arrow), (H&E, 200x)

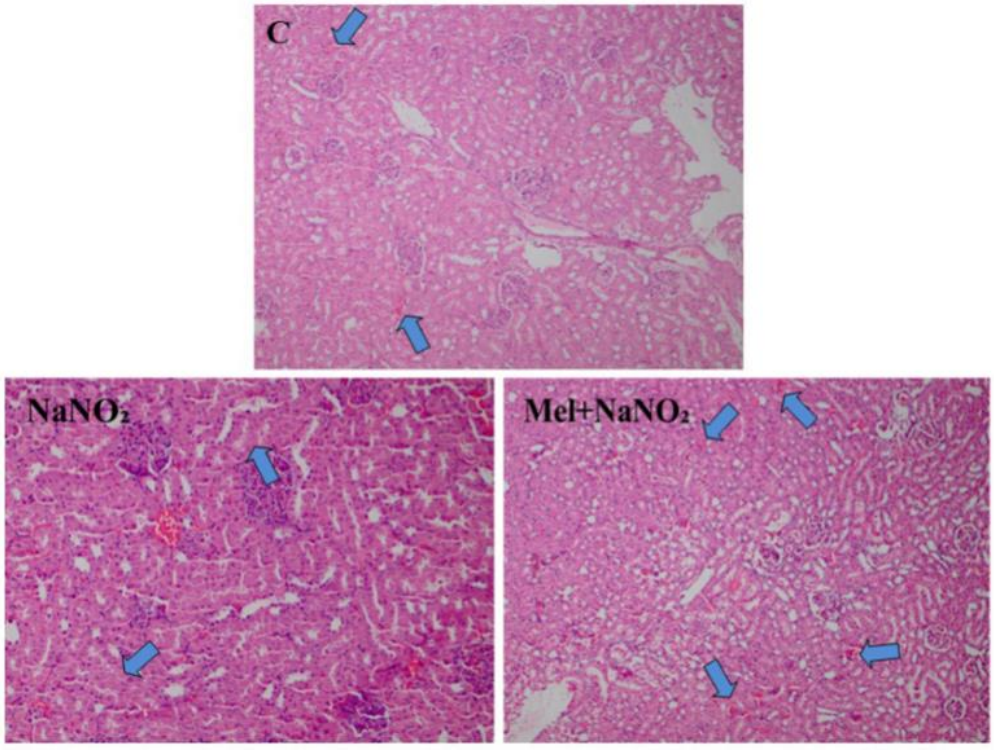

Figure 5. C Group; Kidney tissue showing mild vascular congestion (arrow) (H&E, 100x), $NaNO_2$ group; Focal mild level of tubular epithelial damage (arrow), (H&E, 200x), Mel + $NaNO_2$ group; Inter and intra-tubular bleeding areas (arrow), (H&E, 100x)

Discussion

The World Health Organization (WHO) has declared processed meat products such as salami, sausage, bacon, and sausage as first-rate carcinogenic products, such as cigarettes and asbestos after the studies have focused specifically on additives that are used to delay the breakdown of these processed nutrients and to ensure their longevity (15). One of the important food additives is sodium nitrite. It is stated that sodium nitrite increases the risk of suffering various harms in different organs, such as colorectal cancer, liver-stomach cancer, breast cancer, brain tumor, leukemia, pancreatic cancer (16, 17). Sodium nitrite reacts with amines in the stomach, causing the production of carcinogenic nitrosamines and reactive nitrogen species, induction of lipid peroxidation and depletion of antioxidant enzymes, resulting in free radical induced tissue damage (18-20). In addition to these effects, the nitrite converts hemoglobin to methemoglobin and prevents oxygen transport in the blood and causes tissue hypoxia (5). It was determined in previous studies that oral administration of sodium nitrite resulted in significant kidney and liver damage in rats (3, 21, 22). In that regard the results of this study are consistent with the previous studies.

In the present study serum AST, ALT, urea, and proinflammatory cytokine levels that are effective in liver and renal damage pathogenesis significantly increased in groups treated with sodium nitrite. Previously, melatonin protective effects were determined in various toxicity and tissue damage models. Nevertheless, in the literature it has not been observed in studies on chronic liver and kidney damage formed by applying sodium nitrite. When viewed in this direction, the present study constitutes an important step on this subject. Otha et al. (23) determined that there was an increase in the liver lipid peroxidation (LPO) and a decrease in the level of reduced glutathione in the liver after 6 and 24 hours of injection in carbon tetra chloride (CCl_4) injected rats. The post-melatonin application improves in a dose dependent manner changes that occur 24 hours after the injection of CCl_4. In the study of Nava et al. (24) it significantly alleviates the acute nephrotoxicity produced by inorganic mercury when melatonin beneficial effects is given at least 30 minutes before giving mercury chloride. They expressed that if melatonin is given with or after mercury chloride, it cannot prevent functional and histological damage. In another study, lung tissue damage was generated in rats exposed to chlorpyrifos-etile, and melatonin significantly reduced the effect of chlorpyrifos-ethylintoxic in the lungs in rats (25). Othman et al. (26) investigated the effects of melatonin against hematotoxicity induced by heavy metal (lead acetate) and reported that the levels of bone marrow polychromatic erythroids, neutrophils and lymphocytes were improved in groups where lead and melatonin are administered together. Ding et al. (27) determined that the experimental traumatic brain injury model is formed, application of melatonin increases the activity of antioxidant enzymes, and it is effective in alleviating brain damage by and antioxidant-sensitive element (Nrf2-ARE) pathway system and by factor 2 associated with nuclear factor erythroid 2.

In the study melatonin, a potent endogenous antioxidant, was administered orally two hours before the administration of sodium nitrite for three months. It is determined that it causes a significant decrease in the levels proinflammatory cytokine levels that are effective on the pathogenesis of liver and kidney injury (TNF-α, IL-1α and IL-6). It has also been found that after administration of melatonin, it could be effective for decrease in serum AST, ALT and urea levels and for preventing degenerative changes in the liver and kidney. In conclusion, it was determined that chronic $NaNO_2$ consumption in this study caused significant physiological and histopathology changes in liver and kidney tissues in rats. It is known that melatonin hormone is an effective powerful antioxidant for the regulation of endocrine system, biological rhythms and gonadal functions, and for adjusting smooth muscle tonus and it has been found to have positive effects on chronic $NaNO_2$-induced liver and kidney damage.

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