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PHARMACOLOGY

EFFECT OF NIMESULIDE AGAINST INDOMETHACIN-INDUCED OXIDATIVE LIVER DAMAGE IN RATS

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Abstract: Indomethacin is used in the treatment of ankylosing spondylitis, osteoarthritis, tendinitis, and other inflammations. However, it has been reported indomethacin has nephrotoxic, gastrotoxic, and hepatotoxic effects. The aim of this study is to investigate the effect of nimesulide on indomethacin-induced liver damage in rats biochemically and histopathologically. Experimental animals (21 rats) were divided into 3 groups: the healthy (HG) group receiving distilled water as a solvent, the 25 mg/kg indomethacin administered (IDO) group, and the 50 mg/kg nimesulide + 25 mg/kg indomethacin administered (NIDO) group. Malondialdehyde (MDA), total glutathione (GSH) levels and cyclooxygenase-1 (COX-1), cyclooxygenase (COX-2) enzyme activities were determined from liver tissues. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were also measured in serum. The data obtained in the IDO group were compared with the data obtained from the NIDO and MDA and decreased tGSH levels with indomethacin HG groups. Increased application in liver tissue approached the HG group with nimesulide application, and the difference was found to be insignificant. Nimesulide prevented the decrease in COX-1 and COX-2 levels observed with indomethacin administration. Again, the increase in ALT and AST levels, which increased with indomethacin administration, was prevented by nimesulide. As a result of the histopathological evaluation, it was observed that nimesulide reduced the damage caused by indomethacin. Nimesulide at 50 mg/kg prevented oxidative liver damage induced with indomethacin at 25 mg/kg. Our results suggest that nimesulide may be useful in the treatment of hepatotoxicity and COX-1 inhibition side effects without suppressing analgesic and anti-inflammatory activity due to indomethacin use

Keywords: liver damage, indomethacin, nimesulide, oxidative protection

Indomethacin (1 p- chlorobenzyl- 5 methoxy- 2-methyl- 3- indoleacetic acid) is an indole derivative with anti-inflammatory, analgesic, and antipyretic effects (1). It is used in the treatment of ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, gouty arthritis, bursitis, tendinitis, synovitis, and other inflammations (2). However, the toxic effects of indomethacin during treatment cause its use to be terminated. It has been reported in the literature that indomethacin has nephrotoxic, gastrotoxic, and hepatotoxic effects (1, 3). Inhibition of cyclooxygenase -2 (COX-2) is thought to be responsible for the anti-inflammatory effect of indomethacin, whereas inhibition of cyclooxygenase -1 (COX-1) is responsible for its side effects (2, 4). While inhibition of COX-1 causes side effects as a result of decreased prostaglandin synthesis, it is believed that inhibition of COX-2 is associated with its anti-inflammatory effects (2, 5). In a study by Ahmed et al., it was determined that catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) levels decreased, alanine aminotransferase (AST) and aspartate aminotransferase (ALT) levels increased in the liver tissue with indomethacin administration, and it was stated that the damage in the liver may be due to the increase of indomethacin-induced reactive oxygen species (1). Excessive increases in reactive oxygen species (ROS) production cause lipid peroxidation (LPO) and malondialdehyde (MDA) formation; MDA is an important indicator of oxidative stress. It is known that

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the increase in tissue MDA is associated with damage and its decrease is associated with protection (6, 7). The anti-inflammatory mechanism of action of nimesulide, a nonsteroidal anti-inflammatory drug (NSAID) can be listed as inhibition of COX enzymes and lipoxygenase synthesis, the release of ROS, suppression of lysosomal enzyme functions, and prevention of neutrophil aggregation, adhesion, and chemotaxis (8). On the other hand, nimesulide, which is less selective for COX-2, can inhibit NSAID-induced gastric damage, while celecoxib and rofecoxib (9, 10), while celecoxib and rofecoxib, which are more selective for COX-2 (350 to 800 times as selective), cannot prevent these ulcers(11). Therefore, it would not be correct to attribute the gastrointestinal side effects of indomethacin and other NSAIDs to the inhibition of COX-1 enzyme alone. In a study, nimesulide has been reported to reduce endogenous adrenaline, preventing indomethacin-related ulcers. In addition, it has been stated that in the absence or decrease of endogenous adrenaline, glucocorticoids bind to alpha-2 adrenergic receptors and exert an antiulcer effect on these receptors (12). In another study, it has been shown that alpha-2 receptors are responsible for protective effect related to COX production. Prednisolone cannot bind to a adrenergic receptors in the presence of adrenaline (intact rats) but, in its absence (adrenalectomy), binds to alpha-2 receptors, stimulates them more effectively than adrenaline, suggesting a direct relationship between alpha-2 adrenergic receptors and COX-1 levels, whereas beta-2 receptors are directly related to COX-2 levels (13). Nimesulide has been shown to protect liver tissue from ischemia reperfusion injury by its antioxidant activity and protective effect on COX-1 enzyme (14). This information suggests that nimesulide may be useful in reducing indomethacin-related liver oxidative damage. The aim of this study is to investigate the effect of nimesulide on indomethacin-induced liver damage in rats biochemically and histopathologically.

MATERIALS AND METHODS

Animals

A total of 21 albino male Wistar rats weighing between 245-258 grams were used in the experiment. All animals were received from Ataturk University Medical Experimental Application and Research Center. Animals were housed and fed in the laboratory at normal room temperature (22°C) for one week prior to experimentation, in order to allow them to adapt to the environment. Animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the local animal ethics committee of Ataturk University, Erzurum, Turkey. (Ethics Committee Number: 27.12.2018/13).

Chemicals

Of the chemical substances used for the experiments, thiopental sodium was provided by IE Ulagay (Turkey). Indomethacin and nimesulide were obtained from Deva (Turkey).

Animal groups

Experimental animals were divided into three groups: 25 mg/kg indomethacin administered (IDO), 50 mg/kg nimesulide + 25 mg/kg indomethacin administered (NIDO), and a healthy (HG) group given distilled water as solvent.

Experimental procedure

In the NIDO group of rats (n = 7) 50 mg/kg of nimesulide was administered orally to the stomach by gavage. IDO (n = 7) and HG (n = 7) animal groups were administered orally with the same volume of distilled water as a solvent. One hour after nimesulide and distilled water administration, indomethacin 25 mg/kg was administered orally to NIDO and IDO groups with the same method. This procedure was repeated once a day for 7 days. Drug and solvent (distilled water) were administered in fixed doses at the same time with the same method. Animals were sacrificed using high dose thiopental sodium (50mg/kg) and the levels of MDA, total GSH (tGSH), COX-1, and COX-2 were measured in the excised liver tissues. ALT and AST activity assays were performed in blood samples taken from the tail veins of animals. The evaluation was made by comparing the data obtained from the NIDO and HG groups with the data obtained from the IDO group.

Biochemical analyzes

MDA and tGSH analysis in tissue

MDA measurements are based on the method used by Ohkawa et al., which includes the spectrophotometric measurement of the absorbance of the pink-colored complex formed by thiobarbituric acid (TBA) and MDA (15). Total GSH measurement was made according to the method described by Sedlak J and Lindsay RH (16).

Measurement of COX activity

COX Activity Assay Kit (Item No. 760151), Cayman Chemical Company (Cayman Chemical, MI, USA). We measured the COX activity in rats' sinonasal tissue in this series of experiments using

a COX activity assay kit (Cayman, Ann Arbor, MI, USA). Sinonasal tissue was washed thoroughly with ice-cold Tris buffer, pH 7.4, containing 0.16 mg/mL of heparin, to remove any red blood cells and clots, then stored at -80°C until assayed. For each rat, a sample of sinonasal tissue was homogenized in 5 mL of cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1 mM EDTA) per gram of tissue and centrifuged at 10000 xg for 15 minutes at 4°C. The supernatant was removed for assay and stored on ice. We then measured the protein concentration in the supernatant using the Bradford method (17). (The COX activity assay kit measures the peroxidase activity of COX. This is assayed colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine at 590 nm. We measured COX-2 activity using the COX-1-specific inhibitör (18)**.** Results for COX-1 and COX-2 activity are given as units per milligram of protein. The activity of COX in the tissue was expressed as nmol/min/mg protein (U/ mg protein)

ALT and AST analysis in serum

Venous samples were collected into tubes without an anticoagulant. After clotting, the serum was separated by centrifugation and stored at - 80C until assay. Using a Cobas 8000 (Roche Diagnostics GmBH, Mannheim, Germany) with commercially available kits (Roche Diagnostics), serum AST and ALT activities were measured spectrophotometrically for the liver function tests.

Histopathological study

The liver tissues taken from the rats were fixed in 10% formalin and 5 µm-sections obtained from paraffin blocks were stained with hematoxylin and eosin stain. All blocks were coded and without knowing the group they were involved in, the same histologist examined them one by one on a light microscope (Olympus CX 51, Tokyo, Japan) (x200). For histopathological criteria, a scoring was made between 0-3 points as: 0 = not found, 1 = mild, 2 = moderate, and 3 = severe.

Statistical analysis

Shapiro-Wilk test was used to determine whether the data obtained from the groups were normally distributed. Since the data showed normal distribution, a one-way ANOVA test was applied. Tukey was used as a post hoc test. The results obtained from the experiments were expressed as "mean value ± standard deviation" (x ± SD). The Kruskal-Wallis test was used to evaluate the histopathological grading results. All statistical operations were performed in the "SPSS for Windows, 22" statistical program, and the p-value < 0.05 was considered to be significant.

RESULTS

Biochemical results

MDA and tGSH analysis results

As is seen in Figure 1, indomethacin caused an increase in the MDA of the liver tissue of the animals ($p < 0.001$) (7.6 ± 0.7). Nimesulide significantly reduced ($p < 0.001$) the MDA increase in the liver tissue exposed to indomethacin (2.7 ± 0.4). However, the difference in the amounts of MDA in the NIDO and HG groups (2.1 ± 0.3) was statistically insignificant ($p = 0.064$). Furthermore; the indomethacin imposed on the liver tissue led to an instant decrease in the amount of tGSH ($p < 0.001$) (1.8 ± 0.5). There was a significant difference between indomethacin and nimesulide groups (4.3 ± 0.5) in terms of the amount of tGSH ($p < 0.001$). The difference was insignificant between nimesulide and HG (4.7 ± 0.6) groups ($p = 0.360$) (Figure 1).

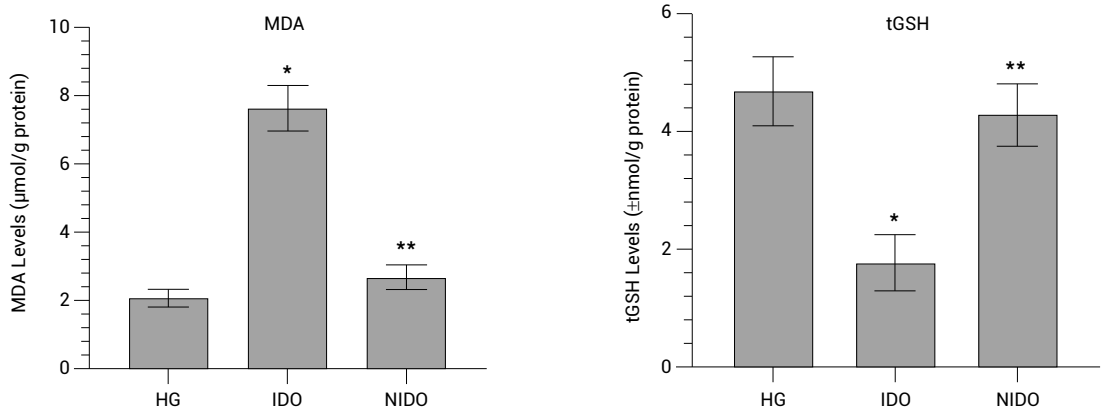


Figure 1. MDA and tGSH levels in the liver tissue of study groups. *p < 0.001 according to NIDO group, **p < 0.001 according to HG group. MDA: malondialdehyde, tGSH: total glutathione, HG: healthy group, IDO: indomethacin group, NIDO: nimesulide + indomethacin group.

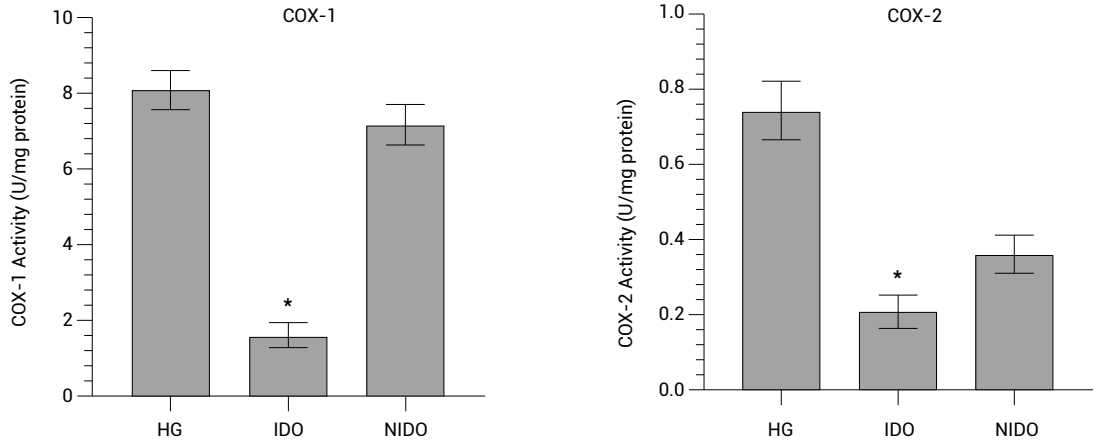


Figure 2. COX-1 and COX-2 levels in the liver tissue of study groups. *p < 0.001 according to NIDO group. COX-1: cyclooxygenase-1, COX-2: cyclooxygenase-2, HG: healthy group, IDO: indomethacin group, NIDO: nimesulide + indomethacin group.

COX-1 and COX-2 analysis results

It resulted in a significant reduction in COX-1 activity compared to the indomethacin HG ((8.1 ± 0.5) group in liver tissue. ($p < 0.001$) (1.6 ± 0.3). There was a significant difference between IDO and NIDO (7.2 ± 0.5) groups with respect to COX-1 activity ($p < 0.001$) (Figure 2). Indomethacin caused a significant decrease in COX-2 activity in liver tissue compared to the HG (0.7 ± 0.1) group. ($p < 0.001$) (1.6 ± 0.3). There was a significant difference between indomethacin and nimesulide (0.4 ± 0.1) groups with respect to COX-2 activity ($p < 0.001$) (Figure 2).

ALT and AST analysis results

In the liver tissue, indomethacin caused a significant increase in ALT level compared to the HG (28.9 ± 4.1) group. ($p < 0.001$) (82.0 ± 5.3). There was a significant difference between indomethacin and nimesulide (35.7 ± 4.5) groups with respect to ALT level ($p < 0.001$) (Figure 3). In the liver tissue, indomethacin caused a significant increase in AST level compared to the HG (46.9 ± 5.5) group ($p < 0.001$) (132.1 ± 6.9). There was a significant difference between IDO and NIDO (59.6 ± 3.4) groups with respect to AST level ($p < 0.001$) (Figure 3).

Histopathological findings:

As a result of the histologic examination in liver tissue, cell cords of hepatocytes, Kupffer cells scattered among the hepatocyte cords, and vascular formation showed normal histological structure in the control group (Figure 4). In the indomethacin group, in the presence of pyknotic nuclei in which some hepatocytes suggested that these cells undergo apoptosis but on the other hand, degeneration was observed in some hepatocytes. Hepatic cords were irregular and it caused disruption of hepatic lobule appearance. Central veins and other blood vessels showed congestion and dilatation (Figures 5, 6). When evaluated in terms of degenerated hepatocytes, pyknotic nuclei, and enlarged and congested blood vessels,

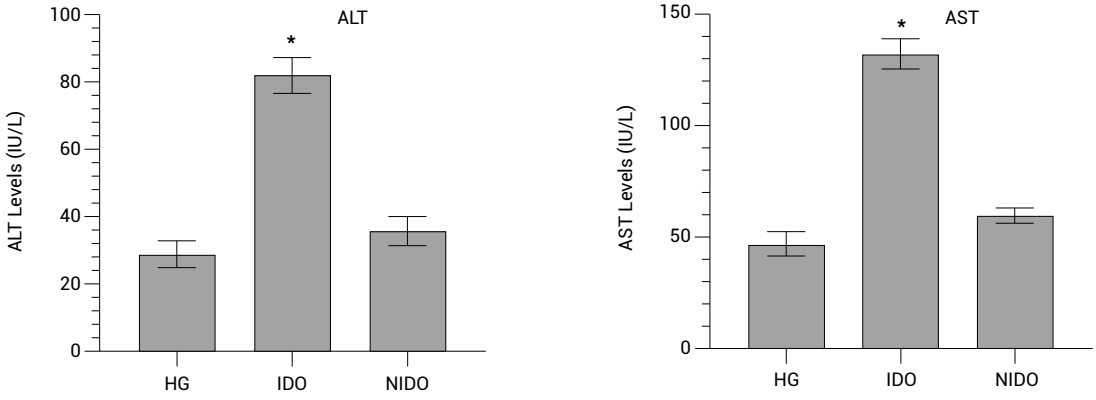


Figure 3. ALT and AST levels in the liver tissue of study groups. *p < 0.001 according to NIDO group. ALT: alanine aminotransferase, AST: aspartate aminotransferase, HG: healthy group, IDO: indomethacin group, NIDO: nimesulide+indomethacin group.

there was a significant difference between IDO and HG groups ($p < 0.05$). In rats treated with nimesulide prior to indomethacin; hepatic cords were normal in shape and degeneration in hepatocytes was decreased. Therewithal blood vessels and general histological architecture were normal (Figure 7). When evaluated in terms of degenerated hepatocytes, pyknotic nuclei, and enlarged and congested blood vessels, there was a significant difference between IDO and NIDO groups ($p < 0.05$).

DISCUSSION

In this study, the effect of nimesulide on indomethacin-induced oxidative damage in rats was investigated biochemically and histopathologically. As is known, organ and tissue oxidative damage is generally evaluated by biochemical and histopathological examination methods. From the previous studies, biochemical findings were correctly understood by histopathological findings (19). In our study, the increase in MDA amount in liver tissue of indomethacin-treated animals was significantly suppressed by the administration of nimesulide. This reveals that nimesulide is an antioxidant. ROS, which is a source of oxidative stress, oxidizes cell membrane lipids, resulting in the formation of toxic products such as MDA from lipids (20). MDA causes further serious damage by inactivating receptors and membrane-bound enzymes in membranes by causing cross-linking and polymerization of cell membrane components (21). In the study of Demiryilmaz et al., it was reported that nimesulide inhibits the increase in MDA quantity and protects the liver tissue from ischemia-reperfusion injury (14). Current studies confirm the hepatoprotective properties of nimesulide in animals, and the oxidative stress, hepatotoxic and genotoxic properties of indomethacin (1, 14). In

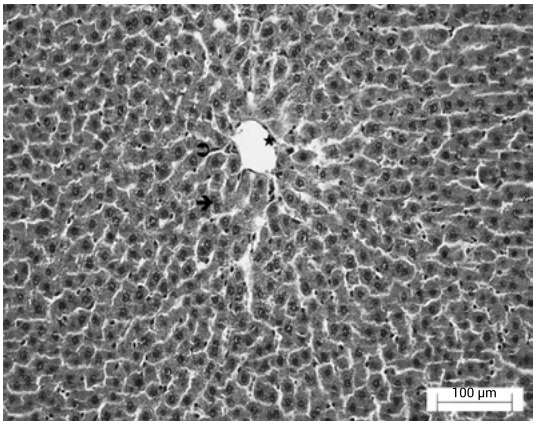


Figure 4. Hematoxylin–eosin staining in liver tissue in the HG; ➔: hepatocyte, ➲: Kupffer cell, ★: blood vessel, x200. HG: healthy group.

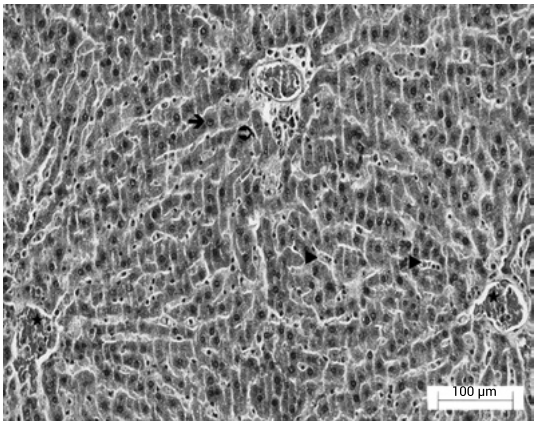


Figure 5. Hematoxylin–eosin staining in liver tissue in the IDO; ➔: degenerated hepatocyte, ➲: Kupffer cell, ►: pyknotic nuclei, ★: dilated and congested blood vessel, x200. IDO: indomethacin group.

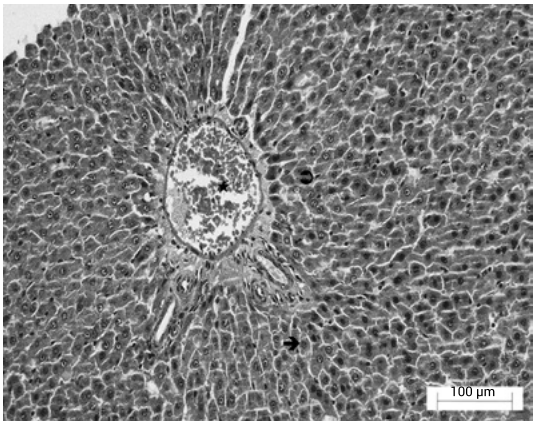


Figure 6. Hematoxylin–eosin staining in liver tissue in the IDO; ➔: degenerated hepatocyte, ➲: Kupffer cell, ★: dilated and congested blood vessel, x200. IDO: indomethacin group.

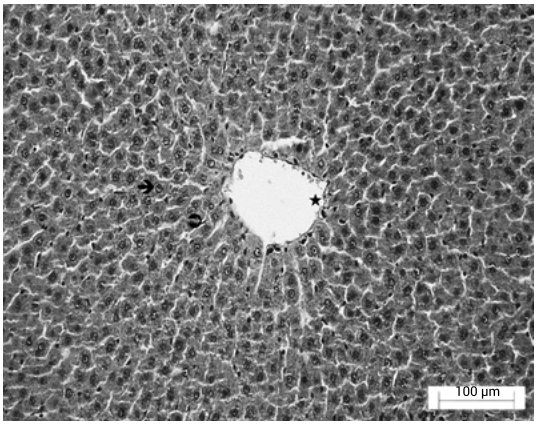


Figure 7. Hematoxylin–eosin staining in liver tissue in the NIDO group; ➔: hepatocyte, ➲: Kupffer cell, ★: blood vessel, x200. NIDO: nimesulide+indomethacin group.

a recent study, it has been shown that indomethacin leads to both liver and multiple organ oxidative damage by increasing lipid peroxidation and reducing antioxidants (22). In this study, indomethacin increased MDA production and decreased tGSH in liver tissue. GSH is an antioxidant tripeptide that eliminates the harmful effects of ROS. GSH detoxifies by reacting with hydrogen peroxide, an ROS, and protects cells from ROS damage (23). It is understood that the oxidant-antioxidant balance in the IDO group changes to the advantage of the oxidants. This is called oxidative stress in the literatüre (24). Because in physiological conditions, oxidant / antioxidant balance is maintained with the superiority of antioxidants (25). In a study supporting our results of this experiment, it was reported that nimesulide prevented the decrease of tGSH in liver and stomach tissue with indomethacin and had a hepato- and gastro-protective effect (14, 26).

In our study, it was also found that the indomethacin group had a significant decrease in protective structural COX-1 and inducible pro-inflammatory COX-2 in liver tissue compared to the control group. While nimesulide significantly inhibited the reduction of COX-1 activity with indomethacin, it did not prevent the reduction of COX-2 activity. This indicates that nimesulide protects liver tissue from the side effects of indomethacin because the inhibition of COX-1 causes tissue damage (27). However, since nimesulide itself is an anti-inflammatory agent, no significant change in COX-2activity was observed. NSAIDs have a therapeutic effect by inhibiting COX-2 and create a toxic effect by inhibiting COX-1 (28). In addition, it has been stated that in the pathogenesis of indomethacin-induced gastric damage, apart from COX-1 enzyme inhibition, ROSs are one of the major components of the damage (29). In addition, Indomethacin has also been documented to cause gastric injury by affecting enzymatic and non-enzymatic oxidant and antioxidant mechanisms (30). Turkyilmaz et al. showed that there is a positive relationship between COX-1 and antioxidants in protective activity (31). Therefore, one of the important mechanisms underlying the hepatoprotective effect of nimesulide is the protective effect on COX-1.

In our study, MDA and COX-2 levels were found to be high, tGSH and COX-1 levels were found to be low in the IDO group compared to the NIDO and HG groups, while serum ALT and AST activities were found to be high. ALT and AST enzymes are commonly used parameters to determine liver toxicity and failure (32). It is understood from the studies that ALT and AST levels are directly related to the increase of oxidant parameters(33). There is also information that ALT and AST are inversely proportional to the decrease of COX-1 and directly proportional to the increase of COX-2 (14).

All of these biochemical test results are consistent with our histopathological findings. In the indomethacin group with high levels of MDA and COX-2 and low levels of tGSH and COX-1, pyknotic nuclei, degeneration, irregular hepatic cords, veins, and other blood vessels showed severe congestion and dilatation. However, nimesulide-administered rats had no pathological findings except mild hepatocyte degeneration. Bagheri et al. reported that indomethacin leads to pathological changes such as hypotrophy in Kupffer cells, dilatation of hepatic sinusoids, and dilation of blood vessels in the portal area (34).

In conclusion, indomethacin administration caused functional, biochemical, and histopathological disorders in the liver tissue of animals. Nimesulide minimizes these functional, biochemical, and histopathological disorders due to indomethacin.

This hepatoprotective effect of nimesulide may be due to its protective effect on the antioxidant and COX-1 enzyme. Our results suggest that nimesulide may be useful in the treatment of hepatotoxicity and COX-1 inhibition side effects without suppressing analgesic and anti-inflammatory activity due to indomethacin use.

Conflict of interest

The authors declare no conflicts of interest.

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