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Research Article
Effect of Thymoquinone on Diclofenac-Induced Liver Injury

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Abstract

Background and Objective: Thymoquinone (TQ) is an active phenolic compound obtained from *Nigella sativa* L. It has anti-inflammatory and antioxidant activity by inhibiting the overproduction of certain inflammatory molecules and lipid peroxidation. TQ also has a hepatoprotective effect. This study, it was aimed to biochemically investigate the effect of TQ on diclofenac (DC)-induced liver damage in rats. **Materials and Methods:** The animals were divided into healthy control (HG), only diclofenac (DG), only thymoquinone (TQG) and diclofenac+thymoquinone (DTQG) groups. The DC was injected intraperitoneally at a dose of 25 mg kg^{-1}. The TQ was administered orally to the stomach at a dose of 20 mg kg^{-1}. This procedure was repeated once a day for 7 days. **Results:** Biochemical test results showed that TQ significantly prevented the increase in oxidant parameters and the decrease in antioxidants in liver tissue, which is formed by DC. Also, DC and TQ inhibited the increase of proinflammatory cytokines in liver tissue. The TQ also prevented the decrease of COX-1 activity by DC and increased the inhibition of COX-2. The TQ also significantly suppressed the elevation of liver function markers such as Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) with DC. **Conclusion:** These findings indicated that the hepatotoxic effect of DC was due to oxidative stress and inhibition of cytoprotective structural COX-1 enzyme. It suggested that TQ may be useful in the treatment of DC-related liver injury.

Key words: Thymoquinone, diclofenac, oxidative stress, hepatotoxicity, COX activity, inflammatory molecules, albino wistar

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Non-steroidal anti-inflammatory medicines (NSAIDs) are the most extensively used pharmacotherapeutic drugs globally due to the wide range of conditions for which they are prescribed[1]. The NSAIDs are preferred in treating inflammatory diseases since they effectively control pain, fever, redness and oedema resulting from the release of inflammatory mediators[2]. The NSAIDs exert their anti-inflammatory, analgesic and antipyretic actions by lowering the activity of the cyclooxygenase (COX) enzyme and reducing prostaglandin synthesis, among other mechanisms[2,3]. The COX-2 inhibition is kept responsible for the therapeutic effects of NSAIDs and inhibition of COX-1, which is cytoprotective, is responsible for gastrointestinal and other side effects. Two NSAIDs are shown as the leading cause of drug-induced liver injury worldwide[4]. It has been reported that NSAIDs are responsible for approximately 10% of drug-induced hepatotoxicity[5]. Studies have shown that diclofenac (DC), an NSAID, has a more severe toxic effect on the liver than other NSAIDs[6]. Metabolism intermediates such as acyl glucuronides and benzoquinone imines are held responsible for this toxic effect of DC[7]. The increased formation of intracellular reactive oxygen species (ROS) has been demonstrated to be a contributing factor to the breakdown of mitochondrial integrity, which is another cause of DC-induced hepatotoxicity[8]. In an animal study, DC increased the levels of oxidative stress markers such as Malondialdehyde (MDA), myeloperoxidase (MPO) and hydrogen peroxide (H_2O_2). At the same time, it reduced the amounts of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH)[9]. It has been also reported that DC increases alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Tumour Necrosis Factor-Alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin 6 (IL-6) levels[9,10]. This information obtained from the literature shows the importance of oxidative stress and increased proinflammatory cytokine in the pathogenesis of DC hepatotoxicity.

In this study, thymoquinone (TQ), the protectiveness of which we will test against DC hepatotoxicity, is an active phenolic compound obtained from the essential oil of nigella (*Nigella sativa* L.) seed[11]. It has been documented that TQ exhibits anti-inflammatory and antioxidant activity by inhibiting the overproduction of inflammatory molecules (TNF-α, NF-κβ, IL-1β, IL-6, COX) and lipid peroxidation (LPO)[12]. It has been reported that TQ has a hepatoprotective effect with its antioxidant, anti-inflammatory and immune-modulatory properties[13]. In the literature, it has been stated that TQ has a selective inhibitory effect on COX-2[14].

All this information suggested that TQ may effectively protect liver tissue against possible oxidative damage of DC. According to the literature review, there was no information about the effect of TQ on DC-induced liver injury in rats. To determine the biochemical effects of TQ on DC-induced liver damage in rats, the researchers plan to conduct a biochemical investigation.

MATERIALS AND METHODS

Study area: This study was carried out at Erzincan Binali Yildirim University, Faculty of Medicine, Erzincan, Turkey from November, 2021 to March, 2022.

Animals: It was decided to experiment using a total of 24 albino Wistar male rats, with their body weight fluctuating between the ranges of 280 and 290 g. It was collected from the Experimental Animals Application and Research Center at Erzincan Binali Yildirim University in Turkey. Immediately before the experiment, the animals were maintained in groups at room temperature (22°C) and fed simultaneously. The local ethics committee approved the study (Date: 11-11-2021, number: E-85748827-050.01.04-122461).

Chemicals: Thymoquinone was acquired from Sigma-Aldrich and utilized in the experiment (Germany), Diclofenac from Deva Holding (Turkey), Thiopental sodium from I.E Ulagay (Turkey).

Animal groups: The test animals were placed into four groups: The healthy group (HG), the diclofenac group (DG), the thymoquinone Group (TQG) and the diclofenac+ thymoquinone group (DTQG).

Experimental procedure: To experiment, thymoquinone was administered to the TQG (n = 6) and DTQG (n = 6) groups at a dose of 20 mg kg^{-1} orally to the stomach with a catheter. The same volume (1 mL) of 0.9% NaCl solution was administered orally to HG (n = 6) and DG (n = 6) as solvent. One hour after administration of thymoquinone and solvent, DC was injected intraperitoneally at a 25 mg kg^{-1} dose to the DTQG and DG groups. This treatment was performed once daily for a total of seven days. After this period, all animals were slain under thiopental sodium anaesthesia at a high dose (50 mg kg^{-1}) and their liver tissues were removed. Biochemical analysis of the removed liver tissues was performed. The enzymes ALT and AST were measured in blood samples collected from the tail veins of the animals just before they were slaughtered to determine how active they were.

Biochemical analyzes

Tissue MDA and tGSH determination: MDA measurements were measured by the spectrophotometric method of the absorbance of the pink colored complex formed by thiobarbituric acid (TBA) and MDA[15]. tGSH measurement was made according to the method used before[16].

Tissue total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) determination: The total oxidant system (TOS, nmol H_2O_2 mg/protein), total antioxidant system (TAS, µmol Trolox Equiv/mg protein) and oxidative stress index (OSI, TOS/TAS×0.1) an innovative automated measurement approach and commercially available kits were used to determine the levels of tissue homogenates in the samples (Rel Assay Diagnostics, Turkey), both developed by Erel[17,18].

Measurement of COX activity: We measured the COX activity in the rat's liver in this series of experiments using a COX activity assay kit (Item No. 760151, Cayman, Ann Arbor, MI, USA). To ensure that no red blood cells or clots were present in the liver tissue before beginning the investigations, it was necessary to carefully remove the liver tissue from the membranes and bathe it with ice-cold Tris buffer, pH 7.4, including 0.16 mg mL^{-1} of heparin before beginning the studies. The sample was then refrigerated at -80°C until it was analyzed.

A gram of stomach tissue was taken from each rat. The solution containing 0.1 M Tris-HCl, pH 7.8, one mM EDTA was prepared and stomach tissue was homogenized in 5 mL of this solution and then centrifuged at 10000 g for 15 min at 4°C. For testing, the supernatant was separated and stored on ice. We then measured the protein concentration in the supernatant using the Bradford method[19].

Peroxidase activity of COX was measured with Cyclooxygenase-1 (COX-1, U mg^{-1} protein) and cyclooxygenase (COX-2, U mg^{-1} protein) activity assay kit. This assay works colourimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine at 590 nm. The COX-2 activity was determined using a COX-1- specific inhibitor[20].

TNF-α, IL-1β and IL-6 analysis: The weight of samples was determined and then chopped all tissues into small pieces, immediately frozen by liquid nitrogen and homogenized by using a pestle and mortar, after melting samples were stored at 2-8°C. The PBS (pH 7.4) at a 1/10 (w/v) concentration was added, vortexed for 10 sec, centrifuged for 20 min at 10000 g and then carefully collected the supernatants. The levels of TNF-α (pg mL^{-1}), IL-1β (pg L^{-1}) and IL-6 (ng L^{-1}) were determined using a commercial kit provided by Eastbiopharm Co., Ltd., ELISA kit, which is located in China.

ALT and AST analysis: Blood samples were drawn from the veins and placed in tubes without anticoagulants. Following clotting, the serum was centrifuged and stored at -80°C until used in the experiment. For the liver function tests, AST (U L^{-1}) and ALT (U L^{-1}) activity were measured spectrophotometrically using a Cobas 8000 autoanalyzer (Roche Diagnostics, Mannheim, Germany) using commercially available kits. (Roche Diagnostics).

Statistical analysis: The IBM SPSS 22 (IBM Corp. Released 2013. Armonk, NY) was employed for the statistical analysis. The data were provided as the Mean±Standard Deviation (SD). The one-way ANOVA was used to compare the two groups. As a *post hoc* analysis after ANOVA, Tukey HSD was utilized. The statistical level of significance for all tests was considered to be $p<0.05$.

RESULTS

Biochemical results

MDA and tGSH analysis: Figure 1a shows MDA level was found to be statistically significantly higher in DG group (3.82±0.12) rats compared to HG (1.25±0.17), TQG (1.14±0.08) and DTQG (1.57±0.44) groups ($p<0.001$). When comparing the TQG and DTQG groups to the HG group, there was no statistically significant difference in MDA levels ($p>0.05$). It was seen that TQ reduces the MDA level that increases with the administration of DC. The level of tGSH in the DG group (2.26±0.12) was found to be significantly lower than the HG (5.67±0.27), TQG (6.26±0.52) and DTQG (5.26±0.04) groups ($p<0.001$). There was no significant difference in the level of tGSH in the DTQG groups compared to the HG ($p = 0.112$). The tGSH levels in the TQG group were higher than in the HG group ($p = 0.015$) (Fig. 1b). The TQ increased the tGSH level that decreased with the administration of DC.

TOS, TAS and OSI analysis: Figure 2a shows TOS level was found to be higher in the DG group (6.10±0.45) compared to the HG (2.21±0.19), TQG (1.80±0.38) and DTQG (3.27±0.20) groups ($p<0.001$). The TOS values in the HG and TQG groups were close to each other ($p = 0.157$). The level of TAS in the DG

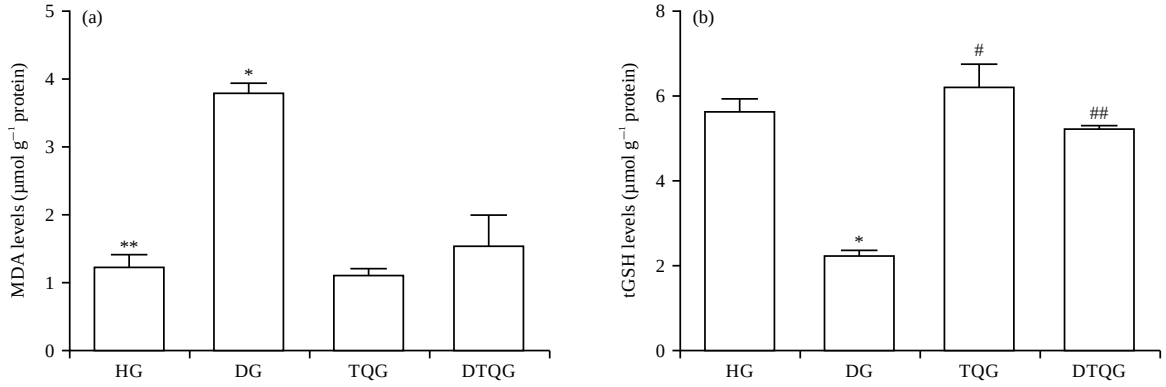

Fig. 1(a-b): MDA and tGSH levels in the liver tissue

*p<0.001, according to HG, TQG and DTQG groups, **p>0.05, according to TQG and DTQG groups, #p = 0.015, according to HG group, ##p = 0.112, according to HG group. MDA: Malondialdehyde, tGSH: Total glutathione, HG: Healthy group, DG: Diclofenac group, TQG: Thymoquinone group, DTQG: Diclofenac+thymoquinone group and One-way ANOVA was followed by Tukey's post-test

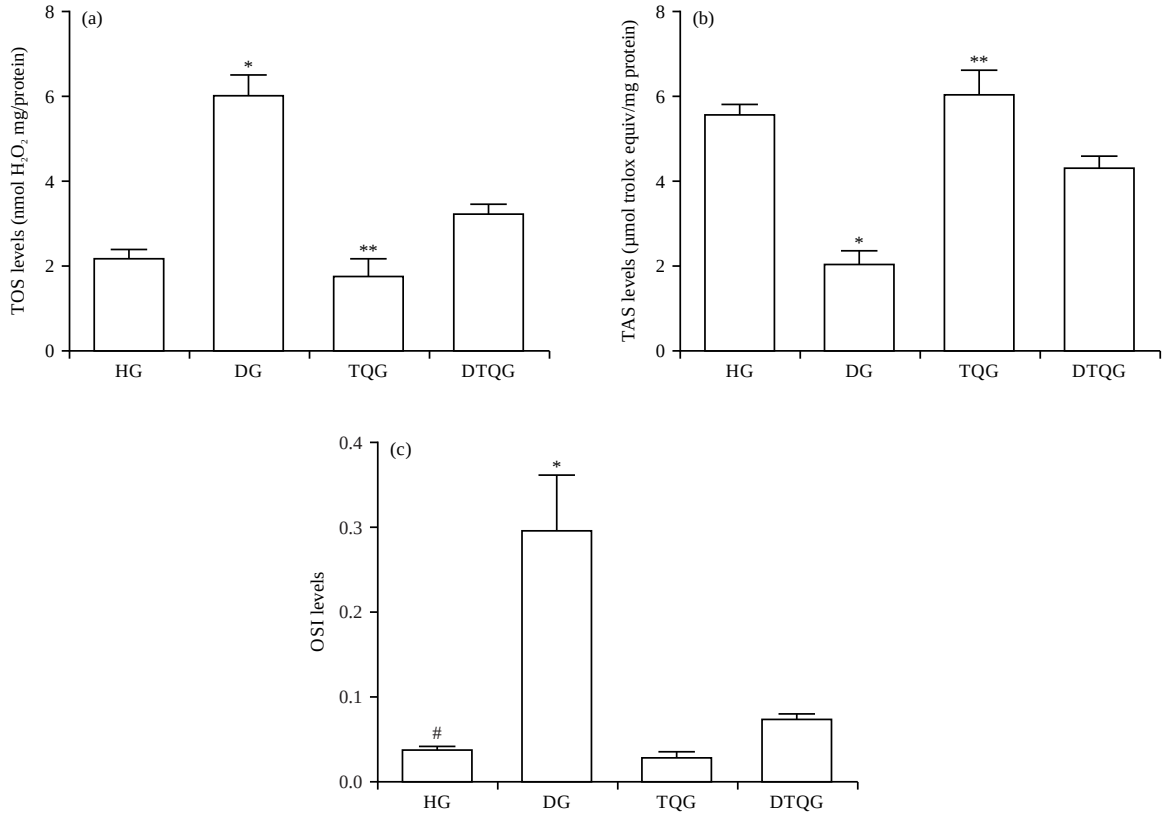

Fig. 2(a-c): TOS, TAS and OSI analysis of liver tissue in study groups

*p<0.001, according to HG, TQG and DTQG groups, **p>0.05, according to HG group, #p>0.05, according to TQG and DTQG groups, COX: Cyclooxygenase, HG: Healthy group, DG: Diclofenac group, TQG: Thymoquinone group, DTQG: Diclofenac+thymoquinone group and One-way ANOVA was followed by Tukey's post-test

group (2.08±0.28) was found lower compared to the HG (5.60±0.20), TQG (6.08±0.56) and DTQG (4.35±0.24) groups (p<0.001). The TAS values in the HG and TQG groups were close to each other (p = 0.117) (Fig. 2b). OSI value was found to be higher in the DG group (0.30±0.01) than in HG (0.04±0.01), TQG (0.03±0.01) and DTQG (0.08±0.01) groups (p<0.001). OSI values in HG, TQG and DTQG groups were close to each other (p>0.05) (Fig. 2c). It is seen that the increase in TOS and OSI values and the decrease in TAS values were prevented by TQ with DC application.

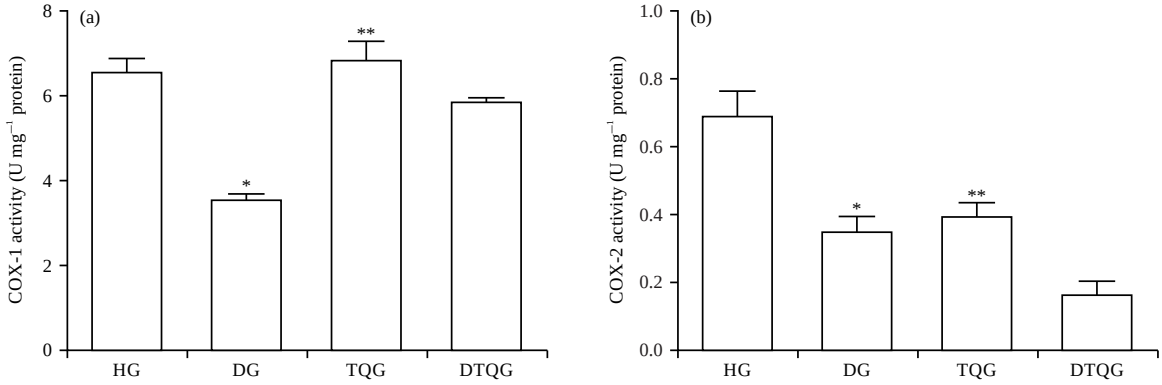

Fig. 3(a-b): COX-1 and COX-2 activity in the liver tissue

*p<0.001, according to HG, TQG and DTQG groups, **p>0.05, according to HG group, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, HG: Healthy group, DG: Diclofenac group, TQG: Thymoquinone group, DTQG: Diclofenac+thymoquinone group and One-way ANOVA was followed by Tukey's post-test

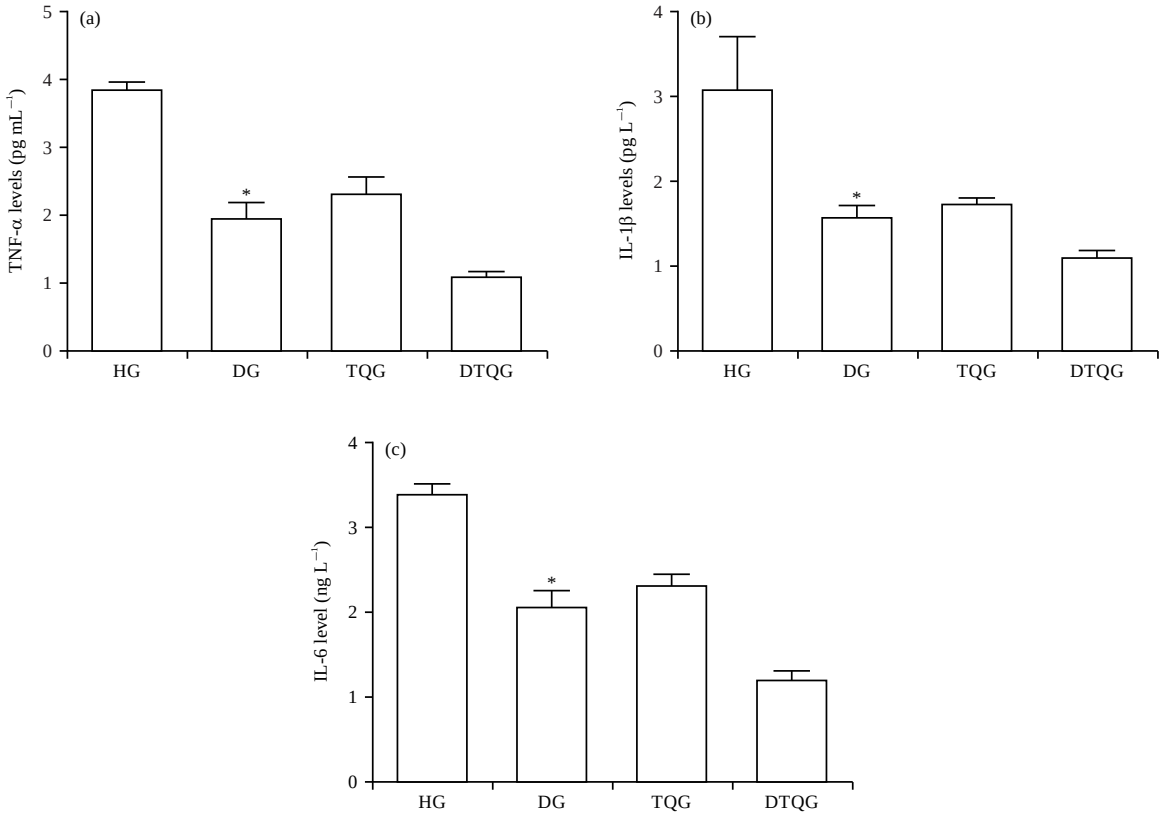

Fig. 4(a-c): TNF-α, IL-1β and IL-6 levels in the liver tissue

*p<0.001, according to HG, TQG and DTQG groups, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1beta, IL-6: Interleukin 6, HG: Healthy group, DG: Diclofenac group, TQG: Thymoquinone group, DTQG: Diclofenac+thymoquinone group and One-way ANOVA was followed by Tukey's post-test

COX-1 and COX-2 analysis: The COX-1 activity was found to be lower in the DG group (3.57±0.10) according to HG (6.56±0.31), TQG (6.84±0.43) and DTQG (5.86±0.08) groups (p<0.001). The TQG and HG groups values were not different in COX-1 activity (p = 0.321) (Fig. 3a). The COX-1 activity was found to be lower in DG (0.35±0.04) according to HG (0.69±0.07), TQG (0.40±0.04) and DTQG (0.17±0.04) groups (p<0.001). The TQG and HG groups values were not different in COX-2 activity (p = 0.366) (Fig. 3b). The addition of TQ to DC application seems to prevent DC-induced reduction in COX-1 and COX-2 levels.

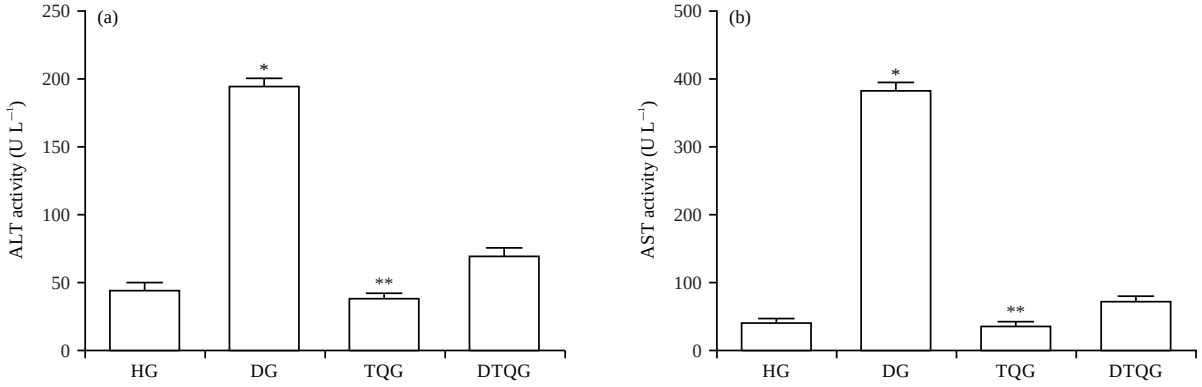

Fig. 5(a-b): ALT and AST levels in serum in blood

*p<0.001, according to HG, TQG and DTQG groups, **p>0.05, according to HG group, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HG: Healthy group, DG: Diclofenac group, TQG: Thymoquinone group, DTQG: Diclofenac+thymoquinone group and One-way ANOVA was followed by Tukey's post-test

TNF-α and IL-6 analysis: The levels of TNF- α were found to be lower in DG (1.97±0.22), TQG (2.33±0.24) and DTQG (1.11±0.06) groups according to HG (3.87±0.09) ($p<0.001$). The group with the lowest TNF-α levels was the DTQG group (Fig. 4a). IL-1β levels were found to be lower in DG (1.59±0.13), TQG (1.75±0.06) and DTQG (1.12±0.07) groups when compared with the HG group (3.09±0.62) ($p<0.001$). In terms of IL-1β, the data in the HG, TQG and DTQG groups were close to each other ($p>0.05$). The group with the lowest IL-6 levels was the DTQG group (Fig. 4b). The IL-6 levels were found to be lower in DG (2.07±0.18), TQG (2.33±0.12) and DTQG (1.20±0.11) groups according to HG (3.40±0.11) ($p<0.001$). The group with the lowest IL-6 levels was DTQG (Fig. 4c).

ALT and AST analysis: Figure 5a shows ALT level was found to be higher in DG (195.17±5.19) compared to the HG (45.00±4.94), TQG (39.33±2.73) and DTQG (70.17±5.50) groups ($p<0.001$). The TQG and HG groups values were not different in terms of AST level ($p = 0.193$). The level of ALT was higher in DG (385.50±10.60) compared to HG (43.17±3.82), TQG (38.50±4.37) and DTQG (74.50±5.79) groups ($p<0.001$). TQG and HG groups were similar in terms of AST level ($p = 0.630$) (Fig. 5b). It is seen that TQ reduces the increase in ALT and AST levels caused by DC administration.

DISCUSSION

The biochemical investigation of the preventive effect of TQ against DC-induced oxidative liver damage in rats was carried out in this work. The findings of biochemical tests revealed that MDA and TOS levels were higher in the DC group but that tGSH and TAS levels were lower in the DC group when compared to the healthy and TQ treated groups in rat liver tissue. As can be understood from previous studies, oxidant/antioxidant status is used to reveal oxidative damage and determine drug-induced toxicity[17,18]. In previous preclinical and clinical studies, it has been reported that DC is hepatotoxic[21,22]. In the literature, there is information that DC inhibits mitochondrial oxidative phosphorylation and ATP synthesis, increasing ROS production and causing damage to hepatocytes[23,24]. In studies conducted in recent years, it has been documented that the level of MDA in the liver tissues of rats administered DC increased[25,26]. The deadly oxidant molecule MDA, which is generated in cells due to the peroxidation of polyunsaturated fatty acids, has long been recognized as a biomarker for the presence of oxidative stress[27]. The MDA plays a role in exacerbating LPO damage and makes this toxic effect by causing cross-linking and polymerization of cell membrane components[28]. In this study, TOS levels, which showed the total effect of all oxidants in the body, were measured to confirm that DC causes oxidative stress. In the DC group where MDA was measured high, the TOS level was also increased.

The current study also found that DC decreased the level of tGSH in the liver tissue. The GSH is an endogenous antioxidant molecule in a tripeptide structure that protects cells from the toxic effects of ROS. The GSH plays a significant role in cellular defense against damage caused by oxidative stress and detoxification reactions (such as hypochlorous acid hydroxyl radicals)[29-30]. This study overlaps with the literature, the amount of MDA increased significantly, while the amount of tGSH decreased in the measured liver tissue[24-27]. Experimental results showed that TAS decreased in correlation with the decrease in tGSH, consistent with the literatüre[10,26].

In this study, the effect of DC on COX-1 and COX-2 levels in liver tissue was investigated. As is known, DC, like all other NSAIDs, inhibits both isoforms of COX. Jung *et al.*[8] reported that although DC showed selectivity to COX-2, it also inhibited COX-1 by 70%. It is known that inhibition of COX-1, which is responsible for synthesizing cytoprotective prostaglandins, triggers tissue damage[31,32]. The COX-2 is activated by proinflammatory cytokines and causes inflammation[15,33]. The fact that DC suppressed COX-1 production in liver tissue suggested that it may have increased the sensitivity of liver tissue to oxidative stress. In a study supporting this theory, it was shown that there is a positive correlation between COX-1 and antioxidants[34].

Current experimental results showed that DC significantly decreased TNF-α, IL-1β and IL-6 levels in liver tissue. However, some researchers argue that DC activates monocytes and macrophages and induces the synthesis and release of various proinflammatory cytokines such as TNF-α and IL-1β, which may cause liver damage[27,35]. However, studies supported current findings showing that DC exerts an inhibitory effect on proinflammatory cytokine production. A recent study reported that DC inhibited the increase in TNF-α, IL-1β and IL-6 levels in the liver[36]. In another study, it has been claimed that DC inhibited nuclear factor-kB activation induced by TNF-α[37]. It has been advocated that DC also reduces the severity of epileptic seizures by suppressing the increase in TNF-α and IL-6 levels in the brain tissue (hippocampus)[38].

In many studies, ALT and AST parameters are widely used to evaluate the hepatotoxicity of DC in test animals and humans[25,27,39]. In this investigation, ALT and AST levels were more significant in the DC group than in the healthy group, which was consistent with the literature. These findings revealed that DC causes damage to hepatocytes Akagunduz *et al.*[40] showed that degeneration and necrosis developed in the hepatocytes of animals with high ALT and AST levels. Yilmaz *et al.*[41], in their studies, documented that liver function markers such as ALT and AST are associated with oxidative stress[41].

As is known, excessively produced ROS are neutralized by endogenous antioxidant defense systems to maintain tissue integrity and functions at normal levels[42]. These antioxidants prevent the destructive effects of the LPO reaction with scavenging reactions[10]. As can be seen from our test results, TQ prevented the increase of MDA amount with DC and the decrease of tGSH. In addition, there was a significant decrease in TOS level and a significant increase in TAS level in the DC and TQ combined group compared to the DC group. It is thought that this antioxidant effect of TQ may have occurred due to its scavenging activity against many ROS, including hydroxyl radical, singlet oxygen and superoxide anion[15,31,32]. In the literature, there is information that TQ has many beneficial biological activities such as anti-inflammatory and hepatoprotective, apart from its antioxidant effect[11]. As current experimental results showed, TQ significantly decreased pro-inflammatory TNF-α, IL-1β and IL-6 levels. The group in which these cytokines were best suppressed was obtained in the TQ and DC combined group. The reason for this can be shown as the inhibitory effect of both TQ and DC on these cytokines. The TQ's decrease in TNF-α, IL-1β and IL-6 levels suggest that it may be one of the mechanisms underlying its anti-inflammatory effect mentioned in the literatüre[39]. In the anti-inflammatory mechanism of action of TQ, besides the inhibition of cytokines, COX-2 inhibition also has a role[39]. Also, it is stated in the literature that TQ has COX-2 selective inhibitory properties[14]. In this study, it was understood that the inhibitory effect of TQ on COX-2 was selective. The fact that TQ increases the level of COX-1 indicated that it is hepatoprotective. There was no information in the literature showing that TQ increased COX-1 activity in liver tissue. However, Demiryilmaz *et al.*[43] showed that nimesulide had a hepatoprotective effect by preventing the decrease of COX-1 activity with ischemia-reperfusion. It is also understood that TQ has a hepatoprotective effect by reducing the ALT and AST activities that rise with DC. The TQ, which suppresses oxidant levels significantly and significantly prevents the increase in ALT and AST activity, was in line with the literature[44].

CONCLUSION

As a result, increased oxidative parameters and decreased antioxidants in liver tissue were considerably reduced when TQ was administered. In addition, DC and TQ were found to decrease the production of proinflammatory cytokines in the liver. While DC decreased COX-1 and COX-2 activities in liver tissue, TQ did not change COX-1 activity but inhibited COX-2 activity. The TQ also prevented the inhibition of COX-1 activity by DC while increasing the inhibition of COX-2. The TQ also significantly suppressed the elevation of liver function markers such as ALT and AST with DC. These findings indicate that the hepatotoxic effect of DC was due to oxidative stress and inhibition of cytoprotective structural COX-1 enzyme. It suggested that TQ may help treat DC-related liver injury.

SIGNIFICANCE STATEMENT

This study discovers that TQ protects the liver from DC-induced damage by decreasing oxidative parameters and increasing antioxidants in rats. It contributes to DC by reducing the production of proinflammatory cytokines. It also reduces the production of ALT and AST. Thus a new theory on TQ may be effective in preventing the toxic effects of DC on the liver. However, to further elucidate the mechanism of action of thymoquinone in diclofenac-induced liver toxicity, it should be investigated histopathologically.

REFERENCES

1. Meunier, L. and D. Larrey, 2018. Recent advances in hepatotoxicity of non steroidal anti-inflammatory drugs. Ann. Hepatol., 17: 187-191.
2. Süleyman, H., B. Demircan and Y. Karagoz, 2007. Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacol. Rep., 59: 247-258.
3. Machado, G.C., C. Abdel-Shaheed, M. Underwood and R.O. Day, 2021. Non-steroidal anti-inflammatory drugs (NSAIDs) for musculoskeletal pain. BMJ, Vol. 372. 10.1136/bmj.n104.
4. Zoubek, M.E., M.I. Lucena, R.J. Andrade and C. Stephens, 2020. Systematic review: Ibuprofen-induced liver injury. Aliment. Pharmacol. Ther., 51: 603-611.
5. Agúndez, J.A.G., M.I. Lucena, C. Martínez, R.J. Andrade, M. Blanca, P. Ayuso and E. García-Martín, 2011. Assessment of nonsteroidal anti-inflammatory drug-induced hepatotoxicity. Expert Opin. Drug Metab. Toxicol., 7: 817-828.
6. Sriuttha, P., B. Sirichanchuen and U. Permsuwan, 2018. Hepatotoxicity of nonsteroidal anti-inflammatory drugs: A systematic review of randomized controlled trials. Int. J. Hepatol., Vol. 2018. 10.1155/2018/5253623.
7. Tang, W., 2003. The metabolism of diclofenac-enzymology and toxicology perspectives. Curr. Drug Metab., 4: 319-329.
8. Jung, S.H., W. Lee, S.H. Park, K.Y. Lee and Y.J. Choi *et al*., 2020. Diclofenac impairs autophagic flux via oxidative stress and lysosomal dysfunction: Implications for hepatotoxicity. Redox Biol., Vol. 37. 10.1016/j.redox.2020.101751.
9. Alabi, Q.K. and R.O. Akomolafe, 2020. Kolaviron diminishes diclofenac-induced liver and kidney toxicity in wistar rats via suppressing inflammatory events, upregulating antioxidant defenses and improving hematological indices. Dose Response, Vol. 18. 10.1177/1559325819899256.
10. Esmaeilzadeh, M., E. Heidarian, M. Shaghaghi, H. Roshanmehr, M. Najafi, A. Moradi and A. Nouri, 2020. Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing *IL-1β* gene expression in male rats. Pharm. Biol., 58: 590-596.
11. Tavakkoli, A., V. Mahdian, B.M. Razavi and H. Hosseinzadeh, 2017. Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. J. Pharmacopuncture, 20: 179-193.
12. Ali, M.Y., Z. Akter, Z. Mei, M. Zheng, M. Tania and M.A. Khan, 2021. Thymoquinone in autoimmune diseases: Therapeutic potential and molecular mechanisms. Biomed. Pharmacother., Vol. 134. 10.1016/j.biopha.2020.111157.
13. Butt, M.S., M. Imran, A. Imran, M.S. Arshad and F. Saeed *et al*., 2021. Therapeutic perspective of thymoquinone: A mechanistic treatise. Food Sci. Nutr., 9: 1792-1809.
14. El Mezayen, R., M. El Gazzar, M.R. Nicolls, J.C. Marecki, S.C. Dreskin and H. Nomiyama, 2006. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol. Lett., 106: 72-81.
15. Qiu, P.L., S.Y. Liu, M. Bradshaw, S. Rooney-Latham and S. Takamatsu *et al*., 2020. Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces* (Ascomycota, Erysiphales), including *G. ambrosiae*, *G. circumfusus* and *G. spadiceus*. BMC Microbiol., Vol. 20. 10.1186/s12866-020-01731-9.
16. Valente, M.J., A.M. Araújo, R. Silva, M. de Lourdes Bastos, F. Carvalho, P.G. de Pinho and M. Carvalho, 2016. 3,4-Methylenedioxypyrovalerone (MDPV): *In vitro* mechanisms of hepatotoxicity under normothermic and hyperthermic conditions. Arch. Toxicol., 90: 1959-1973.
17. Erel, O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin. Biochem., 38: 1103-1111.
18. Erel, O., 2004. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin. Biochem., 37: 112-119.
19. Bedir, Z., K.T.O. Erdem, I. Ates, T.C.O. Karakurt and C. Gursul *et al*., 2022. Effects of ketamine, thiopental and their combination on the rat liver: A biochemical evaluation. Adv. Clin. Exp. Med., 31: 285-292.
20. Suleyman, Z., E. Sener, N. Kurt, M. Comez and T. Yapanoglu, 2015. The effect of nimesulide on oxidative damage inflicted by ischemia-reperfusion on the rat renal tissue. Renal Fail., 37: 323-331.
21. Björnsson, E.S., 2016. Hepatotoxicity by drugs: The most common implicated agents. Int. J. Mol. Sci., Vol. 17. 10.3390/ijms17020224.
22. Licata, A., 2016. Adverse drug reactions and organ damage: The liver. Eur. J. Internal Med., 28: 9-16.
23. Schmeltzer, P.A., A.S. Kosinski, D.E. Kleiner, J.H. Hoofnagle, A. Stolz, R.J. Fontana and M.W. Russo, 2016. Drug-induced liver injury: Liver injury from nonsteroidal anti-inflammatory drugs in the United States. Liver Int., 36: 603-609.

24. Gómez-Lechón, M.J., X. Ponsoda, E. O'Connor, T. Donato, J.V. Castell and R. Jover, 2003. Diclofenac induces apoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS. Biochem. Pharmacol., 66: 2155-2167.
25. Alabi, Q.K., R.O. Akomolafe, O.S. Olukiran, W.J. Adeyemi and A.O. Nafiu *et al*., 2017. The *Garcinia kola* biflavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac. Pathophysiology, 24: 281-290.
26. Ramezannezhad, P., A. Nouri and E. Heidarian, 2019. Silymarin mitigates diclofenac-induced liver toxicity through inhibition of inflammation and oxidative stress in male rats. J. Herbmed Pharmacol., 8: 231-237.
27. Nouri, A., E. Heidarian, H. Amini-Khoei, S. Abbaszadeh and G. Basati, 2019. Quercetin through mitigation of inflammatory response and oxidative stress exerts protective effects in rat model of diclofenac-induced liver toxicity. J. Pharm. Pharmacogn. Res., 7: 200-212.
28. Gaweł, S., M. Wardas, E. Niedworok and P. Wardas, 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci Lekarskie, 57: 453-455.
29. Pompella, A., A. Visvikis, A. Paolicchi, V. de Tata and A.F. Casini, 2003. The changing faces of glutathione, a cellular protagonist. Biochem. Pharmacol., 66: 1499-1503.
30. Goulart, M., M.C. Batoreu, A.S. Rodrigues, A. Laires and J. Rueff, 2005. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis, 20: 311-315.
31. Mitsuoka, H. and G.W. Schmid-Schönbein, 2000. Mechanisms for blockade of *in vivo* activator production in the ischemic intestine and multi-organ failure. Shock, 14: 522-527.
32. Kuduban, O., Z. Suleyman, M. Gulaboglu, G.N. Yazici and H. Suleyman, 2022. Otoprotective effect of nimesulide: Biochemical and histopathologic evaluation. Int. J. Pharmacol., 18: 44-52.
33. Polat, B., H. Suleyman and H.H. Alp, 2010. Adaptation of rat gastric tissue against indomethacin toxicity. Chem.-Biol. Interact., 186: 82-89.
34. Yapca, O.E., M.I. Turan, I. Yilmaz, S. Salman, M. Gulapoglu and H. Suleyman, 2014. Benefits of the antioxidant and anti-inflammatory activity of etoricoxib in the prevention of ovarian ischemia/reperfusion injury induced experimentally in rats. J. Obstetr. Gynaecol. Res., 40: 1674-1679.
35. Peter, S.J., S.K. Basha, R. Giridharan, B.U. Lavinya and E.P. Sabina, 2017. Suppressive effect of *Spirulina fusiformis* on diclofenac-induced hepato-renal injury and gastrointestinal ulcer in wistar albino rats: A biochemical and histological approach. Biomed. Pharmacother., 88: 11-18.
36. Steckling, F.M., F.D. Lima, J.B. Farinha, P.C. Rosa and L.F.F. Royes *et al*., 2020. Diclofenac attenuates inflammation through TLR4 pathway and improves exercise performance after exhaustive swimming. Scand. J. Med. Sci. Sports, 30: 264-271.
37. Fredriksson, L., B. Herpers, G. Benedetti, Q. Matadin and J.C. Puigvert *et al*., 2011. Diclofenac inhibits tumor necrosis factor-α-induced nuclear factor-κB activation causing synergistic hepatocyte apoptosis. Hepatology, 53: 2027-2041.
38. Vieira, V., D. Glassmann, P. Marafon, P. Pereira, R. Gomez and A.S. Coitinho, 2016. Effect of diclofenac sodium on seizures and inflammatory profile induced by kindling seizure model. Epilepsy Res., 127: 107-113.
39. Noorbakhsh, M.F., H. Shaterzadeh-Yazdi, F. Hayati, S. Samarghandian and T. Farkhondeh, 2018. Protective effects of thymoquinon on pulmonary disorders in experimental studies. Tanaffos, 17: 211-222.
40. Akagunduz, B., M. Ozer, F. Ozcıcek, A.V. Kara and S. Lacın *et al*., 2021. Protective effects of taxifolin on pazopanib-induced liver toxicity: An experimental rat model. Exp. Anim., 70: 169-176.
41. Yilmaz, I., I. Demiryilmaz, M.I. Turan, N. Çetin, M.A. Gul and H. Süleyman, 2015. The effects of thiamine and thiamine pyrophosphate on alcohol-induced hepatic damage biomarkers in rats. Eur. Rev. Med. Pharmacol. Sci., 19: 664-670.
42. Urso, M.L. and P.M. Clarkson, 2003. Oxidative stress, exercise and antioxidant supplementation. Toxicology, 189: 41-54.
43. Demiryilmaz, I., M.I. Turan, A. Kisaoglu, M. Gulapoglu, I. Yilmaz and H. Suleyman, 2014. Protective effect of nimesulide against hepatic ischemia/reperfusion injury in rats: Effects on oxidant/antioxidants, DNA mutation and COX-1/COX-2 levels. Pharmacol. Rep., 66: 647-652.
44. Mohany, M., M. El-Feki, I. Refaat, O. Garraud and G. Badr, 2012. Thymoquinone ameliorates the immunological and histological changes induced by exposure to imidacloprid insecticide. J. Toxicol. Sci., 37: 1-11.