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RESEARCH ARTICLE

Protective Effects of Thymoquinone on Doxorubicin-induced Lipid Peroxidation and Antioxidant Enzyme Levels in Rat Peripheral Tissues

Deniz Akpinar[1*], Tanju Mercan[1], Halit Demir[2], Semir Ozdemir[3], Canan Demir[4] and Servet Kavak[5]

[1]Muğla Sıtkı Koçman University Faculty of Medicine Department of Biophysics, 48000 Menteşe/Muğla/Turkey.
[2]Van Yüzüncü Yıl University Faculty of Science Department of Biochemistry, Van/Turkey.
[3]Akdeniz University Faculty of Medicine Department of Biophysics, Antalya/Turkey.
[4]Van Yüzüncü Yıl University Vocational School of Health Services, Van/Turkey.
[5]Bakırçay University Faculty of Medicine Department of Biophysics, İzmir/Turkey.
*Corresponding author: denizakpinar@mu.edu.tr

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A B S T R A C T

We aimed to investigate the effects of thymoquinone (TQ) against Doxorubicin (DOX)-induced toxicity in liver, lung, kidney, testes, diaphragm and stomach tissues of rats. Healthy twenty-eight Wistar albino male rats aged three months, were divided into four groups, each containing seven animals: Group 1: Control (C group); the rats were injected intraperitoneally with saline 5 times every other day and given by gavage to the vehicle solution Group 2 (T group): Rats treated with thymoquinone (10mg/kg given by gavage), Group 3 (D group): Rats treated with doxorubicin were injected intraperitoneally (3mg/kg) and Group 4 (DT group): Rats treated with doxorubicin (injected intraperitoneally with 3mg/kg DOX) and thymoquinone (10mg/kg given by gavage). Group 2 (T group): TQ (10mg/kg) was administered by gavage at 24-hour intervals for 4 weeks. Saline (i.p.) was injected to rats 5 times every other day. Group 3 (D group): The rats were injected with 3 mg/kg DOX (i.p.) 5 times every other day. In addition, vehicle solution was administered by gavage at 24-h intervals for 4 weeks. Group 4 (DT group): Rats were injected with 3mg/kg DOX (i.p.) 5 times every other day. TQ (10mg/kg) was administered by gavage at 24-h intervals for 4 weeks. At the end of the experiment, the animals were euthanized with sodium pentobarbital (50 mg/kg) and their lung, liver, stomach, kidney, diaphragm and testicular tissues were removed for the measurement of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) levels. It was observed that DOX administration (in group D) increased lipid peroxidation and decreased antioxidant enzyme levels in the peripheral tissues of rats. It was determined that TQ treatment (in the DT group) given with DOX decreased lipid peroxidation and increased antioxidant enzyme levels. In our study, it was determined that TQ treatment eliminated the toxic effects of DOX and the protective effects of TQ were revealed.

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INTRODUCTION

The most commonly used methods in the treatment of cancer, which is one of the leading causes of death in the world, are surgery, radiotherapy and chemotherapy (Mortezaee *et al.,* 2021; Sheikholeslami *et al.,* 2021). Chemotherapy is one of the important and effective methods used in cancer treatment. Doxorubicin (DOX), the drug commonly used in chemotherapy, is a quinone-containing anthracycline antibiotic that is used widely for the treatment of various types of cancer (including solid tumors, lymphomas, leukemias, and soft tissue sarcomas) (Kamińska and Cudnoch-Jędrzejewska, 2023). The main problem with the drugs used in chemotherapy is that they target tumor cells as well as other cells and cause the same damage not only to abnormal cells but also to normal cells. For this reason, chemotherapy is applied to patients who do not respond to other treatment methods and are not suitable

for primary radiotherapy or surgery. Clinical use of DOX is limited due to its side effects on various organs (Al-Hussaniy *et al.,* 2023; Zhao *et al.,* 2023).

It is stated that acute (nausea, vomiting, myelosuppression and arrhythmia) and chronic (liver, kidney damage) side effects occur after the use of DOX. Considering the oxidative metabolism and antioxidant defense mechanism of these damaged organs, it is seen that they are more susceptible to damage caused by free radicals. Although low levels of oxidant species are necessary for the normal process, high levels are known to cause pathological conditions. Antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) form the antioxidant defense system of the cell. It is well known that antioxidants inhibit oxidative damage caused by free radicals. SOD is a superoxide radical scavenger that can convert superoxide radicals to hydrogen peroxide (H_2O_2). GSH-Px has an important role in the clearance of H_2O_2. CAT catalyzes the decomposition of H_2O_2 into molecular oxygen and water. A disruption of the balance between ROS and antioxidants causes oxidative stress (Bello *et al.,* 2023). It has been shown that the production of free radicals and oxidative stress are closely related to the toxicity of DOX. ROS are produced mostly in the mitochondria. Enzymes that produce ROS in the mitochondria convert DOX to a semiquinone free radical via an electron reduction of the quinone moiety and this radical reacts readily with oxygen to form superoxide anions (O^{2-}). Superoxide anions are converted to relatively low-toxic and stable H_2O_2 by SOD, and ROS are produced by the generation of toxic and highly reactive hydroxyl radicals ($OH^{\cdot}$) via Fenton reaction. The generated ROS then reacts with surrounding mitochondrial biomolecules, mainly proteins, lipids and nucleic acids. It is known that DOX reacts with mitochondrial DNA (mtDNA) to form adducts that impair normal protein expression, mitochondrial function and lipid oxidation. DOX leads to redox imbalances and hence oxidative stress by increasing the production of free radicals and significantly reducing the levels of endogenous antioxidants (Sangomla *et al.,* 2018; Ahmed *et al.,* 2021; Ismail*,* 2022; Wang *et al.,* 2022).

In recent years, natural products (herbal medicines) play an important role in the treatment of many diseases. Combined therapies are also prominent in cancer treatment due to the side effects that occur due to drugs. TQ is frequently used in combined treatments. Studies show that TQ combined with clinical drugs increases the therapeutic index of drugs used in cancer treatment and/or reduces their cytotoxicity on healthy tissues (Goyal *et al.,* 2017).

Thymoquinone, the most important bioactive component of Nigella sativa seed oil, is used as an anti-inflammatory, anti-tumor, and antioxidant agent (Farooq *et al.,* 2022; Hegazy *et al.,* 2023). TQ is a potent antioxidant due to its scavenging activity against many reactive oxygen species (ROS) (including singlet molecular oxygen, hydroxyl radical, and superoxide anion). TQ antagonizes the negative effects of elevated ROS levels in various diseases. In addition, TQ has been shown to strongly inhibit iron-dependent microsomal lipid peroxidation. TQ also shows its antioxidant effects by increasing endogenous antioxidant enzyme activities such as GSH-Px, SOD, and CAT. TQ has a high antioxidative potential due to the redox properties of the quinone structure of the molecule and its radical scavenging effect by easily accessing subcellular compartments (Goyal *et al.,* 2017). In previous studies, pretreatment administration of TQ has been shown to protect organs against oxidative damage induced by free radical generating agent, including DOX. Combination therapies in cancer treatment are new approaches that especially overwhelm tumor resistance and serious drug-related side effects. Previous studies show that combining TQ with clinical drugs such as DOX improves their therapeutic index and/or reduces their cytotoxicity against non-tumor tissues (Bayat Mokhtari *et al.,* 2017; Goyal *et al.,* 2017).

When previous studies are reviewed, it is seen that the effects of DOX and/or TQ on heart tissue are mostly investigated (Karabulut *et al.,* 2021), but the number of studies investigating the effects on other tissues is relatively less. When we searched the literature, it was seen that in studies where DOX and TQ were given together, the effects on only one tissue were examined, but the effects on other tissues were not investigated together. Therefore, in this study, we aimed to investigate the effects of TQ against DOX-induced toxicity in rats' peripheral organs (liver, lung, kidney, testis, diaphragm and stomach).

MATERIALS AND METHODS

Animals: All experimental protocols were approved by Akdeniz University Animal Care and Use Committee at Akdeniz University Medical School and were performed in accordance with the European Community directive. Rats were maintained at 12-h light/dark cycles, housed in stainless steel cages at standard conditions (temperature 23±1℃, humidity 50±5%) and fed ad libitum with standard rat chow and tap water.

Healthy twenty-eight male Wistar albino rats (aged three months, weighing, 200 to 250g) were divided into four groups, each contained seven animals: Control (C group); the rats were injected intraperitoneally with saline 5 times every other day (15 mg/kg in total) and given by gavage to the vehicle solution by weight at 24-hour intervals for 4 weeks (10mg/kg). Rats treated with thymoquinone (T group); TQ (10mg/kg as described previously by Sener *et al.* (2016)) given by gavage at 24-hour intervals for 4 weeks. The rats were injected with saline intraperitoneally 5 times every other day (15mg/kg in total). Rats treated with doxorubicin (D group); the rats were injected intraperitoneally with 3mg/kg DOX (15mg/kg in total) 5 times, every other day as described previously (Elsherbiny and El-Sherbiny 2014). In addition, carrier solution was given by gavage at 24-hour intervals for 4 weeks (10 mg/kg). Rats treated with doxorubicin and thymoquinone (DT group); the rats were injected intraperitoneally with 3mg/kg DOX (15mg/kg in total) 5 times every other day. TQ (10mg/kg) was given by gavage at 24-hour intervals for 4 weeks.

At the end of the experiment (24-h after administration of the last dose), the animals were euthanized with sodium pentobarbital (50mg/kg) and their lung, liver, stomach, kidney, diaphragm and testicular tissues were dissected for the measurement of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) activities.

Biochemical Analysis

Measurement of MDA level (mg/dL): A tissue specimen of 50mg was homogenized in 200ml (0.15mol/l) KCl. After the centrifugation of homogenate at 1600g, MDA levels in tissue homogenate supernatant were determined by thiobarbituric acid (TBA) reaction according to Yagi (1994). 200ml of supernatant was transferred to the sample tube. 800ml of phosphate buffer, 25ml of BHT solution and 500ml of 30% TCA were added to it. The tubes were mixed by vortex and kept on ice for 2 hours. It was then centrifuged at 2000 rpm for 15 minutes. 1ml of the obtained supernatant was taken and transferred to another tube. Then 75ml of EDTA and 250ml of TBA were added to this mixture. The tubes were mixed by vortex again and kept in a hot water bath for 15 minutes. The tubes were then brought to room temperature. The principle of this method is based on measuring absorbance of pink color produced by the interaction of TBA with MDA at 530nm. Calculation of malondialdehyde level:

C=F× 6.41× A
C: Concentration
F: Dilution factor
A: Absorbance

Measurements of SOD (U/dl) enzyme activities: The tissues were homogenized in physiological saline (1g in 5ml) using a homogenizer (B. Braun Melsungen AG 853202, Germany) and centrifuged at 4000 g for 20 min (Heraus Labofur 200, Germany). GSH-Px activity was determined by following changes in NADPH absorbance at 340nm and by measuring decrease in H_2O_2 absorbance at 240 nm (Paglia and Valentine, 1967; Aebi, 1974). SOD activity was measured by the method based on nitro blue tetrazolium (NBT) reduction rate. One unit of SOD activity was expressed as the enzyme protein amount causing 50% inhibition in NBT reduction rate (Durak *et al.,* 1996).

Measurement of GSH-Px (mlU/mg) enzyme activity: The tissues were homogenized in physiological saline (1g in 5ml) using a homogenizer (B. Braun Melsungen AG 853202, Germany) and centrifuged at 4000 g for 20 min (Heraus Labofur 200, Germany). GSH-Px activity was determined by following changes in NADPH absorbance at 340nm and by measuring decrease in H_2O_2 absorbance at 240 nm (Paglia and Valentine, 1967; Aebi, 1974). Glutathione peroxidase Activity (U/ml) = (ΔOD/t) x [(Vt) / (6.22 x Vö)]
Glutathione peroxidase activity was determined as U/ml.
Readings were taken in the spectrophotometer at 340nm at 0, 2.5, and 5 minutes.
ΔOD: Change in absorbance with time
t: Time
Vt: Total reaction volume (ml)
Vö: Sample volume (ml)
6.22: Optical density of 1 nmol NADPH in a 1cm light path.

Measurement of SOD (U/dl) enzyme activity: SOD activity was measured by the method based on nitro blue tetrazolium (NBT) reduction rate. One unit of SOD activity was expressed as the enzyme protein amount causing 50% inhibition in NBT reduction rate (Durak *et al.,* 1996). Activity Account:
% inhibition: [(Blind OD – Sample OD) / Blind OD] x 100
1 Unit SOD: Enzyme activity that inhibits NBT reduction by 50%.
Activity= (% inhibition) / (50 x 0.1)
Activity; It was calculated in U/ml.

Measurement of CAT (EU/mg) enzyme activity: The Biochemical analysis of tissue CAT activity was performed with a method described by Aebi (1984). Briefly, the supernatant (0.1ml) was added to a quartz cuvette containing 2.95ml of 19mmol/l H_2O_2 solution prepared in potassium phosphate buffer (0.05M, pH 7.00). The change in absorbance was monitored at 240nm for 5 min using a spectrophotometer (Shimadzu UV-1201, Japan). Activity account:
Activity= (2.3/ΔX) × [(log A1 / log A2)]
ΔX: 30 seconds
2.3: 1mmol optical density of H_2O_2 in 1cm light path.

Statistical analysis: Statistical analyses were carried out using the SPSS statistical software package (SPSS for Windows version 22.0, SPSS Inc., Chicago, Illinois, USA). Kruskal-Wallis, which is a nonparametric test, was used for the comparison of groups. Bonferroni-corrected Mann Whitney U test was used for multiple comparison. Significance levels were set at $p<0.05$. The results were expressed as Mean±SE.

RESULTS

Liver tissue levels of SOD, CAT, GSH-Px and MDA are shown in Fig. 1. Liver SOD, CAT and GSH-Px levels were increased significantly in the T group with respect to the C group. Antioxidant enzyme levels also were increased in the DT group compared with the C and D groups. When group D was compared with group C, antioxidant enzyme levels were shown to be decreased significantly. However, when compared with the C group, liver MDA values increased were significantly increased in the D group and decreased in the T and DT groups.

Measured lung tissue levels of SOD, CAT, GSH-Px and MDA are shown in Fig. 2. While thymoquinone treatment caused a significant increase in lung SOD, CAT and GSH-Px activity compared to the control group, it was observed that doxorubicin administration decreased these values. It is observed that antioxidant enzyme levels in the DT group were decreased when compared to the C group, and increased when compared with the D group, approaching the C group value. Lung MDA levels detected in doxorubicin treated rats (Group D) were significantly higher than those detected in the C group, while it was significantly reduced in the thymoquinone treated (T) group. It was determined that the thymoquinone administration with doxorubicin (Group DT) decreased lung MDA levels when compared to doxorubicin treated group alone, approaching the values of the C group.

SOD, CAT, GSH-Px and MDA values of the control and experimental groups in kidney tissue are given in Fig. 3. While thymoquinone treatment caused a significant increase in kidney antioxidant enzyme activities, doxorubicin administration decreased these values compared to the C groups. When the DT group was compared with the C group, it was determined that the SOD and CAT values were increased, while the GSH-Px value was decreased.

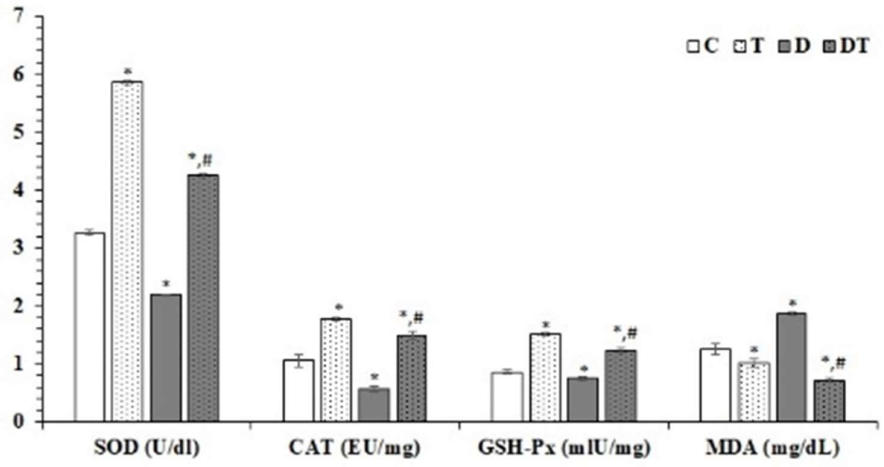

Fig. 1: SOD, CAT, GSH-Px and MDA values of liver tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group.

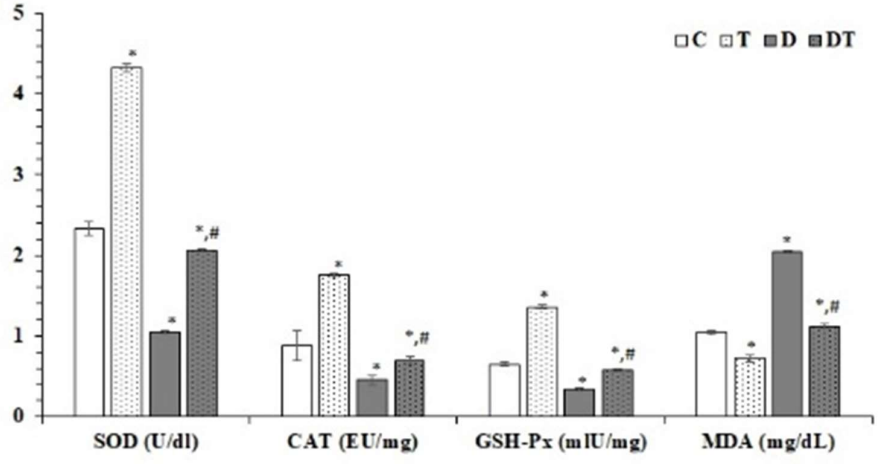

Fig. 2: SOD, CAT, GSH-Px and MDA values of lung tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group.

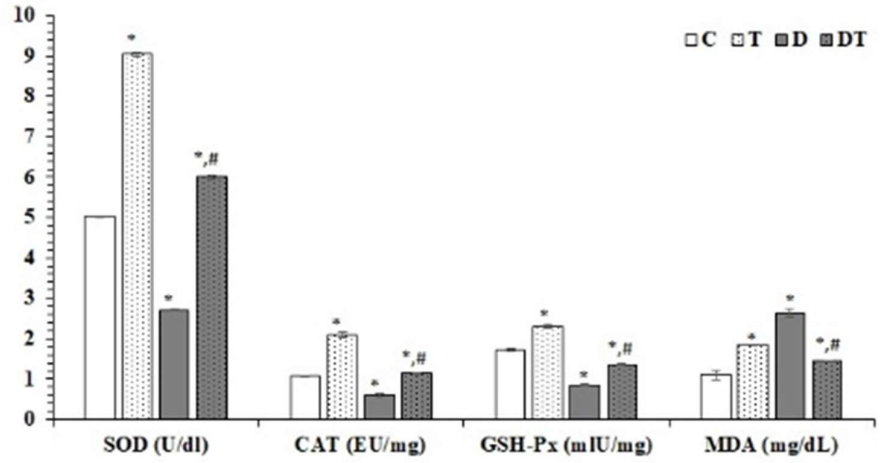

Fig. 3: SOD, CAT, GSH-Px and MDA values of kidney tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group

When compared with the D group, it was observed that the GSH-Px value increased significantly and approached the C group values. It was determined that MDA values were increased in all experimental groups when compared to group C.

Measured testicular tissue levels of SOD, CAT, GSH-Px and MDA are given in Fig. 4. While thymoquinone treatment increased SOD, CAT and GSH-Px activity compared to the C group, it was observed that doxorubicin administration decreased. It is observed that antioxidant enzyme levels in the DT group were increased when compared to the C and DT groups. MDA levels detected in doxorubicin treated rats were significantly higher than those detected in the C and DT groups, while it was significantly reduced in the T and DT groups.

Oxidant and antioxidant enzyme levels of the control and experimental groups in diaphragm tissue are given in Fig. 5. While thymoquinone treatment caused a significant increase in diaphragm SOD, CAT, and GSH-Px levels, doxorubicin administration decreased these values compared to the C groups. When the DT group was compared with the C group, it was determined that the CAT and GSH-Px values

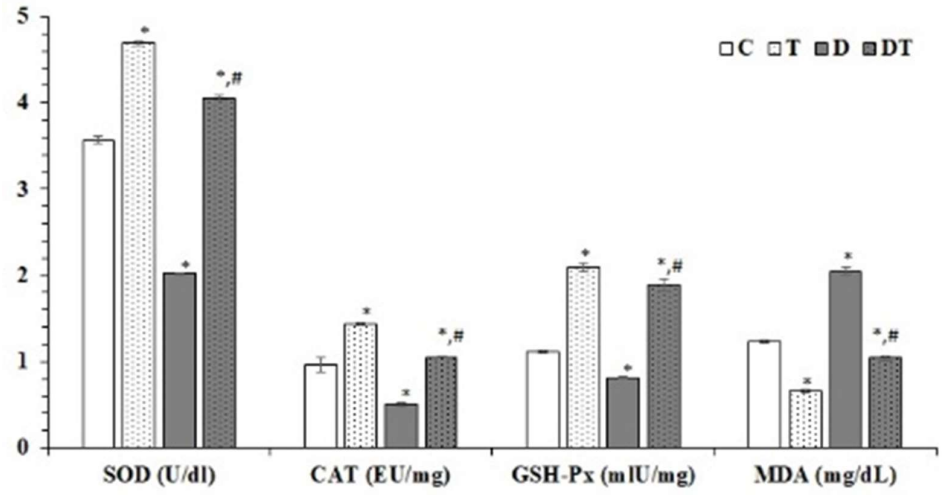

Fig. 4: SOD, CAT, GSH-Px and MDA values of testes tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group.

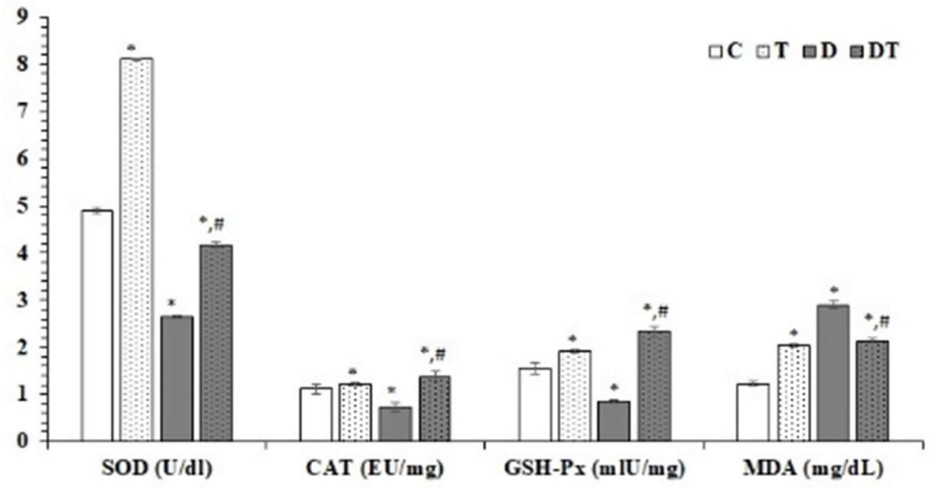

Fig. 5: SOD, CAT, GSH-Px and MDA values of diaphragm tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group.

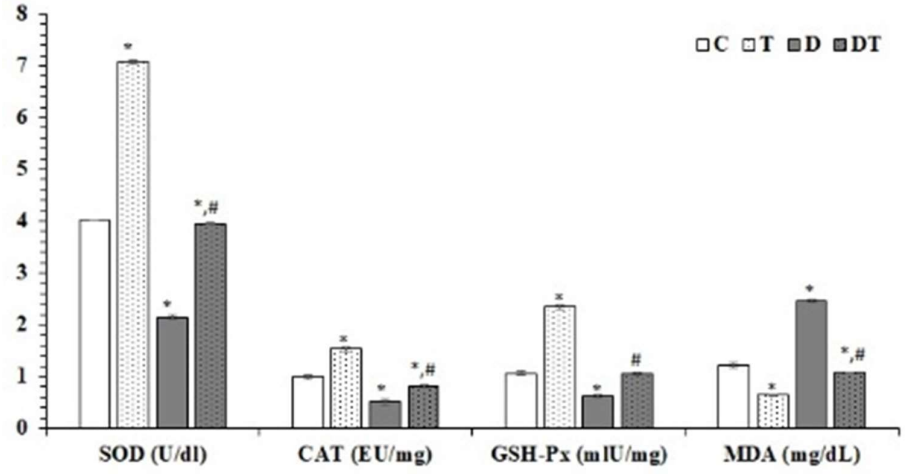

Fig. 6: SOD, CAT, GSH-Px and MDA values of stomach tissue. SOD: Superoxide Dismutase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; MDA: Malondialdehyde; *: p<0.01 vs. C group; #: p<0.01 vs. D group.

were increased, while the SOD value was decreased. MDA levels were increased in all experimental groups in comparison with the group C, but when compared with the D group, it was observed that the MDA values of the DT group were decreased.

The mean values of stomach tissue SOD, CAT, GSH-Px and MDA levels are given in Fig. 6. Compared with the control group, stomach antioxidant enzyme levels were significantly higher in the T group, but lower in the D group. However, when the DT group was compared with the C group, it was determined that the SOD and CAT enzyme levels were increased, and the GSH-Px value did not change. In other words, thymoquinone increased the GSH-Px values which were lowered by doxorubicin to the control values. Doxorubicin significantly increased MDA levels in stomach tissue whereas thymoquinone reduced MDA levels in the T and DT groups in comparison with the control group.

DISCUSSION

In this study, we investigated the levels of SOD, CAT, GSH-Px and MDA in lung, liver, kidney, diaphragm, testis and stomach tissues of rats to determine the effects of TQ and DOX treatment.

The liver is one of the important organs of the body, which regulates various processes (such as detoxification of toxic substances and drugs, protein and lipid metabolism, defense mechanism of the body). The liver is very sensitive to oxidative stress and damage by free radicals. Scavenging free radicals and/or increasing antioxidant defense may result in the elimination of negative effects on liver tissue. In our study, compared to the control group, liver tissue SOD, CAT and GSH-Px values of DOX-treated rats were decreased and MDA values were increased. The decrement in antioxidant enzyme levels and the increment in lipid peroxidation in the liver tissue are also in agreement with previous studies (Wali *et al.,* 2020; Abdel-Daim *et al.,* 2020). Administration of DOX to rats increased lipid peroxidation, which was associated with the increased free radical formation in liver tissue. However, according to the earlier study (Akin *et al.,* 2021), when compared with the DOX-administered group, it was seen that antioxidant enzyme levels were increased and MDA values were decreased in the DT group. Our results determined that TQ both increased antioxidant enzyme levels and decreased lipid peroxidation by inducing antioxidant defense mechanism.

Our data indicated that DOX induced a significant decrease in antioxidant enzyme levels and an increase of lipid peroxidation in the lung tissue. Our results in lung tissue are correlated with earlier studies (Srdjenovic *et al.,* 2010; Tepebaşı *et al.,* 2023). The possible reason for these findings can be associated with the sensitivity of the lung tissue to oxidative stress in which the antioxidant defense mechanism is weak and has distinct oxidative mechanisms (Tektemur *et al.,* 2023). On the other hand, decreased antioxidant enzyme levels in lung tissue of DOX-treated rats returned back to the control levels with the administration of TQ (DT group). When the literature is reviewed, although there is no study in which TQ and DOX are applied simultaneously in lung tissue, many studies have shown that TQ increases antioxidant enzyme levels and decreases lipid peroxidation in lung tissue in cases such as Benzo(a)pyrene-induced lung injury and titanium dioxide nanoparticles induced toxicity. In this study, we obtained consistent results with previous studies (Hassanein and El-Amir*,* 2017; Alzohairy *et al.,* 2021).

Kidney tissue MDA level measured in DOX-treated group (D group) was significantly higher than those measured in the control group and antioxidants were found to be decreased. In the DT group, it was observed that TQ application decreased MDA levels and increased antioxidant levels. Our kidney tissue results are consistent with previous studies (Zidan *et al.,* 2018). One of the main pathophysiologic mechanisms in nephrotoxicity caused by DOX is the increase in oxidative stress (Abdel-Daim *et al.,* 2020; Atta *et al.,* 2023; Boeno *et al.,* 2023). According to our results, the increase in oxidative stress caused a decrease in antioxidant enzyme activities. It was observed that antioxidant enzyme levels increased and lipid peroxidation decreased in the kidney tissue in the DT group given TQ, which is explained by the protective antioxidant effect of TQ. Since TQ increases the antioxidant enzyme levels and is also a strong free radical scavenger, it can protect the kidneys from damage by cleaning the free radicals formed by DOX.

Our data showed that while lipid peroxidation was increased in the testicular tissue in the DOX (D) group, it was decreased in the DOX+TQ (DT) group. On the other hand, antioxidant enzyme levels were decreased in the D group and increased in the DT group. Similar results were obtained in a previous study (Öztürk *et al.,* 2020). In the literature, it is thought that testicular toxicity is caused by lipid peroxidation, oxidative stress and cellular apoptosis, although there is little information about testicular toxicity caused by DOX and its mechanism of action is not known exactly. In our study, it was determined that lipid peroxidation was increased and antioxidant enzyme levels were decreased in the group given DOX. However, in the group treated with DOX and TQ (DT group), it was observed that lipid peroxidation was decreased and antioxidant enzyme levels were increased, which is consistent with previous studies (Öztürk *et al.,* 2020). Due to free radical scavenging and antioxidant enzyme-inducing properties of TQ, it is thought that testicular tissue damage caused by DOX can be prevented by the TQ treatment.

In our study, in which we measured the lipid peroxidation and antioxidant levels of the diaphragm muscle, we observed that lipid peroxidation was increased and antioxidant enzyme levels were decreased in the DOX-administered group (group D) compared to the control group. However, in the DT group, we found that lipid peroxidation was decreased and antioxidant enzyme levels were increased, and these results were consistent with previous studies (Montalvo *et al.,* 2020). DOX binds to the cardiolipin molecule in the inner mitochondrial membrane following the administration. It is stated that this binding causes the formation of superoxide anions and then the production of ROS. Increased ROS production causes the disruption of mitochondrial structure, apoptosis and a decrease in muscle functions. The elimination of mitochondrial ROS caused by DOX can prevent dysfunction of the muscles (Min *et al.,* 2015). The mechanism for the effects of TQ on the diaphragm muscle is unclear. However, the decrease in lipid peroxidation and the increase in antioxidant enzyme levels due to TQ administration in the DT group can be explained by the radical scavenging and protective antioxidant effect of TQ.

Similar to other tissues in our study, it was determined that DOX in stomach tissue increased lipid peroxidation and decreased antioxidant enzyme levels. In the DT group, TQ administration decreased lipid peroxidation and increased antioxidant enzyme levels. To the best of our knowledge, our study is the only study in which DOX and TQ were applied together in stomach tissue in the literature. However, the study (Magdy *et al.,* 2012) showed that lipid peroxidation levels were increased by creating ischemia/reperfusion damage and decreased with the TQ treatment. The same study determined that the decrease in antioxidant enzyme levels caused by ischemia/reperfusion damage were increased to control levels with the TQ treatment. In this study, in which the antioxidant and free

radical scavenging properties of TQ come into prominence, it was determined that TQ protected the gastric tissue from lipid peroxidation and the reduction of antioxidants caused by DOX. TQ non-enzymatically reacts with GSH, Nicotinamide adenine dinucleotide (NADH) and Nicotinamide adenine dinucleotide phosphate **(**NADPH) to form dihydro-thymoquinone, which is a more powerful free radical scavenger. This metabolite prevents lipid peroxidation by removing other free radicals, especially superoxide anions and protects antioxidant defense molecules.

Conclusions: Since acute and chronic side effects occur due to DOX administration, we believe that TQ treatment can alleviate the side effects of DOX caused by lipid peroxidation. In addition, it is necessary to determine the most effective dose of TQ in terms of lipid peroxidation and antioxidant enzyme levels by applying different doses of DOX and/or TQ for each tissue. Besides, since the mechanism related to the effects of DOX and/or TQ treatment on lipid peroxidation and antioxidant enzyme levels is not clear in the literature, more comprehensive studies on the mechanism should be planned for further studies.

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Ethical Approval: Animal experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals and were approved by Akdeniz University Animal Care and Use Committee at Akdeniz University Medical School (24.01.2018, protocol number: 21). At the Akdeniz University Experimental Animals Unit, animals were kept to the highest standards before and during the experiment. The rats were not subjected to any unnecessary and/or painful manipulation. The rats were anesthetized with sodium pentobarbital before being sacrificed to avoid pain. After the tissues were taken, the sacrificed rats were destroyed in accordance with laboratory standards in the Experimental Animals Unit of Akdeniz University.

Declarations: The authors declare that they have no conflicts of interest. The authors did not receive support from any organization for the submitted work. The authors have no relevant financial or non-financial interests to disclose.

Authors contributions: All authors contributed to this present work: DA, SK, HD, and SO participated in the design of the study. TM, SO, and DA performed the experiments. HD, and SK acquired the data. CD, and DA analyzed the data. DA, and TM drafted the manuscript. SK and SO revised the manuscript. All authors read and approved the final manuscript.

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