Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Histopathological evaluation of the effects of thymoquinone and resveratrol on the liver in rats administered doxorubicin

Mehmet Burak Ates[1*], Ozgur Ozdemir[1], Ali Erdem Ozturk[2], Mustafa Numan Bucak[3], Aysegul Bulut[1]

[1]Selcuk University, Veterinary Faculty, Department of Pathology, Konya, Turkey
[2]Erciyes University, Veterinary Faculty, Department of Reproduction and Artificial Insemination, Kayseri, Turkey
[3]Selcuk University, Veterinary Faculty, Department of Reproduction and Artificial Insemination, Konya, Turkey

Received:28.03.2022, Accepted: 30.06.2022
*mehmetburakates@selcuk.edu.tr

Doksorubisin uygulanan ratlarda timokinon ve resveratrolün karaciğer üzerine etkilerinin histopatolojik olarak değerlendirilmesi

Eurasian J Vet Sci, 2022, 38, 3, 150-158
DOI: 10.15312/EurasianJVetSci.2022.377

Öz

Amaç: Ratlarda doksurubisin kaynaklı hepatotoksisite üzerine farklı dozlardaki timokinon ve resveratrolün (5 and 20 mg/kg) etkilerinin patolojik olarak değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çalışmada 80 erkek Wistar Albino rat kullanıldı. Hayvanlar, kontrol (C, Fizyolojik tuzlu su, PO); doksorubisin (Fizyolojik tuzlu su, PO ve 10. günde 15mg/kg Dox, IP); Timokinon-5 (TQ-5, 5 mg/kg TQ, PO); TQ-20 (20 mg/kg TQ, PO); Resveratrol-5 (5 mg/kg Res, PO, günaşırı); Res-20 (20 mg/kg Res, PO); Dox+TQ-5; Dox+TQ-20; Dox+Res-5 ve Dox+Res-20 olmak üzere 10 gruba ayrıldı. 21 günlük deneme sonunda gruplardan rastgele 6 denek seçildi. Vücut ağırlığı tartıldıktan sonra, ötenazi uygulanan ratların karaciğerleri diseke edildi ve tartıldı. Karaciğer örneklerine rutin doku işleme işlemleri uygulandı. Mikroskobik olarak, hepatositlerde dejenerasyon, nekroz/apoptoz, safra kanalı hiperplazisi, disosiasyon, konjesyon, karyomegali, mononükleer hücre infiltrasyonu, çift çekirdekli hepatosit ve mitoz yönünden incelenerek her bir vaka için karaciğer total lezyon skoru hesaplandı.

Bulgular: Dox uygulaması relatif karaciğer ağırlığını arttırırken, TQ ve Res gruplarında Dox kaynaklı artış önlendi ($p<0.05$). Dox ile artan karaciğer total lezyon skorunun TQ ve Res gruplarında daha düşük olduğu gösterildi ($p<0.05$). Ancak Dox+TQ-5, Dox+TQ-20 ve Dox+Res-20 grupları nekroz/apoptozda düzelme saptanmadı.

Öneri: TQ ve Res (5 ve 20 mg/kg), Dox tarafından indüklenen total karaciğer lezyon skorunu azalttı. TQ ve Res, dejenerasyon ve inflamasyonu azaltmasına rağmen, anahtar kriterlerden biri olan nekroz/apoptoz üzerindeki zayıf koruyucu etkileri, kontrolsüz kullanımları için sınırlayıcı bir neden olarak kabul edildi.

Anahtar kelimeler: Karaciğer, histopatoloji, doksurubisin, timokinon, resveratrol

Abstract

Aim: The purpose was to investigate the effects of various dosages of thymoquinone and resveratrol (5 and 20 mg/kg) on doxorubicin-induced hepatotoxicity in rats from a pathological standpoint.

Materials and Methods: Eighty male Wistar Albino rats were used in this study. Animals were divided in to 10 groups: Control (physiological saline, PO); Doxorubicin (physiological saline, PO and Dox,15mg/kg Dox in 10th days, IP); Thymoquinone -5 (TQ-5, 5 mg/kg TQ, PO); TQ-20 (20 mg/kg TQ, PO); Resveratrol-5 (Res-5, 5 mg/kg Res, PO); Res-20 (20 mg/kg Res, PO); Dox+TQ-5 ; Dox+TQ-20; Dox+Res-5; Dox+Res-20. After the 21-day experiment, 6 replicates were randomly selected from the groups. After weighing the body weight, the livers of the euthanized rats were dissected and weighed. Routine tissue processing processes were applied to liver samples. Hepatocyte degeneration, necrosis/apoptosis, bile duct hyperplasia, dissociation, congestion, karyomegaly, mononuclear cell infiltration, binuclear hepatocytes, and mitosis were all examined microscopically, and a liver total lesion score was calculated

Results: Dox treatment increased relative liver weight, but the TQ and Res groups prevented this increase ($p<0.05$). The liver total lesion score, which increased with Dox, was shown to be lower in the TQ and Res groups ($p<0.05$). However, the Dox+TQ-5, Dox+TQ-20, and Dox+Res-20 groups, showed no amelioration in necrosis/apoptosis.

Conclusion: TQ and Res (5 and 20 mg/kg) decreased the total liver lesion score induced by Dox. Although TQ and RES diminish degeneration and inflammation, their poor protective effects on necrosis/apoptosis, one of the key criteria, were considered as a limiting cause for their uncontrolled usage.

Keywords: Liver, histopathology, doxorubicin, thymoquinone, resveratrol

Introduction

Doxorubicin (Dox, Adriamycin) is one of the anthracycline group antineoplastic medications (Carvalho et al 2009). Dox is widely used in many primary cancer therapies (breast, stomach, thyroid, prostate, testis, Hodgkin's lymphoma et al.) (Carvalho et al 2009). However, the side impacts of Dox on the heart, kidney and liver are worrisome. The undesirable side effects of Dox are mostly due to their non-selective cytotoxicity on cells. Even though the exact mechanism of Dox-induced hepatotoxicity is unknown, it has been reported that it may be due to oxidative destruction caused by increased reactive oxygen species (ROS) (Liu et al 2007). In 40% of patients treated with Dox, liver damage is observed (Carvalho et al 2009). It has been reported that a single dose of Dox administration in rats causes hepatocytes degeneration and necrosis, bile duct proliferation and inflammatory cell infiltration in the portal area (Mete et al 2016). All these indicate that the liver is one of the most impacted organs because of Dox administration. However, the decrease in liver functions may result in a decrease or increase in the effectiveness of not only DOX, but also other medical drugs used together, and a change in the dynamics of treatment.

Natural extracts have recently sparked renewed interest as a means of reducing the side effects of anticancer drugs. Thymoquinone (TQ) is one of them, and it acts as an antioxidant by reducing the generation of ROS and subsequent lipid peroxidation. TQ is also reported to have a hepatoprotective effect against toxic substances that can harm the liver (Ates and Ortatatli 2021b). TQ is a popular alternative for liver injury because of its low systemic toxicity and high bioavailability (Darakhshan et al 2015). Resveratrol (Res), another natural compound used to reduce the effects of antineoplastic/hepatotoxic agents, is a polyphenolic substance with antioxidant properties found in some edible plants such as grapes (Giovinazzo et al 2012). Similar to TQ, in Res, if it undergoes one electron reduction as a result of the amount of ROS in the environment and reactions at the molecular level, it can show pro-oxidant properties, and if it undergoes two-electron reduction, it can show antioxidant properties (de la Lastra and Villegas 2007).

Hepatic dysfunction is a condition that requires discontinuing/limiting the use of not only DOX, but also other drugs used to improve complications of disease and adverse signs, or requiring dosage regimen optimization (Nagai et al 2016). This is an indication of the critical importance of investigating various strategies to reduce the incidence and severity of Dox-induced hepatic toxicity. Based on the molecular mechanism of DOX-induced hepatotoxicity and literature information, the use of antioxidant agents can effectively ameliorate hepatic complications induced by this chemotherapeutic agent. Therefore, in the current study, it was aimed to pathologically evaluate the effects of TQ and Res at different doses (5 and 20 mg/kg) administered with Dox on the liver.

Material and Methods

Animals and ethics statement

In the study, livers of 80 albino Wistar rats at 8 weeks of age were used. The care and feeding of the rats were done ad libitum in a special room that was set at 16 hours of light and 8 hours of darkness, a temperature of 23±1 °C with a humidity of 60-65%. The Ethics Committee of the Experimental Animal Production and Research Center of Selçuk University's Faculty of Veterinary Medicine authorized the study's conformity with research ethics (Approval no: 2022/06).

Research design

Animals were divided in to 10 groups: Control [C, physiological saline, per oral (PO), every other day]; Doxorubicin (Dox, physiological saline, PO, every other day, and in 10th days 15mg/kg Dox, IP); Thymoquinone -5 (TQ-5, 5 mg/kg TQ, PO, every other day); Thymoquinone-2 (TQ-20, 20 mg/kg TQ, PO, every other day); Resveratrol-5 (Res-5, 5 mg/kg Res, PO, every other day); Resveratrol-20 (Res-20, 20 mg/kg Res, PO, every other day); Dox+TQ-5 (5 mg/kg TQ, PO, every other day and in 10th days 15mg/kg Dox, IP); Dox+TQ-20 (20 mg/kg TQ, PO, every other day and in 10th days 15mg/kg Dox, IP); Dox+Res-5 (5 mg/kg Res, PO, every other day and in 10th days 15mg/kg Dox, IP); Dox+Res-20 (20 mg/kg Res, PO, every other day and in 10th days 15mg/kg Dox, IP). The experimental period lasted 21 days. Thymoquinone and resveratrol were dissolved in dimethyl sulfoxide (DMSO) and applied 10 times each (every other day). At the end of the trial period, all rats were euthanized under sedation (10 mg/kg xylazine and 90 mg/kg ketamine) after their body weight was determined.

Relative liver weight

Pathological examinations were performed on 6 replicates randomly selected from the experimental groups. The livers of the rats, whose body weight was weighed and then euthanized, were dissected. After the tissues on the livers were removed, they were weighed. Relative liver weights were determined according to the formula:

$$\frac{Liver\ weight \times 100}{Live\ body\ weight}$$

Histopathological examination

Liver samples were fixed in 10% buffered formaldehyde liquid for 24 hours. The tissues were washed in running tap water for 12 hours to remove the fixative. After routine tissue processing on a tissue processing machine (Leica TP1050), all tissues were embedded in paraffin. Sections of 5 μ thickness taken from the paraffin blocks obtained with a microtome (Leica RM2120) were stained with haematoxylin-eosin (HE) (Luna 1968). Microscopic examination was performed in at least five different regions in terms of hepatocytes degeneration (hydropic-vacuolar and fatty changes), necrosis/apoptosis, bile duct hyperplasia, hepatic cord dissociation, congestion, karyomegaly, mononuclear cell infiltration (MCI), and bi-nucleated hepatocytes and mitosis considered as regeneration indicators (Ozdemir et al 2009). Scoring was done according to the severity and prevalence of these findings; (0): no lesion; (1): 1-25%; (2): 26-50%; (3): 51-75%; (4): 76-100. The numerical values given to the degenerative findings were collected, the numerical values given to the regenerative findings were subtracted from the total degeneration score, and finally the total lesion score of that replicate was determined (maximum score is 28). Histopathological examination and scoring were done by two experts who were unaware of the experimental groups.

Statistical analysis

Normal distribution analyzes of relative liver weights and histopathological scoring were done with The Kolmogorov-Smirnov test. The homogeneity of variances was controlled using Levene's test. All pathological data were evaluated by the Duncan analysis following one-way ANOVA (SPSS® program). Statistical importance was described as a value of ($p<0.05$).

Results

Relative liver weights calculated by proportioning the percent live weights of the replicates in the groups are presented in Table 1. According to these data, a statistically insignificant decrease was determined in the TQ-5, TQ-20, Res-5 and Res-20 groups ($p>0.05$). There was a substantial raise in liver weights in the Dox group ($p<0.05$). There was a slight decrease in liver weights in the Dox+TQ-5, Dox+TQ-20 and Dox+Res-5 groups, and a significant decrease in the Dox+Res-20 group ($p<0.05$, Table 1).

In the microscopic examination, swelling due to hydropic degeneration and low-intensity steatosis were detected in hepatocytes. While this situation was most severe in the Dox group ($p<0.05$), no statistical variation was found in the other groups ($p>0.05$, Table 2). In addition to focal necrosis observed more in the centrilobular and mid-zonal regions, cells with pycnotic nuclear fragments and apoptotic hepatocytes characterized by eosinophilic bodies (like Councilman body) were found (Figure 1-2). In the distribution of necrosis/apoptosis, an increase was observed in Dox+Res20, Dox+TQ-5, Dox+TQ-20 and Dox groups, respectively ($p<0.05$). It was determined that bile duct proliferation was drastically boosted in the Dox group ($p<0.05$). Due to the dissociation of liver cords, architectural pattern deterioration was more prominent in the Dox group and milder in the other experimental groups ($p<0.05$). It was noted that the congestion observed in the sinusoids and central vein increased in the Dox compared to the control ($p<0.05$), while there was no substantial variation in the other groups ($p>0.05$). Megalocytosis, which is defined as an enlargement of the nuclei of hepatocytes, sometimes accompanied by their cytoplasm, was found to be more common in the Dox, Dox+TQ-5, Dox+TQ-20, Dox+Res-5, and Dox+Res-20 groups ($p<0.01$). Mononuclear cell infiltrations in the portal area were observed intensively in the Dox group ($p<0.01$) (Figure 3).

Table 1. Relative liver weights

Groups	Relative liver weight
Control	4,23±0,12^{b}
Dox	5,08±0,20^{c}
TQ-5	4,01±0,19^{ab}
TQ-20	4,17±0,16^{b}
Res-5	3,97±0,9^{ab}
Res-20	4,19±0,13^{b}
Dox+TQ-5	3,75±0,27^{ab}
Dox+TQ-20	3,92±0,10^{ab}
Dox+Res-5	3,61±0,38^{ab}
Dox+Res-20	3,41±0,20^{a}

a,b,c Values with different superscripts in the same column indicate that the difference is statistically significant ($p<0.05$, one-way ANOVA *post hoc* Duncan test)

Table 2 Histopathological results

	Hydropic Degeneration/ Fatty changes	Necrosis/ Apoptosis	Bile duct proliferation	Dissociation of liver cords	Congestion	Megalocytosis	Mononuclear cell infiltration	Bi-nucleated hepatocytes/ Mitoses	Total lesion score
Control	1,25±0,17^{a}	0,58±0,11^{a}	0,25±0,11^{a}	0,5±0,22^{a}	0,67±0,17^{abc}	0,33±0,11^{a}	0,25±0,17^{ab}	1,33±0,17^{ab}	2,5±0,34^{a}
Dox	2,25±0,25^{b}	1,17±0,17^{bcd}	0,83±0,11^{b}	1,17±0,17^{c}	1,17±0,17^{d}	0,80±0,2^{bc}	0,83±0,11^{c}	1,08±0,2^{ab}	7,14±0,73^{d}
TQ-5	1,42±0,3^{a}	0,67±0,11^{ab}	0,33±0,21^{ab}	0,42±0,15^{a}	0,67±0,17^{abc}	0,50±0,22^{ab}	0,58±0,08^{bc}	1,00±0,13^{ab}	3,59±0,77^{abc}
TQ-20	1,5±0,26^{a}	0,67±0,11^{ab}	0,33±0,17^{ab}	0,58±0,08^{ab}	0,58±0,15^{abc}	0,58±0,15^{ab}	0,58±0,08^{bc}	1,17±0,17^{ab}	3,65±0,37^{abc}
Res-5	1,17±0,11^{a}	0,58±0,15^{a}	0,33±0,11^{ab}	0,33±0,11^{a}	0,59±0,15^{abc}	0,33±0,11^{a}	0,51±0,13^{bc}	0,92±0,08^{a}	2,92±0,24^{ab}
Res-20	1,58±0,08^{a}	0,67±0,11^{ab}	0,50±0,18^{ab}	0,33±0,11^{a}	0,42±0,15^{ab}	0,25±0,11^{a}	0,00±0,00^{a}	1,17±0,17^{ab}	2,58±0,27^{a}
Dox+TQ-5	1,58±0,24^{a}	1,25±0,17^{cd}	0,25±0,11^{a}	0,67±0,17^{ab}	1,00±0,00^{cd}	0,84±0,17^{bc}	0,00±0,00^{a}	0,92±0,08^{a}	4,67±0,4^{c}
Dox+TQ-20	1,25±0,11^{a}	1,17±0,38^{bcd}	0,5±0,22^{ab}	0,75±0,11^{abc}	0,83±0,17^{bcd}	1,33±0,11^{d}	0,00±0,00^{a}	1,5±0,18^{b}	4,33±0,89^{bc}
Dox+Res-5	1,5±0,18^{a}	0,92±0,15^{abc}	0,33±0,17^{ab}	1,00±0,13^{bc}	0,67±0,17^{abc}	1,17±0,17^{cd}	0,58±0,27^{bc}	1,33±0,17^{ab}	4,83±0,4^{c}
Dox+Res-20	1,67±0,25^{a}	1,5±0,13^{d}	0,17±0,11^{a}	0,75±0,17^{abc}	0,25±0,11^{a}	1,33±0,17^{d}	0,08±0,08^{a}	1,33±0,28^{ab}	4,42±0,27^{bc}
	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p <0.01	p <0.01	p <0.05	p <0.01

a,b,c,d Values with different superscripts in the same column indicate that the difference is statistically significant (p <0.05, one-way ANOVA *post hoc* Duncan test)

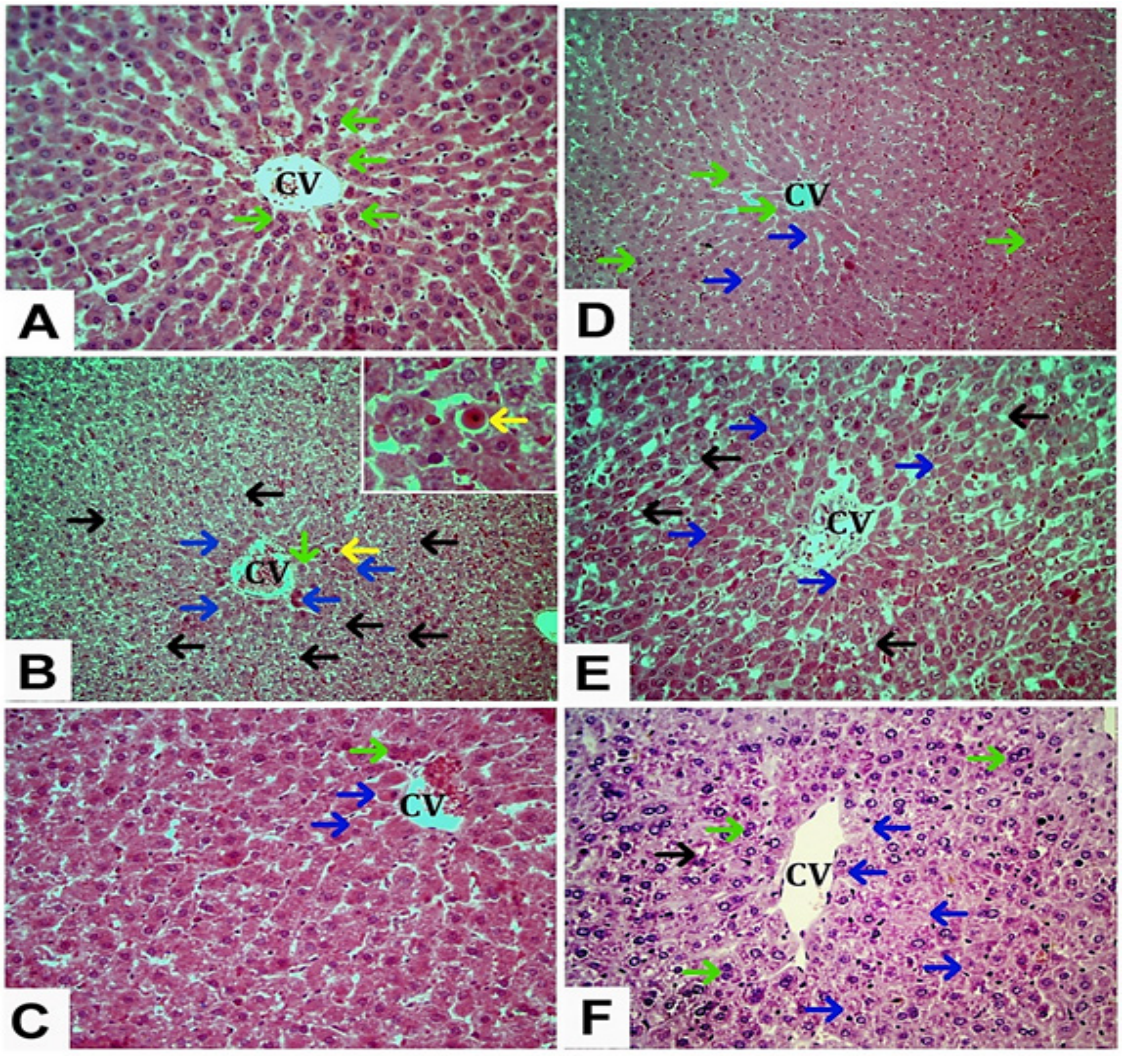

Figure 1. Representative photomicrographs of livers in experimental groups, A: Control; B: Dox; C: Dox+TQ-5; D: Dox+TQ-20; E: Dox+Res-5; F: Dox+Res-20, HE, Objective Magnification: A, C, E, and F: 40X; B and D: 20X. CV: Central vein; Yellow arrows: Councilman body; Green arrows: bi-nucleated hepatocytes; Black arrows: degenerative hepatocytes; Blue arrows: necrotic/apoptotic hepatocytes

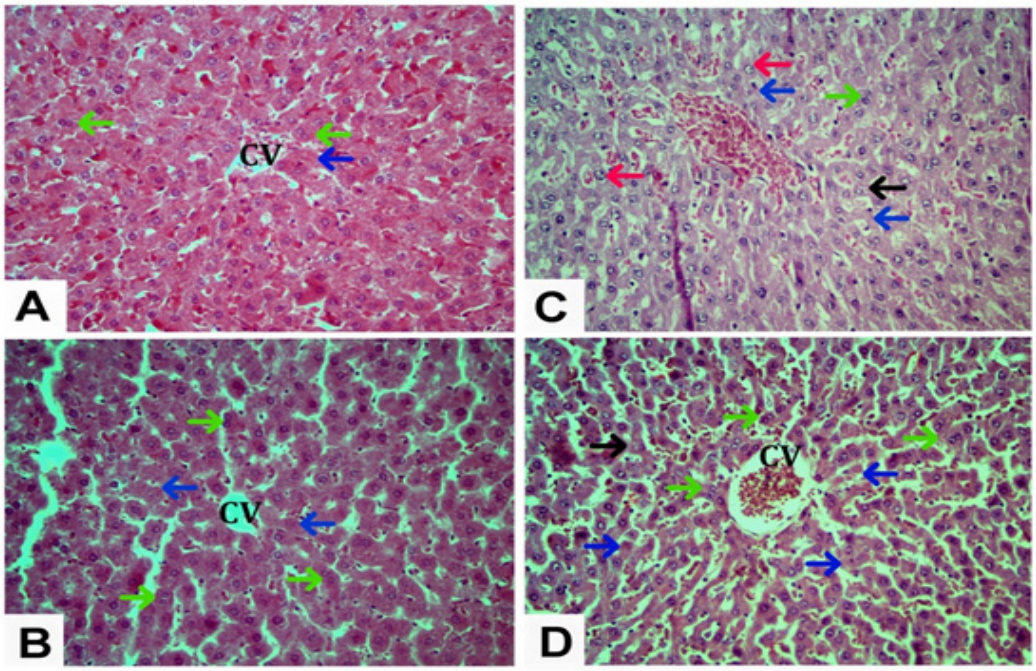

Figure 2. Representative photomicrographs of livers in drug control groups, HE, Objective Magnification: 40X, A: TQ-5; B: TQ-20; C: Res-5; D: Res-20. CV: Central vein; Green arrows: bi-nucleated hepatocytes; Black arrows: degenerative hepatocytes; Blue arrows: necrotic/apoptotic hepatocytes; Pink arrows: megalocytosis

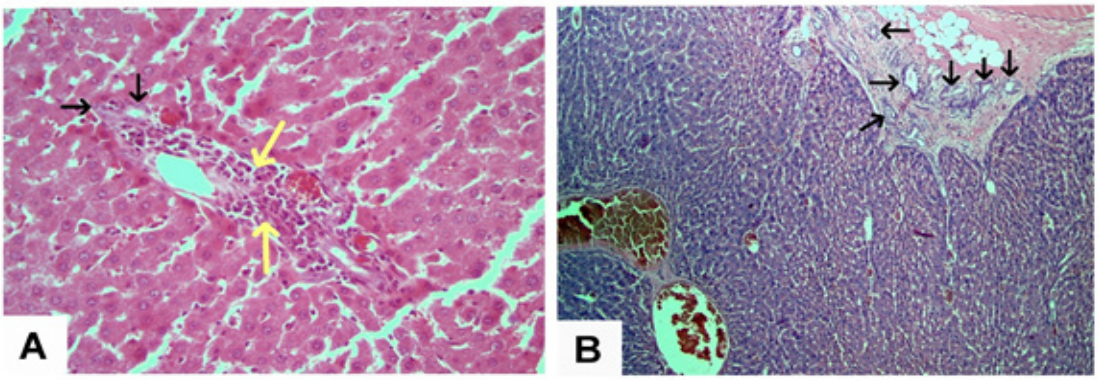

Figure 3. A: Mononuclear cell infiltration (yellow arrows) and bile duct hyperplasia (black arrows) Control, 40X, HE, B: Bile duct hyperplasia (black arrows), Dox+TQ-20, 10X, HE.

Although bi-nucleated hepatocytes and mitosis observed in the liver were observed less frequently in the Dox+TQ-5 group and most intensely in the Dox+TQ-20 group, no substantial variation was detected between the groups ($p>0.05$). In the evaluation made in terms of the total lesion score obtained by subtracting the scores of regenerative indicators (bi-nucleated hepatocytes / mitoses) from the scores of degenerative findings indicated in Table 2, a significant increase was found in the Dox group ($p<0.01$). In the other experimental groups, the total lesion score was noticed to be decreased compared to the Dox group ($p<0.05$).

Discussion

Hepatic dysfunction caused by doxorubicin is an important complication that may occur in disease states where this drug is used for therapeutic purposes and sometimes may cause radical changes in the treatment strategy. For this reason, research on the protective activities of antioxidant natural compounds for such undesirable effects caused by Dox has accelerated in the scientific world. However, these studies are not yet at the level to prove that such treatment supplements are reliable. Therefore, in the present study,

the effects of different doses of thymoquinone, resveratrol and Dox on the liver were pathologically investigated.

The side effects of chemotherapy drugs, which are used extensively in cancer treatment, are a condition that limits their use and must be taken into account during treatment. There are studies showing that Dox, which is one of these chemotherapy drugs and has a strong antineoplastic effect, increases the relative liver weight (RLW) (Kundu et al 2013, Jaćević et al 2018). When our study analyzed the findings, it was found that Dox triggered a substantial increase in RLW (Table 1). It was thought that this situation might be due to hydropic degeneration and fat accumulation, congestion, and inflammatory cell infiltrations in hepatocytes. Based on these data, it was concluded that the congestion was caused by cardiotoxicity produced by Dox (Jaćević et al 2018). Compared to the control group, a significant decrease in RLW was determined in the Dox+Res-20 group and a slight decrease in Dox+TQ-5, Dox+TQ-20 and Dox+Res-5. It was thought that this situation might be due to the signs of Dox-induced inflammation, decreased hepatic degeneration and congestion on the one hand, and increased necrosis and/or apoptosis, which leads to a decrease in liver weight, on the other hand.

TQ and Res did not cause significant liver damage in comparison to the control group, according to the current study's histopathological findings (Table 2). This indicates that at the doses used in the study, TQ and Res have no hepatotoxic effects. The fact that the TQ and Res groups are not different from the control group in terms of bi-nucleated hepatocytes and mitosis density, which are accepted as indicators of regenerative activities in the liver (Ozdemir et al 2009), shows that their regenerative functions are kept within physiological limits.

Dox is known for its potent anticancer properties, but it has also been caused to cardiotoxicity, hepatotoxicity, nephrotoxicity, and gonatoxicity (El-Sayed et al 2017, Jaćević et al 2018, Li et al 2018, Omobowale et al 2018, Öztürk 2021). In the current research, it was determined that Dox significantly boosted the total liver lesion score (Table 2). In experimental studies, it has been reported that Dox application may cause hydropic degeneration, steatosis, necrosis/apoptosis, dissociation, focal parenchymatous and MCI infiltration and congestion in the portal area, in parallel with the findings in the current study (Omobowale et al 2018, Bilgic and Ozgocmen 2019, Akin et al 2021). Free radical formation, oxidative damage, and activation of the intrinsic apoptosis pathway are all linked to Dox's antineoplastic effect and hepatotoxicity. Dox can also directly activate caspases via the extrinsic pathway, causing the cell to continue apoptosis (Mizutani et al 2005). The liver is the major organ for the metabolism of numerous xenobiotics, including Dox. The intense accumulation and metabolization of Dox in the liver causes the liver to be heavily affected (Carvalho et al 2009). As a matter of fact, while Dox increases ROS, superoxide dismutase (SOD), lipid peroxidation, catalase, glutathione peroxidase and DNA damage, it causes a decrease in glutathione level (Carvalho et al 2009). Thus, hepatotoxicity develops as a result of disruption of oxidative balance and cell energy mechanisms (Carvalho et al 2009). Based on this information, it was hypothesized in the current study that Dox-induced hepatocyte degeneration, necrosis, and apoptosis were produced by enhanced oxidative damage due to the drug's mechanism of action. The structure of the hepatic cords was disrupted, and dissociation formed, as a result of the swelling of some hepatocytes due to hydropic degeneration and shrinkage of others due to apoptosis with Dox administration. It is clear that the congestion in the sinusoids also contributes to this situation. It has been reported that the cause of congestion may be Dox-induced cardiotoxicity (Jaćević et al 2018). It has been described that Dox activates nuclear factor-kappa B (NF-kB), which causes the expression of proinflammatory cytokines resulting in cell death in the liver (Carvalho et al 2009). The focal parenchymatous and portal area MCI infiltrates observed in the current study were thought to be due to Dox-induced NF-kB activation. It has been shown that toxic damage caused by chemicals can cause karyomegaly in hepatocytes (Thoolen et al 2010). Increased karyomegaly with Dox administration may be a predictor of increased apoptosis during chemotherapy, according to the current study (Thoolen et al 2010, Al-Rasheed et al 2018).

TQ, which is considered as the pharmacologically active component of Nigella sativa, is reported to have a hepatoprotective effect with its strong antioxidant properties and reduce apoptosis in hepatocytes (Ates and Ortatatli 2021b, Ates et al 2022). TQ was found to prevent Dox-induced hepatoxicity in rats by strengthening the antioxidant system and decreasing inflammation and apoptosis, according to a study (Akin et al 2021). TQ, on the other hand, was discovered to inhibit the PI3K/Akt pathway and boost the production of p53 and p21 proteins through activating PTEN. Thus, it was stated that the combination of Dox and TQ had an antineoplastic effect by inhibiting the G2/M cell cycle and increasing apoptosis (Arafa el et al 2011). The liver total lesion score was shown to be lower in both TQ groups compared to the Dox group in the current investigation. It was thought that this situation occurred with the decrease of degeneration and inflammation findings in the liver. Interestingly, when only necrosis/apoptosis findings were evaluated, no significant change was found in the Dox+TQ-5 and Dox+TQ-20 groups compared to the Dox group (Table 2). Depending on the cellular microenvironment, TQ has been found to function as both an antioxidant and a pro-oxidant (Darakhshan et al 2015). TQ passes through a number of oxido-reduction processes when it reacts with amino or thiol groups in amino acids. TQ, which

loses a single electron at this stage, transforms into a pro-oxidant semiquinone, and exerts an antineoplastic effect. It shows antioxidant properties by the loss of an electron of semiquinone or conversion of TQ to thymohydroquinone and glutathionyl-dihydrothymoquinone by direct double-electron reduction (Darakhshan et al 2015). TQ prevented mitochondrial vacuolization and cell energy system disruption due to increased ROS production and intracellular oxidative destruction caused by Dox in the current study. The alleviation of vacuolar degeneration, which is a marker of mitochondrial damage in hepatocytes, in Dox+TQ-5 and Dox+TQ-20 groups is an indicator of this situation. It has been reported that TQ reduces the metabolism of toxic substances by blocking nuclear receptors (Ahr, PXR, Car) that control the release of cytochrome p450 (CYP450) enzymes (Ates and Ortatatli 2021b). TQ has also been shown to have a hepatoprotective impact by stimulating Phase-2 detoxification processes, which are responsible for the removal of compounds produced by Phase-1 metabolism (Ates and Ortatatli 2021a). As a matter of fact, Dox undergoes Phase-1 metabolism in the liver via CYP450 enzymes and turns into hepatotoxic metabolites. Also, the semiquinone form of Dox that occurs with an electron reduction also causes increased ROS production and oxidative damage (Carvalho et al 2009). However, the limitation of Dox metabolism by TQ's inhibition of nuclear receptors may have contributed to the reduction of ROS production and degenerative effects. Intrinsic apoptosis pathway activation occurs due to increased ROS production and intracellular oxidative / mitochondrial damage with Dox (Mizutani et al 2005). TQ could possibly have achieved inhibition of the intrinsic apoptosis pathway by abolishing these negative effects of Dox as described above. Interestingly, in the present study, it was observed that Dox-induced necrosis/apoptosis was not reduced in the TQ trial groups. However, Dox is also known to trigger apoptosis by directly causing extrinsic pathway activation (Mizutani et al 2005). Although TQ could possibly inhibit the intrinsic apoptosis pathway, the inability to adequately inhibit the extrinsic pathway activation induced by Dox may be the reason why apoptosis could not be prevented in the TQ treatment groups. Also, the decrease in degenerative and inflammatory findings in Dox+TQ-5 and Dox+TQ-20 groups compared to the Dox group, and the increase in necrosis/apoptosis levels compared to the control group also explain the reduce in RLW in these groups.

Resveratrol is an antioxidant natural compound that contributes to protection from oxidative stress-related diseases such as cardiovascular diseases, inflammation, neurological diseases and liver toxicity (de la Lastra and Villegas 2007). Studies showing that Res modulates the effect on the cytotoxic nature of various anticancer drugs and preservation from their toxic impacts are very limited (Bengaied et al 2017). In the current study, Dox-induced boost in liver total lesion score was prevented in Dox+Res-5 and Dox+Res-20 groups (Table 2). This was achieved by the reduction of degenerative changes in hepatocytes, signs of inflammation, and congestion in the Res treated groups, like the TQ treatment groups. Although necrosis/apoptosis was reduced in the Dox+Res-5 group, there was a slight boost in the Dox+Res-20 group that was not statistically significant. Depending on the ratio of free oxygen radicals / Res in the microenvironment, Res becomes a pro-oxidant semiquinone after one electron reduction or an antioxidant quinone after two electron reductions (de la Lastra and Villegas 2007). When the current study findings and this literature information were combined, it was thought that Res used at low doses (5mg/kg) exhibited antioxidant properties, while Res used at high doses might have increased apoptosis by showing pro-oxidant properties.

Conclusion

TQ and Res (5 and 20 mg/kg, respectively) were found to reduce the total liver lesion score from the current study's findings. However, the protective effects of TQ and Res on Dox-induced necrosis/apoptosis observed in hepatocytes were very limited and even a slight increase was detected in the Dox+ Res-20 group. These findings explain the reduction in RLW in the treatment groups in which Dox-induced degeneration and inflammation were reduced but necrosis/apoptosis findings remained unchanged or slightly increased. Because of all these, we think that the use of TQ and Res should be approached more cautiously to alleviate or eradicate the negative impacts of Dox chemotherapy on the liver.

Acknowledgement

This study was made from the tissues of the project named "Determination of Damage Caused by Doxorubicin and Protective Effects of Various Antioxidants in Rat Testicular Tissue".

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

References

Akin AT, Ozturk E, Kaymak E, Karabulut D, et al., 2021. Therapeutic effects of thymoquinone in doxorubicin-induced hepatotoxicity via oxidative stress, inflammation and apoptosis. Anat Histol Embryol, 50, 6, 908-17.

Al-Rasheed NM, El-Masry TA, Tousson E, Hassan HM, et al., 2018. Hepatic protective effect of grape seed proanthocyanidin extract against Gleevec-induced apoptosis, liver Injury and Ki67 alterations in rats. Braz J Pharm Sci, 54, 2, 17391.

Arafa el SA, Zhu Q, Shah ZI, Wani G, et al., 2011. Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. Mutat Res, 706, 1-2, 28-35.

Ates MB, Ortatatli M, 2021a. The effects of Nigella sativa seeds and thymoquinone on aflatoxin phase-2 detoxification through glutathione and glutathione-S-transferase alpha-3, and the relationship between aflatoxin B1-DNA adducts in broilers. Toxicon, 193, 86-92.

Ates MB, Ortatatli M, 2021b. Phase-1 bioactivation mechanisms of aflatoxin through AhR, CAR and PXR nuclear receptors and the interactions with Nigella sativa seeds and thymoquinone in broilers. Ecotoxicol Environ Saf, 208, 111774.

Ates MB, Ortatatli M, Oguz H, Ozdemir O, et al., 2022. The ameliorative effects of Nigella sativa, thymoquinone, and bentonite against aflatoxicosis in broilers via AFAR and Nrf2 signalling pathways, and down-regulation of caspase-3. Br Poult Sci, 63, 3, 332-9.

Bengaied D, Ribeiro A, Amri M, Scherman D, et al., 2017. Reduction of hepatotoxicity induced by doxorubicin. J Integr Oncol, 6, 1-12.

Bilgic S, Ozgocmen M, 2019. The protective effect of misoprostol against doxorubicin induced liver injury. Biotech Histochem, 94, 8, 583-91.

Carvalho C, Santos RX, Cardoso S, Correia S, et al., 2009. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem, 16, 25, 3267-85.

Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S, 2015. Thymoquinone and its therapeutic potentials. Pharmacol Res, 95-96, 138-58.

de la Lastra CA, Villegas I, 2007. Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochem Soc Trans, 35, Pt 5, 1156-60.

El-Sayed EM, Mansour AM, El-Sawy WS, 2017. Protective effect of proanthocyanidins against doxorubicin-induced nephrotoxicity in rats. J Biochem Mol Toxicol, 31, 11.

Giovinazzo G, Ingrosso I, Paradiso A, De Gara L, et al., 2012. Resveratrol biosynthesis: plant metabolic engineering for nutritional improvement of food. Plant Foods Hum Nutr, 67, 3, 191-9.

Jaćević V, Dragojević-Simić V, Tatomirović Ž, Dobrić S, et al., 2018. The Efficacy of Amifostine against Multiple-Dose Doxorubicin-Induced Toxicity in Rats. Int J Mol Sci, 19, 8, 2370.

Kundu B, Ghosh D, Sinha MK, Sen PS, et al., 2013. Doxorubicin-intercalated nano-hydroxyapatite drug-delivery system for liver cancer: An animal model. Ceram, 39, 8, 9557-66.

Li J, Li L, Li X, Wu S, 2018. Long noncoding RNA LINC00339 aggravates doxorubicin-induced cardiomyocyte apoptosis by targeting MiR-484. Biochem Biophys Res Commun, 503, 4, 3038-43.

Liu LL, Li QX, Xia L, Li J, et al., 2007. Differential effects of dihydropyridine calcium antagonists on doxorubicin-induced nephrotoxicity in rats. Toxicology, 231, 1, 81-90.

Luna LG, 1968. Routine Staining Prosedures. In: Manual of histologic staining methods of the Armed Forces Institute of Pathology. Eds, 3.ed. United States of America: McGaw-Hill Book Company, p. 32-44.

Mete R, Oran M, Topcu B, Oznur M, et al., 2016. Protective effects of onion (Allium cepa) extract against doxorubicin-induced hepatotoxicity in rats. Toxicol Ind Health, 32, 3, 551-7.

Mizutani H, Tada-Oikawa S, Hiraku Y, Kojima M, et al., 2005. Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. Life Sci, 76, 13, 1439-53.

Nagai K, Fukuno S, Oda A, Konishi H, 2016. Protective effects of taurine on doxorubicin-induced acute hepatotoxicity through suppression of oxidative stress and apoptotic responses. Anticancer Drugs, 27, 1, 17-23.

Omobowale TO, Oyagbemi AA, Ajufo UE, Adejumobi OA, et al., 2018. Ameliorative Effect of Gallic Acid in Doxorubicin-Induced Hepatotoxicity in Wistar Rats Through Antioxidant Defense System. J Diet Suppl, 15, 2, 183-96.

Ozdemir O, Akalin PP, Baspinar N, Hatipoglu F, 2009. Pathological changes in the acute phase of streptozotocin-induced diabetic rats. Bull Vet Inst Pulawy, 53, 4, 783-90.

Öztürk AE, 2021. Determination of Damage Caused by Doxorubicin and Protective Effects of Various Antioxidants in Rat Testicular Tissue, PhD Thesis, Selcuk University Graduate School Of Health Sciences Konya.

Thoolen B, Maronpot RR, Harada T, Nyska A, et al., 2010. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicologic pathology, 38, 7 Suppl, 5S-81S.

Author Contributions

Motivation / Concept: Ali Erdem Öztürk, Mustafa Numan Bucak
Design: Ali Erdem Öztürk, Mustafa Numan Bucak
Control/Supervision: Mustafa Numan Bucak, Özgür Özdemir
Data Collection and / or Processing: Mehmet Burak Ateş, Ali Erdem Öztürk
Analysis and / or Interpretation: Mehmet Burak Ateş, Özgür Özdemir, Ayşegül Bulut
Literature Review: Mehmet Burak Ateş
Writing The Article: Mehmet Burak Ateş
Critical Review: Özgür Özdemir

Ethical Approval

The Ethics Committee of the Experimental Animal Production and Research Center of Selçuk University's Faculty of Veterinary Medicine 2022/06 Number Ethics Committee Decision

CITE THIS ARTICLE: *Ateş MB, Özdemir Ö, Öztürk AE, Bucak MN et al, 2022. Histopathological evaluation of the effects of thymoquinone and resveratrol on the liver in rats administered doxorubicin. Eurasian J Vet Sci, 38, 3, 150-158.*