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Influence of montelukast on doxorubicin-induced oxidative stress in Rat's Liver

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Abstract
Doxorubicin (DOX) is a chemotherapeutic agent and is widely used in cancer treatment. There are some studies suggesting oxidative stress-induced toxic changes in the liver due to DOX administration. The aim of this study was to reveal the oxidative damage of DOX in liver tissue at molecular level and to evaluate the protective effect of Montelukast (ML) against DOX oxidative damage.
Twenty four male rats were equally divided into 4 groups. The first group was used as control. The second group received a single dose of DOX. The third group received ML for 28 days. The fourth group received a single dose of DOX, followed by ML for 28 days. At the end of the experiment, liver tissues were taken from all animals. Total Antioxidant Capacity (TAC), Total Oxidant Capacity (TOC) levels were determined in these samples by spectrophotometric methods. The histopathological changes of liver tissue were observed routinely in histological staining.
It was determined that TOC level increased, TAC levels decreased in the group given DOX compared to the control group. In addition, TAC levels increased in the DOX+ML group, but did not approach the control group levels. It was showed the occurrence of congestion in portal triad, and pycnotic cells degeneration in DOX group.
It was concluded that DOX administration increased oxidative stress and ML administration could prevent the increased oxidative stress ($p<0.05$). ML caused modulatory effects on oxidative stress and antioxidant redox system in DOX-induced liver toxicity in the rat.

Keywords: Doxorubicin, DOX, Rat's, TOC, doxorubicin-induced

Introduction

Doxorubicin (DOX) is an anti-cancer agent used in the treatment of various types of cancer and some solid tumors. It is used to protect the cell protection of cancer treatment (Aljobaily *et al*., 2020) [2]. DOX is a highly toxic agent that causes toxicity in many organs and tissues in the organism. Studies have reported toxic possibilities on the heart, brain, heart, kidney, kidney, skin, and reproductive organs such as the ovaries and testes. It is known that the pathogen is one of the organs most susceptible to DOX infection. Although the complication rates of the DOX tool are not known exactly, it is stated that the support structure of unusual enzymes, as well as the increase in free radical formation and lipid peroxidation, will play a role (Moslehi, 2016 [17]; Timm *et al*., 2021 [24]; Yu *et al*., 2020) [27].

The anti-inflammatory and antioxidant effects of ML have been emphasized by many authors. Şener *et al*. showed that montelukast protects against oxidative damage caused by reactive oxygen radicals and lipid peroxidation after thermal damage (Sener *et al*., 2005) [22]. Tuğtepe *et al*. concluded that ML has protective effects on the kidney by balancing the oxidant-antioxidant status and regulating the formation of inflammatory mediators (Tuğtepe, Sener, Cetinel, Velioğlu-Öğünç, & Yeğen, 2007). Coşkun *et al*. found that ML had a significant effect on lung, liver, heart and kidney tissues by decreasing or reducing myeloperoxidase (MPO) levels (Coskun *et al*., 2011) [10]. Mohamadin *et al*. showed that ML administration protected the liver from lipopolysaccharide-induced oxidative damage (Mohamadin, Elberry, Elkablawy, Gawad, & Al-Abbasi, 2011) [16].

Lipid peroxidation is the reaction in which fatty acids in the membrane are destroyed by free oxygen radicals. Malondialdehyde (MDA), which can be measured with thiobarbituric acid, is formed in the peroxidation of fatty acids formed by the destruction of lipid hydroperoxides. This method is a frequently preferred method for measuring lipid peroxide levels. Lipid peroxidation plays an important role in disease pathogenesis by inducing changes that lead to cell damage (Hjelle & Petersen, 1983 [14]; Saleh, Mahmoud, Hassan, & Sanad, 2022) [21].

The aim of this study was to reveal the oxidative damage of DOX in liver tissue at molecular level and to evaluate the protective effect of ML against the oxidative damage of DOX on this tissue.

Materıals and methods

Study design and animals

In this experimental study, adult male Wistar Albino rats obtained from Çanakkale Onsekiz Mart University Experimental Research and Application Center were used in this study. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water. The local ethics committee for animal experiments of Çanakkale Onsekiz Mart University Faculty of Medicine approved the current study (Ethics number: 2022-2200189432). All invasive procedures, anesthesia, animal care, etc. were conducted in accordance with international guidelines on experimental animal studies.(Van Sluyters & Obernier, 2004) [26]

G*Power-3.1 program was used in the experimental design. According to the previous studies (Charan & Kantharia, 2013 [8]; Cohen, 1962) [9], the sample size was determined as 24 with an effect size of 0.70, an α error of 0.05, and a power of (1–β) 80%. The groups were formed to include 6 rats in each group.

All rats planned to be used in the experiment were weighed and experimental groups were formed in such a way that the groups were not different from each other. Rats were randomly divided in to four experimental groups (6 rats per group) as follows:

- Group 1: control
- Group 2: DOX (20 mg/kg, IP)
- Group 3: ML (10 mg/kg/day, IP) for 4 weeks
- Group 4: DOX (20 mg/kg Doksorubisin, IP) + ML (10 mg/kg/day IP) for 4 weeks (from the day of DOX administration)

At the end of the experimental period, the rats were anesthetized by ketamine and xylazine hydrochloride. Then, liver was moved for measuring the oxidative stress markers the rats.

Preparation of Tissue Samples

Tissue samples were homogenized in phosphate buffer solution (1:10 w/v, pH: 7.4) using ice-cooled tubes. The homogenate was centrifuged (14,000 rpm, 30 min) and the supernatants were separated for analysis. Protein concentration was estimated by the method of Lowry (Lowry, Rosebrough, Farr, & Randall, 1951) [15]. Tissue samples, taken for malondialdehyde determination, were homogenized and subjected to procedures as outlined before (Ohkawa, Ohishi, & Yagi, 1979) [18]. TAC and TOC levels were measured by a spectrophotometric assay using commercially available kits (Rel Assay Diagnostics, Turkey). The OSI was defined as the ratio of the TAC level to TOC level.

Histopatological Analysis

Liver tissues fixed with 10% buffered formaldehyde were subjected to routine histologic follow-up. The tissues were embedded in paraffin and then 4 micrometer sections were taken from the blocks with a sliding-microtome and deparaffinized. Rehydrated sections were stained with Mayer hematoxylin and eosin stains. After the procedure, the sections were covered with entellan and evaluated histopathologically under light microscope. Liver histopathologic evaluations were performed according to Gibson-Corley KN et. al.(Gibson-Corley, Olivier, & Meyerholz, 2013) In the scoring process performed in 10 random fields in the micrographs at 200x magnification obtained from random sections taken from all samples of each group, 0 was determined as none, 1 as mild, 2 as extensive and 3 as severe (1) (Gibson-Corley *et al*., 2013) [13]. In pyknotic nuclei counting performed in liver tissues, pyknotic nuclei detected in 5 random fields of random sections were counted with the help of ImageJ (NIH, USA, Version 1.53J) program.

Statistical analysis

Statistical analysis of the study data was performed with IBM SPSS Statistics version 19 software. Conformity of the variables to normal distribution was assessed with the Shapiro–Wilks test. Statistical data were stated as mean ± standard deviation (SD) or median and interquartile range for normally and nonnormally distributed variables, respectively. A paired t-test was used to compare two dependent groups. One-way analysis of variance (ANOVA) was used to assess the effect of ML treatment on biochemical parameters. If statistically significant differences were found for the groups, the data were further analyzed using the Tukey multiple comparison test. The differences among the groups were evaluated by using the Kruskal–Wallis test for histological parameters and the comparisons between every two groups were made using the Mann–Whitney U test. Data were considered statistically significant at $P < 0.05$

Results

TAC analysis results

In group 2 given DOX and group 4 given DOX+ML; TAC levels decreased compared to the control group (respectivly p=0.14; p<0.001) (Table 1.)

Table 1: TAC analysis results

Group	TAC (mmole /L)	P Value
Control	2.16±0.87	
DOX	1.61±0.37	§p:0.14,
ML	1.73±0.21	¶p:<0.001
DOX+ML	1.98±0.05	

Group 1: Control group; Group 2: DOX; Group 3: ML; Group 4: DOX + ML. Group Comparisons: §p= grup1 and grup 2; ¶p= grup 1 and grup 3; ‡p= grup1 and grup 4; ♯p= grup 2 and grup 4.

TOC analysis results

In group 2 given DOX, TAC levels increased compared to the control group (p=0.002). In group 4 given DOX+ML, TAC levels decreased compared to the group 2 given DOX (p<0.001) (Table 2.)

Table 2: TOC analysis results

Group	TOC (µmole/L)	P Value
Control	24.58±5.68	
DOX	36.65±2.36	§p:0.002,
ML	23.31±1.35	
DOX+ML	21.75±4.02	♯p:<0.001

Group 1: Control group; Group 2: DOX; Group 3: ML; Group 4: DOX + ML. Group Comparisons: §p= grup1 and grup 2; ¶p= grup 1 and grup 3; ‡p= grup1 and grup 4; ♯p= grup 2 and grup 4.

OSI analysis results

In group 2 given DOX, OSI ratio increased compared to the control group (p=0.001). In group 4 given DOX+ML, OSI ratio decreased compared to the group 2 given DOX (p<0.002) (Table 3.)

Table 3: OSI analysis results

Group	OSI ratio	P Value
Control	1.14±0.26	
DOX	2.37±0.50	§p:0.001,
ML	1.35±0.21	
DOX+ML	1.17±0.44	♯p:<0.002

Group 1: Control group; Group 2: DOX; Group 3: ML; Group 4: DOX + ML. OSI = ((TOC, µmole H_2O_2 Equiv./gram protein)/(TAC, µmole H_2O_2 Equiv./gram protein) × 100. Group Comparisons: §p= grup1 and grup 2; ¶p= grup 1 and grup 3; ‡p= grup1 and grup 4; ♯p= grup 2 and grup 4.

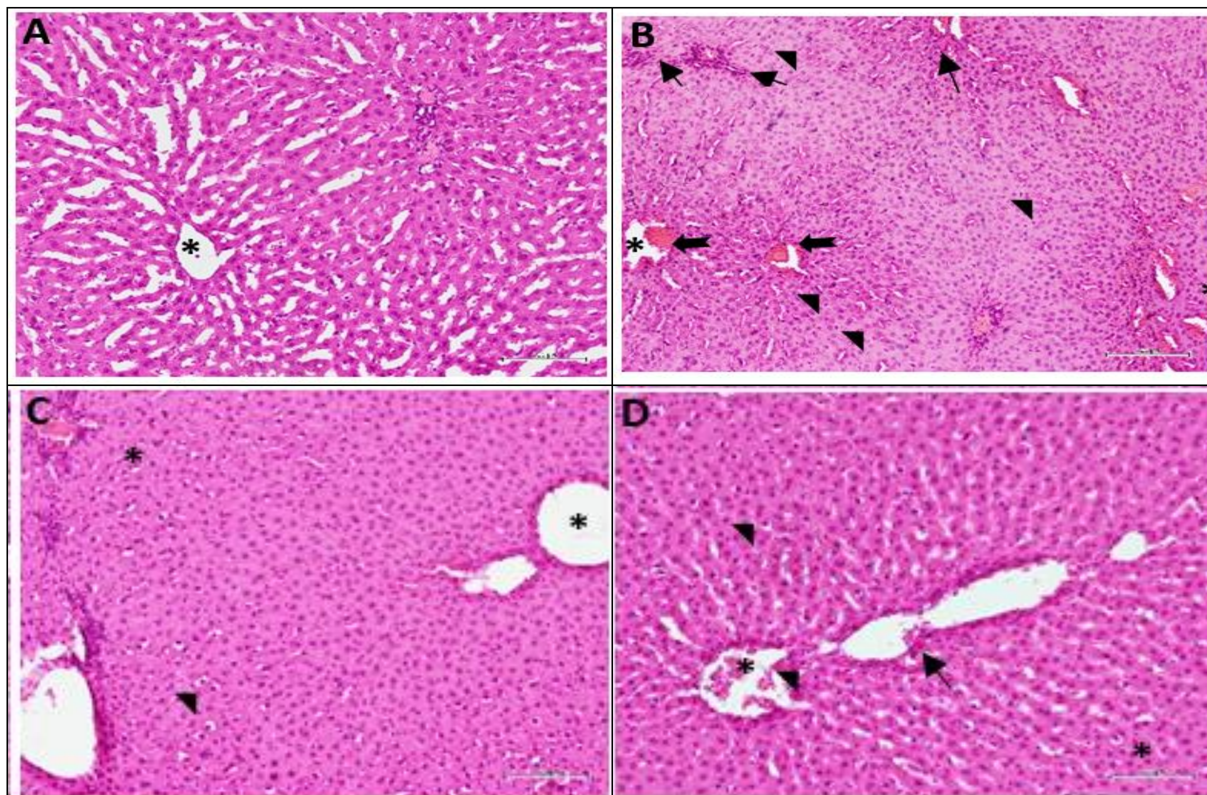
Histopathological results

Inflammatory cell infiltration showing intergroup comparisons was commonly observed in DOX type stroma, while almost none was observed in control types. Congestion and hemorrhagic areas were observed in all groups except the control group (Figure 1A.). Especially hemorrhagic areas were frequently observed in the DOX group.. Hemorrhage was observed in the DOX group both in and around the vessel lumen (Figure 1B). Hepatosteatosis and high amounts were scored in DOX and DOX+ML groups. Steatosis was mostly observed in the connections close to the hepatic triad. The lowest pyknotic nucleus rate was observed in the DOX+ML group after the control group. This result indicates that cell viability and survival are preserved in the DOX+ML group compared to the DOX group. (Table 4. and Fig.1.)

Table 4: Effects of ML on some pathological changes in liver tissue of DOX-treated rats

Group	Inflammatory cell infiltration	Number of pyknotic nuclei	Hepatosteatosis	Hemorragie
Control	0.26±0.07	1.93±0.02	0.20±0.07	0.10±0.04
DOX	2.50±0.02§p	10.13±0.62§p	2.53±0.02§p	2.07±0.02§p
ML	0.50±0.01¶p	2.20±0.35	0.40±0.02¶p	1.06±0.01¶p
DOX+ML	1.23±0.08‡p, ♯p	4.26±0.07‡p, ♯p	0.66±0.17‡p, ♯p	1.20±0.02‡p, ♯p

Group 1: Control group; Group 2: DOX; Group 3: ML; Group 4: DOX + ML. Group Group Comparisons: §p= grup1 and grup 2; ¶p= grup 1 and grup 3; ‡p= grup1 and grup 4; ♯p= grup 2 and grup 4.



These are micrographs belonging to the groups A: control, B: DOX, C: ML, D: DOX+ML. (It belongs to 200x magnification). Asterix indicates central vein, arrowhead indicates steatosis, thick arrows indicate congestion and hemorrhages, and thin arrows indicate inflammatory cell areas.

Fig 1: Liver section micrographs of the groups with H-E staining applied.

Dıscussıon

DOX is widely used in the treatment of various malignant diseases, including breast, ovarian, testicular, thyroid, lung and hematologic cancers. However, its use is limited due to its cytotoxic effect on both normal and cancerous cells (Alshabanah *et al*., 2010 [3]; Biller, 2014 [6]; Gibson-Corley *et al*., 2013; Rivankar, 2014) [20]. In our study, we found that although liver TAC concentrations decreased with DOX administration, TOC levels increased. Therefore, DOX administration in animals is characterized by increased TOC levels and decreased TAC concentrations. Furthermore, liver TOC levels decreased with ML treatment, but TAC concentrations increased with ML treatment. Recent studies have shown that ML is important in terms of antioxidants (Abdel-Raheem & Khedr, 2014 [1]; Beytur, Ciftci, Oguz, Oguzturk, & Yilmaz, 2012 [4]; Mohamadin *et al*., 2011) [16]. Coskun *et al*. (Coskun *et al*., 2011) [10] and Cuciureanu *et al*.(Cuciureanu *et al*., 2009) [11] reported that ML, as an antioxidant, could reduce MDA and MPO levels. To our knowledge, this is the first study to investigate the effects of ML on the antioxidant system in DOX-induced hepatotoxicity in rats. Our results are consistent with the results of previous studies regarding the increase in oxidative stress in the liver after DOX treatment. These data suggest that the mechanism by which DOX induces oxidative stress in these tissues is related to the mechanisms mediated by free radicals and may travel back with ML treatment.

Prasanna *et al*.(Prasanna, Renu, & Valsala Gopalakrishnan, 2020) [19] concluded that oxidative stress is the primary

cause of DOX-induced liver injury. As a result of DOX-induced oxidative stress, electrons are lost from oxygen, leading to the production of superoxide radicals and reactive oxygen species (ROS). High levels of ROS also lead to an increase in lipid peroxidation. This results in damage to hepatocytes and liver. In our study, it was found that DOX+ML group increased TAC levels and decreased TOC levels compared to DOX group.

Mohamadin *et al*. reported that ML administration protects the liver from oxidative damage, as demonstrated by decreased liver marker enzymes, markers of protein oxidation and neutrophilic infiltration, as well as increased antioxidant cascade. These effects of ML may be due to its antioxidant structure, which includes free radical scavenging properties (Mohamadin *et al*., 2011) [16].

Histologic evaluation showed that TAC levels and tissue degeneration decreased with ML administration. ML shows antioxidant properties. As a result of our study, we observed the protective effect of ML on liver antioxidant/oxidant after DOX-induced liver injury. It can be said that ML plays an active role in reducing oxidative stress and shows antioxidant properties (Coskun *et al*., 2011; Cuciureanu *et al*., 2009 [11]; Mohamadin *et al*., 2011) [16].

As a result of the examination of liver tissues removed from the subjects in our study, mononuclear cell infiltration, hyperchromatic nuclei in hepatocytes, dilatation in sinusoids and vacuolar degeneration were reported as important structural changes in the DOX group. In many previous studies conducted with DOX, it was determined that the histopathological changes occurring in the liver tissue were structural changes similar to the findings of our study (Bilgic & Ozgocmen, 2019 [5]; El-Sayyad *et al*., 2009 [12]; Sikandar *et al*., 2020) [23].

When we compared the liver tissue of the DOX+ML group with the group administered DOX alone, a significant decrease in sinusoidal dilatation, inflammation and pyknotic cells was noted. It has been reported that ML application has beneficial effects similar to these findings in different hepatotoxicity models (Mohamadin *et al*., 2011 [16]; Sikandar *et al*., 2020) [23]. The underlying role of ML in these effects is still debated because its molecular mechanisms are quite complex (Beytur *et al*., 2012 [4]; Cevik, Solmaz, Aksoy, & Erbas, 2015) [7].

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Conclusions

As a result, ML shows modulatory effects on oxidative stress and antioxidant redox system in DOX-induced liver toxicity in the rat. More extensive studies are needed on this subject.

References

1. Abdel-Raheem IT, Khedr NF. Renoprotective effects of montelukast, a cysteinyl leukotriene receptor antagonist, against methotrexate-induced kidney damage in rats. Naunyn Schmiedebergs Arch Pharmacol,2014:387(4):341-353. doi:10.1007/s00210-013-0949-x
2. Aljobaily N, Viereckl MJ, Hydock DS, Aljobaily H, Wu TY, Busekrus R, *et al*. Creatine Alleviates Doxorubicin-Induced Liver Damage by Inhibiting Liver Fibrosis, Inflammation, Oxidative Stress, and Cellular Senescence. Nutrients,2020:13(1):41. doi:10.3390/nu13010041
3. Alshabanah OA, Hafez MM, Al-Harbi MM, Hassan ZK, Al Rejaie SS, Asiri YA, *et al*. Doxorubicin toxicity can be ameliorated during antioxidant L-carnitine supplementation. Oxid Med Cell Longe,2010:3(6):428-433. doi:10.4161/oxim.3.6.14416
4. Beytur A, Ciftci O, Oguz F, Oguzturk H, Yilmaz F. Montelukast attenuates side effects of cisplatin including testicular, spermatological, and hormonal damage in male rats. Cancer Chemother Pharmacol,2012:69(1):207-213. doi:10.1007/s00280-011-1692-y
5. Bilgic S, Ozgocmen M. The protective effect of misoprostol against doxorubicin induced liver injury. Biotech Histochem,2019:94(8):583-591. doi:10.1080/10520295.2019.1605457
6. Biller B. Metronomic chemotherapy in veterinary patients with cancer: rethinking the targets and strategies of chemotherapy. Vet Clin North Am Small Anim Pract,2014:44(5):817-829. doi:10.1016/j.cvsm.2014.05.003
7. Cevik B, Solmaz V, Aksoy D, Erbas O. Montelukast inhibits pentylenetetrazol-induced seizures in rats. Med Sci Monit,2015:21:869-874. doi:10.12659/msm.892932
8. Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother,2013:4(4):303-306. doi:10.4103/0976-500x.119726
9. Cohen J. The statistical power of abnormal-social psychological research: a review. J Abnorm Soc Psychol,1962:65:145-153. doi:10.1037/h0045186
10. Coskun AK, Yigiter M, Oral A, Odabasoglu F, Halici Z, Mentes O, *et al*. The effects of montelukast on antioxidant enzymes and proinflammatory cytokines on the heart, liver, lungs, and kidneys in a rat model of cecal ligation and puncture-induced sepsis. ScientificWorldJournal,2011:11:1341-1356. doi:10.1100/tsw.2011.122
11. Cuciureanu M, Căruntu ID, Păduraru O, Stoica B, Jerca L, Crauciuc E, Nechifor M. The protective effect of montelukast sodium on carbon tetrachloride induced hepatopathy in rat. Prostaglandins Other Lipid Mediat,2009:88(3-4):82-88. doi:10.1016/j.prostaglandins.2008.10.004
12. El-Sayyad HI, Ismail MF, Shalaby FM, Abou-El-Magd RF, Gaur RL, Fernando A, *et al*. Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. Int J Biol Sci,2009:5(5):466-473. doi:10.7150/ijbs.5.466
13. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet Pathol,2013:50(6):1007-1015. doi:10.1177/0300985813485099
14. Hjelle JJ, Petersen DR. Metabolism of malondialdehyde by rat liver aldehyde dehydrogenase. Toxicol Appl Pharmacol,1983:70(1):57-66. doi:10.1016/0041-008x(83)90179-5

15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem,1951:193(1):265-275.
16. Mohamadin AM, Elberry AA, Elkablawy MA, Gawad HS, Al-Abbasi FA. Montelukast, a leukotriene receptor antagonist abrogates lipopolysaccharide-induced toxicity and oxidative stress in rat liver. Pathophysiology,2011:18(3):235-242. doi:10.1016/j.pathophys.2011.02.003
17. Moslehi JJ. Cardiovascular Toxic Effects of Targeted Cancer Therapies. N Engl J Med,2016:375(15):1457-1467. doi:10.1056/NEJMra1100265
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem,1979:95(2):351-358. doi:10.1016/0003-2697(79)90738-3
19. Prasanna PL, Renu K, Valsala Gopalakrishnan A. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. Life Sci,2020:250:117599. doi:10.1016/j.lfs.2020.117599
20. Rivankar S. An overview of doxorubicin formulations in cancer therapy. J Cancer Res Ther,2014:10(4):853-858. doi:10.4103/0973-1482.139267
21. Saleh DO, Mahmoud SS, Hassan A, Sanad EF. Doxorubicin-induced hepatic toxicity in rats: Mechanistic protective role of Omega-3 fatty acids through Nrf2/HO-1 activation and PI3K/Akt/GSK-3β axis modulation. Saudi J Biol Sci,2022:29(7):103308. doi:10.1016/j.sjbs.2022.103308
22. Sener G, Kabasakal L, Cetinel S, Contuk G, Gedik N, Yeğen BC. Leukotriene receptor blocker montelukast protects against burn-induced oxidative injury of the skin and remote organs. Burns,2005:31(5):587-596. doi:10.1016/j.burns.2005.01.012
23. Sikandar A, Farhat K, Afzal A, Ajmal K, Laeeq M, Khokhar A. Protective Effects of Trimetazidine Against Doxorubicin-Induced Cardiotoxicity And Hepatotoxicity In Mice. J Ayub Med Coll Abbottabad,2020:32(3):304-309.
24. Timm KN, Ball V, Miller JJ, Savic D, West JA, Griffin JL, Tyler DJ. Metabolic Effects of Doxorubicin on the Rat Liver Assessed With Hyperpolarized MRI and Metabolomics. Front Physiol,2021:12:782745. doi:10.3389/fphys.2021.782745
25. Tuğtepe H, Sener G, Cetinel S, Velioğlu-Oğünç A, Yeğen BC. Oxidative renal damage in pyelonephritic rats is ameliorated by montelukast, a selective leukotriene CysLT1 receptor antagonist. Eur J Pharmacol,2007:557(1):69-75. doi:10.1016/j.ejphar.2006.11.009
26. Van Sluyters R, Obernier JA. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Contemporary Topics in Laboratory Animal Science,2004:43:48-52.
27. Yu X, Ruan Y, Huang X, Dou L, Lan M, Cui J, Shen T. Dexrazoxane ameliorates doxorubicin-induced cardiotoxicity by inhibiting both apoptosis and necroptosis in cardiomyocytes. Biochem Biophys Res Commun,2020:523(1):140-146. doi:10.1016/j.bbrc.2019.12.027