ORIGINAL RESEARCH

Protective effects of molsidomine against doxorubicin-induced renal damage in rats

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Abstract

Purpose: The purpose of this study was to investigate the therapeutic and protective effects of molsidomine (MLS) against doxorubicin (DOX)-induced renal damage in rats.

Methods: Forty rats were randomly divided into five groups (control, MLS, DOX, DOX+MLS and MLS+DOX groups). Thiobarbituric acid reactive substance (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO) and glutathione peroxidase (GPx) levels were determined from kidney tissues and blood urea nitrogen (BUN), creatinine (Cr) and albumin (Alb) levels also determined.

Results: DOX treatment caused a significant increase in TBARS levels and a significant decrease in the GSH and CAT levels compared with the control group. In comparison, MLS administration before DOX injection caused a significant decrease in TBARS levels and also increases in GSH and CAT levels, whereas treatment of MLS after DOX injection did not show any beneficial effect on these parameters. All groups showed a significant increase in NO levels compared to the control group. There were no significant differences among the all groups for BUN and Cr levels. Serum level of Alb decreased in the DOX-treated groups when compared with control and MLS groups. The histopathological findings were in accordance with the biochemical results. MLS treatment reversed the DOX-induced kidney damage in group 4. MLS treatment before DOX injection exerted a protective effect against DOX-induced kidney damage.

Conclusions: MLS shows promise as a possible therapeutic intervention for the prevention of kidney injury associated with DOX treatment. Additional studies are warranted.

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Doxorubicin (DOX), an anthracycline antibiotic, is used for the treatment of human neoplasms, including leukaemias, lymphomas and solid tumors [1]. The use of DOX is limited due to its side-effects such as nephrotoxicity and cardiotoxicity [2]. The mechanism(s) of DOX-toxicity on the kidneys is not yet clear; however, it may be caused by an imbalance of the oxidant-antioxidant systems [3]. The toxicity may be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, membrane lipid peroxidation (LPO) and protein oxidation [4]. It is well established that tissue injury results from the disturbance in the oxidant-antioxidant systems, as demonstrated with LPO and protein oxidation [5].

As a nitric oxide (NO) donor, molsidomine (MLS) is a prodrug and a potent vasodilator that has been used widely as an anti-anginal agent [6]. In the liver, MLS decarboxylases enzymatically to form 3-morpholinosyndnonimine (SIN-1), which spontaneously liberates NO. [7]. MLS was recently shown to have antioxidant and anti-inflammatory properties [6, 8]. Studies demonstrated that the systemic administration of MLS improves survival rates and reduces the renal damage induced by renal ischemia-reperfusion (I-R) in rats, suggesting that it could be a useful agent to protect renal function [9-11]. Chander and Chopra [12] recently reported that MLS showed renoprotective effects against ischemia-reperfusion induced renal damage.

The aim of the current study was to verify the effects of MLS on DOX-induced renal damage in the light of these previously reported biochemical and histopathological findings.

Materials and Methods

Animals

For this study, 40 female Wistar albino rats, aged 10-12 weeks and weighing 250-300 g, were housed in an air-conditioned room with 12-h light and dark cycles, with constant temperature (22±2 °C) and relative humidity (65-70%). The rats were fed standard commercial pellets and water *ad libitum*. All experimental protocols were approved by the Inonu University School of Medicine Animal Care and Use Committee, Malatya, Turkey.

Experimental Protocols

The rats were randomly divided into five groups (n=8/group) as follows: (1) control group; (2) MLS (Molsicor tb® 10 mg, Sandoz, Turkey, 10 mg/kg daily for 21 days per-orally (p.o.)); (3) DOX (Doxorubicin® 50 mg, Farmar, Turkey, single dose 20 mg/kg i.p. at first day); (4) DOX+MLS (single dose of DOX 20 mg/kg i.p. plus 10 mg/kg MLS daily for 21 days p.o., after 3 days injected DOX; and, (5) MLS+DOX (10 mg/kg MLS daily for 21 days p.o. plus single dose of DOX 20 mg/kg i.p. after the last dose of MLS). The dosage of DOX (20 mg/kg) and MLS (10 mg/kg p.o.) were selected according to the levels used successfully in the same experimental models for the oxidant and antioxidant properties, respectively [6, 13].

On the 24th day, rats in all groups were killed with high doses of the anesthesia mixture and the kidney tissues collected. Some kidney tissue was placed into liquid nitrogen and stored at –70°C until assayed for thiobarbituric acid reactive substance (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), NO and glutathione peroxidase (GPx). The remaining kidney tissue was fixed 10% formalin for histopathological analyses. Trunk blood was extracted to evaluated serum levels of blood urea nitrogen (BUN), creatinine (Cr) and albumin (Alb).

Biochemical Analysis

The levels of homogenized tissue TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction using the method of Yagi [14]. The product was evaluated spectrophotometrically at 532 nm and results are expressed as nmol/g tissue.

GSH levels were measured spectrophotometrically at 412 nm using the method of Sedlak and Lindsay [15] and expressed as nmol/ml.

SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2 generated by the xanthine/xanthine oxidase system [16]. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The product was evaluated spectrophotometrically at 560 nm and levels were expressed as U/mg protein.

CAT activity was determined according to the method of Aebi [17]. The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity and the enzyme activities are given in kU/mg protein.

GPx activity was measured by the method of Paglia and Valentina [18]. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured. GPx activity is expressed as IU/mg protein [18].

Total nitrite was considered as an index of NO production [19] so NO levels were measured as total nitrite. The kidney tissues were homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.5) (containing protease inhibitor, phenylmethylsulfonyl flüoride and 1 mM PMSF) with a homogenizer (IKA Ultra Turrax T25 basic) at 16.000 rpm for 3 min at 4°C. The homogenate was centrifuged at 10000 X g for 20 min at 4°C. All nitrate molecules in the supernatant were reduced to nitrites using nitrate reductase, and total nitrite levels were measured by the Griess reaction [20] with minor modifications. Total nitrite levels in the kidney were expressed as nmol/g wet tissue.

Serum levels of BUN, Cr, and Alb were assayed using an Olympus Autoanalyzer (Olympus Instruments, Tokyo, Japan).

Histopathological Analysis

Rat kidney tissue samples were placed in 10% formalin solution and processed by embedding in paraffin. Five-micrometer thick sections were cut, deparaffinized, hydrated and stained with hematoxylin and eosin (H&E) and examined under light microscope. The renal sections from all groups were examined in a blind fashion for tubular cell swelling, cellular vacuolization, pyknotic nuclei, medullary congestion and inflammatory infiltration. Inflammation was evaluated as chronic or acute phase. A minimum of 10 fields for each kidney slide were examined and a score from 0 to 3 was given for each tubular profile involving an intersection: 0; normal histology, 1 (mild); tubular cell swelling, brush border loss, nuclear condensation, with up to one-third of tubular changes and inflammation, 2 (moderate); as for score 1, however greater than one-third and less than two-thirds of tubular changes and inflammation, and 3 (severe); greater than two-thirds of tubular degeneration and inflammation.

Statistical Analysis

For detecting minor effects, the sample size required for this experiment was identified using statistical power analysis. The sample size necessary for a power of 0.80 were estimated using NCSS software. All statistical analyses were conducted using SPSS statistical software (SPSS, version 18.0, for Windows, SPSS Inc., Chicago, IL). Distributions of the groups were analyzed with the Kolmogorov-Smirnov test. According to the results obtained from the normality test, one-way analysis of variance (ANOVA) and the Kruskal–Wallis H test were used for the statistical analysis as appropriate. After a significant Kruskal–Wallis H test, a Conover test was also carried out for Cr. Results were presented as mean ± SD. The Mann-Whitney U test was performed for groups which not show normal distribution. The results were showed as median (min-max). $p<0.05$ was regarded as statistically significant in all data.

Results

Body and Kidney Weight

No animals died during or after the injections or surgical procedures. There were no differences between the body and kidney weights before and after the experiments among the groups (data not shown).

Biochemical results

The kidney TBARS, SOD, CAT, GSH, NO and GPx levels are presented in the Table 1. Briefly, it was determined that DOX induced a significant increase in kidney TBARS levels, whereas CAT and GSH contents decreased significantly as compared with the control group ($p<0.05$). On the other hand, MLS administration before DOX injection (Group 5) caused significant decreases in TBARS production and increases in GSH and CAT levels ($p<0.05$). When compared with the DOX injected group, in group 4 (DOX+MLS) MLS treatment did not show any beneficial effect on these parameters. There were significant increases in NO levels in all groups except the control group ($p<0.05$).

The levels of BUN, Cr, and Alb are shown in Table 2. There were no significant differences among the all group for the BUN and Cr levels. Whereas serum level of Alb decreased in the DOX-treated groups when compared to the control and MLS groups.

Histopathological Results

Histopathological results were presented in the Table 3. In brief, there were no renal histological alterations in control and MLS groups (Figure 1). Tubular damage and moderate inflammation were higher in DOX treated group than control, MLS, and MLS+DOX groups ($p<0.0001$) (Figures 2 and 3). There was no significant difference between MLS and MLS+DOX groups for inflammation ($p=0.0659$); however, treatment with MLS before DOX injection was more effective than treatment with MLS after DOX injection with respect to protection of tubular structures ($p <0.05$) (Figure 4).

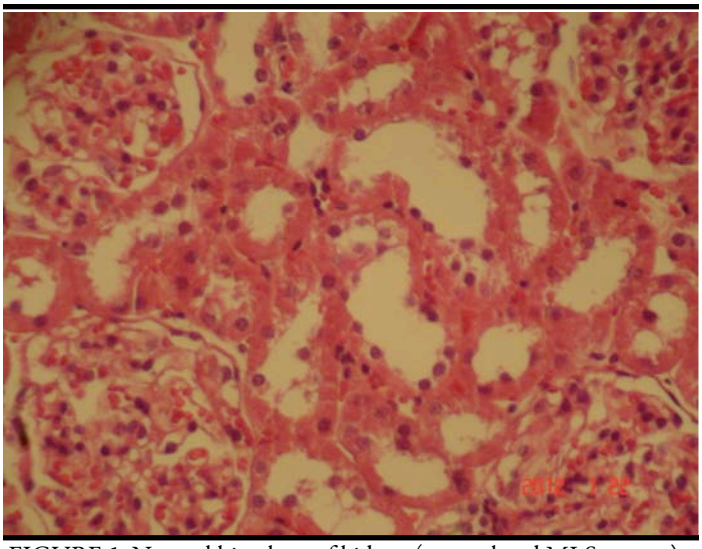

FIGURE 1. Normal histology of kidney (control and MLS groups). H&E 200X.

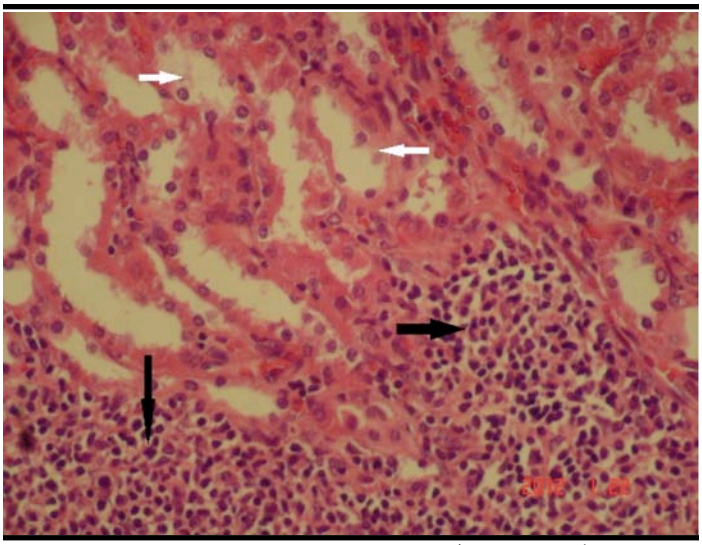

FIGURE 2. Moderate tubular degeneration (white arrow) and moderate inflammation (black arrow) (DOX group). H&E 200X.

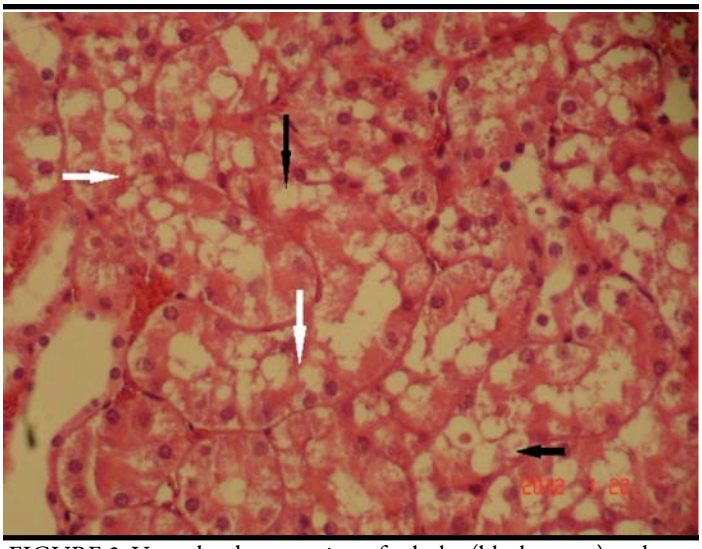

FIGURE 3. Vacuolar degeneration of tubules (black arrow) and desquamation of tubules epithelium (white arrow) (DOX group). H&E 200X.

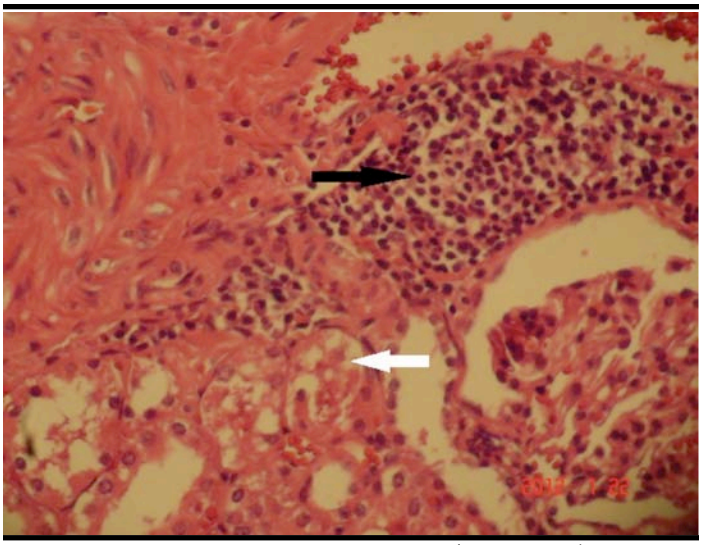

FIGURE 4. Minimal chronic inflammation (black arrow) and mild tubular vacuolization (white arrow) (MLS+DOX group). H&E 200X.

Discussion

In the current study, the effects of MLS on DOX-induced renal injury were investigated in the light of biochemical parameters and histopathological observations. DOX is an effective and widely used broad spectrum chemotherapeutic agent; however, its clinical use is limited because of its serious and dose-dependent cardiotoxicity and nephrotoxicity [21, 2]. Clinical and experimental investigations suggest that the increased oxidative stress associated with an impaired antioxidant defense status initiates the cascade of reactions responsible for DOX-induced cardiotoxicity and nephrotoxicity [22, 23]. DOX-induced cardiotoxicity and its mechanisms have been much investigated [24]; however, DOX-induced nephrotoxicity has not been yet investigated.

The free radical formation of DOX toxicity occurs through two mechanisms [25]: 1) enzymatic (NADPH-reductases), which forms superoxide radicals in the presence of oxygen; and, 2) non-enzymatic, which involves reactions of the iron-DOX complex, which can reduce oxygen

TABLE 1. Levels of biochemical parameters in renal tissue

Parameters	Control	MLS	DOX	DOX+MLS	MLS+DOX
TBARS	175±41	201±36	260±44*	199±46	181±51†
SOD	0.275±0.106	0.299±0.057	0.285±0.067*	0.201±0.037*	0.262±0.045
CAT	155±23	153±12	126±17*	120±16	131±19†
GSH	2.59±0.79	2.79±0.35	1.81±0.45*	2.23±0.37	2.75±0.51†
NO	52±26	137±35*	104±26*	131±30*	132±24*
GPx	1.06 (0.91-1.73)	1.11 (0.92-2.49)	0.99 (0.9-1.5)	0.96 (0.81-1.24)	1.07 (0.88-1.43)

*compared with control group; †compared with DOX group.
TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; NO, nitric oxide; GPx, glutathione peroxidase

TABLE 2. The serum levels of blood urea nitrogen, creatine and albumin levels

Group Names	BUN (mg/dL) Mean+SD	Cr (mg/dL) Median (Min-Max)	Albumin (g/dL) Mean+SD
Control	44.28±5.76	0.53(0.46-0.55)	1.3±0.1
MLS	33.00±5.16	0.48(0.44-0.53)	1.28±0.13
DOX	41.28±5.28	0.47(0.44-0.63)	0.8±0.1*
DOX+MLS	54.00±15.87	0.48(0.43-0.55)	0.74±0.07*
MLS+DOX	50.42±12.29	0.56(0.48-0.65)	0.82±0.16*

*p < 0.05 compared with control group.
BUN, Blood urea nitrogen
Cr, creatine
MLS, molsidomine
DOX, doxorubicin

TABLE 3. Histopathological scores of all groups

Groups	Renal tubule degeneration	Inflammation
Control	1(1-2)	1(1-2)
MLS	1(1-2)	1(1-2)
DOX	3(3-3) †	2(1-3)
MLS+DOX	2(2-2) *, †	1(1-2)
DOX+MLS	3(2-3) *, †	1(1-2)
P value	<0.0001	0.659

* compared with control group; † compared with DOX group.
MLS, molsidomine
DOX, doxorubicin

to H_2O_2 and other reactive oxygen radicals (ROS) [26, 27]. Several conditions are known to disturb the balance between the production of ROS and the cellular defense mechanisms, resulting in dysfunction and cellular destruction. An imbalance between pro- and antioxidant factors plays an important role in many disease processes [24, 28].

In the present study, DOX treatment caused a significant increase in TBARS levels and a significant decrease in GSH and CAT levels, when compared with the control group. These findings are in agreement with previous reports [25, 29]. TBARS, a stable metabolite of the free radical mediated lipid peroxidation cascade, is widely used as a marker of oxidative stress and lipid layer destruction [30]. Elevated TBARS levels show that DOX causes oxidative stress and free radical formation in kidney tissue. GSH plays an important role in the detoxification of xenobiotic compounds and antioxidation of ROS and free radicals. Low levels of GSH are associated with excessive oxidative stress [31]; supporting our findings, in which a decline in GSH levels is combined with an increase in oxidative stress, as evidenced by increased LPO. Our histopathological findings, marked swelling of epithelial cell, tubular dilatation, tubular desquamation and loss of brush border, also support the DOX-induced oxidative stress in the kidney tissue.

Previous studies have stated that MLS, a spontaneous NO donor, is a potent vasodilator and has been used widely as an anti-anginal agent [6]. Based on this relationship, according to our biochemical evaluations, levels of NO in the group receiving MLS alone are also higher than seen in the control and other treatment groups (Table 1). The clinical applications of MLS in the field of cardiology are well established, especially relating to the symptomatic treatment of angina [6-9]. Apart from these properties, it is also well established that MLS exerts antioxidant and anti-inflammatory effects [32-34].

MLS could exert protective effects on the enzymatic defense system and lipid peroxidation. MLS treatment before DOX injection in the rat model decreased TBARS levels and increased GSH, CAT and NO levels, as compared with DOX alone. Also, administration of MLS significantly improved the deteriorated renal architecture. The reduction of TBARS and increase in GSH and CAT levels show that MLS has antioxidant effects. The enhanced GSH level in MLS-treated animals may partially explain its mechanism of protection: the elevated levels of GSH could provide a thiol group for the possible GSH-mediated detoxification reactions of GPx. Moreover, the enhanced CAT activity in the MLS-treated group may be involved in the scavenging of free radicals generated from DOX [6,9,10]. Our results are consistent with these earlier biochemical and histopathological findings.

In the treatment groups, NO levels were found to be higher than those in the control group. NO is a free radical gas that acts as a cytoprotective or a cytotoxic agent. It is generated by either endothelial nitric oxide synthase (eNOS) or inducible nitric oxide synthase (iNOS) [35]. Another radical-forming mechanism in this type of experimental protocol might be NO-producing system. The high levels of NO react with superoxide anions, resulting in the formation of peroxynitrite. Peroxynitrite is a potent and aggressive cellular oxidant [36, 37]. The increased NO levels in the DOX group (Group 3) could be explained by this mechanism. On the other hand, Gurlek *et al.* [38] reported that NO has a dual effect; both protective and toxic. In the current study, treatment with MLS (alone, before and after DOX; Groups 2, 4, and 5) caused an increase in NO levels. According to our biochemical findings, reduction of TBARS and elevated of GSH and CAT levels clearly show that MLS, a pivotal NO donor, has protective effects against DOX-induced renal injury.

In the histopathologically examinations, tubular damage and inflammation were higher in the DOX-treated group than the other groups. MLS treatment (before and after DOX) ameliorated the histopathological damage caused by DOX; however, treatment with MLS before DOX injection was more effective than treatment with MLS after DOX injection, with respect to protection of tubular structures ($p<0.05$). Thus, our histopathological results are in agreement both with our biochemical findings and with previously published data [11, 12, 39, 40].

One of the interesting results of the current study is that there were no significant differences in the levels of BUN and Cr among the five groups. One of the possible explanations of this result is that Cr, as an indicator of glomerular filtration rate (GFR), is based upon its constant production from muscle creatine and its relatively constant renal excretion rates. Cr is removed from plasma by glomerular filtration and then excreted in the urine without significant tubular reabsorption. Early in the course of acute kidney injury, for example, the GFR is markedly reduced; however, there has not yet been time for Cr to accumulate and, therefore, levels of serum Cr do not always accurately reflect the degree of renal dysfunction [39]. Thus, moderate changes in GFR may not be detected by serum Cr levels or BUN. In this study, DOX-injected rats showed low plasma Alb levels, corroborating previous studies reporting hypoalbuminemia in DOX-treated rats [40, 41].

One of the limitations of the current study is that quantitative analysis of an inflammation marker was not done.

We focused especially on the antioxidant properties of MLS, rather than the anti-inflammatory effects [9, 11]. The analysis of kidney histology used a semiquantitative (*vs*. quantitative) method involving grading of the anti-inflammatory effects of MLS (Table 3).

The second limitation of the study is that only 20 mg/kg dose of DOX and 10 mg/kg dose of MLS were used, rather than a range of different doses: only the most effective dosages of DOX and MLS, as reported in the literature, were used.

In summary, this study is the first report regarding the protective effects of MLS against DOX-induced nephrotoxicity as determined by both biochemical parameters and histopathological observations. The preventive effects of MLS on these renal lesions may occur via its antioxidant and anti-inflammatory properties. In addition, MLS treatment, either befor or after DOX injection, had no effect on nephrotoxicity. Further experimental and clinical studies, using longer times and varied dosages and more detailed analysis of the pathways, are required to confirm these findings before applying MLS clinically to treat DOX-related renal injury.

References

1. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013 Feb;65(2):157-70.
2. Jovanovic D, Djukanovic I, Susic D, Funduk G, Jovanovic Z, Dragojlovic Z, et al. The effect of captopril on the development of adriamycin nephropathy in rats with spontaneous arterial hypertension. Srp Arh Celok Lek. 1996; 124 suppl 1: 47-49.
3. Saad SY, Najjar TA, Al-Rikabi AC. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. Pharmacol Res 2001;43: 211–218.
4. Liu LL, Li QX, Xia L, Li J, Shao L. Differential effects of dihydropyridine calcium antagonists on doxorubicin-induced nephrotoxicity in rats. Toxicology. 2007; 231(1):81-90.
5. Karaman A, Fadillioglu E, Turkmen E, Tas E, Yilmaz Z. Protective effects of leflunomide against ischemia-reperfusion injury of the rat liver. Pediatr Surg Int. 2006; 22(5):428-434.
6. Gupta A, Sharma S, Chopra K. Reversal of iron-induced nephrotoxicity in rats by molsidomine, a nitric oxide donor. Food Chem Toxicol. 2008; 46(2):537-543.
7. Kukovetz WR, Holzmann S. Cyclic GMP as the mediator of molsidomine-induced vasodilatation. Eur J Pharmacol. 1986; 122: 130–109.
8. Walder CE, Thiemermann C, Vane JR. The involvement of endothelium-derived relaxing factor in the regulation of renal cortical blood flow in the rat. Br J Pharmacol. 1991; 102: 967–973.
9. Garcia-Criado FJ, Eleno N, Santos-Benito F, Valdunciel JJ, Reverte M, Lozano-Sánchez FS, et al. Protective effect of exogenous nitric oxide on the renal function and inflammatory reaction in a model of ischemia-reperfusion.Transplantation.1998; 66: 982–990.
10. Ozturk H, Aldemir M, Buyukbayram H, Dokucu AI, Otcu S. The effects of the nitric oxide donor molsidomine prevent in warm ischemia-reperfusion injury of the rat renal – a functional and histopathological study. Int Urol Nephrol 2001; 32: 601–607.
11. Rodriguez-Peña A, Garcia-Criado FJ, Eleno N, Arevalo M, Lopez-Novoa JM. Intrarenal administration of molsidomine, a molecule releasing nitric oxide, reduces renal ischemia-reperfusion injury in rats. Am J Transplant. 2004; 4(10):1605-1613.
12. Chander V, Chopra K. Renal protective effect of molsidomine and L-arginine in ischemia-reperfusion induced injury in rats. J Surg Res. 2005; 128(1):132-139.
13. Sahna E, Parlakpinar H, Ozer MK, Ozturk F, Ozugurlu F, Acet A. Melatonin protects against myocardial doxorubicin toxicity in rats: role of physiological concentrations. J Pineal Res 2003;35(4):257-261.
14. Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol. 1998; 108:101–106.
15. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192–205.
16. Sun Y, Oberley LW, Li YA. Simple method for clinical assay of superoxide dismutase. Clin Chem. 1988; 34:497–500.
17. Aebi H. Catalase. In: Bergmeyer HU (Ed.) In Methods of Enzymatic Analysis, New York, NY: Academic Press; 1974: 673–677.
18. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70:158– 169.
19. Parlakpinar H, Sahna E, Acet A, Mizrak B, Polat A. Effect of caffeic acid phenethyl ester (CAPE) on myocardial ischemia-reperfusion induced apoptotic cell death. Toxicology 2005; 207: 169–177.
20. Ozbek E, Turkoz Y, Gokdeniz R, Davarci M, Ozugurlu F. Increased nitric oxide production in the spermatic vein of patients with varicocele. Eur Urol. 2000; 37: 172–175.
21. Ibrahim MA, Ashour OM, Ibrahim YF, El-Bitar HI, Gomaa W, Abdel-Rahim SR. Angiotensin-converting enzyme inhibition and angiotensin AT(1)-receptor antagonism equally improve doxorubicin-induced cardiotoxicity and nephrotoxicity. Pharmacol Res. 2009; 60(5):373-381.
22. Kumar D, Kirshenbaum LA, Danelisen I, Singal PK. Apoptosis in adriamycin cardiomyopathy and its modulation by probucol. Antioxid Redox Signal. 2001; 3:135–145.

23. Nakamura T, Ueda Y, Juan Y, Katsuda S, Takahashi H, Koh E. Fas mediated apoptosis in adriamycin-induced cardiomyopathy in rats. Circulation. 2000; 102: 572–578.
24. Cigremis Y, Parlakpinar H, Polat A, Colak C, Ozturk F, Sahna E, et al. Beneficial role of aminoguanidine on acute cardiomyopathy related to doxorubicin-treatment. Mol Cell Biochem. 2006; 285(1-2):149-154.
25. Abo-Salem OM. The protective effect of aminoguanidine on doxorubicin-induced nephropathy in rats. J Biochem Mol Toxicol. 2012; 26(1):1-9. doi:10.1002/jbt.20422.
26. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology 2002; 180:79–95.
27. Injac R, Radic N, Govedarica B, Perse M, Cerar A, Djordjevic A, et al. Acute doxorubicin pulmotoxicity in rats with malignant neoplasm is effectively treated with fullerenol C60(OH)24 through inhibition of oxidative stress. Pharmacol Rep. 2009; 61:335–342.
28. McCord JM. Human disease, free radicals, and the oxidant/antioxidant balance. Clin Biochem. 1993; 26: 351–357.
29. Usta Y, Ismailoglu UB, Bakkaloglu A, Orhan D, Besbas N, Sahin-Erdemli I, et al. Effects of pentoxifylline in adriamycin-induced renal disease in rats. Pediatr Nephrol 2004; 19:840–843.
30. Sahna E, Parlakpinar H, Cihan OF, Turkoz Y, Acet A. Effects of aminoguanidine against renal ischaemia-reperfusion injury in rats. Cell Biochem Funct. 2006; 24: 137-141.
31. Bray TM, Taylor CG. Tissue glutathione, nutrition, and oxidative stress. Can J Physiol Pharmacol. 1993; 71(9):746-751.
32. Bentli R, Parlakpinar H, Polat A, Samdanci E, Sarihan ME, Sagir M. Molsidomine Prevents Cisplatin-induced Hepatotoxicity. Arch Med Res. 2013; 4(7): 521–528.
33. Kilic T, Parlakpinar H, Polat A, Taslidere E, Vardi N, Sarihan E, Ermis H, Tanbag K. Protective and Therapeutic Effect of Molsidomine on Bleomycin-Induced Lung Fibrosis in Rats.Inflammation. 2014 Aug;37(4):1167-78.
34. Toplu Y, Parlakpinar H, Sapmaz E, Karatas E, Polat A, Kizilay A. The protective role of molsidomine on the cisplatin-induced ototoxicity. Indian J Otolaryngol Head Neck Surgery. 2014 Sep;66(3):314-9
35. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. J Biol Chem. 1994; 269: 13725–13728.
36. Ayla S, Seckin I, Tanriverdi G, Cengiz M, Eser M, Soner BC, et al. Doxorubicin induced nephrotoxicity: protective effect of nicotinamide. Int J Cell Biol. 2011;2011:390238
37. Irmak MK, Fadillioglu E, Sogut S, Erdogan H, Gulec M, Ozer M, et al. Effects of caffeic acid phenethylester and alpha-tocopherol on reperfusion injury in rat brain. Cell Biochem Funct. 2003; 21(3):283–289.
38. Gurlek A, Aydogan H, Parlakpinar H, Bay-Karabulut A, Celik M, Sezgin N, et al. Protective effect of melatonin on random pattern skin flap necrosis in pinealectomized rat. J Pineal Res. 2004; 36(1):58-63.
39. Rule AD, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by cystatin C among different clinical presentations. Kidney Int. 2006;69(2):399-405.
40. Rashikh A, Pillai KK, Ahmad SJ, Akhtar M, Najmi AK. Aliskiren alleviates doxorubicin-induced nephrotoxicity by inhibiting oxidative stress and podocyte injury. J Renin Angiotensin Aldosterone Syst. 2013;14: 14-22.
41. Mansour M, El-Kashef H, Al-Shabanah O. Effect of captopril on doxorubicin-induced nephrotoxicity in normal rats. Pharmacol Res 1999; 39: 239–245.