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ORIGINAL RESEACRH

Investigation of the Effect of Milrinone on Renal Damage in an Experimental Non-Heart Beating Donor Model

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ABSTRACT

Purpose: In our study, it was aimed to investigate the preventive effect of milrinone on renal damage in experimental controlled non-heart-beating donors (NHBDs) model. *Materials and Methods*: Sixteen rats randomly divided into 2 groups, 8 rats in each were used. Group 1 was control, group 2 was milrinone group. Group 1 rats received 1.25 ml 0.09% NaCl intraperitoneally equivalent to the milrinone diluted volume. Group 2 rats were administered intraperitoneally with 0.5 mg/kg of milrinone 2 hours before cardiac arrest. After the cardiac arrest, left nephrectomy was applied to the rats. Malondialdehyde, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) activities, Caspase-3 (apoptotic index) and histopathological evaluation were performed in the tissues. *Results*: In the milrinone group, the total injury score was significantly lower relative to the control group ($p = 0.001$). Caspase-3 staining was moderately strong in the control group but weaker in the milrinone group. Apoptotic index was significantly lower in the milrinone group compared to the control group ($p = 0.001$). In comparison between groups, SOD and GPx in the milrinone group was significantly higher than the control group ($p = 0.008$, $p = 0.006$). *Conclusions*: Milrinone has been shown to be effective in the prevention of tissue damage due to oxidative stress and inflammatory process in the renal of warm ischemia in the experimental NHBDs model and in protecting the renal. Milrinone increases antioxidant activity while reducing apoptosis. Systemic administration of milrinone prior to cardiac arrest may be beneficial. Administration of milrinone to the recipient in the perioperative period may contribute to donor function.

Keywords: Kidney; transplantation; ischemia-reperfusion; apoptosis; milrinone; rat

INTRODUCTION

Renal transplantation is the most effective treatment of recent renal failure. However, necessary number of transplantation cannot occur, because the number of donors does not increase parallel to the increase in the number of patients waiting for organs. The vast majority of organ donors are patients with cerebral death. In recent years, the use of marginal donors and non-heart-beating donors (NHBDs) has been widespread to expand the donor pool [1]. It is estimated that the organ pool is expanding by about 20% with NHBDs. Studies on early NHBDs graft survival show that 73–91% of 1-year surveys and 58–80% of 5-year surveys [2, 3]. These results are comparable to brain-death donors [4]. But the rate of hot ischemia in NHBDs is higher than in brain-death donors. Long hot ischemic period may cause tubular necrosis caused

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by ischemic reperfusion injury [5]. The production of free oxygen radicals increases after reperfusion due to ischemia reperfusion injury. The coagulation process is actively disturbing the renal microcirculation [6, 7]. Free oxygen radicals activate neutrophils and monocytes and cause the release of cytokines such as tumor necrosis factor (TNF)-alpha. TNF-alpha activates intracellular caspase-8, leading to apoptosis and organ damage [8]. Reducing cytokine release may also reduce ischemia-reperfusion injury.

Milrinone is a phosphodiesterase (PDE) 3 inhibitor used in acute congestive heart failure [9]. Milrinone increases heart contraction by increasing intracellular cyclic adenosine monophosphate. Milrinone also provides vasodilatation. Increased cyclic adenosine monophosphate allows reduction of TNF-alpha-induced damage [10]. Milrinone has been shown to reduce ischemic reperfusion damage in the lung, liver, heart and rat renal [11–13]. However, there are not sufficient studies on the effects of milrinone on the kidneys in Experimental NHBDs Model.

In our study, it was aimed to investigate the preventive effect of milrinone on renal damage in rats with experimental controlled NHBDs model.

MATERIALS AND METHODS

Rat Groups

Sixteen female Wistar albino rats, randomly divided into 2 groups as 8 rats in each, weighing between 200 g and 250 g, were used. Control (Group 1) (n: 8), milrinone group (Group 2) (n: 8) were determined.

Anesthesia and Surgical Technique

Rats were kept under 18–24°C (12 hours of day-night cycle) in cages (4 rats in each) with free water and feed intake. Standard rat feed and water were given to the rats. Group 1 was control and group 2 was milrinone group. Group 1 rats received 1.25 ml 0.09% NaCl (saline) intraperitoneally equivalent to the milrinone diluted volume, two hours before cardiac arrest. İntraperitoneally, 0.5 mg/kg of milrinone, whit in the 1.25 ml 0.09% NaCl (saline) was given to the rats two hours before cardiac arrest in Group 2.

In the literature involving the use of milrinone on rats, milrinone was injected both intravenously and intraperitoneally. In the studies of Jung HS et al., Karimfar MH et al., they injected mirlinone intraperitoneally [10, 14]. On the other hand, Dietrichs ES et al, Toyoda T et al. injected milrinone intravenously [15, 16]. In our study, we injected mirlinone intraperitoneally because it is easy to implement and there is no need for anesthesia and catheterization. Also, it is because we followed the protocol in the literature that mirlinone was injected intraperitoneally.

There is no common injection time of milrinone in rats before the procedure. Depending on the aims of studies in the literature, the time for injection of milrinone was varied. For example, Kume M et al. injected milrinone 80 min before the procedure whereas Toyoda T et al. injected milrinone 30 min after reperfusion and collected rats' river 5 hours after reperfusion [16, 17]. However based on the pharmacokinetic features, the effect of milrinone starts within 5 to15 minutes. For the single dosage of milrinone bolus, terminal elimination half-life is 2.3 hours [18]. Thus, we chose 2 hours when the efficiency of milrinone is high.

Nishiki T et al. used the dosage of 0.5 mg/kg in rats while Jung HS et al used lesser dosage for mice [10, 11]. In both studies, they showed that there is a decrease in renal ischemia- reperfusion injury. In the studies not involving renal ischemia, Jafari A et al used the dosage of 0.25 mg/kg in a study investigating the cardioprotective effects of milrinone. They found that in the group of milrinone, there was a decrease in oxidative damage and it preserved from cardiomyocytes apoptosis [19]. Desjardins S et al., in the study entitled "Acute effects of milrinone on the electrocardiogram and the cardiac hemodynamics of rats with pressure overload-induced congestive heart failure", used milrinone in three different bolus dosage groups including 0.1, 0.5, 1, 5 and 10 mg/kg. They showed that 25% of the rats in the groups of 1.5 and 10 mg/kg induced ventricular fibrillation and were death [20]. Therefore, the dosage of 0.5 mg/kg was chosen in our study as in the study of Nishiki T et al. that this dosage did not trigger off ventricular fibrillation.

As the anesthetics, ketamine (Ketalar, Pfizer, Turkey) 75 mg/kg and Xylazine 10 mg/kg (Rompun®, Bayer AG, Leverkusen, Germany) were given intraperitoneally. Cardiac arrest was formed in different ways in previous experimental non-heart beating models. Hu QH, et al made a non-heart beating model by cutting the abdominal aorta, Niu X et al by cutting vena cava [21, 22]. Dutkowski P et al, induced cardiac arrest in male Brown Norway rats by phrenotomy and ligation of the subcardial aorta [23]. Kashiwadate T., created apnea-induced agonal condition and created a non-heart beating model [24]. Cetin MM et al. and Akalın B et al., formed non-heart beating models with intracardiac KCl in their studies [25, 26]. In our study, because of the ease of administration, effectiveness and to create a model similar to the gradual real non-heart beating donors KCI was given to the anesthetized rats by intracardiac infusion very slowly. It is possible to develop sudden cardiac arrest when it is rapidly delivered as milrinone bolus. Group 1 and Group 2 rats underwent cardiac arrest under anesthesia by administering 0.2 mL intracardiac potassium chloride (KCI) according to the American Veterinary Association Guidelines [27]. Surgical

procedure was performed after 30 minutes of cardiac arrest [28, 29].

After the cardiac arrest, 6 cm mid-abdomen incision was performed on the rats. Left nephrectomy was applied to the rats. The kidney is longitudinally divided into two parts. The taken samples were divided into 2 equal parts. One part of the tissue samples was fixed in the Ependrof tup, and kept at 80°C for Malondialdehit (MDA), superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx) determination. The rest of the parts was fixed in formaldehyde for histopathological and immunohistochemical examination.

HISTOPATHOLOGIC ANALYSIS

Histopathological Preparation

The left kidneys of rats were resected with sacrification at 120 minutes after administration of milrinone and it was fixed in 10% formalin. Sagittal renal tissue sections were obtained and it was embedded in paraffin blocks. The sectioned tissue samples of the kidney were 4-μm thick and it mounted on slides. After deparaffinization, each specimen was stained with hematoxylin and eosin (H-E) to examine the level of ischemia-reperfusion (I-R) injury.

Histopathological Evaluation and Grading

Renal tissue sections obtained from a total of sixteen rats were evaluated microscopically in terms of dilatation of proximal tubules, eosinophilic casts in distal tubules, loss of brush borders, detachment of tubular cells, interstitial edema, whole tubular necrosis, neutrophil infiltration, and interstitial hemorrhage. The grade of the injury was described based on the following injury score (scoring range, 0–3):

0 = Frequency of injured proximal tubules was no injury in 10 high-power fields (HPFs), ×400; 1 = Frequency of injured proximal tubules was < 20% in 10 HPFs;

2 = Frequency of injured proximal tubules was between 20% and 50% in 10 HPFs;

3 = Frequency of injured proximal tubules was >50% in 10 HPFs, as well as eosinophilic cast in distal tubules, loss of brush borders, detachment of tubular cells, interstitial edema, whole tubular necrosis, neutrophil infiltration, and interstitial hemorrhage.

All histopathological samples were examined randomly and independently by binocular light microscopes by two experienced pathologists [11].

Immunohistochemical Analysis

Caspase-3 (Apoptotic Index) Evaluation

Localization of Caspase-3 was performed in paraffin-embedded kidney tissues by immunohistochemistry using a standard avidin-biotin peroxidase complex technique as described previously. A polyclonal rabbit anti-human Caspase-3 antibody (Ventana, Roche, USA) recognizing the 32 and 17 kD Caspase-3 subunits with no cross reactivity against other caspase family members (manufacturer's specification) were diluted 1:100 and then applied overnight at 4°C in a humid atmosphere. Thereafter, the sections were stained by an avidin-biotinylated HRP procedure using a commercially available kit (ABC Elite, Vector Laboratories). AEC was used as the substrate. Finally, sections were counterstained with hematoxylin. Negative control sections were incubated with normal mouse IgG or normal rabbit serum at the same protein concentration as the primary antibody. Human tonsil tissue was used as a positive control.

The Apoptotic Index was Calculated using the Formula Below

$$\frac{\text{Mean number of Caspase} - 3 \text{ positive cells in five random fields}}{\text{Mean number of total cells in five random fields}} \times 100$$

Mean number of total cells in five random fields

Biochemical Studies

The left kidney samples were promptly dissected and perfused with 50 mM (pH:7.4) cold phosphate buffer saline solution (PBS). Samples were homogenized in 1/5 (w/V) PBS. The homogenate was sonicated three times for 10 s with intervals and centrifuged at 20,000 × g for 15 min. Supernatants were separated and kept −80°C until all biochemical measurements were performed.

Determination of Protein Levels

The protein concentrations were determined according to the method of Lowry [30]. This method is based on reduction of the phosphotungstate complex to molybdenum blue by the tyrosine and tryptophane amino acids present in the structures of proteins. The reduction reaction is intensified with copper (Cu^{2+}). Absorbance was monitored at 650 nm by a spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan). Bovine serum albumin (BSA) was used as the standard.

Determination of Malondialdehyde Levels

MDA levels were determined according to the method of Mihara and Uchiyama [31]. In this method, the

color change developing from the reaction between MDA, which is an end product of lipid peroxidation, and thiobarbituric acid in hot and acidic medium is measured spectrophotometrically at 532 nm with tetramethoxypropane as the standard. The results were expressed as nmol MDA/mg protein.

Determination of Superoxide Dismutase Activity
SOD activity was determined with the method of McCord and Fridovich [32]. In this method, the activity is measured via SOD-mediated inhibition of cytochrome c reduction, which is induced by superoxide radicals that are produced in the xanthine-xanthine oxidase system. The change in absorbance is monitored for 1 min spectrophotometrically at 550 nm with bovine Cu-Zn SOD as the standard. The results were expressed as U/mg protein.

Determination of Catalase Activity
Catalase activity was determined with the method of Luck [33]. In this method, catalase decomposes hydrogen peroxide (H_2O_2) to water and oxygen (O_2). The decomposition of the H_2O_2 is monitored for 1 min spectrophotometrically at 240 nm. The results were expressed as U/mg protein.

Determination of Glutathione Peroxidase Activity
GPx activity was measured with the method of Lawrence and Burk [34]. GPx is an enzyme that uses reduced glutathione (GSH) to catalyse the conversion of H_2O_2 to water. This reaction also oxidizes the GSH while H_2O_2 is decomposed to H_2O and O_2. The oxidized glutathione (GS-SG) should then be reduced back in order to be used for decomposition of another H_2O_2 to O_2 and H_2O. This reduction requires the presence of reduced NADP (NADPH) and glutathione reductase in the medium. In this case NADPH is oxidized to NADP while GS-SG is reduced to GSH. The change in the absorbance was monitored for 3 min spectrophotometrically at 340 nm. The GPx activity was expressed in micromoles of NADPH consumed per minute per milligram of protein (U/mg protein).

STATISTICAL ANALYSIS

For statistical analysis, Statistical Package for the Social Sciences (version 13.0; SPSS Inc. Chicago, IL, United States of America) was used. The Man-Whitney U test, a nonparametric test that assesses the difference between two independent groups of medians, was used to compare the injury score assessed with hematoxylin eosin. It was assessed whether these two independent groups differed in terms of mean values based on the value of each variable, (SOD (U / mg prt), GPx (U / mgprt), CAT (U / mg prt), MDA (nmol/mg prt)) evaluated in the milrinone group in our study, in control group. The relevant test was performed with independent t test. Previously, the normal distribution in the groups was made with the conditional Shapiro–Wilk test. Normal distribution in the groups was carried out by Shapiro-wilk test. Subsequently, the independent t test was used to determine whether the milrinone variable in the groups (Glomerules, Tubules) differed from the control variables in terms of mean values. In addition, the relationship between two variables is examined by Pearson correlation coefficient. $p < 0.05$ were considered significant.

Ethic Statement

The protocol was approved by the Animal Ethics Review Committee (Permit number 2015/03–02). All experiments were conducted in compliance with the relevant laws and institutional guidelines.

RESULTS

Histopathologic Assessment

Total injury score was statistically significant between the groups ($p = 0.001$). In the milrinone group, the total injury score was significantly lower than the control group (Figure 1, 2).

Immunohistochemical Analysis

Caspase-3 staining was moderate-strong in the control group but weaker in the milrinone group. Caspase-3 staining in the control group was more prominent in both the glomeruli and the tubules and interstitial area than the milrinone group. This was statistically significant. ($p = 0.001$) (Table 1), (Figure 3, 4, 5).

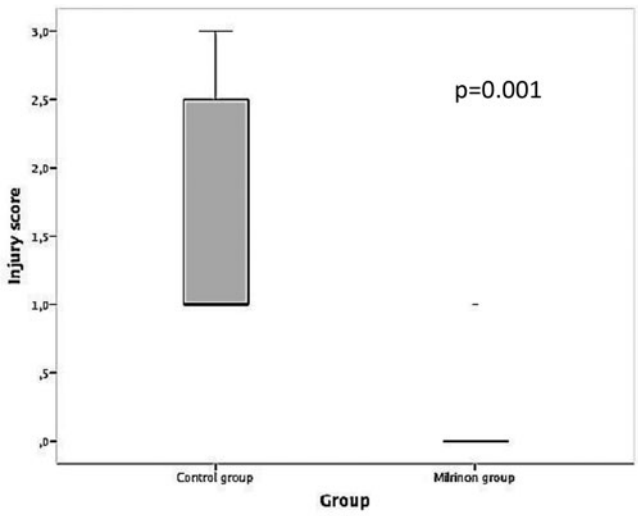

FIGURE 1 Comparison of total injury scores between groups.

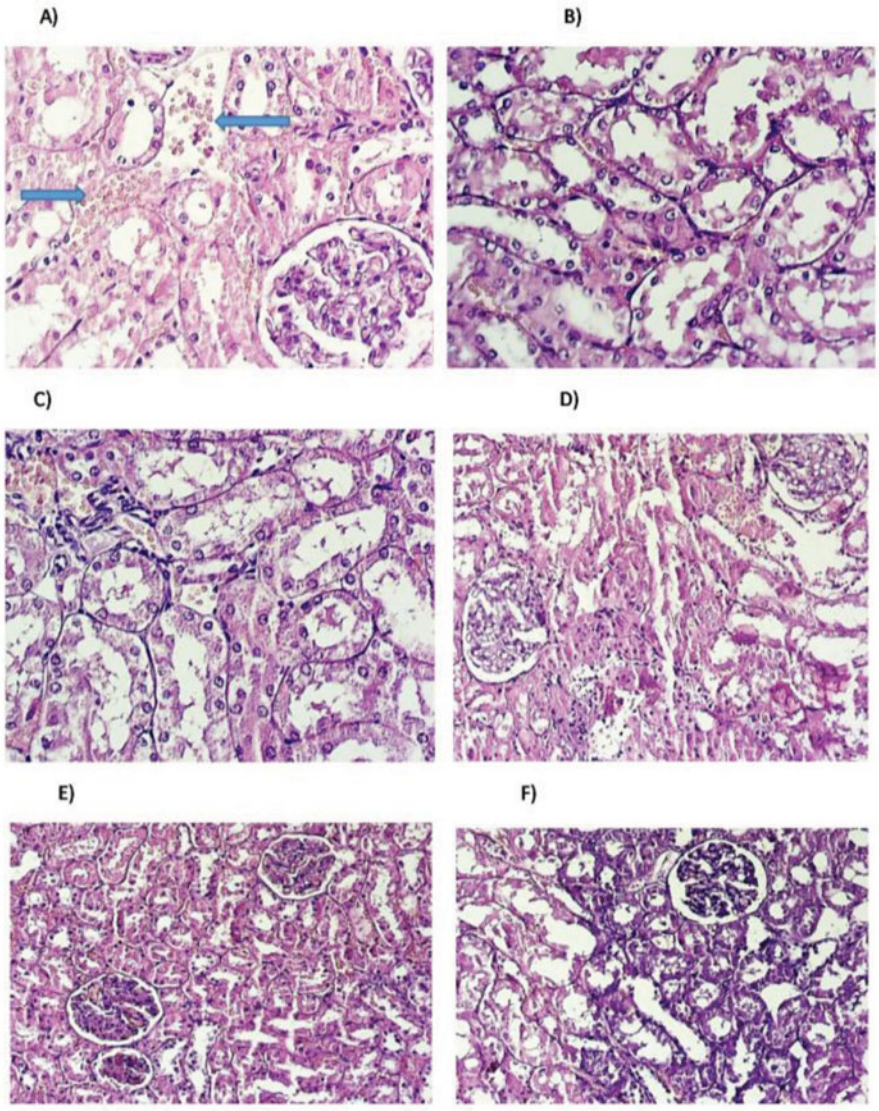

FIGURE 2 Illustrative histopathological findings after ischaemia injury. (A) Control group, sample No.C7. Glomerular and occasional interstitial hemorrhage foci in the renal cortex (arrows), Grade 2 [H&E staining, original magnification 400×]. (B) Control group, sample No.C6. Thyroidisation like eosinophilic casts in the distal tubule lumens of renal medullary sections, Grade 3 [H&E staining, original magnification 400×]. (C) Control group, sample No.C2.Detachment in the proximal tubule cells in the renal cortex, Grade 3 [H&E staining, original magnification 400×]. (D) Control group, sample No.C1. Development of acute tubular necrosis in the proximal tubules and inflammatory cells consisting of neutrophils were observed in the renal cortex, Grade 3, [H&E staining, original magnification 200×]. (E) Milrinon group, sample No.M5. Almost normal tissue morphology was observed in the renal cortex, Grade 1 [H&E staining, original magnification 200×]. (F) Milrinon group, sample No.M1. Completely normal renal tissue morphology was observed, Grade 0 [H&E staining, original magnification 200×].

TABLE 1 Apoptotic index values in renal glomerules and tubulo intertisyum

	Glomerules	Tubuls	*p* value
Groups	range (mean±SD)	range (mean±SD)	
Control ($n = 8$)	13–17 (15.13±1.25)	8–11 (9.5±0.93)	***0.001***
Milrinone ($n = 8$)	4–6 (5±0.76)	3–5 (4.25±0.71)	***0.001***

Biochemical Studies

Superoxide Dismutase Activity

SOD was 2.15 ± 0.30 U/mg prt in the control group and 2.74 ± 0.46 U/mg prt in the milrinone group. In comparison between groups, SOD in milrinone group was significantly higher than the control group ($p = 0.008$) (Table 2).

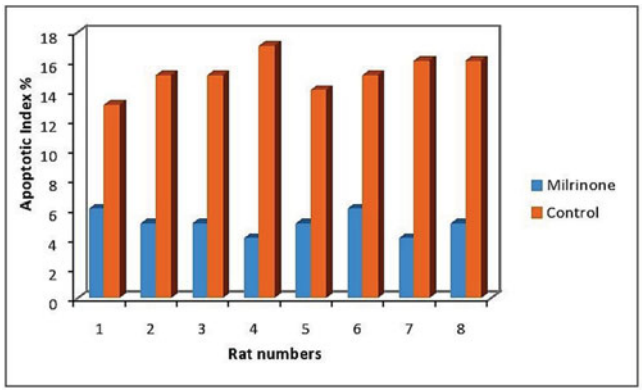

FIGURE 3 Apoptotic index distribution in renal glomerules of rats.

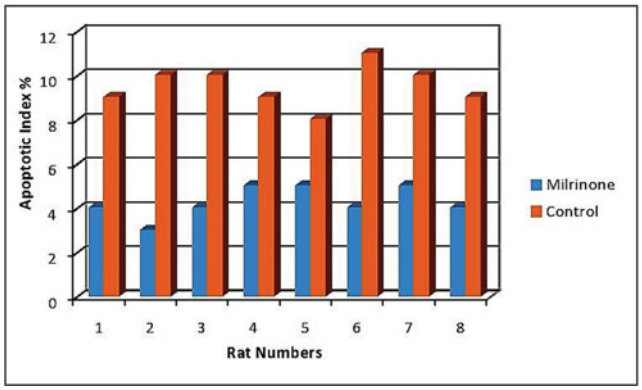

FIGURE 4 Apoptotic index distribution in renal tubules and interstisyum of rats.

Glutathione Peroxidase Activity

GPx was found to be 0.11 ± 0.014 U/mg prt and 0.16 ± 0.04 U/mg prt in the control and the milrinone group, respectively. In comparison between groups, GPx in milrinone group was significantly higher compared with the control group ($p = 0.006$) (Table 2).

Catalase Activity

Catalase activity was 423.38 ± 63.49 U/mg prt in the control group and 471.41 ± 76.73 U/mg prt in the milrinone group. Catalase activity was higher in the milrinone group relative to the control group. However, there was no statistical significance between the groups ($p = 0.194$) (Table 2).

TABLE 2 Biochemical investigations

	Group	Mean ± Std. Deviation	p
SOD (U/mg prt)	Control	2.15 ± 0.30	***0.008***
	Milrinone	2.74 ± 0.46	
GPx (U/mgprt)	Control	0.11 ± 0.014	***0.006***
	Milrinone	0.16 ± 0.04	
CAT (U/mg prt)	Control	423.38 ± 63.49	0.194
	Milrinone	471.41 ± 76.73	
MDA (nmol/mg prt)	Control	5.73 ± 0.48	0.162
	Milrinone	5.23 ± 0.82	

SOD: Superoxide Dismutase, GPx: Glutathione Peroxidase, CAT: Catalase, MDA: Malondialdehyde

Malondialdehyde Levels

MDA was 5.73 ± 0.48 nmol/mg prt and 5.23 ± 0.82 nmol / mg prt in the control and milrinone group, respectively. Although MDA was lower in the milrinone group than in the control group, there was no statistical significance between the groups ($p = 0.162$) (Table 2).

DISCUSSION

Milrinone is a phosphodiesterase 3 inhibitor used in the treatment of congestive heart failure. Milrinone increases intracellular c-AMP concentration. It provides vasodilation in the arteries and venules [35]. It increases blood flow in the tissue and suppresses TNF-alpha production. Increased TNF-alpha release leads to tissue damage by increasing production of eicosanoid analogs and free oxygen radicals [6]. TNF-alpha facilitates coagulation by increasing the production of vasopressor agents such as platelet-activating factor and endothelin-1. Thus, microcirculation deteriorates and tissue blood flow decreases [7]. Tubular obstruction and acute tubular necrosis occur due to increased vasoconstriction [36]. Therefore, suppression of TNF-alpha plays a key role in preventing ischemia-reperfusion injury. Previous studies have shown the efficacy of TNF-alpha inhibitors in preventing ischemia-reperfusion injury [37]. Milrinone is thought to be effective in ischemia reperfusion injury because it is an agent that inhibits TNF-alpha. Milrinone has been shown to reduce ischemic reperfusion injury in the lung, liver, heart and rat kidneys [11, 16, 17]. However, it is predicted that it can also reduce renal failure in the experimental non -heart beating donor model in which the renal arterial and venous blood flow is completely eliminated and does not provide blood flow again.

Milrinone's protective properties against ischemia reperfusion are related to two basic mechanisms. The first mechanism can be summarized as microcirculation enhancement by milrinone and vasodilatation. Milrinone increases tissue blood flow by reducing the release of vasopressive substances and it also protects tissue oxygen source. Thus, the glomerular filtration rate is maintained, preventing histological injury [11]. In our study, the renal injury score in the control group was significantly higher than the milrinone group. In the control group, increased necrosis in the proximal tubules, medullary conjunctiva and tubule cells were damaged. Studies by Jung HS et al. and Nishiki T et al., have shown that histologic injury scores are significantly lower in groups receiving phosphodiesterase

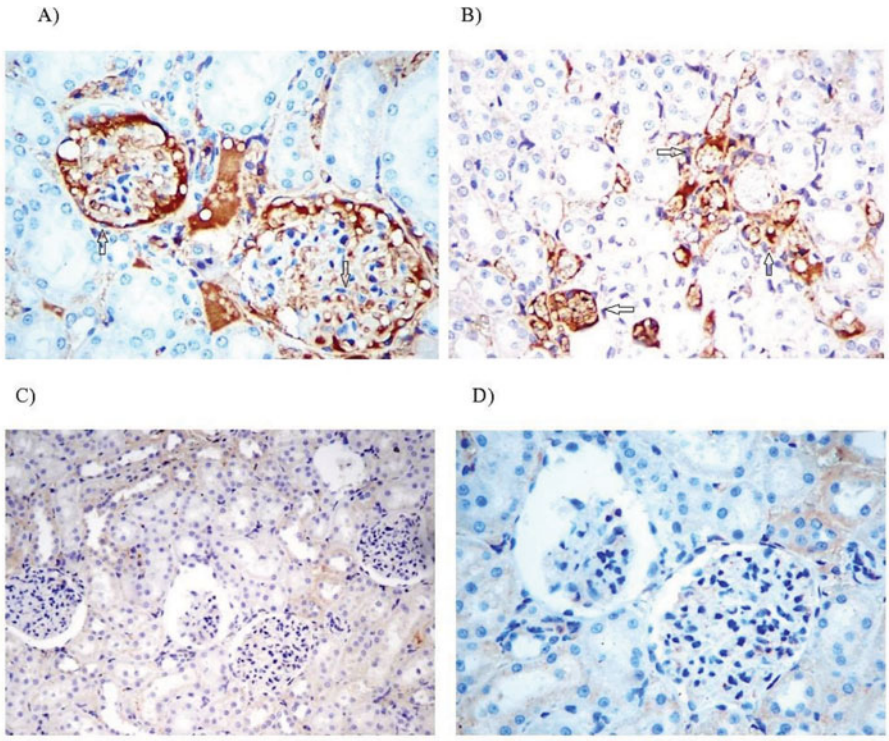

FIGURE 5 A, B: In the control group, glomeruli and interstitial area. Caspase-3 positive cells showing apoptosis (Black arrows; Apoptotic cells) (A;×400, B;×400). C,D: Caspase-3 staining for the apoptosis indicator in the Milrinone group, Note that both the glomeruli and the interstitial area decrease, Please compare with picture 1.(C;x200, D;×400).

3 inhibitors [10, 11]. Findings in terms of histological injury scores in our study were consistent with the literature.

The second protective effect of milrinone on ischemic reperfusion is related to the inflammatory response of c-AMP increased by milrinone and the reduction of apoptosis [10]. Nuclear Factor kappa B (NF-κB) and TNF-alpha lead to an increase in vascular endothelial adhesion molecules such as Intercellular Adhesion Molecule-1 (ICAM-1) and neutrophil chemo attractants (Macrophage inflammatory protein-2 (MIP-2), Monocyte chemoattractant protein- 1) by inducing the inflammatory reaction [38]. As a result, tissue damage occurs due to microcirculation deterioration. Milrinone reduces renal tissue damage by showing anti-inflammatory effect [39]. At the same time, Milrinone treatment attenuates the renal inflammatory response and activation of NF-KB [10].

The apoptosis process is programmed cell death. The mechanism of apoptosis is complex and involves a great number of pathways. Apoptosis has become a popular target in many treatment strategies. Apoptosis also plays an important role in ischemia reperfusion injury [40–42]. Ischemia causes to necrosis and apoptosis in the kidney, whereas reperfusion leads to an increase in reactive oxygen species and apoptotic cell death by regaining blood flow [43]. TNF-alpha, which is one of the molecules responsible for renal injury, is associated with intracellular caspases and apoptosis [8]. Caspases are activated in response to a variety of cell death stimuli and play an important role as initiators of apoptosis mechanisms [44]. Caspase-3 plays a major role in the demolition phase of apoptosis. The apoptotic index was measured using Caspase-3 expression in the study of ischemic damage in our study. The apoptotic index was significantly lower in the milrinone group. Increased apoptosis is associated with cell death and tissue damage. The low apoptotic index in the milrinone group suggests less tissue damage and inflammation. Schumer M. et al., showed molecular, biochemical, and morphological role of apoptosis in the reperfusion phase of renal ischemia [42]. Nishiki T et al., also found that reduced apoptosis contributes to a decrease in TNF- α mRNA in a study conducted on renal ischemia reperfusion injury [11]. Apoptosis in renal ischemia affects both tubular epithelial cells and glomerular cells [45]. In our study, apoptotic index was evaluated separately for tubular cells and glomerular cells. In the milrinone group, apoptotic index decreased significantly in both renal tubules and glomerular cells.

Free oxygen radicals with renal ischemia in the tissue cause cell and tissue damage. Hydroxyl Radical (OH), Hydrogen Peroxide (H_2O_2) and Superoxide Radical (O_2) show an increase during ischemia. Free oxygen radicals cause lipid peroxidation and further increase the damage in the kidney. Damage by free

radicals in the tissue is more prominent during reperfusion [46]. In our study, only ischemia was formed without reperfusion in the experimental non-heart beating donor model. However, both ischemia and reperfusion are known to cause tissue damage [47]. Free radicals in the tissue during ischemia and reperfusion are eliminated by antioxidant defense systems. SOD, GSH-Px and CAT have an important role in the endogenous defense system against free radicals such as O2, H_2O_2 [48]. In normal cases, these free radicals are rapidly eliminated by antioxidant enzymes such as SOD, GSH-Px and CAT [48]. However, the antioxidant system is inadequate in the ischemia and free oxygen radicals accumulate in the tissue causing damage. The inadequacy of the antioxidant system becomes more prominent, especially in the reperfusion process [49, 50]. These events result in cell damage caused by lipid peroxidation.

While there is an increase in MDA levels, a product of lipid peroxidation in the kidney after ischemia reperfusion, a decrease in SOD, CAT and GSH-Px enzyme activation is seen [47]. Cong G et al showed that polysaccharides from the roots of Dipsacus asperoides protected the tissue from oxidative damage after ischemia reperfusion injury. SOD, GSH-Px and CAT activities were significantly higher in the treatment groups, whereas the enzyme activities were lower than the control groups in the untreated ischemia-reperfusion groups [51]. Cakir M et al also found that SOD, GSH-Px and CAT activities were significantly higher in the treatment groups with low oxidative and tissue damage, while MDA levels were found to be decreased in a study investigating the effect of hypericum perforatum on renal failure after ischemia reperfusion. These findings suggest that hypericum perforatum may reduce oxidative stress due to ischemia reperfusion [52]. In our study, SOD and GSH-Px were significantly higher in the milrinone group compared to the control group. Our findings are consistent with the literature. However, although CAT activity did not increase in the milrinone group, there was no significant difference between the groups. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cell. Overproduction is seen in MDA production due to an increase in free radicals. The MDA level is known as the marker of oxidative stress. MDA also shows antioxidant status [53]. Although MDA level in our study was low in the milrinone group, there was no statistical significance found between the groups. Our study in terms of CAT and MDA activities and the reason for the difference between the studies of Cakir et al., and Cong G et al., can be attributed to different ischemic periods and higher oxidative stress caused by reperfusion. Bozlu et al., showed that MDA level in the testicular tissue increased significantly after 4 hours of reperfusion [54]. Not having a significant difference between the groups in terms of MDA in our study may be related to the lack of evaluation in the reperfusion process. Bektas S et al found tissue MDA levels associated with tadalafil dosage in a study conducted on the efficacy of Tadalafil, a phosphodiesterase type-5 inhibitor, on liver ischemia reperfusion [55]. Since the different doses of milrinone were not compared in our study, the relationship between milrinone dox and MDA levels could not be demonstrated.

There were some limitations in our study. For instance, there were no different dose groups of milrinone in our study. There were also no ischemic periods in the milrinone group. Changes in inflammatory response and tissue damage levels may occur at different doses and ischemic periods. Another limitation is that the graft function and long-term outcome of the kidney obtained from the non-heart beating donor after transplantation cannot be assessed. For this purpose, it will be useful to evaluate the post-transplantation outcomes of grafted kidneys obtained from different groups of milrinone doses and groups exposed to different time periods of ischemia.

CONCLUSION

Milrinone has been shown to be effective in the prevention of tissue damage due to oxidative stress and inflammatory process in the renal of warm ischemia in the experimental non-heart-beating donors model and in protecting the renal. Milrinone increases antioxidant activity while reducing apoptosis. Systemic administration of milrinone prior to cardiac arrest may be beneficial. Administration of milrinone to the recipient in the perioperative period may contribute to donor function.

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