

Effects of Losartan on Glycerol-induced Myoglobinuric Acute Renal Failure in Rats

Oktay KAYA *  Nurettin AYDOĞDU ** Ebru TASTEKİN ***
Cetin Hakan KARADAG **** Ozgur GUNDUZ **** Necdet SUT *****

* Department of Physiology, Kafkas University Faculty of Medicine, TR-36000 Kars - TURKEY

** Department of Physiology, Trakya University Faculty of Medicine, TR-22030 Edirne - TURKEY

*** Department of Pathology, Trakya University Faculty of Medicine, TR-22030 Edirne - TURKEY

**** Department of Pharmacology, Trakya University Faculty of Medicine, TR-22030 Edirne - TURKEY

***** Department of Biostatistics and Medical Informatics, Trakya University Faculty of Medicine, TR-22030 Edirne - TURKEY

Makale Kodu (Article Code): KVFD-2012-7620

Summary

Myoglobinuric acute renal failure (mARF) is an uremic syndrome which develops due to damage of skeletal muscle. It was demonstrated that free radicals and nitric oxide (NO) play an important role in pathogenesis of mARF. Our aim was to investigate the effect of losartan, a drug known for its antioxidant effect, on mARF. In our study, a total of 34 male Sprague Dawley rats were divided into four groups. 1st and 2nd groups were injected with saline, 3rd and 4th groups were injected with intramuscular glycerol. One and 24 hours later, 1st and 3rd groups received saline orally and 2nd and 4th groups have taken 10 mg/kg losartan. Urine was collected; the blood samples and kidneys of the rats were taken under the anesthesia. The levels of NO, arginine, asymmetric dimethylarginine (ADMA), glutathione (GSH) and malondialdehyde (MDA) were determined, renal functions and histopathological changes examined. In our study, we found that levels of urea, creatinine, and potassium, in serum samples and MDA and ADMA in renal tissue were increased in 3rd group when it's compared with the 1st group. Levels of sodium, arginine in serum samples and arginine in renal tissue were reduced 3rd group when compared with the 1st group. When 3rd and 4th groups were compared, serum creatinine was higher in the latter group whereas ADMA level in renal tissue was lower in the same group. We think that there is no positive effect of losartan on the pathogenesis of mARF.

Keywords: Losartan, Myoglobinuric Acute Renal Failure, Free radicals, Nitric oxide, Asymmetric dimethylarginine

Ratlarda Gliserol ile İndüklenen Miyoglobinürik Akut Böbrek Yetmezliği Üzerine Losartanın Etkileri

Özet

Miyoglobinürik akut böbrek yetmezliği (mABY) iskelet kaslarının hasarlanmasıyla oluşan üremik bir sendromdur. Nitrik oksit (NO) ve serbest radikallerin mABY patogenezinde önemli rol oynadığı gösterilmiştir. Çalışmamızda antioksidan etkileri olduğu bildirilen losartanın mABY'deki etkilerini araştırmayı amaçladık. Çalışmamızda 4 grupta toplam 34 adet erkek Sprague Dawley sıçan kullanıldı. 1. ve 2. gruba fizyolojik serum, 3. ve 4. gruba gliserol intramüsküler enjekte edildi. 1 ve 24 saat sonra 1. ve 3. gruba distile su, 2. ve 4. gruba 10 mg/kg losartan oral yolla verildi. İdrarları toplandı, anestezi altında kan ve böbrekleri alındı. NO, arginin, asimetrik dimetilarginin (ADMA), glutatyon (GSH), malondialdehit (MDA) düzeyleri, histopatolojik değişiklikler ve böbrek fonksiyonları incelendi. Çalışmamızda 1. grup ile karşılaştırıldığında 3. grupta serumda üre, kreatinin, potasyum, böbrek dokusunda MDA ve ADMA düzeylerinde artma; serum sodyum, arginin, böbrek dokusunda arginin düzeylerinde azalma gözlemlendi. 3. grup ile karşılaştırıldığında 4. grupta serum kreatinin düzeyinde artış, böbrek dokusunda ADMA düzeyinde azalma saptandı. Bu sonuçlar ışığında losartanın mABY patogenezi üzerinde olumlu etkilerinin olmadığını düşünüyoruz.

Anahtar sözcükler: Losartan, Miyoglobinürik Akut Böbrek Yetmezliği, Serbest radikaller, Nitrik oksit, Asimetrik dimetilarginin



İletişim (Correspondence)



+90 474 2251150



oktay680@hotmail.com

INTRODUCTION

Rhabdomyolysis is the clinical and laboratory condition caused by extracellular distribution of intracellular elements from striated muscle due to traumatic and non-traumatic reasons¹. Rhabdomyolysis causes tubular obstruction, tubular necrosis and renal vasoconstriction which results in acute renal failure, a potential complication of rhabdomyolysis^{2,3}.

The most common method of setting up experimental mARF is intramuscular injection of hypertonic glycerol solutions in rats⁴. Intramuscular glycerol injection results in myolysis and hemolysis. Iron released from the degradation of myoglobin and hemoglobin catalyses Haber-Weiss and Fenton reactions and thus resulting in free radical formation, lipid peroxidation and deterioration of renal functions^{3,4}.

Nitric oxide is an intermediate product synthesized from L arginine⁵. NO is reported to have a role in normal renal function⁶, reducing the rate of renal interstitial fibrosis⁷ and in higher concentrations, it is known to participate in proximal renal tubular injury⁶. ADMA is a methylation product of arginine. Studies have shown that ADMA is a good indicator of increased oxidative stress and that it can be a potential marker during the progress of acute renal failure⁸. ADMA, as an inhibitor of nitric oxide synthase, contributes to reduced production of NO⁹. Free radicals and reduced NO levels have important roles in pathogenesis of experimental ARF¹⁰. Endothelial dysfunction is correlated with ADMA levels in patients with ARF¹¹.

Losartan is a non-peptide angiotensin II receptor antagonist¹². Losartan is reported to increase NO release¹³. Losartan is known to decrease the production of reactive oxygen species and suppress lipid peroxidation¹⁴. Losartan is also reported to prevent progression of many chronic renal diseases such as diabetic nephropathy¹². In rats with renal ablation, losartan administration has decreased blood pressure levels and proteinuria¹⁵.

The aim of this study is to evaluate the effects of losartan on oxidative stress, renal functions, NO, arginine, ADMA, histopathological changes of renal tissue.

MATERIAL and METHODS

This study was approved by Trakya University School of Medicine Animal Care and Use Committee (2009/065). The laboratory conditions were standardized as 22±1°C and 12 h light-dark cycles. Male Sprague Dawley rats weighting 370-450 g were fed by standard rat chow and tap water. Rats were randomly divided into 4 groups. There were 7 rats in 1st and 2nd groups, 10 rats in the 3rd and 4th groups. The rats were deprived of water 24 h prior to saline injection for 1st and 2nd groups and im glycerol injection for the other groups.

One and 24 h after the im injection of saline, distilled water was administered to the 1st group (Control) via gavage and 10 mg/kg losartan (Cozaar® tablets, Merck Sharp & Dohme) was administered to the 2nd group (Control+ Losartan) via oral gavage after being dissolved in distilled water. 1 and 24 h after the glycerol injection, distilled water was administered to 3rd group (ARF) and 10 mg/kg losartan was administered to 4th group (ARF+Losartan).

After 24 h urine collection (48 h after glycerol injection) 10 mg/kg xylazine and 50 mg/kg ketamine were used to anesthetize rats. Blood sample were collected by cardiac puncture. Serum and urine samples stored at -80°C. Serum urea, creatinine, Na⁺, K⁺, urine creatinine and urine Na⁺ levels were measured using automated analyzer.

Bilateral nephrectomy was performed to rats and then kidneys were cut longitudinally. One half of the right kidney is fixed in 10% formaline solution. The other half of the right kidney and left kidney were stored at -80°C until tissue analysis.

Renal tissues samples were homogenized in buffer solutions (0.15 M KCl solution for MDA and GSH; 50mM phosphate buffer (pH 7.4) for NO; phosphate buffer (pH 7.0) for arginine and ADMA). Homogenates were centrifuged (1.500xg for 10 min, at 4°C), supernatant of the homogenates were collected for the MDA, GSH, NO, arginine, ADMA measurements.

Protein content of the tissue samples was samples determined according to the method of Lowry¹⁶. Spectrophotometric detection of the color produced by the interaction of free SH groups in the tissue homogenates with Ellman reagent was used to note GSH content¹⁷. Nitrate and nitrite detection was done by the method described by Cortes and Wakid¹⁸. Arginine and ADMA measurements were made by the method described by Teerlink¹⁹.

Kidney slides were stained by Hematoxylin eosin and were blindly analyzed twice by the same pathologist under light microscope. Proximal tubular necrosis was identified and was conveyed as a percent of the total sum of all proximal tubules in x10 magnification. Similarly, proteinaceous casts in the distal tubules were analysed and tubules containing casts were conveyed as a percent of total number of distal tubules.

Statistical Analysis

Statistical analyses were performed using Statistica 7.0 (Licence code: 31N6YUCV38) programme. Quantitative data was given as mean±SD. Normal distribution of variances was determined using Kolmogorov-Smirnov test. Since normality assumption is not satisfied, Kruskal Wallis test was used to investigate inter-group differences. To determine which group was causing the difference, Mann-Whitney U test were used. P<0.05 was considered as statistical significance value.

RESULTS

Renal function markers of the study groups are shown in [Table 1](#). Urine could not be collected in ARF and ARF+Losartan groups. In regards to urine sodium, creatinine, creatinine clearance and fractional sodium excretion no statistically significant difference was detected between the control and losartan groups. Compared to control group, serum urea levels were significantly elevated in ARF and ARF+Losartan groups ($P=0.017$ and $P=0.007$, respectively). No significant difference in serum urea levels was detected between ARF and ARF+Losartan groups. In a similar fashion, serum creatinine levels were significantly elevated in ARF and ARF+Losartan group ($P=0.016$ and $P=0.008$, respectively). Serum creatinine level was higher in ARF+Losartan group than ARF group which was statistically significant ($P=0.034$). Compared to control group, serum sodium levels were significantly decreased in ARF and ARF+Losartan groups ($P=0.016$ and $P=0.029$, respectively). No significant difference in serum sodium levels was detected between ARF and ARF+Losartan groups. Compared to control group, serum potassium levels were significantly elevated in ARF and ARF+Losartan groups ($P=0.016$ and $P=0.008$, respectively). No significant difference in serum potassium levels was detected between ARF and ARF+Losartan groups ($P=0.157$).

Biochemical parameters of the study groups are shown in [Table 2](#). No statistically significant difference was detected between study groups in renal tissue GSH levels. Compared to control group, renal tissue MDA levels were significantly increased in ARF and ARF+Losartan groups ($P=0.017$ and $P=0.008$, respectively). No significant difference in renal tissue MDA levels was detected between ARF and ARF+Losartan groups. No significant difference was detected among groups in regard to serum NO, renal tissue NO, urine NO and serum ADMA levels. When ADMA levels in renal tissue were compared between groups, an increase in ARF group and a decrease in ARF+Losartan group were detected in comparison to control group, both of which were statistically significant ($P=0.030$ and $P=0.014$, respectively). Compared to control group, renal tissue arginine levels were significantly decreased in ARF and

ARF+Losartan groups ($P=0.017$ and $P=0.006$, respectively). No significant difference in renal tissue arginine levels was detected between ARF and ARF+Losartan groups. When serum arginine levels were compared between groups, a decrease in ARF group was detected in comparison to control group ($P=0.017$). There was no significant difference in serum arginine levels between the control, ARF and ARF+Losartan groups.

Histopathological findings of the study groups are shown in [Table 3](#). In our study, no change in glomeruli or tubular necrosis was seen in HE stained kidney slides of control and losartan groups. Tubular necrosis was present in HE stained kidney slides of ARF and ARF+Losartan groups. There was also evident dilatation of the tubules with accumulation of dense, proteinaceous casts in distal tubules. Hydropic cytoplasmic swelling and vacuolisation in distal tubule cells was prominent. Peritubular stroma showed edema and vascular congestion. Tubular cellular regeneration was noted. Glomerular congestion was more pronounced in ARF group ([Fig. 1](#)).

Table 1. Serum renal function markers of the study groups
Tablo 1. Çalışma gruplarının serum renal fonksiyon belirteçleri

Renal Function Markers	Control (n=7)	Losartan (n=7)	ARF (n=3)	ARF+Losartan (n=4)
Urea (mg/dl)	22.71±2.56	24.29±4.19	595.33±31.56*	586.75±48.49*
Creatinine (mg/dl)	0.40±0.06	0.39±0.05	6.87±0.44*	8.23±0.21*#
Na ⁺ (mmol/L)	139.86±4.06	136.71±10.01	123.00±7.93*	132.00±3.92*
K ⁺ (mmol/L)	4.83±0.59	4.89±0.72	8.80±0.92*	7.85±0.76*

Data are given as mean±SD, * $P<0.05$ compared with control group, # $P<0.05$ compared with ARF group

Table 2. Serum and kidney tissue biochemical parameters of the study groups
Tablo 2. Çalışma gruplarının serum ve böbrek dokusu biyokimyasal parametreleri

Biochemical Parameters	Control (n=7)	Losartan (n=7)	ARF (n=3)	ARF+Losartan (n=4)
Serum				
NO (µmol/mg)	2.25±0.63	2.35±0.43	1.55±0.48	4.22±2.66
ADMA (µM)	1.42±0.31	1.64±0.30	3.87±3.97	0.87±0.47
Arginine (µM)	87.97±10.10	95.30±9.90	38.81±32.51*	79.64±12.36
Kidney Tissue				
MDA (nmol/g doku)	0.58±0.12	0.47±0.05	1.37±0.20*	1.20±0.12*
GSH (µmol/g doku)	62.56±10.17	58.89±7.76	59.85±6.02	70.47±9.06
NO (µmol/mg)	97.14±49.00	62.37±28.77	41.21±37.69	44.94±33.02
ADMA (µmol/mg protein)	1.25±0.29	0.72±0.57	3.62±2.84*	0.57±0.25*
Arginine (µmol/mgprotein)	190.10±18.98	181.38±22.37	67.87±25.01*	70.57±10.42*

Data are given as mean±SD, * $P<0.05$ compared with control group

Table 3. Renal histopathological findings of the study groups
Tablo 3. Çalışma gruplarının renal histopatolojik bulguları

Histopathological Findings	Control (n=7)	Losartan (n=7)	ARF (n=7)	ARF+Losartan (n=7)
Necrosis (%)	0.00±0.00	0.00±0.00	78.00±5.29*‡	80.00±7.16*‡
Cast (%)	1.42±0.53	1.29±0.49	77.67±2.51*‡	77.50±2.08*‡

Data are given as mean±SD, * $P<0.05$ compared with control group, ‡ $P<0.05$ compared with losartan group

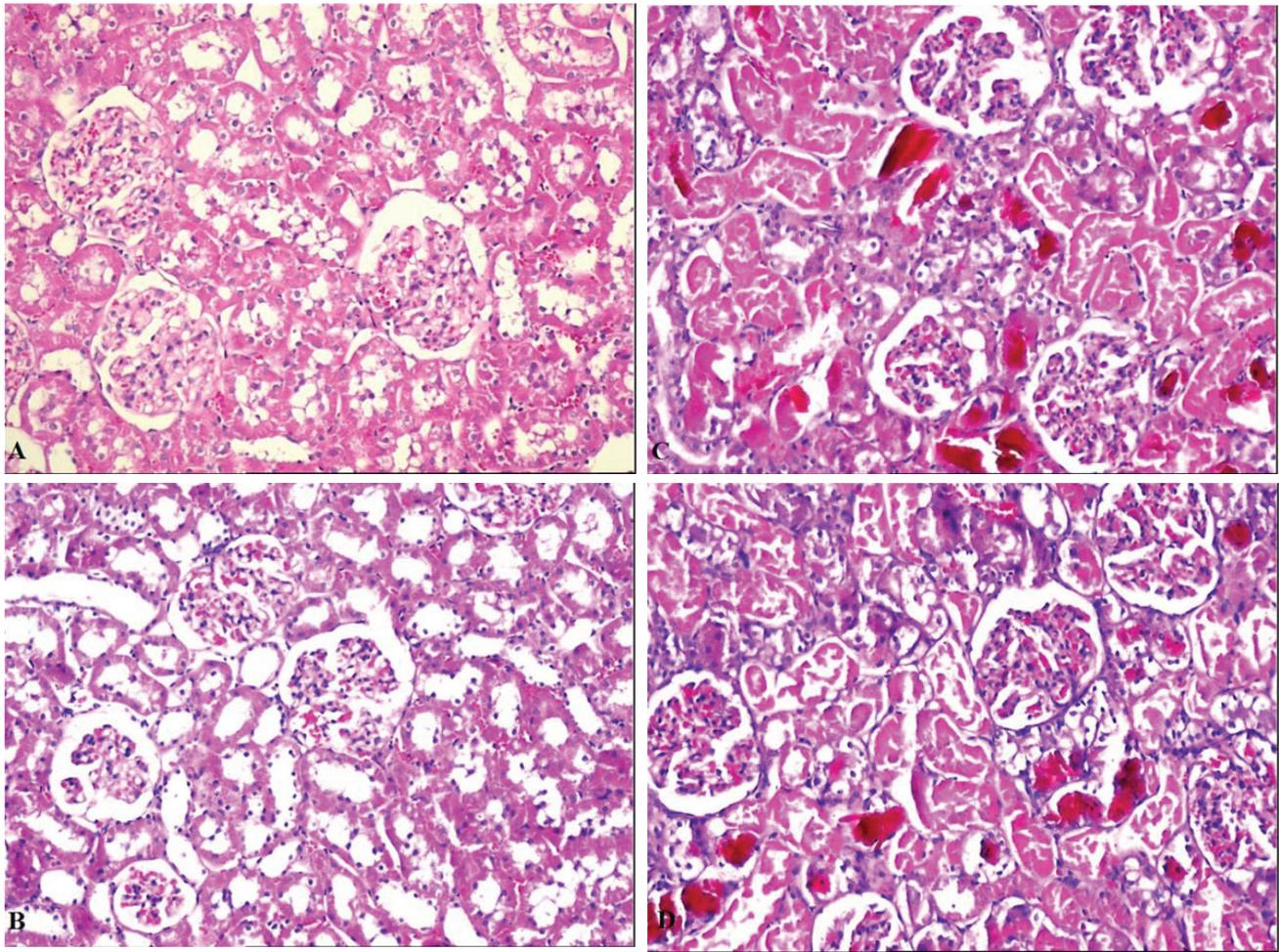


Fig 1. Histopathological appearance of the study groups

A) Control group, B) Losartan group, C) ARF group, D) ARF + Losartan group

Şekil 1. Çalışma gruplarının histopatolojik görünümü

A) Kontrol grubu, B) Losartan grubu, C) ABY grubu, D) ABY + Losartan grubu

DISCUSSION

Oxidative stress and NO play important roles in ARF due to myoglobinuria³. Losartan is a non-peptide angiotensin II receptor antagonist. Losartan has been shown to increase NO release and decrease oxidative stress²⁰. As far as our knowledge, this is the first study in which the effect of losartan on experimental mARF is studied. It is also the first study to investigate ADMA levels in mARF pathogenesis. In our study, rhabdomyolysis was shown to cause renal tubular injury, decrease in renal functions, elevate oxidative stress and ADMA levels and diminish arginine levels. While losartan was shown to decrease ADMA levels, it also increased serum creatinine. Losartan seemed to have no effect on other findings.

In our study, MDA levels were significantly reduced in healthy subjects after administration of losartan. In a study performed by Saleh et al.²¹, investigating the renoprotective effects of losartan, it was reported that in healthy rats given i.p. losartan either as a single dose of 60 mg/kg or in

divided doses (10 mg/kg daily for 6 days), losartan showed no significant effect on renal MDA levels. The decrease of MDA in our study may be due to the differences in dose, timing, method and period of administration.

Seven of the rats in our ARF group died within the first 48 h due to complications. Previous studies reported lower mortality rates in mARF model²²⁻²⁴. Rats used in these studies are reported to weigh between 160 and 300 g whereas the rats in our study weighed between 370 and 450 g. High mortality rate of our study may be a result of high dose administered i.m. glycerol due to higher animal weight. High dose glycerol injection intramuscularly causes muscle damage which results in the release of K⁺, myoglobin and other cytokines into systemic circulation. We believe increased serum K⁺ levels result in cardiotoxicity and released myoglobin promote tubular necrosis and cast formation, thus precipitating renal failure. In our laboratory, the rate of tubular necrosis and cast accumulation was higher than any ARF groups formed in previous studies^{22,25}.

The increase in blood urea and creatinine levels in ARF in control groups is consistent with previous studies performed on this model²⁶⁻²⁸. Elevated K⁺ is known to be a result of decreased urine output in ARF and severe muscle damage²⁹. Decreased blood Na⁺ level may be caused by tubular dysfunction which is consistent with previous studies^{25,30}.

The significant increase in renal MDA levels in ARF group is consistent with previous studies^{28,31}. Free iron ions released from the degradation of significant amount of myoglobin cause free radical formation and lipid peroxidation which may contribute to the increase in MDA levels.

A significant decrease in GSH has been reported in experimental studies on mARF^{4,32}. However, no significant decrease in GSH was noted in our study which may be a result of low number of samples due to high mortality in this group.

Nitric oxide is an intermediate product synthesized from L arginine⁵. NO plays a key role in the regulation of renal blood flow and glomerular filtration, production of renin and angiotensin II and tubular reabsorption³³. Heme proteins produced in mARF model consume NO and cause renal vasoconstriction via cytokines³⁴.

Previous studies stated that L-arginine treatment normalised renal oxidative stress and structural damage and reduced renal function loss^{32,35}. When ARF is formed by myoglobin infusion, L-arginine treatment may prevent renal vasoconstriction and dysfunction via NO. In our study, decrease of L-arginine may have exacerbated renal ischemia and thus aggravated tubular damage^{32,36}. Previous studies performed by this model showed a decrease in renal NO levels³⁷. It has been reported that decrease of NO levels and inhibition of NOS worsens pathogenesis³⁸. There being no significant difference in NO levels may be attributed to high mortality rate seen in this group.

ADMA is a potent endogenous NOS inhibitor. Elevated ADMA levels have been linked to oxidative stress and it is also reported to be a marker for renal failure^{35,39}.

In the experimental model of Volti et al. rats underwent to 45 min of renal ischemia followed by 30 min, 1 h, and 3 h of reperfusion⁴⁰. ADMA levels showed significant difference in the groups which 60 and 180 min reperfusion were performed. This result is consistent with renal ADMA levels of the present study.

Six of the rats in ARF+Losartan group died in 48 h because of the complications. When we reviewed the literature we could not find any study analysing the effects of losartan on experimental mARF models. On the experimental mARF models decreased renal perfusion due to increased renal angiotensin levels was reported. Increased angiotensin II, as a vasoconstrictor in renal vascular bed is known to reduce renal function⁴¹. Losartan used as a therapeutic agent of hypertension and minimal renal changes in renal

transplant recipients, leads to elevated serum creatinine levels. Elevated levels of serum creatinine can be considered a result of low glomerular pressure⁴². In the present study elevated serum creatinine levels can be considered as a result of losartan related to low intraglomerular pressure and hemodynamic effects.

Yavuz et al.⁴³ performed a diabetic nephropathy model on rats. They administered 10 mg/kg losartan by gavage for 8 weeks and reported decreased MDA levels. In the study of Khattab et al.⁴⁴ rats were subjected to hypertension by administering L-NAME. In this study high level of MDA in renal tissue was decreased by a 10 mg/kg losartan treatment lasting 6 weeks. Rashchizadeh et al.⁴⁵ reported that losartan significantly decreased MDA levels in renal transplant recipients. Additionally creatinine levels were detected to be increased in the group is treated by losartan for 6 weeks. Preventive effect of losartan on lipid peroxidation reported in these studies is not correlated with our results. In the study of Raschizadeh et al.⁴⁵, a group of rats were treated with losartan and creatinine levels were increased. This is consistent with our study.

In the study of Ito et al.⁴⁶ untreated patients with essential hypertension were treated with perindopril, losartan, bisoprolol and they reported that perindopril and losartan reduced the ADMA levels. In the study serum MDA levels decreased significantly only in the group treated with losartan.

As mentioned above previous studies suggest that losartan has a protective role in many experimental models. In the present study, losartan is considered to worsen the pathogenesis of mARF and elevated serum creatinine levels are a sign of this entity.

It may also be due to different factors affecting experimental mARF pathogenesis which is not in correlation with other experimental studies. In previous studies, losartan was shown to increase NO levels by decreasing ADMA, which results in fluid accumulation in damaged muscles. We believe this condition exacerbates hypovolemia. We believe that hypovolemia causes renal vasoconstriction which worsens mARF pathogenesis.

We believe the effect of losartan on mARF model should be thoroughly investigated using variations in dosage, administration route, timing and period of administration.

REFERENCES

1. **Sever L:** Ezilme sendromu. *Türk Ped Arşivi*, 44, 43-47, 2009.
2. **Bosch X, Poch E, Grau JM:** Rhabdomyolysis and Acute Kidney Injury. *N Engl J Med*, 361 (1): 62-72, 2009.
3. **Boutaud O, Roberts LJ:** Mechanism-based therapeutic approaches to rhabdomyolysis-induced renal failure. *Free Radic Biol Med*, 51 (5): 1062-1067, 2011.
4. **Aydogdu N, Atmaca G, Yalcin O, Taskiran R, Tastekin E, Kaymak K:** Protective effects of L-Carnitine on myoglobinuric acute renal failure in

rats. *Clin Exp Pharmacol*, 33, 119-124, 2006.

5. Atakışi O, Atakışi E, Atabay T, Uzun M: L-Arjinin ve N ω -Nitro-L-Arjinin Metil Esteri uygulamasının beyin, karaciğer, böbrek dokusu nitrik oksit ve malondialdehit düzeylerine etkisi. *Kafkas Univ Vet Fak Derg*, 15 (1): 71-75, 2009.

6. Milsom AB, Patel NS, Mazzon E, Tripatara P, Storey A, Mota-Flípe A, Sepodes B, Webb AJ, Cuzzocrea S, Hobbs AJ, Thiemermann C, Ahluwalia A: Role of endothelial nitric oxide synthase in nitrite-induced protection against renal ischemia-reperfusion injury in mice. *Nitric Oxide*, 22 (2): 141-148, 2010.

7. Sun D, Wang Y, Liu C, Zhou X, Xiao A: Effects of nitric oxide on renal interstitial fibrosis in rats with unilateral ureteral obstruction. *Life Sci*, 90 (23-24): 900-909, 2012.

8. Sun J, Shannon M, Ando Y, Schnackenberg LK, Khan NA, Portilla D, Beger RD: Serum metabolomic profiles from patients with acute kidney injury: A pilot study. *J Chromatogr B*, 15 (893-894): 107-113, 2012.

9. Pope AJ, Karuppiah K, Cardounel AJ: Role of the PRMT-DDAH-ADMA axis in the regulation of endothelial nitric oxide production. *Pharmacol Res*, 60 (6): 461-465, 2009.

10. Vanholder R, Sever MS, Ereğ E, Lameire N: Rhabdomyolysis. *J Am Soc Nephrol*, 11(8): 1553-1561, 2000.

11. Vallance P, Leiper J: Cardiovascular biology of the asymmetric dimethylarginine: Dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vas Biol*, 24 (6): 1023-1030, 2004.

12. Xu F, Mao C, Liuu Y, Wu L, Xu Z, Zhang L: Losartan chemistry and its effects via AT-1 mechanisms in the kidney. *Curr Med Chem*, 16 (28): 3701-3715, 2009.

13. Kalinowski L, Matys T, Chabielska E, Buczek W, Malinski T: Angiotensin II AT1 receptor antagonists inhibit platelet adhesion and aggregation by nitric oxide release. *Hypertension*, 40 (4): 521-527, 2002.

14. Kamper M, Tsimpoukidi O, Chatzigeorgiou A, Lymberi M, Kamper EF: The antioxidant effect of angiotensin II receptor blocker, losartan, in streptozotocin-induced diabetic rats. *Transl Res*, 156 (1): 26-36, 2010.

15. Lafayette RA, Mayer G, Park SK, Meyer TW: Angiotensin receptor blockade limits glomerular injury in rats with reduced renal mass. *J Clin Invest*, 90 (3): 766-771, 1992.

16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem*, 193 (1): 265-275, 1951.

17. Ellman GL: Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82 (1): 70-77, 1959.

18. Cortas NK, Wakid NW: Determination of inorganic nitrate in serum and urine by kinetic cadmium-reduction method. *Clin Chem*, 36 (8 pt 1): 1440-1443, 1990.

19. Teerlink T, Nijvelt R, Jong S, van Leeuwen PA: Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high performance liquid chromatography. *Anal Biochem*, 303 (2): 131-137, 2002.

20. Pialoux V, Foster GE, Ahmed SB, Beaudin AE, Hanly PJ, Poulin MJ: Losartan abolishes oxidative stress induced by intermittent hypoxia in humans. *J Physiol*, 589 (pt22): 5529-5537, 2011.

21. Saleh S, Ain-Shoka AA, El-Demerdash E, Khalef MM: Protective effects of the angiotensin II receptor blocker losartan on cisplatin-induced kidney injury. *Chemotherapy*, 55 (6): 399-406, 2009.

22. Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, Kaymak K: Effects of exogenous melatonin on myoglobinuric acute renal failure in the rats. *Ren Fail*, 26 (5): 479-486, 2004.

23. Aydogdu N, Atmaca G, Yalcin O, Batcioglu K, Kaymak K: Effects of caffeic acid phenethyl ester on glycerol-induced acute renal failure in rats. *Clin Exp Pharmacol Physiol*, 31 (9): 575-579, 2004.

24. Ustundag S, Sen S, Yalcin O, Ciftci S, Demirkan B, Ture M: L-Carnitine ameliorates glycerol-induced myoglobinuric acute renal failure in rats. *Ren Fail*, 31 (2): 124-133, 2009.

25. İlhan A: Deneysel miyoglobinürük akut böbrek yetmezliğinde nevilololün etkileri *Yüksek Lisans Tezi*, Trakya Üniv. Sağlık Bil. Enst., 2009.

26. Singh AP, Singh AJ, Singh N: Pharmacological investigations of *Punica*

granatum in glycerol-induced acute renal failure in rats. *Indian J Pharmacol*, 43 (5): 551-556, 2011.

27. Vlahovic P, Cvetkovic T, Savic V, Stefanovic V: Dietary curcumin does not protect kidney in glycerol-induced acute renal failure. *Food Chem Toxicol*, 45 (9): 1777-1782, 2007.

28. Ustundag S, Yalcin O, Sen S, Cukur Z, Ciftci S, Demirkan B: Experimental myoglobinuric acute renal failure: The effect of vitamin C. *Ren Fail*, 30 (7): 727-735, 2008.

29. Vanholder R, Sever MS, Ereğ E, Lameire N: Rhabdomyolysis. *J Am Soc Nephrol*, 11 (8): 1553-1561, 2000.

30. Newaz MA, Oyekan AO: Contribution of renal oxygenases to glycerol-induced acute renal failure in the rat. *J Cardiovascular Pharm*, 39 (6): 834-841, 2002.

31. Stefanovich V, Savic V, Vlahovic P, Cvetkovic T, Najman S, Zlatkovic MM: Reversal of experimental myoglobinuric acute renal failure with bioflavonoids from seeds of grape. *Ren Fail*, 22 (3): 255-266, 2000.

32. Chander V, Chopra K: Molsidomine, a nitric oxide donor and L-arginine protects against rhabdomyolysis-induced myoglobinuric acute renal failure. *Biochim Biophys Acta*, 1723 (1-3): 208-214, 2005.

33. Huang HS, Ma MC, Chen J: Chronic L-arginine administration increases oxidative and nitrosative stress in rat hyperoxaluric kidneys and excessive crystal deposition. *Am J Physiol Renal Physiol*, 295 (2): 388-396, 2008.

34. Huerta-Alardin AL, Varon J, Marik PE: Bench-to-bedside review: Rhabdomyolysis-an overview for clinicians. *Crit Care*, 9 (2): 158-169, 2005.

35. Aldamiz-Echevarria L, Andrade F: Asymmetric dimethylarginine, endothelial dysfunction and renal disease. *Int J Mol Sci*, 13 (9): 11288-11311, 2012.

36. Maree A, Peer G, Schwartz D, Serban I, Blum M, Wollman Y, Cabili S, Laina A: Role of nitric oxide in glycerol-induced acute renal failure in rats. *Nephrol Dial Transplant*, 9 (Suppl 4): 78-81, 1994.

37. Gök G: Deneysel miyoglobinürük akut böbrek yetmezliğinde rosiglitazonun etkileri. *Yüksek Lisans Tezi*, Trakya Üniv. Sağlık Bil. Enst., 2009.

38. Zager RA: Rhabdomyolysis and rnyohemoglobinuric acute renal failure. *Kidney International*, 49, 314-326, 1996.

39. Yılmaz Mİ, Eyileten T, Yenicesu M: Kronik böbrek hastalığında yeni bir oyuncu: Asimetrik dimetilarginin (ADMA). *Türk Nefroloji Diyaliz ve Transplantasyon Dergisi*, 16 (3): 93-101, 2007.

40. Li Volti G, Sorrenti V, Acquaviva R, Murabito P, Gullo A, Barcellona ML, Galvano F, Rodella L, Rezzani R, Vanella L, Tringali G, Caruso M, Gazzolo D, Di Giacomo C: Effect of ischemia-reperfusion on renal expression and activity of N^G-N^G-dimethylarginine dimethylaminohydrolase. *Anesthesiology*, 109 (6): 1054-1062, 2008.

41. Newaz MA, Oyekan AO: Vascular responses to endothelin-1, angiotensin-II, and U46619 in glycerol-induced acute renal failure. *J Cardiovas Pharmacol*, 38 (4): 569-577, 2001.

42. Apperloo AJ, Zeeuw DD, Jong PE: A short-term antihypertensive treatment-induced fall in glomerular filtration rate predicts long-term stability of renal function. *Kidney International*, 51 (3): 793-797, 1997.

43. Yavuz D, Küçükkaya B, Haklar G, Ersöz Ö, Akoğlu E, Akalin S: Effects of captopril and losartan on lipid peroxidation, protein oxidation and nitric oxide release in diabetic rat kidney. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 69 (4): 223-227, 2003.

44. Khatlab M, Ahmad M, Al-Shabanah OA, Raza M: Effects of losartan on blood pressure, oxidative stress, and nitrate/nitrite levels in the nitric oxide deficient hypertensive rats. *Resept Channels*, 10 (5-6): 147-157, 2004.

45. Rashtchizadeh N, Aghaeishahsavari M, Argani H, Noroozianavval M, Veisi P, Ghorbanihaghjo A: Enalapril and losartan affect lipid peroxidation in renal transplant recipients with renin-angiotensin system polymorphisms. *Clin Biochem*, 40 (3-4): 194-200, 2007.

46. Ito A, Egashira K, Narishige T, Muramatsu K, Takeshita A: Renin-angiotensin system is involved in the mechanism of increased serum asymmetric dimethylarginine in essential hypertension. *Japan Circul J*, 65 (9): 775-778, 2001.