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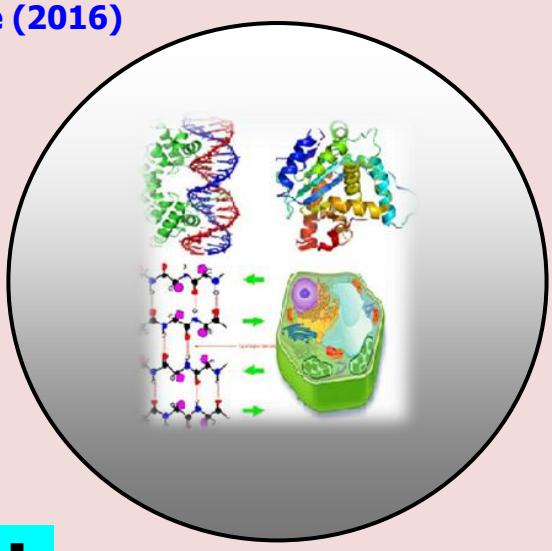

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The Role of Antioxidant Mechanisms and the Protective Effect of L-Arginin in the Nephrotoxicity Induced with Gentamicine on the Rat

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ABSTRACT

It is known that L-arginin, a NO precursor, has protective effects on gemtamycine (GM)-associated kidney failure. At the same time there are few studies suggest that L-arginin's protective effects are not related to only NO pathway and some other mechanisms can be included. In this study we aimed to compare the effects between L-arginin and its inactive isomere, D-Arginin, due to their structural similarities. Nitric Oxide Synthase (NOS) enzyme synthesis NO from L-Arginin. In this study our goal is compare the results between protective effects of L-Arginin on gentamycine-induced kidney damage and L-N-omega-L-arginine methylester (L-NAME), non-selective NOS inhibitor, treatment. There are limited studies about the changes induced by L-Arginin on NOS pathway in gentamycine derived kidney damage. In order to determine the alterations of NOS pathway induced by L-arginin in gentamycine derived kidney damage, Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Malondialdehyde (MDA), Xanthine Oxidase (XO), Nitric Oxide (NO), Glutathione (GSH), TNF-α and TGF-β parameters were measured. For this purpose eight groups were organized as: Control group (n=7), Gentamycine group (n=7), L-Arginin group (n=7), D-Arginin (n=7), GM + L-Arginin group (n=7), L-NAME group (n=7), L-NAME+GM group (n=7), D-Arginin + GM group. Although L-Arginin, GM and L-NAME groups haven't shown any kidney injury, gentamycine combined groups have more kidney injury and increased urea and creatine levels. L-NAME, L-NAME + GM, GM, L-Arginin + GM, D-Arginin + GM groups have decreased GSH levels compared to the control group. L-NAME + GM, L-Arginin + GM, D-Arginin + GM groups have assertive results on kidney damage in terms of NO, MDA, TAS, TOS, OSI levels compared to only gentamycine treated group. The most dramatically decrease on oxidative damage seen in L-Arginin + GM group. When compare the oxidative and nitrosative stress levels between L-Arginin + GM and D-Arginin + GM groups, althought both groups have significantly decrease but L-Arginin + GM group has better results. Both L-Arginin and D-Arginin have positive effects on decreasing of the elevated NO and MDA levels caused by gentamycine treatment. Besides L-NAME, L-Arginin + GM and D-Arginin + GM groups have shown significant decreased XO levels compared to GM group.

Conclusion: As a result, we suggest that L-Arginin can facilitate the removing of gentamycine. Thus, we evaluated the effects of L-arginin by induced NO synthase and L-NAME by blocked NO release in gentamycine associated kidney damage.

Key Words: D-Arginin, Gentamycine, L-Arginin, Nephrotoxicity, Antioxidant and Oxidative stress.

INTRODUCTION

Kidneys are the organs which withdraw the waste products and excess water resulting from the body's metabolic cycle from the organism in the urine. With such a mechanism, electrolyte, water balance is maintained by inspecting the content and the density of tissue fluid (Sharma, 2004, Lakkıs and Maldononado, 1999).

Nitric oxide (NO) defined as a neurotransmitter and vasodilator, is a versatile chemical molecule that have different biological effects. While there are several studies investigating the effect of NO on kidney tissues both physiologically and pathologically (Sharma, 2004, Can et al., 2000, Klahr, 2001), there are many other studies which relate NO's influence in cases of acute renal failure to the use of NO donor and nitric oxide synthase (NOS) inhibitors (Sharma, 2004, Can et al., 2000, Klahr, 2001, Valdivielso et al., 1997, Mazzon et al., 2001, Polat et al., 2006, Ghaznavi et al., 2005).

NO and citrulline is synthesized through a complex free radical reaction via the nitric oxide synthetases (NOS) which is the enzyme creating NO from L-arginine a semi-essential amino acid. Because it is a molecule that is released as NO is synthesized, it is not stored (Moncada et al., 1991).

Gentamicin is an aminoglycoside group antibiotic that is used to treat gram (-) bacterial infections. In the Gentamicin-induced molecular mechanisms of renal failure; it has been suggested that the accumulation of superoxide anions, the lysosomal enzyme changes and the inhibition of microsomal protein synthesis may lead to the nephrotoxic effects.

L-arginine given externally in case of nephrotoxicity has been reported to protect kidney damage by reducing macrophage infiltration in renal tissue, and to protect tubular functions by decreasing the absorption of gentamicin (Kurus et al., 2004, Cherla et al., 2004). It was specified that use of nitric oxide synthetase (NOS) inhibitors in cases leading to the production of NO with physiological levels is harmful (Chaverri et al., 2004).

As it is known that in cases that require long-term gentamicin treatment, excitation of nitric oxide is useful, while the inhibition is harmful, L-arginine provision has been reported in existing studies to work in reducing the nephrotoxicity. In another study, it was revealed that the gentamicin-induced protective effect in nephrotoxicity of L-arginine is not reversed by L-NAME and it was discussed that other mechanisms besides nitric oxide also contribute to the protective effect of L-arginine.

In the light of these studies, we compared the possible effects that may arise from the structural similarities of L-arginine in Gentamicin-induced renal failure and D-arginine, the inactive isomer of it with the effects that L-NAME might create.

MATERIAL AND METHODS

The Experimental Animals Used

In our study, 42 male Wistar albino rats weighing 250-300 grams were used obtained from the Experimental Research Center of Medical Sciences (ERCMS) received. The rats were hosted in metabolic cages in an environment at 24 ± 2 ºC room temperature, following 12 hours light, 12 hours dark cycle. The study was implemented within the framework of the permit received from the ERCMS ethics committee. For 7 days, the amount of water intake and urine output of the animals was followed.

The control group (n=7): The urine of the animals fed by regular food and tap water in metabolic cages was followed for 7 days.

Gentamicin group (n=7): Gentamicin injection (100mg/kg) was administered subcutaneously to the animals for 7 days.

D-Arginine group (n=7): D-Arginine (3 G/L) was added to the animals' drinking water for 7 days.

L-Arginine group (n=7): L-arginine (2 G/L) was added to the animals' drinking water for 7 days.

L-NAME group (n=7): L-NAME (100 mg/l) was added to the animals' drinking water for 7 days.

L-NAME group + Gentamicin (n=8): In addition to the Gentamycin injection, L-NAME (100 mg/l) was added to the rats' drinking water for 7 days.

L-arginine + Gentamicin (n=7): In addition to the Gentamycin injection, L-Arginine (2 g/l) was added to the rats' drinking water for 7 days.

D - Arginine + Gentamicin (n=7): In addition to the Gentamycin injection, D-Arginine (3 g/l) was added to the rats' drinking water for 7 days.

The Acquisition of Blood and Kidney Tissues and Their Preparation for the Analysis
On the 8th day, 24 hours after the last gentamicin injection, animals were anesthetized by ketamine (40 mg/kg) and their intracardiac blood was taken and they were decapitated. The kidney tissues were stored for histopathological evaluation in -70° C deep freezer wrapped in aluminum foil until the day of the biochemical tests to be carried out.
In the blood samples from experimental animals, the required storage conditions of BUN, serum creatinine levels were provided for the TAS, TOS, OSI, GSH, NO, MDA, XO and $TGF_{\beta 1}$, TNF_{α} analysis to be performed.

Renal Histopathology
The kidneys were painted with hematoxylin-eosin by taking 3-5 µm sections after being fixed in 10% formalin solution. Tubular necrosis, degenerative changes and tubular regeneration were evaluated with light microscopy.

Tissue Homogenization and Buffers
By adding 2 ml of Tris-HCl buffer (pH: 7.0), approximately 200 mg weighed tissues were homogenized via homogenizers at a speed of 16000 rpm for 3 minutes. After the process of homogenization, the supernatants separated by the centrifugation at 4000 rpm at +4°C for 10 minutes were stored in a -80°C deep freezer after being proportioned in Eppendorf tubes until the day of the measurement of biochemical parameters.
Serum and Kidney Tissue Homogenization, Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) and TGFβ1, TNFα Analysis
Total Oxidant Status (TOS), Total Antioxidant Status (TAS) levels were studied in Synergy HT Biotech auto analyzer (REL ASSAY brand commercial kit) (Erel, 2005).

Principle of Total Antioxidant Status (TAS) Measurement
Transformation of ferrous ion chelating complexes of oxidants in the sample into ferric ions is based on the directly proportioned measurement at 530 Nm using a spectrophotometer of the oxidant amount of the colored complex formed with ferric ions and chromogen solution. 20 µmol H_2O_2 Equiv./L solution was used as a standard and the results were expressed in µmol H_2O_2 Equiv./L.

Principle of Total Oxidant Status (TOS) Measurement
The antioxidant in the sample is based upon the decolorization of the colored radicals as a result of the molecules' reducing the ABTS cationic radicals (Hu et al., 1993). This decolorization is directly proportional to the concentrations of antioxidant molecules in the sample. Trolox, which is a water-soluble analog of vitamin E, was used as standard. The results were expressed as mmolTrolox Equivalent/L. Calculation of Oxidative Stress Index (OSI).
Oxidative stress index (OSI) is calculated by estimating the total oxidant status (TOS) levels with the level of total antioxidant status (TAS). OSI is used to specify the direction of the oxidant antioxidant balance of the body (Harma et al., 2005). The OSI unit was expressed as AU (Arbitrary Unit).
To calculate oxidative stress index (OSI), the formula, OSI =TOS / (TAS × 100), was used.

Transforming Growth Factor (TGF- β1) Analysis
TGF-β1 levels were determined using commercial rat ELISA kits (BioSource Int., Inc. California, USA, catalog and lot no.: KAC1688 and 043503). The results were calculated with the help of bioelisa reader Elx800 using standard curves.

Tumor Necrosis Factor (TNF α) Analysis
TNF-α levels were determined using commercial rat ELISA kits (IBL Co., Ltd. Gunma, JAPAN, catalog and lot no.: 17194 OL-527). The results were calculated with the help of bioelisa reader El x 800 using standard curves.

GSH, NO, MDA, XO Analysis in Serum and Kidney Tissue Homogenization
Glutathione (GSH) Analysis
GSH determination is based upon the 410 Nm spectrophotometric measurement of the absorbance of yellow colored product resulted from the reaction of sulfhydryl groups in the sample with Elman separator (Karabulut et al., 2002).

Nitric Oxide (NO) Analysis

NO occurred with NOS activity in the environment, the colored composite that occurs when reacting with the Griess reagent over nitrite was measured with a spectrophotometer at 545 Nm using the Cortas and Wakid method (Cortas and Wakid, 1990).

Malondialdehyde (MDA) Analysis

The measurement of lipid peroxidation product MDA was conducted by using Uchiyama and Mihara method (18). Briefly, the method is based upon the evaluation of 532 Nm spectrophotometric measurement of the absorbance of the pink-red color as a result of the reaction of MDA with TBA in the samples that occurs at 95^{o} C. Standards prepared in different concentrations and specimen absorbances obtained by drawing a standard graph were calculated through the standard graph. The amount of MDA was expressed as nmol/L.

Xanthine Oxidase (XO) Analysis

Xanthine Oxidase determination was done after incubation for 30 minutes with a buffer that contained the xanthine of the sample which created uric acid from xanthine. Then, by adding 100% TCA, the reaction was stopped and the amount of uric acid was fixed. Absorbance value was measured at a wavelength of 293 Nm. This value is directly proportional to the activity of the XO (Bergmayer, 1974).

The Statistical Evaluation of Data

In serum and kidney tissues obtained from groups of the generated experimental protocol, TAS, TOS, OSI, GSH, NO, MDA, XO, TGF- β_1 and TNF α levels were investigated. In rat blood samples; BUN, serum creatinine, Na+, K+, in urine samples; GGT, creatinine, Na+ and K+ levels were studied. Also creatinine clearance and fractional Na excretion were calculated by standard methods.

In order to statistically evaluate the data, whether the distribution of the data seemed normal was checked initially. When it was observed that the normal distribution was not existent, non-parametric tests were decided to be used.

Data were summarized by using the median, minimum and maximum values as a descriptive measure.

RESULTS

Compared to the control group, in all other groups except the D-Arginine group, a statistically significant increase in the level of urea was observed. When the control group was compared with the gentamicin group, it was detected that the level of urea was observed to increase significantly. This increase could only be reversed by L-Arginine, not by D-Arginine with a statistically significant manner (Table 8).

In L-Arginine, D-Arginine, L-NAME and the control groups, a statistically significant change wasn't observed in creatinine levels. It was observed that the application of gentamicin caused a statistically significant increase in creatinine levels. It was identified that L-Arginine partially prevented the increase in creatine levels observed with gentamicin application alone (Table 9).

According to the results of this study, a statistically significant increase in xanthine oxidase levels wasn't observed with the gentamicin application. D-Arginine or L-Arginine remained ineffective on this increase observed with gentamicin application (Table 10).

A statistically significant reduction in GSH levels of the Gentamicin group compared to control group was observed. L-Arginine was observed to partially prevent the gentamicin induced decrease in GSH levels. L-Arginine alone led to a statistically significant increase in GSH levels (Table 11).

L-Arginine, or gentamicin application was detected to cause a significant increase in NO levels compared to control group. Application of Gentamicin in combination with L-Arginine or L-NAME was observed to cause a significant decrease in the gentamicin induced increase in NO levels (Table 12).

In gentamicin applied group, MDA levels led to a statistically significant increase compared to control group. It was also observed that the application of L-Arginine partially prevented this increase occurring with gentamicin (Table 13).

While gentamicin application caused a significant decrease in TGF- β_1 levels compared to the control group, it was identified that L-Arginine reversed this decrease thriving with gentamicin statistically in a meaningful way. D-Arginine resulted in a slight decrease in TGF- $\beta_1$levels. D-Arginine use in combination with gentamicin led to partly reverse the decrease in TGF- $\beta_1$levels caused by gentamicin alone (Table 14).

While a statistically significant increase in TNF - α levels was observed when Gentamicin was compared to the control group, L-Arginine was observed to partially reverse this increase caused by gentamicin alone (Table 15). The application of gentamicin caused a statistically significant decrease in TAS levels while it caused a statistically significant increase in TOS levels. Thus, it led to a decline in OSI index which is an indicator of the ratio between TAS/TOS levels. However, L-Arginine application reversed the table caused by gentamicin (Table 16, 17 and 18).

DISCUSSION

In this study, in the protective effect of L-Arginine, a NO precursor amino acid, on gentamicin-mediated nephrotoxicity, it was thought that L-Arginine could contribute through other mechanisms as well as its contribution to the synthesis of nitric oxide. Based upon this hypothesis, the impact of L-Arginine and its inactive isomer D-Arginine on gentamicin nephrotoxicity was investigated comparatively. It was observed that L-Arginine not D-Arginine could cause a protective effect by not only supporting NO synthesis but also reducing oxidative stress.

In the studies, it has been shown that L-Arginine has a protective effect on gentamicin (GM) induced renal failure and this protective effect could be reversed by L-NAME - a nitric oxide synthesis inhibitor and L-Arginine - NO pathway has been reported to be of major importance in renal diseases. In addition, it is known that L-Arginine affects different metabolic pathways by stimulating the release of insulin, glucagon, growth hormone and endogenous steroids.

L-NAME, in cases of GM-induced nephrotoxicity, has been reported to cause exacerbation in the table and result in an escalate in SCR levels. In this study, compared to the control group, in all other groups except the D-Arginine group, a statistically significant increase in urea concentrations was observed. Similarly, creatinine levels in the control group were detected to be significantly higher than those in other groups except D-Arginine and L-NAME groups. In the groups applied L arginine, Gentamicin, L-NAME, L-NAME + gentamicin, L-Arginine + gentamicin and D-Arginine + gentamicin; depending on the application, symptoms of renal damage were identified and with the administration of gentamicin, renal damage was identified to increase as well as the levels of urea and creatinine to be escalated. The finding about the identified negative effect of L-NAME coincides with the findings of the researchers above.

Urinary protein excretion and nitrite (NO2) discharge was measured by hosting rats in metabolic cages which were under nephrotic syndrome characterized by proteinuria through puromicine amino nucleotide (PAN) and glomerular epithelial cell damage. In the subjects of nephrotic syndrome, a significant increase in the levels of urine and plasma of nitrite, nitric oxide was determined when compared to the control group. It was emphasized that oxidative andnitrosative stress increased in gentamicin-induced renal failure and a sharp decline in non - protein sulfhydryl and superoxide dismutase activities was recorded.

In another study, while chronic administration of L-Arginine could cause changes in renal function of rats which were treated by gentamicin, acute L-Arginine administration did not change renal function in any experimental group. It is reported that while L-Arginine has no effect in the excretion of N-acetyl-β-D glukosaminidase, alkaline phosphatase increased in two groups treated by L-NAME.

In rabbits, control in experimental nephrotoxicity of gentamicin, in terms of oral intake of selective and nonselective (AG, L-NAME) nitric oxide synthase inhibitors with nitric oxide donor (L-Arginine), a significant change in biochemical parameters of L-Arginine, aminoguanide, L-ng-Nitro-arginine methyl ester (L-NAME) wasn't determined. An increase in L-Arginine Group was observed in terms of nitrite concentrations and serum while there was a sharp decrease in aminoguanide, L-ng-Nitro-arginine methyl ester (L-NAME) groups. An increase in Gentamicin induced BUN serum levels and a significant decline in creatinine clearance weight were determined. In our study, it was identified that NO, MDA, TAS, TOS, OSI levels of L-NAME + Gentamicin, L-Arginine + gentamicin, D-Arginine + Gentamicin group had a positive effect in terms of renal damage then compared to gentamicin group according to the results obtained with the addition of L-NAME, L-Arginine, D-Arginine as well as gentamicin in gentamicin induced rats. When combined treatments were compared, the biggest decline in oxidative damage was determined to be in L-Arginine + Gentamicin group. As a result, L-Arginine as NO synthesis precursor was considered to facilitate excretion of gentamicin.

In another study in which gentamicin therapy was administered, it was identified that the malondialdehyde levels of kidney tissue of the group with renal failure characterized by a considerable increase in plasma creatinine and urea concentrations of the rats were high; while GSH, gsh-px, and cat levels were low when compared to the control group (9).

In another study in which the effects of aminoguanide were investigated, it was reported that there was an increase in kidney tissue malondialdehyde (MDA), nitric oxide (NO) levels in gentamicin-induced renal failure; however, it was also reported that gsh-px, SOD, cat activities and GSH content were reduced (9). In rats with gentamicin-induced nephrotoxicity, there has been a significant decrease in kidney manganese dependent superoxide dismutase, copper/zinc superoxide dismutase, glutathione peroxidase, glutathione reductase; catalase activities according to the results of the control group.

Table 1. Urealevels of comparison Control, D-arginine, L-arginine, Gentamycin, L- NAME, L NAME + Gentamycin group, L-arginine + Gentamycin group, D arginine + Gentamycin groups.

	Grup	N	Mean	1) SD	2) SEM	p
Ure (mg/dL)	Control	7	11.00	1	0.38	<0,05
	D-arginin	7	12.00	1.29	0.49	
	L-arginine	7	17.86*	0.9	0.34	
	Gentamicin	7	35.29*	3.99	1.50	
	L-NAME	7	15.14	1.77	0.67	
	LNAME+ gentamicin	7	29.50	3.78	1.33	
	Larginine +gentamicin	7	18.57*+	0.98	0.370	
	Darginine+gentamicin	7	32.00*	1.83	0.692	

*** Difference compared to control, + Difference compared to gentamicin (statistically significant).**

Table 2. Creatinine levels of Control, D-arginine, L-arginine, Gentamycin, L-NAME, L NAME + Gentamycin group, L-arginine + Gentamycin group, D arginine + Gentamycin groups.

	Grup	N	Mean	SD	3) SEM	p
Creatin (mg/dl)	Kontrol	7	0,47	0.02	4) 0.008	<0,05
	D-arginine	7	0,47	5) 0.01	6) 0.004	
	L-arginine	7	0,49	7) 0.03	8) 0.011	
	Gentamicine	7	0,93*	9) 0.14	10) 0.053	
	L-NAME	7	0,46	11) 0.02	12) 0.008	
	L-NAME + gentamisin	7	0,94*	13) 0.06	14) 0.021	
	L-arginin + gentamisin	7	0,69*+	15) 0.03	16) 0.011	
	D-arginin + gentamisin	7	0,95	17) 0.05	18) 0.019	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Table 3. Comparison of xanthineoxidase levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L-NAME + Gentamycin group, L-arginine + Gentamycin group, D-arginine + Gentamycin groups.

	Group	N	Mean	19) SD	20) SEM	p
XanthineOxidase (µU/ml)	Kontrol	7	21) 170.59	22) 9.53	23) 3.602	<0.05
	D-arginin	7	24) 170.02	25) 2.63	26) 0.994	
	L-arginin	7	27) 158.54	28) 4.81	29) 1.818	
	Gentamicin	7	30) 189.19*	31) 9.14	32) 3.455	
	L-NAME	7	33) 182.54*	34) 4.4	35) 1.663	
	L-NAME + gentamicin	7	203.89	36) 8.71	37) 3.079	
	L-arginin + gentamicin	7	38) 183.31*	39) 4.26	40) 1.610	
	D-arginin + gentamicin	7	41) 188.90*	42) 7.05	43) 2.665	

- **Differences (statistically significant) with respect to control.**

Table 4. Comparison of glutathione levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L-NAME + Gentamycin group, L-arginine + Gentamycin group, D-arginine + Gentamycin groups.

	Grup	N	Mean	44)	SD	45)	SEM	p
GSH (µmol/l)	Control	7	46) 38.42	47)	0.91	48)	0.344	<0.05
	D-arginin	7	49) 38.02	50)	1.13	51)	0.427	
	L-arginin	7	43.32*	52)	2.08	53)	0.786	
	Gentamicin	7	54) 24.21*	55)	2.97	56)	1.123	
	L-NAME	7	57) 30.28	58)	2.21	59)	0.835	
	L-NAME+ Gentamisin	7	60) 25.61	4.46		61)	1.577	
	L-arginin +Gentamisin	7	62) 28.46*+	63)	0.69	64)	0.261	
	D-arginin+Gentamisin	7	65) 25.39	66)	1.25	67)	0.472	

* Kontrole göre farklılığı, + Gentamisine göre farklılığı (istatiksel olarak anlamlılığı göstermektedir.

Table 6. Comparison of nitricoxide levels of control, D-arginine, L-arginine, Gentamicin, L-NAME, L-NAME + Gentamicin group, L-arginine + Gentamicin group, D-arginine + Gentamicin groups.

	Group	N	Mean	68)	SD	69)	SEM	P
NO (µmol/dl)	Control	7	70) 25,40	71)	0.93	72)	0.352	<0,05
	D-arginine	7	73) 28,03	74)	1.51	75)	0.571	
	L-arginine	7	76) 44,65*	77)	2.38	78)	0.900	
	Gentamicin	7	79) 49,92*	80)	3.37	81)	1.274	
	L-NAME	7	82) 23,31	83)	1.89	84)	0.714	
	L-NAME+ gentamicin	7	85) 42,77*+	86)	3.96	87)	1.400	
	L-arginin +gentamicin	7	88) 34,61*+	89)	2.09	90)	0.790	
	D-arginin + gentamicin	7	91) 47,27	92)	1.92	93)	0.726	

**Difference compared to control, + Difference compared to gentamicin (statistically significant).*

Table 7. Malondialdehyde levels Compared to Control, D-arginine, L-arginine, Gentamycin, L-NAME, L NAME + Gentamicin group, L-arginine + Gentamicin group, D arginine + Gentamicin groups.

	Group	N	Mean	94)	SD	95)	SEM	p
MDA (nmol/ml)	Control	7	96) 20,06	97)	0.99	98)	0.374	<0,05
	D-arginin	7	99) 20,66	0.7		0.265		
	L-arginin	7	21,01	1.03		0.389		
	Gentamicin	7	42,05*	1.33		0.503		
	L-NAME	7	19,58	1.46		0.552		
	L-NAME+ Gentamisin	8	41,83	1.66		0.587		
	L-arginine +Gentamisin	7	33,58*+	1.29		0.488		
	D-arginin +Gentamisin	7	42,05	1.33		0.503		

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Nephrotoxicity developed in Gentamicin-induced nephrotoxicity rat groups and an increase in renal oxidative stress, and a decrease in Mn superoxide dismutase, glutathione peroxidase and glutathione reductase activities were identified.

One of the enzymatic sources of reactive oxygen species in living systems which is actively involved in the formation of xanthine, is converted into oxidase enzymes by oxidizing thiol groups in the case of any stress in the tissues. Xanthine oxidase superoxide (O2-·) also contributes to the formation of radicalshydroperoxide with the production of ROS. It is known that the level of serum increases in cases of causing oxidative damage. Glutathione is a tripeptide that can chemically detoxify many kinds of organic oxide in metabolism. In accordance with all this information, the levels of L-NAME, L-NAME + gentamicin, gentamicin, L-Arginine + gentamicin, D-Arginine + Gentamicin groups were determined to be significantly lower in glutathione when they are compared to the control group.

Gentamicin applied glutathione levels are determined significantly lower as a result of the comparison between the combined treatments. In comparison with the group applied gentamicin solely, glutathione level of the L-Arginine + Gentamicin group is the highest. It can be a sign that the combined application of L-NAME, L-Arginine, D-Arginine to gentamicin reduces the stress of the tissue. It is concluded that nitric oxide inhibition is beneficial in gentamicin associated nephrotoxicity, and L-Arginine would be useful in reducing nephrotoxicity in long term treatments where gentamicin is required considering the results of the harmful effects of the nitric oxide inhibition.

Table 8. Comparison of TGF-B1 levels of control, D-arginine, L-arginine, Gentamicin, L-NAME, L NAME + Gentamycin group, L-arginine + Gentamicin group, D arginine + Gentamicin groups.

	Group	N	Mean	SD	SEM	p
TGF-β1 (pg/ml)	Control	7	741.10	113.71	42.978	<0.05
	D-Arginine	7	656.03	65.6	24.794	
	L-Arginine	7	793.13	23.65	8.939	
	Gentamicin	7	156.32*	26.97	10.194	
	L-NAME	7	679.67	79.34	29.988	
	L-NAME+ Gentamicin	8	139.58	19.94	7.050	
	L-arginine +Gentamicin	7	432.13*+	30.73	11.615	
	D-arginine +Gentamicin	7	207.23	34.33	12.976	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Table 9. Comparison of TNFα levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L NAME + Gentamycin group, L-arginine + Gentamycin group, D arginine + Gentamycin groups.

	Group	N	Mean	SD	SEM	p
TNFα (pg/ml)	Control	7	705.45	77.46	29.28	<0.05
	D-arginine	7	664.28	24.22	9.15	
	L-arginine	7	836.57	70.08	26.50	
	Gentamicin	7	2508.43*	7.72	2.91	
	L-NAME	7	756.26	37.89	14.32	
	L-NAME+ Gentamicin	7	2266.00	133.84	47.32	
	L-arginine +Gentamiscn	7	1283.78*+	61.62	23.29	
	D-arginine +Gentamicine	7	2137.14	186.3	70.41	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Table 10. Comparison of TAS levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L-NAME + Gentamycin group, L-arginine + Gentamycin group, D-arginine + Gentamycin groups.

	Grup	N	Mean	SD	SEM	p
TAS (mMTrolox)	Control	7	0.97	0.02	0.008	<0.05
	D-arginine	7	0.95	0.03	0.011	
	L-arginine	7	1.17	0.35	0.132	
	Gentamicine	7	0.65*	0.07	0.026	
	L-NAME	7	0.89	0.07	0.026	
	L-NAME+ gentamicin	7	0.62	0.04	0.014	
	L-arginin +gentamicin	7	0.79	0.04	0.015	
	D-arginin+gentamicin	7	0.74*+	0.08	0.030	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Table 11. Comparison of TOS levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L-NAME + Gentamycin group, L-arginine + Gentamycin group, D-arginine + Gentamycin groups.

	Group	N	Mean	SD	SEM	p
TOS ($\mu molH_2O_2Equiv/L$)	Control	7	15.07	0.82	0.31	<0.05
	D-arginin	7	15.16	0.53	0.20	
	L-arginin	7	17.09	0.89	0.33	
	Gentamisin	7	27.51*	1.33	0.50	
	L-NAME	7	16.00	1.07	0.40	
	L-NAME+ gentamisin	7	25.70	1.46	0.516	
	L-arginin +gentamisin	7	17,98*+	0.98	0.370	
	D-arginin+gentamisin	7	24.68	1.82	0.688	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Table 12. Comparison of OSI Indices levels of control, D-arginine, L-arginine, Gentamycin, L-NAME, L-NAME + Gentamycin group, L-arginine + Gentamycin group, D-arginine + Gentamycin groups.

	Group	N	Mean	SD	SEM	p
Osi İndex	Kontrol	7	15.59	0.83	0.314	< 0.05
	D-arginin	7	16.00	0.81	0.306	
	L-arginin	7	15.42	3.37	1.274	
	Gentamisin	7	42.71*	5.49	2.075	
	L-NAME	7	18.14	1.77	0.669	
	L-NAME+ gentamisin	7	41.32*	3.73	1.319	
	L-arginin +gentamisin	7	22.73*+	1.86	0.703	
	D-arginin+gentamisin	7	33.85*+	5.25	1.984	

* Difference compared to control, + Difference compared to gentamicin (statistically significant).

Moreover, it is determined that the Xanthine oxidase level of the group in which applied gentamicin is significantly lower when it is compared to the results of xanthine oxidase level of L-NAME, L-Arginine + gentamicin, D-Arginine + Gentamicin. In comparison with the L-NAME + Gentamicin group, it is thought that higher xanthine oxidase levels and lower glutathione level in comparison with the gentamicin group lead to the formation of ROS in the tissue in spite of L-NAME treatment. When combined treatments are compared, it is determined that oxidative damage mostlydecline in L-Arginine + Gentamicin group.

TNFα (pg/ml) levels of L-Arginine, gentamicin, L-NAME, L-NAME + Gentamicin, L-Arginine + gentamicin, D-Arginine + Gentamicin groups were found to be significantly higher when compared to the control group. However; TGFß groups' (pg/ml) levels of D-Arginine, Gentamicin, L-NAME, L-NAME + gentamicin, L-Arginine + gentamicin, D-Arginine + gentamicin groups were significantly lower in comparison with the control group. Tumor necrosis factor Alpha (TNF - α) is important in the processes of inflammation, cell life and cell death. This molecule can increase the production of NO by activating inducible nitric oxide synthetase (iNOS). It is known that while iNOS synthesis is stimulated by necrosis factor - α (TNF - α), it is inhibited bytransforming growth factor - β (TGF –β). This significant increase in TNF α levels suggests a proportional increase in nitrosative andoxidative stress. These findings are seen to be supportive of those argued in previous studies.

Based on the results obtained; it is thought that it could be useful to give gentamicin, one of the aminoglycosides, together with L-Arginine to reduce nephrotoxicity. However, whether L-Arginine could change the antibacterial activity of gentamicin is another issue that needs to be studied.

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REFERENCES

Sharma, S.P. (2004). Nitric oxide and the Kidney. Indian J Nephrol; 14: 77-84.

Lakkıs, F.G., Maldononado, M.M. Jacobson, H.R., Striker, G.E., Klahr, S. (eds) (1999). Conservative management of chronic renal failure and the uremic sendrome. The principles and practice of nephrology. Esence of the science. St Louis: CV, 1999;140-148.

Can, C., Şen, S. and Neşe, B., Işık. (2000). Protectiveeffect of oral L-arginineadministration on gentamicininducedrenalfailure in rats. Eur J Pharmacol; 390: 327-334.

Klahr, S. (2001). The role nitricoxide in hypertensionandrenaldiseaseprogression. Nephrol Dial Transpl; 16 (1): 60-62.

Valdivielso, J.M., Cabanero, L.R., Barriocanal, F.P. and Lopez-Novoa, J.M. (1997). Effect of nitricoxide synthesis modification on renalfunction in gentamicin-induced nephrotoxicity. Environ Toxicol Phar, 1997; 3: 123-128.

Mazzon, E., Britti, D., Sarro, A.D., Caputi, A.P. and Cuzzocrea, S. (2001). Effect of N-acetylcysteine on gentamicin-mediatednephropathy in rats. Eur J Pharmacol; 424: 75-83.

Polat, A., Parlakpinar, H., Tasdemir, S., Colak, C., Vardi, N., Ucar, M., Emre, M.H. and Acet, A. (2006). Proctective role of aminoguanidine on gentamicin-induced acute renal failure in rats. Acta Histochem, 108: 365-371.

Ghaznavi, R., Faghihi, M., Kadkhodaee, M., Shams, S. and Khastar, H. (2005). Effects of nitricoxide on gentamicin toxicity in isolated perfused rat kidneys. J Nephrol; 18: 548-552.

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991). Nitricoxide: physiology, pathophysiology and pharmacology. Pharmacologic Rewiews, 43: 109-141.

Kurus, M., Esrefoglu, M., Karabulut, A.B., Sogutlu, G., Kaya, M., Otlu, and A. Oral (2008). L-A rginine protects against cyclosporine-induced hepatotoxicity in rats, Exp Toxicol Pathol; 60(4-5):411-9.

Cherla, G. and Jaimes, E.A. (2004). Role of L-Arginine in thepathogenesisandtreatment of renaldisease. J Nutr; 134: 2801-2806.

Chaverri, J.P., Barrera, D., Maldonado, P.D., Chirino, Y.I., Macias-Ruvalcaba, N.A., Medina Campos, O.N., Castro, L., Salcedo, M.I. and Hernandes-Pando, R. (2004). Sallylmercaptocysteine scavenges hydroxyl radical and single toxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stres and renal damage in vivo. BMC Clin. Pharmacol, 4(5):1-7.

Erel, O. A. (2005). New automated colorimetric method for measuring total oxidant status. Clin Biochem, 38 (12), 1103-11.

Hu, M.L., Louie, S., Cross, C.E., Motchnik, P. and Halliwell, B. (1993). Antioxidant protection against hydrochlorous acid in human plasma. J Lab Clin Med, 121: 257- 62.

Harma, M., Kocyigit, A. and Erel, O. (2005). Increased DNA damage in patients with complete hydatidiformmole. J. Mutat Res; 583: 49-54.

Karabulut, A.B., Özerol, E. and Temel, İ. (2002). Yaş ve sigara içiminin eritrosit katalaz aktivitesi ve bazı hematolojik parametreler üzerine etkisi. İnönü Üniversitesi Tıp Fakültesi Dergisi; 9(2): 85–88.

Cortas, N.K. and Wakid, N.W. (1990). Determination of inorganicnitrate in serum andurineby a kinetic cadmium reduction method. Clin Chem; 36: 1440-1443.

Zhao, J., Liu, X.J. and Ma, J.W. (2004). DNA damage in healthy termneonate. Early Hum Dev; 77: 89–98.

Bergmayer, H.U. (1974). Methods of Enzymatic Analysis; 644.

Yang, C.W., Yu, C.C., Ko, Y.C. and Huang, C.C. (1998). Amino guanidine reduces glomerular inducible nitricoxid esynthase (İNOS) and transforming growth factor-beta 1 (TGF-β1) mRNA expression and diminishes glomerulosclerosis in NZB/W F1 mice. Clin ExpImmunol; 113: 258-264.

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