The Protective Effects of Pentoxifylline on Contrast Induced Nephropathy in Rats

Ratlarda Pentoksifilinin Kontrast Nefropatisi Üzerine Koruyucu Etkileri

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ABSTRACT

Objective: Pentoxifylline (PTX), which has antioxidant and immunomodulatory effects, has been shown to attenuate renal ischemia-reperfusion injury. We aimed to investigate whether PTX might have a preventive role against the development of contrast induced nephropathy (CIN).

Material and Methods: A total of 24 Wistar-albino female rats were randomly divided into four groups (n=6 each) as follows; control (C), contrast material (CM), PTX, and PTX+CM. Except the control and CM groups, the groups were given PTX once daily orally for 5 days. CIN was induced by administration of intravenous high-osmole contrast material-diatrizoate (6 mL/kg) after 48 hour of dehydration. Basal and post-CIN kidney function parameters, inflammatory parameters, serum and renal tissue oxidative stress markers (serum levels of advanced oxidation protein product and malondialdehyde), and histopathological lesions were assessed.

Results: The increase in serum creatinine and blood urea nitrogen levels was significantly higher only in the CM group (P<0.05). Absolute changes of serum creatinine levels in the PTX, PTX+CM and C groups were significantly lower than those observed in the CM group (P<0.05). There was no significant decrease in creatinine clearance in any group except CM. The differences among the groups regarding the absolute change in creatinine clearance were not significant. Serum levels of advanced oxidation protein product and malondialdehyde were significantly lower in PTX+CM than those in CM (P<0.05). Histopathological lesions in the CM group were more advanced (P<0.05).

Conclusion: In conclusion, the study suggests that PTX may prevent CIN.

Key Words: Contrast nephropathy, Pentoxifylline, Kidney, Preventive, Oxidative stress

ÖZ

Amaç: Antioksidan ve immüno-modülatör etkileri olan pentoksifilinin (PTX), renal iskemi-reperfüzyon hasarını azalttığı gösterilmiştir. PTX'in kontrast kaynaklı nefropatinin (CIN) gelişmesine karşı koruyucu bir rolü olup olmadığını araştırdık.

Gereç ve Yöntemler: Toplam 24 tane wistar-albino dişi sıçan rastgele-altışarlı kontrol (C), kontrast madde (CM), PTX ve PTX + CM şeklinde dört gruba ayrıldı. Kontrol ve CM grupları haricindekilere 5 gün boyunca günde bir kez PTX verildi. CIN, 48 saat dehidrasyondan sonra intravenöz yüksek ozmolar kontrast madde olan -diatrizoatla (6 mL/kg) indüklendi. Bazal ve post-CIN böbrek fonksiyon parametreleri, inflamatuar parametreler, serum ve renal doku oksidatif stres belirteçleri (ileri oksidasyon protein ürünü ve malondialdehid serum seviyeleri) ve histopatolojik lezyonlar değerlendirildi.

Bulgular: Serum kreatinin ve kan üre nitrojen düzeylerindeki artış ve kreatin klerensinde azalma sadece CM grubunda anlamlıydı (P<0.05). PTX, PTX + CM ve C gruplarında serum kreatinin

düzeylerindeki mutlak değişim CM grubundakine göre anlamlı derecede düşüktü (P<0.05). Kreatinin klerensi mutlak değişimi tüm gruplar arasında benzerdi. İleri oksidasyon protein ürünü ve malondialdehidin serum seviyeleri PTX+CM'de CM'den anlamlı olarak daha düşüktü (P<0.05). CM grubunda histopatolojik lezyonlar daha ileri düzeydeydi (P<0.05).

Sonuç: Çalışmadaki bulgular, PTX'in CIN'i önlenmesi için bir aday ajan olabileceğini düşündürmektedir.

Anahtar Sözcükler: Kontrast nefropatisi, Pentoksifilin, Böbrek, Önleyici, Oksidatif stres

INTRODUCTION

Contrast-material Induced Nephropathy (CIN) is characterized as acute renal failure (ARF) arising within 48 hours after administration of intravenous radiographic contrast material (CM) that is not assignable to other reasons (1, 2). It is implicated for most hospital-acquired cases of ARF. Many hospitalized patients treated in intensive care units have impaired kidney function which is the most important underlying risk factor for CIN (3). Other risk factors for iatrogenic nephrotoxicity may be associated with advanced age, diabetes mellitus (DM), heart failure, renal dysfunction, volume depletion, sepsis, or other medications such as concurrent use of several nephrotoxic drugs.

The exact underlying mechanism of CIN is still unclear. Combination of renal ischemia along with direct tubular epithelial cell toxicity was shown to be the possible pathogenesis in experimental studies (1). The release of oxidative stress and free radicals caused by CM and the decrease in the synthesis of renal prostaglandins and nitric oxide have also been shown to be associated with the possible mechanism of CIN (4-6). There is no widely accepted method for preventing contrast-induced nephropathy other than volume expansion with intravenous fluids as yet (7).

PTX is a methylxanthine derivative with potent hemorrheologic properties and is a non-specific phosphodiesterase inhibitor used in the treatment of peripheral vascular diseases (8, 9). PTX exhibits many pharmacological effects such as immuno-modulatory, anti-inflammatory, and antiproliferative effects; inhibition of platelet aggregation, amelioration of the microcirculation, decrease in blood viscosity, increase in erythrocyte deformability, and downregulation of several pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) (10). The anti-oxidative effect of PTX is still uncertain. Recently, pentoxifylline (PTX) has attracted much interest as a scavenger of free oxygen radicals. Several experimental studies have confirmed the probable antioxidant effect of this agent (11, 12). Recent animal studies have shown that PTX precludes progressive kidney injury associated with septic shock, presumably protecting the renal microcirculation (13, 14).

Herein we aimed to investigate in rats the effects of PTX on CM-induced histopathological changes along with biological markers including dialdehyde (MDA), serum creatinine (Cr), and blood urea nitrogen (BUN), which are commonly used to monitor the development of kidney damage due to oxidative stress.

MATERIALS and METHODS

Animals

The study included 24 female Wistar albino rats (6 weeks old with a mean weight of 235 ± 38g). The rats were kept in metabolic cages for the 24-hour urine accumulation periods (on days 1 and 6). During other processes, they were kept in polycarbonate cages. The rats were housed at a temperature of 22–25 °C, with 12 hours of light and dark cycles. The study was approved by the Experimental Animals Ethics Committee of Gazi University.

Experimental design and drugs

Rats were randomly divided into 4 groups (each with 6 animals) as follows: Control, CM, PTX, and PTX+CM (the study design is demonstrated in Figure 1). Considering the average weight of the rats in our study, rats in the PTX group were given PTX at a dose of 20 mg/day for 5 days via oral gavage in order to prevent CIN (drug in microparticle form was prepared from S. cerevisiae yeast-made [Imuneks, Mustafa Nevzat Pharmaceuticals, Turkey] suspension in sterile saline). On day 4, rats in the PTX+CM and CM group were administered diatrizoate intravenously (Urografin 76%, Schering AG, Germany) at a dose of 6 mL/kg by their tail veins under ether anesthesia. All rats were fed with unlimited standard chow and were left thirsty for 2 days (on days 3 and 4).

Biochemical analysis and renal function assessment

In order to minimize circadian alterations, drug administration, blood sampling, and weighing processes were conducted between 09.00 a.m. and 10.00 a.m. Blood samples for blood urea nitrogen (BUN), sodium (Na), creatinine (Cr) and 24-h urine samples for Cr and Na were collected on the 1st and 6th day. On day 6, blood samples were also collected for malondialdehyde (MDA), total thiol, advanced oxidation protein products (AOPP), nitric oxide (NO), tumor necrosis factor-alpha (TNF-a) and interleukin-1-beta (IL-1b). Additionally, on day 6, the kidneys were excised under general anesthesia via intraperitoneal

xylazine (Rompun® flacon, 5 mg/kg, Bayer, Istanbul, Turkey) and ketamine (Ketalar® flacon, 45 mg/kg, Pfizer, Istanbul, Turkey). The right kidney was stored after adding 10% formalin and was preserved for histopathological evaluation. After washing in saline, the left kidney was stored at -70°C for analysis of AOPP, MDA and total thiol in the kidney tissue. For measurements, tissues were weighed and homogenized in 10 mL of cold 0.01 M Tris–HCL. Subsequently, the clear supernatants were kept after 15 minute centrifugation of homogenates at 15.000 rpm at 4°C. Chemicals and kits were from Sigma–Aldrich and Sigma Diagnostic (St. Louis, MO, USA). The Jaffe method was applied in the analyses of serum and urine Cr. BUN was measured by a kinetic ultraviolet assay method in an auto-analyzer. Plasma and urine Na were measured in an auto-analyzer. Creatinine clearance (CrCl) was calculated using the formula of U x V/P (U, urine Cr mg/dl; V, urine volume ml/min per 100 g; P plasma Cr mg/dl), and was corrected as ml/min per 100 g. Fractional Na excretion (FENa) was calculated as (urine Na/plasma Na) x (plasma Cr/urine Cr) x 100.

Measurement of oxidation and inflammatory parameters

AOPP measurement in serum and tissue

Two hundred microliters of supernatant were diluted in 20% phosphate buffered saline or chloramine-T solution. Ten microliters of 1:16 potassium iodide and 20 µL of acetic acid were mixed. The absorbance of the reaction mixture was read at 340 nm. Serum AOPP concentrations are specified as 'lmol/L', and AOPP levels are defined as 'mmol/g tissue'.

MDA measurements in serum and tissue

The lipid peroxidation product-MDA was processed with 2-thiobarbituric acid at 95°C. A pink colored complex absorbing at 532 nm was obtained. 1,1,3,3-tetraethoxy-propane was used as the standard. Results are defined as 'l mol/L' and 'nmol/g tissue'.

Measurement of serum NO levels

The Cayman Elisa Kit (Nitrate/Nitrite Colorimetric Assay Kit item no: 780001, Cayman Chemical Company) was used for NO and results were obtained by comparison with nitrite standards. Values are specified as 'l mol/L'.

Measurement of TNF-a and IL-1b in serum

BioSource Elisa kits were used for TNF-a, and IL-1b. Values are defined as 'pg/mL'.

Histopathological analysis of kidneys

Right renal tissues obtained previously for histopathological analyses were longitudinally cut from the top to the bottom and were preserved in 10% formalin for 72 hours. Samples were studied with routine histological procedures and sections 5 µm thick were cut from the paraffin blocks which were obtained from the tissue. Sections were analyzed

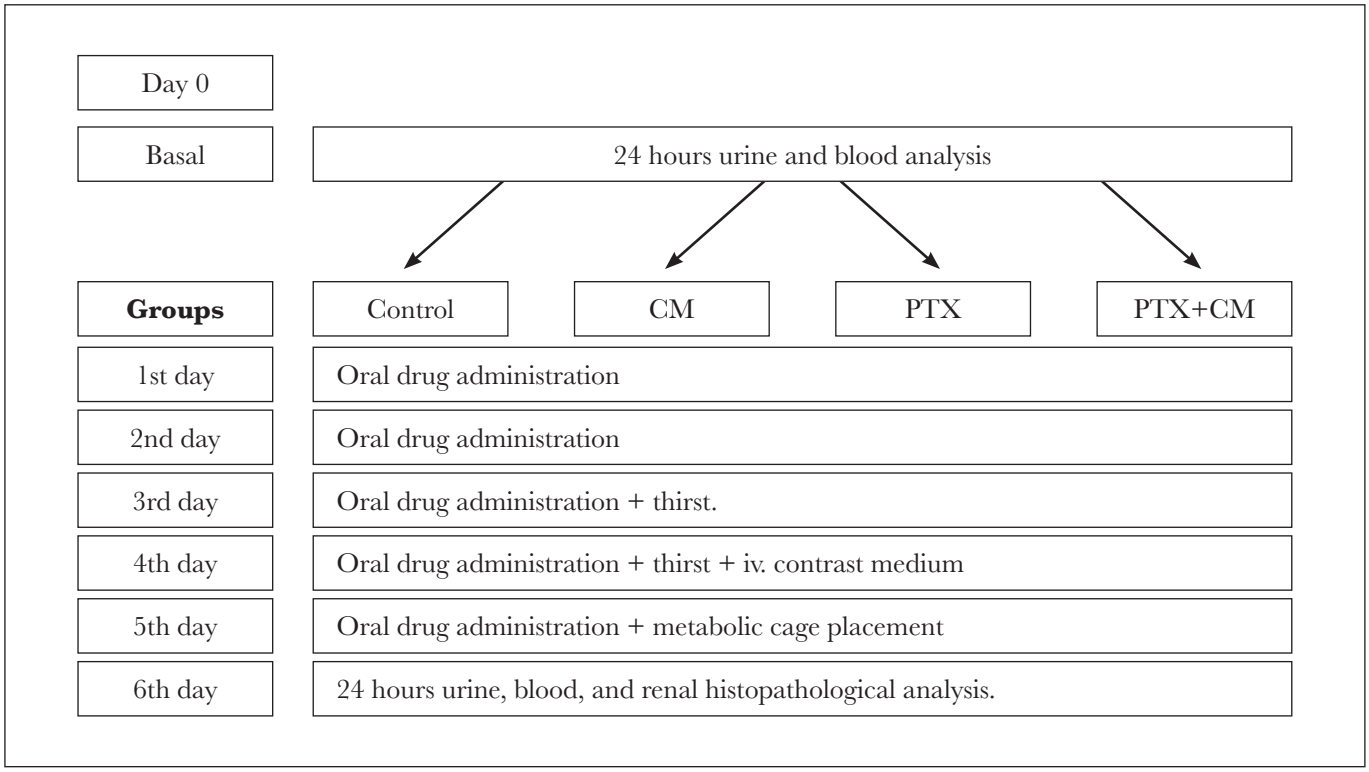

Figure 1: Consort diagram of the study.

using a Leica DM4000 B microscope after staining with Hematoxylin and Eosin (H&E).

Analyses for renal tissue damage; necrotic differences in renal proximal and distal tubules, vacuolation, degeneration and congestion in the interstitial space were scored by assessment of randomly selected microscope areas. Grading was as follows:

a. For tubules: Grade 0: no damage, Grade I: 10% damage, Grade II: 10–25% damage, Grade III: damage between 25–50%, Grade IV: > 50% damage.

b. For interstitial spaces: Grade 0: no congestion, Grade I: + (10% of medulla region in microscope area), Grade II: ++ (10–25% of medulla region in microscope area), Grade III: +++ (25–50% of medulla region in microscope area), Grade IV: ++++ > 50% of medulla region in microscope area).

Statistical analysis

All statistical analyses were performed using SPSS for Windows, Version 10.0 (Chicago, USA). Data were specified as average ± standard deviation. $P < 0.05$ was accepted as significant. Analysis of variance (ANOVA) was applied for the comparison of groups. The Bonferroni test was used for the post-hoc study. The Mann-Whitney U test was conducted for the comparison of two independent groups. We also assessed baseline and 6th day values by using the paired t-test.

RESULTS

There was significant weight loss in all groups after dehydration. On day 6, no significant difference was found in urine volumes among all groups. Serum Cr and BUN levels significantly increased only in the CM group after CM administration ($P < 0.05$), and differences in other groups were not significant (Figure 2,3). CrCl was significantly decreased in the CM group ($P < 0.05$) (Figure 4). Although FENa was decreased in all groups, no significant relationship was found between the groups. The absolute change in microalbuminuria levels was similar in groups. The absolute change in serum Cr and BUN value was found to be the highest in the CM group. However, this relation was not found to be significant among groups. Although the decrease in CrCl values was highest in the CM group, there was no statistical difference in this parameter among the groups. FENa values showed a significant decrease in all groups. However, no significant difference was observed among the groups. On day 1 and 6, values of all groups and comparisons of absolute changes in values including body weight, urine volumes, BUN, CrCl, and FENa between these two days are shown in Table I.

Serum AOPP levels were found to be lower in the PTX, control and PTX + CM groups than in the CM group ($P < 0.05$). Although AOPP levels in renal tissue were highest in the CM group, it showed no significant difference among the groups. Serum and tissue MDA levels were found to be significantly lower in the PTX+CM group than those in the CM group [(2.69 ± 0.31 vs. 4.21 ± 1.04 µmol/L, $P > 0.05$) and (55.9 ± 10.4 vs. 74.2 ± 6.9 nmol/g, $P > 0.05$), respectively]. Total thiol levels in renal tissue of the PTX group were significantly higher only than the CM

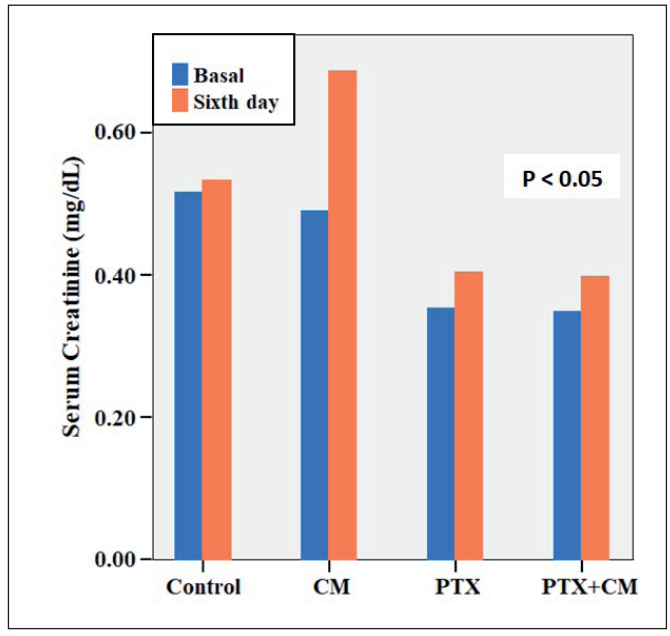

Figure 2: Comparison of serum creatinine (Cr) levels at baseline and 6th day among groups (CM: Contrast Medium, PTX: Pentoxifylline).

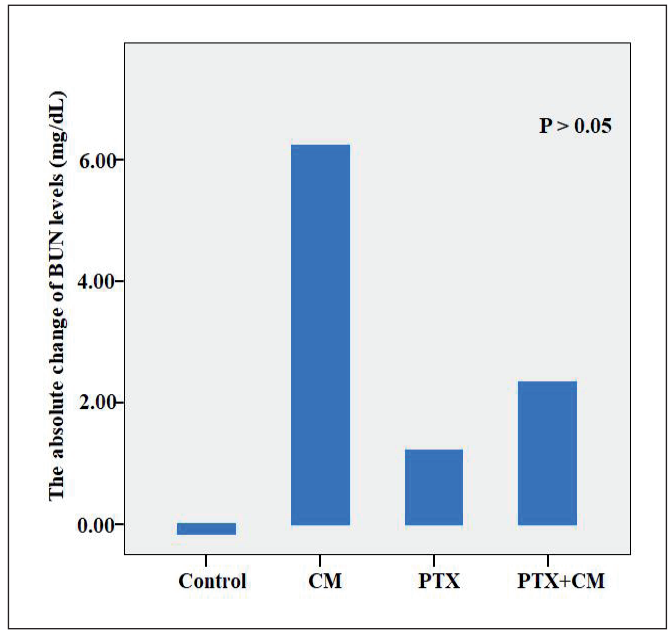

Figure 3: The comparison of absolute BUN change among groups, at baseline and 6th day (BUN: Blood Urea Nitrogen, CM: Contrast Medium, PTX: Pentoxifylline).

group (670.25 ± 166.2 vs. 573.1 ± 53.0 µmol/L, P < 0.05, respectively). The levels of serum thiol, NO, TNF-a, and IL-1b were found to be similar in all groups (Table II).

In the light microscopic visualization of renal tissue, proximal and distal tubules were normal, and there were no findings of vacuolization, degeneration, or necrotic differences in the control group. In transverse and longitudinal sections of the medulla region of the control group, we observed collecting canals to have normal morphology, covered with cubic epithelium cells. No congestion was observed. In CM group; it was observed that some of the glomeruli had a sclerotic appearance. Additionally, there were severe hemorrhagic areas and fibrotic regions between proximal and distal tubules. Peritubular fibrosis and bridging formation severely increased in some areas including the medulla. In the PTX group; Cortex and medulla formation were observed to be normal in structure and appearance. The CM+PTX group had better microscopic changes compared with the CM group; the glomeruli in the cortex had a more normal appearance and less hemorrhagic and fibrotic areas than the CM group. Histopathological findings and comparisons are shown in Figure 7-9 and Table III.

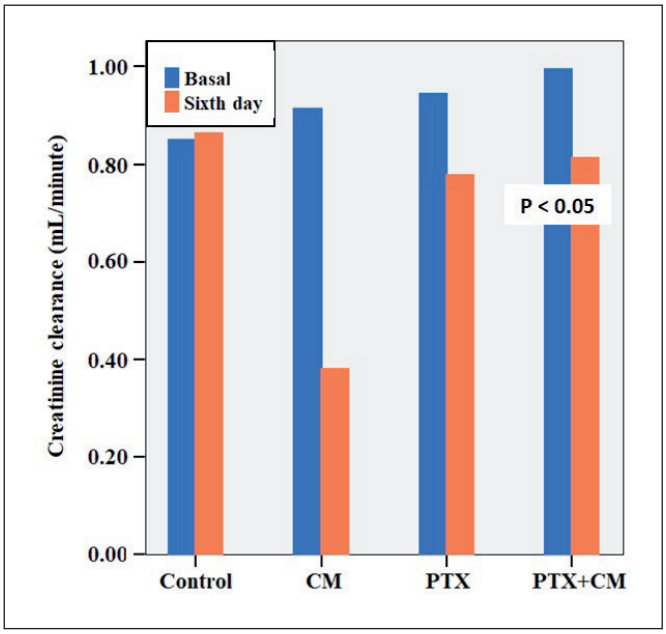

Figure 4: The comparison of creatinine clearance levels among groups, at baseline and 6th day (CM: Contrast Medium, PTX: Pentoxifylline).

DISCUSSION

Several methods have been tried to prevent CIN so far (15). PTX is a relatively new drug which has potential protective effect in CIN (16). In the present study, renal lesions induced by CM administration have been shown to be biochemically and histopathologically improved by oral PTX treatment. Vasoconstriction, renal ischemia, and free radical damage are well-known important factors in the pathophysiology of contrast nephropathy. PTX has recently been shown to have positive effects against these pathophysiological changes (13). Similarly, we observed that PTX significantly protected renal functions from CM damage.

Experimentally establishing CIN has some difficulties. In the literature, researchers have used different methods

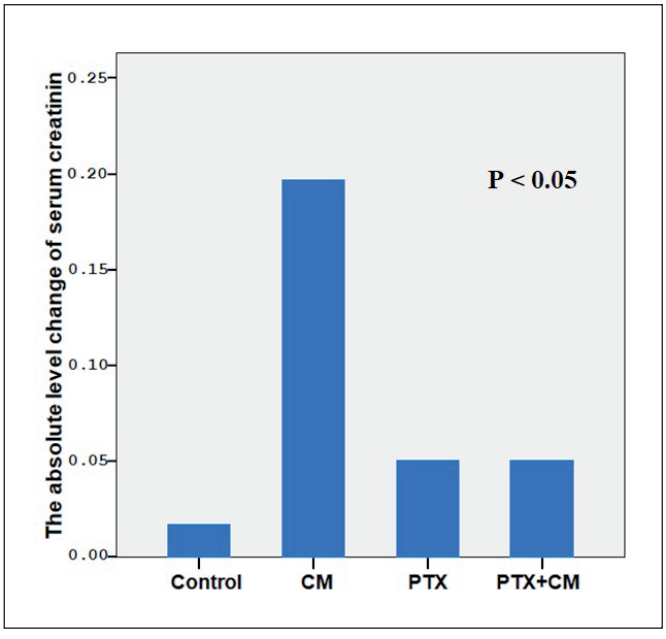

Figure 5: The comparison of absolute change in serum creatinine levels (mg/dL), at baseline and 6th day (CM: Contrast Medium, PTX: Pentoxifylline).

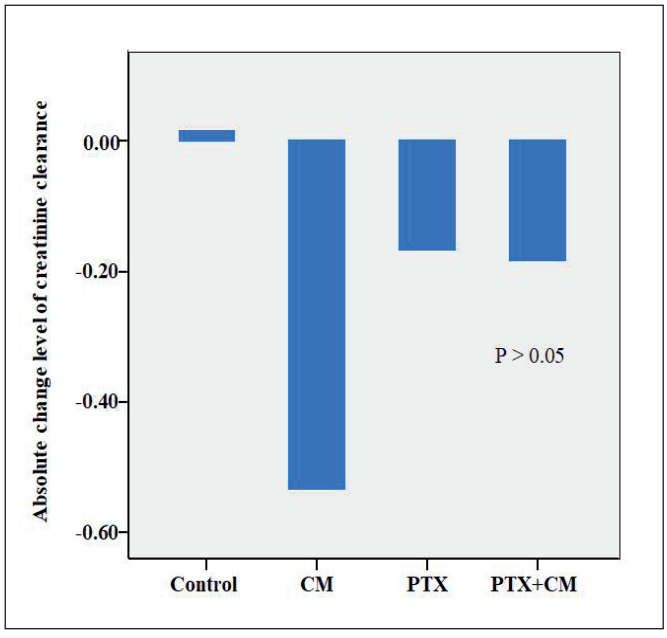

Figure 6: The comparison of absolute change in creatinine clearance (mL/min/100g, at baseline and 6th day. (CM: Contrast Medium, PTX: Pentoxifylline).

Table I: The values of body weight and kidney functions, at the baseline and the sixth day.

Variables		Control	CM	PTX	PTX+CM
Body weight (g)	Basal	276.8 ± 46.1	273.5 ± 35.8	190.5 ± 11.6	200 ± 13.7
	Sixth day	244.0 ± 38.5	232.3 ± 33.8	180.6 ± 12.2	188.8 ± 15.5
	Mean change	-32.8 ± 8.6	-41.2 ± 4.6	-9.8 ± 6.2	-11.0 ± 13.3
	P	0.028	0.027	0.028	0.043
Urine (mL/100g)	Basal	3.60 ± 0.65	3.56 ± 0.74	4.95 ± 0.82	4.46 ± 1.44
	Sixth day	3.47 ± 0.67	3.69 ± 0.85	5.07 ± 0.52	5.05 ± 1.20
	Mean change	-0.12 ± 0.95	0.13 ± 1.19	0.12 ± 0.85	0.56 ± 1.14
	P	> 0.05	> 0.05	> 0.05	> 0.05
Serum creatinine (mg/dL)	Basal	0.51 ± 0.10	0.49 ± 0.03	0.35 ± 0.03	0.36 ± 0.03
	Sixth day	0.53 ± 0.18	0.69 ± 0.03	0.40 ± 0.05	0.40 ± 0.03
	Mean change	0.02 ± 0.10	0.20 ± 0.06	-0.05 ± 0.07	0.05 ± 0.04
	P	> 0.05	0.027	> 0.05	> 0.05
BUN (mg/dL)	Basal	23.0 ± 5.33	23.75 ± 2.04	22.00 ± 4.47	24.40 ± 2.70
	Sixth day	22.83 ± 4.17	29.98 ± 2.56	24.33 ± 3.01	25.83 ± 4.12
	Mean change	-0.17 ± 3.37	6.23 ± 1.43	2.33 ± 5.68	1.20 ± 6.30
	P	> 0.05	0.027	> 0.05	> 0.05
Creatinine Clr[$] (mL/min/100g)	Basal	0.85 ± 0.09	0.91 ± 0.33	0.99 ± 0.12	0.96 ± 0.11
	Sixth day	0.86 ± 0.46	0.38 ± 0.22	0.81 ± 0.18	0.78 ± 0.20
	Mean change	0.02 ± 0.43	-0.53 ± 0.37	-0.18 ± 0.27	-0.17 ± 0.27
	P	> 0.05	0.046	> 0.05	> 0.05
FENa (%)	Basal	0.26 ± 0.08	0.30 ± 0.09	0.51 ± 0.11	0.43 ± 0.20
	Sixth day	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.02
	Mean change	-0.24 ± 0.08	-0.26 ± 0.08	-0.47 ± 0.11	-0.37 ± 0.20
	P	0.028	0.027	0.027	0.027
Microproteinuria (mg/dL)	Basal	9.61 ± 2.34	10.55 ± 4.98	11.17 ± 2.32	9.29 ± 2.36
	Sixth day	8.06 ± 0.74	9.38 ± 2.75	9.89 ± 1.76	10.77 ± 3.01
	Mean change	-1.54 ± 2.75	-1.17 ± 5.49	-1.27 ± 1.51	1.48 ± 4.64
	P	> 0.05	> 0.05	> 0.05	> 0.05

CM: Contrast Medium, **PTX:** Pentoxifylline, **FENa:** Fractional Excretion of Sodium, **BUN:** Blood Urea Nitrogen. [$]Creatinine Clearance

Table II: The comparison of oxidation and inflammation parameters among groups.

Variables	Control	CM	PTX	PTX+CM	P
OPP (µmol/L)[s]	215.6 ± 60.9[a]	348.3 ± 48.6[a,b,c]	177.15 ± 25.19[b]	176.41 ± 64.07[c]	< 0.05
MDA (µmol/L)[s]	2.91 ± 0.94	4.21 ± 1.04[a]	3.28 ± 0.63	2.69 ± 0.31[a]	< 0.05
Total Thiol (µmol/L)[s]	580.5 ± 100	573.1 ± 53.0	670.25 ± 166.2	540.7 ± 100.9	> 0.05
NO (µmol/L)[s]	28.00 ± 8.61	25.46 ± 4.11	25.71 ± 6.03	25.48 ± 12.58	> 0.05
AOPP (mmol/g)[t]	1.22 ± 0.18	1.43 ± 0.29	1.04 ± 0.59	1.01 ± 0.41	> 0.05
MDA (nmol/g)[t]	68.3 ± 11.6	74.2 ± 6.9[a]	59.4 ± 6.7	55.9 ± 10.4[a]	< 0.05
Total thiol (µmol/g)[t]	9.31 ± 0.43	8.41 ± 1.39	11.5 ± 1.54[a]	10.02 ± 1.79	< 0.05
IL-1β (pg/mL)[s]	18.27 ± 4.68	27.24 ± 10.22	21.8 ± 1.92	25.10 ± 4.98	> 0.05
TNF-α (pg/mL)[s]	102.18 ± 11.46	135.71 ± 11.84	113.43 ± 22.57	132.51 ± 24.9	> 0.05

AOPP, Advanced Oxidation Protein Product; **MDA,** Malondialdehyde; **NO,** Nitric Oxide, **IL-1β,** Interleukin-1 Beta; **TNF-α,** Tumor Necrosis Factor Alpha. [s]serum; [t]tissue. [a, b, c]Significant values from post-hoc analysis.

to induce experimental CIN. Some researchers have reported that they noticed renal damage and increase in serum creatinine values by 10 mL/kg contrast agent after a 24 hours of dehydration (17). Dehydration time in experimental studies regarding CIN ranged between 24-72 hours (18) and ionic contrast agents were reported to be more nephrotoxic (19). In this study, dehydration was determined as the period of thirst for 48 hours and CIN was induced by ionic contrast media with a dose of 0.1 mL/kg.

In experimental models, contrast materials were found to decrease antioxidant enzyme activity and show a direct toxic effect by releasing the free radicals (20, 21). Previous studies reported positive results regarding anti-oxidative activities of PTX (11). Among them, PTX provides the down-regulation of various pro-inflammatory cytokines such as TNF-α (22, 23), and shows evidence of free radical scavenging (24) and potential antioxidant properties (11, 25). Serum and tissue AOPP measurements show the

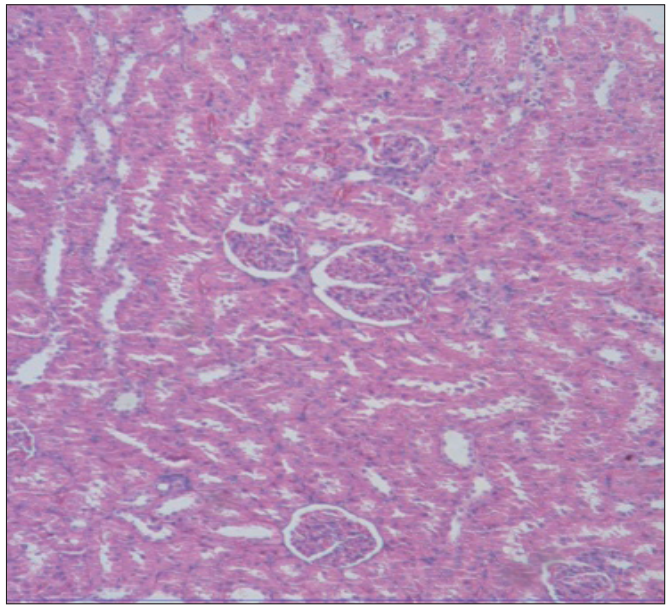

Figure 7: Control group; cortex-containing glomeruli, proximal and distal tubules (HE, X10).

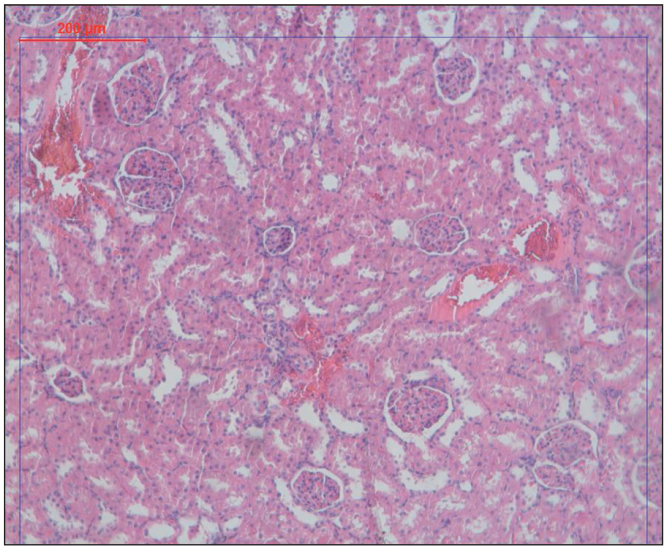

Figure 8: Contrast Nephropathy group; sclerotic appearance of some cortex-derived glomeruli and different sizes, between the proximal and distal tubules. Hemorrhagic areas are seen at the level (HE, X10).

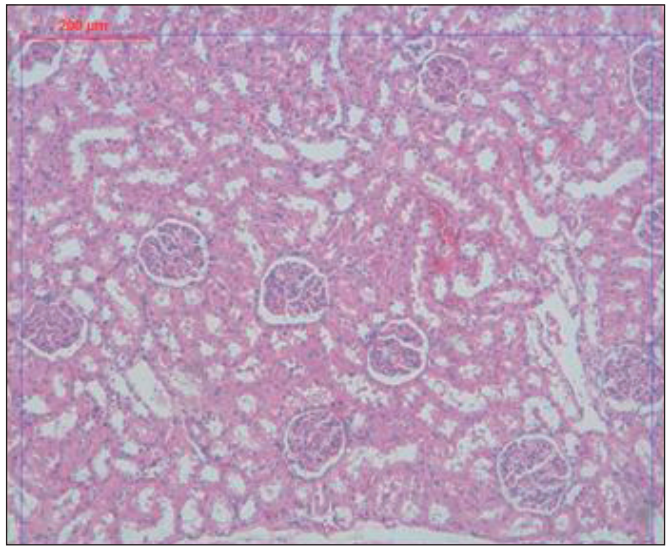

Figure 9: Contrast Nephropathy (CN) + pentoxifylline group; the cortical glomeruli, more normal appearance than group, proximal and distal the hemorrhagic areas around the tubules decreased with respect to the CN group (HE, X10).

Table III: Microscopic glomerular, tubular, and medullary damage rates on light microscopy.

Variables	Control (N=6)				CM (N=6)				PTX (N=6)				CM + PTX (N=6)			
	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Glomerular fibrotic changes	6	0	0	0	0	0	1	5	6	0	0	0	0	4	2	0
Cortical peritubular hemorrhage	6	0	0	0	0	0	2	4	5	1	0	0	0	2	4	0
Medullary peritubular hemorrhage	6	0	0	0	0	0	1	5	5	1	0	0	0	2	4	0
Cortex-medullary peritubular fibrosis	6	0	0	0	0	1	5	0	6	0	0	0	3	1	2	0

CM: Contrast Medium, **PTX:** Pentoxifylline.

oxidation of proteins. In this study, the rats in the CM group had significantly higher serum AOPP levels than those in the PTX group and the CM+PTX group, which indicates oxidation of proteins. AOPP levels in renal tissue were highest in the CM group and there was no difference among the other groups. These results regarding low AOPP levels in the PTX group suggest that PTX may have antioxidant and protective effects on the kidneys.

Oxidative stress and lipid peroxidation are both known to increase following CM administration. MDA levels, which are the product of lipid peroxidation, can be measured to monitor the degree of lipid peroxidation (26). Lipid peroxidation, formed by reactive oxygen free radicals, is the main cause of cell membrane damage and destruction. In a rat study, it was shown that high MDA levels decreased to the levels of the control group with PTX treatment, which indicates that PTX provides a protective effect by maintaining cellular integrity (27). In our study, the rats given PTX had significantly lower serum MDA levels than those in the CM group. Herein our study, these findings regarding lower serum and tissue MDA levels in the CM+PTX group compared to the CM group may suggest antioxidant and free radical scavenger effects of PTX (28). The reduced glutathione levels in blood and tissue samples lead to a decrease in the antioxidant enzyme protection system and an increased sensitivity to the free radicals (29). PTX treatment was shown to increase the degree of glutathione and total antioxidant capacity (10). In the present study, we measured total thiol levels to reflect glutathione levels. Renal tissue total thiol levels were found to be significantly higher in the PTX group. Although not significant, serum and renal total thiol levels were found to be lowest in the CM group, suggesting that PTX might have, at least, a reducing effect in the decrease of these parameters. It has been demonstrated that CMs decrease NO production (30) and increase microproteinuria (31). Proteinuria, which is increased by the decrease of NO synthesis, can be prevented by endogenous NO resources (32). In this research, there was no significant difference in NO or microproteinuria levels among the groups. There is information in the literature that pentoxifylline has protective and therapeutic effects on endothelial functions and the cardiovascular system (33, 34). In addition, creatinine levels were found to be lower than those found in control subjects, even in the absence of a contrast medium effect in rats receiving only pentoxifylline, suggesting a positive effect of pentoxifylline on the microvascular circulation system.

IL-1b, IFN-a, IL-6 and IL-2 are known as the early phase mediators of inflammation. Numerous studies have shown that PTX suppresses the TNF-α production in human and mouse macrophages (35, 36). Further studies have also demonstrated that PTX inhibits the synthesis of pro-inflammatory cytokines such as IL-1b and IL-6 (37-40). No significant difference in TNF- α and IL-1b levels was found among the groups in our study.

Although our study is the one of the experimental studies demonstrating the effectiveness of PTX in preventing contrast material induced nephropathy, it has some limitations. Firstly, we could not design an optimal model for contrast material-induced nephropathy in the present study. Secondly, we could not evaluate the renal tissues by electron microscope, which might have provided further information about the contrast nephropathy-preventive mechanism of pentoxifylline. In addition, it would be more useful to measure some sensitive markers such as kidney injury molecule-1 or neutrophil gelatinase-associated lipocalin in order to detect kidney injury. As mentioned previously, other studies have demonstrated that establishing an experimental contrast nephropathy model has some difficulties. In this study, all rats were kept thirsty for 48 hours to create heavy contrast material-induced nephropathy. However, no method has currently been defined as the best way to establish experimental kidney injury.

CONCLUSION

Pentoxifylline prevents the development of contrast material-induced nephropathy by decreasing the oxidant activity. Furthermore, we have not detected any adverse effect of PTX on the kidneys. Finally, pentoxifylline, a cheap and non-toxic drug, might be suggested as a prophylactic drug that can be practically used in the future for preventing contrast induced nephropathy However, further clinical studies are required to investigate and confirm these effects of pentoxifylline.

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