Effects of Captopril and Ketotifen on Protecting Against Renal Scarring Due to Pyelonephritis Injury

Piyelonefrit Hasarına Bağlı Gelişen Böbrek Skarının Önlenmesinde Kaptopril ve Ketotifenin Etkisi

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ABSTRACT

Objective: Increased levels of Angiotensin II (Ang II) are responsible for the development of hypertension and diabetes-induced nephropathy. In addition, the preventability of renal fibrosis with Ang II blockade is a known entity. Although mast cells are not present in normal kidney, increased mast cell density in the specimens examined for fibrosis has attracted attention and it is thought that fibrotic agents released from these cells and may contribute to the process. Therefore, an experimental study was planned to decrease the ang II levels with captopril, an angiotensin converting enzyme (ACE) inhibitor and to prevent the development of renal scarring due to pyelonephritis using ketotifen, a mast cell stabilizer.

Material and Methods: Fifty rats were divided into five equal groups. In Group A, the renal cortex was injected with SF. In Group B, acute pyelonephritis was induced by 107 E. coli injections into the renal cortex. Group C received antibiotic treatment with E. coli injection, Group D received antibiotic and captopril with E. coli injection, Group E. received antibiotic and ketotifen with E. coli injection. In order to represent the chronic process, rats were sacrificed after 6 weeks, and the contralateral kidney was included in pathological examination and statistical evaluation.

Results: Inflammatory scores were significantly higher in group B compared to group A ($p = 0.001$). Captopril or ketotifen treatment were the only factors that reduced scar development ($p = 0.007$). However, when captopril or ketotifen treatments were compared in preventing scar development, no superiority was found ($p > 0.05$).

Conclusion: We found that captopril and ketotifen has a protective effect in the development of renal fibrosis in a rat model of acute pyelonephritis. We believe that our study may contribute to clinical trials in the prevention of renal scar development.

Key Words: Angiotensin-converting enzyme inhibitors, Captopril, Ketotifen, Renal fibrosis

ÖZ

Amaç: Hipertansiyon ve diabete bağlı nefropati gelişiminden artmış Angiotensin II (Ang II) düzeyleri sorumlu tutulmaktadır. Bunun yanı sıra Ang II blokajı ile renal fibrozis gelişiminin önlenebilirliği bilinen bir antitedir. Böbrek dokusunun doğal bir elemanı olmamasına rağmen fibrosis nedeniyle incelenen spesmenlerde artmış mast hücre yoğunluğu dikkati çekmiş ve bu hücrelerden salınan fibrotik ajanların sürece katkıda bulunabileceği düşünülmüştür. Bu nedenle Ang II düzeylerinin bir anjiotensin converting enzim (ACE) inhibitörü olan kaptopril ile düşürülmesi ve yine bir mast hücre stabilizatörü olan ketotifenin kullanımı ile pyelonefrrite bağlı böbrek skarı gelişiminin önlenebilirliği hakkında deneysel çalışma planlandı.

Gereç ve Yöntemler: Elli rat beş eşit gruba bölündü. Grup A'da renal kortekse SF enjeksiyonu yapıldı. Grup B'de renal kortekse 107 E. coli enjeksiyonu ile akut pyelonefrit oluşturuldu. Grup C'de E. coli enjeksiyonu ile beraber antibiyotik tedavisi, Grup D'de E. coli enjeksiyonu ile antibiyotik ve kaptopril uygulaması, Grup E'de E. coli enjeksiyonu ile antibiyotik ve ketotifen verildi. Kronik süreci temsil etmek amacı ile ratlar altı hafta sonra sakrifiye edildi, karşı böbrek de patolojik inceleme ve istatistiksel değerlendirmeye alındı.

Bulgular: Grup B'de inflamatuar skorların A grubuna (sham) göre istatistiksel olarak anlamlı arttığı görüldü ($p= 0.001$). Skar gelişimini azaltan tek faktörün ise kaptopril ya da ketotifen tedavisi olduğu görüldü ($p= 0.007$). Ancak kaptopril ya da ketotifen tedavilerinin; skar gelişiminin önlenmesi açısından birbirlerine üstünlükleri saptanmadı ($p>0.05$).

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Sonuç: Ratlarda pyelonefrit modelinde kaptopril veya ketotifen tedavisinin renal fibrozis gelişimini önleyici etkisi mevcuttur. Çalışmamızın renal skar gelişimin önlenmesinde klinik çalışmalar için katkı sağlayabileceği düşüncesindeyiz.

Anahtar Kelimeler: Anjiyontensin-dönüştürücü enzim inhibitörleri, Kaptopril, Ketotifen, Renal fibrozis

BACKGROUND

Chronic process of acute pyelonephritis may result renal scarring up to 40-60% and cause serious consequences like hypertension, proteinuria, and chronic renal insufficiency (1, 2). Many studies confirmed the role of angiotensin-converting enzyme inhibitors (ACEIs) reducing risk of cardiovascular events and preserving renal functions in patients with chronic kidney disease (3, 4). Recent data has confirmed the protective effects of renin-angiotensin system inhibitors (RAS-Is) in residual renal function, but the exact mechanism of these agents is still unknown (5). The circulating renin-angiotensin system (RAS) regulates arterial pressure and sodium homeostasis. But inappropriate activation of intrarenal RAS causes increased Angiotensin II (Ang II) levels and contributes the development of hypertension and elicits the tissue damage by the effect of cytotoxins. Increased local activity of Ang II leads to renal injury, proliferation and fibrosis. In addition, there are some clinical studies those showed the efficacy of ACEIs in the patients with severe reflux nephropathy in long term period reporting the decrease of microalbuminuria and keeping stable effect of glomerular filtration and blood pressure (6). In a recent study, urinary angiotensinogen found to be useful for diagnosing the presence of renal scarring in infants who had febrile urinary tract infections (7). According to the protective effect of ACEIs in chronic nephritis, we aimed to prevent renal scarring due to pyelonephritis in a rat model with administration of captopril. But, although Ang II is renin-dependent in human kidney, Hollenberg et al. (8) have shown other responsible pathways than ACE at least 40%. Recent data has shown that inflammatory and fibrotic effects due to Ang II are effectively inhibited by Ang II antagonism than by ACE inhibition, and this suggests that there are alternative pathways forming Ang II than renin-angiotensin system, probably a chymase. Chymase is a proteolytic enzyme which is secreted from mast cells (9). In some studies, increased chymase expression of mast cells correlates with the interstitial fibrosis but the exact role of mast cells in renal fibrosis is not known yet (10, 11). Mast cell secretes numerous fibrogenic factors and is held responsible for the sclerosis in chronic inflammation (12). Mast cell hyperactivity related fibrotic process has been described in many organs such as skin, liver, lung and in kidney (13, 14). Ang II is hold responsible for being trigger for fibrogenic factors. So, we hypothesize that an alternative pathway of Ang II formation originated from mast cells can be hopeful to reverse tubulointerstitial fibrosis. In this study, we performed to investigate the efficacy of ketotifen, which is a mast cell stabilizer for preventing renal fibrosis in the development of renal scar formation. Also, we compared captopril and ketotifen to find out the mechanism of renal fibrosis and related pathways. To our knowledge, there is no study in the literature about the two pathways and their comparison for blockade of Ang II.

MATERIALS and METHODS

Adult 50 female Albino Wistar rats weighing between 200-300 g were used. Animals were housed in pathogen-free conditions at room temperature (22 ± 2 ºC) and subjected to a 12-hour light-dark cycle. The rats aged 12-16 weeks fed standard rat chow and tap water ad libitum. All experiments were performed according to the institutional guidelines for animal care and use.

Escherichia coli strain (ATCC 25922) which was sensitive to cefotaxime had been used in the experimental model. 0.1 ml of E. coli suspension containing 10^7- 10^8 organisms was prepared. After ketamine anesthesia, the rats were laid supine and a median incision was used to enter peritoneal cavity. The right kidney was delivered out and 0.1 ml E. coli suspension was injected in the midpolar region at a depth of 2 mm (Figure 1). Five groups, each consisting of ten rats were organized (Table I).

Table I: The groups, each consisting of ten rats were given in the table of treatment.

Group	n	Given inoculums	Treatment day 1-3	Antibiotics usage day 4-8	Drug over 3 weeks
A	10	0.1 mL SF	-	-	-
B	10	0.1 mL E. coli (10^7-10^8 organisms)	-	-	-
C	10	0.1 mL E. coli (10^7-10^8 organisms)	-	Cefotaxime, (200 mg/kg/day, IP)	-
D	10	0.1 mL E. coli (10^7-10^8 organisms)	-	Cefotaxime, (200 mg/kg/day, IP)	Captopril (50 mg/kg/day, oral)
E	10	0.1 mL E. coli (10^7-10^8 organisms)	-	Cefotaxime, (200 mg/kg/day, IP)	Ketotifen (1 mg/kg/day, oral)

IP: *intraperitoneal*

Table II: Criteria's for histopathalogic evaluation. Maximum total score possible: 9.

Criterion	Score
Degree of inflammation	
None	0
Minimal	1
Moderate	2
Severe	3
Abscess Formation	
None	0
Present	1
Scar Formation	
None	0
Minimal	1
Moderate	2
Severe	3
Destruction of the pelvicalyceal system	
None	0
Present	1
Tubular atrophy	
None	0
Present	1

Group A. 0.1 ml SF inoculum given on day 1

Group B. E. coli inoculum given on day 1.

Group C. E. coli inoculum given on day 1+ cefotaxime treatment (200 mg/kg/day, intraperitoneal, started on day 3, continued for a week.)

Group D. E. coli inoculum given on day 1 and cefotaxime treatment (200 mg/kg/day, intraperitoneal, started on day 3, continued for a week) and captopril (50 mg/kg/day, started on day 3, continued for 3 weeks)

Group E. E. coli inoculum given on day 1 and cefotaxime treatment (200 mg/kg/day, intraperitoneal, started on day 3, continued for a week) and ketotifen (1 mg/kg/day, started on day 3, continued for 3 weeks).

Intraperitoneal cefotaxime was given for 5 days at the dose of 200 mg/kg once a day, starting 72 hours after surgery. Captopril dose in rats ranged from 10 to 100 mg in similar studies and the dose of 50 mg/kg/day was used in this study (15-17). Ketotifen was used in the dose of 1 mg/kg/day by orogastric lavage (18, 19).

In order to represent the chronic process, rats were sacrificed under anesthesia, six weeks after the bacterial inoculation to determine renal scar formation. The contralateral kidneys were included in pathological examination and statistical evaluation.

Tissue samples were processed for routine histological examination with standard formalin fixation and paraffin embedding, and 5µm thin sections were stained with hematoxylin-eosin and Masson's trichrome stain. Histopathological scoring was performed in a blinded manner. The contralateral kidneys were examined for injury and evaluated statistically. Renal injury was defined as degree of inflammation, abscess formation, scar formation, destruction of the pelvicalyceal system and tubular atrophy. The distributions of histopathological evaluations are given in detail in table II. For each section, at least 20 fields were examined under ×400 magnification (Figure 2).

The intensity of inflammation was classified, and their staining scores were evaluated semi quantitatively as 0= none, 1=minimal, 2=moderate, 3=severe. Severity of scar formation was classified based on percent of scaring as follows: zero percent for no scar, less than 5% minimal, 5%–15% moderate, and more than 15% severe (20). A scoring scale of 0 to 9 was used: no injury (0), less than 25% (1), less than 50% (2), less than 75% (3), or more than 75%.

Statistical analyses were performed using SPSS Statistics (Statistical Package for Social Science) for Windows, version 17.0 for Windows. Fisher's exact test was used for statistical analysis. $p<0.05$ was considered statistically significant.

Ethical clearance was taken from Animal Ethics Committee (17-278/2014).

Table III: The results of histopathological evaluation and the comparison of sham and treatment groups.

	Scores		Groups					p
			A (SP)	B (E. coli)	C (E. coli + Cfx)	D (E. coli + Cfx + Capt)	E (E. coli + Cfx+ Kt)	
Degree of inflammation	None	0	10	0	0	0	0	0.001
	Minimal	1	0	0	1	1	3	
	Moderate	2	0	4	8	5	3	
	Severe	3	0	5	1	4	4	
Abscess formation	None	0	10	0	5	6	4	0.001
	Present	1	0	9	5	4	6	
Scar formation	None	0	10	0	0	0	0	0.001
	Minimal	1	0	0	1	5	8	
	Moderate	2	0	4	7	5	2	
	Severe	3	0	5	2	0	0	
Pelvicalyceal destruction	None	0	10	0	4	2	3	0.001
	Present	1	0	9	6	8	7	
Tubular atrophy	None	0	10	0	5	4	2	0.001
	Present	1	0	9	5	6	8	

SP: *serum physiologic,* ***Cfx:*** *cefotaxime,* ***Capt:*** *Captopril,* ***Kt:*** *Ketotifen*

Table IV: The comparison of antibiotic and antibiotic plus treatment (captopril and ketotifen) groups.

	Scores		C (E. coli + Cfx)	D (E. coli + Cfx + Capt)	E (E. coli + Cfx + Kt)	p value
Degree of inflammation	Minimal Moderate Severe	1 2 3	1 5 4	3 3 4	1 8 1	>0.05
Abscess formation	None Present	0 1	6 4	4 6	5 5	>0.05
Scar formation	Minimal Moderate /Severe	1 2 / 3	1 9	5 5	8 2	0.007
Pelvicalyceal destruction	None Present	0 1	4 6	2 8	3 7	>0.05
Tubular atrophy	None Present	0 1	5 5	4 6	2 8	>0.05

SP: *serum physiologic,* ***Cfx:*** *cefotaxime,* ***Capt:*** *Captopril,* ***Kt:*** *Ketotifen*

RESULTS

One rat in group B (E. Coli), died in the early post-inoculation period due to infection of E. coli. The study was completed with 49 rats. Table III shows the results of histopathological evaluation and the comparison of sham and treatment groups. The contralateral kidneys were evaluated, and inflammation criteria were found statistically significant (p=0.001). According to the histopathologic evaluation, the scores of inflammatory criteria increased significantly in the groups of pyelonephritis related with E. coli compared with the sham group (p=0.001) (Figure 3).

In table IV, antibiotic and antibiotic plus treatment (captopril and ketotifen) groups were compared. In three groups, due to the presence of scar, scar was evaluated as minimal or moderate plus severe. When these groups were compared according to the inflammatory criteria's and scar formation, scar formation significantly decreased in the groups of antibiotics plus treatment (captopril and ketotifen) compared with the antibiotic group. Captopril or ketotifen treatment with antibiotic were found to be just factors decreasing scar formation among the inflammatory criteria's (p=0.007). The percentage of scar formation was 20% in the group E (ketotifen) (figure 4), 50% in the group D (captopril), while 90% in the group C (cefotaxime).

When the groups of captopril and ketotifen treatment with antibiotic were compared for preventing scar formation, there was not a significant difference between captopril and ketotifen treatment (p>0.05).

DISCUSSION

Renin-angiotensin system (RAS) has an important role in the regulation of arterial pressure. In recent years, local effects of RAS are also investigated as well as its systemic effects. RAS's components and angiotensin II (Ang II) may cause paracrine effects in kidney (3). Kobori et al. (4) reported that Ang II induces inflammation, cell growth, mitogenesis, and apoptosis, differentiation; all of which contribute to tissue damage. Ang I is converted into Ang II by Angiotensin-Converting Enzyme (ACE). Ang II has several possible functions like acting on cytosolic receptors to stimulate the inositol 1,4,5-triphosphate pathway. Also, there are some hypothesizes related with Ang II, about migrating to the nucleus to exert genomic effects (3,4). In a few studies, it has been shown that the blockade of renin-angiotensin system with ACE inhibitors (ACE i) has effects on preventing renal fibrosis in an experimental acute pyelonephritis model (15). In our experiment, captopril has a significant effect on decreasing renal scarring when compared with the group C (E. coli plus Cfx) (p=0.007).

The infiltrating cells in the cortical interstitial space have a main role in the development of tubulointerstitial fibrosis in chronic renal diseases (10,23). Although lymphocytes and monocytes are believed to be involved, after defining chronic aristolochic acid nephropathy with a few infiltrating inflammatory cells leading to progressive interstitial fibrosis, it supports the idea that mast cells participate in the progression of hypocellular fibrosis independent of lymphocyte and monocytes (10,11). Mast cell hyperactivity in primary fibrotic disorders of liver, lung, and kidney is well defined; but in kidney, the mechanism involved has not demonstrated enough. Mast cells are not present in normal kidney. But in fibrotic disorders; a great number of mast cells were identified through the renal interstitium. There are many reports about the relationship between the increased number of mast cells and the extent of renal fibrosis (10,11,13,14,23). Also, increased mast cells have been investigated in such as Ig A nephropathy, diabetic nephropathy, and renal transplants showing acute and chronic rejection (10,12,14). This suggests that mast cells play a critical role in fibrosis or sclerosis secondary to activation of RAA system related with chronic inflammatory diseases and autoimmune disorders. But it has been reported that at least 40% of Ang I is converted to Ang II by the pathways than ACE. Chymase is also a potent producer of Ang II that secreted from mast cells and act as a protease. As a result, blockage of angiotensin by ACE I may have a special effect in preventing renal fibrosis. Li and col. reported that "an alternative pathway of Ang II formation coming from mast cells can be operative in the tubulointerstitial fibrosis of all renal diseases and mast cells are believed to be a promising therapeutic target in pathophysiology of tubulointerstitial fibrosis in many renal diseases" (10). But the potential role of mast cells has not

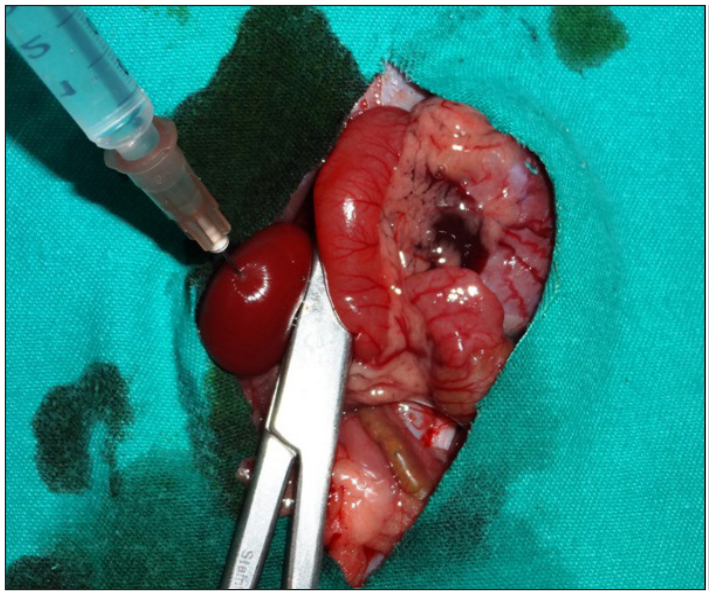

Figure 1: The inoculum of 0.1 ml E. coli suspension to right kidney in the midpolar region at a depth of 2 mm.

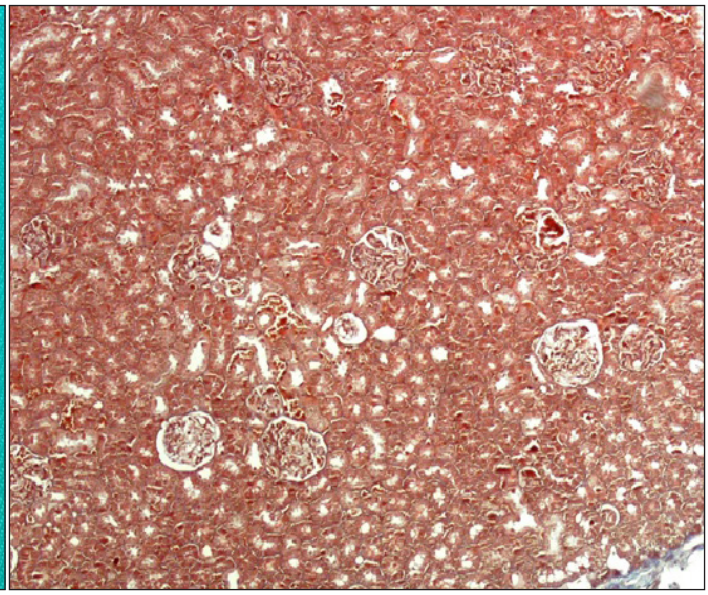

Figure 2: Group A rat kidney, showing normal renal parenchyma (Masson's trichrome stain, X100).

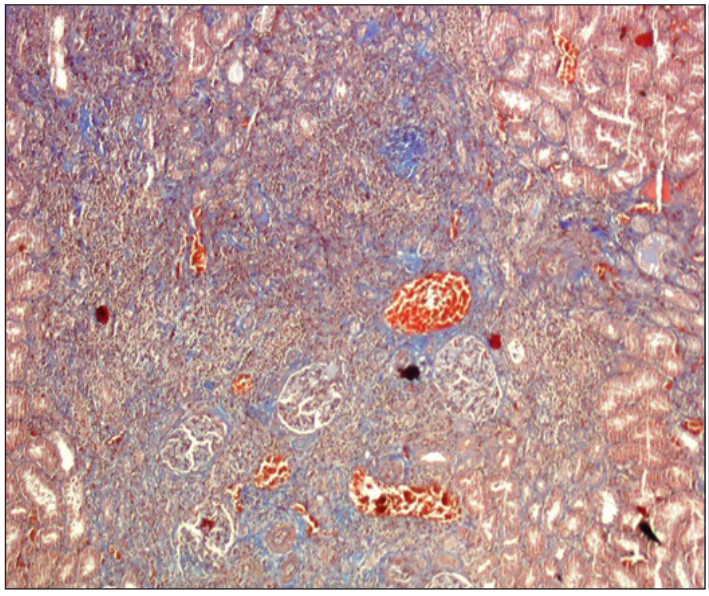

Figure 3: Group B rat kidney, showing dense fibrosis with destruction of the renal parenchyma (Masson's trichrome stain, X100)

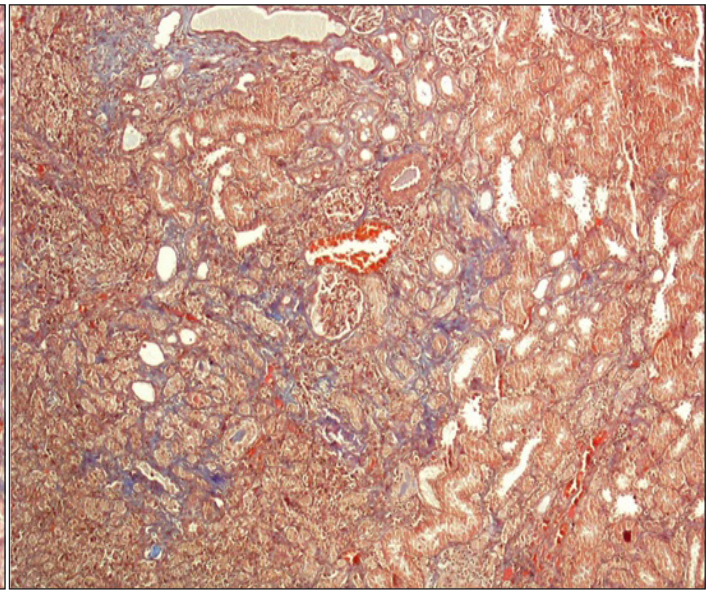

Figure 4: Group E rat kidney showing a smaller scar (Masson's trichrome stain, X100)

known yet (10,11,23). So, we hypothesize that administration of mast cell stabilizer may be effective in the prevention of tubulointerstitial fibrosis. In the group of E (ketotifen) as a mast cell stabilizer, the decrease in scar formation was found significant statistically, like the group of D (captopril) compared with the group of antibiotics (p=0.007). While captopril and ketotifen were compared because of the effect on reducing scar formation, there was no significant difference between the two groups (p>0.05), but the rate of scar formation was 20% in the group of ketotifen, 50% in the group of captopril while 90% in the group of antibiotics only. According to the hypothesis that renal fibrosis develops by the activation of RAS, we think that activated mast cells contribute renal fibrosis by other pathways either.

CONCLUSION

We found that captopril or ketotifen treatment with antibiotic were found to be the only factors decreasing scar formation among the inflammatory criteria's (p=0.007). The percentage of scar formation was 20% in the group of E (cefotaxime + ketotifen), 50% in the group of D (cefotaxime + captopril) while 90% in the group of C (cefotaxime). But there was no significant difference between group D and E in the efficacy of preventing scar formation statistically.

We conclude that captopril and ketotifen have a preventive effect in the development of renal fibrosis in an experimental

model of acute pyelonephritis in rats. Long-term studies are needed to evaluate their roles in reducing renal fibrosis due to pyelonephritis.

Compliance with Ethical Standards:

Conflict of Interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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