IJKD KIDNEY DISEASES

Protective Effect of Curcumin Against Gentamicin-induced Nephrotoxicity Mediated by p38 MAPK, Nuclear Factor-Kappa B, Nuclear Factor Erythroid 2-Related Factor 2

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Keywords. curcumin, gentamicin, nephrotoxicity, nuclear factor erythroid 2-related factor 2, nuclear factor-kappa B, p38 mitogen-activated protein kinases

Original Paper

Introduction. The antioxidant activity of curcumin (CMN) has been evaluated in several studies. We aimed to examine the protective effect of curcumin on gentamicin-induced nephrotoxicity in rats, both at histological and immunohistochemical levels.

Methods. Forty male *Wistar albino* rats were assigned into four groups of 10 as follows: group 1: control, group 2: curcumin for 15 days, group 3: gentamicin for the last 10 days, and group 4: curcumin for 15 days and gentamicin for the last 10 days. Curcumin (100 mg/kg/d) was gavaged, and gentamicin (80 mg/kg/d) was injected intraperitoneally. Kidney tissues and blood were collected for histological, immunohistochemical and biochemical studies. Body weight and kidney weight/body weight changes were recorded.

Results. Gentamicin nephrotoxicity was characterized by a significant rise in serum urea and creatinine levels and a significant reduction in body weight and an increase in kidney weight/body weight. The gentamicin group showed degenerative changes in tubules and glomeruli together with, increased phosphorylated (p)-p38 mitogen-activated protein kinase (p38 MAPK) positive cells in immunohistochemical evaluation, increased immunoreactivity of nuclear factor-kappa B (NFkB), and decreased immunoreactivity of nuclear factor erythroid 2-related factor 2 (Nrf2). Curcumin diminished body weight loss caused by gentamicin administration but, did not change the kidney weight/body weight. Moreover, curcumin ameliorated the histological alterations and reduced the biochemical parameters. Additionaly, curcumin significantly decreased p-p38 MAPK positive cells and NFkB immunoreactivity, while significantly increasing Nrf2 immunoreactivity in the kidney tissue.

Conclusion. We conclude that curcumin may attenuate gentamicin-induced nephrotoxicity by supprresing the p38 MAPK and NFkB, and activating the Nrf2 signaling pathways.

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INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic which is effective on gram-negative bacterial infections.[1,2] One of the main side effect of aminoglycoside antibiotics is nephrotoxicity, and it is responsible for 10 to 20% of cases of acute renal failure.[3] Nephrotoxicity occurs in 30% of the patients treated with aminoglycosides for more than

seven days.[1] The mechanism of GM nephrotoxicity is not fully understood. However, oxidative stress causes apoptosis and inflammation and ultimately may have a crucial role in the pathogenesis of GM nephrotoxicity.[2,4,5] When the synthesis of reactive oxygen species (ROS) increase, the antioxidant defense systems of the cells weakens and oxidative stress occurs, resulting in the antioxidant defense disruption.[6,7] Curcumin (CMN) is an effective component of *Curcuma longa*, and is both used as a popular cooking spice and in traditional medicine since ancient times.[3,8] According to the studies, CMN is a strong antioxidant and has an anti-apoptotic and anti-inflammatory effect on renal tissue.[2-4] The protective effect of CMN against oxidative stress in renal cells has been shown in both *in-vitro* and *in-vivo* studies.[4,9-11] CMN is also reported to have an effect on the function of the transcription factors and signal transduction pathways. p38 mitogen-activated protein kinase (p38 MAPK) is a ubiquitous protein kinase that plays a pivotal role in intracellular physiological processes such as cell cycle progression, differentiation, apoptosis, and inflammatory responses. Phosphorylated p38 (p-p38) translocates into the nucleus, where it activates nuclear transcription factors and initiates the production and secretion of the proinflammatory cytokines.[12] It has been shown that p38 MAPK expression increases in the GM-induced nephrotoxicity, and may play a role in its pathogenesis.[13] On the other hand, CMN is thought to have a reducing effect on p38 MAPK activity and exerts an inhibitory effect on activated p38 MAPK signaling pathways caused by oxidative damage.[14,15] Nuclear factor-kappa B (NFkB), which is a transcriptional factor, takes part in regulating the expression of proinflammatory genes involved in several inflammatory diseases.[6,16] It is also emphasized that factors such as long-term drug intake or radiation exposure may activate the inflammatory pathways by inducing the expression of NFkB.[17,18] Administration of CMN in renal damage reduces NFkB expression, which is responsible for proinflammatory gene transcription. It also prevents kidney failure by increasing the antioxidant enzymes.[6,19] There are considerable reports showing the cross-talk between nuclear factor erythroid 2-related factor 2 (Nrf2) and NFkB. In this cross-talk, Nrf2 is known to control the NFkB levels by suppressing the phosphorylation of inhibitory kappa B (IkB), an NFkB inhibiting protein.[6] Nrf2 is a transcription factor that regulates the expression of many genes encoding antioxidant proteins, enzymes, and stress response proteins.[6] Nrf2 activation plays a role in suppressing the inflammatory pathways via stimulating antioxidant defense system, and protects the cells against oxidative stress.[20] CMN facilitates nuclear translocation of Nrf2 by attenuating Keap1 inhibition of Nrf2 and contributes to the functioning of antioxidant enzymes.[7,9] Balagun *et al.* stated that CMN induces the expression of antioxidant enzymes such as hemeoxygenase-1 (HO-1) in renal epithelial cells via Nrf2/ARE, which is a protective mechanism against oxidative stress.[9] Another study reported that CMN treatment exerted anti-apoptotic and anti-oxidative effects by inducing Nrf2/HO-1.[21]

In the literature review, the antioxidant activity of CMN was mainly evaluated in several studies. However, the studies on intracellular signaling pathways involving the anti-apoptotic and anti-inflammatory effects of CMN against the GM-induced nephrotoxicity are quite limited. In this study, we aimed to examine the protective effect of CMN against GM-induced nephrotoxicity histologically, and to elucidate the p38, NFkB, and Nrf2 signaling pathways that possibly play a role in this protective effect.

MATERIALS AND METHODS

Animals and Experimental Protocol

This study got approval from the Local Ethics Committee for the Animal Experiments of Trakya University (Edirne, Turkey).

Forty male *Wistar albino* rats, (three months old, 200 to 250 g) were provided from Trakya University Experimental Animal Research Unit. The rats were fed daily with pellet foods containing 21% pure protein and tap water under optimal laboratory conditions (temperature, 22 ± 1 °C; light/dark period: 12 h / 12 h).

The CMN was purchased from Sigma-Aldrich (St. Louis, MO, USA), and dissolved in dimethyl sulfoxide (DMSO, Merck, Damrstadt, Germany). GM was supplied from Bilim Pharmaceuticals (Gentreks, Istanbul, Turkey).

Animals were assigned into four groups and treated in the following order:

- Group 1 (Control, n = 10): DMSO (vehicle for

CMN) was gavaged for 15 days.
- Group 2 (Curcumin, n = 10): Curcumin (100 mg/kg/d, dissolving in DMSO) was gavaged for 15 days.
- Group 3 (Gentamicin, n = 10): Gentamicin (80 mg/kg/d), injected intraperitoneally for 10 days.
- Group 4 (Gentamicin + Curcumin, n = 10): Curcumin was gavaged by a dose of 100 mg/kg/d for 15 days, and GM was injected intraperitoneally during the last 10 days of the treatment period.

Doses of CMN and GM were determined based on the previous studies.[1,22] All rats were sacrificed under xylazine (5 mg/kg, Rompun, Bayer, Istanbul, Turkey) and ketamine (80 mg/kg, Alfamine, Alfasan International B.V., Woerden, Holland) anesthesia 24 hours after the last dose application of the agents, and the experiment ended. Blood samples were collected and serum was separated for determining the urea and creatinine levels. Serum levels of urea and creatinine were measured using an autoanalyzer (Siemens Advia 1800 Autoanalyzer, Muenchen, Germany). Additionally, all rats were weighed once at the beginning and once again at the end of the experiment. After sacrification, kidneys were dissected and evaluated for the ratio of kidney weight/body weight and then multiplied by 1000.

Histological Examinations

For histological changes, kidney tissues were fixed in 10% neutral buffered formalin solution (Sigma-Aldrich). Dehydrated tissues were embedded in paraffin. Sections were stained with hematoxylin-eosin (H&E), Masson's trichrome, and Periodic Acid Schiff (PAS) procedure. All sections were examined and photographed by light microscope (Olympus BX51, Tokyo, Japan). Shrunken glomeruli, dilated tubules, tubular vacuolization, loss of brush border, hyalin cast and leukocytic infiltration in the kidney tissue were measured and scored according to the scoring system, used in previous studies, with a minor modification.[18] Histological changes were evaluated as followed: absent (0), mild (1, ≤ 10%), mild to moderate (2, 10 to 25%), moderate (3, 26 to 50%), extensive (4, 51 to 75%), and severe (5, ≥ 75%).

Immunohistochemistry

Immunohistochemical stainings were performed as described by Uz *et al*.[23] Sections were incubated with rabbit monoclonal p-p38 antibody (1/300; Cell Signaling Technology, Beverly, MA, USA) and rabbit polyclonal Nrf2 antibody (1/400; Abcam, Cambridge, MA, USA) at +4 °C overnight or rabbit polyclonal NFkB/p65 antibody (1/100; Thermo Scientific/Lab Vision, Fremont, CA, USA) one hour at room temperature in a humidified chamber. Phosphate-buffered saline (Sigma-Aldrich) was used instead of primary antibodies for the negative control, and 3-amino-9-ethylcarbazole (AEC, Thermo Scientific/Lab Vision) was used as chromogen for visualize immunolabelling. Thereafter, slides were counterstained with Mayer's hematoxylin and were mounted.

Red-stained nuclei of p-p38 positive cells were counted in sections at 400× magnification by light microscope (Olympus BX51) that was equipped with an integrated software analysis system (Argenit Kameram, version 2.11.5.1, Istanbul, Turkey). For each animal, the counted p-p38 positive cells in 10 random fields were recorded, and area unit of renal cortex was measured at 0.1 mm^2. Cytoplasmic immunoreactivity of NFkB and Nrf2 were evaluated semiquantitatively using the HSCORE method. The average HSCORE for each animal was calculated with the formula:

$HSCORE = \Sigma\ i \times Pi$.[23]

These scores were classified according to the percentage of cells with immunoreactivity in the sections (Pi) and the degree of staining (i; 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining). The evaluations were performed in five random areas of each slide at 400× magnification under light microscope (Olympus BX-51). All semiquantitative analyses were made by two independent researchers, and averages were recorded.

Statistical Analysis

All data were presented as mean ± standard deviations (SD). Statistical analyses of data for immunohistochemical evaluation were carried out by one-way analysis of variance. Then, Tukey or Tamhane multiple comparison test was done according to the homogeneity of the group variances. Other data were evaluated by Kruskal Wallis test, comparing the four groups. To specifythe significance of differences between groups, multiple comparison tests with mean ranks were used. The P values < .05 was considered to

be statistically significant.

RESULTS

Animal and Kidney Weights

There was a significant decrease in the body weight and increase in the kidney weight/body weight ratio of GM group compared with the control and CMN groups ($P < .001$, Table 1). CMN treatment increased the body weight, but this change was not statistically significant. Additionally, kidney weight/body weight ratio did not change with CMN administration.($P > .05$, Table 1).

Biochemical Analysis

The serum urea and creatinine levels were increased significantly in GM group as compared with the control and CMN groups ($P < .001$, Table 2). CMN reduced the levels of serum urea and creatinine in GM+CMN group, but the decrease was not statistically significant ($P > .05$, Table 2).

Histological Examinations

The kidney tissues in the control and CMN groups had normal histological structure (Figure 1, A-D; Figure 2, A-D). In the GM group, the most prominent change was dilation of the renal tubules, mostly affecting the distal tubules. Dilated tubules, were surrounded by a line of cells that had lost their rounded appearance and become flattened due to atrophy. In addition, desquamated cells and hyaline cast formations were found in these dilated tubules (Figure 1, E; F). In addition, GM caused proximal tubular damages such as desquamation, cytoplasmic vacuolization, and loss of brush border (Figure 2, E; F). Furthermore, numerous foci of leukocytic infiltration were found around the blood vessels and renal corpuscles in kidney tissue (Figure 1E). A number of the renal corpuscles had lost their normal size and shape. Bowman's space became irregular and enlarged due to shrinkage of the glomerular structure in the corpuscules (Figure, 1F). In addition, increased connective tissue with edema was observed in the interstitial areas around the dilated tubules and degenerated renal corpuscles (Figure 2, E; F).

In the GM + CMN group structure was generally preserved compared with the GM group. The number and diameters of the dilated tubules were not increased, and the hyaline cast formations was observed in their lumen, less commonly than in the GM group. Proximal tubules appeared almost normal (Figure 1, G; H). The brush borders of these tubular cells were preserved, and the thickening of the basement membranes, vacuolization and desquamation in tubular cells were not increased. The number of shrunken glomeruli was not increased and the Bowman spaces were almost normal (Figure 2, G; H). The histological findings described above for all four groups are presented in Table 3.

Immunohistochemistry

The numbers of p-p38 immunopositive cells were low in the control and CMN groups, and was not significantly different. However, p-p38 immunopositive cells were significantly increased

Table 1. Comparison of Changes in Body Weight and the Ratio of Kidney Weight / Body Weight (KW/BW*1000) in Control and Experimental Groups

Parameter	Control (n = 10)	Curcumin (n = 10)	Gentamicin (n = 10)	Gentamicin + Curcumin (n = 10)
BW Change, g	15.4 ± 7.9	15.8 ± 13.9	-37.3 ± 16.8*	-18.1 ± 19.7†
KW/BW*1000	4.1 ± 027	4.1 ± 0.33	5.8 ± 0.52†	5.7 ± 0.53†

Data are expressed as mean ± SD (n = 10).
*$P < .001$ comparison with control and curcumin groups
†$P < .05$ comparison with control and curcumin groups

Table 2. Comparison of Serum Urea and Creatinine Levels in Control and Experimental Groups

Parameter	Control (n = 10)	Curcumin (n = 10)	Gentamicin (n = 10)	Gentamicin + Curcumin (n = 10)
Urea	38.31 ± 2.93	37.45 ± 5.89	254 ± 114.84*	103.58 ± 67.97†
Creatinine	0.27 ± 0.04	0.28 ± 0.04	4.55 ± 1.51*	1.65 ± 1.21‡

Data are expressed as mean ± SD (n = 10).
*$P < .001$ comparison with control and curcumin groups
†$P < .05$ comparison with curcumin group
‡$P < .05$ comparison with control and curcumin groups

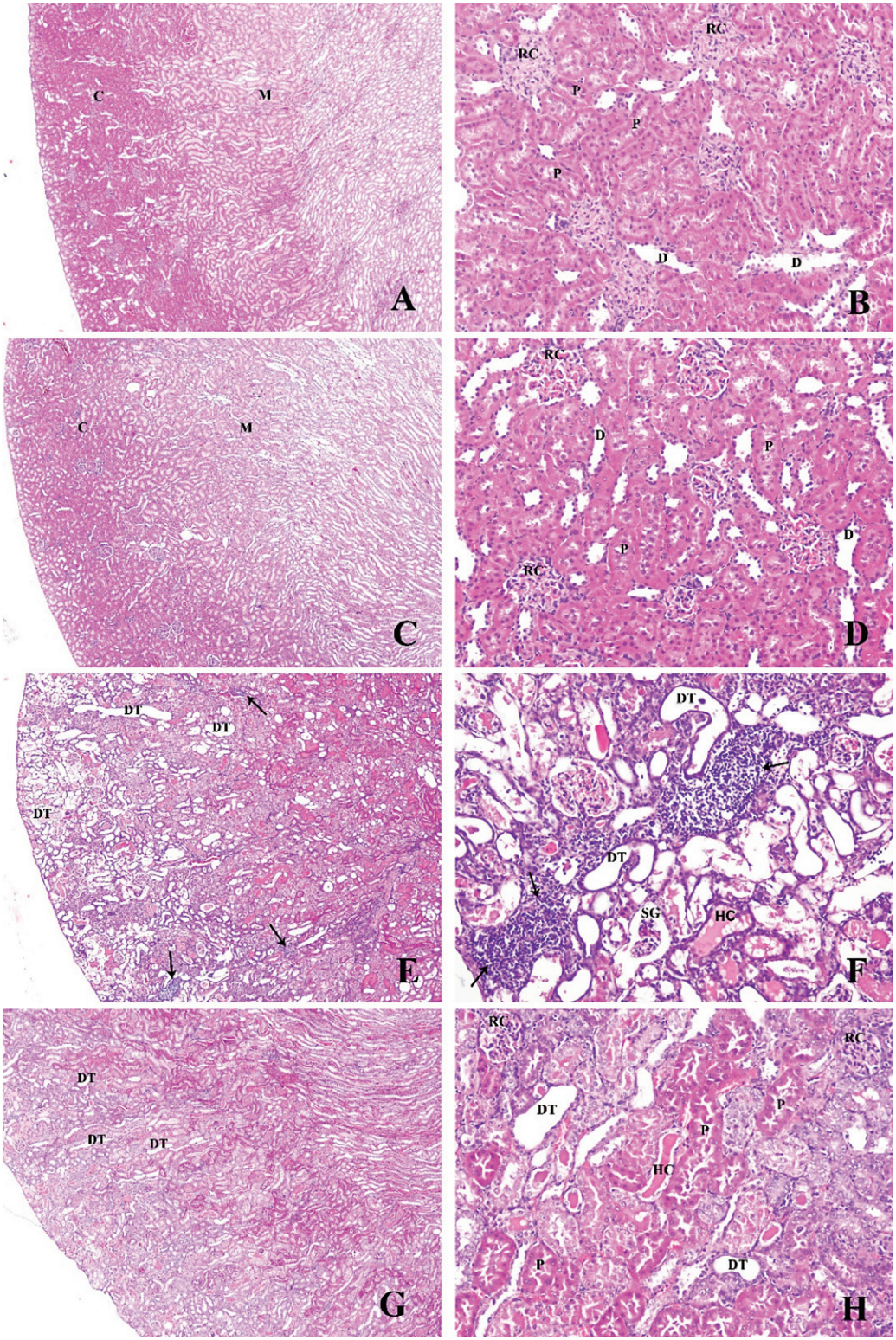

Figure 1. Photomicrographs of the histological changes in the kidney tissues in control and experimental groups.The control group showed normal histological structure of kidney cortex (C) and medulla (M) [A], normal histological structure of the renal corpuscles (RC), proximal tubules (P) and distal tubules (D) in cortex [B]. The CMN group showed normal histological structure similar to control group [C, D]. The GM group demostrated degenerative changes in tubules and renal corpuscles. These changes included dilated tubules (DT), leukocytic infiltration (arrow), hyalin cast (HC) and shrunken glomeruli (SG) in renal cortex [E, F]. In the GM+CMN group, amelioration of renal damage was seen compared with the GM group [G, H]. Staining and magnification of photomicrographs of rat kidney tissue sections: [H&E ×40; A, C, E, G] and [H&E ×200; B, D, F, H].

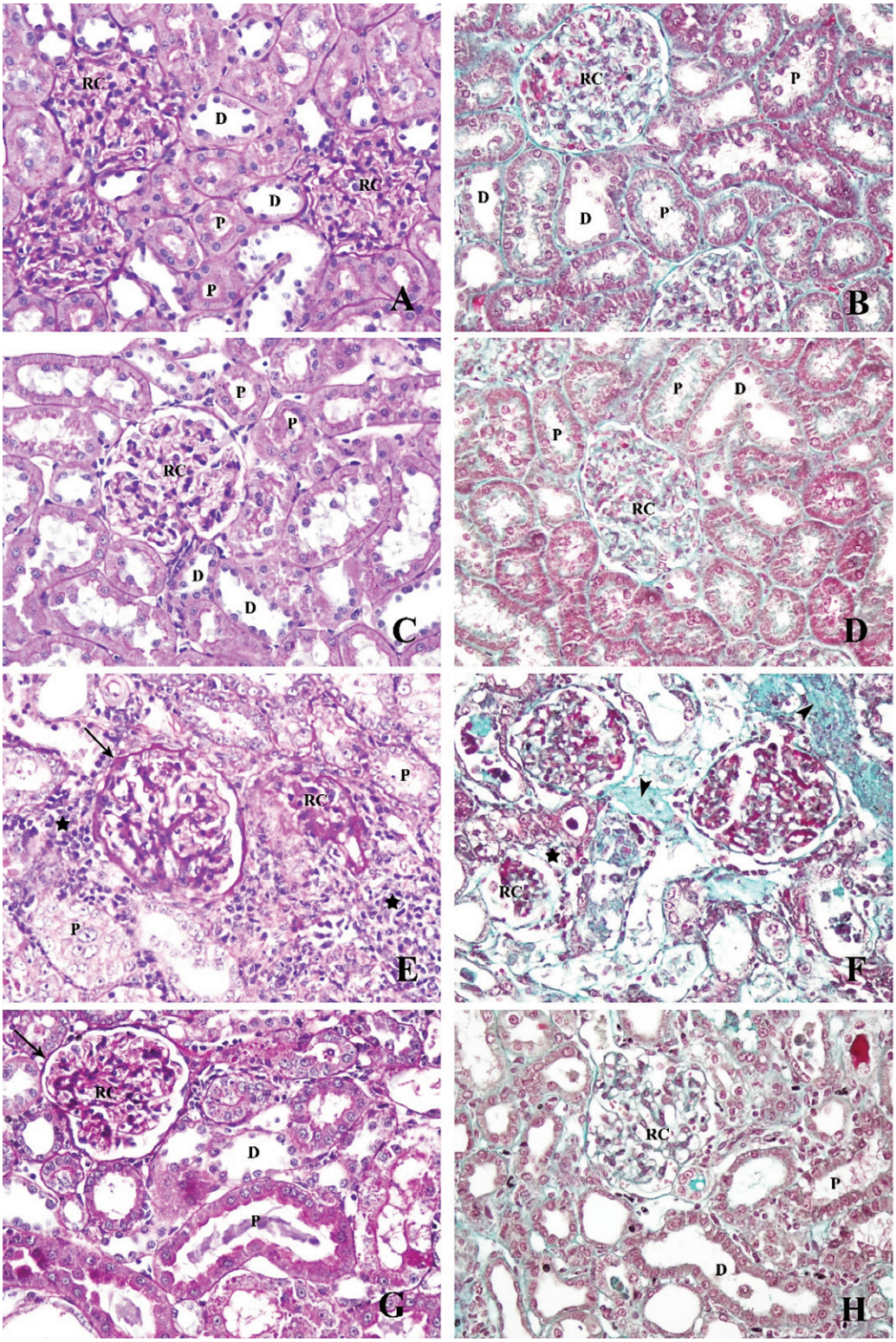

Figure 2. Photomicrographs of kidney cortex sections stained with Periodic Acid-Schiff [A, C, E, G] and Masson's trichrome [B, D, F, H], ×400 magnification. Kidney cortex sections of the control group showed normal histological structure of the renal corpuscles (RC), and normal proximal (P) and distal tubules (D) in cortex [A, B]. The CMN group was similar to control group [C, D]. The GM group showed leukocytic infiltration (asterisks), increased thickness of glomerular basement membrane (arrow), loss of brush borders, increased connective tissue (arrowheads) and partially degenerated renal corpuscles (RC) [E, F]. The GM+CMN group showed almost preserved structures of proximal (P) and distal tubules (D), brush borders, renal corpuscles (RC) and thin glomerular basement membranes (arrow) by comparison with GM group [G, H].

Table 3. Histological Changes in Control and Experimental Groups

Histological Changes	Control (n = 10)	Curcumin (n = 10)	Gentamicin (n = 10)	Gentamicin + Curcumin (n = 10)
Shrunken Glomeruli	0	0	2.9 ± 0.32	0.5 ± 0.53
Dilated Tubules	0	0	4.5 ± 0.53	1.3 ± 0.48
Tubular Vacuolization	0	0	3.8 ± 0.42	0.8 +0.42
Loss of Brush Border	0	0	3.9 ± 0.32	0.9 ± 0.32
Hyalin Cast	0	0	4.3 ± 0.48	1.5 ± 0.53
Leukocytic Infiltration	0	0	3.9 ± 0.32	0.8 ± 0.42

Data are expressed as mean ± SD (n = 10).
No (0), mild (1), mild to moderate (2), moderate (3), extensive (4), and severe (5)

Table 4. Comparison of the p-p38 Positive Cell Count, NFkB, and Nrf2 Immunoreactivities in Control and Experimental Groups

Parameter	Control (n = 10)	Curcumin (n = 10)	Gentamicin (n = 10)	Gentamicin + Curcumin (n = 10)
p-p^{38} positive cell count	31.84 ± 4.32	26 ± 2.95	128.93 ± 9.79*	63.09 ± 10.55
NFkB (HSCORE)	83 ± 10.33	67 ± 8.23†	194 ± 14.3*	135.5 ± 11.65
Nrf2 (HSCORE)	144.5 ± 17.07	158 ± 16.7	41.5 ± 8.18*	96 ± 16.12

Data are expressed as mean ± SD (n = 10).
*P < .001 comparison with control, curcumin, and gentamicin + curcumin group
†P < .001 comparison with control group

(P < .001) in the GM group compared with the control and the CMN groups. CMN + GM treated rats had significantly decreased number of p-p38 immunopositive cell numbers compared with the GM group (P < .001) (Table 4, Figure 3).

We found a weak NFkB immunoreactivity in the control and CMN groups, which was even slightly less in the CMN group. GM group showed a statistically significant increase in NFkB immunoreactivity compared with the control and CMN groups (P < .001). Conversely, CMN treatment with GM injection resulted in statistically significant decreased immunoreactivity of NFkB (P < .001, Table 4; Figure 4).

The immunoreactivity of Nrf2 was moderate in the renal cortex and similar between the control and CMN groups. However, this expression was significantly decreased in GM injected animals compared with the control and CMN groups (P < .001). Combined CMN and GM treatment significantly up-regulated the expression of Nrf2 compared with the GM group (P < .001, Table 4; Figure 5).

DISCUSSION

In our study, we investigated the protective effect of CMN on the GM-induced nephrotoxicity. We observed that pretreatment with CMN in GM treated rats, preserved the blood urea and creatinine levels, and histological changes in the kidney tissues. In addition, CMN had some other beneficial effects such as a significant decrease in p-p38 MAPK and NFkB immunoreactivities, as well as significant increase in Nrf2 immunoreactivity. We believe that these effects resulted from the anti-apoptotic and anti-inflammatory activities of CMN in the intracellular signaling pathways.

Decrease in body weight in the GM group was significant as compared with the control and CMN groups and our findings also support the previous studies.[24,25] The decrease in the body weight in GM group could be attributed to the decrease in the animal's oral food intake due to acidosis along with anorexia caused by acute kidney injury.[25] Reduced body weight may have been also due to impaired water reabsorption by damaged renal tubules and consequent dehydration.[24] Kidney weight/body weight ratio was found to increase significantly in the GM group compared with control and CMN groups. However, CMN did not change this ratio in the GM+CMN group. We could utter that the unchanged kidney weight/body weight ratio might be due to interstitial edema that has not still regressed. Increased serum urea and creatinine levels could be considered as a sign of kidney damage caused by GM.[2,25,26-30] Changes in serum urea and creatinine levels reflects the decrease in glomerular filtration rate.[31] Ustuner *et al.* reported that blood urea and creatinine levels increased significantly after treatment with GM.[27] In our study,

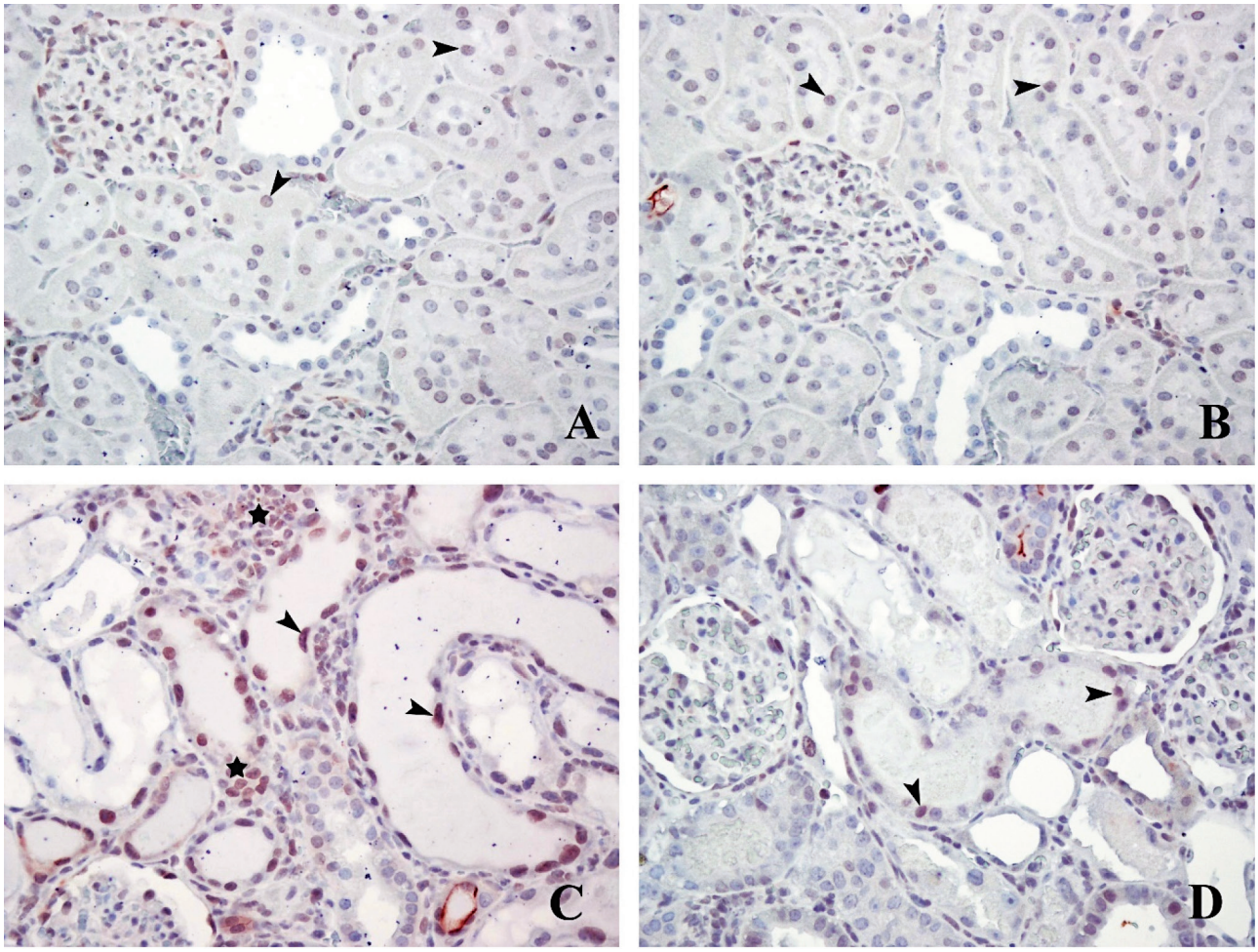

Figure 3. Photomicrographs of p-p38 positive cell nuclei with hematoxylin counterstain, ×400 magnification. P-p38 positive cell count (arrowheads) were similar in the control [A] and CMN group [B], whereas, leukocytic infiltrated positive cells (asterisks) observed and tubular positive cell count (arrowheads) increased in the GM group [C] than the control and CMN groups. In the GM+CMN group, p-p38 positive stained cell count (arrowheads) were significantly diminished [D].

urea and creatinine levels increased significantly in the GM group. These levels decreased dramatically by CMN, however, were not statistically significant.

In GM group, the major morphological change was dilated tubules, similar to what was mentioned in the previous studies.[18,25,32] Hyaline cast formation was seen in both cortical and medullary regions in the lumen of some dilated tubules.[27,33-35] Another tubular change was the loss of brush border of the proximal tubules, and the thickening of basement membranes in a number of proximal tubules.[13,36] Additionally, cytoplasmic vacuolization and desquamation were prominent in the proximal tubular cells, which has been also shown in other studies.[25,32,37,38] Soliman *et al.* stated that tubular vacuolization may result from the damage and dilatation of the cell organelles.[32] We also observed foci of leukocytic infiltration with increased connective tissue and edema in the interstitial areas in GM group, similar to the findings of other studies.[39-41] In the study conducted by Kolgazi *et al.* leukocytic infiltration, intense vasocongestion in the interstitial area, tubular necrosis, and glomerular damage were observed with GM nephrotoxicity.[39] Shrunken glomeruli in the renal cortex were mostly observed within or near the leukocytic infiltration sites. The glomerular shrinkage may occur due to decreased glomerular filtration and cell degeneration.[32] In addition, thickening of the basement membrane surrounding the shrunken glomeruli was observed. Stojiljkovic *et al.* supported this finding in their studies.[42]

In the GM+CMN group, kidney structure was generally preserved compared with the GM group. There are some other studies reporting that CMN

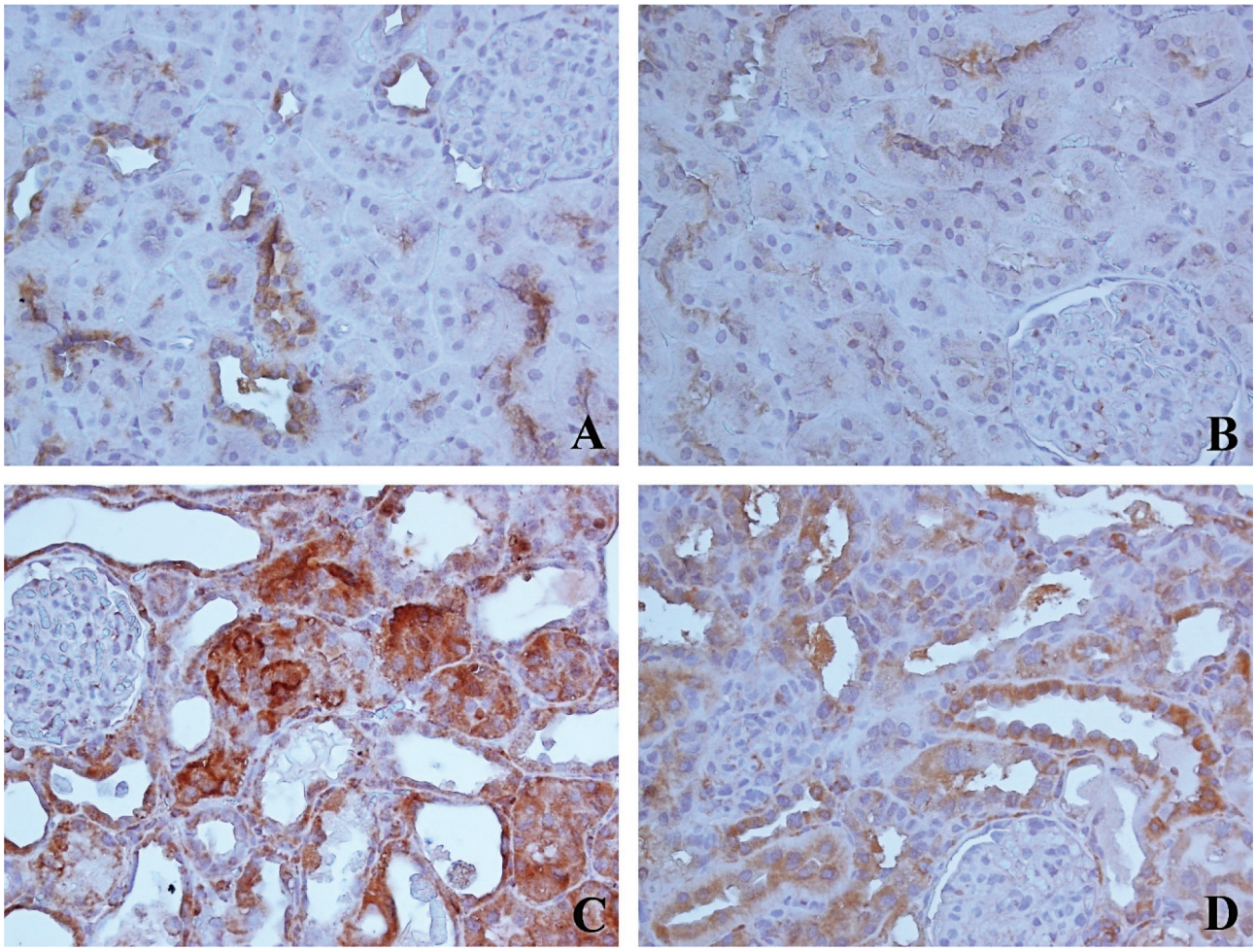

Figure 4. Photomicrographs of NFkB immunoreactivity with hematoxylin counterstain, ×400 magnification. NFkB immunreactivity was weak or very weak in the control group [A] and CMN group [B] respectively, whereas, NFkB immunoreactivity increased in the GM group [C] compared to the control and CMN groups. In the GM+CMN group, NFkB immunoreactivity was significantly diminished [D].

exerts a protective effect against renal oxidative damage.[24,41,43] However, studies elucidating this protective effect on intracellular signaling pathways including apoptosis and inflammation are limited.

Mitogen-activated protein kinase is an important mediator of intracellular interaction proteins that convert extracellular stimuli to intracellular responses.[44] p38 is a member of the MAPK family that may be activated by the various types of cellular stresse, and is involved in intracellular signaling pathways. Additionally, it is known to take part in apoptosis and inflammation.[40,45,46] According to our findings, the number of p-p38 positive cells was significantly increased in the GM group, especially in the dilated tubules, compared with the control and CMN groups. On the other hand, the number of p-p38 positive cells was found to be significantly decreased in the GM + CMN group compared with the GM group. Curcumin reportedly reduces the p38 expression in glomerular mesengial cell cultures.[14] Volpini *et al.* reported that the number of p38 positive cells significantly increased in GM nephrotoxicity, and significantly decreased with the administration of pyrrolidine dithiocarbamate (PDTC), which has an inhibitory effect on NFkB.[13] The point that draws our attention is the existence of a relationship between p38-NFkB signaling pathways, in addition to the significant decrease in the number of p38 positive cells with PDTC administration. Some studies have stated that redox-sensitive cell signaling factors such as NFkB and p38 MAPK play a role in GM-induced nephrotoxicity.[40,47]

Nuclear factor-kappa B is a transcription factor that regulates the gene expression of cytokines involved in various inflammatory diseases. In our study, GM increased the immunoreactivity

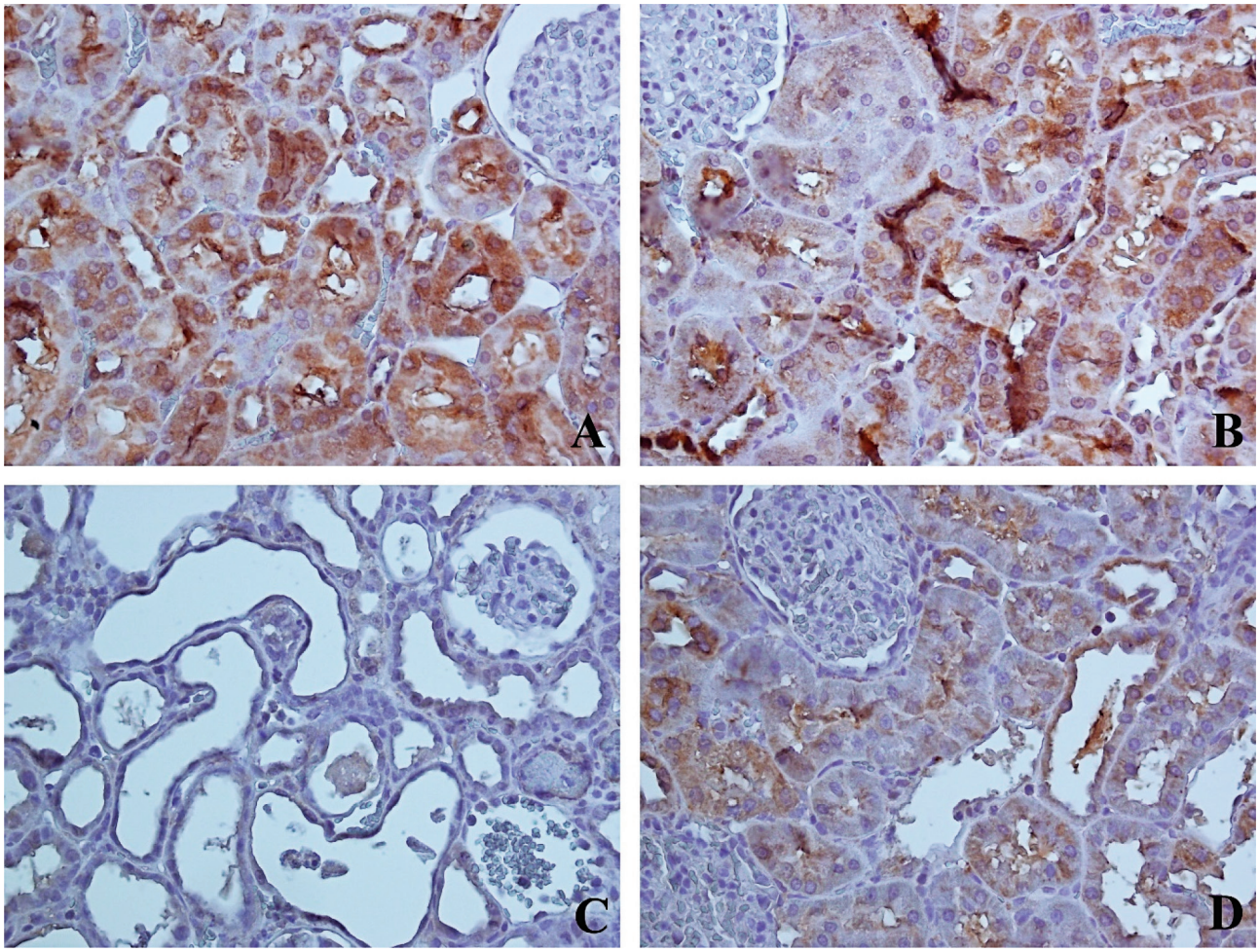

Figure 5. Photomicrographs of Nrf2 immunoreactivity with hematoxylin counterstain, original magnification ×400. Nrf2 immunreactivity was moderate and stained similarly in the control [A] and CMN groups [B], whereas, Nrf2 immunoreactivity significantly decreased in GM group [C] compared to the control and CMN groups. In the GM+CMN group, Nrf2 immunoreactivity was significantly increased [D].

of NFkB compared with the control and CMN groups. However, CMN significantly reduced this immunoreactivity. In another study, expressions of NFkB and p38 protein increased in diabetic mice, and *Houttuynia cordata* liquid extract decreased NFkB p65 and p38 expressions, apoptosis and inflammation.[48] Furthermore, Ozbek *et al.* reported that GM increases NFkB p65 and p38 expressions and there is a relationship between these two proteins.[44] We believe that p38 MAPK may play a crucial role in the production of proinflammatory cytokines by regulating the activation of the p65 subunits of NFkB and consequently in the control of intracellular apoptotic and inflammatory pathways. Manikandan *et al.* showed that CMN decreases NFkB protein expression in GM nephrotoxicity.[19]

The redox-sensitive transcription factor, Nrf2, acts as a sensor of oxidative and electrophilic stress.[49] Although a number of studies have reported that Nrf2 expression increases in GM nephrotoxicity,[49,50] others have indicated that Nrf2 expression decreases in damaged tissues with the activation of apoptotic and inflammatory pathways.[3,21] Curcumin reduces the intracellular ROS formation and stimulates the transcription of genes that induce the expression of the antioxidant system by activating Nrf2 expression.[9,11] These findings indicate that CMN treatment may protect the kidney tissue from oxidative stress injury by activating Nrf2 expression.[21] Our findings of a decrease in Nrf2 immunoreactivity in the GM group and an increase in the GM + CMN group are compatible with the literature. It is known that there is a relationship between NFkB and Nrf2, so that Nrf2 controls NFkB level by suppressing the phosphorylation of IkB, a cytosolic inhibitor protein of NFkB.[6,11] We suppose that CMN may

decrease NFkB expression by suppressing the IkB phosphorylation through Nrf2 activation.

CONCLUSION

In conclusion, we propose that CMN at a dose of 100 mg/kg/d may attenuate GM-induced nephrotoxicity by suppressing the p38 MAPK and NFkB and the activation of Nrf2 signaling pathways. We suggest that this ameloration most likely occurs due to the anti-apoptotic and anti-inflammatory properties of curcumin.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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