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Original Article UROLOGY

Ethanolic extract of *cotinus coggygria* leaves attenuates crystalluria and kidney damage in ethylene glycol-induced urolithiasis in rats

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ABSTRACT

OBJECTIVE: Nephrolithiasis is a common cause of kidney insufficiency. Nephrolithiasis is proven to be the result of various biochemical and inflammatory processes that result in crystal formation and subsequent aggregation. *Cotinus coggygria* L. (CCog) is a plant extract which has been used as a Turkish remedy for kidney stones. With this study, we planned to evaluate the effects of CCog extract in ethylene glycol (EG)-induced nephrolithiasis model in rats.

METHODS: The study group comprised 32 Wistar albino rats which were divided into Control (C), EG, CCog Prophylaxis (CC+EG+CC), and CCog Treatment (EG+CC) groups. Stone formation was induced by adding EG (0.75%) into rat's drinking water. Normal drinking water was given to Control group for 8 weeks. Throughout the study period of 8 weeks, EG group was given only EG (0.75%) and CC+EG+CC group was given both EG and CCog. In EG+CC group, EG (0.75%) was given for 8 weeks whereas CCog was given for the past 4 weeks. After the 8th week, 24-h urine samples were collected. Rats were then sacrificed and kidney tissue samples were harvested.

RESULTS: Metabolites (calcium, citrate) and creatinine in 24 h urine samples were decreased in CC+EG+CC and EG+CC groups. While hyperoxaluria was observed in the EG group, oxalate levels were similar to control levels in the P-CCog and C-CCog groups. The N-acetyl-β-glucosaminidase and myeloperoxidase activities were both increased in EG group and these parameters were significantly decreased on CCog treatment.

CONCLUSION: We can conclude that C. coggygria extract can have beneficial effect on lowering concentration of stone-forming metabolites in urine and consequently protect renal tissues from damage due to nephrolithiasis. C. coggygria extract can be considered as a potential prophylactic and therapeutic option in high-risk stone formers. Furthermore, our data confirm ethnobotanical use of CC against nephrolithiasis.

Keywords: Ethylene glycol, hyperoxaluria; nephrolithiasis; oxidative stress; renal insufficiency.

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Nephrolithiasis is a common and multifactorial disease with a prevalence around 10% and with an incidence around 600,000 people/year in United States [1]. While it is 2 times more common in males than females, the prevalence differs with geographic, genetic, and dietary factors [2]. Nephrolithiasis tends

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to recur, hence the saying "once a stone patient, always a stone patient." Recurrence is seen around 50% of the patients in 10 years [3]. As nephrolithiasis is a common, recurring disease and a major cause of renal insufficiency, it creates a high burden on socioeconomical status of the population [4]. Therefore, preventive strategies for stone formation are among the most studied subjects in the literature.

Pathogenesis of kidney stone formation is a complex process. The initial supersaturation of urine is followed by nucleation, crystal formation, crystal growth, and crystal retention [5]. Although the compositions may differ, the mechanism is more or less the same. Furthermore, with recent studies involving nephrolithiasis and stone formation mechanisms, oxidative stress has been found to be a key contributor to this process [6, 7]. Crystal aggregation is the major step for the cell membrane damage which starts the inflammatory cascade which, in turn, ends up with the ultimate renal epithelial damage. Therefore, studies have focused on alleviating the oxidative stress and decreasing reactive oxygen species formation for the prevention of stone formation [8].

The most common used nephrolithiasis model on rats is the ethylene glycol (EG) model [8–12]. EG is a colorless, odorless, and water-soluble dihydric alcohol. After metabolization in liver with alcohol dehydrogenase, its toxic by-products are formed; glycolaldehyde, glycolic acid, glyoxylic acid, and oxalic acid. These toxic products are responsible for the aggregation of oxalate in kidney in the form of calcium oxalate [13].

Cotinus coggygria L. (CCog) is as a remedy used in Turkish traditional medicine. This species has traditional use especially in the treatment of various urological pathologies such as stones and nephritis. Furthermore, it is proven to have antibacterial, antioxidant, and anti-inflammatory effects [14]. In this study, we aimed to investigate the effects of CCog extract given both during and after the stone formation process in an EG-induced nephrolithiasis model in rats.

Highlight key points

- Nephrolithiasis is one of the major risk factors for end-stage kidney failure.
- The reactive oxygen species formed in the kidney before and after crystal acculumation damage the kidney tissue and also fasten the stone formation processes.
- *Cotinus coggygria* L. plant has antioxidant and anti-inflammatory properties.
- *Cotinus coggygria* L. have been proven to have beneficial effects in rat nephrolithiasis model both in a preventive and therapeutic approach.
- Further clinical studies, using antioxidants and antiinflammatory agents for the prevention and treatment of nephrolithiasis should be conducted to support the findings of pre-clinical studies.

MATERIALS AND METHODS

This study was approved by Marmara University (MU) Animal Experiments Local Ethics Committee with the protocol number "110.2016.mar (Nov 14th, 2016)".

The animals used in the experiment were supplied from Marmara University Experimental Animals Research and Implementation Center. During the study period, optimal conditions were provided for the rats; they were kept in rooms with 12-h dark-light cycles, temperature of 20–22 °C, and humidity of 45–50%. During the experimental period, all animals were kept in transparent cages and fed with standard rat chow *ad libitum*.

Study Groups and Nephrolithiasis Model

Four groups were created for the study with each containing eight male Wistar albino rats, age of 3 months old, weight of 250–300 g:

- Control group (C): Rats were fed with regular drinking water for 8 weeks.
- EG group: Rats were fed with drinking water + 0.75% EG for 8 weeks
- CCog Prophylaxis group (CC+EG+CC): Daily Ethanolic extract of *C. coggygria* (100 mg/kg) was administered simultaneously with 0.75% EG for 8 weeks
- CCog Treatment group (EG+CC): Rats were given drinking water containing 0.75% EG for 8 weeks. 4 weeks after the onset of EG, ethanolic extract of *C. coggygria* (100 mg/kg) was started daily and given for the remaining 4 weeks of the experiment.

After the aforementioned stone formation and treatment period, 24-h urine samples were collected using metabolic cages. Thereafter, under ether anesthesia, intracardiac blood samples were taken, animals were sacrificed, and kidney tissues were harvested for histological and biochemical evaluations.

Preparation of *C. coggygria* Extract

The leaves of C. *coggygria* were collected from Hamidiye village of Kırklareli in June 2002. A few specimens of the plant have been registered at Marmara University Faculty of Pharmacy Herbarium with the number 80926. The leaves of the plant dried in a ventilated environment out of the sun were powdered and approximately 300 g of this powder was weighed. The solvent of the extract obtained using a Soxhlet device with 96% ethanol was evaporated in a rotary evaporator and ethanol extract was obtained. Extract yield was calculated as 41.56% (g/g). This extract was kept at +4 °C until analysis.

Urinary Metabolite Level Measurements

At the end of the experiment, calcium, citrate, oxalate, phosphate, uric acid, and creatinine levels measured from 24 h urine samples collected in metabolic cages of all groups were evaluated. Measurements were performed using special kits for each metabolite; "QuantiChromTM Calcium Assay Kit," "QuantiChromTM Phosphate Assay Kit," "QuantiChromTM Uric Acid Assay Kit," "EnzyChromTM Oxalate Assay Kit," "EnzyChromTM Citrate Assay Kit," and "Rat Cr Creatinine ELISA Kit."

Biochemical Evaluations in Blood Samples

At the end of the experiment, intracardiac blood samples taken under ether anesthesia from all groups were used to measure blood urea nitrogen (BUN), serum creatinine, and the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels. Measurements were performed using special kits for each metabolite; "Rat BUN ELISA Kit," "Rat Cr (Creatinine) Assay Kit,""Quantikine Human TNF-α ELISA Kit," and "Human IL-1β ELISA Kit."

Malondialdehyde Level Measurement

In homogenized kidney tissue samples, MDA levels, as marker of lipid peroxidation, were assayed as products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation and the results are expressed as nmol/g tissue [15].

Oxidative DNA Damage Measurement

8-hydroxy-2'-deoxyguanosine (8-OHdG) levels reflect the oxidative DNA damage in cells. Oxidative DNA damage measurement in tissue samples was performed using "Oxi Select Oxidative DNA Damage Elisa Kit (STA-320, Cell Biolabs)" and DNA isolation from samples using "PureLink® Genomic DNA Mini Kit (K182001, Life Technology)" in accordance with kit procedures. Results are expressed as "8-OHdG ng/mg DNA."

Myeloperoxidase Activity Measurement

Myeloperoxidase (MPO) activity demonstrates the neutrophil infiltration in tissues where tubular epithelial damage occurs due to persistent hyperoxaluria. MPO activity was determined according to Hillegass et al.'s method [16]. The supernatant was discarded after the tissue homogenates were centrifuged for 10 min. 3 mL of 0.5% HETAB was added to the precipitate and homogenized, frozen 3 times, thawed and sonicated, and centrifuged to work with the upper phase. Afterward, 50 mM K_2HPO_4 (pH: 6), o-dianisidin-2 HCl, and 20 mM H_2O_2 (Hydrogen Peroxide) solutions were added and reaction was terminated by adding 2% sodium azide. Centrifugation was done for 10 min at 3000 rpm. The absorbance of the color formed by taking the supernatant was read in the spectrophotometer at 460 nm. The extinction coefficient for MPO was calculated as 42M-1cm-1. The results were expressed in U/g protein.

Caspase-3 Activity Determination

Caspase-3 activity levels reflect the renal cellular apoptosis and were determined using commercial kits (Caspase 3 ELISA Kit 96T). The measurement principle was based on the spectrophotometric measurement of the formation of chromophore p-nitroaniline (pNA) from the caspase-3 substrate N-Acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA). The absorbance of the liberated pNA was found by reading at 405 nm in the ELISA reader.

Measuring Osteopontin Levels and N-acetyl-β-glucosaminidase (NAG) Enzyme Activity

Osteopontin is a marker extracellular structural disruption and measurement of -acetyl-β-glucosaminidase enzyme activity reflects the oxidative stress related renal tubular damage. Measurement of osteopontin levels and NAG enzyme activity in tissues was performed using the OPN ELISA Kit and NAG ELISA Kit, respectively. Results were expressed as ng/g for osteopontin, while NAG activity was expressed as U/mg protein.

Table 1. Measurement of metabolites in 24 h urine collection

(mg/24 h)	Control	EG	CC+EG+CC	EG+CC
Calcium	0.56±0.03	0.24±0.05***	0.50±0.04^{++}	0.54±0.03^{+++}
Citrate	5.38±0.44	1.72±0.25***	4.32±0.45^{+++}	3.29±0.24**$^{+}$
Oxalate	0.59±0.05	1.41±0.20**	0.72±0.08^{++}	0.86±0.12^{+}
Phosphate	0.54±0.05	0.42±0.05	0.53±0.08	0.52±0.06
Uric acid	0.38±0.03	0.69±0.09*	0.38±0.07^{+}	0.49±0.07
Creatinine	9.15±0.88	4.92±0.56**	8.10±0.39^{+}	5.53±0.59*

*: P<0.05; **: P<0.01; ***: P<0.001: Compared to control group; +: P<0.05; ++: P<0.01; +++: P<0.001: Compared to EG group; CCog: Cotinus coggygria; EG: Ethylene glycol group; CC+EG+CC: CCog prophylaxis group; EG+CC: CCog treatment group.

Microscopic Evaluations

The day before the sacrifications were done, unpreserved urine samples were collected from each rat. Microscopic analyses were done as described: 1 mL of fresh urine was centrifuged at 2000 rpm and 950 mL of supernatant was discarded. 10 mL of the vortex mixed precipitate was transferred to a hemocytometer. The number and type of all crystals were determined with an Olympus inverted microscope BH40 (Japan). All samples were examined with an inverted microscope at a small magnification (objective 10× ocular 10× = 100×). The field was then examined at high magnification (objective 40× ocular 10× = 400×). Shaped elements in 20 microscopic fields at 400 magnifications were examined.

Histological Analysis

For histological analyses, 10% formalin solution was used to fix kidney tissues. Dehydration was done degraded ethanol series and tissues were cleared in toluene. Paraffin-embedded samples were cut at 5 µm thickness by rotary microtome and samples were stained with H&E (hematoxylin and eosin). An Olympus BX51 light microscope (Olympus Co., Ltd., Tokyo, Japan) was used for evaluations and photography.

Statistical Analysis

GraphPad Prism 3.0 (GraphPad Software, San Diego, CA) software was used for statistical analyses. Data are expressed as mean±standard error. Analysis of variance followed by Tukey's multiple comparison tests were used for comparisons. Values of $p<0.05$ were considered significant.

RESULTS

24 h Urinary Metabolites

At the end of the experimental period, 24 h urine collection was performed and the urine samples were evaluated for calcium, citrate, oxalate, phosphate, uric acid, and creatinine (Table 1).

In the EG group, the levels of calcium, citrate, and creatinine were significantly decreased compared to control group. In groups treated with CCog, either as a prophylaxis or treatment agent (CC+EG+CC and EG+CC groups, respectively), the levels of aforementioned metabolites increased significantly compared to EG group and approached control group levels.

Similarly, levels of oxalate and uric acid were significantly increased in EG group compared to control group; and levels were significantly decreased in CC+EG+CC compared to EG group.

Levels of phosphate did not achieve any statistical significance in-between groups, although there were quantitative differences.

Biochemical Measurements in Serum Samples

In EG group, BUN, creatinine, TNF-α, and IL-1β levels were significantly decreased compared to control group. In CC+EG+CC group, the levels of all the aforementioned metabolites increased significantly compared to EG group and approached control group levels. In EG+CC group, BUN and creatinine levels increased significantly compared to EG group but TNF-α and IL-1β levels remained similar to EG group levels (Table 2).

Biochemical Measurements in Tissue Samples

In the EG group, levels of MDA (Fig. 1A), 8-OHdG (Fig. 1B), and osteopontin (Fig. 1C) and activities of MPO (Fig. 2A), caspase-3 (Fig. 2B), and NAG (Fig. 2C) significantly increased compared to control group. In CC+EG+CC group, the levels and activities of all the aforementioned measurements decreased significantly compared to EG group and approached control group levels. In EG+CC group, MDA levels and activities of MPO, caspase-3, and NAG decreased significantly compared to EG group (Table 3).

Urine Microscopy

On urinary evaluations under inverted microscope for crystal formation, clustering, and density, EG group had the most advanced and dense crystal formation and clustering. In groups treated with CCog, crystal clustering and density showed a similar pattern compared to control group. It has been observed that crystal density is subjectively less in CC+EG+CC group compared to EG+CC group (Fig. 3).

Histopathological Evaluations

As a result of histopathological examinations, the glomeruli and tubule structures of the control group were normal (Fig. 4A). EG administration caused thickening of the tubular basement membrane, desquamation of tubular cells with distribution into the lumen, and significant degeneration (Fig. 4B). In the CC+EG+CC group, a slight thickening of the basement membrane and a decrease in tubular cell shedding were observed (Fig. 4C). In the EG+CC group, a decrease in basement membrane thickening and a thinning of tubular cell shedding were observed (Fig. 4D).

DISCUSSION

The mechanism of urinary stone formation is a complex process. Biochemical changes, structural changes in kidney tissue, and inflammation mediated by oxidative stress play a role in stone formation. In animal models studying urinary stone disease, EG-induced nephrolithiasis model is one of the most frequently used models [17, 18]. EG is a colorless, odorless, and water-soluble dihydric alcohol. When ingested, EG is rapidly absorbed from the gastrointestinal system and reaches its maximum plasma concentration around 1–4 h. When EG reaches the liver, it is metabolized to organic acids: Gly-

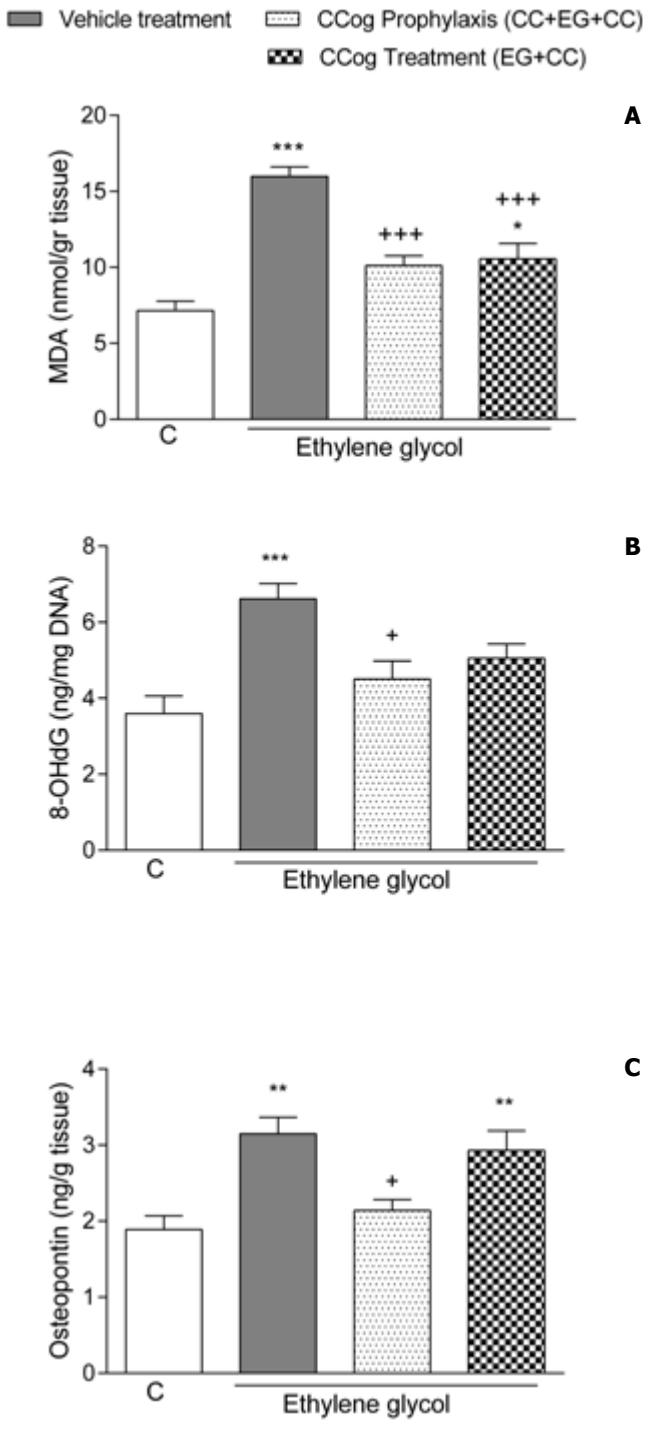

FIGURE 1. **(A)** Measurement of malondialdehyde levels in kidney tissue samples *p<0.05; ***p<0.001: Compared to control group. +++p<0.001: Compared to EG group. **(B)** Measurement of 8-hydroxy-deguanosine (8-OHdG) levels in kidney tissue samples ***p<0.001: Compared to Control group. +p<0.05: Compared to EG group **(C)** Measurement of osteopontin (OPN) levels in kidney tissue samples **p<0.01: Compared to control group. +p<0.05: Compared to EG group.

Table 2. Measurement of metabolites in blood samples

	Control	EG	CC+EG+CC	EG+CC
BUN (mg/dL)	37.35±1.94	53.91±4.15**	36.69±2.06^{++}	41.33±2.48^{+}
Creatinine (mg/dL)	0.49±0.03	0.75±0.03***	0.55±0.03^{++}	0.57±0.04^{++}
TNF-α (pg/mL)	52.00±2.47	84.86±5.34***	60.71±4.28^{++}	68.71±5.67
IL-1β (pg/mL)	110.3±5.54	147.20±9.78**	117.30±7.26^{+}	130.00±4.37

*: P<0.05; **: P<0.01; ***: P<0.001: Compared to control group; +: P<0.05; ++: p<0.01; +++: P<0.001: Compared to EG group; CCog: Cotinus coggygria; EG: Ethylene glycol group; CC+EG+CC: CCog prophylaxis group; EG+CC: CCog treatment group; BUN: Blood urea nitrogen; TNF-α: Tumor necrosis factor-alfa; IL-1β: Interleukin-1β.

Table 3. Biochemical measurements in kidney tissue samples

	Control	EG	CC+EG+CC	EG+CC
MDA (nmol/g)	7.15±0.63	15.98±0.63***	10.10±0.65^{+++}	10.57±1.01*$^{+++}$
8-OhdG (ng/mg DNA)	3.60±0.46	6.62±0.40***	4.50±0.48^{+}	5.05±0.38
MPO Activity (U/g protein)	8.37±0.74	14.97±0.62***	8.18±0.57^{+++}	10.16±0.78^{++}
Caspase-3 Activity (nmolpNA/mg prot)	7.85±0.68	15.38±0.90***	9.77±0.44^{+++}	11.94±0.94**$^{+}$
Osteopontin (ng/g)	1.89±0.18	3.15±0.22**	2.14±0.14^{+}	2.93±0.26**
NAG activity (U/mg protein)	2.30±0.32	4.22±0.38**	2.70±0.20^{++}	3.03±0.23^{+}

*: P<0.05; **: P<0.01; ***: P<0.001: Compared to control group; +: P<0.05; ++: P<0.01; +++: P<0.001: Compared to EG group; CCog: Cotinus coggygria; EG: Ethylene glycol group; CC+EG+CC: CCog prophylaxis group; EG+CC: CCog treatment group; MDA: Malondialdehyde; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; MPO: Myeloperoxidase; NAG: N-acetly-β-glucosaminidase.

coaldehyde, glycolic acid, glyoxylic acid, and oxalic acid. These metabolites are responsible for detrimental effects of EG. In the kidney tissues subjected to these toxic by-products, due to intratubular and medullary crystal deposition, it has been shown that inflammatory process takes place with an end-result of hyperoxaluria with subsequent stone growth and further augmentation of inflammatory process [8].

C. coggygria is a plant known in traditional medicine with anti-inflammatory and antioxidant properties. In the previous animal studies, it has been reported that CCog had anti-inflammatory and anti-oxidative properties against hepatic oxidative stress, neuroinflammation in sepsis-associated encephalopathy, and diabetes mellitus induced inflammatory processes [19–21]. In our study, the effects of CCog plant on the prevention of stone formation and on prevention of kidney injury due to urinary stone disease were investigated in an experimental upper urinary tract stone disease model induced by EG. In the model, we used in our study, similar to previous studies, while hyperoxaluria and hyperuricosuria in the urine were observed in the EG group; urinary concentrations of calcium and citrate decreased [22, 23]. In the CC+EG+CC group, these metabolites were measured at levels similar to the control group values.

EG-induced nephrolithiasis model has been used widely by many authors for evaluation of different anti-oxidant agents. MPO is a heme-peroxidase and is released by neutrophils and monocytes. MPO activities increase in inflammation and oxidative damage [24]. Increased levels of MPO and its pathogenic role have also been demonstrated in different kidney diseases such as pyelonephritis and glomerulonephritis [25]. In Sener et al.'s study [8] on the effects of melatonin on EG-induced nephrolithiasis model, on EG administration, the levels MDA and activities of MPO were reported to be increased and treatment with melatonin reversed the effects of EG and restored these values back to control group values. Malondialdehyde is a marker of lipid peroxidation. In a radia-

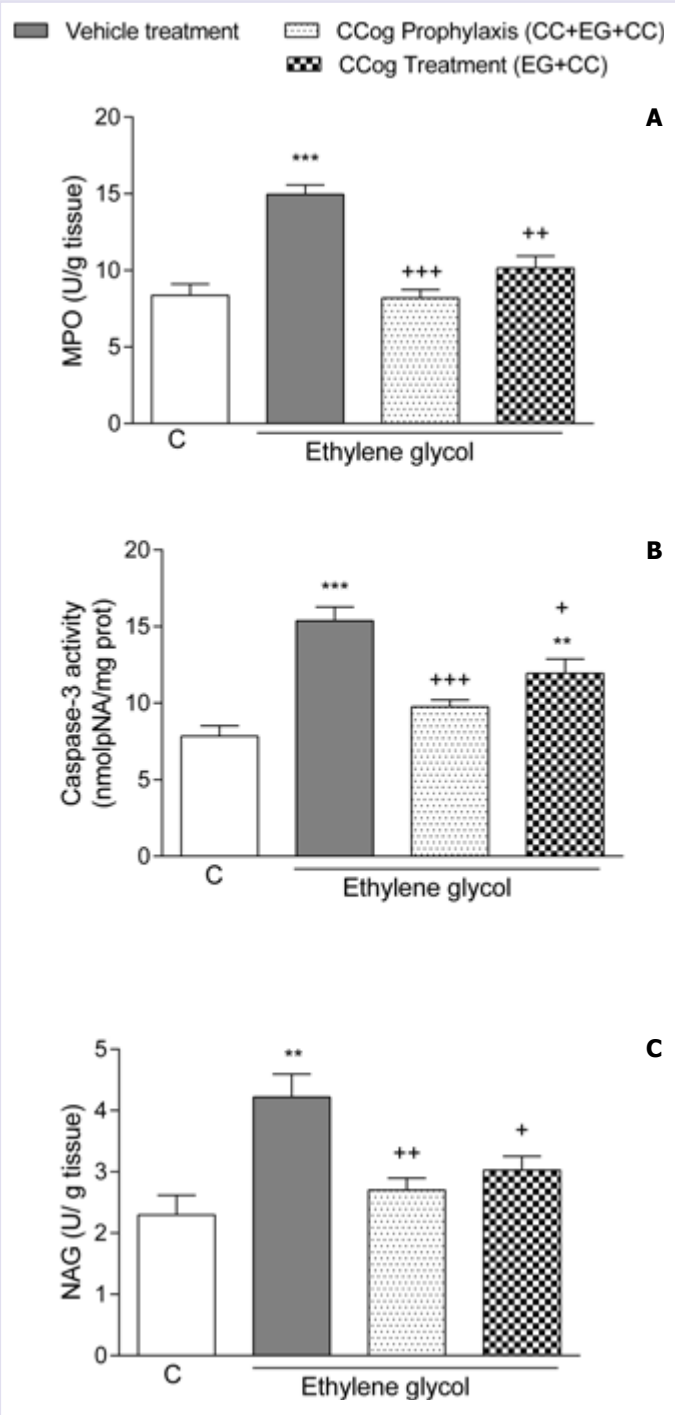

FIGURE 2. **(A)** Measurement of myeloperoxidase (MPO) activity in kidney tissue samples ***p<0.001: Compared to control group. ++p<0.01; +++p<0.001: Compared to EG group **(B)** Measurement of caspase-3 activity in kidney tissue samples **p<0.01: Compared to Control group. +p<0.05; ++p<0.01: Compared to EG group **(C)** Measurement of N-acetyl-β-glucosaminidase activity in kidney tissue samples **p<0.01: Compared to Control group. +p<0.05; ++p<0.01: Compared to EG group.

tion-induced inflammation model, in Ghobadi et al.'s study [26], the authors showed that the inflammatory responses created by scattered radiation were reversed with melatonin as they showed an increase in MDA levels as a response to inflammatory process and a decrease in MDA levels on treatment with melatonin. Similarly, in our study, the increased MDA levels and MPO activities were reversed back to control values in both the CC+EG+CC and EG+CC groups.

Oxidative damage induces apoptosis through mitochondrial damage and increases tubular cell damage. NAG activity, a lysosomal enzyme, can be found in urine at detectable rates as an early indicator of renal tubulointerstitial damage [27]. 8-OHdG is an important indicator of oxidative DNA damage and increases in response to oxidative stress and induces apoptosis. In Taguchi et al's study [28] where a EG-induced stone disease model in rats was used, it was shown that 8-OHdG levels and NAG enzyme activity increased as an indicator of oxidative DNA damage and apoptosis, and pioglitazone reduced these values to control group levels with its antioxidant and anti-inflammatory effects. In another study by Sener et al. [8] using the EG-induced nephrolithiasis model, 8-OHdG levels and NAG activity increased as indicators of oxidative damage and decreased to normal levels in the groups treated with melatonin. Similar to the data provided in these previously mentioned studies, in our study, in EG group, 8-OHdG levels and NAG activity increased and decreased to similar levels to control group in the groups treated with C. *coggygria*.

In our study, caspase-3 activities were evaluated as indicators apoptosis in renal cells for which a significant increase was found in the EG group. It was observed that in the CC+EG+CC group, caspase-3 activity decreased compared to the EG group and reached levels similar to control group. In the EG+CC group, although not significant, there was a decrease in caspase-3 activity.

Osteopontin plays a modulatory role in CaOx crystallization, aggregation, and attachment to tissue in the urine and also attracts monocytes and macrophages to damaged areas [29]. Osteopontin prevents crystallization and also contributes to the elimination of crystals. Due to crystallization and oxidative damage that occurs, an increase in the expression of osteopontin as a mineralization inhibitor is expected [29, 30]. In our study, osteopontin concentrations increased significantly in the EG group, and a significant decrease was observed in CC+EG+CC group compared to EG group.

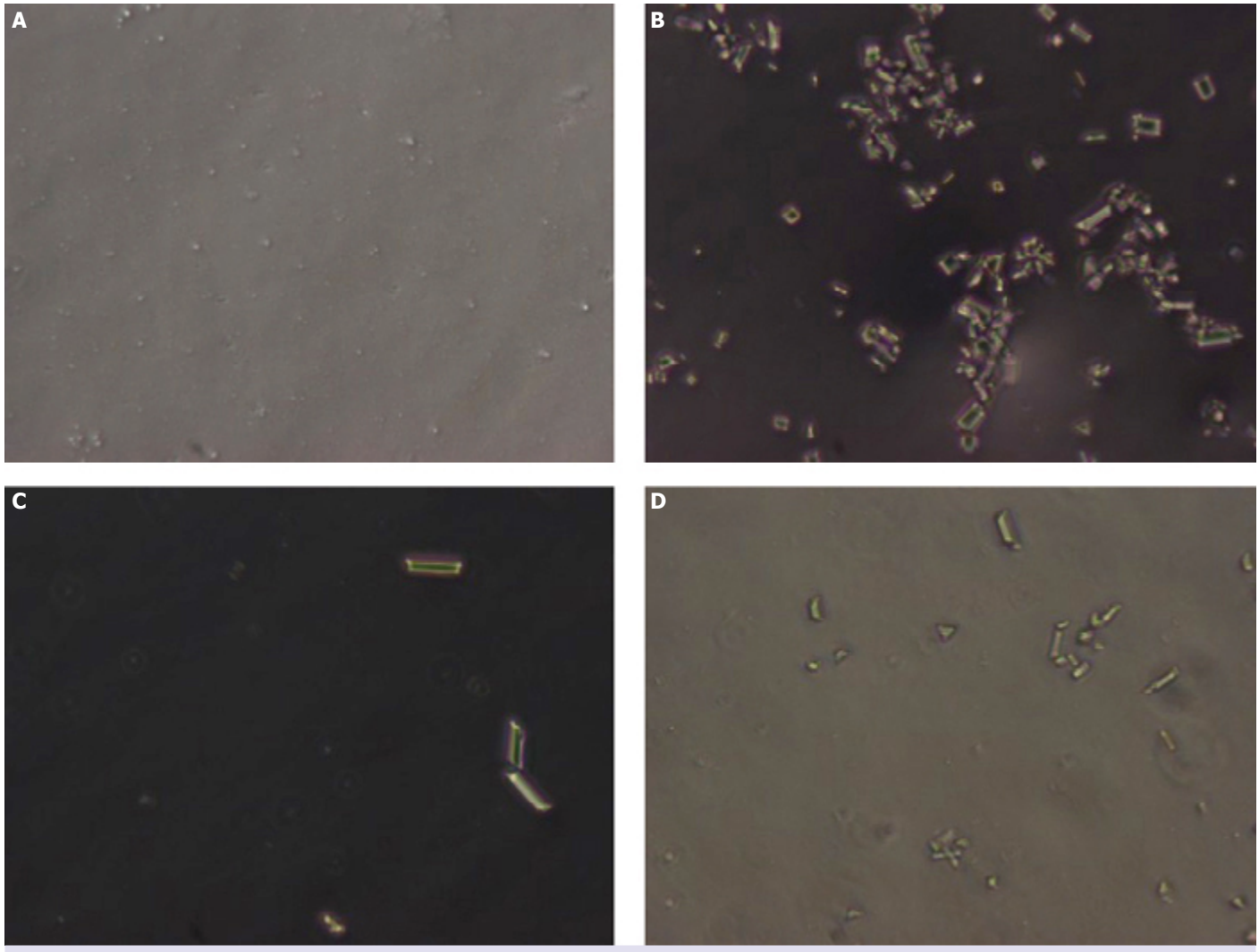

FIGURE 3. Microscopic evaluation in 24-h urine collection samples. **(A)** Control group. **(B)** EG group. **(C)** CC+EG+CC group. **(D)** EG+CC group.

Intrarenal crystals cause inflammation and renal cell necroptosis triggered by stimulation of signaling pathways involving TNF receptors [31]. As a result of caspase activity in the cytoplasm, various enzymatic systems start working to activate cytokines. These enzymatic systems are referred to as "inflammasomes." In the stone model made with hydroxy-1-proline, it has been shown that the levels and activities of NLRP-3 inflammasome, caspase-1, IL-1β, and IL-18 are increased [32]. In our study, it was observed that serum levels of proinflammatory cytokines TNF-α and IL-1β increased in the EG-treated group, and the levels of both cytokines decreased to levels similar to control group in the CC+EG+CC group. However, there was no statistically significant decrease in the EG+CC group.

Nephrolithiasis is a recurrent urological pathology and is one of the leading causes of end-stage renal failure. Furthermore, with recurrent stones, a great financial burden is on the shoulders of the whole burden due to the need for multiple surgeries, emergency hospital admissions, and need for many pharmacological treatments. Due to these issues, there is a relentless search for preventive strategies for stone formation. Apart from metabolic treatments, antioxidant agents have a promising future in preventing stone formation by targeting one of the most important steps in pathogenesis. When the data emerging from different pre-clinical studies about the effectiveness of antioxidant treatments on nephrolithiasis are supported by clinical studies, these agents may be used as supplementary treatments for the prevention and treatment of nephrolithiasis patients in the future.

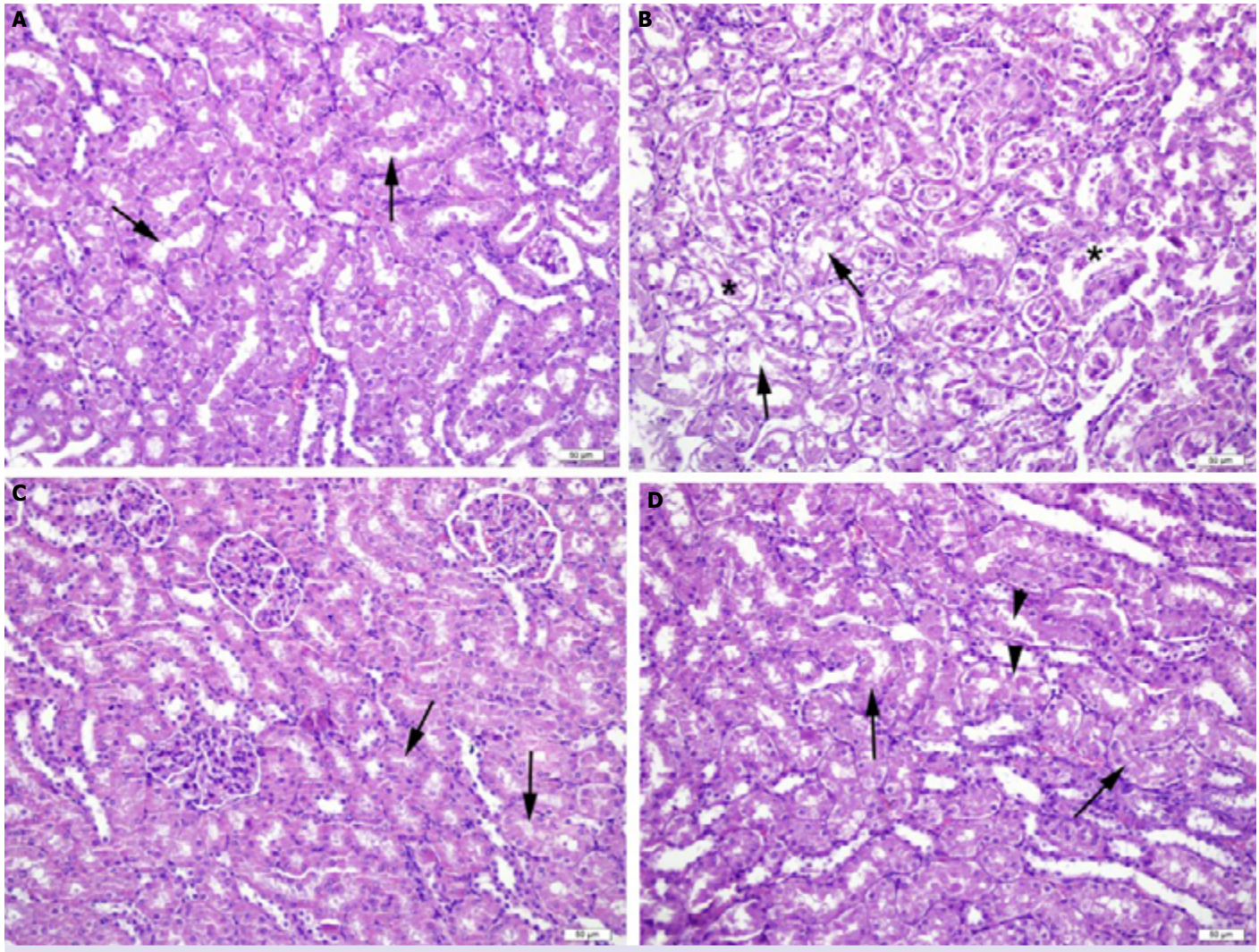

Figure 4. Histopathological evaluation is kidney tissues. **(A)** Control group: The appearance of smooth glomerular and tubular structures (arrows). **(B)** EG group: Thickening of the tubular basement membrane. Massive degeneration of tubular cells with desquamation (arrows) and scattering into the lumen (asterix). **(C)** CC+EG+CC group: Uniform basement membrane structure and decreased tubular cell shedding (arrows). **(D)** EG+CC group: Decreased basement membrane thickening (arrowheads) and dilution of tubular cell shedding (arrows).

Furthermore, *in vitro* antioxidant activity of CC against the DPPH radical was demonstrated in our previous study [33]. In addition, our previous study was found that CC showed a significant *in vitro* anti-inflammatory effect of CC against 5-lipoxygenase enzyme [33]. LC-MS/MS analysis of *C. coggygria* ethanol extract in our previous study showed that the extract contained polyphenolic compounds such as ethyl gallate, ethyl ester of digallic acid, gallic acid, methyl gallate, myricetin, myricetin glucoside, myricetin rhamnoside, protocatechuic acid, quercetin rhamnoside, and quinic acid with pentagalloylglucose being the major compound [33]. The previous studies have shown that pentagalloylglucose had significant potential in the prevention of urolithiasis as well as strong antioxidant and anti-inflammatory activity [34–36]. Therefore, pentagalloylglucose along with other polyphenolic compounds in extract could be responsible for the preventive and curative effects of the extract against urolithiasis.

Conclusion

Urinary system stones are formed in response to increased inflammatory processes, oxidative stress, and insufficient anti-oxidative protective mechanisms.

Oxidative damage accelerates stone formation especially in absence of macromolecular defense mechanisms such as osteopontin in urine supersaturated with various minerals. In our experimental nephrolithiasis model induced by EG in rats, it was determined that CCog prevented inflammation and the damage caused by the oxidative processes in renal tissues and reversed the oxidative and antioxidative parameters to levels similar to control group values. It has been shown that especially preemptive use of CCog supplementation can prevent kidney stone formation in EG-induced nephrolithiasis model in rats. It has also been shown that it may provide a therapeutic effect in rats already subjected to EG, hence with already-induced nephrolithiasis.

With an insight about how ethnopharmacological effects and side effects have been clarified throughout years, it may be suggested that CCog preparations may be used as protective agents against nephrolithiasis especially in high-risk populations. Further, clinical data are needed to create an evidence-based approach to this recurring pathology.

Ethics Committee Approval: The Marmara University Clinical Research Ethics Committee granted approval for this study (date: 14.11.2016, number: 110.2016.mar).

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