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ORIGINAL ARTICLE

Myricetin Attenuates Ethylene Glycol-Induced Nephrolithiasis in Rats via Mitigating Oxidative Stress and Inflammatory Markers

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Abstract

Urolithiasis or nephrolithiasis is a condition of kidney stone formation and is considered a painful disease of the urinary tract system. In this work, we planned to discover the therapeutic roles of myricetin on the ethylene glycol (EG)-induced nephrolithiasis in rats. The experimental rats were treated with 0.75% of EG through drinking water for 4 weeks to initiate the nephrolithiasis and subsequently treated with 25 and 50 mg/kg of myricetin. The body weight and urine volume were measured regularly. After the sacrification of rats, the samples were collected, and serum and urinary biomarkers such as creatinine, urea, Ca2 + ion, and BUN, OPN, oxalate, and citrate levels were determined using assay kits. These biomarkers, the MDA level and CAT, SOD, and GPx activities, were assessed in the kidney tissue homogenates. The IL-6, IL-1β, and TNF-α levels were also quantified using respective kits. The histopathological analysis was done on the kidney tissues. Myricetin treatment did not show major changes in the body weight and kidney weight in the EG-induced rats. The treatment with 25 and 50 mg/kg of myricetin considerably reduced the urea, creatinine, BUN, Ca2 + ion, and oxalate and increased the citrate content in serum and urine samples of EG-induced rats. Further, myricetin depleted the inflammatory cytokines and MDA levels and elevated the CAT, SOD, and GPx activities in the renal tissues. The activities of ALT, AST, ALP, GGT, and LDH were also reduced by the myricetin. Furthermore, the myricetin upheld the histoarchitecture of the kidneys. The outcomes of this investigation propose that myricetin is effective in EG-induced urolithiasis probably because of its antioxidant, anti-inflammatory, and renoprotective activities. In addition, further studies are still required to verify the precise therapeutic mechanism of myricetin.

Keywords Oxalate deposition · Citrate · Interleukin-1β · Myricetin · Ethylene glycol

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Introduction

Urolithiasis is a formation of kidney stones, which has been considered a painful disease of the urinary tract system. It has been reported that nephrolithiasis has a 70–80% of recurrence rate in males and 40–60% in females. It has the occurrence of crystals wherever in the urinary tract including the bladder and kidneys [1]. Urolithiasis has multifactorial pathogenesis, and several factors such as environmental, nutritional, infectious, and metabolic abnormalities play major roles. Urolithiasis is mostly connected with several systemic comorbidities, which mainly affect calcium and lipid metabolism. Among kidney stone patients, calcium-containing stones are predominant, primarily composed of calcium oxalate [2]. According to the chemical composition of stones, the most common types are calcium, magnesium, and uric acid. Furthermore, calcium-containing crystals are responsible for nearly 75% of all kidney stones. A low level of oxalate is essential to induce DNA synthesis and enhance cellular proliferation, whereas high levels of oxalate stimulate cell damage and cell necrosis [3]. The development of calcium oxalate crystals further results in obstruction, infection, and hemorrhage in the urinary tracts [4].

Inflammation and oxidative stress are the vital players in the pathophysiology of urolithiasis. Chronic exposure to oxalate on the renal tubular cells is connected with increased inflammation and oxidative stress by intensified production of inflammatory cytokines and free radicals [5]. Chronic exposure of tubular cells to the oxalate or calcium oxalate leads to damage. The binding of stones to the renal cells induces oxidative stress. The oxalate exposure can also increase ROS production and lipid peroxidation and collapse mitochondrial functions, which eventually stimulates the cell death of kidney epithelial cells [6]. It was reported that the low-antioxidant diet was shown to increase nephrolithiasis by augmenting ROS generation, oxidative stress, and intrarenal oxalate deposition. Furthermore, the antioxidant interventions mitigated oxidative stress and prevented the oxalate deposition [7]. Hence, it is essential to develop a new effective approach with effective antioxidant and anti-inflammatory properties for the successful treatment of nephrolithiasis.

Insufficient water consumption, reduced excretion of urinary citrate, oxidative stress, and inflammation are the major causes of nephrolithiasis. These causative factors should be normalized to prevent stone formation. The major therapeutic approaches for urolithiasis are primarily supportive such as drinking more water and the utilization of anti-inflammatory medications. Though small-sized stones may be released naturally, large-sized stones should be taken out surgically. Additionally, ultrasound shock waves can be applied to break the large-sized stones into small pieces [8]. Nonetheless, the effectiveness of these approaches is poor, and these therapies are reported to possess undesirable aftereffects, such as hemorrhage, tubular necrosis, and renal fibrosis. In recent times, natural-based medications received much interest to evade the side effects of current antiurolithiasis therapies [9].

The experimental animal models play a critical role in examining the therapeutic efficacy of sample drugs for the successful treatment of urolithiasis. Ethylene glycol (EG)-induced urolithiasis in animals is one such widely used experimental model, which mimics the major pathological characteristics of renal stones. It was well reported that the administration of EG in animals develops persistent calcium oxalate crystalluria and reflects a key feature of nephrolithiasis [10]. Myricetin, a 3,3′,4′,5,5′,7-hexahydroxyflavone was isolated from vegetables, herbs, and berries, which are reported to have several biological properties [11]. Myricetin has been renowned to have plentiful biological activities such as antitumor [12], anti-inflammatory, antioxidant, antiseptic [13], cardioprotective

[14], anti-diabetic [15], and antimicrobial [16] properties. Its therapeutic role in non-alcoholic fatty liver disease, ethanol-induced gastric injury [17], and diabetic eye disorders [18] was well reported. However, the beneficial roles of myricetin against urolithiasis were not been systemically studied yet. Therefore, in this work, we planned to discover the therapeutic roles of myricetin on EG-induced nephrolithiasis in rats.

Materials and Methods

Chemicals

Myricetin, EG, and other chemicals were acquired from Sigma-Aldrich, USA. The assay kits for the determination of oxidative stress and inflammatory markers were attained from BioVision and Elabscience, USA.

Experimental Rats

A healthy Wistar albino rats weighing approximately 210 ± 30 g were obtained from the institutional animal house. The rats were housed in well-sanitized polypropylene cabins with a temperature of 22–26 °C, air humidity (50–60%), and 12 h of light/dark sequence. All rats were 1 week acclimatized in the laboratory before conducting the experiments.

Experimental Design and Treatment Protocol

A week acclimatized rats were distributed into four groups of six rats ($n = 6$) as group I–IV. Group I rats were served as normal controls and received only 1 ml of saline. Group II rats were treated with 0.75% of EG through drinking water for 4 consecutive weeks to initiate urolithiasis. Group III and IV rats were administered with EG as specified in group II and then treated with 25 and 50 mg/kg of myricetin, respectively, for 4 consecutive weeks through an oral gavage route. The urine output and body weight of each rat were regularly monitored, and changes were tabulated. After the completion of the works, the rats were sacrificed under anesthesia then blood and renal tissues were taken out and washed with saline solution. Then, kidney tissues were weighed accurately. The blood samples were collected, and serum was prepared for the various biochemical assays.

Quantification of Renal Dysfunction Biomarkers

The serum sample was prepared from the collected blood by centrifuging them at 5000 rpm for 10 min. The prepared serum sample was utilized to assess the renal dysfunction biomarkers such as creatinine, urea, calcium ion (Ca2+), and blood urea nitrogen (BUN) by using the ELISA assay kits. Each assay was done triplicate by using the recommended protocols by the manufacturer (BioVision, Waltham, USA).

Measurement of Urinary Biomarker Levels

The urine samples were collected from the rats and utilized for the measurement of urinary biomarker levels such as oxalate, creatinine, and citrate levels. These biomarkers were

quantified using the respective assay kits. Each assay was performed in triplicates by using the recommended guidelines by the manufacturer (BioVision, Waltham, USA).

Quantification of Osteopontin (OPN) and Superoxide Dismutase (SOD) Activity

The level of OPN and SOD activity in the serum of experimental rats was determined using the assay kits. Each experiment was performed in triplicates using the protocols given by the kit's manufacturer (Elabscience, Houston, USA).

Determination of Oxidative and Antioxidative Biomarkers in the Experimental Rats

The collected renal tissues were homogenized using saline solution and centrifuged at 10,000 rpm for 15 min. The supernatant was utilized to assess the levels of oxidative stress and antioxidant biomarkers such as malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GPx) using the respective assay kits. Each experiment was performed in triplicates by the recommended protocols of the manufacturer (Elabscience, Houston, USA).

Measurement of Pro-inflammatory Cytokines

The interleukin-6, IL-1β, and TNF-α in the kidney tissue homogenates of the experimental rats were determined using the assay kits. The assays were performed using the recommended protocols of the manufacturer (Elabscience, Houston, USA). Each experiment was done in triplicates.

Determination of Renal Function Marker Enzymes

The activities of renal function biomarker enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyl-transferase (GGT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities were measured in both serum and renal tissue homogenates. Each assay was done using the Architect c8000 analyzer.

Histopathological Analysis

The kidney tissues were excised immediately and cleansed with ice-cold saline and then placed in a 10% of formalin solution. Then they were paraffinized using paraffin wax and sliced into pieces at 5μm in diameter. The slides were stained by hematoxylin-eosin and the histological alterations in the kidneys were studied using optical microscopy at 40× magnification.

Statistical Analysis

The data from each assay were statistically analyzed using GraphPad prism software and outcomes were illustrated as a mean±SD of triplicates. The data are analyzed by one-way ANOVA subsequently DMRT assay and statistical significance was set at $p<0.05$.

Results

Effect of Myricetin on the Body Weight, Kidney Weight, and Urine Volume in the Experimental Rats

Figure 1 reveals the effect of myricetin on the body weight, kidney weight, and urine volume in the experimental rats. The outcomes demonstrated no major variances in body weight and kidney weight between the treatment groups. However, the treatment with 25 and 50 mg/kg of myricetin slightly increased the body weight and reduced the kidney weight in the EG-induced rats. The urine level was marginally elevated in the EG-induced rats than in the control. The 25 and 50 mg/kg of myricetin slightly decreased the urine volume in the EG-induced rats (Fig. 1).

Effect of Myricetin on the Creatinine, Urea, BUN, and Ca2+ Ion in the Experimental Rats

The effect of myricetin treatment on the levels of creatinine, urea, BUN, and Ca2+ ion in the serum was evaluated, and the outcomes are shown in Fig. 2. The EG-induced rats revealed a marginal elevation in the creatinine, urea, and BUN in the serum than the control. Also, there were no major changes in the Ca2+ ion level between the treatment groups.

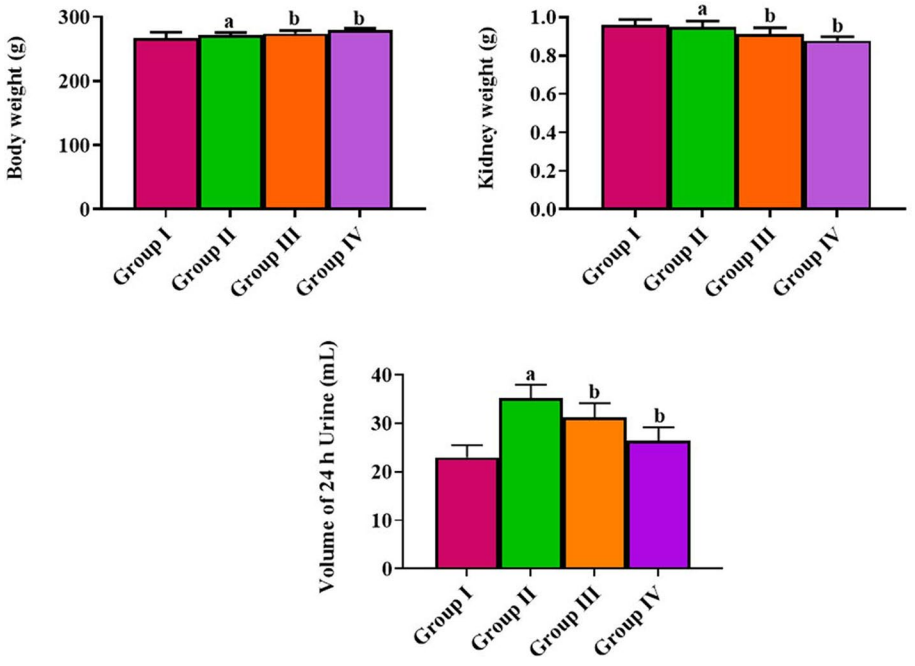

Fig. 1 Effect of myricetin on the body weight, kidney weight, and urine volume in the experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

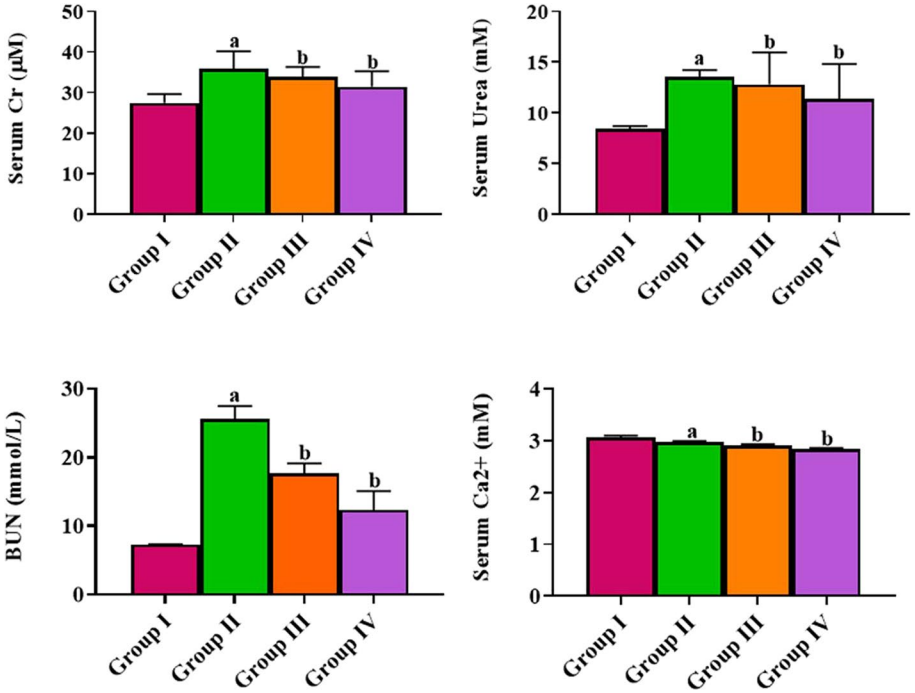

Fig. 2 Effect of myricetin on the creatinine, urea, BUN, and Ca2 + ion in the serum of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

Interestingly, the treatment with 25 and 50 mg/kg of myricetin considerably decreased the creatinine, urea, and BUN in the EG-induced rats (Fig. 2).

Effect of Myricetin on the Oxalate, Creatinine, And Citrate Levels in the Urine of Experimental Rats

The levels of oxalate, creatinine, and citrate in the urine of experimental rats were determined, and the findings are revealed in Fig. 3. The increased level of oxalate and creatinine was observed in the urine of EG-induced rats. The EG rats also revealed a reduction in the citrate level. The 25 and 50 mg/kg of myricetin considerably diminished the oxalate and creatinine levels in the urine of EG-induced rats. The citrate level was considerably elevated by the myricetin treatment in the EG rats (Fig. 3).

Effect of Myricetin on the OPN and SOD Activity in the Serum of Experimental Rats

Figure 4 reveals the effect of myricetin on the OPN level and SOD activity in the serum of experimental rats. The EG-induced rats revealed a drastic reduction in the OPN level and

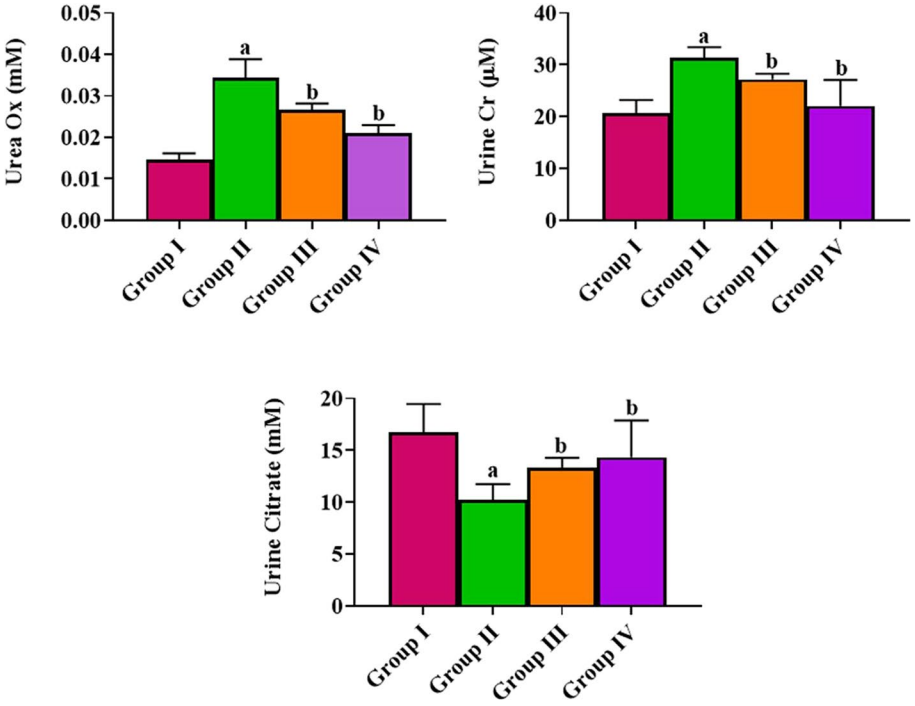

Fig. 3 Effect of myricetin on the oxalate, creatinine, and citrate levels in the urine of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

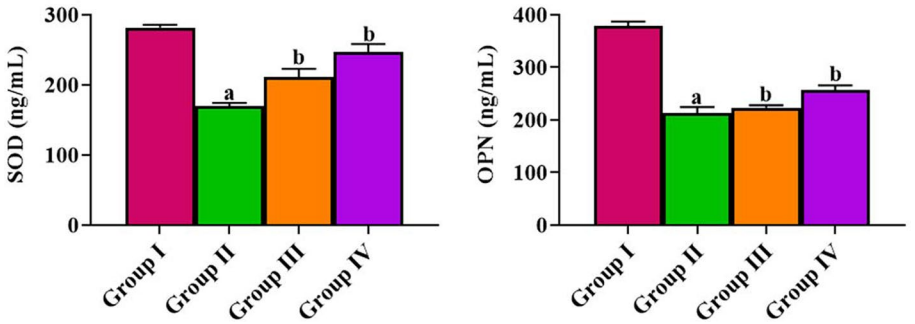

Fig. 4 Effect of myricetin on the OPN and SOD activity in the serum of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

SOD activity in the serum. However, the 25 and 50 mg/kg of myricetin treatment markedly improved the SOD activity and OPN level in the serum of EG-induced rats (Fig. 4).

Effect of Myricetin on the Oxidative and Antioxidative Biomarkers in the Renal Tissues of Experimental Rats

The antioxidant effects of myricetin were assessed by measuring the effect of myricetin on the changes in MDA, SOD, CAT, and GPx in the kidney tissue homogenates. As revealed in Fig. 5, the drastically increased MDA level and decreased CAT, SOD, and GPx activities were noted in the kidney tissue homogenate of EG-induced rats than in the control. Interestingly, the 25 and 50 mg/kg of myricetin substantially diminished the MDA and elevated the GPx, SOD, and CAT in the renal tissue homogenates of EG-induced rats (Fig. 5). These results proved the antioxidant effects of myricetin.

Effect of Myricetin on the Levels of Pro-inflammatory Cytokines in the Kidney Tissues of Experimental Rats

Figure 6 exhibits the effect of myricetin on the changes in the IL-6, IL-1β, and TNF-α in the serum of experimental rats. EG-induced urolithiasis resulted in a severe elevation in the

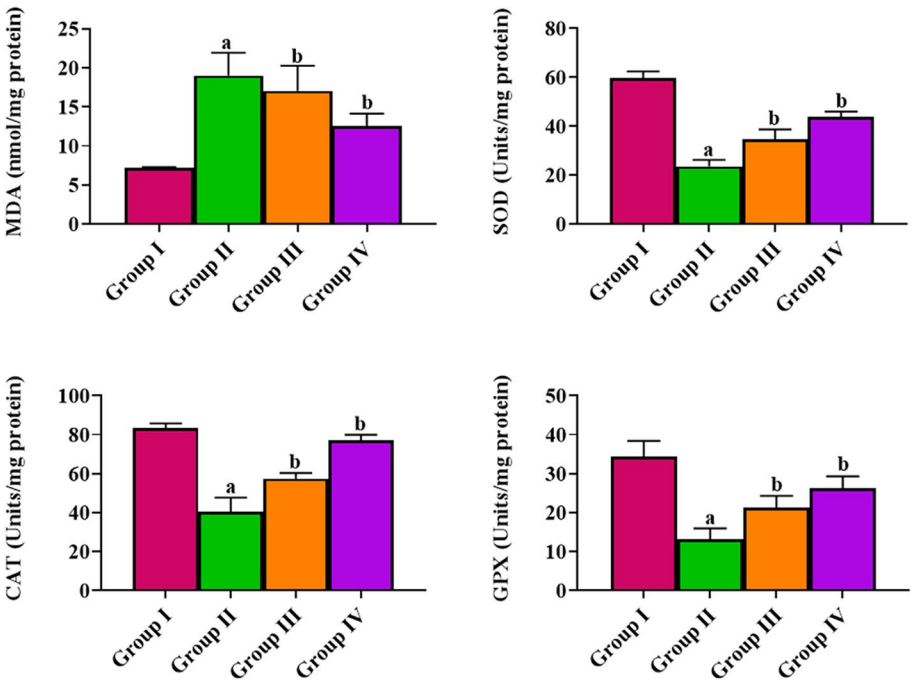

Fig. 5 Effect of myricetin on the oxidative and anti-oxidative biomarkers in the kidney tissues of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

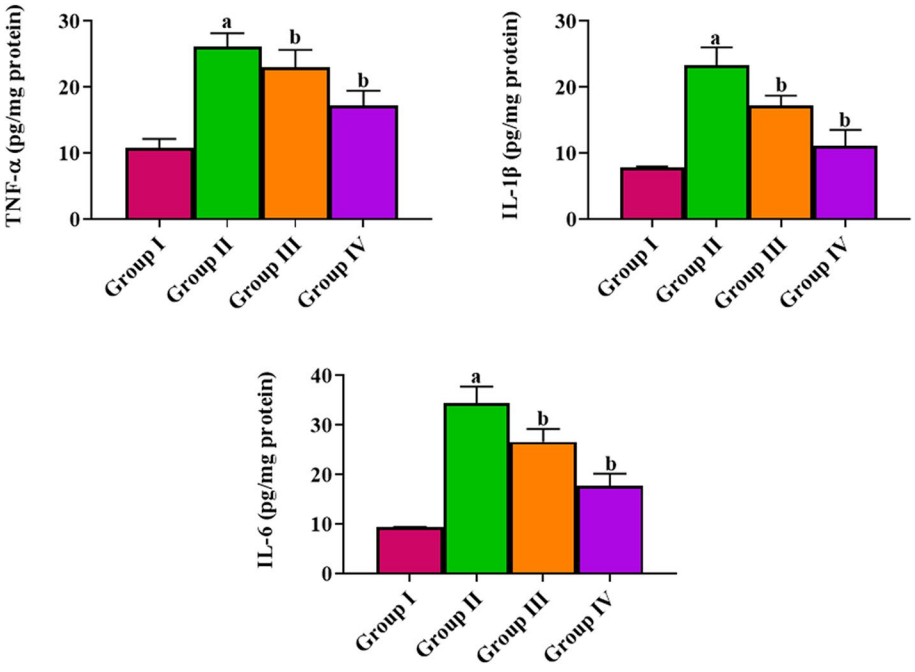

Fig. 6 Effect of myricetin on the levels of pro-inflammatory cytokines in the kidney tissues of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

level of IL-6, IL-1β, and TNF-α in the serum. Whereas, the administration of 25 and 50 mg/kg of myricetin substantially depleted the IL-6, IL-1β, and TNF-α levels in the EG rats (Fig. 6). These results proved the anti-inflammatory properties of myricetin.

Effect of Myricetin on the Renal Function Marker Enzymes in Both Serum and Renal Tissues of Experimental Rats

The effects of myricetin on the ALT, AST, ALP, GGT, and LDH activities were determined, and the findings are presented in Fig. 7. The drastic elevations were observed in the ALT, AST, ALP, GGT, and LDH activities in both serum and renal tissue homogenates of the EG-induced rats. Interestingly, the administration of 25 and 50 mg/kg of myricetin considerably reduced these enzyme activities in both serum and kidney tissue homogenates of EG-induced rats (Fig. 7). These findings revealed that the myricetin ameliorated the renal tissue injury thereby preventing the release of these enzymes into the bloodstream.

Effect of Myricetin on the Renal Histopathology of Experimental Rats

The effect of myricetin on the renal histopathology of the experimental rats was analyzed, and the findings are revealed in Fig. 8. The control rats revealed normal structures, whereas

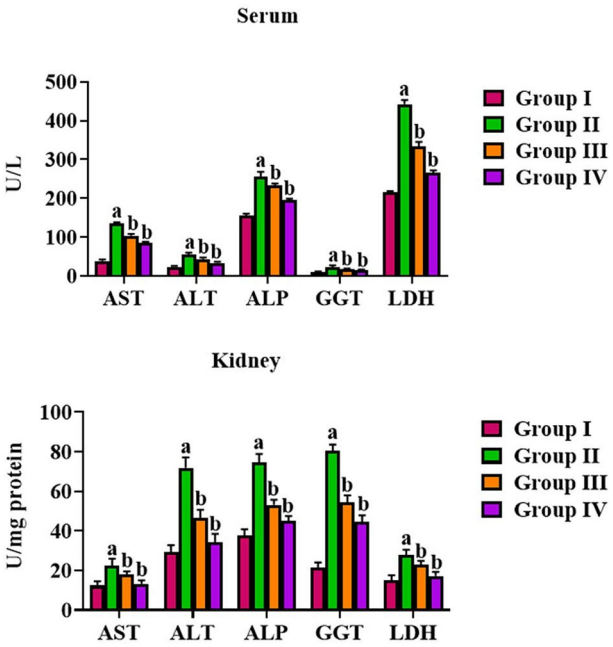

Fig. 7 Effect of myricetin on the activities of renal function marker enzymes in both serum and renal tissues of experimental rats. Each bar reveals the mean ± SD of triplicate values obtained from each assay. All the data were determined statistically by one-way ANOVA and subsequently Dunnett's post hoc assay using the Graphpad Prism software. Note: "a" indicates the statistical significance at $p < 0.01$ from the control, and "b" indicates the statistical significance at $p < 0.05$ from the EG-induced group

the EG-induced rats demonstrated severe histopathological variations in the kidney tissues. The calcium oxalate deposition in the glomerulus, kidney injury, dilation of tubules, matrix expansion, and infiltration of inflammatory cells was noted in the renal tissues of EG rats. Whereas, the 25 and 50 mg/kg of myricetin appreciably reduced the inflammatory response, kidney injury, and crystal deposition in the EG-induced rats (Fig. 8).

Discussion

The incidence and prevalence of urolithiasis are rapidly increasing worldwide each year probably due to the changes in fluid intake, diet, lifestyle, and environment [19]. The pathophysiology of urolithiasis includes nucleation, growth, aggregation, and retention of renal stones. Furthermore, the exact mechanisms of renal stone formation are not fully elucidated yet and thought that inflammation and oxidative stress have been associated with urolithiasis [20]. The outcomes of EG administration were found consistent with the earlier report [21]. The administration of EG through drinking water generated urolithiasis in rats. The oxalate depositions may trigger changes in cellular biochemical mechanisms due to the increased production of free radicals. Oxidative stress in the kidneys is believed to be an imperative cause of renal injury, which further results in several pathological disorders [22]. Furthermore, initiating oxidative stress by EG is believed as a primary source of kidney damage via excessive lipid peroxidation and reducing antioxidant systems [23].

An imbalance between oxidative and anti-oxidative markers further worsens the disease condition. Reduced levels of antioxidants in the kidneys are believed to preserve calcium oxalate in the renal tissues. The decreased antioxidant protective mechanism increases the retention and accumulation of calcium oxalate in the kidneys [24]. Oxidative stress in the kidneys promotes cell membrane damage and inflammatory response.

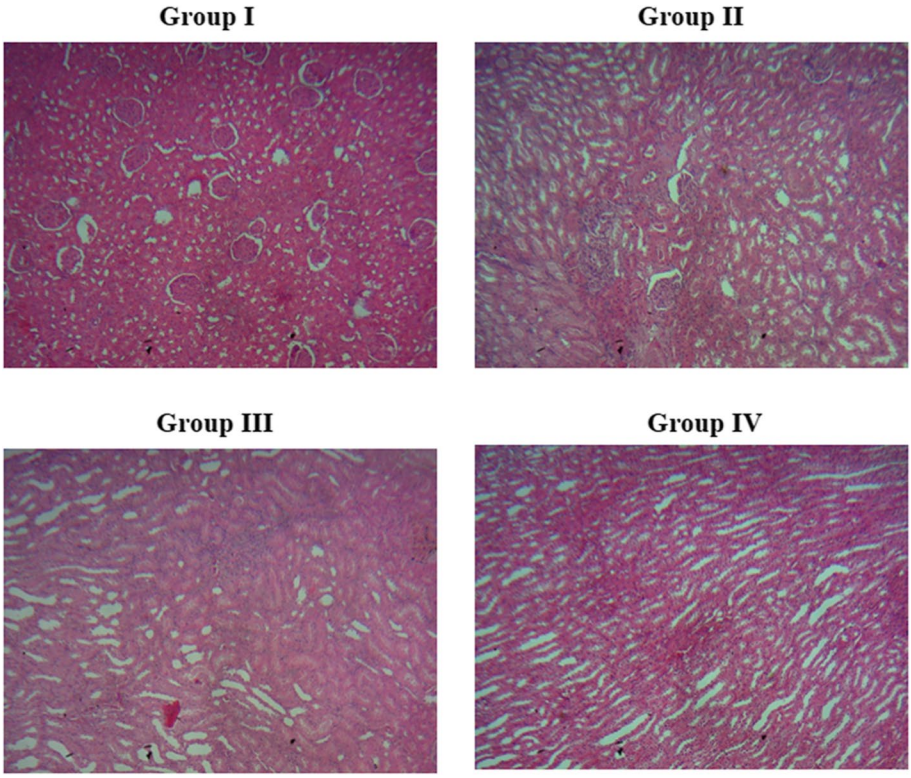

Fig. 8 Effect of myricetin on the renal histopathology of experimental rats. Group I, control rats revealed normal structures; group II, EG-induced rats demonstrated calcium oxalate deposition, renal tissue injury, dilation of tubules, and infiltration of inflammatory cells; group III and IV, the 25 and 50 mg/kg of myricetin treatment appreciably reduced the EG-induced histopathological changes in the kidney tissues

ROS-mediated oxidative stress generates MDA, which is a major indicator of lipid peroxidation. The increased production of ROS facilitates oxidative stress thereby depleting the antioxidant systems [25]. GPx is an imperative intracellular antioxidant, which has the capacity to hunt several free radicals, thereby protecting the cells from oxidative damage [26]. Moreover, SOD offers a first-line antioxidant protective mechanism by decreasing the deleterious effect of superoxide radicals. The reduced SOD and GPx activities disturb the cell redox-homeostasis, which has been reported in kidney failure. Therefore, it was clear that the antioxidants such as GPx, CAT, and SOD have a prominent role in protecting kidney-related diseases as their reduced levels are connected with disturbed intestinal barrier integrity and renal epithelial cell damage [27]. In this work, we observed that the EG-induced rats revealed an elevated MDA and decreased SOD, CAT, and GPx in the renal tissues. Interestingly, the treatment with myricetin considerably depleted the MDA and elevated the SOD, CAT, and GPx in the kidney tissue homogenate in the EG-induced rats. These findings witnessed the antioxidant potential of myricetin.

OPN is an imperative stone matrix protein, which is an inhibitor of abnormal lithogenesis in the kidneys. OPN is expressed in several tissues and is participated in numerous pathological mechanisms like cell signaling, biomineralization, and inflammation.

OPN is likely produced to prevent crystal formation and protect the renal tissues [28]. OPN is an inhibitor of nucleation and aggregation of stones, which is also a chemoattractant that participated in fibrosis and inflammation. It was also reported that drug candidates, which are effective against urolithiasis, should regulate the expression of OPN [29]. Here, we observed that the EG-induced rats showed a decrease in OPN level, whereas the treatment with myricetin effectively increased the OPN level in the serum of EG-induced rats. Hence, it was clear that the myricetin was effective against urolithiasis and promotes renal function.

The increased inflammatory response in kidneys results in renal damage and facilitates other renal failure complications. EG is the most suitable chemical agent to induce urolithiasis in experimental rats. The natural defense mechanisms of rats respond to the EG insult and increase the inflammatory condition, which is escorted by the activation of inflammatory cells and increased production of pro-inflammatory cytokines. TNF-α is a vital pro-inflammatory cytokine, which induces the mononuclear phagocytes to generate other cytokines including IL-6 and IL-1β by macrophages [30]. Mulay et al. [31] have found that calcium oxalate-induced nephropathy is tightly connected with an inflammatory response due to the elevated IL-6, IL-1β, and TNF-α production, which results in renal damage. These inflammatory cytokines could stimulate the generation of other inflammatory regulators and further worsen the inflammatory reactions [32]. Here, our findings evidenced that the EG-induced rats showed a severe increment in the IL-6, IL-1β, and TNF-α in the kidney tissues. Whereas, the treatment with the myricetin appreciably reduced the contents of IL-6, IL-1β, and TNF-α in the renal tissues of EG-induced rats. These findings witnessed the anti-inflammatory effect of myricetin.

The analysis of urine biochemistry is a reliable indicator of renal crystal development and offers a clear understanding of the minerals and biomarkers connected with stone development. A reduction in urine output will decrease the excretion of oxalic acid, which encourages the retention of oxalate in the kidneys and stone development. A reduction in kidney weight or damaged kidney generally stimulates structural and functional hypertrophy. It eventually causes urine supersaturation, which leads to urolithogenesis [33]. Urolithiasis results in increased concentrations of calcium, oxalate, and phosphates and considerable reduction in the membrane integrity because of the excretion of these ions. It has been said that the administration of EG results in calcium oxalate crystalluria in the kidney tissues of rats [34]. It is reported that EG is absorbed by the intestine and underwent metabolism by the liver to generate a high oxalate. In hyperoxaluric conditions, the increased oxalate content obstructs the renal tubules by affecting the epithelial cells [35]. It is necessary to protect the renal cells from injury induced by calcium oxalate. Oxalate can stimulate the renal tubular cells to produce higher free radicals, which results in oxidative stress and inflammation [36]. In this work, we observed that the EG-induced rats demonstrated increased oxalate and Ca2+ ions. Interestingly, the administration of myricetin considerably decreased the oxalate and Ca2+ ions in the EG-induced rats. These findings suggested that myricetin is effective in improving renal function.

Hypocitraturia is an imperative metabolic defect often observed during urolithiasis. The citrate demonstrates an inhibitory effect on kidney stone formation and particularly against calcium oxalate development. Citrate interacts with calcium and develops its complex, thereby decreasing the calcium oxalate level to inhibit the calcium oxalate agglomerate development [37]. Here, we also noted the decreased level of citrate in the urine of EG-induced rats. Nonetheless, the myricetin treatment effectively promoted the citrate level in the EG-induced rats.

The calcium oxalate crystals obstruct the urination process and reduce the glomerular filtration rate. In urolithiasis, the obstructed urine outflow results in the buildup of waste materials in the blood, majorly the non-protein nitrogenous substances including urea, creatinine, and BUN, which is a common hallmark of renal damage and dysfunction [38]. Consequently, the creatinine and BUN were elevated drastically in the EG-administered rats, which denotes the defective renal functions [39]. Similarly, we noted that EG-induced urolithiasis in rats produced renal dysfunction as established by the increased urea, creatinine, and BUN levels. Whereas, the myricetin administration appreciably reduced the urea, creatinine, and BUN in the serum of EG rats. These outcomes witnessed that the myricetin promoted kidney functions in the EG-induced rats.

It was reported that the EG insult increases the activities of renal function marker enzymes such as ALT, AST, ALP, GGT, and LDH in the serum. These increments in the serum are due to the damaged renal tissues, which release these enzymes into the bloodstream [40]. Here, we also observed that the serum activities of AST, ALT, ALP, GGT, and LDH were drastically elevated. This is due to the injured structural integrity of the renal cells that release these enzymes into circulation. The membrane damage of organelles such as mitochondria also releases these enzymes. Our findings also exhibited that myricetin effectively decreased the activities of AST, ALT, ALP, GGT, and LDH in both serum and renal tissues of EG-induced rats. These findings proved that myricetin ameliorated renal tissue injury in the EG rats.

The EG causes histopathological alterations including renal epithelial tissue damage and dilation of tubules. The increased renal epithelial cell injury attracts the inflammatory cells and eventually increases the inflammatory response in the kidneys. Inflammation increases the collagen deposition and transformation of epithelium, which facilitates the biomineralization mechanisms [41]. The outcomes of the histopathological analysis revealed that the myricetin substantially attenuated the histopathological changes in the kidney tissues of EG-induced rats. These outcomes confirmed the beneficial properties of myricetin on EG-induced urolithiasis in rats.

Conclusion

In conclusion, the present findings suggest that myricetin is effective in EG-induced urolithiasis and considerably attenuated renal toxicity. Myricetin treatment exhibited ameliorative effects against EG-induced nephrolithiasis in rats by decreasing urea, creatinine, BUN, Ca2+ ion, and oxalate in serum and urine. The myricetin also reduced the IL-6, IL-1β, and TNF-α and elevated the CAT, SOD, and GPx activities. The activities of ALT, AST, ALP, GGT, and LDH were also reduced by the myricetin. Furthermore, the myricetin administration also decreased the EG-induced kidney toxicity by upholding the histoarchitecture of the kidneys. The fundamental protective mechanisms of myricetin may be regulated by its antioxidant, anti-inflammatory, and renoprotective properties. In addition, further investigation is still needed to verify the precise therapeutic mechanism of myricetin.

Author Contribution The authors contributed equally.

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Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics Approval All work has been done under the guidelines of the Institutional Ethics Committee.

Consent to Participate All authors have their consent to participate.

Consent for Publication All authors have their consent to publish their work.

Competing Interests The authors declare no competing interests.

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