Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Apigenin attenuated ethylene glycol induced urolithiasis in uninephrectomized hypertensive rats: A possible role of bikunin, BMP-2/4, and osteopontin

Ke Zhang, Amit Kandhare[1], Anwesha Mukherjee-Kandhare[1], Subhash L. Bodhankar[1]

Department of Urology, The First People's Hospital of Yunnan Province, Kunming, Yunnan, China, [1]Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India

Submitted: 04-Mar-2020 Revised: 28-Mar-2020 Accepted: 03-Jun-2020 Published: 20-Oct-2020

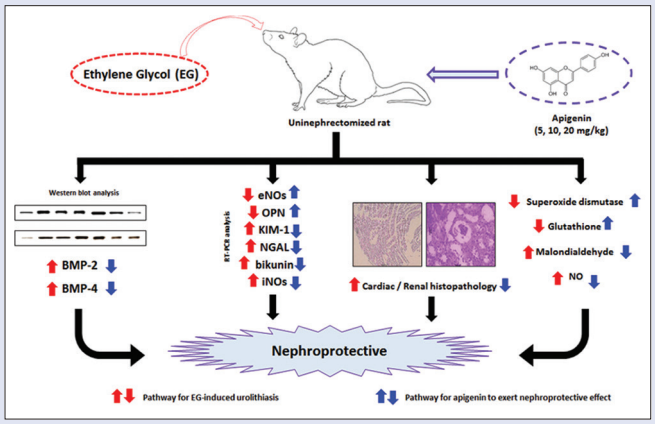
ABSTRACT

Background: Nephrolithiasis is a major prevalent health problem of the urinary system worldwide. Apigenin, a plant flavonoid, reported having nephroprotective and antihypertensive properties. **Aim:** To determine the protective effect of apigenin in experimental ethylene glycol (EG)-induced urolithiasis in uninephrectomized hypertensive animals. **Materials and Methods:** EG was administration in uninephrectomized rats to induce urolithiasis. Then, the rats were administered with apigenin (5, 10, and 20 mg/kg, p.o.) for 28 days followed by the evaluation of behavioral, biochemical, and histological parameters. **Results:** Chronic administration of EG induces alterations in the serum and urine parameters (urine output, pH, urine density and dry weight, urea nitrogen, creatinine, uric acid, calcium, sodium, citrate, albumin, glycosaminoglycans, and lactate dehydrogenase), which was significantly inhibited by apigenin treatment. Apigenin treatment attenuated EG-induced elevated cardiac and renal oxido-nitrosative stress. RT-PCR analysis revealed that apigenin inhibited EG-induced modulations in neutrophil gelatinase-associated lipocalin (NGAL), bikunin, kidney injury molecule-1 (KIM-1), inducible nitric oxide synthase (iNOs), endothelial nitric oxide synthase (eNOs), and osteopontin (OPN) mRNA expressions in the kidney. Western blot analysis showed that elevated levels of renal bone morphogenetic protein (BMP)-2/4 proteins were decreased by apigenin treatment. It also significantly inhibited cardiac and renal histological aberrations. **Conclusion:** Apigenin treatment attenuated EG-induced urolithiasis via the modulation of elevated oxidative stress and altered expressions of KIM-1, NGAL, OPN, bikunin, eNOs, iNOs, BMP-2, and BMP-4 in uninephrectomized hypertensive rats.

Key words: Apigenin, bikunin, bone morphogenetic protein-2, endothelial nitric oxide synthase, ethylene glycol-induced urolithiasis, inducible nitric oxide synthase, kidney stone, osteopontin

SUMMARY

- In the present investigation, the efficacy of apigenin was determined against ethylene glycol (EG)-induced urolithiasis in uninephrectomized animals. Apigenin treatment significantly attenuated EG-induced alterations in hemodynamic and electrocardiographic parameters; urinary and serum (blood urea nitrogen, creatinine, uric acid, calcium, sodium, lactate dehydrogenase, urinary citrate, albumin, and glycosaminoglycans levels) biochemistry; oxido-nitrosative stress in the renal and cardiac tissue; altered mRNA expressions of renal neutrophil gelatinase-associated lipocalin, osteopontin, kidney injury molecule-1, bikunin, inducible nitric oxide synthase, and endothelial nitric oxide synthase; elevated protein expression of bone morphogenetic protein (BMP)-2 and BMP-4, as well as cardio-renal histological aberrations. Thus, apigenin can be considered as an essential therapeutic moiety for the management of urolithiasis in the renal hypertensive patient.



Abbreviations used: ANOVA: Analysis of variance; AP: Apigenin; BMPs: Bone morphogenetic proteins; BPM: Beats per minute; BUN: Blood urea nitrogen; C: Cystone; CaOx: Calcium oxalate; DBP: Diastolic blood pressure; EG: Ethylene glycol; eNOs: Endothelial nitric oxide synthase; GAGs: Glycosaminoglycans; GSH: Reduced glutathione; HR: Heart rate; iNOs: Inducible nitric oxide synthase; KIM-1: Kidney injury molecule-1; LDH: Lactate dehydrogenase; MABP: Mean arterial blood pressure; MDA: Malondialdehyde; NGAL: Neutrophil gelatinase-associated lipocalin, NO: Nitric oxide; OPN: Osteopontin; RT-PCR: Reverse transcription polymerase chain reaction; ROS: Reactive oxygen species; SBP: Systolic blood pressure; SOD: Superoxide dismutase; T: Telmisartan; TNF-α: Tumor necrosis factor-alpha; UC: Urolithiasis control.

Correspondence:

Dr. Subhash L. Bodhankar,
Department of Pharmacology,
Poona College of Pharmacy,
Bharati Vidyapeeth Deemed University,
Erandwane, Paud Road, Pune - 411 038,
Maharashtra, India.
E-mail: drslbodh@gmail.com
DOI: 10.4103/pm.pm_83_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Urolithiasis or kidney stone is the third most predominant disorder of the urogenital system, which needs serious medical attention.[1] Imbalance in the concentration of various macromolecules such as citrate, magnesium, calcium, phosphate, oxalate, and uric acid in the urine leads to the formation of kidney stones. Most of these urinary calculi are arise from either crystal of calcium oxalate (CaOx) or calcium phosphate,

Cite this article as: Zhang K, Kandhare A, Mukherjee-Kandhare A, Bodhankar SL. Apigenin attenuated ethylene glycol induced urolithiasis in uninephrectomized hypertensive rats: A possible role of bikunin, BMP-2/4, and osteopontin. Phcog Mag 2020;16:455-63.

which leads to obstruction, hemorrhage, and infection in the urinary tract system.[2,3] Studies suggested that the crystallization of CaOx in the proximal tubule via intrinsic absorption of oxalate induces toxicity in the renal epithelial cells.[4] Thus, putative inhibition of CaOx or calcium phosphate crystallization has been well documented for the management of nephrolithiasis.

Epidemiological studies documented that genetic factors, altered lifestyles, as well as climate-related changes contributed to a decrease in the onset age and an increase in the prevalence of urolithiasis.[5] If urolithiasis remains untreated, the recurrence rate occurs 10% during the 1st year, which increases to 33% for the next 5 years and reaches 50% by the end of 10 years.[6] Thus, various long-term treatment strategies, including alkali-citrate (K-citrate) and thiazide diuretics, have been attempted to prevent the recurrence of urolithiasis.[7] However, these treatment regimens are less efficacious and provide symptomatic relief in the fraction of patients. Furthermore, removal of urinary calculi by implementing surgical procedures, including lithotripsy and laser of high magnitude, has been used extensively.[7] Nerveless, a significant economic burden and a high recurrence of calculi make these procedures less convincing for the management of urinary calculi.

The relationship between oxidative stress and urolithiasis was previously described by an array of researchers.[1,3] An array of inflammatory cells, including eosinophils and other leukocytes, has an ability to generate reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide radical (O_2), and hydroxyl radical (OH).[8] This elevated the production of ROS in the renal tissue, ultimately resulting in thwart of responsible mechanisms for the consequent phases of oxidative stress during hyperoxaluria and CaOx urolithiasis.[9,10] Thus, investigators have implicated a number of antioxidants with an ability to inhibit stress associated with elevated ROS in the renal tissue via either its scavenging potential or its antioxidant defense mechanism.[2,9,10] Currently, several antioxidant moieties from herbal origin bearing inhibitory potential against CaOx crystal formation have been utilized in the treatment of urolithiasis.[9,10] Various animal models perform a significant part in the evaluation of such alternate beneficial options for the management of nephrolithiasis. Ethylene glycol (EG)-induced urolithiasis is one such animal model that is well established, reproducible, and widely used that mimics key clinicopathological features of kidney stones.[9] However, recent clinical studies demonstrated a significant development of hypertension in some uninephrectomized subjects, along with compromised survival rates.[11] Thus, the administration of EG in uninephrectomized rats develops persistent CaOx crystalluria, along with hypertension within a couple of weeks and reflecting a characteristic of uninephrectomized subjects.[9]

Flavonoids are the naturally-occurring polyphenols, which are the secondary metabolites produced by plants, and they have been well documented for its wide range of pharmacological properties. Apigenin (4',5,7-trihydroxyflavone) is one of such flavonoids which is abundant in vegetables (parsley, celery, and onions), fruits (oranges, grapes, and apples), and plant-based beverages (chamomile tea, beer, and red wine).[12] Apigenin has been well documented for its antihyperglycemic, anti-inflammatory, antiapoptotic, anti-allergic, antihypertensive, antidepressant, antianxiety, antiarthritic, antiviral, and antioxidant potential.[13] The pharmacokinetic study of apigenin suggested its well distribution in the kidney and liver tissue after a single oral administration.[14] Apigenin was shown to suppress p53 accumulation via the modulation of ATM signaling in renal cell carcinoma.[15] The researcher explained that apigenin improves the transcription level of renal angiotensin-converting enzyme-2 mRNA and thus lowers blood pressure in spontaneously hypertensive rats.[16] Clinical studies with apigenin supplementation suggest its beneficial potential against osteoarthritis as well as neuronal disorders, including amnesia, anxiety, depression, and Alzheimer's disease.[13] However, its potential against urolithiasis has not been carried out yet. Thus, the current investigation was undertaken to determine the potential of apigenin in experimental EG-induced urolithiasis in uninephrectomized hypertensive rats.

MATERIALS AND METHODS

Animals

Adult male rats (Sprague–Dawley, 180–220 g) were kept under standard housing conditions (24°C ± 1°C [temperature], 45%–55% [relative humidity], 12:12 h [dark/light cycle], and standard pellet chow food and filtered water [*ad libitum*]) throughout the experimental protocol. Institutional Animal Ethics Committee of Poona College of Pharmacy approved all the experiment-related protocol.

Ethylene glycol-induced urolithiasis

Uninephrectomy was induced in anesthetized rats after removal of the left kidney as described previously.[9] Then, randomly divided rats (n = 8 in each group) were treated with vehicle (10 mg/kg, p.o., distilled water) or cystone (0.5 mg/kg) or telmisartan (0.01 mg/kg) or apigenin (5, 10, and 20 mg/kg, Sigma Chemical Co., USA) for the next 28 days. Urolithiasis was induced in these rats (except normal) by the administration of EG-induced (0.75% in drinking water). Metabolic cages (Tecniplast, Italy) were utilized to determine the daily intake of water and food as well as output of urine.

The systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), mean arterial blood pressure (MABP), left ventricular end-diastolic pressure, and ventricular contractility assessment (dp/dt) were measured using an acquisition data system (AD Instruments Pvt. Ltd. with software LabChart 7.3; AD Instrument Pvt. Ltd).[17] Serum and urine samples were assayed for uric acid, blood urea nitrogen (BUN), sodium, creatinine, lactate dehydrogenase (LDH), citrate, albumin, calcium, and glycosaminoglycans (GAGs) using commercially available reagent kits (Accurex Biomedical Pvt. Ltd., Mumbai, India). The level of endogenous antioxidant enzymes (reduced glutathione [GSH], superoxide dismutase [SOD], and malondialdehyde [MDA]) and nitric oxide (NO) in the kidney and heart was estimated.[18] However, the renal mRNA expressions of neutrophil gelatinase-associated lipocalin (NGAL), bikunin, kidney injury molecule-1 (KIM-1), endothelial nitric oxide synthase (eNOs), osteopontin (OPN), and inducible nitric oxide synthase (iNOs) were analyzed using quantitative Reverse transcription polymerase chain reaction (qRT-PCR) following the manufacturer's instructions [Supplementary Table 1].[18] Western blot assay for bone morphogenetic protein (BMP)-2 and BMP-4 was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the previously reported method.[18] The histopathological studies of the kidney and heart was carried out using hematoxylin and eosin stain and evaluated at ×40 magnification. GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) was used to perform data analysis. Data are expressed as mean ± standard error mean (SEM) and analyzed using one-way analysis of variance followed by Tukey's multiple range *post hoc* analysis for parametric tests. A $P < 0.05$ was considered to be statistically significant.

RESULTS

Relative organ weight, water intake, and urinary parameters

There was a significant decrease ($P < 0.05$) in water intake, urine output, urinary pH, and body weight, whereas urine density, urine dry weight, and relative weights of the heart and kidney were increased significantly

Table 1: Effect of apigenin on EG-induced alterations in body weight, water intake, urine output, urine density, Dry weight of urine, urine pH and relative organ weight in uninephrectomized rats

Treatment	Body weight (g)	Water intake (ml)	Urine output (ml)	Urine density (g/ml)	Dry weight of urine (mg)	Urine pH	Heart/body weight	Kidney/body weight
N	243.50±3.58	10.33±0.56	48.00±3.01	0.69±0.03	7.37±0.33	7.12±0.51	0.0029±0.0002	0.495±0.012
UC	198.00±4.01$^{\#}$	5.00±0.63$^{\#}$	34.50±3.66$^{\#}$	0.99±0.04$^{\#}$	21.86±0.42$^{\#}$	4.03±0.31$^{\#}$	0.0059±0.0002$^{\#}$	0.756±0.016$^{\#}$
T (10)	235.90±4.12$^{*\$}$	9.67±0.71$^{*\$}$	46.00±3.29$^{*\$}$	0.76±0.02$^{*\$}$	9.55±0.59$^{*\$}$	6.17±0.29$^{*\$}$	0.0033±0.0002$^{*\$}$	0.589±0.018$^{*\$}$
C (500)	241.80±2.99$^{*\$}$	8.83±0.48$^{*\$}$	47.17±2.65$^{*\$}$	0.77±0.02$^{*\$}$	9.78±0.39$^{*\$}$	6.52±0.56$^{*\$}$	0.0044±0.0002$^{*\$}$	0.527±0.015$^{*\$}$
AP (5)	210.40±3.88	6.83±0.65	37.83±2.39	0.94±0.03	19.92±0.62	4.28±0.43	0.0058±0.0002	0.702±0.023
AP (10)	217.90±3.92$^{*\$}$	7.33±0.71$^{*\$}$	40.67±3.11$^{*\$}$	0.80±0.03$^{*\$}$	14.47±0.63$^{*\$}$	5.60±0.47$^{*\$}$	0.0043±0.0002$^{*\$}$	0.621±0.011$^{*\$}$
AP (20)	229.20±3.17$^{*\$}$	8.33±0.42$^{*\$}$	49.50±1.81$^{*\$}$	0.79±0.02$^{*\$}$	12.40±0.43$^{*\$}$	6.43±0.53$^{*\$}$	0.0033±0.0001$^{*\$}$	0.557±0.014$^{*\$}$

Results are represented as mean±SEM (n=6) and analyzed by One-way ANOVA followed by Tukey's multiple range test. $^{*}P$<0.05 as compared to UC group, $^{\#}P$<0.05 as compared to normal animals and $^{\$}P$<0.05 as compared to one another group.

Table 2: Effects of apigenin on EG-induced alterations in hemodynamic and electrocardiographic abnormalities in uninephrectomized rats

Treatment	Systolic BP (mmHg)	Diastolic BP (mmHg)	MABP (mmHg)	Heart rate (BPM)	QRS interval (ms)	QTc interval	ST height
N	104.40±2.28	71.03±2.42	79.33±2.30	359.60±5.64	12.68±0.50	113.20±2.42	1.99±0.30
UC	139.50±4.64$^{\#}$	84.99±2.76$^{\#}$	98.87±1.83$^{\#}$	414.30±6.92$^{\#}$	17.59±0.50$^{\#}$	135.70±2.76$^{\#}$	6.11±0.23$^{\#}$
T (0.01)	106.40±4.18$^{*\$}$	74.99±2.89$^{*\$}$	81.34±2.57$^{*\$}$	357.50±6.29$^{*\$}$	14.77±0.51$^{*\$}$	115.70±1.76$^{*\$}$	3.12±0.19$^{*\$}$
Cys (0.5)	118.30±4.32$^{*\$}$	79.50±3.61$^{*\$}$	92.53±2.18$^{*\$}$	399.00±5.88$^{*\$}$	15.48±0.56$^{*\$}$	135.70±2.32$^{*\$}$	3.84±0.25$^{*\$}$
AP (5)	131.10±3.25	81.92±2.11	86.59±1.17	410.30±6.57	16.73±0.51	134.70±2.41	6.28±0.27
AP (10)	122.30±4.49$^{*\$}$	75.84±1.60$^{*\$}$	89.01±2.75$^{*\$}$	384.10±5.99$^{*\$}$	14.98±0.48$^{*\$}$	120.10±2.71$^{*\$}$	5.05±0.24$^{*\$}$
AP (20)	114.50±3.22$^{*\$}$	79.31±2.17$^{*\$}$	82.65±1.35$^{*\$}$	376.40±6.02$^{*\$}$	14.86±0.53$^{*\$}$	120.10±2.71$^{*\$}$	4.71±0.42$^{*\$}$

Results are represented as mean±SEM (n=6) and analyzed by One-way ANOVA followed by Tukey's multiple range test. $^{*}P$<0.05 as compared to UC group, $^{\#}P$<0.05 as compared to normal animals and $^{\$}p$<0.05 as compared to one another group. N: Normal; UC: Urolithiasis Control; T: Telmisartan; C: Cystone; AP: Apigenin; BPM: Beats Per minute; BP: Blood Pressure.

Table 3: Effect of apigenin on EG-induced alterations in serum and urine biochemical parameters in uninephrectomized rats

Treatment		Urea nitrogen (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)	Sodium (mg/g)	Calcium (mg/g)	LDH (mg/dL)	Citrate (mg/g)	Albumin (g/dL)	GAGs (mg/g of creatinine)
N	Serum	2.83±0.61	0.84±0.14	0.20±0.03	143.76±2.80	16.19±1.00	1319±56.26	3.35±0.31	2.40±0.24	9.76±0.44
	Urine	11.22±2.12	1.53±0.12	9.15±1.30	389.47±6.46	20.68±0.46	---	---	---	---
UC	Serum	19.65±0.46$^{\#}$	4.33±0.11$^{\#}$	0.62±0.03$^{\#}$	171.43±3.48$^{\#}$	31.75±0.65$^{\#}$	2208±46.41$^{\#}$	1.77±0.33$^{\#}$	0.61±0.20$^{\#}$	2.73±0.69$^{\#}$
	Urine	57.95±1.88$^{\#}$	4.06±0.11$^{\#}$	44.06±1.36$^{\#}$	308.67±7.40$^{\#}$	34.14±0.86$^{\#}$	---	---	---	---
T (10)	Serum	5.06±0.48$^{*\$}$	1.59±0.13$^{*\$}$	0.28±0.03$^{*\$}$	147.84±2.92$^{*\$}$	20.03±0.89$^{*\$}$	1486±48.16$^{*\$}$	3.26±0.16$^{*\$}$	1.95±0.17$^{*\$}$	9.39±0.50$^{*\$}$
	Urine	20.86±1.52$^{*\$}$	2.27±0.09$^{*\$}$	16.12±1.22$^{*\$}$	375.76±5.61$^{*\$}$	22.95±0.58$^{*\$}$	---	---	---	---
C (500)	Serum	4.30±0.68$^{*\$}$	1.54±0.17$^{*\$}$	0.26±0.02$^{*\$}$	142.61±3.31$^{*\$}$	20.91±1.03$^{*\$}$	1363±52.15$^{*\$}$	2.99±0.34$^{*\$}$	2.15±0.18$^{*\$}$	8.12±0.51$^{*\$}$
	Urine	21.28±2.70$^{*\$}$	2.12±0.09$^{*\$}$	14.80±1.15$^{*\$}$	373.65±7.21$^{*\$}$	23.44±0.76$^{*\$}$	---	---	---	---
AP (5)	Serum	16.54±0.58	3.82±0.12	0.55±0.03	167.55±3.27	28.54±0.53	2186±23.66	1.89±0.35	0.99±0.15	3.79±0.45
	Urine	51.45±2.03	3.77±0.09	42.32±1.47	315.39±8.01	32.58±0.85	---	---	---	---
AP (10)	Serum	12.39±0.60$^{*\$}$	3.35±0.12$^{*\$}$	0.41±0.03$^{*\$}$	163.01±2.26$^{*\$}$	26.36±0.51$^{*\$}$	1760±58.80$^{*\$}$	2.69±0.28$^{*\$}$	1.71±0.19$^{*\$}$	5.55±0.43$^{*\$}$
	Urine	34.3±2.20$^{*\$}$	3.24±0.10$^{*\$}$	33.78±1.06$^{*\$}$	335.25±8.57$^{*\$}$	28.14±1.25$^{*\$}$	---	---	---	---
AP (20)	Serum	7.77±0.68$^{*\$}$	1.88±0.15$^{*\$}$	0.31±0.02$^{*\$}$	151.40±3.81$^{*\$}$	22.93±0.88$^{*\$}$	1510±54.43$^{*\$}$	2.64±0.13$^{*\$}$	2.23±0.19$^{*\$}$	8.09±0.59$^{*\$}$
	Urine	25.79±1.75$^{*\$}$	2.73±0.11$^{*\$}$	22.01±1.31$^{*\$}$	371.66±7.91$^{*\$}$	27.48±0.73$^{*\$}$	---	---	---	---

Results are represented as mean±SEM (n=6) and analyzed by One-way ANOVA followed by Tukey's multiple range test. $^{*}P$<0.05 as compared to UC group, $^{\#}P$<0.05 as compared to normal animals and $^{\$}P$<0.05 as compared to one another group. BUN: Blood Urea Nitrogen; LDH: Lactate dehydrogenase, GAGs: Glycosaminoglycans.

($P < 0.05$) in urolithiasis control (UC) rats compared to normal rats. Telmisartan and cystone treatment markedly ($P < 0.05$) attenuated EG-induced modulation in water intake, relative organ and body weight, and urinary parameters as compared to UC rats. As compared to UC rats, apigenin (10 and 20 mg/kg) treatment markedly increased ($P < 0.05$) water intake, urine output, urinary pH, and body weight, whereas density and dry weight of urine, weight of heart, and kidney weight were markedly decreased ($P < 0.05$) [Table 1].

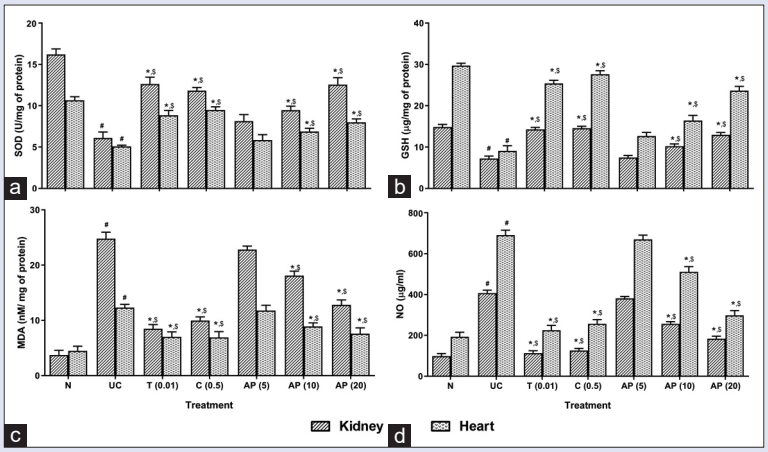


Figure 1: Effect of apigenin on EG-induced cardiac and renal oxido-nitrosative stress (SOD (a), GSH (b), MDA (c) and NO (d)) in uninephrectomized rats. Results are represented as mean ± SEM ($n = 6$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. *$P < 0.05$ as compared to UC group, #$P < 0.05$ as compared to normal animals, and $P < 0.05$ as compared to one another group. N: Normal; UC: Urolithiasis control; T: Telmisartan; C: Cystone; AP: Apigenin; SOD: Superoxide dismutase; GSH: Reduced glutathione; MDA: Lipid peroxidation; NO: Nitric oxide; EG: Ethylene glycol; SEM: Standard error of the mean; ANOVA: Analysis of variance

Table 4: Effect of apigenin on EG-induced alterations in renal KIM-1, NGAL, bikunin, iNOs, eNOs and OPN mRNA expressions in uninephrectomized rats

Treatment	KIM-1 / β Actin Ratio	NGAL / β Actin Ratio	Bikunin / β Actin Ratio	iNOs / β Actin Ratio	eNOs / β Actin Ratio	OPN / β Actin Ratio
N	0.76±0.03	0.23±0.03	0.46±0.09	0.32±0.09	0.84±0.05	1.09±0.09
UC	1.63±0.09#	0.79±0.03#	1.56±0.07#	1.89±0.08#	0.47±0.14#	0.22±0.09#
T (10)	0.90±0.08*$	0.76±0.04	0.66±0.05*$	1.72±0.06	0.44±0.08	0.81±0.06*$
C (500)	1.57±0.19	0.67±0.02	0.73±0.09*$	1.70±0.06	0.46±0.09	0.91±0.08*$
AP (5)	1.55±0.16	0.71±0.05	1.44±0.07	1.73±0.10	0.45±0.08	0.38±0.07
AP (10)	1.31±0.03*$	0.58±0.03*$	1.20±0.07*$	1.14±0.09*$	0.62±0.13*$	0.51±0.05*$
AP (20)	0.95±0.07*$	0.49±0.02*$	0.95±0.10*$	0.96±0.09*$	0.73±0.09*$	0.60±0.06*$

Results are represented as mean±SEM (n=6) and analyzed by One-way ANOVA followed by Tukey's multiple range test. *$P<0.05$ as compared to UC group, #$P<0.05$ as compared to normal animals and $P<0.05$ as compared to one another group. KIM-1: Kidney Injury Molecule-1; NGAL: Neutrophil gelatinase-associated lipocalin; iNOs: inducible nitric oxide synthase; eNOs: endothelial nitric oxide synthase; OPN: Osteopontin.

Hemodynamic and electrocardiographic abnormalities in uninephrectomized rats

A marked increase ($P < 0.05$) in SBP, DBP, MABP, HR, intervals (QRS and QTc), and ST height in UC rats was observed when compared with normal rats. Telmisartan and cystone treatment markedly inhibited ($P < 0.05$) EG-induced elevated SBP, DBP, MABP, HR, intervals (QRS and QTc), and ST height when compared with UC rats. As compared to UC rats, apigenin (10 and 20 mg/kg) treatment showed significant decrease ($P < 0.05$) in SBP, DBP, MABP, HR, intervals (QRS and QTc), and ST height. However, when compared with cystone, treatment with telmisartan and apigenin (20 mg/kg) more markedly ($P < 0.05$) attenuated the EG-induced modulations in hemodynamic and electrocardiographic abnormalities [Table 2].

Serum and urinary biochemical parameters

The levels of BUN, creatinine, uric acid, calcium, sodium, and LDH were increased markedly ($P < 0.05$) in UC rats when compared with normal rats, whereas the levels of citrate, albumin, and GAGs in serum were decreased significantly ($P < 0.05$) in UC rats when compared with normal rats. Telmisartan and cystone treatment markedly ($P < 0.05$) attenuated EG-induced alterations in the levels of BUN, creatinine, uric acid, calcium, sodium, LDH, citrate, albumin, and GAGs in the serum when compared with UC rats. Apigenin (10 and 20 mg/kg) markedly ($P < 0.05$) decreased BUN, creatinine, uric acid, calcium, sodium, and LDH whereas markedly ($P < 0.05$) increased citrate, albumin, and GAGs levels in the serum when compared with UC rats [Table 3].

On the other hand, the levels of urinary urea nitrogen, creatinine, uric acid, and calcium increased significantly ($P < 0.05$) whereas urinary sodium level significantly decreased ($P < 0.05$) in UC rats as compared to normal rats. Telmisartan, cystone, and apigenin (10 and 20 mg/kg) treatment markedly ($P < 0.05$) attenuated EG-induced alterations in urinary urea nitrogen, creatinine, uric acid, sodium, and calcium when compared with UC rats [Table 3].

Cardiac and renal oxido-nitrosative stress

The SOD and GSH levels were markedly decreased ($P < 0.05$) in the kidney and heart tissue of UC rats when compared with normal rats. However, telmisartan and cystone treatment markedly ($P < 0.05$) inhibited EG-induced decreased in the kidney and heart SOD

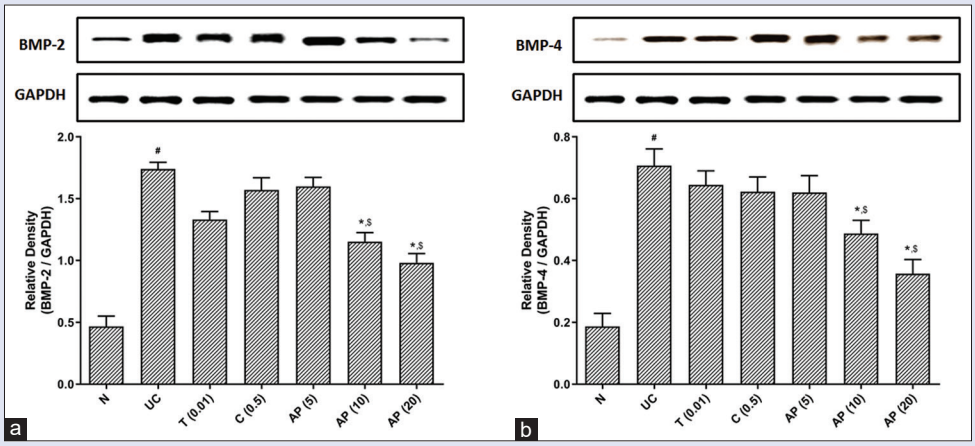


Figure 2: Effect of apigenin on EG-induced alterations in renal BMP-2 (a) and BMP-4 (b) protein expression in uninephrectomized rats. Results are represented as mean ± SEM ($n = 4$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. *$P < 0.05$ as compared to UC group, $^{\#}P < 0.05$ as compared to normal animals, and $^{\$}P < 0.05$ as compared to one another group. BMP: Bone morphogenetic protein; EG: Ethylene glycol; SEM: Standard error of the mean; UC: Urolithiasis control; ANOVA: Analysis of variance

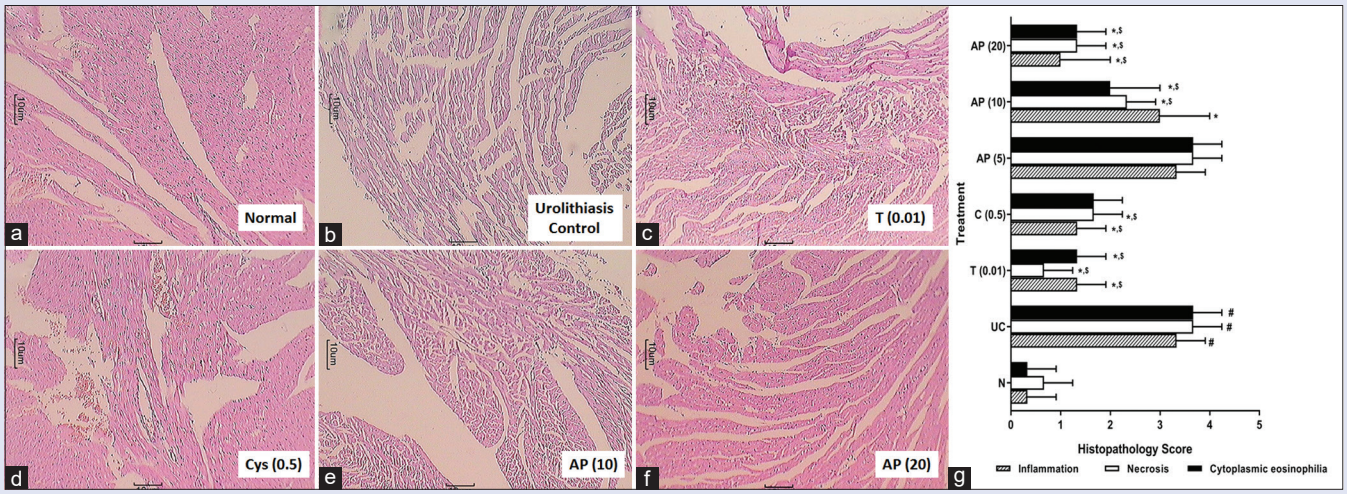


Figure 3: Effect of apigenin on EG-induced alterations in heart histology in uninephrectomized rats. Photomicrograph of sections of heart of normal (a), UC (b), telmisartan (10 mg/kg) treated rats (c), cystone (500 mg/kg) treated rats (d), apigenin (10 mg/kg) treated rats (e), and apigenin (20 mg/kg) treated rats (f) (H and E, ×40). The quantitative analysis of the effect of apigenin on EG-induced alterations in heart histology in uninephrectomized rats (g). Data are expressed as mean ± SEM ($n = 3$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. *$P < 0.05$ as compared to UC group, $^{\#}P < 0.05$ as compared to normal animals, and $^{\$}P < 0.05$ as compared to one another group. EG: Ethylene glycol; ANOVA: Analysis of variance; EG: Ethylene glycol; SEM: Standard error of the mean; UC: Urolithiasis control

and GSH levels when compared with UC rats. Apigenin (10 and 20 mg/kg) treatment also markedly increased ($P < 0.05$) SOD and GSH levels in the kidney and heart tissue as compared to UC rats. However, the administration of telmisartan and cystone showed more marked ($P < 0.05$) inhibition in EG-induced decreased in the GSH and SOD levels in cardiac and renal tissues as compared to apigenin treatment [Figure 1].

Administration of EG resulted in marked ($P < 0.05$) increase in MDA and NO levels in cardiac and renal tissues in UC rats when compared with normal rats. Treatment with telmisartan and cystone markedly ($P < 0.05$) decreased the levels of MDA and NO in heart and kidney tissues when compared with UC rats. Apigenin (10 and 20 mg/kg) administration also markedly decreased ($P < 0.05$) EG-induced increased the levels of MDA and NO levels in heart and kidney tissues as compared to UC rats [Figure 1].

Renal kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, bikunin, inducible nitric oxide synthase, endothelial nitric oxide synthase, and osteopontin mRNA expressions

There was marked upregulation ($P < 0.05$) in the mRNA expressions of KIM-1, NGAL, bikunin, and iNOs in the renal tissue whereas marked downregulation ($P < 0.05$) in the mRNA expressions of eNOs and OPN in the renal tissue of UC rats when compared with normal rats. Administration of telmisartan and cystone significantly ($P < 0.05$) inhibited EG-induced modulation in bikunin and OPN mRNA expressions when compared with UC rats. Telmisartan and cystone treatment did not produce marked downregulation in EG-induced alterations in NGAL, iNOs, and eNOs expressions compared with UC rats. However, apigenin (10 and 20 mg/kg) treatment markedly

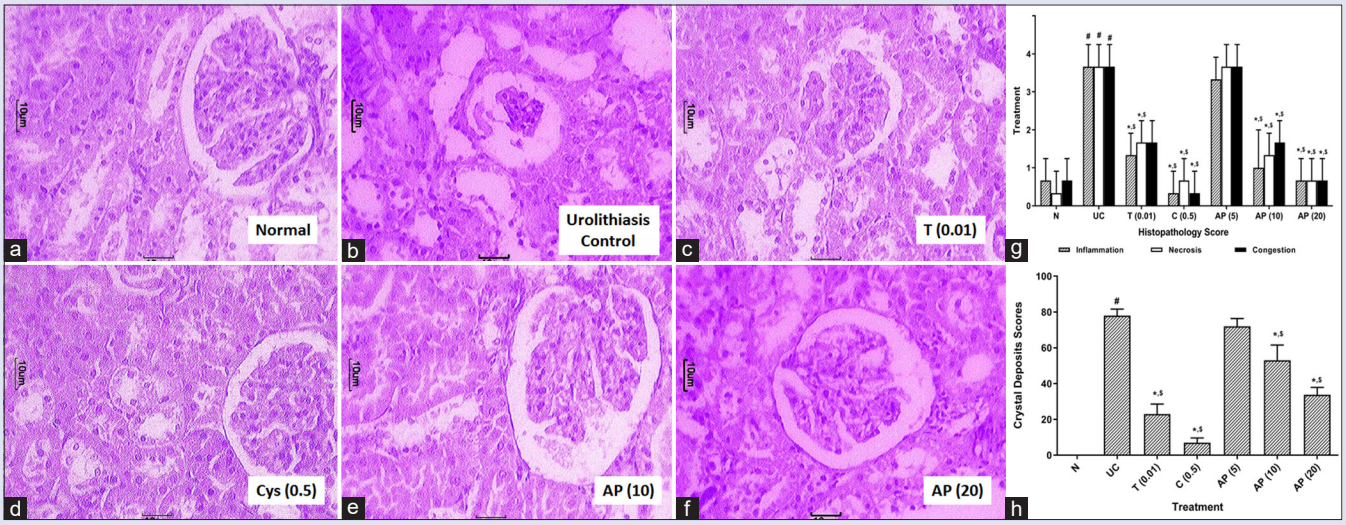


Figure 4: Effect of apigenin on EG-induced alterations in kidney histology in uninephrectomized rats. Photomicrograph of sections of kidney of normal (a), UC (b), telmisartan (10 mg/kg) treated rats (c), cystone (500 mg/kg) treated rats (d), apigenin (10 mg/kg) treated rats (e), and apigenin (20 mg/kg) treated rats (f) (H and E, ×100). The quantitative analysis of the effect of apigenin on EG-induced alterations in kidney histology in uninephrectomized rats (g) and crystal deposition score (h). Data are expressed as mean ± SEM ($n = 3$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. *$P < 0.05$ as compared to UC group, $^{\#}P < 0.05$ as compared to normal animals, and $^{\$}P < 0.05$ as compared to one another group. EG: Ethylene glycol; ANOVA: Analysis of variance; EG: Ethylene glycol; SEM: Standard error of the mean; UC: Urolithiasis control

downregulated ($P < 0.05$) mRNA expressions of KIM-1, NGAL, bikunin, and iNOs in the renal tissues whereas markedly upregulated ($P < 0.05$) the renal eNOs and OPN mRNA expressions as compared to UC rats [Table 4].

Renal bone morphogenetic protein-2 and bone morphogenetic protein-4 expressions

The renal BMP-2 and BMP-4 protein levels were increased significantly ($P < 0.05$) in UC rats when compared with normal rats. However, apigenin (10 and 20 mg/kg) treatment markedly decreased ($P < 0.05$) renal BMP-2 and BMP-4 protein levels as compared to UC rats. Telmisartan and cystone treatment did not produce marked downregulation in EG-induced increased protein levels of renal BMP-2 and BMP-4 as compared to UC rats [Figure 2].

Heart histology

Ingestion of EG induces marked ($P < 0.05$) inflammatory infiltration and necrosis in UC rats when compared with normal rats [Figure 3b]. However, Figure 3a represents a normal architecture of cardiac tissue without any necrosis, inflammatory release, and eosinophilia. Administration of telmisartan and cystone markedly ($P < 0.05$) inhibited EG-induced inflammatory infiltration, eosinophilia release, and necrosis when compared to UC rats [Figure 3c and d]. As compared to UC rats, apigenin (10 and 20 mg/kg) administration also markedly decreased ($P < 0.05$) inflammatory infiltration, eosinophilia release, and necrosis [Figure 3e and f]. However, when compared with cystone treatment, telmisartan and apigenin (20 mg/kg) administration showed more marked ($P < 0.05$) attenuated EG-induced cardiac aberrations [Figure 3g].

Kidney histology

Figure 4a depicts the normal structure of renal tissue, minimal inflammatory release, necrosis, and congestion. However, the administration of EG resulted in a marked increase ($P < 0.05$) in inflammatory release, necrosis, congestion, and crystal deposit score in UC rats [Figure 4b] when compared with normal rats. Telmisartan and cystone administration markedly ($P < 0.05$) attenuated EG-induced inflammatory infiltration, necrosis, congestion, and crystal deposit score when compared with UC rats [Figure 4c and d]. As compared to UC rats, apigenin (10 and 20 mg/kg) administration markedly reduced ($P < 0.05$) inflammatory infiltration, necrosis, congestion, and crystal deposit score [Figure 4e and f]. However, when compared with cystone and telmisartan treatment, apigenin less significantly ($P < 0.05$) decreased the EG-induced crystal deposit score [Figure 4g and h].

DISCUSSION

Nephrolithiasis is a major prevalent health problem of the urinary system worldwide. Studies reported that acute ureteral obstruction occurrence in 12% of the world population, whereas it is estimated to affect less in females (7%) than males (11%).[1,3] Urolithiasis mainly characterized by severe unilateral colicky flank pain that may be accompanied by nausea, vomiting, and hematuria. Moreover, its complications may lead to severe infection and renal failure, which large impact on the quality of life of the affected person. As most of the urinary stone is composed of calcium oxalate (76%), treatment regimen oriented toward the inhibition of CaOx crystallization. In addition to this, advances in modern technology (including percutaneous nephrolithotomy and extracorporeal shock wave lithotripsy) have been profoundly implicated as an alternative in the management of renal calculi.[7] However, long operating times staged procedures, as well as associated complications, including renal vascular trauma, may result in this procedure as a secondary method of choice for the treatment of urinary stones.[7] Thus, the management of urinary calculi using herbal moieties has been widely utilized as a preferred treatment over others. Flavonoids have been well studied for the prevention and treatment of CaOx-induced kidney stones, and thus, in the current study, we have determined the potential of apigenin, a plant flavonoid against urolithiasis induced by EG in uninephrectomized animals. Findings of the current investigation suggest that apigenin administration markedly decreased EG-induced altered serum and urine biochemistry, elevated oxidative stress, and

altered expressions of KIM-1, NGAL, OPN, bikunin, eNOs, iNOs, BMP-2, and BMP-4 expressions to exert its nephroprotective potential.

Studies have well documented that repeated exposure of EG in the experimental rats over a period resulted in the induction of adverse effects qualitatively and quantitatively.[19] These adverse events have been determined using various parameters including body weight, relative organ weights, water intake, urine analysis, and pathological analysis of organs such as kidney and heart, which provide an insight into the toxicity induced by chronic administration of EG. In addition, analysis of urine chemistry is a good indicator of stone formation and provides insights into minerals associated with calculi formation.[9,10] Furthermore, urolithiasis also results in distal renal tubular acidosis, which in turn causes alkaline urinary pH. However, the administration of apigenin exerts its nephroprotective and cardioprotective effect against EG-induced toxicity reflected by a significant attenuation of altered relative organ and body weights, decreased water intake, urine output, urine pH, and histological alterations in the heart and kidney tissues.

It has been well established that administration of EG, a high concentrated precursors of oxalate in experimental animal, results in intratubular CaOx crystallization along with tubular damage, which in turn causes nephrolithiasis.[9] Therefore, nephrotoxicity induced by oral administration of EG is a widely used and ideal model to induce crystal formation in the experimental animals.[9] During the formation of CaOx crystals, the initialization of nucleation is a vital first step, which then grows and results in aggregates formation. Furthermore, elevated levels of calcium in the serum and urine are also important evidence for the deposition of crystalline material.[3] In the present investigation, administration of EG induces aggregation of CaOx which results in the genesis of stones, which is also evident by elevated levels of calcium in serum and urine. However, treatment with apigenin significantly inhibited an essential factor of nucleation, which results in amelioration of CaOx crystal formation. This notion was further supported by decreased calcium levels in serum as well as urine. In addition, histological analysis of the kidney from apigenin-administered rats showed significantly reduced in crystal deposits as compared to polymorphic irregular crystal deposits in UC rats. Furthermore, toxicity induced by CaOx crystal growth results in the release of various inflammatory mediators, which develops an inflammatory reaction which supported by elevated relative weights of the kidneys. This increased relative kidney weight was significantly attenuated by apigenin treatment may be via due of its anti-inflammatory property.

The researcher suggested that hypocitraturia is a major metabolic abnormality in urolithiasis patients.[20] Citrate exhibits inhibitory action against the urinary stone and especially against the CaOx crystal formation.[21] It interacts with calcium and forms its complex, thus reduces the levels of CaOx, and thus prevents the formation of CaOx agglomerates.[5] Furthermore, GAGs exhibit the inhibitory potential against CaOx nucleation, growth, and aggregation as well as the prevention of crystals adherence to the renal surface.[22] However, uric acid interferes with the inhibitory potential of GAGs; thus, it reduces the solubility of CaOx suggests its pivotal role in stone formation.[23] Thus, clinically also, patients with urolithiasis exhibit elevated levels of uric acid and reduced levels of GAGs.[3] Moreover, the formation of CaOx crystals in the urinary system leads to obstruction in urinary outflow and decreases the glomerular filtration rate. The altered urinary filtration is associated with the accumulation of creatinine, BUN, urea, and uric acid in the blood which is generally known as nitrogenous waste products.[23] The results of the current investigation also depicted that the administration of EG altered the levels of calcium, GAGs, urea nitrogen, uric acid, creatinine, citrate, and LDH in the urine and blood. However, treatment with apigenin exerts its nephroprotective via inhibition of EG-induced alterations in the urinary and serum biochemistry.

It has been suggested the oxidative stress plays a pivotal role in the pathogenesis of clinical as well as experimental urolithiasis.[2] Induction of nephrotoxicity due to the administration of various chemical agents such as aminoglycosides, mercury, lead, and EG that results in an increased level of ROS leads to elevated oxidative stress.[19] Exaggerated renal oxidative stress causes an imbalance in the cellular antioxidative responses reflected by a reduction of total intracellular GSH levels.[24] GSH, an intracellular antioxidant molecule, has the ability to quench electrophilic chemical species, thus protecting the cells from oxidative injury.[25] Depletion in the level of GSH causes a decrease in the development of T-cells, which results in elevated inflammatory responses.[26] Furthermore, SOD serves as a first-line antioxidant defense system by reducing the damaging effect of superoxide radicals through its conversion to H_2O_2.[27] The depleted activity of SOD and GSH has also been documented in the subject with renal failure.[2] Thus, SOD and GSH have paramount importance in nephropathy as its depleted levels are associated with disrupted intestinal barrier integrity along with intestinal epithelial cells injury. Moreover, oxidation of polyunsaturated fatty acids leads to the overproduction of MDA, which contributes to renal damage.[28] In the present study, urolithiasis control rats showed a decline in the SOD and GSH levels along with increased inactivity of MDA in renal and heart tissue, whereas treatment with apigenin augmented GSH and SOD levels and thus advocated its inhibitory potential against oxidative stress. The findings of the current investigation corroborated with the findings of the previous researchers where apigenin administration increased the renal SOD and GSH levels to ameliorate nephrotoxicity.[15]

Recent advances in molecular pharmacological techniques have reported KIM-1 and NGAL as the essential hallmarks of acute kidney injury.[29] NGAL, a lipocalin superfamily member, has been documented to elevate during inflammation, hypertension, and renal tubular damage.[9,10] During various clinical settings such as contrast-induced nephropathy, kidney transplantation, septic shock, and critical care in the intensive care unit, the role of NGAL has been well defined.[9,10] Thus, the researcher has documented the 0.815 (95% confidence interval: 0.732–0.892) as a sensitivity of NGAL toward the prediction of acute kidney injury.[9,10] Furthermore, KIM-1 plays an essential role in phagocytosis and expression explicitly in the proximal tubule.[29] It has been documented that intrarenal inflammatory influx causes upregulation in the KIM-1 and NGAL expressions in the renal tissue, and in the current study also, EG administration resulted in elevated renal expressions of NGAL and KIM-1. However, treatment with apigenin significantly attenuated these elevated renal NGAL and KIM-1 expressions, which might be via its anti-inflammatory potential.

OPN is a secreted integrin-binding protein that plays a diverse role in various biological functions, including nephrotoxicity.[30] Numerous studies have reported that oxalate initiates and maintains pathogenesis of renal stone formation, and OPN inhibits the formation of calcium oxalate monohydrate; thus, OPN considers as an essential hallmark during urolithiasis.[30,31] The researcher showed that the administration of EG in OPN-knockout mice resulted in the formation of renal calculi.[30] Therefore, high expression of OPN plays a beneficial role in the prevention of urinary stone formation. In addition, bikunin, which is a member of the light chain of inter-alpha-trypsin inhibitor family, also considers a vital marker for monitoring kidney function. Clinically, the expression of bikunin significantly increased in patients with renal impairment due to proteinuria.[22] During the various conditions such as renal tissue injury due to kidney disease or surgery or infection, the concentrations of bikunin elevated in the plasma and urine. Thus, bikunin considered

rapid markers during kidney injuries.[22,32] In the present study, treatment with apigenin significantly inhibited EG-induced downregulated OPN and upregulated bikunin expression. Previous investigators showed that apigenin exerts its potential via upregulating osteoblast differentiation genes, including OPN,[33,34] and the outcomes of the current study are in accordance with the result of previous investigators.[33,34]

BMPs, a transforming growth factor-β cytokine superfamily members, play an essential role during the embryonic development of kidney and nephron.[31,34] The researcher has well established the molecular linkage of BMP-2 with vascular calcification and oxidative stress during kidney diseases.[31] The BMP receptor-IA (BMPR-IA) and BMPR-IB are the domain on type I and type II serine-threonine kinase receptors to which BMPs bind results in the formation of specific complexes. This complex initiates and regulates Smad1/5 phosphorylation and combine with Smad4, which further translocates to the nucleus, where it brings out molecular perturbation that results in vascular calcification. BMP-4 also plays a vital role during embryonic development by modulating cell proliferation, differentiation, and apoptosis.[31,35] Furthermore, patients with chronic kidney disease exhibits the elevated levels of BMP-4.[35] In the current study also, EG administration caused a marked upregulation in the BMP-2 and BMP-4 expressions, whereas administration of apigenin attenuated these elevated expressions of BMPs. The previous research also showed that apigenin treatment exerts its protective efficacy against oxidative stress-induced damage via modulating the expression of BMPs (BMP-2 and BMP-4), and the outcomes of the current study are according to the results of this researcher.[34]

Currently, moieties from herbal origin play an important role in the treatment of urolithiasis and cystone is one such polyherbal medicine from Ayurveda origin, which was approved by the Drug Regulatory Authority of India. Its potential against the management of various urinary tract complications has been well studied. Thus, in the current study, we have also implicated this as a positive control to compare the efficacy of apigenin. Findings of the current investigation suggest that the cystone showed prominent efficacy during nephrotoxicity however, it has less efficiency during cardioprotection. However, the administration of apigenin showed significant protection against both EG-induced nephrotoxicity and cardiotoxicity. Thus, it can be considered as an important moiety for the management of complications associated with unilateral nephrectomy patients along with hypertension.

CONCLUSION

Findings of the current study suggest that apigenin attenuated EG-induced nephrolithiasis in uninephrectomized hypertensive rats. This nephroprotective potential of apigenin may be orchestrated via the modulation of altered serum and urine biochemistry, elevated oxidative stress, and altered expressions of KIM-1, NGAL, OPN, bikunin, eNOs, iNOs, BMP-2, and BMP-4 in the renal tissue.

Acknowledgements

The authors would like to acknowledge Dr. S. S. Kadam, Chancellor, and Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, for providing necessary facilities to carry out the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Trinchieri A. Epidemiology of urolithiasis: An update. Clin Cases Miner Bone Metab 2008;5:101-6.
2. Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J Med Life 2016;9:259-62.
3. Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H, *et al.* Epidemiology of urolithiasis in Asia. Asian J Urol 2018;5:205-14.
4. Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharm Biol 2014;52:814-28.
5. Mandel EI, Taylor EN, Curhan GC. Dietary and lifestyle factors and medical conditions associated with urinary citrate excretion. Clin J Am Soc Nephrol 2013;8:901-8.
6. Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU Update Series 2007;5:126-36.
7. Shafi H, Moazzami B, Pourghasem M, Kasaeian A. An overview of treatment options for urinary stones. Caspian J Intern Med 2016;7:1-6.
8. Li W, Kandhare AD, Mukherjee AA, Bodhankar SL. Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats: Role of TGF-ß/Smads and Ang-1/Tie2 signaling pathways. EXCLI J 2018;17:399-419.
9. Kandhare AD, Patil MV, Bodhankar SL. L-Arginine attenuates the ethylene glycol induced urolithiasis in ininephrectomized hypertensive rats: Role of KIM-1, NGAL, and NOs. Ren Fail 2015;37:709-21.
10. Mukherjee A, Zhang J, Kandhare AD, Bodhankar SL. Curcumin ameliorates vitamin A deficiency-induced urolithiasis in neonatal rats via inhibition of KIM-1/NGAL, Nrf2, and iNOs pathways. Lat Am J Pharm 2018;37:2502-11.
11. Weir MR, Burgess ED, Cooper JE, Fenves AZ, Goldsmith D, McKay D, *et al.* Assessment and management of hypertension in transplant patients. J Am Soc Nephrol 2015;26:1248-60.
12. Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action. Cell Biosci 2017;7:50.
13. Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, *et al.* The therapeutic potential of apigenin. Int J Mol Sci 2019;20(6):1305. doi: 10.3390/ijms20061305.
14. Gradolatto A, Basly JP, Berges R, Teyssier C, Chagnon MC, Siess MH, *et al.* Pharmacokinetics and metabolism of apigenin in female and male rats after a single oral administration. Drug Metab Dispos 2005;33:49-54.
15. Meng S, Zhu Y, Li JF, Wang X, Liang Z, Li SQ, *et al.* Apigenin inhibits renal cell carcinoma cell proliferation. Oncotarget 2017;8:19834-42.
16. Sui H, Yu Q, Zhi Y, Geng G, Liu H, Xu H. Effects of apigenin on the expression of angiotensin-converting enzyme 2 in kidney in spontaneously hypertensive rats. Wei Sheng Yan Jiu 2010;39:693-6, 700.
17. Ghule AE, Kandhare AD, Jadhav SS, Zanwar AA, Bodhankar SL. Omega-3-fatty acid adds to the protective effect of flax lignan concentrate in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis. Int Immunopharmacol 2015;28:751-63.
18. Zhang L, Wu T, Kandhare A, Mukherjee A, Guo G, Bodhankar SL. Elucidation of the molecular mechanism of tempol in pentylenetetrazol-induced epilepsy in mice: Role of gamma-aminobutyric acid, tumor necrosis factor-alpha, interleukin-1β and c-Fos. Pharmacognosy Magazine 2018;14:520.
19. Tzou DT, Taguchi K, Chi T, Stoller ML. Animal models to study urolithiasis. In: Conn PM, editor. Animal Models for the Study of Human Disease. 2nd ed., Ch. 17, TX, United States. Academic Press; 2017. p. 419-43.
20. Mukherjee AA, Kandhare AD, Bodhankar SL. Evaluation of health-related quality of life in hemolytic uraemic syndrome patients treated with eculizumab: A systematic evaluation on basis of EMPRO. Ren Fail 2018;40:107-18.
21. Caudarella R, Vescini F. Urinary citrate and renal stone disease: The preventive role of alkali citrate treatment. Arch Ital Urol Androl 2009;81:182-7.
22. Lepedda AJ, Fancellu L, Zinellu E, De Muro P, Nieddu G, Deiana GA, *et al.* Urine bikunin as a marker of renal impairment in Fabry's disease. Biomed Res Int 2013;2013:205948.
23. Honmore V, Kandhare A, Zanwar AA, Rojatkar S, Bodhankar S, Natu A. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharm Biol 2015;53:571-81.

24. Kandhare AD, Aswar UM, Mohan V, Thakurdesai PA. Ameliorative effects of type-A procyanidins polyphenols from cinnamon bark in compound 48/80-induced mast cell degranulation. Anat Cell Biol 2017;50:275-83.
25. Visnagri A, Kandhare AD, Kumar VS, Rajmane AR, Mohammad A, Ghosh P, *et al.* Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed Aging Pathol 2012;2:157-72.
26. Yin H, Bodhankar S, Zhang G, Kandhare A, Mukherjee A. Ameliorative effect of morin, a plant flavonoid against Freund's complete adjuvant-induced polyarthritis in rats. Pharmacognosy Magazine 2019;15:43.
27. Kumar VS, Rajmane AR, Adil M, Kandhare AD, Ghosh P, Bodhankar SL. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J Biomed Res 2014;28:132-45.
28. Zhou Z, Kandhare AD, Kandhare AA, Bodhankar SL. Hesperidin ameliorates bleomycin-induced experimental pulmonary fibrosis via inhibition of TGF-β1/Smad3/AMPK and IκBα/NF-κB pathways. EXCLI J 2019;18:723-45.
29. Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: Role of FXR and KIM-1. Ren Fail 2016;38:1007-20.
30. Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, *et al.* Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. J Am Soc Nephrol 2003;14:139-47.
31. Salama RH, Alghasham A, Mostafa MS, El-Moniem AE. Bone morphogenetic protein-2 will be a novel biochemical marker in urinary tract infections and stone formation. Clin Biochem 2012;45:766-9.
32. Liao WL, Chang CT, Chen CC, Lee WJ, Lin SY, Liao HY, *et al.* Urinary proteomics for the early diagnosis of diabetic nephropathy in Taiwanese patients. J Clin Med 2018;7:483. doi:10.3390/jcm7120483.
33. Zhang X, Zhou C, Zha X, Xu Z, Li L, Liu Y, *et al.* Apigenin promotes osteogenic differentiation of human mesenchymal stem cells through JNK and p38 MAPK pathways. Mol Cell Biochem 2015;407:41-50.
34. Jung WW. Protective effect of apigenin against oxidative stress-induced damage in osteoblastic cells. Int J Mol Med 2014;33:1327-34.
35. Stahls PF 3rd, Lightell DJ Jr., Moss SC, Goldman CK, Woods TC. Elevated serum bone morphogenetic protein 4 in patients with chronic kidney disease and coronary artery disease. J Cardiovasc Transl Res 2013;6:232-8.