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Comparative Evaluation of Aqueous Extracts of Peel, Seed and Whole Fruit of *Punica granatum* L. on Ethylene Glycol Induced Urolithiasis in Rats

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ABSTRACT

Objective: The present study was aimed to investigate the anti-urolithiatic activity of aqueous extract of peel, seed and whole fruit of *Punica granatum* L. on ethylene glycol (EG) induced urolithiasis in male Wistar albino rats. **Materials & Methods:** Urolithiasis was induced in male Wistar albino rats by incorporating 0.75% v/v EG in drinking water for 28 days. The aqueous extracts of peel, seed and whole fruit of *Punica granatum* (200 mg/kg p.o) was administered for 28 days. Various renal functional parameters such as calcium, phosphorus, uric acid, BUN and creatinine were evaluated inserum and urine sample. Tissue antioxidant parameters such as superoxide dismutase, catalase and lipid peroxidation were also determined. **Results:** 28 days administration of EG significantly increased the levels of calcium, phosphorus, uric acid, BUN and creatinine in urine sample. Oxidative parameters like increased MDA, decreased SOD and catalase levels and histological changes indicated the presence of urolithiasis in EG group when compared to control group. However, 28 days treatment with aqueous extract of peel, seed and peel+seed of PG (200 mg/kg) orally restored the parameters of serum & urine to normal levels in urolithiatic rats. A significantly improved pathological change was observed in EG induced group. **Conclusion:** EG induced urolithiasis is an effective model to study and evaluate the new therapeutic modalities of urolithiasis. The present study suggested that pomegranate may reduce the risk of urolithiasis and compared to seed, peel is found to be more effective in controlling urolithiasis.

INTRODUCTION

Urolithiasis is a complex disease in which stones or calculi are formed at any site inside the urinary tract because of interruption in the equilibrium between promoters and inhibitors.[1] The pervasiveness of urolithiasis is 5 - 19.1 % in developing Asian countries and some developed nations.[2] The rate of occurrence is three times higher in men (70-81%) than women (47-60%).[3]

Punica granatum L. (PG) is commonly known as pomegranate belonging to the family Puniacaceae, generally developed and cultivated in tropical and subtropical area of the world.[4] PG comprises of many flavonoids, the fruit which alone account for nearly 0.2 - 1.0 %. Peel comprises about 30% of all anthocyanidins. Various metabolic products such as estradiol, diadzein, isoflavones, genistin, and diadzin can be isolated from the seeds. Isopelletierine, pseudopelletierine, anthocyanidins, ellagotannins, pelargonidin, gallic and ellagic acid were also found in the stem and roots of pomegranate.[5]

Traditionally, PG is utilized in curing arthritis, coughs, urinary infections (UTIs), skin & digestive disorders.[6] On the other hand, no scientific data exists to ascertain the use of peel, seed and whole fruit of *Punica granatum* L. in treating urolithiasis. Therefore, the present study was carried out to set up the scientific legitimacy of the anti-urolithiatic activity of peel, seed and whole fruit of *Punica granatum* L. aqueous extract using ethylene glycol induced urolithiasis in male Wistar albino rats.

MATERIAL AND METHODS

Drugs and Chemicals

Ethylene glycol was obtained from SD Fine Chem. Ltd., India. All other chemicals and reagents used were of analytical grade and obtained from standard companies. Biochemical kits for the measurement of serum and urine markers (calcium, phosphorus, BUN, uric acid and creatinine) were obtained from ARKRAY Healthcare Pvt. Ltd., Mumbai.

Experimental Animals

Thirty male Wistar rats (150-200 g) were procured from Sainath Agency, Hyderabad, India. The animals were acclimatized for one week in 12:12 h light and dark cycle, 25 ± 2^{0}C temperature and 50 ± 5% humidity controlled room. Animals were allowed to free access food and water *ad libitum*. After one week of acclimatization period, they were randomly

selected for different experimental groups. All the experimental procedures were carried out in accordance with committee for the purpose of Control and Supervision of Experiments on Animal guidelines (320/CPCSCEA dated 03-01-2001), Government of India. The study was reviewed and approved by the Institutional Animal Ethics Committee (GPRCP/IAEC/23/19/02/PCL/AE-5-Rats-M-30.), G. Pulla Reddy College of Pharmacy, Hyderabad, India.

Preparation of Plant Extract

Fruits of *Punica granatum* L. were procured from herbal garden of Osman Sagar Lake, Hyderabad, Telangana, India.

Preparation of Aqueous Peel Extract

150g of peel was grounded, air dried and boiled in 3000 ml of distilled water for 15 minutes with continuous stirring. The resultant solution was passed through filter paper for filtered. The filtrate was evaporated completely under reduced pressure at 60ºC.[7]

Preparation of Aqueous Seed Extract

The seeds were crushed, squeezed, grounded and blended with electronic blender and dried in an oven adjusted at 40ºC for 24 hrs. The obtained fine powder was sieved with the help of 24mesh. 10 gm of the obtained fine powdered sample was extracted with 100ml of distilled water at 25ºC for 24 h in a shaking water bath. After extraction, the extract was filtered under vaccum with the help of millipore filter of having 0.45µm nylon membrane at 25ºC. The extract was then pasteurized, concentrated and stored at 4ºC till used.[8]

Preparation of Aqueous Peel + Seed Extract

The extracts obtained from the peel and seed was taken in 1:1 ratio for the peel + seed extract.

The phytochemical screening analysis of aqueous extract of peel and seed of *Punica granatum* was conducted according to standard procedures.[9]

Experimental Design

Urolithiasis was induced in animals by administration of 0.75% v/v ethylene glycol in their drinking water for 28 days. 30 male Wistar rats were taken divided into 5 groups (n=6).

Group, I served as normal control group which received only vehicle (CMC). Group II-V received ethylene glycol (0.75% v/v) in drinking water for 28 days to induce urolithiasis. Group III received PG peel extract (200 mg/kg p.o.) daily for 28 days. Group IV received PG seed extract (200 mg/kg p.o.) daily for 28 days. Group V received PG whole fruit extract (200 mg/kg p.o.) daily for 28 days.

Analysis of Serum &Urine Markers

All animals were kept in individual metabolic cages during the experiment. The urine samples of 24hrs duration were collected and measured for calcium and phosphorus content on the last day of experiment. During the urine collection period, animals were allowed for free access to drinking water. Before storing at 4^0C, the collected urine was mixed with a drop of conc. HCl.

The animals were anesthetized on 29th day of experiment with diethyl ether. Blood was withdrawn from retro-orbital plexus and centrifuged at 10000 rpm for 15 mins to separate serum. Serum was analysed for uric acid, blood urea nitrogen (BUN) and creatinine.

Estimation of Kidney Markers &Endogenous Tissue Antioxidants

All the animals were sacrificed on 29th day of experiment by overdose of isoflurane. Kidneys were isolated and homogenised to estimate the calcium, phosphorus, and oxidative stress parameters (MDA, SOD, and CAT). SOD activity in renal tissues was assessed by the method described by Marklund & Marklund and catalase enzyme activity was measured by the method of Hadwan & Abed.[10,11] The concentration of lipid peroxides was analyzed by estimating MDA using the Ammen & Moayad procedure.[12]

Histopathological Study

Kidney tissue samples were kept in 10% formalin and fixed with paraffin wax. Small sections were stained for analysis of histopathological findings by means of light microscope.

Statistical Analysis

Data expressed as mean ± SEM (n=6) and analysed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graph Pad Prism Software (8.0 version).$P < 0.001$ and $P< 0.0001$ were considered significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

In the present study, phytochemical screening of aqueous extract of peel of *Punica granatum* L. was reported to have glycosides, alkaloids, flavonoids, saponins, tannins, phenols and carbohydrates. The aqueous extract of seed of *Punica granatum* L. was found to contain glycosides, alkaloids, flavonoids, tannins, carbohydrates and betacynins mentioned in Table 1.

Table No. 1: Phytochemical Screening of Aqueous Extracts of Peel and Seed of *Punica granatum* L.

S. No.	Test	Peel Extract	Seed Extract
1	**Glycosides** Keller Killians Test	+	+
2	**Alkaloids** Hager's Test	+	+
3	**Flavonoids** Ferric Chloride Test	+	+
4	**Saponins** Foam Test	+	-
5	**Tannins** Gelatin Test	+	+
6	**Steroids & Triterpenoids** Libermann Burchard	-	-
7	**Proteins** Biuret test	-	-
8	**Free Amino Acid** Ninhydrin Test	-	-
9	**Phenols**	+	-
10	**Carbohydrates** Benedict's Test	+	+
11	**Coumarins**	-	-
12	**Anthocyanins & Betacynins**	-	+

Effect of *Punica granatum* L. on Serum and Urine Markers

In the present study, administration of 0.75% v/v ethylene glycol through water for 28 days to male Wistar rats has significantly increased the serum uric acid, BUN and creatinine levels in ethylene glycol group. However, supplementation with aqueous extract of peel, seed and peel+seed of *Punica granatum* L. have significantly ($P< 0.0001$) lowered the elevated levels of serum uric acid, BUN and creatinine when compared to the ethylene glycol group as mentioned in Table 2.

The urine calcium and phosphorus levels were significantly increased in ethylene glycol group. However, treatment with aqueous extract of peel, seed and peel + seed of *Punica granatum* L. have significantly decreased the urine calcium and phosphorus levels indicating a decrease in the development of stones in kidneys as mentioned in Table 3.

Table No. 2: Effect of Aqueous Extracts of Peel, Seed and Peel + Seed of PG on Serum Uric Acid, BUN and Creatinine Levels

S. No.	Groups (n=6)	Uric acid (mg/dL) ± SEM	Blood Urea Nitrogen (mg/dL) ± SEM	Creatinine (mg/dL) ± SEM
1.	Normal Control	2.563 ± 0.125	59.77 ± 1.225	2.056 ± 0.055
2.	Ethylene glycol (0.75% v/v)	5.208 ± 0.164[α]	111.2 ± 9.123[α]	3.701 ± 0.104[α]
3.	Peel (200 mg/kg)	3.788 ± 0.078[a]	74.53 ± 2.482[a]	2.191 ± 0.107[a]
4.	Seed (200 mg/kg)	4.089 ± 0.133[a]	82.32 ± 1.350[b]	2.512 ± 0.150[b]
5.	Peel + Seed (200 mg/kg)	3.678 ± 0.081[a]	73.75 ± 1.483[a]	1.985 ± 0.291[a]

Values are expressed as mean ± SEM (n=6), [α]$p < 0.0001$ compared with Normal control, [a]$p < 0.0001$ and [b]$p < 0.001$ compared with EG Group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Table No. 3: Effect of Aqueous Extracts of Peel, Seed and Peel + Seed of PG on Urine Calcium and Phosphorus Levels

S. No	Groups (n=6)	Calcium (mg/24 hrs) ± SEM	Phosphorus (mg/dL) ± SEM
1.	Normal Control	0.176 ± 0.022	0.810 ± 0.091
2.	Ethylene glycol (0.75% v/v)	0.933 ± 0.047$^{\alpha}$	1.354 ± 0.027$^{\alpha}$
3.	Peel (200 mg/kg)	0.390 ± 0.006^{a}	0.833 ± 0.038^{a}
4.	Seed (200 mg/kg)	0.551 ± 0.025^{a}	1.011 ± 0.023^{b}
5.	Peel + Seed (200 mg/kg)	0.326 ± 0.027^{a}	0.845 ± 0.038^{a}

Values are expressed as mean ± SEM (n=6), $^{\alpha}p < 0.0001$ compared with Normal control, $^{a}p < 0.0001$ and $^{b}p < 0.001$ compared with EG Group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Effect of *Punica granatum* L. on Kidney Markers & Endogenous Tissue Antioxidants

The deposition of the crystalline components in the renal tissue, namely calcium and phosphorus was increased in ethylene glycol group. However, treatment with aqueous extract of peel, seed and peel + seed of *Punica granatum* L. have significantly decreased the calcium and phosphorus levels when compared to the ethylene glycol group as mentioned in Table 4.

Ethylene glycol treatment significantly ($P< 0.0001$) increased the MDA levels and decreased SOD and catalase levels in ethylene glycol induced animals compared to normal control group. The treatment with aqueous extract of peel, seed, and peel + seed of *Punica granatum* L. have significantly ($P< 0.0001$) reduced the MDA and improved the level of antioxidant enzymes like SOD and catalase levels when compared to the ethylene glycol group alone as mentioned in Table 5.

Table No. 4: Effect of Aqueous Extracts of Peel, Seed and Peel + Seed of PG on Kidney Calcium and Phosphorus Levels

S. No	Groups (n=6)	Calcium (mg/ dL) ± SEM	Phosphorus (mg/dL) ± SEM
1.	Normal Control	3.445 ± 0.279	7.268 ± 0.108
2.	Ethylene glycol (0.75% v/v)	6.467 ± 0.139[α]	11.17 ± 0.124[α]
3.	Peel (200 mg/kg)	4.406 ± 0.103[a]	8.912 ± 0.134[a]
4.	Seed (200 mg/kg)	4.681 ± 0.030[a]	10.12 ± 0.103[b]
5.	Peel + Seed (200 mg/kg)	4.098 ± 0.092[a]	9.502 ± 0.174[a]

Values are expressed as mean ± SEM (n=6), [α]p < 0.0001 compared with Normal control, [a]p < 0.0001 and [b]p < 0.001 compared with EG Group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Table No. 5: Effect of Aqueous Extracts of Peel, Seed and Peel + Seed of PG on Catalase, MDA and SOD Levels

S. No	Groups (n=6)	Catalase (mg/dL) ± SEM	MDA (mg/dL) ± SEM	SOD (mg/dL) ± SEM
1.	Normal Control	3.781 ± 0.084	1.920 ± 0.226	4.092 ± 0.136
2.	Ethylene glycol (0.75% v/v)	1.566 ± 0.144[α]	5.580 ± 0.184[α]	1.570 ± 0.154[α]
3.	Peel (200 mg/kg)	2.870 ± 0.167[a]	2.962 ± 0.156[a]	3.270 ± 0.148[a]
4.	Seed (200 mg/kg)	2.523 ± 0.160[b]	3.920 ± 0.065[a]	2.949 ± 0.164[b]
5.	Peel + Seed (200 mg/kg)	3.425 ± 0.082[a]	3.321 ± 0.218[a]	3.377 ± 0.283[a]

Values are expressed as mean ± SEM (n=6), [α]p < 0.0001 compared with Normal control, [a]p < 0.0001and [b]p < 0.001 compared with EG Group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Histopathological Studies

Histopathological analysis revealed severe tubular degeneration and inflammation in renal pelvis region in EG induced urolithiatic rats. However, the treatment with aqueous extract of PG has shown to reverse the ethylene glycol induced pathological changes observed.

DISCUSSION

The current study was planned to evaluate the aqueous extracts of peel and seed *in vivo* models of urolithiasis in rats. The phytochemical screening of peel and seed extract of *Punica granatum* L. revealed the presence of glycosides, alkaloids, flavonoids, saponins, tannins, phenols and carbohydrates.

Urolithiasis is generally the result of an imbalance between inhibitors and promoters of stone formation substances in the kidneys. Stones are generally formed in men than in women with a 70-81% recurrence rate in males than47-60% in females.[3,13] The principal approach in the treatment of urolithiasis includes NSAIDs, antimuscarinic agents, Ca^{2+} channel blockers and diuretics. However, this line of treatment leads to various impediments like intracellular acidosis, gastrointestinal disorders and musculoskeletal symptoms. The surgical treatment to remove stones includes lithotripsy, percutaneous nephrolithotomy, and ureteroscopy but, leads to acute renal injury, decreased renal function and increased stone recurrence. Thus, alternative treatment methods with phyto-therapeutic agents have become the foundation of safe medical therapy.[14]Peel extract of *Punica granatum* L. is rich in phytochemical constituents than seed extract.[15]Reported studies revealed that flavonoids, polyphenol and saponins have antioxidant and diuretic activity. Pomegranate rich in polyphenols, anthocyanins and many alkaloids causes relaxation of smooth muscles of the urinary and billiary tract that facilitate the expulsion of stones from kidneys.[16-18]

Male rats were used for *in vivo* studies as males are more prone to urolithiasis than females.[19] Urolithiasis was assessed by measuring the serum, kidney and urine biochemical parameters, oxidative stress markers and histological examination.

Administration of ethylene glycol (0.75% v/v) for 28 days leads to increased urine and kidney calcium and phosphorus levels in Group II rats. It was reported earlier that ethylene glycol causes hypercalciuria, hyperphosphaturia and hyperoxaluria leads to calcium oxalate crystals in kidney urolithiasis. The mechanism for this process may be due to an increase in the urinary concentration of oxalates. The increased urinary calcium is a factor favouring the

nucleation and precipitation of calcium oxalate from urine and subsequently crystal growth.[20,21] The aqueous extract of peel, seed and peel + seed of *Punica granatum* L. at 200 mg/kg significantly decreased the urinary calcium levels.

Increased urinary and kidney phosphorus excretion is observed in EG group seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition.[22] Treatment with aqueous extract of *Punica granatum* L. restored phosphorus to normal thus reducing the risk of stone formation.

In urolithiasis, nonprotein nitrogenous (NPN) substances such as urea, uric acid, and creatinine accumulate in the blood. In this study, the concentration of NPN substances significantly increases in the serum of EG Group rats. This suggests that the EG causes renal tubular damage and decreases Glomerular Filtration Rate (GFR).[23] Treatment with aqueous extracts of *Punica granatum* L. significantly decreased the serum levels shows anti-urolithiatic activity.

This study also revealed the increased lipid peroxidation and decreased levels of antioxidant potential in the kidneys of rats supplemented with ethylene glycol. Oxalate, the major stone forming constituent, has been reported to induce lipid peroxidation and cause tissue damage by generating ROS.[24] Treatment with aqueous extracts of *Punica granatum* L. significantly decreased MDA and increased SOD and catalase concentrations. Phenolic compound present in *Punica granatum* L. prevent the lipid peroxidation induced renal damage caused by calcium oxalate deposition in the kidney. Hence, *Punica granatum* L. prevents calcium oxalate crystal attachment as well as stone formation. These results indicate the protective effect of PG against the oxidative changes induced by ethylene glycol.

The histopathological studies revealed severe tubular degeneration and inflammation in renal pelvis region in EG induced urolithiatic rats. However, treatment with aqueous extract of *Punica granatum* L. has shown to reverse the ethylene glycol induced pathological changes.

CONCLUSION

The present study suggests that ethylene glycol induced urolithiasis is an effective model to study and evaluate various new therapeutic modalities. It also suggests that pomegranate could reduce the risk of urolithiasis and compared to seed, peel is found more effective in controlling urolithiasis. Consumption of juice of *Punica granatum* could be effective in treating urolithiasis and also could minimise the progression of stone formation and damage

to the kidneys. It also suggests that the consumption of whole fruit juice could be more effective than seed juice alone in treating urolithiasis. Further studies are required to explore the mechanism involved and dose dependent antiurolithiatic effect of aqueous extract of *Punica granatum* in treatment of urolithiasis.

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REFERENCES:

1. Sweta B, Archana NS, Tewari D. Urolithiasis: An Update on Diagnostic Modalities and Treatment Protocols. Indian Journal of Pharmaceutical Sciences. 2017;79(2):164-174.
2. Yu L, Yuntian C, Banghua L, Deyi L, Wang K, Hong L, Guohua Z. Epidemiology of Urolithiasis in Asia. Asian Journal of Urology. 2018;5:205-214.
3. Vijaya T, Sathish KM, Ramarao NV, Naredra B, Ramarao N. Urolithiasis and Its Cause – Short Review. The Journal of Phytopharmacology. 2013;2(3):1-6.
4. Anita M, Amrita A, Jayant KM, Pooja M, Preeti G, Sheweta S. Pomegranate (*Punica granatum*) – Review article. Indian Journal of Drugs. 2018;6(2):128-132.
5. Archana K, Jyotsna D, Anil K, Ashok K. Pomegranate (*Punica granatum*) – Overview. International Journal of Pharmaceutical and Chemical Science. 2012;1(4):1218-1222.
6. Debjit B, Harish G, Pragathi KB, DuraivelS, Aravind G, Sampath KPK. Journal of Pharmacognosy and Phytochemistry. 2013;1(5):28- 35.
7. Qnais EY, Elokda AS, Abu GYY, Abdulla FA. Antidiarrheal Activity of the Aqueous Extract of *Punica granatum* (Pomegranate) Peels. Pharmaceutical Biology. 2007;45(9):715-720.
8. Ghulam JK, Muhammad OO, Muhammad A, Habib UR, Zaheer UDK. Effect of *Punica granatum* (Pomegranate) Fruit Extract on Angiogenesis. J App Pharm 2013;5(3):764-780.
9. Satheesh KB, Suchetha KN, Vadisha SB, Sharmila KP, Mahesh PB. Preliminary Phytochemical Screening of Various Extracts of *Punica granatum* peel, Whole Fruit and Seeds. Nitte University Journal of Health Science. 2012;2(4):34-38.
10. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 1974;47:469-474.
11. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief 2016;6:194-199.
12. Ameen T, Moayad NM. Measurement of Serum Malondialdehyde (MDA) levels as a marker of Lipid Peroxidation in Newnatal Sepsis. Thi- Qar Medical Journal. 2011;5(2):9-17.
13. Jagannath N, Somashekara SC, Damodaram G, Devasankaraiah G. Study of antiurolithiatic activity of *Asparagus racemosus* on albino rats. Indian Journal of Pharmacology. 2012;44(5):576-579.
14. Veronika B, Khan S. Herbal Medicines in the management of urolithiasis: Alternative or Complementary. HHS Public Access. 2009;10:1095-1103.
15. Jayaprakash A, Sangeetha R. Phytochemical Screening of *Punica granatum* Linn. Peel extracts. Journal of Academia and Industrial Research. 2015;4:160-162.
16. Vidhan J, Ara DM, John RP. Anthocyanins and polyphenol oxidase from dried aerials of pomegranate (*Punica granatum* L.). Food Chemistry. 2010;118:11-16.
17. Nirumand MC, Hajialyani M, Rahimi R, Farzaei MH, Zingue S, Nabavi SM, Bishayee A. Dietary Plants for the Prevention and Management of Kidney Stones: Preclinical and Clinical Evidence and Molecular Mechanisms. Int J Mol Sci. 2018 Mar 7;19(3):765.
18. Michael T. Herbal Monograph – *Asparagus racemosus*. Phytomedicine 2000;38:119-128.
19. Bano H, Jahan N, Makbul SAA, Kumar BN, Husain S, Sayed A. Effect of *Piper cubeba* L. fruit on ethylene glycol and ammonium chloride induced urolithiasis in male Sprague Dawley rats. Integr Med Res. 2018 Dec;7(4):358-365.

20. Verma NK, Patel SS, Saleem TSM, Christina AJM, Chidambaranathan N. Modulatory effect of NONI – Herbal formulation against ethylene glycol-induced nephrolithiasis in albino rats. Journal of Pharmaceutical Sciences and Research. 2009;3:83-89.
21. Shehzad A, Saleem U, Shah MA, la Cruz CV, Khan AH, Ahmad B. Antiurolithic evaluation of *Cucurbita pepo* seeds extract against sodium oxalate-induced renal calculi. Phcog Mag. 2020;16(S1):174-80.
22. Patel PK, Vyas BA. Evaluation of Antiurolithiatic Effect of Pedalium murex Fruit Extract in Ethylene Glycol-induced Nephrolithiasis in Rat. Indian Journal of Pharmaceutical Sciences. 2016;78(2).
23. Selistre LDS, Cochat P, Rech DL, Parant F, Souza VCD, Dubourg L. Association between glomerular filtration rate (measured by high-performance liquid chromatography with iohexol) and plasma oxalate. Brazilian Journal of Nephrology. 2018;40(1):73–6.
24. Khan SR. Reactive oxygen species, inflammation and calcium oxalate nephrolithiasis. Transl Androl Urol. 2014 Sep 1;3(3):256-276.