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Research Article

ANTI-LITHIATIC EFFECT OF LYCOPENE IN CHEMICALLY INDUCED NEPHROLITHIASIS IN RATS

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ABSTRACT

Objective: The search for anti-calculi drugs from natural sources has been believed of greater importance. Hence, the present study explored the effectiveness of lycopene against experimentally induced nephrolithiasis.

Methods: The experimental study lasted for 28 days. Adult male Wistar rats were divided into six groups. Group I (Normal control) received drinking water. Group II (Disease control) received 0.75% ethylene glycol and 1% ammonium chloride in drinking water to induce nephrolithiasis. Group III–V was treated with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg, p.o.) along with 0.75% ethylene glycol and 1% ammonium chloride. Group VI treated standard (750 mg/kg, p.o.) along with 0.75% ethylene glycol and 1% ammonium chloride.

Results: The study results showed significantly high levels of urinary and serum creatinine, urea, calcium, and uric acid levels and a decrease in magnesium levels in Group II (Disease control) compared with Group I (Normal control). Treatment with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg) restored the elevated urinary and serum parameters in Group III–VI compared with Group II. Ethylene glycol administrations lead to the production of oxidative stress and decrease superoxide dismutase, reduced glutathione, and catalase activity. Lycopene treatment restored the elevated oxidative stress parameters to normal. Histologically, lycopene has alleviated the damaged integrity of the renal structure.

Conclusion: Supplementation with lycopene (100 mg/kg and 200 mg/kg) reduces and prevents the toxicity caused by ethylene glycol administration and protects the renal cells from damage.

Keywords: Ethylene glycol, Ammonium chloride, Lycopene, Nephrolithiasis.

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INTRODUCTION

Nephrolithiasis is the most-wide spread, painful, and multifactorial disorder. Nephrolithiasis is represented with a recurring rate of 50% in 5–10 years [1]. Nephrolithiasis is mainly associated with various systemic comorbidities, primarily affecting calcium metabolism, lipid metabolism, and diabetes mellitus. Calcium-containing stones are most prevalent among the patients, mainly composed of calcium phosphate and calcium oxalate (CaOx) [2].

CaOx stone is prominent toxicity of ethylene glycol, producing hyperoxaluria and inducing acute kidney injury by accumulating intrarenal CaOx deposition. CaOx deposition triggers a series of inflammatory events in renal tubule cells, triggering structural and functional damage to the tubules. Triggering oxidative stress by ethylene glycol is considered the primary source for renal injury through lipid peroxidation and depleting antioxidant defence mechanisms, which further progressed to the inflammatory response [3].

Researchers' main aim and objective are to develop drug therapy with lesser side effects that do not affect patients' quality of life. Hence, they are moving toward using an alternative treatment to cure stones. Kidney stones have been treated with a variety of herbs, including *Piper cubeba* L., *Terminalia arjuna* Roxb., *Bombex ceiba* Linn., *Borrhaavia diffusa* L., *Bryophyllum pinnatum*., *Daucus carota* Linn., and *Herniaria hirsute* Linn. Apart from this, several nutraceuticals have been used to treat nephrolithiasis, including probiotic products, fish oil, and vitamin B6 supplements [4].

Lycopene is a natural pigment in tomatoes and belongs to the carotenoid family. Lycopene's antioxidant activity protected cellular and tissue damage in various *in vivo* or *in vitro* experiments against harmful effects produced by biological reaction oxygen species (ROS) [5-9]. Further, interventional studies of lycopene against various animal models of renal diseases have been suggested the nephron protective potential of lycopene [10,11].

Concerning the nephroprotective potential of lycopene, we hypothesized that lycopene might be effective to treat nephrolithiasis conditions. There is no scientific evidence suggesting the anti-urolithiatic activity of lycopene in ethylene glycol and ammonium chloride-induced kidney stone in laboratory animals. Therefore, an attempt is made to establish the putative beneficial role of lycopene in experimentally induced nephrolithiasis in Wistar albino rats.

METHODS

Plant material

The gratis of lycopene was procured from Medheal Pharmaceuticals, Ahmedabad, India. Cystone was procured from Himalaya pharmaceuticals limited. Diagnostic kit for various biochemical analyses was procured from Coral Clinical System, Goa.

Pharmacological screening of anti-lithiatic activity

Animals selection

Male Wistar albino rats were procured from Zydus Research Centre, Ahmedabad, India. Animals were placed in polypropylene rat cages as three animals per cage per sex. The animals were adjusted to standard laboratory conditions (temperature: 25±2°C) and habituated on a 12 h light: 12 h dark cycle, and laboratory rat pellet and pure drinking water were supplied. The experimental protocol was approved by the Institutional Animal Ethics committee (Protocol No: RPCP/IAEC/2018-2019/R30).

Ethylene glycol induced nephrolithiasis model

Male Wistar albino rats weighing 200–250 g were selected. Animals were segregated into six groups, each having six animals. Group I (Normal control) served as a normal rat diet and drinking water *ad libitum*. The induction of renal calculi was carried out by supplementing ethylene glycol (0.75%) and ammonium chloride (1%) through water in Group II–Group VI for 28 days. Group II (Disease control) served as ethylene glycol (0.75%) and ammonium chloride (1%) for 28 days. Group III (50 mg/kg) received lycopene (50 mg/kg; p.o) for 28 days. Group IV (100 mg/kg) received lycopene (100 mg/kg; p.o) for 28 days. Group V (200 mg/kg) received lycopene (200 mg/kg; p.o) for 28 days. Group VI (Standard) received cystone (50 mg/kg; p.o) for 28 days. All the drugs were administered once daily through the oral route.

Assessment of anti-lithiatic activity

Collection and analysis of urine

At the end of 28 days, 24 h urine was collected and stored at 4°C. Urine was examined for creatinine, urea, and magnesium content.

Serum analysis

End of an experiment, retro-orbital blood was collected from each animal, and serum was separated for the estimation of creatinine, magnesium, urea, uric acid, and calcium content.

Kidney homogenate analysis

Animals were sacrificed using a high dose of anesthesia and kidneys were isolated. Out of two kidneys, one kidney was separated and washed in phosphate buffer (pH 7.4) and homogenized in phosphate buffer (pH 7.4). The supernatant was collected and was used to determine malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase activity [12-15].

Histopathological study

The isolated kidney was fixed in neutral buffered formalin (10%) and thereafter embedded in paraffin. The section was taken and stained by hematoxylin and eosin dye, and the prepared slides were observed under a microscope (×40) [16,17].

Statistical analysis

Differences in the mean of different experimental groups were evaluated by statistical analysis tool, one-way analysis of variance followed by Dunnett's test. The values were expressed as mean±SEM for n=6 rats of each group. $p<0.05$ was considered statistically significant.

RESULTS

Effects of lycopene on urine output and various urinary parameters in ethylene glycol-induced nephrolithiasis

On the 28th day, the urine output of all six animals was collected. The urine output of the disease group was significantly decreased ($p<0.05$) as compared with the normal control group (Fig. 1a). The treatment with lycopene (100 mg/kg, 200 mg/kg) showed a significant increase in urine output, but there was no significant increase in urine output at 50 mg/kg dose compared with the disease group. The creatinine and urea levels in urine were remarkably increased ($p<0.05$) compared to the normal group. However, treatment with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg) significantly ($p<0.05$) lowered the elevated levels of creatinine and urea (Fig. 1b and c). The excretion of magnesium was significantly ($p<0.05$) reduced compared to the normal group. The administration of lycopene (100 mg/kg, 200 mg/kg) produced a significant elevation in urinary magnesium levels as compared to the disease group ($p<0.05$) but, lycopene administration at the dose of 50 mg/kg, showed no elevation in magnesium excretion (Fig. 1d).

Effect of lycopene on various biochemical parameters

A significant ($p<0.05$) elevation in levels of creatinine, calcium, urea, uric acid, and a decrease in magnesium levels was observed in the disease group as compared with a control group. However, treatment

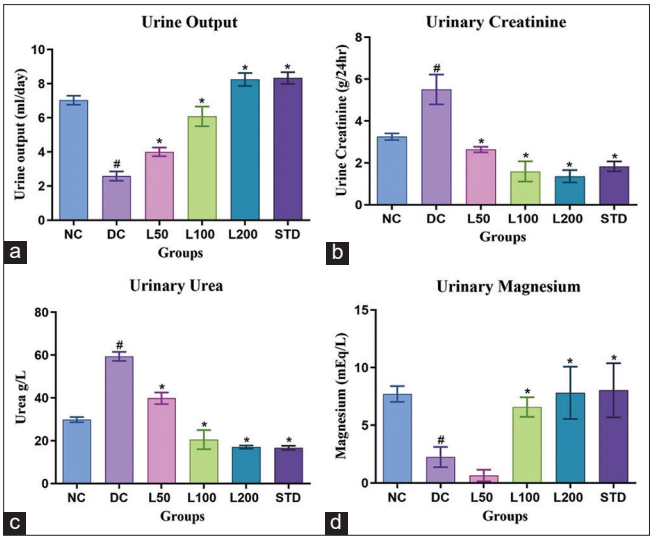

Fig. 1: Effect of lycopene on urinary renal function indices in ethylene glycol-induced nephrolithiasis. (a) Urine output, (b) urinary creatinine, (c) urinary urea, and (d) urinary magnesium. Each bar represents mean±SEM (n=6). NC: Normal Control, DC: Disease Control (EG+NH4CL), L50: EG+NH4CL along with lycopene (50 mg/kg), L100: EG+NH4CL along with lycopene (100 mg/kg), L200: EG+NH4CL along with lycopene (200 mg/kg), STD: EG+NH4CL along with cystone (750 mg/kg). #p<0.05 compared to normal group; *p<0.05 when compared with disease group

with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg), the elevated level of creatinine, calcium, urea, and uric acid was remarkably ($p<0.05$) lowered as compared with the disease group (Fig. 2a-d). While in the case of magnesium, after treatment with lycopene (50 and 100 mg/kg; p.o.), the magnesium level was restored to normal (Fig. 2e).

Effect of lycopene on oxidative markers

Ethylene glycol-induced nephrolithiasis rats exhibited higher MDA levels in tissue than normal control rats. Treatment with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg) significantly ($p<0.05$) decreased MDA level compared with disease group (Fig. 3a). Ethylene glycol administration significantly ($p<0.05$) decreased the levels of GSH, SOD, and catalase levels as compared with a normal group. However, treatment with lycopene (50 mg/kg, 100 mg/kg, and 200 mg/kg) significantly ($p<005$) elevated the levels when compared with the disease group (Fig. 3b-d).

Histopathological studies

The histopathological evaluation reported normal architecture of kidney tissue in the normal group (Fig. 4a). There is no damage to the renal tubular cells. Histopathological analysis revealed deposition of CaOx crystals in the disease group (Fig. 4b) and it also represents severe damage to the normal structure of kidney tissue. In the disease control group (Fig. 4b), the entire structure of the kidney is damaged, leading to hypercellularity of glomerulus, matrix expansion, infiltration of inflammatory cells, and cellular necrosis. However, simultaneous treatment with lycopene (50 mg/kg) (Fig. 4c) showed mild restoration of tubular cells to their normal structure. Higher doses of lycopene (100 mg/kg and 200 mg/kg) (Fig. 4d and e) lowered the damage to tubules and cellular derangement by inhibiting the inflammatory responses, reducing oxidative renal injury, and maintaining the normal functioning of the cells in comparison with the disease control group. Furthermore, the standard cystone group (Fig. 4f) lowered the inflammation of tubular cells and cellular derangement compared to the disease control group.

DISCUSSION

The limitation of modern surgical techniques and drugs used to treat nephrolithiasis pertaining to the quality of life and associated

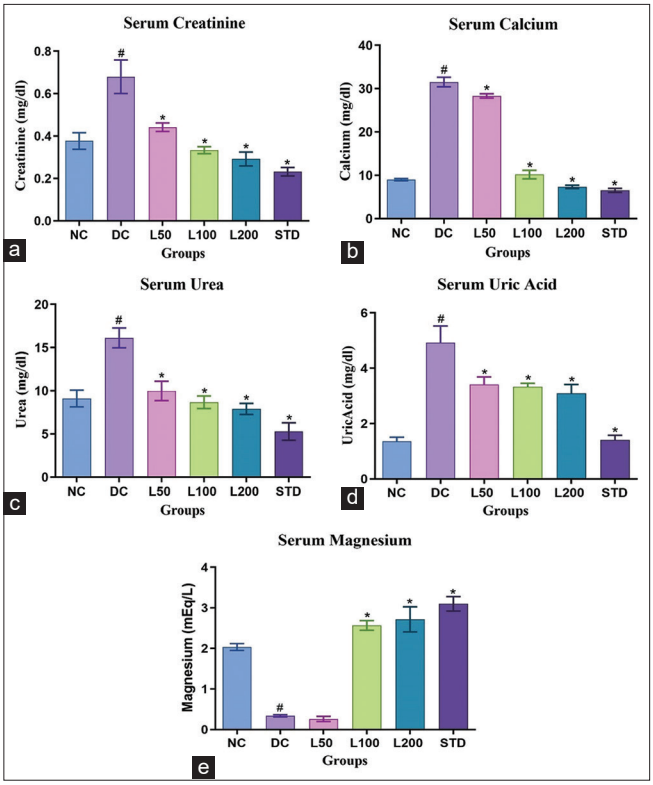

Fig. 2: Effect of lycopene on serum renal function indices in ethylene glycol-induced nephrolithiasis. (a) Creatinine, (b) calcium, (c) urea, (d) uric acid, and (e) magnesium. Each bar represents mean±SEM (n=6). NC: Normal Control, DC: Disease Control (EG+NH4CL), L50: EG+NH4CL along with lycopene (50 mg/kg), L100: EG+NH4CL along with lycopene (100 mg/kg), L200: EG+NH4CL along with lycopene (200 mg/kg), STD: EG+NH4CL along with cystone (750 mg/kg). #p<0.05 compared to normal group; *p<0.05 when compared with disease group

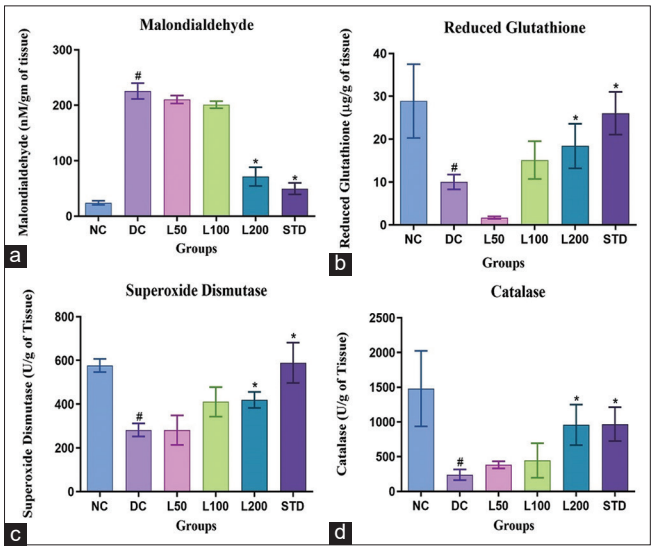

Fig. 3: Effect of lycopene on oxidative markers in ethylene glycol-induced nephrolithiasis. (a) Malondialdehyde, (b) reduced glutathione, (c) superoxide dismutase, and (d) catalase. Each bar represents mean ± SEM (n=6). NC: Normal Control, DC: Disease Control (EG+NH4CL), L50: EG+NH4CL along with lycopene (50 mg/kg), L100: EG +NH4CL along with lycopene (100mg/kg), L200: EG+NH4CL along with lycopene (200 mg/kg), STD: EG+NH4CL along with cystone (750 mg/kg). #p<0.05 compared to normal group; *p<0.05 when compared with disease group

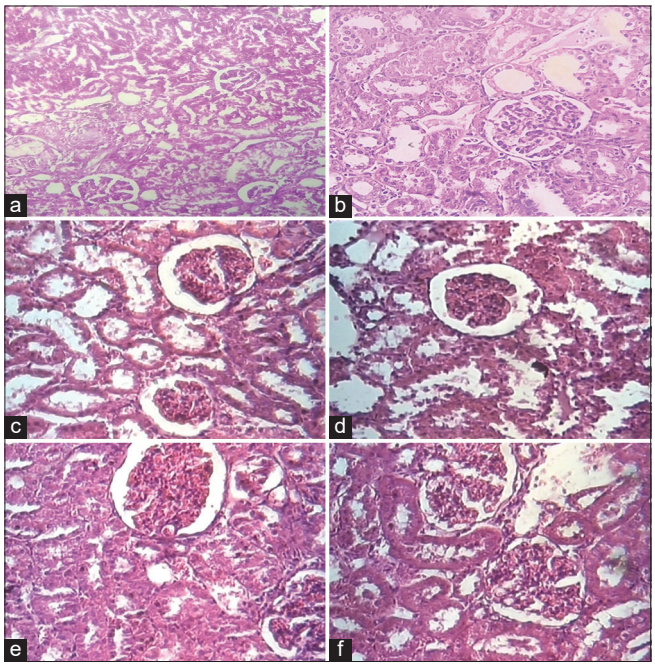

Fig. 4: Microscopic images of kidney sections (a) normal control showing the normal structure of tubular cells and glomerulus functions. (b) Disease control showing cells derangement, glomerulus hypercellularity, leukocyte infiltration, and matrix expansion. (c) Treatment with lycopene (50 mg/kg) showed less protection against toxicity. (d) Treatment with lycopene (100 mg/kg) showed changes in the inflammatory responses and tubular damages. (e) Treatment with lycopene (200 mg/kg) lowered the cellular necrosis. (f) Standard cystone (750 mg/kg) lowers the cellular damage

complications warrants the search for alternative evidence-based treatment options. Various experimental evidence suggested the natural products' usefulness in different animal models of nephrolithiasis [17]. Hence, the present study was designed to assess the probable beneficial action of lycopene against experimentally induced nephrolithiasis.

The effects of ethylene glycol and ammonium chloride were consistent with the previous findings reported elsewhere [12,16,18,19]. The addition of ethylene glycol and ammonium chloride in drinking water produced nephrolithiasis more significantly in male Wistar rats as testosterone intensifies oxalate production by the liver and promotes CaOx supersaturations and electrolytes abnormalities [17,20]. Ethylene glycol is known to absorb from the intestine and undergo significant metabolism by the liver to produce excessive oxalate, glycolaldehyde, and glycolate. Hyperoxaluric conditions precipitate oxalate in the urine in the form of a poorly soluble form of CaOx, which is likely to produce heterogeneous nucleation and produced obstruction to renal tubules by damaging the epithelial cells. Moreover, acidic urine produced by supplementation of ammonium chloride accelerates the deposition of CaOx and thereby nephrolithiasis [21].

Nephrolithiasis induced by ethylene glycol and ammonium chloride produced marked renal damage as manifested by the increase in creatinine, urea, and uric acid in blood and urine. The existence of CaOx stones in the kidney is thought to be the cause of renal damage, which obstructs urine flow and thereby reduces glomerular filtration rate (GFR), leading to the accumulation of nitrogenous waste in blood and urine. Supplementation of lycopene in nephrolithiasis rats showed significant improvement in the renal function markers in urinary and serum. The reduction in inflammation and damage of renal tissues may contribute to the recovery in GFR on therapy with lycopene. Subsequently, the increased levels are restored to normal levels [17,22].

Earlier reports suggested the occurrence of metabolic acidosis due to the metabolism of ethylene glycol into metabolic acids, which produced acid-base imbalance by the reduction in the levels of HCO_3^-. Metabolic acidosis produces hypercalcemia due to increased calcium absorption from the gut and the release of calcium from bones. The nucleation and precipitation of CaOx in hypercalcemia stimulate crystal development in the urinary system. Because of poor calcium absorption from the injured renal tubular epithelium, increased levels of oxalate in urine caused by ethylene glycol administration stimulate the production of CaOx crystals. Increased calcium deposition in renal tissues may be due to the activation of second messengers due to inflammatory mediators such as nitric oxide. In our study, treatment with lycopene effectively reduced the elevated serum calcium levels in nephrolithiasis animals, suggesting increased excretion of calcium in urine or improved calcium absorption by osteoblast may be attributed to the alleviation of damaged renal tubules [23,24].

Magnesium is one of the CaOx crystallization inhibitors. Many reports have suggested that the reduced magnesium levels in serum and urine contribute significantly to the pathogenesis of nephrolithiasis in experimental animals and patients with renal stones. Furthermore, the studies revealed that potassium magnesium citrate consumption prevented the occurrence of stone in patients and CaOx accumulation in the kidneys of Vitamin B6 deficient animals by forming a complex with oxalate, lowering supersaturation of CaOx and thus preventing the process of crystal growth and nucleation of CaOx. In this study, lycopene therapy restored magnesium excretion to near-normal levels, reducing the development of CaOx crystals [21].

Administration of ethylene glycol and ammonium chloride promotes oxidative stress to the renal tissues. Oxidative damage to the renal tissues leads to the production of superoxide, hydroxyl free radicals, oxidative stress-induced cell membrane rupture, apoptosis, and various inflammatory responses. ROS-induced oxidative stress produces MDA, one of the important oxidative markers. In the present study, the ethylene glycol control group showed increased MDA level indicates oxidative stress. Treatments with lycopene showed a decrease in the levels of MDA, while SOD, GSH, and catalase work as protectants against oxidative stress. They are present in the cell and protect the cells from oxidative damage. SOD and catalase are the enzymes that convert the superoxide radical and hydrogen peroxide into water, thereby protecting the cells from damage. In the study, in ethylene glycol control groups, there was a decrease in SOD, GSH, and catalase activity levels. SOD, GSH, and catalase levels increased after the treatment with lycopene and, subsequently, prevented the cells from oxidative stress renal injury [12,25].

The microscopic examination of the disease group tissue section showed cellular derangement, hyper-cellularity of the glomerulus, leukocyte infiltration, and cell necrosis. Treatment with a higher dose of lycopene (100 mg/kg, 200 mg/kg) notably improves tubular cells' deformation, inhibits the inflammatory responses, and protects the cells from oxidative injury [17].

Lycopene is effective in many animalnephrotoxicity models and clinical studies of renal complications. The participating mechanisms of nephroprotection are attributed to its anti-oxidant property, free radical scavenging action, chelating, and anti-apoptotic properties which may be involved in the attenuation of inflammatory response produced by ethylene glycol administration [5,9,11,26].

CONCLUSION

The results obtained from the present study conclude that lycopene is beneficial in ethylene glycol-induced renal toxicity and effectively prevents the progression of the disease. Lycopene administration (100 mg/kg, 200 mg/kg) showed a protective effect against ethylene glycol-induced nephrolithiasis by restoring creatinine, urea, magnesium, calcium, and uric acid levels in urine and serum. Further, treatment with lycopene also reduced the ethylene glycol-induced renal toxicity by preserving histoarchitecture of the renal system. The underlying biochemical mechanism of this potential effect of lycopene is mediated through antioxidant, diuretic, and nephroprotective properties. However, further investigation is required to clarify the exact mechanism of this action.

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AUTHOR'S CONTRIBUTIONS

Priyal Patel: Methodology, Investigation, Data Curation, Formal analysis, and Writing-Original Draft.

Sandip Patel: Conceptualization, Visualization, and Writing - Review and Editing.

Veena Patel: Review and editing of the manuscript

COMPETING INTERESTS

There is no conflict of interest.

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REFERENCES

1. Nirumand MC, Hajialyani M, Rahimi R, Farzaei MH, Zingue S, Nabavi SM, *et al*. Dietary plants for the prevention and management of kidney stones: Preclinical and clinical evidence and molecular mechanisms. Int J Mol Sci 2018;19765. doi: 10.3390/ijms19030765, PMID 29518971
2. Bushinsky DA, Asplin JR, Grynpas MD, Evan AP, Parker WR, Alexander KM, *et al*. Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. Kidney Int 2002;61:975-87. doi: 10.1046/j.1523-1755.2002.00190.x, PMID 11849452
3. Aggarwal D, Gautam D, Sharma M, Singla SK. Bergenin attenuates renal injury by reversing mitochondrial dysfunction in ethylene glycol induced hyperoxaluric rat model. Eur J Pharmacol 2016;791:611-21. doi: 10.1016/j.ejphar.2016.10.002, PMID 27717728
4. Nagal A, Singla RK. Herbal resources with antiurolithiatic effects: A review. Indo Glob J Pharm Sci 2013;3:6-14.
5. Kaya C, Karabulut R, Turkyilmaz Z, Sonmez K, Kulduk G, Gülbahar Ö, *et al*. Lycopene has reduced renal damage histopathologically and biochemically in experimental renal ischemia-reperfusion injury. Ren Fail 2015;37:1390-5. doi: 10.3109/0886022X.2015.1064742, PMID 26161692
6. Cohen LA. A review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. Exp Biol Med (Maywood) 2002;227:864-8. doi: 10.1177/153537020222701005, PMID 12424327
7. Heber D, Lu QY. Overview of mechanisms of action of lycopene. Exp Biol Med (Maywood) 2002;227:920-3. doi: 10.1177/153537020222701013, PMID 12424335
8. Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med 2003;24:345-51. doi: 10.1016/s0098-2997(03)00030-x, PMID 14585305
9. Matos HR, Di Mascio P, Medeiros MH. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. Arch Biochem Biophys 2000;383:56-9. doi: 10.1006/abbi.2000.2035, PMID 11097176
10. Atessahin A, Yilmaz S, Karahan I, Ceribasi AO, Karaoglu A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicology 2005;212:116-23. doi: 10.1016/j.tox.2005.04.016, PMID 15946783
11. Karahan I, Ateşşahin A, Yilmaz S, Çeribaşi AO, Sakin F. Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicology 2005;215:198-204. doi: 10.1016/j.tox.2005.07.007, PMID 16125832
12. Sridharan B, Michael ST, Arya R, Roopan SM, Ganesh RN, Viswanathan P. Beneficial effect of Citrus Limon peel aqueous methanol extract on experimentally induced urolithic rats. Pharm

Biol 2016;54:759-69. doi: 10.3109/13880209.2015.1079724, PMID 26452728

13. Cand F, Verdetti J. Superoxide dismutase, glutathione peroxidase, catalase, and lipid peroxidation in the major organs of the aging rats. Free Radic Biol Med 1989;7:59-63. doi: 10.1016/0891-5849(89)90101-9, PMID 2753396
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810
15. Veena CK, Josephine A, Preetha SP, Rajesh NG, Varalakshmi P. Mitochondrial dysfunction in an animal model of hyperoxaluria: A prophylactic approach with fucoidan. Eur J Pharmacol 2008;579:330-6. doi: 10.1016/j.ejphar.2007.09.044, PMID 18001705
16. Makasana A, Ranpariya V, Desai D, Mendpara J, Parekh V. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats. Toxicol Rep 2014;1:46-52. doi: 10.1016/j.toxrep.2014.03.006, PMID 28962225
17. Bano H, Jahan N, Makbul SA, 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;7:358-65. doi: 10.1016/j.imr.2018.06.005, PMID 30591890
18. El Menyiy N, Al Waili N, Bakour M, Al-Waili H, Lyoussi B. Protective effect of propolis in proteinuria, crystaluria, nephrotoxicity and hepatotoxicity induced by ethylene glycol ingestion. Arch Med Res 2016;47:526-34. doi: 10.1016/j.arcmed.2016.12.010, PMID 28262194
19. Shah JG, Patel BG, Patel SB, Patel RK. Antiurolithiatic and antioxidant activity of *Hordeum vulgare* seeds on ethylene glycol-induced urolithiasis in rats. Indian J Pharmacol 2012;44:672-7. doi: 10.4103/0253-7613.103237, PMID 23248392
20. Partovi N, Ebadzadeh MR, Fatemi SJ, Khaksari M. Antilithiatic effect of aqueous and ethanolic extracts of cactus prickly pear in chemically induced urolithiasis in rats. Toxin Rev 2018;37:166-70. doi: 10.1080/15569543.2017.1325906
21. Pawar AT, Vyawahare NS. Protective effect of standardized extract of *Biophytum sensitivum* against calcium oxalate urolithiasis in rats. Bull Fac Pharm Cairo Univ 2015;53:161-72. doi: 10.1016/j.bfopcu.2015.10.002
22. Alenzi M, Rahiman S, Tantry BA. Antiurolithic effect of olive oil in a mouse model of ethylene glycol-induced urolithiasis. Investig Clin Urol 2017;58:210-6. doi: 10.4111/icu.2017.58.3.210, PMID 28480348
23. Ardawi MS, Badawoud MH, Hassan SM, Rouzi AA, Ardawi JM, AlNosani NM, *et al*. Lycopene treatment against loss of bone mass, microarchitecture and strength in relation to regulatory mechanisms in a postmenopausal osteoporosis model. Bone 2016;83:127-40. doi: 10.1016/j.bone.2015.10.017, PMID 26549245
24. Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of *Acorus calamus* L. rhizome in experimental animal models. J Tradit Complement Med 2016;6:431-6. doi: 10.1016/j.jtcme.2015.12.004, PMID 27774431
25. Ghaeni FA, Amin B, Hariri AT, Meybodi NT, Hosseinzadeh H. Antilithiatic effects of crocin on ethylene glycol-induced lithiasis in rats. Urolithiasis 2014;42:549-58. doi: 10.1007/s00240-014-0711-y, PMID 25173352
26. Hedayati N, Naeini MB, Nezami A, Hosseinzadeh H, Hayes AW, Hosseini S, *et al*. Protective effect of lycopene against chemical and natural toxins: A review. Biofactors 2019;45:5-23. doi: 10.1002/biof.1458, PMID 30339717