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EVALUATION OF ANTI-UROLITHIATIC EFFECT OF AQUEOUS EXTRACT OF PARSLEY (*PETROSELINUM SATIVUM*) USING ETHYLENE GLYCOL-INDUCED RENAL CALCULI

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ABSTRACT

Objective: The present study was undertaken to evaluate the antiurolithiatic effect of *petroselinum sativum* (Parsley) at a dose (25 mg /kg body weight /day) on ethylene glycol induced renal calculi in male albino rats and was compared with cystone as reference drug at a dose (750mg/kg body weight /day). Urolithiasis was induced by ethylene glycol (0.75% v/v) and ammonium chloride (1% w/v) in drinking water. **Methods:** Animals were divided into five groups; namely, healthy control rats, Lithiatic control rats, Cystone treated, preventive regimen and curative regimen. **Results:** Treatment with the parsley aqueous extract (preventive or curative regimen)restored all the elevated biochemical parameters including serum and urine (calcium, oxalate, phosphate , protein , creatinine and BUN).In addition, parsley aqueous extract groups restored the urine pH to normal and significantly increased the urine volume and magnesium ($P < 0.05$) associated with improved in renal function. Lipid peroxidation in the kidneys was increased following the lithogenic treatment; however, preventive and curative regimens with parsley reduced the elevated levels of MDA, this evident attributed with the increased kidney reduced glutathione and antioxidant enzymes. Microscopic and histopathological examinations confirmed the above results and revealed that the potential prophylactic and curative parsley aqueous extract on inhibition renal stone associated with decreased calcium kidney content. **In conclusion:** Results of this study suggest the presence of antiurolithic effects in parsley against calcium oxalate stones mediated through a combination of antioxidant, diuretic, urinary alkalinizing and hypocalciuric effects.

KEYWORDS: stones, antiurolithiatic, Parsley, antioxidant, diuretic, Cystone.

INTRODUCTION

Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys.[1] Even though the technological developments in the present medical practice the formation and growth of renal calculi continues to afflict human kind. Though various kinds of stone have been identified, calcium stones are the most common in human as well as rats.[2] Urinary stone disease is a common disorder estimated to occur in approximately 12% of the world population, with a recurrence rate of 70-81% in males and 47- 60% in females.[3]

The recurrence of urolithiasis represents a major problem as patients who have formed one stone are more likely to form another. The standard drugs used to prevent urolithiasis are not effective in all patients, and many of them have adverse effects that compromise their long term use.[4] The present day management of nephrolithiasis with open renal surgery is unusual and rarely used only since the introduction of Extracorporeal Shock Wave Lithotripsy (ESWL) which has almost become the standard procedure for eliminating kidney stones.[5]

However, in addition to the traumatic effect of shockwaves, persistent residue stone fragments and the possibility of infection suggests that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence.[6] Hence the search for antilithiatic drugs to be effective without side effects from natural sources has gained great potential.[7,8] Certain herbs such as parsley might help maintain kidney health. The *petroselinum sativum* or parsley, a member of the family of Umbelliferae, have been reported to be anti-inflammatory, antiedema antihypertensive, antidiabetic, antimicrobial ,laxative in digestive tract, antioxidant, balance enzyme activities, increase glutathione in the kidney and reconstruct kidney tissue after nephrotoxicity.[9,10]

Parsley tea is essentially made with the green leaves and is one of the medicinal herbs. Parsley is rich with an antioxidant arsenal that includes glycoside a flavonoids such as apiine, apiol, saponins, myristisine, alkaloids that searches out and eradicates free radicals in the body that cause oxidative stress in cells.[11,112] The present study was planned to evaluate the antilithiatic activity of aqueous extract of Parsley on ethylene glycol induced calcium oxalate urolithiasis in male rats.

2. MATERIALS AND METHODS

Chemicals

• Cystone : Each tablet contains 223 mg of cystone - (Multipharma 6C Taksim Asmaa Fahmy, Heliopolis, Cairo, Egypt.) as standard antiurolithiatic drug. Cystone drug was dissolved in distilled water at a dose 750mg /kg body weight of rat using a stomach tube.[13]

• All other chemicals (Ethylene glycol and ammonium chloride) were purchased from El-Gomhouria Company, Cairo, Egypt and were of analytical grade.

• Kits used for the determination of biochemical parameters were obtained from Bio diagnostic company, Cairo, Egypt.

Plant materials

The fresh parsley was obtained from local market in Cairo. The fresh parsley was washed with tap water, chopped into small pieces, dried with hot air oven (40–60°C) and grinded to powder.[10] The aqueous extract was prepared by boiling 5 g of parsley powder in 100 ml of distilled water for 10 min and left for 15 min to infuse then cooled and filtered before use to remove particular matter. parsley extract was given at a dose of (25 mg /kg body weight /day) according to Nabila.[12]

Animals

Thirty healthy male adult albino rats Spargue- Dawley strain were obtained from Laboratory Animal Colonies, Helwan, Egypt. The average weight was 150±10g. They were maintained under standard conditions of temperature, humidity and light (12 h dark, 12 h light) and provided with standard commercial pellets diet and having free access to water.

Methodology

Stone induction

Ethylene glycol plus ammonium chloride model was used to induce urolithiasis.[14] Kidney stone formation were induced in rats by , comprised of 0.75 % v/v ethylene glycol and 1 % w/v ammonium chloride in drinking water ad libitum for 3 days. Following this, treatment was switched to only 0.75 % ethylene glycol for 25 days.

Experiment design

Rats under study were randomly divided into five groups (6 rats each):

Group I (healthy control rats): Rats in this group were considered as control and received only distilled water 1ml/100 g body weight once a day for 28th.

Group II (Lithiatic control rats): Rats in this group were received ethylene glycol (0.75 % v/v) and ammonium chloride (1 % w/v) in drinking water as mentioned before from 1st day till 28th day for the induction of kidney stone .

Group III (Cystone treated) : Rats in this group were treated with ethylene glycol and ammonium chloride in drinking water as mentioned before, then orally treated with standard antiurolithiatic drug, cystone (750mg/kg body weight) once daily using gastric tube from 15th day till 28th day.

Group IV (preventive regimen) : Rats in this group treated with ethylene glycol and ammonium chloride in drinking water as mentioned before with orally administered parsley aqueous extract (25 mg /kg body weight) once daily using gastric tube from 1st day till 28th day as Preventive group .

Group V (curative regimen) : Rats in this group treated with ethylene glycol and ammonium chloride in drinking water as mentioned before then orally treated with parsley aqueous extract (25 mg /kg body weight) once daily using gastric tube from 15th day till 28th day as curative group.

Collection and analysis of urine

On day 28 animals of all the groups were kept in metabolic cages and urine samples were collected for 24h . Animals had free access to water during the urine collection period. The urinary volume and pH was determined.[15] A drop of concentrated HCl was added to the urine before being stored at 4°C. Urine was analyzed for calcium, magnesium, oxalate, inorganic phosphate, protein and creatinine using standard methods.[16-20] The serum creatinine levels and urinary output volumes of all groups were also noted. Creatinine clearance (CC) was calculated using the formula[20]

$$CC\ (ml/min) = \frac{(\text{mg creatinine /dl urine}) \times (\text{ml urine / 24h})}{(\text{mg creatinine /dl serum}) \times 1440}$$

The sample was also subjected to microscopic examination for calcium oxalate crystals and for the confirmation of urolithiasis.

Serum analysis

After the 28 day experimental period, the animals were sacrificed. Blood samples were collected via cardiac puncture. Blood was allowed to stand for 15 minutes at temperature of 37°c, then was centrifuged at 4000 rpm for 20 min by EBA8 centrifuge (obtained from china)

for the separation of serum. The serum collected and analyzed for level of calcium, creatinine and blood urea nitrogen (BUN) concentrations.[21, 20,22]

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was finely minced and 10 % homogenate was prepared in Tris-Hcl buffer (0.02 mol/l, pH 7.4). The homogenate was used for measurement of various biochemical parameters.

Determination of kidney oxidative stress markers

Oxidative stress markers were measured in kidney tissue homogenates included super oxide dismutase (SOD)[23] activity, Glutathione Peroxidase (GSPx) activity[24], reduced glutathione (GSH) content[25] and malondialdehyde (MDA)[26] concentration.

Determination of Kidneys Calcium

The left kidneys were removed from the rats for calcium determination. The kidneys were dried in an oven at 100°C .Each kidney was then weighed and subsequently minced in a beaker containing 7 mL of 0.5 N nitric acid , The mixture was then heated until the liquid became transparent. After calibration using the standard calcium solution, the calcium content was determined by atomic absorption spectroscopy. The calcium content of the kidney was expressed as mg/g wet tissue of the kidney.[27]

Histopathological Examination

kidney morphology were assessed by light microscopy. The other Part of right kidney was sliced and tissue was fixed in 10% buffered-neutral formalin for 6 hours. Fixed kidney tissue was processed and embedded in paraffin. Sections of 4 mm in thickness were subjected to Hematoxylin and Eosin (H&E) staining before examination.

Statistical analysis

The data were statistically analyzed by Statistical Package for Social Science (SPSS) version 17.0 statistical packages. Values were presented as mean ± standard deviation (S.D.). Statistical differences between groups were performed using one way ANOVA, the mean difference was significant at the ($p<0.05$) level.

3. RESULTS

The urinary output of the control and experimental rats on day 28 are shown in table1. The urinary volume of normal rats was 3.25±0.40 ml/day/rat whereas in the ethylene glycol alone treated rats it was statistically reduced (P<0.05) 1.57±0.22 ml/day/rat. However in the parsley extract treated (curative or preventive regimens) groups, the urinary outputs increased significantly (P<0.05) to 5.27±0.48 and 5.73±0.22 ml/day/rat respectively. In addition, Table 1 shows the pH reading of the lithiatic control group, which was acidic. The acidity was significantly reduced ($P < 0.05$) in cystone and parsley extract treated groups.

The chronic administration of 0.75%v/v ethylene glycol aqueous solution to male rats resulted in hyperoxaluria. And the calcium, Oxalate, phosphate and protein excretion were grossly increased. However, supplementation with cystone or parsley extract regimens significantly (P<0.05) lowered the elevated levels of calcium, oxalate, phosphate and protein excretion in urine when compared to lithiatic control group (Table 2).

Contrary to this, in lithiatic control group the magnesium excretion was gradually decreased following ethylene glycol treatment. Subsequent administration of cystone or parsley extract enhanced significantly (P<0.05) the magnesium excretion in cystone and both regimens of parsley extract as compared to lithiatic control group (Table 2).

The data on serum analysis showed significant Increase (P<0.05) in creatinine , BUN and Calcium levels in lithiatic control rats when compared to normal rats. The parsley extract treated groups (curative or preventive regimen) were restored serum creatinine ,BUN and Calcium levels to normal limits (Table3). Similarly, cystone treated group evident an improved in previous serum parameters as compared to lithiatic control rats .Furthermore, The creatinine clearance of lithiatic control rats were decreased, but it was improved significantly (P<0.05) in cystone and parsley extract treated groups.

Lithiatic control rats showed a significant decrease in kidney SOD, GSP_X and GSH (P<0.05) and a significant increase in kidney MDA (P<0.05) while other treated groups which consumed parsley either preventive or curative regimen showed a significant increase in kidney SOD, GSP_X and GSH (P<0.05) and a significant decrease in kidney MDA (P<0.05) compared with lithiatic control group. Similarly, cystone treated group showed a significant increase in kidney SOD, GSP_X and GSH (P<0.05) and a significant decrease in kidney MDA(P<0.05) compared with lithiatic rats. The highest values of kidney SOD, GSP_X and

GSH were recorded in parsley preventive regimen compared with other treated groups as shown in Table 4.

Kidney content of calcium is shown in Table 5 for the different experimental groups. It is significantly ($P < 0.05$) higher in lithiatic control group when compared to healthy control rats. However in the cystone and parsley extract treated groups (curative or preventive regimens), kidney content of calcium significantly decreased ($P<0.05$) and were comparable to healthy control rats.

The microscopic examination of urine samples for the studied groups shows high density of calcium oxalate crystals in the lithiatic control rats that was completely absent in both parsley extract regimens (preventive or curative) and cystone treated group as compared to healthy control rats (Figure 1). The pictures were randomly selected due to the similarity of results.

Histopathological studies of kidneys clearly revealed that the tissue samples from the control group shows tubules with single epithelial lining along the margin and were of normal size. In lithiatic control, all the tubules showed the presence of crystals calcium oxalate CaOx, there was marked dilation of the tubules and total degeneration of epithelial lining with infiltration of inflammatory cells in to the interstitial space. But kidney specimen from cystone and parsley extract treated groups (curative and preventive regimen) showed characters similar to the normal control group (Figure 2).

Table 1: Effect of parsley aqueous extract on pH and Urine Volume of different groups of rats .

Parameters / Groups	Urine pH	Urine volume (ml)
Healthy control rats	**8.15±0.16** [a]	**3.25±0.40** [c]
Lithiatic control rats	**5.88±0.27** [c]	**1.57±0.22** [d]
Cystone treated	**7.70±0.18** [b]	**5.20±0.39** [b]
Preventive regimen	**7.98±0.28** [a]	**5.73±0.22** [a]
Curative regimen	**7.67±0.25** [b]	**5.27±0.48** [b]

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same Column (P<0.05)

Table2: Effect of parsley aqueous extract on Urinary biochemical parameters of different groups of rats .

Parameters / Groups	Calcium (mg/day)	Oxalate (mg/day)	Phosphate (mg/day)	Magnesium (mg/day)	Protein (mg/day)
Healthy control rats	4.82±0.26 ^{c}	0.53±0.08 ^{b}	5.84±0.31 ^{e}	0.98±0.05 ^{a}	3.02±0.15 ^{e}
Lithiatic control rats	10.78±1.04 ^{a}	2.56±0.41 ^{a}	11.38±0.43 ^{a}	0.46±0.04 ^{d}	7.03±0.21 ^{a}
Cystone treated	5.70±0.72 ^{b}	0.72±0.09 ^{b}	7.09±0.07 ^{c}	0.88±0.03 ^{bc}	3.59±0.16 ^{c}
Preventive regimen	5.45±0.68 ^{bc}	0.63±0.04 ^{b}	6.72±0.25 ^{d}	0.92±0.03 ^{b}	3.34±0.12 ^{d}
Curative regimen	6.21±0.69 ^{b}	0.75±0.06 ^{b}	7.45±0.27 ^{b}	0.85±0.03 ^{c}	3.88±0.27 ^{b}

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same Column (P<0.05)

Table 3: Effect of parsley aqueous extract on urinary and serum parameters of different groups of rats .

Parameters / Groups	Serum Creatinine (mg/dl)	Creatinine clearance (ml/min)	Serum BUN (mg/dl)	Serum Calcium (mg/dl)
Healthy control rats	0.58±0.03^{b}	0.66±0.02^{a}	24.33±2.82 ^{b}	8.48±0.19 ^{a}
Lithiatic control rats	1.99±0.15^{a}	0.05±0.02^{d}	35.52±1.40 ^{a}	6.25±0.10 ^{d}
Cystone treated	0.64±0.06^{b}	0.56±0.02^{b}	25.54±2.01 ^{b}	7.56±0.29 ^{c}
Preventive regimen	0.63±0.06^{b}	0.58±0.01^{b}	24.32±2.34 ^{b}	8.00±0.17 ^{b}
Curative regimen	0.66±0.06^{b}	0.52±0.01^{c}	25.70±1.39 ^{b}	7.43±0.12 ^{c}

Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same Column (P<0.05)

Table 4: Effect of parsley aqueous extract on some oxidative stress status of different groups of rats .

Parameters / Groups	MDA- Kidney (nmol/g tissue)	GSH- Kidney (mg/g tissue)	SOD-Kidney (U/g tissue)	GSP_X- Kidney (U/g tissue)
Healthy control rats	14.56±1.20 ^{c}	24.13±1.13 ^{a}	15.95±0.52 ^{a}	6.72±0.38 ^{a}
Lithiatic control rats	30.14±2.23 ^{a}	12.60±1.91 ^{c}	8.10±0.80 ^{d}	3.47±0.42 ^{d}
Cystone treated	16.73±0.65 ^{b}	22.77±1.22 ^{ab}	14.97±0.27 ^{bc}	6.06±0.21 ^{bc}
Preventive regimen	15.66±0.62 ^{bc}	23.11±1.03 ^{ab}	15.37±0.65 ^{ab}	6.33±0.32 ^{ab}
Curative regimen	16.72±0.37 ^{b}	21.66±0.72 ^{b}	14.61±0.51 ^{c}	5.81±0.37 ^{c}

Values are expressed as means ± S.D, n=6

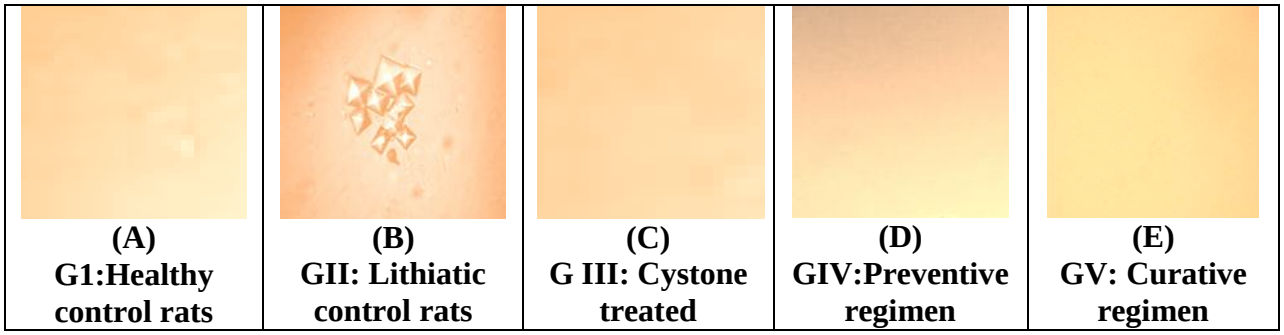
There is no significant difference between means have the same letter in the same Column (P<0.05)

Table 5: Effect of parsley aqueous extract on Contents of calcium in dry kidney tissue of different groups of rats

Parameters / Groups	Kidney Calcium (mg/g)
Healthy control rats	0.12±0.03 [c]
Lithiatic control rats	0.72±0.11 [a]
Cystone treated	0.18±0.03 [b]
Preventive regimen	0.17±0.02 [bc]
Curative regimen	0.20±0.02 [b]

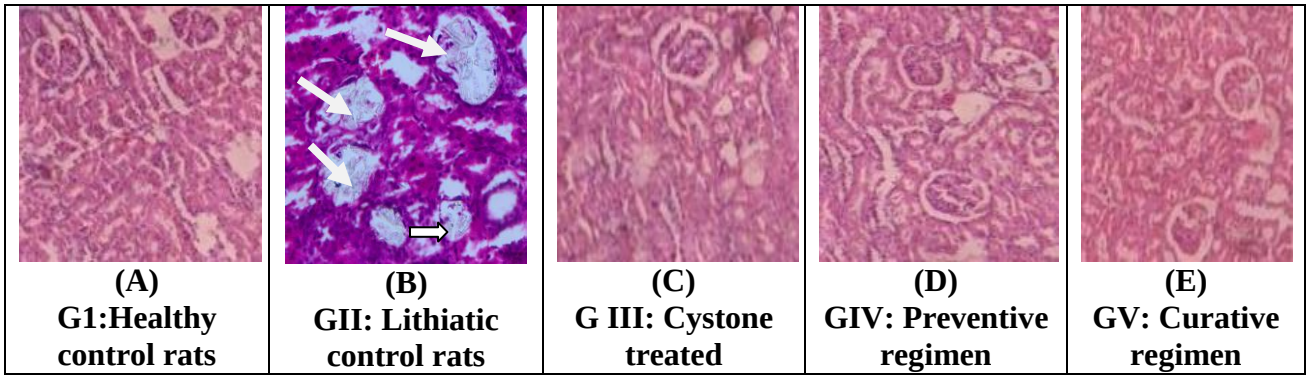
Values are expressed as means ± S.D, n=6

There is no significant difference between means have the same letter in the same Column (P<0.05)



(A) G1:Healthy control rats | **(B) GII: Lithiatic control rats** | **(C) G III: Cystone treated** | **(D) GIV:Preventive regimen** | **(E) GV: Curative regimen**

Fig.1: Microscopic examination of urine samples of Healthy and urolithiatic groups. (x40)



(A) G1:Healthy control rats | **(B) GII: Lithiatic control rats** | **(C) G III: Cystone treated** | **(D) GIV: Preventive regimen** | **(E) GV: Curative regimen**

Fig. 2. Histopathology of kidney tissues of Healthy and urolithiatic rats groups, stained with (H&E-x40)

4. DISCUSSION

It has been well documented that calculi disease affects 12% of the world population. Herbal drugs claim many promising remedies in urolithiasis.[2] In the present study, hyperoxaluria was induced in rats by employing ethylene glycol (EG) and ammonium chloride (AC) in drinking water orally for 28 days. Studies indicate that oral administration of EG (a metabolic precursor of oxalate), induce oxalate lithiasis in rats by being converted to endogenous oxalic acid in the liver and AC when ingested, induce urinary acidification, thus favoring adhesion and retention of calcium oxalate CaOx crystals within the renal tubules.[12] Supersaturation of

urine with calcium oxalate (CaOx), the most common component of kidney stones is an important factor in crystallization and enhanced urinary creatinine levels are indicators of renal impairment.[27]

The type of stone formed in human subjects can be predicted from the pH of the fasting urine. Crystalluria is pH dependent, thus by changing urinary pH, dissolution of calculi can be attained. Urinary pH of 5.0-6.5 promotes mostly CaOx type of stones.[15] The calcium salts are insoluble at physiological pH inducing (CaOx) nephrolithiasis. In the present study, the decrease in urine pH from 8.15±0.16 (Group I) to 5.88±0.27 (Group II), supports the formation of CaOx type of stones. In the cystone group and parsley aqueous extract administered rats (preventive or curative groups), restored urinary pH to normal limits, indicates prevention of CaOx stone formation which was evident through the marked decrease in urinary excretion of calcium and oxalate. The mechanism of parsley extract on nephrolithiasis related to increased dieresis via an inhibition of the $Na^+ – K^+$ pump in renal epithelial cell.[11] It probably that the parsley extract can disperse Caox crystals in the solution and eliminate them from the kidney easily.

Diuretic action is also needed to increase the amount of fluid going through the kidneys and flush out the deposits. Increase in urine volume decreases the saturation of the salts and prevents the precipitation of the crystals at physiological pH[15] . In the treatment of kidney stones, plants are used as antilithics either to dissolve the stones or to aid their passing to guard against further retention. There are reports in the literature attributing the antilithiatic activity to the diuretic property of the plants.[7,8] In our study , the urinary output was markedly decreased in lithiatic control rats on day 28, however in cystone group and preventive or curative regimens the urinary volumes were increased when compared to that lithiatic Group (table2). This suggested that parsley extract might have mild diuretic effect. Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic control group while in cystone , curative and preventive groups these levels were significantly decreased ($P<0.05$) . Elevated levels of calcium and oxalate in urine and even its retention in kidney may be one of the causative factors for the peroxidative degeneration of renal epithelia.

Thus, the antiurolithiatic activity of *Petroselinum sativum* may be due to its diuretic activity which is attributed to the presence of high percentage of flavonol glycoside and to the presence of saponins.[11]

On contrary to this the magnesium level was decreased in lithiatic group while in Cystone , curative and preventive groups those levels were increased significantly (P<0.05) (table2). Olivier et al. [28] and Celik et al.[29] have shown that magnesium deficiency accelerates renal stone formation in rats and administration of magnesium results in prevention of calcium oxalate crystallization in their kidneys. These findings indicate that rats supplemented with parsley extract were mostly recovered from nephrolithiasis. Magnesium complexes with oxalate, thus reducing Caox super saturation in urine.[30] Prevention or curative with aqueous extract of parsley reduced the level of serum calcium in rats. This indicates that parsley materials mainly glycoside flavonoids act as inhibiting some steps of oxalate synthesis from glycolic acid.[8]

In urolithiasis, the glomerular filtration rate (GFR) decreases, due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and BUN get accumulated in blood. The markedly elevated serum levels of BUN, creatinine and calcium in stone-forming animals are indicative of prominent necrosis of renal epithelia.[31] On treatment with aqueous extract of parsley(preventive or curative) the elevated serum levels of calcium, creatinine and BUN were significantly (P<0.05) reduced and comparable to those of cystone treated animals (Table 3). The creatinine clearance was also found to be improved.

In addition, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with ethylene glycol and ammonium chloride. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane. In calculi-induced rats (Group II, Table 4). Renal cellular exposure to oxalate (Ox) and/or CaOx crystals leads to the production of Reactive Oxygen Species (ROS), development of oxidative stress followed by injury and inflammation[32]. Renal injury and inflammation appear to play a significant role in stone formation. An over production of ROS and a reduction in cellular antioxidant capacities, due to down-regulated expression of the antioxidant enzymes (superoxide dismutase and glutathione peroxidase) as well as radical scavengers (reduced glutathione) leads to the development of Oxidative Stress (OS) followed by renal cell injury and inflammation due to lipid peroxidation. Loss of membrane integrity subsequently facilitates the retention of calcium oxalate crystals and growth of stones in renal tubules[29]. In the present study, the increased concentration of MDA as well as reduced activities of SOD

and glutathione peroxidase with depletion reduced glutathione levels in the Lithiatic control rats is a manifestation of facilitated lipid peroxidation and over production of free radicals resulting in renal cell injury . Recent studies evidenced that treatment with anti-oxidants and free radical scavengers reduced CaOx crystal induced renal injuries[31]. These antioxidant therapies restore the activity of antioxidant enzymes and free radical scavengers. Therefore, treatments with natural antioxidants and free radical scavengers, seems to be possible therapeutic strategy for ameliorating hyperoxaluria induced oxidative stress and renal cell injury in urolithiasis[30].

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies. It is a ubiquitous compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract[33]. In animal cells, glutathione acts as a substrate for glutathione peroxidase, which reduces lipid peroxides that are formed from polyunsaturated fatty acids (PUFA) in the diet and as a substrate for glutathione-S transferase, which conjugates electrophilic compounds. Many evidences showed that glutathione obtained from the diet is directly absorbed by the gastrointestinal tract and thus dietary glutathione can readily increase the antioxidant status in humans. However, treatment with the aqueous extract of parsley prevents oxalate induced lipid peroxidation and causes regeneration of renal epithelium owing to its antioxidant potential. Decreased levels of BUN and creatinine may be attributed to its antioxidant potential[12] . In the present study, the significant reduction of MDA coupled with marked increase in the GSH levels and activity of SOD and glutathione peroxidase in rats administrated with parsley aqueous extract is an obvious indication of antiperoxidative potential and thus antioxidative potential.

On other hand, Kidney deposits of CaOx crystals were also significantly lower in groups treated with cystone and parsley aqueous extract when compared to lithiatic control group. The histopathological study supported these results. The kidney sections from lithiatic control rats which was treated with ethylene glycol and ammonium chloride showed extensive hypertrophy and large crystalline deposits in most parts of the kidney but those from treated rats were apparently of near normal architecture to healthy control rats. All these observations enabled us to confirm the inhibitory and curative potential of parsley extract on ethylene glycol induced lithiasis.

CONCLUSION

In conclusion, the aqueous extract of parsley has both prophylactic as well as curative property in urolithiasis of rats, the prophylactic effect of parsley aqueous extract is stronger than its curative effect. These finding , thus prompt the necessity for further study to carry out the antilithiatic effect of *Petroselinum sativum* by isolation of constituents and find out the actual constituent that active against stone formation. By which more effective treatment for lithiasis with *Petroselinum sativum* can be achieved.

REFERENCES

1. Gandhi M, Aggarwal M, Puri S, Singla SK. Prophylactic effect of coconut water (*Cocos nucifera* L.) on ethylene glycol induced nephrocalcinosis in male wistar rat. Int Braz J Urol., 2013; 39: 108-17.
2. Fauzia Y, Muhammad A, Waqar.. Effect of indigenous plant extracts on calcium oxalate crystallization having a role in urolithiasis. Urol Res, 2011; 39: 345-350.
3. Atef M and Al-Attar.. Antilithiatic Influence of Spirulina on Ethylene Glycol-Induced Nephrolithiasis in Male Rats. Am J Biochem Biotechnol, 2010; 6: 25-31.
4. Wiessner J., Hasegawa A., Hung L., Mandel G., and Mandel N. Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. Kidney International., 2001; 59: 637–644.
5. Kishimoto T, Yamamoto K, Sugimoto T, Yoshihara H, Maekawa M. Side effects of extracorporeal shock-wave exposure in patients treated by extracorporeal shock-wave lithotripsy for upper urinary tract stone. European Urology., 1986; 12: 308– 313.
6. Begun FP, Knoll CE, Gottlieb M, Lawson RK. Chronic effects of focused electro hydraulic shock-waves on renal function and hypertension. The Journal of Urology., 1991; 145: 635–639.
7. Shirfule AL, Racharla V, Qadri SS, Khandare AL. Exploring antiurolithic effects of gokshuradi poly herbal ayurvedic formulation in ethylene-glycolinduced urolithic rats. Evid Based Complement Alternat Med., 2013; 2013: 763720.
8. Bahareh A., Hanieh M. F., Alireza T. H., Naser T. M.and Hossein H. Protective effects of the aqueous extract of crocus satIvus against ethylene glycol induced nephrolithiasis in rats. EXCLI Journal., 2015; 14: 411-422.
9. Wahba N., Ahmed A. ,and Ebraheim Z.. Antimicrobial Effects of Pepper, Parsley, and Dill and Their Roles in the Microbiological Quality Enhancement of Traditional Egyptian Kareish Cheese. Food borne Pathog., 2009; Dis. Nov 17.

10. Nielsen, S.E., J.F. Young, B. Daneshvar, T.S. Lauridsen, P. Knuthsen, B. Sandstrom and L.O. Dragsted, 1999. Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. British J. Nutr., 1999; 81: 447-455.
11. Sawsan I. and Julnar U.2002. Diuretic effect and mechanism of action of parsley.Journal of Ethnopharmacology., 2002; 79: Issue 3.
12. Nabila M. Rashwan. Biological Study on the Effect of Arginine and Parsley on Renal Toxicity in Rats World Journal of Medical Sciences., 2012; 7(4): 264-269.
13. Mitra SK, Gopumadhavan G, Venkataranganna MV and Sundaram R. Effect of cystone, a herbal formulation on glycolic acid induced urolithiasis. Phytotherapy research., 1998; 12: 372-374.
14. Ravindra V Karadi, Navneet B Gadge,Alagawadi KR, Rudraprabhu V Savadi. Ethylene glycol induced urolithiasis in effect of Moringa oleifera Lam. Root -wood on rats. Journal of Ethnopharamacology., 2006; 105(1): 306-311.
15. Wagner CA, Mohebbi N Urinary pH and stone formation. J Nephrol., 2010; 23(16): S165-S169. Pub Med: 21170875.
16. Bai Y, Ouyang JM, Chen ML (2004) Simultaneous determination of calcium and magnesium in urines by flame atomic absorption spectrometry. Guang Pu Xue Yu Guang Pu Fen Xi., 2004; 24: 1016-1019. Pub Med: 15766134.
17. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. Clin Chim Acta., 1972; 36: 127-132.
18. Goldenberg H, Fernandez A Simplified method for the estimation of inorganic phosphorus in body fluids. Clin Chem., 1966; 12: 871-882. Pub Med: 5926907.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement using Folia-ciocalteau reagent. J Biol Chem., 1951; 193: 265-275.
20. . Slot C creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest, 1965; 17: 381-387. PubMed: 5838275.
21. N. I. Khan, J. S. Shinge, N. S. Naikwade. Antilithiatic Effect of Helianthus Annuus Linn. Leaf Extract in Ethylene Glycol and Ammonium Chloride Induced Nephrolithiasis. International Journal of Pharmacy and Pharmaceutical Sciences, 2010; 2(4).
22. . Wybenga DR, Di Giorgio J, Pileggi VJ Manual and automated methods for urea nitrogen measurement in whole serum. Clin Chem., 1971; 17: 891-895. PubMed: 5571487.

23. 23-Nishikimi M, Roa NA, Yogi K. Measurement of superoxide dismutase. Biochem. Biophys. Res. Commun, 1972; 46: 449–454.
24. Rotruck J, Pope A, Ganther H, Swanson A, Hafeman D, Hockstra W. (Selenium: Biochemical role as a component of glutathione peroxidase). Science, 1973; 179: 588-598.
25. Ellman GL. (Tissue sulfhydryl groups). Archieves Biochem Biophys, 1959; 82: 70 – 77.
26. Satoh K. lipid peroxide in cerebro vascular disorders determined by a new colorimetric method. ClinChimActa, 1978; 90(1): 37–43.
27. J.Lemann, E.Worcestor, R .Gray. Hypercalciuria and stones .Am. J. Kidney Dis., 1991; 27: 839.
28. Olivier B., Adam R. , Chou-Long H., William H. F. , Charles Y.C. , and Orson W. M. Mechanism of Urinary Calcium Regulation by Urinary Magnesium and Ph. J Am Soc Nephrol , 2008; 19: 1530–1537
29. Celik, I. Suzek.2007. Effects of sub acute treatment of ethylene glycol on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Chem Biol Interact., 2007; 167: 145-152.
30. DG. Baheti* and SS. Kadam. Antiurolithiatic activity of some traditional medicinal plants against calcium oxalate induced urolithiasis in rats. IJPCBS., 2013; 3(4): 1276-1285
31. Padma N. P., Sahadeb D. and Subash C. J. Urolithiasis: Critical Analysis of Mechanism of Renal Stone Formation and Use of Medicinal Plants as Antiurolithiatic Agents .Asian J. Anim. vet. Adv., 2016; 11(1): 9-16
32. Wiessner J., Hasegawa A., Hung L., Mandel G., and Mandel N.2001. Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. Kidney International., 2001; 59: 637–644.
33. Halici M., F. Odabasoglu, H. Suleyman, A. Cakir, A. Aslam, Y. Bayir, Effect of water extract of Usnealongissima on antioxidant enzymeactivity and mucosal damage caused by indomethacin in rats, Phytomedicine, 2005; 12: 656–662.