

Effect of Citrus Limon (L.), Citrus Aurantium And Citrus Medica On Ethylene Glycol Induced Urolithiasis In Rats

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Abstract

Introduction: Renal stone formation and the predominant chemical stone composition are age and gender dependent. The most common stones are struvite (magnesium ammonium phosphate), calcium oxalate, urate, cystine and silica. The most common type of kidney stones worldwide contains calcium. The process of forming stones in the kidney, bladder, and/or urethra (urinary tract) is called as Urolithiasis. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. Citrus species were used in traditional practices as remedies for the urolithiasis have been well established in vivo-vitro test, clinical trial and effective use but are lacking well documented record. Citrus limon (L.) is tree with evergreen leaves and yellow edible fruits from the family Rutaceae. Citrus aurantiumL commonly called as bitter orange. Citrus medica Linn.,commonly known as a Citron in English and bijapura in Ayurvedic literature is shrub or small tree. All three are reported to use in treatment of wide panel of diseases like antiurolithic activity, stomach ache, vomiting antifungal and antibacterialetc. Urolithiasis was introduced by administration of ethylene glycol in rats. Standard drug treated group, the urinary outputs increased significantly. Body weight of all animals measured on day 0, 14 and 28 of calculi induction to assess the effect of treatment on body weight. The collection of urine from rats scheduled to be every seven days from calculi induction. C. aurantium extract showed significantly increase in urinary output. The collected urine stored in 4°C. The analysis of calcium, phosphate, magnesium, creatinine, uric acid, protein, urea nitrogen and oxalate recorded.300 mg/kg of combined extract of all three plants show significant result compare to individual plant response.

Keywords: Citrus limon, Citrus aurantium, Citrus medica Linn, Ethylene Glycol, Calculi Induction, Urine Parameters.

1. INTRODUCTION

Urolithiasis

The process of forming stones in the kidney, bladder, and/or urethra (urinary tract) is called as Urolithiasis. Stones form twice as often in men as women. The stone type is named after its mineral composition. The most common stones are struvite (magnesium ammonium phosphate), calcium oxalate, urate, cystine and silica. The most common type of kidney stones worldwide contains calcium[1].

Renal stone formation and the predominant chemical stone composition are age and gender dependent. Most stones are formed in older patients. However, clinical observations have indicated not only a changing frequency and composition of urinary calculi but also a shift in gender and age-related incidences. Urinary stone disease remains rare in children with a stable overall incidence in most series. As in adults, factors implicated in the metabolic syndrome complex such as obesity pose risks for urinary stone formation in children. Although some authors have suggested the impact of climate change, changing life style and dietary choices are the more probable cause of the increasing incidence and prevalence of Urolithiasis [2].

Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years [3].

Causes of urolithiasis

Dietary factors that increase the risk of stone formation include low fluid intake and high dietary intake of animal protein, sodium, refined sugars, fructose and high fructose corn syrup, oxalate, grapefruit juice, apple juice, and cola drinks. Stone formation commonly occur due to inadequate urinary drainage, foreign bodies in urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities like vitamin A deficiencies, excess vitamin D, and metabolic diseases like hyperthyroidism, cystinuria, gout, intestinal dysfunction etc. Calcium oxalate is considered as main constituent in the renal calculi [4,5].

Signs and symptoms of urolithiasis

Symptoms of kidney stones include

- Colicky pain, Nausea/vomiting
- Hematuria, Pyuria, Dysuria, Oliguria
- Pain
- Frequent urination

As per the indigenous system of medicine, the various citrus species such as citrus medica, citrus limon, citrus aurantium etc. have been traditionally claimed for their Antiurolithic activities.

Citrus species were used in traditional practices as remedies for the urolithiasis have been well established in vivo-vitro test, clinical trial and effective use but are lacking well documented record [6].

Use of natural remedies for treatment of disease has a long history because of their safety, effectiveness and low cost medication [1]. The ancient system of Indian recommended a number of herbal remedies. The genus Citrus is one of the most important taxonomic subunits of the family Rutaceae. Citrus plants are commonly known for their valuable nutritional, pharmaceutical and cosmetic properties. One of the best known and most used species of the genus Citrus limon (L.), Citrus aurantium and Citrus medica.

Citrus limon Linn.,

Citrus limon (L.) is tree with evergreen leaves and yellow edible fruits from the family Rutaceae. C. limon has traditionally been used as a remedy for scurvy before the discovery of vitamin C [2]. Other uses for lemon include treatment of high blood pressure, the common cold, irregular menstruation and coughs [3–5]. Other activities include, antibacterial, antifungal, anti-inflammatory, anticancer, hepatoregenerating and cardioprotective activities [7–12]. Leaves have been administered for womb infection and kidney stones [4].

Citrus aurantium Linn.,

Citrus aurantium commonly called as bitter orange. Its leaves are traditionally known to be useful for the treatment of wide panel of diseases like stomach ache, vomiting etc, used for emmenagogue, blood pressure, cough, cold, bronchitis, ear ache, dysentery, diarrhea, UI ailments, dysmenorrhea, influenza, insomnia, anti-inflammatory, headache, nervousness, weakness, kidney stone, hypoglycaemic, carminative, fever, sedative, digestive. The leaves used as cytotoxic, antiyeast, antiurolithic activity, antifungal and antibacterial. [6,13-32].

Citrus medica Linn

Citrus medica Linn., commonly known as a Citron in English and bijapura in Ayurvedic literature is shrub or small tree. Ripe fruits are potent antiscorbutic, stomachic, cardiac tonic, stimulant, sedative, analgesic and used in dyspepsia, bilious vomiting, cold, fever, palpitation, sore throat, cough, asthma, thirst, hiccough and earache; root is analgesic, antispasmodic and used in

diarrhea, piles and constipation; seeds are anthelmintic, stomachic, sedative, cardiac tonic and useful in palpitation; flowers and buds are astringent and used in blood disorders and peels are anthelmintic [33-41].

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant

Collection and authentication of Leaves of Citrus limon, Citrus aurantium, Citrus medica L. collected from Shri Agri Clinic, Rajawas, Sikar road, Jaipur, Rajasthan, India and identified and authenticated by the Botanical survey of India(BSI) (No. BSI/AZRC/1.12012/Tech./2020-21-138), Jodhpur, India. Leaves were washed, cleaned and dried in shade for several days and then powdered coarsely with the help of grinder.

2.2 Preparation of extract

Ten grams of dried powder of leaves of Citrus limon, Citrus aurantium, and Citrus medica L were first defatted with petroleum ether and then individually extracted with hydro alcoholic solution using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extracts were stored in air tight bottle at 4°C.

2.3 Experimental groups

Group I: Normal animals received rat food and water ad libitum.

Group II: Urolithic animals received only ethylene glycol in drinking water (0.75%) till 28th day.

Group III: Urolithic animals received standard antiurolithiatic drug, cystone(750 mg/kg, p.o.) from Ist day till 28th day.

Group IV: Urolithic animals received plant (Citrus Medica) extract from Ist day till 28th day.

Group V: Urolithic animals received plant (Citrus Limon) extract from Ist day till 28th day.

Group VI: Urolithic animals received plant (Citrus Aurantium) extract from Ist day till 28th day.

Group VII: Urolithic animals received combination of plants (Citrus medica, citrus limon and citrus Aurantium) extract from Ist day till 28th day.

All extracts and cystone were given once daily by oral route to the respective group as shown above.

Group I served as control group and received rat food and drinking water ad libitum. All animals of Group II-VII received ethylene glycol (0.75%) in drinking water for induction of renal calculi for 28 days. Group II served as calculi-induced control group and received only ethylene glycol.

2.4 Assessment of body weight

Body weight of all animals measured on day 0, 14 and 28 of calculi induction to assess the effect of treatment on body weight.

2.5 Collection and analysis of urine

The collection of urine from rats scheduled to be every seven days from calculi induction. The collected urine stored in 4°C. The analysis of calcium, phosphate, magnesium, creatinine, uric acid, protein, urea nitrogen and oxalate recorded.

2.6 Statistical Analysis

The results were expressed as mean \pm SD. Statistical analysis was performed by

ANOVA test for multiple comparisons followed by Dunnett's test and $P < 0.05$ was considered as significant.

3. RESULTS

3.1 Body weight

Treated Groups	Body weight (gm)		
	0 day	14 day	28 day
Control	165	168	174
Ethylene Glycol treated	158	166	178
EG + Standard	163	155	151
EG + limon extract 200 mg/kg	165	157	154
EG + aurantinum extract 200 mg/kg	162	156	152
EG + medica extract 200 mg/kg	168	164	159
EG + combined extract 300 mg/kg	166	159	155

Ethylene glycol treated group of rats showed significant increase in body weight which was observed at time duration of 0, 14 and 28 days because of calculi induction in kidney. There was significant decrease in animal weight of groups of control treated and groups of animal treated with combined hydroalcoholic extract of all three plants. Out of all three plants *C. aurantinum* found to reduce more body weight of animals. *C. limon* showed more reduction in body weight of animals as compared to *C. medica*.

3.2 Urine volume

Treated Groups	Urine volume (ml)			
	7 day	14 day	21 day	28 day
Control	5.05	5.05	4.50	5.25
Ethylene Glycol treated	2.9	2.3	2.1	1.45
EG + Standard	5.1 ***	5.75 ***	6.35 ***	6.7 ***
EG + limon extract 200 mg/kg	3.85 **	3.75 **	4.0 **	4.6 **
EG + aurantinum extract 200 mg/kg	3.9 ***	4.3 ***	4.9 ***	5.2 ***
EG + medica extract 200 mg/kg	3.0	3.1	3.25	3.5
EG + combined extract 300 mg/kg	4.7 ***	5.0 ***	5.75 ***	6.1 ***

Standard drug treated group, the urinary outputs increased significantly. Out of all chronic administration of 200mg/kg of hydroalcoholic extract of limon, aurantinum and medica, *C. aurantinum* extract showed significantly increase in urinary output. 300 mg/kg of combined extract of all three plants show significant result compared to individual plant response.

3.3 Urine Parameters

Treated Groups	Urine Parameters (mg/dl)							
	Magnesium	Calcium	Creatinine	Oxalate	Phosphorus	protein	BUN	Uric acid
Control	1.8 \pm 0.2	3.26 \pm 0.14	0.33 \pm 0.01	0.36 \pm 0.09	6.76 \pm 0.15	3.07 \pm 0.12	37.99 \pm 0.44	1.38 \pm 0.007
Ethylene Glycol treated	4.5 \pm 0.4	6.45 \pm 0.07	8.79 \pm 0.1	1.39 \pm 0.14	12.01 \pm 0.69	6.82 \pm 0.15	52.02 \pm 0.62	3.53 \pm 0.008
EG + Standard	2.34 \pm 0.42*	3.4 \pm 0.15***	3.7 \pm 0.05***	0.48 \pm 0.09***	6.91 \pm 0.12	3.28 \pm 0.21**	43.21 \pm 0.69***	1.80 \pm 0.005***

EG + limon extract 200 mg/kg	2.84 ± 0.24*	4.5 ± 0.08***	8.15 ± 0.04**	1.00 ± 0.03**	6.86 ± 0.13	3.65 ± 0.25**	47.94 ± 0.62***	2.07 ± 0.01* **
EG + aurantium extract 200 mg/kg	2.68 ± 0.1*	3.2 ± 0.07***	4.8 ± 0.04***	0.7 ± 0.03***	6.92 ± 0.19	3.39 ± 0.06**	44.02 ± 0.54***	1.92 ± 0.01* **
EG + medica extract 200 mg/kg	2.9 ± 0.11*	4.2 ± 0.07***	7.91 ± 0.06***	0.98 ± 0.04**	6.94 ± 0.08	3.89 ± 0.28**	50.02 ± 0.57**	2.23 ± 0.01* *
EG + combined extract 300 mg/kg	2.45 ± 0.28	3.4 ± 0.07***	4.02 ± 0.07***	0.64 ± 0.02***	6.78 ± 0.21	3.31 ± 0.21**	43.09 ± 0.69***	1.90 ± 0.06* **

Magnesium

Group I showed lower mean value of magnesium as compare to all other groups. As group I served as normal control. Group II showed higher mean value of magnesium as compare to all other groups. It is due to administration of ethylene glycol. Group III served as standard control and showed lower mean value of magnesium as compare to all other groups. Group V and VII showed significant decrease in mean value of magnesium as compare to group IV. In V and VII groups mean values of magnesium is significant lower than group III and group VII, but it is not in the range of group I. It indicates that the standard and combined form of all three plants extracts protects against induction of renal damage following ethylene glycol administration. Group V showed highly significant decrease in mean values of magnesium as compare to group IV and VI.

Calcium

Group I showed lower mean value of Calcium as compare to all other groups. As group I served as normal control. Group II showed higher mean value of Calcium as compare to all other groups. It is due to administration of ethylene glycol. Group V served with hydroalcoholic extract of *C. aurantium* and showed lower mean value of Calcium as compare to all other groups. Group III served as standard control and showed and group VII lower mean value of Calcium as compare to group IV and VI. In these groups mean values of Calcium is significant lower in group V and mean values of calcium is similar in both group III and group VIII. Hydroalcoholic extract of *C. Medica* shows slightly good effect than *C. limon*.

Creatinine

Group III showed lower mean value of creatinine as compare to all other groups. Group V and VII showed significant decrease in mean value of creatinine as compare to group IV and VI. The standard and combined form of all three plants extracts protects against induction of renal damage following ethylene glycol administration. Group V showed highly significant decrease in mean values of creatinine as compare to group IV and VI. It suggests that hydroalcoholic extract *C. aurantium* is more effective than hydroalcoholic extract *C. limon* and *C. medica* at the same dose rate of 200 mg/kg. Same time *C. medica* extract shows slightly good effect than *C. limon*.

Oxalate

Group II (1.39 mg/dl) as negative control showed higher mean value of oxalate as compare to all other groups. Group III served as standard control and showed lower mean value of oxalate as compare to all other groups. Group V and VII showed significant decrease in mean value of oxalate as compare to group IV and VI. Group VII showed highly significant decrease in mean values of oxalate as compare to group V, IV and VI.

Phosphorus

Group VII served with hydroalcoholic extract combination of all three plants and showed lower mean value of phosphorus as compare to all other groups.

Protein

Group III showed lower mean value of protein as compare to all other groups. Group V and VII showed significant decrease in mean value of protein as compare to group IV and VI. Group VII showed highly significant decrease in mean values of protein as compare to group IV, V and VI. Group IV showed significant effect than group V.

BUN

Group VII served with hydroalcoholic extract of all three plants extract and showed lower mean value of BUN as compare to all other groups.

Uric acid

Group III showed lower mean value of uric acid as compare to all other groups. Group V and VII showed significant decrease in mean value of uric acid as compare to group IV and VI. The standard and combined form of all three plants extracts protects against induction of renal damage following ethylene glycol administration. Group V showed highly significant decrease in mean values of uric acid as compare to group IV and VI.

4. DISCUSSION

Pathological diseases of kidney including Ca Ox renal stones have resulted due to the oxalate-induced damage to the renal cells. Enhanced levels of oxalate are accountable for the toxic effects on the renal epithelial cells via alteration in membrane integrity, production of reactive oxygen species and minimal resource of antioxidant enzymes. In the present study, rats were selected to induce urolithiasis because their urinary system resembles that of humans [25]. Leaves extract of Citrus limon, Citrus aurantium and Citrus medica L was studied to screen its potential as antiurolithic agent in EG induced urolithiasis.

From the results, it was noted that hydroalcoholic extract of Citrus limon, Citrus aurantium and Citrus medica L leaves respectively shown curative effect in urolithiasis induced rats by preventing the formation and decreasing number and disruption of Ca Ox stone formed in the kidneys. The basis for calcium stone formation is super saturation of urine with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities can alter the saturation of the urine that increases stoneforming property.

The urinary volume of rats was collected and in the ethylene glycol alone treated rats it was statistically reduced. However in the standard drug treated group, the urinary outputs increased significantly. Out of all chronic administration of 200mg/kg of hydroalcoholic extract of limon, aurantium and medica, C. aurantium extract showed significantly increase in urinary output. 300 mg/kg of combined extract of all three plants show significant result compare to individual plant response. Treatment with extract improved the kidney function which was proved by increasing urinary output.

From urine parameters it was found that out of all groups in standard treated group and combined extract treated groups mean values of magnesium was lower, but it is not in the range of normal control animals. It indicates that the standard and combined form of all three plants extracts protects against induction of renal damage following ethylene glycol administration. Results also showed that hydroalcoholic extract C. aurantium is more effective than hydroalcoholic extract C. limon and C. medica at the same dose rate of 200 mg/kg. At the same time C. limon shows slighty good effect than C. medica.

The standard drug and hydroalcoholic extract of C. aurantium showed lower mean value of Calcium as compare to all other groups. Hydroalcoholic extract of C. Medica shows slighty good effect than C. limon to reduce the calcium level which was increased by administration of ethylene glycol.

For Creatinine level extract of c. aurantium showed as significant effect as standard drug. It suggests that hydroalcoholic extract C. aurantium is more effective than hydroalcoholic extract C. limon and C. medica at the same dose rate of 200 mg/kg. Same time C. medica extract shows slighty good effect than C. limon. In renal damage the glomerular filtration rate (GFR) reduced due to obstruction to the outflow of urine by stones in urinary system. Due to this the waste product, particularly nitrogenous substances such as creatinine get accumulated in blood.

The hydroalcoholic extract of combination of all three plants showed significant reduction in value of oxalate, Phosphorus, Protein and Uric acid. Out all three extract *C. aurantium* was found with significant effect for antiurolithic activity.

In the present study, hydroalcoholic extract of *Citrus limon*, *Citrus aurantium* and *Citrus medica* L treated rats exhibited enhanced urinary output, which dilutes the urinary electrolytes concentration. As a result, calcium and uric acid are flush out via the urine leaving a lesser possibility of precipitation with a reduced formation as well as the growth of urinary calculi. The elimination of calcium and uric acid were gradually increased in stone induced rats that are in accordance with the previous reports. Most calculi in the urinary system come up from a common component of urine such as Ca Ox and hypercalciuria, indicating up to 80% of analyzed stones. Enhanced urinary calcium facilitates the nucleation and precipitation of Ca Ox from urine and subsequent crystal growth. However, hydroalcoholic extracts regulate the levels of calcium, phosphorous, protein, uric acid and others which is helpful in preventing calculi formation.

Magnesium strongly inhibits the crystallization of Ca Ox in vitro, magnesium attaches to oxalate to form a soluble complex, consequently decreasing the concentration available for Ca Ox precipitation. Low urinary magnesium content is a common feature in stone formers. Experiments in animal models have shown increased levels of magnesium offers protection against Ca Ox deposition in kidneys, but clinical studies have not shown any such beneficial effects in impeding the formation of Ca Ox kidney stones. Treatment with hydroalcoholic extracts significantly enhanced the levels of magnesium in urine but significantly decreased. In the present work, hydroalcoholic extract was studied for its antilithiatic activity. The study it suggest that the combination of all three extract showed the best result than the individual plant extract.

5. CONCLUSION

Results exhibited that hydroalcoholic extract of *Citrus limon*, *Citrus aurantium* and *Citrus medica* L have shown a considerable antilithiatic effect against ethylene glycol renal stone producing agents. The present study supports and rationalizes the basis for traditional use of leaves of *Citrus limon*, *Citrus aurantium* and *Citrus medica* L for antilithiatic and nephroprotective activity.

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