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Pharmacological evaluation of traditional claims of Himalayan *Citrus medica* L.

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In India, *Citrus medica* L. (*Citron*; Family: Rutaceae) is used traditionally in the treatment of many ailments like urinary calculus, tumours, constipation, carries of the teeth and as vermifuge. The present study investigated the antiurolithiatic effect of hydroalcoholic extract of root and fruit juice and anthelmintic activity of root and seed extracts of *C. medica* L. Ethylene glycol was used to induce urolithiasis in male Wistar rats. Treatment of urolithiatic rats with hydroalcoholic extract of *C. medica* L. root and fruit juice significantly lowered the elevated calcium, oxalate and phosphate levels in urine. They also increased the level of stone inhibitor (magnesium) and improved the impairment of renal functions. The mechanism of this activity may be the synergism of its diuretic activity and its ability to maintain balance between stone promoters and inhibitors. In another experiment ethanolic extract of *C. medica* L. root, its fractions and petroleum ether extract of its seeds were investigated for their activity against *Pheretima posthuma*. Two concentrations of ethanolic root extract (50 and 100 mg/ml) and its two fractions, chloroform and ethanolic (each 50 mg/ml) along with petroleum ether extract of seeds (10 % v/v emulsion) were studied in the *in vitro* assay, which involved determination of paralytic and death time. All the tested extracts exhibited considerable anthelmintic activities; root extract was observed more active than the seed extract.

Keywords: *Citrus medica* L., Citron, Urolithiasis, Ethylene glycol, Anthelmintic.

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Citrus medica L. (Family: Rutaceae), commonly known as '*citron*' in English and '*bara nimbu*' in Hindi is mostly found in Kumaun regions of Himalaya, Uttarakhand. Flower, peel, seed, root and fruits of *citron* have been claimed in traditional Indian system in a wide variety of ailments. Roots of *citron* are known to be useful in the treatment of urinary calculus, tumours, constipation and carries of the teeth and as an anthelmintic[1]. Its root contains campesterol, stigmasterol, sitosterol, cholesterol and volatile oil. In previous studies, fruit juice, peels, seeds and leaves of many varieties of *C. medica* L. have been evaluated for various pharmacological effects including antioxidant[2], hypoglycemic[3], anti-inflammatory[4], anticancer[5,6], antifungal[7] and anthelmintic activities. Yi *et al.,* in 2010 investigated bioassay-guided anti-inflammatory principles from the stem and root barks of *C. medica* L. var. *sarcodactylis* Swingle[8]. Urinary calculus is the third prevalent disorder of the urinary system; the prevalence is estimated to be 1-5 %. However, its frequency varies with differences in dietary habits, food and water contamination, their level of development, environmental pollution, etc.[9]. It may cause obstruction, hydronephrosis, infection and haemorrhage in the urinary tract. Most of the renal calculi are composed of calcium as calcium oxalate and calcium phosphate which represent about 80 % of all cases[10]. Approximately 10-15 % of urinary calculi are composed of ammonium magnesium phosphate[11] and 5-10 % of uric acid. Surgical procedures, lithotripsy and calculus disruption by high power laser are commonly used techniques to remove the calculi. However, these procedures are costly with chances of recurrence[12]. The recurrence rate without preventive treatment is approximately 10 % at one year, 33 % at 5 yrs and 50 % at 10 yrs[13]. Various therapies including thiazide diuretics and alkali-citrates are being used to prevent recurrence but their efficacy is not fully convincing[14]. Many plant drugs have been used in India and elsewhere to cure and prevent kidney stones. The marketed polyherbal formulations Cystone, Calcuri, Chandraprabhavati, etc., have been widely used clinically in India to dissolve urinary calculi. The present study was designed to evaluate traditional claims of *C. medica* L. In this study, we evaluated

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antiurolithiatic activity of *C. medica* L. root and fruit juice against ethylene glycol induced urolithiasis model and anthelmintic activity of root and seed extracts. Fruit juice was selected for evaluation of antiurolithiatic activity because of its citrate content which acts as stone inhibitor like other citrus juices.

Methodology

Evaluation of antiurolithiatic activity

Plant material and preparation of extract

The samples of *C. medica* L. were collected locally from Bhimtal, Uttarakhand, India and authenticated at National Botanical Research Institute, Lucknow, India with accession number 97840. The plant specimen was deposited in the herbarium of same institute for future reference. Fruit juice and hydroalcoholic extract of root were used for the evaluation of antiurolithiatic activity. The roots were dried under sun and coarsely powdered. The extract was prepared by macerating 100 gm powdered root in 500 ml of 70 % v/v ethanol in water for 96 hrs. The mixture was stirred every 24 hrs using a sterile glass rod. The extract obtained was concentrated under vacuum at 40 ºC using Rotavapour, dried and stored at 4-6 ºC until used. The yield of *Citrus medica* root extract (CMRE) was found to be 7.43 %.

Animals

Male Wistar rats weighing 150-200 gm were used in the experiment. The animals were acclimatized to standard laboratory conditions and maintained at 27 ± 2 ºC in 12 hrs light/dark cycles. They were provided with standard rat food and drinking water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee.

Chemicals and apparatus

Ethylene glycol was obtained from Sigma Chemicals, Cystone from the Himalaya Drug Company, Bangalore (India). All other chemicals and reagents used were of analytical grade and procured from approved chemical suppliers. Apparatus such as autoanalyser (Carelab 200 Autoanalyser, Model No S/N-9012154), UV-visible spectrophotometer (UV-1601, Shimadzu), centrifuge (RM 12C, Remi Motors Ltd., Mumbai, India) were used in the study.

Ethylene glycol induced urolithiasis model

For evaluation of antiurolithiatic activity, ethylene glycol induced hyperoxaluria model was used[15,16]. The selected experimental animals were divided into seven groups from Group I–VII with five animals in each group. Group I served as the control group and was provided with standard rat food and drinking water *ad libitum*. For inducing renal calculi, all animals of Group II–VII received 0.75 % v/v ethylene glycol in drinking water for 28 days. Group II was the lithiatic control group and did not received any treatment. Group III animals were treated with standard drug cystone (750 mg/kg body weight) from 15th day till 28th day[15]. Group IV received 200 mg/kg body weight and Group V received 400 mg/kg body weight *C. medica* L. root extract from 15th day till 28th day. Animals of Group VI received fruit juice 1 ml/100 gm body weight from 15th day till 28th day and served as curative group, Group VII animals received the same dose of fruit juice but from 1st day till 28th day and served as preventive group. All the extracts and cystone were given once daily by oral route.

Assessment of body weight and urine collection

Body weight of all animals was measured on 0, 14 and 28th day of calculi induction to assess the effect of treatment on body weight. Urine samples (24 hrs) of all animals were collected by keeping them in individual metabolic cages on 0, 7, 14, 21 and 28th day of calculi induction. Before storing urine samples at 4 ºC, a drop of concentrated hydrochloric acid was added to each urine sample. The volume of 24 hrs urine was measured to assess diuretic effect of the samples. Urine samples of 0, 14 and 28th day were analyzed for calcium (Kit of Preci Chem Calcium, Kruise Pathline Pvt. Ltd., India), magnesium (Kit of Coral Clinical Systems, India), uric acid (Kit of Beacon Diagnostics Pvt. Ltd., India), other parameters (oxalate and phosphate) were estimated as per standard protocols.

Serum and kidney homogenate analysis

After 28 days of treatment, blood was collected from the retro-orbital sinus of each rat under anaesthetic condition. The serum was separated from the blood samples by centrifugation at 4000 rpm for 10 min and analyzed for creatinine and uric acid levels. The creatinine kit (Agappe Diagnostics, Kerala, India) and uric acid kit (Beacon Diagnostics Pvt. Ltd., India) were used to estimate creatinine and uric acid. The abdomen of each animal was cut open to remove both kidneys. After cleaning, isolated kidneys were preserved in 10 % neutral formalin. The kidneys were dried in a hot air oven at 80 ºC. Further, dried kidneys (100 mg) were boiled in 10 ml of 1N hydrochloric acid for 30 min and then homogenized. The kidney homogenate was centrifuged at 2000 rpm for 10 min and the supernatant

was separated. Then it was used for assaying calcium (Kit of Preci Chem Calcium, India), oxalate and phosphate; estimated as per standard protocols.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The statistical analysis was done by using one way analysis of variance (ANOVA) followed by Dunnett multiple comparison test; $p < 0.05$ was considered significant.

Evaluation of anthelmintic activity

Plant material and preparation of extracts

Roots and seeds of *C. medica* L. were used for the evaluation of anthelmintic activity. Ethanolic extract (EtOH) of root was prepared by continuous hot extraction method using Soxhlet Extractor in 70 % ethanol at 50 ºC for 3 hrs. Prepared extract was further concentrated under vacuum using Rotavapour at 40 ºC and then dried. This ethanolic/ hydroalcoholic extract was further fractionated with chloroform and acetone successively and the remaining fraction was marked as ethanolic fraction. The dried and coarsely powdered seeds were extracted with petroleum ether by using Soxhlet Extractor for 3-4 hrs and the solvent was recovered at 40 ºC under reduced pressure using Rotavapour. The extract obtained was an oily pale yellow coloured liquid that was stored at room temperature till used.

Drug and chemicals

Piperazine citrate was used as a standard anthelmintic drug and procured from Glaxo Smithkline Pharmaceutical Ltd. All other chemicals used were of analytical grade.

Animals

Anthelmintic activity was performed on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite *Ascaris lumbricoides* of human beings. Anthelmintic activity was carried out against earthworms by using *in vitro* model. Because of easy availability, earthworms have been widely used for the initial *in vitro* evaluation of anthelmintic compounds[17]. These *in vitro* screenings are important as they give basis for further *in vivo* studies. Earthworms of nearly equal size (about 8 cm) were collected locally and identified at the Department of Zoology, Kumaun University Nainital, Uttarakhand.

Anthelmintic activity of root extract

Initially four groups were selected with 6 animals in each group. Animals of group I were placed in 20 ml normal saline with 0.1 % v/v of Tween 80 (vehicle). Group II animals were placed in 20 ml solution of 15 mg/ml piperazine citrate in vehicle. Group III and IV animals were kept in formulations of 50 mg/ml and 100 mg/ml concentrations of ethanolic root extract in vehicle, respectively. The time taken by worms to become motionless was considered as paralytic time. The death time was recorded by observing the time taken to become motionless on application of external stimuli, by pricking with a pin[17,18]. Ethanolic extract of root was found as an effective anthelmintic agent. So the same extract was further fractionated with chloroform and acetone; the chloroform and ethanolic fractions were screened for anthelmintic activity while the acetone fraction was not evaluated because of its insolubility in the vehicle. Animals of group V and VI were treated with 50 mg/ml concentration of chloroform and ethanolic fractions, respectively. Time taken for paralysis and death were recorded.

Anthelmintic activity of seed extract

Three groups were selected with 6 animals in each group. Animals of group I were placed in 20 ml normal saline with 0.5 % v/v of Tween 80 (vehicle). Group II animals were placed in 20 ml solution of 15 mg/ml piperazine citrate in vehicle. Group III animals were placed in 10 % v/v emulsion of petroleum ether extract of seed in vehicle with 0.5 % v/v of Tween 80. Paralytic time and death time were recorded.

Results and discussion

Antiurolithiatic activity

The body weight of normal (Group I), calculi induced (Group II) and cystone treated (Group III) rats consistently increased till the completion of the experiment. The body weight of Groups IV, V and VI increased till 14th day of lithiasis induction and decreased on treatment with 200 and 400 mg/kg body weight doses of CMRE and curative dose of fruit juice; while body weight continuously decreased with preventive dose of the fruit juice in group VII, results are shown in Table 1. The urinary output of the normal rats (Group I) was 9.28 ± 0.53 ml/24 hrs/rat on 28th day and significantly increased to 17.5 ± 2.30 and 16.50 ± 1.50 ml/24 hrs/rat in CMRE treated Groups IV & V, respectively; also increased to 15.5 ± 1.50 and 20.750 ± 0.78 ml/24hrs/rat in fruit

juice treated groups VI & VII, respectively. Hyperoxaluria was produced by administration of 0.75 % v/v ethylene glycol in male Wistar rats (Group II) as indicated by increased excretion of oxalate, phosphate, calcium and uric acid levels in their urine. CMRE treatment by 200 and 400 mg/kg body weight doses (Group IV & V) and curative dose of fruit juice (Group VI) significantly reduced these increased levels of ions and uric acid dose-dependently. While there was no increase in the excretion of above ions and uric acid levels by preventive dose of fruit juice (Group VII), so it was found to be able to prevent the formation of urinary stones. Stone inhibitor magnesium was observed to be significantly reduced in calculi induced rats (Group II) compared to normal rats (Group I). However, treatment with cystone (Group III), 400 mg/kg root extract (Group V) and 1 ml/100 gm fruit juice (Group VI & VII) significantly increased the reduced level of magnesium compared to calculi-induced group (Table 2). The

Table 1- Effect of *C. medica* L. root extract and fruit juice on body weight of wistar rats

Days	Group I control	Group II Lithiatic control	Group III Cystone treated 750 mg/kg	Group IV Root extract 200 mg/kg	Group V Root extract 400 mg/kg	Group VI Fruit juice 1ml/100 gm (curative)	Group VII Fruit juice 1ml/100 gm (preventive)
Body weight (g)							
0	150.0 ± 10.72	152.0 ± 13.15	155.0 ± 10.30	150.5 ± 5.47	154.0 ± 5.83	160.0 ± 9.27	160 ± 8.78
14	158.8 ± 13.33	166.4 ± 10.39	164.6 ± 12.35	159.7 ± 5.61	174.8 ± 5.78	171.0 ± 9.02	154 ± 9.96
28	174.0 ± 10.83	181.7 ± 12.15	179.4 ± 11.38	137.7 ± 6.00	154.0 ± 6.63	160.0 ± 6.78	150 ± 11.8

Values are expressed as mean ± SEM. # $P < 0.01$= very significant, * $P < 0.05$ = significant. Number of animals = 5.
Comparisons are made against Group I (Control)[a] and Group II (Lithiatic control)[b].

Table 2- Effect of *C. medica* L. root extract and fruit juice on urinary parameters of wistar rats

Days	Group I control	Group II Lithiatic control	Group III Cystone treated 750 mg/kg	Group IV Root extract 200 mg/kg	Group V Root extract 400 mg/kg	Group VI Fruit juice 1 ml/100 gm (curative)	Group VII Fruit juice 1 ml/100 gm (preventive)
Oxalate							
0	1.54 ± 0.07	1.40 ± 0.06	1.56 ± 0.09	1.46 ± 0.04	1.54 ± 0.03	1.49 ± 0.05	1.71 ± 0.06
14	1.57 ± 0.04	2.41 ± 0.08 [a#]	2.50 ± 0.10 [a#]	2.31 ± 0.08 [a#]	2.00 ± 0.06 [a#, b#]	2.01 ± 0.03 [a# b#]	1.66 ± 0.06 [b]
28	1.55 ± 0.06	3.02 ± 0.09 [a#]	2.14 ± 0.05 [a#, b#]	1.23 ± 0.06 [a*, b#]	1.06 ± 0.06 [a#, b#]	1.71 ± 0.03 [b]	1.51 ± 0.13 [b]
Phosphate							
0	5.77 ± 0.09	6.06 ± 0.06	5.22 ± 0.13	6.29 ± 0.09	6.16 ± 0.03	6.27 ± 0.14	6.64 ± 0.07
14	5.71 ± 0.04	7.11 ± 0.06 [a#]	6.97 ± 0.09 [a#]	6.90 ± 0.06 [a#]	6.98 ± 0.12 [a#]	7.05 ± 0.04 [a#]	6.72 ± 0.05 [a#]
28	5.70 ± 0.05	7.93 ± 0.12 [a#]	6.39 ± 0.07 [a#, b#]	6.42 ± 0.07 [a#, b#]	6.38 ± 0.04 [a#, b#]	6.43 ± 0.08 [a# b#]	6.52 ± 0.10 [a]
Calcium							
0	0.61 ± 0.06	0.66 ± 0.07	0.67 ± 0.05	0.58 ± 0.08	0.61 ± 0.06	0.65 ± 0.11	0.66 ± 0.10
14	0.67 ± 0.13	1.68 ± 0.09 [a#]	1.60 ± 0.10 [a#]	1.62 ± 0.06 [a#]	1.87 ± 0.05 [a#]	1.67 ± 0.10 [a#]	0.72 ± 0.06 [b]
28	0.69 ± 0.14	2.49 ± 0.09 [a#]	1.40 ± 0.10 [a#, b#]	1.87 ± 0.08 [a#, b#]	1.59 ± 0.07 [a#, b#]	0.59 ± 0.03 [b#]	0.68 ± 0.12 [b]
Uric acid							
0	1.76 ± 0.05	1.80 ± 0.11	1.74 ± 0.06	1.43 ± 0.08	1.68 ± 0.12	1.75 ± 0.07	1.79 ± 0.02
14	1.81 ± 0.09	2.24 ± 0.02 [a#]	2.16 ± 0.04 [a#]	2.32 ± 0.10 [a#]	2.21 ± 0.06 [a#]	2.29 ± 0.04 [a#]	1.58 ± 0.09 [b]
28	1.76 ± 0.07	2.72 ± 0.06 [a#]	2.09 ± 0.04 [b#]	1.72 ± 0.12 [b#]	1.16 ± 0.08 [a#, b#]	1.35 ± 0.13 [a*b#]	1.30 ± 0.07 [a]
Magnesium							
0	2.66 ± 0.18	2.51 ± 0.26	2.67 ± 0.09	2.69 ± 0.19	2.83 ± 0.19	2.64 ± 0.12	2.76 ± 0.09
14	2.52 ± 0.05	1.83 ± 0.31 [a*]	1.92 ± 0.14 [a*]	1.65 ± 0.12 [a#]	1.76 ± 0.07 [a#]	1.68 ± 0.09 [a#]	2.09 ± 0.10
28	2.55 ± 0.20	1.48 ± 0.28 [a#]	2.53 ± 0.08 [b#]	1.98 ± 0.30	2.45 ± 0.11 [b#]	2.23 ± 0.05 [b*]	2.39 ± 0.09 [b]

Values are expressed as mean ± SEM. # $P < 0.01$= very significant, * $P < 0.05$ = significant. Number of animals = 5.
Comparisons are made against Group I (Control)[a] and Group II (Lithiatic control)[b].

Table 3- Effect of *C. medica* L. root extract and fruit juice on kidney and serum parameters.

Parameter (unit)	Group I control	Group II Lithiatic control	Group III Cystone treated 750 mg/kg	Group IV Root extract 200 mg/kg	Group V Root extract 400 mg/kg	Group VI Fruit juice 1 ml/100 gm (curative)	Group VII Fruit juice 1 ml/100 gm (preventive)
Kidney (mg/g)							
Calcium	2.48 ± 0.06	4.09 ± 0.04 $^{a\#}$	2.80 ± 0.10 $^{a*, b\#}$	3.39 ± 0.09 $^{a\#, b\#}$	1.36 ± 0.07 $^{a\#, b\#}$	1.29 ± 0.04 $^{a\#b\#}$	0.75 ± 0.11 $^{a\#b\#}$
Oxalate	1.10 ± 0.03	4.65 ± 0.09 $^{a\#}$	1.40 ± 0.07$^{b\#}$	2.34 ± 0.05 $^{a\#, b\#}$	0.99 ± 0.08 $^{b\#}$	1.29 ± 0.11 $^{b\#}$	1.17 ± 0.13 $^{b\#}$
Phosphate	2.58 ± 0.07	4.23 ± 0.05 $^{a\#}$	2.82 ± 0.08$^{b\#}$	2.89 ± 0.10 $^{a*, b\#}$	2.83 ± 0.08 $^{b\#}$	3.16 ± 0.09 $^{a\#b\#}$	3.02 ± 0.05 $^{a\#b\#}$
Serum (mg/dl)							
Creatinine	0.42 ± 0.07	0.93 ± 0.09 ^{a*}	0.47 ± 0.19 $^{b\#}$	0.51 ± 0.06	0.39 ± 0.06 $^{b\#}$	0.42 ± 0.18 $^{b\#}$	0.40 ± 0.08 $^{b\#}$
Uric acid	1.86 ± 0.16	4.58 ± 0.08 $^{a\#}$	1.97 ± 0.16 $^{b\#}$	3.99 ± 0.25 $^{a\#, b*}$	3.58 ± 0.06 $^{a*, b\#}$	2.75 ± 0.18 $^{a\#b\#}$	1.95 ± 0.06 $^{b\#}$

Values are expressed as mean ± SEM. $^{\#}$ $P < 0.01$= very significant, * $P < 0.05$ = significant. Number of animals = 5.
Comparisons are made against Group I (Control)[a] and Group II (Lithiatic control)[b].

deposition of crystalline components in the renal tissue, namely calcium, oxalate and phosphate was significantly ($p < 0.01$) increased in the calculi induced rats (Group II). Treatment with CMRE (Groups IV & V) significantly ($p < 0.01$) reduced the renal content of these stone forming constituents in dose-dependent manner. Fruit juice also significantly ($p < 0.01$) reduced the renal content of calcium, oxalate and phosphate in both the regimens, but the preventive regimen (Group VII) reduced the level of these constituents more than the curative regimen (Group VI) (Table 3). The serum uric acid and creatinine levels were significantly increased in calculi induced animals (Group II), indicating marked renal damage. However, treatment with CMRE (Group IV & V) and fruit juice (Group VI & VII) in calculi induced rats significantly reduced the elevated serum levels of uric acid and creatinine dose-dependently, thus preventing renal tissue damage (Table 3). Till date there is no satisfactory drug in allopathic system of medicine to dissolve renal stones and to prevent their recurrence, so sufferers depend on alternative systems of medicine for better relief. The present study is an effort in the evaluation of a folk medicine for treatment of renal calculi. There are several types of renal stones, most commonly consisting of calcium oxalates and calcium phosphates; others are composed of magnesium ammonium phosphate, uric acid or cystine[19]. Stone formation occurs when urinary concentration of stone forming salts exceed the limit of meta stability for that salt in solution. This reflects excessive excretion of one or more stone constituents, deficient inhibitory activity in urine, or a low urine volume resulting in excessively concentrated urine[20]. Calcium oxalate stone formation is a multistep process, which includes nucleation, crystal growth, crystal aggregation and crystal retention[21]. Endoscopic stone removal and Extracorporeal Shock Wave Lithotripsy (ESWL) have revolutionized the treatment of urolithiasis but do not prevent the recurrence of renal stones. Thiazide diuretics and alkali-citrates are being used to prevent recurrence but their efficacy is less convincing[14]. These recent treatment procedures are not only costly to the common man of developing countries but are also unable to reduce the chances of recurrence. Plant based medicines are found to be more efficacious and have lesser side effects compared to modern medicines and are also known to reduce the recurrence rate of renal stones[12]. Folk medicines are generally administered by oral route, so same route was followed for evaluation of urolithiasis in present study. Previous studies indicated that ethylene glycol (0.75 % v/v) administration for 14 days induces formation of renal calculi composed mainly of calcium oxalate[15,16,22]. It is reported that hyperoxaluria is a far more significant risk factor than hypercalciuria in the pathogenesis of renal calculi. Increased concentration of calcium in urine is responsible for nucleation, precipitation and crystal growth of calcium oxalate or calcium phosphate in urine. Uric acid is also a promoter of renal stone formation along with calcium, oxalate and phosphate while citrate and magnesium are inhibitors[23]. However, hydroalcoholic extract of *C. medica* L. root reduced the levels of calcium, oxalate, phosphate and uric acid in calculi induced groups as well as increased the level of inhibitor magnesium. In urolithiasis, glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and

uric acid get accumulated in blood. In lithiatic control group (Group II), renal damage was observed by the increased level of serum creatinine and uric acid. However, accumulation of waste products like creatinine and uric acid in serum was reduced by *C. medica* L. fruit juice and root extract (CMRE). This effect may be because of diuresis and improved GFR of both fruit juice and root extract. Antilithogenic effect of some herbal remedies is due to antimicrobial properties. In the process of urinary stone formation, the anti-adherent layer of glycosaminoglycans (GAGs) acts as a protective barrier against urinary stone disease. If this layer is damaged due to bacterial infection, a stone nucleus might develop, leading to stone formation in the urinary tract. At this point, extracts that show antimicrobial properties can be considered antilithogenic. Renal stones are often accompanied by urinary tract infections (UTIs)[21]. Urease producing organisms such as those of the genus *Proteus* may provoke the formation of stones in the urinary tract. Strains of *Proteus mirabilis* are prominent cause of UTI in children and in domiciliary. In a previous study *C. medica* L. root have shown antibacterial activity against *Proteus vulgaris, Staphylococcus aureus* and *Enterococcus faecalis*[24].

The present study showed that the administration of hydroalcoholic extract of *C. medica* L. root and fruit juice effectively reduced the ethylene glycol induced urolithiasis in rats. As discussed above, the antiurolithiatic activity of CMRE may be due to the synergism of its diuretic activity, its ability of maintaining balance between stone promoters and inhibitors. These findings thus support the traditional use of *C. medica* L. root in urolithiasis. Like lemon, citron juice is rich in flavonoids, limonoids, citric acid, vitamin C and many more vitamins. Some of these compounds are responsible for their antioxidant activity. Citrus juices have long been considered to have anti-lithogenic potential because of increased citraturia. Citrate is the most abundant organic ion in urine and is a potent inhibitor of calcium oxalate and calcium phosphate nucleation. Lower concentration of citrate ions have been documented in stone formers. In a study, a Unani formulation which has been used since long time in the management of urinary tract stones was found to have a significant citraturic effect[25]. In the present study citron juice prevented and reduced the growth of urinary stones. Preventive regimen was observed more effective than its curative regime. Therefore, the citron fruit is helpful to prevent the recurrence of stone formation. The mechanism of this effect may be attributed to the rich antioxidant activity of fruit juice, increased diuresis and the presence of citrate may also take part on that.

Anthelmintic activity

The results are shown in Figs. 1&2. Ethanolic extract of root showed promising anthelmintic activity and the effect was dose dependant. It was observed that with 100 mg/ml concentration of the extract, whole body of tested animals was shrunken and beaded structures were formed in it. After observing the promising anthelmintic effects of ethanolic extract, its fractions were evaluated for the same to find out the more active fraction. It was observed that as compared to the ethanolic fraction chloroform fraction showed better anthelmintic activity at

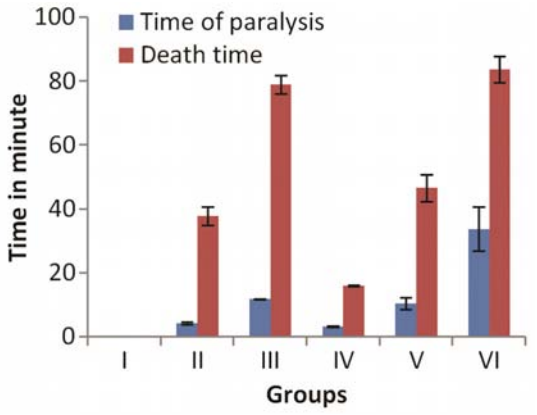

Fig. 1- Anthelmintic activity of *C. medica* L. root extract. Group I- control, II- piperazine citrate (15 mg/ml), III- ethanolic root extract (50 mg/ml), IV- ethanolic root extract (100 mg/ml), V- chloroform fraction (50 mg/ml), VI- ethanolic fraction (50 mg/ml)

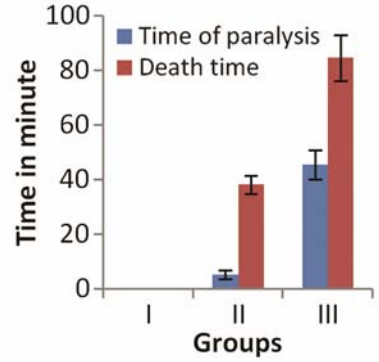

Fig. 2- Anthelmintic activity of *C. medica* L. seed extract. Group I- control (vehicle), II- piperazine citrate (15 mg/ml), III- petroleum ether extract of seed (10 % v/v in vehicle); vehicle- normal saline with 0.5 % v/v of Tween 80.

50 mg/ml concentration. The qualitative phytochemical investigation of root extract and its fractions indicated the presence of alkaloids, carbohydrates, cardiac glycosides, triterpenoids, steroids, phenolic compounds and tannins. Qualitative phytochemical screening indicated the presence of almost same constituents in fractions and ethanolic extract, but they may vary quantitatively, so responsible for variation in their potency. Petroleum ether extract of seeds showed anthelmintic activity by paralyzing and causing death of earthworms with time of paralysis 45.5 min and death time 84.66 min at 10 % v/v concentration of seed extract compared to piperazine citrate having paralytic time 5.13 min and death time 38.24 min with 15 mg/ml concentration (Fig. 2). The present study thus justifies the traditional uses of *C. medica* L. roots and seeds as anthelmintic. In this study, it was also observed that compared to seed extract its root extract was much more effective as anthelmintic. The promising results of root extract as an anthelmintic indicated future work on bioassay guided isolation of active anthelmintic compounds from *C. medica* L. root.

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