

Evaluation of Antiurolithiatic Activity of *Lawsonia inermis* Linn. in Rats

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Received January 5, 2017; accepted March 26, 2017

ABSTRACT

This study was conducted to evaluate the potential antiurolithiatic effects of *Lawsonia inermis* Linn. in rat models. Animals were divided into seven groups and urolithiasis was induced by ethylene glycol (0.75% v/v) in drinking water to all groups (Groups II-VII) except normal control (Group I) for 28 days. Methanolic extracts of *Lawsonia inermis* (MELI) bark (300 & 500 mg/kg, p.o.) were administered once daily, from 15th day to 28th day as curative regimen and from 1st day to 28th day as preventive regimen. Cystone (750 mg/kg, p.o.) was used as a standard drug for comparison. After 28 days, various biochemical parameters like urine volume, pH were

measured. Calcium, phosphate and oxalate were measured in urine and kidney homogenate. Serum creatinine, uric acid and urea nitrogen were estimated. Histopathology of kidney was investigated. Treatment with the MELI extract significantly ($p < 0.05$) restored all elevated parameters including calcium, phosphate and oxalate in urine and kidney homogenate; creatinine, uric acid and urea nitrogen in serum when compared to model control group. The histopathological study of the kidney also supported the above results. It was concluded that methanolic extract of bark of *Lawsonia inermis* Linn. has significant antiurolithiatic effect in experimental rats.

KEYWORDS: *Lawsonia inermis* Linn.; Ethylene glycol; Urolithiasis; Cystone.

Introduction

Urolithiasis is derived from the Greek words “ouron” (urine) and “lithos” (stone) (Agarwal and Verma 2014). Urolithiasis, which is referred to as the process of formation of calculi in the urinary system including ureter (Ureterolithiasis), urinary bladder (Cystolithiasis) and kidney (Nephrolithiasis) as well as in the gallbladder (Khaling et al., 2014). It is considered as the third most common affliction of the urinary tract (Agarwal et al., 2014). The stones may be classified on the basis of their constituent i.e. Calcium-containing stones (more than 80%), specially calcium oxalate monohydrate, calcium oxalate dihydrate and 15-25% is basic Calcium phosphate, 15-30% is struvite, 6-10% is cystine and 2-10% is uric acid stones and 2.6% is miscellaneous stones (Alok et al., 2013; Narayan and Panda et al., 2014). Urinary stone is a common disorder with a recurrence rate of 70-81% in males and 47-60% in females (Galani et al., 2014a). The overall probability of forming stones differs in various parts of the world and is estimated at 1-5% in Asia, 5-9% in Europe, 13% in North America and 12% population in India (Galani et al., 2014b; Karadi et al., 2006). In India, 5.7 million people are estimated to be suffering from this disease and the incidence of urolithiasis is higher in northern India, compared to that of Southern India (Hegde et al., 2014; Atodariya et al., 2013).

The pathogenesis of calcium oxalate stone formation is a physicochemical event includes nucleation, crystal growth, crystal aggregation and crystal retention in the urinary system. Promoters facilitate the stone formation while inhibitors prevent it (Aggarwal et al., 2013). The treatment of urolithiasis is mainly considered with the dissolution of existing stones and preventing the reoccurrence of stones. Standard pharmaceutical drugs like thiazide diuretics, orthophosphate, alkali-citrates and magnesium reduce the reoccurrence rate of stones. But standard pharmaceutical drugs used to prevent and cure urolithiasis are costly, not effective in all cases, have potential side effects like reoccurrences and risk of infertility (Joy et al., 2012).

Treatment of stone disease depends on the size and location of the stones present in urinary tract. If stones are larger than 5 mm, it fails to pass and it should be treated by some interventional procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS) or percutaneous nephrolithotomy (PCNL) (Galani et al., 2014b). Surgical treatment causes some problems like reoccurrence of stones and causes side effects like haemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury (Shelke et al., 2014).

Hence, Medicinal plants are having curative properties and therapeutic values due to the presence of various complex phytochemical constituents. This traditional medicines are having greater important

because of very effective, safer, locally available and little side effects (Padmalochana and Dhana 2015). According to the World Health Organization, approximately 75% of the global population, in most of the developing world, depends on botanical medicines for their basic healthcare needs (Khan and Pradhan 2012).

In the Ayurvedic system of medicine, 'Pashanabheda' group plants, claimed to be useful in the treatment of urinary stones. Many plants like *Ammi visnaga*, *Bergenia ligulata*, *Achyranthus Aspera*, *Vediuppu chunnam*, *Dolichos biflorus*, *Aerva lanata*, *Raphanus sativus*, *Quercus salicina*, *Tribulus terrestris*, *Phyllanthus niruri*, *Cranberry juice*, *Herniaria hirsute* are used as antiurolithic agent (Yadav et al., 2011). There are many marketed formulations are available which having antiurolithiatic activity, some of them are **Cystone** (Himalaya Drug Company, India); **Calcuri** (Charak Pharmaceuticals, Bombay, India) and **Chandraprabha bati** (Baidyanath, India) prevents the formation of stones and dissolve urinary calculi in the kidney and urinary bladder (Nagal and singla et al., 2013).

Lawsonia inermis Linn., commonly called as Henna belongs to family *Lythraceae*. Traditionally, it has been reported to have therapeutic activity in bronchitis, diabetes, antimicrobial, antibacterial, trypsin inhibitory, cytotoxicity, wound healing, antioxidant, anti-inflammatory, analgesic and hepatoprotective (Kirtikar and Basu 2005; Agarwal et al., 2014). Traditionally *Lawsonia inermis* bark is also used as diuretic, dysuria and calculus affection (Kirtikar et al., 2005). It has been reported that the plant contains triterpenoids-hennadiol, betulin, betulinic acid, lupeol, flavonoids and phenolic compounds (Semwal et al., 2014; Singh et al., 2014). However, so far no scientific study has been reported regarding the antiurolithiatic activity of the bark extract of *L. inermis* Linn.

In this study, we investigated protective effect of the alcoholic extract of *L. inermis* bark against ethylene glycol induced urolithiasis and its possible underlying mechanisms using male Wistar albino rats.

Materials and Methods

Collection and Authentication of Plant

The plant of *Lawsonia inermis* Linn. was collected from the Botanical Garden, Anand Agriculture University, Anand, India and was authenticated by Dr. D. B. Patel, Botanist, Anand Agriculture University, Anand, India.

Preparation of methanolic extract of *Lawsonia inermis*

The fresh barks of *Lawsonia inermis* were collected and dried for one week and pulverized into a coarse powder with the help of a suitable grinder. About 500 g of powdered material was taken in a clean, flat bottomed glass container and soaked in 700 ml of 85% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was filtered by a piece of

clean, white muslin cloth. The obtained methanolic extract of *Lawsonia inermis* (MELI) bark was evaporated using a rotary evaporator (Nesa et al., 2014). Percentage yield of the extract was 7.5%W/W.

Phytochemical investigations

The extract was used for qualitative determination of phytoconstituents like tannins (phenolic compound), steroids, triterpenoids, flavonoids, alkaloids, carbohydrates and proteins (Kokate et al., 1990).

Experimental Animals

Healthy male Wistar Albino rats (150-200 g, 9-10 week age) were used in the experiments. The animals were acclimatized to standard laboratory conditions (temperature: $22 \pm 5^\circ$ C), humidity ($55 \pm 5\%$) and maintained on 12-hr light: 12-hr dark cycle. They were provided with regular rat chow and drinking water *ad libitum*. Experiment was conducted according to the CPCSEA guidelines and the study was approved by the Institutional Animal Ethics Committee (SPCP/IAEC/RP-02/2014).

Ethylene Glycol Induced Urolithiasis

Model in Albino Rats (Ashok et al., 2010)

In this study, a total of 42 male albino rats were used. The rats were divided into seven groups of six rats each and study for 28 days. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups except normal control for induction of renal calculi till the 28 days.

- Group-1 animals were received regular rat chow diet and drinking water *ad libitum* as normal control.
- Group-2 animals were received regular rat chow diet + ethylene glycol (0.75%) in drinking water as model control for 28 days.
- Group-3 was received regular rat chow diet + ethylene glycol (0.75%) in drinking water + Methanolic extract of *Lawsonia inermis* (300 mg/kg, b.wt.) from 15th day till 28 days.
- Group-4 was received regular rat chow diet + ethylene glycol (0.75%) in drinking water + Methanolic extract of *Lawsonia inermis* (500 mg/kg, b.wt.) from 15th day till 28 days. Group-3 and Group-4 served as curative regimen (CR-300 mg/kg and 500 mg/kg).
- Group-5 was received regular rat chow diet + ethylene glycol (0.75%) in drinking water + Methanolic extract of *Lawsonia inermis* (300 mg/kg, b.wt.) from 1st day till 28 days.
- Group-6 was received regular rat chow diet + ethylene glycol (0.75%) in drinking water + Methanolic extract of *Lawsonia inermis* (500 mg/kg, b.wt.) from 1st day till 28 days. Group-5 and Group-6 served as preventive regimen (PR-300 mg/kg and 500 mg/kg).
- Group-7 was received regular rat chow diet + ethylene glycol (0.75%) in drinking water + standard antiurolithiatic drug, Cystone (750 mg/kg, b.wt.) from 15th day to 28 days.

Rats were sacrificed on 28th day. All doses were given once daily by oral route and standard drug (Cystone) was administered orally as a suspension in 3 % w/v tween 80.

Assessment of Antiuro lithiatic Activity

Measurement of volume and pH of urine: Urine samples (24 hr) were collected on 28th day by keeping the animals in metabolic polypropylene cages. Animals had free access to drinking water during urine collection period. The volumes of urine from each group of animals were measured (Dixit et al., 2014). The acidity of the urine was tested using the pH meter (Baheti and Kadam 2013).

Stone observation in fresh urine: The rats were kept separately in metabolic cages and 24 hr urine samples were collected on the 28th day. A drop of concentrated hydrochloric acid was added to the urine prior to storage at 4°C. The collected urine samples were centrifuged at 3000 rpm for 10 min. After centrifugation, the urine samples were examined under light microscope at 100 X lens to identify the presence of crystals (Dharmalingam et al., 2014).

Urine analysis: Urine samples (24 hr) were collected on 28th day by keeping the animals in metabolic polypropylene cages. Animals had free access to drinking water during urine collection period. Urine was used for the estimation of calcium and phosphate using commercially available kits (Span Diagnostics Ltd., India) and oxalate by colorimetric method (Hodgkinson, 1970).

Kidney homogenate analysis: The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys are cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was separated and the calcium and phosphate contents in kidney homogenate were determined using commercially available kits (Span Diagnostics Ltd., India) and oxalate by colorimetric method (Hodgkinson, 1970).

Serum analysis: On last day of experiment (Day 28), rats were anaesthetized using ether and blood was withdrawn by retro-orbital puncture using micro capillary tubes. Serum was separated by centrifugation at 7000 rpm for 15 min and used for estimation of creatinine, uric acid and urea nitrogen using commercially available kits (Span Diagnostics Ltd., India).

Histopathology of kidney: To confirm the incidence of urolithiasis the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. The kidneys were cleaned off from extraneous tissue and transferred to 10% neutralized formalin solution (pH 7.4). Sections of kidney was fixed in paraffin, stained with hematoxylin and eosin and observed for histopathological studies (Chinnala et al., 2013).

Statistical analysis: Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *Dunnnett's post-hoc* test using Graph Pad Prism Version 5.03. All values were expressed as Mean \pm SEM and $p < 0.05$ was considered as significant.

Results

Preliminary Phytochemical Investigations

Phytochemical screening of MELI revealed the presence of tannins (phenolic compound), steroids, triterpenoids, flavonoids, alkaloids, carbohydrates and proteins.

Measurement of Volume and pH of Urine

There was a significant decrease in the urine volume as well as pH of urine in ethylene glycol treated group when compared with normal control (NC) group. However, treatment with *Lawsonia inermis* extract in curative regimen (CR) (300 mg/kg and 500 mg/kg, p.o.) significantly increased urine output as well as pH of urine as compare to model control (MC) group. Also treatment with *Lawsonia inermis* extract in preventive regimen (PR) (300 mg/kg and 500 mg/kg, p.o.) more significantly increased urine volume as well as pH of urine as compare to model control (MC) group. The animals receiving standard treatment Cystone 750 mg/kg, p.o.) showed significant increased urinary volume as well as pH of urine when compared to normal and calculi induced model control group (Table 1).

TABLE 1

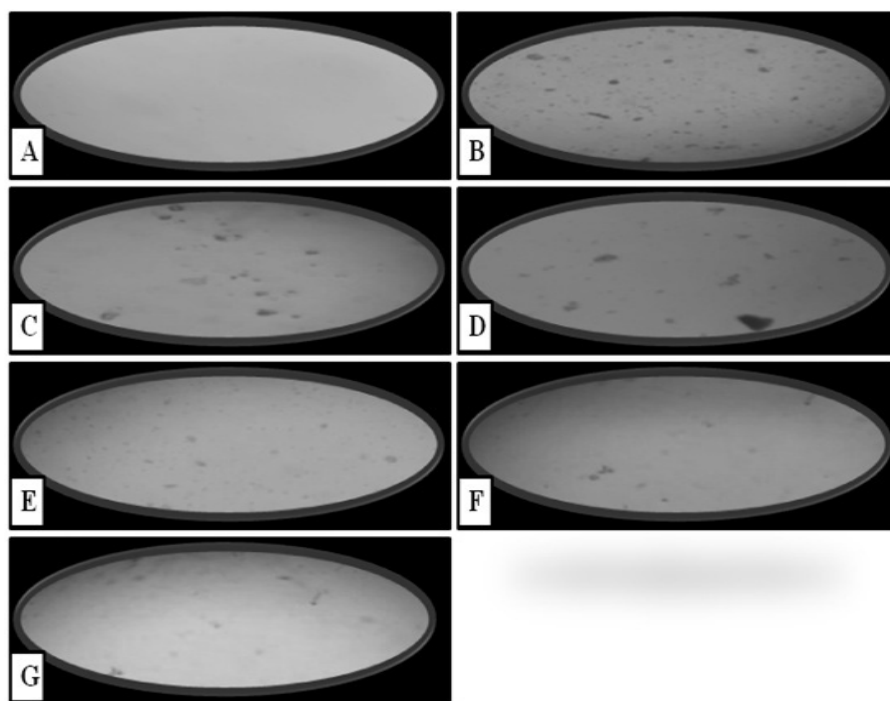
Effect of Methanolic extract of *Lawsonia inermis* Linn. on Volume and pH of Urine analysis.

S. No.	Treatment	Volume (ml/24 hrs)	pH
1.	NC	10.53 \pm 0.270	7.700 \pm 0.123
2.	MC	3.333 \pm 0.304*	6.167 \pm 0.098*
3.	CR-300	6.583 \pm 0.179#	7.150 \pm 0.076#
4.	CR-500	6.967 \pm 0.095#	7.250 \pm 0.088#
5.	PR-300	8.350 \pm 0.152#	7.450 \pm 0.071#
6.	PR-500	8.633 \pm 0.111#	7.583 \pm 0.087#
7.	STD-750	8.833 \pm 0.450#	7.717 \pm 0.070#

NC-Normal Control; MC-Model Control; CR-Curative Regimen; PR-Protective Regimen; STD-Standard. All values are expressed as Mean \pm SEM for each group (n=6). One way ANOVA followed by *Dunnnett post-hoc test*. * $P < 0.05$ Model control Vs Normal control, # $P < 0.05$ Treatment group Vs Model control.

Stone Observed in Fresh Urine

The microscopic examination of urine of normal group of animal showed the absence of crystal or similar structure (Fig. A), while in case of calculi induced group, the urine sample showed abundant, large crystals of Calcium oxalate (Fig. B). In Curative Regimen both groups (CR-300 & CR-500 mg/kg, p.o.) showed better dissolution of the preformed crystal of Calcium oxalate (Fig. C, Fig. D). However, small fragments of crystals were seen in both the groups. In Preventive Regimen, both groups (PR-300 & PR-500 mg/kg, p.o.) effect clearly showed better prevention of stone formation along with the dissolution of stones (Fig. E, Fig. F). The Cystone (750 mg/kg, p.o.) treated animals showed very less or almost dissolved small crystals (Fig. G) as compared to model control group (Figure 1).



Calcium oxalate crystals, viewed under microscope (100x) of fresh urine. A=NC, B=MC, C=CR-300, D=CR-500, E=PR-300, F= PR-500, G= STD-750

Fig. 1. Stone observed in fresh urine.

Urine Analysis

In the present study, administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Stone forming promoters like calcium, oxalate and phosphate excretion were grossly increased in calculi-induced model control animals. Both curative and preventive regimen groups at (300 and 500 mg/kg, p.o.) by MELI treatment and Cystone (750 mg/kg, p.o.) were significant (*p<0.05) lowered the elevated levels of these stone forming promoters in urine (Group III-VI & VII) as compared to calculi-induced model control group (Table 2).

TABLE 2
Effect of Methanolic extract of *Lawsonia inermis* Linn. on Calcium, Oxalate and Phosphate in Urine analysis.

S. No.	Treatment	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
1.	NC	1.767 ± 0.111	8.000 ± 0.112	2.250 ± 0.356
2.	MC	7.033 ± 0.149 [*]	13.00 ± 0.326 [*]	5.350 ± 0.172 [*]
3.	CR-300	6.033 ± 0.042 [#]	9.133 ± 0.255 [#]	3.867 ± 0.160 [#]
4.	CR-500	5.633 ± 0.135 [#]	8.617 ± 0.200 [#]	3.650 ± 0.274 [#]
5.	PR-300	4.433 ± 0.168 [#]	8.167 ± 0.182 [#]	3.283 ± 0.244 [#]
6.	PR-500	3.700 ± 0.081 [#]	8.083 ± 0.231 [#]	2.933 ± 0.160 [#]
7.	STD-750	3.167 ± 0.102 [#]	7.383 ± 0.170 [#]	2.500 ± 0.319 [#]

NC-Normal Control; MC-Model Control; CR-Curative Regimen; PR-Protective Regimen; STD-Standard. All values are expressed as Mean ± SEM for each group (n=6). One-way ANOVA followed by *Dunnnett post-hoc test*. *P<0.05 Model control Vs Normal control. #P<0.05 Treatment group Vs Model control.

Kidney Homogenate Analysis

The deposition of the crystalline components like calcium, oxalate and phosphate in the renal tissue (kidney homogenate) was increased in calculi-induced

model control group. Both CR and PR at 300 and 500 mg/kg, p.o. by MELI treatment and Cystone (750 mg/kg, p.o.) were significant (*p<0.05) reduced. The kidney homogenate contents of these stone forming constituents (Group III-VI & VII) as compared to calculi-induced model control group (Table 3).

TABLE 3
Effect of Methanolic extract of *Lawsonia inermis* Linn. on calcium, oxalate and phosphate in kidney homogenate.

S. No.	Treatment	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
1.	NC	1.317 ± 0.205	1.717 ± 0.119	1.683 ± 0.116
2.	MC	5.750 ± 0.164 [*]	6.200 ± 0.085 [*]	5.250 ± 0.143 [*]
3.	CR-300	4.433 ± 0.154 [#]	5.700 ± 0.112 [#]	3.150 ± 0.111 [#]
4.	CR-500	3.867 ± 0.201 [#]	4.717 ± 0.157 [#]	2.783 ± 0.065 [#]
5.	PR-300	3.083 ± 0.079 [#]	3.833 ± 0.080 [#]	2.300 ± 0.136 [#]
6.	PR-500	2.750 ± 0.133 [#]	3.483 ± 0.122 [#]	1.867 ± 0.055 [#]
7.	STD-750	2.700 ± 0.085 [#]	3.233 ± 0.138 [#]	1.667 ± 0.122 [#]

NC-Normal Control; MC-Model Control; CR-Curative Regimen; PR-Protective Regimen; STD-Standard. All values are expressed as Mean ± SEM for each group (n=6). One-way ANOVA followed by *Dunnnett post-hoc test*. *P<0.05 Model control Vs Normal control. #P<0.05 Treatment group Vs Model control.

Serum Analysis

Serum analysis showed that creatinine, uric acid and urea nitrogen levels were significantly increased in 0.75 % (v/v) ethylene glycol treated model control group when compared to normal group. Both CR and PR at 300 and 500 mg/kg, p.o. by MELI treatment and Cystone (750 mg/kg, p.o.) significantly (*p<0.05) lowered the elevated levels of serum creatinine, uric acid and urea nitrogen when compared to model control group (Table 4).

TABLE 4

Effect of Methanolic extract of *Lawsonia inermis* Linn. on Creatinine, Uric acid and Urea nitrogen in Serum analysis.

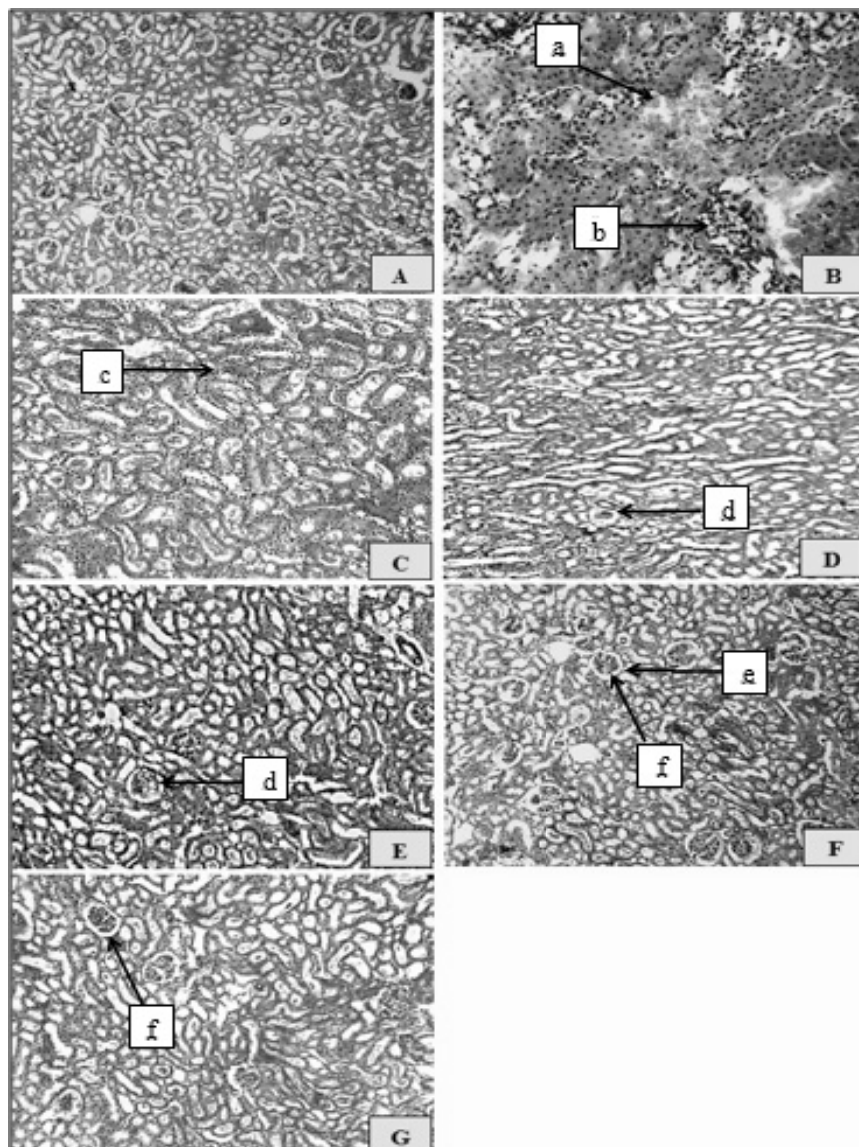
S. No.	Treatment	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
1.	NC	0.750 ± 0.056	3.183 ± 0.174	12.02 ± 0.317
2.	MC	2.833 ± 0.220 ^a	8.750 ± 0.108 ^a	30.87 ± 0.577 ^a
3.	CR-300	1.300 ± 0.085 [#]	6.583 ± 0.162 [#]	18.87 ± 0.248 [#]
4.	CR-500	1.100 ± 0.089 [#]	5.067 ± 0.164 [#]	18.20 ± 0.208 [#]
5.	PR-300	0.983 ± 0.101 [#]	4.683 ± 0.215 [#]	18.10 ± 0.320 [#]
6.	PR-500	0.950 ± 0.071 [#]	3.800 ± 0.211 [#]	17.57 ± 0.214 [#]
7.	STD-750	0.933 ± 0.055 [#]	3.567 ± 0.262 [#]	16.85 ± 0.183 [#]

NC-Normal Control; MC-Model Control; CR-Curative Regimen; PR-Protective Regimen; STD-Standard. All values are expressed as Mean ± SEM for each group (n=6). One-way ANOVA followed by *Dunnett post-hoc test*. ^a*P*<0.05 Model control Vs Normal control. [#]*P*<0.05 Treatment group Vs Model control.

Histopathology of Kidney

Histopathological analysis of rat kidneys revealed that tubules of normal size with single epithelial lining

along the margin and no calcium oxalate deposits were observed in normal control group (A). In ethylene glycol induced model control group showed severe degeneration and necrosis of tubular epithelium, presence of casts in the lumen and focal haemorrhage, presence of polymorphic calcium oxalate crystals, marked dilatation of tubules and total degeneration of epithelial lining with infiltration of inflammatory cells into the interstitial space were observed (B). On administration with MELI (300 and 500 mg/kg, p.o.) in CR gradually decreased deposition of calcium oxalate crystals with decreased dilatation of tubules, less tissue damage (C & D) and in PR no deposition of crystals and no haemorrhage and showed characteristics similar to the normal control group (E & F). In Cystone (750 mg/kg, p.o.) treated group, no deposition of calcium oxalate crystals were observed in any parts of the renal tubule and showed characteristics similar to the normal control group (G) (Figure 2).



A-Normal Control, **B**-Model Control, **C**-Curative Regimen 300 mg/kg, **D**-Curative Regimen 500 mg/kg, **E**-Protective Regimen 300 mg/kg, **F**-Protective Regimen 500 mg/kg, **G**-Standard 750 mg/kg; a- cell necrosis; b- infiltration of inflammatory of cell; c- less infiltration of inflammatory of cell; d- less healed necrosis; e- little tubular dilation; f- healed necrosis.

Fig. 2. Histopathology of kidney.

Discussion

In the present study, dried powder of barks of *Lawsonia inermis* Linn. were extracted with 85 % v/v methanol. The preliminary phytochemical study of methanolic extract of *Lawsonia inermis* bark showed presence of tannins (phenolic compound), steroids, triterpenoids, coumarine, flavonoids, alkaloids, carbohydrates and proteins.

Naturally occurring triterpenoids of plants like Lupeol and Betulin present in *Mimusops elengi* and *Costus igneus* have been identified as possessing a wide range of pharmacological effects by reducing the risk of stone formation by way of preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents (Ashok et al., 2010; Manjula et al., 2012). Previous study showed that Flavonoids containing plant *Cynodon dactylon* prevents supersaturation of calcium oxalate and decrease calcium oxalate deposition in renal tubules (Hajzadeh et al., 2011). In this study, the antiurolithiatic activity may be apparently due to triterpenes, lupeol, betulin, flavonoids and phenolic compounds present in bark of *Lawsonia inermis*.

In the present study, ethylene glycol was used as inducing agent for formations of renal calculi model. Male Wistar rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less. In addition, oxalate metabolism and excretion is almost similar to human (Shukla et al., 2014).

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 (Pareta et al., 2011). In the present study, the urinary volume was significantly decreased in ethylene glycol model control group due to obstruction of stones in the kidney. Treatment with Methanolic Extract of *Lawsonia inermis* (MELI) and Cystone (750 mg/kg) treated group showed significant increased in urinary volume as compared to model control group.

The type of stone formed in human subjects can be predicted from the pH of the urine. Crystalluria is dependent on the pH, thus by change in urinary pH, dissolution of calculi can be attained. Urinary pH of 5.0-6.5 promotes mostly Calcium oxalate type of stones (Gupta et al., 2012). In the present study, the urinary pH of urine was significant decreased in ethylene glycol model control group due to obstruction of stones in the kidney. Treatment with MELI and standard drug showed significant increased in urinary pH as compared to model control group.

The microscopic examination of urine of normal group of animal showed the absence of crystal or similar structure, while in case of calculi induced model control group, the urine sample showed abundant, large crystals of Calcium Oxalate. In Curative Regimen, both groups (CR-300 and CR-500 mg/kg) showed dissolution of the

preformed crystal of Calcium Oxalate. In Preventive Regimen, both groups (PR-300 and PR-500 mg/kg) effect clearly showed better prevention of stone formation along with the dissolution of stones when compared to model control group. The Cystone (750 mg/kg) treated animals showed very less or almost dissolved small crystals when compared to model control group.

Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of calcium. Urinary chemistry is one of the important factors in determining the type of crystal formed and the nature of macromolecules included on the surface of the crystals. Hence, the study of the urinary chemistry related to the calculi forming minerals will provide a good indication of the extent of stone formation (Divakar et al., 2010). Stone induction by EG caused an increase in calcium urinary excretion in the model control group. The rate of decrease in calcium excretion was significant reduced by MELI in CR and PR at 300 and 500 mg/kg in a dose-dependent manner and in Cystone at 750 mg/kg treatment as compared to model control group.

Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate play an important role in stone formation and has about 15-fold greater effect than urinary calcium. Animal model studies have provided evidence for the hyperoxaluria-induced activation of the Renin-Angiotensin System (RAS); a major player in renal disease progression. RAS activate the NADPH oxidase in renal cells which is responsible for Reactive Oxygen Species (ROS) production. Reduction of angiotensin II production by inhibiting Angiotensin Converting Enzyme or blocking angiotensin receptors has been shown to significantly reduce renal Calcium oxalate crystal deposition as well as the development of interstitial inflammation. The Reactive Oxygen Species responsible for phospholipase A2 activation through transcription factor NF- κ B (nuclear factor NF- κ B) as NF- κ B can be activated by the stress of oxidants and oxalate exposure also promotes rapid degradation of I κ B α (an endogenous inhibitor of the NF- κ B) (Pareta et al., 2011).

Many constituents of plants like flavonoids reported to inhibitory activity on NF- κ B gene expression. Some plants having antiurolithiatic property also reported to have ACE inhibition activity (Pareta et al., 2011). The reduction in oxalate excretion was observed by MELI in CR and PR at 300 and 500 mg/kg treatment and in Cystone at 750 mg/kg treatment as compared to model control group.

An increase in urinary phosphorus excretion was observed in ethylene glycol induced model control group. Increased excretion of phosphorus has been reported in stone formers. Increased urinary phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Divakar et al., 2010). Treatment of MELI at 300 and 500 mg/kg was significant lowered the excretion of phosphorus and in Cystone at

750 mg/kg treatment as compared to model control group.

In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the flow of urine by stones in urinary system. Due to this, the nitrogenous waste products such as creatinine, uric acid and urea nitrogen get accumulated in blood (Divakar et al., 2010). In calculi-induced rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid which are markers of glomerular and tubular damage. Treatment of MELI and standard drug showed significant decreased the elevation of serum levels of these markers as compared to model control group.

Histopathological observation of the kidney sections of ethylene glycol induced model control group showed severe degeneration and necrosis of tubular epithelium, presence of casts in the lumen, presence of polymorphic irregular calcium oxalate crystals in lumen of tubules causes dilation of proximal tubules; this might be attributed to oxalate formation. On administration of MELI at different doses (300 and 500 mg/kg b.wt) in CR moderate to few crystals were observed along the mild dilation in tubules, less tissue damage and crystals are present focally indicating the ability of MELI to dissolve the preformed stones to some extent. In PR at the doses (300 and 500 mg/kg) showed no deposition of crystals, less tissue no haemorrhage and showed characteristics similar to the normal control group. Similarly on administration of Cystone (750 mg/kg) no deposition of crystals and showed characteristics similar to the normal control group.

Results indicate that administration of bark extract of *Lawsonia inermis* Linn. reduced and prevented the growth of urinary stones. It also seems that the preventive regimen is more effective than curative regimen. The present study concluded that methanolic extract of bark of *Lawsonia inermis* has exhibited a significant antiurolithiatic effect against urolithiasis in experimental rats induced by ethylene glycol. Further studies were required to isolate the chemical moiety which is showing potent antiurolithiatic activity.

Acknowledgements

The authors are thankful to the Chairman, Principal and Co-guide of the Sardar Patel College of Pharmacy, Bakrol, Anand for providing facilities to carry out the work and Dr. D. B. Patel, Botanist, Anand Agriculture University, Anand, for the Authentication of plant and Dr. Ghodasara, for the Histopathology of kidney, Anand Agriculture University, Anand.

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