

Evaluation of *Lantana camara* Linn. (*Verbenaceae*) for Antiurolithiatic and Antioxidant Activities in Rats

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ABSTRACT

The roots of *Lantana camara* Linn. are recommended for kidney stone disorders in the Indian traditional system of medicine. Ethanolic extract of *Lantana camara* Linn. leaves were evaluated for antiurolithiatic activity against 0.75% v/v ethylene glycol and 2% w/v ammonium chloride induced calcium oxalate urolithiasis and for antioxidant activity against hyperoxaluria induced oxidative stress in male albino rats. Ethylene glycol and ammonium chloride administration increased the deposition of calcium and oxalate in the kidneys; also increased urinary excretion of calcium oxalate and creatinine in the preventive and curative control rats. In these groups, increased levels of malondialdehyde, depleted levels of antioxidant enzymes, reduced glutathione and catalase were observed. On treatment with the extract, a significant reduction in the deposition of calcium, oxalate and also urinary excretion of calcium, oxalate and creatinine was observed, indicating its antiurolithiatic effect. The extract administration also decreased the extent of lipid peroxidation and hence enhanced the levels of antioxidant enzymes in the kidneys of urolithic rats, reflecting its antioxidant efficacy against hyperoxaluria induced renal oxidative stress. Results of the present study support the traditional claim of *Lantana camara* Linn. leaves in treating renal calculi.

Keywords: *Lantana camara* Linn, calcium oxalate urolithiasis, ethylene glycol, ammonium chloride, oxidative stress, antiurolithiatic and antioxidant activities.

INTRODUCTION

Kidney stone affects up to 5% of the world population.^[1] In normal conditions, Oxalate (Ox), an end product of metabolism is excreted in urine. However, under certain pathological conditions, Ox interacts with calcium within the renal tubular lumen to form calcium oxalate (CaOx). In addition, studies show that renal cells on exposure to Ox and/or CaOx crystals generate reactive oxygen species (ROS), develop oxidative stress (OS) and associated cellular injury.^[2] In spite of advances in the present practice of medicine, the formation and growth of calculi continues to trouble mankind as there is no satisfactory drug to treat kidney stones. In India, many indigenous drugs are in use for the treatment of urinary calculus disease.

Lantana camara Linn. (*Verbenaceae*) is a plant which is commonly known as wild sage - a notorious weed and is a popular garden plant. It is an annual plant 1.2-2.4 m high and has various uses in folklore medicine in many parts of the world.^[3-4] The leaves are reported to be useful in the treatment of urinary stone, tetanus, malaria and epilepsy. They are also used as carminatives and antispasmodics. Hence, the present study was planned to evaluate the leaves

of *Lantana camara* Linn. for antiurolithiatic and antioxidant activities as a preventive and curative plant against ethylene glycol and ammonium chloride induced CaOx kidney stones and hyperoxaluria induced renal OS.

MATERIALS AND METHODS

Plant material

Lantana camara Linn. was collected from authentic source i.e. Karad, District Satara, Maharashtra in the month of August 2004. The plant was authenticated by the Department of Botany, Yashwantrao Chavan College of Science Karad Dist. Satara, Maharashtra and the voucher specimen was preserved.

Preparation of the extract

Leaves of *Lantana camara* Linn were shade dried at room temperature for 2-3 days. These dried leaves were then powdered in a mixer so as to get a coarse powder for extraction. Leaves of *Lantana camara* Linn. were extracted in a soxhlet extractor, successively with Petroleum ether (60°-80°), Chloroform, Acetone and Ethanol (95%) for 36-65 hrs for each solvent. After extraction with each solvent, the solvent was evaporated and residue was air dried. The residues from each extract were dried in the desiccator and the resultant extract was stored in an air tight container for further use. A 10% w/v aqueous suspension was prepared from the extract daily before administration to rats. The extract was subjected to preliminary phytochemical testing.

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Experimental animals

Healthy adult male albino rats of Wistar strain weighing 150-220g were used in the present study. The approval from the institutional animal Ethics committee was obtained before starting the experiment. Healthy adult albino rats were housed in a well ventilated room under natural Photoperiodic conditions, [2-3] along with access to food and water *ad libitum* throughout the study.

Acute toxicity and gross behavioral changes study

Rats were divided into five groups of six animals each and were fasted overnight with free access to drinking water. Group I animals served as normal and received distilled water (10 ml/kg, orally). Group II to V animals received 0.5, 1.0, 2.0 and 4.0 g/kg of ethanolic extract of *Lantana camara* Linn (ELC) respectively, orally by gastric intubations using a soft catheter. After administration of the extract, the animals were observed continuously for 2 hours and then intermittently at one hour interval up to 4 hours and at the end of 48th hour the number of deaths was recorded to calculate LD₅₀. [5] The animals were also observed for gross behavioral changes during acute toxicity studies. [6]

Antiuro lithiatic activity**Induction of urolithiasis**

In the present study, CaOx urolithiasis was induced in rat by free access to drinking water containing 0.75% (v/v) Ethylene glycol (EG) and 2% (w/v) Ammonium chloride (AC) for 15 days. [7]

Study protocol

The rats were divided into seven groups consisting of six per group and were put on the following treatment. Group I: (Normal) - Received distilled water, 10 ml/kg, orally and drinking water *ad libitum* from 1-15th day. Group II: (Preventive control) - Received distilled water 10ml/kg, orally and drinking water containing EG and AC *ad libitum* from 1 - 15th day. Group III: (Preventive treated) - Received ELC 0.5 g/kg, orally and drinking water containing EG and AC *ad libitum* 1-15th day. Group IV: (Preventive treated) - Received ELC 1.0 g/kg, orally and drinking water containing EG and AC *ad libitum* 1-15th day. Group V: (Curative control) - Received drinking water containing EG and AC *ad libitum* from 1 -15th day and distilled water 10 ml/kg, orally from 16-30th day. Group VI: (Curative treated) - Received drinking water containing EG and AC *ad libitum* from 1 -15th day and ELC 0.5 g/kg, orally from 16-30th day. Group VII: (Curative treated) - Received drinking water containing EG and AC *ad libitum* from 1 -15th day and ERT 1.0g/kg, orally from 16-30th day.

Evaluation of urinary parameters

Rats were hydrated with 5 ml of distilled water orally, placed in separate metabolic cages and 24 hours urine samples were collected from overnight fasted rats on day 15 from normal, preventive control and preventive treated groups. Whereas, from curative control and curative treated groups, urine samples were collected on day 30. The samples were centrifuged at 2,500 rpm at 30±2°C for 5 min. The supernatant was used to determine pH and quantitative estimation of calcium, [8] oxalate [9] and creatinine.

Kidney homogenate analysis

The rats were sacrificed by cervical decapitation after respective treatment schedules. Kidneys were perfused with ice-cold saline (0.9% w/v sodium chloride) and carefully isolated. One kidney from each animal was washed in ice-cold 0.15 M KCl and weighed. Then a 10% w/v of the

kidney homogenate was prepared in 10NHCl. The homogenate was centrifuged at 2,500 rpm at 30±2°C for 3 min and the supernatant was used to estimate calcium [8] and oxalate. [9]

Assessment of oxidative stress (OS)

The other kidney was washed with chilled normal saline and weighed. A 10% w/v homogenate was prepared in ice cold phosphate buffer (pH 7.4) using a glass homogenizer. The homogenate was centrifuged at 800 rpm at 4°C for 5 min to separate nuclear debris. The resultant supernatant was centrifuged at 10,000 rpm at 4°C for 20 min to get post mitochondrial supernatant (PMS), which was used to estimate lipid peroxidation as malondialdehyde (MDA) [10], and antioxidant enzymes reduced glutathione (GSH) [11] and catalase [12].

In-vitro antioxidant studies**DPPH free radical scavenging**

The free radical scavenging activity of the extract was examined in vitro using 1, 1- diphenyl-2-picrylhydrazyl (DPPH) stable free radical. [13] Solutions of the extract at different concentrations 100, 200, 400, 800 and 1000 µg/ml were added to 100µM of DPPH in ethanol, the tubes were kept at ambient temperature for 20 min and the absorbance was measured at 517 nm. The difference in absorbance between the control and the test was taken and expressed as percentage scavenging of the DPPH by the extract. Ascorbic acid was used as the standard. Results were expressed as means of triplicates. IC₅₀ was also determined for the extract.

Nitric oxide free radical scavenging

Sodium nitroprusside (10mM) in phosphate buffer (pH 7.7) was incubated with 100, 200, 400, 800 and 1000 µg/ml of the extract dissolved in ethanol in triplicate, and the tubes were incubated at 25°C for 120 min. After incubation, 0.5 ml of the reaction mixture was diluted with 0.5 ml of Griess reagent (2% o-phosphoric acid, 1% sulphanilamide, 0.1% N-naphthylethylenediamine). Ascorbic acid was used as the standard. The absorbance of the pink chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with N-naphthylethylenediamine was measured at 546 nm against the corresponding blank solution. The degree of free radical scavenging in the presence and absence of different concentrations of the extract was measured. The difference in absorbance between the control and the test was taken and expressed as percentage free radical scavenging of the Nitric Oxide by the extract. [14] IC₅₀ was also determined for the extract.

Statistical analysis

The results were expressed as mean ± SEM. The inter-group variation was measured by one way analysis of variance (ANOVA) followed by Scheffe's test for multiple comparisons and statistical significance was considered at Pd" 0.01.

RESULTS**Preliminary phytochemical studies**

On preliminary phytochemical screening, the extract showed the presence of alkaloids, flavonoids, tannins, glycoside, protein and carbohydrates.

Acute toxicity and gross behavioral changes

Lantana camara Linn. extract was found to be safe, as there was no animal death up to 4.0 g/kg orally and there were no gross behavioral changes except for an increase in urination.

Antiuro lithiatic activity Kidney weight

On EG/AC administration, a significant (P<0.001) increase in kidney weight was observed in both the preventive and curative control groups, when compared to the normal group. On treatment with ELC for 15 days, a dose dependent significant reduction in the kidney weight was observed in both the preventive and curative treated groups, when compared to their respective controls (Table 1).

Urine pH

In normal rats, urine pH was 7.0 to 7.5. In the preventive and curative control groups a sharp decline in urinary pH to 5.0 - 6.0 was observed. ELC administration increased the urine pH to 6.5 - 7.0 in both the preventive and curative treated groups.

Deposition of calcium and oxalate in the kidney

In the preventive and curative control groups, free access to drinking water containing EG and AC resulted in a significant enhancement in the kidney calcium and oxalate deposition. Treatment with ELC at 0.5 and 1.0 g/kg produced a significant dose dependent reduction in kidney calcium and oxalate deposition in the preventive and curative treated groups (Table 1).

Urinary excretion of calcium, oxalate and creatinine

Urinary calcium, oxalate and creatinine excretion was enhanced significantly (P<0.001) in the preventive and curative control groups on EG/AC administration. A significant (P<0.001), dose dependent reduction in urinary calcium, oxalate and creatinine excretion was observed in the preventive and curative treated groups, on treatment with ELC at 0.5 and 1.0 g/kg orally, when compared to their respective control groups (Table 2).

Oxidative stress

In-vivo LPO

In group II and V animals, EG/AC induced lithogenesis produced a significant enhancement in the renal MDA levels (119.30±3.02) and (93.20±4.03) respectively, when compared to the normal group (67.00±4.3). After treatment with ELC (0.5 and 1.0 g/kg, a dose dependent significant (P<0.001) reduction in the kidney MDA levels was observed in the preventive and curative treated groups, when compared to their respective control groups (Table 3).

In-vivo antioxidant enzymes

GSH and catalase levels of the kidney were significantly decreased in the preventive and curative control groups on EG/AC administration for 15 days, when compared to the normal group. On treatment with ELC, a significant rise in the renal GSH and catalase levels was observed in the preventive and curative treated groups (Table 3).

In-vitro antioxidant parameters

DPPH scavenging

The extract exhibited a concentration dependent DPPH free radical scavenging effect. IC₅₀ of ELC is 578.63µg/ml comparable with IC₅₀ of ascorbic acid (58.45µg/ml).

Nitric oxide free radical scavenging

ELC showed a concentration dependent inhibition of NO induced free radicals. (IC₅₀ -789.37µg/ml), compared to ascorbic acid (IC₅₀ -7.04µg/ml)

DISCUSSION

Urinary stone disease is a common, painful and expensive medical condition. [15] Though extracorporeal shock wave lithotripsy has facilitated the stone removal and reduced the morbidity associated with urinary stone, recurrence is common. [16] Several experimental and clinical studies on some of the plants used in the Indian traditional system of

medicine proved their efficacy in the management of renal stone disease. Therefore, it is advisable to evaluate plants used in the traditional medicine to treat kidney stone disease for Antiurolithiatic activity, which might be also useful in reducing stone recurrence rate.

Rats are commonly used to study the pathogenesis of human CaOx kidney stone disease, as Ox metabolism is regarded almost similar in rats and humans. [17] Ingestion of EG/AC has been found to be a reliable inducer of Ox lithiasis in rats. [18] EG is converted to endogenous oxalic acid by the liver enzyme glycolate oxidase [19] and AC induces urinary acidification, is supposed to upset the enzyme sorting mechanism in the tubular cells in the kidney [18], thus favors adhesion and retention of CaOx particles within the renal tubules.

Table 1: Effect of ELC on kidney weight, deposition of calcium and oxalate in the kidney

Gro up	Treatment	Kidney weight (g/1000g)	Calcium (mg/g kidney tissue)	Oxalate(mg/g kidney tissue)
I	Normal	0.33 ± 0.02	0.32 ± 0.02	0.95 ± 0.09
II	Preventive control	0.48 ± 0.02 ^a	0.95 ± 0.02 ^a	3.74 ± 0.18 ^a
III	ELC 0.5g/kg	0.37 ± 0.02 ^c	0.51 ± 0.02 ^d	2.04 ± 0.18 ^d
IV	ELC 1.0g/kg	0.36 ± 0.02 ^c	0.42 ± 0.02 ^d	1.65 ± 0.06 ^d
V	Curative control	0.44 ± 0.02 ^b	0.93 ± 0.02 ^a	3.65 ± 0.17 ^a
VI	ELC 0.5g/kg	0.35 ± 0.02 ^c	0.79 ± 0.03 ^e	2.21 ± 0.52 ^f
VII	ELC 1.0g/kg	0.34 ± 0.02 ^e	0.62 ± 0.02 ^f	1.91 ± 0.05 ^f

Values are expressed as mean ± SEM, n=6
 a- P<0.001, b-P<0.01 compared to normal group I
 c- P<0.01, d-P<0.001 compared to preventive- control group II
 e- P<0.01, f-P<0.001 compared to curative -control group V
 ELC- Ethanolic extract of *Lantana camara* Linn.

Table 2: Effect of ELC on urinary creatinine, calcium and oxalate excretion

Gro up	Treatment	Creatinine (mg/dl)	Calcium (mg/dl)	Oxalate (mg/dl)
I	Normal	7.83 ± 0.48	8.56 ± 0.19	1.70 ± 0.14
II	Preventive control	28.83 ± 0.40 ^a	21.75 ± 0.66 ^a	9.12 ± 0.23 ^a
III	ELC 0.5g/kg	17.17 ± 0.48 ^b	14.92 ± 0.09 ^b	4.12 ± 0.12 ^b
IV	ELC 1.0g/kg	16.00 ± 0.37 ^b	13.15 ± 0.08 ^b	2.69 ± 0.13 ^b
V	Curative control	26.17 ± 0.54 ^a	20.66 ± 0.17 ^a	8.47 ± 0.29 ^a
VI	ELC 0.5g/kg	22.17 ± 0.40 ^c	17.43 ± 0.18 ^c	6.09 ± 0.28 ^c
VII	ELC 1.0g/kg	20.00 ± 0.45 ^c	15.47 ± 0.07 ^c	4.42 ± 0.19 ^c

Values are expressed as mean ± SEM, n=6
 a. P<0001 when compared to normal group I
 b. P<0.001 when compared to preventive- control group II
 c. P<0.001 when compared to curative-control group V
 ELC- Ethanolic extract of *Lantana camara* Linn.

Table 3: Effect of ELC on in vivo lipid peroxidation and antioxidant parameters

Gro up	Treatment	MDA (nm/mg tissue)	GSH (nm/mg tissue)	Catalase (µm/mg tissue)
I	Normal	67.00 ± 4.28	74.99 ± 4.14	365.70 ± 14.15
II	Preventive control	119.30 ± 3.02 ^a	24.50 ± 1.64 ^a	81.80 ± 4.34 ^a
III	ELC 0.5g/kg	41.19 ± 0.76 ^b	48.52 ± 2.92 ^c	212.50 ± 12.35 ^b
IV	ELC 1.0g/kg	32.22 ± 0.98 ^b	89.70 ± 7.43 ^b	272.90 ± 9.60 ^b
V	Curative control	93.20 ± 4.03 ^a	26.95 ± 2.57 ^a	109.80 ± 2.66 ^a
VI	ELC 0.5g/kg	53.97 ± 1.13 ^d	46.52 ± 2.79 ^e	165.10 ± 8.37 ^c
VII	ELC 1.0g/kg	43.54 ± 0.66 ^d	62.70 ± 5.25 ^d	217.90 ± 12.41 ^d

Values are expressed as mean ± SEM, n=6
 a. P<0.001 when compared to normal group I
 b. P<0.001, c-P<0.01 when compared to preventive- control group II
 c. P<0.001, e-P<0.01 when compared to curative-control V
 ELC- Ethanolic extract of *Lantana camara* Linn.

Hence, in the present study, EG/AC in drinking water was employed to induce hyperoxaluria in rats.

Urinary supersaturation in relation to stone forming constituents, mainly urinary oxalate is important in renal calculi formation^[20], as urinary oxalic acid complexes with calcium and forms insoluble CaOx crystals.^[21] Enhanced deposition and urinary excretion of calcium and oxalate in the preventive and curative control group animals indicate that administration of EG/AC induced hyperoxaluria. An increase in the kidney weight and enhanced urinary creatinine excretion in the control group animals also substantiated these results.

On administration of ELC, the dose-dependent reduction in calcium and oxalate deposition in the kidneys and their urinary excretion in both the preventive and curative treated groups implies the potential of *Lantana camara* Linn. in both preventing the formation and dissolving the preformed CaOx stones. On treatment with the extract, the significant reduction in the elevated urinary creatinine in both the treated groups reflects the improvement in hyperoxaluria induced renal impairment. Dissolution of calculi can be achieved by alteration in urinary pH.^[22] If the pH is 5.0 or below, the stones likely to form are of uric acid type, if 5.0-6.5, calcium oxalate type and if above 7 indicates crystals of magnesium ammonium phosphate. In the present study, a decrease in the normal urine pH of 7.0-7.5 to 5.5-6.0 in both the control groups, indicates hyperoxaluria induced CaOx stone formation. In the preventive and curative treated groups, ELC administration restored the pH to 6.5-7.5, supporting the decrease in the deposition and excretion of calcium and oxalate.

Mucoproteins have significant affinity for CaOx surface and promote the growth of crystals and cement them.^[23] Flavonoids act by disintegrating the mucoproteins, thereby prevent calcium and oxalate deposition and excretion.^[24] In the present study also, preliminary phytochemical screening of ELC revealed the presence of flavonoids. Thus, in the ELC treated groups, flavonoids might have reduced calcium and oxalate deposition by pre-coating CaOx crystals and disintegrating the mucoproteins. The stone forming effects of EG are also ascribed to its hyperoxaluria induced oxidative damage. Oxalate has been reported to induce LPO and to cause renal tissue damage. As kidney is rich in polyunsaturated fatty acids, is susceptible to ROS attack.^[27] Excessive generation of ROS and/or a reduction in cellular antioxidant levels results in the development of OS. Moreover, low concentration of renal cellular glutathione favors LPO and subsequent retention of calcium and oxalate in the kidneys.^[28]

MDA is one of the most common byproducts of ROS induced OS.^[2] In the present study, increased levels of MDA, diminished levels of GSH and catalase in the preventive and curative control groups indicate that EG/ AC administration promoted extensive generation of ROS. The resultant ROS may have consumed GSH and catalase excessively and impaired antioxidant protection. Thus, the unquenched ROS may have provoked cellular damage and resulted in enhanced OS, which might have further favored the accumulation and retention of oxalate and subsequent deposition of CaOx. Studies show that treatment with antioxidants prevents CaOx deposition in the kidney and reduce Ox excretion.^[29] Daily consumption of tea reduced the risk of kidney stone formation in women by 8%.^[30]

Health benefits of tea are due to its antioxidant properties of flavonoids^[31] which act by quenching ROS and also by chelating metal ions like iron and copper. Lupeol and betulin were proposed to act by scavenging oxalate promoted free radicals and enhancing body antioxidant status, thus reducing oxalate induced renal peroxidative tissue damage.^[32]

In the present study, lowered levels of MDA and enhanced levels of antioxidant enzymes, GSH and catalase in the kidneys of the preventive and curative treated animals indicate attenuation of hyperoxaluria induced LPO and oxidative damage. Flavonoids may have minimized ROS by free radical scavenging and prevented further generation, by metal chelating property, as flavonoids like camaraside, linarioside, lantanoside, verbascoside, martynoside, lantanaside were reported in *Lantana camara* Linn. In vitro antioxidant studies also supported the hyperoxaluria induced free radical scavenging effect of ELC. Thus, the flavonoid principles of *Lantana camara* Linn might have been responsible for the inhibition of CaOx crystal aggregation and stone formation. The results support the use of *Lantana camara* Linn Leaves as an effective alternative in treating CaOx urolithiasis. Disintegration of the mucoproteins and pre-coating of CaOx crystals by antioxidant effect of flavonoid principles may be responsible for the possible antiurolithiatic activity of *Lantana camara* Linn. Further studies are necessary to find out the chemical components responsible for the antiurolithiatic activity of *Lantana camara* Linn.

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