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Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats

Atul Makasana[a,*], Vishavas Ranpariya[b], Dishant Desai[a], Jaymin Mendpara[a], Vivek Parekh[a]

[a] *Department of Biochemistry, Saurashtra University, Rajkot 360 005, Gujarat, India*
[b] *Purple Remedies Pvt. Ltd., Ahmedabad, Gujarat, India*

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ABSTRACT

Launaea procumbens Linn. is a plant commonly found in the west India and has been reported to decrease the renal calculi. This study investigated the anti-urolithiatic activity of *L. procumbens* against ethylene glycol-induced urolithiasis and its possible underlying mechanisms. The crude methanolic extract of *L. procumbens* leaves was studied using ethylene glycol-induced renal calculi in rat model. Results indicate that ethylene glycol feeding to rats resulted in to hyper oxaluria, hypercalciuria, as well as increased renal excretion of phosphate. Supplementation with methanolic extract of *L. procumbens* leaves (MELP) significantly prevented changes in urinary calcium, oxalate and phosphate excretion dose-dependently. The increased calcium and oxalate level and number of calcium oxalate crystal in the kidney tissue of calculogenic rats were significantly reverted by supplementation with MELP. The MELP supplementation also prevents the impairment of renal functions. The mechanism underlying this effect is mediated possibly through antioxidant nephroprotection and its effect on urinary concentration of stone forming constituents and risk factor.

Conclusion: These results indicate that methanolic extracts of *L. procumbens* leaves are effective against the urolithiasis.

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1. Introduction

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors. It occurs both in men and women but the risk is generally higher in men and is becoming more common in young women [16]. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are expensive and recurrence is also common [14]. The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 years and 50% at 10 years [4]. Various therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing [2].

In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale

* Corresponding author. Tel.: +91 9727423031.
E-mail addresses: atulmksn2008@yahoo.com (A. Makasana), vishwash811@yahoo.co.in (V. Ranpariya), desai_dishant21@yahoo.com (D. Desai), j.p.mendpara@gmail.com (J. Mendpara), vivek_parekh1988@yahoo.in (V. Parekh).

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behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects [14].

As per the indigenous system of medicine, the leaves of *Launaea procumbens* Linn. were reported to be useful in the treatment of a wide range of aliments including urinary stones [11,18]. However, so far no scientific study has been reported regarding the anti-urolithiatic activity of methanolic extract of *L. procumbens* leaves (MELP). In this study, we investigated evaluation for the anti-urolithiatic activity of *L. procumbens* against ethylene glycol-induced renal calculi and its possible underlying mechanisms using male Wistar albino rats.

2. Materials and methods

2.1. Plant material and preparation of extract

The leaves of *L. procumbens* were collected from the local area of Saurashtra University, Rajkot in the month of August and shade dried. The plant was authenticated by Dr. A.S. Reddy, Sardar Patel University, Vallabh Vidhyanagar, Gujarat and a voucher specimen was deposited in the herbarium of the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for future reference. The dried leaves were coarsely powdered (passed through sieve no. 40) and packed in to the Soxhlet column and extracted with 95% (v/v) methanol at 40–45 °C for 24 h. The extract obtained was evaporated at 45 °C, then dried and stored in airtight container (yield 27.69%, w/w).

2.2. Chemicals and apparatus

Ethylene glycol was purchased from Merck Ltd., Mumbai, India. Various kits for biochemical estimation of urine and serum were purchased from Span Diagnostics, Mumbai, India. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers. Apparatus such as the metabolic cage (INCO, Ambala, India), semiautoanalyzer (model Microlab-300, Merck, Germany), cold centrifuge (Remi, compufuge, Korea), UV-spectrometer (model UV 1800, Shimadzu, Japan) were used in the study.

2.3. Animals

Male Wistar albino rats (8 weeks old having body weight 150–200 g) were used for the present study. They were housed in polypropylene cages (five per cage) and maintained under temperature (27 ± 2 °C) and light (12 h light/dark cycles; lights on at 0700 h) controlled environment. The animals were given standard diet (Amrut, Pranav Agro Ind Ltd., Vadodara, India). The study protocol was approved (approval no. SU/DPS/IAEC/2011/13) by the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, India.

2.4. Ethylene glycol-induced urolithiasis model in rats

Urolithiasis was induced by ethylene glycol and ammonium chloride in experimental animals [5,9]. Briefly, thirty animals were randomly divided into five groups ($n = 6$) as follow:

Group I: Vehicle Control group; received 0.5% (w/v) gum acacia solution (5 ml/kg p.o.) and maintained on regular rat food and drinking water *ad libitum* for 21 days
Group II: Ethylene glycol (EG) group; received 0.75% (v/v) EG in drinking water alone for 21 days
Group III: Cystone group; received standard drug cystone (750 mg/kg, p.o.) for 21 days
Groups IV and V: Treatment group; fed orally with methanolic extract of *L. procumbens* leaves (MELP) 150 and 300 mg/kg, respectively, for 21 days
Groups II–V received urolithiasis inducing treatment (0.75%, v/v ethylene glycol in drinking water) for 21 days. Further, ammonium chloride (1%, w/v in drinking water) was co-administered with EG for the first 3 days in order to augment lithiasis effect of EG.

2.5. Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 0, 7, 14, 21st day of calculi induction treatment period. The volume of urine was measured and calcium content estimated by diagnostic kit (Span Diagnostics Pvt. Ltd., India) in clinical semiautoanalyser. Various urine parameters such as oxalate [8], magnesium [7,12], phosphate [6], uric acid [20], and total protein [21] were performed as per the manuals provided with various kits.

2.6. Serum analysis

The blood was collected from the retro-orbital sinus under anaesthetic condition (diethyl ether used for anaesthesia) and serum was separated by centrifugation at 10,000 × *g* for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Span Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

2.7. Kidney histopathological study

Histopathological study of kidney was followed as per the method of K. Divakar et al. (2010). Briefly, the abdomen was cut open to isolate both kidneys from each animal. Isolated kidneys were then cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin (H) and eosin (E) for histopathological examination. The slides were

examined under light microscope (magnification 10×) to study light microscopic architecture of the kidney and calcium oxalate deposits. The left kidney was finely minced and 20% homogenate was prepared in Tris–HCl buffer (0.02 mol/l, pH 7.4). Total kidney homogenate was used for assaying tissue calcium, oxalate [8] and lipid peroxidation activity [17].

2.8. Statistical analysis

The results are expressed as mean ± SEM. The statistical significance was assessed using One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test and value of $p < 0.05$ was considered significant.

3. Results

3.1. Urine analysis

The urinary output was increased significantly ($p < 0.01$) in calculi-induced rats. The urinary output of the vehicle treated group was 5.68 ± 0.91 ml/day/rat on the 21st day, which was increased to about 212.32% in renal stone induced Group II. In the prophylactic groups, the urine output was lower than that of the calculi-induced rats but significantly higher than vehicle treated rats (Table A1).

Urinary calcium, and magnesium excretion was decreased by stone inducing treatment group. However, supplementation with MELP significantly prevented these changes (Table A2).

In the present study, renal stone inducing treatment to male Wistar rats resulted in hyperoxaluria. There was an increase in oxalate phosphate and uric acid excretion in Group II. However, supplementation with MELP significantly prevented these changes in urinary oxalate, phosphate and uric acid excretion dose-dependently in Groups IV and V (Table A3).

3.2. Kidney homogenate analysis

Renal stone induction caused significant increase ($p < 0.01$) in lipid peroxidation of kidney tissue of the Group II (Table A4), which was dose-dependently prevented in the animals receiving 150 and 300 mg/kg treatment with MELP.

The deposition of the calcium in the renal tissue was increased in the Group II (Table A4). However, 150 and 300 mg/kg doses of methanolic extract significantly ($p < 0.01$) reduced the increase of calcium in renal tissue in prophylactic groups, whereas, only 300 mg/kg of methanolic extract in prophylactic group significantly ($p < 0.01$) reduced the elevation of renal calcium content.

3.3. Serum analysis

Renal stone induction caused impairment of renal function of the untreated rats as was evident from the markers of glomerular and tubular damage: elevated serum creatinine, uric acid and also urinary protein loss ($p < 0.01$), which were dose-dependently prevented in the animals receiving a simultaneous treatment with MELP at dose of 150 mg/kg and 300 mg/kg (Tables A4 and A5).

3.4. Kidney histopathology

Histopathological analysis revealed no calcium oxalate deposits or other abnormalities in the nephron segments of vehicle treated group. On the other hand, many calcium oxalate deposits inside the renal tubules and dilation of the proximal tubules along with interstitial inflammation were observed in the renal tissue of urolithiatic rats. The number of calcium oxalate deposits in the renal tubules of Groups IV and V rats was significantly less than the Group II (Fig. B1).

4. Discussion

As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of antiurolithiatic activity of *L. procumbens* against ethylene glycol induced renal calculi in rats.

Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies have shown that kidney stone formation in female rats was significantly less than male rats [9] The phytochemical studies of MELP showed the presence of alkaloids, glycosides, flavonoids, tannins, anthraquinones, fixed oils and fats, carbohydrates, terpenoids and steroids.

Urinary super saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that upon 14 days administration of ethylene glycol to young albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate. The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed rats is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate [9]. Renal calcium oxalate deposition by EG (ethylene glycol) and ammonium chloride in rats is frequently used to mimic the urinary stone formation in humans. Ammonium chloride reported to accelerate the lithiaisis [1,5]. Therefore, this model was used to evaluate the protective effect of *L. procumbens* against urolithiasis.

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.

Consistent with some previous reports [2], stone induction by EG caused an increase in oxalate and decrease in calcium urinary excretion in the Group II. Calcium excretion was reduced by MELP treatment in a dose-dependent manner compared with Group II.

Normal urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming

rats. The magnesium levels return to normal on drug treatment [16]. Diets enriched with high magnesium have been found to protect against deposition of calcium oxalate in the kidneys of vitamin B6 deficient rats. Promising results in preventing recurrence have been shown in patients treated with potassium magnesium citrate. Magnesium complexes with oxalate and reduce the super saturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals [16,19]. Urinary magnesium was significantly diminished in ethylene glycol induced urolithic rats. The MELP treatment restored the magnesium excretion compared to EG treatment group.

An increase in urinary phosphorus excretion was observed in ethylene glycol induced urolithic rats. Increased excretion of phosphorus has been reported in stone formers [19]. 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 [9,16,19].

The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyper oxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans [16]. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation [16,19].

The present observation showed increased protein excretion in ethylene glycol induced urolithic rats [16,19]. Proteinuria reflects proximal tubular dysfunction. Supersaturation of urinary proteins results in precipitation as crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth. This is associated with proteinuria [16]. Administration of MELP had profound effects on minimizing the excretion of protein in Group IV and thus might have prevented the nidus formation for crystal nucleation.

In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the flow of urine by stones in urinary system. So, the waste products particularly nitrogenous substances such as urea, creatinine and uric acid accumulate in blood. Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet. Elevated oxalate concentration in urine has been reported to induce lipid peroxidation and cause renal damage by reacting with polyunsaturated fatty acids in cell membrane [9]. 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 MELP showed to prevent the elevation of serum levels of these markers and inhibits the lipid peroxidation.

Increase in calcium levels in the renal tissue of EG treated rats was observed. The MELP treatment suppresses this increase in intracellular calcium. The exact reason of this effect is not clear, however it might be due to the increased bioavailability of NO (nitric oxide) which in turns activates cGMP (3′,5′ cyclic guanosine monophosphate) that controls the increase in intracellular calcium levels. Previous studies reported that NO donors have the capacity to control the intracellular rise in calcium levels. Thus, plant extract could effectively control the levels of both the salts by the mechanism such as inhibiting the oxalate or increasing the bioavailability of NO to sequester calcium through the cGMP pathway [15].

Microscopic examination of kidney sections derived from ethylene glycol induced-urolithic rats showed polymorphic irregular crystal deposits inside the tubules which cause dilation of the proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with the MELP decreased the number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damages to the tubules and calyxes.

5. Conclusion

The current study was able to show the antiurolithiatic effect of leaf extract of *L. procumbens* in ethylene glycol induced renal calculi model. Therefore, MELP may prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation), which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

Also, these results indicate administration of leaf extract of *L. procumbens* reduced and prevented the growth of urinary stones. Earlier study reported that *L. procumbens* is useful in renal disorder amelioration (RA. et al., 2010). Therefore, the leaf extract of *L. procumbens* is helpful to prevent the recurrence of the disease as it showed its effect on early stages of stone development.

The mechanism underlying this effect is possibly mediated through antioxidant nephroprotective properties and also lowering the concentration of urinary stone-forming constituents. However, it requires more investigation to clarify the exact mechanism of this action.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A.

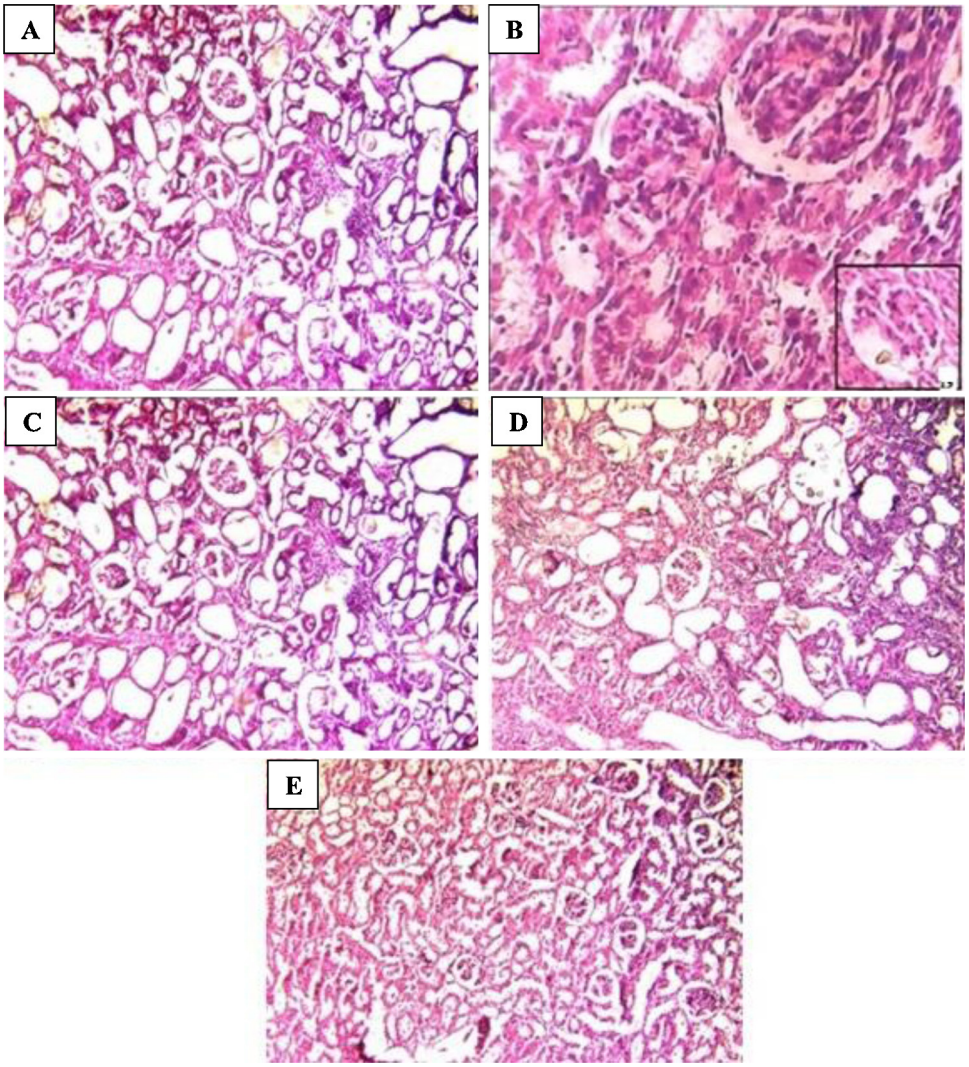

Fig. B1. Histopathology of kidney tissue of (A) vehicle control, (B) calculi induced group, crystal of calcium oxalate shown in the box (C) cystone treated, (D) prophylactic treatment with MELP at the dose 150 mg/kg, (E) prophylactic treatment with MELP at the dose 300 mg/kg. Microscopic magnification: 40×.

Table A1
Effect of methanolic extract of *Launaea procumbens* leaves on urinary output (ml/24 h) of different groups of rat.

Days	Group I	Group II	Group III	Group IV	Group V
0	7.38 ± 0.87	7.50 ± 0.34	4.67 ± 0.44^{a**b**}	7.83 ± 0.48	8.00 ± 0.37
7	9.31 ± 0.11^{b**}	12.36 ± 0.42^{a**}	10.42 ± 0.40	9.00 ± 0.52^{b**}	13.17 ± 0.70^{a***}
14	7.723 ± 0.39^{a***}	17.56 ± 1.77^{a***}	17.69 ± 0.50^{a***}	10.66 ± 0.50	13.56 ± 0.76^{a***}
21	8.77 ± 0.65^{b***}	17.74 ± 1.13^{a***}	17.98 ± 0.86^{a***}	11.65 ± 0.67^{b***}	13.74 ± 0.54^{a***b**}

Values are expressed in ml/24 h urine volume as mean ± SEM ($n = 6$). One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett test. Group I: Vehicle control, Group II: EG group, Group III: Cystone group, Group IV: 150 mg/kg MELP treatment group, Group V: 300 mg/kg MELP treatment group. Comparisons are made against Group I (vehicle control)[a] and Group II (lithiatic control)[b].

** $p < 0.01$.

*** $p < 0.001$.

Table A2
Effect of methanolic extract of *Launaea procumbens* leaves on urinary calcium and magnesium excretion rate (mg/24 h) of different groups of rat.

Days	Group I	Group II	Group III	Group IV	Group V
Calcium (mg/24 h)					
0	$0.06 \pm 0.005^{b***}$	$0.18 \pm 0.0^{a***}$	$0.11 \pm 0.01^{a*b***}$	$0.06 \pm 003^{b***}$	$0.04 \pm 0.004^{b***}$
7	$0.22 \pm 0.004^{b*}$	$0.17 \pm 0.01^{a*}$	$0.22 \pm 0.02^{b*}$	$0.03 \pm 0.005^{a***b***}$	$0.02 \pm 0.005^{a***b***}$
14	$0.16 \pm 0.003^{b*}$	$0.24 \pm 0.03^{a*}$	$0.34 \pm 0.03^{a***b**}$	$0.02 \pm 0.004^{a***b***}$	$0.03 \pm 0.004^{a***b***}$
21	$0.14 \pm 0.005^{b*}$	$0.09 \pm 0.01^{a*}$	$0.30 \pm 0.02^{a***b***}$	$0.02 \pm 0.004^{a***b***}$	$0.02 \pm 0.003^{a***b***}$
Magnesium (mg/24 h)					
0	$0.33 \pm 0.05^{b***}$	$0.77 \pm 0.06^{a***}$	$0.86 \pm 0.05^{a***}$	$0.05 \pm 0.002^{a***b***}$	$0.03 \pm 0.003^{a***b***}$
7	$1.29 \pm 0.004^{b***}$	$0.17 \pm 0.008^{a***}$	$0.22 \pm 0.02^{a***b*}$	$0.03 \pm 0.005^{a***b***}$	$0.03 \pm 0.005^{a***b***}$
14	$0.79 \pm 0.04^{b**}$	$0.52 \pm 0.06^{a**}$	$0.94 \pm 0.07^{b***}$	$0.24 \pm 0.02^{a***b**}$	$0.43 \pm 0.01^{a***}$
21	$0.54 \pm 0.01^{b**}$	$0.34 \pm 0.03^{a**}$	$0.69 \pm 0.08^{b***}$	$0.23 \pm 0.02^{a***}$	$0.34 \pm 0.03^{a***}$

Values are expressed in mg/24 h urine sample as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett test. Number of animals (N) = 6. Group I: Vehicle control, Group II: EG group, Group III: Cystone group, Group IV: 150 mg/kg MELP treatment group, Group V: 300 mg/kg MELP treatment group. Comparisons are made against Group I (vehicle control)[a] and Group II (lithiatic control)[b].
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table A3
Effect of methanolic extract of *Launaea procumbens* leaves on urinary oxalate, phosphate and uric acid excretion rate (mg/24 h) on different groups of rat.

Days	Group I	Group II	Group III	Group IV	Group V
Oxlate (mg/24 h)					
0	0.56 ± 0.11	0.42 ± 0.04	$0.18 \pm 0.03^{a***b*}$	$0.30 \pm 0.01^{a*}$	0.38 ± 0.03
7	$0.67 \pm 0.17^{b***}$	$1.09 \pm 0.03^{a***}$	$0.63 \pm 0.05^{b***}$	$0.46 \pm 0.06^{a***b***}$	$0.80 \pm 0.04^{b***}$
14	$0.32 \pm 0.05^{b***}$	$1.63 \pm 0.16^{a***}$	$1.20 \pm 0.06^{a***b**}$	$1.38 \pm 0.04^{a***}$	$1.37 \pm 0.03^{a***}$
21	$0.20 \pm 0.04^{b***}$	$2.05 \pm 0.16^{a***}$	$1.26 \pm 0.07^{a***b***}$	$1.38 \pm 0.04^{a***b***}$	$1.55 \pm 0.05^{a***b**}$
Phosphate (mg/24 h)					
0	0.15 ± 0.08	0.2 ± 0.02	0.19 ± 0.02	$1.30 \pm 0.02^{a***b***}$	$0.75 \pm 0.11^{a***b***}$
7	0.24 ± 0.05	0.35 ± 0.04	0.25 ± 0.02	$1.34 \pm 0.04^{a***b***}$	$0.81 \pm 0.05^{a***b***}$
14	$0.16 \pm 0.03^{b**}$	$0.55 \pm 0.08^{a**}$	0.28 ± 0.03	$1.45 \pm 0.05^{a***b***}$	$1.35 \pm 0.15^{a***b***}$
21	$0.15 \pm 0.01^{b**}$	$0.58 \pm 0.07^{a**}$	0.32 ± 0.01	$1.53 \pm 0.05^{a***b***}$	$1.42 \pm 0.15^{a***b***}$
Uric acid (mg/24 h)					
0	$0.11 \pm 0.02^{b***}$	$0.40 \pm 0.06^{a***}$	$0.41 \pm 0.02^{a***}$	$0.29 \pm 0.02^{a**}$	$0.31 \pm 0.02^{a**}$
7	0.45 ± 0.1	0.71 ± 0.06	0.43 ± 0.01	0.46 ± 0.03	$1.06 \pm 0.17^{a**b*}$
14	$0.36 \pm 0.05^{b***}$	$1.36 \pm 0.15^{a***}$	$0.77 \pm 0.08^{a*}$	$1.27 \pm 0.10^{a***b***}$	$1.38 \pm 0.02^{a***}$
21	$0.31 \pm 0.01^{b***}$	$1.42 \pm 0.09^{a****}$	$0.77 \pm 0.06^{a***b***}$	$1.58 \pm 0.09^{a***}$	$1.50 \pm 0.02^{a***}$

Values are expressed in mg/24 h urine sample as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett test. Number of animals (N) = 6. Group I: Vehicle control, Group II: EG group, Group III: Cystone group, Group IV: 150 mg/kg MELP treatment group, Group V: 300 mg/kg MELP treatment group. Comparisons are made against Group I (vehicle control)[a] and Group II (lithiatic control)[b].
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table A4
Effect of methanolic extract of *Launaea procumbens* leaves on serum and kidney parameters on different groups of rat.

Parameter	Group I	Group II	Group III	Group IV	Group V
Kidney (mg/g)					
Calcium	$0.11 \pm 0.01^{b**}$	$0.65 \pm 0.22^{a**}$	$0.16 \pm 0.05^{b**}$	0.35 ± 0.02	0.32 ± 0.03
% Lipid peroxidation	$24.43 \pm 1.43^{b***}$	$100 \pm 0.00^{a***}$	$45.22 \pm 2.77^{a***b***}$	$69.00 \pm 3.87^{a***b***}$	$59.07 \pm 4.38^{a***b***}$
Serum (mg/dl)					
Creatinine	$0.91 \pm 0.02^{b***}$	$1.87 \pm 0.04^{a***}$	$1.38 \pm 0.02^{a***b***}$	$1.87 \pm 0.08^{a***}$	$1.12 \pm 0.09^{b***}$
Uric acid	$3.23 \pm 0.58^{b***}$	$8.50 \pm 0.55^{a***}$	$5.77 \pm 0.75^{a**b**}$	$2.52 \pm 0.28^{b***}$	$1.49 \pm 0.24^{b***}$

Values are expressed in mg/g in kidney parameter and mg/dl in serum sample as mean ± SEM. One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett test. Number of animals (N) = 6. Group I: Vehicle control, Group II: EG group, Group III: Cystone group, Group IV: 150 mg/kg MELP treatment group, Group V: 300 mg/kg MELP treatment group. Comparisons are made against Group I (vehicle control)[a] and Group II (lithiatic control)[b].
** $p < 0.01$.
*** $p < 0.001$.

Table A5
Effect of methanolic extract of *Launaea procumbens* leaves on urinary total protein excretion rate (mg/24 h) on different groups of rat.

Days	Group I	Group II	Group III	Group IV	Group V
0	0.21 ± 0.01	0.93 ± 0.06^{a**}	0.87 ± 0.06^{a**}	0.96 ± 0.19^{a***}	0.77 ± 0.17^{a*}
7	1.19 ± 0.03^{b***}	1.85 ± 0.06^{a**}	0.96 ± 0.07^{b***}	1.52 ± 0.11	1.30 ± 0.14^{b***}
14	0.67 ± 0.04^{b**}	3.33 ± 0.34^{a***}	1.14 ± 0.06^{a***}	1.60 ± 0.12	0.78 ± 0.25^{a**}
21	0.75 ± 0.11^{b***}	3.66 ± 0.24^{a***}	2.60 ± 0.16^{a***b***}	3.01 ± 0.20^{a***b*}	4.58 ± 0.06^{a***b**}

Values are expressed in mg/24 h urine sample as mean ± SEM ($n = 6$). One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett test. Group I: Vehicle control, Group II: EG group, Group III: Cystone group, Group IV: 150 mg/kg MELP treatment group, Group V: 300 mg/kg MELP treatment group. Comparisons are made against Group I (vehicle control)^{a} and Group II (lithiatic control)^{b}.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

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