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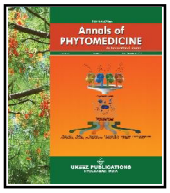

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Lepidium sativum L. seeds extract as a preventive agent in experimentally induced urolithiasis model

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Abstract

Lepidium sativum L. is an annual herb, belonging to Brassicaceae family and is used as a diuretic, anti-oxidant and nephroprotective agent. In the present study, we investigated the antiurolithiatic activity of aqueous and ethanolic extract of *L. sativum* (LS) seeds in 0.75% ethylene glycol (EG) and 1% ammonium chloride (AC) induced urolithias is model in rats. An *in vitro* assay was used to determine whether both extracts of LS prevent the formation of calcium oxalate (CaOx) or promote CaOx dissolution. Animals model of calcium oxalate urolithias was induced in the rat by adding EG 0.75% and 1% AC in drinking water. Aqueous extract of *L. sativum* (AELS) and ethanolic extract of *L. sativum* (EELS) orally at 100 and 200 mg/kg and cystone 750 mg/kg were administered along with EG and AC for 14 days (from 8th to 21st days). Various urine, serum and kidney parameters were assessed at the end of the study.

The ethylene glycol group had a significant increase in levels of urine oxalate, calcium and phosphate, serum creatinine, urea and uric acid and renal tissues oxalates, as compared to normal. Histopathology shows significant changes. The treatment of AELS and EELS at 100 and 200 mg/kg doses, significantly decreased the urine oxalate, calcium, phosphate, serum creatinine, urea, anduric acid levels, in EG+AC-induced urolithiasis after 21 days. From the above results, it can be concluded that both extracts of *L. sativum* seeds have a significant inhibitory effect on calcium oxalate urolithiasis due to their diuretic and antioxidant activity.

1. Introduction

Urolithiasis is the third most normal problem of the urinary plot. It is the presence of calculi in the urinary plot with a male-to-female frequency proportion multiple times and more among white guys than in dark guys (Venkateswarlu *et al*., 2016). As per studies, calcium oxalate and calcium phosphate make up 80% of renal stones, while struvite, which contains magnesium ammonium phosphate, makes up 10%. Uric acid (9%) and cystine or ammonium acid urate are the reasons for drug-related renal stones (1%) (Pandhare *et al*., 2021).

The development of stones is believed to be brought about by a strange expansion in urinary degrees of calcium, oxalate and uric acid, which prompts a decrease in urinary citrate levels. Citrate and magnesium are the principal inhibitors of stone development in the urinary tract and diminished levels or an absence of these inhibitors in the pee cause stone arrangement (Ilhan *et al*., 2014). Urinary calculi may cause obstruction, hydronephrosis, infection and haemorrhage in the urinary tract system (Dharmalingam *et al*., 2016).

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The therapy for this infection incorporates clinical and careful treatment. Be that as it may, careful evacuation of stones by lithotripsy and percutaneous nephrolithotomy strategies can cause a few incidental effects like rounded rot, hypertension, drain and resulting fibrosis of the kidney, prompting cell injury and a repeat of renal stone development (Bayir *et al*., 2011).

L. sativum (Garden cress) is an annual spice, belonging to the Brassicaceae family. In certain locales, garden cress is known as garden pepper cress, pepper grass, or pepperwort. It is also known as Asalio or chandrasur in India and it is a significant medicinal yield in India. The qualitative analysis of *L. sativum* seeds showed that the seeds contained protein, lipids, carbohydrates, fiber, ash, moisture, alkaloids, flavonoids, saponin, tannin and phenol (Al-Snafi *et al*., 2019). In the traditional arrangement of Indian medication, different pieces of the plant have been utilized to treat different human ailments like the runs, looseness of the bowels, leprosy, skin and eye illnesses, leucorrhoea, scurvy, liver diseases, renal illnesses, dyspepsia, asthma, cough, cold and seminal shortcoming, also thought to be as harsh, diuretic, tonic, abortifacient, Spanish fly, thermogenic, galactagogue, emmenagogue, depurative and ophthalmic. It is also used to treat tenesmus and secondary syphilis (Sharma *et al*., 2020).

L. sativum seeds have diuretic (Patel *et al*., 2009), antioxidant (Fayez Aboelmaati *et al*., 2016; Mulla *et al*., 2019), anti-spasmodic (Rehman *et al*., 2011) and nephroprotective activity (Sawsan *et al*., 2021). We have selected this plant for urolithiasis activity. No examinations researching this impact have been accounted for in the literature yet. Consequently, the purpose of our current study is to assess the effects of *L. sativum* seed extract as a preventive agent in an experimentally induced urolithiasis model in rats.

2. Materials and Methods

2.1 Drug and chemicals

Ethylene glycol was purchased from Chem Think Lab, Ankleshwar, Gujarat, India. Cystone was obtained from Himalaya Health Care Pvt. Ltd. Various kits for biochemical estimation of urine and serum were purchased from Arkray Healthcare Private Limited, Sachin, Surat, India. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

2.2 Plant material

L. sativum seeds were procured in Jan 2022 from Yucca Enterprises; Barkat Ali Naka, Wadala (East), Mumbai-400037, Maharashtra, India and authenticated by Harshad M. Pandit, Ph.D. (Botany), (Formerly Head and Associate Professor of Botany). Andheri (West), Mumbai 400058, Maharashtra, India.

2.3 Preparation of extract

1 g of dried powdered seeds of *L. sativum* were boiled in 100 ml of distilled water for 10-15 min. Cooled the extract and filtered it before using it to remove specific matter. The filtrate was preserved at 4°C till further use (AE, 34.6 %) (Maghrani *et al.*, 2005). And for ethanolic extract, 100 g of dried powder of *L. sativum* seeds was added to one liter of ethanol for 8 h. The extract was filtered using sterile filter paper (Whatman paper No.1) into a clean conical flask. After filtration, a rotatory evaporator was used to evaporate the solvent to yield a semi-solid mass. The obtained extract was collected and stored at 4°C until use (EE 25.3%) (Yahla *et al*., 2021; Rosaria *et al*., 2018).

2.4 Experimental animals

Experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The experimental protocol in the study was approved by the Institutional Animal Ethical Committee (CPCSEA/SNLPCP/IAEC/22/01/131). The male albino rats of the Wistar strain, weighing 200-250 g, were obtained from the Jay Research Foundation, Vapi, Gujarat. The rats were kept at a temperature of 25 ± 1°C and relative humidity of 50 ± 55%. Rats were fed a standard chow diet and water *ad libitum*. Animals were acclimatized in institutional animal houses and were exposed to a 12 h day and night cycle.

2.5 Evaluation of the *in vitro* antiurolithiatic activity

The classical model for the study of oxalate crystallization was chosen because of its simplicity and satisfactory reproducibility, according to the method, crystallization without inhibitors and with them to assess the inhibiting capacity of the test material. The plant extracts, antiurolithiatic activity was evaluated *in vitro* by inhibiting calcium oxalate nucleation, aggregation and growth in the presence of inhibitors (standard drug and extracts) and the absence of inhibitors. A UV/Visible spectrophotometer (Shimadzu UV-1700) was employed to measure the turbidity changes in each assay. Five different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) of the plant extracts were tested in each assay.

2.5.1 Nucleation assay

The solution of calcium chloride (5 mmol/l) and sodium oxalate (7.5 mmol/l) was prepared in a buffer containing Tris-HCl 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5. 9 ml of calcium chloride solution was blended in with 1ml of at various concentrations of plant extracts (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml). Crystallization was begun by adding 950 ml of sodium oxalate solution. The temperature was kept up at 37°C. The optical density (OD) of the solution was observed at 620 nm after 30 min. The rate of nucleation was assessed by contrasting the acceptance time within the sight of plant removal with that of control (Kaushik *et al*., 2019). The development of crystals was supposed to be because of the accompanying response:

$$CaCl_2 + Na_2C_2O_4 \; CaC_2O_4 + 2NaCl$$

Percentage inhibition of nucleation was calculated using the following formula:

$$\% \text{ Inhibition of nucleation} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.5.2 Aggregation assay

Calcium oxalate monohydrate (COM) crystals were ready by blending calcium chloride and sodium oxalate at 50 mmol/l. Both solutions were equilibrated to 60°C in a water shower for 1 h and afterward cooled to 37°C overnight. The crystals were gathered by centrifugation and afterward vanished at 37°C. The CaOx crystals were utilized at the last centralization of 0.8 mg/ml, supported with Tris 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5. The examinations were led in the nonappearance and presence of the plant separate (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) after halting blending. Cystone tablets were utilized as a standard drug solution. The percentage aggregation inhibition rate was calculated by comparing the turbidity in the presence of the extract with that obtained in the control using the following formula (Kaushik *et al*., 2019; Patel *et al*., 2012).

% Aggregation = (1 Turbidity sample/Turbidity control) × 100

2.6 Evaluation of the *in vivo* antiurolithiatic activity

Screening of the antiurolithiatic potential of AELS and EELS was performed on rats using an ethylene glycol (EG) and ammonium chloride (NH_4Cl) induced model of urolithiasis. Forty-two male Wistar rats were divided into seven groups (n = 6).

Group I: Normal control group received regular drinking water *ad libitum* throughout the study.

Group II: Model control group received EG 0.75% (V/V) and AC 1% (W/V) for 7 days (p.o.).

Group III: Standard group received cystone (750 mg/kg) from the 8th to 21st day (p.o.).

Groups IV-V: AELS 100 and 200 mg/kg p.o. respectively, for the 8th to 21st day.

Groups VI-VII: EELS 100 and 200 mg/kg p.o.respectively, for the 8th to 21st day.

The groups II-VII, received EG and AC in their drinking water *ad libitium* for 7 days, respectively, to induce urolithiasis and generate CaOx deposition into the kidneys. The body weight of all the animals was noted on the 0, 8th and 22nd days of the experiment. All the animals were sacrificed at the end of the experiments **(**Bano *et al*., 2018).

2.6.1 Analysis of urine samples

All of the animals were housed in individual metabolic cages and a 24 h urine sample was collected on the 21st day. Body weight, water intake, volume and pH of urine and number of Caox crystal in urine (Kumar *et al.,* 2016; Bouanani *et al.,* 2010) were determined.

Urine was acidified with a drop of concentrated HCl and stored at –20ºC for determination of calcium, oxalate, magnesium, phosphate, and uric acid using commercially available kits. The oxalate and citrate were estimated by the method described previously (Hodgkinson *et al.,* 1970).

2.6.2 Collection and analysis of serum

After the experimental period, blood was collected from the retroorbital under light ether anesthesia and animals were sacrificed under high doses of anesthetics. Serum was separated by centrifugation at 10,000 g for 10 min and analyzed for calcium, creatinine, uric acid, urea and blood urea nitrogen (BUN) using commercially available diagnostic kits, while oxalate was measured by the method of Hodgkinson (1970).

2.6.3 Kidney histopathology and homogenate analysis

The abdomen was incised and opened and both kidneys were taken out from each animal. Detached kidneys were cleaned of unessential tissue, gauged and washed with super cold typical saline. The left kidney was fixed with 10% v/v neutral formalin and after gathering, cut on a level plane and shipped off histology administrations (Samarth Pathology Lab, Surat) for hematoxylin and eosin staining.

The same histology slides were subjected to microscopic examination for the presence of glomerular congestion, tubular casts, peritubular congestion, epithelial adhesion, blood vessel congestion, interstitial edema and inflammatory cells.

The right kidney was finely cleaved and 20% homogenate was ready in Tris-HCl buffer (pH7.4). Absolute kidney homogenate was utilized for testing tissue calcium and oxalate, malondialdehyde (MDA) (Ohkawa *et al*.,1979), reduced glutathione (GSH) (Beutler *et al*., 1963) and catalase (Hugo, 1984).

2.7 Statistical analysis

Results are presented as mean ± SEM, using the software GraphPad Prism 8.0. The statistical significance of data was assessed by analysis of variance (one-way-ANOVA), followed by Dunnett's Multiple Comparison test. Significance was considered at $p<0.05$.

3. Results

The qualitative chemical analysis revealed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds in both extracts. from the literature of acute oral toxicity studies of ethanolic and aqueous extracts of *L. sativum* seeds. At 2000 mg/kg, we discovered no toxic effect (Yadav *et al*., 2009; Olotu *et al.,* 2018). Two doses were selected: 100 and 200 mg/kg of body weight.

The aqueous and ethanol extract of LS inhibited crystal nucleation as well as aggregation in a dose dependent manner and were comparable to the activity of cystone (Table 1).

3.1 *In vitro* study

Table 1: Inhibitory effects of AELS and EELS on nucleation and aggregation of calcium oxalate

Concentration (µg/ml)	Percentage inhibition of nucleation	Percentage inhibition of aggregation
Aqueous extract		
100	31.71	41.69
200	47.21	50.97
300	56.43	62.40
400	69.18	73.25
500	75.60	85.62
Ethanolic extract		
100	24.91	39.81
200	30.09	47.18
300	36.13	50.51
400	38.42	62.89
500	42.51	68.37
Cystone		
100	52.68	58.11
200	56.68	66.01
300	58.67	74.91
400	64.43	80.34
500	68.90	83.24

3.2 Urine analysis

There was a critical ($p<0.01$) decline in urine volume with no significant changes in pH in model control rats in contrast with normal rats. Cystone-treated rats showed a significant ($p<0.05$) increase in urine volume when contrasted with the model controlgroup. LS-treated rats at dosages of 100 and 200 mg/kg body weight caused a significant ($p<0.05$ to 0.01) increase in urine volume with practically no massive change in pH when contrasted with model control rats (Tables 2, 3).

Table 2: Effects of AELS and EELS on body weight and water intake

Groups	Body weight			Water in take		
	Day 0	Day 8	Day 22	Day 0	Day 8	Day 22
Normal control	252.50 ± 0.76	254.16 ± 0.65	255.83 ± 0.40	24.66 ± 0.88	20.83 ± 0.65	24.00 ± 0.57
Model control	253.33 ± 0.88	203.500 ± 0.99###	213.00 ± 0.96***	18.16 ± 0.60	13.16 ± 0.94###	14.50 ± 1.11***
Standard	254.16 ± 0.60	216.83 ± 2.18###	243.50 ± 0.76***	23.00 ± 0.57	18.83 ± 0.47###	20.00 ± 0.57***
AELS-100	259.16 ± 2.24	209.33 ± 1.68#	242.50 ± 0.76**	22.16 ± 0.60	17.50 ± 0.56#	18.83 ± 0.47**
AELS-200	257.50 ± 2.29	211.83 ± 4.65##	247.50 ± 0.42***	21.83 ± 0.60	18.50 ± 0.76##	20.16 ± 0.83***
EELS-100	255.83 ± 2.52	213.50 ± 0.76##	232.50 ± 0.76*	23.00 ± 0.57	16.66 ± 0.66#	17.16 ± 0.70*
EELS-200	259.16 ± 2.08	214.50 ± 0.76##	236.50 ± 0.56**	23.16 ± 0.60	17.50 ± 0.56#	18.83 ± 0.47**

The values are expressed as mean ± SEM (n=6); Statistical analysis by ANOVA, followed by Dunnett's Multiple Comparison test. ***p<0.001, **p<0.01, *p<0.05. # Day 8 compared with Day 0 * Day 22 compared with Day 8.

Table 3: Effects of AELS and EELS on urine volume and No. of urine Caox crystal

Groups	Urine volume			No. of urine Caox crystal	
	Day 0	Day 8	Day 22	Day 8	Day 22
Normal control	4.94 ± 0.00	4.93 ± 0.00	4.91 ± 0.00	10.13 ± 0.00###	7.83 ± 0.30*
Model control	4.94 ± 0.00	4.65 ± 0.00###	4.65 ± 0.01***	10.22 ± 0.00###	4.83 ± 0.30***
Standard	4.93 ± 0.00	4.66 ± 0.01###	5.12 ± 0.00***	10.25 ± 0.02##	7.00 ± 0.36**
AELS-100	4.94 ± 0.00	4.68 ± 0.01#	4.90 ± 0.00**	10.18 ± 0.00###	5.83 ± 0.30***
AELS-200	4.93 ± 0.00	4.68 ± 0.01#	5.03 ± 0.02***	10.21 ± 0.00##	8.33 ± 0.33*
EELS-100	4.93 ± 0.00	4.68 ± 0.01#	4.77 ± 0.00*	10.20 ± 0.01##	7.33 ± 0.33**
EELS-200	4.94 ± 0.00	4.68 ± 0.01#	4.87 ± 0.00**	10.13 ± 0.00###	7.83 ± 0.30*

The values are expressed as mean ± SEM (n=6); Statistical analysis by ANOVA, followed by Dunnett's Multiple Comparison test. *** p<0.001, ** p<0.01, * p<0.05 # Day 8 compared with Day 0 * Day 22 compared with Day 8.

In the current examination, administration of EG and AC as a renal stone inducer caused hyperoxaluria, which was recognized by an expansion in the urinary discharge of oxalate, uric corrosive, creatinine, phosphorous and a decline in magnesium. However, repeated administration of cystone 750 mg/kg and AELS and EELS at portions of 100 and 200 mg/kg body weight for 14 days successfully stayed away from this expansion in urinary oxalate, uric corrosive, creatinine, phosphorous and decline in magnesium in a dose-dependent manner when compared with the EG-AC treated control group and recovered it to normal levels Table 4.

Table 4: Effect of AELS and EELS on various urinary parameters in ethylene glycol and ammonium chloride-induced urolithiasis

Groups	Calcium (mg/24 h)	Phosphorus (mg/24 h)	Creatinine (mg/24 h)	Sodium (mEq/dl)	Oxalate (mg/24 h)	Uric acid (mg/24 h)	Magnesium (mg/24 h)
Normal control	4.00 ± 0.36	4.83 ± 0.47	0.76 ± 0.008	170 ± 0.76	3.22 ± 0.29	3.37 ± 0.47	10.18 ± 0.45
Model control	7.17 ± 0.47###	7.50 ± 0.42###	0.60 ± 0.005###	179 ± 1.39###	13.95 ± 0.87###	9.69 ± 0.73###	18.34 ± 1.04###
Standard	4.73 ± 0.13***	5.00 ± 0.36***	0.72 ± 0.007***	170 ± 0.20***	7.86 ± 0.61**	4.91 ± 0.71**	12.26 ± 0.72**
AELS-100	5.67 ± 0.33*	5.67 ± 0.33*	0.77 ± 0.004**	176 ± 0.88**	11.32 ±1.25**	6.91 ± 0.28**	15.68 ± 0.56**
AELS-200	4.83 ± 0.30***	5.17 ± 0.30***	0.75 ± 0.006***	173 ± 0.47***	8.57 ± 1.28**	5.82 ± 0.27**	13.96 ± 0.47**
EELS-100	5.67 ± 0.33*	6.00 ± 0.36*	0.77 ± 0.004*	175 ± 0.47**	11.09 ±1.38*	7.93 ± 0.82**	14.98 ± 0.86*
EELS-200	5.50 ± 0.42*	5.50 ± 0.42**	0.76 ± 0.004**	174 ± 0.57**	8.96 ± 1.56**	6.62 ± 0.53**	14.26 ±0.72**

The values are expressed a smean ± SEM (n=6); Statistical analysis by one way-ANOVA, followed by dunnett's multiple comparis on test. ***p<0.001, **p<0.01, *p<0.05. Compared with the Model control group.### p<0.001 when compared to normal control group.

3.3 Serum analysis

The induction of renal stone by EG-AC treatment brought about disabled renal capability as confirmed by raised serum sodium, potassium, creatinine and uric corrosive, which are markers of glomerular and tubular injury. However, repeated administration of cystone 750 mg/kg and AELS and EELS at portions of 100 and 200 mg/kg body weight for 14 days kept away from these progressions when contrasted with the EG-AC-treated control group (Table 5).

Table 5: Effect of AELS and EELS on various serum parameters in ethylene glycol and ammonium chloride-induced urolithiasis

Groups	Calcium (mg/dl)	Phosphorus (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Oxalate (mg/dl)	Uric acid (mg/dl)	BUN (mg/dl)
Normal control	8.90 ± 0.39	2.00 ± 0.020	1.11 ± 0.02	45.4 ± 2.04	0.82 ± 0.03	2.29 ± 0.14	41.86 ± 2.16
Model control	11.00 ± 0.36###	5.48 ± 0.273###	1.32 ± 0.02###	54.3 ± 0.04###	2.21 ± 0.12###	6.38 ± 0.39###	72.26 ± 4.23###
Standard	8.80 ± 0.20***	4.75 ± 0.006***	1.10 ± 0.05***	44.1 ± 0.02***	0.83 ± 0.01**	2.88 ± 0.16**	47.81 ± 2.56**
AELS-100	9.19 ± 0.25**	4.88 ± 0.007**	1.15 ± 0.02*	47.2 ± 0.05**	1.29 ± 0.09*	4.60 ± 0.27*	53.28 ± 1.36**
AELS-200	8.97 ± 0.25***	4.77 ± 0.024***	1.12 ± 0.04***	45.4 ± 0.14***	0.92 ± 0.03**	3.47 ± 0.18**	48.19 ± 2.28**
EELS-100	9.50 ± 0.22*	4.89 ± 0.016*	1.14 ± 0.01*	48.3 ± 1.46*	1.46 ± 0.16*	4.87 ± 0.35*	56.64 ± 2.31*
EELS-200	9.17 ± 0.30**	4.87 ± 0.015**	1.13 ± 0.01**	46.9 ± 1.88**	0.93 ± 0.08**	4.00 ± 0.27**	51.78 ± 2.89**

The values are expressed as mean ± SEM (n=6); Statistical analysis by One way- ANOVA, followed by Dunnett's Multiple Comparison test. *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Compared with the Model control group. ### $p<0.001$ when compared to normal control group.

3.4 Kidney homogenate analysis

EG-AC administration significantly ($p<0.01$) increased the MDA level and diminished GSH content and exercises of CAT and SOD in EG-AC-treated model control rats when contrasted with normal control rats. The treatment with cystone 750 mg/kg significantly ($p<0.01$) diminished the degrees of MDA and expanded GSH content, CAT and SOD activities when contrasted with model control rats. However, repetitive administration of AELS and EELS at dosages of 100 and 200 mg/kg body weight for 14 days significantly ($p<0.05$) decreased MDA and increased GSH content, CAT and SOD activities in a dose-dependent manner when contrasted with EG-AC model control rats. In the EG-AC-treated model control rats, calcium deposition in the renal tissue was also increased (Table 6).

Table 6: Effect of AELS and EELS on kidney parameters in ethylene glycol and ammonium chloride-induced urolithiasis

Groups	Calcium (mg/gm)	Oxalate (mg/gm)	GSH (n moles/mg protein)	SOD (U/mg protein)	LOP (nm of MDA/mg)
Normal control	0.31 ± 0.006	1.91 ± 0.007	63.8 ± 0.01	12.7 ± 0.11	10.5 ± 0.06
Model control	0.62 ± 0.006###	5.92 ± 0.006###	45.7 ± 0.00###	4.71 ± 0.01###	19.0 ± 0.57###
Standard	0.32 ± 0.004***	3.79 ± 0.006***	64.4 ± 0.04***	11.1 ± 0.29***	9.33 ± 0.04***
AELS-100	0.60 ± 0.007**	5.88 ± 0.007**	67.2 ± 0.01*	7.80 ± 0.00**	9.38 ± 0.00**
AELS-200	0.58 ± 0.005***	5.86 ± 0.006***	68.9 ± 0.01***	6.50 ± 0.05***	9.31 ± 0.03***
EELS-100	0.60 ± 0.005*	5.89 ± 0.008*	65.3 ± 0.00*	9.23 ± 0.00*	9.42 ± 0.00**
EELS-200	0.59 ± 0.007**	5.88 ± 0.007**	66.8 ± 0.01**	8.13 ± 0.00**	9.38 ± 0.00**

The values are expressed as mean ± SEM (n=6); Statistical analysis by One way-ANOVA, followed by Dunnett's Multiple Comparison test. *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Compared with the Model control group. # # # $p<0.001$ when compared to normal control group.

3.5 Kidney histopathology

The induction of renal stone by EG-AC treatment caused marked histological changes, for example, tubular dilatation and beginning cystic changes, tubular atrophy, calcium oxalate crystal deposits and interstitial mononuclear cell infiltration in the kidneys. However, repeated administration of cystone 750 mg/kg and AELS and EELS at dosages of 100 and 200 mg/kg body weight for 14 days substantially reduced the amount of calcium oxalate deposits and other anomalies in the renal tubules in a dose-dependent manner (Figure 1).

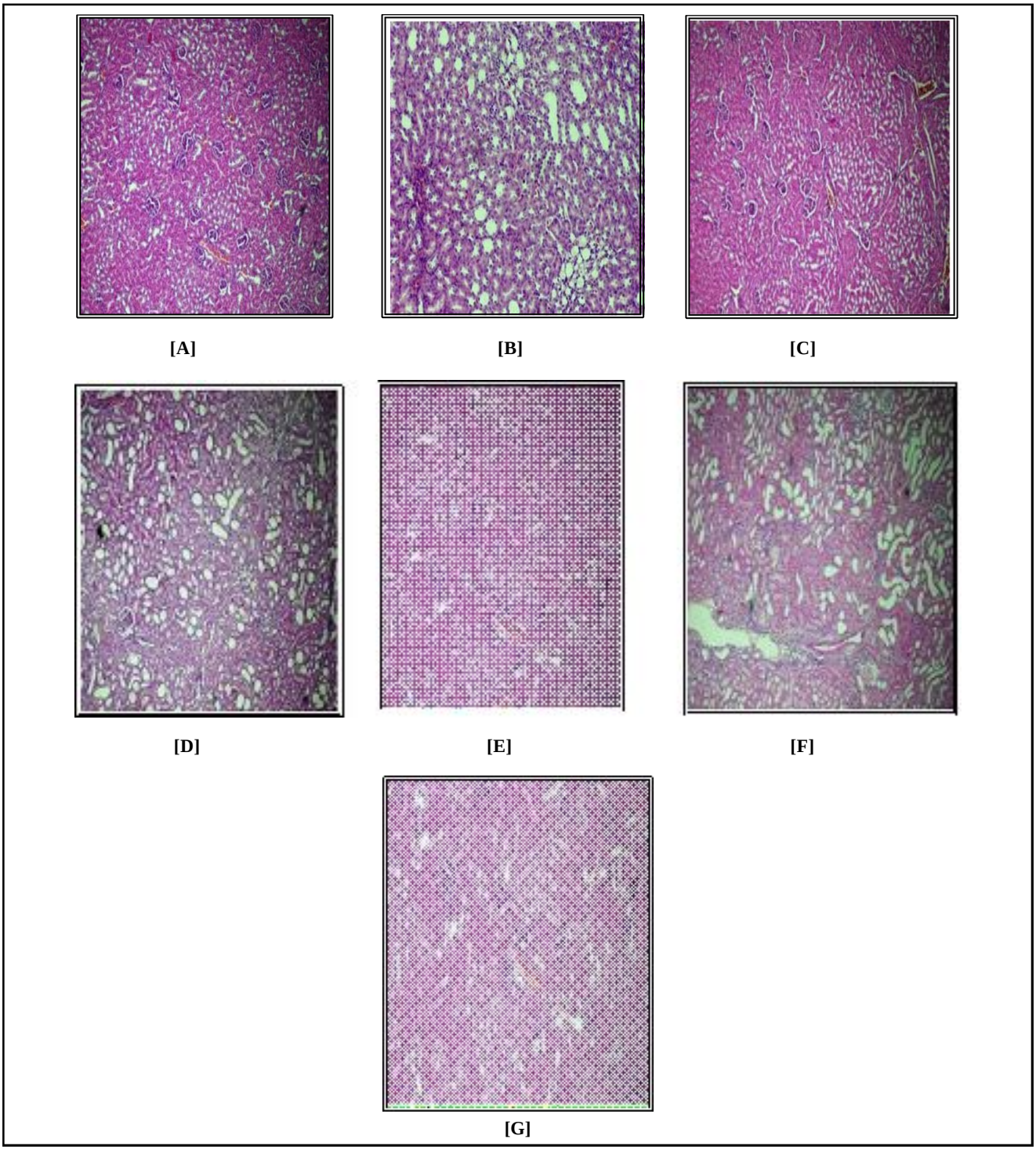

Figure 1: Histopathological analysis of kidney sections: [A] Normal group, [B] Model control group, [C] Standard group, [D] AELS-100, [E] AELS-200, [F] EELS-100, [G] EELS-200.

4. Discussion

The occurrence of calculus in the kidney and different segments of the urinary tract, chiefly the bladder and ureter, is known as urolithiasis. Urolithiasis is a successive stepwise obsessive occasion that begin from the supersaturation of urine, nucleation, crystal development, conglomeration and crystal retention (Aggarwal *et al*., 2013). The rat is the most reasonable and generally involved exploratory animal for calcium oxalate induced urolithiasis. There are numerous likenesses between human renal stone arrangement and that of experimental urolithiasis-induced rats (Tzou *et al*., 2016).

Ethylene glycol (EG) ingestion into rats has been generally utilized as a trial model for the investigation of nephrolithiasis. however, when EG is utilized alone, kidney crystal deposition can be very conflicting. To accomplish a consistently high rate of kidney crystal deposition, ammonium chloride (AC) has been utilized in a mix with ethylene glycol. It has been accounted that EG is oxidized to oxalic acid by dehydrogenase, which prompts hyperoxaluria key element for urolithiasis. EG metabolizes into CaOx monohydrate and produces renal mitochondrial toxicity like clinical CaOx renal calculi (Bano *et al*., 2018).

The aqueous and ethanolic extract of *L. sativum* inhibited crystal formation as well as promoted crystal dissolution in a dose-dependent manner and were comparable to the activity of cystone. From all the extracts, the highest percentage of nucleation inhibition was obtained from aqueous extract at a concentration of 500 µg/ml (75.60%). The aqueous (100 to 500 µg/ml) and ethanolic extracts (100 to 500 µg/ml) were found to possess significant % inhibition of nucleation when compared to standard cystone (100 to 500 µg/ml). In the aggregation assay of different extracts of *L. sativum* the highest percentage of aggregation and inhibition was obtained from aqueous extract at a concentration of 500 µg/ml (85.62%). The aqueous (100 to 500 µg/ml) and ethanolic extracts (100 to 500 µg/ml) were found to possess significant % inhibition of aggregation when compared to standard cystone (100 to 500 µg/ml) (Patel *et al*., 2012).

In the present study, the body weight, urinevolume and water intake of animals on the 7th day were found to be decreased in all the groups. It is because of ingestion of EG 0.75% with AC 1% for 7 days, due to this animal became sick, appeared lethargic, drank less water, stopped eating and lost body weight. After treatment with test extracts like AELS and EELS with a dose of 100 mg/kg and 200 mg/kg, the body weight, urine output and water intake of animals increased comparatively (Bouanani *et al*., 2010).

Considerably low urine volume, pH, mild hyperoxaluria, hypercalciuria, hypocitraturia, hyperuricosuria and hypomagnesuria are significant risk factors in stone formers. Urine pH (<5.5 for uric acid stones and >6 for calcium stones) is a substitute marker for deciding the type of calculi. Especially, in CaOx urolithiasis, the commented basicity of the urine beyond pH 7.2 starts the nucleation of phosphate and oxalate with calcium (Gadge *et al*., 2012). In this study, albino rats showed expanded pH (7.82) and formed renal calculi made mostly out of CaOx in response to 14 days of the oral supplement of EG.

Due to the effect of inducing material on the 8th day, CaOx crystals were observed in all groups of animals (Patel *et al.*, 2022). After treatment with plant extract, number of calcium oxalate crystals decreased which confirms that AELS and EELS were having an anti-urolithiatic effect. The dose of 200 mg/kg of AELS showed more significance in CaOx crystal about (5.83 ± 0.30, p<0.001).

Increase durinary calcium is a key factor, that favors then ucleation and precipitation of calcium oxalate from urine and consequent crystal growth. Increase durinary phosphorus excretion and oxalate stress appear to provide an appropriate environment for the formation of calcium phosphate crystals which induces calcium oxalatede position. Increased sodiumand decreased creatinine levels also result in the formation of kidney stones (Ratkalkar *et al*., 2011). Treatment with AELS and EELS at two different doses (100 mg/kg and 200 mg/kg) resto redurine calcium, phosphorus, creatinine and sodium levels, thus reducing the risk of stone formation.

AELS with a dose of 200 mg/kg restored all the levels more significantly. EG is accounted for to be nephrotoxic, renal capability was surveyed toward the finish of the concentrate by assessing the serum creatinine, urea, calcium and phosphorus levels. In urolithiasis, the stones in the urinary system obstruct the outflow of urine, cause diminished GFR and eventually leading to gathering of nitrogenous substances like urea and creatinine in the blood. The administration of test extracts inhibited these changes and brought down the serum creatinine and urea, prompting an improved GFR.Treatment with AELS and EELS (100 mg/kg, 200 mg/kg) extracts also balance the serum calcium and phosphorus level. As compared to EELS, AELS with a dose of 200 mg/kg showed a more significant balance in serum calcium, phosphorus, creatinine and urea level.

When urolithiasis is produced, it causes the deposition of calciuman doxalate crystals on kidney homogenate in all groups. After treatment with standard drugcystone, their number decreased significantly. Treatment with AELS and EELS (100 mg/kg and 200 mg/kg) also reduced calcium and oxalate level.The 200 mg/kg dose of AELS showed a more significant decrease in calcium (0.59 ± 0.007) and oxalate (5.88 ± 0.007) crystal in the kidney.

Oxidative stress plays an active role in urolithiasis and human studies, it has been shown that stone formers have increased oxidative stress and renal tubular injury relative to the non-stone formers ROS plays an important role in the formation of CaOx kidney stones by regulating crystal formation, growth and retention and antioxidant treatments reduce crystal and oxalate-induced injury (Singh *et al*., 2021). After treatment with cystone, AELS and EELS, their levels were restored comparatively. As compared to all extracts only, AELS (200 mg/kg) showed more significant restoration in the level of GSH, SOD and LOP (Ashok *et al*., 2010).

The histopathological investigation of the kidney showed unpredictable eosinophilic material, hardly any blocked veins and scattered mononuclear inflammatory infiltration in positive control. While the test drug and the cystone-treated group showed mild changes as compared with the model control. These discoveries showed the efficacy of test extrication when compared with positive control as no undeniable insanity in renal architectures was tracked down in test and standard groups. Oxalate is the existing precursor molecule for lipid peroxidation, responds with polyunsaturated unsaturated fats in the cell layer and causes renal tissue harm. This undeniable renal harm was observed in the histological architecture of the kidney in model control rats. In AELS and EELS with a dose

of 100 mg/kg and 200 mg/kg and cystone showed that the damage was found to be almost recovered except a few blood vessels appeared congested and some tubular epithelial cells showed necrosis. Test drug AELS with a dose of 200 mg/kg reported a more significant recovery in kidney structure (Makasana *et al*., 2014). Therefore, aqueousande thanolic extracts (100 mg/kg and 200 mg/kg) possess antiurolithiatic activity due to the irdiuretic, antioxidant and nephroprotective activity.

5. Conclusion

Antiurolithiatic activity of *L. sativum* is mediated, possibly due to the prevention of urinary supersaturation, inhibition of mineralization of stone-forming constituents and normalization of the cellular function by neutralizing the effect of ROS, which could have caused oxidative stress in renal tubules. The study suggests that flavonoids, steroids, alkaloids, andphenolic compounds of LS are therapeutically effective for the treatment of calcium oxalate stones, exhibiting effects through a combination of a diuretic and antioxidant action, which could be responsible for its antilithiatic activity. Further studies are necessary to determine the mechanism of action of LS in the treatment of urolithiasis.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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