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BIOLOGICAL EVALUATION OF *IN-VIVO* DIURETIC AND ANTIUROLITHIATIC ACTIVITIES OF LEAF EXTRACTS OF *MELOCHIA CORCHORIFOLIA*

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ABSTRACT: The present study was investigated to establish the diuretic and antiurolithiatic activities of chloroform and ethanolic leaf extract of *Melochia corchorifolia*. The chloroform and ethanolic leaf extract were administered to experimental rats orally at a dose of 200 mg/ kg and 400 mg/kg. Furosemide (5 mg/kg) was used as reference standard for diuretic activity. The parameters measured for diuretic activity was total urine volume; urine electrolyte concentrations such as sodium, potassium, chloride, and bicarbonates. In the *in-vitro* antiurolithiatic activity, Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The effect of chloroform and ethanolic leaf extract of *Melochia corchorifolia* (100, 200 and 500 µg/ml) was studied by time course measurement of absorbance at 620 nm for ten minutes using a spectrophotometer. Both the extracts show good *in-vitro* antiurolithiatic activity. In the *in-vivo* antiurolithiatic activity, urolithiasis was induced in male rats by administering ethylene glycol (0.75% v/v) in drinking water for 28 days, and the parameters such as oxalate, calcium, and phosphate were estimated in urine. Serum creatinine, calcium, and uric acid were also estimated. Treatment with the leaf extract of *Melochia corchorifolia* restored all biochemical, urinary parameters. The results obtained justified the importance of the leaf extract of *Melochia corchorifolia* as a diuretic and antiurolithiatic agent.

INTRODUCTION: Diuretics are agents which augment the renal excretion of sodium and either chloride or bicarbonate primarily and water excretion secondarily. The term saluretic is sometimes used to describe a drug that increases the renal excretion of sodium and chloride ions ¹.

Diuretics play an important role in situations like hypercalciuria, edema, acute and chronic renal failure, cirrhosis of the liver, and acts as an antihypertensive agent.

Several diuretics like thiazides, furosemide, mannitol, and ethacrynic acid are used in practice ². Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive nephritic syndrome, renal failure, heart failure, cirrhosis, hypertension, and pregnancy toxemia ³. Urolithiasis is the formation of stones in the urinary tract that prominently causes the variable degree of pain, bleeding, and further may lead to secondary

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infection. It is one of the third most common afflictions found in humans⁴. The process of formation of kidney stones may be due to nucleation, aggregation, and crystal growth phenomena⁵.

Melochia corchorifolia Linn. Malvaceae is a wild crop and grows in most parts of India as a weed. Some species of the genus *Melochia* have been used in folk medicine, such as dysentery, abdominal swellings, and water-snake bites, bronchitis and cough⁶.

MATERIALS AND METHODS:

Collection of Plant Parts: The whole plants of *Melochia corchorifolia* were collected from the surroundings of Surampalem, East Godavari district, Andhra Pradesh. The plants were identified and authenticated by the taxonomist Mr. T.V. Raghavarao, Department of Botany SRVBSJB Maharane College, Peddapuram, E.G., District. Andhra Pradesh.

Preparation of *Melochia Corchorifolia* Extract:

The leaves were shade dried, pulverized and sieved through 40 mesh. The powder leaves were extracted with ethanol in soxhlet apparatus for 72 hours at 50 °C. The extract obtained was evaporated under vacuum to remove the solvent completely and obtained gummy exudates, stored at low temperature in the refrigerator for further studies.

Diuretic Activity: Diuretic activity was determined following Lipschitz method⁷. The Institutional Animal Ethical Committee approved the experimental protocol (1269/a/10/CPCSEA). The rats [36 no.] were fasted for 18 h and deprived of water before the experiment. A priming dose of 25 ml/kg of normal saline was given to all rats. The rats were grouped into 6 groups [6 rats in each].

Group I: Control group and treated with vehicle, 0.5% acacia orally.

Group II: Treated with standard drug Furosemide (5 mg/kg p.o) dissolved in the vehicle.

Group III and IV: Were treated with chloroform leaf extract of *Melochia corchorifolia* (200 mg/kg and 400 mg/kg p.o respectively).

Group V and VI: Were treated with ethanolic leaf extract of *Melochia corchorifolia* (200 mg/kg and 400 mg/kg p.o) respectively.

Immediately after the administration, the rats were placed in metabolic cages, one rat per cage. The metabolic cages provided with a funnel for urine collection and a mesh to separate the feces from the urine. The bladder was emptied by pulling the base of the tail of each rat⁸. The urine was collected into a beaker covered with aluminum foils to avoid evaporation. The volume of urine collected was recorded after 5 h, and urine was subjected to analysis for determination of sodium, potassium ions by Flame photometry⁹ and chloride, bicarbonate by titrimetric analysis¹⁰ after 24 hrs. The Saluretic, Natriuretic and Diuretic Indices were also calculated.

Antiuro lithiatic Activity:

In-vitro Antiuro lithiatic Activity:

Experimental Protocol: The effect of the extract on calcium oxalate crystallization was determined by the time course measurement of turbidity changes due to the crystallization in artificial urine on the addition of 0.01M sodium oxalate solution. The Precipitation of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer¹¹.

Preparation of Synthetic Urine: For preparation of synthetic urine 3.8 gm of potassium chloride, 8.5 gm of sodium chloride, 24.5 gm of urea, 1.03 gm of citric acid, 0.34 gm of ascorbic acid, 1.18 gm of potassium phosphate, 1.4 gm of creatinine, 0.64 gm of sodium hydroxide, 0.5 gm of calcium chloride, 0.47 gm of sodium bicarbonate and 0.28 ml of sulphuric acid were added in 500 ml of deionised water and stirred for 1 h. The synthetic urine was stored at -4 °C until further use¹².

Study without Inhibitor: A volume of 1.0 ml of artificial urine was transferred into the cell and 0.5 ml of distilled water added to it, and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume and the measurement is determined immediately and recorded for ten minutes.

Study with Inhibitor: The extract was dissolved in distilled water filtered through a membrane filter, and the concentration of 100, 300 and 500 µg/ml were obtained. A mixture of 1ml of artificial urine and 0.5 ml of extract solution was taken in the cell.

A blank reading was taken, and then 0.5 ml of 0.01M sodium oxalate solution was added and immediately absorbance was measured for a period of the 10 minutes with 2 min interval at 620 nm¹³. The % of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

In-vivo Antiuro lithiatic Activity: Ethylene glycol-induced hyperoxaluria method¹⁴ was used to assess antiuro lithiatic activity in male albino rats. Animals were divided into four groups containing six animals in each group. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups for induction of renal calculi for 28 days. The extract was assessed for antiuro lithiatic activity for its curative action in urolithiasis.

In the Curative regimen, the extract was given from 15th day to 28th day.

- **Group I:** Normal Control group received regular rat food and drinking water.
- **Group II (Calculi-Induced):** Positive control received ethylene glycol (0.75% v/v) in Drinking Water for induction of renal calculi for 28 days.
- **Group III:** Received ethylene glycol and standard anti-Urolithiasis drug Cystone (750 mg/kg b.w) from 15th day to 28th day¹⁵.
- **Group IV and V:** Received *Melochia corchorifolia* (chloroform extract 200 mg/kg and 400 mg/kg p.o) respectively;
- **Group VI and VII:** Received *Melochia corchorifolia* (ethanol extracts 200 mg/kg and 400 mg/kg p.o) respectively, from 15th day to 28th day.

Estimation of Biochemical Parameter: At the end of the experiment (28th day), urine was collected, and urine was subjected to analysis for determination calcium, phosphate, and oxalate. Blood was collected through a tail vein for determination of calcium, creatinine and uric acid in serum.

RESULTS:

Diuretic Activity: The chloroform extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced a volume of urine was 0.44 and 0.61 ml after 5 h. The excretion of sodium by both doses of chloroform extract was found to be 178.14 and 182.14 μ moles/kg. Similarly, the excretion of potassium, chlorides, and bicarbonates were markedly increased in chloroform treated groups compared to the control group.

But when compared to chloroform treated groups the ethanolic extract of *Melochia corchorifolia* treated group showed better results. The ethanolic extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced volumes of urine was 0.51 and 0.70 ml after 5 hrs. The excretion of sodium by both doses of ethanolic extract was found to be 172.23 and 212.14 μ moles/kg which is higher than the chloroform treated group. Similarly, the excretion of potassium, chlorides, and bicarbonates was markedly increased in ethanolic extract treated groups compared to other groups. Furosemide was used as standard (group II).

The volume output and the electrolytes excretion with the standard drug were found to be excellent. When compared to chloroform extract the ethanolic extract shows good diuretic activity. Saluretic, Natriuretic and Diuretic indexes were calculated for all the groups and results were tabulated in **Table 2**. The results of the diuretic effect by the control, extract treated groups and standard drug Furosemide 5 mg/kg were given in **Table 1**.

TABLE 1: DIURETIC EFFECT OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

Parameters	Control	Standard Furosemide 5 mg/kg	Chloroform extract in mg/kg		Ethanolic extract in mg/kg	
			200	400	200	400
Volume of urine (mL/Hr) after 5hrs	0.18 ±0.26	0.74±0.14	0.44±0.62	0.61±0.04	0.51±0.56	0.70±0.52
Sodium (Na ⁺) μ moles/Kg	173.3 ±0.35	232.14±0.65**	178.14±0.45**	182.14±0.36**	172.14±0.24**	212.14±0.35**
Potassium (K ⁺) μ moles/Kg	121.48±0.06	144±0.23**	124±0.64**	129±0.24**	118±0.76**	134±0.29**
Chloride (Cl ⁻) μ moles/Kg	98.69±0.59	152±0.329**	76±0.42**	102±0.32**	97±0.12**	132±0.09**
Bicarbonate (HCO ³⁻) μ moles/Kg	9.97±0.17	26±0.22**	10±0.56	16±0.42**	13±0.14**	21±0.08**

Values are Mean ± SEM; n=3; ** p<0.01; All comparisons are made with control.

TABLE 2: SALURETIC, NATRIURETIC AND DIURETIC INDICES OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

S. no.	Parameters	Control	Standard	Chloroform extract in mg/kg		Ethanol extract in mg/kg	
				200	400	200	400
1	Saluretic Index [Na ⁺ + Cl ⁻]	156.5±0.3	384±0.44*	254±0.06*	284±0.06*	269±0.14*	344±0.10*
2	Natriuretic Index [Na ⁺ /K ⁺]	1.04±0.18	0.88±0.16	1.21±0.16	1.41±0.42	1.45±0.12	1.58±0.14
3	Volume of urine in ml after 5 h	0.15±0.08	0.74±0.08	0.44±0.14	0.61±0.03	0.51±0.24	0.70±0.056
4	Diuretic Index	-	4.11±0.8	2.44±0.20	3.38±0.14	2.83±0.24	3.88±0.18

Values are Mean ± SEM n = 3 ** = p>0.01

Diuretic Index = volume of urine in test group/volume of urine in control.

TABLE 3: IN-VITRO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA EXTRACTS

S. no.	% inhibition at a time in Sec.	Chloroform extract in µg/ml			Ethanol extract in µg/ml		
		100	300	500	100	300	500
1	0 sec	24.32±0.504	32.46±0.16	43.38±0.18	27.03±0.114	36.12±0.14	46.34±0.27
2	120 sec	26.48±0.54**	35.34±0.18**	49.41±0.42**	30.84±0.13**	41.21±0.32**	54.12±0.27**
3	240 sec	29.90±0.620	39.91±0.18	55.80±0.18	33.69±0.142	45.02±0.18	57.57±0.18
4	360 sec	32.13±0.666	42.88±0.18	59.94±0.18	36.07±0.152	48.19±0.18	60.47±0.18
5	480 sec	34.25±0.71**	45.72±0.14**	63.91±0.48**	39.70±0.167	53.05±0.37**	63.24±0.19**
6	600 sec	36.29±0.52**	48.44±0.15**	66.71±0.12**	42.02±0.7**	56.15±0.18**	67.16±0.16**

Values are mean ± SEM n = 3

The maximum inhibition of chloroform (500 µg/ml) was observed at 66.71%. But when

compared with these 2 extracts the ethanolic extract showed maximum % inhibition at 67.16%.

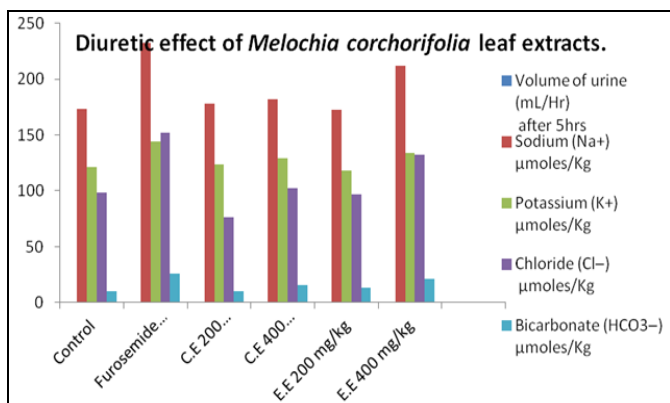


FIG. 1: DIURETIC EFFECT OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

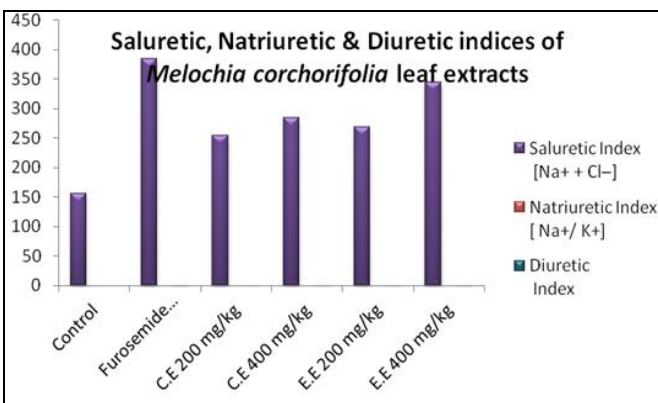


FIG. 2: SALURETIC, NATRIURETIC AND DIURETIC INDICES OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

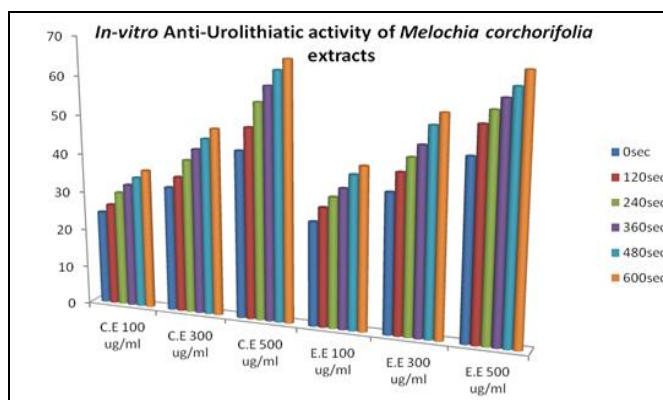


FIG. 3: IN-VITRO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA EXTRACTS

Anti-Urolithiasis Activity:

In-vitro Anti-Urolithiasis Activity: The results of *In-vitro* Anti-Urolithiasis activity of chloroform and ethanolic leaf extracts of *Melochia corchorifolia* exhibits dose and time-dependent % inhibition were given in the below **Table 3**.

In-vivo Anti-Urolithiasis Activity: The ethanolic and chloroform extracts of *Melochia corchorifolia* exhibited a marked decrease in the levels of oxalate, phosphate, and calcium in urine and also in serum. The oxalate, phosphate and calcium levels

in urine after induction of calculi (group II) were found to be 7.63, 40.0 and 14.67 mg/dl. The rats treated with chloroform extract of *Melochia corchorifolia* with a dose of 200 and 400 mg/kg has decreased the level of oxalate, phosphate, and calcium.

But when compared to group V (chloroform 400 mg/kg treated group) the ethanolic extract treated groups (group VII) has decreased the levels of oxalate, phosphate, and calcium to a greater extent. The results are 2.49, 15.87 and 3.37 mg/dl.

TABLE 4: IN-VIVO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

S. no.	Parameters	Group I (Control)	Group II (Calculi Induced)	Group III (Cystone 750 mg/kg)	Group IV	Group V	Group VI	Group VII
					CE-200 mg/kg	CE-400 mg/kg	EE-200 mg/kg	EE-400 mg/kg
1	Urine Calcium mg/dl	3.53	14.67	3.33	5.40	3.59	4.96	3.37
		± 0.058	± 0.574	± 0.058	± 0.100	± 0.067	± 0.059	± 0.153
		18.40	40.00	15.30	29.63	19.17	10.55	15.87
2	Phosphate mg/dl	± 0.529	± 0.600	± 0.608	± 0.232	± 0.764	± 0.196	± 0.231
		4.30	7.63	3.20	4.03	2.68	3.70	2.49
		± 0.100	± 0.058	± 0.100	± 0.153	± 0.050	± 0.100	± 0.029
3	Oxalate mg/dl	8.13	16.33	3.43	11.97	7.96	14.53	9.78
		± 0.231	± 0.537	± 0.153	± 0.451	± 0.148	± 0.306	± 0.116
		0.65	38.33	0.80	17.65	11.53	11.98	1.90
4	Serum Calcium mg/dl	± 0.050	± 0.579	± 0.100	± 0.216	± 0.503	± 0.015	± 0.100
		3.23	7.70	3.30	3.33	2.21	3.25	2.20
		± 0.058	± 0.100	± 0.265	± 0.153	± 0.041	± 0.038	± 0.153
5	Creatinine mg/dl	3.23	7.70	3.30	3.33	2.21	3.25	2.20
		± 0.058	± 0.100	± 0.265	± 0.153	± 0.041	± 0.038	± 0.153
		6	Uric acid mg/dl	± 0.058	± 0.100	± 0.265	± 0.153	± 0.041

CE-chloroform extract, EE-ethanol extract, Values are Mean \pm SEM; n = 3 (number of animals in each group)

All comparisons are made with that of control.

The results of all three extract treated groups are significant and comparable to the standard drug (750 mg/kg). Cystone treated group's shows oxalate, phosphate and calcium levels are 3.20, 15.30 and 3.33 mg/dl respectively.

Similarly, chloroform and ethanolic treated groups of *Melochia corchorifolia* leaf extract markedly decreased the uric acid, calcium and creatinine levels in serum. The maximum uric acid, creatinine, and calcium levels were found in calculi induced rats (group II) 7.70, 38.33 and 16.33 mg/dl. The chloroform extract of *Melochia corchorifolia* at the dose 200 and 400 mg/kg body wt has decreased level of. But when compared with these 2 extracts, the ethanolic treated groups have decreased the level of oxalate, phosphate, and calcium to a greater extent. The results are 3.30, 0.80 and 3.43 mg/dl where as Cystone treated group's shows uric acid, calcium and creatinine levels are 2.20, 1.90 and 9.78 mg/dl, respectively. All these results are mentioned in **Table 4**.

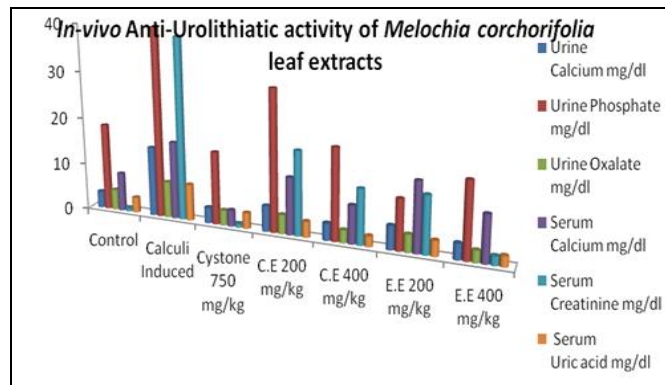


FIG. 4: IN-VIVO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

DISCUSSION:

In Diuretic Activity: The chloroform extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced diuresis, and the volume of urine is 0.44 and 0.61 ml after 5 h. The excretion of sodium by both doses of chloroform extract was found to be 178.14 and 182.14 μ moles/kg. Similarly, the excretion of potassium, chlorides, and bicarbonates was markedly increased in

chloroform extract treated groups when compared to control group. When compared with chloroform treated groups the ethanolic extract of *Melochia corchorifolia* treated group showed better results.

The ethanolic extract of *Melochia corchorifolia* at 200, 400 mg/kg body weight dose produced better diuresis, and the volume of urine is 0.51 and 0.70 ml after 5 h. The excretion of sodium by both doses of ethanolic extract was found to be 172.23 and 212.14 μ moles/kg which is higher than the chloroform treated groups. Similarly, the excretion of potassium, chlorides, and bicarbonates was markedly increased in ethanolic extract treated groups when compared to other groups. The results of diuretic effect by the control, extract treated groups and standard drug Furosemide 5 mg/kg were given. Natriuretic, Saluretic and Diuretic indices were calculated for all the groups and obtained results so were found to be significant.

In-vitro Anti-Urolithiasis Activity: The results of *In-vitro* Anti-Urolithiasis activity of chloroform and ethanolic leaf extracts of *Melochia corchorifolia* exhibits dose and time-dependent % inhibition. The maximum inhibition with chloroform (500 μ g/ml) was observed at 66.71%. But when compared with chloroform extracts the ethanolic extract showed maximum inhibition at 67.16 %.

In In-vivo Anti-Urolithiasis Activity: The *Melochia corchorifolia* leaf extracts exhibited a marked decrease in the levels of oxalate, phosphate, and calcium in urine and also in serum. The oxalate, phosphate and calcium levels in urine after induction of calculi (group II) were found to be 7.63, 40.0 and 14.67 mg/ml. The results of all three extracts treated groups are significant and comparable to standard drug Cystone (750 mg/kg).

Previous studies have demonstrated that there are several compounds which could be responsible for the plants diuretic effects such as flavonoids, saponins¹⁶. The effect may be produced by stimulation or regional blood flow or initial vasodilation¹⁷ or by producing inhibition of tubular reabsorption of water and ions¹⁸, the result in both cases being diuresis. The increased sodium and water excretion activity also provide a strong basis for its proved anti-hypertensive action¹⁹.

The kidney is the principal target for Ethylene glycol induced toxicity. Its administration to rats for 28 days resulted in substantial excretion of oxalate and deposition of microcrystal in kidney²⁰. An increased urinary calcium concentration is a factor favoring nucleation and precipitation of Calcium oxalate or apatite (calcium phosphate) and subsequent crystal growth²¹. Apart from urinary calcium excretion, a decrease in serum calcium was evident in treated urolithiasis rats²². Another possible mode of action includes excessive excretion or decrease in the concentration of urinary salts that prevent the supersaturation of the crystallizing salts. Based on the results obtained Treatment with ethanol leaf extract of *Melochia corchorifolia* decrease the re-absorption of water and electrolytes results in diuresis, and also it lowers the levels of calcium, phosphate and oxalate excretion. With support to histological features, inflammatory changes were improved in Cystone and ethanol extract treated rats which exhibited normal renal architecture. Phytochemical screening of extracts of *Melochia corchorifolia* revealed the presence of phytosterols, tannins, flavonoids, saponins, glycosides²³, etc.; these constituents may contribute to its diuretic and anti-urolithiasis activities.

CONCLUSION: From the study, it was concluded that *Melochia corchorifolia* extract 400 mg/kg p.o significantly reduce the elevated calcium, oxalate phosphate and creatinine concentrations in urine confirming the stone inhibitory effect.

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CONFLICT OF INTEREST: Nil

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