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Effect of *Dichrostachys cinerea* (Linn.) Root Extract on Ethylene Glycol Induced Urolithiasis in Rats

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Abstract – *Dichrostachys cinerea* (Linn.) is commonly known as Vadatalla and used as phytotherapeutic agent. Tender shoots of the plant bruised and applied to the eyes in case of ophthalmia. The root is astringent and used in rheumatism, urinary calculi and renal troubles. The effect of the Ethanolic and aqueous extract of the root of *D. cinerea* were studied for its anti-urolithiatic and diuretic activity at 200 mg/kg dose level in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Supplementation with aqueous and ethanolic extract of the plant significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. Compared to ethanolic extract, aqueous extract exhibited significant anti-urolithiatic activity. Both the extracts showed significant diuretic activity. The results of our present study supports folklore claim of *D. cinerea*.
Keywords – *Dichrostachys cinerea*, Urolithiasis, Ethylene glycol, Diuretic activity

Introduction

Dichrostachys cinerea (Mimosaceae) is commonly known as Vaadatalla (Nadkarni, 1982). It is a medium sized thorny shrub occurs in tropical regions of India. Earlier claims reported that the roots are bitter, astringent, acrid, anti-inflammatory, anodyne, lithnotriptic and diuretic and are useful in vitiated conditions of Kapha and Vata (Vaidyaratnam, 1998). It is also reported that, roots are also used in urinary calculi and renal troubles, disease of vagina, uterus, pain in joint (Kirthikar and Basu, 1975). Tender shoots of the Plant bruised and applied to eyes in case of opthalmia (Anonymous, 1985).

Earlier phytochemical investigations showed that the root contains β-amyrin, friedelin-3-β-ol, friedelin-3-one, β-sitosterol and *n*-octacosanol (Krishna and Tara, 1977). Earlier studies showed that friedelin, friedelin-3-β-ol, β-amyrin and β-sitosterol from bark, octacosanol and sitosterol from heartwood, hextriacontanol and sitosterol from leaves were isolated (Krishna and Tara, 1974). Free radical scavenger property of novel-3-*O*-acyl mosquitol analogues was studied from the plant (Jagadeeswar *et al*., 2003). Anti-bacterial activity of leaves and fruits of plant were reported (Eisa *et al*., 2000). Methanolic extract of root of *D. cinerea* was investigated for its sexually transmitted diseases (STD) (Kambizil *et al*., 2001). A thorough literature survey showed that no work was carried out on the anti-urolithaiatic activity and diuretic activity attributed to the plant, so an attempt has been made to establish the scientific validity fort the anti-urolithiatic property and diuretic activities of vacuum dried alcoholic and aqueous extract using ethylene glycol induced hyperoxaluria model and diuretic activity by Lipschitz method (Lipschitz *et al*., 1943)

Experimental

Plant Material – The root of *D. cinerea* was collected from Kanchipuram district, Tamil Nadu , South India and it was authenticated by Dr. P.Jayaraman, Director, plant anatomy research center, Chennai. A voucher specimen of the plant was deposited.at the herbarium for reference.

Extraction – Air dried coarsely powdered root was extracted separately with 70% ethanol and water by soxhlet method at a temperature of 60 - 70 °C and 100 °C, respectively. The extracts were dried using vacuum flash evaporator to yield solid yield of ethanolic extract (EEDC) of 2.6% and aqueous extract (AEDC) of 3.5% w/w. A suspension of EEDC and AEDC in 10% aqueous Tween 80 was prepared for oral administration by gastric intubation method.

Animals – For acute toxicity studies, Wistar albino mice of either sex weighing between 25 - 30 g were

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selected and healthy adult male Wistar albino rats weighing between 150 - 200 g were selected for the urolithiatic activity and diuretic activity. The animals were acclimatized to standard laboratory conditions (temperature 25 ± 2 °C) and maintained on 12 hours light; 12 hours dark cycle. They were fed with standard animal feed (Hindustan Lever Limited) and water *ad libitum*.

Acute toxicity study – The acute oral toxicity study was carried out as per the 423 guide line set by Organisation for Economic Co-operation and Development (OECD) (Donald, 1997). The ethanolic and aqueous extracts were administered at the dose level of 2000 mg/kg. One tenth of the median lethal dose (LD50) was taken as an effective dose (Anupama, 1990).

Ethylene glycol induced urolithiasis model – Ethylene glycol induced hyperoxaluria model (Atmani *et al*., 2003) was used to assess the antiurolithiatic activity in albino rats. Animals were divided into five groups containing six animals each. Group I served as vehicle control and received 10 ml/kg of 10% aqueous Tween 80. Ethylene glycol (0.75%) in drinking water was fed to Group II-VII for induction of renal calculi till 28th day. Group II served as lithiatic control, Group III received standard drug, Cystone (750 mg/kg body weight) from 1st day till 28th day. Group IV received ethanolic extract (200 mg/kg) and Group V received aqueous extract (200 mg/kg body weight) from 1st day till 28th day. All extracts and standard drug were given once daily by oral route.

Assessment of antiurolithiatic activity

Urine analysis – All animals kept in individual metabolic cages and urine sample was collected on 28th day. Urine was analysed for calcium, phosphate, oxalate and protein content (Robert, 1993).

Serum analysis – After the experimental period, blood was collected from the retro-orbital under anaesthetic condition and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 gm for 10 minutes and analysed for uric acid, urea, creatinine and albumin content (Robert, 1993).

Diuretic activity – Animals were divided into four groups of six animals each. All animals were fasted overnight and received an initial dose of normal saline (25 ml/kg) orally one hour prior to treatment. Group I (Vehicle control) received distilled water10 mL/kg of 10% aqueous solution Tween 80. Group II was treated with standard (Frusemide) (10 mg/kg orally).Group III and Group IV were treated with ethanolic and aqueous extract of plant at dose of 200 mg/kg orally. Animals were placed in metabolic cages, extreme care was taken to avoid contamination of urine with fecal matter (Turner, 1965).

At the end of the 5th hour and 24th hour, urine volume was measured and estimated for sodium and potassium by using Flame Photometer (Elico Pvt.Ltd., model CL 22D). Chloride was estimated by schales and schales method (Robert and Carman, 1993).

Statistical analysis – All data were expressed as mean ± SEM. Statistical significance test was done by One way ANOVA followed by TUKEY KRAMER multiple comparison test (Spiegel and Meddis, 1980). Differences between the data were considered significant at $p < 0.05$.

Table 1. Effect of *Dichrostachys cinerea* root extracts on urolithiatic rats (urine analysis)

Dose (mg/kg)	Treatment	Calcium (mg/dL)	Oxalate (mg/dL)	Phosphorous (mg/dL)	Protein (mg/dL)
10ml 10% Twen 80	Vehicle Control	0.58 ± 0.03	0.26 ± 0.02	12.87 ± 0.62	19.76 ± 2.68
0.75% E.G	Lithiatic Control	$2.33 \pm 0.48_a$***	$4.45 \pm 0.17_a$**	$23.12 \pm 1.64_a$**	$56.36 \pm 5.02_a$**
750	Standard (Cystone)	$0.66 \pm 0.03_b$***	$1.64 \pm 0.06_b$***	$12.16 \pm 1.70_b$***	$28.86 \pm 2.62_b$***
200	EEDC	$0.86 \pm 0.24b_b$***	$1.86 \pm 0.35_b$***	$17.66 \pm 1.88_b$***	$25.3 \pm 2.12_b$**
200	AEDC	$0.66 \pm 0.10_b$***	$1.90 \pm 0.43_b$*	$16 \pm 0.57_b$*	$26 \pm 2.46_b$***

Data were analysed by one-way ANOVA followed by Tukey kramer
Multiple comparison test
Values are expressed as mean ± SEM. (n = 6)
a-comparison made between Vehicle control and lithiatic control group.
b-comparison made between lithiatic control and drug treated group.
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$
EG: Ethylene glycol

Table 2. Effect of *Dichrostachys cinerea* in serum of urolithiatic rats

DOSE (mg/kg)	Treatment	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	Albumin (mg/dL)
10 ml of 10% Tween 80	Vehicle Control	16.70 ± 1.45	0.70 ± 0.02	1.3 ± 0.03	4.4 ± 1.25
0.75% EG	Lithiatic control	36.4 ± 2.10$_a$***	2.64 ± 0.76$_a$**	3.17 ± 0.68$_a$**	18.6 ± 1.54$_a$***
750	Standard (Cystone)	20.82 ± 1.86***	0.89 ± 0.208*	1.36 ± 0.05**	5.8 ± 0.52***
200	EEDC	24.12 ± 2.06$_b$**	0.92 ± 2.007$_b$*	1.86 ± 0.33$_b$	5.15 ± 0.47$_b$***
200	AEDC	22.56 ± 1.98$_b$***	0.90 ± 0.006$_b$*	1.65 ± 0.19$_b$*	5.7 ± 0.50$_b$***

Data were analysed by one-way ANOVA followed by Tukey kramer Multiple comparison test
Values are expressed as mean ± SEM. (n = 6)
a-Comparison made between normal and urolithiatic group.
b-Comparison made between urolithiatic group and drug treated group.
Significance level: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$
EG: Ethylene glycol

Table 3. Effect of *Dichrostachys cinerea* in serum of urolithiatic rat

Dose (mg/kg)	Treatment	Calcium (mg/dL)	Phosphorous (mg/dL)	Magnesium (mg/dL)	Potassium (mg/dL)
10 ml of 10% Tween 80	Vehicle Control	11.32 ± 0.51	9.22 ± 0.62	1.68 ± 0.22	21.04 ± 1.44
0.75% EG	Lithiatic Control	19.86 ± 1.44$_a$***	21.83 ± 1.86$_a$***	3.64 ± 0.24$_a$**	46.62 ± 2.11$_a$***
750	Standard (Cystone)	10.98 ± 1.62$_b$***	10.11 ± 1.48$_b$***	1.08 ± 0.22$_b$**	23.22 ± 1.78$_b$***
200	EEDC	14.86 ± 1.50	12.43 ± 0.68$_b$***	1.72 ± 0.06$_b$***	26 ± 1.54$_b$***
200	AEDC	16.47 ± 1.21	13.28 ± 0.79$_a$***	1.8 ± 0.076$_b$**	23.42 ± 1.18$_b$

Data were analysed by one-way ANOVA followed by Tukey kramer Multiple comparison test
Values are expressed as mean ± SEM. (n = 6)
a-Comparison made between normal and urolithiatic group.
b-Comparison made between urolithiatic group and drug treated group.
Significance level: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$
EG: Ethylene glycol

Results

From the acute toxicity study, the LD_{50} cut-off dose was found to be 2000 mg/kg body weight for both extracts. Hence, the therapeutic dose was taken as 200 mg/kg body weight for both extracts.

In the present study, chronic administration of 0.75% (w/v) aqueous solution of ethylene glycol to male wistar rats resulted in hyperoxaluria, which is shown by increased elevation of calcium, phosphate and oxalate by control group (Table 1)

However, test sample (Group IV treated with alcoholic extracts and Group V treated with aqueous extract) significantly ($p < 0.001$) lowered the elevated levels of oxalate, phosphate and calcium .The results are shown in Table 1 and Table 2.

The serum levels of (urea, uric acid and albumin) where remarkably increased in urolithatic rats (control) (Table 3) while creatine was only slightly elevated in lithiatic control group (Group II) indicating marked renal damage. However, test animals showed significant reduction in urea, uric acid and albumin as compared to control. Compared to ethanolic extract of plant, the aqueous extract is more significant action in reducing lithiasis at dose level of 200 mg/kg.

The extracts also exhibited significant diuretic activity at experimental dose levels. Both ethanolic and aqueous extracts showed significant increase in volume of urine ($p < 0.001$) and increased urinary sodium, potassium and chloride levels (Table 4).

Discussion

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans (Vermeulen, 1962) and also earlier studies have shown that the amount of stone deposition in female rats was significantly less (Prasad *et al*., 1993).

Table 4. Diurectic activity of *Dichrostachys cinerea*

Dose (mg/kg)	Treatment	Volume of Urine (mL)		Sodium (mEq/L)		Potassium (mEq/L)		Chloride (mEq/L)	
		5hrs	24hrs	5hrs	24hrs	5hrs	24hrs	5hrs	24hrs
10 mL 10% Tween 80	Vehicle Control	2.76 ± 0.07	3.3 ± 0.10	0.5796 ± 0.03	0.7100 ± 0.04	0.2562 ± 0.01	0.1538 ± 0.01	96.712 ± 30687	95.303 ± 3.09
10	Standard (Frusemide)	3.13 ± 0.07**	1.98 ± 0.04**	1.8043 ± 0.01***	0.5412 ± 0.006**	0.5469 ± 0.001**	0.1221 ± 0.002	237.05586 ± 1.9***	40.3755 ± 2.43
200	EEDC	3.55 ± 0.11***	5.03 ± 0.06***	1.0325 ± 0.01***	0.2173 ± 0.02	0.4867 ± 0.005**	0.1256 ± 0.005	149.295 ± 3.31***	124.4130 ± 3.27***
200	AEDC	4.55 ± 0.17***	4.3 ± 0.09***	1.9202 ± 0.02***	0.4680 ± 0.01	0.4081 ± 0.0025**	0.0916 ± 0.004	143.6619 ± 2.09***	669.2018 ± 84.69***

Data were analysed by one-way ANOVA followed by Tukey kramer
Multiple comparison test, n = 6, value are expressed as mean ± SEM
Significance level: *** $p < 0.001$, ** $p < 0.01$.

Urinary super-saturation with respect to stone-forming consitutents is generally considered to be one of the causative factors in calculogenesis. Evidence in pervious studies indicated that in response to 14 day period of ethylene glycol (0.75% v/v) administration , young male albino rats form renal calculi composed mainly of calcium oxalate (Selvam *et al.,* 2001; Huang *et al.,* 2002). The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria ,which causes increased renal retention and excretion of oxalate (Selvam *et al.,* 2001).

In the present study oxalate and calcium excretion are progressively increased in calculi-induced animals (group II). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria (Tisseliu, 1996). The changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or calcium phosphate from urine and subsequent crystal growth (Lemann *et al*., 1991). However, EE and AE of *D. cinerea* root lower the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi-induced rats (group II). Increased urinary phosphate 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 (Roger *et al*., 1997). Treatment of *D. cinerea* root extract restores phosphate level, thus reducing the risk of stone formation.

In urolithiasis, the glomerular filteration rate (GFR) decreases due to the obstruction to the out flow of urine by stones in urinary system. Due to this the waste products such as urea and creatinine and uric acid get accumulated in blood (Ghodkar, 1994).

In calculi induced rats (group II) marked renal damage was seen as indicated by the elevated serum levels of creatine and uric acid. However prophylatic treatment of EEDC and AEDC causes diuresis and hastens the process of dissolving the free form stones and prevents new stone formation. Compared to ethanolic extract of the plant (EEDC), aqueous extract produced significant anti-urolithiatic property and diuretic property.

In conclusion, the presented data indicate that administration of EE and AE of *D. cinerea* root to urolithiatic rat, reduced and prevented the growth of urinary stone supporting folk information regarding the anti-urolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis . This effect could conclude the anti-urolithatic property and diuretic activity of *D. cinerea* Linn.

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