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EVALUATION OF ANTILITHIATIC ACTIVITY ON WHOLE PLANT OF MURRAYA KOENIGII.

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ABSTRACT

The ethanolic extract of whole plant of *murraya koenigii Linn.* was evaluated for its antilithiatic activity in mices. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%) in adult male Swiss albino mices for 28 days. The ionic chemistry of urine was altered by ethylene glycol (EG), which elevated the urinary concentration of crucial ions, viz. calcium, phosphorus and protein thereby contributing to renal stone formation. However treatment with ethanolic whole plant extract of *murraya koenigii* (200 and 300 mg / kg body weight) in group III and IV significantly ($p<0.05$) reduced the elevated level of these ions in urine. The levels of serum urea, uric acid and creatinine were significantly increased ($p < 0.05$) in urolithiatic mices. Treatment with plant extract restored the levels and it brought back the values to near normal range in urolithiatic mices. All these observations revealed that ethanolic whole plant extract of *murraya koenigii* has curative effect on stone formation induced by ethylene glycol.

KEYWORDS: Antilithiatic, *murraya koenigii*, Ethylene glycol, Calcium, Phosphate and Protein.

INTRODUCTION

Urolithiasis is derived from the Greek words “ouron” (urine) and “lithos” (stone). It is considered as the third most common affliction of the urinary tract.[1]

Lithiasis is the process of formation of stone and urolithiasis are the solid nonmetallic minerals in the urinary tract.[2] Stones result due to phase change whereby dissolved salts condense into solids because of super saturation.[3] Among several types of kidney stones, the

most common are calcium oxalate. The formation of these stones occurs with crystal nucleation, aggregation and retention within urinary tract. If a stone blocks the flow of urine, excruciating pain may result.[4] Recurrent stone formation is a common part of the medical care of patients with stone disease.[5] Calcium containing stones, especially calcium oxalates and phosphate are the most commonly occurring ones.[6] In Ayurveda, the medicinal use of plants decrease the recurrence rate of urolithiasis without any potential side effects.[7] Surgery, lithotripsy and local calculus disruption using a high power laser are also used to treat calculi.[8] However, these procedures are expensive and recurrence is quite common, requiring preventive treatment.[9]

Murraya koenigii is a common plant with medicinal properties it belongs to family Rutaceae & used in Indian Cuisine in a tropical to sub-tropical tree, which is native to India distributed throughout Bangladesh, Nepal, Malaysia, Srilanka and Burma. The plant is used as a stimulant, stomachic, febrifuge, analgesic and for treatment of diarrhea, dysentery and insect bite. The leaves can be used in many other dishes to add spice. *Murraya koenigii* can be used as a tonic. They are also available dried, through the aroma is largely inferior. The leaves are rich in monotorponoids and sesquiterpenoids which exhibited antifungal activities. Minor furanocumarines are also reported from seeds. Carbazole alkaloids, the major constituent of the plant are known to possess cyotoxic, antioxidative, antimutagenic and anti- inflammatory activities.[10]

Several methods were used for induction of urolithiasis, which cause predominantly two types of hyperoxaluria, one acute, when the mices is challenged by a large single dose of lithogen and secondly chronic, when the mices is continuously challenged by small doses of lithogen for a period of time. In the present study, ethylene glycol-induced hyperoxaluria model was used to assess the antilithiatic activity in albino mices. Chronic administration of 0.75% ethylene glycol aqueous solution to male Swiss albino mices resulted in hyperoxaluria. The study of the urinary chemistry with respect to the stone forming minerals will provide a good indication of the risk of stone formation. In general, the crystallization of stone forming salts is due to an abnormal urinary composition that is either higher in crystallization promoters (e.g. calcium, oxalate and uric acid) or lower in inhibitors (e.g. citrate, glycosaminoglycans, kidney proteins such as nephrocalcin, Tamm- Horsfall mucoprotein uropontin), or both. As traditional medicines are usually taken by the oral route, same route

of administration was used for evaluation of antilithiatic effect of the *murraya koenigii* against ethylene glycol induced urolithiasis in mices.

MATERIALS AND METHODS

Collection of plant material

Leaves, roots and stems of *murraya koenigii* were selected for Evaluation of antilithiatic activity on whole plant of *murraya koenigii Lin.* and were collected from the Munna nursery, narayan bagh road, Jhansi, U.P, India. The leaves, stems and roots were washed and dried in shade. The leaves, stems and roots were crushed in coarse powder and stored in closed container further use.

Authentication and identification

The leaves, stems and barks of *murraya koenigii* - Linn. belonging to family Rutaceae were authenticated by Dr. Gaurav Nigam, Asstt. Professor, Botany department, Bundelkhand University, Jhansi, Uttar Pradesh, India. A voucher specimen no. BU/Bot./Sep./Phar./05-2015/01 was submitted at Botany Department, Bundelkhand University, Jhansi, U.P, India.

PHYTOCHEMICAL INVESTIGATION

PRELIMINARY PHYTOCHEMICAL TEST

The freshly prepared extract of the leaves, stems and roots of *murraya koenigii* was subjected to phytochemical screening tests for the detection of various constituents. It revealed the presence of alkaloids, Carbohydrates, amino acids, proteins, saponins, steroids, Triterpenoids, Tannins, Flavonoids, Phenolics compound, sugars, reducing sugars, Glycosides, Quinones, Volatiles oil, Vitamine-C, Phytosterols, Gum and mucilage.

THIN LAYER CHROMATOGRAPHY

The extract dissolved in their mother solvent were taken in a capillary tube and spotted on TLC plates, 1cm above its bottom and were put in various solvent system. The best result was given by solvent system -Benzene: chloroform: Formic acid (7:2:1). The five spots were observed during day light and seven spots were observed after treating with iodine vapours.

COLUMN CHROMATOGRAPHY

For packing column slurry of silica gel (60-120 mesh size) was proposed using n-Hexane as a solvent and poured into the column. After packing the column ethanolic extract of *murraya koenigii's* leaves, stem and roots which was previously coated on silica gel (60-120 mesh

size) was loaded on the top of column and start elution using n-Hexane, Benzene, chloroform and formic acid as an eluting solvent in different composition with increasing polarity. The eluents are collected in fractions in separate beakers and analyzed by TLC.

Table1: Pure fraction of ethanolic extracts of murraya koenigii's leaves, stems and roots.

No. of fraction	Solvent	Color of the fraction
F1	90:10	Orange

The isolation of the constituent of ethanolic extract was carried out by mass spectrometry analytical technique.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

HPTLC of ethanolic extract of leaves, stem and roots of *murraya koenigii* was carried out by PG Tech Research institute Indore, India. Ethanolic extract shows sixteen peaks which gave confirmation that sixteen compounds may be present in ethanolic extract.

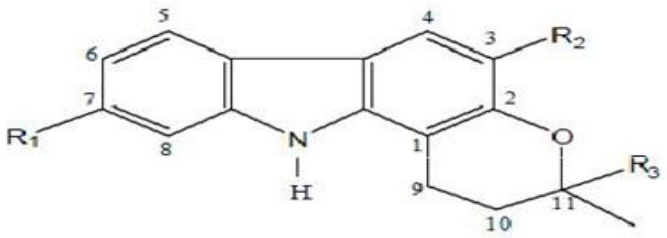
MASS SPECTROMETRY

The mass spectrometry graph of isolated compound of ethanolic extracted leaves, stems and roots of crude drug was done from the IIT Kanpur, Kalyanpur, Kanpur, Uttar Pradesh, India-208016.

Mahanimbine category of Alkaloid has been confirmed for which

R1= H

R2= -C CH R3= -CH2CH2CH2CH=CH-CH=C



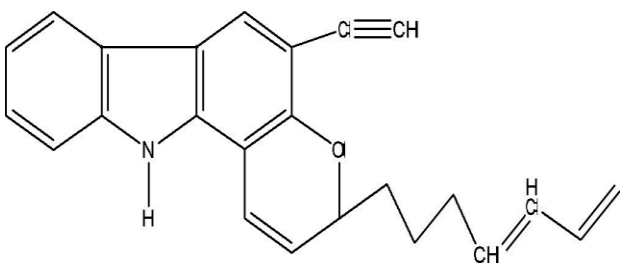


Table 2: Mass expected and observed values.

S.NO.	ELEMENT	EXPECTED PEAK value	OBSERVED PEAK Value
1	Carbon	84.90 m/z	84.92 m/z
2	Hydrogen	4.11 m/z	4.71 m/z

Chemical Formula: $C_{24}H_{21}NO$

Exact mass: 339.16

Molecular mass: 339.44

m/z: 339.16 (100%), 340.17 (26.0%), 341.17 (2.7%)

Elemental analysis: C., 84.92 and H., 6.2

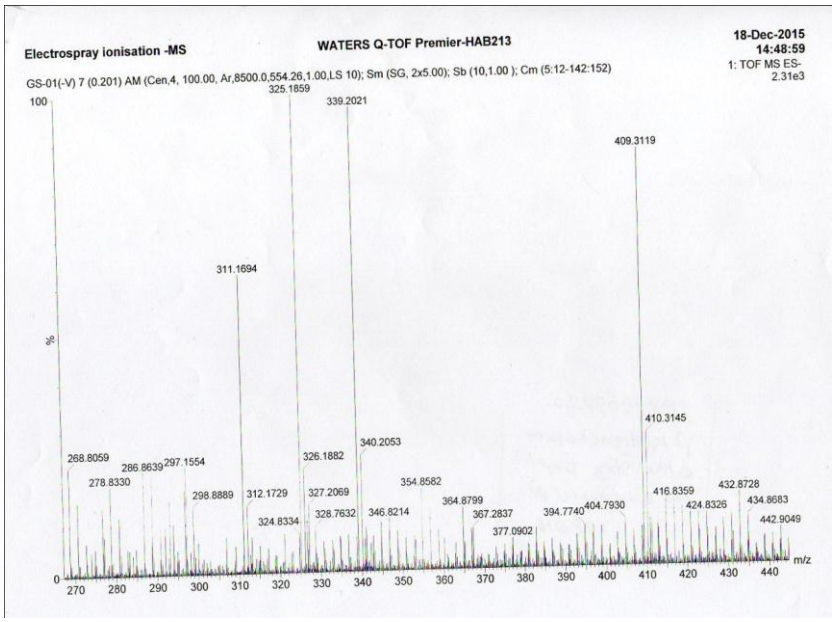


Fig 1: Mass spectra

REPORT OF HEAVY METALS TESTING

Table 3: Showing Determination of Heavy Metals in given sample.

S. N.	Parameter	Actual Conc. Unit	APLI Limits
1.	Lead (Pb)	3.4103 ppm	10 ppm
2.	Cadmium (Cd)	0.0593 ppm	0.3 ppm
3.	Arsenic (As)	1.4321 ppm	03 ppm
4.	Mercury (Hg)	0.5432 ppm	01 ppm

Method of Test

CLL-IDR-STP-AGR-028.

REPORT OF METALS TESTING

Table 4: Showing Determination of Metals in given sample.

S. N.	Parameter	Actual Conc. unit
1	Sodium	790.5 ppm
2	Potassium	313.0 Ppm

Preparation of the ethanolic root extract for *in vivo* studies

The powdered dried leaves, stems and roots material was extracted with ethanol as a solvent by soxhlet apparatus. After complete extraction, the extract was dried. The extract was suspended in distilled water, for oral administration to animals.

Selection of animals for *In vivo* studies

For the purpose of antilithiatic studies, adult male mice weighing about 150 to 200 g were collected from animal breeding centre. The ethical committee permission license number is CPCSEA (Reg. no.716/02/a/CPCSEA). The mices were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hr. light and dark cycle at 28^{o}C ± 2^{o}C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted at animal house, Depatrtment of pharmacy, Bundelkhand university, Jhansi. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC). Paddy husk was used as beding material and changed twice a week.

Experimental design of animals for *in vivo* studies

The method of M.Sathya *et al.* (2012) was followed to evaluate the antilithiatic effect. The acclimatized animals were divided into five groups of six each designated as Group I, II, III, IV and V. The animals of Group I served as the normal control. Group II animals received 0.75% ethylene glycol in drinking water *ad libitum* for 28 days and served as the lithiatic control.

The Group III and Group IV group animals received 0.75% ethylene glycol in drinking water *ad libitum*; along with ethanolic leaves, stems and roots extract of (200 and 300mg/ kg body weight and Group-V group animals received 0.75% ethylene glycol in drinking water *ad libitum*; along with Pentylenetetrazole (PTZ) (150µg/ kg body wt) by oral route for 28 days.

Biochemical parameters assayed for pharmacological screening studies

The 24-h urine samples were collected in metabolic cages, on the 7th, 14th, 21st and 28th days and the volume noted. Urinary calcium, phosphorus and protein and the serological parameters were estimated on 28th day of lithiasis. In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male mices resulted in hyperoxaluria. As mentioned in table 5, 6 and 7. The urinary excretion of calcium, oxalate and phosphorus are increased ($p < 0.05$) significantly on the 14th day in calculi- induced (group II) animals when compared with normal control mices. Maximum levels of excretion were observed with group II on the 28th day.

However the calcium and phosphorus excretion was reduced significantly in the extract treated group (group III and IV), though normal values were reached. When *murraya koenigii* extract treated mices (Group III and IV) were compared with Pentylenetetrazol (PTZ) treated mices (Group V), there was no significant difference between these groups of mices.

The present observation showed proteinuria in ethylene glycol induced urolithic mices on the 14th and 28th day (Table7). Administration of the extract had profound effects on minimizing the excretion of protein and thus has prevented the nidus formation for crystal nucleation (Group III and IV).

Table 5. Effect of ethanolic whole plant extract of *murraya koenigii* on calcium excretion in experimental nephrolithiasis (urine analysis)

GROUP	CALCIUM ΨΨ BEFORE EG TREATMENT	CALCIUM ΨΨAFTER EG TREATMENT (DAYS)			
		Days 7	Day 14	Day 21	Day 28
I	1.52±0.06	1.52±0.08	1.58 ±0.24	1.65±0.11	1.75±0.06
II	1.68±0.27	2.98±0.04 a*	3.09±0.71 a*	4.40±0.11a*	5.24 ±0.20a*
III	1.47±0.55	2.01±0.54 b*e^{ns}	2.05±0.03b*e^{ns}	1..97±0.10 b*	1.88±0.11 b*e^{ns}
IV	1.59±0.20	2.07±0.20c*f^{ns}	2.03±0.26 c*^{fns}	2.05±0.19c*f^{ns}	1.89±0.10 c*f^{ns}
V	1.62±0.26	2.15±0.85 d*	2.10±0.50 d*	2.12±0.85 d*	1.95±0.06 d*

Values are expressed as mg/ 24 hr. urine sample.

Experimental design

Group I: Control mices – received normal pelleted diet

Group II: Received 0.75% ethylene glycol in water for 28 days

Group III: Treated mices –Urolithiasis induced mices received *murraya koenigii* extract (200 mg / kg body weight) by oral administration for 28 days at a rate of 1.0 ml / mice / day

Group IV: Treated mices – Urolithiasis induced mices received ethanolic leaves, stem and roots extract of *murraya koenigii* (300 mg / kg body weight) by oral administration for 4 weeks at a rate of 1.0 ml / mice / day

Group V: Standard drug Pentylenetetrazol (PTZ) treated mices – Urolithiasis induced mices received Pentylenetetrazol (PTZ). (150 µg / kg body weight) by oral administration for 30 days at a rate of 1.0 ml / mice / day.

Comparison between the groups

'a' represents comparison between II and I

'b' represents comparison between III and II

'c' represents comparison between IV and II

'd' represents comparison between V and II

'e' represents comparison between III and V

'f' represents comparison between IV and V

Table 6. Effect of ethanolic whole plant extract of *murraya koenigii* on phosphorus excretion in experimental nephrolithiasis

GROUP	PHOSPHORUS$^{\Psi\Psi}$ BEFORE EG TREATMENT	PHOSPHORUS $^{\Psi\Psi}$AFTER EG TREATMENT (DAYS)			
		Days 7	Day 14	Day 21	Day 28
I	6.52±0.20	6.80±0.14	6.81±0.11	6.81±0.11	6.75±0.14
II	6.70±0.14	8.18±0.04 a*	9.76±0.33 a*	10.40±018a*	12.01±0.69a*
III	6.59±0.15	7.0±0.06 b*e^{ns}	6.94±0.06b*e^{ns}	6.97±0.16b*	6.95±0.08b*e^{ns}
IV	6.74±0.07	6.95±0.12c*f^{ns}	6.92±0.14 c*^{fns}	6.85±0.16c*f^{ns}	6.85±0.13c*f^{ns}
V	6.79±0.02	7.03±0.11d*	6.94±0.15 d*	6.96±0.07d*	6.90±0.12d*

Values are expressed as mg/ 24 hr. urine sample

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 5.

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

ψψmg/ 24 hr. urine sample.

Table 7. Effect of ethanolic whole plant extract of *murraya koenigii* on protein excretion in experimental nephrolithiasis.

GROUP	BEFORE EG TREATMENT	AFTER EG TREATMENT (DAYS)			
		Days 7	Day 14	Day 21	Day 28
I	1.02.±0.05	1.05±0.11	1.08±0.10	1.19±0.08	1.15±0.07
II	1.06±0.02	4.12±0.24 a*	5.96±0.22 a*	7.65±0.18a*	9.30±0.06a*
III	1.14±0.03	1.75±0.98 b*e^{ns}	1.47±0.06b*e^{ns}	1.61±0.06b*	1.28±0.05b*e^{ns}
IV	1.16±0.03	1.78±0.13c*f^{ns}	1.47±0.02 c*f^{ns}	1.56±0.16c*f^{ns}	1.25±0.05c*f^{ns}
V	1.19±0.02	1.68±0.10d*	1.57±0.02 d*	1.50±0.04 d*	1.29±0.02d*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 5.

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

ψψmg/ 24 hr. urine sample.

SERUM BIOCHEMICAL PARAMETERS

From the table 8 it is evident that the levels of serum urea, uric acid and creatinine were significantly increased (p < 0.05) in urolithiatic mices (Group II).

Treatment with plant extract restored the levels and it brought back the values to near normal range in group III and IV mices. When *murraya koenigii* extract treated mices (Group III) were compared with Pentylenetetrazol (PTZ) treated mices (Group V), there was no significant difference between these groups of mices. This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract of *murraya koenigii* similar to standard drug Pentylenetetrazol (PTZ).

Table 8. Effect of ethanolic whole plant extract of *murraya koenigii* on serological Parameters on 28th day of lithiasis.

serological Parameters	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
Urea (mg/dl)	10.36±0.17	25.07±1.32a*	10.74±0.17b*e^{ns}	10.73±0.16c*f^{ns}	10.75±0.13 d*
Uric acid (mg/dl)	4.29±0.17	10.84±016a*	4.86±0.08b*e^{ns}	4.86±0.12 c*f^{ns}	4.92±0.24 d*
Creatinine(mg/dl)	0.82±0.12	2.73±0.20a*	0.95±0.01 b*e^{ns}	0.96±0.02 c*f^{ns}	0.99±0.08d*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 5.

The symbols represent statistical significance p* < 0.05, ns – not significant

RESULT AND DISCUSSION

Changes in ionic pattern of urine are the major determinant of stone formation. In this study, the ionic pattern was found disturbed by treatment with ethylene glycol. It has been reported that daily oral administration of ethylene glycol for more than 4 weeks resulted in a significant increase in oxalate excretion and that kidneys are the targets for ethylene glycol toxicity. Ethylene glycol gets oxidized to oxalic acid leading to hyper oxaluria.

Hyperoxaluria is reported to be a more significant risk factor in the pathogenesis of stone formation. Likewise, ethylene glycol administration increased the urinary calcium level. It has been stated that hyper calciuria favors precipitation of calcium oxalate from urine. Thus the high oxalate and calcium ion concentration in urine tends to form calcium oxalate crystals.

Calcium and oxalate excretion are progressively increased in calculi induced animals (Group II). Oxalate plays an important role in stone formation and has about 15 fold greater effect than urinary calcium. Calcium oxalate crystals and high oxalate levels in nephrons can produce damages in the epithelial cells and consequently, the cells may produce some products, as well as free radicals, inducing heterogeneous crystal nucleation and causing aggregation of crystals.

A gradual increase in urinary phosphorus excretion was observed in ethylene glycol induced urolithic mices. 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 CaOx deposition. Increased excretion of phosphorus has been reported in stone formers and hyper oxaluric mices. Increased phosphorus excretion along with the oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition.

Soundararajan *et al*. (2006) showed that calcium oxalate excretion was significantly increased in urine of ethylene glycol induced urolithic rats. Additionally, they stated that ethylene glycol disturbs oxalate metabolism by way of increase the substrate availability that increase the activity of oxalate synthesizing enzymes in rats. Moreover, several investigations demonstrated that ethylene glycol treatment increased urinary calcium excretion significantly in lithiatic rats (Christina *et al*., 2002; Karadi *et al*., 2006; Verma *et al*., 2009).

Christiana *et al.* (2006) showed that aqueous extract of *Melia azedarach* Linn. reduced calcium and oxalate and elevated magnesium levels in serum of urolithiatic rats.

Christiana *et al.* (2002) showed that *Cyclea peltata* root powder increased serum magnesium and phosphorous levels in urolithiatic rats.

Karadi *et al*. (2008) reported that the root bark of *Moringa oleifera* Lam. normalized the serum levels of urea, uric acid and creatinine in experimental animals.

Anand *et al.* (1993) showed that alcoholic extract of *Crataeva nurvula* has reversed the levels of biochemical parameters in blood and serum to normal levels in urolithiatic rats.

From the above results it was evident that the levels of the serum mineral constituents were restored to its near normal range on treatment with the plant extract.

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