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EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF WHOLE PLANT OF *VERONIA CINEREA* LINN

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ABSTRACT

This study was aimed to evaluate the effectiveness of the *Veronia cinerea* on albino rats as a preventive agent against the development of kidney stones and urinary bladder stones. Activity of *Veronia cinerea* was studied using the ethylene glycol-induced urolithiasis model. Standard drug used was Cystone. Several parameters were used including urinary volume, urine pH, urine analysis, and serum analysis to assess the activity. The results indicated that the administration of *Veronia cinerea* to rats with ethylene glycol-induced lithiasis significantly reduced and prevented the growth of urinary stones ($P < 0.01$). Also, the treatment of lithiasis-induced rats by *Veronia cinerea* restored all the elevated biochemical parameters (creatinine, uric acid, and blood urea nitrogen), restored the urine pH to normal, and increased the urine volume significantly ($P < 0.01$) when compared to the model control drug. This study supports the usage of *Veronia cinerea* in urolithiasis and the utility could further be confirmed in other animal models.

Keywords: *Veronia cinerea*, Ethylene glycol, Urolithiasis, Whole plant.

INTRODUCTION

The most painful urologic disorder is calculi or stone formation in the kidneys and urinary bladder due to imbalance between promoters and inhibitors of crystallization in urine. Stone formation is documented from traditional periods and is considered as a medical challenge due to its multifactorial etiology. Stone formation commonly occur due to inadequate urinary drainage, foreign bodies in urinary tract, microbial, gout, intestinal dysfunction etc., Herbal remedies are gaining their importance due to inefficiency of standard pharmaceutical drugs, and reoccurance is possible by treating with ultrasonic energy and surgery. As investigations proved that phytotherapy is potent in preventing and curing renal calculi with less side effects and produced satisfactory results in preventing reoccurance of renal stones, the present study is mainly focused on providing information on potent herbal wealth with litholytic property.

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The current study was aimed to evaluate the effectiveness of the *Veronia cinerea* capsule on albino rats as a preventive agent against the development of kidney stones and urinary bladder stones.

MATERIAL AND METHODS

The whole plant of *Veronia cinerea* were collected from PIMS campus, Ganapathichettykulam, Pondicherry, India. Care was taken to collect only the healthy plant. The collected plants were authenticated at the Department of Ecology and environmental sciences Puducherry, India. The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with Ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless.The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 14.5%w/w.

Animals

Pharmacological evaluation of ethanolic extract of *Veronia cinerea* Linn. was carried out in the Department of Pharmacology, Adhibhagawan College of Pharmacy, Rantham, Cheyyar, Tamilnadu, India. Animal facility of this institute is approved by CPCSEA, New Delhi. The experimental protocols for the antiurolithiatic activity have been approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. IAEC approved this proposal with approval number PCP/IAEC/2014. The animals were maintained at a well ventilated, temperature controlled 30oC±1oC animal room for 7days prior to the experimental period and provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once.

Toxicity studies

1) Acute oral toxicity - Guideline number 423 [1]

2) The set out in this guideline is a stepwise procedure and depending on the mortalityand/or the morbid status of the animals, an average of 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The test substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure using three animals of a single sex (normally females) per step.

Absence or presence of compound related mortality of the animals dosed at one step
determined the next step i.e.
- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level Healthy young female adult animals were used.

The test substance was administered in stomach tube. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The time interval between treatment groups was determined by the onset, duration and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours with special attention given during the first 4 hours and daily thereafter for a total of 14 days.

Observations should include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. In addition behavioural changes, biochemical parameters and histopathological studies were also observed.

Ethylene Glycol Induced Urolithiasis

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats following procedures as under. Animals were divided into 5 groups containing 6 animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water ad libitum. All the remaining groups (Groups V) received calculi inducing treatment, comprised of ethylene glycol (0.75% v/v) in drinking water ad libitum for 15 days to accelerate lithiasis. Groups III, IV and V were administered cystone (750 mg/kg body wt.) and extract at doses of 200 and 400 mg/kg body wt. from day 1 to day 15 of calculi induction, respectively. Extract and standard drug were suspended in distilled water and given by gastric intubation once daily. Urine was collected on the 15th day for 24 h by keeping the animals in polypropylene metabolic cages. The collected urine was analyzed for calcium, oxalates and inorganic phosphates using standard methods. The volume of urine collected from all groups was recorded. The rats were sacrificed by cervical dislocation after 24 h of above treatment. The blood was collected by cardiac puncture and the serum uric acid and creatinine levels were estimated. Finally, the prevalence of lithiasis was confirmed by histopathological studies of the kidneys isolated from the sacrificed animals.

Histopathological studies

The isolated kidneys were weighed and transferred to 10% neutralized formalin (pH 7.4). Pathological changes were observed in the sections of kidney fixed in paraffin that were stained with hematoxylin and eosin.

Statistical analysis

Data are presented as Mean ± SEM. The data was analysed using one way analysis of
variance (ANOVA). The statistical significance of the difference of the means was evaluated
by Dunnett's multiple comparision

Effect of antioxidant activity of Ethanolic Extract of *Veronia cinerea*

***In vitro* evaluation**

Assay for nitric oxide (NO)scavenging activity

Procedure

SNP (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (100-1000µg/ml) of the drug dissolved in ethanol and water and incubated at 25ºc for 180 minutes. The samples from the above are reacted with Griess reagent. The absorbance of the chromophores formed during the diazotization of nitric with sulphanilamide and subsequent coupling with naphthylethylene diamine dichloride was read at 546 nm

and referred to the absorbance of ascorbic acid used as appositive control treated in the same way with Griess reagent..

Diphenyl 2-picryl hydrazyl (DPPH) assay [2]

Procedure

The reaction mixture contained methanol-50ml. DPPH (diphenyl 2-picryl hydrazyl radical) 0.3mM. 1ml of 0.3mM DPPH in methanol was added to 100µl of compound concentrations ranging from 20µg to 100µg. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidants in the sample, it was reduced and the colour changed from deep violet to light yellow. This was measured at 517nm. Ascorbic acid was used as a standard.

RESULTS

The results showed significant increase in uric acid, calcium, phosphate levels in serum and urine in the EG contol group compared to normal control. The levels decreased after treatment with EEVc and cystone therby hastening the process of dissolving the performed stones and prevention of new stone formation in the urinary system.

Table 1. Effect of Ethanolic extract of whole plant of *Veronia cinerea* on Serological parameters

Groups	Creatinine mg/24hrs	Uric acid mg/24 hrs	Oxalates mg/24hrs	Calcium mg/24 hrs	Magnesium mg/24 hrs	Sodium mg/24 hrs	Potassium mg/24hrs
Normal	0.48±0,09	0.94±0,06	2.29±0.02	3.51±0.19	0.60±0.08	119.20±2.25	5.08±0.08
Control	1.15±0.14	1.99±0.20	2.95±0.02	0.72±0.14	1.77±0.06	151.30±1.88	6.15±0.07
Cystone	0.5±0.08	2.13±0.03	2.14±0.03	3.36±0.53	0.80±0.07	123.00±2.81	5.28±0.10
EEVc 200mg	0.63±0.10	2.46±0.01	2.47±0.01	2.26±0.26	1.18±0.09	136.50±3.78	5.75±0.15
EEVc 400mg	0.53±0.06	1.95±0.05	1.98±0.01	3.25±0.67	0.73±0.21	126.40±3.00	5.21±0.07

Table 2. Effect of *Veronia cinerea* on Urine output and pH in Ethylene glycol induced urolithiasis

	Group	Study	Volume of urine	pH of urine
1.	Group I: Normal	Normal	12.16±0.83	7.98±0.04
2.	Group II: Control	Ethylene glycolated waterol	5.97±0,71	6.47±0.28
3.	Grou.p III:Cystone	Standard	11.86±0.35 ***	7.74±0.14**
4.	Group IV:EEVc200mg/kg	Preventive regimen	9.05±0.29 **	7.45±0.15**
5.	Group V: EEVc 400 mg/kg	Curative regimen	8.95±0.072**	7.44±0.10 **

Figure 1. Effect of Ethanolic extract of whole plant of *Veronia cinerea* on Serological parameters

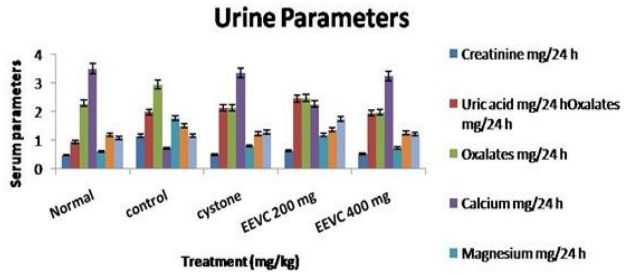

Figure 2. Isolation of Rat Kidney HISTOPATHOLOGY

Figure 3. T.S. of Kidney - Control Group

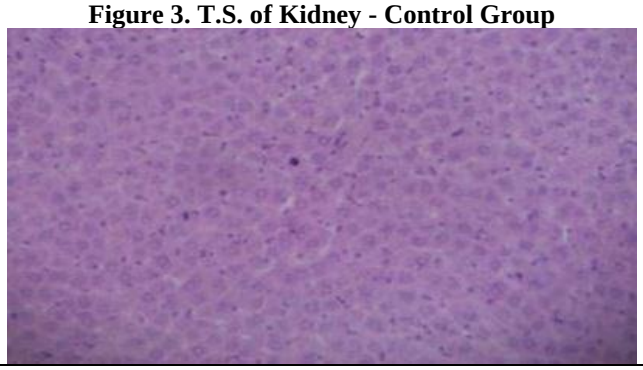

Figure 4. T.S. of Kidney - Standard Group

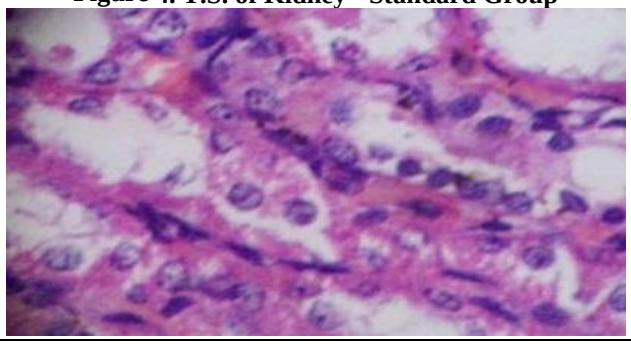

Figure 5. T.S. of Kidney - EEVc (200 mg/kg)

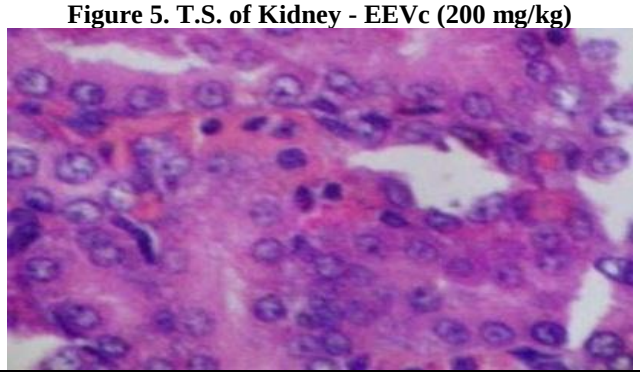

Figure 6. T.S. of Kidney - EEVc (400 mg/kg)

DISCUSSION

As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of protective effect of *Veronia cinerea* against ethylene glycol induced urolithiasis in rats. The discoveries of the clinical roles of these herbal remedies have made important contribution to the treatment of urinary stone disease as an alternative or adjunct therapy. Kidney stones develop as a result of a complicated interaction of biological events that are most likely triggered by genetic susceptibility coupled to dietary factors and lifestyle. In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats that of humans and also earlier studies have shown that the amount of stone deposition in female rats as significantly less.

In the present study chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats resulted in hyperoxaluria . Oxalate and calcium excretion were grossly increased in calculi induced animals. Since it is accepted that hyperoxaluria is far more significant risk factor in the pathogenesis of renal stones than hypercalciuria, the changes in urinary oxalate levels are relatively much more important than those of calcium. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth. However supplementation with ethanolic extract 200mg and ethanolic extract 400mg of *Veronia cinerea* significantly lowered the elevated levels of oxalate as well as calcium excretion in urine.

Normal urine contains many organic and inorganic inhibitors of crytallisation, 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 with ethanolic extract of *Veronia cinerea* 200 mg and ethanolic extract of *Veronia cinerea* 400mg but the preventive regimen study increases the magnesium level more comparing the curative regimen study.

The increase in urinary uric acid excretion as observed in urolithiatic rats. Increased excretion of uric acid has been reported in stone formed and hyperoxaluria rats.uric acid interfers with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans [3]. The predominance of uric acid crystals in calcium oxalate and modulate its crystallization also suggests its primary role in stone formation.

The present study examined the effect of various extracts, doses and studies *Veronia cinerea* on creatinine clearance in urine .Inducing agent showing a decrease in creatinine level which increased by cumin this indicates the antilithiatic action of *Veronia cinerea*.

In urolithiasis the glomerular flitration rate decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this the waste products particularly nitrogenous substances such as creatinine and uric acid get accumulated in blood [4]. In the present study the positive control calculi induced rats were found to have marked renal damage consistent with the elevated serum levels of creatinine and uric acid. however the curative and prophylactic treatment with ethanolic extract of *Veronia cinerea* 200mg and ethanolic extract of *Veronia cinerea* 400mg inhibited these changes that would otherwise promote new stone formation in the urinary system in rats treated with *Veronia cinerea* we attribute the lower serum creatinie and the acid levels to an enhanced GFR.

The mechanism of antilithiatic activity of ethanolic extract of 200mg and 400 mg of *Veronia cinerea* may involve the inhibition of oxalte induced toxic manifestation and free radical production along with enhancement of the body defence system. drug treated group showing cytoprotection due to its effect on prevention of deposition or aggretion of calcium oxalate in tubules so the mechanical disruption of epithelium is less or protection against free radicals rearrangements.

The alterations in membrane lipid and protein resulting from peroxidation can lead to increased permeability of calcium resulting in a loss of enzyme activity. Decreased ca2+ ATPase activity was noted in the kidneys of patients with nephrolithiasis [5]. Upon treatment with ethanolic extract of *Veronia* 200mg and ethanolic extract of *Veronia* 400 mg there was an elevation in the levels of calcium in both curative groups when compared to control and this effect was dose dependant. In this study 28 days administration of 0.75% (v/v) ethylene glycol induced nephrotoxicities. These toxicities were characterized by

marked elevation of blood magnesium and oxalates are remarkable decrease to normal value by treating with ethanolic extract of 200mg of *Veronia cinerea* and ethanolic extract of 400mg of *Veronia cinerea*. Prophylactic treatment was showing more treating efficiency than curative treatment.

In case urolithiasis, elimination of the normal electrolytes like sodium and potassium will be reduced due to the deposition of crystals in distal tubule of the kidney where those parameters will get excreted. Hence due to the reduced elimination serum level of those normal electrolytes will be elevated. In our present investigation reveals that ethanolic extract of *Veronia cinerea* 200mg and ethanolic extract of *Veronia cinerea* 400mg increases the electrolyte elimination and hence reduced their blood levels to normal

Microscopic examination using polarized light of kidney sections derived from nephrolithiatic rats showed intratubular and interstitial crystal deposits consistent with the findings of others. In the present investigation histopathological evaluation showed the maximum prevention of crystal deposition at the preventive study which may be due to the active compounds which is present in various doses of ethanolic extract of *Veronoia cinerea*. Ethanolic extract of *Veronia cinerea* have a high antioxidant capacity may be due to the presence of important phytoconstituents like phenolic compounds, steroids and flavonoids may be prevents the calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria -induced peroxidative damage to the renal tubular membrane surface which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones [6]. In the present investigation Ethanolic extract of 200mg and 400mg of *Veronia cinerea* shows potent antiurolithiatic activity may be due to their antioxidant activity.

CONCLUSION

The presented data indicate that administration of ethanolic extract of 200mg and 400mg of *Veronia cinerea* to the rats with ethylene glycol induced urolithiasis reduced the formation regarding antiurolithiatic activity of the plant .Exact mechanism underlying this effect is not clear but apparently related to antioxidant effect and lowering of stone forming constituents. Hence further research was suggested to explore the exact pharmacology of the drug and the study was closed with delight.

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