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ANTIUROLITHIATIC ACTIVITY OF *Scoparia dulcis* IN ETHYLENE GLYCOL INDUCED UROLITHIASIS IN MALE ALBINO WISTAR RATS

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ABSTRAT

The use of herbal medicines (medicinal plants or phytotherapy) has recently gained popularity in world wide. The research is lead to the new drug discovery or advances the use of indigenous herbal medicines for Urolithiasis. This revival of interest in plant derived drugs is mainly due to the current widespread belief green medicine is safe and more dependable than the costly synthetic drugs many of which have adverse side effects. The study lights up on the effect of *Scoparia dulcis* plant extract in ethanol. The plant extract is acting on the urolithiatic albino wistar rats induced by ethylene glycol. Urolithiasis was induced in rats by administrating 0.75 % of ethylene glycol orally for 30 days and analysed by the serum marker enzymes as ACP, ALP, AST, ALT, Creatinine and Uric acid. The toxic rats were treated with the ethanolic leaf extract of *Scoparia dulcis* was administered in 250 mg/kg body weight of rats orally for 30 days . The variations in the haematological profile of the urolithiasis results in the elevated levels of serum marker enzymes and increased level of Creatinine and Uric acid. This study summarized that the treatment with *Scoparia dulcis* is capable of counteracting the urolithiasis and it can be developed into antiurolithiatic drug.

Key Words: Urolithiasis, *Scoparia dulcis*, Ethylene glycol.

INTRODUCTION

Plants are one of the oldest living things on Earth, and yet they are also one of the most simple. Plants will grow naturally with no human input, though many have been

domesticated for human needs. Plants have been one of the major sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics in the recent days. Plants are rich in a variety of compounds including the secondary metabolites, aromatic substances. Stone disease is a multifactorial disease, ingestion of excessive amounts of purines,oxalates, calcium phosphate,sodium and other elements often results in excessive excretion of these components in urine thus increase in the risk of calculi formation.[1] Urinary stone disease is a problematic illness of human since antiquity and can persist, with serious medical consequences, throughout a person's life time. In addition to the incidence of kidney stones has been increased in western societies, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones[2]. Urolithiasis is defined as the presence of one or more calculi in any location within the urinary tract. The disease affects 1% to 5% of the population in developed countries mostly between 20 and 50 years of age. Men are three times more likely to be affected than women and the lifetime risk of developing a calculus. [3]

Kidney stones are composed of inorganic and organic crystals combined with proteins. Urinary stones can be classified according tostone composition as calcium stone, struvite stone,urc acid stone, cystine stone;some other types include xanthine stone. Approximately 80% of the kidney stone is composed of Calcium oxalate and calcium phosphate, 10% struvite ie Magnesium ammoniumphosphate produced during infection with bacteria that posses the enzyme urease.[4] *Scoparia dulcis* is a species of flowering plant in the plantain family. Common names include goatweed, scoparia-weed and *tipychä kuratu* in Guarani. It is native to the Neotropics but it can be found throughout the tropical and subtropical world. The theory of stone formation was concluded as an imbalance between promoter (calcium, oxalate, uric acid, inorganic phosphate etc) and inhibitors (citrates, magnesium, potassium, pyrophosphate and urinary glycoprotein etc), due to oxidative stress the reactive oxygen species or free radicals (species with one or more unpaired electrons) were generated and damages epithelium of kidney or bladder, thereby producing a favourable environment for crystal attachment to surface. As a result of these, the stone may not be able to travel through the ureter, causing pain and possibly an obstruction, blocking the flow of urine out of the kidney. [5] The worldwide incidence of urolithiasis is quite high and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid of this painful disease [6] Various

therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing. In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects.[7]

The present day medical management of urolithiasis is either costly or with side effects. Hence, the search for antiurolithiatic drugs from natural sources has assumed greater importance. Many Indian plants have been quoted to be useful as antiurolithiatic agents. They are effective with fewer side effects and are also less costly. Hence; the Indian plants are constantly being evaluated for possible antiurolithiatic effects in a systematic manner[8]. The plant *Scoparia dulcis*, which is used as anti-inflammatory is here evaluated for the anti urolithiasis.

MATERIALS AND METHOD

Animal Model Used: Male albino white rats of wistar strain with an average weight of 130-150 grams were purchased from RVS College of Pharmacy, Sulur, Coimbatore. The rats were then housed in large spacious cages. The animal room was ventilated. The temperature was maintained between 20° to 30°C.

Experimental Design

Rats were divided into four different groups comprising of six animals each.

Table 1 Experimental design

Groups	Experimental Design
Group I	Rats given normal diet for 30 days.
Group II	0.75 %Ethylene glycol for 30 days through drinking water.
Group III	Hyperoxaluria induced rats were received *Scoparia dulcis* leaf extracts (250 mg/ Kg body weight) by oral administration for 30 days at the rate of 1.0 ml / rat / day
Group IV	*Scoparia dulcis* leaf extract (250 mg / Kg weight administrated at the rate of 1.0 ml / rat / day) for 30 days.

Collection of Plant Species: The leafs of *Scoparia dulcis* were collected from Thenkurissi, Palakkad, Kerala and were shade dried and powdered and used for the analysis.

Preparation of Leaf extract: The fresh leaves collected were washed with distilled water and shade dried. The dried plant material was powdered and extracted with ethanol by immersing for 72 hours. The extract was filtered and air dried to obtain the residue. The residue was suspended in water and administrated orally (250mg per Kg body weight was administrated at the rate of 1 ml/rat/day).

Experimental induction of Urolithiasis by Ethylene glycol

Ethylene glycol induced hyperoxaluria model was used to assess the antiurolithiatic activity in albino rats. Animals were divided into four groups containing six animals in each. Group I served as control and received regular rat food and drinking water. Ethylene glycol (0.75%) in drinking water was fed to Groups II and III for induction of renal calculi till 28th day. [5]

Collection of Biological samples

At the end of the experimental period, the animals were sacrificed by cervical decepitation, under mild anesthesia. The blood was carefully collected by pumping the heart after the rat was killed. From the collected blood, the serum was separated by centrifugation at 3000 rpm for 20 minutes.

Biochemical Serum analysis

The serum parameters Creatinine, Uric acid, Acid phosphatase, Alkaline phosphatase, Aspartate amino transferase and Alanine amino transferase was assessed.

Statistical Analysis

All values were expressed as Mean ± Standard Deviation. While analysis of students t test was used to analyze the extent of variation between groups and values significant at 5% ($P<0.05$), 1%($P<0.01$), 0.1%($P<0.001$), levels were found.

RESULT AND DISCUSSION

Urolithiasis is a condition in which crystals in the urine combine to form stones, also called calculi or uroliths. These can be found anywhere in the urinary tract, where they cause irritation and secondary infection. Most end up in the bladder or urethra. [3]

Kidney stone formation or urolithiasis is a complex process that results from a succession of several physicochemical events including super saturation, nucleation, growth, aggregation, and retention within the kidneys. Epidemiological data have shown that calcium oxalate is the predominant mineral in a majority of kidney stones .

Furthermore, in spite of substantial progress in the study of the biological and physical manifestations of kidney stones, there is no satisfactory drug to use in clinical therapy. Data from *in vitro, in vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of Urolithiasis .

Among many in vivo models developed to evaluate antiurolithiatic effect, Ethylene glycol induced calculi are widely used. Rat has been found a suitable and frequently used animal to induce CaOx deposition in kidney because of its close resemblance of urinary system to human. Selection of male rats was based on previous studies showing higher rate of crystal depositions in male as compared to female rats [9]

The basic mechanism behind ethylene glycol induced calculi is hypercalciuria and hyperoxaluria leading to CaOx crystal formation. In addition to development of oxalate crystal, it is also associated with severe oxidative stress to renal tissue. Oxalate has been reported to induce lipid peroxidation and causes renal tissue damage by reacting with polyunsaturated fatty acids in cell membranes and by generation of reactive oxygen species like hydroxyl and superoxide ions. Due to renal papillary hypertrophy and crystal depositions, kidney weight is increased in ethylene glycol treated rats.. It increases blood urea and creatinine levels significantly due to impairment in renal function[9]

A renal calculus due to ethylene glycol in the drinking water has been widely used for inducing urolithiasis. Ethylene glycol is metabolized by alcohol dehydrogenase to glycolaldehyde, which is then oxidized to glycolic acid and finally to oxalic acid. Oxalic acid binds with calcium to form calcium oxalate crystals.[10]

Nature is the best combinatorial chemistry and has possible answers to all diseases for mankind. Medicinal plants play a vital role in stone diseases. The undesirable effect of the modern medicine has already diverted the attention of the people towards herbal medicines. To increase the acceptability and awareness among the people, there is an urgent need to develop trust and faith towards the safer indigenous system by establishing its validity in

treatment for various diseases. Health care systems are going to become more and more expensive, therefore we have to introduce herbal medicine systems in our health care. Lets us hope that in future natural products will be competing modern medicines with added advantages of more safety and lower costs.[11]

As traditional medicines are usually taken by the oral route, same route of administration was used for valuation of antiurolithiatic effect of the *Scoparia dulcis* against ethylene glycol induced urolithiasis in rats.

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and also earlier studies have shown that the amount of stone deposition in female rats was significantly less.

The present study focuses on the antiurolithiatic properties of the formulation "*Scoparia dulcis* "against Ethylene glycol induced Urolithiatic rats. The alteration shows in the serum biochemical marker enzymes in uroiolithiatic rats showed the elevated levels of the ACP, ALP, AST, and ALT in the serum.

Table 2 EFFECT OF *Scoparia dulcis* IN SERUM BIOCHEMICAL PARAMETERS

Group	ACP	ALP	AST	ALT
I	60.05 ± 0.17	65.37 ± 0.11	43.57 ± 0.12	39.73 ± 0.14
II	100.15 ± 0.52a*	142.02 ± 0.75a*	132.70 ±0.14a*	99.20 ± 0.72a*
III	73.26 ± 0.51b*	82.01 ± 0.02b*	45.50 ± 0.12b*	40.20 ± 0.52b*
IV	80.02 ± 0.51	76.13 ± 0.20	44.71 ± 0.46c^{ns}	43.24 ± 0.16

Values are expressed as mean ± standard division of six animals each

Treatment of Groups are as in Table 1

Units are expressed as:

- ACP, ALP : µ moles of phenols liberated per liter
- AST,ALT : µ moles of pyruvate liberated per liter

The comparison between groups and the statistical significance are as follows:

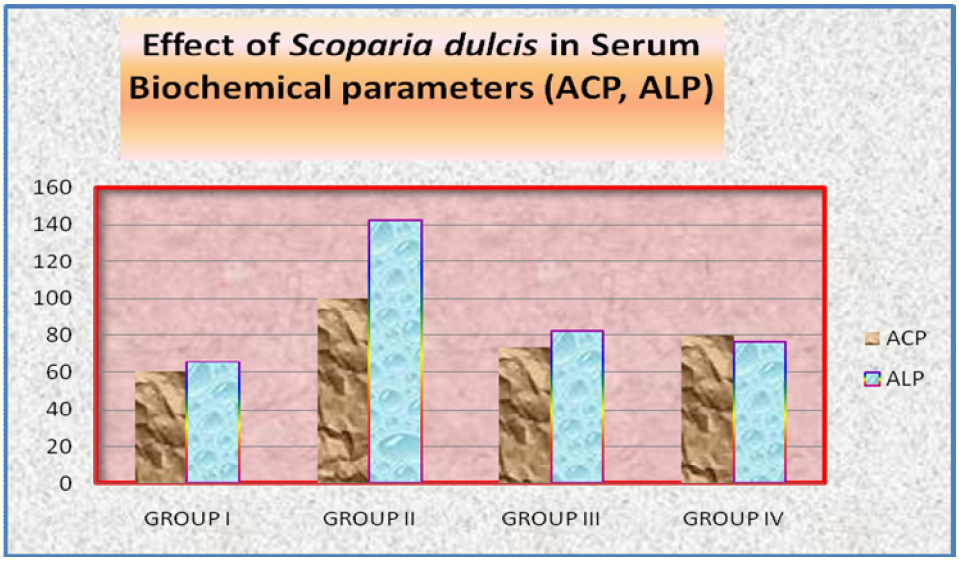
a represents the comparison between Group II and Group I

b represents the comparison between Group III and Group II

c represents the comparison between Group IV and Group I

Symbols of statistical significance:

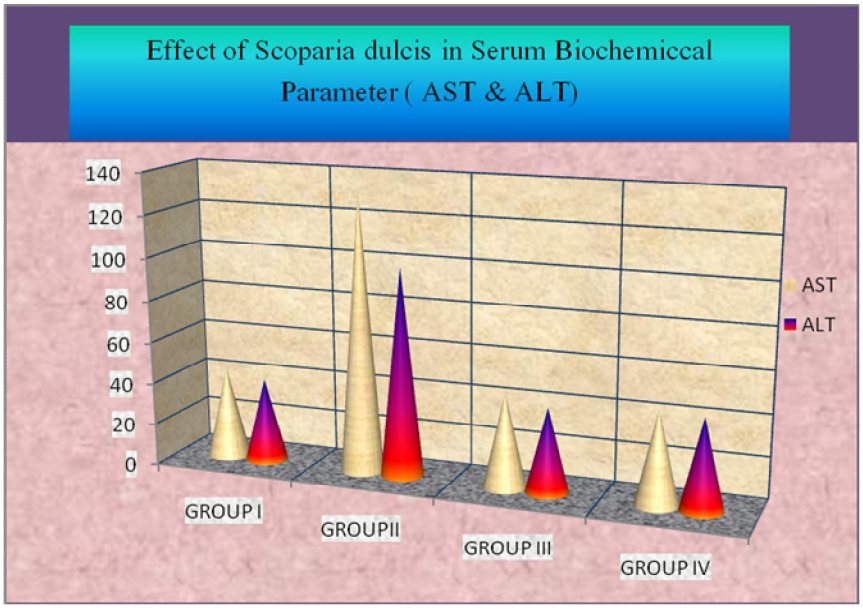
*P <0.01; **p <0.05; $p< 0.001; ns- not significant



Graph 1 Effect of *Scoparia dulcis* in Serum Biochemical parameters (ACP, ALP)

The serum biochemical analysis results in the increased level of the marker enzymes ACP, ALP, AST and ALT in Group II rats, due to the administration of ethylene glycol for the induction of Urolithiasis. The toxic urolithiatic rats eliminates the Kidney marker enzymes into the blood and thereby it circulates through the blood stream. These enzymes values were reverted in a controlled manner in the Group III animals because of the administration of Plant *Scoaparia dulcis* extract. The animals which were given with plant extract alone in Group IV were showing some slight difference from the control rats.

Activity of serum biochemical enzymes in serum were significantly increased ($P < 0.05$) in serum in Group II rats when compared with Group I rats. The activities of the enzyme were near to normal in *Scoparia dulcis* ethanolic extract(Group III) administered rats in serum .The ethanolic extract of Scoparia dulcis administered in Group III rats compared with Group IV was found to have no significant difference between the animals. The stone formation may block the ureter reading to an increasing pressure in the renal pelvis and damage of the tubular cells. It shows the *Scoparia dulcis* has an antiurolithiatic activity in ethanolic extract ethylene glycol induced rats.



Graph 2 Effect of *Scoparia dulcis* in Serum Biochemiccal Parameter (AST & ALT)

Table 3 Effect of *Scoparia dulcis* in Serum Biochemical Parameter

GROUP	CREATININE	URIC ACID
I	0.72 ± 0.02	7.39 ± 0.02
II	2.71 ± 0.03a*	10.81 ± 0.01a*
III	1.31 ± 0.02b*	7.79 ± 0.03 b*
IV	0.89 ± 0.03 c^{ns}	7.98 ±0.07 c^{ns}

Values are expressed as mean ± standard division of six animals each

Treatment of Groups are as in Table 1

Units are expressed as:

Creatinine and Uric acid expressed in mg/ per dl

The comparison between groups and the statistical significance are as follows:

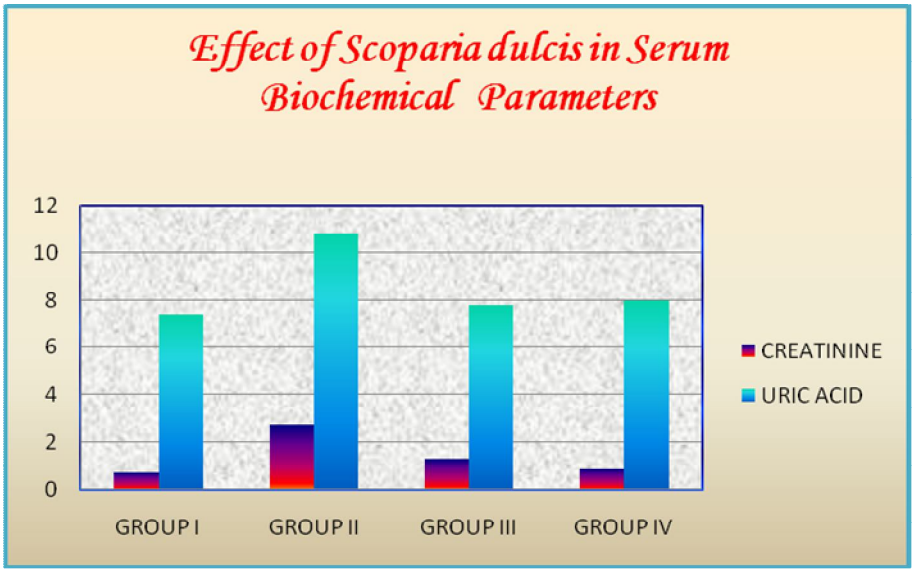
a represents the comparison between Group II and Group I

b represents the comparison between Group III and Group II

c represents the comparison between Group IV and Group I

Symbols of statistical significance:

*P <0.01; **p <0.05; $p< 0.001; ns- not significant



Graph 3 Effect of *Scoparia dulcis* in Serum Biochemical Parameters

It is clear that from the above values , that the levels of biochemical parameters Uric acid and Creatinine in serum were significantly increased ($P < 0.05$) in Group II rats (Urolithiatic rats) when compared with Group I rats (Control rats) .Treatment with *Scoparia dulcis* extract brought back the values to near normal values in Group III rats.

Uric acid is the end product of Purine metabolism produced with peroxisomes and excreated in urine and can be crystallizable to form stones. Pure uric acid stone formation is rare and it is accomplished by urates and phosphates. The result makes us clear that the biochemical parameters were reverted back to the normal range on administration with the plant extract.

Thus the result of our present study indicate that the treatment with *Scoparia dulcis* is capable of counteracting the toxic effect caused by Ethylene glycol in serum and it can be used as an anti Urolithiatic drug. It shows the *Scoparia dulcis* has an Antiurolithiatic activity in ethanolic extract ethylene glycol induced rats.

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