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Antiurolithiatic activity of *Stephania japonica* Linn., leaves

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ABSTRACT

Stephania japonica Linn. Leaves commonly known as Molakaranaikkodi, belonging to family Menispermaceae, has been investigated for its anti urolithiatic activity in models (in vivo) of urolithiasis in rats. The method chosen were ethelene glycol 0.75% w/v and ammonium chloride 2% w/v induced urolithiasis respectively. The ethanol extract (400 mg/kg/bw) reduced the elevated levels of serum calcium (7.26 +/- 0.076) and urine calcium (5.8 +/- 0.13) significantly. ($P<0.05$), employing 0.75 % w/v ethylene glycol and 2% w/v of ammonium chloride induced urolithiasis model. The ethanol extract 400 mg/kg/bw reduced urine uric acid level significantly employing this method, viz. The results shown by ethanol extract (400 mg/kg/bw) group compared to standard polyherbal drug cystone 750 mg/kg/bw treated group and thus exibited potent anti urolithiatic activity.

Keywords: Ammonium chloride, Leaves, *Stephania japonica,* Ethylene glycol, Cystone, Urolithiasis.

1. INTRODUCTION

Nephrolithiasis (renal stone formation) is worldwide indistibution and a common disorder estimated to occur in approximately 12% of the population. With recurrence rate of 70-80% on males and 47-60% on females. The majority of stones, upto80% are composed mainly of calcium oxalate. Many remedies have been employed during ages to treat renal stones. Most of remedies were taken from plants and proved to be useful, though the rationale behind their use is not established except for a few plants and some proprietory composite herbal drugs and they are reported to be effective with no side effects.[1]

The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for anti lithiatic drug from natural sources has assumed greater importance.[2] In recent times, focus on plant research has increased all over the world under and a large body of evidence has collected to show immence potential of medicinal plants used in various traditional systems. *Stephania japonica* Linn. Leaves belonging to the family menispermaceae, has many therapeutic benefits.[3] such as its use in tribal area as anti urolithiatic, diuretic, fever, diarrhoea, asthma and to treat kidney stones.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The leaves of *Stephania japonica*.Plate (1) was collected from slender wirly climber from kollimalai during the month of May. And identified by the Botanist of department of Botany, Chennai, Tamilnadu. After authentification of plant materials were collected in bulk, washed under running tap water to remove adhering dust, shad dried and pulvarised in a mechanical grinder. The course powder was used for further studies. [4]

Figure - 1: *Stephania japonica* Leaves.

2.2. Extraction

About 1kg of coarse powder of leaves was taken in the Soxhelet apparatus and extracted. Successively using 95% ethanol.The extraction for each solvent was carried out to 24 hours. The extract was collected by evaporating. The solvents and percentage yield was calculated. [5]

2.3. Experimental design

Wister albino rats weighing 150-200gm of either sex were used. (Verma,N.K.,1976) The experimental protocol was approved by the Institutional animal ethics committee and animals were period of 15days before performing the housed in standard conditions experiment of temperature (25'c +/- 2'c),relative humidity of 45-55% and maintain on 12hour light dark cycle in animal cycle. The condition in the animal house was approved by committee for the purpose of control and supervision on experiments on animals. (Regd.no: 263/C/05/CPCSEA). The acute oral toxicity study was done according to OECD guideline at the dose range 100 to 400mg/kg. No mortality of animals was observed at the dose range and hence two different doses 200mg/kg and 400mg/kg was taken for the study. [6]

2.4. Antiurolithiatic activity

Nephrolithiasis (renal stone formation) is worldwide in distribution and a common disorder estimated to occur in approximately 12 % of the population, with a recurrence rate of 70-80% on males and 47-60% females. The majority of stones, upto 80%, are composed mainly of calcium oxalate. Many remedies have been employed during ages to treat renal stones. Most of remedies were taken from plants and proved to be useful, though the rational behind their use is not established except for a few plants and some proprietary composite herbal drugs and they are reported to be effective with no side effects. [7] The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for antilithiatic drugs from natural sources has assumed greater importance. [8]

Oxalate, a by – product of metabolism, is normally excreted in the urine and at low concentrations is harmless to the renal epithelial cells. However, elevated oxalate levels and/ or calcium oxalate crystals are injurious. In addition, exposure of renal epithelial cells in vitro to high levels of oxalate and/ or calcium oxalate crystals upregulates the production of chemoattractants osteopontin and monocyte- chemoattractant protein – 1. Mild idiopathic is associated with Calcium oxalate nephrolithiasis, whereas hyperoxaluria resulting from jejunal bypassfor obesity or genetic defects in oxalate metabolism is nephrotoxic and produces tubulointerstitial lesions. Tubulointerstitial damage is recognised as one of the most important risk factors for the development of chronic renal diseases and eventual renal failure [9].

2.5. Animals Used

Albino rats of Wistar strain, of either sex, aged around 2 to 3 months and weighing 150-200 gm were used. They were housed in standard conditions of temperature (25 ± 2°C), relative humidity of 45- 55%, and maintained on 12 hour light : dark cycle in animal house. Experiments were conducted in accordance with internationally accepted standard guidelines for the use of animals. Animals were fed ad libitumon normal commercial chow and had free access to water. [10]

2.6. Ethylene glycol-induced urolithiasis model

Twenty rats were divided into five groups comprising four animals per group. Each group underwent a different treatment protocol for 28 days. Animals of Group 1 were untreated and served as normal control. Group 2, 3, and 4 ad libitumaccess to regular food and ad libitumaccess to drinking water containing 0.75% [v/v] ethytlene glycol (EG) and 2% [w/v] ammonium chloride (AC) in order to promote hyperoxaluria and CaOx deposition in the kidneys.

Group I : Normal Control

Group II : Standard durg (Cystone tablet 750 mg/kg)

Group III : Ethanolic extract of leaves of *Stephania japonica*(200mg/kg)

Group IV : Ethanolic extract of leaves of *Stephania japonica*(400mg/kg)

Group V : Rats were administered 6µl distilled water / body weight by gavage (Positive control);

2.7. Assessment of antiurolithiatic Activity

2.7.1. Collection of urine sample

The rats were placed in metabolic cages and urine was collected for 24 hours. Urine was freed from faecal contamination. Rats were provided with water but no feed. The collected urine samples were centrifuged for 10 minutes and any sediment present was discarded. The urine was used for further analysis.

2.7.2. Serum analysis

After the 10-day experimental period, rats were anaesthetized and blood was collected from the retro – orbital under anesthetic condition on the 28thday. Serum was separated by

centrifugation at 10,000 X g for 10 min and was analyzed for creatinine, urea nitrogen and uric acid using calcium (Biolab Diagnostics), phosphorus (Coral Clinical Systems), urea (Pathozyme Diagnostics), creatinine (Coral Clinical Systems) diagnostic kits. Kidney Homogenate Analysis The rats were then sacrificed by cervical dislocation, the abdomen opened and both kidneys removed. The isolated kidneys were cleaned off extraneous tissue and right kidney was preserved in 10% neutral formalin. The left kidney was dried in an oven at 100°C for 24 h, after which the kidney was weighed and then minced in a beaker containing 7 ml of 0.5N nitric acid. The mixture was then heated until the liquid became transparent. The calcium content of the mixture was determined using calcium Kit (Biolab Diagnostics). The amount of calcium is expressed as mg/gm dry kidney. The right kidney was fixed in bouin liquid, soaked in paraffin, cut at 3-4 mm intervals, and the slices stained using hematoxylin and eosin 18, 19. Tissue slices were photographed using optical microscopy under polarized light. [11]

2.7.3. Statistical analysis

The result were expressed as mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by the Dunnets test. P<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Stone formation in ethylene glycol – fed rats is caused by hyperoxaluria, which cause increased renal retention and excessive excretion of oxalate in urine. Urinary lithiasis is generally the results of an imbalance between inhibitors and promoters in the kidneys.

Rats are the most frequently used animals in models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Rat models of calcium oxalate urolithiasis induced by either EG alone or in combination with other drugs such as AC, are often used to study the pathogenesis of kidney crystal deposition. Using the accelerated model, in the present study rats were treated with 0.75% EG 24 days (Figure 1 and 2). All positive control rats (Group 5) developed calcium oxalate depositions during that time. In this study, calcium excretion was increased in calculi – induced animals (Table 1 and figure 3). An increase in urinary phosphate is also observed in calculi induced rats (Table 2 and 3). Increased urinary phosphate excretion along with oxalate stress seems to provide phosphate crystals, which induce calcium oxalate deposition (Tale 3 and figure 4). Treatment with the test extracts of *Stephania japonica* restored the phosphate level, thus reducing the risk of stone formation (Figure 5).

Table -1: Effect of Leaves Extract of *Stephania japonica* on Ethylene Glycol Induced Lithiasis on Urinary Eletrolyte Concentration in Rats

Groups	Calcium (mg/dL)	Phosphorous (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control(Vehicle)	3.4±0.26	0.8±0.02	0.8±0.04	7.24±0.46
Positive control	7.2±0.16	0.2±0.03	9.1±0.16	12.29±0.16
Standard Cystone (25mg/kg)	4.6±0.12*	0.74±0.07*	6.4±0.02*	9.7±0.08*
Ethanolic extract (200mg/kg)	6.4±0.09*	0.68±0.06*	7.2±0.16*	10.6±0.20*
Ethanolic extract (400mg/kg)	5.8±0.13*	0.78±0.04*	6.9±0.16*	8.4±0.20*

Values are expressed as mean ± SEM; P*<0.05 was considered statistically significant

Table -2: Serum Biochemical Data on leaves extract of *Stephania japonica* in Wistar Rats

Groups	Calcium (mg/dL)	Phosphorus (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control (Vehicle)	6.45±0.06	8.21±0.04	0.35±0.01	3.15±0.05
Positive control	9.52±0.10	8.65±0.10	0.60±0.03	37.65±0.10
Standard (Cystone) (25mg/kg)	7.07±0.06*	7.31±0.06*	0.32±0.02*	27.68±0.03*
Ethanolic extract (200mg/kg)	7.84±0.08*	8.42±0.09*	0.46±0.01*	26.28±0.07*
Ethanolic extract (400mg/kg)	7.26±0.076*	8.32±0.07*	0.42±0.01*	21.52±0.05*

Values are expressed as Mean ± SEM; P*<0.05 was considered statistically significant

Table - 3: Kidney Calcium Homogenate (µg/gm)

Groups	Kidney homogenate (Calcium) (µg/gm)
Normal (Vehicle)	7.65±0.04
Positive control	25.62±0.10
Standard (Cystone 25mg/kg)	7.58±0.07*
Ethanolic extract (200mg/kg)	12.58±0.09*
Ethanolic extract (400mg/kg)	6.86±0.07*

Values are expressed as mean ± SEM; P*<0.05 was considered statistically significant.

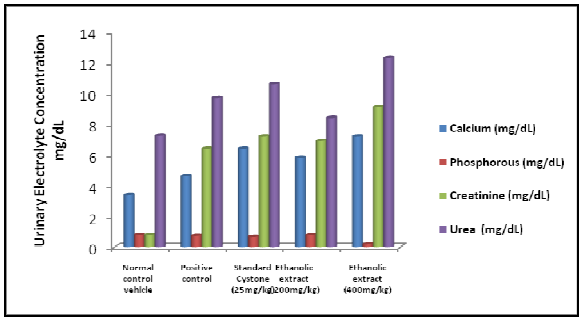

Figure - 2: Effect of leaves extract on ethylene glycol induced lithiasis on urinary electrolyte concentration in wistar rats.

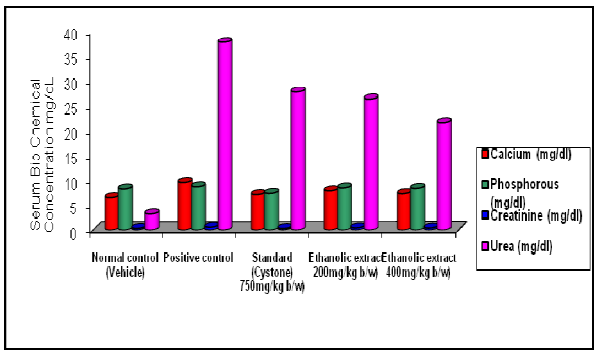

Figure - 3: Serum bio chemical data in leaves extract of *Stephania japonica* in wistar rats.

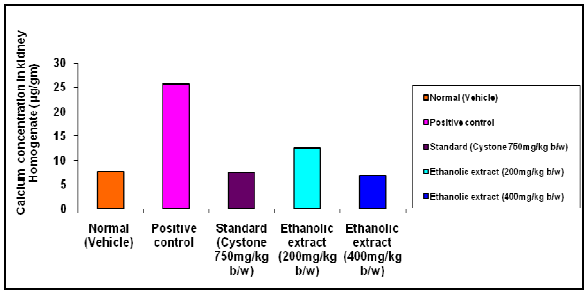

Figure - 4: Kidney calcium homogenate (µg/gm) on leaves extract of *Stephania japonica* in wistar rats.

4. CONCLUSION

The results exhibited by the ethanol extract of *stephania japonica* leaves (400mg/kg/ bw) showed significant antiurolithiatic activity when compared with standard drug cystone 750mg/kg. Further studies will be aimed at extensive investigation, isolation and purification of active phytoconstituents with potent antiurolithiatic activity.

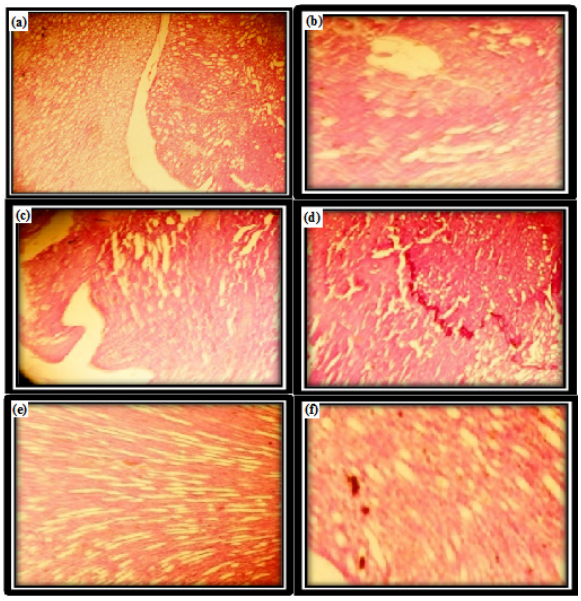

Figure - 5: Histological Section.

((a)Group – I : Histological section of kidney Crystalline formation in Normal; (b)Group – II : Crystalline Formation in the Rat kidney of Postive Control(A); (c)Group – III : Crystalline Formation in the Rat kidney of Postive Control(B); (d)Group – IV : Histological Section of Kidney in Standard Cystone 750mg/kg bw; (e)Group – V : Crystalline Formation in the Rat Kideny of Ethanolic Extraact of *Stephania japonica* 200mg/kg bw; (f)Group – V : Crystalline Formation in the Rat Kideny of Ethanolic Extraact of *Stephania japonica* 400mg/kg bw).

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