

Antiuro lithiatic activity of Triphala Karpa Chooranam

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ABSTRACT

Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition which are found as secondary metabolites in one or more parts of these plants. *Triphala Karpa Chooranam* a traditional medicine used for curing kidney stones contains *Terminalia chebula*, *Phyllanthus emblica*, *Terminalia bellerica*, Karungali sathu and Vengai sathu. Preclinical evaluation of the herbal formulation shows significant antiuro lithiatic action against ethylene glycol and calcium oxalate induced urolithiasis in rat model. The histopathology of the kidney section shows no damaged cells of formulation treated and Cystone treated (750mg/kg) compared with that of the control animals. The urine analysis showed reduction in the excretion of oxalate crystals in both test and the standard treated groups.

Keywords: *Triphala Karpa Chooranam*, Glycolic acid, calcium oxalate, cystone

INTRODUCTION

Urolithiasis is an extremely painful disease that afflicts the human population since ancient times¹. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent, is known to induce lipid peroxidation which causes disruption of the cellular membrane integrity^{2, 3}. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion transport across the cellular organelle^{4, 5}.

Benefits of Triphala correct constipation, cleanses digestive tract, cleanses colon, detoxifies body and treats irritable bowel syndrome and ulcerative colitis. Triphala is a matchless herbal formulation that has been a gift of ayurveda to this world. Triphala since centuries has been an herb of choice for an ayurvedic physician to cure any kind of disease⁴. Since ages it

has been a house hold name in Indian civilization as it is the most easily available herbal supplement that has the potential to treat any kind of disorder especially related to the gastro intestinal tract.

MATERIAL AND METHODS

The herbal formulation *Triphala Karpa Chooranam* was purchased from SKM Siddha and Ayurvedha Company (India) Limited, Erode, Tamil Nadu, India. The purchased formulation was standardised, evaluated for toxicity assessment and preclinically for antiuro lithiatic action in the suitable animal models.

Herbal standardisation and phytochemical analysis

The herbal formulation *Triphala Karpa Chooranam* was standardised for Ash values, extractive values, crude fibre content and loss on drying as per the procedure. Phytochemical tests on the extract were performed using standard procedures⁵ (Kokate, 1991).

Experimental animals

Male albino rats of wistar strain weighing between 150-200gm were used, the animals were fed with commercial rat feed pellets (Amrut laboratory animal feed Ltd, Bangalore) and were given water *ad libitum*. Animals were housed in plastic cages with filter tops under controlled conditions of 12:12 light dark cycle, 50% humidity and 28°C. All animal experiments and

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maintenance were carried out according to the ethical guidelines suggested by the IAEC of Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad.

Toxicity study

Toxicity study was performed to find out the toxic dose and therapeutic dose confirmation. The human therapeutic dose (1gm) was converted into animal therapeutic dose (90mg/kg) and acute toxicity test was performed for that particular dose⁶.

Pharmacological screening

Ethylene glycol induced urolithiasis⁷

Animal were divided in to five groups containing six animals in each group. Group I served as normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to group II for induction of renal calculi till 28 day. Group III received standard anti urolithiatic drug, Cystone (750mg/kg, p.o) from 15th day till 28th day. Group IV received herbal formulation of *Triphala karpa chooranam* (90mg/kg, p.o) from 15th day till 28th day. On day 28 animals of the entire group were kept in metabolic cages and urine samples were collected for 24h and analysed for inorganic sodium, potassium and chloride using standard methods. The serum creatinine levels and urinary output volumes of all groups were also noted.

Calcium oxalate induced urolithiasis⁸

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce calcium oxalate urolithiasis. Group I served as normal group and received 1ml/kg distilled water. All the remaining groups received calculi-inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for three days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 25 days. Group II served as induction control group and received distilled water 1ml/kg. Groups III received Cystone (750mg/kg) and Group IV received *Triphala karpa chooranam* (90mg/kg, p.o) from first day to 28th day of calculi induction.

Urine analysis and biochemical parameters

On the 28th day of calculi induction treatment, all animals were kept in individual metabolic cages and urine samples of 24 h were collected. Urine was analysed for calcium, oxalates and total proteins. On the next day all animals were sacrificed, blood samples were taken and analysed for calcium, phosphate, magnesium, oxalate and protein⁹.

Histopathological studies

To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. The kidneys were washed, weighed and fixed rapidly with 10% neutralized formalin (pH7.4), and soaked in paraffin, cut at 5 μ m intervals and the slices were stained with hematoxylin and eosin. Tissue slices were photographed using optical microscopy and observed the pathological changes¹⁰.

STATISTICAL ANALYSIS

Values are presented a MEAN \pm SEM of 6 rats in each group. All the data were statistically evaluated by one way ANOVA followed by Dunnet's test.

RESULTS

In the preliminary phytochemical investigation on *Triphala karpa choornam* revealed the presence of triterpenes, flavonoids, saponins, alkaloids, carbohydrates in the herbal formulation. Ash values, extractive values, crude fibre content and loss on drying were within the official pharmacopoeial limits. In the present study chronic administration of 0.75% ethylene glycol aqueous solution to albino rats resulted in hyperoxaluria. Ethylene glycol induced urolithiatic rats exhibited significant increase in liver glycolic acid oxidase activity when compared to that of the normal control rats. Treatment with *Triphala karpa choornam* reduced the activities of GAO, oxalate levels to near normal control. Calcium and oxalate excretion were significantly increased while citrate and magnesium excretion were significantly decreased in 24 h urine of ethylene glycol induced urolithiatic rats when compared with the normal

control rats. Calcium, oxalate, phosphate and protein excretion were grossly increased. However supplementation with *Triphala karpa choornam* significantly ($p < 0.01$) lowered the elevated levels of calcium, oxalate, phosphate and protein excretion in urine of curative and preventive regimen groups. In lithiatic control group the magnesium excretion was gradually decreased following ethylene glycol treatment. Subsequent administration of the formulation enhanced the magnesium excretion significantly ($p < 0.01$) in both regimen.

Supplementation with *Triphala karpa choornam* significantly ($P < 0.01$) lowered the elevated levels of

oxalate, calcium and phosphate in urine and kidney as compared to cystone- treated animals. The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate and calcium, was increased in the stone forming rats *Triphala karpa choornam* treatment significantly ($P < 0.01$) reduced the renal content of these stone forming constituents. The serum uric acid and BUN were remarkably increased in calculi-induced animals, while serum creatinine was elevated in Group II, indicating marked renal damage. However, *triphala karpa choornam* treatment significantly ($P < 0.01$) lowered the elevated serum levels of Creatinine, Uric acid and BUN.

Table 1: Effect of Triphala karpa choornam on Ethylene glycol induced urolithiasis in rats

Group	Treatment	Volume of Urine (ml/4hrs)	Sodium (mMol/l)	Potassium (mMol/l)	Chloride (mMol/l)
1	Normal saline	4.7±0.40	51.33±2.33	45.83±1.72	1.120
2	Calculi-induced control	2.08±0.26	99.7±8.4	114.7±9.8*	122±14.2*
3	Calculi-induced+ TKC (90mg/kg)	3.05±0.18**	68±2.0**	59.83±2.48**	1.136
4	Cystone (750 mg/ kg)	6.24±0.32**	87.16±3.31**	72.66±2.44**	1.251

Values are mean ±SEM, n=6, **P<0.001, *P<0.05

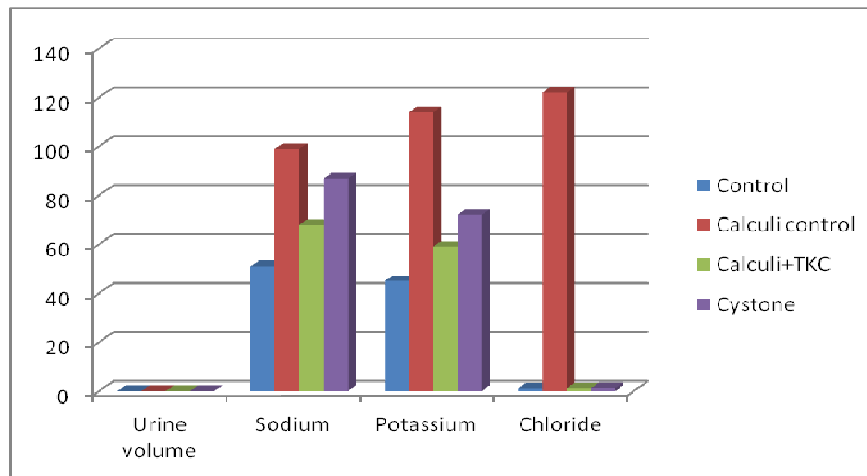


Table 2: Effect of Triphala karpa choornam on Urinary and Serum Parameters in Rats

Parameter	Group I Normal	Group II Calculi-induced Control	Group III Calculi -induced+ TKC (90mg/kg)	Group IV Cystone treated (750mg/kg)
Urine (mg/dl)	0.37 ± 0.03	3.64 ± 0.11	1.60 ± 0.03*	0.53 ± 0.04**
Calcium oxalate	1.27 ± 0.07	4.51 ± 0.10	2.21 ± 0.05*	1.50 ± 0.06**
Phosphate	3.67 ± 0.04	7.29 ± 0.06	4.13 ± 0.05*	3.81 ± 0.09**
Serum (mg/dl)	37.61 ± 0.15	49.97 ± 0.48	32.14 ± 0.17*	39.30 ± 0.48**
Creatinine	0.75 ± 0.01	0.94 ± 0.03	3.12 ± 0.05*	0.81 ± 0.02**
Uric acid	1.49 ± 0.07	3.64 ± 0.11	1.95 ± 0.03*	1.71 ± 0.04**

Values are mean ±SEM, n=6, **P<0.001, *P<0.05,

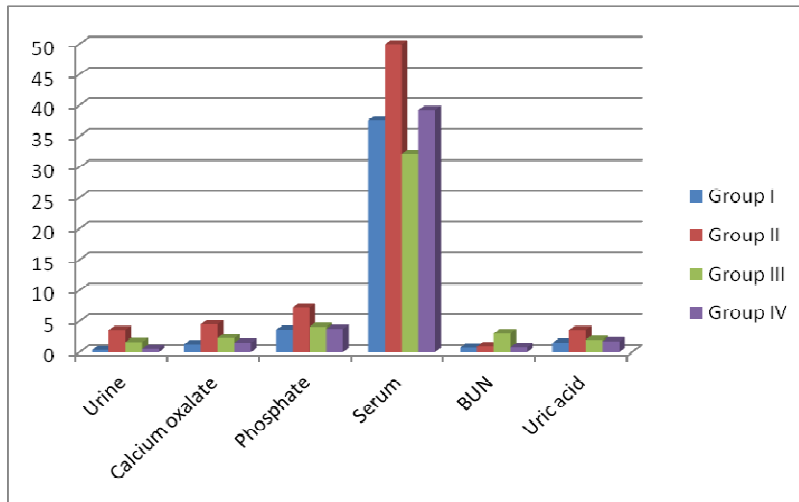
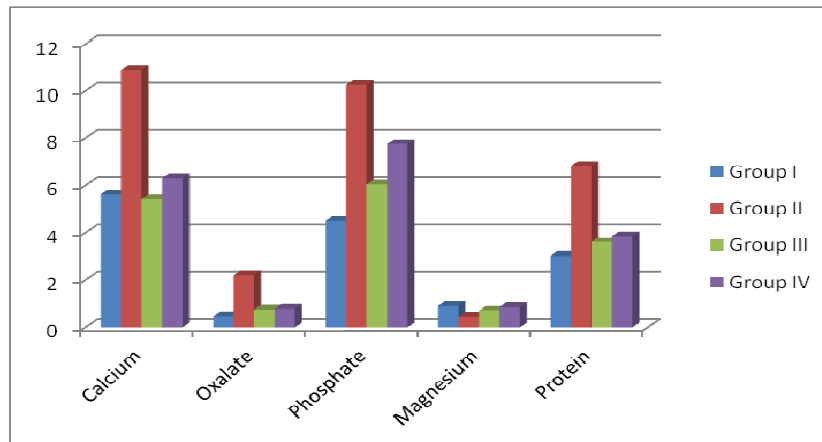


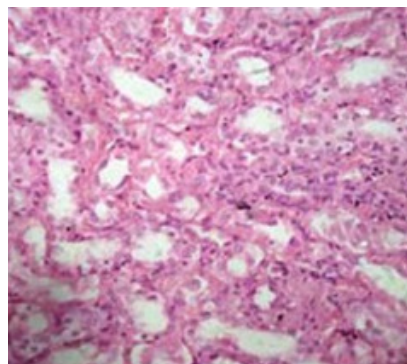
Table 3: Effect of Triphala karpa choornam on urinary Biochemical Parameters

Urinary excretion level (mg/24h)	Group I Normal control	Group II Lithiatic control	Group III Cystone treated (750mg/kg)	Group IV TKC (90mg/kg)
Calcium	5.6±0.35	10.92±0.25	5.4±0.20*	6.31±0.11**
Oxalate	0.45±0.05	2.22±0.30	0.75±0.03*	0.78±0.09**
Phosphate	4.5±0.10	10.25±0.29	6.05±0.18*	7.74±0.12**
Magnesium	0.90±0.01	0.44±0.02	0.7±0.02*	0.85±0.04**
Protein	3.02±0.11	6.84±0.15	3.60±0.21*	3.85±0.04**

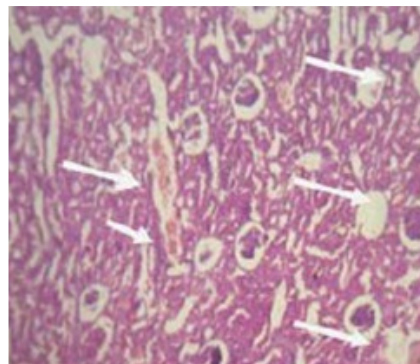
Values are mean±SEM, n=6, **P<0.001, *P<0.05



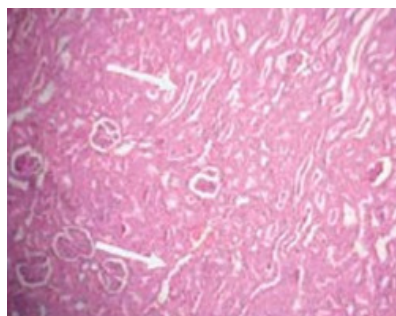
T. S Kidney showing the histopathology of animals in Calcium oxalate induced urolithiasis



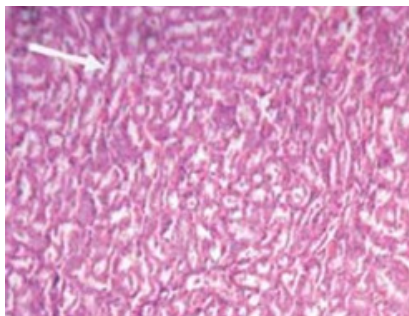
Control



Lithiatic control



Triphala Karpa Chooranam treated



Cystone treated

DISCUSSION

In the present study, rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 days period of ethylene glycol (0.75%, V/V) administration, young albino rats form renal calculi composed mainly of calcium oxalate¹¹. The biochemical mechanisms for this process are 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. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate¹². In the present study, oxalate and calcium excretion and progressively increased in calculi-induced animals. Since it is accepted that hyperoxaluria is a 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 increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth¹³. However, the formation of urinary tract stones is world wide, sparing no geographical, cultural or racial groups. Those composed of calcium oxalate, either alone or mixed with calcium phosphate, are forming the most common urolithiasis accounting for more than 80% of the stones. The mechanisms involved in the formation of calcific stones are not fully understood but it is

generally agreed that urinary lithiasis is a multifaceted process involving events leading to crystal nucleation, aggregation and growth of insoluble particles. Various therapies like diuretics are being used in attempt to prevent recurrence of hypercalciuria- and hyperoxaluria-induced calculi but scientific evidence for their efficacy is less convincing¹⁴. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population¹⁵. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the in vitro or in vivo models. In the present investigation, a polyherbal formulation constituted with *Terminalia chebula*, *Phyllanthus emblica*, *Terminalia bellerica*, Karungali sathu and Vengai sathu was evaluated for antiurolithiatic activity against commonly occurring calcium oxalate urolithiasis. Ethylene glycol disturbs oxalate metabolism by way of increase in substrate availability that increase the activity of oxalate synthesizing enzymes in the rats. In the present study, significantly increase activities of GAO in liver and LDH in liver and kidney of ethylene glycol induced urolithiatic rats that may be due to substrate mediated induction of the enzymes. A similar increase was also observed in glyoxylate, pyridoxine, deficient diet and glycolate administered rats. Administration of Triphala karpa chooranam brought about a significant reduction in GAO activity in liver and LDH activity in liver and kidney of urolithic rats. Increase activity of GAO and LDH confirmed their direct link to endogenous oxalate deposition in ethylene glycol

induced urolithiasis. Administration of Triphala karpa chooranam reduced the oxalate level in liver and kidney of urolithic rats.

CONCLUSION

Together these data suggest that the presence of antiurolithic effect in Triphala Karpa chooranam against renal calcium oxalate crystal deposits is mediated possibly through a combination of CaOx crystal inhibitory, diuretic, antioxidant, epithelial cell protective, hypocalciuric and hypercitrauric effects thus acting on multiple sites. This study rationalizes its medicinal use in the treatment of urolithiasis.

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