

ANTIUROLITHIC ACTIVITY OF AQUEOUS EXTRACT OF ROOTS OF *CISSAMPELOS PAREIRA* IN ALBINO RATS

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ABSTRACT

Objective: To evaluate the antiurolithic activity of aqueous extract of roots of *Cissampelos pareira* (AQERCP) in 2% Ammonium chloride (AC) and 0.75% Ethylene glycol (EG) induced urolithiasis in albino rats.

Methods : Urolithiasis was induced in rats by supplying drinking water mixed with 2 % (AC) and 0.75 % (EG) for 10 days. Calculi were confirmed by the high urinary levels of calcium, uric acid and low levels of magnesium and high levels of serum creatinine and calcium. The animals were treated with 03 doses of AQERCP i.e., 100 mg/kg, 200 mg/kg, 400 mg/kg respectively orally in different groups of rats once daily for 10 days along with 2 % (AC) and 0.75% (EG) mixed drinking water. On 11th day 3 rats from each group were kept in one metabolic cage and urine (pooled) collected for 24 h was subjected for estimation of various biochemical parameters. Blood was collected on the same day and analysed for various parameters. Kidneys were observed for the histopathological changes.

Results: Rats treated with 03 doses of AQERCP significantly ($P \leq 0.05$) reduced urinary calcium, uric acid and increased urinary magnesium levels, reduced serum calcium, creatinine and increased serum magnesium. Histopathology of kidneys in groups treated with AQERCP at 200 mg/kg and 400 mg/kg doses revealed less tissue damage and the cytology of nephrotic tissue was almost similar to the control Group I rats.

Conclusion: Results showed AQERCP has shown significant antiurolithic effect against chemical induced urolithiasis in rats.

Keywords: *C.pareira*, Roots extracts, Antiurolithic activity, Ammonium chloride, Ethylene glycol.

INTRODUCTION

Urolithiasis is defined as the formation of sediment anywhere within the urinary tract and consisting of one or more of the poorly soluble crystalloids of urine. It is the 3rd most common disorder of the urinary tract. Cases of urinary calculi are present worldwide but are particularly common in some geographic locales such as in parts of United States, South Africa, India and South East Asia. It is estimated that approximately 2% of the world population experiences renal stone disease at sometime in the lifespan with a male-female ratio of 2:1. The peak incidence is observed in 2nd to 3rd decade of life. Renal calculi are characterised clinically by colicky pain (renal colic) as they pass down along the ureter and manifest by hematuria. Major risk factors responsible for the nephrolithiasis are inadequate urinary drainage, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities i.e; deficiency of Vitamin-A, excess of vitamin D, metabolic diseases like hyperparathyroidism, cystinuria, gout, intestinal dysfunction [1] and environmental factors related to regions with hot and dry climatic conditions [2]. Despite various advantages and numerous methods available for the treatment of urolithiasis in the allopathic system of medicine, it suffers from few disadvantages that force the patients to other forms of medicine like Ayurveda, Homeopathy, Unani, Folklore medicine etc. A vast number of medicinal plants mentioned in ayurvedic system of medicine are known to possess antiurolithic properties some of the antiurolithic agents are derived from medicinal plants such as of *Didymocarpus pedicellata*, *Saxifraga ligulata*, *Rubia cordifolia*, *Cyperus scariosus*, *Achyranthes aspera*, *Cissampelos pareira*, *Onosma bracteatum*, *Lanata camara*, *Pinus eldarica*, *Pergularia daemia*, *Cynodon dactylon*,

Hordeum vulgare, *Veronica cinerea* and herbomineral preparations *Shilajeet* and *Hajrul yahood bhasmas* etc.

Plant Description

The *Cissampelos pareira* [3] an extensively spreading, glabrous to soft pubescent, perennial climbing shrub found all over India and is commonly known as Padha and other synonyms are Padvel, Padvali, Aaknadi, Venivel, Poda and Patha belongs to the family of Menispermaceae [3]. In Ayurvedic system of medicine, the leaves and roots are used in the treatment of indolent ulcers (Kirtikar and Basu,2001) and diarrhea (Amresh *et al*,2003). The plant is used in the treatment of urinary tract infections since it is considered as antiseptic (Dandiya and Chopra,1970). Juice of *C. pareira* is given in migraine and the plant has a long history of use for inflammation of muscles, snakebite, rheumatism, diarrhea, dysentery and menstrual problems. *C.pariera* is widely employed in herbal medicine today as a diuretic, tonic as well as to reduce fever and to relieve pain. It is often employed for menstrual cramps, dysmenorrhoea, excessive bleeding and uterine hemorrhages, fibroid tumors, pre and post natal pain, colic, constipation, poor digestion and dyspepsia. Hence midwives in Amazon always carry the *C.pareira* for the above mentioned ailments (Mukerji and Bhandari,1959).

Some scientific studies revealed its antinociceptive [5], antiarthritic [5], cardi tonic [9], anticancer [10], anti-inflammatory [7], anti diarrheal [8], anti-hemorrhagic, antifertility [6] antioxidant, neuroprotective [11], hepatoprotective [12], antioxidant [4], immunomodulatory [4], anti trypanosomal activities. The major constituents of roots of *C.pareira* include [13] Pelosin, O-methylcurine, l-curine Cissamine, Cissampareine, Hyatin, Bebeerine, Cycleanine, Tetrandine and Beriberine, Cissampeline, Cissampoline, Dicine, Insularine, Pareirine, Hyatinine, Pareirubrine A, Pareirubrine B, Pareitropone, Norimelutaine, Cissampeloflavone, D-

Quercitol and Grandirubrine [13]. The roots of *C.paireira* are traditionally used as a antiurolithic but scientifically not evaluated as a antiurolithic agent. The main aim of the present study was to evaluate antiurolithic activity of aqueous extract of roots of *C. pareira* in Ammonium chloride (2%) AC and (0.75%) Ethylene glycol induced urolithiasis in albino rats.

METHODOLOGY

Collection of Plant

The roots of *C.paireira* were collected from the forest of Tirupati, AP and were identified and authenticated by Dr. Pramod Kumar, Pharmacognocist V.L.College of Pharmacy, Raichur, Karnataka.

Preparation of extract: Roots were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation of aqueous extract of roots of *C.paireira* was done by using maceration. About 200 g of root powder was subjected to cold maceration with chloroform water in a conical flask for about 7 days at room temperature. The flask was securely plugged with absorbent cotton and shaken periodically. Then the material was filtered through a muslin cloth and mark was pressed. The filtered was refiltered through whatman filter paper to get the clear filtrate. The filtrate was concentrated to dry residue in a desiccator over anhydrous sodium sulphate. The resulting extract was weighed and filled into the sample containers. Phytochemical evaluation for the extract was performed using standard procedures [14].

Experimental Animals

Male Albino rats (54) weighing between 140-200 g used in the study (9 groups; n = 6) were obtained from the Central Animal House, V.L. College of Pharmacy, Raichur, Karnataka. The experimental protocol was approved by the Institutional Animal Ethical Committee. The animals were maintained under standard husbandry conditions temperature 22±2°C, humidity 45-55%, light: dark cycle (12:12h) for an acclimatization period of 15 days before performing the experiments. All rats were placed in metallic cages 3 in each.

Ethics

The experiments compiled with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethical Committee (IAEC), VLCP, Raichur.

Drugs and Chemicals Used

Cystone 5 ml/kg (Himalaya drug company, Bangalore, India), Ethylene glycol (S.D Fine chemicals, Hyderabad, Andhra Pradesh, India), Ammonium chloride (S.D Fine chemicals, Hyderabad, Andhra Pradesh, India), CMC (S.D Fine chemicals, Hyderabad, Andhra Pradesh, India).

Acute Toxicity Study

Determination of LD₅₀ The acute toxicity [15,16] of AQERCP was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 h study period (short term toxicity). Based on the short term toxicity profile, the next dose of the extract was determined as per OECD guidelines No.420 upto the maximum dose level of 2000 mg/kg. From the LD₅₀, dose of the individual extract, doses like 1/20th, 1/10th and 1/5th were selected and considered as low, medium and high dose i.e: 100 mg/kg, 200 mg/kg, 400 mg/kg respectively to carry out this study.

Experimental Design

The antiurolithic activity of AQERCP in albino rats was studied in Ammonium chloride (2% AC) and (0.75%) Ethylene glycol induced urolithiasis [17,18, 19]. Healthy male albino rats weighing between 140-200 g were randomly divided into 09 groups with each consisting of 6 animals and the treatment with AC, EG mixed water was continued for 10 days.

Group-1: Fed with standard rat chow diet and tap water only *ad libitum* for 10 days.

Group-2: Fed with normal rat diet + drinking water containing 0.75% EG v/v + 2% AC w/v for 10 days to induce urolithiasis.

Group-3: Fed with normal rat diet + drinking water containing 0.75% v/v EG + 2% w/v AC + Standard drug cystone (5ml/kg) for 10 days.

Group-4: Fed with normal rat diet + AQERCP lower dose (100 mg/kg) for 10 days.

Group-5: Fed with normal rat diet + AQERCP medium dose (200 mg/kg) for 10 days.

Group-6: Fed with normal rat diet + AQERCP high dose (400 mg/kg) for 10 days.

Group-7: Fed with normal rat diet + drinking water containing 0.75v/v EG + 2% w/v AC with AQERCP lower dose (100 mg/kg) for 10 days.

Group-8: Fed with normal rat diet + drinking water containing 0.75 v/v EG + 2% w/v AC with AQERCP medium dose (200 mg/kg) for 10 days.

Group-9: Fed with normal rat diet + drinking water containing 0.75v/v EG + 2% w/v AC with AQERCP high dose (400 mg/kg) for 10 days.

Collection and Analysis of Urine

On 11th day 3 rats from each group was kept in single metabolic cage and urine (pooled) collected for 24 h. HCL was added to the urine before being stored at 4° C. Urine was measured for volume and analysed for biochemical parameters i.e; calcium, magnesium and uric acid.

Serum Analysis

Blood was also collected on 11th day by retro orbital puncture under ether anaesthesia and the animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min and analysed for calcium, magnesium and creatinin

Histopathological Studies

Kidneys collected from rats were weighed individually and fixed rapidly with 10% formalin. This sections of kidneys fixed in paraffin were prepared and stained with eosin and hematoxylin and observed for histopathological changes.

Statistical Analysis

Experimental results were expressed as mean + SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnetts 't' test.

RESULTS

The AQERCP was subjected to qualitative phytochemical tests to identify the phytoconstituents and the tests revealed the presence of carbohydrates, alkaloids, sterols, phenolic compounds, tannins, flavonoids and resins.

In acute toxicity study all the animals were survived even after 14 days indicates the non toxicity of the extract even up to the maximum permitted dose level of 2000 mg/kg. No major behavioural changes were observed during this period of study.

The results obtained with antiurolithic activity studies with AQERCP was shown in Table No.1 and Figure No.1. From the results when compared to normal control it can be observed that AQERCP has shown a significant antiurolithic activity by increasing urinary output, magnesium and decreasing calcium, uric acid and decreasing serum creatinine, calcium and increasing magnesium levels. The antiurolithic effect observed after treatment with AQERCP was found to be significant and comparable to standard cystone in terms of increase in urinary output and reduction in the tendency for crystallization.

Biochemical parameters noted with groups of rats treated only with extract (AQERCP) at different dose levels are compared with normal control & toxicant control. No significant difference in biochemical parameters observed confirming the non toxicity nature of this extract on the biological system of rats.

The rats treated with AQERCP at doses 100 mg/kg, 200 mg/kg and 400 mg/kg significantly (P≤ 0.05) reduced serum calcium and creatinine but increased magnesium. Further urinary calcium, uric acid levels were significantly decreased but urinary magnesium increased.

Table 1: Effect of AQERCP on Urine Volume, Urine Calcium, Urine Uric acid, Urine Magnesium, Serum Calcium, Serum Creatinine, Serum Magnesium against Ammonium chloride (AC 2%) and Ethylene glycol (EG 0.75%) induced urolithiasis

S.No	Group	Total Urine Volume	Serum Creatinine	Serum Calcium	Serum Magnesium	Urine Uric Acid	Urine Magnesium	Urine Calcium
1	Normal Control	16.30 ± 0.03	0.65±0.02	9.82±0.03	2.23±0.03	9.23±0.03	5.38±0.03	7.66±0.04
2	Toxicant Control	7.55 ± 0.03	1.85±0.01	17.14±0.04	0.98±0.04	17.84±0.02	1.51±0.02	16.60±0.03
3	Standard	15.20±0.04***	0.77±0.03**	10.04±0.04***	2.19±0.02*	11.24±0.03***	4.15±0.02*	9.90±0.04***
4	AQERCP 100mg/Kg (Perse effect)	18.02±0.03***	0.76±0.01**	9.83±0.02**	2.20±0.02*	9.42±0.02**	5.44±0.01*	7.88±0.01***
5	AQERCP 200mg/Kg (Perse effect)	20.68±0.03***	0.81±0.02**	9.81±0.02**	2.17±0.01*	9.77±0.02**	5.57±0.02*	8.24±0.01***
6	AQERCP 400mg/Kg (Perse effect)	24.26±0.02***	0.85±0.001	9.80±0.02**	2.14±0.01*	10.00±0.02	5.70±0.02*	8.61±0.02***
7	AQERCP 100 mg/Kg + Toxicant	7.87 ± 0.03***	1.72±0.01 ^{ns}	15.42±0.02	1.20±0.02*	16.49±0.05	1.87±0.02*	16.22±0.03***
8	AQERCP 200 mg/Kg + Toxicant	10.70±0.05***	1.54±0.11**	13.58±0.03	1.49±0.04*	14.24±0.04	2.66±0.03*	13.06±0.05***
9	AQERCP 400 mg/Kg + Toxicant	13.97±0.06***	1.13±0.09**	11.86±0.04	2.19±0.02*	12.04±0.03	3.97±0.04*	11.66±0.04***

Values expressed as mean ± SEM, n=6, Significance at p<0.001***, ns = not-significant, AQERCP- Aqueous extract of roots of *C.parieta*

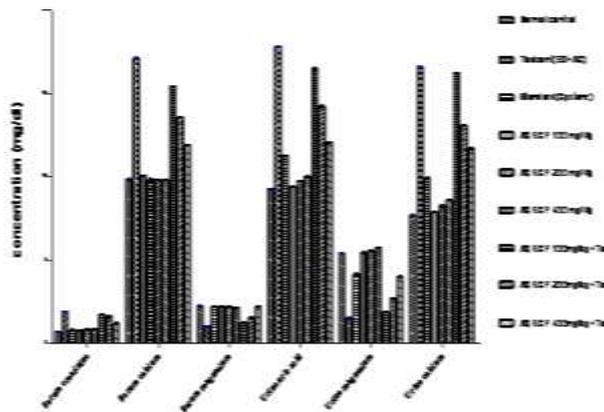


Fig.1 Effect of CYSTONE, AQERCP and AQERCP+Toxicant on Urinary Calcium, Uric acid, Magnesium, Serum Calcium, Creatinine, Magnesium (mg/dl) against Ammonium chloride (AC 2%) and Ethylene glycol (EG 0.75%) induced urolithiasis

In control group (Group-I) histopathology of kidneys revealed no calcium oxalate deposits or other abnormalities in the nephron segment. In urolithic rats (Group-II) several calcium oxalate crystal deposits inside the tubules and dilation of the proximal tubules along with interstitial inflammations and degeneration of epithelial cells were observed in the renal tissue. The groups treated with AQERCP (groups VII -IX) and cystone treated rats (group III) the number of calcium oxalate deposits in the tubules were less than group II. In groups treated with AQERCP at 200 mg/kg and 400 mg/kg dose levels revealed less tissue damage and the cytology of the nephrotic tissue was almost similar to Group I (normal) control rats.

DISCUSSION

A number of pathological diseases of kidney including calcium oxalate kidney stones, have resulted due to the oxalate-induced damage to the renal cells [20,21]. Increased levels of oxalate are responsible for the toxic effects on the renal epithelial cells via modification in membrane integrity, production of reactive oxygen

species and depleted resource of antioxidant enzymes [22,23]. In the present study, male rats were selected to induce urolithiasis because their urinary system resembles that of humans [24].

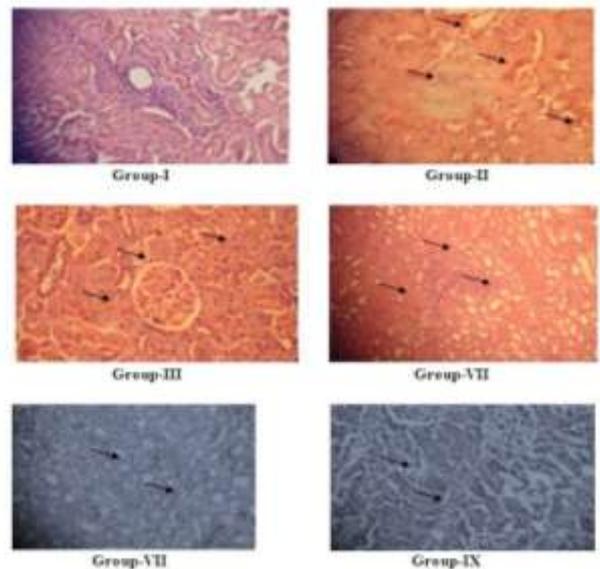


Fig No: 2 Histopathology of Kidneys with AC (2%) and EG(0.75%) induced urolithiasis in albino rats :

Ethylene glycol increases the risk of urolithiasis by increasing urinary levels of stone constituents (calcium, oxalate, phosphate and uric acid) and facilitate an optimal environment like low citrate level for stone growth. Ethylene glycol increases oxalate production by increasing substrate availability which induces the activity of oxalate synthesizing liver enzyme, glycolate oxidase.

In view of its traditional use in renal calculi, *C.parieta* root extracts were studied to explore its potential as antiurolithic agent in (AC 2%) Ammonium chloride and (0.75%) Ethylene glycol induced urolithiasis. This is the first kind of the scientific work for the first

time studied to show the antiurolithic effect of AQERCP in urolithiasis model.

From the results it was observed that AQERCP exhibited curative effect in urolithiasis induced rats by preventing the formation, reducing number and disruption of calcium oxalate calculi formed in the kidneys. The base for calcium stone development is super saturation of urine with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities can change the composition or saturation of the urine that enhance stone-forming tendency. Among the metabolic conditions are hypercalciuria, hypocitraturia and hyperoxaluria.

Renal calcium oxalate deposition induced by ammonium chloride and ethylene glycol in rats is commonly used as a model to mimic the urinary stone development in humans (Thamilselvan et al., 1997; Atmani et al., 2003; Tsai et al., 2008). Hence this model was used to evaluate the potential antiurolithic effect of AQERCP on calcium oxalate urolithiasis.

In the present study AQERCP treated animals showed increased in urine output which dilutes the urinary electrolytes concentration. As a result, calcium and uric acid are flushed out via the urine leaving a lesser chance of precipitation with a decreased formation as well as the growth of urinary stone. The excretion of calcium and uric acid were gradually enhanced in stone induced animals which is in accordance with the earlier reports [25]. Most stones in the urinary system come up from a common component of urine such as calcium oxalate (Ca Ox) and hypercalciuria, representing up to 80% of analyzed stones [26]. Increased urinary calcium favours the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth [27]. However, AQERCP lowered the levels of calcium as well as uric acid, which is beneficial in preventing calculi formation.

Calcium oxalate crystal growth is promoted by uric acid either by direct induction of calcium oxalate precipitation by colloidal uric acid [28] or by acting as promoter by binding to glycosaminoglycans and thereby reducing their inhibitory activity against calcium oxalate crystallization.

Magnesium powerfully inhibits the crystallization of Calcium Oxalate *in vitro*, magnesium binds to oxalate to form a soluble complex, consequently reducing the concentration available for Ca Ox precipitation [29]. Low urinary magnesium content is a common feature in stone formers [30]. Magnesium deficiency accelerates the deposition of renal tubular calcium oxalate in rats. Experiments in animal models have shown increased levels of magnesium offers protection against Ca Ox deposition in kidneys but clinical studies have not shown any such beneficial effects in impeding the formation of Ca Ox kidney stones. Treatment with AQERCP significantly increased the levels of magnesium in urine and serum but significantly reduced in ethylene glycol and ammonium chloride treated (group-II) animals.

Animal experiments have revealed that exposure to high levels of oxalate and Ca Ox crystals produce cellular injury mediated by membrane lipid peroxidation through intracellular oxygen free radical generation. It has been confirmed that epithelial cell injury facilitates the events of Ca Ox crystal nucleation, aggregation by lowering concentration at which crystal forms and promotes crystal retention in renal tubules for subsequent stone development (Khan and Hackett, 1991; Khan 1995; Moro et al., 2005). Recently collected human data are also suggestive of the development of oxidative stress responsible for kidney stone formation (Huang et al., 2003).

In the present work AQERCP was studied for its antiurolithic activity. The phytochemical studies reveals that the roots of *C.paireira* contains flavonoids, alkaloids, carbohydrates, sterols, phenolic compounds, tannins, resins. From the earlier studies it has been reported that flavanoids [31,32,33,34] alkaloids [34], saponins have antiurolithic activity. Earlier studies reported phytochemical substances like flavonoids, saponins, organic acids, steroids, carbohydrates, tannins, phenolic compounds, terpenoids, alkaloids, glycosides, sterols, sesquiterpenes & aminoacids, carotenoids in different plant extracts. AQERCP was identified with most of these

phytochemical substances mentioned above. Hence it can be reported that the observed antiurolithic activity is due to these above phytoconstituents.

CONCLUSION

Results showed that AQERCP have exhibited a significant protective (antiurolithic) effect against urolithiasis producing agents. Phytoconstituent like berberine is already reported for its antiurolithic activity. Berberine is an important bioactive constituent present in *C.paireira*. So here benzyl isoquinoline alkaloid berberine is responsible for antiurolithic activity because it was therapeutically effective for both prevention as well as curative aspect of calcium oxalate urolithiasis, exhibiting these effects through a combination of antioxidant, diuretic, hypocalciuric, hypermagnesiemia and urine alkalinizing activities. Thus the present study supports and justify the basis for folklore use of roots of *C.paireira* for antiurolithic activity.

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