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Research Article

ANTILITHIATIC EFFECT OF *CISSAMPELOS PAREIRA* LEAVES IN AMMONIUM CHLORIDE AND ETHYLENE GLYCOL INDUCED UROLITHISIS IN RATS

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ABSTRACT

Objective: To study the antilithiatic activity of ethanolic extract of leaves of Cissampelos pareira (EELCP) in 2% ammonium chloride (AC) and 0.75% ethylene glycol (EG) induced urolithiasis in albino rats.

Methods: Kidney stones were induced in rats by feeding drinking water mixed with 2% (AC) and 0.75% (EG) for 10 days. Stones were confirmed by the increased urinary levels of calcium, uric acid and decreased levels of magnesium and increased levels of serum creatinine and calcium. The rats were treated with 03 doses of EELCP i.e. 100 mg/kg, 200 mg/kg, 400 mg/kg respectively, orally in different groups of albino rats once daily for 10 days along with 2% (AC) and 0.75% (EG) containing drinking water. On 11th day, 3 rats from each group were kept in one metabolic cage and urine (pooled) collected for 24 hrs was subjected for assessment of various biochemical parameters. Blood was collected on the same day and analyzed for various parameters. Kidneys were observed for the histopathological changes.

Results: Rats treated with 03 doses of EELCP significantly ($p \leq 0.05$) decreases the urinary calcium, uric acid and enhanced urinary magnesium levels, decreased serum calcium, creatinine and enhanced serum magnesium. Histopathology of kidneys in groups treated with EELCP 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 EELCP has shown significant antilithiatic effect against chemical induced urolithiasis in rats.

Keywords: *Cissampelos pareira*, Leaf extract, Antilithiatic activity, Urolithiasis, Ethylene glycol.

INTRODUCTION

Urolithiasis is defined as the formation of urinary calculi or the condition associated with urinary calculi. Urolithiasis is the third 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, India, South Africa, and South East Asia. It is estimated that around 2% of the world population experiences renal calculi at sometime in the lifespan with a male-female ratio of 2:1. The peak occurrence is observed in 2nd-3rd decade of life. Kidney stones were characterized clinically by colicky pain as they pass down along the ureter, and noticeable by hematuria. Risk factors accountable for the nephrolithiasis are insufficient urinary drainage, microbial infections, food 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]. In spite of various advantages and several methods available for the treatment of urolithiasis in the allopathic system of medicine, it suffers from little disadvantages that compel the patients to other forms of medicine like Ayurveda, Homeopathy, Unani, Folklore medicine etc. 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 *Cissampelos pareira, Onosma bracteatum, Lanata camara, Pinus eldarica, Pergularia daemia, Cynodon dactylon, Hordeum vulgare, Didymocarpus pedicellata, Saxifraga ligulata, Rubia cordifolia, Cyperus scariosus, Achyranthes aspera, Vernonia cinerea* and herbomineral preparations Shilajeet and Hajrul yahood bhasmas etc.

Plant description

The *C. 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, Poda, Aaknadi, Venievel, 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. pareira* 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 [4], antiarthritic [4], cardiotonic [5], anticancer [6], anti-inflammatory [7], antidiarrheal [8], anti-hemorrhagic, antifertility [9], antioxidant, neuroprotective [10], hepatoprotective [11], antioxidant [12], immunomodulatory [12], antiurolithic, [13] anti trypanosomal activities. The major constituents of roots of *C. pareira* include [14] pelosin, O-methylcurine, l-curine cissamine, cissampareine, Hyatin, bebeerine, cycleanine, tetrandine and beriberine, cissampeline, cissampoline, dicentrine, insularine, pareirine, hyatinine, pareirubrine A, pareirubrine B, pareitropone, norimeluteine, cissampeloflavone, D-quercitol and grandirubrine [15]. The leaves of *C. pareira* are traditionally used as a antilithiatic but scientifically not evaluated as a antiurolithic agent. The main aim of the present study was to evaluate antilithiatic activity of leaves of *C. pareira* in ammonium chloride (AC) (2%) and (0.75%) ethylene glycol (EG) induced urolithiasis in albino rats.

METHODS

Collection of plant

The leaves of *C. pareira* were collected from the forest of Tirupati, Andhra Pradesh, and were identified and authenticated by Dr. Pramod Kumar, Pharmacognocist V. L. College of Pharmacy, Raichur, Karnataka.

Preparation of extract

Leaves were thoroughly washed under fresh tap water, and shade dried and powdered using a mechanical grinder. The ethanolic extract of leaves of *C. pareira* (EELCP) was prepared by soxhletation. About 200 g of leaves powder was taken into the soxhlet apparatus, and extracted using (95%) ethanol. The extraction process was carried out for 18-20 hrs till the appearance of the colorless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at <50°C and percentage yield of EELCP was recorded with respect to the total quantity of powder used for the extraction. Then the extract was evaluated for its phytochemicals by following standard procedures [15].

Experimental animals

Male Albino rats (36) weighing between 140 and 200 g used in the study (6 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 (IAEC). The animals were maintained under standard husbandry conditions temperature 22±2°C, humidity 45-55%, light:Dark cycle (12:12 hrs) for an acclimatization period of 15 days before performing the experiments. All rats were placed in metallic cages three in each.

Ethics

The experiments compiled with the guidelines for animal experimentation of our laboratory, and were approved by the IAEC, VLCP, Raichur.

Drugs and chemicals used

Cystone 5 ml/kg (Himalaya Drug Company, Bangalore, India.), EG (S. D Fine Chemicals, Hyderabad, Andhra Pradesh, India), AC (S. D Fine Chemicals, Hyderabad, Andhra Pradesh, India), CMC (S. D Fine Chemicals, Hyderabad, Andhra Pradesh, India).

Acute toxicity study

Determination of LD_{50}: The acute toxicity [16,17] of EELCP was determined using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 hrs prior to the experiment, and the extract was administered as a single dose and observed for the mortality up to 48 hrs 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 up-to the maximum dose level of 2000 mg/kg. From the LD_{50}, dose of the individual extract, doses like $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ 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 antilithiatic activity of EELCP in albino rats was studied in AC (2% AC) and (0.75%) EG induced urolithiasis [18-20]. Healthy male albino rats weighing between 140 and 200 g were randomly divided into 06 groups with each consisting of six animals and the treatment with AC, EG mixed water was continued for 10 days.

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

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

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

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

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

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

Collection and analysis of urine

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

Serum analysis

Blood was also collected on 11^{th} day by retro orbital puncture under ether anesthesia, and the animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 minutes and analyzed for calcium, magnesium and creatinine.

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±standard error of the mean (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnetts *t*-test by using Graph Pad Prism software version 5.00.

RESULTS

The EELCP 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 behavioral changes were observed during this period of study.

The results obtained with antilithiatic activity studies with EELCP was shown in Table 1. From the results when compared to normal control it can be observed that EELCP has shown a considerable antilithiatic activity by enhancing urinary output, magnesium and decreasing calcium, uric acid and decreasing serum creatinine, calcium and increasing magnesium levels. The antilithiatic effect observed after treatment with EELCP was found to be significant and comparable to standard drug cystone in terms of increase in urinary output and reduction in the tendency for crystallization.

The rats treated with EELCP at doses 100 mg/kg, 200 mg/kg and 400 mg/kg significantly ($p \leq 0.05$) reduced serum calcium and creatinine but increased magnesium. Further urinary calcium, uric acid levels were significantly decreased but urinary magnesium increased (Fig. 1).

In Group-I histopathology of kidneys revealed no calcium oxalate (Ca Ox) deposits or other abnormalities in the nephron segment. In lithiatic rats (Group-II) several Ca Ox 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 EELCP (Groups IV-VI) and cystone treated rats (Group III) the number of Ca Ox deposits in the tubules were less than Group II. In groups treated with EELCP 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 (Fig. 1).

DISCUSSION

Pathological diseases of kidney including Ca Ox renal stones, have resulted due to the oxalate-induced damage to the renal cells [21,22].

Table 1: Effect of EELCP on urine volume, urinary calcium, uric acid, magnesium, and serum calcium, creatinine, magnesium in AC (2%) and EG (0.75%) induced urolithiasis

S. no.	Group	Total urine volume	Serum creatinine	Serum calcium	Serum magensium	Urine uric acid	Urine magnesium	Urine calcium
1	Normal control	14.30±0.02	0.47±0.01	7.80±0.01	1.95±0.01	7.24±0.02	4.31±0.04	5.61±0.03
2	Toxicant	5.51±0.03	1.52±0.02	5.10±0.02	0.73±0.01	15.69±0.01	1.34±0.03	14.59±0.03
3	Standard	13.25±0.03***	0.59±0.01***	8.04±0.03***	1.86±0.02***	9.27±0.03***	3.59±0.04***	7.80±0.03***
4	EELCP 100 mg/kg+EG plus AC	6.80±0.02***	1.43±0.06***	13.47±0.04***	1.12±0.02***	14.49±0.02***	1.36±0.03***	14.21±0.03***
5	EELCP 200 mg/kg+EG plus AC	8.76±0.03***	1.34±0.01***	11.54±0.02***	1.20±0.02***	12.43±0.03***	1.59±0.03***	11.07±0.03***
6	EELCP 400 mg/kg+EG plus AC	11.84±0.02***	1.04±0.01***	9.76±0.04***	1.43±0.01***	10.10±0.03***	2.04±0.04***	9.81±0.05***

Values expressed as mean±SEM, n=6, Significance at p<0.05*, p<0.01**, and p<0.001***. EELCP: Ethanolic extract of leaves of *Cissampelos pareira*, AC: Ammonium chloride, EG: Ethylene glycol, SEM: Standard error of the mean

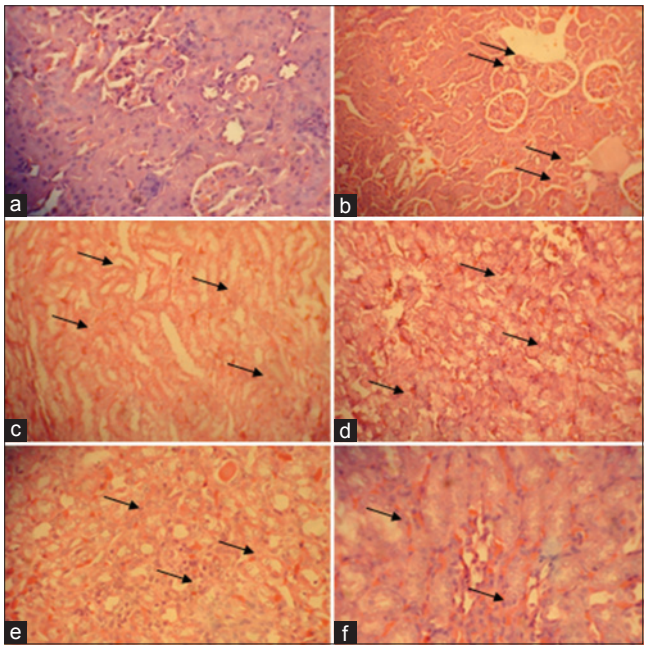

Fig. 1: (a-f) Histopathology of kidneys with ammonium chloride (2%) and ethylene glycol (0.75%) induced urolithiasis in albino rats

Enhanced levels of oxalate are accountable for the toxic effects on the renal epithelial cells via alteration in membrane integrity, production of reactive oxygen species and minimal resource of antioxidant enzymes [23,24]. In the present study, male rats were selected to induce urolithiasis because their urinary system resembles that of humans [25].

In view of its traditional use in renal stones, *C. pareira* leaves extract was studied to screen its potential as antiurolithic agent in (AC 2%) AC and (0.75%) EG induced urolithiasis. This is the first kind of the scientific work for the first time studied to show the antiurolithiatic effect of EELCP in urolithiasis model.

From the results, it was noted that EELCP shown curative effect in urolithiasis induced rats by preventing the formation, decreasing number and disruption of Ca Ox stone formed in the kidneys. The basis for calcium stone formation is super saturation of urine with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities can alter the saturation of the urine that increases stone-forming property. Among the metabolic conditions are hypercalciuria, hyperoxaluria and hypocitraturia.

Renal Ca Ox deposition induced by AC and EG 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). Thus, this model was used to screen the possible antilithiatic effect of EELCP on Ca Ox urolithiasis.

In the present study, EELCP treated rats exhibited enhanced urinary output, which dilutes the urinary electrolytes concentration. As a result, calcium and uric acid are flush out via the urine leaving a lesser possibility of precipitation with a reduced formation as well as the growth of urinary calculi. The elimination of calcium and uric acid were gradually increased in stone induced rats that are in accordance with the previous reports [26]. Most calculi in the urinary system come up from a common component of urine such as Ca Ox and hypercalciuria, indicating up to 80% of analyzed stones [27]. Enhanced urinary calcium facilitates the nucleation and precipitation of Ca Ox from urine and subsequent crystal growth [28]. However, EELCP reduced the levels of calcium as well as uric acid, which is helpful in preventing calculi formation.

Calcium oxalate crystal development is facilitated by uric acid either by direct induction of Ca Ox precipitation by colloidal uric acid [29] or by acting as promoter by binding to glycosaminoglycans, and thereby decreasing their inhibitory activity against Ca Ox crystallization.

Magnesium strongly inhibits the crystallization of Ca Ox *in vitro*, magnesium attaches to oxalate to form a soluble complex, consequently decreasing the concentration available for Ca Ox precipitation [30]. Low urinary magnesium content is a common feature in stone formers [31]. 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 EELCP significantly enhanced the levels of magnesium in urine and serum but significantly decreased in EG and AC treated (Group-II) animals.

In the present work, EELCP was studied for its antilithiatic activity. The phytochemical studies reveal that the leaves of *C. pareira* contains flavonoids, alkaloids, carbohydrates, sterols, phenolic compounds. From the previous studies, it has been reported that flavonoids [13,32-35] alkaloids [13,35], saponins have antilithiatic activity. Previous studies reported phytochemical substances like flavonoids, saponins, organic acids, steroids, carbohydrates, phenolic compounds, terpenoids, alkaloids, glycosides, sterols, sesquiterpenes and aminoacids, carotinoids in different plant extracts. EERCP was identified with most of these phytochemical substances mentioned above. Hence, it can be reported that the observed antilithiatic activity is due to these above phytoconstituents.

CONCLUSION

Results exhibited that EELCP have shown a considerable protective effect (antilithiatic) against renal stone producing agents. Phytoconstituent like berberine is already reported for its antilithiatic activity. Berberine is an important bioactive constituent present in *C. pareira*. So here benzyl isoquinoline alkaloid berberine is responsible for antilithiatic activity because it was therapeutically effective for both prevention as well as curative aspect of Ca Ox urolithiasis, exhibiting these effects through a combination of antioxidant, diuretic, hypocalciuric, hypermagnesiemia and urine alkalanizing activities. Thus, the present study supports and rationalizes the basis for traditional use of leaves of *C. pareira* for antilithiatic activity.

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