Journal of Applied Pharmaceutical Science Vol. 8 (01), pp 129-135, January, 2018
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2018.8119
ISSN 2231-3354

Evaluation of antiurolithiatic effects of *Parmelia perlata* against calcium oxalate calculi in hyperoxaluric rats

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ARTICLE INFO

Article history:
Received on: 12/09/2017
Accepted on: 17/11/2017
Available online: 28/01/2018

Key words:
Parmelia perlata, Urolithiasis, Hyperoxaluria, Ethylene glycol, Oxalate calculi

ABSTRACT

This manuscript was aimed to evaluate the antiurolithiatic potential of *Parmelia perlata* extract (PPE) against calcium oxalate calculi in experimental rats. The drinking water containing 0.75% v/v Ethylene Glycol (EG) and 1% w/v Ammonium Chloride (AC) was used to induce hyperoxaluria in Wistar rats. Thirty-six rats divided into six groups (each containing six animals) were treated with vehicle (Normal control), EG + AC (Urolithiatic control), Cystone (Standard), and 100, 300 and 500 mg/kg, PPE (Tests). Administration of EG + AC produced significant hyperoxaluria and altered biochemical parameters of urine, serum and kidney tissue homogenates in lithiatic group. It caused glomerular atrophy, tubular deposition of oxalate crystals, altered renal architecture and impaired renal functions. PPE (100, 300 and 500 mg/kg, p.o., once daily for four weeks) significantly ($p < 0.05$) reversed the biochemical changes in urine, serum, and kidney tissue homogenates. It restored the normal renal functions and biochemical parameters like urinary pH, volume, creatinine clearance, BUN levels, uric acid concentrations and some inorganic parameters like oxalate, calcium, phosphorus contents, etc. The histopathological studies revealed that PPE restored the normal renal architecture in lithiatic rats. Conclusively, the experimental findings showed that PPE exhibited significant antiurolithiatic potential against calcium oxalate calculi in experimental rats.

INTRODUCTION

Urolithiasis or nephrolithiasis, presence of calculi in kidney or any part of urinary tract, is the third most prevalent renal disease that afflicting humankind since antiquity. It jeopardised the public health worldwide, sparing no geographical area. More than 80% of renal stones are composed of calcium oxalate (CaOx) and occurs in two forms i.e. mono and di-hydrates of CaOx (Aggarwal *et al*., 2013; Goyal *et al*., 2017; Khan, 1997). Urinary stone formation occurs as a result of cascade of physiochemical events started with supersaturation of urine and followed by nucleation, growth, aggregation and retention of crystals (Li *et al*., 2017; Moe, 2006). Despite of recognition of urolithiasis since stone-age and tremendous advances in the field of urology, in modern system of medicines, except few alkalizers and diuretics, there is no clinically satisfactory medicine that can either dissolve and/or prevent the formation or recurrence of urinary stones (Goyal *et al*., 2017). It is usually treated by some surgical and interventional techniques like Percutaneous Nephrolithotomy, Ureteroscopy, Extracorporeal Shock Wave Lithotripsy, etc. Such techniques cannot hamper, even facilitate, the chances of stone recurrence and have some serious side effects like haemorrhage, renal fibrosis, infections, etc. (Li *et al*., 2017). Therefore, after adopting these treatment procedures, the patients have to be subjected to careful follow up for long time, and moreover these procedures are prohibitively costly for a common man. So there is a constant need to identify some more clinically useful antiurolithiatic therapies that alter the rate of recurrences, have minimum or no side effects, and affordable for a common man.

In alternative systems of medicines, many herbal remedies are available for urolithiasis which are quite efficacious and have comparatively lesser side effects. There are many herbal drugs which are traditionally prescribed for renal calculi, and even

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used as the ingredient in some composite herbal formulations prescribed for urolithiasis patients, but, rationale behind their use has not been scientifically substantiated using modern methodology.

One such herbal lichen *Parmelia perlata* was identified and focussed in this manuscript. *P. perlata* (Huds.) Ach., belonging to family Parmeliaceae, is commonly known as Chharila, Some other common names like Stone flower, Pattharphool, etc., given to this are probably because of its traditional therapeutic uses in urinary calculi (Goyal *et al*., 2016). It contains several phytoconstituents including lichen acids like lecanoric acid, atranorin, usnic acid (Khare, 2007; The Ayurvedic Pharmacopoeia of India, 2001), dibenzofuran (Sharma *et al*., 2014), terpenes (Abdullah *et al*., 2007), phenolic compounds, etc. (Sharma *et al*., 2012).

P. perlata has traditionally been recommended in sevaral renal diseases like urinary obstruction, calculi, dysuria, burning sensation, etc, and seminal weakness, spermatorrhoea, nocturnal emission, inflammations, general pains, etc. (Khare, 2007; Nadkarni, 2002; The Ayurvedic Pharmacopoeia of India, 2001). It is also used as the ingredient of some herbal composite formulations like Calcury (Charak Pharma Pvt. Ltd.), Pathrina (Shri Baidyanath Ayurved Bhawan Pvt. Ltd.), and Neeri (Aimil Pharmaceuticals, India Ltd.) prescribed for urolithiasis and improving renal functions (Goyal *et al*., 2016). It has been scientifically substantiated for antiulcer (Lakshmi *et al*., 2013), hepatoprotective (Shailajan *et al*., 2014), antidiabetic (Jothi and Brindha, 2013), antioxidant, hypolipidemic (Rahman *et al*., 2014), antimicrobial (Thippeswamy *et al*., 2013; Vidyalakshmi and Kruthika, 2012) effects, etc. There is no scientific report for its antiurolithiatic effects. The present manuscript is focussed to evaluate the hydroethanolic extract of *P. paelata* for antiurolithiatic potential against CaOx calculi in experimental rats.

MATERIALS AND METHODS

Plant material

The dried whole plant of *Parmelia perlata* (Huds.) Ach., (Family: Parmeliaceae), authenticated by the botanist Dr. H. B. Singh (Former Chief Scientist, Raw Materials Herbarium, and Museum, NISCAIR, New Delhi) was obtained as a gift sample from Aimil Pharmaceutical India Limited, New Delhi (Ref. No. AIMIL/PD/2015). A voucher specimen was deposited in the Department of Pharmacognosy, Hindu College of Pharmacy, Sonepat.

Chemicals and reagents

Ethylene glycol was purchased from Loba Chemie, Mumbai, India. Ammonium chloride was purchased from Thermo Fisher Scientific India Pvt Ltd. Mumbai, India. All diagnostic kits used for estimating the biochemical parameters were obtained from ERBA Diagnostic Mannheim GmbH, Germany. The reference herbal formulation Cystone (hereinafter designated as CST) was purchased from Himalaya Drug Company, Mumbai, India. All other chemicals and reagents used were of the at least analytical grade.

Experimental animals

Healthy adult Wistar rats, weighing 150-250 g and equivalent age (4-5 months) groups, were procured from Panacea Biotec Limited, Lalru (India). They were kept in polypropylene cages and housed in standard laboratory conditions at 25 ± 2ºC with alternate light and dark cycle of 12 hours each. All the experimental animals were allowed a free access to standard rat pellet diet and water. Prior to start the experimental work, the rats were acclimatized to experimental laboratory conditions for one week. The study protocol was duly approved by the Institutional Animal Ethical Committee [CTIPS/2014/IV/0020(PCL-D)]. The care and experimental handling of animals were taken as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests, Government of India, New Delhi.

Preparation of extract

The hydroethanolic extract of *P. perlata*, was prepared using the extraction procedure as previously carried out in our laboratory (Goyal *et al*., 2017). Briefly, 100 g coarsely powdered drug was mixed with 2500 ml 60% ethanol, in a stoppered glass container and kept at 45°C for five days with occasional shaking. The contents were filtered through Whatman No. 1 filter paper and the filtrate obtained was concentrated under vacuum using a rotary evaporator. The concentrated contents were dried to constant weight at 45°C in a hot air oven and labeled as PPE i.e. *P. perlata* extract. PPE was stored in a sterile glass container and kept in a refrigerator.

Animal model for inducing hyperoxaluria

Ethylene glycol (EG) and ammonium chloride (AC) induced hyperoxaluria in rat model was employed to assess the antiurolithiatic potential. The rats were treated with 0.75% v/v EG and 1% w/v AC in drinking water *ad libitum* for first three days to accelerate lithiasis followed by only 0.75% v/v EG in drinking water for the remaining period of six week study (Goyal *et al*., 2017; Prabhu *et al*., 2016).

Experimental design

Thirty-six Wistar rats were randomly divided into six groups, each containing six animals. The different groups were treated and designated as follows:

Group I (Normal control): The rats were maintained on a standard pellet diet and water *ad libitum* for six weeks. The vehicle (10% v/v Tween 80 solution) was orally administered once daily.

Group II (Urolithiatic control): The rats were allowed a free access to EG + AC containing drinking water for 1-3 days followed by only EG containing water for the remaining period of study. After two weeks of treatment, the vehicle was orally administered once daily for remaining four week period. The rats were allowed a free access to standard rat diet through the whole study tenure.

Group III (Standard groups): For the first two weeks, the rats were treated same as in the urolithiatic control group II. After two weeks of the treatment period, the vehicle, 10% v/v Tween 80 solution was replaced with standard drug CST (500 mg/kg; p.o.) for remaining periods of four weeks.

Group IV to VI (Test groups): For first two weeks, the rats were treated same as in the urolithiatic control group II. After two weeks of the treatment period, the rats of different groups were administered with PPE (100, 300 and 500 mg/kg; p.o. respectively) instead of vehicle for next four weeks.

The extract was emulsified in 10% v/v Tween 80. After two weeks treatment, the rats of group II-VI were individually kept in metabolic cages for 24 h to collect the urine samples. The urine samples were microscopically examined for the presence of CaOx crystals and the animals showing the presence of urinary crystals were selected for further study.

Biochemical analysis

Collection and analysis of urine

After six week treatment period, the animals were individually kept in metabolic cages for 24 hours and urine samples were collected. After measuring the urine volume and pH, the collected urine volumes were divided into two parts. One part was acidified with 1-2 drops of 1N Hydrochloric acid. In order to remove the debris, both the acidified and non-acidified urine samples were centrifuged at 1500 rpm for 10 minutes (Goyal *et al*., 2017). The calcium, magnesium and phosphorus contents were estimated in acidified urine sample using standard reagent kits (Erba Diagnostics Mannheim, GmbH, Germany). The non-acidified urine sample was subjected to estimate the oxalate contents by Hodgkinson's method (Hodgkinson, 1970). The urinary creatinine, urea, uric acid, and total protein contents in the non-acidified sample were estimated using commercially available standard reagent kits (Erba Diagnostics Mannheim, GmbH, Germany).

Collection and analysis of serum

After collecting the urine, the blood samples were collected from retro-orbital sinus of each animal and serum was separated by centrifugation at 2000 rpm for 10 minutes. The serum was quantitatively analyzed for creatinine, uric acid, urea nitrogen, magnesium, calcium, phosphorus, and lactate dehydrogenase (LDH) using spectrophotometric methods by employing commercially available standard reagent kits (Erba Diagnostics Mannheim, GmbH, Germany).

Kidney tissue homogenate analysis

After collecting the blood samples, the animals were sacrificed by cervical dislocation. The abdomen was cut opened and both the kidneys were carefully isolated. The isolated kidneys were cleaned of the extraneous tissues in ice-cold saline and weighed. One kidney was preserved in 10% buffered neutral formalin for the purpose of histological studies. The other kidney was sliced into two equal halves. One-half was dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1.0 N hydrochloric acid for about 30 minutes and homogenized. The homogenate was then centrifuged at 2000g for 10 minutes and the supernatant was separated (Chow *et al*., 1975; Gadge and Jalalpure, 2012; Goyal *et al*., 2017). The supernatant was used for estimating the calcium and phosphorus contents with commercially available kits (Erba Diagnostics Mannheim, GmbH, Germany). The oxalate contents were measured by Hodgkinson's method (Hodgkinson, 1970). The other half of kidney was minced and a 10% homogenate was prepared in tris-HCl buffer pH 7.0. The homogenate was then subjected to LDH estimation by employing commercially available standard reagent kits for spectrophotometric methods (Dodoala *et al*., 2010; Goyal *et al*., 2017; Soundararajan *et al*., 2006).

Histopathological studies

The kidneys, fixed in 10% buffered formalin (pH 7.0) solution, were dehydrated with ascending grades of ethanol and embedded in paraffin. The 4-6 µm thick sections of paraffin kidney were cut, mounted on slides, deparaffinised and rehydrated with descending grades of ethanol. After staining with hematoxylin and eosin, the kidney sections were examined (at 400x), under a light microscope equipped with a digital camera, for the presence of crystals of CaOx and various renal pathological changes like tubular necrosis, glomerular and tubular architecture etc. (Atmani *et al*., 2009; Goyal *et al*., 2017; Pareta *et al*., 2011).

Data analysis

All the values were expressed as mean ± SEM (standard error mean). The data were analyzed by employing One-way ANOVA followed by Tukey's multiple comparison tests using GraphPad Prism 5.0 software. The values of $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

EG + AC induced hyperoxaluria in rats is the highly reliable and widely used experimental model for preclinical evaluation of antiurolithiatic agents. The oxalate metabolism in rats is almost similar to that of human beings, so the rats were preferred in this study (Goyal *et al*., 2017; Pawar and Vyawahare, 2015). EG, when administered to rats in drinking water, is readily absorbed, and increase the substrate availability for oxalate synthesizing enzymes like glycolic acid oxidase (GAO) in liver and LDH in liver and kidney. This leads to hyperoxaluria which is one of the major risk factors for CaOx urolithiasis (Liao and Richardson, 1972; Soundararjan *et al*., 2006). Administration of AC along with EG acidify the urine and facilitate crystallization of oxalate (Fan *et al*., 1999). The oxalates, as poorly soluble, readily precipitate as CaOx and damage the epithelial lining of renal tubules that leads to adhesion and retention of crystals (Scheid *et al*., 2004; Thamilselvan *et al*., 2003).

Effects of PPE on urine output and pH

In the present study, administration of EG + AC significantly reduced the urine output and acidify the urine in lithiatic group when compared with normal control as shown in Table 1. The reduced urine output supersaturates the urine and favors the crystallization of oxalates. The acidification of urine also facilitates the stone formation (Vermeulen *et al*., 1951). PPE significantly enhanced the urine volume and pH as represented in Table 1. The enhanced urine volume hampered the crystal formation as well as facilitated the flushing out of crystals. The raised pH also hindered the stone formation. The best effects were seen in the PPE 500 mg/kg treated group.

Effects of PPE on oxalate and LDH levels

A significant increase in the oxalate levels of urine and kidney tissue homogenates was observed in EG + AC treated rats. The hyperoxaluria is considered as the major risk factor of CaOx urolithiasis (Khan, 2004). LDH is an oxalate synthesizing enzyme present in liver and kidney. The enhanced LDH levels in EG + AC treated rats also indicated the prominent hyperoxaluric con-

ditions. PPE significantly prevented the hyperoxaluric conditions, in a dose-dependent manner, by decreasing the elevated oxalate and LDH levels in the urine and kidney tissue homogenates as depicted in Table 1.

Table 1: Effects of PPE on urine output, urine pH, oxalate, and LDH levels in hyperoxaluric rats.

Parameters	Normal Control	Lithiatic Control$^{\$}$	CST 500 mg/kg$^{\#}$	PPE 100 mg/kg$^{\#}$	PPE 300 mg/kg$^{\#}$	PPE 500 mg/kg$^{\#}$
Ur. Vol. (ml/24 hrs)	19.70 ± 0.71	10.62 ± 0.42***	21.77 ± 0.66***	11.58 ± 0.60^{ns}	12.53 ± 0.63^{ns}	15.10 ± 0.43***
Ur. pH	7.77 ± 0.27	6.25 ± 0.12***	8.17 ± 0.19***	7.95 ± 0.26***	7.50 ± 0.18***	8.02 ± 0.12***
Ur. Ox (mg/dl)	0.34 ± 0.05	4.82 ± 0.42***	0.98 ± 0.15***	3.98 ± 0.13^{ns}	3.22 ± 0.20***	1.98 ± 0.12***
KH. Ox (mg/g)	2.80 ± 0.19	7.34 ± 0.57***	2.74 ± 0.18***	5.82 ± 0.42^{ns}	4.56 ± 0.38***	4.08 ± 0.26***
Sr. LDH (U/I)	848.92 ± 108.20	2396.34 ± 217.50***	1023.25 ± 65.32***	1982.84 ± 135.18^{ns}	1622.14 ± 118.41**	1295.56 ± 89.36***
KH. LDH (U/g)	1.98 ± 0.26	5.04 ± 0.44***	1.92 ± 0.09***	4.07 ± 0.29^{ns}	3.33 ± 0.21**	2.98 ± 0.22***

All the values for each group (n = 6) were represented as mean ± standard error mean. Data were analyzed by using One-way ANOVA followed by Tukey's multiple comparison test.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} not significant; $^{\$}$ compared with normal control; $^{\#}$ compared with lithiatic control
Ur. - Urine, Vol. - Volume, KH. - Kidney Tissue Homogenate, Ox - Oxalate, Sr. - Serum, LDH - Lactate dehydrogenase

Effects of PPE on renal functions assessing parameters

Once the urinary crystals of CaOx produced, they agglomerate and tend to retain in renal tubules. They tend to damage the renal tissues, decrease the glomerular filtration, obstruct the urine outflow and facilitate the accumulation of various waste products especially nitrogenous substances in blood (Karadi *et al*., 2006; Pareeta *et al*., 2011; Rathod *et al*., 2012).

In the present study, administration of EG + AC significantly altered the renal functions in lithiatic rats and indicated by increased serum concentration of creatinine, uric acid, BUN, and decreased creatinine clearance. The increased BUN, serum creatinine, and reduced creatinine clearance are the markers of tubular and glomerular damage in the kidney (Karadi *et al*., 2006). PPE significantly decreased the serum concentration of creatinine in a dose-dependent manner as shown in Table 2. It improved the creatinine clearance and showed significant effects at 500 mg/kg. PPE (100, 300, and 500 mg/kg) significantly decreased the BUN levels in a dose-dependent manner. The elevated uric acid is reported to promote the growth of CaOx crystals (Grover *et al*., 1990). PPE (500 mg/kg) significantly decreased the serum concentration of uric acid and altered the growth of crystals. EG + AC treatment increased the urinary protein levels and caused proteinuria. The proteinuria reflected the dysfunctions of proximal convoluted tubules (Resnick *et al*., 1979). PPP significantly ($p < 0.001$) reduced the urine levels of proteins and improved tubular functions. The effects of PPE (500 mg/kg) on serum creatinine, creatinine clearance, BUN, and urinary protein levels were found to be comparable with that of reference drug CST.

Table 2: Effects of PPE on renal function assessing parameters in hyperoxaluric rats.

Parameters	Normal Control	Lithiatic Control$^{\$}$	CST 500 mg/kg$^{\#}$	PPE 100 mg/kg$^{\#}$	PPE 300 mg/kg$^{\#}$	PPE 500 mg/kg$^{\#}$
Sr. Cre. (mg/dl)	0.46 ± 0.08	1.56 ± 0.11***	0.57 ± 0.03***	0.99 ± 0.04***	0.75 ± 0.02***	0.58 ± 0.04***
Cre. Clr (ml/min.)	0.59 ± 0.02	0.42 ± 0.01*	0.63 ± 0.02**	0.54 ± 0.03^{ns}	0.56 ± 0.04^{ns}	0.65 ± 0.04***
BUN (mg/dl)	19.66 ± 0.87	48.86 ± 3.77***	20.99 ± 0.75***	34.91 ± 1.87***	25.62 ± 1.17***	22.08 ± 0.99***
Sr. UAC (mg/dl)	0.39 ± 0.03	2.04 ± 0.27***	0.40 ± 0.05***	1.322 ± 0.369^{ns}	1.317 ± 0.255^{ns}	0.703 ± 0.137***
Ur. Pro. (g/dl)	0.13 ± 0.02	0.79 ± 0.04***	0.20 ± 0.03***	0.37 ± 0.02***	0.35 ± 0.02***	0.24 ± 0.02***

All the values for each group (n = 6) were represented as mean ± standard error mean. Data were analyzed by using One-way ANOVA followed by Tukey's multiple comparison test.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} not significant; $^{\$}$ compared with normal control; $^{\#}$ compared with lithiatic control
Sr. - Serum, Cre - Creatinine, Clr. - Clearance, BUN - Blood Urea Nitrogen, UAC - Uric acid, Ur. - Urine, Pro. - Protein

Effects of PPE on other stone promoting and inhibiting factors

Formation of urinary stone is considered to be affected by various stone promoting and inhibiting factors. The calcium and phosphorus are considered as the stone promoting inorganic factors while the magnesium is stone inhibiting (Basavaraj *et al*., 2007). In the present study, EG + AC treatment significantly elevated the calcium and phosphorus level in urine, serum, and kidney tissue homogenates of hyperoxaluric rats as shown in Table 3. It also significantly decreased the urinary and serum concentration of magnesium. Hypercalciuria favors the nucleation and precipitation of CaOx in urine that leads to crystal growth (Lemann *et al*., 1991). Hypercalcemia was also reported in urolithiasis patients (Bhale *et al*., 2013). Increased urinary phosphorus level has also been reported in hyperoxaluric rats (Subha and Varalakshmi, 1993). In hyperoxaluric condition, multiple cations tend to complex with oxalates to form urinary salts of oxalate. The magnesium salts are soluble while the calcium salts are insoluble and facilitate the precipitation of calculi of CaOx (Marshall and Robertson, 1976). PPE significantly reversed the urine, serum and kidney tissue homogenate levels of calcium towards normal range in a dose-dependent manner as shown in Table 3. The significant effects of PPE on magnesium levels in urine were seen at 300 and 500 mg/kg, while in serum at only 500 mg/kg. It significantly

reduced the elevated serum phosphorus toward normal but did not produce any significant effect on urine and kidney tissue homogenate levels. The remarkable effecsts of PPE on these stone promoting/inhibiting factors were observed at 500 mg/kg, and found to be comparable with that of reference drug CST.

Table 3: Effects of PPE on inorganic stone promoting/inhibiting factors in hyperoxaluric rats.

Parameters	Normal Control	Lithiatic Control$^{\$}$	CST 500 mg/kg$^{\#}$	PPE 100 mg/kg$^{\#}$	PPE 300 mg/kg$^{\#}$	PPE 500 mg/kg$^{\#}$
Ur. Ca (mg/dl)	1.19 ± 0.17	4.86 ± 0.42***	0.91 ± 0.08***	3.13 ± 0.22***	2.13 ± 0.10***	1.32 ± 0.23***
Sr. Ca (mg/dl)	8.78 ± 0.38	14.09 ± 0.88***	7.64 ± 0.39***	9.10 ± 0.41***	8.30 ± 0.35***	7.53 ± 0.53***
KH. Ca (mg/g)	4.34 ± 0.37	11.70 ± 0.89***	5.02 ± 0.26***	8.85 ± 0.54*	7.22 ± 0.35***	6.96 ± 0.32***
Ur. Ma (mg/dl)	4.70 ± 0.10	3.62 ± 0.15***	4.91 ± 0.11***	4.13 ± 0.11^{ns}	4.33 ± 0.18*	4.77 ± 0.20***
Sr. Mg (mg/dl)	2.51 ± 0.23	1.38 ± 0.06*	3.18 ± 0.27***	2.01 ± 0.13^{ns}	2.05 ± 0.31^{ns}	2.53 ± 0.27*
Ur. P (mg/dl)	1.37 ± 0.10	3.92 ± 0.20***	1.10 ± 0.07***	3.69 ± 0.15^{ns}	3.28 ± 0.13^{ns}	3.19 ± 0.30^{ns}
Sr. P (mg/dl)	4.71 ± 0.27	7.44 ± 0.38***	4.31 ± 0.19***	5.64 ± 0.29***	5.53 ± 0.21***	4.11 ± 0.40***
KH. P (mg/g)	1.49 ± 0.18	4.63 ± 0.52***	2.47 ± 0.14***	4.28 ± 0.36^{ns}	4.44 ± 0.28^{ns}	4.02 ± 0.34^{ns}

All the values for each group (n = 6) were represented as mean ± standard error mean. Data were analyzed by using One-way ANOVA followed by Tukey's multiple comparison test.

*** $p < 0.001$; * $p < 0.05$; ^{ns} not significant; $^{\$}$ compared with normal control; $^{\#}$ compared with lithiatic control

Ur. - Urine, Sr. - Serum, KH. - Kidney Tissue Homogenate, Ca - Calcium, Mg - Magnesium, P - Phosphorus

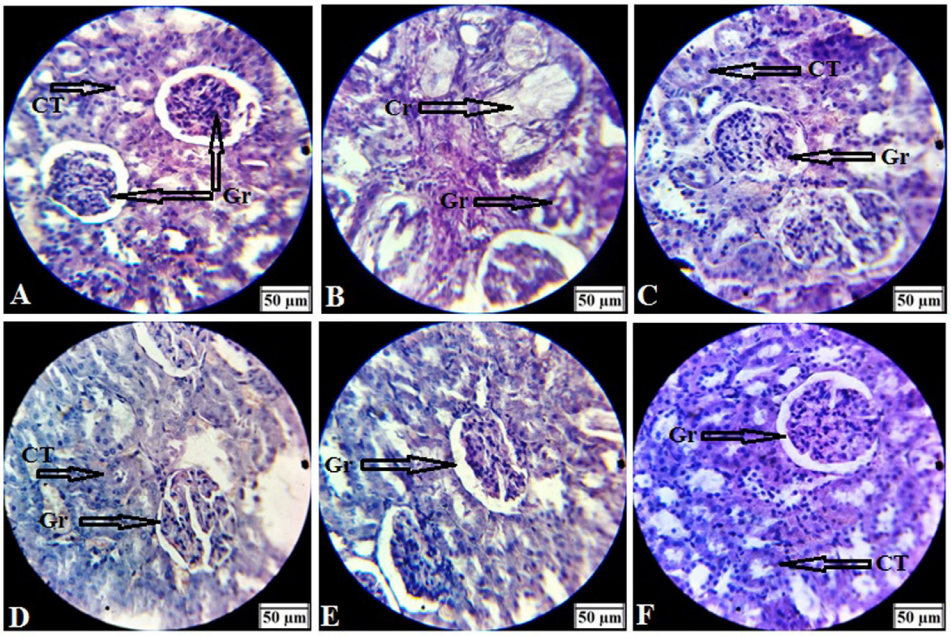

Fig. 1: Histological images (400 x) of normal, hyperoxaluric and treated rat kidney. (A) Normal control treated with vehicle, (B) Urolithiatic (hyperoxaluric) control treated with EG + AC, (C) Standard group: hyperoxaluric rats treated with CST. (D-E) Test groups: hyperoxaluric rats treated with 100, 300, and 500 mg/kg PPE respectively.

Cr – CaOx crystals; CT – Convoluted tubules; Gr – Glomerulus

Histopathological study

The microscopic examinations of histopathological slides shown in Figure 1 supported the results of the biochemical analysis. The rats in the control group (Figure 1 A) showed a normal renal architecture without any crystal deposition. The slides of lithiatic group showed the presence of large sized crystals of CaOx, glomerular atrophy, and altered renal architecture (Figure 1B). Crystal deposition is usually associated with cellular injury and caused a variety of changes in renal architecture that facilitate crystal retention. The interactions between injured tubular epithelial cells and oxalate crystals are considered to have a significant role in the development of urinary stones (Aggarwal *et al*., 2010; Bijarnia *et al*., 2008; Grover *et al*., 1990; Khan, 2004; Khan *et al*., 2000). PPE (100, 300 and 500 mg/kg), when compared with urolithiatic group, significantly restored the normal renal architecture as shown in Figure 1 D, E, and F respectively. No CaOx crystals were observed and the normal cellular organizations of proximal and distilled convoluted tubules were restored. The normal glomerular sizes and renal architectures were recovered. The

effects of PPE were found to be comparable with that of the reference drug as shown in Figure 1 C.

CONCLUSION

The present study concluded that *P. perlata* extract exhibited significant antiurolithiatic potentials against CaOx calculi in experimental rats. It significantly restored the normal renal architecture and improved the renal functions by restoring the EG + AC mediated biochemical changes in urine, serum and kidney tissue homogenate parameters of experimental rats towards normal.

CONFLICT OF INTEREST

The authors have declared no conflict of interests.

ACKNOWLEDGEMENT

The authors are thankful to I.K.G. Punjab Technical University, Jalandhar (Punjab), India for providing a platform to carry out this research work. The authors are also thankful to Aimil Pharmaceutical India Limited, New Delhi for providing gift samples of authenticated *P. perlata*.

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How to cite this article:
Goyal PK, Verma SK, Sharma AK. Evaluation of antiurolithiatic effects of *Parmelia perlata* against calcium oxalate calculi in hyperoxaluric rats. J App Pharm Sci, 2018; 8 (01): 129-135.