ORIGINAL ARTICLE

Vol. 41 (3): 511-520, May - June, 2015
doi: 10.1590/S1677-5538.IBJU.2014.0357

Antilithiatic Activity of phlorotannin rich extract of *Sarghassum Wightii* on Calcium Oxalate Urolithiais – *In Vitro* and *In Vivo* Evaluation

D. Sujatha [1], Kiranpal Singh [1], Mursalin Vohra [1], K. Vijay Kumar [1], S. Sunitha [1]

[1] *Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Hyderabad, Andhrapradesh, India*

ABSTRACT

Purpose: Urolithiasis is a common urological disorder responsible for serious human affliction and cost to the society with a high recurrence rate. The aim of the present study was to systematically evaluate the phlorotannin rich extract of *Sargassum wightii* using suitable in vitro and in vivo models to provide scientific evidence for its antilithiatic activity.

Materials and Methods: To explore the effect of *Sargassum wightii* on calcium oxalate crystallization, in vitro assays like crystal nucleation, aggregation and crystal growth were performed. Calcium oxalate urolithiasis was induced in male Sprague dawley rats using a combination of gentamicin and calculi producing diet (5% ammonium oxalate and rat pellet feed). The biochemical parameters like calcium, oxalate, magnesium, phosphate, sodium and potassium were evaluated in urine, serum and kidney homogenates. Histopathological studies were also done to confirm the biochemical findings.

Results: The yield of *Sargassum wightii* extract was found to be 74.5 gm/kg and confirmed by quantitative analysis. In vitro experiments with *Sargassum wightii* showed concentration dependent inhibition of calcium oxalate nucleation, aggregation and growth supported by SEM analysis. In the in vivo model, *Sargassum wightii* reduced both calcium and oxalate supersaturation in urine, serum and deposition in the kidney. The biochemical results were supported by histopathological studies.

Conclusion: The findings of the present study suggest that *Sargassum wightii* has the ability to prevent nucleation, aggregation and growth of calcium oxalate crystals. *Sargassum wightii* has better preventive effect on calcium oxalate stone formation indicating its strong potential to develop as a therapeutic option to prevent recurrence of urolithiasis.

ARTICLE INFO

Key words:
Calcium Oxalate; Calculi; Phaeophyta

Int Braz J Urol. 2015; 41: 511-20

Submitted for publication:
July 18, 2014

Accepted after revision:
October 18, 2014

INTRODUCTION

The risk of developing urolithiasis in adults appears to be higher in the western hemisphere (5-9% in Europe, 12% in Canada, and 13-15% in the USA) (1), than in the eastern hemisphere (1-5%), although the highest risks have been reported in some Asian countries such as Saudi Arabia (20.1%) with lifetime recurrence rates of up to 50% (2).

The formation of the most common type of calculi, the calcium containing calculi, is more complex and surprisingly not yet understood completely. Current evidence suggests that both free and fixed stone formation is possible. The

long accepted simple explanation of formation of calcium oxalate (CaOx) stones is supersaturation (3). Deviating from the hypothesis new insights suggest a primary plaque formation in the interstitial space of the renal papilla. This plaque acts as support commonly referred as nidus made of either calcium phosphate crystals or organic matrix derived from damaged membranes. This membrane damage is mainly because of various apoptotic pathways as ROS, oxidative stress, altered pH and co-morbid conditions damaging the kidney.

Hence, it has been hypothesized that compounds that either alter supersaturation or prevent membrane damage can be a useful aid to treat and prevent recurrence of urolithiasis. Phlorotannins, important secondary metabolites obtained from many marine sources are structurally similar to tannins, can easily complex these divalent ions and accordingly reduce supersaturation of urine. On the other hand, phlorotannins are claimed and proved to be good antioxidants from earlier reports (4). Hence, they may also reduce the renal tubular damage and prevent stone formation.

Sargassum wightii is an important marine species (Family: Phaeophyceae), widely distributed in tropical and temperate oceans. *S. wightii* shows presence of good amount of flavonoids, alkaloids, phenolics, phlorotannins and steroids with various pharmacological activities like antibacterial and antioxidant activity (5-8). Still many pharmaceutical and therapeutical applications of *S. wightii* are untapped. Hence, the present study has been initiated with an objective to obtain phlorotannin rich extract of *S. wightii* (PTSW) and to evaluate whether PTSW has any preventive or curative affect against calcium oxalate stones using suitable in vitro crystallization methods and animal model.

MATERIALS AND METHODS

Collection of *S. Wightii* and Extraction

The brown algae *S. wightii* was collected in November from sea shore of MANDAPAM region Rameshwaram coast. The brown algae was authenticated by Dr. B. Seetharam, Professor, Sri Venkateswara Ayurvedic Medical College Tirupathi, Andhra Pradesh, India and a voucher specimen (M-001) was deposited in the department of pharmacology and toxicology of National Institute of Pharmaceutical Education and Research, Hyderabad, India.

Air dried S.wightii was extracted to obtain phlorotannin rich extract as explained by Young et al. with some modifications (9). Briefly, air dried S.wightii was kept for maceration at room temperature with 70% methanol (v/v) for 24 hrs under nitrogen environment. Methanolic extract was then collected by using rota evaporator (Rotavac, Heidolf, Germany) at 40°C and fractioned thrice with distilled water and n-hexane for 24 hr (1:1). All the aqueous portions were pooled and acetylated with ethyl acetate in pyridine environment. The acetylated aqueous extract was then dialyzed against distilled water using dialyzing membrane (3000 kd cutoff). The obtained phlorotannin rich S.wightii extract (PTSW) was collected and stored at 2-8°C.

Quantification of PTSW

For qualitative estimation of phlorotannins, TLC was carried out on 10×20 cm silica gel plate as per the procedure of Jeeva et al. (10). The chloroform and methanol (9:1) served as mobile phase. Folin-Ciocalteu reagent was used as spraying agent to detect the phenolic compounds. Quantification of phlorotannins in PTSW was done according to modified Folin-Ciocalteu method, using phloroglucinol as standard (11). Total phlorotannin content was expressed as gram equivalents of phloroglucinol.

In vitro crystallization methods

The method used to study the effect of PTSW on CaOx nucleation, aggregation and crystal growth was described by Hennequin et al. (12), Atmani and Khan (13) and Nakagawa (14) respectively but with some modifications. Calcium chloride (12mmol/L) and sodium oxalate (NaOx) (2mmol/L) were used for nucleation assay and simultaneously, morphological characterization of the calcium oxalate monohydrate (COM) crystals was done using scanning electron microscopy (SEM) (SEM-3700N). The crystals were viewed at the voltage of 15 kv, 5 eV and SE of range 37-270 at 0 and 60 min in the crystal growth assay.

Antilithiatic activity of S. wightii

Animals

Male Sprague Dawley (SD) rats (150-200g,) were obtained from Teena laboratories and housed under conditions of optimum light, temperature and humidity (12 h light–dark cycle, 22±2˚C and relative humidity of 45 to 55%), with food and water provided ad libitum. The animal experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC No: NIP/10/2013/PC/66). Acute toxicity study for *S. wightii* (PTSW) was performed as per OECD guideline no 425 to determine the dose for antilithiatic study.

Experimental design

Hyperoxaluria and calcium oxalate deposition was induced using gentamicin and calculi producing diet (CPD) (15). The standard rat pellet feed was powdered and mixed with ammonium oxalate (5%), then made into pellets used as CPD.

Male SD rats were randomly grouped in to 7 groups (n=8). The group Normal (N) received only vehicle (distilled water, 2 mL/kg/p.o.). The remaining six groups received gentamicin (40 mg/kg/s.c., 1-8th day) and CPD from 5-15th day. The preventive-control (PC) and curative-control (CC) groups received only vehicle (distilled water, 2 mL/kg/orally) from day 1 to 15 and day 16 to 30 respectively. The preventive groups (P1 and P2) received the PTSW (200 and 400 mg/kg/p.o. respectively) from day 1 to 15, whereas curative groups (C1 and C2) received the PTSW (200 and 400 mg/kg/p.o. respectively) from day 16 to 30. As the extract was administered from 1st day, P1 and P2 assess ability of PTSW to prevent stone formation for which the PC group serves as control. The purpose of groups C1 and C2 was to assess the ability of the extract to treat already developed renal calculi for which CC group serves as control.

Parameters monitored

Biochemical parameters were measured for preventive groups on the day 15 and for curative groups on the day 30. At regular intervals, as mentioned above the animals were individually housed in metabolic cages for collection of urine samples for 24 hr. The urine volume was measured and then centrifuged at 3000 rpm for 5 min to collect the supernatant for estimation of urinary parameters like sodium, potassium, calcium, oxalate, magnesium and phosphate.

Blood was collected from the retro-orbital region at the same time points and allowed to clot at room temperature and serum was separated by centrifugation at 3000 rpm for 5 minutes. The serum was used to analyze the serum level of sodium, potassium, calcium, oxalate, magnesium and phosphate.

Animals were sacrificed at the end of the experimental period day 15 (PC, P1 and P2) and day 30 (CC, C1 and C2) by decapitation. The kidneys were carefully excised, weighed and one kidney of each animal was put in 10% formalin for histological studies. The other kidney was sliced into two equal halves. One half was homogenized in 10% HCl and another in 10 % Tris buffer. The supernatant from HCl homogenate was used for estimation of calcium and oxalate and Tris buffer homogenate for estimation of magnesium and phosphate. All the biochemical parameters mentioned were measured using commercial analytical kits (Accurex Pvt Ltd) except oxalate. The urine, serum and kidney levels of oxalate were estimated manually using procedure of Hodgkinson and Williams (16).

Histopathological evaluation

For microscopic evaluation, kidneys were fixed in 10% formalin. Tissue sections of 5µm thickness were stained with hematoxylin-eosin. A minimum of 10 fields for each kidney slide were examined for necrosis, calcium oxalate crystals deposits, stone size, membrane damage and other histopathological parameters. Tissue slices were photographed using research microscope (Nikon Eclipse Ti-U, USA) at 10X magnification.

Statistical analysis

All values were expressed as mean ± SD of three observations for in vitro studies and 8 observations for in vivo studies. The data was analyzed using Graph pad prism software version 5.0. One way ANOVA and two ANOVA were used depending upon the need followed by Bonferroni's test as post hoc test. The statistical significance was set at $p<0.05$.

RESULTS

PTSW yield and quantification

The yield of methanolic extract of *S. wighttii* was found to be 74.5 gm/kg of dry material. The yield of phlorotannins rich extract (PTSW) was found to be 20g / 100 g of methanolic extract of S. wightii. Total phlorotannin content of PTSW was determined from the calibration curves of phloroglucinol ($y=0.002x-0.022$, $R^2=0.989$) by Folin-Ciocalteu method. The total phlorotannin content of PTSW was found to be 74.5µg/mg phloroglucinol equivalents.

Nucleation assay

The simultaneous incubation along with the different concentrations of PTSW significantly reduced the nucleation of calcium oxalate at 30 and 60 min significantly ($p<0.05$), when compared to control setup. The % inhibition of nucleation, by PTSW was found to be dose dependent (Table-1).

Crystal aggregation assay

The simultaneous incubation of COM seed along with different concentrations of PTSW (t_1-50µg, t_2-100µg, t_3-250µg and t_4-500µg) had shown a significant reduction in aggregation of COM crystals. Results showed a significant inhibitory effect of PTSW in all the different concentration when compared to the control COM slurry ($p<0.05$), but the effect was dose independent. The % aggregation inhibition rate was calculated and found to be dose independent effect as shown in Table-1.

Crystal growth assay

A significant deposition of 6.18% of oxalate was observed at 400 s, whereas an additional 5.71 % was deposited by 800s indicating significant growth of COM crystal in control samples. When COM crystals were incubated with different concentrations of PTSW, it was observed that the oxalate deposition was significantly reduced in dose and time dependent manner as shown in Table-1.The SEM analysis of calcium oxalate growth assay at 0 and 60 min showed the reduction in size and the shape of COM crystals in the incubation mixtures having different concentrations of PTSW when compared with control (Figure-1), which clearly indicate ability of PTSW to reduce crystal growth supporting the results of crystal growth assay.

Table 1 - Effect of PTSW on in vitro crystal nucleation, aggregation and growth assay.

Concentrations	Nucleation assay	Aggregation assay	Crystal growth assay			
	% inhibition={(1-(T_{Si}/T_{Sc})} X 100	% aggregation inhibition rate, Ir=(1-T_{Si}/T_{Sc}) X 100	% Reduction of free oxalate		% Relative inhibitory activity {% Relative inhibitory activity =((C-S)/C)×100}	
	60 min	60 ⇒ in	(0–400) seconds	(400-800) seconds	400 seconds	800 seconds
Control	Nil	Nil	6.18±0.45	5.71±0.32	Nil	Nil
T1 (50µg/mL)	91.95±6.25	31.65±1.25	4.50*±0.34	2.38*±0.23	27.18±1.25	58.31±3.14
T2 (100µg/mL)	92.90±4.56	27.66±2.01	1.47*±0.04	1.20*±0.06	76.21±8.21	78.98±6.54
T3 (250µg/mL)	97.04±6.89	48.28±4.56	1.08*±0.1	0.78*±0.09	82.52±9.14	86.34±4.89
T4 (500µg/mL)	98.97±5.61	42.6±3.58	0.56*±0.05	0.55*±0.05	90.93±6.24	90.36±7.28

All values were mean ± SD of 3 observations. Where; *=$p<0.05$, when compared with control and

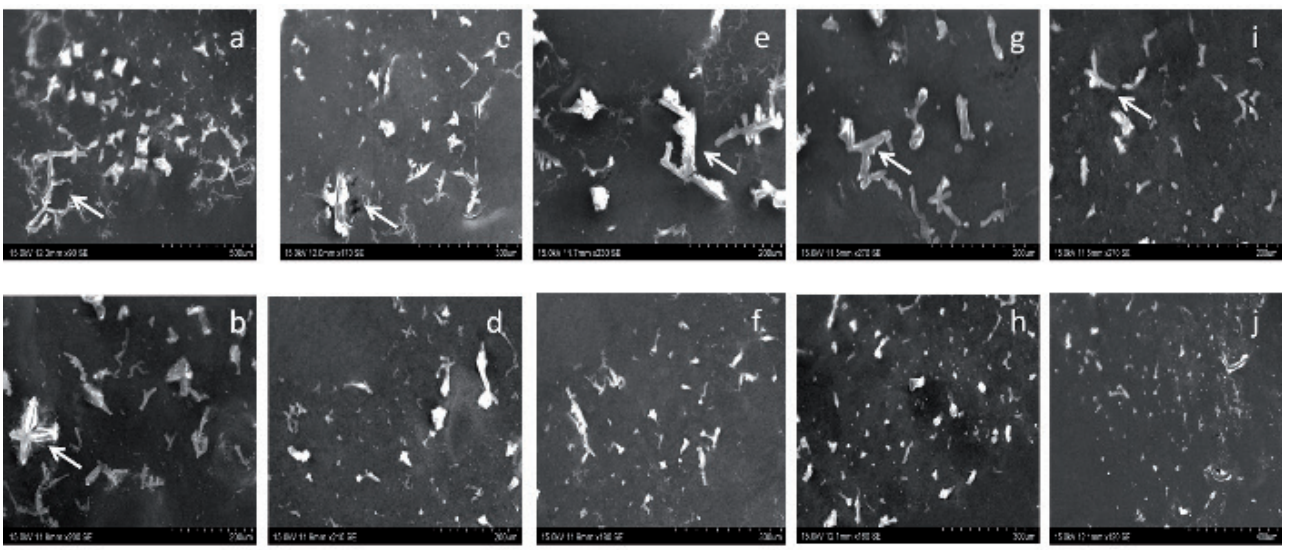
T_{Si} = Turbidity slope in the presence of the test sample;

T_{Sc} = Turbidity slope of the control;

C= Rate of reduction of free oxalate without test sample;

S= Rate of reduction of free oxalate with test sample.

Figure 1 - SEM Images of Crystal growth assay taken at 0 and 60 min.



a, b = Control (0 and 60 min respectively); **c, d =** t_1-50µg/mL (0 and 60 min respectively); **e, f** = t_2- 100µg/mL (0 and 60 min respectively);**g, h** = t_3- 250 µg/mL (0 and 60 min respectively); **i, j =** t_4-500µg/mL (0 and 60 min respectively); Arrows indicate sharp edged COM crystals.

Acute toxicity study

PTSW was found to be safe up to 2 g/kg, p.o. as no mortality was observed in 48 hrs followed by 14 days observation except increase in urination in the first 6 hrs. The dose for pharmacological evaluation was fixed at 200 and 400 mg/kg p.o as per standard guidelines.

Effect of PTSW on biochemical parameters

With administration of CPD and gentamicin, significant ($p<0.05$) increase in urinary excretion of calcium and oxalate was observed in PC and CC groups when compared with normal group (N) confirming that CPD and gentamicin treatment has caused supersaturation of urine with calcium and oxalate. After treatment with PTSW a significant ($p<0.05$) decrease in calcium and oxalate was observed in P1, P2, C1 and C2 groups when compared with their respective control groups, indicating the ability of PTSW to reduce super saturation of urine caused by CPD and gentamicin administration. PC and CC has not shown any significant increase in magnesium and phosphorus excretion when compared with normal animals (N), but with PTSW treatment a significant ($p<0.05$) decrease in urinary excretion of magnesium and phosphorus was observed in P1, P2, C1 and C2 groups when compared with their respective control groups.

Similar significant ($p<0.05$) increase was observed in serum concentrations of calcium, oxalate and magnesium, in PC and CC, when compared with normal group (N). Whereas on treatment with PTSW for 15 days, a significant ($p<0.05$) decrease in serum concentrations of calcium and oxalate alone was found P1, P2, C1 and C2 groups when compared with their respective control groups, without any effect on magnesium levels (Table-2).

While on treatment with gentamicin and CPD for 15 days, a significant ($p<0.05$) deposition of calcium, oxalate, magnesium and phosphorous in kidney was observed in PC and CC groups when compared to the normal group (N). Administration of PTSW in two different doses for 15 days, significantly ($p<0.05$) decreased deposition of calcium, oxalate and magnesium in P1, P2, C1 and C2 groups when compared to their respective control groups. A significant decrease in phosphate deposition was also observed but only with higher concentration of PTSW i.e., only in P2 and C2 groups as shown in Table-2.

Though urinary, serum and kidney deposition of sodium and potassium were also measured, they were not documented as no significant chan-

Table 2 - Effect of PTSW on biochemical parameters in urine, blood and kidney homogenates.

Groups	Urinary Excretion				Serum Levels			Kidney deposition			
	Calcium (m Mol/L)	Oxalate (µg/dL)	Magnesium (m Mol/L)	Phosphorus (m Mol/L)	Calcium (m Mol/L)	Oxalate (µg/dL)	Magnesium (m Mol/L)	Calcium (mg/g wet tissue)	Oxalate (mg/g wet tissue)	Magnesium (mg/g wet tissue)	Phosphorous (mg/g wet tissue)
N	0.618±0.15	80.84±11.4	4.52±0.78	18.33±2.51	14.17±1.2	8.8±4.4	0.23±0.03	1.59±0.37	0.98±0.03	11.21±1.0	2.26 ± 0.76
PC	10.6±6.11^{a}	105.8±12.2^{a}	3.92±0.98^{ns}	14.27±3.26^{ns}	23.31±0.5^{a}	23.18±2.0^{a}	2.30±0.6^{a}	5.85±0.6^{a}	6.43±0.7^{a}	28.99±9.01^{a}	8.88 ± 2.7^{a}
P1	3.76±0.79^{b}	24.2±5.2^{b}	1.20±0.51^{b}	6.5±2.4^{b}	14.77±1.2^{b}	13.76±1.6^{b}	1.97±0.4^{ns}	2.59±1.2^{b}	2.52±0.9^{b}	12.16±6.2^{b}	6.77 ± 2.6^{ns}
P2	3.32±0.91^{b}	32.2±4.9^{b}	2.03±0.49^{b}	3.27±0.9^{b}	14.55±0.71^{b}	15.36±3.6^{b}	2.01±0.5^{ns}	2.17±0.9^{b}	1.46±0.2^{b}	14.8±3.2^{b}	3.83 ± 1.1^{b}
CC	16.01±3.5^{a}	155.67±9.3^{a}	5.86±2.2^{ns}	15.4±2.04^{ns}	29.48±0.7^{a}	24.67±1.8^{a}	2.15±0.42^{a}	5.05±0.5^{a}	6.04±0.7	21.61±5.2^{a}	9.05 ±0.7^{a}
C1	6.24±1.77^{b}	35.2±8.5^{b}	5.65±0.41^{ns}	2.5±0.76^{b}	10.15±0.6^{ns}	17.17±6.4^{b}	2.08±0.22^{ns}	1.3±0.3^{b}	1.27±0.27^{b}	11.79±1.1^{b}	8.04 ±0.3^{ns}
C2	4.79±2.28^{b}	28.4±2.3	5.29±1.01^{ns}	6.50±1.08^{b}	10.65±0.8^{ns}	12.88±3.7^{b}	2.06±0.45^{ns}	1.3±0.8^{b}	1.12±0.07^{b}	15.43±4.1^{b}	2.85 ±0.3^{b}

All values were mean ± SD of 8 observations. Where, a=p<0.05, when compared with normal group, b=p<0.05 when compared to rescpective control groups (P1 and P2 Vs PC; C1 and C2 Vs CC), ns = not significant, using one way ANOVA.

ges were observed between the groups and serum levels of phosphorous have also not been included because of their non significant changes.

Histopathology

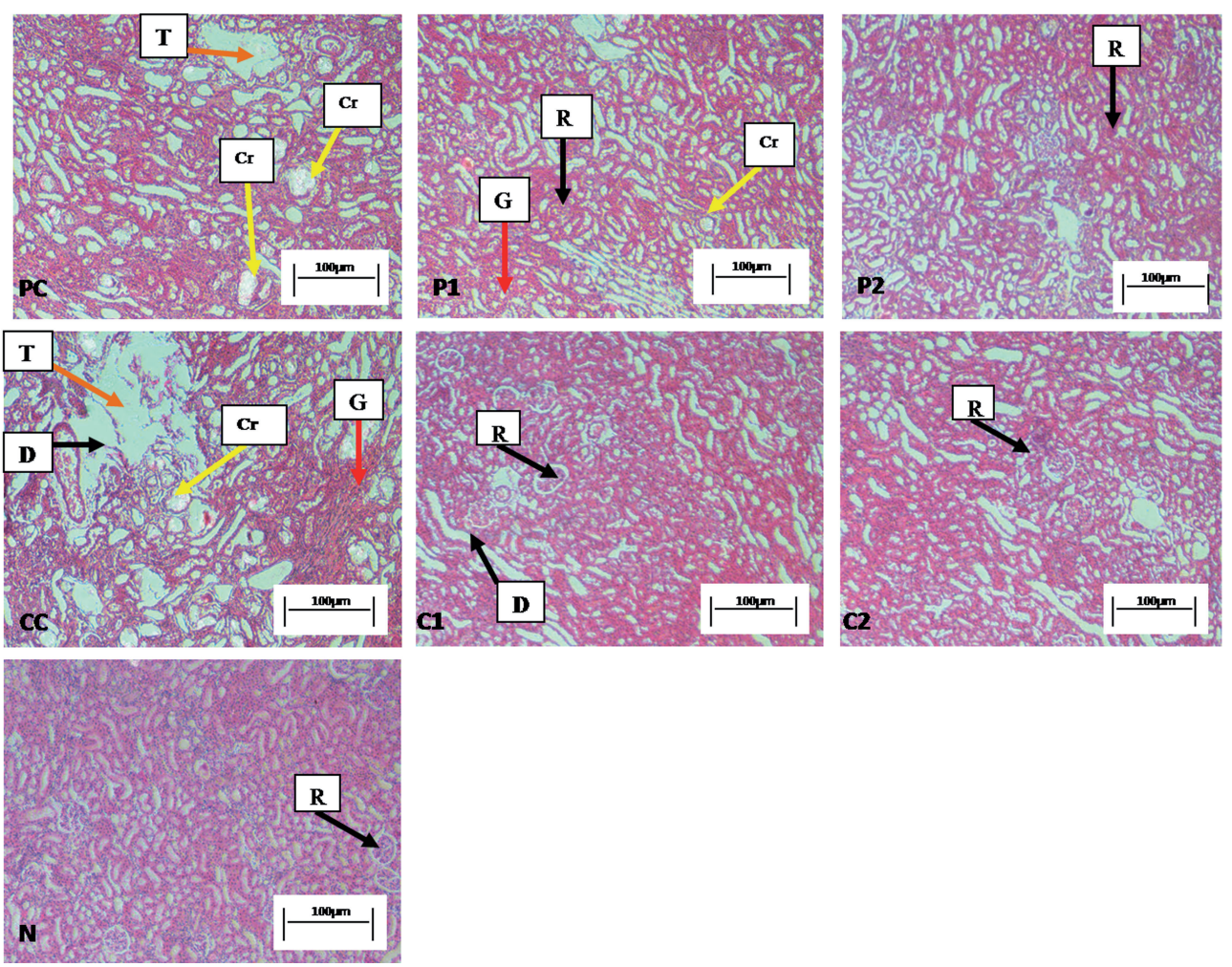
The sections photographed highlights the junction of medulla and cortex region in kidney as both in humans and rats CaOx deposition mainly occurs in this region. Gentamicin and CPD administration has caused severe glomerular damage, tubular dilation, membrane damage and deposition of CaOx crystals as highlighted in the photomicrographs of PC and CC.

Treatment with PTSW at both the doses clearly ameliorated the nephrotoxic damage caused by gentamicin and CaOx deposition by CPD as noted in the sections of P1, P2, C1 and C2.These results not only support the biochemical estimations but also testify that PTSW has antilithiatic property (Figure-2).

DISCUSSION

In clinical scenario, irrespective of the chemical nature of kidney stones they are treated by various surgical procedures like extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy etc.,. Beyond doubt the surgical procedures cause immediate relief from the kidney stones but cannot overcome their recurrence, a very common

Figure 2 - Photomicrographs of kidney sections at 100 X magnification where N,PC,P1,P2,CC,C1 and C2 corresponds to groups.



Cr = Calcium oxalate crystals; **D** = Dilation of tubules; **G** = Glomerular damage; **R** = Normal glomeruli; **T** = Tubular damage

problem associated with kidney stones and considered unmet clinical need in the area of urology.

Phlorotannins, important secondary metabolites are obtained from many marine sources. These being structurally similar to tannins can easily complex the divalent ions and might reduce supersaturation of urine (17).On the other hand, phlorotannins like tannins/polyphenols obtained from plant sources are claimed and proved to be good antioxidants from earlier reports (18). Hence, the present study focuses on the systematic evaluation of PTSW for its benefit as antilithiatic agent.

Extraction of phlorotannins is not always easy and uniform to isolate phlorotannins due to the susceptible chemical structure of phlorotannins with interphloroglucinol linkages which can be oxidized easily (19). Hence, ascorbic acid and acetylation of phlorotannins was used during the extraction procedure which might be responsible for increase in the yield of phlorotannins.

The rationale behind the selection of $CaCl_2$ and NaOx solution for in vitro studies is their swift solubility and they rapidly complex to form CaOx crystals uniformly, which can be used as probes for in vitro studies as mentioned in many former reports (17). Results of nucleation assay indicate that PTSW complexes the free Ca and Ox ions available in the incubation mixture, thus causing dose dependent inhibition of nucleation. After nucleation, agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in the urinary tract. If the extract keeps CaOx particles dispersed in solution for longer time they are more easily eliminated. Thus, from aggregation assay results it can be affirmed that phlorotannins can maintain divalent ions in dispersed condition for longer durations such that they can be eliminated out through urine.

Antilithiatic substances can also prevent the growth of COM by inhibiting oxalate deposition on the COM crystals surface present in incubation mixture (20). PTSW has shown similar dose dependent ability to inhibit the growth of CaOx crystals. The growth assay was followed by SEM analysis of crystals which clearly testified ability of PTSW to reduce crystal growth. PTSW incubated samples showed comparatively less number and smaller size smooth surfaced crystals indicating PTSW ability of converting COM to CaOx dehydrate crystals, which may adhere less to renal membranes and can be easily excreted along with urine.

In the in vivo studies, from the results of urinary biochemical parameters, we can explain that PTSW clearly reduces supersaturation of urine with divalent cations with much specific effect on calcium and oxalate. Sodium and potassium excretion were not altered, but those ions are neither induced nor regulated by gentamicin and CPD diet, which was confirmed once again by our studies. Moreover, in urolithiasis oxalic salts are soluble when formed with magnesium but when complexes with calcium forms insoluble calcium oxalate thus causing crystalline precipitation of renal calculi of calcium oxalate type (21). Accordingly, hyperoxaluria is far more significant risk factor in the pathogenesis of renal stones than hypercalciuria and changes in urinary oxalate levels are relatively much more important than those of magnesium, phosphorus, sodium and potassium (22). As, PTSW lowered the levels of both oxalate and calcium in urine it can be suggested that PTSW reduces both hyperoxaluria and hypercalciuria.

After serum studies, it was clear that supersaturation of urine with certain urinary salts such as calcium and oxalate was comparative with their serum levels. CPD and gentamicin clearly increased serum levels of calcium and oxalate which can be attributed to the enzymatic disturbances caused by gentamicin and increased absorption of both calcium and oxalate by feeding with CPD as mentioned earlier (23). PTSW ability to reduce hyperoxaluria and hypercalciuria can thus also be attributed to its ability to reduce absorption of calcium and oxalate from dietary sources and also might rectify the enzymatic disturbances leading to idiopathic hyperoxaluria, evident by decreased serum levels of calcium and oxalate. Further mechanistic studies are warranted to explain the exact effect of PTSW on absorption of divalent ions.

In the present study, gentamicin along with CPD increased kidney deposition of calcium and oxalate as a consequence of increased calcium and oxalate levels both in serum and urine.

But PTSW at both the doses clearly diminished the deposition of calcium and oxalate in kidney. These results were further supported by histological findings, where preventive control and curative control groups showed marked glomerular damage, widened gaps in tubular duct, deposition of honey colored CaOx crystals, inflammatory damage and collecting duct dilatation. The different concentration of PTSW has shown the marked reduction in glomerular damage, membrane damage and infiltration of inflammatory cells. CaOx deposition was significantly reduced as shown in both preventive treatment groups and curative treatment groups. These findings support the in vitro results and biochemical changes caused by PTSW, suggesting the antilithiatic activity of PTSW.

CONCLUSIONS

The findings of the present study highlight the ability of PTSW to prevent nucleation, aggregation and growth of calcium oxalate crystals as proved in in vitro studies. PTSW has also shown remarkable decrease in supersaturation of urine and serum with calcium, oxalate and magnesium. All these conditions put together will create an environment to prevent formation of kidney stone rather than dissolving them. Though PTSW has shown both the properties, i.e.; ability to prevent stone formation and dissolved already formed stones, the preventive effect was more predominant. This study encourages isolation of active constituents of PTSW responsible for antilithiatic activity.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Soucie JM, Thun MJ, Coates RJ, McClellan W, Austin H. Demographic and geographic variability of kidney stones in the United States. Kidney Int.1994;46:893-9.
2. Hughes P; Caring for Australians with Renal Impairment (CARI). The CARI guidelines. Kidney stones epidemiology. Nephrology (Carlton). 2007;12:S26-30.
3. Khan A, Bashir S, Khan SR, Gilani AH. Antiurolithic activity of Origanum vulgare is mediated through multiple pathways. BMC Complement Altern Med. 2011;11:96.
4. Pareta S K, Patra KC, Harwansh R: In-vitro calcium oxalate crystallization inhibition by Achyranthes indica linn. Hydroalcoholic extract: An approach to antilithiasis. International Journal of Pharma & Bio Sciences. 2011;2:432-7.
5. Josephine A, Nithya K, Amudha G, Veena CK, Preetha SP, Varalakshmi P. Role of sulphated polysaccharides from Sargassum Wightii in Cyclosporine A-induced oxidative liver injury in rats. BMC Pharmacol. 2008;8:4.
6. Vallinayagam K, Arumugam R, Kannan R, Thirumaran G, Anantharaman P: Antibacterial activity of some selected seaweeds from pudumadam coastal regions. Global J Pharmacol. 2009;3:50-2.
7. Syad AN, Shunmugiah KP, Kasi PD. Antioxidant and anticholinesterase activity of Sargassum wightii. Pharm Biol. 2013;51:1401-10.
8. Jang KH, Lee BH, Choi BW, Lee HS, Shin J. Chromenes from the brown alga Sargassum siliquastrum. J Nat Prod. 2005;68:716-23.
9. Young MH, Jong SB, Jin WH, Nam HL. Isolation of a New Phlorotannin, Fucodiphlorethol G, from a Brown Alga Ecklonia cava. Bull. Korean Chem. Soc. 2007;28:1595-7.
10. Jeeva S, Antonisamy JM, Domettila C, Anantham B, Mahesh M. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. Asian Pacific Journal of Tropical Biomedicine. 2012;2:S30-3.
11. Waterman PG, Mole S: Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications, Oxford. 1994;pp.85.
12. Hennequin C, Lalanne V, Daudon M, Lacour B, Drueke T. A new approach to studying inhibitors of calcium oxalate crystal growth. Urol Res. 1993;21:101-8.
13. Atmani F, Khan SR. Role of urinary bikunin in the inhibition of calcium oxalate crystallization. J Am Soc Nephrol. 1999;1:S385-8.
14. Nakagawa Y, Abram V, Parks JH, Lau HS, Kawooya JK, Coe FL. Urine glycoprotein crystal growth inhibitors. Evidence for a molecular abnormality in calcium oxalate nephrolithiasis. J Clin Invest. 1985;76:1455-62.
15. Kumar S, Sigmon D, Miller T, Carpenter B, Khan S, Malhotra R, et al. A new model of nephrolithiasis involving tubular dysfunction/injury. J Urol. 1991;146:1384-9.
16. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. Clin Chim Acta. 1972;36:127-32.
17. Murdiati TB, McSweeney CS, Lowry JB. Complexing of toxic hydrolysable tannins of yellow-wood (Terminalia oblongata) and harendong (Clidemia hirta) with reactive substances: an approach to preventing toxicity. J Appl Toxicol. 1991; 11:333-8.

18. Li Y, Qian ZJ, Ryu B, Lee SH, Kim MM, Kim SK. Chemical components and its antioxidant properties in vitro: an edible marine brown alga, Ecklonia cava. Bioorg Med Chem. 2009;17: 1963-73.
19. Peng S, Jay-Allemand C. Use of antioxidants in extraction of tannins from walnut plants. J Chem Ecol. 1991;17:887-96.
20. Tayal S, Duggal S, Bandyopadhyay P, Aggarwal A, Tandon S, Tandon C. Cytoprotective role of the aqueous extract of Terminalia chebula on renal epithelial cells. Int Braz J Urol. 2012;38:204-13; discussion 213-4.
21. Pak CY, Adams-Huet B, Poindexter JR, Pearle MS, Peterson RD, Moe OW. Rapid Communication: relative effect of urinary calcium and oxalate on saturation of calcium oxalate. Kidney Int. 2004;66:2032-7.
22. Robertson WG, Hughes H. Importance of mild hyperoxaluria in the pathogenesis of urolithiasis--new evidence from studies in the Arabian peninsula. Scanning Microsc. 1993;7:391-401.
23. Jonassen JA, Cao LC, Honeyman T, Scheid CR. Mechanisms mediating oxalate-induced alterations in renal cell functions. Crit Rev Eukaryot Gene Expr. 2003; 13:55-72.

Correspondence address:
D. Sujatha, MD
Assistant Professor,
Department of Pharmacology and Toxicology,
National Institute of Pharmaceutical Education and Research, Hyderabad, Andhra Pradesh, India
Fax: +91 040 2307-3751
E-mail: drsujathasai@gmail.com