



Control of urinary risk factors of stones by *Padina boergesenii* (Allander and Kraft), a brown algae in experimental hyperoxaluria

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Abstract

Objective: In view of the continued screening of seaweeds of the Gulf of Mannar for biological activity *Padina boergesenii*, the most common species along the Mandapam coast was identified to study the various biological effects. *Padina spp.*, exhibits diuretic effect hence, the antiurolithiatic effect of *Padina boergesenii* was tested in hyperoxaluric male albino rats. **Methodology:** Urolithiasis was induced in rats by feeding 3% glycolic acid along with pyridoxine deficient diet. The effect of the seaweed extract (ethanolic) at different doses was determined by comparing with the controls. **Results:** The ethanolic extract of *Padina boergesenii* at a dose of (150 & 200mg /kg p.o) significantly reduced the calcium excretion to normal level and significantly decreased oxalate excretion, thus reducing the risk of calcium oxalate super-saturation in urine as compared to the pyridoxine deficient control rats. The extracts also slightly elevated phosphorous, decreased uric acid and raised the magnesium excretion. Protein and creatinine elimination was effectively normalized by the extracts. **Conclusion:** The antiurolithiatic activity exhibited by the ethanolic extract is related to the chemical constituents in the algae which is discussed.

Key words: *Padina boergesenii*, brown algae, urolithiatic activity, pyridoxine, magnesium.

1. Introduction

Urolithiasis, the process of formation of stones in the kidney and the urinary tract, is the major clinical manifestation of hyperoxaluria.

Hyperoxaluria is caused due to two major pathogenic mechanisms - increased endogenous production of oxalate and increased intake or

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gastrointestinal absorption of oxalate. So, the treatment of hyperoxaluria should be aimed at reducing the oxalate excretion and thereby, diminishing stone formation. Species of the marine algal genus *Padina boergesenii* (Dictyotales, Phaeophyta) are good sources of alginic acid, mannitol, and iodine [1].

So far seven species *viz.*, *P.boergesenii*, *P. boyana*, *P.distromatica*, *P.dubia*, *P.glabra*, *P. pavonica* and *P.tetrastromatica* have been reported [2] from the Indian coasts. Among these *Padina boergesenii* (Allander & Kraft) is the most common species along the Gulf of Mannar coast especially around the Mandapam coast [3]. Species of *Padina* exhibit greater antimicrobial activity against both gram negative and gram positive bacteria [4, 5].

Diethyl extract of *Padina boergesenii* specifically inhibits the growth of *Staphylococcus aureus* [4]. *P.tetrastromatica* collected from Baga exhibits spasmogenic and infertility activity [6]. Likewise *P. gymnospora* (Kuetz) Vick., of Dwaraka exhibits diuretic effect [7]. In the continued screening of the seaweeds of the Gulf of Mannar for various biological activities, the present investigation was designed to explore the urolithiatic activity of the marine brown algae *Padina boergesenii* keeping in mind the diuretic effect exhibited by the *Padina* species.

2. Materials and Methods

The marine algae *Padina boergesenii* was collected in June 2001, from the inter-tidal region of the coastal waters around Mandapam coast. Algal samples were thoroughly cleaned to remove the epiphytes, quick washed with fresh water, dried in shade, powdered and the ethanolic extract was prepared using a soxhlet apparatus. The extract obtained was evaporated to dryness by a rotary evaporator and used for the animal experiments.

Antiurolithiasis activity of the alcoholic extracts of the seaweeds was evaluated in hyperoxaluria induced in adult wistar albino rats [8]. Adult wistar albino rats of either sex weighing 100 – 130 gm were divided into eight groups of six rats each. Group I (normal control) pyridoxine supplemented rats (PSR) were maintained on pellet feed (8 gm/day/rat) supplemented with pyridoxine (4 mg/kg pellet feed).

Group II pyridoxine deficient rats (PDR) were maintained on pyridoxine deficient diet containing 3 % glycolic acid for 21 days (8 gm diet/rat). Group III to group V were maintained on pyridoxine deficient diet and 100,150, 200 mg/kg b.w / day of seaweed extract was given for 21 days parentally.

At the end of the experimental period, the animals were housed in metabolic cages to collect 24 h urine sample. The urine was centrifuged and dialysed. The dialysate was used to estimate the stone forming constituents like calcium [9], magnesium [10], oxalate [11], phosphorous [12], uric acid [13] and other metabolites such as protein [14], creatinine [15] and urea [16]. The shade dried powdered seaweeds were subjected to chemical analysis [17,18] for the nutrients related to stone formation namely calcium, magnesium and pyridoxine. All quantitative measurements were expressed as mean \pm S.D. The results were assessed statistically by the Student's *t* - test.

3. Results

The ethanolic extracts of *Padina boergesenii* showed promising effect on calcium oxalate urolithiasis by decreasing urinary risk factors diminishing tubular dysfunction and preventing further damage to the renal tubules. Hyperoxaluric rats that maintained on pyridoxine deficient diet exhibited tremendous increase in calcium and oxalate excretion. The ethanolic extracts of *Padina boergesenii* (150 mg/kg and

Table.1

Effect of seaweed extracts on the urinary excretion pattern of stone forming constituents in pyridoxine deficient rats

Groups	Excretion (mg/24 h)			
	Oxalate	Calcium	Phosphorus	Uric acid
Control (Normal)	2.85 ± 0.14	0.29 ± 0.02	8.73 ± 0.14	1.41 ± 0.06
Control (Hyperoxaluric)	8.76 ± 0.45	0.95 ± 0.01	5.9 ± 0.21	1.89 ± 0.07
<i>Padina boergesenii</i> Extract (100 mg/kg)	6.53 ± 0.55	0.59 ± 0.02	6.1 ± 0.41	1.62 ± 0.02
<i>Padina boergesenii</i> Extract (150 mg/kg)	3.71 ± 0.18**	0.34 ± 0.02**	7.3 ± 0.32*	1.49 ± 0.04*
<i>Padina boergesenii</i> Extract (200 mg/kg)	4.3 ± 0.33*	0.39 ± 0.04*	6.8 ± 0.34*	1.54 ± 0.07*

Values are in mean ± S.E.; n=6 ; * P<0.001 vs Hyperoxaluric control; ** P<0.01 vs Hyperoxaluric control

200 mg/kg p.o) lowered the calcium excretion to normal level and significantly decreased oxalate excretion, thus reducing the risk of calcium oxalate super-saturation in urine as compared to the pyridoxine deficient control rats.

The extracts also slightly elevated phosphorous, decreased uric acid and raised the magnesium excretion. Protein and creatinine elimination were effectively normalized by the extracts. However the alcoholic extracts of *Padina boergesenii* at (100 mg/kg p.o) did not exhibit significant changes in the excretion pattern of the various stone forming constituents.

The nutrients related to the lithiatic activity analysed exhibits a considerable content of pyridoxine in *Padina boergesenii* (13.3mg/g). The magnesium content (278 ppm) is higher than calcium (180 ppm) in *Padina boergesenii*.

4. Discussion

Calcium oxalate crystals are formed due to hypercalcuria and hyperoxaluria. Calcium oxalate is highly insoluble in aqueous solution. Hence hyperoxaluria, which increases urinary saturation

with respect to calcium oxalate is an important risk factor for kidney stone formation. Pyridoxine (vitamin B₆) is a co-factor in the transamination of glyoxylate to glycine. Pyridoxine deficiency leads to hyperoxaluria probably, related to the shunting of glycolic acid to oxalate synthesis [19]. Likewise, oxidation of ascorbic acid accounts for a significant proportion of the endogenous production rate of oxalate.

Hence, mega doses of ascorbic acid (Vit. C) might lead to hyperoxaluria. This fact combined with increased urinary calcium leads to their super-saturation in urine and finally, formation of calcium oxalate stones

Padina boergesenii significantly reduced the risk of calcium oxalate nephrolithiasis by increasing the urinary volume, which results in reduction in calcium oxalate supersaturation in the urine. This might be due to the diuretic effect exhibited by *Padina boergesenii* [7]. Loading with pyridoxine represents an attempt to force the transamination of glyoxalate to glycine, thereby, decreasing the conversion of glyoxalate to oxalate.

Table.2

Effect of seaweed extract on the urinary excretion pattern of other constituents in control and experimental rats

Groups	Excretion (mg/24 h)			
	Magnesium	Protein	Creatinine	Urea
Control (Normal)	1.34 ± 0.08	14.51 ± 0.94	10.8 ± 0.86	22.3 ± 0.96
Control (Hyperoxaluric)	0.89 ± 0.03	18.24 ± 0.86	4.71 ± 0.21	26.4 ± 1.91
<i>Padina boergesenii</i> Extract (100 mg/kg)	1.02 ± 0.09	15.2 ± 0.11	6.93 ± 0.42	25.1 ± 0.54
<i>Padina boergesenii</i> Extract (150 mg/kg)	1.31 ± 0.07**	12.98 ± 0.64**	9.47 ± 0.46**	24.3 ± 1.24
<i>Padina boergesenii</i> Extract (200 mg/kg)	1.32 ± 0.09*	12.2 ± 0.81*	8.93 ± 0.41*	24.1 ± 0.14

Values are mean ± S. E.; n=6; * P<0.001 vs Hyperoxaluric control; ** P<0.01 vs Hyperoxaluric control
Extracts were administered at respective doses for 21 days.

The considerable content of pyridoxine in *Padina boergesenii* (13.3 mg/g) might also be a reason for the pronounced activity exhibited by this algae. Reduction in oxalate excretion as observed on treatment with triterpenes, indicates that these act by inhibiting some steps of oxalate synthesis from glycolic acid [20,21]. Seaweeds possess a number of terpenes, which are responsible for a number of biological activity.

Another factor, which plays an important role in the risk of urinary stone formation, is alteration in the 'inhibitory activity' in the urine. The only significant inhibitor of calcium oxalate crystallization is the acid mucopoly-saccharides namely hyaluronic acid, chondroitin sulfate, heparin sulfate and heparin. Sulphated polysaccharides (SPS) reported to have wide pharmacological properties [22] are commonly found in marine algae and higher animals.

The sulfated polysaccharides from marine algae are of highly diverse nature and there exists similarities between their structure with that of

heparin. Heparinoid active sulfated polysaccharides are present in some Indian marine algae [23]. The promising biological activity in urolithiasis might also be due to the heparinoid active sulphated polysaccharides present in the seaweed extracts.

Proteinuria reflects proximal tubular dysfunction [24], Crystal induced tubular damage to the kidney results in decreased creatinine excretion [25]. *Padina boergesenii* at a dose of 200mg/kg brought back the parameters favouring stone formation and tubular dysfunction to normal indicating the beneficial effect in urolithiasis.

Magnesium complexes with oxalate thus, reducing calcium oxalate supersaturation in urine and as a consequence, growth and nucleation rate of calcium oxalate crystals is decreased. Therefore, the hypomagnesuria observed in the pyridoxine deficient rats would increase the available oxalate for calcium binding thus, accelerating calcium oxalate crystal deposition [26]. Magnesium increases the amount of vitamin B₆, which is able to penetrate into the cells.

Likewise, vitamin B6 increases the bio-availability of magnesium. The synergistic effect exhibited by both the vitamin and the mineral element might be the reason for the promising activity exhibited by *Padina boergesenii*. This has further being confirmed by the strong correlation exhibited by the vitamin and the metal.

A general assumption has been made that bioactivity displayed by a particular alga is due to one or few chemical entities [27]. Hence apart from the nutrients the secondary metabolites contributing to the antilithiatic activity should

be isolated and characterized for better understanding of the mechanism of action of this seaweed.

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