EFFECT OF ETHANOLIC FRUIT EXTRACT OF *Pedalium murex* Linn. IN ETHYLENE GLYCOL INDUCED UROLITHIASIS IN MALE WISTAR ALBINO RATS

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ABSTRACT

The ethanolic fruit extract of Pedalium murex to ethylene glycol intoxicated rats reverted the levels of the liver and kidney markers to near normal levels protecting liver and renal tissues from damage and also prevents the crystal retention in tissues. The levels of ACP, ALP, AST, ALT in serum and urine were significantly increased due to the damaged structural integrity of renal and hepatic cells causing the enzymes which are located in the cytoplasm to be released into the circulation. The levels of ACP and ALP, AST, ALT in renal and hepatic tissues of ethylene glycol induced rats might be due to leakage of the enzyme into the general circulation from the collateral circulation.LDH levels in serum, urine and tissues were increased on ethylene glycol intoxication is due to the oxalate induced renal and hepatic cellular damage.

Key words: *Marker enzymes, urolithiasis, ethylene glycol, Pedalium murex*

INTRODUCTION

Urolithiasis or urinary calculi or stones refers to calcifications that form in the urinary system, primarily in the kidney called nephrolithiasis or ureter is called ureterolithiasis, and may also form in or migrate into the lower urinary system like bladder or urethra (Bernier, 2005). Urinary tract stone disease has been documented historically as far back as the Egyptian mummies (Wolf, 2004).The experimental intoxication induced by ethylene glycol is widely used for kidney stone formation in rats. When ethylene glycol is metabolized by the body, it produces four toxic metabolites: glycoaldehyde, glycolate, and glyoxylate. These metabolites cause tissue destruction, primarily from calcium oxalate crystal deposition, and metabolic abnormalities, specifically a high anion-gap metabolic acidosis, lactic acidosis, and hypocalcemia. Oxalic acid combines with calcium to form calcium oxalate crystals, which deposit in the kidneys. This can result in hematuria, and proteinuria, increased creatinine and renal failure (Brent, 2001).

Many developing countries including China use herbal medicines which have gained popularity in Europe and are becoming increasingly in the United States as well. As far as urolithiasis is concerned, several herbal treatments seem to cure lithiasis in patients (Atmani, 2003).

The recent resurgence of plant remedies results from several factors like effectiveness of plant medicines and lesser side effects compared to modern medicines. In the present scenario, the need for basic scientific investigations on medicinal plants used in the indigenous systems becomes imminent. This is evident by the increase in number of reports by various investigators supporting the claims of medicinal plants and a dramatic increase in the share of plant products in pharmaceutical market. The present review aims to give data highlighting the present trends in research of medicinal plants accredited with antiurolithiatic activity. This may help investigators to identify and develop appropriate lead compounds or plant products beneficial in the management of urolithiasis (Prasad *et al*., 2007).

Materials and Methods

Collection of plant materials

Pedalium murex Linn. Fruits was collected from Senbagathoppu area of virudhunagar district, Tamil Nadu, during the month of September to November, 2009. The plant was identified and authenticated by Taxonomist Dr.K. Arumugasamy, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu. Voucher specimen was deposited at herbarium collection in the Department of Botany, Kongunadu Arts and Science College, Coimbatore.

Preparation of the ethanolic fruit extract

Fruits of *Pedalium murex* were washed and then shade dried, powdered and stored in tight containers under refrigeration. 100g of *P. murex* powder was taken in a conical flask. To this 500ml of 99% ethanol was added. The content of the flask was soaked over night. This suspension was filtered and residue was resuspended in an equal volume of 99% ethanol for 48 hours and filtered again. The two filtrates were pooled and the solvents were dried in an oven at 37ºC and a crude residue was obtained. The yield was 18g and the residue was suspended in water and administered orally to experimental rats at the rate of 1.0ml/day.

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Selection of animals for toxicity studies

Healthy adult male wistar albino rats weighing about 150 to 200 g were collected from Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hours light and dark cycle at 28°C ± 2° C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the institutional animal ethics committee (IAEC).

Experimental induction of urolithiasis

Calcium oxalate stones were induced in experimental animals 0.75% (0.75ml of ethylene glycol in 100 ml of drinking water) of rats for a period of 28 days for the production of calcium oxalate stone in rats.

Experimental design of animals

The rats were divided into four groups of six animals each as given n the table 1.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages and urine was collected for 24 hours. Urine was freed from faecal contamination. Rats were provided with water but no feed. Urine collected in 50 ml beaker maintained at 0°C in an ice bath. The collected urine samples were centrifuged for 10 minutes and any sediment present was discarded. The urine was used for further analysis.

Collection of serum sample

After the experimental regimen, the animals were sacrificed by cervical decapitation under light ether anesthesia. Blood was collected and centrifuged for 10 min. at 2500 rpm. The serum supernatant was collected and then diluted with water in the ratio of 1:10. Aliquots of the diluted serum were then used for the determination of serum constituents and serum enzymic activities.

Collection of liver and kidney samples

The experimental animals were sacrificed, liver and kidney were removed immediately washed with ice cold saline. 10% tissue homogenate was prepared by homogenizing 1.0g of chopped liver or kidney tissue in 10ml of 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for assaying the enzyme activities.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Estimation of biochemical markers

The serum, urine and tissue homogenate was used to assay the marker enzymes in serum, urine and tissue constituents like ACP, ALP, AST, ALT and LDH according to the method of King (1965a), King & Armstrong (1934), Reitman & Frankel (1957), Reitman & Frankel (1957) and King (1965b) respectively.

Experimental design

Group	Experimental design
I	**Control rats**- received normal pelleted diet.
II	**Ethylene glycol intoxicated rats** - Urolithiasis was induced by administrating 0.75% (0.75ml ethylene glycol + 100 ml drinking water) of rats for a period of 28 days for the production of calcium oxalate stone in rats
III	**Fruit extract -** Urolithiasis induced rats received ethanolic fruit extract of *P.murex* (250 mg / kg body wt) by oral administration for 28 days at a rate of 1.0 ml / rat / day.
IV	**Standard drug thiazide treated rats -** Urolithiasis induced rats received thiazide (150µg/ kg body wt) by oral administration for 28 days at the rate of 1.0 ml / rat / day.

Table 1

Effect of ethanolic fruit extract of *P.murex* on marker enzymes in serum of control and experimental rats

Groups	ACP[#]	ALP[#]	AST[$]	ALT[$]
I	66.05 ± 0.96	72.69 ± 0.17	31.57 ± 0.22	32.73 ± 0.41
II	116.15 ± 0.60a*	157.44 ± 0.95a*	112.80 ± 0.57a*	95.60 ± 0.74a*
III	72.36 ± 0.61b*	74.40 ± 0.09b*	32.40 ± 0.46b*	33.20 ± 0.54b*
IV	73.94 ± 0.53c*d^{ns}	75.02 ± 0.40c* d^{ns}	32.82 ± 0.46c* d^{ns}	35.34 ± 0.61c* d^{ns}

Values are expressed as mean ± SD of six animals (n = 6).

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. The data obtained from acute toxicity studies was analyzed using Student's paired t-test. P values less than 0.05 were considered significant

Results

The effect of ethanolic fruit extract of *Pedalium murex* on ACP, ALP, AST and ALT in serum, urine and tissue homogenate of control and experimental rats are shown in the table 2, 3 &4. From the table it was evident that the levels of these enzymes were significantly increased ($P<0.05$) in serum and urine whereas in liver and kidney the levels were significantly decreased ($P<0.05$) of ethylene glycol intoxicated (group II) rats. The treatment (group III) with *P. murex* extract after ethylene glycol induction showed a significant ($p<0.05$) decrease in the activity of these enzymes in serum and urine when compared to group II rats. As evident from the above results, the ethanolic fruit extract of *P.murex* seems to contain antiurolithiatic activity. However, when the standard drug, thiazide treated rats (group IV) were compared with ethanolic fruit extract administered group (group III),there is no significant difference ($p<0.05$) was observed between these two groups of animals. This indicates that the hepato, nephroprotective and antiurolithiatic activity of ethanolic fruit extract of *P.murex* was comparable with the standard drug thiazide, a commercially available drug for treating kidney stones.

Experimental design

Group I : **Control rats** - received normal pelleted diet.

Group II: **Ethylene glycol intoxicated rats** - Urolithiasis was induced by administrating 0.75% (0.75ml ethylene glycol + 100 ml drinking water) of rats for a period of 28 days for the production of calcium oxalate stone in rats.

Group III: **Fruit extract treated rats** - Urolithiasis induced rats received *P.murex* fruit extract (250 mg / kg body weight) by oral administration for 28days at a rate of 1.0 ml / rat / day.

Group IV: **Stadard drug thiazide treated rats** - Urolithiasis induced rats received thiazide (150 µg / kg body weight) by oral administration for 28 days at a rate of 1.0 ml / rat / day.

Comparison between the groups

'a' represents comparison between group II and group I

'b' represents comparison between group III and group II

'c' represents comparison between group IV and group II

'd' represents comparison between group III and group IV

The symbols represent statistical significance $p^* < 0.05$,

ns - not significant

Units

[#] µ moles of phenol liberated / L

[$] µ moles of pyruvate liberated / L

Table 2

Effect of ethanolic fruit extract of *P.murex* on marker enzymes in urine of control and experimental rats

Groups	ACP$^{###}$	ALP$^{###}$	AST$^{\$\$\$}$	ALT$^{\$\$\$}$
I	0.48 ± 0.01	6.82 ± 0.11	1.77 ± 0.19	2.73 ± 0.20
II	0.77 ± 0.01a*	9.67 ± 0.18a*	5.27 ± 0.28a*	5.69 ± 0.32a*
III	0.53 ± 0.01b*	6.90 ± 0.18b *	1.84 ± 0.21b*	2.79 ± 0.19b*
IV	0.56 ± 0.02c*d^{ns}	6.96 ± 0.20c* d^{ns}	1.99 ± 0.11c* d^{ns}	2.85 ± 0.18c* d^{ns}

Values are expressed as mean ± SD of six animals (n = 6)
Experimental design and statistical comparisons s are as in table 1.
The symbols represent statistical significance p* < 0.05, ns - not significant.

Units

$^{###}$µ moles of phenol liberated / 24 hr urine.
$^{\$\$\$}$ µ moles of pyruvate liberated / 24 hr urine.

Table 3

Effect of ethanolic fruit extract of *P.murex* on marker enzymes in liver of control and experimental rats

Groups	ACP$^{##}$	ALP$^{##}$	AST$^{\$\$}$	ALT$^{\$\$}$
I	16.61 ± 0.29	11.95 ± 0.11	17.83 ± 0.19	17.66 ± 0.22
II	9.66 ± 0.19a*	8.47 ± 0.16a*	11.80 ± 0.22a*	12.66 ± 0.16a*
III	16.54 ± 0.19b*	11.88 ± 0.19b *	17.60 ± 0.26b*	17.44 ± 0.22b*
IV	16.42 ± 0.24c*d^{ns}	11.73 ± 0.31c* d^{ns}	17.59 ± 0.30c* d^{ns}	17.35 ± 0.19c* d^{ns}

Values are expressed as mean ± SD of six animals
Experimental design and comparison between the groups are as in table 1
The symbols represent statistical significance p* < 0.05, ns – not significant

Units

$^{##}$µ moles of phenol liberated / min / mg protein.
$^{\$\$}$µ moles of pyruvate liberated / min / mg protein.

Table 4

Effect of ethanolic extract of *P.murex* on marker enzymes in kidney of control and experimental rats

Groups	ACP(K)$^{\#\#}$	ALP(K)$^{\#\#}$	AST(K)$^{\$\$}$	ALT(K)$^{\$\$}$
I	11.72 ± 0.18	6.51 ± 0.26	11.55 ± 0.21	12.57 ± 0.15
II	8.54 ± 0.28a*	3.66 ± 0.27a*	6.72 ± 0.23a*	9.71 ± 0.15a*
III	11.69 ± 0.24 b*	6.49 ± 0.22b *	11.53 ± 0.33b*	12.31 ± 0.20b*
IV	11.59 ± 0.10c* d^{ns}	6.25 ± 0.12c* d^{ns}	11.25 ± 0.47c* d^{ns}	12.22 ± 0.20c* d^{ns}

Values are expressed as mean ± SD of six animals
Experimental design and comparison between the groups are as in table 1
The symbols represent statistical significance p* < 0.05, ns – not significant

Units

$^{\#\#}$µ moles of phenol liberated / min / mg protein
$^{\$\$}$ µ moles of pyruvate liberated / min / mg protein

Table 5

Effect of ethanolic fruit extract of *P.murex* on lactate dehydrogenase in control and experimental rats

Groups	LDH(S)$^{\#}$	LDH(L)$^{\#\#}$	LDH(K)$^{\#\#}$	LDH(U)$^{\$\$\$}$
I	145.64 ± 0.42	1.86 ± 0.10	3.71 ± 0.16	132.96 ± 0.43
II	206.00 ±0.51a *	3.76 ±0.15a *	7.73 ±0.15a *	195.91 ±0.43a *
III	147.60 ± 0.31b*	1. 99 ± 0.32b*	3.87 ± 0.07b*	136.69 ± 0.98b*
IV	147.69 ± 0.20c* dns	2.03 ± 0.18c* dns	3.93 ± 0.09c* dns	137.01 ± 0.74c* dns

Values are expressed as mean ± SD of six animals (n =6)
Experimental design and comparison between the groups are as in table 1
The symbols represent statistical significance p* < 0.05, ns – not significant

Units

$^{\#}$µ moles of phenol liberated / L
$^{\#\#}$µ moles of phenol liberated / min / mg protein
$^{\$\$\$}$ µ moles of pyruvate liberated / 24 hr urine

Table 5 represent the levels of lactate dehydrogenase levels in the serum,urine and tissue homogenate. Levels of these enzymes were significantly increased in serum, urine, liver and kidney of ethylene glycol intoxicated (group II) rats. LDH is an oxalate synthesizing enzyme; its activity was increased on ethylene glycol administration. It was released into the blood serum and urine. This may be attributed to oxalate induced renal and hepatic cellular damage. Renal damage is particularly confined to the proximal tubule, a part of the nephron closely involved in handling urinary oxalate (Hackett *et al*., 1990). Further damage to proximal tubular epithelium is generally associated with the shedding of brush border membrane thereby causing crystal retention (Khan *et al*., 1989). The treatment (group III) with *P. murex* fruit extract after ethylene glycol induction showed a significant ($p<0.05$) decrease in the activity of these enzymes in serum, urine and tissue homogenate when compared to group II rats. As evident from the above results, the ethanolic fruit extract

of *P. murex* seems to contain antiurolithiatic activity. However, when the standard drug, thiazide treated rats(Group IV) were compared with ethanolic fruit extract administered group (group III), no significant difference ($p<0.05$) was observed between these two groups of animals. This indicates that the hepato, nephroprotective, antiurolithiatic activity of ethanolic fruit extract of *P.murex* was comparable with the standard drug thiazide, a commercially available drug for treating kidney stones.

Discussion

Increased activities of serum and urine AST and ALT levels in ethylene glycol intoxicated rats were observed. This can be attributed to the damaged structural integrity of the renal and hepatic cells causing the enzymes which are located in the cytoplasm to be released into the circulation (Senthilkumar *et al.,*2003).If membrane of other organelles such as mitochondria is damaged, soluble enzymes such as compartmentalized AST will also released. The release of these enzymes into the circulation will indicate both plasma and organelle membrane damage.

The above results are in agreement with the findings of Farooq *et al*. (2004), who reported that serum ACP and ALP levels were increased due to administration of oxalate and their levels were maintained near normal in phycocyanin supplementation.

Reduced, ACP and ALP, AST, ALT activities in renal and hepatic tissues of ethylene glycol induced rats might be due to leakage of the enzyme into the general circulation from the collateral circulation. The stone formation may acclude the ureter, leading to an increase in back pressure in the renal pelvis and because of ischeamia, may ultimately damage the tubular cells (Thind and Nath 1978).

The above results are in agreement with the findings of Poonkuzhali *et al*. (1994) marker enzymes and their restoration to near normal levels by uric acid administration to sodium glycolate fed urolithiatic rats who reported that kidney and liver marker enzymes were decreased and their restoration to near normal levels by uric acid administration to sodium glycolate fed urolithiatic rats.

LDH is an oxalate synthesizing enzyme; its activity was increased on ethylene glycol administration. It was released into the blood serum and urine. This may be attributed to oxalate induced renal and hepatic cellular damage. Renal damage is particularly confined to the proximal tubule, a part of the nephron closely involved in handling urinary oxalate (Hackett *et al*., 1990). Further damage to proximal tubular epithelium is generally associated with the shedding of brush border membrane thereby causing crystal retention (Khan *et al*., 1989).

Varalakshmi *et al*. (1990) has showed a curative effect of Betulin from the plant *Crataeva nurvula* which is used to minimize the renal tubular damage and normalized the renal marker enzymes like LDH by decreasing crystal deposition in kidneys.

In conclusion, our findings clearly state that the ethanolic fruit extract of *Pedalium murex*, is a potent hepato and nephroprotective agent by preventing the crystal retention in the tissues and can also be used as an antiurolithiatic agent .

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