International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-3, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of Anti Diuretic and Antiuroliathiatic Activity of Methanolic Leaf Extract of *Pandanus Fascicularis* on Rats

Shakti Prasanna Sahoo[*1], N. Sucharitha[1], S. Ramachandran[1], M.D. Dhanaraju[1]

[1]Department of Pharmacology, GIET School of Pharmacy, Chaitanya Knowledge City, Rajahmundry, Andhra Pradesh, India.

Manuscript No: IJPRS/V2/I3/00148, Received On: 11/09/2013, Accepted On: 17/09/2013

ABSTRACT

*Pandanus fascicularis Lam. (*pandanaceae) is claimed as ananti-diuretic by some traditional practitioners. Purpose of the study was to evaluate the anti-diuretic and anti urolithiatic activity of methanolic extract of *P.fascicularis.* The present study was undertaken to evaluate the anti-diuretic and anti urolithiatic activity in ethylene glycol induced urolithiasis and furosemide induced diuresis. Vasopressin was used as standard for anti-diuretic activity and cystone was used as standard for anti urolithiatic activity. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and oxalate. In furosemide induced diuresis there was increase in urinary output and renal excretion of urinary electrolyte concentration. The methanolic extract of *P.fascicularis* significantly lowered the stone forming constituents in the kidney of calculogenic rats and also decreased urinary output and urinary volume and urinary electrolyte concentration in furosemide induced diuretic rats. Supplementation with methanolic leaves extract of *P.fascicularis* significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The results indicate that the methanolic leaves extract of *P.fascicularis* is endowed with anti-diuretic and anti urolithiatic activity.

KEYWORDS

Pandanus fascicularis lam, antidiuretic, antiurolithiatic, methanol extract, furosemide, cystone.

INTRODUCTION

Anti-diuretic hormone release helps to maintain the optimum amount of water in the body, when there is an increase in the concentration of blood serum or decrease in blood volume, physical stress, surgery and high levels of anxiety can also stimulate ADH[1]. ADH (Anti-diuretic hormone) helps maintain water balance in the blood by making the membranes of the distal tubule and collecting ducts in the nephron of kidneys more permeable to water.

***Address for Correspondence:**
Shakti Prasanna Sahoo
Associate Professor, Department of Pharmacology,
GIET School of pharmacy, Rajahmundry,
Andhra Pradesh, India.
E-Mail Id: sahoo.shakti@gmail.com

Various factors such as the ethanol consumption reduces ADH production in a temporarily increase in the production of urine. This may also occur in diabetes insipidus, when the pituitary gland produces insufficient ADH, or rarely, when the kidneys fail to respond to ADH[2].Urolithias is a common chronic disorder in humans and the most common type of renal stone is made up of calcium oxalate[3]. Pathophysiologically, urolithiasis occurs whenever the urine becomes super saturated with insoluble material, because excretion rates are excessive and/or reduced water conservation, crystals are formed, grow and aggregate to form a stones. The impact of these

stones increases by many folds in the presence of other complaints like hypertension, obesity hepatic dysfunction etc[4,5].

Pandanus fascicularis Lam commonly referred as screw pines are palm like evergreen trees or shrubs belong to the genus-pandanus, order-pandanales, class-Lilliopsida, and division-mangliophyta. Pandanus comprises 500-600 species and is distributed mainly in subtropical and tropical region. *P.fascularis* is native to south Asia and has a significant particularly in mangrove swamps. Indian pandanaceae in particular are characterized by having good fiber content, fragrant spadix and edible fruits in some species. Inner parts of leaves are eaten as vegetables[6]. In present study investigation methanolic leaves extract of *p. fascicularis* was used to evaluate anti urolithiatic and anti diuretic activity in ethylene glycol induced urolithiasis and furosemide induced diuresis.

MATERIALS AND METHOD

Collection of the Plant Material and Authentication

Pandanus fascicularis a branched leaves collected from the Tirupathi region, A.P.India, in January 2013. It was confirmed by Dr. K. Madhavachetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupathi. The plant is very much well known and widely distributed throughout India especially in weed areas.

Selection of Animals

Wistar rats (150-200g) were obtained from GIET School of Pharmacy animal house. Animals were housed under standard condition of temperature ($24^0\pm1^0$c), relative humidity (65±10%), light & dark cycle (12:12h) and fed with standard pellet food and water adlibitum.

Preparation and Route of Drug Administration

The extract was prepared in 1% (w/v) CMC as a uniform suspension using mortar & pestle. The dosages of the suspension were adjusted so that maximum volume of the drug administered was within the range of 1ml/100g. Reference drugs, methanolic extract of *Pandanus fascicularis* plants are administered orally to rats to all experiments.

Institutional Ethics Committee Approval

The experimental design & research plan along with animals handling and disposal procedure were placed before the institutional ethics committee. The committee granted approval after carefully evaluating research project during their meeting held in January 2012. Animal house registration no 1069/PO/ac/07/CPCSEA

Preparation of Extract

P. fascularis plant was dried in shade and powdered. Methonalic extract was prepared by extracted with methanol 95% v/v in soxhlet apparatus. The extract was evaporated to dryness under vaccum and dried in vacuum desiccators.

Drugs and Chemicals

Furosemide (20mg/kg)(diuretic) and vasopressin (antidiuretic), potassium chromate indicator, silvernitratre, Ethylene glycol(0.75%) cystone (750mg/kg).

Pharmacological Study on Diuretic Activity

Different groups of rats were used to study the effect of methnolic extract of *Pandanus fascicularis*. Diuresis has been induced to all groups of animals by Furosemide 20mg/kg. The animals were divided into four groups (I–IV) each of six animals: group (I) received with normal saline solution (25ml/kg), group (II) received vasopressin (3-5 milli unit), group (III) and group IV received the methanolic extract of *P. fascicularis* at 250mg/kg body weight, respectively. After oral administration, each animal was placed in an individual metabolic cage specially designed to separate faeces and urine at room temperature. The volume of urine collected was measured at the end of 5 hrs and the total urine volume and concentrations of Na+, K+, and Cl− in the urine were determined. The concentrations of the electrolytes in urine were expressed in terms of mmol/L and the urine volume was expressed in ml/5hr. Na+ and

K+ concentrations were measured by Flame photometer and Cl− concentration was estimated by titration with silver nitrate solution (N/50) using 3-5 drops of 5% potassium chromate as an indicator[7,8].

Estimation of Urine Output

Metabolism cage is designed with a plastic circular frame. The upper portion is covered with a lid, which has several holes to allow ventilation. The cage rests on a plastic funnel. The whole structure is fixed to a metal frame in upright position. A plastic fine mesh is placed at the exit of the funnel, which prevents the contamination of the urine with faecal matter. Conical flask is kept to collect the urine, at the bottom exit of the funnel for a period of 5 hours[9].

Ethylene glycol Induced Urolithiasis Model

Ethylene glycol induced hyperoxaluria model (Atmani et al., 2003) was used to assess the antilithiatic activity in albino rats. Animals were divided into four groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II–IV induction of renal calculi till 28th day. Group III received standard antiurolithiatic drug, cystone (750 mg/kg body weight) from 15th day till 28th day (Mitra et al., 1998). Group IV received methonolic extract (200 mg/kg) body weight for 15th day till 28th day. All extracts were given once daily by oral route.

Measurement of Anti Uroliathiatic

Assessment of Anti Urolithiatic Activity Collection and Analysis of Urine

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4◦C. Urine was analysed for calcium and oxalate content[10,11].

RESULTS AND DISCUSSION

Diuretics are used to relieve pulmonary congestion, edema, hypertension, volume over load and peripheral edema. This decreases the cardiac work and play important role in the treatment of hypertension. These drugs inhibit symports, antiports and ion channels in nephron and increase the urine and electrolyte excretion. But most of the diuretics produce Hypokalemisea adverse effect. To overcome this plants are best choice. *Pandanus fascicularis* administration decreases the urine output, sodium, potassium and chloride excretion. The present study establishes the antidiuretic activity of the methanolic leaves extracts in rats. The results of different anti diuretic parameters are shown in Table 1. Methanolic extract treated animals significantly ($p < 0.05$) decreased the urinary output (2.5ml) and electrolytic excretion of Na+ , K+ and Cl– when compared to control.

The formation of urinary tract stones is world-wide, sparing no geographical, cultural or phosphate, are forming the most common urolithiasis accounting for more than 80% of the stones[12]. The mechanisms involved in the formation of calcific stones are not fully understood but it is generally agreed that urinary lithiasis is a multifaceted process involving events leading to crystal nucleation, aggregation and growth of insoluble particles[13]. In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male wistar rats resulted in hyperoxaluria. Oxalate, calcium excretion were grossly increased in calculi-induced animals (Table 2). However, supplementation with methanolic leaves extract of *p. fascicularis* significantly lowered the elevated levels of oxalate, calcium in urine in compared to control.

According to the survey carried out in the plant of *Pandanus* group, they have healthful properties including antidiabetic, anti-inflammatory, antiviral, anti-allergy, antiplatelet, antioxidant and antitumor[14].

Table 1: Estimation of Na^+, K+, Cl^- ions by flame photometry(ELICO CL22D) and titration with silver nitrate solution using 3 to 5 drops of 5% potassium chromate indicator.

Design of treatment	Dose	Urine volume(ml/rat)	Electrolyte Excretion		
			Na	K	Cl
Control	25ml/kg	0.68±0.06	68.83±0.40	68.95±0.32	67.65±0.43
Vasopressin	3-5 milliunits	1.20±0.08*	52.83±0.47*	52.50±0.42*	52.50±0.42*
Methanolic extract	250 mg/kg	1.16±0.11**	59.33±0.66**	58.50±0.76**	57.67±0.66**

**P<0.05- significant with control, **P<0.05-significant with standard*

Parameters	Control	Cystone treated	Methanol extract
Urine calcium	0.54±0.02	0.56±0.03*	0.20±0.013*
Oxalate	0.17±0.004	0.28±0.02**	0.29±0.01**

*Values for urine parameters are assessed in 24hr urine sample. All values are expressed as mean±SEM, for six animals in each group, *P<0.05- significant with control, **P<0.05significant compared with control.*

The role of vasopressin as the principal factor regulating renal water handling is supported by experience with ADH receptor antagonists. The antidiuretic effects of methanolic extract of leaves causes decrease in both water excretion and excretion of sodium and potassium. In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and also earlier studies have shown that the amount of stone deposition in female rats was significantly less. Urinary super saturating with respect stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (0.75%, v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalates. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention of oxalate. This alteration was significantly reversed in the *p. fascicularis* treated group and shows that it has significant anti urolithiatic activity.

CONCLUSION

In conclusion the sub-acute treatment of *P. fascicularis* on ethylene glycol induced urolithiasis animals showed significant decrease in stone forming constituents (Fig-1 and Fig-2)

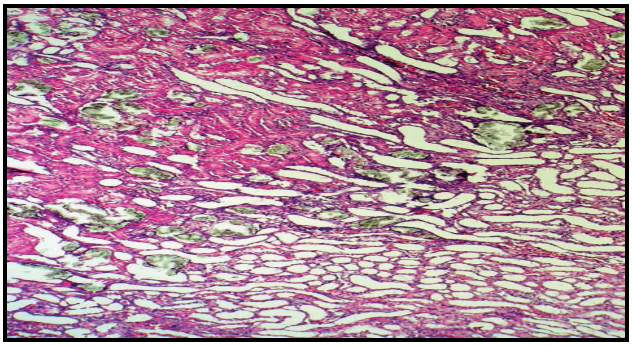

Figure 1: Histopathologic appearances of many stones in an ethylene glycol-treated rat (X 100, Hematoxylin and Eosin).

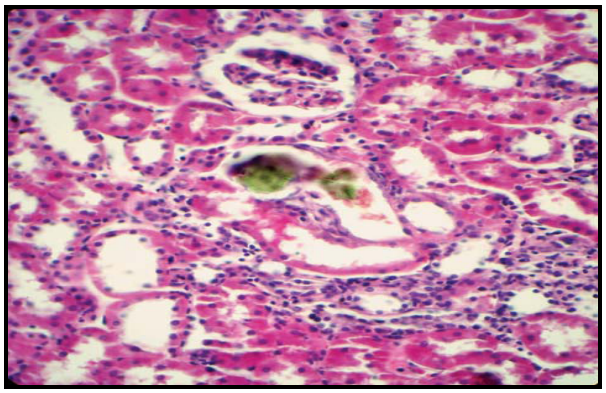

Figure 2: Histopathologic appearances of one cortical tubule in *P. fascicularis* extract-pretreated rat (X 200, Hematoxylin an Eosin).

as well as significant decrement in urinary output, urine volume and urinary electrolyte excretion on furosemide induced diuresis. Based on the results methanolic leaves extract of *P. fascicularis* at a dose of 250mg/kg were effective and exhibited potent anti-diuretic and anti urolithiatic activity. The present study also provide basis for traditional use in treatment of diabetic inspidus.

ACKNOWLEDGEMENTS

The authors sincerely thank the management of GIET School of Pharmacy, Chaitanya Knowledge City, Rajahmundry, 533296, Andhra Pradesh, India, for providing the necessary facilities for carrying out this work.

REFERENCES

1. Jyothimani R, Karthikeyan K, Balasundaram. "Anti-diuretic activity of aqueous and ethanol extract of prop roots of pandanus fascicularis lam", International journal of pharmaceutical sciences and research, 2012, 3(4), 1061-1064
2. Pagana, Kathleen Deska. Mosby's Manual of Diagnostic and Laboratory Tests. St. Louis: Mosby, Inc., 1998.
3. Duffy JC, Dearden JC, Rostron C, "Design, Synthesis and biological testing of a novel series of anti- inflammatory drugs", J Pharm Pharmacol, 2001, 53, 1505-1515.
4. Yasin Bayir, zekai halisi, suat colak. Helichrysum plicatum DC sub sp. Plicatum extract as a prevent agent in experimentally induced urolithiasis model, Journal of Ethnopharmacology, 2011, 138, 404-408.
5. Leonetti F, Lechevallier E, Dussol B, Berland Y. Lithiase Urinaire—La Revue du Praticien, 1996, 26, 1557–1567.
6. Economical Importance of Indian pandanus Species-Springer, page 3of 10.
7. Ediriweera ERHSS and Ratnasoorya WD, "Anti diuretic effect of scoparia dulcis in rats", J Trop Med Plants, 2002, 3, 55-58.
8. Patel U, Kulkarni M, "Evaluation diuretic activity of methanolic extracts of lepidiem sativum graden cress in rats", Tropical journal of pharmaceutical sciences 2009; 8(3) 215-219.
9. Aruna D, Chakarvarthy K, Sarath Babu, "Evolution of Diuretic activity of cynodon dactylon in rats with comparision of hydrochlorothiazide", International Journal of Research in pharmaceutical and biomedicine sciences.ISSN:2229-3701.
10. Ravindra V. Karadi, Navneet B, Gadge, V. Savadi, "Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats", Journal of Ethno pharmacology, 2006, 105, 306-311.
11. Anil T, Patwari, GD, "Effect Terminalia chebula fruit extract on ethylene glycol induced urolithiasis in rat", Biomedicine and aging pathology, 2012, 2, 99-103.
12. Moe OW, "Kidney stones: Pathophysiology and medical management", Lancet, 2006, 367, 333-344.
13. Baumann JM, "Stone prevention: Why so little Progress?", Urol Res, 1998, 26, 77-81.
14. Jong TT, Chau SW, "Antioxidative activities of constituents Isolated from pandanu sdoratissimus", phytochemistry, 2000, 40, 2145-2148.

