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In-Vitro Anti-Urolithiatic Evaluation of Tamsulosin against Urolithiasis Induced in Wistar Albino Rats

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Abstract

Urolithiasis is formation of stony concretions in the kidney or bladder or in any part of urinary tract and stone recurrence is most frequently observed. Literature reveals there is no drug or treatment available to inhibit the formation of these caliculi. The present study aims at evaluating the anti-urolithiatic activity of tamsulosin on experimentally induced urolithiasis on wistar albino rats. Urolithiasis was induced by oral administration of 1% v/v ethylene glycol in drinking water for 15 days in rats. Ingestion of Ethylene glycol results in hypercalcemia, hyperoxaluria, increased excretion of creatinine, phosphate and uric acid. Oral administration of Tamsulosin at doses of 0.5 mg/kg and 1 mg/kg for 7 days from 8th day to 15th day served as curative regimen significantly reduced the elevated levels of calcium, phosphate, creatinine and uric acid levels. Animals belonging to standard group received Allopurinol 500 mg/kg, p.o. for a period of 7 days 8th day to 15th day. Parameters such as urine volume, pH, urinary calcium output, serum creatinine, serum uric acid and calculi weight were measured at the end of the study. The histopathological studies were performed by sacrificing the animal; these represented disrupted renal parenchymal cells, degenerative changes in glomeruli, calcification in glomerulo tubular structures and microcrystal deposition in the sections of kidney from animals treated with ethylene glycol. In animals treated with Tamsulosin, reduction in calcium, phosphate, creatinine and uric acid levels, microcrystal deposition and calcification in glomerulo tubular structures was less compared to urolithiatic control group. These observations made us conclude that Tamsulosin showed anti-urolithiatic activity against ethylene glycol induced urolithiasis in rats.

Keywords: Urolithiasis; Tamsulosin; Ethylene glycol; Allopurinol

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Introduction

Urolithiasis is formation of renal stones and more prevalent disorder of the urinary system. Urinary calculi, if untreated, may cause extreme obstruction, hemorrhage in the urinary system associated with the risk of acute renal injury impacting renal function [1]. The symptoms include obstruction to urine flow, renal colic, person suffers pain radiating from the flank to the groin, inner thigh and genitals, restlessness, nausea, vomiting, sweating and hematuria, if untreated, it leads to sarcoidosis, thyrotoxicosis, and vitamin D toxicity, granulomatous diseases like histoplasmosis. Moreover, an increase in renal calculi recurrence is most frequently encountered [2].

Occurrence of urolithiasis is high in recent days; most of the urinary calculi are calcium oxalate crystals alone or mixed with calcium phosphate, struvite or cystine. Epidemiological studies revealed that the nephrolithiasis is more prevalent in men (12%) than in women (6%) and is more prevalent between the ages of 20 to 40 in both sexes [3].

Causes of stone formation include inadequate drinking of water, systematic dehydration, high dietary intake of calcium, sodium, oxalate, magnesium, sugars like glucose, fructose, vitamin C, corn syrups, vitamin abnormalities like vitamin A deficiency, vitamin D deficiency. Other possible reasons include excess intake of alcohol, obstruction to urine flow by microbial infections or foreign substances. Rare cases include certain drugs like loop diuretics, acetazolamide, ephedrine, ciporofloxacin and products with silica and sulfa medications [4].

Kidney stones can be as small as a grain of sand or as large a pearl. Formation of stone is a complex process, involving crystalline phase (formation of a crystalline or mineral) and non-crystalline phase (kidney stone matrix). There are three main issues contributing relevant to crystallization: super saturation of urine with respect to ions (such as calcium and oxalate), crystal nucleation, crystal growth and aggregation which further results in precipitation of certain substances within the urine.

Table 1: Urine analysis.

Group	Urine volume	pH	Urinary calcium in mg/dl	Urinary phosphate in mg/dl
Group 1	3.1 ± 0.21	6.2 ± 0.21	6.7 ± 0.12	3.78 ± 0.19
Group 2	2.2 ± 0.12	4.1 ± 0.19	9.4 ± 0.37	4.79 ± 0.26
Group 3	2.6 ± 0.21	6.4 ± 0.24	8.2 ± 0.23	3.2 ± 0.88
Group 4	2.8 ± 0.26	6.8 ± 0.18	7.9 ± 0.82	2.8 ± 0.22
Group 5	4.6 ± 0.17	7.0 ± 0.15	6.4 ± 0.74	2.9 ± 0.52

Values are given as mean ± standard deviation, all the values in the column are statistically significant ($p<0.05$)

This process is favored in presence of super saturation is necessary for precipitating crystals. Thus super saturation acts as a driving force for the stone formation. The imbalance between inhibitors (citrate, magnesium, potassium, and pyrophosphate) and promoters (calcium, oxalate, uric acid, inorganic phosphate) in the kidney forms calculi. This cause obstructs the flow of urine [5].

Diagnosis includes physical examination, urinary analysis, and histopathological studies. Clinical diagnosis includes severity of the pain and typical nature of pain. Some techniques of renal stone removal include surgical operation, lithotripsy, and local calculus disruption using high-power laser [2].

Tamsulosin belongs to a class of α-1 adrenergic blockers. Tamsulosin is used by men to treat the symptoms of a prostatic hyperplasia (enlarged prostate). It exhibits selectivity for α-adrenoceptor in the lower urinary tract by inhibiting them it relaxes the muscles in the prostate and the bladder. Tamsulosin helps to relieve symptoms weak stream, flow of obstructed urine and the need to urinate often or urgently (during night) at a single dose of 0.4 mg/kg as reported [6]. This property would aid in rush of adhered stone in renal tubules or bladder as well.

Materials

Normal saline from Gliven life sciences, Hyderabad, India. Ethylene glycol (AR Grade), Central Drug House Pvt. Ltd., New Delhi, India. Ketamine hydrochloride and Tamsulosin Hydrochloride (Flomax 0.4 mg, Mylan USA) was gifted by Omega Hospitals, Department of Oncology, Kothapet, Hyderabad, Allopurinol.

Equipments

Shimadzu Electronic balance procured from Toshvin Analytical Pvt. Ltd., Mumbai, India. Ultrasonic homogenizer from Biologics Inc. USA. Centrifuge from Remi equipments Pvt. Ltd, Hyderabad, India. Biochemical analyzer from (BSI) Biochemical Systems International Ltd, India. pH Meter pH 142 from Cyber Labs, Mumbai, India. Indikrom pH strips from Glaxo India Limited, India.

Experimental animals

Healthy adult albino wistar male rats weighing between 200 gm to 220 gm were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air conditioning. A 12 h light/dark cycle was maintained. Room temperature was maintained between 22+2°C and relative humidity 40% to 65%. They were provided with food and water ad libitum. All the animals were acclimatized to the laboratory about 7 days prior to experimentation. The experimental animal handling and protocols were in accordance with CPCSEA [7].

Experimental protocol

Male rats were randomly divided into 5 groups containing five rats in each.

Table 2: Blood -serum analysis.

Group	Serum Creatinine in mg/dl	Serum uric acid in mg/dl
Group 1	1.44 ± 0.12	
Group 2	3.65 ± 0.54	
Group 3	2.14 ± 0.28	
Group 4	2.03 ± 0.22	
Group 5	0.62 ± 0.98	

Values are given as mean ± standard deviation, all the values in the column are statistically significant ($p<0.05$)

Table 3: Weight of calculi.

Group	Weight in mg
Group 1	-
Group 2	98
Group 3	24
Group 4	19
Group 5	10

Group 1: Served as normal control group received normal drinking water.

Group 2: Served as urolithiatic control group received 1% ethylene glycol in drinking water for 15 days.

Group 3: Served as group received Tamsulosin (0.4 mg/kg, p.o) from 8th day to 15th day, urolithiasis was induced from 1st day to 7th day.

Group 4: Served as group received Tamsulosin (0.8 mg/kg, p.o) from 8th day to 15th day, urolithiasis was induced from 1st day to 7th day.

Group 5: Served as standard drug received Allopurinol (5 mg/kg, p.o) for 15 days.

Urine collection

At the end of the treatment, individual animal was placed in the metabolic cage for collection of 24 h urine samples. Initially a drop of concentrated hydrochloric acid was added to the test tubes as a preservative [6,7]. Samples were tested total urine volume in Milliliter (mL), pH of each sample using pH meter [8] (Table 1).

Blood collection

On the day 15th 0.5 mL of blood was collected by capillary tubes from each animal through retro orbital puncture under mild anesthetic conditions with ketamine hydrochloride, serum was separated by centrifugation at 2500 rpm for 20 min and was stored at -4°C. Sample can retake after 2 h of sampling [9] (Table 2).

Weight of calculi

The weight of urinary calculi determined by sacrificing the animals

Table 4: Tissue homogenate.

Group	Volume of homogenate in ml	pH	Creatinine in mg/dl	uric acid in mg/dl
Group 1	1.02 ± 0.11	6.8 ± 0.41	0.22 ± 0.11	2.7 ± 0.51
Group 2	0.66 ± 0.51	5.2 ± 0.79	1.02 ± 0.32	5.7 ± 0.51
Group 3	0.92 ± 0.22	6.4 ± 0.24	0.78 ± 0.37	3.7 ± 0.13
Group 4	0.98 ± 0.34	6.7 ± 0.56	0.65 ± 0.41	3.4 ± 0.32
Group 5	1.6 ± 0.11	6.4 ± 0.15	0.21 ± 0.69	2.4 ± 0.19

Values are given as mean ± standard deviation, all the values in the column are statistically significant ($p<0.05$)

with excess dose of pentobarbitone sodium at the end of study. The urinary bladders were opened by incision and exposed along with the adhered crystals was removed and wrapped in separated polyethylene bags containing 10% formaldehyde solution. Weight is estimated by digital balance (Table 3).

Histopathological studies

The sections of collected kidney were observed under microscope 40x magnification for histopathological changes. The tissue homogenate is collected by homogenization using concentrated hydrochloric acid, the homogenate was centrifuged at 2,500 rpm for 5 min and the supernatant was assayed using biochemical analyzer (Table 4).

Results

Group 1 received Normal saline. Group 2 received 1% Ethylene glycol-urolithatic control. Group 3 received Tamsulosin (0.4 mg/kg, p.o)-drug treatment low dose. Group 4 received Tamsulosin (0.8 mg/kg, p.o)-drug treatment low dose. Group 5 received Allopurinol (5 mg/kg, p.o)-standard treatment.

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