International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Volume 6 | Issue 1 | Jan-Mar – 2022
www.ijpcr.net

Research article *Clinical research*

ISSN: 2521-2206

Effect of hydro-alcoholic extract of pisonia alba root against ethylene glycol-induced urolithiasis in rats

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ABSTRACT

The goal of this study is to assess the antiurolithiatic potential of a whole plant hydro-alcoholic (30:70) extract of Pisonia alba root, which has been claimed in ancient literature and is also one of the constituents in cystone, a marketed urolithiasis treatment. 0.75 percent v/v ethylene glycol was given orally for 14 days to produce urolithiasis. From the 15th to the 28th day, and from the 1st to the 28th day, respectively, the therapeutic dose of 400 mg/kg b.w. and preventive doses of 100, 200, and 400 mg/kg b.w. were given. The reference standard for both curative and preventative doses was cystone 750 mg/kg b.w. On the 28th day, urine from the previous 24 hours was collected and tested for calcium, oxalate, and phosphates. The content of renal oxalate was determined using serum biochemistry and kidney homogenate analysis. The levels of urine calcium, oxalate, and phosphate in the sick Group II were significantly higher (P 0.001 vs. normal Group I). The levels of serum creatinine, urea, and uric acid were also elevated. Significant alterations were discovered in histopathological investigations of kidney sections. The dose-dependent action of the hydro-alcoholic extract of P.alba was substantial (P 0.01 vs. calculi-induced Group II). There was a gradual increase in urine output, body weight, and a decrease in stone-forming component concentrations such as calcium, oxalates, and phosphates. P.alba root appears to be beneficial in the treatment of ethylene glycol-induced urolithiasis and may have the capacity to prevent and cure urolithiasis.

Keywords: Antiurolithiatic, cystone, ethylene glycol-induced urolithiasis, Pisonia alba root

INTRODUCTION

The production and retention of stone (s) in various regions of the urinary tract, the kidney, ureter, and bladder, where the size and kind of calculi varies greatly, is the most consistent feature of urolithiasis. According to statistics, urolithiasis affects 12% of the world's population. Between the ages of 25 and 50, the frequency is higher in the working class. It is more common in men than in women, possibly due to increased testosterone and estrogen's inhibitory effect on the development of uroliths. [1] Urolithiasis is a multifaceted illness including a variety of endogenous and external etiological variables as well as multivariate etiology. Improper calcium and phosphorous metabolism, as well as oxalic acid, uric acid, and cystine metabolism, are all endogenous contributors. Furthermore, hyperthyroidism, difficulties in excretion of nitrogenous waste products may also be a major contributing factor in stone formation. Exogenous factors comprise food habits, dehydration, less fluid intake, hot climatic conditions, excessive consumption of some vitamins like Vitamin A and D, unsafe or unnecessary use of certain drug substances and hard water usage for drinking purpose.[2]

Calcium oxalate stones are most common neproliths, accounting for more than 80% of stones, whereas 5–10% of uric acid stones are present. The other types are cystine, struvite, and urate stones, have very less percentage. Urolithiasis is the outcome of various physicochemical changes such as super-saturation of urine, nucleation, growth of crystal and aggregation. Urine is invariably saturated with the stone-forming components such as calcium, oxalate, urate, cystine, xanthenes, and phosphate. However, the natural tendency to inhibit crystallization prevents stone formation, whereas this natural inhibition capacity varies person to person and which is poor in stone formers.[3] Revolutionary developments in surgical procedures and extracorporeal shock wave lithotripsy (ESWL) in the treatment of kidney stone has taken place, but the expenses, and recurrence or relapse rate still remains the challenge. Moreover, renal damage and decreased kidney function becomes the major concern in these procedures.[4] Diverse therapeutic approaches to treat urolithiasis were adopted such as diuretics, chelating agents (magnesium citrate, magnesium, and citrates), and diet therapy (Increased water intake, reduced intake of Vitamin C, guidance regarding calcium intake, etc).[5]

Despite these advancements in treatment of kidney stone, no satisfactory medication is available. The evidence-based information suggests that the drugs and formulations derived from herbal source are widely preferred as a promising therapy in treating urolithiasis. The hypothetical underlying mechanism of these medicinal plant materials is believed to be, the effect on kidney and urinary blood circulation, diuresis, changes the pH of urine and also exerts spasmolytic and analgesic effect. These are interrelated with the liver, pancreas, and intestines through metabolism and removal of stone-forming components.[2] Emphasis was given by the WHO (2002) on the development and standardization of herbal medicine due to its cost effectiveness and less or no side effects. As the advancement took place, the gap between the modern and traditional medicine widened due to lack of scientific evidence and its less convincing therapeutic effects, where safety remains a major concern.

Pisonia alba also known as *Pisonia alba spanoghe*, *pisonia umbellifera*, belongs to the family of Nyctaginaceae. It is found on many of the Seychelles Islands that have had habitat restoration and subsequently is a key part of the habitat associated with high biodiversity and a complex food web. It is therefore not as easy as replacing Pisonia with other native tree species; it was discovered by(6) that Pisonia is the most common nest tree for the Seychelles warbler an endemic land bird brought back from near extinction by careful habitat management and translocation, thus showing that careful consideration of the entire island ecosystem is essential. P. alba is a large evergreen shrub. It is originally from the beach forests of Andaman Islands. Leaves: Long, bounty, and fresh green in color. If planted in good sunlight, the leaves may acquire a light yellow color. Flowers: The tree rarely flowers in India. The flowers are small, green, and inconspicuous. Uses: The leaves are edible. Young leaves are used as a vegetable. Leaves make good cattle feed too and are mostly used to treat rheumatism or arthritis. In traditional Indian medicine, they are used as an anti-diabetic; Leaves , of course , are used by natives as cattle feed; They are cooked and eaten for arthritis; The leaves are also carminative; Leaves are an antidote for snake bites; Researches have revealed that favonoids, steroids and phenolic compounds are present in the leaf. (7-10) The aim of this study undertaken is to evaluate and validate the antiurolithiatic potential of hydro-alcoholic extract of P.alba Less against ethylene glycol-induced urolithiasis in rats.

MATERIALS AND METHODS

Chemicals

The chemicals and solvents used were of analytical grade procured from Sigma-Aldrich Co., USA. The kits used for urine estimation and serum analysis purchased from Erba Mannheim, Transasia Biomedicals Ltd., Solan. Ethlyene glycol was obtained from S D Fine Chemicals. The standard marketed preparation – Cystone tablets (Himalaya Herbal Healthcare – Bengaluru, Karnataka, India) were purchased from the market.

Collection and Authentication of Plant Material

P.alba Less. plant was collected from botanical garden of Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi and nearby areas of Belagavi. The plant material was identified and authenticated by Scientist B, RMRC, Belagavi. The herbarium was prepared and stored at RMRC, Belagavi, Karnataka, India.

Preparation of Plant Extract

The collected whole plant P.alba was cleaned properly, dried in shade and further pulverized to obtain a coarse powder. The powder, thus, obtained was extracted with hydro-alcohol [30:70] water and 95% ethanol) using soxhlet apparatus. The extract was concentrated using rotary evaporator (IKA RV 10 Digital) under reduced pressure at 40°C to obtain (Yield - 9.6% w/w) a semisolid mass. It was labeled and stored in a glass bottle for further studies. A suspension of the extract was formulated using 5% Tween-80 for oral administration.[3] The extract was analyzed for various phytochemical constituents (flavonoids, alkaloids, tannins, phytosterols, glycosides, terpenoids, and saponins) using standard procedures.[11-14]

Antiurolithiatic Activity

Considering various parameters, the hydro-alcoholic extract of P.alba Less was screened for its antiurolithiatic potential.

Animal selection

Wistar albino rats weighing about 150–200 g, of either sex, were selected for acute toxicity study and male adult rats were used for screening of antiurolithiatic activity. The experimental protocol was approved by Institutional Animal Ethical Committee. The animals were kept in transparent polypropylene cages at a temperature 25°C ± 2°C and 12–12-day night cycle. The animals were provided with standard pellets and drinking water ad libitium. They were acclimatized to the laboratory conditions and randomly divided into eight groups.

Acute oral toxicity study

The acute oral toxicity study was performed as per OECD No. 425 guidelines. For the hydro-alcoholic extract of P.alba, a starting dose of 2000 mg/kg b.w. was selected. After overnight fasting, the animals were administered with extract orally with gastric intubation method. The animals were observed for any physiological and behavioral changes for first 4 h continuously, then at every 4 h for 12 h, followed by for first 14 days daily once. The LD50 cut off dose was determined, and 1/10th of it was considered for antiurolithiatic activity.[15]

Experimental protocol

Animals were divided into eight groups containing six animals in each group. During the experimental protocol, animals were allowed free access to food and water. Group I: Normal control, Group II: Calculi-induced, received 0.75% ethylene glycol in drinking water from 1st to 14th day and were left untreated, Group III: Standard curative-received 400 mg/kg b.w. standard drug cystone from day 15th to 28th, Group IV: Standard preventive-received standard drug cystone from day 1st to 28th, Group V: Curative– received 400 mg/kg b.w. hydro-alcoholic extracts from day 15th to 28th, Group VI: Preventive I-received 100 mg/kg b.w. hydro-alcoholic extracts from day 1st to 28th, Group VII: Preventive II-received 200 mg/kg b.w. hydro-alcoholic extracts from day 1st to 28th, Group VIII: Preventive III-received 400 mg/kg b.w. hydro-alcoholic extracts from day 1st to 28th.

Collection and Analysis of Urine

The animals were kept in polypropylene cages separately, and 24 h urine was collected on the 28th day. The volume of urine collected from each animal of all the groups was measured. During the study, the animals were having free access to drinking water. The collected urine was stored at 4°C by adding a drop of concentrated. HCl.

Using UroColor 10 strips (SD Bio Standard Diagnostics) routine urine analysis was done for the presence of protein, glucose, blood, nitrite, etc., Furthermore, quantitative determination of specific gravity and pH was done. Urine was analyzed for calcium, phosphate, and oxalate using semi-auto analyzer [16,17] [Table 1].

Urine Volume

On 14th and 28th day, 24 h urine was collected of all the eight groups, measured the urine volume and recorded.

Change in Body Weight

The difference in initial body weight (day 0) and final body weight (day 14 and day 28) was noted, and further comparative analysis was done among different groups.

Urine Microscopy

Urine microscopy was done on a 28th day to observe the presence of crystals of CaOX and phosphates at ×40, with characteristic size and shape. For this, an electron microscope (Labomed TCM 400) fitted with a Canon digital camera (12 megapixel) was used, and photographs were taken.

Serum Analysis

From the anesthetized animals, the blood sample was withdrawn from the retro-orbital sac. It was centrifuged at 14,500 rpm (14,100 ×g) for 10 min in a centrifuge machine (Eppendorf, Minispin plus); serum was separated and analyzed for blood, urea and nitrogen (BUN), creatinine and uric acid.

Kidney Homogenate Analysis

After the animals were sacrificed, the abdomen was cut open to remove both the kidneys from each animal. The kidneys were cleaned and preserved in formalin (10%). A sample of one kidney (100 g) was boiled for ½ h in 10 mL of 1 N HCl and homogenized. It was further centrifuged at 2000 rpm (1930 ×g) for 10 min; supernatant was analyzed for calcium, oxalate, and phosphate content.

Histopathological Analysis of Kidney

Examination of histopathological changes such as tubular congestion, tubular necrosis, glomerular congestion, peritubular inflammation, hemorrhage, and presence of calculi was done. The remaining kidney was embedded in liquid paraffin, 5 µm sections were taken, stained with hematoxylin and eosin and mounted in diphenyl xylene. The microscopic examination of thus prepared slides was done using compound microscope (×50). To determine the nephritic damage and recovery, photographs were taken and scored in the format as, no damage (−), Mild (+), Moderate (++), and Severe (+++) damage. For this, at least 10 fields were analyzed and marked.[18]

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of the mean. One-way analysis of variance, followed by Dunnett's multiple comparison test (GraphPad Prism software for Windows, Version 5.01, GraphPad Software, Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA) was used to analyze the statistical significance among different groups. The $P < 0.05$ was considered to be statistically significant.

RESULTS

The phytochemical study of P.alba confirmed the presence of alkaloids, saponins, triterpenoids, glycosides, steroids, and flavonoids.

Table 1: Effect of *P.alba* on urine and serum parameters in experimental animals-curative/preventive dose

Parameters	Group I Normal	Group II Calculi induced	Group III Standard curative	Group IV Standard preventive	Group V PA curative	Group VI PA preventive I	Group VII PA preventive II	Group VIII PA preventive III
Urine:Calcium	4.27±0.14	8.62***,a±0.55	4.79±0.15	5.34***,b±0.14	5.14±0.06	6.08NS±0.16	5.61**,b±0.01	5.29±0.13
Phosphate	140.7±1.3	346***,a±2.01	180.6±3.55	194.2***,b±4.71	176.2±4.41	216.2*,b±5.91	202.1±1.79	195.7±2.16
Oxalate	1.63±0.11	4.68**,a±0.12	1.88±0.098	1.85***,b±0.07	1.68±0.09	2.36*,b±0.11	1.9±0.07	2.02±0.10
Serum BUN	22.4±0.75	38.4***,a±0.75	25±0.62	26.5***,b±0.60	25.4±0.98	31NS±1.02	28.4**,b±1.0	27.2±0.66
Creatinine	0.53±0.011	0.89***,a±0.013	0.55±0.014	0.57***,b±0.012	0.57±0.021	0.70NS±0.012	0.60**,b±0.013	0.59±0.009
Uric acid	1.55±0.06	5.45***,a±0.14	1.97±0.07	2.02***,b±0.07	1.86±0.06	3.0*,b±0.12	2.34**,b±0.11	2.14±0.07

Kidney homogenate Calcium	**5.75±0.13**	**12.55***,a±0.36**	**6.27±0.12**	**6.27***,b±0.11**	**6.20±0.15**	**7.06**,b±0.21**	**6.27**,b±0.18**	**6.6±0.12**
Phosphate	**1.34±0.12**	**5.37***,a±0.14**	**1.58±0.09**	**1.64***,b±0.10**	**1.65±0.12**	**2.34**,b±0.25**	**1.87**,b±0.06**	**1.85±0.07**
Oxalate	**2.94±0.03**	**8.67***,a±0.11**	**3.35±0.07**	**3.63***,b±0.13**	**3.31±0.10**	**3.19**,b±0.01**	**3.7**,b±0.06**	**3.62±0.12s**

P<0.05, **P<0.01, ***P<0.001: Significant as compared with calculi induced, a Compared with normal group, b Compared with calculi induced group, NSNot significant. PA=P. alba, BUN=Blood, urea, and nitrogen

In acute toxicity study, the extract does not show any adverse clinical signs and mortality as well. Therefore, 2000 mg/kg b.w. was considered as LD50 cut off dose and 1/10th of it was taken as therapeutic dose for the study, i.e. 200 mg/kg b.w.

The preventive and curative antilithiatic effects of hydro-alcoholic extract of *P.alba* were evaluated considering various parameters. In this study, ethylene glycol (0.75% v/v) in drinking water was administered to induce calculi except normal control Group I. The preliminary confirmation was done by doing urine microscopy where the presence of various stones was observed. As per the data reported, urine calcium, oxalate, and phosphate excretion significantly increased (P< 0.001) in calculi-induced (Group II) as compared to normal control (Group I). However, in both the preventive and curative dose regimen, there was an increased degree (P< 0.001–0.05) of reduction in the levels of urine calcium, oxalate, and phosphate, relevant to the dose of hydro-alcoholic extract administered, and highly significant decrease was observed in cystone treated group [Table 1], Group III to VIII]. Increase in dissolution and significant reduction (P< 0.001 vs. Group II) in the concentration of calcium, phosphate, and oxalates results in better prevention of stone formation.

In the analysis of other routine urine parameters, it was observed that there was increase in urinary pH of 8.3 in calculi-induced Group II, but it was brought down gradually toward normal value in the groups treated with hydro-alcoholic extract of P.alba and cystone. In other groups, the parameters such as ketone bodies, nitrites, bilirubin, urobilinogen, and leukocytes were statistically insignificant. All the groups showed absence of glucose in urine. In calculi-induced group, the severe nephritic damage was confirmed by the presence of protein in urine. There was a considerable recovery recorded with a decrease in protein in the groups treated with both preventive and curative dose regimen of cystone and hydro-alcoholic extract of P.alba [Table 2].

Table 2: Effect of P.alba hydro-alcoholic extracts on urine analysis parameters in rats in curative/preventive dose regimen

Parameters	Group I Normal	Group II Calculi induced	Group III Standard curative	Group IV Standard preventive	Group V PA curative	Group VI PA preventive I	Group VII PA preventive II	Group VIII PA preventive III
Blood (RBC/µL)	**Absent**	**181.3±42.1**	**1.63±1.66**	**1.63±1.66**	**1.63±1.66**	**16.5±10.52**	**3.31±2.0**	**1.65±1.65**
Bilirubin	**0.08±0.07**	**0.74±0.10**	**0.07±0.07**	**0.07±0.07**	**0.07±0.07**	**0.32±0.15**	**0.24±0.16**	**0.15±0.10**
Urobilinogen	**0.25±0.15**	**1.0±0**	**0.25±0.15**	**0.55±0.20**	**0.4±0.18**	**0.7±18**	**0.7±18**	**0.25±0.15**
Ketone	**0.82±0.81**	**15.7±6.77**	**0.82±0.82**	**Absent**	**0.82±0.83***	**4.2±1.0**	**2.5±1.6**	**1.6±1.1**
Protein	**Absent**	**15±4**	**1.6±1.7**	**1.6±1.7**	**1.6±1.7**	**8.2±4.6**	**3.2±2.0**	**1.6±1.7**
Nitrite	**Absent**	**0.75±0.10**	**0.66±0.16**	**0.66±0.16**	**0.16±0.10**	**0.43±0.22**	**0.26±0.16**	**0.18±0.11**
Glucose	**Absent**	**Absent**	**Absent**	**Absent**	**Absent**	**Absent**	**Absent**	**Absent**
pH	**6.4±0.11**	**8.2±0.25**	**6.74±0.21**	**6.5±0.21**	**6.7±0.15**	**7.2±0.23**	**6.7±0.1**	**6.6±0.12**
Specific gravity	**1.003±0.002**	**1.03±0.002**	**1.004±0.002**	**1.006±0.003**	**1.004±0.002**	**1.01±0.002**	**1.005±0.002**	**1.005±0.002**
Leucocytes (WBC/µL)	**Absent**	**37.4±12.3**	**4.16±4.0**	**7 8.4±5.1**	**4.18±4.16**	**4.16±4.16**	**4.16±4.16**	**4.15±4.14**

**Blood (RBC/µL) +10, ++50, +++250, **Leucocytes (WBC/µL) +25, ++75, +++500. Absent: No color change observed. RBC=Red blood cell, WBC=White blood cell, PA=P. alba*

In diseased Group II, urine output was drastically reduced (P< 0.001 vs. Group I). There was increased (P< 0.001) urine output observed in the groups treated with cystone and hydro-alcoholic extract of P.alba [Table 3]. The increase in urine volume was appreciable on day 28 when compared with day 14.

Table 3: Effect of hydro-alcoholic extract of P.alba on urine volume in urolithiatic animals-curative/preventive dose

Group	Day 14 (mL)	Day 28 (mL)
Normal	9.4±0.94	10.2±0.48
Calculi induced	4.4±0.41***	3.6±0.32***
Standard curative	6.4±0.41##	9.1±0.30
Standard preventive	6.4±0.41	8.±0.42###
PA curative	6.1±0.56##	7.7±0.46##
PA Preventive-I	5.1±0.35	5.2±0.32
PA Preventive-II	5.2±0.32	6.1±0.71##
PA Preventive-III	6.1±0.30	7.6±0.41###

******P<0.001=Significant as compared with normal, ##P<0.01=Significant as compared with calculi induced. PA=P. alba.***

A consistent growth in body weight was recorded [Table 4] in normal control (Group I) animals, whereas significant (P< 0.001) weight loss was observed in calculi-induced Group II. Cystone treated animals (Group III and IV) gained body weight significantly (P< 0.01). The progressive increase in body weight was recorded on day 14 and day 28, in animals treated with extract (Group V, VI, VII, and VIII).

Table 4: Effect of hydro alcoholic extract of P.alba on change in body weight in urolithiatic animals curative/preventive dose

Group	**Day 0 (g)**	**Day 14 (g)**	**Day 28 (g)**
Normal	181±4.45	203±3.25	225.8±7.33
Calculi induced	191.8±3.91	168.7±3.48	147.3±3.91***
Standard curative	193.5±5.94	167.2±5.85	189.7±6.353##
Standard preventive	195.2±10.5	197±11.06	197.2±10.65
PA curative	162.2±7.08	146±5.64	155±6.35
PA Preventive-I	208.2±3.50	193.2±5.55	202.7±3.33
PA Preventive-II	196.7±9.73	181.8±10.18	194±9.57
PA Preventive-III	199.7±13.0	191±12.46	201±12.29*

******P<0.001=Significant as compared with normal, ##P<0.01=Significant as compared with calculi induced. PA=P. alba.***

The urine of normal control animals (Group I) revealed no renal calculi, but the urine of calculi-induced animals (Group II) revealed rectangular, bigger calcium oxalate crystals. Cystone-treated animals (Groups III and IV) had no or nearly no stones in their urine, whereas hydro-alcoholic extract of P.alba therapeutic dosage had the same number of crystals. In the preventative therapy groups, the lack of tiny crystals and comparable structures increased when the dose was raised (Group VI VII VIII). In smaller doses, however, tiny crystal pieces were found (Group VI).

In comparison to normal control animals, calculi-induced animals (Group II) had higher BUN, creatinine, and uric acid levels in their blood (Group I). Severe nephritic damage and reduced renal function ensue as a result of this. The rats given cystone and hydro-alcoholic extracts of P.alba had significantly lower corresponding values, indicating that they were protected from renal damage and had enhanced kidney function.

Stone-forming components calcium, phosphate, and oxalate were found in higher amounts (P 0.001) in diseased animals' kidney homogenates (Group II vs. Group I). Cystone-treated animals (Groups III and IV) had lower levels of stone-forming components. Animals given a hydro-alcoholic extract of P.alba as a curative (Group V) or preventive (Group VI, VII, and VIII) dosage regimen had lower calcium, phosphate, and oxalate levels.

In normal control animals, histopathology of the kidney reveals the lack of performed stones and related significant abnormalities (Group I). In the calculi-induced group II, there was a considerable (P 0.001) CaOX crystal deposition. Peritubular congestion, glomerular congestion, epithelial desquamation, blood vessel congestion, and cell inflammation were shown to be related abnormalities. Treatment with cystone and extract for both curative and preventative purposes.

DISCUSSIONS

Kidney stone development is a debilitating illness that affects humans. Alkalizers, diuretics, anti-inflammatory, analgesics, and antispasmodic medications will be provided to individuals seeking treatment today. Not only that, but technical improvements have made urolith removal therapies such as ESWL, surgery, and percutaneous nephrolithotripsy possible.[19] These approaches, however, are linked to a number of negative side effects, including bleeding, hypertension, tubular necrosis, and cell harm. Furthermore, these procedures do not prevent the production of stones, as recurrences of stones have been seen.

Many herbs and herbal preparations are used in traditional medicine to prevent and cure kidney stones. According to the research, alternative medicine, including plant-based medications, has a substantial influence in preventing and treating urolithiasis. Standardizing these phytotherapeutic compounds and establishing a justification for their usage is critical. [2]

The antiurolithiatic properties of a hydro-alcoholic extract of P.alba were evaluated in this study. The study employed male Wister albino rats, which were chosen for their physiological similarities to the human system. According to previous research, due to the inhibitory effect of female sex hormones, male rats are more prone to stone formation than female rats.

The ethylene glycol-induced urolithiasis model was employed in this investigation. The metabolic route implicated in stone formation, according to previous findings, is the easy absorption and conversion of ethylene glycol into glycolic acid in the liver via alcohol dehydrogenase or aldehyde dehydrogenase. Glyoxylic acid is formed when glycolic acid is oxidised, and lactate dehydrogenase or glycolate oxidase converts it to oxalic acid. [5]

The major risk factors in urolithiasis are decreased urine output, elevation in pH, hyperoxalurea, and hypercalciurea. To determine the characteristic types of kidney stones, urine analysis plays an important role.

There was a significant reduction in urine production in Group II when compared to the vehicle-treated Group I, which is symptomatic of the performed stones. Animals treated with Cystone (curative Group III and preventative Group IV) and hydro-alcoholic extract of P.alba (curative Group V and preventive Group VI, VII, and VIII) showed a gradual recovery. In urolithiatic disease, a reduction in glomerular filtration rate (GFR) is caused by a blockage in urine production. This explains why urine is supersaturated with calcium, oxalate, and phosphates. The improvement in GFR shows that hydro-alcoholic extract has both preventative and curative therapeutic effect.

The nucleation and aggregation of diverse forms of stones in urolithiasis is pH dependent. Uric acid stones are more common at pH 5.5, and calcium oxalate and calcium phosphate stones are more common at pH >7.2. The pH of urine in normal control Group I was determined to be 6.5 0.12.

The existence of kidney stones is confirmed by the considerable change in pH (8.3 0.25) in calculi-induced Group II. While the animals given hydro-alcoholic extract do not exhibit any statistically significant changes in pH, Group VI does show a modest rise, which might be due to the lower dosage. The presence of aberrant elements such as protein and blood in urine, which is the result of nephritic damage produced by stones, further supported the creation of stones. In the extract-treated group, the proportion of protein and red blood cells (RBCs) is dosage dependent, whereas it is greatest in the calculi-induced Group II (15 5 and 183.3 42.2, respectively). The hydro-alcoholic extract's inhibitory activity may be responsible for the dose-dependent reduction in protein and RBC content.

Kidney stones are a key cause of mental and bodily discord. This is indicated by a considerable fall in body weight (P 0.001) in the calculi-induced Group II. This weight loss is linked to a reduction in food intake, which might be the result of a physiological imbalance and emotional stress. Treatment with the usual medicine cystone and extracts, on the other hand, returned the unfavourable physiological changes and mental stress to a normal level. Cystone and hydro-alcoholic extracts had a greater diuretic and/or antiurolithiatic action, preventing the development of stones. This leads to a change in food and, as a result, a rise in body weight.The characteristic shape, size observed in microscopic study, and earlier observation confirms the predominant CaOX crystals present in calculi-induced Group II. It was evident from the observation that the groups treated with cystone and extract (higher dose) show the absence of stones. This absence and gradual decreased amount of crystals was in accordance with the dose administered. The effect observed may be due to the prevention of urine saturation with stone-forming components or dissolution of performed crystals. This observation was suggestive of promising lithotriptic effect.

Crystal deposition caused reduced GFR and obstructed urine outflow, which resulted in decreased urine excretion. This decrease in urine production results in a buildup of nitrogenous waste products in the blood, such as creatinine, urea, and uric acid, as measured by serum analysis. A significant rise in serum BUN, creatinine, and uric acid was reported in Group II (diseased). This indicates glomerulus and renal tubule damage.[20] However, there was no statistically significant increase in the concentration of nitrogenous waste products in the groups treated with ordinary cystone and hydro-alcoholic extract. The probable underlying cause for this may be due to diuresis or

dissolution of performed crystals or prevention of nucleation and aggregation of stones. It is well-defined that the major cause for urolithiasis is believed to be hyperoxalurea. There was a significant rise ($P< 0.001$) in kidney homogenate contents of oxalates and phosphates in calculi-induced Group II. This accelerates the process of stone formation. Increased oxalate concentration is more crucial factor than calcium in urolithiasis. The enhanced stone formation is the result of increased urinary calcium, causing aggregation, and crystal growth. However, this process of stone formation was hindered by decreased oxalate levels, reduced calcium excretion, and maintained phosphate levels in urine, which was observed in the animals treated with hydro-alcoholic extract.

Histopathological analysis of kidney sections revealed an accumulation of irregularly formed polymorphic crystals in the tubules. As shown in calculi-induced Group II, elevated oxalate and calcium concentrations produce dilatation of proximal tubules and interstitial inflammation in the kidney. Animals given hydro-alcoholic extracts of P.alba showed a great reduction in kidney damage as well as a spectacular recovery.

CONCLUSION

The antiurolithiatic action described in the data backs up the claims, and it's one of the main constituents of a well-known commercial treatment called cystone. Despite the fact that the specific process is unknown, a hypothetical conclusion can be formed. The efficiency of the P.alba hydro-alcoholic extract was demonstrated by a decrease in the concentration of stone-forming components, which inhibited lithogenesis. The therapeutic impact of plant extracts might be attributable to diuretic properties, the dissolving of kidney stones, or a synergistic effect of all of these factors.

REFERENCES

1. Joy JM, Prathyusha S, Mohanalakshmi S, Praveen Kumar AV, Ashokkumar CK. Potent herbal wealth with litholytic activity: a review. Int J Innov Drug Discov. 2012;2:66-75.
2. Smyslova OA, Markaryan AA, Evdokimova OV, Glazkova IU, Yaroshenko MA. Characteristics of the new comprehensive herbal medicine for the treatment and prevention of urolithiasis. Biol Med. 2015;7:4.
3. Rathod NR, Biswas D, Chitme HR, Ratna S, Muchandi IS, Chandra R. Anti-urolithiatic effects of Punica granatum in male rats. J Ethnopharmacol. 2012;140(2):234-8. doi: 10.1016/j.jep.2012.01.003, PMID 22285521.
4. Butterweck V, Khan SR. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Med. 2009;75(10):1095-103. doi: 10.1055/s-0029-1185719, PMID 19444769.
5. Chavada K, Fadadu KN, Patel KV, Patel KG, Gandhi TR. Effect of flavanoid rich fraction of Citrus medica Linn. on ethylene glycol induced urolithiasis in rats. J Drug Deliv Ther. 2012;2:109-16.
6. Singha S, Bawari M, Choudhury MD. Hepatoprotective and antipyretic effect of bark of Nyctanthes arbortristis Linn. Int J Pharm Pharm Sci. 2014;6;Suppl 2:110-4.
7. Available from: https://commons.wikimedia.org/wiki/File. retrived; 2019.
8. Esau K. Plant anatomy. Soil Science. 1953;75(5):767. doi: 10.1097/00010694-195305000-00014.
9. Kokate CK. Practical pharmacognosy. 1st ed. 2002;405-406, 426-489:107-14, 123-5, 130. 2002.
10. Kokate CK. Evaluation of crude drug, Practical Pharmacognosy. 1st ed; 1985. p. 122-35.
11. Kokate CK. Practical pharmacognosy – evaluation of crude drugs. 9th ed. Pune: Nirali Prakashan; 2000. p. 122-4.
12. Kokate CK. Practical pharmacognosy – techniques and experiments. 9th ed. Pune: Nirali Prakashan; 2002. p. 149-53.
13. WHO. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO. Press: World Health Organization; Geneva 27. Switzerland; 2007. p. 118.
14. Khandelwal KR. Practical pharmacognosy, techniques and experiments. 22nd ed. Pune: Nirali Prakashan; 2012. p. 23.1-5.9.
15. OECD, OCDE. Acute oral toxicity – up-and-down-procedure (UDP). OECD Guidelines for the testing of chemicals. Vol. 425. Paris: OECD Publishing; 2008. p. 1-27. doi: 10.1787/9789264071049-en.
16. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniaria hirsuta on experimentally induced nephrolithiasis in rats. BJU Int. 2003;92(1):137-40. doi: 10.1046/j.1464-410x.2003.04289.x, PMID 12823398.
17. Gadge NB, Jalalpure SS. Curative treatment with extracts of Bombax ceiba fruit reduces risk of calcium oxalate urolithiasis in rats. Pharm Biol. 2012;50(3):310-7. doi: 10.3109/13880209.2011.604332, PMID 22321032.
18. Singh D, Chopra K. The effect of naringin, a bioflavonoid on ischemia-reperfusion induced renal injury in rats. Pharmacol Res. 2004;50(2):187-93. doi: 10.1016/j.phrs.2004.01.007, PMID 15177308.

19. Selvam R, Kalaiselvi P, Govindaraj A, Bala Murugan V, Sathish Kumar AS. Effect of A. lanata leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res. 2001;43(1):89-93. doi: 10.1006/phrs.2000.0745, PMID 11207071.
20. Ghodkar PB. Chemical tests in kidney disease. Mumbai: Textbook of Medical Laboratory Technology. Bhalani Publishing House; 1994. p. 118-32.