ARTICLE INFO:
Article history:
Received: 30 October 2014
Received in revised form: 18 November 2014
Accepted: 30 November 2014
Available online: 31 December 2014

Keywords:
Ethylene glycol, calcium and oxalate crystals, urolithiasis, free radicals, Antiurolithiatic activity

ABSTRACT
The effect of ethanolic extract of Aristolochia indica was studied on experimentally induced nephrolithic and urolithiasis in rats. Oxalate urolithiasis was produced by the addition of 0.75% ethylene glycol in the diet for a period for 30 days. Ethylene glycol treatment resulted in a significant increase in the levels of calcium and oxalate. Treatment of ethanolic extract of Aristolochia indica 100mg/kg body weight for 30 days revealed a dose-related effect in the reduction of lithogenic substances, following glycolic acid induced urolithiasis. Simultaneous oral treatment with at a dose of ethanolic extract of Aristolochia indica 100mg/kg for 30 days significantly reversed the ethylene glycol induced nephrolithiasis and urolithiasis. Presumably by preventing the urinary supersaturation of lithogenic substances. Especially of oxalate and calcium. These observation indicate that ethanolic extract of Aristolochia indica can play an important role in the prevention of disorders associated with kidney stone formation.

Introduction
Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors. A kidney stone is a hard, crystalline mineral material formed within the kidney or urinary tract. Kidney stones are a common cause of blood in the urine (hematuria) and often serve pain in the abdomen, flank, or groin. Kidney stones are sometimes called as renal calculi. The condition of having kidney stones is termed nephrolithiasis. Having stones at any location in the urinary tract are referred to as urolithiasis, and the term ureterolithiasis is used to refer to stones located in the ureters. Urolithiasis, referred to the formation of a stones in urinary tract, is one among the diseases that afflicted humans since antiquity. Despite tremendous advance accomplished in understanding the mechanisms governing to formation of such concretion, the disease remains an enigma since several factors intervene and interfere each other. As many forms of mineralization, urolithogenesis forms of mineralization, urolithogenesis encompasses several physico-chemical steps which occur either sequentially or concurrently that start with supersaturation, then nucleation, growth and aggregation. of course based on their size, aggregated particles can be attached and retained within urinary tubule epithelium constituting an additional step in calculogenesis. According to clinical and epidemiological studies, calcium oxalate followed by calcium phosphate is the most frequently encountered crystalline components found in analyzed stones. Most stone do not contain on single crystal phases. This leads to wonder which phase was initiated the crystallization process. It is well known that kidneys reabsorb water and contribute to the concentration of different solutes that might crystallize and precipitate. Therefore any subject is susceptible to form calculi. The simplest explanation for the occurrence of kidney stones would be that the urine of stone formers is supersaturated with stone minerals, which consequently precipitate is their urine. Equally, healthy persons have supersaturated urine as well and occasionally pass crystals in their urine kidneys stones are of four types. The overall probability of forming stones differs in various parts of the world and is estimated as 1.5% in Asia 5.9% in Europe. 13% in North America and the recurrence rate of renal stones about 75% in 20 years spa. It occurs both in men and women but the risk is generally high in men and is becoming more common in young women. The world wide incidence of urolithiasis is quite high and in spite of tremendous advances in the field of medicine. There is
no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to be rid to this painful disease.[3] The goals of management of urolithiasis are to decrease the pain when present and to maintain function. Pharmaceutical agents are used to minimize the formation of calculi. The Pharmacological drug is used such as spironolactone, Thiazides[4] and thiazide-like agents, alkaline citrate, orthophosphate, magnesium. Allopurinol, Pyridoxine administration is the preferable and widely used for management of urolithiasis.

Therapy with spironolactone is associated with potential side effect like Drowsiness, lightheadedness. Stomach upset. Diarrhea, nausea, vomiting or headache may occur. Allopurinol [5] has serious side effects like numbness/tingling of arms/legs. Easy bleeding /bruising signs of infection (e.g fever, persistent sore throat). Unusual tiredness, Painful/bloody urination, changes in the amount of urine. Yellowing eyes/skin, severe stomach/abdominal pain. Persistent nausea /vomiting, dark urine, unusual weight loss. Eye pain, vision changes etc. Not only these adverse effects and side effects of current pharmacological options of urolithiasis have made to think on use of alternative and complementary medicines but also their inability to complete removal of stone formation. Ayurvedic system of medicines is one of the oldest system of medicine having a history of more than 20 years. Several prototype derived from these herbal medicine are in use for various kind of disease and disorders more than 200 drugs have been medicated in ancient texts for have been scientifically evaluated importantly jasminum driculatum [6], Bergenia ligulata. Crataeva magma lour [7], tamarindus indicus, Eysenhardtia Polystachya, Musa paradisiacal, Aerva Lanta [8]. And Aristolochia indica has been known traditionally in Indian system of medicine for the treatment of various maenolic disease and disorders. Since, the present study was carried out to know its applicability in urolithiasis condition. Extract of Aristolochia indica was prepared with Ethanol and used for evaluation of antiurolithiatic activity. In this study we systematically evaluated its property by using Ethylene Glycol induced Albino rats. The present study also highlights effect of ethanolic extract of plant on body weight, kidney weights, urine, serum. This study also involves stare of the rat animal model to elucidate its probable mechanism of industrial guidelines of FDA, USA for pre- clinical evaluation of anti-Nephrolithiasis drug.

Materials and methods

Plant selection

The plant is distributed throughout all the provinces of India and in Shri Lanka, Nepal and Bangladesh. It is usually found scrambling over hedges and bushes. Its synonyms are as follows Ahwaganda, Ishwari, Ruhimula, Indian Birthwort,Sanskrit name; Gandhi-unakuli English Name; Birth wort; Common Name: Isarmool, Ishwarmool. It constitutes aromatic oil, colouring, principles, alkaloid, steroids, triterpenoids. Its Roots, Rhizomes and Leaves are used tonic, Stimulant, Emmenagouge. Alexiteric and Anti-arthritic, snake bite, carminative in diarrhea. Literature surevey reveals Aristolochia indica awes not evaluated for its antiurilithiatic activity, so in this work preliminary screening is carried out.

Collection and authentification of plant leaves

In the present study, the root of Aristolochia indica was collected in warananagar. The plant was authenticated by Prof. S. Y. Jadhav, H.O.D. of Botany, Yashwantrao Chavan Mahavidyalay, Warananagar. The study includes organoleptic tests, and macroscopic and microscopic observations were done in by, Prof. S. Y. Jadhav Lecturer. Soon after authentication, all leaves were dried at room temperature. Until they were free from the moisture. Finally the root was subjected to get course powder and then passed through sieve no.44 to get uniform powder. The sieve powder was stored in air light, high- density polyethylene container before extraction.
Extraction

The powdered root (500g) was subjected to hot continuous extraction (soxhlet) with ethanol. After the residue extraction, solvent was distilled off, and excess solvent was completely removed by using a rotary flash evaporator to get reddish-brown semi solid extract (yield: 28.17%). The obtained extracts were then evaluated for anti-urolithiasis activity.

Animal selection

Eight male Wistar albino rats, weighing between 150 to 280g were selected and allowed to acclimatize for a minimum ten days prior to the study. The rats were housed in room maintained of 21±1 C, relative humidity50-55% and 12hr light-dark cycle. The rats were caged with one animal in each polypropylene cage and were fed with standard food pellets and water ad libitum throughout the study. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the institutional Animal ethical committee.

Experimental section

After ten days the rats were divided into four groups, two rats in each group. Four groups include normal group control (Induced and no treatment given), Standard group (induced and treated with spironolactone), and test group (induced and treated with plant extract) The rats from all groups were administered with the ethylene glycol except group I. Group-II serve as a control induced but no treatment was given, group III induced and treated with standard spironolactone. Group IV were maintained on normal diet, along with oral administration of ethanol extract 100mg/kg. The extract treatment was started after the administration of ethylene glycol and continued for 30 days. But the body weights of all animals were recorded at the beginning and at weekly intervals throughout the experiment. Twenty-four hour urine samples were collected using separator by placing each group in a metabolic cage during the last week. Urine samples were acidified with 2ml of 1MHCL and centrifuged for 10 min at 4 C to remove contaminating Sediments. Then aliquots were stored at 20°c until they were assayed. After 30 days treatment with ethanolic extract of Aristolochia indica, experimental blood samples from all the group were withdrawn by retro orbital route (see in fig no. 2), blood samples were allowed to clot at room temperature and the serum was separated by centrifugation for 20 min. Serum samples were stored at 70°c until analysis. The left and right kidneys were dissected out for the measurement of dry and wet weight. The left and right kidneys were immediately fixed in 10% neutral buffered formalin for histopathological examination.

1. Serum Analysis: Blood urea nitrogen, uric acid

The test was carried out using diagnostic reagent kit (Span diagnostic Ltd, Surat, India) for the In vitro determination of Blood urea nitrogen, uric acid analyzed by using an auto analyzer.

2. Serum Analysis: Creatinine

This method is based on the deproteinisation of samples with picric acid and the determination of creatinine in the supernatant by the addition of NaOH solution. The assay was performed exactly described in Arzneibuch (D. L.) DDR-83. To 250 µl of samples, 1.5ml of picric acid were added, mixed and centrifuged for 10 min at 3000rpm. The supernatant was mixed with 50µl of NaOH solution (final concentrations:41 mmol/l picric acid, 69 mmol/l NaOH ) and the absorbance was measured between the 20th and 30th min at 530nm.

3. Urine analysis: Calcium oxalate crystals

The test was carried out using diagnostic reagent kit (Span diagnostic Ltd, Surat, India) for the In vitro determination of calcium oxalate. The calcium present in the serum was precipitated with naphtyl hydroxamic acid (calcium reagent).
The precipitate was then dissolved in EDTA reagent and calcium from this solution was complexed with color reagent to give a colored complex that was measured calorimetrically, and analyzed using an autoanalyzer.

3. Renal Tissue Samples analysis

All the end of the experiment, on day 30. The rats were killed by cervical dislocation and kidneys excised, washed with normal saline and weighed. The kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min.

Statistical analysis: All the data collected in the present study are expressed as Mean± SE and were analyzed by students’ t test for coming to conclusion.

Results

Effect of Ethanolic Extract *Aristolochia indica* on body weight in Nephrolithiasis rats (0.2 ml/kg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>261.57</td>
</tr>
<tr>
<td>Group-II</td>
<td>250.00</td>
</tr>
<tr>
<td>Group-III</td>
<td>257.00</td>
</tr>
<tr>
<td>Group-IV</td>
<td>253.00</td>
</tr>
</tbody>
</table>

Effect of Ethanolic Extract *Aristolochia indica* on Urine sample in Nephrolithiasis rats (0.2 ml/kg)

Calcium and oxalate excretion were significantly increases in 24h urine of ethylene glycol induced Nephrolithiasis rats when compared with normal rats. It was also decreased significantly when treated with ethanolic extract of *Aristolochia indica* compared to Group II and shows similar significance result with standard (see in table no.2).

Table 2: Effect of Extract on Urine Constituents

<table>
<thead>
<tr>
<th>Groups</th>
<th>Crystals in urine</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Calcium oxalate</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Crystals are not seen</td>
<td>--</td>
</tr>
<tr>
<td>Group-II</td>
<td>Calcium oxalate</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Crystals are seen</td>
<td>**</td>
</tr>
<tr>
<td>Group-III</td>
<td>Calcium oxalate</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Crystals are seen</td>
<td>*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>Calcium oxalate</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Crystals are seen</td>
<td>*</td>
</tr>
</tbody>
</table>

Effect of Ethanolic Extract *Aristolochia indica* on Serum sample in Nephrolithiasis rats (0.2 ml/kg)

Table No.3: Effect of Extract on Blood Serum

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>8.13±0.38</td>
<td>1300±0.48</td>
<td>10±0.14</td>
<td>9.12±0.11</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.58±0.06</td>
<td>4.30±0.11</td>
<td>2.78±0.02</td>
<td>1.98±0.13**</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.70±0.01</td>
<td>0.90±0.03</td>
<td>0.82±0.05</td>
<td>0.75±0.03**</td>
</tr>
</tbody>
</table>
Effect of *Aristolochia indica* extract on Nephrolithiasis rats was studied by serum biochemical analysis. Result obtained from serum biochemical analysis (see in table no.3) expressed as Mean ± SE and evaluated by student’s test. Serum biochemical results were collected from each group and statistically evaluated by chi-square test for coming to conclusion P value less than 0.05 was considered as significant.**p<0.01.

Effect of Ethanolic Extract *Aristolochia indica* on Dry and wet Kidney weight in Nephrolithiasis rats (0.2 ml/kg)

**Table 4: Effect of Extract on Kidney Weight (gm)**

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Dry kidney weight (gm)</th>
<th>Wet Kidney weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>0.094±0016</td>
<td>0.421±0.0054</td>
</tr>
<tr>
<td>Group-II</td>
<td>0.112±0.0020</td>
<td>0.925±0.0019*</td>
</tr>
<tr>
<td>Group-III</td>
<td>0.096±0.0022</td>
<td>0.902±0.0012</td>
</tr>
<tr>
<td>Group-IV</td>
<td>0.087±00018</td>
<td>0.735±0.009*</td>
</tr>
</tbody>
</table>

Values are mean ± SE evaluated by student’s test. P value less the 0.05 was considered as significant. *p<0.01. Results were as shown in table no.4.

**Discussion**

In the past, since increased calcium intake was considered to be an important risk factor for stone formation, calcium restriction was recommended as an obvious intervention to prevent kidney stones in calcium stone-forming patients suffering from hypercalciuria. However, this practice has been questioned due to a large epidemiologic study which reported an increased risk for stone formation in subjects consuming a low calcium diet due to secondary hyperoxaluria, although these data had been largely indirect. Furthermore, several investigators have observed that calcium restriction has a deleterious effect on bone in hypercalciuria patients. In healthy subjects, observed that an increased calcium intake can totally abolish the hyperoxaluria induced by a oxalate load. However, in an experimental model of genetic hypercalciuric rats, observed that, although urinary calcium
proportionally increased with increasing dietary calcium content, a parallel decrease in urinary oxalate did not occur.

By knowing the biochemical studies primary stone formation and recurrence of stone formation is one of the biggest challenges faced by urologists today and remain a major source of morbidity in humans. Despite intensive studies in the last decade many aspects of Nephrolithiasis/ urolithiasis, the complete pathogenesis and thus prevention still remains to be clarified. Studies have concentrated on the metabolic defects in urine and mechanisms of supersaturation, reasons for cytotoxic damage of renal tubular cells and interference with some natural inhibitors. It is believed that, like urinary metabolic defects, biochemical variation in blood is less likely to occur in first time stone formers than in patients with recurrent disease. Increased excretion of oxalate and deposition of calcium oxalate crystals in the renal tubules is associated with renal epithelial injury and that products of cellular damage can act as heterogeneous nucleators of both calcium oxalate and calcium phosphate crystals in animal mode.

The body weights of rats at the beginning of the study were not significantly different between the four groups. However, at the end of the study. The rats treated with 0.75% Ethylene Glycol weighed less than those on the normal diet. The Group-III and Group IV treatment with standard and extract of Aristolochia indica significantly increase the level of body weight in urolithic rat.

The present study were urinary chemistry with respect to stone forming minerals will provide a good indication of risk if stone formation. In the present study observed hypercalciuria in ethylene glycol induced urolithic rat might be a factor favoring the nucleation and precipitation of calcium oxalate of apatite (calcium phosphate) from urine and subsequent crystal growth. The Aristolochia indica significance reduced the level of calcium oxalate in urolithic rat.

Uric acid is known to promote calcium oxalate crystal growth, predominance of the uric acid crystals in calcium oxalate stones and the observation that uric acid binding protein are capable of binding to calcium oxalate and modulate it crystallization also suggests its primary role in stone formation. In the present study, higher concentration of urinary uric acid was observed in ethylene glycol induced urolithiasis rats. Aristolochia indica treatment restored the uric acid level to normal thus reducing the risk of stone formation.

The serum uric and BUN were remarkably increased in ethylene Glycol induced animal while serum creatinine was only slightly elevated in group-II indicating marked renal damage. However ethanolic extract of Aristolochia indica treatment in curative and prophylactic regimen significantly lowers the elevated serum level of creatinine, uric acid and BUN in Group IV.

There was a significant increase in the kidney weight of animals receiving 0.75% ethylene glycol which was almost normalized in the Aristolochia indica treated animals (table 4). Ethylene Glycol feeding for 30 days resulted in renal tissue deposition of calcium and oxalate, the increased disposition of calcium and oxalate in the renal tissue leads to papillary calcification and eventual calculi formation. Aristolochia indica administration significantly reduced both calcium and oxalate levels in kidneys, which is known to prove beneficial in preventing calculi formation due to supersaturation of these lithogenic substances.

The earlier photochemical analysis is demonstrated the presence of the alkaloids, Quinines, Glycosides, Saponin, fat and oils, triterpenes and Steroids. It has also been evaluated for antioxidant, anti-inflammatory, antimicrobial, anti-fungal, antistress, anti-diarrheal, and antiulcer properties.

Recently, studies have implicated the generation of oxygen derived free radicals and lipid peroxidiation as one of the most important mechanisms involved in the pathogenesis of nephrolithiasis. By knowing the above pathophysiology of nephrolithiasis and the plant profile of Aristolochia indica is having a essential chemical constituents that reduce the formation to calculi in kidney as well as ureter and urinary bladder.

The use of ethanolic extract Aristolochia indica is also appeared to be safe for long-term usage, as there were no short term and toxicity reports. Therefore, it can be considered that use of Aristolochia indica extract will be beneficial, safe and effective in management of nephrolithiasis.

Conclusion

On detail study, the alcohol extracts Aristoochia indica was found to be effective than Spironolactone in reducing stone forming constituents both in urine and renal tissues and also reduced, the calcium and oxalate. The result exhibited by ethanolic extracts of Aristoochia indica shown significant anti urolithiasis activity. Further studies will extensive investigation, isolation, purification of active phytoconstituents with potent antiurolithiatic activity. Based on these results, we conclude that the Aristolochia indica dhanolic extract is more potent in protecting the animals from nephrolithiasis. All of its effects observed in this study are similar to Spirinolactone treatment. Hence, this Aristolochia indica is considered as an anti nephrolithiasis drug in treatment of nephrolithiasis.

Acknowledgment

I wish to acknowledge my team for their consistent support during the work. I am thankful to Girnar laboratory jaysingpur and Yashwantrao Chavan Mahavidyalay, Warananagar for their valuable help.
Conflict of interest statement

We declare that we have no conflict of interest.

References


10. Dr. K. M. Nadkarni’s: Indian Material Medica: Published by Ramdas Bhatkal: Vol-1908.pg no.139.