FORMULATION AND EVALUATION OF HERBAL SYRUP USED IN THE MANAGEMENT OF CHOLESTEROL AND KIDNEY STONES

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ABSTRACT

Hypercholesterolemia is the presence of high levels of cholesterol in the blood and it is the major risk factor for coronary heart disease, a cause of heart attacks. Hypercholesterolemia is typically due to a combination of environmental and genetic factors. Kidney stone is the formation of stones in the urinary tract, causing pain and bleeding, and may lead to secondary infection. Of many types of stones that are formed, the most common are calcium oxalate. Urolithiasis (renal stone formation) is a recurrent disorder predominant in males. The present day medical management of urolithiasis is either costly or not without side effects. Hence, the search for antilithiatic drugs from natural sources has assumed greater importance and the prepared natural syrup playing a significant role in the management of kidney stones. Formulation and evaluation of herbal syrup and testing the syrup in animals by invivo manner. Syrup showed promising results in the size reduction of kidney stones by invivo method by ethylene induced urolithiasis in albino wistar rats (P < 0.001). The treatment of lithiasis- induced rats by natural syrup also restored all the elevated biochemical parameters (creatinine, blood urea nitrogen and uric acid) restored the urine pH of normal and increased the urine volume significantly (P < 0.001) when compared to the model control drug. Natural syrup showed prominent results in the management of renal calculi by invivo methodology. Studies in humans can further confirm and revalidate the use of these agents in real time clinical settings.

KEYWORDS: Herbal syrup, Albino wistar rats, Phytotherapy.
INTRODUCTION

Many Indian plants have been quoted to be useful as antilithiatic agents. They are effective with fewer side effects and are also inexpensive. One of the important phenomena that characterize renal calculi is its high recurrence. Thus, a protective system is required including Extra corporeal shock wave lithotripsy (ESWL) and medicament treatment. Unfortunately, these means remain costly and in most case are invasive and with side effects. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants or phytotherapy. Therefore, it is highly recommended to explore new drugs coming from medicinal plants to treat and prevent the formation of kidney stones. Ideally, conventional and phytotherapy should supplement one another and have all the need available for renal calculi patients.[1]

Banana plants (Musa species) are of the family Musaceae. They are economic, easily available and found native to the tropical region. Plantain juice is used as an antidote for snake bite. Studies in rats demonstrate effectiveness for stone lysis.[2] The roots can arrest hemoptysis and possess strongly astringent and anthelmentic properties. Musa paradisiaca is available in tincture or capsule No toxicities and contraindication are reported in human yet. The easy digestibility and nutritional content make ripe banana an excellent food, particularly suitable for young children and elderly people.[3] Bananas contain considerable amounts of vitamin B6, vitamin C, and potassium. The latter makes them of particular interest to athletes who use them to quickly replenish their electrolytes.[4] In India, juice is extracted from the corm and used as a home remedy for jaundice, sometimes with the addition of honey, and for kidney stones. Moringa olifera, Mimosa pudica are also used. Kidney stones are a painful disorder of the urinary tract. Stones occur four times more often in men than in women. If the crystals remain tiny enough, they will travel through the urinary tract and pass out of the body in the urine without being noticed.[5]

Urolithiasis is the medical term used to describe stones occurring in the urinary tract. Other frequently used terms are urinary tract stone disease and nephrolithiasis. The kidney filters waste products from the blood and adds them to the urine that the kidneys produce. When waste materials in the urine do not dissolve completely, crystals & kidney stone are likely to form. Kidney stone form when there is a high level of calcium (hypercalciuria), oxalate (hyperoxaluria) and uric acid (hyperuricosuria) in the urine; a lack of citrate in the urine or insufficient water in the kidneys to dissolve waste products. The kidneys must maintain an
adequate amount of water in the body to remove waste products. If dehydration occurs, high level of substances that do not dissolve completely (eg. Calcium, oxalate, uric acid) may form crystal that slowly build up into kidney stones. Urine normally contain chemicals, Citrate, Magnesium, Pyrophosphate, Glycosaminoglycans. These prevent the formation of crystals & low level of these inhibitors can contributes to the formation of kidney stones. Often these citrate is thought to be most important because citrate, or citric acid, is an ordinary component of our diet, present in high amounts in citrus fruits. Citrate binds with calcium in the urine, thereby reducing the amount of calcium available to form calcium oxalate stone. It also prevent tiny calcium oxalate crystals from growing and massing together into larger stones. Finally, it makes the urine less acidic, which inhibits the developments of both calcium oxalate and uric acid stone. Magnesium is also one of the crystal inhibitor which present in urine. It act by increasing calcium solubility (especially in the urine) and reducing calcium absorption, magnesium can help to prevent kidney stone specially those composed of calcium oxalate. It is thought that calcium oxalate stone are most likely to form in people who are magnesium deficient, so it may just correct that deficiency. Inorganic pyrophosphate is a potent inhibitor which appears to affect calcium phosphate more than calcium oxalate crystals. Other urine inhibitors in urine that appear are glycoproteins, which strongly inhibit the growth of calcium oxalate crystals. As a consequence of the presence of these inhibitors, crystal growth in urine is very slow. Potassium nitrate and magnesium nitrate are the major constituents present in Banana stem juice and was confirmed by chemical test and UV spectroscopy Literature has proved the explosive and solubilizing property of potassium nitrate. Extracts of stem showed the presence of alkaloids, steroids like β-sitosterol, saponins, flavonoids like quercetin, reducing sugar, tannins and anthraquinones by chemical tests, UV, IR, Flame photometric studies and HPTLC determination. All plants showing promising cholesterol lowering property.

MATERIALS AND METHODS
Preparation and evaluation of herbal syrup

*Mimosa pudica, Moringa olifera* and Plantain stem were authenticated and identified. The plants were dried under shade, powdered and then subjected for extraction processes. Herbal syrup was prepared according to standard formulae and all evaluation techniques have been carried out as per standards.
Procurement of materials
Allopurinol (Zyloric) tablets was procured from Glaxo Smithkline Beecham Pharmaceuticals, Bangalore. The urea kit, creatinine monoreagent test kit and triglycerides test kit were procured from laboratory. Natural syrup were prepared in Moulana College of Pharmacy, Angadippuram, Malappuram, Kerala using suitable equipments. Preformulation and post formulation studies were carried out.

Animal selection
Healthy adult male albino wistar rats weighing between 150-225g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions and maintained as per standard guidelines. Animals were provided with regular rat chow and drinking water ad libitum. The animal care and experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) Reg. No: (CADD/30/282/CPCSEA).

Ethylene glycol induced urolithiasis model\[^{11,12,13}\]
Animals were divided into five groups containing three animals in each. Group I served as control and received regular rat chow and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II to V for induction of renal calculi for 28 days. Group III received standard antiurolithiatic drug, Allopurinol (500mg/kg body weight) from 15\(^{th}\) till 28\(^{th}\) day. Group IV and V received Herbal syrup at high dose (500mg/kg) and low dose (250mg/kg) from 15\(^{th}\) till 28\(^{th}\) day.

Biochemical analysis
Collection and analysis of urine
The urine samples of 24 hour were collected on 28\(^{th}\) day from all animals and they were priorly kept in individual metabolic cages. 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\(^{\circ}\)C. Urine was analysed for calcium, phosphate and oxalate content using the method of Bahuguna et. al.

Serum analysis
After the experimental period, blood was collected from the retro-orbital under anesthetic condition and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 100000 \(x\) g for 10 minutes and analysed for creatinine, uric acid and urea nitrogen using the method of Atef and Attar.
Urine Volume and Urine pH
All animals were placed in separate metabolic cages for 24 hours and the total urinary volume was measured using the measuring cylinder and reported in ml. Uric acid crystals were found to be deposit most frequently in the concentrated urine. Thus the acidity of the urine was tested using the pH meter.

Histopathology studies
All rats were anaesthetized by diethylether at the end of the experiment say, 28th day and the kidneys were removed. For histological processing, the kidneys were fixed in 10% formalin, dehydrated in a gradient of ethanol, embedded in paraffin and cut into 5 µm serial sections. Ten slides containing five sections from each kidney were deparaffinized, stained with hematoxylin-eosin and examined by light microscope. Aggregations of calcium oxalate deposits were counted in 50 microscopic fields.\[14\]

Statistical Analysis
This was done by using one-way analysis of variance (ANOVA) followed by the Bonferroni test. The statistical significant was set at P < 0.05. The results were presented as mean± standard error of mean (SEM).

RESULTS
Administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino wistar rats produced hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in the calculi induced animals. However supplementation of herbal syrup significantly lowered the increased levels of calcium, oxalate and phosphate in urine when compared to model control group (Table I). Level of creatinine, blood urea nitrogen and serum were found to increase in calculi-induced animals (Table II). In case of herbal syrup and Allopurinol (Standard drug) the treatment significantly (P < 0.001) lowered the elevated level of creatinine, uric acid and blood urea nitrogen. Urine volumes were increased by herbal syrup and standard drug Allopurinol compared to the model control group. Urinary pH was significantly increased in the animals treated with the 0.75%v/v of ethylene glycol. Herbal syrup and Allopurinol significantly decreased the pH (Table III). Not much differences were observed in the activity of herbal syrup between low dose and high dose. Examination of kidney sections in control group showed no calcium oxalate deposits or other abnormalities in various segments of nephrons. Whereas in case of ethylene glycol group calcium oxalate deposits were found abundantly in different segments of nephron including proximal tubules,
loop of Henle, distal tubules, collecting ducts and even in kidney calyxes (Figure I and II). Renal tubular dilation with epithelial damage and leukocyte reaction were also observed on pathology examination (Figure III). The average number of calcium oxalate deposits in 50 microscopic fields in the kidney specimens of ethylene group was significantly higher than control group (P < 0.001).

It was found that herbal syrup has good antiurolithiatic (kidney stone dissolving) property. From the two weeks *invitro* studies it was found that the size of kidney stone reduced to a greater extent. In general, herbal syrup was successful to reduce kidney stone in *invitro* condition. The benefit of this was nontoxic and cheaply available (the plant is commonly available in India, especially in Kerala). In present investigation there was a significant decrease in the size of kidney stone under *invitro* condition. This is due to the presence of organic constituents like β-sitosterol, quercetin, tannins, saponins and inorganic constituents like magnesium, potassium and nitrate. The result from these experiment demonstrate the potential of herbal syrup was a good natural remedy against kidney stone. Antioxidant property of herbal syrup have been proved by both superoxide scavanging activity and thiobarbituric acid methods. Cholesterol lowering property was also observed.

**Table: I Effect of herbal syrup on urinary salts of experimental animals (mg/g):**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Group</th>
<th>Oxalate (mg/g)</th>
<th>Calcium (mg/g)</th>
<th>Phosphate (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>0.31±0.07</td>
<td>0.32±0.01</td>
<td>3.62±0.02</td>
</tr>
<tr>
<td>2</td>
<td>Model control</td>
<td>3.46±0.03</td>
<td>4.15±0.09</td>
<td>6.88±0.08</td>
</tr>
<tr>
<td>3</td>
<td>Allopurinol</td>
<td>0.53±0.07</td>
<td>1.40±0.06</td>
<td>3.36±0.04</td>
</tr>
<tr>
<td>4</td>
<td>Herbal Syrup(250mg/kg)</td>
<td>0.65±0.09</td>
<td>1.44±0.08</td>
<td>3.99±0.04</td>
</tr>
<tr>
<td>5</td>
<td>Herbal Syrup (500mg/kg)</td>
<td>0.66±0.02</td>
<td>1.45±0.01</td>
<td>4.02±0.05</td>
</tr>
</tbody>
</table>

n = 3, *P < 0.05, **P < 0.01, ***P < 0.001 vs model control; values are expressed in mean ± SEM; Statistics: oneway ANOVA followed by the Bonferroni test.

**Table: II Effect of herbal syrup on serum parameters of experimental animals (mg/dl).**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Group</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>32.60±1.01</td>
<td>0.72±0.11</td>
<td>1.45±0.07</td>
</tr>
<tr>
<td>2</td>
<td>Model control</td>
<td>46.90±0.01</td>
<td>1.39±0.02</td>
<td>2.11±0.04</td>
</tr>
<tr>
<td>3</td>
<td>Allopurinol</td>
<td>34.88±0.32</td>
<td>0.92±0.01</td>
<td>1.48±0.03</td>
</tr>
<tr>
<td>4</td>
<td>Herbal Syrup(250mg/kg)</td>
<td>38.62±0.70</td>
<td>0.94±0.03</td>
<td>2.52±0.01</td>
</tr>
<tr>
<td>5</td>
<td>Herbal Syrup (500mg/kg)</td>
<td>34.20±0.06</td>
<td>0.93±0.09</td>
<td>2.19±0.01</td>
</tr>
</tbody>
</table>
n = 3, * P < 0.05, ** P < 0.01, *** P < 0.001 vs model control; values are expressed in mean ±SEM; Statistics: oneway ANOVA followed by the Bonferroni test.

Table: III Total urinary volume and pH of urine observed in herbal syrup.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Groups</th>
<th>Total urinary volume (ml)</th>
<th>pH of urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1.89±0.13 ***</td>
<td>7.04±0.06 ***</td>
</tr>
<tr>
<td>2</td>
<td>Model control</td>
<td>1.36±0.08</td>
<td>9.21±0.11</td>
</tr>
<tr>
<td>3</td>
<td>Allopurinol</td>
<td>4.29±0.03 ***</td>
<td>7.21±0.06</td>
</tr>
<tr>
<td>4</td>
<td>Herbal Syrup (250mg/kg)</td>
<td>4.18±0.02 ***</td>
<td>7.27±0.02 *</td>
</tr>
<tr>
<td>5</td>
<td>Herbal Syrup (500mg/kg)</td>
<td>4.16±0.11 ***</td>
<td>7.27±0.05 ***</td>
</tr>
</tbody>
</table>

n = 3, * P < 0.05, ** P < 0.01, *** P < 0.001 vs model control; values are expressed in mean ±SEM; Statistics: oneway ANOVA followed by the Bonferroni test.

T.S. of Kidney

Figure: I (Normal rats).

Figure: II (Ethylene glycol alone).

Figure: III (Allopurinol).
INTERPRETATION AND CONCLUSION

Stone formation in ethylene glycol-fed rats is caused by hyperoxaluria, which cause increased renal retention and excessive excretion of oxalate in urine. In the present study, calcium and oxalate excretion were increased in calculi induced animals. Increased urinary phosphate excretion along with oxalate seems to provide an environment appropriate for stone formation by forming calcium oxalate deposition.\(^{[18,19,20]}\) Herbal syrup restored the phosphate level, thus reducing the risk of stone formation. In urolithiasis, the glomerular filtration rate decreases due to the obstruction to the outflow of urine by stones in the urinary system because of that waste products, particularly nitrogenous substances such as creatinine, urea and uric acid get accumulated in blood. In calculi-induced rats (Group II), marked renal damage was seen by the elevated serum levels of creatinine, uric acid and blood urea nitrogen. Curative treatment of herbal syrup and Allopurinol caused diuresis and hastened the process of dissolving the preformed stones and prevention of new stone formation in the urinary system. The diuretic effect of herbal syrup was evident from urine volumes collected when compared to the model control group. Histopathological studies also supporting the evidence of antiurolithiatic property of Herbal Syrup (Figure I-V). Overall, the results indicated that the administration of herbal syrup to albino wistar rats with ethylene glycol induced lithiasis reduced the growth of the urinary stones. The present study aims to give data highlighting the present trends in research of medicinal plants accredited with antiurolithiatic
activity. The recent treatment of urolithiasis involves NSAID’s, Antidiuretics and Extracorporeal Shock Wave Lithotripsy (ESWL). Recurrence is quite common with these therapies. Herbal syrup may be useful to overcome the major drawback of surgical procedures which is recurrence of stones. The present study will be an initiative towards the commercialization of Herbal preparations in the Pharmaceutical market. The objective of investigation was to evaluate urolithiatic property of herbal syrup. For this purpose carried out chemical test, UV analysis and two months invitro studies. All plants were widely distributed through India (Kerala). Antioxidant property of herbal syrup have been proved by both superoxide scavanging activity and thiobarbituric acid methods. The present investigation shows that herbal syrup have antiurolithiatic property. The experiment is carried out in invitro condition. The chemical and analytical studies shows that the organic and inorganic constituents present in the stem juice which are responsible for the activity. The plant products and derivatives of their lead compounds as such may not replace the ESWL and surgical removal procedures but may surely help in decreasing the recurrence rate of renal calculi. Herbal Syrup may be useful to overcome the major drawback of surgical procedures which is recurrence of stones and the herbal syrup maintains the cholesterol level in the blood.

REFERENCES