EFFECT OF MARKER ENZYMES IN UROLITHIATIC RATS TREATED WITH *ENSETE SUPERBUM ROXB.* SEED EXTRACT

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ABSTRACT
Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been revealed to have valid utility and about 80% rural population depends on its efficacy for their primary health care. The research is lead to the advance usage of indigenous herbal medicines for Urolithiasis. Medicinal plants can be valuable therapeutic resources. The study enlightens the effect of *Ensete superbum* Roxb. ethanolic seed extract in the urolithiatic male albino rats. The administration of Ethylene glycol (0.75%) to the male albino rats were resulting the urolithiasis to the rats. The induction of ethylene glycol is of 30 days and after that the analysis of serum and kidney marker enzymes. The level of marker enzymes AST, ALT, ACP and ALP was showing a decreased level in the kidney because of the cell destruction of tissues caused by urolithiasis. In serum, the level of these enzymes were elevated by the release of enzymes from the organ. It concludes that the treatment with ethanolic seed extract of *Ensete superbum* Roxb. is capable of counteracting the urolithiasis.


INTRODUCTION
Urolithiasis is a common multi-factorial disease that has been recognized and documented in medical literature by the Greek and Roman physicians. Urolithiasis encompasses the renal, bladder and ureteric stones. (Kachchhi NR et al., 2012). Urinary calculi are the third prevalent disorder in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause the obstruction, hydronephrosis,
infection and hemorrhage in the urinary tract system. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common. (Patel et al., 2011).

Kidney stones are composed of inorganic and organic crystals amalgamated with proteins. Urinary stones can be classified according to stone composition as calcium stone, uric acid stone, struvite stone and cystine stone. Some of the other types are calcium phosphate stone, xanthine stone, DHA stone, and crixivian stone. The major predisposing factors that create an imbalance between levels of promoters and inhibitors of stone formation are low urine volumes, diet, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia, hypomagnesuria, low urinary pH, cystinuria, and distal renal tubular acidosis. (Kachchhi NR et al., 2012).

The Indian Traditional Medicine like ayurvedic, siddha and unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases.

Herbal medicine has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources as a good choice, because these natural resources have ordinarily fewer side effects. (Koushik Nandan Dutta et al., 2014).

Drug therapy for the treatment of urinary stone includes, antibiotics, allopurinol, opiates and NSAID’S and diuretics. kidney stones that do not pass on their own by pharmacological management, the most widely preferred technique is the lithotripsy. In this procedure, shock waves are used to break up a large stone into smaller pieces that can then pass through the urinary system. In case of failure with all other treatments, surgical invasive techniques have also been used like percutaneous nephrolithotomy or through ureteroscopy (Kachchhi NR et al., 2012).

*Ensete superbum* Roxb.(wild banana or raan keli) belongs to family Musaceae and it is 12 feet tall evergreen perennial shrub. Plants may grow up to 12 ft in height and the pseudostem may be up to half the height with a swollen base of up to 8 ft in circumference at the base.
The leaves are bright green in colour on both sides with a deeply grooved and short petiole. The leaf sheaths are persistent at the base and leave closely set scars on the corm. The fruits are about 3 inches long and more or less triangular with dark brown seeds.

The urolithiatic treatment is costly and having side effects. Hence the better choice is to be the drug from the natural sources. Many Indian plants have been quoted to be useful as antiurolithiatic agents. They are effective with fewer side effects and are also less costly. The plant *Ensete superbum* Roxb. is here evaluated for antiurolithiasis.

**MATERIALS AND METHOD**

**Animal Model Used**
Male albino white rats of wistar strain with an average weight of 130-150 grams were purchased from RVS College of Pharmacy, Sulur, Coimbatore. The rats were then housed in large spacious cages. The animal room was ventilated. The temperature was maintained between 20° to 30°C.

**Experimental Design**

Rats were divided into four different groups comprising of six animals each.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experimental Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Rats given normal diet for 30 days.</td>
</tr>
<tr>
<td>II</td>
<td>0.75 %Ethylene glycol for 30 days through drinking water</td>
</tr>
<tr>
<td>III</td>
<td>Hyperoxaluria induced rats were received <em>Ensete superbum</em> Roxb. seed extracts (250 mg/ Kg body weight) by oral administration for 30 days</td>
</tr>
<tr>
<td>IV</td>
<td>Hyperoxaluria induced rats were received the standard drug cystone (250 mg/ Kg body weight) by oral administration for 30 days.</td>
</tr>
</tbody>
</table>

**Collection of Plant Species**
The seeds of *Ensete superbum* Roxb. were collected from Palakkad, Kerala and were shade dried and powdered and used for the analysis.

**Preparation of Seed extract**
The fresh seeds collected were washed with distilled water and shade dried. The dried plant material was powdered and extracted with ethanol by immersing for 72 hours. The extract was filtered and air dried to obtain the residue. The residue was suspended in water and administrated orally (250mg /kg body weight) was administrated.
Experimental induction of Urolithiasis by Ethylene glycol

Ethylene glycol induced hyperoxaluria model was used to assess the antiurolithiatic 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. Ethylene glycol (0.75%) in drinking water was fed to Groups II, III and IV for induction of renal calculi till 28th day. (Biren N. et.al., 2011).

Collection of Biological samples

At the end of the experimental period, the animals were sacrificed by cervical decepitation, under mild anesthesia. The blood was carefully collected by pumping the heart after the rat was killed. From the collected blood, the serum was separated by centrifugation at 3000 rpm for 20 minutes. The kidney of these experimental were also taken out through the process and preserved in the buffer.

Biochemical analysis

The serum and kidney marker enzymes like Acid phosphatise (ACP), Alkaline phosphatise (ALP), Aspartate amino transferase(ASP) and Alanine amino transferase (AST) was assessed.

Statistical Analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett’s Multiple Comparison Test using Graph Pad Prism software .The mean values ± SEM were calculated for each parameter. The differences in marker enzymes between the calculi induced group and standard drug treated group were compared. Level of significance was kept at $P<0.05$. (Biren N.Shah.et.al., 2011).

RESULT AND DISCUSSION

Medicinal plants can be valuable therapeutic resources. The treatment of infections with plant derived compounds is an age old practice that is employed throughout the world, especially in developing countries where traditional medicines are used to treat variety of diseases (Yousef M Abouzeed.et.al.2013).

Urolithiasis is a condition in which crystals in the urine combine to form stones, also called calculi or uroliths. These can be found anywhere in the urinary tract, where they cause
irritation and secondary infection. Most end up in the bladder or urethra. (Hossein Hosseinzadeh et.al.2010).

EFFECT OF LYSOMAL AND CYTOSOLIC ENZYMES IN KIDNEY

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>12.19 ± 0.01</td>
<td>7.12 ± 0.01 a*</td>
<td>12.09 ± 0.01 b*</td>
<td>12.11 ± 0.01c* dns</td>
</tr>
<tr>
<td>ALT</td>
<td>13.56 ± 0.01</td>
<td>10.49 ± 0.01 a*</td>
<td>13.32 ± 0.01 b*</td>
<td>13.39 ± 0.01 c* dns</td>
</tr>
<tr>
<td>ACP</td>
<td>11.67 ± 0.01*</td>
<td>8.94 ± 0.01 a*</td>
<td>11.12 ± 0.01 b*</td>
<td>11.23 ± 0.01 c* dns</td>
</tr>
<tr>
<td>ALP</td>
<td>6.82 ± 0.01*</td>
<td>3.92 ± 0.01 a*</td>
<td>6.31 ± 0.01 b*</td>
<td>6.71 ± 0.01 c* dns</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard division of six animals each

AST- Aspartate Transaminase
ALT – Alanine Transaminase
ACP – Acid Phosphatase
ALP – Alkaline Phosphatase

COMPARISON

‘a’ represents comparison between Group II and Group I
‘b’ represents comparison between Group III and Group II
‘c’ represents comparison between Group IV and Group II
‘d’ represents comparison between Group III and Group IV

SYMBOL: * This symbol represents the statistical significance P* < 0.05 ns represents non significance.

UNITS ACP, ALP: μ moles of phenols liberated per liter
AST, ALT: μ moles of pyruvate liberated per liter

The above given table shows the effect of kidney marker enzymes in the experimental groups. The ethylene glycol induced urolithiatic rats has a drastic decrease in the level of the
kidney marker enzymes AST, ALT, ACP and ALP. The formation of the stone will cause some tissue damage and the marker enzymes synthesized by the kidney tissues were exposed to the blood stream. This is the reason were at the urolithiatic condition causing the drastic decrease in the marker enzymes in the tissues. In the mean time the Group III which is considered as the Eneste superbum seed extract administered Group, it shows a highly commendable recovery of the levels of marker enzymes in the kidney tissues. The Group IV, the urolithiatic animals treated with the standard drug Cystone is also showing the neighbouring levels of the marker enzymes as of the control Group I.

The kidney tissue damage in the urolithiatic condition leads to the increased level of these cystolic enzymes in the circulation. So this can also be considering as an after effect of the urolithiagnosis. These cystolic enzymes absences were create a major obstacle in the organ function.

**EFFECT OF LYSOMAL AND CYTOSOLIC ENZYMES IN SERUM**

<table>
<thead>
<tr>
<th></th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
<th>GROUP IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>45.17 ± 0.01</td>
<td>106.54 ± 0.01*</td>
<td>48.72 ± 0.01b*</td>
<td>47.32 ± 0.01c* d**s</td>
</tr>
<tr>
<td>ALT</td>
<td>47.56 ± 0.01</td>
<td>95.12 ± 0.01a*</td>
<td>49.34 ± 0.01b*</td>
<td>48.57 ± 0.01c* d**s</td>
</tr>
<tr>
<td>ACP</td>
<td>59.19 ± 0.01</td>
<td>131.60 ± 0.01a*</td>
<td>62.39 ± 0.01b*</td>
<td>61.21 ± 0.01c* d**s</td>
</tr>
<tr>
<td>ALP</td>
<td>72.53 ± 0.01</td>
<td>132.71 ± 0.01a*</td>
<td>74.19 ± 0.01b*</td>
<td>73.71 ± 0.01c* d**s</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard division of six animals each

AST- Aspartate Transaminase
ALT – Alanine Transaminase
ACP – Acid Phosphatase
ALP – Alkaline Phosphatase

**COMPARISON**

‘a’ represents comparison between Group II and Group I
‘b’ represents comparison between Group III and Group II
‘c’ represents comparison between Group IV and Group II
‘d’ represents comparison between Group III and Group IV

**SYMBOL:** * This symbol represents the statistical significance P* < 0.05
ns represents the non significance .

**UNITS**
- ACP, ALP : μ moles of phenols liberated per liter
- AST, ALT : μ moles of pyruvate liberated per liter
The above mentioned table shows the marker enzyme levels in the serum of the experimental animals. The Group II animals showing highly increased level of the marker enzymes because of the release of these enzymes from the kidney. In urolithiatic conditions the kidney tissue getting damaged and the enzymes are released to the circulating system. Hence the enzymes level in the serum will increase in the serum. By the administration of ethanolic seed extract of the plant *Ensete superbum* Roxb were reduce the level of these enzymes in the serum. The Group IV, the urolithiatic animals treated with the standard drug Cystone is also showing a similarity level of the marker enzymes in Group I.

The study concluded that the ethanolic seed extract of *Ensete superbum* Roxb is able to control the urolithiasis. The standard drug and the plant drug is giving a near normal values compared with normal rats. The study focussed on the marker enzymes in the kidney and serum. The level of marker enzymes in kidney were decreasing in the urolithiatic group, but in case of serum the Group II showing increasing level. The tissue damage in the kidney leads to the release of the marker enzymes to the circulating system. The plant extract is able to regulate the level of these marker enzymes in both kidney and serum. Considering the level of marker enzymes in Group IV which is treated with the standard drug Cystone we can clearly assure that the plant extract treated group were regulating the level of these marker enzymes. It concludes that the treatment with ethanolic seed extract of *Ensete superbum* Roxb. is capable of counteracting the urolithiasis.

REFERENCES


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