EVALUATION OF THE ANTIUROLITHIATIC ACTIVITY OF METHANOLIC EXTRACT OF CELOSIA ARGENTEA ROOTS IN RATS

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ABSTRACT

Urolithiasis is a common disorder with higher recurrence rate in men. Supersaturation of crystals with imbalance between levels of promoters and inhibitors of stone formation results in urolithiasis. Current medical management of urolithiasis is either costly or not without side effects. Therefore, traditionally reported more effective and safer antiurolithiatic medicinal plants need to be studied. Thus, this study was aimed to evaluate the antiurolithiatic activity of methanolic extract of Celosia argentea roots (CaME) in male albino wistar rats. Ethylene glycol (0.75% v/v in drinking water; 28 days) induced urolithiasis preventive model was used to study the effect of CaME low dose (250 mg/kg; p.o.) and high dose (500 mg/kg; p.o.). Cystone (750 mg/kg; p.o.) was used as a standard. At the end of the treatment changes in various physical parameters, promoters, inhibitors, renal function markers in urine and serum samples and antioxidant parameters and histopathology of kidneys were observed. Treatment groups significantly prevented improvement in urinary pH, diuresis and body weight. All the treatments significantly prevented the rise in promoters like calcium, oxalate, uric acid, and inorganic phosphate and increased the levels of magnesium and citrate like inhibitors in various biological samples. Renal function impairment and oxidative stress was also prevented by the treatment as observed by BUN and creatinine analysis and analysis of MDA, proteins, catalase and histopathology respectively. Thus methanolic extract of Celosia argentea roots has proved to be an effective drug in prevention of urolithiasis.

Key words: Urolithiasis, Celosia argentea, Ethylene glycol, Hyperoxaluria, Histopathology.

INTRODUCTION

Urolithiasis is a common multi-factorial disease that has been recognized and documented in medical literature even by the Greek and Roman physicians. Urolithiasis encompasses all the renal, bladder and ureteric stones (Lawrence A and Koya MP, 2009). The lifetime risk is about 10–15% in the developed world, but can be as high as 20–25% in the Middle East. Urolithiasis is a recurrent renal disease affecting 4-8% in UK, 15% in US, 20% in gulf countries and 11% population in India with a relapse rate of 50% in 5–10 years and 75% in 20 years (Paul A. Dawson et al., 2010, Rahul Deo Yadav et al., 2011, Orson W Moe, 2006). 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 crixivan stone. Approximately 80% of kidney stones are primarily composed of calcium oxalate (Stamatiou KM et al., 2006, Heilberg P and Schor, 2006, Coe F L et al., 1992). 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,

Presently, the available drug therapy for the treatment of urinary stone includes, antibiotics (for struvite stones), allopurinol (for uric acid stone), opiates and NSAID’S (for relieving pain), and diuretics (for renal stone removal) (Heilberg P and Schor, 2006). For 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 (Lawrence A and Koya MP, 2009, Tiselius HG et al., 2011).

_Celosia argentea_ is commonly known as plumed cockscomb or the feathery amaranth. It is a tropical herbaceous plant which is widely known because of its beautiful and unique color of flowers. _Celosia argentea_ is from family Amaranthaceae and is an annual shrub with a multiple branching. The seeds and roots yield triterpenoid saponins, alkaloids and flavonoids. Traditionally it is reported to have diuretic activity which can be useful in the management of urolithiasis. Thus, aim of the study was to evaluate antiurolithiatic activity of methanolic extract of _Celosia argentea_ roots in male albino wistar rats (Khare CP, 2007, Santosh ghule et al., 2010).

**MATERIALS AND METHODS**

**ANIMALS**

Healthy adult male Wistar Albino rats (250-300gm) were selected for study of antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum.

**PLANT MATERIAL AND EXTRACTION**

The roots of _Celosia argentea_ were collected from the medicinal garden of R. K. College of pharmacy, Rajkot. The collected roots were identified and authenticated by head of botany department, Christ College, Rajkot. The root samples were shade-dried and grounded to fine powder. About 0.5 kg powdered sample was defatted with petroleum ether. The defatted root powder was then used for the preparation of methanolic extract. The methanolic extract has been prepared by using soxhlet apparatus at 60°C for 24 hours. Methanolic extract was concentrated by evaporation to dryness and yield was calculated (Santosh ghule et al., 2010).

**EXPERIMENTAL PROTOCOL**

**ETHYLENE GLYCOL INDUCED UROLITHIASIS PREVENTIVE STUDY**

Healthy male Wistar albino rats (30 rats) were divided into five groups containing six rats in each and the preventive study was conducted for 28 days. All animals were weighed before and after the study period. All groups received regular rat food and drinking water ad libitum. Except group I, all animals received 0.75% v/v ethylene glycol in drinking water ad libitum throughout study period. Group I and group II served as normal control and disease control respectively. Group III received standard antiurolithiatic drug, Cystone (750 mg/kg), while Group IV and group V received low dose (250mg/kg) and high dose (500 mg/kg) of methanolic extract of roots of _Celosia argentea_ (CaME). The treatment was given orally once daily throughout study period for 28 days. Various biological samples like blood, urine and kidney were collected at the end of the treatment period for the analysis of different parameters (Gadgeb N B et al., 2006).

Group 1: Normal control
Group 2: Disease control (0.75% v/v EG in drinking water)
Group 3: Standard (0.75% v/v EG in drinking water +750mg/Kg Cystone; p.o)
Group 4: CaME-250mg/kg (0.75% v/v EG in drinking water + 250 mg/kg of CaME; p.o)
Group 5: CaME-500mg/kg (0.75% v/v EG in drinking water + 500 mg/kg of CaME; p.o)

**COLLECTION OF VARIOUS BIOLOGICAL SAMPLES**

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 29th day. Animals had free access to drinking water during the urine collection period. After the experimental period, blood was collected retro-orbitally under anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 15000 RPM for 20 min. Rats were sacrificed by cervical dislocation at the end of the experimental period. The abdomen was cut open to isolate both kidneys from each animal (Gadgeb N B et al., 2006).

**ESTIMATION OF BIOCHEMICAL PARAMETERS**

Various biochemical parameters were estimated by using erba diagnostic kit and analytical method. These parameters are urolithiasis promoters (calcium, oxalate, uric acid and inorganic phosphate), urolithiasis inhibitors (magnesium and citrate), BUN, Urea nitrogen, creatinine and antioxidant parameter (MDA, protein, and catalase) (Newman D J and Price C P, 1999).

**HISTOPATHOLOGY EXAMINATION**
At the end of the experiment (day 28), all rats were anesthetized by diethyleter and the kidneys were removed for histopathological examination.

**STATISTICAL ANALYSIS**

**RESULTS**

Table 1. Effect of methanolic extract of *Celosia argentea* root on various physical parameters in ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHANGE IN BODY WEIGHT (gm)</td>
<td>276.66±6.69</td>
<td>216.66±5.59</td>
<td>248.33±4.02**</td>
<td>255±6.21</td>
<td>253.33±4.96*</td>
</tr>
<tr>
<td>VOLUME OF URINE IN (ml)</td>
<td>17.66±0.21</td>
<td>15.5±0.42*</td>
<td>28.33±0.42*</td>
<td>23.16±0.70*</td>
<td>25.66±0.42*</td>
</tr>
<tr>
<td>pH</td>
<td>6.5±0.22</td>
<td>8.66±0.21**</td>
<td>7.33±0.21*</td>
<td>7.83±0.16</td>
<td>7.66±0.21**</td>
</tr>
<tr>
<td>WET KIDNEY WEIGHT (gm)</td>
<td>0.82±0.03</td>
<td>1.38±0.08*</td>
<td>0.77±0.02*</td>
<td>0.78±0.02*</td>
<td>0.76±0.01*</td>
</tr>
<tr>
<td>DRY KIDNEY WEIGHT (gm)</td>
<td>0.14±0.01</td>
<td>0.36±0.01*</td>
<td>0.18±0.01*</td>
<td>0.19±0.01*</td>
<td>0.17±0.01*</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group. Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

- # Highly significant difference from normal p ≤ 0.001
- ## Significant difference from normal p ≤ 0.05
- * Highly significant difference from control p ≤ 0.001
- ** Significant difference from control p ≤ 0.05

CaME-LD: *Celosia argentea* methanolic extract-Low dose (250mg/kg)

CaME-HD: *Celosia argentea* methanolic extract-High dose (500mg/kg)

Table 2. Effect of methanolic extract of *Celosia argentea* root on various promoters in urine by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCIUM (mg/dl)</td>
<td>5.7±0.24</td>
<td>7.39±0.53*</td>
<td>4.28±0.37**</td>
<td>5.04±0.23</td>
<td>4.80±0.22*</td>
</tr>
<tr>
<td>OXALATE (mg/dl)</td>
<td>1.89±0.34</td>
<td>6.49±0.17*</td>
<td>0.45±0.05*</td>
<td>2.52±0.30*</td>
<td>2.32±0.20*</td>
</tr>
<tr>
<td>INORGANIC PHOSPHATE (IP) (mg/dl)</td>
<td>0.17±0.01</td>
<td>0.87±0.05*</td>
<td>0.15±0.01*</td>
<td>0.58±0.08**</td>
<td>0.46±0.07*</td>
</tr>
<tr>
<td>URIC ACID (mg/dl)</td>
<td>10.13±0.51</td>
<td>25.73±0.76*</td>
<td>9.25±0.43*</td>
<td>22.71±0.84**</td>
<td>18.45±0.90*</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group. Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

- # Highly significant difference from normal p ≤ 0.001
- ## Significant difference from normal p ≤ 0.05
- * Highly significant difference from control p ≤ 0.001
- ** Significant difference from control p ≤ 0.05
Table 3. Effect of methanolic extract of *Celosia argentea* root on various promoters in serum by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCIUM (mg/dl)</td>
<td>4.01±0.15</td>
<td>5.05±0.21**</td>
<td>3.09±0.13*</td>
<td>4.30±0.36</td>
<td>3.88±0.23**</td>
</tr>
<tr>
<td>INORGANIC PHOSPHATE (I.P.) (mg/dl)</td>
<td>0.92±0.01</td>
<td>1.27±0.09**</td>
<td>0.77±0.02*</td>
<td>1.00±0.05</td>
<td>0.79±0.09*</td>
</tr>
<tr>
<td>URIC ACID (mg/dl)</td>
<td>2.49±0.19</td>
<td>4.11±0.25*</td>
<td>1.80±0.13*</td>
<td>2.08±0.26*</td>
<td>1.04±0.16*</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group.

Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

# Highly significant difference from normal p ≤ 0.001
## Significant difference from normal p ≤ 0.05
* Highly significant difference from control p ≤ 0.001
** Significant difference from control p ≤ 0.05

Table 4. Effect of methanolic extract of *Celosia argentea* root on various promoters in kidney by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCIUM (mg/gm)</td>
<td>0.22±0.007</td>
<td>0.4±0.02*</td>
<td>0.19±0.003*</td>
<td>0.25±0.01*</td>
<td>0.21±0.01*</td>
</tr>
<tr>
<td>OXALATE (mg/gm)</td>
<td>4.11±0.06</td>
<td>6.13±0.16*</td>
<td>1.17±0.11**</td>
<td>3.02±0.23*</td>
<td>1.74±0.20*</td>
</tr>
<tr>
<td>INORGANIC PHOSPHATE (I.P.) (mg/100mg)</td>
<td>4.52±0.07</td>
<td>8.93±0.05*</td>
<td>5.44±0.04*</td>
<td>5.56±0.16*</td>
<td>6.57±0.08*</td>
</tr>
<tr>
<td>URIC ACID (mg/100mg)</td>
<td>1.18±0.08</td>
<td>2.56±0.03*</td>
<td>1.49±0.05*</td>
<td>1.65±0.08*</td>
<td>2.10±0.16*</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group.

Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

# Highly significant difference from normal p ≤ 0.001
## Significant difference from normal p ≤ 0.05
* Highly significant difference from control p ≤ 0.001
** Significant difference from control p ≤ 0.05

Table 5. Effect of methanolic extract of *Celosia argentea* root on various inhibitors in urine and serum by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CITRATE (mg/dl)</td>
<td>5.94±0.06</td>
<td>2.87±0.12*</td>
<td>4.55±0.22*</td>
<td>5.14±0.29*</td>
<td>4.51±0.32*</td>
</tr>
<tr>
<td>MAGNESIUM (URINE) (mg/dl)</td>
<td>1.24±0.16</td>
<td>0.68±0.13</td>
<td>2.48±0.18*</td>
<td>1.49±0.29</td>
<td>2.01±0.25**</td>
</tr>
<tr>
<td>MAGNESIUM (SERUM) (mg/dl)</td>
<td>0.93±0.03</td>
<td>0.62±0.03*</td>
<td>1.23±0.03*</td>
<td>1.10±0.09*</td>
<td>1.14±0.05*</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group.

Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

# Highly significant difference from normal p ≤ 0.001
## Significant difference from normal p ≤ 0.05
* Highly significant difference from control p ≤ 0.001
** Significant difference from control p ≤ 0.05
Table 6. Effect of methanolic extract of *Celosia argentea* root on renal function in urine and serum by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA NITROGEN IN URINE (mg/dl)</td>
<td>12.25±0.58</td>
<td>18.47±0.95*</td>
<td>10.28±0.56*</td>
<td>14.58±0.65^</td>
<td>11.15±0.55*</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>40.13±0.71</td>
<td>61.60±0.66*</td>
<td>37.41±0.78*</td>
<td>42.70±0.94*</td>
<td>39.10±0.98*</td>
</tr>
<tr>
<td>CREATININE CLEARANCE IN URINE (mg/dl)</td>
<td>14.78±0.95</td>
<td>27.48±0.77*</td>
<td>13.56±0.78*</td>
<td>17.16±0.60*</td>
<td>15.58±0.81*</td>
</tr>
<tr>
<td>CREATININE CLEARANCE IN SERUM (mg/dl)</td>
<td>0.54±0.02</td>
<td>0.99±0.06*</td>
<td>0.39±0.02*</td>
<td>0.75±0.05**</td>
<td>0.73±0.08**</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group.

Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

# Highly significant difference from normal p ≤ 0.001
## Significant difference from normal p ≤ 0.05
* Highly significant difference from control p ≤ 0.001
** Significant difference from control p ≤ 0.05
^ Significant difference from control p ≤ 0.01

Table 7. Effect of methanolic extract of *Celosia argentea* root on oxidative stress in kidney by ethylene glycol induced urolithiasis preventive study

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL</th>
<th>CONTROL</th>
<th>STANDARD</th>
<th>CaME-LD</th>
<th>CaME-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROTEIN (mg/ml)</td>
<td>3.60±0.12</td>
<td>2.86±0.08**</td>
<td>3.54±0.15**</td>
<td>3.39±0.14**</td>
<td>3.69±0.14**</td>
</tr>
<tr>
<td>CATALASE (µmol/mg)</td>
<td>77.62±0.30</td>
<td>43.80±0.27*</td>
<td>72.96±0.28*</td>
<td>58.22±0.29*</td>
<td>70.22±0.30*</td>
</tr>
<tr>
<td>MDA (nmol/mg)</td>
<td>0.84±0.08</td>
<td>1.61±0.11*</td>
<td>0.90±0.08</td>
<td>1.27±0.14</td>
<td>1.09±0.13**</td>
</tr>
</tbody>
</table>

All the values are expressed in terms of mean ± SEM; n = 6 in each group.

Statistical analysis of various physical and biochemical parameters is carried out using the one-way ANOVA test.

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## Significant difference from normal p ≤ 0.05
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^ Significant difference from control p ≤ 0.01

HISTOPATHOLOGY OF KIDNEY
DISCUSSION

Urinary stone is a hard, crystalline mineral material formed within the kidney or urinary tract. Urolithiasis is a common disease with an increasing incidence and prevalence worldwide that appears even more pronounced in industrialized countries (Hesse A et al., 2003).

Ethylene glycol on intake is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase or aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which is further oxidized to oxalic acid/oxalate by glycolate oxidase or lactate dehydrogenase, thus promoting hyperoxaluria. Further, increase in calcium, oxalate, uric acid and inorganic phosphate levels in the urine and serum enhances the crystallization. Crystals in urine combine to form stone which is commonly because of supersaturation of urine with crystalloids which depends on salt concentration, urinary volume and pH (Maurice A and Erick L, 2000). Same results were also observed in ethylene glycol induced disease control group whereas treated groups showed significant prevention in formation of hyperoxaluric and thus stone formation. However, high dose of test drug (500 mg/kg) gave comparatively more effect. Intense pain may lead to decrease in the food consumption which may further result into decrease in the body weight. We also found decrease in body weight in the disease control group. However, the standard (750mg/kg; p.o.), low dose of CaME (250mg/kg; p.o.) and high dose of CaME (500mg/kg; p.o.) treatment showed a good diuretic activity and so clearance of calculi which would have prevented the stone formation and subsequent pain and reduction in body weight.

Urine is usually acidic which is inhibitory to crystallization but, hyperoxaluria results in alkalinization of urine pH as observed in disease control group. Treatment with standard and test groups prevented a shift of pH from acidic to alkaline suggesting that it prevents the precipitation of calcium oxalate.

Ethylene glycol intake leads to increase in levels of promoters like calcium, oxalate, uric acid, and inorganic phosphate and decrease level of inhibitors like magnesium and citrate as observed in disease control groups. However, promoters and inhibitors in various biological samples were significantly prevented by the treatment with standard drug and low and high dose test samples.

Due to the obstruction to the urine outflow by stones and due to severe oxalate induced nephrotoxicity, waste nitrogenous substances like BUN accumulates resulting in decreased excretion of urea nitrogen in urine and creatinine clearance (Ramasamy Selvan, 2002).
However, both standard and test treatments significantly prevented the formation of stone and thus reduced the damage to kidney as observed by the inhibited decline in urea and creatinine clearance. Thus, administration of methanolic extract of Celosia argentea prevented the impairment of renal function.

Renal impairment and kidney stone itself can result in oxidative stress which can be compensated by elevated antioxidant enzymes in the kidney. But failure to compensate it result in cellular damage as evidenced by histopathology and significant increase in lipid peroxidation and decrease in catalase levels in disease control group. Administration of standard and test drugs resulted in significant decrease in lipid peroxidation and so significantly prevented decrease in the catalase levels in kidney which suggests its efficacy in preventing oxidative stress induced damage.

Thus we can say that Celosia argentea root is good and effective in prevention of crystal aggregation which may be because of its diuretic ability to alter promoters and inhibitors level.

CONCLUSION
Treatment with low dose of CaME (250mg/kg body weight) and high dose of CaME (500mg/kg body weight) significantly prevented decrease in body weight, water intake, urine output and shift of pH to alkaline. Test drugs significantly prevented increase in urolithiatic promoters (calcium, oxalate, uric acid and inorganic phosphate) and decrease in urolithiatic inhibitors (citrate and magnesium) as observed in various biological samples. Oxidative stress as observed by increased MDA level and decreased protein and catalase levels. Histopathology results were also significantly prevented by treatment groups.

In conclusion, treatment with both low dose (250mg/kg, p.o.) and high dose (500mg/kg, p.o.) of CaME have prophylactic effect in renal stone, confirming its antiurolithiatic activity. However, comparatively 500 mg/kg dose of CaME is more efficacious.

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