BIOLOGICAL EVALUATION OF IN-VIVO DIURETIC AND ANTIUROLITHIATIC ACTIVITIES OF LEAF EXTRACTS OF MELOCHIA CORCHORIFOLIA

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ABSTRACT: The present study was investigated to establish the diuretic and antiurolithiatic activities of chloroform and ethanolic leaf extract of Melochia corchorifolia. The chloroform and ethanolic leaf extract were administered to experimental rats orally at a dose of 200 mg/ kg and 400 mg/kg. Furosemide (5 mg/kg) was used as reference standard for diuretic activity. The parameters measured for diuretic activity was total urine volume; urine electrolyte concentrations such as sodium, potassium, chloride, and bicarbonates. In the in-vitro antiurolithiatic activity, Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The effect of chloroform and ethanolic leaf extract of Melochia corchorifolia (100, 200 and 500 μg/ml) was studied by time course measurement of absorbance at 620 nm for ten minutes using a spectrophotometer. Both the extracts show good in-vitro antiurolithiatic activity. In the in-vivo antiurolithiatic activity, urolithiasis was induced in male rats by administering ethylene glycol (0.75% v/v) in drinking water for 28 days, and the parameters such as oxalate, calcium, and phosphate were estimated in urine. Serum creatinine, calcium, and uric acid were also estimated. Treatment with the leaf extract of Melochia corchorifolia restored all biochemical, urinary parameters. The results obtained justified the importance of the leaf extract of Melochia corchorifolia as a diuretic and antiurolithiatic agent.

INTRODUCTION: Diuretics are agents which augment the renal excretion of sodium and either chloride or bicarbonate primarily and water excretion secondarily. The term saluretic is sometimes used to describe a drug that increases the renal excretion of sodium and chloride ions ¹. Diuretics play an important role in situations like hypercalciuria, edema, acute and chronic renal failure, cirrhosis of the liver, and acts as an antihypertensive agent.

Several diuretics like thiazides, furosemide, mannitol, and ethacrynic acid are used in practice ². Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive nephritic syndrome, renal failure, heart failure, cirrhosis, hypertension, and pregnancy toxaemia ³. Urolithiasis is the formation of stones in the urinary tract that prominently causes the variable degree of pain, bleeding, and further may lead to secondary
infection. It is one of the third most common afflictions found in humans. The process of formation of kidney stones may be due to nucleation, aggregation, and crystal growth phenomena.

Melochia corchorifolia Linn. Malvaceae is a wild crop and grows in most parts of India as a weed. Some species of the genus Melochia have been used in folk medicine, such as dysentery, abdominal swellings, and water-snake bites, bronchitis and cough.

MATERIALS AND METHODS:
Collection of Plant Parts: The whole plants of Melochia corchorifolia were collected from the surroundings of Surampalem, East Godavari district, Andhra Pradesh. The plants were identified and authenticated by the taxonomist Mr. T.V. Raghavarao, Department of Botany SRVBSJB Maharani College, Peddapuram, E.G., District. Andhra Pradesh.

Preparation of Melochia Corchorifolia Extract: The leaves were shade dried, pulverized and sieved through 40 mesh. The powder leaves were extracted with ethanol in soxhlet apparatus for 72 hours at 50 °C. The extract obtained was evaporated under vacuum to remove the solvent completely and obtained gummy exudates, stored at low temperature in the refrigerator for further studies.

Diuretic Activity: Diuretic activity was determined following Lipschitz method. The Institutional Animal Ethical Committee approved the experimental protocol (1269/a/10/CPCSEA). The rats [36 no.] were fasted for 18 h and deprived of water before the experiment. A priming dose of 25 ml/kg of normal saline was given to all rats. The rats were grouped into 6 groups [6 rats in each].

Group I: Control group and treated with vehicle, 0.5% acacia orally.
Group II: Treated with standard drug Furosemide (5 mg/kg p.o) dissolved in the vehicle.
Group III and IV: Were treated with chloroform leaf extract of Melochia corchorifolia (200 mg/kg and 400 mg/kg p.o respectively).
Group V and VI: Were treated with ethanolic leaf extract of Melochia corchorifolia (200 mg/kg and 400 mg/kg p.o) respectively.

Immediately after the administration, the rats were placed in metabolic cages, one rat per cage. The metabolic cages provided with a funnel for urine collection and a mesh to separate the feces from the urine. The bladder was emptied by pulling the base of the tail of each rat. The urine was collected into a beaker covered with aluminum foils to avoid evaporation. The volume of urine collected was recorded after 5 h, and urine was subjected to analysis for determination of sodium, potassium ions by Flame photometry and chloride, bicarbonate by titrimetric analysis after 24 hrs. The Saluretic, Natriuretic and Diuretic Indices were also calculated.

Antiurethiatic Activity: In-vitro Antiurethiatic Activity:
Experimental Protocol: The effect of the extract on calcium oxalate crystallization was determined by the time course measurement of turbidity changes due to the crystallization in artificial urine on the addition of 0.01M sodium oxalate solution. The Precipitation of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer.

Preparation of Synthetic Urine: For preparation of synthetic urine 3.8 gm of potassium chloride, 8.5 gm of sodium chloride, 24.5 gm of urea, 1.03 gm of citric acid, 0.34 gm of ascorbic acid, 1.18 gm of potassium phosphate, 1.4 gm of creatinine, 0.64 gm of sodium hydroxide, 0.5 gm of calcium chloride, 0.47 gm of sodium bicarbonate and 0.28 ml of sulphuric acid were added in 500 ml of deionised water and stirred for 1 h. The synthetic urine was stored at -4 °C until further use.

Study without Inhibitor: A volume of 1.0 ml of artificial urine was transferred into the cell and 0.5 ml of distilled water added to it, and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume and the measurement is determined immediately and recorded for ten minutes.

Study with Inhibitor: The extract was dissolved in distilled water filtered through a membrane filter, and the concentration of 100, 300 and 500 µg/ml were obtained. A mixture of 1ml of artificial urine and 0.5 ml of extract solution was taken in the cell.
A blank reading was taken, and then 0.5 ml of 0.01M sodium oxalate solution was added and immediately absorbance was measured for a period of the 10 minutes with 2 min interval at 620 nm. The % of inhibition was calculated using the following formula:

\[
% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100
\]

**In-vivo Antiurolithiatic Activity:** Ethylene glycol-induced hyperoxaluria method was used to assess antiurolithiatic activity in male albino rats. Animals were divided into four groups containing six animals in each group. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups for induction of renal calculi for 28 days. The extract was assessed for antiurolithiatic activity for its curative action in urolithiasis.

In the Curative regimen, the extract was given from 15th day to 28th day.

- **Group I:** Normal Control group received regular rat food and drinking water.
- **Group II (Calculi-Induced):** Positive control received ethylene glycol (0.75% v/v) in Drinking Water for induction of renal calculi for 28 days.
- **Group III:** Received ethylene glycol and standard anti-Urolithiasis drug Cystone (750 mg/kg b.w) from 15th day to 28th day.
- **Group IV and V:** Received *Melochia corchorifolia* (chloroform extract 200 mg/kg and 400 mg/kg p.o) respectively;
- **Group VI and VII:** Received *Melochia corchorifolia* (ethanol extracts 200 mg/kg and 400 mg/kg p.o) respectively, from 15th day to 28th day.

**Estimation of Biochemical Parameter:** At the end of the experiment (28th day), urine was collected, and urine was subjected to analysis for determination calcium, phosphate, and oxalate. Blood was collected through a tail vein for determination of calcium, creatinine and uric acid in serum.

**RESULTS:**

**Diuretic Activity:** The chloroform extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced a volume of urine was 0.44 and 0.61 ml after 5 h. The excretion of sodium by both doses of chloroform extract was found to be 178.14 and 182.14 µ moles/kg. Similarly, the excretion of potassium, chlorides, and bicarbonates were markedly increased in chloroform treated groups compared to the control group.

But when compared to chloroform treated groups the ethanolic extract of *Melochia corchorifolia* treated group showed better results. The ethanolic extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced volumes of urine was 0.51 and 0.70 ml after 5 hrs. The excretion of sodium by both doses of ethanolic extract was found to be 172.23 and 212.14 µ moles/kg which is higher than the chloroform treated group. Similarly, the excretion of potassium, chlorides, and bicarbonates was markedly increased in ethanolic extract treated groups compared to other groups. Furosemide was used as standard (group II).

The volume output and the electrolytes excretion with the standard drug were found to be excellent. When compared to chloroform extract the ethanolic extract shows good diuretic activity. Saluretic, Natriuretic and Diuretic indexes were calculated for all the groups and results were tabulated in Table 2. The results of the diuretic effect by the control, extract treated groups and standard drug Furosemide 5 mg/kg were given in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Standard Furosemide 5 mg/kg</th>
<th>Chloroform extract in mg/kg</th>
<th>Ethanolic extract in mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>Volume of urine (mL/Hr) after 5 hrs</td>
<td>0.18±0.26</td>
<td>0.74±0.14</td>
<td>0.44±0.62</td>
<td>0.61±0.04</td>
</tr>
<tr>
<td>Sodium (Na⁺) µ moles/Kg</td>
<td>173.3±0.35</td>
<td>232.14±0.65**</td>
<td>178.14±0.45**</td>
<td>182.14±0.36**</td>
</tr>
<tr>
<td>Potassium (K⁺) µ moles/Kg</td>
<td>121.48±0.06</td>
<td>144±0.23''</td>
<td>124±0.64''</td>
<td>129±0.24''</td>
</tr>
<tr>
<td>Chloride (Cl⁻) µ moles/Kg</td>
<td>98.69±0.59</td>
<td>152±0.39**</td>
<td>76±0.42''</td>
<td>102±0.32''</td>
</tr>
<tr>
<td>Bicarbonate (HCO₃⁻) µ moles/Kg</td>
<td>9.97±0.17</td>
<td>26±0.22''</td>
<td>10±0.56</td>
<td>16±0.42''</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; n=3; ** p<0.01; All comparisons are made with control.
TABLE 2: SALURETIC, NATRIURETIC AND DIURETIC INDICES OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Parameters</th>
<th>Control</th>
<th>Standard</th>
<th>Chloroform extract in mg/kg</th>
<th>Ethanolic extract in mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>1</td>
<td>Saluretic Index [Na⁺ + Cl⁻]</td>
<td>156.5±0.3</td>
<td>384±0.44*</td>
<td>254±0.06*</td>
<td>284±0.06*</td>
</tr>
<tr>
<td>2</td>
<td>Natriuretic Index [Na⁺/ K⁺]</td>
<td>1.04±0.18</td>
<td>0.88±0.16</td>
<td>1.21±0.16</td>
<td>1.41±0.42</td>
</tr>
<tr>
<td>3</td>
<td>Volume of urine in ml after 5 h</td>
<td>0.15±0.08</td>
<td>0.74±0.08</td>
<td>0.44±0.14</td>
<td>0.61±0.03</td>
</tr>
<tr>
<td>4</td>
<td>Diuretic Index</td>
<td>-</td>
<td>4.11±0.8</td>
<td>2.44±0.20</td>
<td>3.38±0.14</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM n = 3 ** = p>0.01
Diuretic Index = volume of urine in test group/volume of urine in control.

The maximum inhibition of chloroform (500 µg/ml) was observed at 66.71%. But when compared with these 2 extracts the ethanolic extract showed maximum % inhibition at 67.16%.

FIG. 1: DIURETIC EFFECT OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

FIG. 2: SALURETIC, NATRIURETIC AND DIURETIC INDICES OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS

FIG. 3: IN-VITRO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA EXTRACTS
Anti-Urolithiasis Activity:

**In-vitro Anti-Urolithiasis Activity:** The results of in-vitro Anti-Urolithiasis activity of chloroform and ethanolic leaf extracts of *Melochia corchorifolia* exhibits dose and time-dependent % inhibition were given in the below Table 3.

**In-vivo Anti-Urolithiasis Activity:** The ethanolic and chloroform extracts of *Melochia corchorifolia* exhibited a marked decrease in the levels of oxalate, phosphate, and calcium in urine and also in serum. The oxalate, phosphate and calcium levels in urine after induction of calculi (group II) were found to be 7.63, 40.0 and 14.67 mg/dl. The rats treated with chloroform extract of *Melochia corchorifolia* with a dose of 200 and 400 mg/kg has decreased the level of oxalate, phosphate, and calcium.

But when compared to group V (chloroform 400 mg/kg treated group) the ethanolic extract treated groups (group VII) has decreased the levels of oxalate, phosphate, and calcium to a greater extent. The results are 2.49, 15.87 and 3.37 mg/dl.

![Image](image_url)

**FIG. 4: IN-VIVO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS**

**DISCUSSION:**

**In Diuretic Activity:** The chloroform extract of *Melochia corchorifolia* at dose 200 and 400 mg/kg body weight produced diuresis, and the volume of urine is 0.44 and 0.61 ml after 5 h. The excretion of sodium by both doses of chloroform extract was found to be 178.14 and 182.14 µmoles/kg. Similarly, the excretion of potassium, chlorides, and bicarbonates was markedly increased in

**TABLE 4: IN-VIVO ANTI-UROLITHIASIS ACTIVITY OF MELOCHIA CORCHORIFOLIA LEAF EXTRACTS**

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Calculi Induced)</th>
<th>Group III (Cystone) 750 mg/kg</th>
<th>Group IV CE-200 mg/kg</th>
<th>Group V CE-400 mg/kg</th>
<th>Group VI EE-200 mg/kg</th>
<th>Group VII EE-400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>Calcium mg/dl</td>
<td>3.53</td>
<td>14.67</td>
<td>3.33</td>
<td>5.40</td>
<td>3.59</td>
<td>4.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.058</td>
<td>±0.574</td>
<td>±0.058</td>
<td>±0.100</td>
<td>±0.067</td>
<td>±0.059</td>
<td>±0.153</td>
</tr>
<tr>
<td>2</td>
<td>Phosphate</td>
<td>mg/dl</td>
<td>18.40</td>
<td>40.00</td>
<td>15.30</td>
<td>29.63</td>
<td>19.17</td>
<td>10.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.529</td>
<td>±0.600</td>
<td>±0.608</td>
<td>±0.232</td>
<td>±0.764</td>
<td>±0.196</td>
<td>±0.231</td>
</tr>
<tr>
<td>3</td>
<td>Oxalate</td>
<td>mg/dl</td>
<td>4.30</td>
<td>7.63</td>
<td>3.20</td>
<td>4.03</td>
<td>2.68</td>
<td>3.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.100</td>
<td>±0.058</td>
<td>±0.100</td>
<td>±0.153</td>
<td>±0.050</td>
<td>±0.100</td>
<td>±0.029</td>
</tr>
<tr>
<td>4</td>
<td>Serum</td>
<td>Calcium mg/dl</td>
<td>8.13</td>
<td>16.33</td>
<td>3.43</td>
<td>11.97</td>
<td>7.96</td>
<td>14.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.231</td>
<td>±0.537</td>
<td>±0.153</td>
<td>±0.451</td>
<td>±0.148</td>
<td>±0.306</td>
<td>±0.116</td>
</tr>
<tr>
<td>5</td>
<td>Creatinine</td>
<td>mg/dl</td>
<td>0.65</td>
<td>38.33</td>
<td>0.80</td>
<td>17.65</td>
<td>11.53</td>
<td>11.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.050</td>
<td>±0.579</td>
<td>±0.100</td>
<td>±0.216</td>
<td>±0.503</td>
<td>±0.015</td>
<td>±0.100</td>
</tr>
<tr>
<td>6</td>
<td>Uric acid</td>
<td>mg/dl</td>
<td>3.23</td>
<td>7.70</td>
<td>3.30</td>
<td>3.33</td>
<td>2.21</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.058</td>
<td>±0.100</td>
<td>±0.265</td>
<td>±0.153</td>
<td>±0.041</td>
<td>±0.038</td>
<td>±0.153</td>
</tr>
</tbody>
</table>

CE-chloroform extract, EE-ethanol extract, Values are Mean ± SEM; n = 3 (number of animals in each group)

All comparisons are made with that of control.

The results of all three extract treated groups are significant and comparable to the standard drug (750 mg/kg). Cystone treated group’s shows oxalate, phosphate and calcium levels are 3.20, 15.30 and 3.33 mg/dl respectively.

Similarly, chloroform and ethanolic treated groups of *Melochia corchorifolia* leaf extract markedly decreased the uric acid, calcium and creatinine levels in serum. The maximum uric acid, creatinine, and calcium levels were found in calculi induced rats (group II) 7.70, 38.33 and 16.33 mg/dl. The chloroform extract of *Melochia corchorifolia* at the dose 200 and 400 mg/kg body wt has decreased level of. But when compared with these 2 extracts, the ethanolic treated groups have decreased the level of oxalate, phosphate, and calcium to a greater extent. The results are 3.30, 0.80 and 3.43 mg/dl where as Cystone treated group’s shows uric acid, calcium and creatinine levels are 2.20, 1.90 and 9.78 mg/dl, respectively. All these results are mentioned in Table 4.
The kidney is the principal target for Ethylene glycol induced toxicity. Its administration to rats for 28 days resulted in substantial excretion of oxalate and deposition of microcrystal in kidney. An increased urinary calcium concentration is a factor favoring nucleation and precipitation of Calcium oxalate or apatite (calcium phosphate) and subsequent crystal growth. Apart from urinary calcium excretion, a decrease in serum calcium was evident in treated urolithiasis rats. Another possible mode of action includes excessive excretion or decrease in the concentration of urinary salts that prevent the supersaturation of the crystallizing salts. Based on the results obtained Treatment with ethanol leaf extract of Melochia corchorifolia decrease the re-absorption of water and electrolytes results in diuresis, and also it lowers the levels of calcium, phosphate and oxalate excretion. With support to histological features, inflammatory changes were improved in Cystone and ethanol extract treated rats which exhibited normal renal architecture. Phytochemical screening of extracts of Melochia corchorifolia revealed the presence of phytosterols, tannins, flavonoids, saponins, glycosides; these constituents may contribute to its diuretic and anti-urolithiasis activities.

CONCLUSION: From the study, it was concluded that Melochia corchorifolia extract 400 mg/kg p.o significantly reduce the elevated calcium, oxalate phosphate and creatinine concentrations in urine confirming the stone inhibitory effect.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:


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