Antiurolithiatic activity of *Crataeva magna* Lour. bark

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*Crataeva magna* Lour. bark, commonly known as *Baruna*, belonging to family Capparaceae, has been investigated for its antiurolithiatic activity in two conventional models (*in vivo*) of Urolithiasis in rats. The two methods chosen were lactose (30%) + ethylene glycol (1%) and ammonium chloride (2%) + ethylene glycol (0.75%) induced urolithiasis, respectively. The ethanol extract (400 mg/kg bw) reduced the elevated levels of serum calcium (3.25 ± 0.30) and urine calcium (2.33 ± 0.18) significantly (*P*<0.05), employing lactose (30%) + ethylene glycol (1%) induced urolithiasis model. The ethanol extract (400 mg/kg bw) reduced the urine uric acid level significantly employing both models, viz. lactose (30%) + ethylene glycol (1%) (0.82 ± 0.07; *P*<0.001) and ammonium chloride (2%) + ethylene glycol (0.75%) (0.85 ± 0.12; *P*<0.001) when compared to toxic group. The ethanol extract (400 mg/kg bw) employing both models resulted in reduced serum creatinine and calcium, urine oxalate and kidney weight significantly with a marked increase in final body weight and urine volume output when compared to toxic group. The results shown by the ethanol extract (400 mg/kg bw) group was compared to standard polyherbal drug (Cystone; 5 ml/kg bw) treated group and thus exhibited potent antiurolithiatic activity.

**Keywords**: Ammonium chloride, Bark, Baruna, *Crataeva magna*, Ethylene glycol, Lactose, Urolithiasis.

**IPC code**: Int. cl. (2011.01) — A61K 36/18, A61K 129/00, A61P 13/04

**Introduction**

Urolithiasis in its different forms is frequently encountered during urological complications. Some common causes are inadequate urinary drainage, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities, viz. Vitamin A deficiencies, Vitamin D excess, metabolic diseases like hyperparathyroidism, cystinuria, gout and intestinal dysfunction¹. Generally stones of two types i.e., non calcium and calcium stones are formed². Calcium, albumin, creatinine, urate and oxalate are some necessary analytical markers in serum and urine for clinical diagnosis of this type of urological disorders³⁻⁴.

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. *Crataeva magna* Lour. bark belonging to family Capparaceae, has many therapeutic benefits such as its use in tribal area as antispasmodic, anti-hypertensive, anti-inflammatory, to treat kidney stones, anti-hyperglycemic, anti-protozoal, antibacterial, anthelmintic and as an analgesic agent⁵. The present study is focused on the investigation of the antiurolithiatic activity of ethanol and aqueous extracts of *C. magna* bark.

**Materials and Methods**

**Collection of plant materials**

The bark of *C. magna* (Plate 1) was collected from young matured plant from Utkal University campus during the month of May and identified by the Botanist of Department of Botany, Utkal University, Bhubaneswar by comparing with the specimen present in the Departmental herbarium. After authentification fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, shade dried and pulverized in a mechanical grinder. The coarse powder was used for further studies.

**Extraction**

About 200 g of coarse dried powder of bark was taken in the Soxhlet apparatus and extracted...
successively using ethanol (95%) and water\textsuperscript{7,8}. The extraction for each solvent was carried out for 18 to 20 hours. The extract was collected by evaporating the solvents and percentage yield was calculated.

Experimental design

Wistar albino rats weighing 140-200 g of either sex were used. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions for an acclimatization period of 15 days before performing the experiment. All rats were housed individually in metabolic cages and temperature maintained at 22±2°C. The condition in the animal house was approved by Committee for the purpose of Control and Supervision on Experiments on Animals (Regd. No. 990/c/05/CPCSEA). The acute oral toxicity study was done according to OECD guideline at dose range 100 to 4000 mg/kg. No mortality of animals was observed at the dose range and hence two different doses 200 and 400 mg/kg was taken for the study\textsuperscript{9}.

Lactose (30%) and Ethylene glycol (1%) induced urolithiasis\textsuperscript{10}

Forty two healthy adult Wistar albino strain rats of either sex weighing 140-200 g were randomly divided into seven groups. Each group consists of 6 animals. The treatment period was considered for 10 days. Group 1: Normal rats were fed with standard rat chow diet and tap water \textit{ad libitum} for 10 days. Group 2: EG and ammonium chloride intoxicated rats were given normal lab diet + drinking water containing 0.75% [v/v] ethylene glycol (EG) and 2% [w/v] ammonium chloride (AC) for 10 days to induce urolithiasis. Group 3: Standard group were fed with normal lab diet + drinking water containing 0.75% [v/v] EG and 2% [w/v] AC + Cystone (5 ml/kg) for 10 days. Groups 4 and 5: the test groups treated with aqueous extract 200 mg/kg and 400 mg/kg of body weight were fed with normal lab diet + drinking water containing 0.75% [v/v] EG and 2% [w/v] AC with aqueous extract 200 and 400 mg/kg body weight, respectively for 10 days. Groups 6 and 7: the test groups, treated with ethanol extract 200 mg/kg and 400 mg/kg of body weight were fed with normal lab diet + drinking water containing 0.75% [v/v] EG and 2% [w/v] AC with ethanol extract of \textit{C. magna} at dose of 200 and 400 mg/kg body weight, respectively for 10 days.

Urine and blood sampling

The crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. The urine samples of the test animals in different groups were collected in their respective end day of the experiment [on 28\textsuperscript{th} day in lactose rich diet + (1%) EG model and on 10\textsuperscript{th} day in (0.75%) EG + (2%) AC model]. The collected urine sample volume were measured followed by centrifugation at 3000 rpm for.
10 minutes. After centrifugation the urine samples were examined under light microscope (LAICA, DME Germany 400X) to ensure the presence of oxalate microcrystal followed by biochemical analysis (urine oxalate, calcium and uric acid). The blood samples were collected from the animals under anaesthesia (ether) before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium.

**Kidney analysis**

The kidney was fixed in bouin liquid soaked in paraffin, cut at 3-4 µm intervals and the slices stained using hematoxylene and eosin. Tissue slices were photographed using optical microscopy under polarized light (LAICA, DME Germany 400 X).

**Statistical analysis**

Results were indicated in terms of mean ± SEM. Statistical significance of data were assessed by analysis of variance (One way-ANOVA), followed by comparison between different groups using ‘Tukey-Kramer’ multiple comparison test. The significance was considered at the level of P<0.05.

**Results and Discussion**

The results of serum and urine biochemistry is indicated in Tables 1 and 2. Table 1 comprises: the urine and serum biochemistry data of Lactose (30%) + Ethylene glycol (1%) induced urolithiasis model along with the body weight, urine volume and kidney weight data. Table 2 comprises: the urine and serum biochemistry, body weight, urine volume and kidney weight data of Ammonium chloride (2%) + Ethylene glycol (0.75%) induced urolithiasis model.

The acute urolithiasis in both the conventional models was evidenced by the significant elevation in urine and serum biochemical parameters along with the reduced urine output as compared to the normal rats.

The ethanol extract (400 mg/kg bw) employing Lactose (30%) + Ethylene glycol (1%) induced urolithiasis resulted a significant reduction (P<0.001) in urine uric acid (0.82 ± 0.07) and oxalate (1.54 ± 0.26) level as compared to toxic group. Serum calcium (3.25 ± 0.30) and urine calcium (2.33 ± 0.18) level was significantly (P<0.05) lowered when compared with the toxic group along with significant elevation in urine volume (13.38 ± 1.12; P<0.05) output, However, the extract showed a reduction in serum creatinine level employing both models but the reduction is not significant when compared with urolithic control group.

The ethanol extract (400 mg/kg bw) employing ammonium chloride (2%) + Ethylene glycol (0.75%) induced Urolithiasis model exhibited significant

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<th>Table 1—Urine and serum biochemistry of 30% lactose and 1% EG induced urolithiasis on 28th day of experiment</th>
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Values are given in mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. For n=6; Group 2 (Toxic group) was compared with Group 1 (Normal group) and all the other groups were compared with Group 2 (toxic group). Group 1: Normal control group (Normal lab diet with normal water ad libitum); Group 2: Urolithic control group (Lactose rich diet + 1% EG in drinking water ad libitum); Group 3: Standard group (treated with Cystone 5 ml/kg); Group 4: Treated with aqueous extract 200 mg/kg; Group 5: treated with aqueous extract 400 mg/kg; Group 6: Treated with ethanol extract 200 mg/kg; Group 7: Treated with ethanol extract of 400 mg/kg. I: Initial body weight, F: Final body weight.
The results of urine and serum biochemistry showed significant reduction in urine calcium, uric acid and oxalate level, serum calcium with significant elevation in urine volume output, the markers previously reported which affirmed potent antiurolithiatic activity.

The photomicrograph exhibiting anatomical changes of kidney is shown in Plate 2 (a-g). The photo-micrograph of normal control rat kidney (Plate 2a) showing normal organization of tubular epithelial cells and glomeruli (X-400). Plate 2 (b, c) represents the section of Urolithic control rat kidney showing glomerular atrophy and deposition of crystals (X-400) treated with Lactose rich diet + (1%) EG in drinking water and (0.75%) EG + (2%) AC in drinking water, respectively. Plate 2 (d, e) showing sections of rat kidney treated with standard Cystone (5 ml/kg) showing regenerative changes in glomeruli and tubules (X-400) employing both models. The section of rat kidney treated with ethanol extract 400 mg/kg in 30% lactose + (1%) EG showed significant reduction in urine calcium, uric acid and oxalate level, serum calcium with significant elevation in urine volume output (12.5 ± 1.0; P< 0.01) output as compared to toxic rat group.

Ethanol extract 200 mg/kg and the aqueous extracts 200 and 400 mg/kg of body weight in both the experimental models exhibited minimal antiurolithiatic activity which was considered below the significant level.

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Plate 2 (a-g)—Histological sections of rat kidney: a: Section of normal control rat kidney showing normal organization of tubular epithelial cells and glomeruli (X-400); b: Section of urolithic control rat (treated with 30% lactose rich diet + 1% EG in drinking water) kidney showing glomerular atrophy and deposition of crystals (X-400); c: Section of urolithic control rat (treated with 0.75% EG + 2% AC in drinking water) kidney showing glomerular atrophy and deposition of crystals (X-400); d: Section of standard rat (treated with cystone; 5 ml/kg) kidney in 30% lactose + 1% EG model, showing regenerative changes in glomeruli and tubules (X-400); e: Section of standard rat (treated with cystone; 5 ml/kg) kidney in 0.75% EG + 2% AC model, showing regenerative changes in glomeruli and tubules (X-400); f: Section of rat kidney treated with ethanol extract (400 mg/kg) in 30% lactose + 1% EG model, showing normalcy of tubular epithelial cells and glomeruli (X-400); g: Section of rat kidney treated with ethanol extract (400 mg/kg) in 0.75% EG + 2% AC model, showing normalcy of tubular epithelial cells and glomeruli (X-400).
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References