A Promising Natural Approach: Caesalpinia bonduc Seed Extracts for Calcium Oxalate Urolithiasis in Rats

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ABSTRACT

Background: It is essential to identify a therapy for hyperoxaluria that specifically focuses on reducing oxalate excretion since current therapies for urolithiasis have drawbacks. It is noteworthy to observe that the seeds of Caesalpinia bonduc (Family: Caesalpiniaceae) have been historically used by several Indian tribes for the purpose of treating renal diseases.

Purpose: The primary purpose of this study was to assess the efficacy of C. bonduc seed extracts as therapeutic agents in rats with experimentally generated calcium oxalate urolithiasis.

Methodology: The experimental animals were given 0.75% of ethylene glycol orally for fourteen consecutive days in order to produce calcium oxalate lithiasis. C. bonduc seed extracts, 400 mg/kg of body weight in aqueous and ethanolic forms, were administered in the same way for a further 14 days in succession. The standard antiurolithiatic medicine used was cystone, administered at a dose of 750 mg/kg of body weight. The research primarily examined the quantification of serum biochemical markers and the elimination of salt constituents from urine and renal deposits, which were shown to be challenging.

Results: Ethylene glycol administered orally caused hyperoxaluria and augmented calcium, phosphate, and oxalate excretion in the kidneys. Nevertheless, the administration of C. bonduc seed extracts effectively decreased the elevated levels of oxalate in the urine, showing an inhibitory effect on the synthesis of oxalate inside the human body. The renal accumulation of components that cause stone formation in rats with calculogenic conditions decreased considerably after the administration of curative treatments with aqueous and ethanolic extracts.

Discussion and Conclusion: Based on the findings, it has been shown that C. bonduc seeds have lithontriptic action, which deserves further investigation as a potential remedy for urolithiasis. Further research is necessary to elucidate the specific phytoconstituents present in seeds that are responsible for their antiurolithiatic activity.

Keywords: Rats, Hyperoxaluria, Ethylene glycol, Urolithiasis, Caesalpinia bonduc.


Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A large number of people rely on medicinal herbs to alleviate a variety of human ailments. Medicinal plants have been used for millennia, especially in developing nations, to treat a variety of human illnesses. The third most common renal disease that has afflicted people since antiquity is urolithiasis, sometimes known as nephrolithiasis, which is brought on by kidney or other urinary system stones. The main component of about 80% of kidney stones is calcium oxalate. It can exist in both mono- and di-hydrate forms. Urinary stones are the result of several physiochemical processes, starting with the supersaturation of urine and proceeding to crystal nucleation, growth, aggregation, and retention.

As of right now, other than a few alkalizers and diuretics, there is no therapeutically useful drug that can dissolve and/or stop the growth of calculi in the urine. This condition is frequently treated with surgery and interventional techniques such as ESWL, PCNL and ureteroscopy. These procedures do not impede but rather enhance the likelihood of stone recurrence and are associated with significant adverse consequences such as hemorrhage, renal fibrosis, and infections. Because of this, patients who get such treatments have to endure a lengthy period of attentive follow-up, and the expense of such treatment is unaffordable for everyone else. Therefore, there is a continuing need to search for more therapeutically

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beneficial antiurolithic treatments that are inexpensive for the average person.

_Caesalpinia bonduc_ (Linn.) Roxb., a prickly shrub belonging to the Caesalpiniaeae/Fabaceae Family, is also known by its scientific name _C. bonducella_ (L.) Fleming. It is abundantly distributed over Africa, the Andaman and Nicobar Islands, Pakistan, India, Myanmar, and other tropical areas. The whole plant, including the root, leaf, seed, bark, and stem, serves as a natural remedy for a variety of diseases. _C. bonduc_ is utilized in medicine because of its numerous health benefits, including antidiabetic, anti-inflammatory, antipyretic, analgesic, immunomodulatory, wound healing, anti-estrogenic activities.

Currently, there is a lack of evidence addressing the effectiveness of _C. bonduc_ seed for treating urolithiasis. However, despite the extensive exploration of its therapeutic potential, scientific research regarding the antiurolithic activity of _C. bonduc_ remains scarce. Thus, the intent of the research was to investigate the antiurolithic activity of _C. bonduc_ seed extracts on rats that had urolithiasis caused by ethylene glycol.

**MATERIALS AND METHODS**

_C. bonduc_ seeds collection

The seeds of _C. bonduc_ were procured from the Solapur region of Maharashtra in March 2021. Dr. D. L. Shirodkar, a research scientist at the Botanical Survey of India, Pune, Maharashtra, led the process of authentication (MSSCB1).

Chemicals

The EG was bought from the Merck Laboratories located in Mumbai. The standard antiurolithic drug used in this study was cystone tablets, which are a commercially available pharmaceutical manufactured by Himalaya Healthcare Company in Bangalore, India. The urine test strips-UroColor 10 were procured from Abbott Diagnostics Korea Inc.

Extract Preparation

About 250 g of coarsely powdered seeds of _C. bonduc_ were extracted with absolute ethanol using a continuous hot extraction process to obtain the ethanolic extract. Another batch of 250 g of coarsely powdered seeds underwent a cold maceration technique in water for 7 days to prepare the aqueous extract.

The two extracts were concentrated individually under reduced pressure employing a rotary flash evaporator. Subsequently, the residue was dried over sodium sulfate using a desiccator. The percentage yields of the aqueous and ethanolic extracts were found to be 13.33 and 12.92, respectively. The aqueous and ethanolic extracts were suspended in aqueous sodium carboxymethylcellulose (0.5% w/v) solution for oral administration in rats.

**Qualitative Analysis**

Each extract underwent phytochemical analysis to confirm the presence of primary and secondary metabolites in _C. bonduc_ seed extracts.[12]

**Antiurolithic Activity**

*Animal selection*

In the context of acute toxicity experiments, albino Wistar rats, regardless of gender, with weights ranging from 150 to 200 gm, were chosen. Wistar albino rats weighing 150 to 200 gm were utilized to test the antiurolithic effect. Rats were acclimated to laboratory settings and then placed on a 12:12-hour light-dark cycle. The rats were given unrestricted access to standard rat food as well as water to drink. The handling and care of the animals during experiments was conducted in line to NIH standards. The Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra, approved the research protocol (CPCSEA Registration no.: IAEC/Sangli.2020-21/19).

*Acute toxicity studies*

In accordance with OECD Guidelines No. 423, a study on acute oral toxicity was done.[13] The effective therapeutic dose was calculated on the basis of a threshold value of the median lethal dose (LD50).

*Ethylene glycol-induced urolithiasis model*

The antiurolithic activity was evaluated using the Atmani et al. approach with the necessary modifications.[14] The rats were then split into five groups, each comprising six rats. Group I served as normal control. In experimental groups II to V, Oral delivery of a 0.75% dose of EG in DW caused hyperoxaluria in rats for up to 14 days. Rats in group II acted as negative control; they were not exposed to any kind of treatment from days 15 to 28. Curative treatments were administered to groups III, IV, and V using cystone at a dosage of 750 mg/kg of body weight, AECB at a dosage of 400 mg/kg of body weight, and EECA at a dosage of 400 mg/kg of body weight, respectively. Each of the dosages was administered orally once a day via the gastric intubation technique.

*Urine collections and examination*

All rats were housed in separate metabolic cages after the experiment and 24 hours of urine were collected. The UroColor test strips were used to carry out routine urinalysis from urine samples in rats. The test comprised measurement of pH and specific gravity, in addition to the detection of proteins, glucose, bilirubin, urobilinogen, nitrite, occult blood, leucocytes and
ketone bodies. A small quantity of strong hydrochloric acid was introduced into the urine prior to its storage at a temperature of 4°C. The urine was examined to analyze the level of calcium, phosphate, and oxalate.

Microscopic examination of urine

On day 28, fresh collected urine samples were inspected under a microscope at a magnification of 50X to check for the existence of distinctive CaOx and CaPh crystals. A digital camera, compatible with the Avercap program, was used to capture their photomicrographs.

Serum analysis

Subsequent to the experimental duration, blood was taken from the orbital vein retrogradely of the rats after they had been slightly anesthetized with ether. Finally, greater doses of ether anesthesia were used for sacrificing the rats. The blood sample was centrifuged at 10,000 g for 10 minutes to extract the serum, which was then tested for blood urea nitrogen, uric acid, and creatinine.

Kidney homogenate analysis

Both kidneys from every animal were extracted by cutting the abdomen. Once superfusives tissue was removed from isolated kidneys, they were stored in 10% neutral formalin. A hot air oven was used to dry (at 80°C) each of the extracted kidneys. About 100 mg sample of a dry kidney was homogenized subsequent to 30 minutes of boiling in 10 mL of 1 N HCl solution. The homogenate was separated from the supernatant after centrifuging it for 10 minutes at 2000 g. The levels of calcium, phosphate, and oxalate in the kidney homogenates were analyzed.

Histopathological analysis of the kidney

Using conventional methods, another extracted kidney was paraffin-embedded, sectioned into 5 μm thick pieces, stained with hematoxylin and eosin dye, and then mounted using diphenyl xylene. Each piece of the section was examined for histopathological alterations in kidney architecture using a compound microscope set to 50X magnification. A digital camera was used to capture photomicrographs of the sections. A general scoring system was used to visualize several fields in order to monitor the degree of nephritic injury and the progression of recovery. At least ten fields per kidney slide were examined and scores on a scale of none (NS), mild damage (+), moderate damage (++), and significant damage (+++) were used to indicate the severity of the alterations.

Statistical analysis

The Tukey’s post-hock test is applied after One-way Anova, and the findings are represented as mean ± SEM. The p-value < 0.05 was regarded as statistically significant.

RESULTS

Qualitative chemical analysis of AECB and EECB revealed the presence of proteins, carbohydrates, amino acids, terpenoids, glycosides, tannins, and steroids (Table 1). Based on acute ingestion toxicity trials in rats, a safe dose of 400 mg/kg body weight for AECB and EECB was selected.

In the current investigation, oral delivery of ethylene glycol for a continuous period of 14 days led to the occurrence of hyperoxaluria (Figure 1). Rats with negative control, i.e., calculi-induced (Group II) had substantially higher excretion of oxalate in their urine compared to the group under control (Table 2, Group I). The excretion of phosphate and calcium (p < 0.01) was also elevated in group II. Calcium, oxalate, and phosphorus were found in higher concentrations in the renal tissue of rats that had calculi. The therapeutic use of AECB and EECB seed was also shown to be beneficial in reducing the increased levels of harmful salts in the urine and kidneys. The raised urinary amount of phosphate, calcium, and oxalate were considerably normalized after administering cystone (Table 2, Group III). The delivery of EECB seed led to a statistically significant decrease in the excretion of calcium, phosphorus, and oxalate in urine in contrast to group II (Table 2, Group V). Elevated blood levels of BUN, creatinine, and uric acid were indicative of significant damage to the renal architecture caused by kidney stones (Table 2, Group II). EECB seed showed a substantial decrease in BUN, creatinine and uric acid, whereas AECB seed showed a substantial decrease in BUN, creatinine and uric acid. The administration of aqueous and ethanolic extract (Group IV and V) was found to be substantial and equivalent to that of cystone (Table 2, Group III) not withstanding the non-significant decrease in these amassed waste materials seen in the intergroup comparison between the treatments of aqueous and ethanolic extracts.

<table>
<thead>
<tr>
<th>Table 1: Qualitative chemical analysis of C. bonduc seed extracts</th>
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</thead>
<tbody>
<tr>
<td><strong>Metabolites</strong></td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Amino acids</td>
</tr>
<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Fats and oils</td>
</tr>
<tr>
<td>Steroids</td>
</tr>
<tr>
<td>Terpenoids</td>
</tr>
<tr>
<td>Volatile oils</td>
</tr>
<tr>
<td>Glycosides</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Tannins</td>
</tr>
<tr>
<td>Alkaloids</td>
</tr>
</tbody>
</table>

`+` Present, `-` Absent
The mean pH of the calculi-induced rats was 7.60, according to the findings of routine urinalysis (Table 3, Group II). The raised urine pH was, however, found to drop to almost normal levels with the administration of *C. bonduc* seed extracts (AECB and EECB). There were non-significant differences in the urine of bilirubin, urobilinogen, leucocytes, ketone bodies, nitrite concentrations, and specific gravity across all experimental groups, although all groups showed negative results for urinary excretion of sugar. Additionally, the calculi-induced group showed proteinuria, indicating a severe cellular injury to the nephritic kidney. In contrast, the administration of ethanolic extracts of *C. bonduc* seed (EECB) resulted significant reduction in protein excretion in the urinary system, similar to the rats treated with cystone (Table 3, group III).

The urine from the control group showed no signs of crystals or other comparable structures when examined under a microscope (Figure 2A). The urine sample from rats with calculi-induced conditions exhibited a significant presence of sizable crystals showing the distinctive morphology of calcium oxalate and triple phosphate (Figure 2B). By contrast, urine samples collected from rats treated with cystone (Figure 2C), aqueous extract, and ethanolic extract (Figure 2D and E, respectively) exhibited a lower prevalence of crystal fragments, which were notably smaller in size. The breakdown pattern of crystal shown by seed extracts of *C. bonduc* was found to be indistinguishable as that of cystone. Nevertheless, there were solitary and smaller crystal pieces that were observed within this group.

The histopathological analysis of the kidney provided normal glomeruli and a modest recuperation of renal histology, ethanolic extracts treated groups (Figure 3D and E) revealed tubules and peritubular interstitium. However, the aqueous and ethanolic extracts of *C. bonduc* seed showing the extent of dissolution of pre-formed calcium crystals (CaPh and CaOx) were administered just 0.75% ethylene glycol, (C) rats were administered 0.75% ethylene glycol and then treated with cystone, (D) rats were administered 0.75% ethylene glycol and then treated with AECB seed and (E) rats were administered 0.75% ethylene glycol and then treated with EECB seed showing the extent of dissolution of pre-formed calcium crystals (CaPh and CaOx). Collecting system, and peritubular interstitium across all the rats forming stones (Figure 3B), compared to the normal rat’s kidney anatomy (Figure 3A). The tubules had an inflammatory infiltrate around them and were focally ectasic (Figure 3B). The tubules were surrounded by flattened epithelium with localized vacular degeneration and necrosis of individual cells. These tubules contained hyaline casts in certain areas. Mature lymphocytes invading the tubular epithelium constituted the majority of the inflammatory infiltration. Irregular crystals appeared along the nephron, at the papillary level, and in the tubules and peritubular interstitium. However, the aqueous and ethanolic extracts treated groups (Figure 3D and E) revealed normal glomeruli and a modest recuperation of renal histology, analogous to the group receiving cystone treatment (Figure 3C, Table 4).

### Table 2: Effect of *C. bonduc* extracts of seeds on kidney homogenate, serum, and urine parameters in both experimental and control groups of animals

<table>
<thead>
<tr>
<th>Parameters (Unit)</th>
<th>Group I normal control</th>
<th>Group II calculi induced</th>
<th>Group III cystone drug</th>
<th>Group IV AECB treated</th>
<th>Group V EECB treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg, p.o.)</td>
<td>-</td>
<td>-</td>
<td>750</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Urine (milligrams/decilitre)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>3.01 ± 0.49</td>
<td>5.51 ± 0.79***</td>
<td>3.49 ± 0.61**</td>
<td>3.69 ± 0.20</td>
<td>3.29 ± 0.19***</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>140.70 ± 47.29</td>
<td>349.70 ± 51.83***</td>
<td>194.10 ± 54.67</td>
<td>248.20 ± 23.56</td>
<td>182.00 ± 21.67***</td>
</tr>
<tr>
<td>Oxalate</td>
<td>1.44 ± 0.19</td>
<td>4.65 ± 1.11***</td>
<td>1.76 ± 0.29**</td>
<td>2.29 ± 0.89</td>
<td>1.79 ± 0.29**</td>
</tr>
<tr>
<td>Serum (milligrams/decilitre)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>21.80 ± 0.81</td>
<td>34.21 ± 4.20***</td>
<td>25.41 ± 0.81</td>
<td>24.11 ± 0.89**</td>
<td>23.45 ± 0.69***</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.60 ± 0.04</td>
<td>0.81 ± 0.03**</td>
<td>0.59 ± 0.02**</td>
<td>0.59 ± 0.03**</td>
<td>0.56 ± 0.03***</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.69 ± 0.19</td>
<td>5.07 ± 0.29**</td>
<td>1.97 ± 0.49**</td>
<td>2.10 ± 0.19**</td>
<td>1.79 ± 0.39***</td>
</tr>
<tr>
<td>Kidney homogenate (milligram/gram)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>5.99 ± 1.39</td>
<td>12.12 ± 2.67**</td>
<td>6.23 ± 1.09**</td>
<td>8.54 ± 1.00</td>
<td>7.19 ± 0.56**</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>1.20 ± 0.19</td>
<td>4.15 ± 1.29**</td>
<td>1.60 ± 0.28**</td>
<td>1.99 ± 0.53</td>
<td>1.68 ± 0.69**</td>
</tr>
<tr>
<td>Oxalate</td>
<td>2.99 ± 0.55</td>
<td>8.38 ± 0.79**</td>
<td>3.42 ± 0.49**</td>
<td>4.40 ± 0.69**</td>
<td>3.57 ± 0.49**</td>
</tr>
</tbody>
</table>

Urinary parameters were measured in 24-hour urine samples, with mean ± SEM values from six animals per group; *p < 0.05, **p < 0.01, ***p < 0.001; xComparisons are made with the Group I (Normal control); yComparisons are made with the Group II (Disease induced).
Anti-urolithiatic Activity of *Caesalpinia bonduc* Seed Extracts in Rats

Table 3: Effect of seed extracts of *C. bonduc* on various urine parameters in both experimental and control groups of animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Group I normal control</th>
<th>Group II calculi induced</th>
<th>Group III cystone drug</th>
<th>Group IV AECB treated</th>
<th>Group V EECB treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>6.89 ± 0.13</td>
<td>7.60 ± 0.17</td>
<td>7.04 ± 0.22</td>
<td>6.99 ± 0.09</td>
<td>6.70 ± 0.20</td>
</tr>
<tr>
<td>Specific gravity</td>
<td></td>
<td>1.01 ± 0.00</td>
<td>1.02 ± 0.00</td>
<td>1.01 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.01 ± 0.00</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>1.66 ± 1.70</td>
<td>23.49 ± 4.19</td>
<td>6.59 ± 2.08</td>
<td>9.98 ± 3.92</td>
<td>3.29 ± 1.98</td>
</tr>
<tr>
<td>Nitrite</td>
<td>mg/dL</td>
<td>0.32 ± 0.16</td>
<td>0.68 ± 0.20</td>
<td>0.43 ± 0.14</td>
<td>0.49 ± 0.20</td>
<td>0.39 ± 0.20</td>
</tr>
<tr>
<td>Ketone</td>
<td></td>
<td>0.80 ± 0.79</td>
<td>4.99 ± 1.19</td>
<td>3.31 ± 1.06</td>
<td>2.49 ± 1.68</td>
<td>2.48 ± 1.21</td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
<td>-</td>
<td>0.32 ± 0.12</td>
<td>0.16 ± 0.12</td>
<td>0.20 ± 0.09</td>
<td>0.09 ± 0.1</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td></td>
<td>0.10 ± 0.00</td>
<td>1.21 ± 0.61</td>
<td>0.10 ± 0.00</td>
<td>0.11 ± 0.00</td>
<td>0.11 ± 0.00</td>
</tr>
<tr>
<td>Leucocyte†</td>
<td>WBC/µL</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blood††</td>
<td>RBC/µL</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Leucocyte (WBC/µL) +25, ++ 75; †Blood (RBC/ µL) +10, ++50, +++ 250; ‘-’ No change in color after qualitative test

DISCUSSION

The chemistry of urine greatly influences the type of crystal that forms and the tiny particles that stick to their surfaces. The main risk factors for stone formers are significantly reduced volume of urine, pH, moderate hyperoxaluria, elevated calcium levels, hypocitraturia, hyperuricosuria, and hypomagnesuria. The urine’s pH provides an additional clue to identify the kind of calculi (less than 5.5 in cases of calcium stones and higher than 6 in cases of uric acid stones). In particular, phosphate and oxalate nucleate with calcium when urine is found to be basic, more than pH 7.2 in calcium oxalate urolithiasis. In this study, rats had elevated pH levels (7.60) and formed kidney stones primarily made of calcium oxalate after administering ethylene glycol orally for 14 days. The available evidence from previous studies indicates that the molecular mechanisms behind this phenomenon are attributed to an elevation in the urine concentration of oxalate. EG causes hyperoxaluria
in rats because it quickly converts glycolate to oxalate.\textsuperscript{24} Rats treated with ethylene glycol have shown comparable results.\textsuperscript{25} Within rats with calculi-induced conditions (Group II), the elimination of phosphorus, calcium, and oxalate increases gradually in the urine, possibly due to reduced reabsorption from the tubes in the kidneys. Nevertheless, the variations in oxalate content found in the urine are much more significant than changes in calcium levels,\textsuperscript{26} because it is thought that hyperoxaluria poses a greater risk of kidney stones than hypercalcemia.\textsuperscript{27} The growth and production of calcium oxalate crystals are facilitated by higher levels of calcium in the urine.\textsuperscript{28} Nonetheless, the therapeutic administration of AECB and EECB seed extracts results in a decrease in the amounts of excretion of oxalate, phosphorus, and calcium.

In addition to oxalate load, a gradual rise in urinary phosphorus, as seen in calculi-induced rats, appears to foster the formation of calculi through the epitaxial accumulation of calcium oxalate and the formation of a nidus composed of calcium and triple phosphate crystals.\textsuperscript{29} On the other hand, by raising the phosphate level, supplementing with extracts from \textit{C. bonduc} seeds lowers the chance of stone formation.

Urolithiasis impairs renal function by decreasing the GFR, which is caused by the blockage of urine flow due to the presence of calculi inside the urinary system. As a result, waste products, specifically nitrogenous molecules, build up in the bloodstream.\textsuperscript{30} It is well established that consuming glucose raises lipid peroxidation and lowers the kidneys’ antioxidant capability.\textsuperscript{31} Renal tissue deterioration is exacerbated by the chemical oxalate, which acts as the initial catalyst for lipid peroxidation and interacts to polyunsaturated fatty acids found in cell membranes.\textsuperscript{32} In the present scenario, the elevated serum concentration of creatinine, uric acid, and BUN (Group II, Table 2) in the calculi-induced rats are attributed to extensive kidney damage (Table 4, Group II). Substantial proteinuria and haematuria confirm the severity of nephritic injury (Group II Table 3). \par

CONCLUSION

Finally, the findings show that \textit{C. bonduc} seed extracts decreased and reversed the formation of urinary stones in rats with experimentally induced urolithiasis, confirming traditional beliefs about the plant’s antiurolithic activity. Although the precise mechanism of the action is unknown, it seems to be connected to both increased urine output and decreased levels of components that cause stones to develop in the urine. Renal injury may heal in part due to the preventive action against oxalate-induced peroxidation of lipids. It is possible that the observed antiurolithic activity might be due to the presence of tannins, steroids, flavonoids, and triterpenoids.\textsuperscript{33} These findings could validate the antiurolithic activity of \textit{C. bonduc} seed.

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