EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF THE AQUEOUS AND ALCOHOLIC EXTRACTS OF ROOTS OF **BOERHAAVIA DIFFUSA**

Balaji L G¹*, David Banji², Otilia J. F. Banji²

¹Research Scholar, JNTU, Hyderabad, India
²Nalanda College of Pharmacy, Nalgonda, Telangana, India

**ARTICLE INFO**

**Article history**
Received 08/01/2015
Available online
30/01/2015

**Keywords**
Boerhaavia Diffusa,
Punarnava,
Kidney Stones,
Urolithiasis,
Urinary Calculi,
Calcium Oxalate.

**ABSTRACT**

Objective of the current study is to evaluate the antiurolithiatic activity of the root extracts of *Boerhaavia diffusa* and validating the indigenous use of *B. diffusa* in propulsion of calculi (urinary stones). Calculi were induced in Wistar male rats using calculi producing diet (CPD, ethylene glycol in drinking water for 28 days). Urolithiatic rats were treated with various doses of aqueous extract of *B. diffusa* and alcoholic extracts of *B. diffusa* for 30 days. The 24-hour urine samples were collected for analyzing the stone forming constituents calcium, oxalate and phosphorous. Also, magnesium concentration was estimated in the urine samples. Cystone, an ayurvedic medicine approved for management of calculi was used as a standard reference. Administration of CPD induced calculi in all experimental rats. Treatment with aqueous extract of *B. diffusa* and alcoholic extracts of *B. diffusa* significantly decreased the concentrations of stone forming constituents calcium, oxalate and phosphorous in the urine. In addition, both aqueous and alcoholic extracts of *B. diffusa* increased the magnesium levels in urolithiatic rats. Changes in stone forming constituents and magnesium levels were considerably higher (although not significant) with alcoholic extracts than aqueous extract of *B. diffusa*. Therefore we conclude that the root extracts of *B. diffusa* possess antiurolithiatic activity and justifies its use in indigenous medicine for propulsion of urinary stones.

**Corresponding author**

Balaji L G
No.26, Gowri Nilaya,
Sulikunte village, Sarjapura main road,
Bangalore-562125, Karnataka, India.
91-9986500398; +91-9880840854
lgbalaji.pharma@gmail.com

*Please cite this article in press as Balaji L G et al. Evaluation of Antiurolithiatic Activity of the Aqueous and Alcoholic Extracts of Roots of Boerhaavia diffusa. Indo American Journal of Pharm Research.2015:5(01).*

Copy right © 2015 This is an Open Access article distributed under the terms of the Indo American Journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION

Urolithiasis is the presence of stones in the urinary tract. Formation of urinary stones is a complex physico-chemical process resulting in accumulation of minerals and their retention in renal tubules[1]. Urinary stone disease is the third most common disorder[2] of urinary tract and is estimated to occur in approximately more than $10^6$ of population[3]. Urolithiasis is an age old condition and its description in the literature of Hippocrates suggests that it is a condition affecting mankind from centuries[4]. Despite of efforts from centuries and tremendous advances in this field of medicine, even today the problem of urinary stones remains completely unresolved. Major problem with urolithiasis is recurrence and with currently available medical procedures as well as medical treatments this problem can’t be completely solved. The current scenario demands the need of a drug that can be used alone or in conjunction with other therapies to overcome the limitations of current treatment approaches. Our objective was to identify a plant that possesses antiurolithiatic activity that can also be used as a vegetable so that it can be used as required to flush the newly formed stones.

*Boerhaavia diffusa*, known as Punarnava (Punah punarnava bhawati iti, which in Sanskrit means “which becomes fresh again and again”) is a herb belonging to the family Nyctaginaceae and is commonly found in tropical and subtropical regions. Probably, the name punarnava is conferred owing to its habit as well the therapeutic property. Punarnava remains dry and dormant in summer and regenerates from the same old root stalk in rainy season. Punarnava is known to possess the therapeutic property of rejuvenating the body. It is used in India as a medicinal herb and the leaves as well as roots of the herb are used as a vegetable. Medicinal value of the herb in treatment of various ailments is described in Ayurveda and other forms of medicine. It possesses diuretic property and is used in treatment of urinary disorders. Punarnava is used for propulsion of kidney stones in indigenous medicine[5]. In the present study, the antiurolithiatic activity of *B. diffusa* was evaluated in ethylene glycol induced urolithiatic male rats.

METHODOLOGY

Materials and methods

Plant:

*B. diffusa* is collected from the outskirts of Bangalore and Nalgonda, India. Collected herb was authenticated by Dr Lalitha, Professor, Department of PG studies Dravyaguna, Government Ayurvedic Medical College, Bangalore. A voucher specimen (NLG/02/09/025) was deposited in the herbarium of Nalanda College of pharmacy, Nalgonda, Andhra Pradesh. The fresh roots were dried in shade, powdered and 1000 g of the powder was used for extraction using Soxhlet apparatus. Final yield obtained was 8g/100g of crude material.

Animals:

Study design is outlined in figure 1. Healthy, male, Wistar strain rats weighing from 150 to 200g were obtained from a local supplier. The animals were acclimatized to standard laboratory conditions of temp (22±3°C) and maintained on 12:12 hours natural light:dark cycle. They were provided with regular rat chow (Lipton India Ltd, Mumbai) and distilled drinking water ad libitum[6]. The animals care and experimental protocol were in accordance with CPCSEA/IAEC guidelines. [Ref: KCP/IAEC-59/2009-10 dated 09/03/10].

![Figure 1: Study design.](image-url)

*B. diffusa*: *Boerhaavia diffusa*
CPD: calculi producing diet
Induction of urolithiasis:
Ethylene glycol induced urolithiasis model, 0.75% ethylene glycol in distilled drinking water[6], was selected for the study. Analytical grade chemicals were used in the study. The doses of aqueous and alcoholic extracts of *B. diffusa* were fixed between 10 mg/kg and 1000 mg/kg body weight.

Treatment:
Rats were divided into 9 groups of 6 rats each and all animals were given standard rat diet throughout the study period.
Group-1 – Normal: Regular diet and no drug treatment
Group II – Positive control: 0.75% ethylene glycol for 28 days and no drug treatment
Group-III – 0.75% ethylene glycol for 28 days followed by 10 mg/kg aqueous extract of *B. diffusa* for 30 days
Group-IV – 0.75% ethylene glycol for 28 days followed by 100 mg/kg aqueous extract of *B. diffusa* for 30 days
Group-V – 0.75% ethylene glycol for 28 days followed by 1000 mg/kg aqueous extract of *B. diffusa* for 30 days
Group-VI – 0.75% ethylene glycol for 28 days followed by 10 mg/kg alcoholic extracts of *B. diffusa* for 30 days
Group-VII – 0.75% ethylene glycol for 28 days followed by 100 mg/kg alcoholic extracts of *B. diffusa* for 30 days
Group-VIII – 0.75% ethylene glycol for 28 days followed by 1000 mg/kg alcoholic extracts of *B. diffusa* for 30 days
Group-IX – 0.75% ethylene glycol for 28 days followed by 750 mg/kg Cystone for 30 days

Assay procedures
Analysis of urine samples:
Animals were housed in metabolic cages to collect urine samples. Twenty-four hour urine samples were collected on day 1, day 29 (day after completion of induction period) and day 60 (day after completion of *B. diffusa* treatment). The urine samples were centrifuged and the supernatant was used to estimate the mineral constituents—calcium, magnesium, and phosphorous using AR-601 semi auto analyzer [Qualisystem, GlaxoSmithkline] and Span diagnostic kits. Urinary oxalate was estimated using procedure of Hodgkinson and Williams[7].

Statistical analysis
Data of the urinary parameters were expressed as mean value ± SEM. The results were analyzed by one way analysis of variance (ANOVA) and student’s unpaired t-test using graphpad prism 6.00 software (Graphpad software Inc. Version 6.00). The minimum level of significance was fixed at *P*<0.05.

RESULT AND DISCUSSION
Concentration of the urinary stone forming constituents at baseline, after induction of calculi and after completion of treatment with aqueous extract of *B. diffusa* and alcoholic extracts of *B. diffusa* are presented in figure 2 (figure 2A, 2B, 2C and 2D) and table 1.
Biochemical analysis of 24-hour urine samples demonstrated significant changes in the mineral constituents. Urinary calcium increased from baseline value of 1.723±0.05 mg/dL to 4.651±0.02 mg/dL (p<0.0001) following the administration of CPD for 28 days. Urine oxalate concentration increased tremendously from baseline (0.935±0.035 to 5.310±0.02; p<0.0001) in all animals following administration of CPD for 28 days. Treatment with aqueous extracts and alcoholic extracts of B. diffusa for 30 days decreased both urinary calcium and oxalate considerably in all the groups. Urinary levels of these minerals decreased with increase in dose of aqueous extract of B. diffusa and maximum decrease was observed in group treated with 1000 mg/kg body weight of aqueous extract of B. diffusa (calcium 3.287±0.34, p=0.0061 and oxalate 3.186±0.13, p=0.0001). Similar results were seen with alcoholic extracts of B. diffusa administered for 30 days (calcium 2.452±0.32, p<0.0001 and oxalate 1.831±0.39, p<0.0001). Dose dependent decrease in calcium and oxalate levels were observed with maximum effect seen at 1000 mg/kg body weight.

Phosphorous levels were also increased from baseline in rats after induction of stones with calculi producing diet for 28 days. Urine phosphorous levels increased from baseline value of 1.723±0.05 mg/dL to 4.651±0.02 mg/dL (p<0.0001) following the administration of CPD for 28 days. Treatment with aqueous extracts and alcoholic extracts of B. diffusa for 30 days decreased the calcium levels (from 4.651±0.02 to 2.091±0.09, p<0.0001), oxalate levels (from 5.310±0.02 to 1.354±0.12, p<0.0001), phosphorous levels (from 5.264±0.18 to 1.753±0.08, p<0.0001) and also increased the magnesium levels (from 5.132±0.31, p<0.0001) as compared to positive control.

Cystone treatment was used as standard reference to compare the effectiveness of the aqueous and alcoholic extracts of B. diffusa. Treatment with cystone 750 mg for 30 days decreased the calcium levels (from 4.651±0.02 to 2.091±0.09, p<0.0001), oxalate levels (from 5.310±0.02 to 1.354±0.12, p<0.0001), phosphorous levels (from 5.264±0.18 to 1.753±0.08, p<0.0001) and also increased the magnesium levels (from 5.132±0.31, p<0.0001) as compared to positive control.

Table 1: Concentration of mineral forming constituents and magnesium in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Positive control</th>
<th>B. diffusa 10 mg/kg body weight</th>
<th>B. diffusa 100 mg/kg body weight</th>
<th>B. diffusa 1000 mg/kg body weight</th>
<th>Cystone 750 mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Positive control</td>
<td>Aqueous Extract</td>
<td>Alcoholic Extract</td>
<td>Aqueous Extract</td>
<td>Alcoholic Extract</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.723±0.05</td>
<td>4.651±0.02</td>
<td>4.317±0.14</td>
<td>3.955±0.08</td>
<td>3.891±0.29</td>
<td>3.344±0.15</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.935±0.035</td>
<td>5.310±0.02</td>
<td>4.945±0.15</td>
<td>4.038±0.38</td>
<td>4.190±0.08</td>
<td>3.282±0.23</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>1.831±0.39</td>
<td>5.264±0.18</td>
<td>4.857±0.15</td>
<td>3.964±0.30</td>
<td>3.904±0.38</td>
<td>3.047±0.27</td>
</tr>
<tr>
<td>Magnesium</td>
<td>5.638±0.18</td>
<td>5.310±0.02</td>
<td>5.132±0.09</td>
<td>5.264±0.18</td>
<td>4.857±0.15</td>
<td>4.344±0.30</td>
</tr>
</tbody>
</table>

a: p<0.0001; compared to normal group
b: p=0.0061; c: p=0.002; d: p<0.001; e: p=0.0003; f:p<0.0001; (compared to positive control group)

Figure 2: Concentration of minerals in experimental rats across the treatment groups.
Urinary stone formation is a complex physiological process initiated by supersaturation with stone forming constituents, followed by crystallization, nucleation, growth, aggregation and retention. Supersaturation of urine with stone forming constituent oxalate, the state of hyperoxaluria, is the initiating factor for development of calcium oxalate urolithiasis[8]. Hyperoxaluria complemented with calcium supersaturation mounts the further physiological events including crystallization, nucleation, growth, and aggregation[9,10]. Aggregated stone forming crystals retain on the surface of renal papillae and this is favored by ethylene glycol induced renal damage[9,11]. In the present study treatment with 0.75% ethylene glycol in drinking water resulted in hyperoxaluria and subsequent development of stones. Rats are the most commonly used animals in urolithiasis studies because they mimic the etiology of formation of stones in humans[12]. Furthermore, male rats have been selected for our study because various researches have suggested that incidence of urolithiasis is more in males than their counterparts[13].

Calcium oxalate crystals are the most common constituent of kidney stones[14]. Results of our study demonstrated that the treatment with aqueous extracts of B. diffusa lowered urinary oxalate and calcium levels in all the treatment groups thus minimizing the risk of calcium oxalate crystal formation. Remarkable increase in urinary phosphate was observed in calculi induced rats. Increased phosphorous excretion along with oxalate stress predisposes formation of calcium-phosphate crystals that induces calcium-oxalate deposition[15,16]. Our study showed that treatment with 1000 mg/kg body weight of aqueous extracts and alcoholic extracts of B. diffusa also lowered phosphorous excretion and hence reducing the risk of formation of urinary stones.

Treatment with CPD increased the urinary mineral constituents from base line and the crystals were formed within the kidney. Crystals excreted in urine was observed in microscopic analysis of urine (figures 3A, 3B and 3C). This confirmed the formation of crystals with CPD. Following treatment with aqueous extracts and alcoholic extracts of B. diffusa, urinary mineral constituents were decreased significantly. This confirms the antiurolithiatic potential of B. diffusa. Decrease in urinary constituents was significantly higher in group treated with 1000 mg/kg body weight of extracts as compared to other groups. In addition, microscopic analysis of urine samples after treatment with extracts showed significant decrease in the quantity as well as size of the crystals. Compared with aqueous extracts, the alcoholic extracts of B. diffusa showed better results in improving the urinary mineral constituents with a significant change in oxalate and magnesium.

Figure 3: Qualitative microscopic analysis of crystals in urine in rats (3A) Positive control (3B) Groups treated with 1000 mg/kg b.w of aqueous extracts of B. diffusa and (3C) Groups treated with 1000 mg/kg b.w of alcoholic extracts of B. diffusa. Reduction in the quantity of crystals is evident in groups treated with B. diffusa extracts as compared with positive control.

Root extracts of B. diffusa possess anti-inflammatory activities and plant possess kidney-regeneration activity[17]. Alcoholic extracts showed presence of alkaloids, steroids, glycosides, flavonoids[18]. Earlier researchers have demonstrated that decreasing the stone forming constituents in the urine prevent the stone recurrence[9]. Administration of extracts of B. diffusa decreased the urinary excretion of calcium and oxalate, the chief components responsible for formation of stones.

CONCLUSION
In conclusion B. diffusa, a potent diuretic, demonstrated the antiurolithiatic activity. The aqueous extracts and alcoholic extracts of B. diffusa treatment significantly decreased the urinary levels of stone forming constituents, calcium and oxalate, and therefore minimized the formation of calcium oxalate crystals. The extracts of B. diffusa also decreased urinary phosphorous concentrations and avoided the formation of calcium phosphate stones. In addition, B. diffusa also improved urinary magnesium concentration which is a potent inhibitor of crystal aggregation and stone formation. These effects demonstrated the antiurolithiatic activity of B. diffusa and validate its traditional use in indigenous medicine. Although the exact underlying mechanism is yet to be revealed, the effects observed may be attributed to increased excretion of stone forming constituents. Hence we recommend future research.

ACKNOWLEDGEMENTS
We would like to express our gratitude to Dr. Amit Kumar Das, then Principal of Krupanidhi College of Pharmacy and Dr. Premkumar, Professor of Pharmacology, Krupanidhi College of Pharmacy, Bangalore, Karnataka for providing the facilities necessary to carry out our research work. We also thank Dr. Lalitha, Professor, Government Ayurvedic Medical College, Bangalore for authentication of the plant material.

www.iajpr.com
Competing Interests
We declare that we have no conflict of interest.

REFERENCES