ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC DOLICHOS BIFLORUS LEAF EXTRACT

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ABSTRACT

Objective: The objective of this study is to investigate the antiurolithiatic potential of ethanolic Dolichos biflorus L. leaf extract in swiss albino mice. Methods: The antiurolithiatic potential of Dolichos biflorus L. ethanolic leaf extract was investigated by using Zinc disc foreign body insertion technique model. Antiurolithiatic activity of the leaves was evaluated on the basis of decrease in the weight of stones. 36 swiss albino mice of either sex weighing between 25-30gm were randomly selected and divided into 6 equal groups. Group-I received Prophylactic control (1% ethylene glycol for 4 weeks), Group-II received Prophylactic treatment (1% ethylene glycol + ethanolic extract of leaves of Dolichos biflorus 400 mg/kg orally for 4 weeks), Group-III received Prophylactic treatment (1% ethylene glycol + ethanolic extract of leaves of Dolichos biflorus 800 mg/kg orally for 4 weeks), Group IV received Curative control (1% ethylene glycol for 4 weeks followed by water for 4 weeks), Group V received Curative treatment (1% ethylene glycol for 4 weeks followed by ethanolic extract of leaves of Dolichos biflorus 400 mg/kg for 4 weeks) and Group VI received Curative treatment (1% ethylene glycol for 4 weeks followed by ethanolic extract leaves of Dolichos biflorus 800 mg/kg for 4 weeks). Results: It was observed that there was significant decrease in the weight of the stones in the groups treated with leaf extract of Dolichos biflorus in comparison to the control group. Conclusion: The results of the present study indicate that ethanolic extract of Dolichos biflorus possesses significant Antiurolithiatic activity.

KEYWORDS: Dolichos biflorus leaves, Antiurolithiatic activity, urinary bladder.

INTRODUCTION

The formation of stones in the urinary tract is known as urolithiasis. Pain, bleeding and secondary infection may be caused by Urolithiasis. About 70-80% of the stones are of calcium oxalate. The calculi can be removed by using higher power laser through lithotripsy, local calculus disruption and surgery.¹ There is about 5-10% of risk of stone formation which is more common in males than females. For the treatment of kidney stones various natural remedies are available which may act by one of the mechanisms like enhancing the urine volume, pH and anti-calcifying activity (Diuretic activity) helps in spontaneous passage. Restricts the binding mucin of calculi (lithotriptic activity); Control of oxalate metabolism; Improving kidney function; Reveals marked improvement in symptoms of urinary calculi like pain, burning micturition and haematuria (Analgesic and anti-inflammatory activity).²

Dolichos biflorus L. belongs to Fabaceae family. It is commonly called Kulthi and is an edible pulse crop. It has small trifoliate leaves, thin stem and flat-curved pods. There are 5-6 flat and ellipsoid shaped seeds within the pods. It has been reported that the precipitation of calcium hydrogen phosphate dihydrate crystals was inhibited by seeds of Dolichos biflorus. The seeds are useful for the cure of leukorrhea, menstrual troubles and ulcer. The seeds are also useful as anti-hepatotoxic, hypolipidemic, antioxidant and anti-nephrotoxic.³ Dolichos biflorus is also known as Horse gram. It is used in treating asthma, cough, oedema, anti-allergic and anti-anaphylactic activity of Dolichos biflorus.⁴ It's seeds are mostly used as feed for cattle. It consists of 4- hydroxybenzoic, sinapic, caffeic, ferulic, 3,4-dihydroxybenzoic, syringic and vanillic acids. It is also useful in the treatment of hyperglycemia as it degrades carbohydrate digestion and also decreases insulin release.⁵

The leaves of Dolichos biflorus are alternate, trifoliate having slender and hairy petiole. Flowers solitary or paired, axillary calyx campanulate, the upper two connate, other linear, hirsute, corolla of 5 pale-yellow petals, standard oblong, shortly clawed; fruit pod, recurved, sub globose or flat, beaked and straw brown. It
is mainly composed of β-sitosterol, bulbiformin, kievitone, linoleic acid, polyphenols, oxalates, genistein, 5-hydroxy-7,3,4-trimethoxy-8-methylisoflavone-5-neohesperidose, dalbergioidin, genistein, phaseollidin and 5-o-a-Lrhamnopyranosyl (1-2)-β-D-glucopyranoside.[6] Earlier the antiurolithic activity has been conducted on Dolichos biflorus seed extract [7]. The present study has been conducted to investigate the antiurolithic activity on ethanolic leaf extract of Dolichos biflorus in mice.

MATERIALS AND METHODS

Approval for the Project
Approval for the experiment was obtained from the Institutional animal ethics committee (IAEC), Shri Ram Murti Smarak, CET (Pharmacy), Bareilly (715/02/a/CPCSEA).

Method of Extraction
The fresh leaves were washed, dried and coarsely powdered. Then extraction was performed with ethanol. The extract was stored in desiccators and used for further experiments after dissolving it in distilled water.

Animals
Albino mice of both sex weighing 20-25 g were used. They were housed in standard conditions of temperature (25 ± 2°C), relative humidity of 45-55%, and maintained on 12-hour light: 12-hour dark cycle in animal house of Shri Ram Murti Smarak, CET (Pharmacy), Bareilly. They were fed standard pellet diet and water ad libitum.

Antiurolithiatic Study: Method of Induction of Urolithiasis by Insertion of Zinc Disc
Mice were anaesthetized with ketamine (50 mg/kg i.p.). The abdomen was opened by making suprapubic incision. After that, the urinary bladder was exposed and aspirated with sterile syringe. A preweighed sterile zinc disc was inserted by making a small nick at the apex of urinary bladder, followed by stitching the urinary bladder by chromic catgut (4-0). The abdomen was closed with chromic catgut and skin was closed with silk thread. The rats recovered from anaesthesia. Food and 1% ethylene glycol in water was administered ad libitum. The stone was allowed to form and grow inside the urinary bladder during the study. After completion of the study, the mice were sacrificed and zinc disc implants/stones were removed from the bladder and dried. The weight of the stones was recorded. The amount of stone formed was calculated by subtracting the initial weight from the final weight [8].

Grouping of Animals for Different Treatments and Procedure of the Study
Adult albino mice, weighing between 20-25 g were selected for the study. In this study the mice were divided into 6 groups with 6 animals in each group receiving different treatments.

Table 1: Various study groups and their treatments

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Prophylactic control (1% ethylene glycol for 4 weeks)</td>
</tr>
<tr>
<td>Group II</td>
<td>Prophylactic treatment (1% ethylene glycol + ethanolic extract of leaves of Dolichos biflorus 400 mg/kg orally for 4 weeks)</td>
</tr>
<tr>
<td>Group III</td>
<td>Prophylactic treatment (1% ethylene glycol + ethanolic extract of leaves of Dolichos biflorus 800 mg/kg orally for 4 weeks)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Curative control (1% ethylene glycol for 4 weeks followed by water for 4 weeks)</td>
</tr>
<tr>
<td>Group V</td>
<td>Curative treatment (1% ethylene glycol for 4 weeks followed by ethanolic extract of leaves of Dolichos biflorus 400 mg/kg for 4 weeks)</td>
</tr>
<tr>
<td>Group VI</td>
<td>Curative treatment (1% ethylene glycol for 4 weeks followed by ethanolic extract leaves of Dolichos biflorus 800 mg/kg for 4 weeks).</td>
</tr>
</tbody>
</table>

Prophylactic activity against urolithiasis was investigated using Groups I to III in this study and after 4 weeks, animals were sacrificed and vesicle calculi were collected, weighed and statistically evaluated. Curative property was investigated in using Groups IV to VI in the study and, at the end of eight weeks, animals were sacrificed and vesicle calculi were collected, weighed and evaluated statistically.

Weight of Stones
The weight of deposited stone was indicated by calculating the difference between the weight of the implanted zinc discs at the time of implantation and final weight of the dried calculi taken out from the bladder at the end of the 4th and 8th week period.

Statistical Analysis
The data obtained from the study was statistically evaluated using a parametric test One way-ANOVA (Analysis of Variance) and Dunnet’s test as post hoc test.

RESULTS
Weight of stone material deposit on zinc discs in control and after using ethanolic extract of leaves of Dolichos biflorus 400 mg/kg & 800 mg/kg treated rats is represented in Table 2.
Table 2: Weight of stones in various groups.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>No. of mice</th>
<th>Weight of stone (mg) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I – prophylactic control</td>
<td>6</td>
<td>282.46±0.62</td>
</tr>
<tr>
<td>2</td>
<td>Group II – prophylactic treatment (400 mg/kg)</td>
<td>6</td>
<td>144.08±1.12</td>
</tr>
<tr>
<td>3</td>
<td>Group III – prophylactic treatment (800 mg/kg)</td>
<td>6</td>
<td>112.28±0.99</td>
</tr>
<tr>
<td>4</td>
<td>Group IV – curative control</td>
<td>6</td>
<td>273.55±1.14</td>
</tr>
<tr>
<td>5</td>
<td>Group V – curative treatment (400 mg/kg)</td>
<td>6</td>
<td>173.8±1.20</td>
</tr>
<tr>
<td>6</td>
<td>Group VI – curative treatment (800 mg/kg)</td>
<td>6</td>
<td>121.46±0.98</td>
</tr>
</tbody>
</table>

* p < 0.001, df = 2, SEM = Standard error of mean, n = 6

In this study, the ethanolic extracts of leaves of *Dolichos biflorus* showed significant decrease in the weight of stones compared to control after the study.

DISCUSSIONS

In the present study, ethanolic extract of leaves of *Dolichos biflorus* 400 mg/kg & 800 mg/kg were investigated for the antiurolithic activity in Swiss albino mice. Stone formation was induced by zinc disc foreign body insertion technique supplemented with 1% ethylene glycol.

For estimating the curative or preventive effect of the ethanolic extract of the leaves of *Dolichos biflorus* weight of the stones was calculated. The use of extract in both the groups has shown a significant decrease in the weight of stones in comparison to the control group. It has been observed that there is reduction in the bladder stone formation with increase in the dose of extract.[8]

CONCLUSIONS

It is concluded from the study that the ethanolic leaf extract of *Dolichos biflorus* possesses antiurolithic activity as it was reported that there was decrease in the stone size. Further studies are needed to prove the stone dissolving property of ethanolic extract of leaves of *Dolichos biflorus* (400 mg/kg & 800 mg/kg) in other animal models.

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