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Research Article

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Phytochemical and Pharmacological Study on the Leaves of Bauhinia Purpurea L. for Antilithiatic Activity

Dilip Kumar Chanchal[1]*, Mayank Kumar[2], Archana Dongray[3], Sakshi Tiwari[4], Saurabh Gupta[1] and Shahbaz Khan[1]

[1]*Department of Pharmacy, Smt. Vidyawati Group of Institutions, Jhansi - 284128, Uttar Pradesh, India*

[2]*Department of Pharmacognosy, Shri RLT Institute of Pharmaceutical Science and Technology, Ekdil, Etawah - 206126, Uttar Pradesh, India*

[3]*College of Pharmacy, SRGI Ambabai, Jhansi - 284002, Uttar Pradesh, India*

[4]*Department of Pharmacy, Bundelkhand University Jhansi - 284128, Uttar Pradesh, India*

***Corresponding author**

Dilip Kumar Chanchal, Assistant Professor, Department of Pharmacy, Smt. Vidyawati Group of Institutions, Jhansi-284128, Uttar Pradesh, India

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Abstract

The main purpose of this study was to investigate the impact of ethanolic concentrate of Bauhinia purpurea L. leaves on lithiatic action in rat. Twenty four rats were divided into 6 groups comprising four animals per groups. The blood was collected from the retro-orbital sinus; serum was separated by centrifugation and analyzed for creatinine and uric acid. Both kidneys from each animal were removed and sectioned for histopathological examination. The results were expressed as mean ± standard error mean (SEM) and the statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test and $p<0.05$ was considered significant. In the present in-vivo study, renal stone inciting treatment to rodents came about in hyper-oxaluria. Ethanolic concentrate of B. purpurea L. was exposed to antilithiatic action in rodents where ethylene glycol 0.75% (v/v) utilized as the causing lithiasis specialist. Ethanolic concentrate of B. purpurea (at 100 and 200 mg/kg) showed a portion subordinate noteworthy enemy of lithiatic action on treatment. The concentrate portion of 100 mg/kg likewise caused decrease of Ca, oxalates, P and creatinine in blood serum level the outcomes were found factually noteworthy. The impact of ethanol concentrate of this movement was discovered critical than the reference standard. By and large investigation uncovers that the ethanolic concentrate of B. purpurea L. demonstrates the mellow to direct antilithiatic movement with correlation with cystone.

Keywords: Antilithiatic activity, Blood, *Bauhinia purpurea*, Ethylene glycol, Histopathology, Renal

Introduction

Nature has given a total storage facility of solutions for fix all diseases of humanity [1]. India is the 8th biggest nations having an aggregate of around 47,000 plants species, out of which 7,500 species are referred to as therapeutic plants. Among these therapeutic plants just 800 species are professed to be being used and around 120 species are utilized in huge amounts, which establishes to about 1.6% of all our number of restorative plants, and around 0.25% of the absolute number of plant species in India. The situation demonstrates that there is immense undiscovered potential and larger part of the restorative plants yet to be investigated experimentally [1, 2].

Urinary stone illness has distressed mankind since artifact and can persevere, with genuine therapeutic results, all through the life expectancy of a patient. Urolithiasis known as Kal Adaippu in Siddha arrangement of drug is a typical issue found in people of any age because of changing way of life and dietary decisions, prompting expanded rate and commonness around the world [3]. It is a multi-factorial procedure caused because of unevenness between the advertisers and the inhibitors of renal calculi lastly may cause kidney disappointment moreover. About 75% of renal stones are made out of calcium oxalate precious stones [4]. Flow present day prescriptions incorporate α-1-blockers and calcium channel blockers and advances, for example, percutaneous water system chemolysis and Extracorporeal Shock Wave Lithotripsy give successful treatment. Be that as it may, antagonistic medication responses, for example, drain; hematuria, cylindrical corruption, and consequent fibrosis of the kidney are distinguished [5].

Conventional drugs, principally Chinese meds and Indian prescriptions are ending up increasingly more prevalent as option and advantageous cures over ongoing years in light of its minimal effort and nontoxic nature [6]. For quite a long time, an enormous number of natural and herbo-mineral arrangements have been utilized in customary arrangement of medication and somewhere

else which guarantee a proficient remedy for urinary stones [7, 8]. Prior entire plant of *A. lanata*, base of *C. nurvala*, product of *T. terrestris*, and base of *P. odorata* in the detailing of *B. purpurea* have been accounted for their different natural properties, for example, cancer prevention agent, diuretic and antilithiatic properties [9-12]. Despite the fact that the individual herbs have both logical and conventional case for their diuretic, cancer prevention agent and analytics controlling property, there is no legitimate logical information accessible for the antilithiatic property of *B. purpurea* plan. A few models for prompting urolithiasis are accessible off late, to assess the medication reaction however they have a few impediments, for example, hematuria, and mortality [5, 11]. In the present investigation, an altered enlistment technique was embraced to instigate urolithiasis utilizing ethylene glycol and sodium oxalate to deliver renal stone effectively with lesser mortality. Subsequently, in the present examination, the antilithiatic capability of *B. purpurea* was logically assessed against tentatively incited urolithiasis in rodents utilizing ethylene glycol and sodium oxalate and along these lines likewise checks the lessening of nephrotoxicity initiated by ethylene glycol.

Figure 1: Leaves and flower of *B. purpurea* L.

Materials and Methods

Collection and Authentication of Plant

B. Purpurea L. leaves were gathered from the Bundelkhand Region. The plant was distinguished by nearby individuals of that park and verified by Dr. Gaurav Nigam (Asst. Teacher) Department of Botany, Bundelkhand University, Jhansi (UP) India. A Voucher example number is BU/Bot./Spe./Pha./11-2015/07. The leaves were isolated and dried under shade, pounded by mechanical processor, went through 40 number work sifters and put away in a shut vessel for further use.

Extraction of Plant Materials

Confirmed leaves were shade dried at room temperature and make leaves to coarse powder structure with the assistance of processor. The coarsely powder leaves is filled in Soxhlet mechanical assembly and ceaselessly removed with ethanol at temp at 60-80 °C till every one of the constituents is isolated out. The accomplishment of the extraction with ethanol is legitimately identified with the degree that chlorophyll is expelled into the dissolvable. At the point when the tissue on rehashed extraction is totally free of green shading, it tends to be guessed that all the low sub-atomic weight mixes have been extricated.

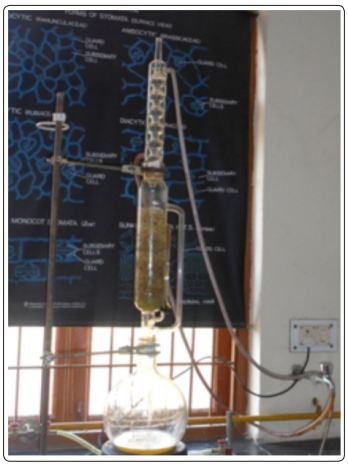

Figure 2: Extraction from Soxhlet apparatus

Characteristics of Extract

Colour: Dark green
Odour: Characteristics
Taste: Characteristics

Results

Ethanolic concentrate of the *B. purpurea* L. leaves demonstrates the nearness of carbohydrates, proteins, amino acids, steroids, phenolics mixes, flavonoids and saponin glycosides, alkaloids, and fixed oil.

Preparation of dose

The portion of 100 mg/kg, 200 mg/kg, and 300 mg/kg of ethanol concentrate was chosen for the test. Every one of the portions was given orally in the wake of making suspension in vehicle for example 1% Tween 80 and the standard medication for example Cystone was given orally (750 mg/kg) in vehicle.

Ethylene glycol (0.75%) prompted hyperoxaluria model was utilized to initiated stone in rat.

Animal Selection

Male albino rats of wistar strain weighing between 150-200g were chosen for the antiuro-lithiatic action. Animals were housed in polypropylene confines with the channel beat and kept up at 25 ± 2 ºC, relative moistness 55 ± 10% under controlled states of 12-h light: 12-h dim cycle. The animals were fed up with commercial rat chow and were given water *ad libitum*. All protocols of the study was approved by the Institutional Animal Ethical Committee with reference number BU/PHARM/IAEC/2a/16/07. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with registration number 716/02/a/CPCSEA.

Acute Oral Toxicity

The limit test of acute oral toxicity was carried out and the relatively high LD_{50} value is determined by administering dose of 50, 100, 150, 200, 400, 800, and 1000 mg/kg and mortality is observes after 24 hrs. The LD_{50} 6 was determined graphically as describe earlier. It suggested that within this dose range is relatively safe and non-toxic to rats as no observable acute toxicity effect were produced.

Animal Groups

Here, 24 rats were divided into 6 groups comprising 4 animals per groups.

Group 1 Normal, *ad libitum* access to regular food and drinking water administered.
Group 2, 3, 4, 5 and 6, *ad libitum* access to regular food and *ad libitum* access to drinking water containing 0.75% (v/v) ethylene glycol (EG) in order to promote hyperoxaluria and CaoX deposition in the kidneys

Group 2 Ethylene glycol 0.75% (v/v)
Group 3 Standard Drug (Cystone 750 mg/kg)
Group 4 Ethanolic extract of *Bauhinia purpurea* Linn. (100 mg/kg)
Group 5 Ethanolic extract of *Bauhinia purpurea* Linn. (200 mg/kg)
Group 6 Ethanolic extract of *Bauhinia purpurea* Linn. (300 mg/kg)

Design of Work

The rats were housed in cages and divided into 6 groups of four animals each. Group 1 serve as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to group 2 to 6 for induction of renal calculi till 28th day. Group 2 receive only Ethylene glycol 0.75% (v/v), Group 3 receive standard antilithiatic drug, cystone (750 mg/kg) from 15th day till 28th day. Group 4 to 6 received as curative regimen, group 4 received ethanol extract 100 mg/kg, group 5 received ethanol extract 200 mg/kg and group 6 received ethanol extract 300 mg/kg from 15th day till 28th day.

Assessment of Antiurolithiatic Activity

Collection and Analysis of Urine

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40ºC. Urine was centrifuged and the crystals of urine were analyzed under optical microscope at 10x to 40x resolution. Urine was analyzed for calcium oxalate and phosphate content.

Serum Analysis

The blood was gathered from the retro-orbital sinus under soporific condition and serum was isolated by centrifugation at 10,000g for 10 min and analyzed for creatinine and uric acid. The creatinine pack (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit Span Diagnostics Ltd., were applied.

Table 1: Serum Biochemical Data

Parameter Unit (mg/dl)	Group 1 Control	Group 2 (EG)	Group 3 Cystone	Curative regimen (BPEE)		
				Group 4 (100mg)	Group 5 (200mg)	Group 6 (300mg)
Blood Urea	2.69±0.42	10.21±0.25	4.18±0.30	6.64±0.32	7.60±0.39	5.94±0.26
Creatinine	0.42±0.04	0.89±0.03	0.59±0.04	0.72±0.02	0.54±0.04	0.31±0.03
Calcium	7.13±0.4	13.39±0.22	8.28±0.34	11.15±0.16	10.35±0.30	8.86±0.19
Inorg. Phosphorus	7.84±0.06	10.91±0.22	7.27±42	9.79±0.22	8.54±0.18	7.83±0.23

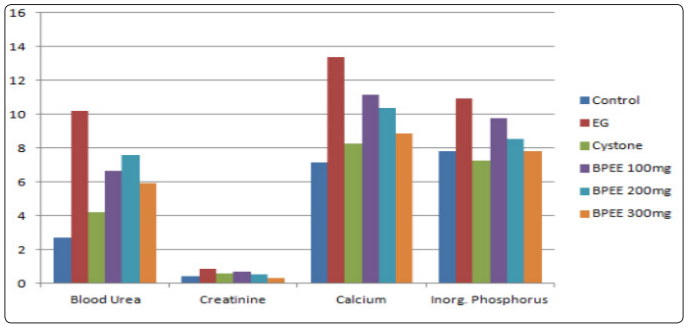

Figure 3: Graphical Representation of Antilithiatic Activity of *B. purpurea* n=6, Values are Expressed as Mean±SEM P< 0.05 When Compared with Control Group

Kidney Histopathology and Homogenate Analysis

The mid-region was sliced open to expel both kidneys from every creature. Secluded kidneys were cleaned up incidental tissue and washed in freezing physiological saline. The right kidney was fixed in 10% nonpartisan cradled formalin, handled in a progression of reviewed liquor and xylene, installed in paraffin wax, segmented at 5lm and recolored with H and E (Haematoxylin and Eosin) for histopathological assessment. The slides were inspected under light magnifying instrument to concentrate light minuscule engineering of the kidney and calcium oxalate stores. The left kidney was finely minced and 20% homogenate was set up in Tris-HCl support (0.02mol/l, pH 7.4). Absolute kidney homogenate was total kidney homogenate was utilized for examining tissue calcium, oxalate and lipid peroxidation movement.

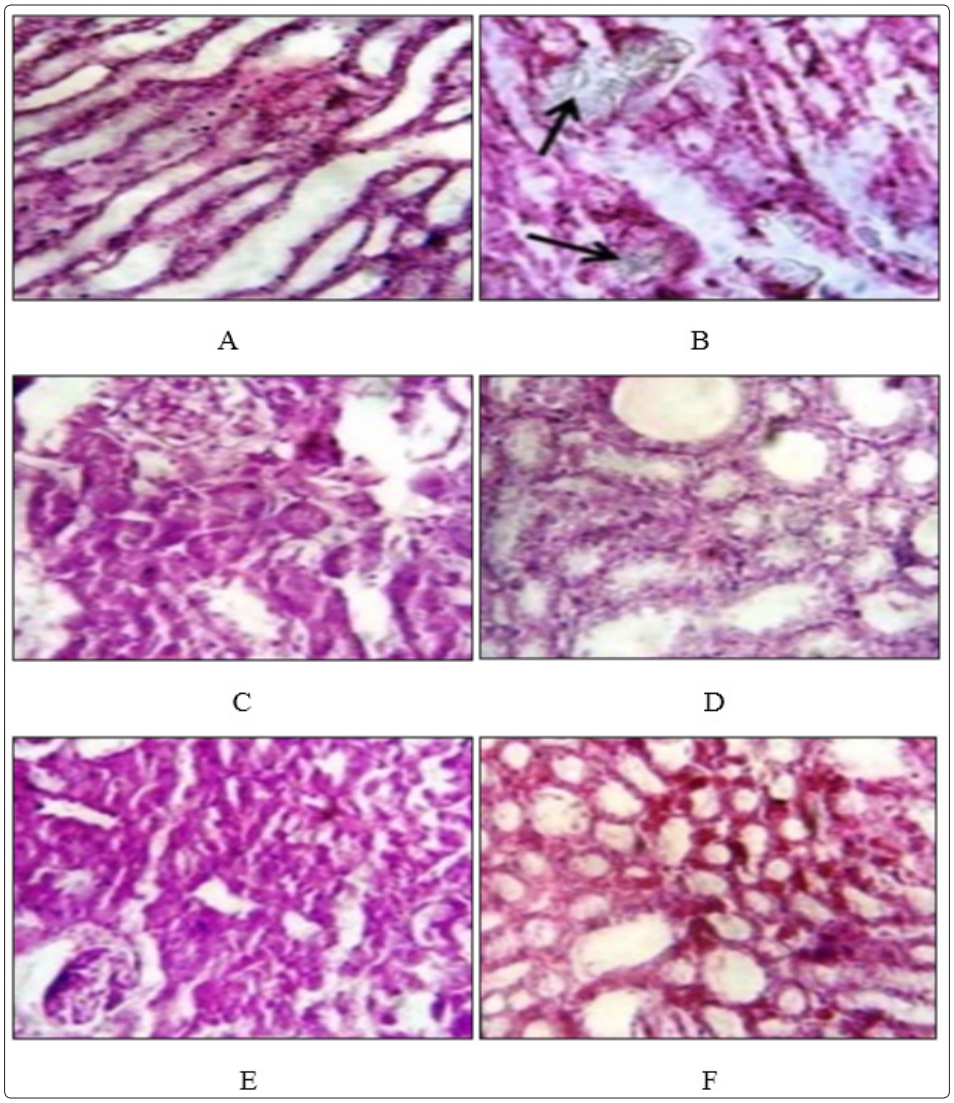

Figure 4: Microscopic architecture and calcium oxalate deposits in the kidney section. Kidney sections of (A) Vehicle control (B) Urolithic (C) Cystone treated (D) Treatment with BPEE at the dose of 100 mg/kg (E) Treatment with BPEE at the dose of 200 mg/kg (F) Treatment with BPEE at the dose of 300 mg/kg.

Result

Ethanolic concentrate of *B. purpurea* L. was exposed to antilithiatic action in rats where Ethylene Glycol (0.75% v/v) was utilized as the causing lithiasis operator. A checked ascent in calcium, bull alates, phosphorus and creatinine in blood serum level saw in lithiatic contrast standard with various curative doses of rats. Ethanolic concentrate of *B. purpurea* at (100 and 200 mg/kg) displayed a portion subordinate huge enemy of lithiatic movement on treatment. The concentrate portion of 100 mg/kg additionally caused decrease of Calcium, oxalates, Phosphorus and creatinine in blood serum level the outcomes were found measurably huge. The antilithiatic impact of ethanol separate at was discovered critical than the reference standard.

Discussion

The present work was to completed with the goal of ethanolic concentrate of leaves *B. purpurea* L., phytochemical examination, assessment of the antilithiatic movement (in-vivo) against ethylene glycol incited lithiatic model primer phytochemical screening of *B. purpurea* L. leaves of demonstrates the nearness of flavonoids mixes as significant dynamic rule constituents in ethanol concentrate of leaves of *B. purpurea* L. Accordingly; there is probability that ethanol concentrate of leaves of *B. purpurea* L. may have the antilithiatic movement. Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less.

The biochemical instrument for this procedure is identified with an expansion in the urinary centralization of oxalate. Stone development in ethylene glycol bolstered is brought about by hyperoxaluria, which causes in-wrinkled renal maintenance and discharge of oxalate renal calcium oxalate testimony by EG (ethylene glycol) in rodents is oftentimes used to copy the urinary stone arrangement in people. Thus, this model was utilized to assess the defensive impact of leaves of *B. purpurea* L. against urolithiasis. Typical pee contains numerous

inorganic and natural inhibitors of crystallization, magnesium is one such surely understood inhibitors. Low degrees of magnesium are experienced in stone formers just as in stone-framing rats.

The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans the predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment of CIEE lowered the excretion of uric acid and reduces the risk of stone formation.

The expansion in urinary acid excretion was seen in urolithic rodents. Expanded discharge of uric acid has been accounted for in stone formers and hyperoxaluric rodents. Uric acid meddles with calcium oxalate solvency and it ties and diminishes the inhibitory movement of glycosaminoglycans the prevalence of uric corrosive precious stones in calcium oxalate stones and the perception that uric acid restricting proteins are equipped for official to calcium oxalate and regulate its crystallization likewise proposes its essential job in stone development. Treatment of CIEE brought down the discharge of uric acid and lessens the danger of stone arrangement.

The present observation showed increased protein excretion in ethylene glycol induced urolithic rats. Proteinuria reflects proximal tubular dysfunction. Supersaturation of urinary colloids results in precipitation as crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth administration of BPEE had profound effects on minimizing the excretion of protein and thus might have prevented the nidus formation for crystal nucleation.

The result showed the nephroprotective effect of leaves of *B. purpurea* L. in ethylene glycol induced urolithiatic model. Earlier study reported a high hypoglycemic and anti-inflammatory capacity of leaves of *B. purpurea* L. Therefore, BPEE may prevent cal-cium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation), which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

Conclusion

The present work was completed with the goal of ethanolic concentrate of leaves of Bauhinia purpurea Linn., phytochemical study, assessment of Antilithiatic action (*in vivo*) against ethyl-ene glycol instigated lithiatic model. The leaves of the plant was removed with ethanol and after that oppressed for phytochemical screening for the discovery of different plant constituents, it is discovered that flavonoids and phenolic mixes are available as real dynamic guideline. In general examination uncovers that the ethanol concentrate of Bauhinia purpurea L. demonstrates the mellow to direct antilithiatic action with correlation with cystone.

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