

In-vivo* antiurolithiatic activity of *Thunbergia erecta

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ABSTRACT

Objective: The aim of the present study was to evaluate *in-vivo* antiurolithiatic activity of a methanolic extract from the leaves of *Thunbergia erecta*. **Method:** The antiurolithiatic assay was performed by Ethylene Glycol alone induced urolithiasis and Ethylene glycol + Ammonium chloride induced urolithiasis model. Blood was collected through retro orbital puncture from animals under ether anaesthesia for serum separation in order to assess serum creatinine and BUN. Animals were sacrificed and both the kidneys will be excised, rinsed in ice cold physiological saline and weighed. Further analysis parameter also performed estimation of urea, kidney stone analysis, phosphate analysis, urea nitrogen analysis calcium oxalate analysis. **Results:** *Thunbergia erecta* 400 shows the maximum potential compared to other groups and standard. Effect of *Thunbergia erecta* extracts on kidney and urinary parameters in ethylene glycol and ammonium chloride induced wistar rats the amount of calcium, phosphates and oxalate decrease in kidney likewise in the urine. Similarly the level of BUN, uric acid and creatinine decrease at the dose of *Thunbergia erecta* 400. **Conclusion:** Effect of *Thunbergia erecta* extracts on serum parameters in ethylene glycol and ammonium chloride induced wistar rats. Same result exhibits in case of ethylene glycol alone with Kidney and Urinary Parameters and serum parameters. Thus we claimed that the *Thunbergia erecta* 400 shows best result for this research work which will help to researches for further analysis and studies.

Keywords: *Thunbergia erecta*, Antiurolithiatic activity, Phytochemical analysis, nucleation method

INTRODUCTION

Urolithiasis refers to the formation of calculi anywhere in the urinary tract afflicting the kidneys, ureters, bladder or urethra (Vijaya, *et al.*, 2013). It is the third most prevalent urological disorder after urinary tract infection and prostate conditions (Al-Jebouri & Atalah 2012). Approximately 12% of the world population is affected by the incidence of urinary stones with a comparatively higher frequency of recurrence in males (70-81%) than in females (47-60%) (Joy, *et al.*, 2102). Depending upon the chemical nature, there are various types of stones such as calcium oxalate monohydrate, calcium oxalate dihydrate, basic calcium phosphate (brushite), magnesium ammonium phosphate (struvite), uric acid and cystine stones (Nagal & Singla 2013). Manifestations include colicky pain, vomiting, dysuria, haematuria, pyuria and oliguria (Tiwari *et al.*, 2012). These calculi arise as an outcome of supersaturation of urine with stone

forming constituents resulting in crystallization followed by crystal nucleation, aggregation and growth leading to their adherence to the renal tubules (Agarwal *et al.*, 2013). Numerous surgical strategies exist in scenario for management of urinary calculi such as shock wave lithotripsy, ureteroscopic lithotripsy, digital endoscopy, percutaneous nephrolithotomy and robotic surgery (Rosa *et al.*, 2013). However, many of these techniques bring along with them several drawbacks such as renal casualties in the long run, hypertension and repeated episodes of stones (Began *et al.*, 1991). Several allopathic interventions are available but are increasingly being overtaken by herbal therapeutics on account of their safety, minimal side effects, comparatively greater efficacy in dissolving stones and preventing the chances of recurrence (Alok *et al.*, 2013). Moreover, the applications of medicinal plants in combating urinary stones have been extensively mentioned in the ancient medical literature as well as Ayurvedic system of medicine in addition to their relevance as folk remedies (Chitme *et al.*, 2010). *Thunbergia erecta* (Syn: *Meyenia erecta*, Acanthaceae) is also known as King's Mantle, Blue Bell and Bush Clock Vine (Manokari & Shekhawat, 2017). It is woody,

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straight shrub which has 2-2.5 m altitude. It contains flower that blooms throughout the year and has blue to purple color range (Kitajima *et al.*, 2010). *Thunbergia* is native to the tropical part of Madagascar, Australia, Africa, India and South Asia. Leaves, roots and stems of *Thunbergia* species have the traditional history to be used for the treatment of inflammation and pyrexia (Jeeva *et al.*, 2011). *Thunbergia* species have been reported to show antibacterial effects against both gram positive and gram negative bacteria (Oonsivilai *et al.*, 2008). *Thunbergia* species also possess analgesic, antipyretic, anthelmintic, antidiabetic, cytotoxic, antioxidant, hepatoprotective (Oonsivilai *et al.*, 2008 & Wonkchalee *et al.*, 2008), antitumor and antinociceptive activities (Jetawattana *et al.*, 2015). Chemical ingredients separated from *Thunbergia* genus include alkaloids, glucosides, naphthalene, coumaroylmalic acid, iridoidglucosides, grandifloric acid, benzyl beta glucopyranoside, delphinidin, apigenin and phenolic compounds such as tannin, feruloylmalic, flavonoids, phenolic acids, rosmarinic acid (Kanchanapoom *et al.*, 2002 & Areekul *et al.*, 2009). Therefore, current investigation was carried out for the screening of the antilithic potential of *Thunbergia erecta* against calcium oxalate crystallization in an in vitro setting.

MATERIAL AND METHOD

Selection and Collection of Plant

The leaves of *Thunbergia erecta* were collected in the month of July 2019 from the Pinnacle Biomedical Research Institute, Bhopal Campus.

Authentication of Plant

The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number 195/Saif./Sci./Cig/Bpl. was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Pharmacological study (In vivo studies)

Animals were selected randomly from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7 % at 22±2 °C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal has approved all animal experiments with CPCSEA

Reg. No.-1824/PO/ERe/S/15/CPCSEA. Protocol reference No. is PBRI/IAEC/PN-16027.

Animals used

- Strain - Albino Wistar rats
- Age - 5-6 weeks
- Sex - either sex
- Body weight - 200±20 g

Acute oral toxicity

Acute toxicity was performed as per OECD 423 guidelines and three albino rats per step were selected. Two to four steps may be necessary to allow for making a judgment on the acute toxicity of the test substance depending on the mortality and/or the morbidity status of the animals.

In-vivo Urolithiasis Activity

Ethylene Glycol alone induced urolithiasis model

Ethylene glycol induced hyperoxaluria model (Atmani *et al.*, 2003) was used to assess the antilithiatic activity in albino rats. Ethylene glycol (0.75%) in drinking water was fed to different groups for induction of renal calculi till 28th day.

Ethylene glycol + Ammonium chloride induced urolithiasis

To study the effect of test material on CaC₂O₄ urolithiasis, group I rats, serving as vehicle treated control, received vehicle once in 24 h, whereas, the untreated rats (group II) received stone inducing treatment for 21 days, comprised of 0.75% (w/v) Ethylene Glycol with 1% (w/v) ammonium chloride for 5 days, following this the water supply will be switched to 0.75% EG alone in water, along with vehicle treatment. Other treatment group was given test sample/ standard anti-urolithic drug dissolved/suspended in vehicle once in 24 h and simultaneously received stone inducing treatment similar to the untreated groups.

Analysis Parameters (For in-vivo model)

The activity was regularly monitored to assess their overall health so that any animal looking lethargic or excessively losing weight could be excluded from the study.

Blood was collected through retro orbital puncture from animals under ether anaesthesia for serum separation in order to assess serum creatinine and BUN. Animals were sacrificed and both the kidneys were excised, rinsed in ice cold physiological saline and weighed.

Kidney Homogenate Analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys will be dried at 80°C in a hot air

oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000×g for 10 min and the supernatant were separated. The calcium phosphate and oxalate content in kidney homogenate was determined.

Urea nitrogen

Blood urea nitrogen was estimated by Berthelot method (Fawcett & Scott, 1960) using the commercially available kit (Kamineni Life Sciences Pvt. Ltd. Hyderabad, India). Working reagent-I up to 1000 µl of containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were mixed well and incubated at 37 °C for 5 min. Then 1000 µl of reagent-II containing alkaline buffer, was added to all the test tubes, which were incubated at 37 °C for 5 min. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield indophenol, a blue-green coloured compound. The intensity of the colour produced is directly proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm.

The blood urea was calculated using the following formula:

Blood urea nitrogen (mg/dl) = Serum urea X 0.467.

Estimation of Calcium oxalate

Calcium has numerous functions within the body, not only as a structure factor in bones and teeth but also in normal neuromuscular function and the clotting of blood. Estimation of calcium was estimated by O-Cresolphthalein complexone (OCPC) method (Moorehead & Briggs, 1974) using the commercially available kit (Transasia Biomedicals Ltd. baddi). Reagent 1 containing 2-Amino-2-methyl-1-propanol and reagent 2 containing OCPC, 8-hydroxy Quinoline and HCl, add in equal volume and allow to stand at room temperature. Then take absorbance at 578 nm.

Calcium (mg/dl) = Abs. of Test/Abs of standard × conc. of standard (mg/dl)

Estimation of Phosphate

Phosphate was estimated by Berthelot method (Daly & Ertingshausen, 1972) using the commercially available kit (vital Diagnostics Pvt. Ltd. Thane, India). Molybdate reagent using as a working reagent for estimation of phosphate present in urea. And take absorbance at 340 nm.

Estimation of Urea

Urea was estimated by Berthelot method (Gutmann & Bergmeyer, 1974) using the commercially available kit ((Transasia Biomedicals Ltd. baddi), India). Reagent 1 and aqua 4 added equally and attain the temperature 15-30° C. reagent and serum was added in appropriate volume then take absorbance at 340nm.

Biostatistical interpretation

Data was calculated as Mean ± SD/SEM. One-way ANOVA was used to interpret the results followed by Bonferroni's test. P<0.001 was considered as level of significance.

Statistical Analysis

The data are represented as mean ± S.E.M, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Dunnett t-test where p<0.05 was considered statistically significant using Graph pad 5 software.

RESULT & DISCUSSION

Acute Oral Toxicity

Toxicity testing of new compounds is significant for drug development process. The toxicity of the substances can be determined on experimental animals by analyzing accidental exposures to an in vivo substance. Acute oral toxicity study of methanolic extract of *Thunbergia erecta* according to OECD-423 guidelines in mice.

Table 1: Acute oral toxicity of extract of selected plants (in albino wistar rat)

S. No.	Dose	No. of animal	Mortality* Methanolic
1.	5 mg/kg	Three	0/3
2.	50 mg/kg	Three	0/3
3.	300 mg/kg	Three	0/3
4.	2000 mg/kg	Three	0/3
5.	5 mg/kg	Three	0/3
6.	50 mg/kg	Three	0/3
7.	300 mg/kg	Three	0/3
8.	2000 mg/kg	Three	0/3

*x/3: x animals dead in 3 test animals

To ascertain safety of any component its acute oral toxicity is an important parameter to assess. Toxicity was assessed at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. As per OECD 423 guideline up to the dose of 2000 mg/kg safety was assessed. All two extracts were found to be safe at all selected doses. As all two extracts were safe up to the dose of 2000 mg/kg and no mortality was observed (Table), dose

was selected accordingly. $1/10^{\text{th}}$ and $1/5^{\text{th}}$ of 2000 mg/kg was selected as dose for present investigation. Doses selected were 200 mg/kg and 400 mg/kg (Rosa *et al.*, 2013; Wonkchalee *et al.*, 2012).

In vivo anti urolithiasis activity

Urolithiasis or kidney stone formation is a complex process which take place due to supersaturation of stone forming constitute like calcium, oxalate, uric acid, urea, phosphate in urine and an imbalance of inhibitors like glycosaminoglycans, magnesium. Herbal medicines have many phytoconstituents

which may exert their beneficial effect alone or in combination in kidney stone treatment. Plant extracts contain phytochemicals that inhibit stone formation by inhibiting synthesis and agglomeration of crystals (Vijaya *et al.*, 2013).

Ethylene glycol and ammonium chloride induced model (EG+NH₄Cl Model)

In the present study, methanolic extracts of the leaves of *T. erecta* were assessed for its anti-urolithiatic activity in ethylene glycol and ammonium chloride induced urolithiasis in rats and the results obtained were recorded.

Table 2: Effect of *Thunbergia erecta* extracts on Kidney and Urinary Parameters in Ethylene Glycol and ammonium chloride Induced Wistar Rats

Group No.	Treatment	In kidney			In urine		
		Oxalate	Calcium	Phosphate	Oxalate	Calcium	Phosphate
I	Control	1.21±0.157	1.94±0.333	1.53±0.174	0.29±0.078	1.12±0.085	2.44±0.359
II	EG+NH ₄ Cl Control	6.72±0.306*	5.48±0.407**	5.30±0.544	4.13±0.528**	5.28±0.256	7.03±0.500
III	Standard	1.37±0.356	2.77±0.543	1.95±0.464	0.77±0.121	1.52±0.264	3.08±0.214
IV	TE 100	3.40±0.623	4.07±0.356**	3.30±0.681	2.77±0.524	3.30±0.482	4.56±0.149
V	TE 200	2.43±0.339*	3.33±0.524	2.72±0.172	2.02±0.256	1.95±0.383	3.53±0.378
VI	TE 400	1.83±0.294	2.85±0.729	2.67±0.513	1.30±0.155	1.68±0.519	3.12±0.838

*Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Table 3: Effect of *Thunbergia erecta* extracts on Serum Parameters in Ethylene Glycol and ammonium chloride Induced Wistar Rats

Group No.	Treatment	In Serum		
		BUN	Uric acid	Creatinine
I	Control	25.53±1.324	1.03±0.040	0.58±0.065
II	EG+NH ₄ Cl Control	59.53±1.544	4.77±0.532	2.48±0.376*
III	Cystone 750	27.27±3.653	1.52±0.349**	0.70±0.050
IV	TE 100	48.60±4.590**	2.67±0.412	1.32±0.109*
V	TE 200	41.48±5.601	2.45±0.345	1.10±0.055
VI	TE 400	33.28±2.193	1.91±0.802	0.96±0.122

*Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Kidney and Urine Analysis

The kidney parameters of the rats were assessed by evaluating the oxalate, calcium and phosphate concentrations in the kidney homogenate. For kidney, EG+NH₄Cl control gave the value of oxalate, calcium and phosphate as 6.72±0.306, 5.48±0.407, 5.30±0.544 respectively. Negative control showed decrease in value as 1.21±0.157, 1.94±0.333, 1.53±0.174 of oxalate, calcium and phosphate respectively. TE 100 showed increase in value of oxalate, calcium and phosphate i.e., 3.40±0.623, 4.07±0.356, 3.30±0.681 respectively. TE 400 was not found to be effective as it gave lowest increase

level of oxalate, calcium and phosphate as 1.83±0.294, 2.85±0.729, 2.67±0.513 respectively (Wesson *et al.*, 1998).

Similarly for urine, EG+NH₄Cl control gave the value of oxalate, calcium and phosphate as 4.13±0.528, 5.28±0.256, 7.03±0.500. Negative control showed decrease in value as 0.29±0.078, 1.12±0.085, 2.44±0.359 of oxalate, calcium and phosphate respectively. TE 100 showed increase in value of all three constituents as 2.77±0.524, 3.30±0.482, 4.56±0.149 whereas in TE 400 all the three constituents showed decrease in value i.e., 1.30±0.155, 1.68±0.519, 3.12±0.838.

Ethylene Glycol Induced Alone model

In the present study, methanolic extracts of the leaves of *T. erecta* were assessed for its anti-

urolithiatic activity in ethylene glycol alone induced urolithiasis in rats and the results obtained were recorded (Hennequin *et al.*, 1993).

Table 4: Effect of *Thunbergia erecta* leaves Extract on Kidney and Urine Parameters in Ethylene Glycol Alone

Group No.	Treatment	In kidney			In urine		
		Oxalate	Calcium	Phosphate	Oxalate	Calcium	Phosphate
I	Control	1.00±0.018	1.71±0.136	1.55±0.131	0.24±0.053	1.18±0.053	2.53±0.271
II	EG Control	7.37±0.464	6.00±0.260	5.93±0.319	4.60±0.358	5.63±0.149	7.30±0.249
III	Standard	1.79±0.147	2.70±0.293	1.98±0.263	0.79±0.093	1.65±0.187	3.11±0.115
IV	TE 100	4.39±0.517*	4.33±0.301	3.84±0.264	2.82±0.217**	3.72±0.162	4.94±0.397
V	TE 200	3.74±0.312	3.66±0.108**	3.26±0.093	2.46±0.113	2.52±0.166**	3.90±0.156
VI	TE 400	2.96±0.153*	2.82±0.386	2.35±0.201	2.11±0.187	1.98±0.143	3.14±0.125

*Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Table 5: Effect of *Thunbergia erecta* leaves Extract on Serum Parameters in Ethylene Glycol Alone

Group No.	Treatment	In Serum		
		BUN	Uric acid	Creatinine
I	Control	30.39±1.993	1.08±0.063	0.62±0.088
II	EG Control	61.33±7.394	4.82±0.307	2.51±0.267
III	Cystone 750	36.17±3.061	1.68±0.407	0.79±0.113
IV	TE 100	50.99±3.481*	2.74±0.266**	1.51±0.221*
V	TE 200	42.87±4.741**	2.48±0.190	1.16±0.073**
VI	TE 400	37.77±4.840**	1.97±0.580*	1.04±0.123

*Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

CONCLUSION

From the present research work we conclude that the TE 400 shows the maximum potential compared to other groups and standard. Effect of *Thunbergia erecta* extracts on Kidney and Urinary Parameters in Ethylene Glycol and ammonium chloride induced Wistar Rats the amount of calcium, phosphates an oxalate decreases in kidney likewise in the urine. Similarly the level of BUN, uric acid and creatinine decreases at the dose of TE 400 in effect of *Thunbergia erecta* extracts on serum parameters in ethylene glycol and ammonium chloride induced wistar rats. Same result exhibits in case of ethylene glycol alone with Kidney and Urinary Parameters and serum parameters. Thus we may claimed that the TE 400 shows best result for this research work and go for further analysis and studies.

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