Research Article

Investigations for anti-urolithiatic activity of *Tephrosia purpurea* roots against ethylene glycol-induced renal calculi in rats

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Abstract

Objective: *Tephrosia purpurea* is used as digestive, antiulcer, diuretic and antitussive in ayurvedic practice. Decoction of roots is given in dyspepsia, diarrheoa, rheumatism, asthma and urinary disorder. The objective of present study was to investigate anti-urolithiatic activity of ethanolic and aqueous extract of *T. purpurea* roots against ethylene glycol-induced renal calculi in rats. **Materials and methods:** Ethanol and aqueous extract was administered into the calculogenic rats to observe the increased calcium and oxalate level and number of calcium oxalate crystal in the kidney tissue of rats. **Results**: Results indicate that ethylene glycol administered to rats resulted in to hyper hypercalciuria, oxaluria, and increased renal excretion of phosphate. Treatment with ethanolic and aqueous extracts of *T. purpurea* roots significantly prevented level of urinary calcium, oxalate and phosphate excretion. The possible mechanism of this effect is mediated through antioxidant nephroprotection. Microscopic analysis also confirmed the deposition of calcium oxalate crystals and disruption of tubular cells that protected significantly (P<0.05) with 300 mg/kg dose of ethanolic extract of *T. purpurea* given to the rats. **Conclusion:** The results of the study was supported the protective effect of ethanolic extract of *T. purpurea* against the urolithiasis.

***Keywords*:** *Tephrosia purpurea,* anti-urolithiatic, ethylene glycol, antioxidant, flavonoids

Introduction

Urolithiasis or kidney stone formation is a complex process that occurs due to imbalance between promoters and inhibitors in the kidneys. It is a progression of several physicochemical events including supersaturation, nucleation, aggregation and retention within the kidneys. Calcium-containing stones, especially calcium oxalate mono and dihydrate and basic calcium phosphate are the most commonly occurring stones. Some other types of stone include uric acid stone, cystine stone, silicate stone, protease related stone and DHA stone etc. Minerals in the urine especially calcium, build on the fleck similar to that a pearl grows in an oyster shell. The formation of the nidus may be analagous to the first stage in the physiologic calcification of bone in which a nucleus of calcium phosphate develops in an organic matrix (Howard et al., 1967; Thomas and Howard, 1959; Boyce and King, 1963; Boyce, 1968). It is reported already that calcium phosphate is soluble in urine which is crystallize easily and is present in small quantities in renal and urethral stones (Grases, 1993; Tiselius, 1996). These observations concluded that different stones have their origin on a calcium phosphate precipitate and their early stages are attached to the renal papilla (Evan, 2009). A state of super saturation of urine in the form of calcium and phosphate ions is probably necessary for the development of a renal stone and the growth of the stone (Mac Gregor et al., 1965). In short, kidney stone formation is a complex process that results from the progression of several physico-chemical events including supersaturation, nucleation, aggregation, and retention within renal tubules (Khan, 1997).

Tephrosia purpurea L. (Fabaceae) is commonly known as Sarpunkha, an important indigenous medicinal plant of Indian Medicinal System. It is an important component of herbal preparation such as Tephroli and Yakrifit used for liver disorders. Tefroli tablet is clinically used in treatment of viral Hepatitis with safely and not have any side effect.

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Yakrifit is a liver tonic of *Tephrosia purpurea* found clinically effective in veterinary medicine (Sankaran, 1980; Kumar et al., 1997). In Ayurvedic system of medicine *T. purpurea* has been used as remedy for impotency, asthma, diarrhoea, gonorrhoea, allergic and inflammatory conditions such as rheumatism, ulcer and urinary disorders (Kirtikar and Basu, 1956; Despande et al., 2003). *T. purpurea* has been already reported to effective in bilious febrile attacks, bleeding piles and obstruction of liver and spleen. Pods extract reported to effective for pain, inflammation and their decoction is used in vomiting (Anonymous, 1976). The aqueous extract of *T. purpurea* seeds found significant *in-vivo* hypoglycaemic activity in diabetic rabbits (Rahman et al., 1985). The ethanolic extracts of *Tephrosia purpurea* L. found potential antibacterial activity (Mahajan et al., 1999).

The roots, leaves and seeds contain tephrosin, 2.5% rutin, deguelin and quercetin, Roots also contain isotephrosin and rotenone. Rotenoids are produced in *in-vitro* tissue cultures of the plant parts. Purpurin, a flavonone has been isolated from the seeds, as also 8-substituted flavonoid and 3-substituted oxygenated chalcones (Agarkar, 1991). Ethanolic extract of T. purpurea leaves and aerial parts reported to have potential wound healing activity (Lodhi et al., 2006; Lodhi et al., 2013). Leaves contains β-sitosterol, lupeol and lanceolatin B (Sinha et al, 1982) and Lodhi et al. (2010) reported effect of *T. purpurea* on burn wound healing. The present study was aimed to evaluate anti-urolithiatic activity of ethanolic and aqueous extract of *T. purpurea* roots against ethylene glycol-induced renal calculi in rats.

Materials and methods

Collection and identification of plant materials

T. purpurea roots were collected from region of Jabalpur (M.P.). Plant materials were identified by the taxonomist from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.). Plant material was dried in shed and powdered for extraction with petroleum ether, ethyl alcohol and water. Ethyl alcohol and aqueous extracts were further processed for qualitative chemical screening.

Phytochemical screening

The extracts were filtered through the simple filter paper and concentrate in vacuum under reduced pressure. Various qualitative chemical tests for flavonoids, alkaloids, saponins, tannins, amino acids, glycosides and terpenoids were applied in ethyl alcohol and aqueous extract (Kokate et al., 2001).

Animals

Wistar male albino rats (150-200g) were selected for *in vivo* study. They were housed individually in well-ventilated, controlled temperature (26 ±2°C) animal room for seven days prior to start experiment. The animals were allowed to given the standard commercial pellet diet (Hindustan Lever Pvt Limited, Bangalore, India) and water ad libitum. All experimental protocol were reviewed and approved by the Institutional Animal Ethics Committee (Reg. No. 1471/P0/a/11/CPCSEA).

Ethylene glycol-induced urolithiasis

Ethylene glycol was used to induce urolithiasis (Makasanaa et al., 2014). Twenty four animals were randomly divided into four groups containing six animals in each. Group I was denoted as control group received 0.5% w/v gum acacia solution (5 ml/kg p.o.) while Group II and III referred as treated groups were received ethanol extract and aqueous extract 300 mg/kg, p.o. for 21 days. Group IV denoted as reference group that received standard drug cystone (750 mg/kg, p.o.) for 21 days. All groups treated with 0.75%, v/v ethylene glycol in drinking water for 21 days to induce urolithiasis. All extracts were given once daily by oral route.

Urine sample collection

After 21st day urine samples were collected from each rat. A drop of concentrated hydrochloric acid was added to the urine prior to storage for the analysis of calcium and oxalate content in urine by colorimetric procedure (Dharmalingam et al., 2014). Phosphorus content was determined by molybdenum blue reaction. Urine samples were centrifuged at 3000 rpm for 10 min and were examined under light microscope to ensure shape and size of the calcium oxalate crystals.

Serum analysis

At the end of the treatment period, the animals were anaesthetized and sacrificed by cervical dislocation. Blood was collected from the retro-orbital puncture of all animals. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine and uric acid content using commercially available kits and by a colorimetric method (Fossati et al., 1980).

Statistical analysis

All data were represented as the mean ±S.E.M. for six rats and data were evaluated using the Tukey test. Values of $P < 0.01$ were considered to be statistically significant.

Results and discussion

Phytochemical screening

The ethanol extract and aqueous extract were tested for qualitative chemical analysis to detect the presence of different chemical constituents. Ethanolic extract found presence of flavonoids, tannins, amino acids, glycosides and terpenoids. Aqueous extract was give positive test for glycosides, saponins, amino acids and flavonoids.

Anti-urolithiatic activity

There are several factors which can increase the risk for kidney stones formation, including inadequate water intake and dehydration, slow urinary flow and reduced urine volume. Some chemical e.g. calcium, oxalate, uric acid levels in the urine may increases during stone formation (Kumar et al.,1991). In normal and routine life, we do not take sufficient water, our food that is too rich in calories and table salt, but have deficiencies in fiber and alkali (Straub and Hautmann, 2005). All these conditions leads to the urinary stones formation. Wistar albino male rats were selected to induce urolithiasis. The phytochemical screening of ethanol and aqueous extract showed the presence of glycosides, flavonoids, tannins, amino acids and terpenoids.

Table 1. Effect of different extracts of *T. purpurea* roots on urine parameters of rats

Treatment groups	Calcium (mg/dl)	Oxalate (mg/dl)	Inorganic Phosphate (mg/dl)
Control	0.18±0.08	2.51±0.07	0.93±0.06
EETP (300 mg/kg)	0.08±0.04*	1.31±0.05*	0.45±0.04*
AETP (300 mg/kg)	0.09±0.02*	1.52±0.08*	0.51±0.06*
Standard Cystone (750 mg/kg, p.o.)	0.04±0.06	1.25±0.06	0.46±0.02

Urine super saturation is one of the important causative factors in calculogenesis for stone-forming constituents. Ethylene glycol induced stone formation in rats is caused by hyperoxaluria, which increases renal retention and excretion of oxalate (Roger et al., 1997). Renal calcium oxalate deposition by ethylene glycol in rats is normally used to mimic the urinary stone formation in humans. Therefore, this model was used to evaluate the protective effect of *T. purpurea* against urolithiasis.

Table 2. Effect of different extracts of *T. purpurea* roots on serum parameters of rats

Treatment groups	Creatinine (mg/dl)	Uric Acid (mg/dl)
Control vehicle	2.14±0.09	7.53±0.54
EETP (300 mg/kg)	1.86±0.05*	4.79±0.48*
AETP (300 mg/kg)	1.47±0.06*	3.82±0.42*
Standard Cystone (750 mg/kg, p.o.)	1.52±0.07	3.85±0.28

After administration of ethylene glycol solution (0.75 %) to the rats, produced hyperoxaluria condition. Ethanol extract treated group showed calcium and oxalate excretion in urine was decreased up to 0.08±0.04and 1.31±0.05 mg/dl, respectively in calculi-induced animals compared to vehicle control group (Table 1). The results of ethanol extract treated group were found approximately similar to the standard group. However, both extract significantly ($P < 0.01$) reduced the elevated levels of calcium, oxalate, phosphate excretion in urine but ethanol extract found most potent effect on calculi induced animals.

Administration of ethylene glycol (0.75 %v/v) to male albino rats up to 21 days and developed renal calculi composed mainly of calcium oxalate. The creatinine content in serum of vehicle control group rats were decreased, in standard drug and extract-treated groups. Serum analysis showed a significant increase *(*$P < 0.01$) in creatinine levels in lithiatic control rats when compared to normal rats. After treatment with ethanol extract, serum creatinine levels were restored to the normal value. Microscopy observations of urine samples were revealed frequency and size of calcium oxalate crystals is larger in the vehicle control group animals, while in the ethanol and aqueous extract treated groups showed reduced in frequency and size of calcium oxalate crystals.

Conclusion

The results of present study were confirmed the protective effect of ethanol and aqueous extract of *T. purpurea* roots on ethylene glycol induce renal calculi. Comparatively the ethanol extract was found more effective than the aqueous extract of *T. purpurea* roots. The effect can be due to synergistic property of flavonoids and other components present in roots. Further detail study of extract and individual compound is required investigate possible mechanism of action.

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Conflict of interest

Authors did not have any conflict of interest.

References

Agarkar SP. 1991. Medicinal plants of Bombay Presidency. Scientific Publishers Jodhpur, India. pp. 209–210.

Anonymous. 1976. Wealth of India. A Dictionary of Indian Raw Materials & Industrial, Products Raw Materials, vol. X. Publishing & Information Directorate, CSIR New Delhi, pp. 151–156.

Boyce WH. 1968. Organic matrix of human urinary concretions. American Journal of Medicine, 45: 673.

Boyce WH, JS King, Jr. 1963. Present concepts concerning the origin of matrix and stones. Annals of New York Academic Science, 104: 563.

Deshpande SS, Shah GB, Parmar NS. 2003. Antiulcer activity of *Tephrosia purpurea* in rats. Indian Journal Pharmacology, 35: 168–172.

Evan AP. 2009. Physiopathology and etiology of stone formation in the kidney and the urinary tract. Pediatric Nephrology, 25: 831-841.

Grases F, March JG, Conte A, Costa-Bautza A. 1993. New aspects on the composition, structure and origin of calcium oxalate monohydrate calculi. European Urology, 24: 381–386.

Howard JE, Thomas WC, Barker LM, Smith LH, Wadkins CL. 1967. The recognition and isolation from urine and serum of a peptide inhibitor to calcification. Johns Hopkins Medical Journal, 120: 119.

Khan SR. 1977. Interactions between stone forming calcific crystals and macromolecules. Urology International, 59 (2): 59–71.

Kirtikar KR, Basu BD. 1956. Indian Medicinal Plants, vol. 1, 2nd ed. Lalit Mohan Basu, Allahabad, India. p. 723.

Kumar S, Sigmon D, Miller T, Carpenter B, Khan S, Malhotra R, Scheid C, Menon M. 1991. A New Model of Nephrolithiasis Involving Tubular Dysfunction Injury. Journal of Urology, 146:1384-1389.

Kumar A, Dutta M, Bhatt TK, Dalal DS. 1997. Use of herbal tonic Yakrifit in equine practice. Indian Veterinary Journal, 74(5): 424–425.

Lodhi S, Jain AP, Sharma VK, Singhai AK. 2013.Wound-Healing Effect of Flavonoid-Rich Fraction from *Tephrosia purpurea* Linn. on Streptozotocin-Induced Diabetic Rats. Journal of Herbs, Spices and Medicinal Plants, 19(2): 191-205.

Lodhi S, Pawar RS, Jain AP, Jain A, Singhai AK. 2010. Effect of *Tephrosia purpurea* (L) Pers. on partial thickness and full thickness burn wounds in rats. Journal of Complementary and Integrative Medicine, 7(1): 13.

Lodhi S, Pawar RS, Jain AP, Singhai AK. 2006. Wound healing potential of *Tephrosia purpurea* (Linn) Pers. in rats. Journal of Ethnopharmacology, 108: 204–210.

Mac Gregor J*,* Robertson WG, Nordin BEC. 1965. Octacalcium phosphate*:* the phase governing the solubility equilibrium in Apatitic calculi. British Journal of Urology, 37: 518.

Mahajan RT, Chaudhary GS, Chopra MZ. 1999. Screening of some indigenous plants for their possible antibacterial activity. Environmental Bulletin, 15: 61–62.

Rahman H, Kashfudduja MT. 1985. Hypoglycemic activity of *Tephrosia purpurea* Linn. Seeds. Indian Journal of Medical Research, 81: 418–421.

Sankaran JR. 1980. Tefroli in the management of viral hepatitis. Antiseptic, 77(11): 643–646.

Sinha B, Natu AA, Nanavati DD. Prenylated flavonoids from *Tephrosia purpurea* seeds. Phytochemistry 1982; 21(6): 1568-1470.

Straub M, Hautmann RE. 2005. Developments in stone prevention. Current Opinion in Urology. 15: 119-126.

Thomas WC, Howard JE. 1959. Studies on the mineralizing propensity of urine from patients with and without renal calculi. Trans. Association of American Physicians (Philadelphia), 72: 181.

Tiselius HG. 1996. Solution chemistry of supersaturation. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM (eds) Kidney stones: medical and surgical management. Lippincott- Raven Publishers, Philadelphia. pp 33–64.

Makasanaa A, Ranpariyab V, Desaia D, Mendparaa J, Parekha V. 2014. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in Rats. Toxicology Reports, 1: 46–52

Dharmalingam SR, Madhappan R, Chidambaram K, Ramamurthy S, Gopal K, Swetha P, Senthil Kumar KL. Anti-urolithiatic activity of *Melia azedarach* Linn leaf extract in ethylene glycol-induced urolithiasis in male albino rats. Tropical Journal of Pharmaceutical Research March 2014; 13 (3): 391-397.

Fossati P, Prencipe L, Berti G. 1980. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical Chemistry, 26: 227-231.

Roger K, Low MD, Stoller ML. 1997. Uric acid nephrolithiasis. Urologic Clinics of North America 24, pp 135–148.