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ORIGINAL PAPER

Preliminary investigation on ultra high diluted *B. vulgaris* in experimental urolithiasis

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Purpose: **The study focuses on the anti-urolithiasis potential of ultra-diluted homeopathic potency of *Berberis vulgaris* (*B. vulgaris*) root bark, commonly used in homeopathic system to treat renal calculi.**
Methodology: ***B. vulgaris*** **root bark (200c, 20 μl/100 g body weight/day, p.o, for 28 days) was tested in an animal model of urolithiasis. Urolithiasis was induced in male Wistar rats by adding 0.75% ethylene glycol (EG) to drinking water. Urine and serum samples were analyzed for calcium, magnesium, phosphorus, uric acid and creatinine. Enzymic makers of renal damage (alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase and γ-glutamyl transpeptidase) were assessed in kidney and urine. Renal tissues were analyzed for oxalate content.**
Results: **Administration of EG to rats increased the levels of the stone-forming constituents calcium, phosphorus and uric acid, in urine. Levels were normalized by *B. vulgaris* treatment. The decrease in the urolithiasis inhibitor magnesium in urine was prevented by treatment with *B. vulgaris*. Serum creatinine levels were largely normalized by *B. vulgaris* treatment. Hyperoxaluria induced renal damage was evident from the decreased activities of tissue marker enzymes and an apparent escalation in their activity in the urine in control animals; this was prevented by *B. vulgaris* treatment.**
Conclusion: **Homeopathic *B. vulgaris* root bark has strong anti-urolithiasis potential at ultra-diluted dose.** *Homeopathy* (2013) **102**, 172–178.

Keywords: Urolithiasis; Hyperoxaluria; Homeopathy; *Berberis vulgaris*; Renal damage

Introduction

Urolithiasis has afflicted mankind since antiquity.[1] It is the third most common disorder of the urinary tract, after urinary tract infections and benign prostatic hyperplasia.[2] Modern medical treatments for urolithiasis risk of adverse health-effects such as loss of renal function, hypertension and an increased rate of new stone occurrence.[3] It is therefore interesting to consider novel treatments such as homeopathy for non-toxic anti-urolithiasis treatments.

Homeopathy is a 200 year old therapeutic system based on the 'Principle of Similars' and the use of 'minimum' dose to stimulate autoregulatory and self-healing processes. *Berberis vulgaris* is one of the most widely used homeopathic medicines for kidney pain and kidney stones. *Berberis vulgaris* Linn. (family, Berberidaceae) commonly known as 'barberry' is a common garden bush, native to Europe, North America and the British Isles.[4] Several *in vitro* and *in vivo* studies on medicinal plants used in traditional antiurolithic therapy have proven that these remedies are effective.[5,6] Inhibition and dissolution of calcium oxalate crystals in solutions containing a homeopathic medicine *Berberis vulgaris*-mother tincture has been reported.[7] In

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the traditional system of medicine, the root bark is reported to be useful in the treatment of urinary stones. However, there are no records of systematic pharmacological studies that support the claimed effectiveness of *B. vulgaris* roots for treatment of urinary stone. The active principles of *B. vulgaris* include alkaloid components such as berberine, oxyacanthine, berbamine and palmatine.[8] Berberine, an isoquinoline plant alkaloid, belongs to the structural class of protoberberines and is one of the most studied naturally occurring protoberberine alkaloids.[9] In addition to *B. vulgaris*, berberine is present in many other plants including *Hydrastis canadensis* (goldenseal) (Ranunculaceae), *Coptis chinensis* (Coptis or golden thread) (Ranunculaceae), *Arcangelisia flava* (Menispermaceae), *Berberis aquifolium* (Oregon grape) and *Berberis aristata* (tree turmeric). It possesses a spectrum of biochemical and pharmacological activities including: inhibition of DNA and protein synthesis, arrests cell cycle progress, including anti-inflammation. Berberine has been found to have anti-cancer effects in HeLa and leukemia cells by inducing DNA topoisomerase poisoning and hence apoptotic cell death.[10] It also has been documented to inhibit mediastinal lymph node metastasis by suppressing activator protein 1 (AP-1) activity.[11] Berberine inhibits TPA (12-O-tetradecanoylphorbol-13-acetate)-mediated inflammation and cyclooxygenase-2 (COX-2) expression through the regulation of AP-1.[12] It suppresses LPS (lipopolysaccharide)-induced COX-2, TNF-alpha, and iNOS (inducible nitric oxide synthase) productions in lung macrophage.[13] It also inhibits acetaldehyde-induced cytokine production in HepG2 cells.[14] Berberine also suppresses inflammatory agents-induced cytokine production by inhibiting the IkB-alpha (inhibitor of NF-kappaB alpha) phosphorylation and degradation of lung cells. The anti-inflammatory action of Berberine may arise in part from the inhibition of DNA-synthesis in activated lymphocytes.[15] Bashir and Gilani[16] have shown that Berberine acts as an antiurolithic drug in a multifaceted way by its antioxidant, diuretic, urinary alkalinizing and hypocalciuric effects.

In the present study, an effort has been made to establish the scientific validity for the antiurolithic property of homeopathic formulation of *B. vulgaris* using ethylene glycol (EG) induced hyperoxaluria model in rats.

Material and methods

Drugs and chemicals

Homeopathic preparation of *B. vulgaris* (200c, Batch No: 7970) was procured from Hahnemann Publishing Co. Pvt. Ltd., Kolkata, India. All other chemicals and solvents used in the present study were procured from standard agencies and were of analytical grade.

Animal model

Male albino rats of Wistar strain weighing 120 ± 20 g were obtained from Institutional Central Animal House Facility. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house with constant 12 h light/dark cycle. Rats were permitted free access to drinking water throughout the experimental period. The animals were fed with standard rat pellet diet (Lipton India Ltd., Mumbai, India). The experiment was approved by the Institutional Animal Ethical Committee (IAEC No. 01/012/2010).

B. vulgaris treatment

The rats were randomly divided into four groups consisting of six rats ($n = 6$) each. Group I rats served as untreated control. Group II received EG (0.75% in drinking water) for 28 days, to induce a chronic low grade hyperoxaluria and generate calcium oxalate deposition in kidneys. Group III rats received homeopathic preparation of *B. vulgaris* (200c, 20 μl/day/100 g body weight, oral gavage) along with 0.75% EG as in group II. Group IV rats served as drug controls and were given 200c *B. vulgaris* alone for 28 days.

Sample collection

Rats were housed in individual metabolic cages. Urine was collected in ice-jacketed flasks at 4°C for assessment of renal function. The urine free from fecal contamination was collected for 24 h at the 7th, 14th, 21st and 28th day with sodium azide as preservative. Cell debris and particulate matter were then removed from the urine samples by 1000*g* centrifugation at 4°C for 15 min. The supernatant was recovered. Aliquots of the supernatant were dialyzed against deionized water at 4°C (Sigma Chemicals, USA, No: D9277) and used for the assay of enzymes and protein. Estimation of stone-forming constituents and other parameters were carried out with ideal undialyzed sample.

On completion of the 28-day experimental period, the rats were killed by decapitation and the kidneys from each rat were immediately dissected out, washed with ice-cold physiological saline. The washed tissues were homogenized in 0.01 mol/l Tris−HCl buffer (pH 7.4) and aliquots of the 10% homogenate were used for the assays. Blood samples were obtained, centrifuged, and sera were used for biochemical parameters.

Analysis of stone-forming constituents

Estimation of oxalate: Oxalate was estimated by the method of Hodgkinson and Williams[17]. To 2.0 ml of the acidified urine, 1.5 ml of water was added and the pH was adjusted to 7.0. To this, 2.0 ml of saturated aqueous solution of calcium sulphate was added followed by 14.0 ml of ethanol, mixed well and allowed to stand at room temperature for 3 h. The mixture was centrifuged at 2000 rpm for 10 min. The precipitate was dissolved in 2.0 ml of 2 N sulphuric acid. Electrolytic zinc was added and heated in a boiling water bath for at least 30 min, till the final volume became less than 0.5 ml due to evaporation. The zinc piece was removed and 0.3 ml of chromotropic acid was added and tubes were placed in cold condition. 5.0 ml concentrated sulphuric acid was added mixed and heated in a boiling water bath for 30 min. Tubes were cooled, diluted to 20 ml with 10 N sulphuric acid and the color was read at 570 nm.