Journal of Ayurveda and Integrative Medicine xxx (2016) 1–7

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article

Antiurolithiatic activity of natural constituents isolated from *Aerva lanata*

Basavaraj M. Dinnimath [a], Sunil S. Jalalpure [b, c, *], Umesh K. Patil [d]

[a] Department of Pharmachemistry, KLEU'S College of Pharmacy, Belagavi, Karnataka, India
[b] Department of Pharmacognosy, KLEU'S College of Pharmacy, Belagavi, Karnataka, India
[c] Dr Prabhakar Kore Basic Science Research Centre (BSRC), KLE University, Belagavi, Karnataka, India
[d] Dept. of Pharmaceutical Sciences, Dr H S Gour Central University, Sagar, M.P., India

ARTICLE INFO

Article history:
Received 3 June 2016
Accepted 4 November 2016
Available online xxx

Keywords:
Urolithiasis
Ayurveda
Quercetin
Betulin
Pashanabheda
Kidney stones

ABSTRACT

Background: *Pashanabheda* is used as antiurolithiatic in Ayurveda. In the present study, *Aerva lanata* (L) Juss. ex. Schult (Amaranthaceae) from Western Ghats of India was selected for isolation of active constituents and screening for antiurolithiatic potentials.
Objective: Screening of compounds isolated from *A. lanata* for antiurolithiatic potentials.
Materials and methods: Ethylene glycol (0.75% v/v) induced urolithiasis model was used to study the antiurolithiatic activity in male Wistar albino rats. The animals were divided into five groups containing six each. Based on the LD_{50} of the plant extract (2000 mg/kg b.w) equivalent dose was calculated from their yield. Two isolated compounds (quercetin and betulin) of *A. lanata* were screened for antiurolithiatic potentials in calculi induced (ethylene glycol 0.75% v/v) male Wistar albino rats by administering 2 mg/kg b.w/day orally as test dose for 28 days.
Results: The urine volume was found to be significantly increased from 12.76 ± 0.10 ml to 21.35 ± 0.20 ml in the rats treated by quercetin and 21.50 ± 0.21 ml in rats treated by betulin. Urine microscopy revealed significant reduction ($p < 0.001$) in the size of calculi and significantly enhanced ($p < 0.001$) excretion of calcium, oxalate, phosphate, whereas the level of magnesium was increased. SEM of kidney sections has revealed reduction in the calculi in treated animals. Serum analysis has revealed significant reduction in the level of BUN and creatinine in treated rats.
Conclusion: The isolated quercetin and betulin from *A. lanata* have shown mild diuretic effect as well as antiurolithiatic effect by significantly reducing the size of calculi in the kidneys and enhancing the excretion of calcium, phosphate, oxalate while maintaining the level of magnesium, which is reported to be one of the calculi inhibiting factors.

1. Introduction

Pashanabheda (stone breaking) plants are a group of medicinal plants which are used in Indian traditional medicinal system by Ayurvedic practitioners as antiurolithiatic drugs. Traditionally *Aerva lanata* (L) also known as *Pashanabheda*, belonging to the family Amaranthaceae, used for various medicinal uses including both antiurolithiatic and diuretic [1–4].

The traditional medicine system of India is a rich source of valuable medicinal plants but there is no scientific data reported to establish the activity of these plants. Hence these plants need to be evaluated, based on their biological efficacy and chemical constituents for the drug development [5]. So we have selected *Aerva lanata* whole plant, cultivated in Western Ghats region of India for the present study. The whole plant was subjected to bioactivity guided isolation and screening for antiurolithiatic activity in order to investigate and justify the traditional claim.

It is reported that flavanoids, triterpenoids and saponins such as α-amyrin, β-amyrin, lupeol from different plants showed antiurolithiatic and diuretic activity. Many plant extracts and different fractions possessing these active constituents have been screened for antiurolithiatic activity [6,7]. It is reported that flavanoids are

* Corresponding author. Department of Pharmacognosy, KLEUS College of Pharmacy, Belagavi, Karnataka, India.
E-mail addresses: basavaraj_dm@yahoo.co.in (B.M. Dinnimath), jalalpuresunil@rediffmail.com (S.S. Jalalpure).
Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2016.11.006

Please cite this article in press as: Dinnimath BM, et al., Antiurolithiatic activity of natural constituents isolated from *Aerva lanata*, J Ayurveda Integr Med (2016), http://dx.doi.org/10.1016/j.jaim.2016.11.006

found to be present in stem bark and triterpenoids are present in root bark portions of some plants such as *Crataeva nurvala*, *Crataeva magna* (Capparaceae) which acts as antiurolithiatic [8]. Hence the whole plant is selected for the study as different parts of the plant *Aerva lanata* are reported to be potent [10–12].

The reported phytochemical constituents present in *Aerva lanata* are responsible for various biological activities. These constituents include alkaloids (ervine, methylervine, ervoside, aervine, methylaervine, aervoside, ervolanine, and aervolanine), flavanoids (kaempferol, quercetin, isorhamnetin, persinol, persinosides A and B), methyl grevillate, lupeol, lupeol acetate benzoic acid, β-sitosteryl acetate and tannic acid [9].

Although *Aerva lanata* is used traditionally by Ayurvedic physicians, however, there is no report on the antiurolithiatic potentials by any of the active constituents isolated from *Aerva lanata* (L) cultivated in Western Ghats of Khanapur region from Belagavi district of Karnataka. Hence this plant was selected for isolating and developing new lead molecules for urolithiasis.

2. Material and methods

2.1. Collection of plant

The whole plant *Aerva lanata* was collected from Western Ghats region of Khanapur (Belagavi Dist, Karnataka) in March 2011. The plant was authenticated by taxonomist Dr. Harsha Hedge, Scientist B, RMRC, Belagavi (Specimen No-RMRC-507).

2.2. Extraction and fractionation

The plant material was shade dried and then it was ground to coarse powder. The powdered dry material was used for the extraction using hydroalcoholic (80-20%) and followed by fractionation with different organic solvents such as dichloromethane (fraction I), ethyl acetate (fraction II), *n*-butanol (fraction III) to separate different groups of polar compounds like flavanoids, triterpenoids and saponins following the technique of liquid–liquid separation. As polar compounds would come in polar solvents, these two fractions (ethyl acetate fraction and *n*-butanol fraction) which were found to be rich with flavanoids and triterpenoids were analyzed by phytochemical study and spectral analysis. Based on these study results, ethyl acetate and *n*-butanol fractions which were found to contain flavanoids and triterpenoids were subjected to *in vivo* analysis for antiurolithiatic potential on ethylene glycol (0.75% v/v) induced urolithiatic model of Wistar albino male rats [13,14].

2.3. Isolation and characterization

Based on the *in vivo* bioactivity study results of fractions, (ethyl acetate and *n*-butanol) the fractions were found to be potent, may be due to the presence of polar compounds. Hence these two fractions were subjected to isolation of active constituents using column chromatography technique followed by purification of the isolated constituents by preparative Thin Layer Chromatography (TLC). The fraction (II) and fraction (III) were loaded separately in the glass column by dissolving in chloroform (5 g in 500 ml each fraction) and separation was carried out. The isolated compound was identified with preparative TLC. This isolation technique has yielded two compounds AEF 1(isolated compound 1) and AEF 2.3 (isolated compound 2) from ethyl acetate and *n*-butanol fractions respectively and these two compounds were characterized as quercetin and betulin using Infra-Red, Nuclear Magnetic Resonance and Liquid Chromatography Mass Spectroscopy [15].

2.4. Animals

Male Wistar albino rats weighing 150–200 g were purchased from Sri Venkateshwara traders, Bengaluru. They were housed in acryl fiber cages at 23 ± 2 °C, humidity 50 ± 1% and were kept on a 12 h light/dark cycle. They were fed with standard chow feed (Amrut laboratories, Sangali) and water *ad libitum* and acclimatized for 15 days before the study. Experimental protocol reported in this study was approved by the Institutional Animal Ethical Committee of CPCSEA, Govt. of India (IAEC-Resolution No-13, 31-07-2010) and carried out in accordance with OECD guidelines.

2.5. Chemicals and drugs

Ethylene glycol (0.75% v/v) and sodium carboxy methyl cellulose were purchased from Himedia Lab (Mumbai) and Loba Chemie (Mumbai) respectively. Reference drug Cystone was purchased from Himalaya Herbal Healthcare, Bangalore. Demineralized water and analytical grade chemicals/solvents were procured from local market.

Electron microscope–Inverted microscope, Lobamade, model no-TCM-400 was used for urine microscopy study.

2.6. Drug administration

The reference drug and isolated compounds (quercetin and betulin) were administered orally through stainless steel oral feeding tube. Sodium CMC 1% of the weight was added to the isolated compounds for preparing the test doses.

2.7. Acute toxicity assay

Acute toxicity assay was conducted for hydroalcoholic extract as per OECD guideline 423 (limit test-standard protocol). Six male Wistar albino rats (three animals in each step) and total 12 animals were randomly selected. The extract was found to be safe up to 2000 mg/kg b.w. No separate toxicity study for the test compound was done since the hydroalcoholic extract was found to be safe as per IAEC [16].

2.8. Evaluation of antiurolithiatic activity of isolated compounds on ethylene glycol (0.75% v/v) induced albino rats

Ethylene glycol (0.75% v/v) induced urolithiasis model was used to study the antiurolithiatic activity in male Wistar albino rats. The animals were divided into five groups containing six each. The group I served as control and fed with normal rat food and water *ad libitum*. Group II to V received ethylene glycol (0.75% v/v) orally in drinking water from day 1 to day 28 for the induction of renal calculi (day 1 to day 14-induction period). Group II served as disease induced group. Group III received reference drug Cystone (750 mg/kg b.w) from 14th day to 28th day (treatment period). Group IV received quercetin-2 mg/kg b.w. from 14th day to 28th day (equivalent dose), group V received betulin-2 mg/kg b.w (equivalent dose) from 14th day to 28th day (treatment period) [17–19]. This equivalent dose was calculated based on the yield of the isolated compound from the corresponding fraction/s (as per IAEC).

Urine and serum analysis was carried out at the end of the study. The blood sample (1 ml) was collected on 28th day from each animal through retro-orbital plexus under anesthetic conditions. Urine samples were collected from all the animals on 14th and 28th day. Biochemical investigations of calcium, oxalate, magnesium, phosphate from urine sample and BUN and creatinine from blood serum sample was carried out. Both the kidneys were isolated from all the animals of different groups and subjected to histopathological study for the detection of calculus in kidneys [20].

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