

Diuretic & Anti-urolithic activity of Some Crude Extracts

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ABSTRACT

Eight crude extracts were evaluated for the presence of diuretic and anti-urolithiasis activity. Diuretic activity was carried out on rats and all extracts exhibited significant diuretic activity while *A. montana* (300 mg) and *A. uva-ursi* (300 mg) showed better diuretic activity i.e. 2.59 ± 0.0033 and 2.65 ± 0.0033 respectively in comparison to other test drugs, *A. mellifica*, *C. virosa*, *D. purpurea*, *S. nigra*, *T. occidentalis*, *U. urens* and control drug, Furosemide. Prominent anti-urolithic activity was shown by *A. uva-ursi* (95.7%), *A. montana* (80.2%), *D. purpureae* (91.2%) and *C. virosa* (91.2%) extracts while no anti-urolithic activity was found in the extracts of *U. urens*, *A. mellifica*, *S. nigra* and *T. occidentalis*. Our present study revealed the usefulness of crude extracts as safe and efficacious diuretic and anti-urolithic drugs.

Keywords: diuretic, anti-urolithiasis, herbal drugs, insect drug

INTRODUCTION

This research work is a part of our toxicity determination tests in herbal and insect crude drugs¹⁻³. Here we are reporting the diuretic and anti-urolithic activity in crude extracts of seven medicinal plants (*Arnica montana*, *Cicuta virosa*, *Digitalis purpurea*, *Sambucus nigra*, *Thuja occidentalis*, *Urtica urens*, *A. uva-ursi*) and one insect drug (*Apis mellifica*).

MATERIAL & METHOD

Crude Extracts The seven plants and one insect crude extracts were obtained from authorized dealers of local market and stored in cool, dry place for further studies. All the chemicals and reagents were procured from Merck (Germany) and Sigma-Aldrich (USA).

Diuretic activity: The diuretic effect of crude extracts of *A. mellifica*, *A. montana*, *C. virosa*, *D. purpureae*, *S. nigra*, *T. occidentalis*, *U. urens* and *U. ursi* in mice were evaluated by the method of Umang *et al.* (2009). Aqueous extract of the above mentioned eight drugs were administered to experimental mice orally at doses of 300mg/kg. Furosemide (10 mg/kg) was used as a standard drug. The diuretic effects of the extracts were assessed by measuring urine volume in ml as compared with the standard and control⁴.

Calcium oxalate crystallization inhibition activity (Anti-urolithiasis)

Experimental Protocol: The time dependent effects of turbidity changes in artificial urine on addition of 0.01M sodium oxalate solution alone and in combination with each of the following extracts, *A. montana*, *A. mellifica*, *C.*

virosa, *D. purpureae*, *S. nigra*, *T. occidentalis*, *U. urens*, *A. uva-ursi* extracts were observed on calcium oxalate crystallization. The Precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer⁵.

Preparation of artificial urine

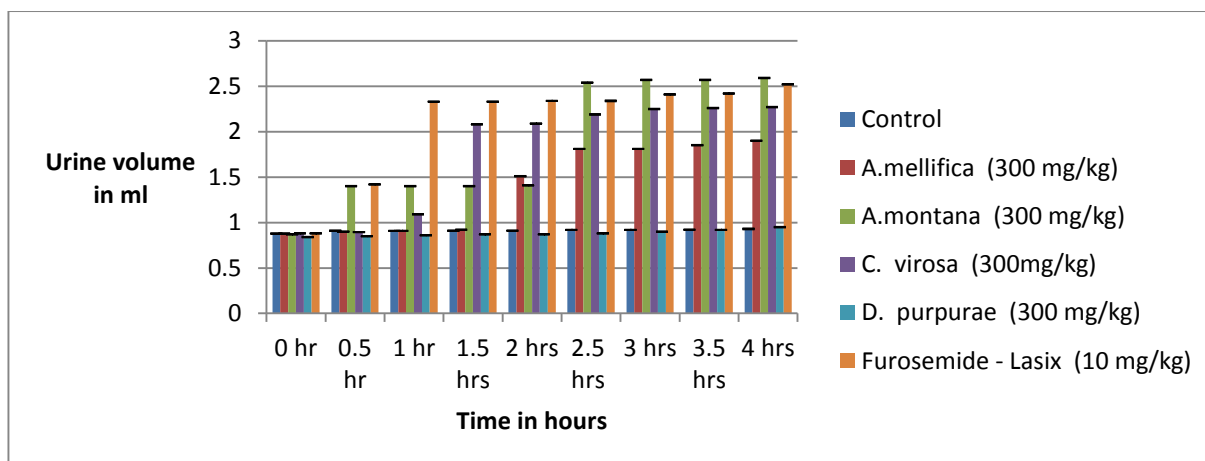
The artificial urine was prepared according to the method Burns and Finlayson⁶ and had the following composition: sodium chloride 105.5 mmol/l, sodium phosphate 32.3 mmol/l, sodium citrate 3.21 mmol/l, magnesium sulfate 3.85 mmol/l, sodium sulfate 16.95 mmol/l, potassium chloride 63.7 mmol/l, calcium chloride 4.5 mmol/l, sodium oxalate 0.32 mmol/l, ammonium hydroxide 17.9 mmol/l, and ammonium chloride 0.0028mmol/l. The artificial urine was prepared fresh each time and pH adjusted to 6.0.

Study without inhibitor

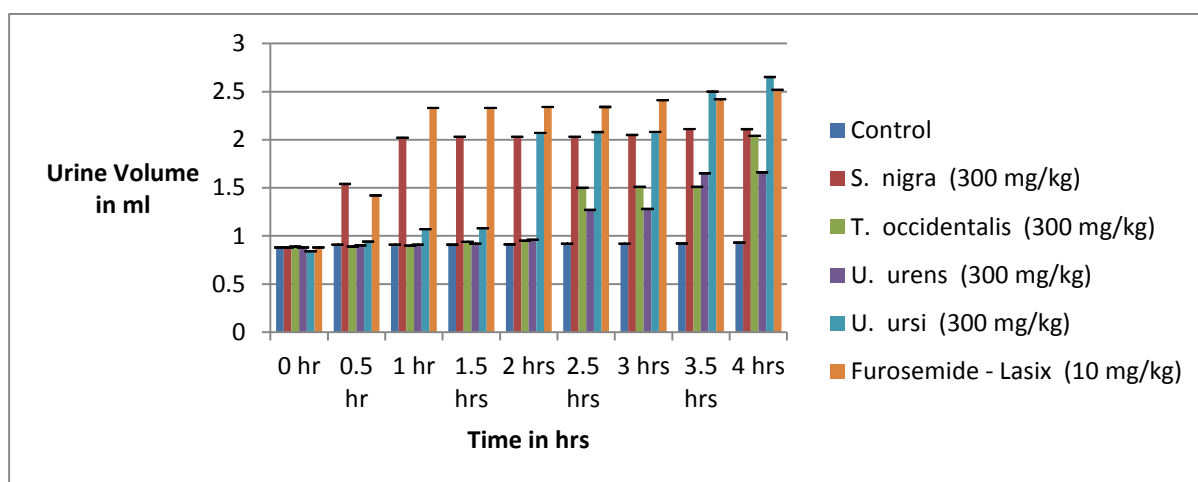
A volume of 1.0 ml of artificial urine was transferred into the cell and 0.5 ml of distilled water added to it and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement is immediately started for a period of ten minutes. For each experiment, six replicates were taken.

Study with inhibitor

The extract was dissolved in distilled water, filtered through membrane filter and the concentration of 50, 100, 150, 200 and 250µg/ml was obtained. A mixture of 1 ml of artificial urine and 0.5 ml of plants and insect crude extract solution is versed in the cell. A blank reading was taken and then 0.5 ml of 0.01M sodium oxalate solution was added and immediately the absorbance was measured for



Graph 1: Diuretic activity of *A. mellifica*, *A. montana*, *C. virosa* and *D. purpureae* in comparison with control and standard drug



Graph 2: Diuretic activity of *S. nigra*, *T. occidentalis*, *U. urens* and *A. uva-ursi* in comparison with control and standard drug

a period of ten minutes at 620nm. For each experiment, six replicates were taken. The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \{1 - [S_i / S_c]\} \times 100$$

Where; S_i : slope of graph in the presence of inhibitor (Extract), S_c : slope of graph without inhibitor (Control).

Table 1: Time-dependent synthesis and aggregation of Calcium oxalate crystals in synthetic urine

Time (mins)	Number of Calcium oxalate crystals /mm ³	Calcium oxalate aggregation/mm ³	Total
5	499	76	575
10	500	88	588
15	512	110	622
20	542	100	642
25	577	143	720
30	630	121	751
35	653	150	803
40	680	143	823

Microscopic study

The crystals of calcium oxalate (with and without inhibitors) were observed using a light microscope (Labomed) equipped with a digital camera. The photographs of calcium oxalate were taken using the objective of 40X and eye piece of 10X⁷.

Table 2: Effect of 25%, 50%, 75% and 100% of *D. purpureae*, *C. virosa*, *A. uva-ursi* and *A. montana* extracts on calcium oxalate crystallization inhibition

Percentages of drug extracts	<i>D. purpureae</i>	<i>C. virosa</i>	<i>U. ursi</i>	<i>A. montana</i>
25%	63.2	71.1	78.2	61.2
50%	70.9	73.5	80.1	70.0
75%	72.3	80.1	88.9	72.4
100%	80.4	91.2	95.7	80.2

RESULTS

Pronounced diuretic activity was exhibited on administration of 300mg/kg of oral dose of following drug extracts; *A. uva- ursi* (2.65±0.0033); *U. urens* (1.66±0.0033); *S. nigra* (2.11±0.0033); *A. montana* (2.59±0.0033), *A. mellifica* (1.90±0.0024); *D. purpureae*

(0.95 ± 0.0024); *C. virosa* (2.27 ± 0.0024); *T. occidentalis* (2.04 ± 0.0024) in comparison to control and standard drug. Maximum inhibition of calcium oxalate crystallization was observed in case of *A. uva-ursi* 95.7%, *A. montana* 80.2%, *D. purpurea* 91.2%, *C. virosa* 91.2%; while no anti-urolithic activity was observed in *U. urens*, *S. nigra*, *A. mellifica* and *T. occidentalis* extracts (Tables 1-3).

Table 3: Effect of *D. purpurea*, *C. virosa*, *A. uva-ursi* and *A. montana* extracts on different phases of crystallization

Drug extracts	Nucleation	Growth	Aggregation
<i>D. purpurea</i>	+	+	-
<i>C. virosa</i>	+	+	-
<i>A. uva-ursi</i>	+	+	-
<i>montana</i>	+	+	+

DISCUSSION

Worldwide kidney stone formation is a problem. Pakistan comes under "stone belts" area. 60% of the majority kidney stones consist of calcium oxalate and they exist in the form of calcium oxalate monohydrate and calcium oxalate dihydrate⁸. The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes: nucleation, crystal growth, crystal aggregation and crystal retention. Stone-formation takes place due to supersaturated urine⁹. Urolithiasis is a complex process that occurs due to imbalance between promoters and inhibitors in the kidneys. The factors involved in stone formation include urine output, concentration of urine, urine pH, infection or damage within the urinary tract^{10, 11}. Many anti-lithic medicinal plants are available that contain chemical constituents that have an inhibitory effect in the crystallization of calcium oxalate⁸. *A. uva-ursi* exhibited pronounced diuretic (2.65 ± 0.0033) and anti-urolithic activity (95.7%) may be due to the presence of arbutin, a phenolic glycoside along with ursolic acid and isoquercetin are the main constituent that has been reported by many researchers to be effective in relieving pain associated with kidney stones, cystitis, nephritis as well as a diuretic^{12, 13}. *U. urens* showed significant diuretic activity (1.66 ± 0.0033) and no anti-urolithic activity as reported by *U. urens* has the property of increasing the urine flow^{14, 15}. Our results support the diuretic activity (2.11 ± 0.0033) of *S. nigra* as reported by other researcher^{16, 17} but anti-urolithic activity was not found that was contradictory to the traditionally reported use of *S. nigra* in removal of kidney stones¹⁸. *A. montana* extract revealed potent diuretic (2.59 ± 0.0033) and anti-urolithic activity (80.2%) in similarity to the previously reported results indicating its usefulness in the treatment of wounds, reducing inflammation and urolithiasis^{19, 20}. Potent diuretic activity (1.90 ± 0.0024) and no anti-urolithic activity were observed in *A. mellifica* extract. Our results support the efficacy of *A. mellifica* extract in the treatment of urine retention. The chemical constituents of *A. mellifica* like phospholipase A2, phospholipase B, Hyaluronidase, phosphatase and α -glucosidase may play a significant role in symptomatic

treatment of kidney diseases like retention of urine and kidney stones^{21, 22}. *D. purpurea* extract showed prominent diuretic (0.95 ± 0.0024) and anti-urolithic activity (91.2%) as it is already well-known for its usefulness as a cardiac, diuretic, stimulant and tonic²³. It helps urination by improving the blood supply to the kidneys and helpful in removing obstructions within the kidneys may be due to its glycosides content²⁴. Diuretic (2.27 ± 0.0024) and anti-urolithic activity (91.2%) was found in *C. virosa* extract may be due to its pseudoalkaloids content. Our *C. virosa* results are in conformity to already report diuretic activity along with its specific effect on treating convulsions associated with dialysis in end-stage renal failure patients²⁵. Diuretic activity was present (2.04 ± 0.0024) whereas no anti-urolithic activity was found in *T. occidentalis* extract. Diuretic activity of *T. occidentalis* may be due to its volatile oil constituents²⁶.

CONCLUSION

Diuretic are useful in treatment of diseases associated with retention of urine like chronic renal failure, congestive heart disease, nephritis, toxemia of pregnancy, premenstrual tension, hypertension and pulmonary congestion. Synthetic diuretics like loop and thiazide cause inhibition of potassium secretion leading to potassium retention that has some toxic effects^{26, 27}. On contrary, many herbs have been explored and found to possess potent diuretic activity with lesser toxic effects²⁸. Our research work exposed the efficacy of crude extracts for the treatment of urolithiasis and urinary retention associated with cardiac, renal or other diseases.

CONFLICTS OF INTEREST

None

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