Diuretic activity of *Spilanthes acmella* flowers in rats

W.D. Ratnasooriya\(^\text{a,}\)\(^*\), K.P.P. Pieris\(^\text{b}\), U. Samaratunga\(^\text{c}\), J.R.A.C. Jayakody\(^\text{a}\)

\(^\text{a}\) Department of Zoology, University of Colombo, Colombo 03, Sri Lanka

\(^\text{b}\) Department of Surgery and E.N.T. Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Colombo, Sri Lanka

\(^\text{c}\) Department of Anatomy, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Colombo, Sri Lanka

Received 22 September 2003; received in revised form 4 January 2004; accepted 4 January 2004

Abstract

In the Sri Lankan traditional medicine, *Spilanthes acmella* Murr. (Family: Compositae) flowers are claimed to possess powerful diuretic activity. However, as yet, the diuretic potential of these flowers is not investigated by scientifically controlled studies. The aim of this study was to evaluate the diuretic potential of *Spilanthes acmella* flowers in rats using a cold-water extract (CWE). Different concentrations of CWE (500, 1000, 1500 mg/kg) or vehicle or furosemide (13 mg/kg) were orally administered (\(N = 6\) per each treatment group) to hydrated rats and their urine output was monitored at several intervals of time (1–5 h). The highest dose of CWE significantly (\(P < 0.05\)) and markedly increased the urine output. The onset of this diuretic action was extremely prompt (within 1 h) and lasted throughout the studied period (up to 5 h). The peak effect was evident between 1 and 2 h. Further, the intensity of diuresis induced by the CWE in the first hour was almost similar to that of furosemide. *Spilanthes acmella* CWE also caused marked increase in urinary \(\text{Na}^+\) and \(\text{K}^+\) levels and a reduction in the osmolality of urine suggesting that it is mainly acting as a loop diuretic. It may also inhibit ADH release and/or action. It is concluded that the *Spilanthes acmella* CWE has strong diuretic action as is claimed.

© 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Spilanthes acmella*; Diuretic; Loop diuretic; Urine output

1. Introduction

*Spilanthes acmella* Murr. (Family: Compositae). Acmella in Sinhala and Akkarakaran in Tamil, is an annual or short-lived perennial herb, 20–60 cm tall, with a prostrate or ascending branched cylindrical hairy stem and simple ovate opposite leaves without stipules. The flowers are yellow, non-fragrant with five petals on long glabrous peduncles. This herb is found in India, Sri Lanka and other tropical countries (Jayaweera, 1981). In Sri Lanka, it is common in moist places up to 1800 m altitude (Jayaweera, 1981).

Sri Lanka traditional physicians, especially, in the Uva province claim that the cold infusion of the flowers of *Spilanthes acmella* have potent diuretic activity and the ability to dissolve urinary calculi. The flowers are chewed or used in the form of a tincture for toothache and to stimulate flow of saliva (Jayaweera, 1981). It is also recommended for paralysis of the tongue and in stammer and sore mouth in children (Jayasinghe, 1994). Further, the flowers are used locally against itching and psoriasis (Jayaweera, 1981). Experimentally, it is shown to have larvicidal potential against *Culex quinquefasciatus* (Pitasawat et al., 1998). Phytochemically, flowers of *Spilanthes acmella* are reported to contain amino acids (Mondal et al., 1998; Pieris et al., 2001), alkaloids (Pieris et al., 2001) and N-isobutylamides (spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide and undeca-2E-en-8,10-dynoic acid isobutylamide) (Ramsewak et al., 1999).

Since the advocated diuretic potential of *Spilanthes acmella* flowers were not tested rigorously by scientifically controlled experiments, this study was undertaken to investigate the diuretic potential of a cold water extract (CWE) of *Spilanthes acmella* flowers using rats.

2. Materials and methods

2.1. Experimental animals

Healthy adult crossbred male albino rats (weighing 200–225 g) from our own colony were used. They were housed in standard environmental conditions (temperature: 28–30°C; photoperiod: approximately 12 h natural light per day and 12 h darkness).
day, relative humidity: 50–55%) with free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and water.

2.2. Collection of flowers

Yellow coloured fresh flowers were collected from mature Spilanthes acmella plants at the Ayurvedic medicinal garden, Haldumulla (Uva Province), Sri Lanka, in June 2002. The identification an authentication was done by Mr. S. B. Weerakoon, Department of Ayurveda, Colombo, Sri Lanka. A voucher specimen (Am01, 2002) is deposited at the museum of the Department of Zoology, University of Colombo, Sri Lanka.

2.3. Preparation of the cold water extract (CWE)

Fresh flowers (482 g) were homogenised in distilled water (400 ml) using a domestic blender (National Model MX-7, Matsushita Ltd., Tokyo, Japan) for 10 min and filtered through eight layers of muslin cloth. The resulting brown coloured filtrate was freeze-dried (17.5 g; yield: 3.6% w/w) and stored air tight at 4°C. The freeze-dried powder was dissolved in distilled water to obtain the required dosage concentration (in terms of fresh weight) in 1 ml solution (500, 1000, or 1500 mg/kg). The highest dose tested was 7.5 times higher than that is normally recommended by the traditional practitioners of the Uva province, which is within the accepted range for the rat model (Dhawan and Srimal, 2000).

2.4. Evaluation of the diuretic activity

Thirty rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally administered with 15 ml of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water load. Forty-five minutes later, these rats were randomly assigned into five groups (N = 6 per group) and treated orally in the following manner. Group 1: 1 ml of distilled water, group 2: 500 mg/kg of CWE, group 3: 1000 mg/kg of CWE, group 4: 1500 mg/kg of CWE and group 5: 13 mg/kg of furosemide (State Pharmaceutical Corporation, Colombo, Sri Lanka), the reference drug (Rang et al., 1995; Dharmasiri et al., 2003). Each of these rats was individually placed in metabolic cages and cumulative urine output was determined at hourly intervals for 5 h. The colour of urine was also noted.

In an attempt to ascertain the broad mechanisms of action, the urine collected from group 1 (control) and group 4 (1500 mg/kg of CWE) were subjected to the following investigations: pH (by pH meter, Toa Electronics Ltd., Tokyo, Japan), Na⁺ and K⁺ levels by flame photometry (compact atomic absorption spectrometer, GFS Scientific Equipment Pvt. Ltd., Sydney, Australia), osmolality (by Osmometer, Type TW2, Advanced Instrument Inc., Massachusetts, USA), specific gravity and, glucose and proteins (using Combistrix®, Reagent strips, Bayer Diagnostics Manufacturing Ltd., Bridgend, UK). Na⁺/K⁺ ratio was then computed.

2.5. Evaluation of acute and sub chronic toxicity

Twelve rats were randomly assigned into two equal groups (n = 6). The first group was orally treated daily for 7 days with the highest dose of CWE and the other with 1 ml of distilled water. During this period, each rat was observed for overt signs of toxicity (salivation, lachrymation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exophthalmia), behavioural abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changes) and aversive behaviour (biting and scratching behaviour, licking of tail, paw and penis, intense grooming behaviour and vocalization) and diarrhoea. On day 1 post treatment, these rats were anaesthetised with ether (BDH Chemical Co., Poole, UK). Blood was collected from tails using aseptic precautions, serum separated and, urea and creatinine (to examine renal toxicity), and GOT and GPT (to judge liver toxicity) levels determined using respective assay kits (Randox Laboratory Ltd., UK).

2.6. Statistical analysis

Data are represented as mean ± S.E.M. Statistical comparisons were made using Mann–Whitney, U-test. Significance was set at P ≤ 0.05.

3. Results

As shown in Table 1, the highest dose of the CWE significantly (P < 0.05) and profoundly increased the cumulative urine out put (by 426%). Further, the increase in urine output induced by the highest dose of CWE was evident from the first hour and lasted until the termination of the experiment: 1 h (by 523%), 2 h (by 526%) 3 h (by 451%) 4 h (by 388%) and 5 h (by 348%) (see Fig. 1). The peak effect was seen at the first and the second hour. The reference drug, furosemide significantly (P < 0.05) increased (by 405%) the urine out

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total urine output (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>10.2 ± 2.0</td>
</tr>
<tr>
<td>500 mg/kg of CWE</td>
<td>8.6 ± 1.7</td>
</tr>
<tr>
<td>1000 mg/kg of CWE</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>1500 mg/kg of CWE</td>
<td>53.4 ± 8.0*</td>
</tr>
<tr>
<td>13 mg/kg of furosemide</td>
<td>8.1 ± 0.8</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared with control (Mann–Whitney, U-test).
put only at the first hour. Further, the diuresis induced by the CWE at 1 h was almost similar to that of furosemide.

As shown in Table 2, the highest dose of CWE slightly but significantly ($P < 0.05$) reduced the urinary pH. It also provoked a massive and significant ($P < 0.05$) increase in urinary excretion of Na$^+$ (by 51%) and K$^+$ (by 213%). However, the Na$^+$/K$^+$ ratio remained unaltered. Accompanying the increase of urine output was a significant ($P < 0.05$) reduction (by 22%) in urinary osmolality. Additionally, the highest dose did not induce proteinuria or glucosuria. The colour of the urine of rats in treated groups appeared almost identical to that of the control group.

In the toxicity study, the CWE did not provoke any overt signs of toxicity, stress or aversive behaviour. There was also no sign of diarrhoea and none of the treated rats died. Moreover, of the serum parameters tested only serum urea significantly ($P < 0.05$) altered: (reduced by 54%) (urea: control versus treatment: 31.8 ± 6.6 versus 14.6 ± 1.6 mg/dl, creatinine: 0.7 ± 0.2 versus 0.6 ± 0.1 mg/dl, SGOT: 22.3 ± 5.7 versus 22.4 ± 4.7 mg/dl and SGPT: 14.5 ± 1.4 versus 11.8 ± 2.3 mg/dl).

4. Discussion

This study examined the diuretic potential of *Spilanthes acmella* flowers using a CWE. The results showed that the highest dose of CWE of flowers tested possesses strong diuretic activity when given orally in a single dose. A similar pattern of diuresis is reported with some other plant diuretics: for instance, leaves and stems of *Anisomeles indica* (Dharmasiri et al., 2003) and *Strechos potatorum* seeds (Biswas et al., 2001). The CWE was well tolerated with an encouraging safety profile even following subchronic administration (as judged by absence of mortality, overt signs of toxicity, stress, behavioural abnormalities and increased levels of serum GOT, GPT creatinine and urea). However, hexanic extract of *Spilanthes acmella* plant in rats is reported to induce full tonic-clonic convulsions accompanied by typical electrographic seizures in the EEG (Moreira et al., 1989). It is well recognized that hexane, extracts predominately nonpolar constituents whilst CWE isolates polar constituents. This may account for the discrepancy observed between these two studies. In addition, Moreira et al. (1989) have used the whole plant while we have used fresh flowers. Interestingly, the CWE also appears to be renoprotective (in terms of serum urea levels). If the results are applicable to humans, then this is an important and clinically useful finding both locally and globally as it provides scientific evidence in favour of its claimed diuretic potential by controlled experimentation; after all, 35% of Sri Lankan (Mahindapala, 2000) and 80% of the people in developing countries of the world (Farnsworth, 1999) rely on traditional medicine, and about 85% of traditional medicine involves the use of plant extracts (Farnsworth, 1999). Undoubtedly, this would widen the options of potential diuretic therapies available to traditional practitioners.

The CWE-induced diuresis was strong and was not accompanied with a reduction in urinary K$^+$ levels. Further, there was no alkalinisation of urine. Collectively, these observations suggest that the CWE is not acting as a potassium-sparing diuretics (Rang et al., 1995; BNF, 2000; Kreidly and Jehal, 2002). The CWE is also unlikely to be acting as thiazide diuretics: these only increase the urinary K$^+$ level and alter the urinary Na$^+$/K$^+$ ratio (Rang et al., 1995; BNF, 2000). But, in this study, both urinary Na$^+$ and K$^+$ levels were increased without any alteration in the Na$^+$/K$^+$ ratio.

On the other hand, the diuresis induced by the CWE of *Spilanthes acmella* flowers was strong with an intensity similar to that of furosemide and accompanied by marked increases in both urinary Na$^+$ and K$^+$ levels.
Further, the urine was slightly acidified. These features strongly suggest that the CWE is acting as a loop diuretic. Loop diuretics are the most powerful of all diuretics and these inhibit the Na\(^+\)/K\(^+\)/Cl\(^-\) co-transporter system in the thick ascending loop of the nephron, thereby increasing natriuresis and kaliuresis (Rang et al., 1995; BNF, 2000; Kreydiyyeh and Julnar, 2002). These diuretic also cause acidification of urine (Rang et al., 1995; Osorio and Teitelbaum, 1997; BNF, 2000). Further, the onset of the diuretic activity of the CWE was extremely rapid (within 1 h of administration) as observed with clinically used synthetic loop diuretics (Rang et al., 1995; BNF, 2000). Interestingly, in spite of the heavy loss of urinary Na\(^+\) and K\(^+\), there was a significant reduction in the osmolarity of urine in CWE treated rats. Thus, it is possible that the CWE, in addition, may impair the basal secretion of ADH and/or diminished the responsiveness of uriniferous tubules to the action of ADH: inhibition of ADH causes polyuria with low osmolarity (Mayne, 1994; Osorio and Teitelbaum, 1997). Physicochemically, Spatholobus acmella flowers are shown to contain N-isobutylamides (Ramsewak et al., 1999), alkaloids, (Peiris et al., 2001) and amino acids (Mondal et al., 1998; Peiris et al., 2001). Amino acids are resorbed in the proximal convoluted tubules of nephrons (Rang et al., 1995) and cannot function as diuretics. Thus, the diuretic activity of the CWE may be attributed to its alkaloids.

Loop diuretics are clinically used in patients with salt and water overload due to host conditions such as pulmonary oedema, heart failure asciates, hypertension (Rang et al., 1995; BNF, 2000). Loop diuretic mode of action of the CWE of the Spatholobus acmella flowers indicate that Spatholobus acmella flowers may be useful as a non toxic natural therapeutic agent in the treatment of such conditions by traditional practitioners. The onset of the diuretic action of the CWE was extremely rapid and it also had a fairly long duration of action. This is an appealing diuretic profile as it would curtail the frequency of administration. However, there is one major limitation: an increased risk of hypokalaemia as with other therapeutically used loop diuretics.

In conclusion, this study provides first scientific evidence in favour of claimed diuretic potential of CWE of Spatholobus acmella flowers. It further shows that the CWE of Spatholobus acmella flowers mainly acts as a loop diuretic in inducing diuresis.

References