Diuretic activity of hot flower infusion of nyctanthes arbo-tristis in rats

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Universidad de Santiago de Chile
Santiago, Chile

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ARTICULOS
DIURETIC ACTIVITY OF HOT FLOWER INFUSION OF NYCTANTHES ARBO-TRISTIS IN RATS

W. D. RATNASOORIYA 2
AND
J. R. A. C. JAYAKODY 3

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Summary: The objective of this study was to evaluate the diuretic potential of Nyctanthes arbo-tristis Linn. (Oleaceae) flowers using a hot water infusion in rats. Different concentrations of the infusion (3.7, 7.5, 12.5, or 18.7 mg/kg), or vehicle, or furosemide (the reference drug at 13 mg/kg), was orally administered to hydrated rats. Their cumulative urine output was monitored at hourly intervals for 6 hours. The flower infusion induced a significant (P<0.05) diuresis in a dose-related manner. The onset of this diuretic action was extremely rapid (within 1 hr) and lasted throughout the study period. Further, the flower infusion caused a significant impairment (P<0.05) of the urinary Na+ and K+ levels and caused slight but significant (P<0.05) alkalinisation. However, it had no significant (P>0.05) effect on urine specific gravity or Na+/K+ ratio of urine. Collectively, these data suggest that the infusion induces diuresis by basically acting as a potassium sparing diuretic. It is concluded that these flowers have a promising potential as a safe, effective and cheap diuretic.

Key words: Nyctanthes arbo-tristis, diuresis, potassium sparing diuretic, urine output

INTRODUCTION

Nyctanthes arbo-tristis Linn. (Family Oleaceae), night flowering jasmine in English, Sepalika in Sinhala, is a small tree which is introduced to Sri Lanka (1), but which is now commonly found in Buddhist temples and home gardens almost throughout the country. The tree flowers at night all year round and the flowers start to fall in large numbers from mid night on. These flowers can be collected, if required, with no payment.

In the traditional and folk medicine of Sri Lanka the flowers are claimed to have a diuretic action (2), which has not been so far tested by scientifically controlled investigations. Phytochemically, N. arbo-tristis flowers are reported to contain appreciable amounts of flavonoids (Ratnasooriya et al, unpublished data) and an essential oil similar to that of jasmine (3). The presence of these classes of compounds suggested that this flower may have diuretic potential (4). Further, it is now known that many of the medicinal claims for plants are indeed valid (5). This prompted us to investigate the diuretic potential of N. arbo-tristis flowers. If the claimed diuretic potential is established, then it could be used as an effective and cheap herbal diuretic in Sri Lanka, as in Sri Lanka, about 35% of the population are mainly dependent on Ayurvedic and traditional systems for their health care (6).

MATERIALS AND METHODS

Animals: Healthy adult cross bred male albino rats (weighing 200-250 g) from our colony were used. They were housed in standard environmental conditions (temperature: 28-31°C; photoperiod: approximately 12 hrs natural light per day; relative humidity: 50-55%) with free access to pelleted food (Master Feed Ltd, Colombo, Sri Lanka) and water.

Collection of flowers: Fresh flowers from a mature N. arbo-tristis tree which were fallen on the ground, were collected in the early morning (5.00-6.00 h) from a home garden at Hunupitiya, Colombo 02, Sri Lanka, between September and December 2002. The identification and authentication was done by Prof. R.N. De Fonseka, Department of Botany,
Preparation of infusion: The fresh flowers were oven dried (60°C) for 24 h and powdered. 4.5 g of this material was soaked in 24 mL boiling distilled water (DW) for 30 minutes. The dark brown infusion (I) was then filtered through a muslin cloth (yield: 25.7% w/v), and used directly for experimental work at doses of 3.7, 7.5, 12.5 or 18.7 mg/kg in 1 mL. These doses are identical to what we have used previously in the evaluation of sedative potential of the flower infusion (Ratnasooriya et al, unpublished data, submitted to Pharmaceutical Biol.).

Evaluation of diuretic activity: The evaluation was done as described previously (7). 43 rats were deprived of water but not food for 18 hrs. Their bladders were emptied by gentle compression of the pelvic area and by pulling their tails. Each rat was then orally administered with 15 mL of isotonic saline (NaCl 0.9% w/v) to impose a uniform liquid load. The rats were assigned to 6 groups and treated orally in the following manner: 1 (n=8) 1 mL of DW, 2 (n=8) 3.7 mg/kg of I, 3 (n=8) 7.5 mg/kg of I, 4 (n=8) 12.5 mg/kg of I, 5 (n=5) 18.7 mg/kg of I and 6 (n=6) 13 mg/kg of furosemide, the reference drug. Each of the rats was individually placed in a metabolic cage and the cumulative urine output determined at hourly intervals, for 6 hrs. The colour of urine was noted. The pH of urine was determined using a pHmeter (Toa Electronics Ltd., Tokyo, Japan). The specific gravity, presence or absence of glucose and proteins were determined using Combistix® reagent strips (Bayer Diagnostics Manufacturing Ltd., Bridgenl, UK). Urinary Na+ and K+ levels were determined flame photometrically (Compact Atomic Absorption Spectrometer, GFC Scientific Equipment Pvt. Ltd., Sydney, Australia).

Statistical Analysis: Data are given as mean ±SEM. Statistical comparisons were made using Mann-Whitney U-test. Significance was set at P ≤ 0.05.

RESULTS

As shown in Table 1, 3.7 (by 61%), 7.5 (by 57%) and 12.5 (by 66%) mg/kg doses of I significantly (P<0.05) and markedly increased the cumulative urine output. Furosemide, the reference diuretic, also increased the total urine output significantly (P<0.05) (by 46%). The highest dose of I failed to show significant (P>0.05) diuretic effects. The diuretic effects of the infusion exhibited a curvilinear dose-response relationship (r²=0.91, P<0.05). EC50 value for the diuretic effect was 1.14 mg/kg. The onset of the I-induced diuresis (Figure 1) was extremely rapid (in the first hour). The peak diuresis was also evident in the first hour, as with furosemide. However, at the peak diuresis furosemide increased diuresis by 262%, while the 12.5 mg/kg dose of the I increased diuresis by 441%. The diuretic effects of the I persisted up to the termination of the experiment.

Table 1: Cumulative urine output in rats over 5 hour period following oral administration of infusion of Nyctanthes arbo-tristis flowers. (mean ± SEM).

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Total urine output (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ±0.45</td>
</tr>
<tr>
<td>3.7</td>
<td>9.0 ±1.4</td>
</tr>
<tr>
<td>7.5</td>
<td>8.8 ±1.1</td>
</tr>
<tr>
<td>12.5</td>
<td>9.3 ±1.3</td>
</tr>
<tr>
<td>18.7</td>
<td>6.6 ±0.8</td>
</tr>
<tr>
<td>Furosemide</td>
<td>8.2 ±0.9</td>
</tr>
</tbody>
</table>

* P<0.05 as compared with control (Mann-Whitney, U-test)
As shown in Table 2, 12.5 mg/kg dose of I significantly (P<0.05) decreased the urinary output of both Na\textsuperscript{+} (by 64\%) and K\textsuperscript{+} (by 60\%). However, Na\textsuperscript{+}/K\textsuperscript{+} ratio remained unaltered. The pH of the urine was slightly (by 17\%) but significantly (P<0.05) increased by I, but not its specific gravity. Further, the colour of the urine of the treated rats appeared to be similar to that of control and had no glucose or proteins at detectable level in it.

**DISCUSSION**

The results show, for the first time that, *N. arbo-tristis* flowers can function as an orally active moderately strong diuretic agent. The onset of the diuretic action was extremely rapid, almost similar to that of strong loop diuretics (within 1h), indicating quick absorption from the gastrointestinal tract. Interestingly, the peak activity was also evident at the first hour and the diuretic action lasted throughout the study period (6h), suggesting a slow clearance. The diuretic action of *N. arbo-tristis* showed a curvilinear dose-response relationship. The existence of a dose-related activity suggest that the observed effect was intrinsic and causal, and may not have been result from a non-specific action, such as high salt content of the flower, as reported for some Thai indigenous medicinal plants (8). *N. arbo-tristis* flowers have extremely low concentrations of electrolytes (3). Some herbal drugs induce diuresis by stimulating thirst and thereby enhancing fluid intake (9). Such a mode of action is unlikely here as rats had no access to water after the administration of I. The *N. arbo-tristis* flower-induced urinary output was not accompanied by a corresponding increase in Na\textsuperscript{+}, K\textsuperscript{+}, Na\textsuperscript{+}/K\textsuperscript{+} ratio or the specific gravity. Collectively, these observations suggest that the I is not acting as an osmotic diuretic (10). Osmotic diuretics are usually given intravenously and are pharmacologically inert (11,12). In contrast, this flower is orally active and contains flavonoids, an essential oil, anthocyanines and modified diterpenoids, which have other biological effects (3). These evidences provide additional support in favour of the previous notion. ADH plays a vital role in the regulation of urinary output (11,12). A possibility exists that the flower I may stimulate diuresis by inhibiting ADH release, or its action on the uriniferous tubules. However, such a mechanism seems unlikely to be operative, as there was no change in the specific gravity of urine. Inhibition of ADH action causes polyurea with low osmolality (12). *N. arbo-tristis* flower I could produce diuresis by stimulating the release of endogenous natriuretic peptides, which promote sodium and water secretion (11,12). Such a mode of action is also unlikely, as there was no increase in urinary Na\textsuperscript{+} level although the urinary volume was raised. The flower I did not promote an increase in natriuresis and kaleuresis with its diuretic action. Therefore the flower is not acting as a loop diuretic (11,12). Loop diuretics are the most powerful of all diuretics and they inhibit the Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} co-transporter system of the ascending limb of Henle’s loop (11,12). The flower I is not acting as thiazides and related diuretics, as these act by inhibiting the Na\textsuperscript{+}/Cl\textsuperscript{−} co-transporter in the distal convoluted tubule and increase urinary Na\textsuperscript{+} and K\textsuperscript{−} loss (11,12). However, in this study there was profound reduction in urinary Na\textsuperscript{+} and K\textsuperscript{−} levels.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONTROL GROUP</th>
<th>TREATED GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.06 ± 0.31</td>
<td>7.18 ± 0.2</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.013 ± 0.0</td>
<td>1.011 ± 0.0</td>
</tr>
<tr>
<td>Na\textsuperscript{+} (ppm)</td>
<td>10336.3 ± 1539.7</td>
<td>3697.5 ± 258.7</td>
</tr>
<tr>
<td>K\textsuperscript{+} (ppm)</td>
<td>4490.6 ± 844.6</td>
<td>1737.7 ± 232.2</td>
</tr>
<tr>
<td>Na\textsuperscript{+}/K\textsuperscript{+} ratio</td>
<td>2.5 ± 0.3</td>
<td>2.33 ± 0.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

Table 2: Effects of orally administrated infusion of *Nyctanthes arbo-tristis* flowers, 12.5 mg/kg on some urine parameters (up to 5h) of rats (mean±SEM). P<0.05 compared to control (Mann Whitney, U-test, T-test).

On the other hand, the reduction of urinary K\textsuperscript{+} level accompanied with an increased urinary output strongly suggest that the flower I is acting as a potassium-sparing diuretic. Clinically used potassium-sparing diuretics on their own are weak diuretics and act in the collecting tubules by blocking the Na\textsuperscript{+} channels (11,12). They also impair Na\textsuperscript{+}/H\textsuperscript{+} exchange mechanisms, resulting in slight alkalization of urine (12). Although an alkalization of urine was evident in this study, supporting a potassium-sparing diuretic mechanism of the flower I, there was no increase in Na\textsuperscript{+} excretion. All the therapeutically used diuretics are known to increase Na\textsuperscript{+} excretion (11,12). In this aspect it is noteworthy that recently we showed another plant claimed as a diuretic in traditional medicine in Sri Lanka, *Anisomeles indica* (Limiaceae), has a powerful diuretic action which was mediated by an identical mechanism (13). This K\textsuperscript{−} retention ability of *N. arbo-tristis* flowers is an important feature as it indicates the potential of this extract as an antihypertensive, for a negative correlation exists between total body potassium and blood pressure in hypertensive patients (14). *N.
arbo-tristis flowers are shown to have an encouraging safety profile even with sub-chronic administration (in terms of overt clinical signs of toxicity, serum GOT, GPT, alkaline phosphatase, creatinine, urea, total protein levels). These flowers have no effects on blood tryglycerides and glucose levels or on male libido and fertility (Ratnasooriya et al., unpublished data). On the other hand, an elevated tryglyceride concentration in blood, hyperglycemia, and impotence are common side effects of several clinically used diuretics (11).

In conclusion, this study provides the first scientific evidence in support of the claimed diuretic potential of N. arbo-tristis flowers. It also shows that the diuresis is mediated via a potassium-sparing mechanism.

REFERENCES


FRASES

No te afanes en las cosas que el mundo se afana. Afánate en las cosas en que el mundo no se afana. Confucio

Descifrar los signos del mundo quiere decir siempre luchar contra cierta inocencia de los objetos. Roland Bartles

La fortuna vende muy caro lo que parece regalar. Vincent Voiture

Para obrar, el que da debe olvidar pronto, y el que recibe, nunca. Séneca

Jamás en la vida encontraréis ternura mejor, más profunda, más desinteresada ni verdadera que la de vuestra madre. Honoré de Balzac

Nunca prediques porque tienes que decir algo, sino porque tienes algo que decir. Richard Whately

Desde el lugar más reducido del mundo, cualquier hombre puede contemplar la inmensa grandeza del firmamento. Vicente Risco

Así como el hierro se oxida por falta de uso, también la inactividad destruye el intelecto. Leonardo da Vinci

El hombre honrado es el que mide su derecho por su deber. Henri Lacordaire

Un padre sirve para dejar que te equivoques y ayudarte después. Daniel Múgica