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Effect of Extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) Spreng. in Rats

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Aqueous extracts of *Sambucus nigra* and *Arctostaphylos uva-ursi* and hydroalcohol extracts of *Orthosiphon stamineus* and *Hieracium pilosella* were tested for their diuretic activities in rats; pharmacological evaluation revealed that they led to an increase in urine flow. Urinary sodium excretion in rats was increased with *O. stamineus* and *S. nigra*. © **1998 John Wiley & Sons, Ltd.**

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Keywords: Orthosiphon stamineus; *Hieracium pilosella*; *Sambucus nigra*; *Arctostaphylos uva-ursi*; diuretic activity.

INTRODUCTION

Orthosiphon stamineus (Os) (Lamiaceae) aerial parts, *Hieracium pilosella* (Hp) (Asteraceae) aerial parts, *Sambucus nigra* (Sn) (Caprifoliaceae) flowers and *Arctostaphylos uva-ursi* (Au) (Ericaceae) leaves are traditionally used as herbal remedies for their diuretic properties (Fournier, 1947–1948; Bezanger-Beauquesne *et al.*, 1990), and as phytomedicine according to the French regulations (Bulletin Officiel, 1990). These drugs contain di and triterpene glycosides, and many phenolic compounds such as flavonoids, tannins and coumarins (Bruneton, 1993).

The purpose of this report is the pharmacological evaluation of Os, Hp, Sn and Au as a diuretic in order to validate their popular use in traditional preparations. This study followed the procedure developed by Petkof and Markovska (1981), modified later by Beaux (1991). Diuresis was first evaluated in rats which had received a hypotonic saline overload and extracts or hydrochlorothiazide (used as a reference product); urinary volume and electrolytes were calculated.

MATERIALS AND METHODS

Plant material. Flowers of Sn and Au were supplied by Phyto Est (France). The samples were identified by P. Valk (director of the Botanical Garden of Nancy, France).

Extract preparation and route of administration. Dry powdered drug of Sn was extracted with boiling water and then macerated for 24 h at room temperature. These extracts were then filtered and finally freeze-dried. Commercial aqueous extracts of leaves of Au were provided by Laboratoire Extra Norm (Expansion Aromatique Française, France). Commercial hydroalcohol extracts of Os and Hp were supplied by Laboratoire Vernin (Melun France). Yields were respectively: Os, 20%; Hp, 6.6%; Sn, 18.6%; Au, 16.6%. The extracts were administered intraperitoneally in a hypotonic saline solution (0.45%). The doses indicated refer to the initial weight of dry drugs.

Animals. Sprague Dawley male rats (Iffa Credo, l'Arbresle, France) weighing 280–380 g were used. All animals were housed in individual metabolic boxes for 3 days before experiment. They were fed laboratory diet (croquettes Extralabo, Provins, France) *ad libitum* and allowed free access to drinking water before experiments; animals were starved 24 h before and during the experiment. They were kept in a 12/12 h light/dark cycle at 20° ± 1°C.

Chemicals. Hydrochlorothiazide (HCT) was used (Esidrex, Ciba-Geigy, France).

Experimental. Five groups of animals were treated; extracts were dissolved in hypotonic saline solution (NaCl 0.45%, 5mL/100 g i.p). A single dose (50 mg/kg of animal body weight) was administered for Os, Sn and Au

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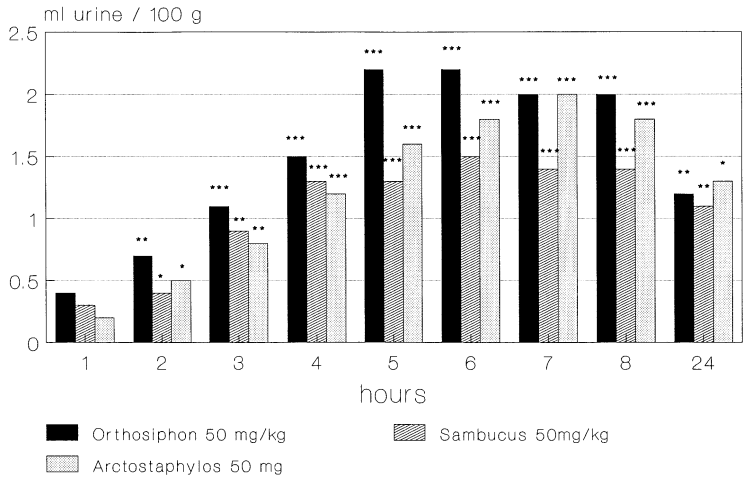

Figure 1. Influence of Os (*Orthosiphon*), Sn (*Sambucus*) and Au (*Arctostaphylos*) extracts on urinary secretion, compared with control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

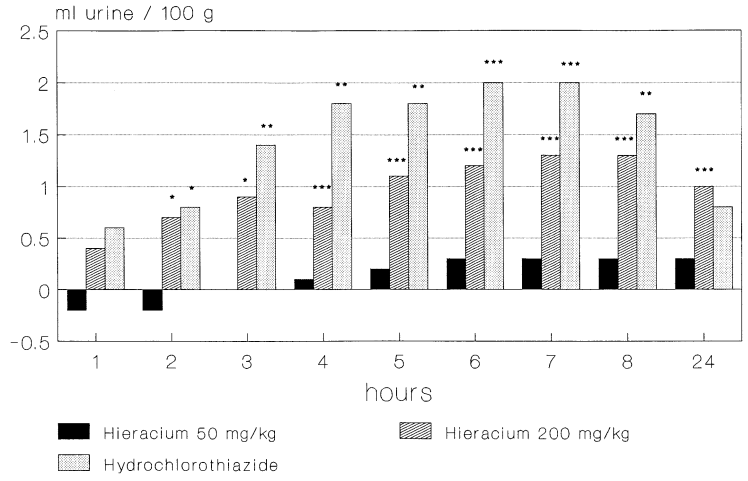

Figure 2. Influence of Hp (*Hieracium*) extracts and hydrochlorothiazide on urinary secretion, compared with control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

and two doses for Hp (50 and 200 mg/kg). Another group received the diuretic compound: hydrochlorothiazide (10 mg/kg). Five control groups, corresponding to each extract and hydrochlorothiazide, received hypotonic saline solution.

Immediately after treatment, each animal was housed in its metabolic box; urine was collected in graduated vials, and the volume was measured every hour for 8 h and at 24 h. Electrolytes (Na^+ and K^+) were determined after 8 h and 24 h in the urine samples by flame photometer (Delhomme, II 243-05, F.); pH was evaluated by a pH meter (pH Meter Schott Gerate Ger.)

Statistical analysis. After confirming the variance homogeneity (Bartlett's test), F_{max} was used to control the significance of difference; means were compared using Student's *t*-test.

RESULTS AND DISCUSSION

The results presented in Figs 1 and 2 and Tables 1 and 2 indicate that extracts of Os, Sn and Au administered to rats i.p. at 50 mg/kg caused a significant diuresis from 2–24 h compared with the control. A dose of 200 mg/kg of Hp was necessary to induce a significant diuresis from 2–24h, at 50 mg/kg the Hp extract was ineffective. A sizeable rise in Na^+ and K^+ excretion with respect to the control was also noticed with Sn at 8 h and 24 h and with K^+ only with Os at 8 h (Table 2).

After hydrochlorothiazide (10 mg/kg) was administered, a noticeable increase of the urinary excretion was obtained from 2–8 h and also an increase of Na^+ and K^+ urinary excretion was recorded after 8 h.

The pH remained unchanged (pH 8.4–8.8) whatever the extract of plants or hydrochlorothiazide.

Table 1. influence of Os, Hp, Sn and Au and hydrochlorothiazide on urine secretion in rats and their related control

		Time (h)								
Dose (mg/kg)	*n*	1	2	3	4	5	6	7	8	24
Control	14	1.3 (0.2)	1.5 (0.2)	1.9 (0.2)	2.5 (0.2)	3.0 (0.2)	3.4 (0.2)	3.8 (0.2)	3.9 (0.2)	5.7 (0.3)
Os (50 mg)	13	1.7 (0.2)	2.2[b] (0.2)	3.0[c] (0.2)	4.0[c] (0.3)	5.2[c] (0.3)	5.6[c] (0.4)	5.8[c] (0.4)	5.9[c] (0.4)	6.9[b] (0.3)
Control	28	1.5 (0.1)	1.8 (0.1)	2.1 (0.2)	2.7 (0.2)	3.3 (0.2)	3.7 (0.2)	4.0 (0.2)	4.1 (0.2)	6.0 (0.2)
Hp (50 mg)	14	1.3 (0.1)	1.6 (0.2)	2.1 (0.2)	2.8 (0.2)	3.4 (0.2)	3.9 (0.2)	4.3 (0.2)	4.4 (0.2)	6.3 (0.4)
Hp (200 mg)	14	1.9 (0.1)	2.5[a] (0.1)	3.0[a] (0.1)	3.5[c] (0.2)	4.4[c] (0.2)	4.9[c] (0.2)	5.3[c] (0.2)	5.4[c] (0.2)	7.0[c] (0.2)
Control	15	1.6 (0.1)	2.0 (0.1)	2.2 (0.2)	2.8 (0.2)	3.7 (0.2)	4.0 (0.2)	4.4 (0.2)	4.6 (0.2)	6.4 (0.3)
Sn (50 mg)	9	1.9 (0.2)	2.4[a] (0.1)	3.1[b] (0.3)	4.1[c] (0.3)	5.0[c] (0.3)	5.5[c] (0.3)	5.8[c] (0.3)	6.0[c] (0.3)	7.5[b] (0.3)
Control	8	1.4 (0.1)	1.6 (0.1)	1.8 (0.2)	2.4 (0.2)	2.9 (0.2)	3.4 (0.2)	3.6 (0.2)	3.9 (0.2)	5.8 (0.4)
Au (50 mg)	10	1.6 (0.1)	2.1[a] (0.2)	2.6[b] (0.2)	3.6[c] (0.2)	4.5[c] (0.3)	5.2[c] (0.4)	5.6[c] (0.4)	5.7[c] (0.3)	7.1[a] (0.3)
Control	10	1.5 (0.2)	2.0 (0.2)	2.1 (0.2)	2.5 (0.2)	3.0 (0.2)	3.2 (0.2)	3.5 (0.2)	3.9 (0.2)	6.3 (0.4)
Hydrochlorothiazide (10 mg)	10	2.1 (0.2)	2.8[a] (0.3)	3.5[b] (0.3)	4.3[b] (0.4)	4.8[b] (0.5)	5.2[c] (0.4)	5.5[c] (0.4)	5.6[b] (0.4)	7.1 (0.4)

Values are mean ± SEM; [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$. *n* = number of rats.

Table 2. influence of Os, Hp,Sn and Au and hydrochlorothiazide on sodium and potassium excretion (mmol/100 g) in pooled urines (at 8 and 24 h) of rats

		Time (h)			
		8	24		
Dose (mg/kg)	*n*	Na^+ 8	K^+ 8	Na^+ 24	K^+ 24
Control	14	0.186 (0.014)	0.174 (0.010)	0.337 (0.024)	0.350 (0.028)
Os (50 mg/kg)	13	0.258 (0.033)	0.244[a] (0.026)	0.384 (0.032)	0.361 (0.029)
Control	28	0.156 (0.012)	0.155 (0.010)	0.296 (0.019)	0.348 (0.018)
Hp (50 mg/kg)	12	0.190 (0.034)	0.204 (0.019)	0.349 (0.044)	0.369 (0.023)
Hp (200 mg/kg)	14	0.159 (0.015)	0.201 (0.014)	0.300 (0.026)	0.363 (0.034)
Control	15	0.178 (0.025)	0.171 (0.017)	0.244 (0.025)	0.235 (0.014)
Sn (50 mg/kg)	9	0.280[a] (0.034)	0.260[b] (0.026)	0.354[a] (0.035)	0.314[a] (0.028)
Control	15	0.199 (0.029)	0.234 (0.037)	0.331 (0.023)	0.382 (0.031)
Au (50 mg/kg)	14	0.238 (0.020)	0.217 (0.018)	0.340 (0.023)	0.345 (0.020)
Control	10	0.208 (0.033)	0.192 (0.015)	0.444 (0.050)	0.430 (0.028)
Hydrochlorothiazide (10 mg/kg)	10	0.416[c] (0.036)	0.293[c] (0.018)	0.493 (0.045)	0.480 (0.035)

Values are mean ± SEM mmol/100 g. *n* = number of rats. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

In this experiment, 100% of the hypotonic saline solution overload was eliminated within 4.75 h, 5.0 h, 5.75 h, 14 h and 6.0 h in the case of extracts of Os, Sn, Au, Hp (50 mg/kg) and Hp (200 mg/kg) respectively, and within 5.33 h with hydrochlorothiazide (Fig. 3).

These experiments clearly indicate that aqueous extracts of Sn and Au and hydroalcohol extracts of Os (at 50 mg/kg) can be compared to the diuretic effect of the reference product hydrochlorothiazide. The hydroalcohol extract of Hp required a higher dose of plant

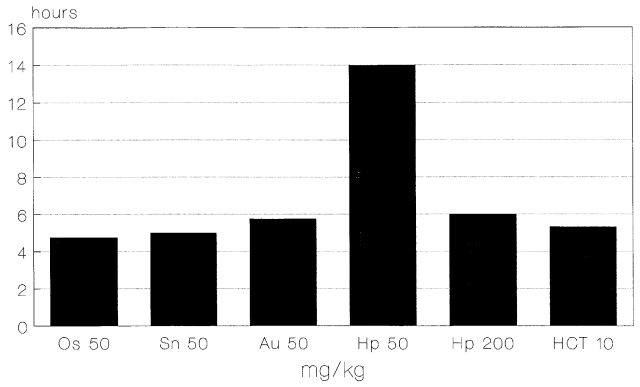

Figure 3. Influence of Os, Hp, Sn and Au extracts and hydrochlorothiazide on time (hours) necessary to eliminate the hypotonic saline overload.

(200 mg/kg) to be effective. Another experiment demonstrated that the aqueous extract of Os induced diuretic effects in rats (Burtin, 1983).

Only Sn and hydrochlorothiazide exerted a natriuretic effect. It is interesting to note that the doses of dried plants for infusion recommended for humans by Fournier (1947–1948) are 35 – 175 mg/kg for Sn, 70 mg/kg for Au and 35 mg/kg for Hp.

The diuresis observed with these extracts cannot be attributed to the K^+ concentration in the extracts as a similar K^+ concentration in an aqueous solution of KCl (1.05 mmol/L) did not increase diuresis (Beaux, 1991).

Similar diuretic effects were obtained previously with 50, 100 and 200 mg/kg of hydroalcohol extracts of *Foeniculum vulgare* var *dulce* roots (Beaux *et al.*, 1997). These experiments justify the use of *Orthosiphon stamineus* (aerial parts), *Sambucus nigra* (flowers), *Hieratium pilosella* (aerial parts) and *Arctostaphylos uva-ursi* (leaves) as diuretic agents in traditional medicine and also as a phytomedicine according to the French regulations (Bulletin Officiel, 1990).

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