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Journal of Ethnopharmacology 100 (2005) 205–209
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Diuretic activity of *Artemisia thuscula*, an endemic canary species

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Received 3 November 2004; received in revised form 2 March 2005; accepted 4 March 2005
Available online 28 April 2005

Abstract

A pharmacological evaluation for diuretic activity of infusions at 5, 10 and 15% of *Artemisia thuscula* Cav. was carried out. Urinary excretion of water, pH, density, conductivity and Na^+, K^+ and Cl^- content were investigated in saline-loaded rats. The infusions showed a dose-dependent decrease diuretic effect, but augmented significantly with respect to the control group for the urinary excretion of water and sodium. Furthermore, a potassium-sparing effect at 5 and 10% was showed. The diuretic effect does not seem to be related to the potassium content of the starting material. The results justify the use of *Artemisia thuscula* as diuretic agent by the canary traditional medicine.

Keywords: *Artemisia thuscula*; Diuretic activity; Medicinal plant; Infusion; Rats; Folklore medicine

1. Introduction

Artemisia thuscula Cav. (Asteraceae), a synonym of *Artemisia canariensis* (Bess.) Lessing, is an endemic Canary Islands species, widespread in all the islands in which different species of nitrophilous vegetation are dominant. It is most often found in coastal and mid zones of Western Canary Islands (100–350 m). The plant has the characteristics of a ramified shrub, 1 m in height with grey–silver branches and yellow flowers. It gives off a strong aroma that in the local language it is called “incienso” or “insensio” or also “mol” or “ajenjo” (Pérez de Paz and Medina, 1988; Bramwell and Bramwell, 1990; Kunkel, 1992).

This species has been used by the Canary folklore medicine as panacea for a great diversity of health problems. The part used as medicine is all the aerial part and habitually it is employed as an infusion or boiled (Darias et al., 1986; Pérez de Paz and Hernández Padrón, 1999; Darias et al., 2001).

Traditionally, this plant has been used as diuretic, hypoglycaemic, antidiarrhoeic, uricosuric, spasmolytic stomachic, carminative, vermifuge, tranquillizer, pectoral, and anticatarrhal, and is still used today (Bethencourt, 1985; Darias et al., 1986; Pérez de Paz and Hernández Padrón, 1999). Furthermore, it is used as a mosquito repellent and for other harmful insects or plagues for people or harvest crops (lice, fleas, bedbugs, and scarabs), by means of aromatic smoke (Jaén, 1984; Jaén, 1989).

Many *Artemisia* species of the world have been the subject of numerous chemical and biological studies due to their interesting properties (Tan et al., 1998). These species give sesquiterpene lactones, coumarins and acetylenes as the main metabolites (Marco and Barbera, 1990).

Partial studies have been carried out on the chemical composition of *Artemisia thuscula*, having isolated eudesmanolides, a type of sesquiterpene lactone (González et al., 1983; Breton et al., 1985; Mansilla and Palenzuela, 1999), and essential oil (Bellomaria et al., 1993).

However, no studies on biological activities have been carried out with *Artemisia thuscula*, in order to confirm its assumed beneficial properties. Therefore, the present study was undertaken to verify the efficacy of the infusion of the *Artemisia thuscula* as diuretic drug in experimental rats. So, we assayed three doses of *Artemisia* infusions, measuring urinary volume and electrolytes (Na^+, K^+ and Cl^-), pH, density

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0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.03.005

and conductivity on the urine samples. Furthermore, electrolytes and pH were measured on infusion samples in order to know if the potassium salt contents of the sample could play a certain role in the potential diuretic effect of this species (Loew et al., 1991).

2. Materials and methods

2.1. Plant material

Artemisia thuscula was harvested from the La Laguna Coast in a place known as Punta del Hidalgo in Tenerife, Canary Islands (Spain) at 50 m, in March 2003. Exp. N. UTM: E 371098-N3161213. Voucher specimen was deposited in the La Laguna University Herbarium (TFC 44301).

2.2. Extracts preparation

Artemisia thuscula fresh aerial part was carefully air-dried in an oven at a temperature of 40 °C for four days, and then the dry plant was cut and ground to powder mechanically in a mill. Then, three infusions at 5, 10 and 15% from the dried powdered plant material were prepared by mean traditional method applied in Canaries. So, 5, 10 and 15 g of pulverized plant material were placed in 100 ml of distilled boiling water and left at room temperature for 15 min to infuse and filter. 5 ml/kg (body weight) of each filter was then given orally to the rats (equivalent doses at 0.25, 0.50 and 0.75 g/kg with respect to the weight of starting dry material). These infusions were freshly prepared just before administration.

2.3. Animals

Male albino Sprague–Dawley rats (180–210 g) and male and female albino Swiss mice (20–24 g), obtained from the Central Animal House, University of La Laguna, were used for the experiments, according with the guidelines of the European Community Council Directive 86/609.

2.4. Drugs

Hydrochlorothiazide (HCTZ) from Sigma was used as reference diuretic drug.

2.5. Acute toxicity test

Groups of 10 mice, five male and five female, weighing 20–24 g were used. The animals had free access to standard commercial diet and water ad libitum in a 12 h/12 h light–dark cycle at 22 °C. Infusion of *Artemisia thuscula* was given at a dose of 2.5 g/kg body weight (0.4 ml/20 g body weight) and administered orally by means of a gastric catheter. Food was withdrawn 16 h before the day of the experiment. Toxicity was evaluated over 15 d in terms of weight loss, autonomic and neurobehavioral profile. On the fifteenth day, animals were killed by cervical dislocation and vital organs were observed.

2.6. Diuretic activity

The method of Kau et al. (1984), with modification was employed in the determination of diuretic activity. In short, male rats were randomly assigned five groups of eight each, and were conditioned in standard cages. They were fed laboratory diet ad libitum and allowed free access to drinking water and kept in 12 h/12 h light–dark cycle at 22 °C. Eighteen hours before testing, the animals were fasted overnight, with free access to tap water only. Then, all animals were given an oral loading of normal saline (5% *bw*). Subsequently, three groups of rats were orally administered 5 ml/kg *bw* of the infusions of *Artemisia thuscula*, at concentrations 5, 10 and 15%, respectively, and two other groups were administered 5 ml/kg *bw* p.o. of HCTZ at doses 10 and 25 mg/kg of weight, respectively. Control rats received the same amount of deionised water (5 ml/kg *bw*). Immediately after administration, the rats were paired and placed in metabolism cages. Urine was recollected in a graduated cylinder and its volume was recorded at 2-h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g *bw*. Electrolytes (Na^+, K^+, Cl^-) concentrations, pH, density and conductivity were estimated from pooled urine sample of each pair of rat at the end of the experiment, 8 h after administration, and expressed as mEq/100 g *bw*.

Controls were carried out on the same animals, seven days before infusions or HCTZ were administered.

2.7. Analytical procedures

Na^+ and K^+concentrations were measured by Jenway PFP7 flame photometer. The instrument was calibrated with standard solution containing different concentrations of Na^+ and K^+. Cl^- concentrations were determined by direct potentiometry, using clorure electrode ion-selective (Orion 9417B) and an Ag/AgCl reference electrode with double junction (Orion 90-02). The potentials were measured with an Ionalyzer Orion 901. KNO_3 (2 M) was used as ISA in all the determinations. pH and conductivity were directly determined on fresh urine samples using a HI-8424 Hanna Instruments pH-meter and a LF-320 WTF conductivity meter, respectively. Density estimation was made weighing in a Mettler AE163 (±0.1 mg) analytical balance on urine volume measured with a Nichiryo micropipette.

2.8. Statistical analyses

Results are expressed as the mean values ±S.E.M. (standard error of mean) of four pairs of rats. The statistical evaluation was carried out by means of analysis of variance. The difference between the means of treatment groups and the control group (values taken in the rats, one week prior to

Table 1
Effects of oral administration of *Artemisia thuscula* infusions on urinary volume excretion

Group	*n*	*V* (ml/100 g/8 h)	Diuretic index
Control	16	4.43 ± 0.36	-
HCTZ (10 mg/kg)	4	6.14 ± 0.45*	1.39
HCTZ (25 mg/kg)	4	6.07 ± 0.15*	1.37
Artemisia thuscula (5%)	4	5.56 ± 0.27*	1.25
Artemisia thuscula (10%)	4	5.42 ± 1.79**	1.22
Artemisia thuscula (15%)	4	4.89 ± 0.36	1.10

The results show the mean values and standard errors; *n* = number of pairs used in each group.
* $p < 0.01$.
** $p < 0.05$ compared with the control group (Student's unpaired *t*-test).

taking test values) was evaluated by the Student's unpaired *t*-test.

3. Results

3.1. Diuretic activity

The different diuretic parameters tested for infusions from *Artemisia thuscula*, HCTZ and control groups are shown in Tables 1–3.

Table 1 shows the urinary volume (mL/100 g/8 h) for *Artemisia thuscula* infusions, HCTZ and control groups, and Table 2 shows the content (mEq/100 g/8 h) of Na^+, K^+ and Cl^- in the sample urines for *Artemisia thuscula* infusions, HCTZ and control. Other urinary excretion parameters as density, pH and conductivity of the sample urine at 8 h are shown in Table 3.

HCTZ, saluretic drug reference had values of water urinary excretion near to 40%, with respect to the control group and between 40 and 70% for the Na^+ and K^+ electrolytic excretion. We can also appreciate that the lower dose reaches the highest excretion values (Tables 1 and 2).

With respect to the results for *Artemisia thuscula* infusion, we can say that it shows a water-excretor effect dose-dependant decrease with values between 25 and 10%, which supposes an interesting excretor effect of water for a phytodiuretic.

With regards to the *Artemisia thuscula* electrolytic excretion (Na^+, K^+ Cl^-), we observed that compared with those of HCTZ, reference saluretic drug, they are sensitively inferior, but compared to the control group, they produce a significative increase in Na^+ excretion for the three *Artemisia thuscula* concentrations, although not for K^+ (with the exception of the 15% concentration) nor for Cl^-. On the other hand, no parallelism exists with respect to the aqueous excretion which was dose-dependant, since ionic excretion is almost the same for the three *Artemisia thuscula* concentrations used.

The comparison against the control of *Artemisia thuscula* K^+ excretion values at 5 and 10% confirm a K^+-saving effect, since their values are very near with those of the control group (in fact, there are not significant differences); this can be better appreciated if we compare these excretion levels with those of HCTZ which are significant.

With regards to the quantitative analysis of ions present in the *Artemisia thuscula* infusions (data not shown), this gave moderately low-value potassium salt results.

Regarding the conductivity—an indirect measure of total ionic contents—a raise of its values was observed when in-

Table 2
Effects of oral administration of *Artemisia thuscula* infusions on urinary electrolytic excretion

Group	*n*	Na (mEq/100 g/8 h)	K (mEq/100 g/8 h)	Cl (mEq/100 g/8 h)	Saluretic index			Na/K
					Na	K	Cl	
Control	16	0.49 ± 0.06	0.18 ± 0.05	0.82 ± 0.15	–	–	–	2.72
HCTZ (10 mg/kg)	4	0.70 ± 0.09*	0.29 ± 0.01*	1.37 ± 0.12*	1.52	1.70	1.67	2.41
HCTZ (25 mg/kg)	4	0.69 ± 0.08*	0.27 ± 0.02*	1.51 ± 0.36*	1.40	1.50	1.84	2.55
Artemisia thuscula (5%)	4	0.60 ± 0.05*	0.21 ± 0.05	0.98 ± 0.09	1.22	1.16	1.20	2.85
Artemisia thuscula (10%)	4	0.60 ± 0.03*	0.20 ± 0.01	0.94 ± 0.11	1.22	1.11	1.15	3.00
Artemisia thuscula (15%)	4	0.59 ± 0.03*	0.24 ± 0.02**	0.98 ± 0.18	1.20	1.33	1.20	2.46

The results show the mean values and standard errors; *n* = number of pairs used in each group.
* $p < 0.01$.
** $p < 0.05$ compared with the control group (Student's unpaired *t*-test).

Table 3
Effects of oral administration of *Artemisia thuscula* infusions on conductivity, pH and density

Group	*n*	Conductivity (mS/cm)	pH	Density (g/ml)
Control	16	17.47 ± 1.76	6.43 ± 0.50	0.99 ± 0.01
HCTZ (10 mg/kg)	4	17.92 ± 0.52	7.26 ± 0.25**	0.99 ± 0.01
HCTZ (25 mg/kg)	4	17.76 ± 0.69	7.10 ± 0.24	0.98 ± 0.01
A.thuscula (5%)	4	15.72 ± 1.06	7.71 ± 0.51	0.96 ± 0.01*
Artemisia thuscula (10%)	4	16.47 ± 4.60	7.00 ± 0.13	0.97 ± 0.04
Artemisia thuscula (15%)	4	17.34 ± 0.67	6.71 ± 0.18	1.00 ± 0.01

The results show the mean values and standard errors; *n* = number of pairs used in each group.
* $p < 0.01$.
** $p < 0.05$ compared with the control group (Student's unpaired *t*-test).

creasing the concentration used, but its values are less than that of the control group. On the contrary, values of pH and density decrease when the *Artemisia thuscula* concentration is increased. However, no statistical differences for these parameters were observed between the infusion group and the control group, except for density of *Artemisia thuscula* at 15%.

3.2. Acute toxicity

We also found that the infusions of *Artemisia thuscula* did not produce any sign of toxicity at the dose employed. No mortality was observed during the study period. No macroscopic changes in viscera could be detected in the treated group.

4. Discussion and conclusions

HCTZ reaches its maximum effect at a dose of 10 mg/kg, coinciding with scientific literatura (Kawashima and cols, 1985). It reveals a remarkable excretor effect so much in water as in electrolytes, typical in saluretic diuretics.

The infusions of *Artemisia thuscula* showed a significant water excretor effect, dose-dependent decrease, reaching the lowest concentration (5%), a notable increase in the urinary volume (25%) confirming, therefore its popular use as diuretic (Bethencourt, 1985; Darias et al., 1986; Pérez de Paz and Hernández Padrón, 1999).

Concerning electrolytic excretion, we observed something different, since although an increase in ionic excretion is produced at 5% in agreement with water excretion. From this concentration - that of 10 and 15% - ionic excretion keeps more or less constant, but water excretion begins to diminish. Therefore, no parallelism exists between urinary volume and electrolyte excretion. Therefore, it seems reasonable to think that diuresis produced by *Artemisia thuscula* at 5% is more related to saluretic mechanisms than aquaretic ones.

With respect to *Artemisia thuscula* electrolytic excretion values (Na^+, K^+ and Cl^-) compared to the control group, a significant increase in Na^+ excretion is produced in the three *Artemisia thuscula* concentrations, although not in K^+ (with the exception of the 15% concentration) nor in Cl^-.

Respect to the conductivity, although the dates are not significative, they seem to support these facts, since they tend to increase as the concentration of *Artemisia thuscula* increases, or as the urinary volumes decreases; something reasonable since urine tends to be more concentrated (the water volume excreted diminishes, but ion concentration keeps up). However, these values were lower than those belonging to the control. This could be explained by a marked increase in urinary excretion of water for *Artemisia thuscula*, which was more important than the urinary electrolyte excretion (Jouad et al., 2001).

Regarding the K^+ excretion, we observe that *Artemisia thuscula* at concentrations of 5 and 10% show an interesting K^+-saving effect, whose values are near to those of the control group (no significant differences) and it is easy to appreciate if we compare these K^+ excretion levels of *Artemisia thuscula* with these presented by HCTZ. However, at a concentration of 15% it can be seen with respect to the control group an important increase in K^+ excretion. Although we must take into account that this *Artemisia thuscula* concentration seems to be excessively concentrated and it is therefore difficult to reach under the form of popular preparation. In fact, the habitual infusion preparations used by the traditional canary people is between 2.5 and 5% w/v.

The quantitative analyses of the ion content in the *Artemisia thuscula* infusions samples shows a scant amount in potassium salts; therefore, diuretic effect does not seem to be due to the potassium content in the infusion samples. It is known that with an overload of potassium, if the renal tubules are not capable of absorbing it, it appears in the urine with extra water (Loew, 1991), but an assay with an aqueous solution of KCl (1.06 mmol/L) of similar potassium concentration to the infusion sample at 15% did not increase the diuresis (results not shown).

We should associate the *Artemisia thuscula* diuretic effect to the active principles present in the plant where we would point out the presence of eudesmanolides (sesquiterpene lactones), essential oils and flavonoids as the main active components, although data do not exist in literature consulted which support this activity for the first of these types of products.

On the other hand, the fact that when we increase the concentration of *Artemisia thuscula* infusion, water excretion decreases whereas ionic excretion more or less remains constant, which raises the possibility of the existence of more than one mechanism of diuretic action. It is possible that *Artemisia thuscula* at low concentrations exerted its diuretic activity by inhibiting tubular reabsorption of water and sodium, and where the role or the influence of polar compounds, as the flavonoids present in the plant, cannot be neglected.

However at higher concentrations of *Artemisia thuscula*, the decrease of urinary volume produced without a parallel reduction of ion excretion, could be explained through a decrease of the glomerular filtration rate (perhaps by renal blood flow decrease); maybe the essential oils contained by the plant (Perfumi et al., 1995) are related to this effect (Stanic and Samarzija, 1993).

On the other hand, the absence of acute toxicity allows us to confirm the security also to take this botanic species, since at a dose even 10 times over that used in popular medicine, no sign of toxicity was shown.

In short, we can say that infusions of *Artemisia thuscula* showed a notable diuretic effect, with a different profile respect to the HCTZ, and not due to anosmotic mechanism type related with the plant contents of potassium salts. Furthermore, *Artemisia thuscula* is very safe without toxicity and with a K^+-saving effect very interesting at concentrations 5 and 10%.

These results justify the use of *Artemisia thuscula* as diuretic agent by the canary traditional medicine, but furthermore, this species could be indicated for the urinary bacterial infections, through the so-called "therapeutic washing" while simultaneously taking sufficient amounts of liquid, due to its content of eudesmanolides (sesquiterpene lactones), a group of substances which exhibit a high antibacterial activity (Mansilla and Palenzuela, 1999) and its potassium-sparing effect.

More studies will be necessary to get more information about the potential diuretic value of this botanic species, and in particular on the role of some plant component chemicals, and to evaluate the effects of long-term administration on diuretic activity.

Acknowledgements

This work was supported by a Grant from Consejería de Educación, Cultura y Deportes del Gobierno Autónomo de Canarias (No. 53/90).The authors thanks to the technician G. Clemente for his help in the Flame Photometric determination.

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