Anti-allergic effects and oedema inhibition caused by the extract of *Drymis winteri*

K. S. Tratsk¹, M. M. Campos¹, Z. R. Vaz¹, V. C. Filho³, V. Schlemper³, R. A. Yunes² and J. B. Calixto¹

¹Department of Pharmacology, Universidade Federal de Santa Catarina, Rua Ferreira Lima 82, 88015-420 Florianópolis, Brazil, Fax +55 482 224164, e-mail: calixto@ccb.ufsc.br
²Department of Chemistry, Universidade Federal de Santa Catarina, Rua Ferreira Lima 82, 88015-420 Florianópolis, Brazil
³Department of Chemistry, Núcleo de Investigações Químico-Farmacêuticas, Universidade do Vale do Itajai, 88302-202 Itajaí, Brazil

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Abstract. **Objective:** To study the acute anti-inflammatory and anti-allergic properties of an extract of *D. winteri*. **Material and Methods:** Paw oedema induced in rats with various stimuli and anaphylactic shock in mice. **Results:** The hydroalcoholic extract (HE) of *D. winteri* (Winteraceae) (30 to 100 mg/kg, p.o., 1 h prior) inhibited carrageenan (300 µg/paw) and dextran (100 µg/paw)-induced paw oedema formation in a dose-dependent manner, with mean ID₅₀ values of 49 and <30 mg/kg, respectively. The HE of *D. winteri* (30 to 100 mg/kg) also inhibited paw oedema induced by bradykinin (BK) (3 nmol), substance P (SP) (10 nmol) and PAF-acether (PAF) (10 nmol), in a dose-dependent manner, with mean ID₅₀ values of 56, 63, and 58 mg/kg, respectively. However, the HE inhibited the rat paw oedema induced by prostaglandin E₂ (PGE₂) (10 nmol) (29 ± 7 and 33 ± 2% at 60 and 240 min) to a smaller extent, and had no effect on oedema elicited by histamine (100 nmol). In adrenalectomized animals, the inhibition by the HE of *D. winteri* (100 mg/kg, p.o., 1 h prior) of BK-elicited oedema (30 mg/paw) was significantly smaller when compared with that observed in control animals. When assessed in rats actively sensitised to ovalbumin (OVO), the oedema caused by OVO (6 µg/paw) was significantly inhibited by the HE of *D. winteri* (30 to 100 mg/kg, p.o.), with a mean ID₅₀ of about 65 mg/kg. The HE of *D. winteri* (100 and 200 mg/kg, p.o.) significantly increased survival rate when assessed in anaphylactic shock in mice actively sensitised to the antigen. The protective effect was long-lasting, being observed for up to 15 h. Dexamethasone, used as positive control (0.5, 1 and 2 mg/kg) produced a long-lasting (up to 24 h) increase in the survival rate of the animals.

Conclusions: These results confirm and extend our previous studies, and demonstrate the clear oral anti-inflammatory and anti-allergic properties of the active principle(s) present in the barks of *D. winteri*, thus confirming its reported medicinal use in folk medicine for the management of airway diseases.

**Key words:** *Drymis winteri* extract – Medicinal plant – Allergy – Anti-inflammatory – Paw oedema

Introduction

*Drymis winteri* is a plant belonging to the Winteraceae family, native to the south of Brazil and some other countries of South America. Folk medicine recommends the infusion of its barks or leaves for the treatment of different inflammatory diseases, such as asthma, allergy and bronchitis. This plant is also used as an antispasmodic and antipyretic [1], and is sometimes used for the treatment of cancer [2].

Although a great amount of evidence supports the traditional use of this plant in folk medicine, allied to chemical isolation and identification of several constituents in this and other species of the genus such as flavonoids [3], sesquiterpenoids [4–6] and terpenoids [7,8], very little information is available regarding the pharmacological actions of its extract or active principles.

In a recent study [9] we showed that the HE of barks of *D. Winteri* antagonists, in a concentration-dependent and reversible manner, the trachea contractions induced by several mediators involved in asthma and allergy, namely tachykinins (through NK₂ but not NK₁ receptors), bradykinin, the stable analogue of thromboxane A₂, U46619 and prostaglandin E₂ and partially contraction elicited by histamine, without affecting the contractions caused by acetylcholine. In addition, the HE of *D. winteri* inhibited, in a concentration-dependent manner, contractions induced by ovalbumin and compound 48/80 in guinea pig trachea from actively-sensitised and normal animals, respectively [9]. Such data confirms, at least partly, the medicinal properties of this plant reported in folk medicine, and strongly suggests a useful potential therapeutic application of its active principle(s) as an anti-inflammatory, anti-allergic and anti-asthmatic preparation. We therefore decided to investigate, in the present series of experiments, the in vivo oral effect of...
the HE of *D. Winteri* on rat paw oedema induced by several mediators involved in the inflammatory and allergic processes, and also some phlogistic substances such as carrageenan and dextran. Attempts have also been made to determine whether the active principle(s) present in the HE of this plant exhibit in vivo anti-allergic properties in animals which have been actively sensitised to ovalbumin.

**Materials and methods**

**Plant material and preparation of hydroalcoholic extract**

Botanical material was collected in Bom Retiro, State of Santa Catarina, Brazil, and was classified by Prof. Leila da Graça Amaral (Department of Botany, Universidade Federal de Santa Catarina). Samples of the plant were deposited in the Herbarium Flor at this University. The barks of *D. winteri* were minced and extracted with 50% ethanol-water in the proportion of 1:3 (W/V), being maintained at room temperature (21 ± 3°C) for 15 days. The solvent was fully evaporated and the extract was concentrated to the desired level and stored at −20°C. The extract was dissolved in 0.9% NaCl solution to the desired concentration just before use.

**Animals**

Non-fasted male Wistar rats (120–150 g) or Swiss mice (20–30 g) from our department, housed at 22 ± 2°C under a 12 h:12 light-dark cycle, were used. Food and water were freely available. The experiments reported were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [10].

**Measurement of rat paw oedema**

Experiments were conducted using non-fasted male Wistar rats (140–180 g) kept in a room controlled for temperature (22 ± 2°C) and illumination (12 h on and 12 h off). Under anaesthesia with 2,2,2-tribromoethanol (0.25 mg/kg, i.p.), animals received a 0.1 ml intraplantar injection, in one hindpaw, of phosphate-buffered saline (PBS; composition: NaCl 137 mmol/l, KCl 2.7 mmol/l and phosphate buffer 10 mmol/l) containing carrageenan (300 μg/paw), dextran (100 μg/paw), compound 48/80 (12 μg/paw), bradykinin (BK, 3 nmol/paw), histamine (H, 3 nmol/paw), platelet-aggregating factor (PAF, 10 nmol/paw), prostaglandin E2 (PGE2, 30 nmol/paw) or histamine (His, 100 nmol/paw). The contralateral paw received the same volume of PBS alone and served as a control. In experiments with bradykinin, animals were pre-treated with captopril (5 mg/kg, s.c.) 1 h prior to prevent the action of kininases [11]. For allergic oedema, the animals were actively sensitised by subcutaneous injection of 50 μg of OVO dispersed in 5 mg of Al(OH)3 dissolved in 0.1 ml of saline solution [12]. After 14 days, oedema was induced by injection of 6 μg of OVO into the left hindpaw. Animals were treated orally with HE of *D. winteri* (30 to 100 mg/kg) 60 min before. In other group of experiments, the animals were treated with dexamethasone (0.5 mg/kg, s.c., 24 h) that was used as a positive control. Oedema was measured by use of a plethysmometer (Ugo Basile) at several time-points after injection of irritant substances or inflammatory mediators, and was expressed as the difference in ml between both paws.

**Adrenalectomy**

In order to investigate the possible participation of endogenous glucocorticoids in the oedema inhibition caused by the HE of *D. winteri*, animals were anaesthetised with 2,2,2-tribromoethanol (0.25 mg/kg, i.p.), the dorsal region was incised (approximately 2 cm), and both adrenal glands were removed [13–15]. After surgery, animals were returned to their cages, with free access to food and drink, but water was substituted by 0.9% NaCl solution to maintain physiological sodium plasma concentrations. After seven days, animals received the HE of *D. winteri* (100 mg/kg, p.o.) or saline solution (10 ml/kg, p.o.) 60 min before the experiments. The oedema induced by bradykinin (3 nmol/paw) was managed and evaluated as described above.

**Evaluation of anti-allergic activity**

Anaphylactic shock in sensitised mice caused by chicken egg albumin. Male Swiss mice (18–20 g) were actively sensitised by subcutaneous injection of 0.2 ml of saline solution (0.9%) containing 20 μg of OVO dispersed in 1 mg of Al(OH)3 [12]. Fourteen days after this, animals received a new injection of 20 μg of OVO. Seven days after the last injection of OVO, animals were treated with different doses of HE of *D. winteri* (100 to 200 mg/kg p.o.) or with saline solution (0.1 ml/10 g, control group). In another group of experiments, the animals were treated with HE of *D. winteri* (100 mg/kg), 1, 4, 15, or 24 h before i.v. injection of OVO to analyse the time-course of the anti-allergic effect of this plant. Animal death was defined as the instant at which respiration ceased. The number of animals surviving was quantified after a period of 60 min after injection of OVO (500 μg/kg, i.v.). In a separate series of experiments, the animals were treated with dexamethasone (0.5, 1 and 2 mg/kg, s.c.) 2, 24 or 48 h before, and it was used as a positive control.

**Drugs**

The following drugs were used: bradykinin, prostaglandin E2, lambda carrageenan grade IV, dextran, substance P, histamine, captopril, chicken egg albumin (ovalbumin), 2,2,2-tribromoethanol, dexamethasone, compound 48/80 (Sigma Chemical Co., St. Louis, MO, USA) and platelet aggregating factor (PAF) (Bachem, Switzerland). The stock solutions for all peptides and drugs used were prepared in PBS (1–10 mM) in siliconized plastic tubes, maintained at −18°C, diluted to the desired concentration just before use. The other drugs were prepared daily in 0.9% w/v NaCl.

**Statistical analysis**

The results are presented as the mean ± SEM, except for the ID₅₀ values (i.e. the dose of the extract that reduced oedema formation by 50% relative to control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The ID₅₀ values were determined by use of the least-square method for individual experiments. Statistical comparison of the data was carried out by the use of analysis of variance followed by Dunnett’s test or unpaired Student’s test, when indicated. The x²-test was used to compare the survival rate in the group treated with the vehicle and the groups treated with drugs. p-Values of less than 0.05 were considered significant [16].

**Results**

The results of Figure 1 show the effect of HE of *D. Winteri* (30 to 100 mg/kg p.o.) on rat paw oedema caused by intraplantar injection of carrageenan at different intervals of time. The treatment of animals with HE of *D. winteri* (30 to 100 mg/kg, p.o.), 1 h beforehand, inhibited in a dose- and time-dependent manner, carrageenan (300 μg/paw)-induced oedema (percentage of inhibitions of 72 ± 1.88 ± 1.73 ± 1.67 ± 1.1% at 30, 60, 120 and 240 min after the irritant injection, respectively). The calculated mean ID₅₀ value (and 95% confidence limits) determined at the 4 h interval point,