Analgesic and Anti-inflammatory Properties of *Plantago australis* Hydroalcoholic Extract

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SUMMARY. The hydroalcoholic extracts of leaves, roots, and fruits of *Plantago australis* Lam. (Plantaginaceae) were investigated regarding their possible anti-inflammatory and analgesic properties. Carrageenan-induced rat hind paw edema was significantly inhibited (P<0.05) by oral administration of these extracts. All studied extracts also reduced the total number of writhes induced by acetic acid. The results indicated that the hydroalcoholic extracts of leaves, roots, and fruits of *P. australis* are endowed with anti-inflammatory and analgesic activities.

RESUMEN. “Propiedades analgésicas y antiinflamatorias del extracto hidroalcohólico de *Plantago australis*”. Fueron investigados los extractos hidroalcohólicos de hojas, raíces y frutos de *Plantago australis* Lam. (Plantaginaceae) para determinar sus posibles propiedades antiinflamatorias y analgésicas. La inducción del edema de pata por carragenina fue significativamente inhibida (P<0,05) por la administración oral de estos extractos. También hubo una reducción en el número total de contorsiones inducidas por el ácido acético con todas las partes de la planta estudiadas. Los resultados indican que los extractos hidroalcohólicos de hojas, raíces y frutos de *P. australis* estarían dotados de actividad antiinflamatoria y analgésica.

INTRODUCTION

*Plantago australis* Lam. is a perennial plant that belongs to the Plantaginaceae family. This plant is known as tansagem, transagem or trançagem in Southern Brazil and it is used in folk medicine as laxative, diuretic, anti-inflammatory, anti-bacterial, cicatrizing, and other medicinal treatments 1.

Several species of the genus Plantago (P. minor L., P. lanceolata L., P. major L.) are used to treat pain and inflammation, in diseases as rheumatism, fever, gastric ulcers, and hemorrhoids 2-4. The pharmacological activity of some of these species was determined by scientific studies, as the anti-inflammatory action of *P. major* 5. However, studies on pharmacological activities of *P. australis* are scarce, and therefore the present study aimed to investigate analgesic and anti-inflammatory properties of the hydroalcoholic extract (HAE) of *P. australis* leaves, roots, and fruits on the carrageenan-induced paw edema in rats and acetic acid induced writhing test in mice.

MATERIAL AND METHODS

*P. australis* was collected in Santa Maria, Rio Grande do Sul state, Southern Brazil. A voucher specimen is deposited in the herbarium of the Department of Biology of the Universidade Federal de Santa Maria under number 6369. The leaves, roots, and fruits were maintained in a ventilated oven (40 °C) for drying and stabilization. The material was then pulverized in a Wiley mill. The HAE of leaves, roots and fruits was prepared at ethanol 70%, respectively. The obtained of each plant portion was lyophilized and stored at -12 °C. The yield of this process was...
18.74 % for leaves, 17.08% for roots, and 10.48% for fruits.

**Antiedematogenic Activity**

The method of carrageenan-induced paw edema in rats to evaluate the anti-inflammatory activity described by Winter et al. 6 was used. Male rats were treated orally with the vehicle (water), HAE of leaves, roots, and fruits (250, 500, and 1000 mg/kg) or indomethacin (10 mg/kg) 60 min before injection of 0.05 mL of 1% carrageenan into the rat hind paw plantar surface. The measurements of foot volume were taken before carrageenan injection and in every hour for 4 h. The inhibition of the inflammation was calculated by measuring the volume difference between the control and the tested paw.

**Acetic Acid Induced Writhing Test**

Adult mice (25-35 g) were treated with either the vehicle (water) or HAE of leaves, roots, and fruits (60, 125, and 250 mg/kg) v.o., 60 min prior to i.p. injection of 1.2% acetic acid (0.1 mL/10g body weight). Indomethacin (10 mg/kg, v.o.) treated animals were used for positive control. The number of writhes was counted for 30 min 7,8.

**Statistical Analysis**

Data were expressed as mean ± S.E.M. and the different treatments were compared by analysis of variance and the post-hoc Duncan’s multiple range test, with the aid of the statistical software SPSS (version 1986). The minimum significance level was set at P < 0.05. Differences between two means were detected using the Student’s t test.

**RESULTS**

**Effect of the HAE of P. australis on the Rat Paw Edema Induced by Carrageenan**

Rats treated with HAE of leaves at the dose of 250 mg/kg inhibited the edema in 29.91% (0.89 ± 0.07 mL) and 21.80 % (1.14 ± 0.07 mL) after 180 min and 240 min when compared with the control group. The doses of 500 and 1000 mg/kg of the HAE of leaves inhibited the edema after 120 min in 41.92% (0.42 ± 0.06 mL) and 20.46% (0.58 ± 0.05 mL), after 180 min in 31.74% (0.87 ± 0.05 mL) and 26.52% (0.94 ± 0.04 mL), and after 240 min in 24.53% (1.10 ± 0.05 mL) and 21.66% (1.15 ± 0.03 mL), respectively, when compared with the control group (Figure 1 A).

Pretreatment with HAE of roots at dose of 500 mg/kg inhibited the edema after 120 min in 42.46% (0.42 ± 0.07 mL), after 180 min in 29.02% (0.90 ± 0.09 mL), and after 240 min in 19.07% (1.18 ± 0.10 mL), when compared with the control group. No significant difference with relation to control was detected at the doses of 250 and 1000 mg/kg (Figure 1 B).

Administration of HAE of fruits at dose of 250 mg/kg inhibited the edema after 120 min in 27.16% (0.53 ± 0.07 mL), 180 min in 34.88%...
and 250 mg/kg) reduced the writhes by 50.12% (38.4 ± 5.26) and 56.88% (33.2 ± 3.36). No significant difference was detected to the dose of 60 mg/kg (72.6 ± 7.31) (Figure 2 B). Previous administration with HAE of fruits (60, 125 and 250 mg/kg) reduced the writhes by 28.58% (55 ± 5.16), 45.46% (42 ± 4.40) and 44.42% (42.8 ± 4.09), respectively (Figure 2 C). Indomethacin pretreatment reduced the number of writhes to 52.17% of control.

DISCUSSION

An analgesic and anti-inflammatory effect was reported for Plantago species 5,9. Latin American folk medicine refers also to the anti-inflammatory activity of Plantago australis, but no studies validating its use were reported. Although people utilize the aqueous infusion, this study has assessed the pharmacological effects of the HAE prepared with the leaves, roots, and fruits separately, aiming to obtain the major presence of the active compounds.

Oral treatment of rats with the HAE of leaves, roots, and fruits inhibited the paw edema induced by carrageenan in a non-dependent manner. The same was observed on the anti-inflammatory effect of the aqueous extract of leaves of Sida cordifolia L.10 The mechanism involved on the genesis of the carrageenan-induced edema may cause the release of prostaglandins and kinins, among other substances 11. Pretreatment of mice with the HAE of leaves, roots, and fruits of P. australis inhibited writhing induced by acetic acid. When this acid is administrated i.p. it induces the release of prostaglandins and sympathetic system mediators 12. Writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluating mild analgesic non-steroidal anti-inflammatory compounds 13.

The anti-edematogenic and analgesic mechanisms of the HAE of P. australis may also be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of indomethacin 14. Although this study suggests the existence of a common mechanism for the analgesia and inflammation inhibition by the HAE of P. australis, higher doses were needed for a significant anti-inflammatory effect. Additional studies with other experimental models must be done to verify why the pain inhibition occurred with minor doses when compared with those used in edema inhibition.

Figure 2. Number of writhes (in 30 min) induced by intraperitoneal injection of acetic acid in mice pretreated orally with the vehicle (C), the HAE of P. australis leaves (A), roots (B), and fruits (C) at doses of 60, 125, and 250 mg/kg or indomethacin (Ind). Vertical bars are means ± SEM (n=6) * significantly different from control (P < 0.05)

Effect on the Acetic Acid Induced Writhing in Mice

Mice injected with 0.1 mL/10g of 1.2% acetic acid i.p. presented 77 ± 4.49 writhes (n=6) in 30 min. Previous treatment with HAE of leaves (60, 125 and 250 mg/kg) reduced the number of writhes by 27.46% (55.85 ± 2.73), 48.70% (39.5 ± 2.18) and 48.71% (39.5 ± 5.27), respectively (Figure 2 A). Treatment with HAE of roots (125
CONCLUSIONS

The results indicated that the HAE of leaves, roots, and fruits of P. australis shows anti-inflammatory and analgesic actions. Consequently, a detailed study of this plant in order to determine its active compounds and their mechanism of action certainly will produce interesting results.

REFERENCES