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Investigation of the Antiinflammatory Activity of Liquid Extracts of *Plantago lanceolata* L.

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***Plantago lanceolata* L. extracts are used against inflammatory diseases. In this study we have demonstrated the ability of four liquid extracts of *Plantago lanceolata L.* to inhibit membrane irritation on the chick chorioallantoic membrane. We used a modified hen's egg chorioallantoic membrane test (HET-CAM), in which the membrane irritation was induced with sodium dodecyl sulphate. The antiinflammatory activity of the extracts was compared with the activity of some antiinflammatory active drugs. These extracts showed a potent activity in the inhibition of membrane irritation.** © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Plantago lanceolata L. extracts present, besides antiinflammatory (Shipochliev *et al.*, 1981; Murai *et al.*, 1995), several other properties. In remedies *Plantago lanceolata* L. extracts are the active ingredient against inflammatory states for example in tussive irritation or bronchial catarrh. But there are no systematic pharmacological examinations that prove the antiinflammatory activity of the extracts and trace their active principle. In the present work, the antiinflammatory properties of these extracts were tested using the modified hen's egg chorioallantoic membrane test (HET-CAM). Moreover the antiinflammatory activity was compared to that of hydrocortisone, phenylbutazone and sodium diclofenac.

MATERIALS AND METHOD

Test materials. For the investigation freeze-dried liquid extracts (28% ethanol) were used. The first liquid extract (E1) (Ch.-Nr 150633F Charge 5000) was provided from Asta Medica, Frankfurt, Germany. The second liquid extract (E2) (Ch-B.: 504470) was provided from Müggenburg Extrakt-Gesellschaft, Germany. The third liquid extract (E3) was self-produced from dried herb (Ch.-B.: 6500, Typ: 127165) provided from Asta Medica, Frankfurt, Germany. The fourth liquid extract (E4) was self-produced from dried herb harvested in October 1996 in Regensburg, Germany.

Modifed hen's egg chorioallantoic membrane test

Phase 1: Pellet preparation. 5 mg sodium dodecyl sulphate was dissolved with or without 5 mg test compound (hydrocortisone, phenylbutazone or sodium diclofenac) or 50 mg of the freeze-dried extracts in 1 mL of a hot (about 60 °C) 2.5% agarose solution. 10 μL of these gelling solutions were used for the pellet preparation (Dobson *et al.*, 1990).

Phase 2: Execution. The modifed method of D'Arcy and Howard (1967) was used. The fertile hens' eggs were incubated for 65–70 h at 37°C and a relative humidity of 80%. The eggs were placed in a horizontal position and rotated several times.

The eggs were opened on the snub end after aspiration of 10 mL of albumin from a hole on the pointed end. At two-thirds of the height (from the pointed end) the eggs were traced with a scalpel and after that the shells were removed with forceps. The aperture was covered with keep-fresh paper and the eggs were incubated at 37 °C at a relative humidity of 80% for 75 h. One pellet per egg was put on the formed chorioallantoic membrane (CAM) which was about 2 cm in diameter and one pellet was put on it (1 pellet/egg). The eggs were incubated for 1 day and then evaluated.

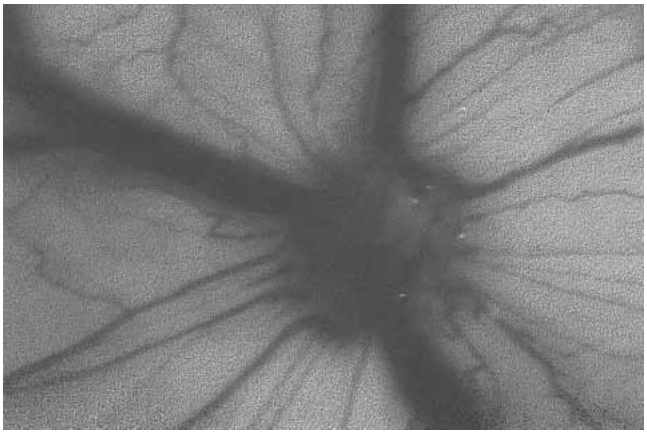

Figure 1. Membrane irritation with sodium dodecyl sulphate (50 μg/pellet).

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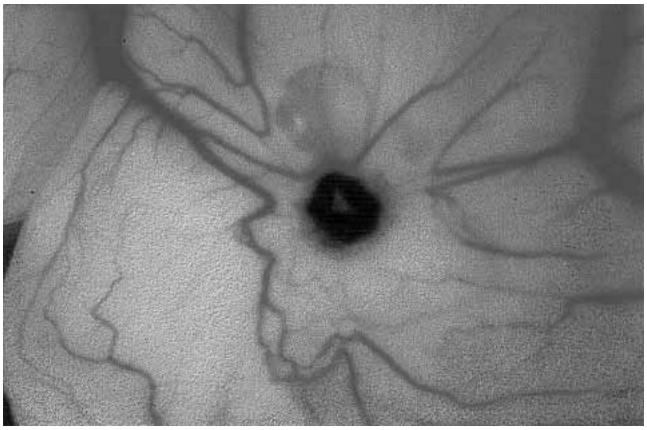

Figure 2. Inhibition of membrane irritation by E1 (500 μg/pellet).

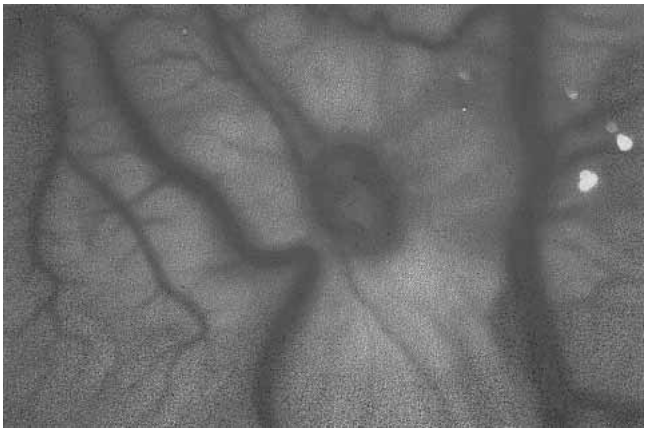

Figure 3. Inhibition of membrane irritation by hydrocortisone (50 μg/pellet).

For every test 15–20 eggs were utilized. To evaluate the effects, as positive-irritation controls CAMs were treated with sodium dodecyl sulphate only. As positive controls diclofenac, phenylbutazone and hydrocortisone were tested at a concentration of 50 μg/pellet in the presence of sodium dodecyl sulphate at a concentration of 50 μg/pellet. As negative controls the extracts were tested at a concentration of 500 μg/mL without sodium dodecyl sulphate. As a blank CAMs were treated with agarose solution only.

Phase 3: Interpretation of experiments. The inhibition of the membrane irritation was observed. A positive effect, corresponding to antiinflammatory activity, exists if the irritation of membrane induced by sodium dodecyl sulphate decreases and the blood-vessel net appears normal. The number of experiments with a positive effect was given in per cent. This percentage is a measure of the antiinflammatory activity of the substance tested.

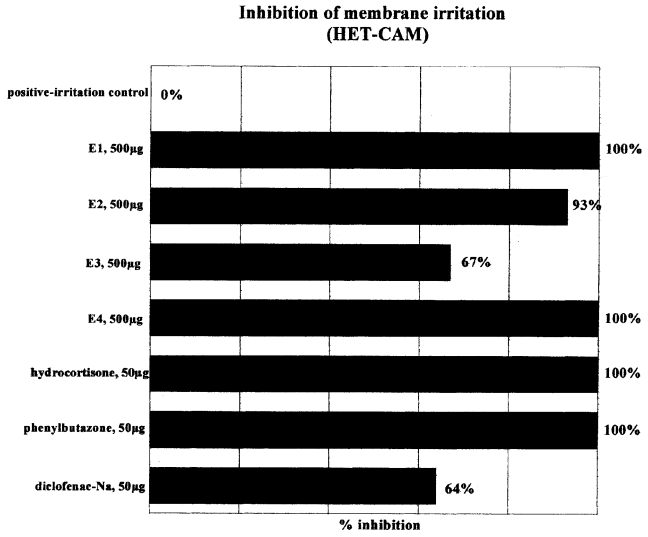

Figure 4. Inhibition of membrane irritation. The irritation was stimulated with 50 μg sodium dodecyl sulphate per pellet. As a positive-irritation control CAMs treated with sodium dodecyl sulphate only at 50 μg/pellet were taken.

RESULTS AND DISCUSSION

The typical membrane irritation induced by sodium dodecyl sulphate is shown in Fig. 1. In this inflammatory state the number of blood vessels is high and there is formation of a granuloma. In all cases the blood vessels have a star form around the granuloma. The four extracts at a concentration of 500 μg/pellet neutralized the formation of blood vessels (no observation of a star form) around the granuloma and the total blood-vessel net appeared normal. An inhibition of membrane irritation (inhibition of 100%) was registered for E1 (Fig. 2) and E4 in all experiments. For E2 and E3 this inhibition was 67% and 93%, respectively. At a 10-fold higher concentration (500 μg/pellet instead of 50 μg) the antiinflammatory activity of the extracts was comparable to that of hydrocortisone (Fig. 3), phenylbutazone and sodium diclofenac. All results are summarized in Fig. 4.

These finding confirm the antiinflammatory activity of *Plantago lanceolata* liquid extracts.

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