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# Anti-inflammatory and antioxidant properties of *Helichrysum italicum*

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**Abstract**

The anti-inflammatory and antioxidant activities of the aerial part of *Helichrysum italicum* extracts have been established in various in-vivo and in-vitro experimental models. The results obtained on the acute oedemas induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and ethyl phenylpropiolate in the mouse ear, by serotonin and phospholipase $A_2$ ($PLA_2$) in the mouse paw, on chronic inflammation induced by repeated application of TPA in the mouse ear and on the delayed-type hypersensitivity induced by sheep red blood cells suggest that said anti-inflammatory activity is due to the effects of compounds expressed via a corticoid-like mechanism. In addition, the antioxidant activity of the extracts seems to be implicated in this anti-inflammatory activity, as the former inhibits enzymatic and non-enzymatic lipid peroxidation and has free-radical scavenger properties. We conclude that the anti-inflammatory activity of *Helichrysum italicum* can be explained by multiple effects, including inflammatory enzyme inhibition, free-radical scavenging activity and corticoid-like effects.

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## Introduction

*Helichrysum italicum* (Roth) G. Don fil (Asteraceae) is a woody shrub characteristic of the Mediterranean area. Its flowers and aerial parts are used as an anti-inflammatory and anti-allergic phytomedicine (Peris et al 1995). Taken in the form of a fluid extract or as an infusion, they are effective in respiratory disorders with allergic or infectious components, in skin diseases such as psoriasis or eczema and in other inflammatory processes. The medicinal properties of this species are thought to be owed to its flavonoid, sesquiterpene lactone and essential oil content. Several studies on the anti-inflammatory activity of closely related species and on related topics have been published (Recio et al 1991; de la Puerta et al 1999), but there are few reports on the anti-inflammatory (Máñez et al 1990) and radical-scavenging (Facino et al 1990; Schinella et al 2002) properties of *H. italicum*.

The aim of this study was to investigate the anti-inflammatory and antioxidant activity of the methanolic extract of *H. italicum* and the fractions obtained from it, to establish its potential therapeutic value.

## Materials and Methods

### Plant material, extraction and identification

Aerial parts of *Helichrysum italicum* (Roth) G. Don fil (Asteraceae) were collected in Chiva (Valencia, Spain) in June 1996. A specimen was deposited in the herbarium of the Department of Pharmacology, University of Valencia (Burjassot, Spain).

Air-dried and powdered aerial parts of *H. italicum* (400 g) were percolated with methanol (3.5 L) at room temperature, and the solution was evaporated under reduced pressure, leaving a residue of 55 g. This was redissolved in water and fractionated with solvents of increasing polarity to obtain four subextracts: *n*-hexane (10 g), dichloromethane (22 g), ethyl acetate (7 g) and *n*-butanol (4 g). The fractions were subjected to a chromatographic analysis by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), and the major compounds were identified by comparison with standards previously isolated and identified by Recio et al (1991) from *H. stoechas*. TLC analysis was performed on $SiO_2$ using dichloromethane–methanol (90:10) or dichloromethane–ethyl acetate (70:30; 97:3) as mobile phases. HPLC–diode array detector (HPLC-DAD) analysis was performed using a Merck Hitachi HPLC system (L-6200 pump) equipped with an L-3000 Photodiode array detector and a pre-packed analytical column (12.5×0.7 mm) of Lichrospher RP-18 (5 $\mu$m). The following conditions were used: as eluents, $H_2O$+trifluoroacetic acid 0.05% (A), methanol+trifluoroacetic acid 0.05% (B). Elution profile: 0–5 min 70% A, 5–15 min 50% A, 15–29 min 30% A, 29–30 min 70% A; flow rate 1 mL $min^{-1}$, column pressure 60–80 bar and the UV detector was set at 280 nm.

### Chemicals and animals

*Chemicals*

Ascorbic acid, arachidonic acid 99%, butylated hydroxytoluene, cyproheptadine hydrochloride, dexamethasone, dimethyl sulfoxide (DMSO), 2,2-dinitrofluorobenzene (DNFB), 2,2-diphenyl-1-picrylhydrazyl (DPPH), hexadecyltrimethylammonium bromide (HTAB), hypoxanthine, indometacin, nitro blue tetrazolium, nordihydroguaiaretic acid (NDGA), oxazolone, phospholipase $A_2$ ($PLA_2$) from *Naja mossambica* venom, pyrogallol, sheep red blood cells (SRBC), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), 2-thiobarbituric acid, trichloroacetic acid, xanthine and xanthine oxidase were purchased from Sigma Chemical Co. (St Louis, MO); ethyl phenylpropiolate (EPP) and Tween 80 from Fluka Chemika-Biochemika (Buchs, Switzerland); methanol (HPLC grade) from Merck (Darmstadt, Germany); acetone, butanol, dichloromethane, ethanol 96°, ethyl acetate, hexane, methanol, and trifluoroacetic acid from Panreac (Barcelona, Spain).

*Animals*

Groups of six Swiss female mice, 25–30 g, were used. All mice had free access to a standard diet. Housing conditions and all in-vivo experiments were approved by the institutional Ethical Committee of the Faculty of Pharmacy according to the guidelines established by the European Union on Animal Care (CEE Council 86/609).

### Extracts and reference drug dissolution

For topical application, the hexane, dichloromethane and ethyl acetate extracts (1 mg/ear), NDGA (2 mg/ear), indometacin (0.5 mg/ear) and dexamethasone (0.05 mg/ear) were dissolved in 20 $\mu$L acetone, and methanol and butanol extracts (1 mg/ear) in 20 $\mu$L 80% aqueous ethanol. For subcutaneous application, the hexane, dichloromethane and ethyl acetate extracts (100 mg $kg^{-1}$) were dissolved in olive oil, and methanol and butanol extracts (100 mg $kg^{-1}$) and dexamethasone (0.5 mg $kg^{-1}$) in ethanol–saline (1:19). For oral application, the extracts (200 mg $kg^{-1}$) and cyproheptadine (10 mg $kg^{-1}$) were dissolved in Tween 80–ethanol–$H_2O$ (1:1:10). The antioxidant activity of the extracts dissolved in DMSO was tested at a concentration of 100 $\mu$g $mL^{-1}$. Extracts in the hydroxyl radical test were dissolved in 2% Tween 80.

### 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)-, arachidonic acid-, and ethyl phenylpropiolate (EPP)-induced mouse ear oedema

Experimental methods have been previously described by Recio et al (2000). An oedema was induced in the right ear by topical application with a micropipette of an irritant agent dissolved in acetone (20 $\mu$l). The oedema was expressed as the difference between the ear's thickness before and after induction of inflammation.

### Phospholipase $A_2$ ($PLA_2$)- and serotonin-induced hind-paw mouse oedema

The $PLA_2$ from *Naja mossambica* venom (1.18 U) in saline (25 $\mu$L) was injected subcutaneously into the mouse right hind paw. The left paw received the same volume of the saline solution. Samples were admin-

istered orally 60 min before challenge, and the oedema was measured 30, 60 and 90 min after challenge (Neves et al 1993).

Serotonin was injected into the right hind paw 3 h after administration of the test compounds. The left paw received the same volume of the saline solution. The oedema was measured 12 min after its induction (Recio et al 2000).

### Mouse ear inflammation induced by multiple topical applications of TPA

Inflammation was induced in each ear by topical application of 2 $\mu$g of TPA (20 $\mu$L) on alternate days (5 applications). Extracts and dexamethasone were applied topically twice daily for four days. On the last day the compounds were applied only in the morning. The mice were killed by cervical dislocation, and two ear punches were taken from each mouse (n = 5 mice). Eight samples placed in HTAB were frozen for the myeloperoxidase assay. Details of the methods have been described in an earlier study (Recio et al 2000).

### Oxazolone- and DNFB-induced contact-delayed-type hypersensitivity (DTH)

The oxazolone-induced DTH test was performed according to Recio et al (2000). The DNFB-induced DTH test was performed according to Góngora et al (2000). Ear swelling was assessed 24 and 96 h after each challenge. Samples and dexamethasone were applied topically 2, 24, 48 and 72 h after challenge.

### Sheep red blood cell (SRBC)-induced DTH

The experiment was performed according to the protocol referred to previously (Góngora et al 2000). The oedema was measured 18, 24 and 48 h after challenge. The extracts and dexamethasone were administered intraperitoneally immediately before and 16 h after challenge.

### Oedema measurements

Ear swelling was measured using a micrometer (Mitutoyo Series 293). The oedema was expressed as the increase in ear thickness due to the oedematous agent. The percentage of oedema inhibition was expressed as the reduction in thickness with respect to that of the control group treated only with the oedema inductor. The paw oedema was measured by means of a plethysmometer (Ugo Basile) and was expressed as the difference between the right and left paw.

### Lipid peroxidation

Liver microsomes were prepared using a standard differential centrifugation technique (Schinella et al 2000). Protein content was quantified by Bradford's method using bovine serum albumin as the standard (Bradford 1976).

Non-enzymatic peroxidation was induced by $FeSO_4$ (5 $\mu$M) and ascorbate (500 $\mu$M) (Schinella et al 2000). The products of lipid peroxidation were detected measuring the absorbance at 535 nm using the 2-thiobarbituric acid method. Butylated hydroxytoluene was used as a positive control. In enzymatic lipid peroxidation, the reaction mixture contained microsomal protein and an NADPH-generating system (Schinella et al 2000). Peroxidation was started by 10 $\mu$L $CCl_4$ 1:4 (v/v) in DMSO. After 15 min incubation at 37°C, thiobarbituric acid reactive substances were determined as above.

To determine cytochrome P-450 2E1 activity, microsomal protein was pre-incubated for 10 min in 100 $\mu$g mL$^{-1}$ of each extract, and the catalytic activity was then assessed using *p*-nitrophenol hydrolase as the selective enzyme assay (Lee & Forkert 1994).

### Free radical generation

Superoxide radical was generated by enzymatic oxidation of hypoxanthine with xanthine oxidase grade I and was detected by nitro blue tetrazolium reduction viewed spectrophotometrically at 560 nm (Schinella et al 2000). The influence on enzyme activity was evaluated by uric acid formation from xanthine and absorbance was measured at 295 nm. Pyrogallol was used as a positive control.

The hydroxyl radical was produced in a Fenton reaction (Schinella et al 2000) with different concentrations of test material at a volume of 1 mL. Deoxyribose degradation by hydroxyl radicals was measured by using the thiobarbituric acid method. DMSO was used as a positive control.

A 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution in methanol was added to the extract solutions and activity was determined by spectrophotometry at 517 nm after 10 min (Schinella et al 2000). Butylated hydroxytoluene was used as reference compound.

### Statistics

Statistical analysis was performed using one-way analysis of variance followed by Dunnett's *t*-test for multiple comparisons, and Student's *t*-test for single comparisons.

## Results

Ursolic acid, 4′-hydroxy-3′-(3-methyl-2-butenyl)acetophenone and the flavonoid gnaphaliin were identified by TLC and HPLC-DAD analysis as the main compounds in the dichloromethane fraction by comparison with previously isolated standards (Recio et al 1991). Other phenolics were detected by HPLC-DAD analysis, and had a UV spectrum profile characteristic of flavonoids. The ethyl acetate and butanol fractions are formed mainly by high-polarity flavonoids and acetophenone derivatives. In the hexane fraction, only lipids, sitosterol and other analogous compounds were detected by chromatographic analysis.

The results obtained with the total methanol extract and its fractions – hexane, dichloromethane, ethyl acetate and butanol – on different mouse models of topical acute inflammation are presented in Figure 1. All the samples inhibited the TPA-induced ear oedema, whereas only the ethyl acetate and butanol extracts were active against the EPP-induced ear oedema. None of these extracts, however, reduced the inflammation provoked by topical application of arachidonic acid (data not shown).

Figure 2 shows the effect of the extracts and dexamethasone on vascular leakage induced by serotonin, 3 h after administration to mice. All the extracts reduced the oedema formation, but the ethyl acetate extract was the most active and gave a percentage reduction in oedema volume similar to that of dexamethasone.

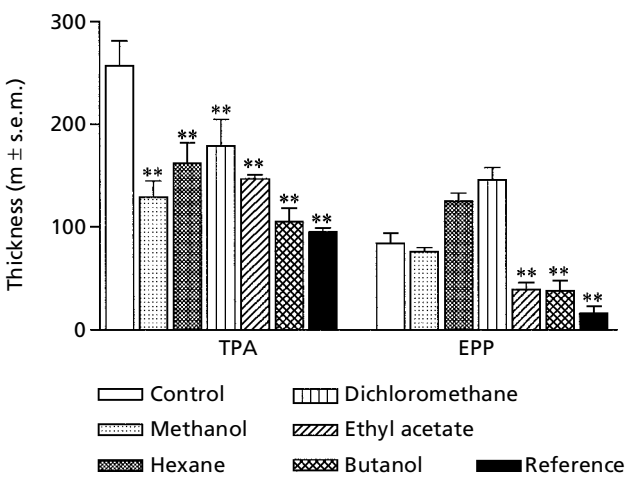


**Figure 1** Anti-inflammatory effect of *Helichrysum italicum* extracts (1 mg/ear) on acute 12-*O*-tetradecanoylphorbol 13-acetate (TPA)- and ethyl phenylpropiolate (EPP)-induced ear oedemas in mice. Values are mean±s.e.m. (n = 6); $^{**}P < 0.01$ (Dunnett's *t*-test) compared with control. Reference drugs: indometacin (0.5 mg/ear) in TPA test, dexamethasone (0.05 mg/ear) in EPP test.

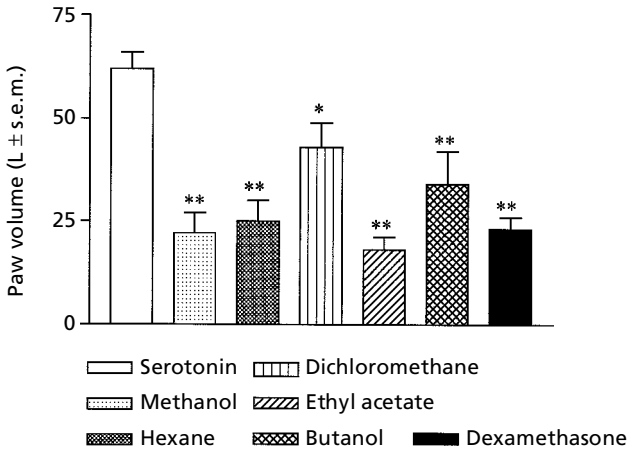


**Figure 2** Anti-inflammatory effect of *Helichrysum italicum* extracts (100 mg kg$^{-1}$) and dexamethasone (0.5 mg kg$^{-1}$) on serotonin-induced paw oedema in mice. Values are mean±s.e.m. (n = 6); $^{**}P < 0.01$, $^{*}P < 0.05$ (Dunnett's *t*-test) compared with control.

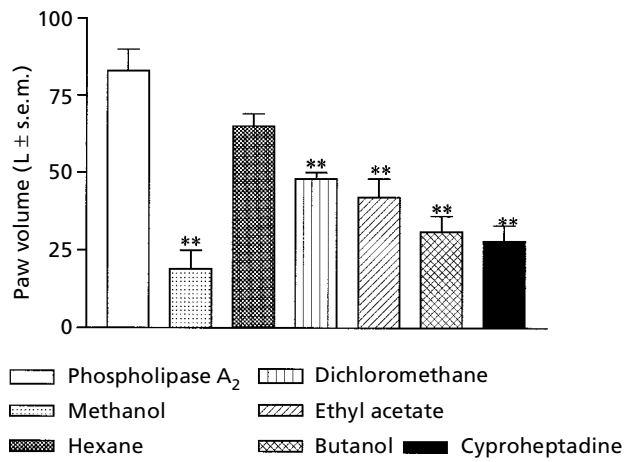


**Figure 3** Anti-inflammatory effect of *Helichrysum italicum* extracts (200 mg kg$^{-1}$) and cyproheptadine (10 mg kg$^{-1}$) on $PLA_2$-induced paw oedema in mice 60 min after subcutaneous injection of $PLA_2$. Values are mean±s.e.m. (n = 6; $^{**}P < 0.01$ (Dunnett's *t*-test) compared with $PLA_2$.

Subplantar injection of $PLA_2$ produced a time-dependent hind paw oedema that reached a maximum after 60 min. All the samples (200 mg kg$^{-1}$, p.o.), except the hexane extract, inhibited the paw oedema (Figure 3), the methanol and butanol extracts being the most active.

In chronic inflammation induced by TPA, the methanol extract inhibited the oedema (65% inhibition) and leucocyte infiltration (58% inhibition) to a lesser extent than dexamethasone (85% and 86% inhibition, respectively). After fractioning the methanol extract, only those fractions obtained with hexane (44% inhibition)

**Table 1** Anti-inflammatory effect of *Helichrysum italicum* extracts (100 $\mu$g mL$^{-1}$) on lipid peroxidation in rat liver microsomes stimulated with $Fe^{2+}$/ascorbate or $CCl_4$/NADPH.

| | **$Fe^{2+}$/ascorbate** | | **$CCl_4$/NADPH** | |
|---|---|---|---|---|
| | Malondialdehyde (nmol) | % Inhibition | Malondialdehyde (nmol) | % Inhibition |
| Control | 40.7±0.7 | – | 7.4±0.4 | – |
| Hexane | 34.4±1.0$^{ns}$ | 16 | 2.8±0.1** | 62 |
| Dichloromethane | 1.5±0.6** | 96 | 1.0±0.0** | 87 |
| Ethyl acetate | 1.5±0.1** | 96 | 1.5±0.1** | 80 |
| Butanol | 2.1±0.3** | 95 | 4.0±0.2** | 46 |
| Butylated hydroxytoluene | 1.2±0.1** | 97 | 0.9±0.1** | 88 |

Values are mean±s.e.m. (n = 3); **$P < 0.01$ (Dunnett's *t*-test) compared with control.

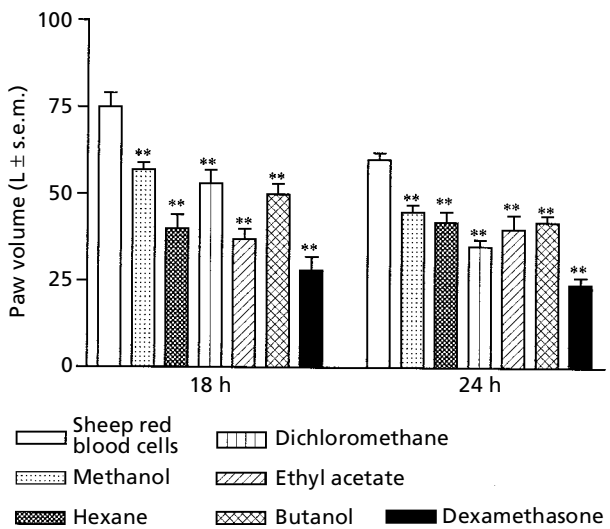


**Figure 4** Anti-inflammatory effect of *Helichrysum italicum* extracts (50 mg kg$^{-1}$) and dexamethasone (10 mg kg$^{-1}$) on sheep red blood cell (SRBC)-induced paw oedema in mice at 18 and 24 h, respectively. Values are mean±s.e.m. (n = 5); **$P < 0.01$ (Dunnett's *t*-test) compared with $PLA_2$.

**Table 2** Anti-inflammatory effect of *Helichrysum italicum* extracts (100 $\mu$g mL$^{-1}$ in DMSO) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction.

| | **DPPH A** | **% Inhibition** |
|---|---|---|
| Control | 0.340±0.010 | – |
| Hexane | 0.229±0.001** | 33 |
| Dichloromethane | 0.037±0.005** | 89 |
| Ethyl acetate | 0.019±0.001** | 94 |
| Butanol | 0.023±0.001** | 93 |
| Butylated hydroxytoluene | 0.031±0.006** | 91 |

A = absorbance units. Values are mean±s.e.m. (n = 3); **$P < 0.01$ (Dunnett's *t*-test) compared with control.

and dichloromethane (48% inhibition) significantly reduced the ear oedema, while all inhibited leucocyte recruitment (40–66%, depending on the type of extract).

The SRBC-induced DTH inflammation reached its maximum at 18 h and disappeared 48 h after challenge. Treatment with the drugs immediately before, and 16 h, after challenge was effective in reducing the paw oedema (Figure 4). However, none of the drugs displayed activity against the contact-DTH induced by oxazolone or DNFB.

The activity of the methanol extract has been reported by Schinella et al (2002) in a previous screening. In this study we demonstrate the activity of the different fractions obtained by liquid–liquid extraction from the methanol extract. Table 1 shows the activities of the extracts against enzymatic and non-enzymatic lipid peroxidation in rat liver microsomes. When tested in the system dependent on $Fe^{2+}$/ascorbate at a concentration of 100 $\mu$g mL$^{-1}$, all the fractions except the hexanic showed anti-peroxidative activity, and all the fractions, without exception, inhibited lipid peroxidation in the $CCl_4$/NADPH system.

The effects of the extracts on *p*-nitrophenol hydroxylase (cytochrome P-450 2E1) activity were evaluated, but the fractions did not inhibit this enzymatic activity (data not shown). The potential antioxidant activity of the plant extracts was assessed on the basis of the scavenging activity of the stable DPPH free radical, and all the fractions, except the hexanic, reduced this radical in the same way as did the reference compound (Table 2).

Superoxide scavenging was only observed with the dichloromethane and butanol fractions. The effect observed in this test was not related to the inhibition of

xanthine oxidase activity, except in the case of the ethyl acetate fraction, which was able to inhibit the activity of the enzyme.

The extracts were unable to scavenge the hydroxyl radical produced in the $Fe^{3+}$-EDTA + $H_2O_2$ system in the presence of ascorbate. The inclusion of ethyl acetate and butanol extracts in the reaction mixture increased deoxyribose degradation in this system, both in the presence and absence of ascorbate (data not shown).

## Discussion

In this study, the anti-inflammatory activity of the methanol extract from *Helichrysum italicum* and its four sub-extracts was demonstrated in different experimental models of inflammation. The most active extract was dichloromethane in the chronic TPA test, ethyl acetate in the EPP and serotonin tests and butanol in the acute TPA and $PLA_2$ tests.

Interesting data were obtained from the EPP test. The total methanol extract showed no inhibitory activity and the two extracts with less polarity (hexane and dichloromethane) increased the oedema, whereas the two polar extracts significantly reduced the oedema formation. These data suggest the possible presence of compounds in the ethyl acetate and butanol extracts which have a mechanism of action similar to that of corticoids. This would explain why the total extract (methanol) was not effective in this experimental model of inflammation.

In the acute TPA assay all the extracts inhibited formation of the oedema, the effect noted being similar in part to that obtained in the EPP test. In fact, the ethyl acetate and butanol extracts were once again the most effective, but in this case the extracts with less polarity were also active, though to a lesser extent than the methanol extract. In two previous papers, the anti-oedematous effect of ursolic acid and 4′-hydroxy-3′-(3-methyl-2-butenyl)acetophenone isolated from *H. stoechas* in the TPA test (Recio et al 1991) was reported, as was the inhibition of leucocyte eicosanoid generation and the radical scavenging activity of gnaphaliin (de la Puerta et al 1999). These compounds are present in the dichloromethane extract, and appear to be among the constituents implicated in these pharmacological properties. With respect to the ethyl acetate and butanol extracts, their activity may be due to the glycosidic flavonoids and acetophenones detected as major compounds.

All the extracts inhibited the paw oedema induced by serotonin and $PLA_2$. The paw oedema induced by $PLA_2$ is mediated by mast cell degranulation and histamine/serotonin release. For this reason, anti-$PLA_2$ drugs which block the effect of the enzyme against the membrane phospholipids and $H_1$-antihistamine and anti-serotonin drugs are effective in this test. In our study all the extracts were active, while the methanol extract was again the most effective and only the butanol fraction had a similar effect. However, in the serotonin-induced paw oedema, the ethyl acetate fraction was the most active.

The methanol extract clearly inhibited the chronic inflammation induced after application of TPA on the mouse ear, but had no effect on acute arachidonic-acid-induced ear oedema and the DTH reaction induced by oxazolone or DNFB. However, SRBC-DTH was clearly decreased by the methanol extract and some of its fractions which we assayed. All the fractions improved the results obtained with the total extract at 18 and 24 h, but only the butanol fraction was clearly more effective at 48 h. In addition, this extract showed similar effects at the different times points assayed.

Taken together, these results suggest that the anti-inflammatory activity of *H. italicum* is due to different compounds, some of which are polar principles and are present in the butanol fraction. In addition, they seem to have some corticoid-related mechanisms. A direct interaction of some compounds with $PLA_2$ or serotonin receptor could be considered for further study.

The role of oxygen-derived free radicals in the inflammatory process is well known. Free radicals are implicated in the activation of nuclear factor $\kappa$B (NF$\kappa$B) and p38-mitogen-activated protein kinase, which induces the transcription of pro-inflammatory enzymes such as cyclooxygenase 2 and nitric oxide synthase, and inflammatory cytokines such as interleukin-1$\beta$ (IL-1$\beta$), IL-2 and tumour necrosis factor $\alpha$ (TNF$\alpha$). On the other hand NF$\kappa$B is itself activated by some of these cytokines (Winrow et al 1993; Sahnoun et al 1998; Bowie & O'Neill 2000; Schoonbroodt & Piette 2000).

Previously, we showed that the methanol extract inhibits the enzymatic and non-enzymatic lipid peroxidation in model membranes and also acts as a superoxide radical scavenger (Schinella et al 2002). In this study, we demonstrated that all the fractions significantly inhibited the microsomal lipid peroxidation. The hexane extract was a weak scavenger of the stable DPPH radical, but had no scavenging effect on anion superoxide and hydroxyl radicals. The dichloromethane and butanol extracts showed significant scavenging activity on superoxide anion and DPPH radicals. The ethyl acetate extract also showed antioxidant activity against the DPPH radical. When examined in the $Fe^{3+}$-

EDTA+$H_2O_2$ system, the butanol and ethyl acetate extracts increased deoxyribose degradation in the presence and absence of ascorbate. The ability to stimulate deoxyribose degradation as a result of $Fe^{3+}$ reduction would indicate the pro-oxidant properties of some compounds present in the extracts, probably due to redox cycling of the iron (Aruoma et al 1992; Puppo 1992). In a previous paper, Facino et al (1990) described the radical scavenger activity of three flavonoids isolated from *H. italicum*, tetrahydroxychalcone-2′-glucoside, kaempferol-3-glucoside and naringenin-glucoside, all of which inhibited lipid peroxidation induced by ADP/$Fe^{2+}$ or $CCl_4$ in the presence of NADPH, while the total extract was clearly more active than the isolated flavonoids. We obtained the most notable effect in the $CCl_4$/NADPH system when the dichloromethane and ethyl acetate extracts were assayed. Cytochrome P450-linked activities are involved in NADPH-induced microsomal lipid peroxidation. The extracts did not inhibit the hydroxylation of *p*-nitrophenol, which suggests that the preventive action of the extracts are due to their inhibition of the initiation of lipid peroxidation by acting as scavengers of free radical species.

### Conclusions

The anti-inflammatory activity of our plant extracts could be due to the synergistic effect of pro-inflammatory enzyme inhibition, free radical scavenging activities or corticoid-like effects. The results of this study suggest that the anti-inflammatory activity of the same extracts can be explained, at least in part, by their antioxidant properties. Although the active principles responsible for the antioxidant activity of the tested extracts have not yet been identified, we propose these extracts as a useful source of compounds that help to increase the overall antioxidant capacity of an organism, and thereby protect it against lipid peroxidation induced by oxidative stress.

## References


Aruoma, O. I., Halliwell, B., Aeschbach, R., Löligers, J. (1992) Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica* **22**: 257–268

Bowie, A., O'Neill, L. A. J. (2000) Oxidative stress and Nuclear Factor-κB activation. *Biochem. Pharmacol.* **59**: 13–23

Bradford, M. M. (1976) A rapid and sensitive method for quantification of micrograms quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254

de la Puerta, R., Forder, R. A., Hoult, J. R. S. (1999) Inhibition of leukocyte eicosanoid generation and radical scavenging activity by gnaphaliin, a lipophilic flavonol isolated from *Helichrysum picardii*. *Planta Med.* **65**: 507–511

Facino, R. M., Carini, M., Franzoi, L., Pirola, O., Bosisio, E. (1990) Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). *Pharm. Res.* **22**: 709–721

Góngora, L., Máñez, S., Giner, R. M., Recio, M. C., Ríos, J. L. (2000) On the activity of trifluoperazine and palmitoylcarnitine in delayed hypersensitivity models. *Life Sci.* **66**: 183–188

Lee, R. P., Forkert, P. (1994) *In vitro* biotransformation of 1,1-dichloroethylene by hepatic cytochrome P-450 2E1 in mice. *J. Pharmacol. Exp. Ther.* **270**: 371–376

Máñez, S., Alcaraz, M. J., Payá, M., Ríos, J. L., Hancke, J. L. (1990) Selected extracts from medicinal plants as anti-inflammatory agents. *Planta Med.* **56**: S656

Neves, P. C. A., Neves, M. C. A., Bella Cruz, A., Sant'ana, A. E. G., Yunes, R. A., Calixto, J. B. (1993) Differential effects of *Mandevilla velutina* compounds on paw oedema induced by phospholipase $A_2$ and phospholipase C. *Eur. J. Pharmacol.* **243**: 213–219

Peris, J. B., Stübing, G., Vanaclocha, B. (1995) *Fitoterapia aplicada.* MICOF, Valencia, pp 470–471

Puppo, A. (1992) Effect of flavonoids on hydroxyl radical formation by Fenton type reactions; influence of the iron chelator. *Phytochemistry* **31**: 85–88

Recio, M. C., Giner, R. M., Terencio, M. C., Sanz, M. J., Ríos, J. L. (1991) Anti-inflammatory activity of *Helichrysum stoechas*. *Planta Med.* **57**: A56–57

Recio, M. C., Giner, R. M., Uriburu, L., Máñez, S., Cerdá, M., De la Fuente, J. R., Ríos, J. L. (2000) *In vivo* activity of pseudoguaianolide sesquiterpene lactones in acute and chronic inflammation. *Life Sci.* **66**: 2509–2518

Sahnoun, Z., Jamoussi, K., Zeghal, K. M. (1998) Free radicals and antioxidants: physiology, human pathology and therapeutic aspects. *Therapie* **53**: 315–339

Schinella, G. R., Troiani, G., Davila, V., de Buschiazzo, P. M., Tournier, H. A. (2000) Antioxidant effects of aqueous extract of *Ilex paraguariensis*. *Biochem. Biophys. Res. Com.* **269**: 357–360

Schinella, G. R., Tournier, H. A., Prieto, J. M., Mordujovich de Buschiazzo, P., Ríos, J. L. (2002) Antioxidant activity of anti-inflammatory plant extracts. *Life Sci.* **70**: 1023–1033

Schoonbroodt, S., Piette, J. (2000) Oxidative stress interference with the nuclear factor-κB activation pathways. *Biochem. Pharmacol.* **60**: 1075–1083

Winrow, V. R., Winyard, P. G., Morris, C. J., Blake, D. R. (1993) Free radicals in inflammation: second messengers and mediators of tissue destruction. *Br. Med. Bull.* **49**: 506–522