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Fitoterapia 75 (2004) 557–565

FITOTERAPIA

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Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*

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Received 1 July 2003; accepted in revised form 11 May 2004
Available online 4 August 2004

Abstract

Oral administration (200 mg/kg) of *Foeniculum vulgare* fruit methanolic extract exhibited inhibitory effects against acute and subacute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect. Moreover, it significantly increased the plasma superoxide dismutase (SOD) and catalase activities and the high density lipoprotein–cholesterol level. On the contrary, the malondialdehyde (MDA) (as a measure of lipid peroxidation) level was significantly decreased in *F. vulgare* fruit methanolic extract group compared to the control group ($P<0.05$). These results seems to support the use of *F. vulgare* fruit methanolic extract in relieving inflammation.

Keywords: *Foeniculum vulgare*; Antiinflammatory activity; Analgesic activity; Antioxidant activity

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic or antipyretic agents for the clinical treatment of inflammatory diseases such as arthritis, lumbago and rheumatism. These agents exhibit an inhibitory action on the cyclooxygenase

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doi:10.1016/j.fitote.2004.05.005

that catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid. It has been also reported that reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues [1].

In skin, ROS can be produced not only by chemical ionization and/or UV radiation but also enzymatically by polymorphonuclear leukocytes that infiltrate the sites of infection [2]. In both cases, the excessively produced ROS can injure cellular biomolecules such as nucleic acids, proteins, carbohydrates and lipids, causing cellular and tissue damage, which in turn augments the state of inflammation [3,4].

Although the synthesis of leukotrienes and prostaglandins has been reported to be involved in arachidonic acid- and TPA-induced ear edema [5], it has been assumed that ROS also play an important role in the edema formation in these models. As evidence in support of this hypothesis, several antioxidative compounds have been reported to show anti-inflammatory action either on arachidonic acid-induced ear edema or on TPA-induced ear edema [6]. The result that CX-659-052, which showed no antioxidative activity in vitro, failed to inhibit arachidonic acid-induced ear edema [7] is an additional data supporting the correlation between reactive oxygen species and edema formation.

In addition to their role in acute inflammation, ROS may also contribute to several chronic cutaneous inflammatory diseases such as psoriasis, atopic dermatitis, and contact dermatitis [8]. In a chronological sequence of reactions, various cytokines, which participate in the pathogenesis of inflammatory reactions, are produced. Therefore, compounds that have scavenging activities toward these radicals and/or suppressive activities on lipid peroxidation may be expected to have therapeutic potentials for several inflammatory diseases [1]. Cyclooxygenase products are well known to play an important role in pain, and it has been reported that extracellular calcium is involved in the formation of prostaglandins [9,10]. Prostaglandins have been reported to act as a Ca^{2+} ionophore, through L-type voltage-sensitive calcium channels in brain synaptosomes [11]. Peripheral sensory stimulation has been reported to induce prostaglandin release from the cerebral cortex [12].

Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side-effects are required to substitute chemical therapeutics.

Foeniculum vulgare Mill. has been known as a medicinal and aromatic herb and its fruit is commonly used as a natural remedy against digestive disorders [13]. It is also used to flavor foods, liqueurs and in the perfumery industry. Essential oils are mainly concentrated in the mericarps (fruits) and provide the unique aroma and taste. They are composed of several monoterpenes and phenylpropanoids, where *trans*-anethole, estragole, fenchone and limonene exist as main constituents. *trans*-Anethole, often the most prevalent constituents, counts for the anise taste, fenchone provides the bitterness, and estragole (methylchavicol) the sweetness [14]. The present study was carried out to evaluate the effect of *F. vulgare* fruit methanolic extract (*F. vulgare* FME) on

inflammatory reactions and nociception. Moreover, we also evaluated the effect of the extract on the antioxidant defense systems.

2. Experimental

2.1. Plant material

F. vulgare fruits, collected from the Biofarmaka Research Center of Bogor Agricultural University (Indonesia), in July 2001, were identified by Dr. I. Latifah, Department of Pharmacy.

2.2. Preparation of extract

Dried and powdered *F. vulgare* fruits were soaked in 80% MeOH. The methanolic extract was filtered and evaporated in vacuo to give a residue (*F. vulgare* FME) (yield: 6.4% w/w).

2.3. Animals

Male ICR mice (6 weeks old), BALB/c mice (7 weeks old) and Sprague–Dawley rats (5 week old) were purchased from Jungang Lab Animal (Korea). Animals were maintained under constant environmental conditions and fed a standard laboratory diet (Jungang Lab Animal) with water ad libitum. All procedures used in these studies were approved by the Ethical Committee of the Medical School of Yonsei University (Korea).

2.4. Antiinflammatory activity

Test samples were prepared by suspending them in 0.5% sodium carboxymethyl cellulose (CMC) solution. Mice were administered orally with *F. vulgare* FME at dose 200 mg/kg, while control group received 10 mg/kg of 0.5% CMC. Indomethacin (10 mg/kg) was used as standard.

2.4.1. Carrageenan-induced paw edema

The method of Winter et al. [15] was used. One hour after oral *F. vulgare* FME administration, edema was induced by injecting in the right hind paw 0.02 ml of 1% λ-carrageenan in sterile saline. The pad thickness of hind paw was measured with a Dial Thickness Gauge (Mitutoyo, Japan) before and at 1 and 3 h after carrageenan injection. Indomethacin (10 mg/kg) was used as a standard.

2.4.2. Arachidonic acid-induced ear edema assay

The *F. vulgare* FME at dose of 200 mg/kg was orally administered 1 h prior to the topical application of 2% arachidonic acid in acetone (0.02 ml/ear) to right ear of mice. The ear thicknesses were measured using a Dial Thickness Gauge before and at 1 and 3 h after arachidonic acid treatment [16]. Indomethacin (10 mg/kg) was used as a standard.

2.4.3. Formaldehyde-induced arthritis assay

Formaldehyde (2% v/v) solution, 0.02 ml, was injected in the first and third day into the left hind paw just beneath the plantar aponeurosis to induce arthritis. *F. vulgare* FME were administered orally, once a day, for 7 days at the dose of 200 mg/kg. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured according to the method described by Reitman and Frankel [17]. Indomethacin (10 mg/kg) was used as a standard.

2.4.4. Type IV anti-allergic activity

Anti-allergic activity (type IV) was tested using 2,4-dinitrofluoro benzene (DNFB)-induced contact hypersensitivity reaction [18]. The dorsal skin of the mice was shaved the day before the experiment, and the mice were sensitized with 0.02 ml of 0.5% DNFB in acetone–olive oil (4:1) applied to the dorsal skin on days −1 and 0. *F. vulgare* FME (200 mg/kg) were administered orally once a day for 7 days. At the end of treatment, the mice were challenged on the right ear with 0.25% DNFB in acetone–olive oil (4:1) (0.02 ml/ear). Ear thickness was measured with Dial Thickness Gauge before and 24 h after the DNFB challenge, and the differences in the thickness were calculated. The degree of ear swelling was expressed as increase in ear thickness (mm).

2.5. Thermal nociception

The analgesic activity was determined using the hot-plate test in mice according to Eddy and Leimback [19]. Mice were placed in a glass beaker placed on a hot plate maintained at 55 °C 1 h after oral administration of *F. vulgare* FME at the dose of 200 mg/kg. Latency to exhibit the nociceptive response such as licking paws or jumping was determined. A cut-off time of 40 s was selected to avoid tissue damage.

2.6. Plasma antioxidant system and lipid levels in rats

Experimental design: two groups of seven rats each were used: the CMC (control group) and *F. vulgare* FME (FV group). Animals of the FV group were treated, once a day, with 200 mg/kg, while the control group was treated with CMC (10 ml/kg) After 21 days, blood was collected into heparinized tubes from rats fasted during overnight. Samples were immediately centrifuged at 1500 rev./min for 5 min and the plasma was separated. Superoxide dismutase, catalase, malonedialdehyde, triglyceride, and cholesterol levels were determined as follow.

2.6.1. Measurement of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol [20]. At 25 °C and 320 nm, the rate of pyrogallol oxidation was recorded with a Shimadzu UV 1201 spectrophotometer (Shimadzu). Activity was expressed as the amount of enzyme that inhibits the oxidation of pyrogallol by 50%, which is equal to 1 unit. The result for SOD activity was expressed as U/mg protein.

2.6.2. Measurement of catalase activity

Catalase activity was determined according to Beutler [21]. Briefly, 1 mol/l Tris–HCl, 5 mol/l EDTA (pH 8) and 10 mol/l H_2O_2 were added to plasma and the mixture was incubated at 37 °C. The change in absorbance of the system at 230 nm was followed for 10 min. The result for catalase activity was expressed as the mol of H_2O_2 degraded per min/mg/proteins. Proteins were determined according to Read and Northcole [22] using bovine serum albumin as a standard.

2.6.3. Lipid peroxidation intermediates

Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA). levels on the base of reaction with thiobarbituric acid according to Buege and Aust [23]. Briefly, 0.2 ml of serum was mixed with thiobarbituric acid reagent (0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 N HCl). The reaction mixture of serum and thiobarbituric acid reagent was placed in boiling water for 15 min, cooled, centrifuged, and then the optical density of the supernatant was recorded at 532 nm. A standard curve was obtained with a known amount of 1.1.3.3.-tetraethoxypropane, using the same assay procedure.

2.6.4. Triglyceride and cholesterol levels

The concentrations of triglyceride (TG), total cholesterol and HDL-cholesterol in plasma were determined enzymatically using commercial available kit reagents (Boehringer, Mannheim, Germany). LDL-cholesterol was calculated by Friedewald formula: LDL cholesterol=total cholesterol−HDL cholesterol−TG/5 [24].

2.7. Statistical analysis

The results are expressed as mean±S.E.M. (n=7). Statistical significance was determined by analysis of variance (P<0.05). The analysis was performed using SAS statistical software.

3. Results and discussion

The anti-inflammatory activity of *F. vulgare* FME was evaluated by three screening protocols widely used for testing the non-steroidal anti-inflammatory drugs (NSAIDs); namely, carrageenan-induced paw edema, arachidonic acid-induced ear edema and formaldehyde-induced arthritis.

For the acute inflammation, *F. vulgare* FME at dose of 200 mg/kg caused a significant inhibition of paw edema (69%) as compared to the control group 3 h after carragenan injection (Fig. 1).

F. vulgare FME also inhibited by approximately 70% the ear-edema induced by arachidonic acid in mice (Fig. 2).

These overall results seems to suggest that *F. vulgare* FME may act on both the cyclooxygenase and lipoxygenase pathways.

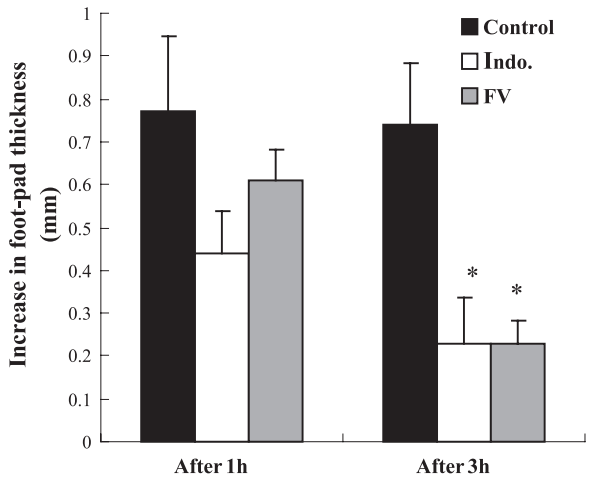

Fig. 1. Effect of *F. vulgare* FME on carrageenan-induced paw edema in rats. FV: *F. vulgare* FME 200 mg/kg; Indo: indomethacin 10 mg/kg. Results are mean±S.E.M. (n=7). *P<0.05 vs. control.

As a result of inflammation induced by formaldehyde, the level of serum transaminase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is increased. Accordingly, the assessment of the level of AST and ALT provides a good and simple tool to measure the anti-inflammatory activity of the target compounds [25].

As shown in Fig. 3, plasma ALT activity of group given *F. vulgare* FME was significantly lower than that of control group, while AST activity was not significantly affected.

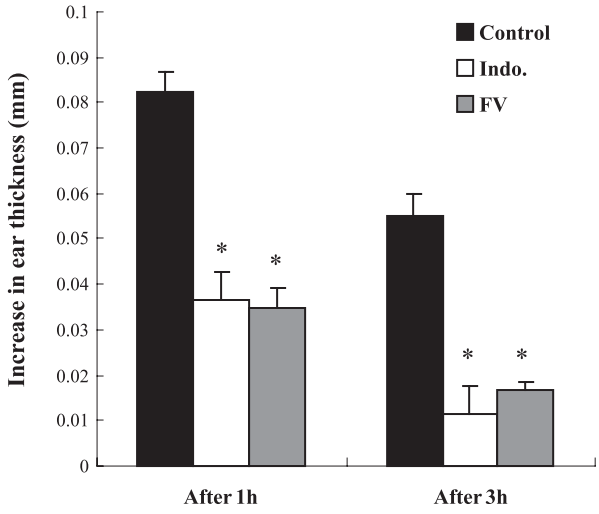

Fig. 2. Effect of *F. vulgare* FME on arachidonic acid-induced ear edema in mice. FV:*F. vulgare* FME 200 mg/kg; Indo: indomethacin 10 mg/kg. Results are mean±S.E.M. (n=7). *P<0.05 vs. control.

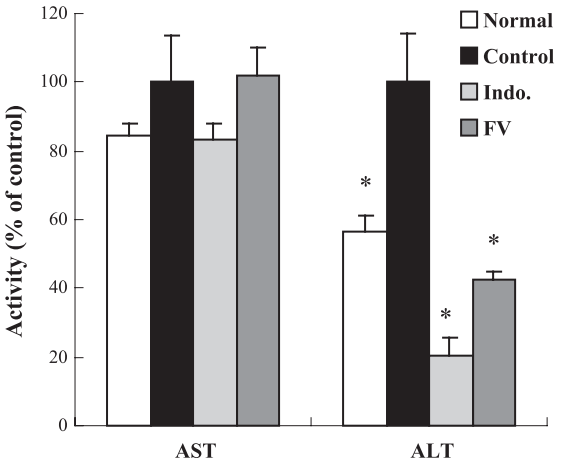

Fig. 3. Antiinflammatory activity of *F. vulgare* FME in formaldehyde-induced arthritis in rats. FV: *F. vulgare* FME 200 mg/kg; Indo: indomethacin 10 mg/kg. Results are mean±S.E.M. (n=7). *P<0.05 vs. control, Fig. 1.

We further investigated the effect of *F. vulgare* FME on DNFB-induced delayed-type hypersensitivity (type IV allergy). It showed significant inhibitory effect on DNFB-induced delayed-type hypersensitivity after oral administration of 200 mg/kg once a day For 7 days (Table 1). The inhibitory effect on immunologically induced swelling suggests the possible immunosuppressive properties of *F. vulgare* FME.

The result of Table 1 also indicates that oral administration 200 mg/kg *F. vulgare* FME significantly reduced the hot-plate thermal stimulation. Hot-plate test is normally used to study the central analgesic effects of drugs. Therefore, it is probable that *F. vulgare* FME could be producing their analgesic effects centrally or, as already known for NSAIDs [26], it inhibited the pain receptors at the inflammation site. Further studies are necessary to fully elucidate the mechanism of action of the analgesic activity of the *F. vulgare* FME.

After 3 weeks of administration of 200 mg/kg/day of *F. vulgare* FME to rats, SOD and catalase activities were increased significantly while lipid peroxidation (measured as TBARS) was decreased significantly. An increase in the antioxidant enzyme activity and a reduction in the lipid peroxidation by *F. vulgare* FME may result in reducing a number of deleterious effects due to the accumulation of oxygen radicals, which could exert a beneficial action against pathological alterations, especially in inflammatory diseases.

Table 1
Effect of *F. vulgare* FME on delayed type hypersensitivity and thermal nociception in mice

Group	Delayed type hypersensitivity		Thermal nociception	
	Increase in ear thickness (mm)	% Inh.	Latency (s)	% Control
Control	0.16±0.04	–	19.5±1.77	–
F. vulgare FME (200 mg/kg)	0.01±0.01*	94	34.3±4.54*	176

Results are mean±S.E.M. (n=7).
* P<0.05 vs. control.

Table 2
Effects of *F. vulgare* FME on antioxidant enzymes, malondialdehyde and lipid levels in the plasma of rats

	Group	
	Control	*F. vulgare* FME[a]
Superoxide dismutase (U/mg protein)	10.8±0.167	19.867±0.011*
Catalase (nmol H_2O_2 degraded/min/mg protein)	10.14±3.43	33.41±4.05*
Malondialdehyde (nmol/ml)	1.2±0.09	0.696±0.147*
Triglyceride (mg/100 ml)	2.278±0.054	2.267±0.126
Total cholesterol (mg/100 ml)	233.766±25.25	221.591±1.61
LDL cholesterol (mg/100 ml)	231.7±25.22	219.397±1.56
HDL cholesterol (mg/100 ml)	1.611±0.029	1.741±0.027*

Results are mean±S.E.M. (n=7).
[a] Animals were treated with 200 mg/kg of extract once a day for 21 days.
* P<0.05 vs. control.

Table 2 also showed the plasma lipid profiles following administration of *F. vulgare* FME at 200 mg/kg in rats. No significant difference in concentration of TG, total- and LDL-cholesterol was found. On the contrary, HDL-cholesterol were significantly increased in the *F. vulgare* FME group. This effect of HDL may be due in part to an inhibition of the oxidative modification of LDL [27].

In conclusion, results of the present study showed a significant anti-inflammatory, anti-type IV allergic and central analgesic activities *F. vulgare* FME when given at dose of 200 mg/kg in mice and rats. Moreover, we showed that plasma antioxidant enzyme activities, lipid peroxidation and HDL cholesterol levels are affected by administration of *F. vulgare* FME in rats. Therefore, we can speculate that *F. vulgare* fruit methanolic extract may reduce the risk of inflammation-related diseases.

Acknowledgements

The work was supported by the Korea Research Foundation Grant (KRF-2002-037-F00013).

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