# Antioxidant and Antiinflammatory Activities of Oregano Extract

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**The antioxidant activity of the crude extract prepared from oregano (*Origanum vulgare* L.) and its antiinflammatory activities in mouse models of stress-induced gastritis and contact hypersensitivity were investigated. Oregano extract was a substrate for peroxidase, similar to phenol. Oregano extract exhibited iron-reducing activity, although its strength was approximately one-fifth of that of ascorbic acid. Oral administration of oregano extract significantly prevented mouse gastritis induced by cold-restraint stress. Percutaneous administration of oregano extract also significantly prevented mouse contact hypersensitivity induced by oxazolone. These antiinflammatory activities of oregano extract tended to be weaker than those of hydrocortisone. The antioxidant activities of oregano extract appear to contribute to its preventive effects against inflammatory diseases, such as stress-induced gastritis and contact hypersensitivity in mice.**

**Key words** ——— oregano, mouse, inflammation, gastritis, contact hypersensitivity, peroxidase

## INTRODUCTION

Recently, increases in the incidence of various inflammatory diseases, such as digestive tract ulcers, due to lifestyle changes including dietary habits, have become serious social problems. Hydrochloric acid and digestive enzymes, such as pepsin, in gastric juice and *Helicobacter pylori*, bacteria living in the gastric mucosa, are direct causative factors responsible for human gastric ulcers, chronic gastritis, and/or gastric cancer.[1–3] Injury of the cells of the mucous membranes may provoke the production of active oxygen species, such as nitrogen monoxide and superoxide anion radicals, by macrophages and neutrophils permeating into them. Active oxygen species can directly injure the surrounding cells and extracellular matrices, such as hyaluronic acid, and produce lipid peroxides and metabolites of arachidonic acid. In general, active oxygen species are thought to promote inflammation through these processes.[4] It was reported that some active oxygen species produced in the Fenton reaction or Harber-Weiss reaction, such as superoxide anion radicals and hydrogen peroxide, play important roles in the inflammatory process.[5] Antioxidants, such as superoxide dismutase and tea catechins, are known to suppress inflammation in a rat arthritis model and in a mouse model of contact hypersensitivity.[6,7] Some antioxidants can not only directly scavenge radicals but also act as electron donors for various peroxidases (PODs), which catalyze the decomposition of hydrogen peroxide. Glutathione POD is a well-known internal POD, and salivary POD (s-POD) and microperoxidase (m-POD) are known to be present in the digestive tract. The usual substrates for these enzymes are thought to be glutathione for glutathione POD and $SCN^-$ for s-POD and m-POD in the mouth, and $Cl^-$ for s-POD and m-POD in the stomach. Additional dietary electron donors are desirable, especially for s-POD and m-POD.

Many herbs ingested with ordinary meals are known to include some antioxidant components. Oregano (*Origanum vulgare* L.) is one such herb, which is known to include many effective antioxidants, such as rosmarinic acid, caffeic acid, and various flavonoids.[8] These compounds also function as substrates for POD.

In the present study, the antioxidant activity of the crude extract prepared from oregano and its antiinflammatory activities were investigated in mouse models of stress-induced gastritis and contact hypersensitivity.

## MATERIALS AND METHODS

**Materials and Animals** ——— Horseradish POD and oxazolone (4-ethoxymethylene-2-phenyl-oxazolin-5-one), which is commonly used as a sensitizer for type IV allergy, were purchased from Wako

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Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals used were of reagent grade. Samples of 10 g of commercial oregano leaves were comminuted with a food mill, added to 90 ml of 60%(v/v) ethanol, and shaken at 50°C for 1 hr. The mixture was centrifuged at 5000 rpm for 10 min. The supernatant was evaporated *in vacuo* and lyophilized to obtain oregano extract. Four-week-old male ddY mice and male ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan). Mice were maintained under conventional conditions throughout the experimental period and given tap water and commercial laboratory chow (5L37; Japan SLC Inc.) *ad libitum*.

**Measurement of Activity as Substrate for POD**[9] ——— The reaction mixture consisted of 2.0 mg/ml of oregano extract, horseradish POD 0.1 U/ml, hydrogen peroxide 0.35 mM, and 4-aminoantipyrine 0.25 mM in phosphate buffer 84 mM (pH 7.0), in a total volume of 1.0 ml. The reaction mixtures were incubated at 37°C for 10, 20, or 30 min. To the reaction mixtures was added 1 N hydrochloric acid 100 $\mu$l to stop the reaction, and the absorbance at 500 nm was determined immediately. Phenol was assayed as a positive control. The concentration of phenol was 0.235 mg/ml in the reaction mixture. The concentration of oregano extract used in this experiment was rather high, because the extract was crude and the contents of active components in it were thought to be low. For the blank, phosphate buffer was added instead of oregano extract and/or phenol.

**Measurement of Antioxidant Activity**[10] ——— The reaction mixture consisted of $1.0 \times 10^{-3}$–$6.6 \times 10^{-2}$ mg/ml of oregano extract, 0.32%(w/v) $\alpha,\alpha'$-dipyridyl/ethanol, and 0.12%(w/v) ferric chloride in acetate buffer 2.13 M (pH 3.3), in a total volume of 3.75 ml. The mixture was stored at room temperature for 30 min, and the absorption at 525 nm was determined to estimate the iron-reducing activity of oregano extract. The well-known iron reductant ascorbic acid was also assayed. The concentration of ascorbic acid was $4.7 \times 10^{-4}$–$9.4 \times 10^{-3}$ mg/ml in the reaction mixture.

**Mouse Model of Stress-Induced Gastric Inflammation**[11] ——— Four-week-old male ddY mice were starved for 24 hr but given tap water *ad libitum*. Oregano extract was dissolved in 0.5 ml of 0.5%(w/v) tragacanth gum solution and administered orally to mice with a gastric sonde at doses of 50, 100, and 200 mg/kg body weight. Mice were immobilized in stress cages and left at 4°C for 90 min under close observation to avoid death. After stress-loading, the mice were immediately killed under anesthesia with diethyl ether and the stomach was removed. The stomachs were incised in line with the greater curvature and the contents were washed out with chilled saline. Injury to the inner mucosa of the mouse stomach was examined, and the number of bleeding points was counted. A control experiment was performed with 0.5%(w/v) tragacanth gum solution alone. Hydrocortisone, a well-known steroid-type antiinflammatory agent, was administered orally to mice as a positive control at the same doses as oregano extract. The number of mice in each group was 8. Counting the number of bleeding points was a convenient and appropriate method to estimate the degree of injury in the mouse stomach, because the data obtained with this method showed a significant positive correlation with the data obtained using the ranking method reported by Desai *et al.*[12] in 93 random samples of mice subjected to cold-immobilization stress ($\gamma = 0.77$, $p < 0.05$, F-test).

**Mouse Model of Contact Hypersensitivity** ——— Contact hypersensitivity was induced by the following procedures.[13,14] The hair of the abdominal region of the mice was shaved off carefully, and 0.1 ml of 0.5%(w/v) oxazolone solution in ethanol was applied to the skin. This operation was called sensitization. Five days after sensitization, 20 $\mu$l of 0.5%(w/v) oxazolone solution in acetone was applied to both sides of the mouse right ear. This operation was called challenge. Twenty-four hours after the challenge, the mice were killed under anesthesia with diethyl ether. Circular parts (5.0 mm in diameter) were removed from both ears using a punching apparatus, and the right ear (WR) and left ear (WL) specimens were weighed.

For percutaneous administration, oregano extract was dissolved in oxazolone/acetone solution at the challenge, and doses of 0.10 or 0.20 mg/ear were adminitered. Hydrocortisone was also assayed at the same doses. A control experiment was performed with only the solvents used for dissolution of the samples. The number of mice in each group was 8.

The ear swelling ratio was calculated using the following equation:

$$\text{Ear swelling ratio (\%)} = \frac{\{(\text{WR sample} - \text{WL sample})/\text{WL sample}\}}{\{(\text{WR control} - \text{WL control})/\text{WL control}\}} \times 100.$$

**Statistics** ——— Statistical analyses were performed with the nonparametric Mann-Whitney U-test for differences between the groups, and $p < 0.05$ was

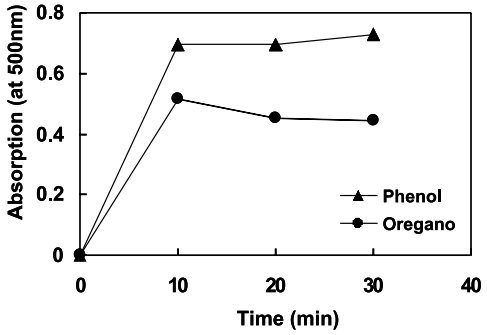


**Fig. 1.** Activities of Oregano Extract and Phenol as Substrates for Horseradish Peroxidase

Concentrations of phenol and oregano extract used in this study were 0.235 and 2.0 mg/ml, respectively. Values are means of three independent experiments.

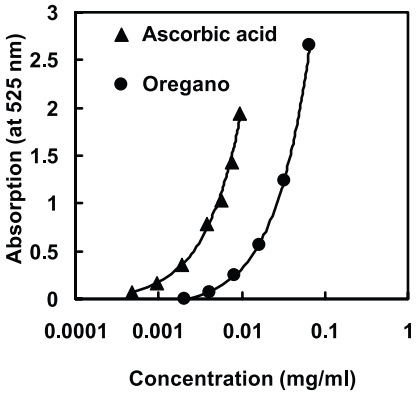


**Fig. 2.** Antioxidant Activity of Oregano Extract

Iron-reducing activities of ascorbic acid and oregano extract. Values are means of three independent experiments.

considered statistically significant.

## RESULTS AND DISCUSSION

### Activity of Oregano Extract as Substrate for POD

The yield of the extract from oregano leaves was 33.4%(w/w). The activities of oregano extract as a substrate for POD are shown in Fig. 1. At 30 min, the absorbance of oregano extract was 60.6% of that of phenol. We also examined the activities of the extracts of two other herbs, laurel (*Laurus nobilis*) and marjoram (*Origanum majorana*). Their absorbance at 30 min were 28.1 and 25.8% of that of phenol, respectively (data not shown). Therefore oregano extract was suggested to be an electron donor for POD and is also expected to be one of the substrates of s-POD and m-POD in the digestive tracts of animals.

### Antioxidant Activity of Oregano Extract

The iron-reducing activities of oregano extract and ascorbic acid are shown in Fig. 2. Oregano extract exhibited concentration-dependent antioxidant activity, although the activity of oregano extract was lower than that of ascorbic acid. The concentrations that showed the same absorption, *i.e.*, 1.5, at 525 nm were $3.9 \times 10^{-2}$ mg/ml of oregano extract and $7.6 \times 10^{-3}$ mg/ml of ascorbic acid, respectively. This activity of oregano extract was thought to be due to its phenolic constituents. These results indicate effective antioxidant activitiy of oregano extract as an iron reductant and an electron donor for POD. Its antiinflammatory activity was also investigated in two mouse models.

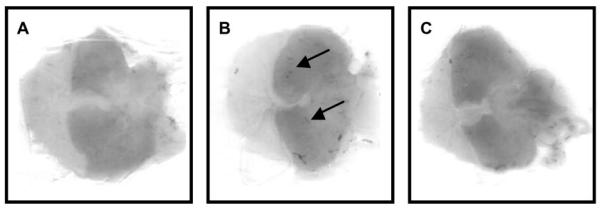


**Fig. 3.** Inhibitory Effects of the Oral Administration of Oregano Extract on Mouse Gastritis Induced by Cold-Restraint Stress

A, Normal; B, control; C, oregano extract-supplemented mouse stomach. Dose of oregano, 200 mg/kg body weight. Arrows show the bleeding points.

### Inhibitory Effects of Oregano Extract on Stress-Induced Gastric Inflammation in Mice

In this study, cold-restraint stress was used to promote mouse gastritis, because it did not require induction with agents, such as ethanol and hydrochloric acid, thus excluding any direct interactions between the extract and inflammation-inducing agents. The inhibitory effects of oregano extract on mouse gastric inflammation are shown in Figs. 3 and 4. As shown in Fig. 3, many bleeding points (indicated by arrows) were observed in the gastric mucosa of mice subjected to cold-restraint stress, while no bleeding points were observed in normal healthy mice. Oral administration of oregano extract at a dose of 200 mg/kg body weight reduced the number of bleeding points in the gastric mucosa. As shown in Fig. 4, oral administration of oregano extract at doses of 50, 100, and 200 mg/kg body weight significantly reduced the number of bleeding points in the gastric mucosa of mice subjected to cold-restraint stress. The inhibition rates at these doses of oregano ex-

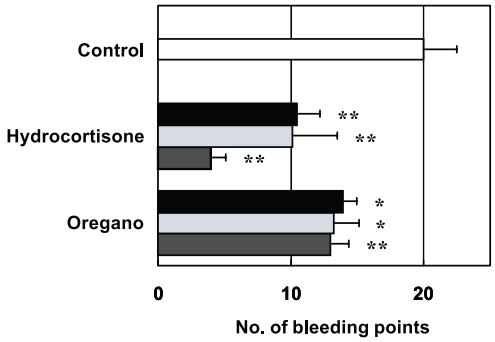


**Fig. 4.** Inhibitory Effects of the Oral Administration of Oregano Extract on Mouse Gastritis Induced by Cold-Restraint Stress

Values are mean ± SEM ($n$ = 8). Significant difference from the control: *$p$ < 0.05, **$p$ < 0.01. Dose: ■, 50 mg/kg body weight; □, 100 mg/kg body weight; ▩, 200 mg/kg body weight.

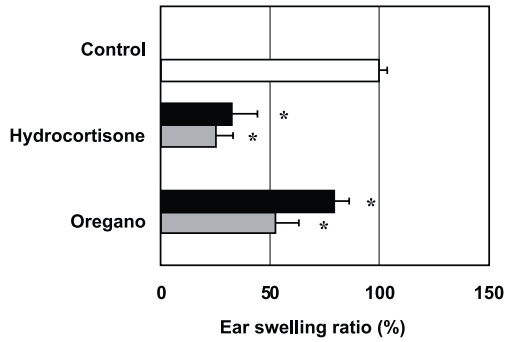


**Fig. 5.** Inhibitory Effects of the Percutaneous Administration of Oregano Extract on Mouse Contact Hypersensitivity Induced with Oxazolone

Values are mean ± SEM ($n$ = 8). Significant difference from the control: *$p$ < 0.01. Dose: ■, 0.10 mg/ear; ▩, 0.20 mg/ear.

tract were almost the same at 30.5, 34.0, and 35.0%, respectively. Oral administration of hydrocortisone, a positive control, at the same doses also significantly reduced the number of bleeding points in the gastric mucosa of mice subjected to cold-restraint stress. The inhibition rates at these doses were 47.5, 49.5, and 80.0%, respectively. The number of bleeding points in the gastric mucosa in mice administered hydrocortisone at a dose of 200 mg/kg body weight was significantly lower than that in mice treated with oregano extract at the same dose ($p$ < 0.01).

**Inhibitory Effects of Oregano Extract on Contact Hypersensitivity in Mice**

The inhibitory effects of oregano extract on mouse contact hypersensitivity are shown in Fig. 5. Percutaneous administration of oregano extract at doses of 0.10 and 0.20 mg/ear significantly reduced the ear swelling ratios in mice as compared with controls. The inhibition rates at these doses of oregano extract were 20.4 and 47.4%, respectively. The difference between these ear swelling ratios was significant ($p$ < 0.05). Hydrocortisone administered percutaneously at the same doses also significantly reduced the ear swelling ratios in mice as compared with controls. The inhibition rates at these doses of hydrocortisone were 67.4 and 74.7%, respectively. The ear swelling ratio in mice administered hydrocortisone at a dose of 0.10 mg/ear was significantly lower than that in mice administered oregano extract at the same dose ($p$ < 0.05).

Mouse contact hypersensitivity is known to be suppressed by tea catechins, which are strong antioxidants.[7] On the other hand, it was reported that the lipid peroxide levels in mice with gastritis induced by water immersion-restraint stress were increased as compared with those in normal controls.[15] This observation indicates a close relationship between the generation of stress-induced gastritis and the promotion of oxidative stress in mice. Therefore antioxidant activities of oregano extract were thought to contribute in part to the preventive effects against mouse contact hypersensitivity and mouse stress-induced gastritis observed in the present study. However, in this experiment on mouse contact hypersensitivity, there could have been some direct interactions between oxazolone and oregano extract in the mixed solution. Further investigations are necessary to clarify the active components in oregano extract responsible for its antiinflammatory activity and its mechanisms of action.

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