Antioxidant and Hepatoprotective Actions of the Medicinal Herb
Artemisia campestris from the Okinawa Islands

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The antioxidant action of Artemisia campestris was examined in vitro and in vivo. A water extract of A. campestris showed a strong scavenging action of 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radicals. When the extract was given intraperitoneally to mice prior to carbon tetrachloride (CCL4) treatment, CCL4-induced liver toxicity, as seen by an elevation of serum aspartate aminotransferase and alanine aminotransferase activities, was significantly reduced. Depression of the elevation of serum enzyme levels after CCL4 treatment was also observed by oral administration of the extract. In that case, CCL4-derived lipid peroxidation in the liver was decreased by the extract treatment. These results suggest that the extract of A. campestris scavenges radicals formed by CCL4 treatment resulting in protection against CCL4-induced liver toxicity.

Key words antioxidant; medicinal herb; carbon tetrachloride; free radical; Artemisia

Artemisia campestris L. (Japanese name; Ryukyuyomogi) grows wild in a seaside district in the Okinawa Islands and has traditionally been used as a folk medicine for the treatment of jaundice, diabetes, and kidney disorders. However, biological and pharmacological actions of the herb have not been studied well. As preliminary experiments, we screened the antioxidant action of various medicinal herbs by measuring the scavenging action of a stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and found that A. campestris has a strong antioxidant action.

Reactive oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases3–5 and the aging process.6–8 Thus, antioxidants which can scavenge ROS are expected to improve these disorders. It is known that carbon tetrachloride is biotransformed to a trichloromethyl radical by the cytochrome P450 system in liver microsomes, and consequently causes lipid peroxidation of membranes followed by liver injury.5–7 In the present study, the radical scavenging and hepatoprotective actions of A. campestris against carbon tetrachloride-induced liver injury were examined.

MATERIALS AND METHODS

Chemicals Reduced glutathione (GSH), glucose 6-phosphate, and β-nicotinamide adenine dinucleotide phosphate were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). 1-Chloro-2,4-dinitrobenzene (CDNB), DPPH and 2-thiobarbituric acid (TBA) were from Wako Pure Chemicals (Osaka, Japan). Glucose 6-phosphate dehydrogenase was obtained from Oriental Yeast (Tokyo, Japan). Aniline was used after distillation. All regents were of analytical grade.

Preparation of the Herbal Extract and Measurement of Radical Scavenging Activity Dried A. campestris was supplied by a company (Nakazen Co., Ltd.) which is cultivating medicinal herbs in Okinawa, Japan. One gram of the herb was extracted with 10 ml of water or ethanol at 37°C for 2 h and filtrated by filter paper. The filtrate thus obtained was used as the original herbal extract. The antioxidant activity of the extract was examined using the DPPH radical. The reaction mixture consisted of 1 ml of 0.1 mM DPPH in ethanol, 0.95 ml of 0.05 mM Tris–HCl buffer (pH 7.4), 1 ml of ethanol and 50 μl of the herbal extract or deionized water (control). The absorbance of the mixture was measured at 517 nm exactly 30 s after adding the extract.

The radical trapping ability of the extract from A. campestris for superoxide anion (O2·−) and hydroxyl radical (·OH) was determined by an electron spin resonance (ESR) spectrometer (JES-REIX, Tokyo, Japan), as described previously.9

Hepatoprotective Action of the Herbal Extract Mice (ddY, 8 weeks) from Nihon SLC, Co. (Shizuoka) were divided into three groups at random. The CCl4 group of mice were given carbon tetrachloride (CCl4; 2 ml/kg in 50% corn oil solution) subcutaneously (s.c.). In the herbal extract and CCl4-treated group, the herbal extract (5 ml/kg) with 50% DPPH scavenging action was given intraperitoneally (i.p.) 1 and 15 h before CCl4 treatment. Control mice were given corn oil (2 ml/kg, s.c.) in place of CCl4. Mice were killed by decapitation 72 h after CCl4 treatment after overnight starvation. To evaluate the effect of the herb alone on the parameters studied, an extract of A. campestris with 50% DPPH scavenging activity (5 ml/kg) or water was given to mice intraperitoneally twice at 1 and 15 h before corn oil (2 ml/kg, s.c.) injection, then the mice were killed 72 h after the last injection.

In the case of oral administration, the herbal extract (5 ml/kg) was given to mice by a gastric tube once a day for 3 d and 1 h before CCl4 (25 μl/kg, i.p., in corn oil solution) treatment. Control mice were given corn oil in place of CCl4. Mice were killed 20 h after CCl4 treatment by decapitation after overnight starvation. The herbal extract (5 ml/kg, p.o.) alone was given to mice for 3 d with corn oil in the place of CCl4.

In all cases the blood was collected from the stump and the serum was isolated by centrifugation. Enzyme activity in the serum was measured within 2 d after storage at 4°C. The liver was perfused with 1.15% potassium chloride solution.

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after removal, followed by homogenization in 3 volumes of 0.05 M Tris–HCl buffer (pH 7.4) with a teflon-glass homogenizer. The cytosol and microsomes were prepared as reported previously. All experiments were conducted in conformity with the guidelines set by the Animal Care and Use Committee of University of the Ryukyus.

**Measurement** The activity of GSH S-transferase for CDNB in liver cytosol and microsomes was measured by the method of Habig et al. Aniline hydroxylase activity in microsomes was evaluated by measuring p-aminophenol. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the serum were measured using an Assay Kit (Kainos, Tokyo). Lipid peroxide in liver homogenates was determined by the TBA assay according to the method of Buege and Aust with some modifications, and was expressed as malondialdehyde (MDA) equivalents as described previously. The protein concentration in each fraction of the liver was determined by the method of Lowry et al.

**Statistical Analyses** Data were expressed as the mean ± S.D. The significance of difference was calculated by Student's t-test and values of p<0.05 were used as significant.

**RESULTS**

**Radical Scavenging Action of A. campestris** Figure 1 shows the DPPH scavenging action of *A. campestris*. The DPPH radical was scavenged by the water extract of *A. campestris* dose dependently, and the extract with 50% DPPH scavenging action was 5.3-times the dilution of the originally prepared herbal extract (1 g/10 ml H₂O). In contrast with the water extract, only 36% of the DPPH radical was scavenged when the ethanol extract (1 g/10 ml ethanol) was mixed with the DPPH solution. Superoxide anion and the hydroxyl radical scavenging actions of *A. campestris* were measured by ESR spectrometer. As shown in Fig. 2, scavenging of superoxide anion of 95%, 80% and 50%, respectively, when extracts of the original and at 40 times and 320 times dilution were used. Hydroxyl radicals of 75% and 42% were lost by the addition of twice the original extract (×0.5) and the original (×1) extract, respectively.

**Effect of the Herbal Extract on Carbon Tetrachloride-Induced Hepatotoxicity** Figure 3 shows the effect of the intraperitoneal injection of *A. campestris* extract on CCl₄-induced liver toxicity. Serum AST and ALT activities were increased to 617% and 615% of the control, respectively, by CCl₄ treatment, and the increases were significantly decreased to 154% and 193%, respectively, when the herbal extract was given intraperitoneally twice before CCl₄ treatment. Cytosolic and microsomal GSH S-transferase activities were not altered significantly either by CCl₄ or by CCl₄ plus the herbal extract-treated mice. Aniline hydroxylase activity was significantly decreased to 55% of the control by CCl₄-treatment and depressed to 69% of the control by pretreatment with the *A. campestris* extract. Lipid peroxidation in the liver homogenate was slightly increased by CCl₄ treatment and also by pretreatment with the herbal extract. When the extract was given orally for 3 d before CCl₄ (i.p.) treatment, CCl₄-induced increases in serum AST (775% of the control)

![Fig. 1. DPPH Radical Scavenging Activity of the Extract of A. campestris Water (○) or ethanol (■) extracts of *A. campestris* were diluted with each vehicle, then mixed with DPPH (100 μM). Scavenging activity of the extracts for the DPPH radical was measured as described in Materials and Methods.](image1)

![Fig. 2. Effect of *A. campestris* on the ESR Signals Formed via Hypoxanthine/Xanthine Oxidase (A) and H₂O₂/FeSO₄ (B)](image2)

A: hypoxanthine (500 μM), xanthine oxidase (0.002 unit) and 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) (230 μM) were mixed in 0.1 M phosphate buffer (pH 7.8) in the presence of diethylenetriamine penta acetic acid (DETAPAC) (960 μM) with the water extract at room temperature, and the ESR signal for DMPO•OH after 40 s was monitored as described in Materials and Methods (g=2.006, δρ=1.418 mT, δλ=1.164mT, δρ=0.137 mT). B: FeSO₄ (250 μM), H₂O₂ (250 μM) and DMPO (92 μM) were mixed in 0.1 M phosphate buffer (pH 7.8) in the presence of DETAPAC (250 μM) with the extract, and the ESR signal for DMPO•OH after 40 s was monitored (g=2.007, δρ=1.488 mT, δλ=1.488 mT). C: control, A.C.: *A. campestris*. Dilution of the extract was as follows. 320 (×320) and 80 (×80) dilution, original extract (×1), twice the original extract (×0.5).
and ALT (5322% of the control) activities were also depressed (Fig. 4). The decrease in aniline hydroxylation (72% of the control) caused by CCl₄ treatment was recovered to the control level by the herbal extract. Lipid peroxidation was increased to 113% of the control by CCl₄ treatment and to 105% by pretreatment with the herbal extract.

The liver and serum parameters were not changed by either intraperitoneal or oral treatment of mice with the herbal extract alone (Figs. 3 and 4).

**DISCUSSION**

The antioxidant action of *A. campestris* was evaluated using an ESR spectrometer, and it was found that the extract from the herb could scavenge markedly the superoxide anion and DPPH radicals and moderately the hydroxyl radical. DPPH can abstract labile hydrogen,53 and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation.54 In our laboratory, it was confirmed that the DPPH scavenging activity was parallel to the inhibitory action of lipid peroxidation using an extract of mold or medicinal herbs.17 In our laboratory, it was confirmed that the DPPH scavenging activity was parallel to the inhibitory action of lipid peroxidation using an extract of mold or medicinal herbs.17,18 Since hydroxyl radicals or superoxide anions are generated under oxidative stress caused by various chemicals or by various disorders, it was suggested that *A. campestris* may ameliorate oxidative stress-induced disorders. In the present study, the effect of the herbal extract on CCl₄-induced liver injury was examined.

CCl₄ is known to cause liver injury via metabolic activation by the microsomal cytochrome P450-dependent monooxygenase system. CCl₄ is converted to the reactive intermediate, trichloromethyl radical (·CCl₃) and peroxy radical (·OOCCl), which in turn reacts with macromolecules such as lipid and protein, leading to lipid peroxidation and cell injury.5,7 When the extract from *A. campestris* was given intraperitoneally prior to CCl₄ treatment, CCl₄-induced hepatotoxicity seen in the elevation of serum AST and ALT levels was significantly depressed (Fig. 3). Lipid peroxidation was slightly increased by CCl₄ treatment and was scarcely decreased by pretreatment with *A. campestris* extract. Similar protection against CCl₄-induced liver toxicity was observed following oral administration of the herbal extract. Thus, it was suggested that antioxidant(s) in the aqueous extract of *A. campestris* is absorbed from the intestines of mice, resulting in protection against liver toxicity.

Phenolic derivatives such as flavonoids are known to be antioxidantive components of various herbs, and it is known that flavonoids are hydrolyzed by intestinal glycosidase, then the subsequent aglycone is absorbed.19 Thus, if the glycoside
moiety is essential for the antioxidant action, the oral administration of the flavonoid may be ineffective. It is postulated that the protective action of the extract of A. campestris against CCl₄-toxicity by oral administration may be due to antioxidant(s) without a glycoside moiety in the herbal extract. Indeed, such a flavone (without glycoside) has been isolated as an antioxidant from the extract of A. campestris (Aniya, unpublished data). It is likely that this antioxidant flavone is absorbed by either peritoneal injection or oral administration, resulting in protection against CCl₄-induced liver injury.

Although it has been reported that GSH S-transferase activity is increased or inhibited by plant phenols, in our experiments liver cytosolic and microsomal GSH S-transferase activities were not changed by treatment of mice with an herbal extract alone. Aniline hydroxylase activity was decreased by CCl₄ treatment, and the decrease was recovered by oral treatment with A. campestris. Since it is known that reactive metabolites from CCl₄ destroy liver microsomal cytochromes P450 by producing irreversibly bound heme-derived products, the decrease in aniline hydroxylase activity in cytochrome P450 by CCl₄ treatment is reasonable. Thus, recovery of the aniline hydroxylase activity by the herbal treatment is assumed to be due to scavenging of CCl₄-derived reactive metabolites by antioxidants in the herbal extract. In our study, we administered CCl₄ to mice subcutaneously (2 ml/kg as 50% corn oil solution) or intraperitoneally (25 μl/kg in corn oil solution). Repeated experiments confirmed that the pretreatment (p.o. or i.p.) of mice with an extract of A. campestris was protective against CCl₄-induced liver injuries caused by either intraperitoneal or subcutaneous injection. Taken together, the extract from A. campestris could prevent CCl₄-induced liver toxicity by either parenteral or oral administration, suggesting that antioxidant(s) of A. campestris absorbed scavenge CCl₄-derived radicals.

A. campestris has been used in the Okinawa Islands in Japan as a folk medicine for the treatment of liver and kidney disorders. The antioxidant action of A. campestris may contribute to the treatment of these diseases. Further investigations involving analyses of active components in the herb are in progress in our laboratory.

In summary, the medicinal herb A. campestris has a strong scavenging action of hydroxyl radicals, superoxide anions and DPPH radicals. This herb was protective against liver injury caused by CCl₄, suggesting that CCl₄-derived free radicals are scavenged by A. campestris.

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