Arch Pharm Res Vol 26, No 9, 719-722, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Anti-Oxidant Activities of Fucosterol from the Marine Algae *Pelvetia siliquosa*

Sanghyun Lee, Yeon Sil Lee[1], Sang Hoon Jung, Sam Sik Kang, and Kuk Hyun Shin[1]

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea and [1]Seokwon Life Science Research Institute, World Sea Green Co. Ltd., Paju 413-832, Korea

(Received May 28, 2003)

The anti-oxidant activities of fucosterol isolated from the marine algae *Pelvetia siliquosa* were investigated. Fucosterol exhibited a significant decrease in serum transaminase activities elevated by hepatic damage induced by CCl_4-intoxication in rats. Fucosterol inhibited the sGOT and sGPT activities by 25.57 and 63.16%, respectively. Fucosterol showed the increase in the anti-oxidant enzymes such as hepatic cytosolic superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-px) activities by 33.89, 21.56 and 39.24%, respectively, in CCl_4-intoxicated rats. These results suggest that fucosterol possess not only the anti-oxidant, but also the hepatoprotective activities in rats.

Key words: *Pelvetia siliquosa*, Fucaceae, Fucosterol, Anti-oxidant enzymes, s-Transaminases CCl_4-intoxication.

INTRODUCTION

Reactive oxygen species (ROS) are generated as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). The involvement of ROS in the pathogenesis of a large number of diseases is well documented (Cross, 1987). It is suggested that free radical damage to cells leads to the pathological changes associated with aging (Beckman and Ames, 1998). Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Chandra, 1995). Cooperative defense systems that protect the body from free radical damage include the anti-oxidant nutrients and enzymes. The anti-oxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-px) and indirectly glutathione reductase. Their roles as protective enzymes are well known and have been investigated extensively both *in vivo* and *in vitro* model systems. The first three enzymes directly catalyze the transformation of peroxides and superoxides to nontoxic species. Glutathione reductase reduces oxidized glutathione to glutathione, a substrate for glutathione peroxidase. The consequences of oxidative stress are serious, and in many cases are manifested by increased activities of enzymes involved in oxygen detoxification. Identification of new anti-oxidants remains a highly active research area because anti-oxidants may reduce the risk of various chronic diseases caused by free radicals.

Pelvetia siliquosa has been reported to be peculiar to the Korean peninsula and self-grown on the craggy surfaces near to the seashores of the southern area (Yoon, 1995). It has traditionally been used as seasoned sea greens for religious services or as health food (Oh *et al.*, 1990). In the previous paper, we reported that the ether fraction from *P. siliquosa* exhibit the hepatoprotective and anti-diabetic effects (Lee *et al.*, 2002).

This paper deals with the isolation and characterization of an anti-oxidant as well as hepatoprotective principle from *P. siliquosa*.

MATERIALS AND METHODS

Instruments and reagents

IR spectra were recorded with Jasco FT/IR-300E instrument on KBr disc. ^1H- and ^{13}C-NMR spectra were recorded with BRUKER AVANCE 400 NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS spectra were measured with JEOL JMS-AX505WA mass spectrometer. Sodium azide, ethylenediamine tetraacetic acid (EDTA),

Correspondence to: Dr. Kuk Hyun Shin, Seokwon Life Science Research Institute, World Sea Green Co. Ltd., Paju 413-832, Korea
E-mail: kshin4079@hanmail.net

β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), cumene hydroperoxide, glutathione reductase, carbon tetrachloride (CCl_4), xanthine, potassium cyanide (KCN), sodium dodecylsulfate, cytochrome C and pyridine were purchased from Sigma Chem. Co. (St. Louis, MO). All other chemicals and reagents were analytical grade.

Materials

Pelvetia siliquosa Tseng et Chang (Fucaceae) was collected at Jindo area, Jeonnam Province in 2002 and botanically identified by Prof. Jong-Ahm Shin, Yosu National University, Korea. The voucher specimen has been deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Korea.

Extraction and isolation

The air-dried powdered whole parts (4 kg) of *P. siliquosa* were extracted three times with *n*-hexane and methanol (1:5) under reflux. The resultant extract was combined and concentrated under reduced pressure to afford 59 g of the residue. The methanol extract was suspended in water and then extracted successively with equal volumes of *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Each fraction was evaporated *in vacuo* to obtain *n*-hexane (39 g), chloroform (1 g), ethylacetate (1 g) and *n*-butanol (1 g) fractions, respectively.

A portion of the *n*-hexane fraction (30 g) was chromatographed on silica gel column eluting with a gradient of *n*-hexane-EtOAc to afford compound **1** (756 mg). Compound **1**; EI-MS *m/z* (70 eV, rel. int. %): 412 $[M]^+$ (11.9), 397 (3.8), 394 (2.8), 379 (3.7), 314 (100), 299 (23.9), 296 (16.7), 281 (24.5), 271 (12.6), 255 (5.6), 253 (5.1), 229 (21.4), 213 (11.5), 145 (10.7), 55 (25.3); IR ν_{max} (KBr) cm^{-1}: 3439, 2936, 1626, 823; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 5.36 (1 H, br d, J = 5.2 Hz, H-6), 5.19 (1 H, q, J = 6.7 Hz, H-28), 3.53 (1 H, m, H-3), 1.58 (3 H, d, J = 6.7 Hz, H-29), 1.02 (3 H, s, H-19), 1.01 (3 H, br s, H-21), 1.00 (3 H, d, J = 1.2 Hz, H-27), 0.98 (3 H, d, J = 1.2 Hz, H-26), 0.70 (3 H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (ppm): 146.9 (C-24), 140.7 (C-5), 121.6 (C-6), 115.5 (C-28), 71.7 (C-3), 56.7 (C-14), 55.7 (C-17), 50.1 (C-9), 42.3 (C-13), 42.2 (C-4), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.4 (C-20), 35.2 (C-22), 34.7 (C-25), 31.8 (C-7,8), 31.5 (C-2), 28.2 (C-16), 25.6 (C-23), 24.3 (C-15), 22.2 (C-26), 22.1 (C-27), 21.0 (C-11), 19.4 (C-19), 18.7 (C-21), 13.1 (C-29), 11.8 (C-18).

Anti-oxidant assay *in vivo*

Male Sprague-Dawley rats weighing 200-250 g were used in all experiments. Animals were maintained on 12 h light/dark cycle at approximately 22°C and allowed food and water *ad libitum.* Six animals were used in each group. All treatments were conducted between 9:00 and 10:00 h to minimize variations in animal response due to circadian rhythm. Rats were injected i.p. with a mixture of CCl_4 in olive oil (1:1) at a dose of 0.6 mL/kg to induce hepatotoxicity. Control animals were given the vehicle alone. Rats were pretreated with silymarin and fucosterol given p.o. at a dose of 30 mg/kg/day for seven consecutive days prior to the administration of CCl_4. Animals were sacrified 24 h after CCl_4 dosing and blood was collected by decapitation for the determination of serum transaminases.

Hepatic tissues were carefully excised and homogenized in cold 1.15% KCl-10 mM phosphate buffer with EDTA (pH 7.4) and centrifuged at 10,000 rpm for 10 min. The supernatant was further centrifuged at 40,000 rpm for 60 min to obtain cytosolic extract for the measurement of liver cytosolic SOD, catalase, and GSH-px activities. The protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Hepatocellular damage was estimated by measuring sGOT and sGPT activities (Reitman and Frankel, 1957). Blood was collected from the abdominal aorta of each rat. The blood was centrifuged at 3,000 rpm at 4°C for 10 min to separate the serum. sGOT and sGPT activities were expressed as Karmen unit.

SOD was assayed by the method of McCord and Fridovich (1969). The reaction mixture containing 0.5 mM xanthine as substrate (300 μL), 0.05 mM KCN (100 μL), 1% sodium deoxycholate (100 μL), xanthine oxidase (20 μL), cytosolic extract (20 μL) and 0.1 mM cytochrome C (300 μL) was placed in a 1 cm cuvette and the rate of increase in absorbance at 550 nm was recorded for 5 min. SOD activity was expressed as unit/mg protein. Catalase was assayed by the method of Rigo and Rotilio (1977). The cytosolic extract of liver (40 μL) diluted 10 times was added with 0.13 mM phosphate buffer (pH 7.0, 500 μL), distilled water (660 μL) and 15 mM H_2O_2 (1800 μL), and thoroughly mixed. The rate of changes in the absorbance at 240 nm for 5 min was recorded. Catalase activity was expressed as unit/mg protein. GSH-px was assayed by the method of Burk *et al.* (1978). The reaction mixture containing 0.3 mM phosphate buffer with 4.0 mM EDTA (pH 7.2, 1000 μL), 26.56 mM sodium azide (500 μL), 294.37 mM GSH (60 μL), 8.4 mM NADPH (110 μL), 1 mM cumene hydroperoxide (320 μL), glutathione reductase (5 μL) and cytosolic solution (30 μL) was placed in 1 cm cuvette and the rate of changes in absorbance was recorded at 340 nm for 5 min. GSH-px activity was expressed as unit/mg protein.

Statistical analysis

Multiple comparison test was applied for detecting the significance of difference between different groups.

RESULTS AND DISCUSSION

A chromatographic separation of the *n*-hexane fraction from *P. siliquosa* led to the isolation of compound **1**. Compound **1** was obtained as white crystals. The IR spectrum showed absorptions at 3439 (OH), 2936, 1626, and 823 cm^{-1} ($\Delta^{24(28)}$ ethylidine sterol). In the EIMS, molecular ion peak showed at *m/z* 412 corresponding to the molecular formula $C_{29}H_{48}O$. In the ^{1}H-NMR spectrum, the angular methyl singlet signals of 18-Me and 19-Me at δ 0.70 and 1.02, and the doublet of 21-Me, 26-Me, 27-Me and 29-Me at δ 1.01, 0.98, 1.00 and 1.58 were observed, respectively. The quartet signal at δ 5.19 showed H-28 of ethylidine sterol. The broad doublet at δ 5.36 showed a H-6 signal of (B) ring. Accordingly, the structure of **1** was elucidated as fucosterol (Fig. 1). Atta-ur-Rahman *et al.* reported the isolation of fucosterol from marine algae (1999).

It has been reported that fucosterol decrease angiotensin-converting enzyme levels with reduction of glucocorticoid receptor in endothelial cells (Hagiwara *et al.*, 1986). Also it inhibited the lymphatic absorption of cholesterol in rats (Ikeda *et al.*, 1988). It exhibited antifungal activity against *Curvularia lunata, Stachybotrys atra* and *Microsporum canis* (Atta-ur-Rahman *et al.*, 1997). It also exhibited cytotoxic activity against P-388 cancer cell lines (Tang *et al.*, 2002).

Fucosterol obtained from the *n*-hexane fraction of *P. siliquosa* was tested for their free radical scavenging effects, and the effects on hepatocellular damage in CCl_4-intoxicated rats were evaluated. Carbon tetrachloride (CCl_4) is widely used to induce lipid peroxidation and toxicity. CCl_4 is metabolized by cytochrome P450 2E1 (CYP2E1) to the trichloromethyl radical (CCl_3·), which is assumed to initiate free radical-mediated lipid peroxidation leading to the accumulation of lipid-derived oxidation products that cause liver injury (Recknagel *et al.*, 1989). A number of investigators have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation, and increasing antioxidant enzyme activity (Kumaravelu *et al.*, 1995).

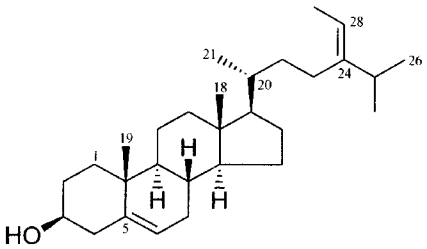

Fig. 1. Structure of fucosterol (**1**)

The effects of fucosterol of *P. siliquosa* on serum transaminases in CCl_4-intoxicated rats were estimated and the results are shown in Table I. In the CCl_4-intoxicated control group, the sGOT and sGPT activities increased significantly when compared with the normal group. In contrast, the group treated with fucosterol decreased significantly these elevated transaminase activities. Fucosterol inhibited the sGOT and sGPT activities by 25.57 and 63.16%, respectively. Silymarin inhibited the sGOT and sGPT activities by 18.85 and 52.72%, respectively.

Fucosterol cause a significant elevation of free radical scavenging enzyme activities such as SOD, catalase and GSH-px. As shown in Fig. 2, silymarin caused significant elevation of SOD, catalase and GSH-px activities by

Table I. Effect of fucosterol (1) from *P. siliquosa* on the sGOT and sGPT activities

Treatments	sGOT (Karmen unit)	sGPT (Karmen unit)
Normal	95.89 ± 4.50	18.78 ± 1.68
Control	205.83 ± 8.78	117.38 ±16.75
Silymarin	167.04 ± 10.25*	55.50 ± 6.60*
Fucosterol (1)	153.20 ± 14.43**	43.24 ± 5.52**

Rats were pretreated with silymarin and fucosterol given at a dose of 30 mg/kg for 7 days, prior to the administration of CCl_4. Rats were sacrified 24 hr after CCl_4. Heparinized blood sample was collected. Hepatocellular damage was estimated by measuring sGOT and sGPT activities. Significantly different from the control; *p<0.05, **p<0.01.

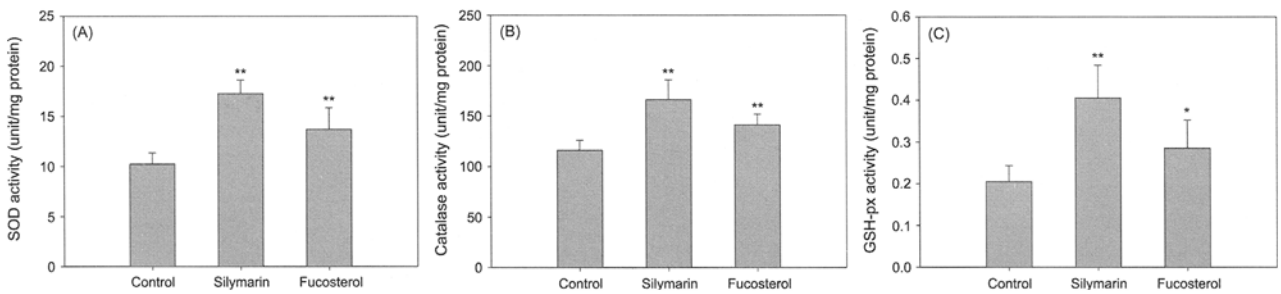

Fig. 2. Effect of fucosterol (**1**) from *P. siliquosa* on the liver cytosolic SOD (**A**), catalase (**B**) and GSH-px (**C**) activities. Rats were pretreated with silymarin and fucosterol given at a dose of 30 mg/kg for 7 days, prior to the administration of CCl_4. Hepatic tissues were carefully excised, homogenized, and centrifuged to obtain cytosolic fractions for the measurement of liver cytosolic SOD, catalase, GSH-px activities. The test method is the same as that described in the determination of anti-oxidant enzyme activities. Significantly different from the control; *p<0.05, **p<0.01.

68.65, 43.19 and 97.56%, respectively. Fucosterol increased the SOD, catalase and GSH-px activities by 33.89, 21.56 and 39.24%, respectively. Increase in the catalase activity with respect to CCl_4 treatment indicates that fucosterol can play an important role in scavenging hydrogen peroxide. Restoration of SOD activity indicates that fucosterol can help in cellular defense mechanisms by preventing cell membrane oxidation. Similarly, an increase in glutathione peroxidase activity indicates that fucosterol also helps in the restoration of vital molecules such as NAD, cytochrome, and glutathione.

In conclusion, fucosterol showed the recovery of liver function by radical-scavenging activities such as SOD, catalase and GSH-px in CCl_4-intoxicated rats. The present study demonstrated that fucosterol possess not only the anti-oxidant, but also the hepatoprotective activities in rats.

ACKNOWLEDGMENT

This study was supported by a grant from the Korea Health Industry Development Institute (KHIDI), the Ministry of Health & Welfare, Korea (No. 03-PJ1-PG11-VN01-SV04-0016).

REFERENCES

Atta-ur-Rahman, Choudhary, M. I., Hayat, S., Khan, A. M., Ahmad, and A., Malik, S., Spatozoate and verninasterol from the brown alga *Spatoglossum variabile*. *Phytochemistry*, 52, 495-499 (1999).

Atta-ur-Rahman, Choudhary, M. I., Majeed, A., Shabbir, M., Ghani, U., and Shameel, M., A succinylanthranilic acid ester and other bioactive constituents of *Jolyna laminarioides*. *Phytochemistry*, 46, 1215-1218 (1997).

Beckman, K. B. and Ames, B. N., The free radical theory of aging matures. *Physiol. Rev.*, 78, 547-581 (1998).

Burk, R. F., Nishiki, K., Lawrence, R. A., and Chance, B., Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin free perfused rat liver. *J. Biol. Chem.*, 253, 43-46 (1978).

Cerutti, P. A., Oxidant stress and carcinogenesis. *Eur. J. Clin. Invest.*, 21, 1-11 (1991).

Cross, C. E., Oxygen radicals and human disease. *Ann. Intern. Med.*, 107, 526-545 (1987).

Hagiwara, H., Wakita, K., Inada, Y., and Hirose, S., Fucosterol decreases angiotensin converting enzyme levels with reduction of glucocorticoid receptors in endothelial cells. *Biochem. Biophys. Res. Commun.*, 139, 348-352 (1986).

Ikeda, I., Tanaka, K., Sugano, M., Vahouny, G. V., and Gallo, L. L., Inhibition of cholesterol absorption in rats by plant sterols. *J. Lipid Res.*, 29, 1573-1582 (1988).

Kumaravelu, P., Dakshinamoorthy, D. P., Subramaniam, S., Devaraj, H., and Devaraj, N. S., Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochem. Pharmacol.*, 49, 17031707 (1995).

Lee, Y. S., Jung, S. H., Lee, S., Choi, Y. J., and Shin, K. H., Hepatoprotective and anti-diabetic effects of *Pelvetia siliquosa*, a marine algae, in rats. *J. Appl. Pharmacol.*, 10, 165-169 (2002).

Lowry, O. H., Posenbrough, N. N., Farr, A. L., and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

McCord, J. M. and Fridovich, I., Superoxide dismutase: An enzymatic function for erythrocuprein (hemocoprein). *J. Biol. Chem.*, 244, 6049-6055 (1969).

Oh, Y. S., Lee, I. K., and Boo, S. M., An annotated account of Korean economic seaweeds for food, medical and industrial uses. *Kor. J. Phycol.*, 5, 57-71 (1990).

Pike, J. and Chandra, R. K., Effect of vitamin and trace element supplementation on immune indices in healthy elderly. *Int. J. Vitamin Nutr. Res.*, 65, 117-120 (1995).

Recknagel, R. O., Glende Jr., E. A., Dolak, J. A., and Waller, R. L., Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Therapeut.*, 43, 139-154 (1989).

Reitman, S. and Frankel, S., A chlorotrimetric method for the determination of sGOT and sGPT. *Am. J. Clin. Pathol.*, 28, 56-63 (1957).

Rigo, A. and Rotilio, G., Simultaneous determination of superoxide dismutase and catalase in biological materials by polarography. *Anal. Biochem.*, 81, 157-166 (1977).

Tang, H. F., Yang-Hua, Y., Yao, X. S., Xu, Q. Z., Zhang, S. Y., and Lin, H. W., Bioactive steroids from the brown alga *Sargassum carpophyllum*. *J. Asian Nat. Prod. Res.*, 4, 95-101 (2002).

Yoon, J. T., Developmental morphology on the regeneration of *Pelvetia siliquosa* Tseng et Chang, Phaeophyta in Korea. Chonbuk National University. Ph. D. Thesis (1995).