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Hepatoprotective and Anti-diabetic Effects of *Pelvetia siliquosa*, a Marine Algae, in Rats

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Abstract–The effects of various fractions from the whole plant of *Pelvetia siliquosa* Tseng et Chang (Fucaceae) on the CCl_4-induced hepatotoxicity as well as on streptozotocin (STZ)-induced diabetes in rats were investigated. The ether fraction exhibited a potent rat lens aldose reductase (RLAR) inhibition *in vitro* and showed a significant inhibition of not only serum glucose concentrations but also sorbitol accumulations in the lens, red blood cells and sciatic nerves in the STZ-induced diabetic rats. When administered orally in Sprague-Dawley rats, H_2O fraction was found to cause a significant inhibition of the rise in the serum transaminase activities in CCl_4-intoxicated rats. These results suggested that this plant might possess constituents with hepatoprotective, anti-diabetic effects and those effects on diabetic complications.

Key words □ *Pelvetia siliquosa,* Fucaceae, Hepatotoxicity, Diabetic Complication

Genus *Pelvetia* is a typical marine algae, only four species of which have been known to be distributed world wide. Among them, *Pelvetia siliquosa* Tseng et Chang (Fucaceae) 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 foods (Oh *et al.*, 1990), however, studies on its biological activities have yet been carried out.

In the course of the evaluation of biological activities and its bioactive principles from this plant, the effects of various fractions from the whole plant on CCl_4-induced hepatotoxicity as well as on sorbitol accumulation in the tissues of streptozotocin (STZ)-induced diabetic rats *in vivo* and on rat lens aldose reductase *in vitro* were investigated.

MATERIALS AND METHODS

Materials

Pelvetia siliquosa Tseng et Chang was collected at Jindo area, Jeonnam Province and botanically identified by Prof. Jong-Ahm Shin, Yosu National University, Korea. A voucher specimen was deposited at the Herbarium of the institute. DL-Glyceraldehyde, sodium phosphate, nicotinamide adenine dinucleotide (NAD), adenine dinucleotide phosphate reduced form (NADPH), sorbitol dehydrogenase, glycine, citric acid, glucose, streptozotocin (STZ) were purchased from Sigma Chem. Co. (St. Louis, MO). Diethyl ether (Et_2O), ethylacetate (EtOAc), methanol (MeOH), *n*-butanol (*n*-BuOH) and tetrachloromethane (CCl_4) were from Duk San Pure Chemical Co., LTD. All other chemicals and reagents were analytical grade commercially available.

Extraction and fractionation

The air-dried powdered whole plant (4 kg) of *P. siliquosa* was extracted three times with MeOH (5 L each) for five hours under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 59 g of the residue. The MeOH extract was suspended in water and then fractionated successively with equal volumes of Et_2O, EtOAc and *n*-BuOH. Each fraction was evaporated *in vacuo* to yield the residues of Et_2O fraction (40 g), EtOAc fraction (1 g) and *n*-BuOH fraction (1 g), respectively. The other air-dried powdered whole plant (2 kg) of *P. siliquosa* was extracted with water (3 L each) for 5 hr under reflux and then lyophilized to yield the residue of H_2O extract (74.4 g).

Animals

All experiments were performed on male Sprague-Dawley (SD) rat obtained from Daehan Biolink Co., LTD. Chungbuk,

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Korea. The animals were acclimatized for 1-2 weeks before being used for the experiments. Standard pelleted diet and water were given *ad libitum*. Animals were maintained under a constant 12 hr light and dark cycle and an environmental temperature of 21-23°C.

Measurement of aldose reductase activity *in vitro*

Crude aldose reductase (AR) was prepared as follows: rat lens were removed from SD rats weighing 250-280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman and Kinoshita (1965). Partially purified enzyme with a specific activity of 6.5 U/mg was routinely used to test enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots and stored at −40°C. AR activity was assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm over a 4 min period with DL-glyceraldehyde as a substrate (Sato and Kador, 1990). Each 1.0 ml cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH with or without 10 mM substrate and inhibitor.

Induction of STZ-induced diabetes and sample treatments *in vivo*

Diabetes mellitus was induced in male SD rats by a single intraperitoneal injection STZ dissolved in phosphate buffered saline acidified to pH 4.5 with 0.05 M citric acid (66 mg/kg). Normal rats were injected with the vehicle only. Two weeks after the induction of diabetes, the animals showing the serum glucose level higher than 600 mg/dl were selected, then epalrestat (ONO Co. Ltd.) and *P. siliquosa* fractions were administered *via* an intragastric tube once a day at a dose of 200 mg/kg for 2 weeks. *P. siliquosa* fractions were suspended in saline containing 0.5% carboxymethyl cellulose (CMC). The animals were then sacrificed under ether anesthesia. Glucose levels were determined in serum by using a commercial kit based on glucose oxidase method (Trinder, 1969). The amount of glucosylated hemoglobin in red blood cells (RBC) was measured using Drabkins reagent kit 525 (Drabkin and Ausin, 1932). The content of sorbitol in the lens, RBC and sciatic nerves was determined enzymatically.

Determination of tissue sorbitol contents

Sorbitol was measured by a modification of the enzymatic assay of Clements *et al.* (1969). Protein-free filtrate 0.5 ml of tissues was added to a reaction mixture, which consisted of 1.0 ml of 0.05 M glycine buffer, pH 9.4 containing 0.2 mM NAD and 0.64 U of sorbitol dehydrogenase. Blanks with either filtrate, NAD or sorbitol dehydrogenase deleted, were run simultaneously. The relative fluorescence due to NADH was measured with an excitation wavelength of 366 nm and an emission wavelength of 452 nm.

Oral glucose tolerance test *in vivo*

Prior to an oral glucose tolerance test (OGTT), rats were fasted for 16 hr. Saline (control), a reference drug metformin (250 mg/kg) or each fraction of *P. siliquosa* (250 mg/kg) suspended in saline containing CMC was then orally administered to groups of 5 rats. Thirty minutes later, glucose (2 g/kg) was orally administered to each rats with a feeding syringe (Aybar *et al.*, 2001). Blood samples were collected from carotid artery at 0 (just before the oral administration of glucose), 60, 120 and 180 min after glucose load for the assay of glucose. Glucose levels were estimated by commercially available glucose kits based on glucose oxidase method.

Hepatoprotective activity of *P. siliquosa* fractions *in vivo*

Hepatoprotective activity was measured by a modification of the assay of Macay *et al.* (1984). Male SD rats weighting 230-260 g were divided into six groups. Two groups, used as normal and control, received an isotonic saline solution, while the other groups received MeOH extract, Et_2O, EtOAc and H_2O fraction Except the normal group, all the groups were given i.p. with CCl_4 solution [CCl_4 : Olive oil = 3:2 (v/v), 0.6 ml/kg]. Each test sample (200 mg/kg) was given p.o. 18 hr and 30 min before and 6 hr after administration of CCl_4. At the end of the sample treatment, animals were fasted for at least 16 hr and sacrificed. Blood samples were obtained to determine GOT and GPT activities.

Statistical analysis

Statistical comparisons between groups were performed using the Students *t*-test. *P*-values of < 0.05 were considered to be statistically significant. The results are presented as means ± S.E.M.

RESULTS AND DISCUSSION

It has been reported that aldose reductase (AR), the key enzyme in the polyol pathway, plays a central role in the reduction of aldose to polyol and cataract formation and other complications in diabetes are triggered by the accumulation in the tissues of excessive sorbitol synthesized by the action of AR

Table I. Inhibitory effect of *P. siliquosa* fractions on rat lens aldose reductase activity

Treatments	Concentration (μg/ml)	Inhibition[a] (%)	IC_{50}[b] (μg/ml)
TMG*	1.86	87.4	0.67
	0.19	42.4	
	0.02	22.4	
Et_2O	100	95.9	17.6
	10	49.4	
	5	31.4	
EtOAc	100	91.6	38.0
	10	49.0	
	5	10.1	
n-BuOH	100	82.5	65.9
	50	27.4	
	1	02.1	
H_2O	100	84.7	40.8
	50	47.8	
	1	32.6	

[a]Inhibition rate was calculated as percentage with respect to the control value.
*TMG: tetramethylene glutaric acid, a reference compound as one of typical aldose reductase inhibitors.
[b]IC_{50} values were calculated from the least-squares regression equations in the plot of the logarithm of at three graded concentrations vs % inhibition.

(Kinoshita, 1974; Kinoshita *et al.*, 1979). Various fractions from *P. siliquosa* were estimated by their effects on rat lens aldose reductase (RLAR) enzyme using DL-glyceraldehyde as a substrate and the results were shown in Table I. All fractions inhibited the AR activity in a concentration dependent manner. The inhibitory potencies of Et_2O, EtOAc and H_2O fraction, as indicated in IC_{50} values, were determined to be 17.6, 38.0 and 40.8 μg/ml, respectively, while the IC_{50} value of TMG, known as one of typical AR inhibitor, was 0.67 μg/ml.

The development of lenticular cataracts and defects in nerve conduction velocity are associated with increases in lens, RBC and peripheral nerve sorbitol and fructose level (Malone *et al.*, 1980; Raskin and Rosentstock, 1987). To confirm the efficacy of *P. siliquosa* fractions *in vivo*, we also studied the effects on glucose concentration as well as on sorbitol accumulation in STZ-induced diabetic rat tissues. The results of the effects on serum glucose concentration and sorbitol contents in the lens, RBC and sciatic nerves are shown in Table II. In diabetic control rats, the serum glucose level and the sorbitol content of the lens, RBC and sciatic nerves were significantly elevated compared to those of the normal rats. Treatment with Et_2O fraction

Table II. Effect of *P. siliquosa* fractions and epalrestat on the accumulation of sorbitol in the lens, red blood cell and sciatic nerves and serum glucose concentration in STZ-diabetic rats

Treatments	Serum glucose (g/l)	Sorbitol content		
		Lens (nmol/mg)	Sciatic nerve (nmol/mg)	RBC (nmol/g Hb)
Normal[a]	1.2±0.1	0.05±0.02	2.0±0.3	63.2 ± 3.9
Control[b]	5.7±0.3	0.80±0.04	10.4±1.4	403.6 ± 61.3
Epalrestat	3.9±0.2**	0.38±0.05**	4.3±0.5**	108.7 ± 11.6**
Et_2O	4.0±0.3*	0.38±0.1**	7.2±0.5*	145.3 ± 12.8*
EtOAc	4.3±0.2*	1.24±0.1	6.4±0.7*	128.1 ± 19.1**
H_2O	5.9±0.3	0.67±0.07	8.7±0.7	198.0 ± 14.6**

[a]Normal: Non-diabetic rats
[b]Control: Diabetic rats
STZ was administered as in methods. Two weeks after STZ administration, epalrestat and the fractions were given orally to diabetic rats (each 6 rats) at the dose of 200 mg/kg for 15 days. Sorbitol content was measured in lens, red blood cell and sciatic nerves. Significantly different from the control: $^{**}p < 0.01$, $^{*}p < 0.05$.

Table III. Effect of *P. siliquosa* fractions and metformin on glucose tolerance in normal SD-rats

Treatments	0 (min)	60 (min)	120 (min)	180 (min)
Control	55.5±1.9	160.3±4.8	189.7±4.0	132.1 ± 3.4
Metformin	54.5±2.2	140.1±7.0*	122.8±1.4**	139.6 ± 4.5
Et_2O	47.6±3.8	191.5±12.9	139.3±8.8**	124.3 ± 4.4
EtOAc	47.5±3.3	166.4±4.6	135.0±3.0**	121.9 ± 5.3
H_2O	44.0±3.3	171.0±4.7	135.7±4.6**	130.5 ± 3.3

Rats were fasted for 16 hr. Saline (control), a reference drug metformin (250 mg/kg), or each fraction of *P. siliquosa* (250 mg/kg) were then orally administered to groups of 5 rats. Thirty minutes later, glucose (2 g/kg) was orally administered to each rat with a feeding syringe. The change in blood glucose at 0, 60, 120 and 180 min was measured in each group. Significantly different from the control: $^{**}p < 0.01$, $^{*}p < 0.05$.

of *P. siliquosa* in rats not only caused a significant decrease in serum glucose level but also the accumulation of sorbitol in tissues comparable to those of epalrestat, as a reference drug.

Serum glucose level was lowered by 30% and sorbitol contents in the the lens, RBC and sciatic nerves decreased by 52.5, 31.0 and 66.6%, respectively. Also, in rats treated with EtOAc fraction, the accumulation of sorbitol in the RBC and sciatic nerve except the lens was inhibited by 70.5 and 38.1%. However, H_2O fraction of *P. siliquosa* inhibited sorbitol accumulation in RBC alone.

Associated with reduction in serum glucose concentration in STZ-induced diabetic rats, all of the fractions from *P. siliquosa*,

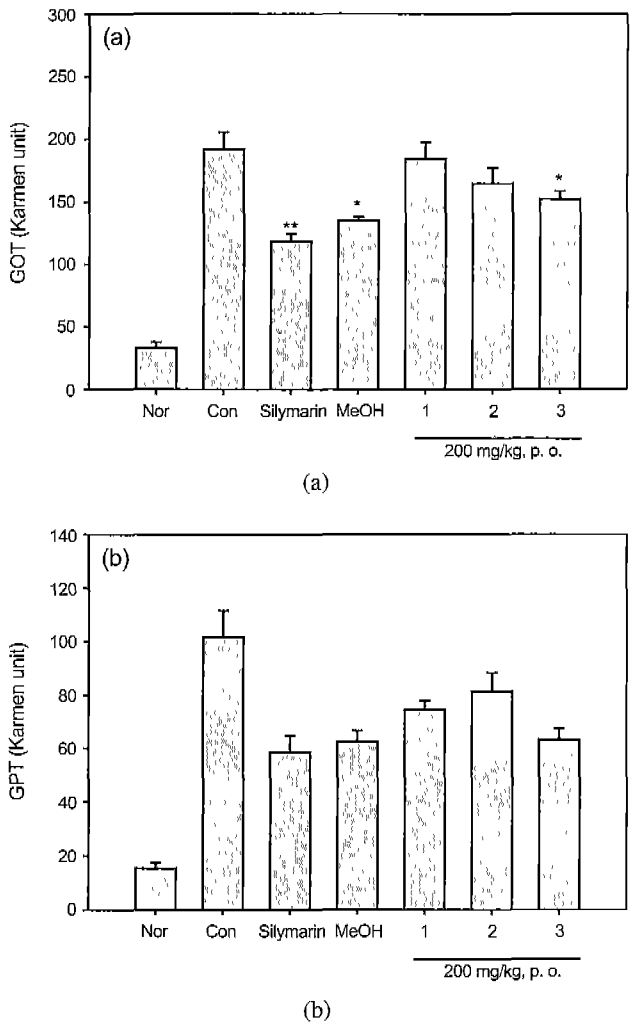

Fig. 1. Effects of *P. siliquosa* MeOH extract and each fraction on serum GOT and GPT activities in CCl_4-induced hepatic damage Control group was given with CCl_4 0.6 ml/kg [CCl_4: Olive oil= 3 : 2 (v/v)] i.p. Each sample was given p.o. 18 hr and 30 min before and 6 hr after administration of CCl_4. Significantly different from the control group; $^{**}p < 0.01$, $^{*}p < 0.05$. 1: Et_2O fraction, 2: EtOAc fraction, 3: H_2O fraction

when administered orally in normal SD rats (250 mg/kg, p.o.), were found to cause a significant glucose tolerance effect, as indicated in Table III. Blood glucose level of the control group was markedly increased 60 min after the oral glucose load and reached a peak at 120 min. All of the groups treated with various fractions showed a significant decrease in glucose concentrations 120 min after an oral glucose load compared to those of the control, the tendency of which was similar to those of metformin, a reference drug.

The hepatoprotective effects of *P. siliquosa* were evaluated by measuring serum GOT and GPT activities in CCl_4 intoxicated rats and the results are shown in Fig. 1a and 1b. The MeOH extract from *P. siliquosa* caused a significant decrease in serum GOP and GPT activities by 30.6 and 40.4% inhibition, respectively. Based on these results, the effect of various fractions obtained from the MeOH extract on serum transaminases was tested to evaluate active fractions. Among various fractions tested, H_2O fraction was demonstrated to be most active and was shown to decrease in elevated serum GOT and GPT activities by 20.8 and 37.9%, respectively.

The present study was carried out in search for fractions useful for the treatment of diabetic diseases and hepatic damage as well as for the identification and the elucidation of the active principles from *P. siliquosa*. We found that Et_2O fraction has the most promising activity because this fraction not only inhibited AR *in vitro* but also suppressed serum glucose concentration and sorbitol accumulation in the tissues of STZ-induced diabetic rats. Meanwhile, H_2O fraction is considered to show a significant liver protective effect against CCl_4-induced hepatotoxicity. The isolation of active principles from active fractions is in progress and the results will be reported elsewhere.

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