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Antimutagenic and anticarcinogenic effect of methanol extracts of *Petasites japonicus* Maxim leaves

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The methanol extract from the leaves of *Petasites japonicus* Maxim (PJ) was studied for its (anti-)mutagenic effect with the SOS chromotest and reverse mutation assay. The (anti-)carcinogenic effects were evaluated by the cytotoxicity on human cancer line cells and by the function and the expression of gap junctions in rat liver epithelial cell. PJ extracts significantly decreased spontaneous β-galactosidase activity and β-galactosidase activity induced by a mutagen, ICR, in *Salmonella* (*S.*) *typhimurium* TA 1535/pSK 1002. All doses of the extract (0.08-100 mg/plate) decreased the reversion frequency induced by benzo (α)pyrene (BaP) in *S. typhimurium* TA 98. It decreased not only the spontaneous reversion frequency but also that induced by BaP in *S. typhimurium* TA 100. PJ extract showed greater cytotoxic effects on human stomach, colon and uterus cancer cells than on other cancer cell types and normal rat liver epithelial cells. Dye transfers though gap junctions were significantly increased by PJ extracts at concentrations greater than 200 μg/mL and the inhibition of dye transfer by 12-*O*-tetradecanoylphorobol-13-acetate (TPA) was obstructed in all concentrations of PJ. PJ significantly increased the numbers of gap junction protein connexin 43, and increased the protein expression decreased by TPA in a dose-dependent manner. Based on these findings, PJ is suggested to contain antimutagenic and anticarcionogenic compounds.

Keywords: anticarcinogenicity, antimutagenicity, gap junction, *Petasites japonicus* Maxim

Introduction

In the last three decades, many studies have focused on the evaluation of the antimutagenic and anticarcinogenic activities of vegetables, fruits, and plants in order to develop functional food or drugs to prevent cancers [2,4,9]. *Petasites japonicus* Maxim (PJ) is an herb of the tribe senecioneae in the family compositae. It is a perennial grass, and the lower stalk has been used as food in some Asian countries. Petasin and isopetasin, identified in extracts of *Petasites hybridus*, inhibit the biosynthesis of the vasoconstrictive peptide leukotriene, and they are thus used in traditional medicine to improve gastrointestinal pain [1,3]. Petasiphenol, an extract of PJ, was reported as a bio-antimutagen isolated from scapes of PJ [12]. On the other hand, petasitenine from young flower stalks of PJ can induce neoplasia in the livers of rats, but the mature terrestrial part of the cultivated plant does not show carcinogenic activity [9,10]. PJ is also known to reduce the neurotoxicity induced by kainic acid in mice and to have anti-histaminic and anti-allergic effects [12,34,35].

A gene mutation is a permanent DNA sequence change and the accumulation of genetic errors results in cancer development. The Ames test, which detects frame shift mutation or base substitution of DNA, and the SOS chromotest (*umu* test), which detects expression of the SOS gene (*umu*C) caused by DNA damage, have been developed for the screening of chemical carcinogenicity [21,28].

Cancer is characterized by uncontrolled division and malignant growth of cells that present tumor specific promoters and mutations of certain types of genes that regulate cell growth. Materials that have selective cytotoxic effects on cancer cells can be considered as candidate drugs for cancer therapy [30].

Gap junctions are intercellular plasma membrane domains enriched in channels that allow direct exchange of ions and small molecules between adjacent cells. Gap junctions are essential for cell growth, proliferation and physiological function through communication in normal cells [19]. Cancer cells usually have down-regulated levels of gap junctions, and the induction of gap junctional intercellular communication (GJIC) leads to cell growth inhibition of some cancer cells [11]. So, studies on the effects of test

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compounds on the function of gap junctions can be used as prescreening tools for the potential carcinogenicity or anticarcinogenicity of compounds [17]. To our knowledge, there have been few studies into the effect of PJ on anticarcinogenicity involving GJIC.

The present paper studies the (anti-)mutagenic activities of the methanol extract from leaves of PJ through the *umu* test and Ames' test using *Salmonella* TA 98 and TA 100. The anticarcinogenic activity of the extract was also investigated by MTT assay for cytotoxicity on cancer line cells and gap junctional intercellular communication assay in rat liver epithelial cells.

Materials and Methods

Preparation of methanolic extracts

PJ leaves were collected during the spring season in the Korean Peninsula. They were washed with distilled water and then ground using mortar. Ten times methanol in volume was poured to the ground PJ leaves and let extract some ingredients from PJ for 48 h by shaking at 240 rpm, en times in volume and mixed with to extract. Sonication was then applied for 30 min. The solution was filtered through filter paper (φ 185 mm; Advantec, USA) and vacuum dried at 40°C to produce a dark gray colored residue. The mean recovery was 2.66% of the initial weight. The methanol extract of PJ was dissolved in 50% dimethylsulfoxide (DMSO) at concentrations of 0.8, 4, 20, 100, 250, 500 and 1,000 mg/mL for the (anti-)mutagenic assay and 8, 40, 200, 1,000 and 5,000 μg/mL for the (anti-) carcinogenicity assay and then sonicated for 1 h to sterilize the solution.

Bacterial strains, cell types and chemicals

The test strains of *S. typhimurium* TA 98 and TA 100 were provided by Dr. Bruce Ames (University of California, USA) and *S. typhimurium* TA 1535/pSK 1002 was supplied by Dr. Oda (Perfectual Institute of Public Health, Japan). Human gastric cancer cells, colon cancer cells, uterine cancer cells, lung cancer cells, and hepatoma cells were obtained from the Natural Product Research Institute of Seoul National University, Korea. Rat liver epithelial cells (WB-F344) were kindly provided by Dr. Trosko JE (Michigan State University, USA). 6-chloro-9-[3-(2-chloroethylamino)proylamino]-2-methoxyacridine dihydrochloride (ICR 191), 12-*O*-tetradecanoylphorobol-13-acetate (TPA), benzo(α)pyrene (BaP) and DMSO were obtained from Sigma-Aldrich (USA).

Umu test for (anti-)mutagenic activity

The *umu* test was performed to detect DNA damage by monitoring the expression of the SOS gene (*umuC*) fused with a *lacZ* gene (β-galactosidase) according to the procedure developed by Oda *et al*. [28]

Briefly, *S. typhimurium* TA 1535/pSK 1002 was cultured at 37°C overnight in Luria- Bertani broth medium supplemented with ampicillin at 20 μg/mL and diluted 50-fold with TGA medium (1% bacto tryptone, 0.5% NaCl, 0.2% glucose, 20 μg/mL ampicillin). Cultures were incubated at 37°C until the bacterial density reached an absorbance of 0.25 ~ 0.3 at 600 nm. The bacterial cultures was subdivided into 2.4 mL portions in test tubes, and 100 μL of test sample, 100 μL of positive control (ICR), and 0.5 mL of S_9 mixture or phosphate buffered saline (PBS) were added. The mixtures were incubated at 37°C for 2 h. The expression of *umu* gene was calculated by β-galactosidase activity according to Miller [24]. S_9 mixture was prepared by the method of Maron and Ames [21] and 1 mL of the mixture was mixed with 9 mL of the cofactors.

Reverse mutation assay for (anti-)mutagenic activity

The pre-incubation method of Maron and Ames [21] was applied to evaluate the (anti-)mutagenic effect of PJ extract. Briefly, 0.1 mL of *S. typhimurium* TA98 or TA100 cultured overnight were added to 0.1 mL of sample, 0.1 mL of positive control (BaP) or vehicle, and 0.5 mL of S_9 mixture or PBS. The entire mixture was incubated at 37°C in a rotary shaker (125 rpm) for 20 min. After incubation, 2.0 mL of 0.5M his/bio top agar was added, and mixtures were poured onto minimal glucose agar and further incubated for 48 h. The toxicity of the test sample was determined by examination of the background lawn. The S_9 mixture was prepared as mentioned above.

MTT assay for cancer cell viability

After the addition of PJ extract to human gastric cancer cells, colon cancer cells, uterine cancer cells, lung cancer cells and normal rat liver epithelial cells in 96-well plates for 4 days, the cytotoxicity was determined by the MTT assay using the method described by Mosmann [27]. Briefly, cells were washed once with 37°C PBS after the sample treatment and then 0.1 mL of serum-free medium containing 0.1% MTT was added to each well. After incubation for 4 h, the cells were centrifuged at 450 × g for 5 min and the culture medium removed. 0.1 mL of DMSO was added to each well to solubilize the formazan formed. The plates were shaken gently for 10 min and the absorbance was measured at 570 nm. The absorbance of treated cells was compared with that of the controls, which were exposed only to the vehicle and were considered to have a viability value of 100%. The determination of the 50% inhibition concentration of the extract for each cancer cell was carried out by the sigmoidal fitting method (Origin 6.0; Microcal Software, USA).

Scrape-loading and dye transfer (SLDT) assay

GJIC was determined through the SLDT technique according to the method described by El-Fouly *et al*. [6].

WB-F344 cells confluently grown in 35-mm tissue culture dish were exposed to the test compound with or without TPA for 1, 4, 8, or 24 h. After washing with Ca^{2+}, Mg^{2+}-free PBS 3 times, 2 mL of 0.05% Lucifer yellow CH dye solution in PBS was added, and then several scrapes were made on the monolayer using a surgical blade. The cells were incubated for 3 min at room temperature and then washed three times with Ca^{2+}, Mg^{2+}-free PBS. The cells were fixed with 1 mL of 10% buffered formalin solution. Dye transferred cells were observed with an inverted epifluorescence microscope (×100).

Immunofluorescent staining for gap junction protein

Immunofluorescent staining for a gap junction protein, connexin 43, was carried out according to the method described by Matesic [22]. After treatment with test sample with or without 0.01 μg/mL of TPA for 8 h, the WB-F344 cells were fixed with cold methanol/acetone (95 : 5, v/v) for 30 min and then rehydrated with PBS. Nonspecific binding sites were blocked with 1% normal rabbit serum (Jackson, USA) in PBS for 30 min at room temperature. The cells were incubated for 2 h with mouse monoclonal anti-connexin 43 (Zymed, USA) diluted 1:100 in PBS, and then washed with PBS. The cells were treated with fluorescence isothiocyanate-conjugated rabbit anti-mouse IgG (Jackson, USA) diluted 1 : 100 in PBS for 1 h. The numbers of stained gap junction proteins per cell were counted (×1,000) using a fluorescence microscope (Nikon, Japan).

Statistics

Statistical analyses of the data were performed with one-way ANOVA and Duncan's multicomparison test using PC-STAT Version 1A (StatSoft, USA).

Results

Effect of the extract on β-galactosigase activity in *S. typhimurium* TA 1535/pSK 1002

Methanol extract of PJ produced dose-dependent inhibition of spontaneous β-galactosidase activity at concentrations of more than 2 mg/0.1 mL in *S. typhimurium* TA 1535/pSK 1002. The β-galactosidase activity induced by mutagen ICR (0.03 mg/0.1 mL) was also decreased significantly ($p < 0.01$) by simultaneous treatment of the extract at doses greater than 10 mg/0.1 mL (Table 1).

Table 1. Inhibitory effect of *Petasites japonicus* Maxim (PJ) extract on expression of SOS *gene* (*umu C'-'lac* Z fusion gene) induced spontaneously and by ICR in *Salmonella* (*S.*) *typhimurium* TA1535/pSK1002

Treatment	Dose (mg/0.1 mL)	Activity of β-galactosidase[a]	Inhibition ratio[b]
Control	0	140.8 ± 7.8	1.00
PJ	0.08	139.0 ± 4.5	0.99
	0.4	131.4 ± 3.9	0.93
	2	126.3 ± 2.7*	0.90
	10	122.5 ± 2.7†	0.87
	25	111.4 ± 2.0†	0.79
	50	106.6 ± 5.1†	0.76
	100	114.4 ± 3.8†	0.81
ICR	0.03	364.7 ± 16.9†	1.00
ICR+ PJ	0.03 + 0.08	372.9 ± 15.9	1.07
	0.03 + 0.4	370.9 ± 16.0	1.04
	0.03 + 2	372.0 ± 17.4	1.10
	0.03 + 10	307.2 ± 10.7†	0.82
	0.03 + 25	216.2 ± 6.1†	0.47
	0.03 + 50	154.3 ± 5.0†	0.21
	0.03 + 100	128.6 ± 2.6†	0.06

ICR: 2-methoxy-6-chloro-9-(3-(2-chlorethyl) aminopropylamino) acridine·2HCl. a: Activities of β-galactosidase were presented in units and the values are mean ± SE of 6 replicas. b: The ratios are defined as the value of the β-galactosidase units of PJ divided by those of controls in the case of PJ alone and as the value of β-galactosidase unit of ICR+PJ divided by that of ICR alone. Significantly different (*$p < 0.05$ and †$p < 0.01$) from control (in the case of PJ or ICR only) or ICR (in the case of ICR + PJ), respectively.

Effect on the reversions of *S. typhimurium* TA 98 and TA 100

All extract concentrations (0.08 ~ 100 mg/plate) had no effect on spontaneous reversions in *S. typhimurium* TA 98 and TA 100. Simultaneous treatment of BaP (2 mg/plate) with all extract concentrations had no significant effect on spontaneous reversions in *S. typhimurium* TA 100 colonies, while extract concentrations of more than 25 mg/plate were associated with bacterial toxicity. In *S. typhimurium* TA 98, however, BaP-induced reversions decreased dose-dependently with PJ extract concentrations of more than 25 mg/0.1 mL ($p < 0.01$) and inhibition ratios were less than 0.35 (Table 2).

Inhibition of the viability of cancer cell lines

The mean 50% inhibitory concentration (IC_{50}) of the extract for cellular viability was 550 μg/mL [95% confidence interval (CI) 357 ~ 846 μg/mL] for stomach cancer cells, 503 μg/mL (95% CI 392 ~ 644 μg/mL) for colon cancer cells, and 870 μg/mL (95% CI 798 ~ 948 μg/mL) for uterine cancer cells, all of which were lower than than the IC50 for normal rat liver epithelial cells (2,468 μg/mL; 95% CI 2,088 ~ 2,918 μg/mL). In case of liver and lung cancer cells, the IC_{50} values were 1,913 μg/mL (95% CI 1,001 ~ 3,516 μg/mL) and 2,781 μg/mL (95% CI 1,493 ~ 5,108 μg/mL), respectively - similar to those of normal rat liver epithelial cells (Table 3).

Table 2. Inhibitory effect of *Petasites japonicus* Maxim (PJ) extract on the reversion frequency induced spontaneously and by benzo(α)pyrene (BaP) in *S. typhimurium* TA98 and TA100

	Dose[a]	TA98		TA100	
		Revertant[b]	Ratio[c]	Revertant[b]	Ratio[c]
Control	0	45.3 ± 1.5	1.00	128.5 ± 7.3	1.00
PJ	0.08	50.3 ± 3.3	1.11	128.0 ± 5.8	1.00
	0.4	46.4 ± 3.3	1.02	126.5 ± 2.8	0.98
	2	53.6 ± 4.5	1.18	136.5 ± 3.6	1.06
	10	51.7 ± 5.1	1.14	128.8 ± 4.9	1.00
	25	50.3 ± 3.7	1.11	BK	
	50	49.7 ± 4.7	1.10	BK	
	100	45.2 ± 2.8	1.00	BK	
BaP	2	351.7 ± 25.3*	1.00	634.4. ± 32.1*	1.00
BaP+ PJ	2 + 0.08	358.7 ± 23.7	1.01	565.3 ± 9.1	0.86
	2 + 0.4	330.4 ± 23.8	0.93	572.3 ± 28.5	0.88
	2 + 2	332.0 ± 19.8	0.91	652.6 ± 20.9	1.02
	2 + 10	310.4 ± 26.9	0.84	678.3 ± 15.8	1.09
	2 + 25	157.1 ± 10.4*	0.35	BK	
	2 + 50	92.4 ± 6.5*	0.14	BK	
	2 + 100	53.0 ± 3.5*	0.03	BK	

a: Units of doses for PJ and BaP are mg/plate and μg/plate, respectively. b: Revertants are the number of revertants/plate and the values of mean ± SE of 9 replicas. c: The ratios are defined as the value of the revertant of PJ divided by that of control in the case of PJ alone and as the revertants of BaP + PJ divided by those of BaP alone. BK: Revertants could not be counted because of bacterial killing effect. Significantly different (*$p < 0.01$) from control (in the case of BaP) or BaP (in the case of BaP + PJ).

Table 3. Inhibitory effect of *Petasites japonicus* Maxim (PJ) extract on the viability of normal rat liver epithelial cells and five human cancer line cells

Cell type	IC_{50} (μg/mL)
Norma rat liver epithelial	2,468 (2,088~2,918)
Human stomach cancer	550 (357~846)
Human colon cancer	503 (392~644)
Human uterine cancer	870 (798~948)
Human liver cancer	1,903 (1,001~3,616)
Human lung cancer	2,781 (1,493~5,180)

IC_{50}s are the 50% growth inhibition concentration obtained by MTT assay. ( ): 95% confidence interval.

Effect of the extract on GJIC

GJIC was assessed by dye transfer through gap junctions after treatment with PJ extract. After treatment with the extract at concentrations of more than 200 μg/mL for 1 h, transfer of dye through the gap junction increased significantly ($p < 0.01$), but was not significantly different from that of the control at all treatment concentrations after 1 h of treatment. The simultaneous treatment of extract with TPA (0.01 μg/mL) effectively prevented the inhibition of dye transfer by TAP 1 h after treatment at all concentrations (Fig. 1, Table 4).

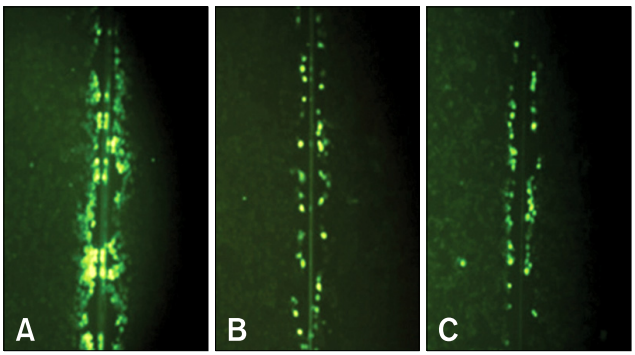

Fig. 1. Gap junctional intercellular communication assayed with Lucifer yellow CH dye solution in rat liver epithelial cells. The cells were exposed to 0.1% methanol (A), 0.01 μg/mL 12-*O*-tetradecanoylphorobol-13-acetate (TPA) (B) and 0.01 μg/mL TPA + 40 μg/mL *Petasites japonicus* Maxim (C) extract for 1 h. The cells were observed under epifluorescence microscope. ×100.

Effect of the extract on the expression of gap junction protein

The number of gap junction proteins (connexin 43) expressed was significantly ($p < 0.01$) increased by

Table 4. Effect of *Petasites japonicus* Maxim (PJ) extract on gap junctional intercellular communication in WB-F344 rat liver epithelial cells treated with/without TPA

Treatment	Dose (μg/mL)	No. of dye-transferred cellsa			
		1	4	8	24 h
Control	0	169.9 ± 9.0	189.9 ± 15.1	188.2 ± 8.3	226.6 ± 19.2
PJ	8	173.2 ± 4.8	197.4 ± 17.3	202.7 ± 8.6	223.0 ± 14.2
	40	187.3 ± 9.5	198.9 ± 15.0	208.3 ± 6.5	229.6 ± 20.8
	200	217.9 ± 14.4†	206.6 ± 7.0	192.4 ± 9.7	263.3 ± 24.7
	1,000	234.4 ± 10.0†	230.5 ± 19.2†	242.4 ± 15.2†	258.4 ± 19.7
	5,000	261.2 ± 12.7†	246.5 ± 12.7†	226.7 ± 11.2†	230.3 ± 23.7
TPA	0.01	28.5 ± 2.6†	27.2 ± 1.7†	31.3 ± 3.0†	54.2 ± 6.0†
TPA+ PJ	0.01 + 8	40.0 ± 3.1*	48.2 ± 4.5†	88.4 ± 8.2†	125.3 ± 10.4†
	0.01 + 40	43.4 ± 3.7†	58.8 ± 6.7†	108.3 ± 5.8†	152.1 ± 14.8†
	0.01 + 200	47.4 ± 3.5†	58.6 ± 3.9†	145.0 ± 15.5†	214.8 ± 19.2†
	0.01 + 1,000	58.9 ± 3.9†	64.4 ± 3.3†	144.4 ± 18.9†	189.9 ± 9.2†
	0.01 + 5,000	78.6 ± 6.2†	68.8 ± 5.0†	160.4 ± 10.0†	174.2 ± 14.8†

TPA: 12-O-tetradecanoylphorbol-13-acetate. a: The values presented are the mean ± SE of 12 (1 h) or 9 (4, 8 and 24 h) replicas. Significantly different (*$p < 0.05$ or †$p < 0.01$) from control (in the case of PJ and TPA) or TPA (in the case of TPA + PJ). Dye-transferred cells are determined under epifluorescence microscope. ×100.

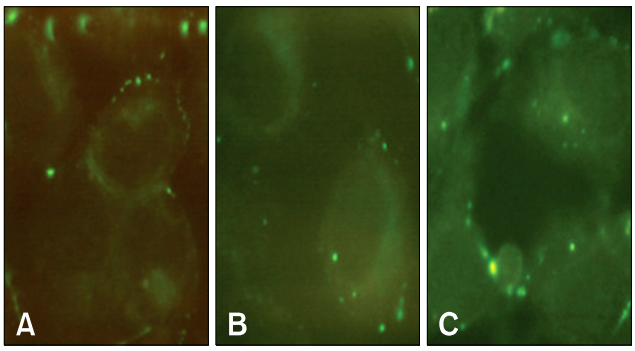

Fig. 2. Gap junction proteins in rat liver epithelial cells. The cells were exposed to 0.1% methanol (A), 0.01 μg/mL TPA (B) and 0.01 μg/mL TPA + 40 μg/mL *Petasites japonicus* Maxim (C) extract for 1 h. The cells were observed under epifluorescence microscope. ×1,000.

Table 5. Effect of *Petasites japonicus* Maxim (PJ) extract on the expression of gap junction protein in WB-F344 rat liver epithelial cells at 8 h after the treatment with/without TPA

Treatment	Dose (μg/mL)	No. of gap Junction protein/cell[a]	Induction ratio[b]
Control	0	11.3 ± 0.7	1.00
PJ	8	12.4 ± 0.2	1.10
	40	12.5 ± 0.3	1.11
	200	13.6 ± 0.7	1.20
	1,000	16.0 ± 0.8†	1.42
	5,000	25.9 ± 1.8†	2.29
TPA	0.01	3.3 ± 0.1†	0.29
TPA+ PJ	0.01 + 8	5.5 ± 0.3*	0.49
	0.01 + 40	6.9 ± 0.3*	0.61
	0.01 + 200	7.7 ± 0.9**	0.68
	0.01 + 1,000	9.6 ± 0.5**	0.85
	0.01 + 5,000	12.7 ± 0.9†	1.12

a: The values presented are the mean ± SE of 6 replicas. b: The ratio is defined as the value of the gap junction numbers per cell of PJ, TPA and TPA+PJ divided by those of control. Significantly different (*$p < 0.05$ or †$p < 0.01$) from control (in the case of PJ and TPA) or TPA (in the case of TPA + PJ), respectively.

treatment with extracts at concentrations of more than 1,000 μg/mL. Inhibition of gap junction expression by treatment with extract decreased dose-dependently in the presence of TPA (0.01 μg/mL) (Fig. 2, Table 5).

Discussion

It is well known that plants suitable for human diet such as garlic, broccoli, hot chili peppers and soybean contain a variety of natural antimutagenic and anticarcnogenic compounds [31,33]. Controversial results have been reported regarding the antimutagenic effect of PJ [9,10,12]. In this experiment, PJ methanol extract did not show mutagenic activity. Conversely, it showed antimutagenic activity by preventing spontaneous DNA damage or DNA damage induced by the mutagen ICR in *S. typhimurium* TA

1535/pSK1002. Antimutagens can be classified as "desmutagens" which can inactivate mutagens by directly binding them before gene damage occurs, and "bioantimutagens" which can affect the recovery or replication of damaged genes [18]. Thus our data show that PJ extract could have both bioantimutagenic and desmutagenic activity in *S. typhimurium* TA 1535/pSK 1002. In the present study, the extract influenced neither spontaneous reversions nor mutagen-induced reversions in *S. typhimurium* TA 100, but it decreased BaP-induced reversions whilst having no effect on spontaneous reversions in *S. typhimurium* TA 98. Antimutagens generally exhibit their activities by either correction of the error-prone SOS response or enhancement of an error-free recombinational repair system [16]. We suggest that the different results of the two test methods come from the fact that different targets are detected by each test; the *umu* test detects activation of the SOS repair system using β-galactosidase activity, whereas *S. typhimurium* reversion tests measure the final revertants involved in various type of gene mutations, such as base pair substitution or frame shift [13,16,28,29]. The difference between S .typhimurium TA 98 and TA 100 is that *S. typhimurium* TA 98 detects the reversions caused by frame shift mutation, while *S. typhimurium* TA 100 detects reversions originating from base pair substitution [21]. These data suggest that PJ extract may inhibit the mutagenesis aroused by frame shift mutation rather than base pair substitution as it inhibits mutagenic activity in *S. typhimurium* TA 98 but not in *S. typhimurium* TA 100. Furthermore, PJ extract has desmutagenic effects, as apposed to bioantimutagenic effects, as it inhibits only BaP-induced frame shift mutation whilst having no effect on spontaneous reversion. Considering the results of the two batteries of mutagenicity tests, PJ extract appears to act as a bioantimutagen and desmutagen in the activation of the SOS DNA repair system and as a desmutagen in the prevention of BaP-induced frame-shift mutation.

The present results show that IC_{50} for human gastric cancer cells, uterine cancer cells, and colon cancer cells is lower than that for human liver cancer cells, lung cancer cells and rat normal liver epithelial cells. These data suggest that the effect of PJ extract can vary according to the tissue origin of cancer; there might be a somewhat specific relationship between PJ extract and cancer inhibition. There have been several controversial results reported about the anticarcinogenic effect of PJ depending on the growth stage and site of sampling. Petasiphenol, a phenolic compound from PJ was reported to be a potent antiangiogenic agent [23]. Petasiphenone, a phenol isolated from *Climicuifuga racemosa* also demonstrated inhibitory effects on the proliferation of a human prostate cancer line cell [14]. However, petasitenine, a pyrrolizidine alkaloid from the young flower stalk of PJ was associated with liver cell adenoma and hemangioendothelial sarcoma in rats [9,10]. Liver sarcoma induced by the flower stalk of PJ in the rat was enhanced by simultaneous treatment with carbon tetrachloride [26]. Activation of apoptosis pathways is understood as a key mechanism by which cytotoxic compounds kill tumor cells and blockage of the apoptosis-inducing pathway could be an important mechanism for resistance to chemotherapy [20]. In this study, PJ extract exerted apparent cytotoxic effect on human stomach cancer cells and colon cancer cells. Change of apoptosis by PJ extract in those cancer cell lines needs to be investigated to demonstrate the anti-cancer effect.

The inhibition of GJIC in cells is suggested to be related to the carcinogenesis of non-genotoxic chemicals as well as to the induction of developmental effects [15,32]. Cancer cells are devoid of gap junctions, so that contact inhibition does not occur in cancer cells, and growth control is more difficult than in normal cells [7,8]. GJIC showed an inverse relationship with cell proliferation in a rat hepatoma cell line [5]. In this experiment, the methanol extract alone increased the transfer of dye and it also obstructed the inhibition of dye transfer by TPA in normal rat liver epithelial cells. The extract alone increased the number of gap junction proteins, and it also increased TPA-induced gap junction protein expression. These results show that PJ may have some active components which stimulate GJIC between cells in normal cells. The tumorigenic or antitumorigenic effect of PJ depends on whether the major component is petasiphenol or petasitenine [9,10,12]. This experiment supports the leaves of PJ containing petasiphenol because we collected leaves of mature PJ in late spring in the Korean peninsula. We speculate that this component may contribute to the anticarcinogenic activities of PJ. Petasiphenol inhibits DNA polymerase lambda activity, which is specifically expressed in developmentally regulated cells such as spermatocytes in the testis and directly binds to proliferating cell nuclear antigens [25]. Therefore, we speculate that the anticarcinogenic effect may be related to the inhibition of DNA polymerase lambda activity. However, there are to our knowledge no studies on the effect of petasiphenol on gap junctions. Further study is required to clarify whether the mechanism of the anticarcinogenicity of PJ involves the function of DNA polymerase lambda in gap junction induction and to elucidate the relationship with petasiphenol.

In conclusion, methanol extract from leaves of PJ appears to contain bioactive compounds which have antimutagenic and anticarcinogenic activities *in vitro*.

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