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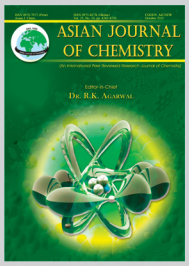

Protective Effect of *Panax quinquefolium* Against Chemical-Induced Gastric Mucosal Injury

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The American ginseng (*Panax quinquefolium* L.) is widely used in the oriental traditional medicine. The present study investigates the protection effects of *Panax quinquefolium* extract granule (PQEG) against chemical-induced gastric mucosal injury. *In vitro* cell damage was induced by incubating rat gastric mucosal cells, RGM1, with indomethacin (INDO) and quantitated by methylthiazole-tetrazolium (MTT) assay. *In vivo* gastric damage was induced by 70 % ethanol 10 mL/kg and the ulcer index was calculated the gastric mucosal damage percentage. *In vitro* study, *Panax quinquefolium* extract granule revealed significant protection on rat gastric mucosal cells, RGM1, from indomethacin-caused cytotoxicity, with an EC_{50} of 694.4 mg/kg (95 % CL: 582.2-872.6). *In vivo* studies showed pretreatment with *Panax quinquefolium* extract granule caused a significant and dose-dependent inhibition of ethanol-induced gastric mucosal damage, with an ED_{50} of 588.7 mg/kg (95 % CL: 426.8-850.3). In conclusion, *Panax quinquefolium* treatment shows protective role in indomethacin-induced gastric mucosa cell injury *in vitro* and ethanol-induced gastric mucosa lesions *in vivo*.

Key Words: *Panax quinquefolium*, American ginseng, Ethanol, Indomethacin, Gastric mucosal injury, RGM1.

INTRODUCTION

Millions of people worldwide suffer from peptic ulcer, which is caused by various factors such as emotional stress, heavy drinking, smoking, caffeinated drinks, infection of *Helicobacter pylori* and ingestion of non-steroidal antiinflammatory drugs[1,2]. In addition to neutralization of acid and interference of acid secretion, reinforcement of gastric mucosal protection is an effective way for therapy to decrease the occurrence of peptic ulcers[1]. Due to most drugs' several adverse effects, plant extracts have been applied as one of the most attractive alternate source of medicine[3]. A number of plant extracts have been known to have promising results in gastroprotective effects, which is beneficial for prevention and/or treatment of peptic ulcers[4-6].

Panax quinquefolium L. (American ginseng, Araliaceae) has long been cultivated extensively in North America and is widely used in the oriental medicine[7]. Several studies have reported that *Panax quinquefolium* possesses various pharmacological activities including antioxidant activity[8], reduction of skeletal muscle cell membrane damage[9], inhibition of thrombin-induced endothelin release[10], immunomodulation[11], reduction of low density lipoprotein oxidation[12] and hyperglycemia[13]. It has also been reported that *Panax ginseng* C.A. Meyer (Asian ginseng) is classed within the same genus possess gastroprotective activity[14]. In the case of *Panax quinquefolium*, however, little is known on its biological effect. The aim of this study is to evaluate the protection effect of *Panax quinquefolium* on indomethacin-induced gastric mucosa cell injury *in vitro* and ethanol-induced gastric mucosa lesions *in vivo*.

EXPERIMENTAL

Fetal bovine serum (FBS), trypsin-EDTA (0.5 %-5.3 mM), antibiotic-antimycotic (penicillin G sodium 10,000 units/mL/streptomycin sulfate 10,000 µg/mL/amphotericin B 25 µg/mL) and L-glutamine (200 mM) were purchased from Gibco (Grand Island, NY, USA). Dulbecco's modified Eagle medium (DMEM, HyQ®DME/High) and nutrient mixture F-12 Ham's (HamF12) were from Hyclone (Logan, Utah, USA). Methylthiazole tetrazolium, dimethyl sulfoxide and indomethacin were from Sigma chemicals (St. Louis, MO, USA). The rat

gastric mucosal cell line, RGM1 (RCB 0876), was obtained from RIKEN Cell Bank (Tsukuba, Japan). All other chemicals were of analytical reagent grade.

Compounding of *Panax quinquefolium* extract granule: *Panax quinquefolium* extract granule (PQEG) was supplied by Taiwan Biotech Co., Ltd. and it was prepared under standardized conditions by a GMP certified pharmaceutical company (Science Park Branch, Taiwan Biotech Co., Ltd.). The preparation procedures were as follows: extracted the *Panax quinquefolium* radix with hot water, separated by membrane and spring dry the liquid, mixed the extract powder with xylitol, alginic acid, glycyrrhiza radix powder, hydroxypropyl cellulose, essence, silicon dioxide and then granulated the mixed materials. The content of *Panax quinquefolium* extract granule is 500 mg in 1300 mg of granules.

Determination of ginseng saponins in *Panax quinquefolium* extract granule: Ginseng saponins in *Panax quinquefolium* extract granule were analyzed using a high performance liquid chromatographic method with a 4.0 mm × 250 mm RP18 column and detected with UV at 203 nm at the program system of methanol and phosphate buffer. Quantification was accomplished by the comparison of peak areas from the sample with those of the reference standards. The ginsenosides in each 1300 mg of *Panax quinquefolium* extract granule are 79.39 mg (calculated as the total of Rb1: 44.99 mg, Re: 26.00 mg, Rc: 3.76 mg and Rd: 4.64 mg).

Effect of *Panax quinquefolium* extract granule on gastric mucosal cytoprotection *in vitro*: Effect of *Panax quinquefolium* extract granule on gastric mucosal cytoprotection *in vitro* was conducted as previous report[15,16]. For routine maintenance, rat gastric mucosal cells, RGM1, were grown in a 75-cm^2 cell culture flask in a 1:1 mixture of DMEM and HamF12 medium supplemented with 20 % FBS, 2 mM glutamine and 1 % antibiotic-antimycotic in incubator with 5 % CO_2 at 37 ºC.

RGM1 cells were harvested with 0.25 % trypsin-EDTA and were further seeded into a 96-well microplate (NUNC, Roskilde, Denmark) at a density of 2 × 104 cells/200 mL/ well. After incubation for 24 h, cells were subjected to exposure of *Panax quinquefolium* extract granule in various concentrations (125, 250, 500 and 1000 ppm) at 37 ºC for 2 h. Each concentration was tested in eight replicates. After incubation, the cells were then treated with 250 ppm indomethacin at 37 ºC for 5 h. The cell viability was performed by methylthiazole-tetrazolium assay using the following procedure: medium were removed and then 50 µL of methylthiazole-tetrazolium reagent was added at a concentration of 2 mg/ mL in medium for 3 h. Medium were removed and then 100 µL DMSO was added with gentle shaking for 10 min. The optical density at 560 nm was estimated by an ELISA reader (ThermoLabsystems, Cheshire, UK).

Effect of *Panax quinquefolium* extract granule on ethanol-induced gastric mucosal damage *in vivo*: A total of 32 male Wistar rats (6-8 weeks old) were purchased from BioLASCO Taiwan Co. Ltd. Animals were maintained under standard laboratory conditions [12 h light/dark cycle, temperature (22 ± 2) ºC]. Standard chow and water were available and the rats were randomly divided into four groups of 8 each and fasted for 24 h before the experiment. All the rats had free access to water and this study was approved by the appropriate animal care and use committees at Tajen University (Pingtung, Taiwan). Groups of animals received *Panax quinquefolium* extract granule (250 or 500 or 1250 mg/kg) or vehicle, the tap water (10 mL/kg, control) as gastric gavages. After 1 h, 70 % ethanol was given orally to each animal at a dose of 10 mL/kg to induce gastric ulceration. After 4 h of ethanol administration, the animals were sacrificed with CO_2 and the stomachs were removed, opened along the greater curvature and rinsed with saline to remove gastric contents and blood clots.

Histopathological evaluation: The opened stomach was stretched on a sheet of cork with mucosal surface so as to obtain a clear macroscopic view of the gastric mucus. The gastric lesions were assessed by 2 observers unaware of the treatments. Macroscopic gastric injuries observed in the stomach was photographed with a digital camera (Nikon, Tokyo, Japan) and measured using computer software and analyzed with a software of USB digital scale 1.0 E (Myguard, Taiwan). Semi-quantitative analysis of gastric lesion were examined macroscopically and classified according to a scoring system of Shackelford *et al.*[17]; 1 = minimal (< 1 %); 2 = mild (1-25 %); 3 = moderate (26-50 %); 4 = moderately severe (51-75 %); and 5 = severe (76-100 %). The ulcer index (UI) is percentage of lesion area in relation to total stomach area. Gastroprotection (%) was calculated according to: % gastroprotection = (UIC-UIT) × 100/UIC, where UIC is ulcer index in control and UIT is ulcer index in test[18].

Statistical analysis: For the *in vitro* and *in vivo* studies, data were analyzed by one-way analysis of variance (one-way ANOVA) and Fisher's LSD for multiple comparisons as the post hoc test. Statistical tests for cell viability *in vitro* and ulcer index in histopathological evaluation were one-tailed tests because the hypotheses concerning these variables were a priori. The 50 % effective concentration (EC_{50}) or doses (ED_{50}) and their associated 95 % confidence limits (95 % CL) were estimated according to standard linear regression analysis. Data are expressed as the mean ± S.D. The results were analyzed by a computerized statistical package (SPSS 12.0). Values of significance were set at $p < 0.05$ for both tests.

RESULTS AND DISCUSSION

Effect of *Panax quinquefolium* extract granule on indomethacin-induced cytotoxicity: The cell viability of the group without indomethacin treatment was expressed as 100 %. The percentage of cell death was abruptly increased to 71.62 % after treatment with indomethacin at the concentration of 250 mg/mL. Results indicated that indomethacin was harmful to rat gastric mucosal cells, RGM1. After the treatment of *Panax quinquefolium* extract granule at various concentrations ranging from 125 to 1000 mg/mL, the percentage of cell death was decreased significantly in a concentration-response manner. The results indicated that *Panax quinquefolium* extract granule was able to protect the rat's gastric mucosal cell from indomethacin-caused damage. Table-1 reveals pretreatment with *Panax quinquefolium* extract granule (125 to 1000 mg/ L; n = 8 for each concentration) caused a significant and concentration-dependent protection of indomethacin-induced cytotoxicity to rat gastric mucosal cells, RGM1, with an with an EC_{50} of 694.4 mg/kg (95 % CL: 582.2-872.6).

TABLE-1
PROTECTION EFFECT OF *Panax quinquefolium* EXTRACT GRANULE ON INDOMETHACIN-INDUCED CYTOTOXICITY IN RAT GASTRIC MUCOSAL CELLS, RGM1

Groups	Concentration (μg/mL)	Cell death (%) (mean ± SD)	Cell protection (%)	ED_{50} (95 % CL) (μg/mL)
Control (indomethacin treated only)	250	71.62 ± 3.79	–	–
Panax quinquefolium extract granule	125	68.11 ± 4.79	4.9	694.4 (582.2 – 872.6)
	250	56.25 ± 4.39[a]	21.5	
	500	40.83 ± 8.37[a]	43.0	
	1000	29.60 ± 5.08[a]	58.7	

The values are presented as mean ± SD (n = 8); [a]$p < 0.001$ *vs.* control group.

TABLE-2
EFFECTS OF *Panax quinquefolium* EXTRACT GRANULE ON ETHANOL-INDUCED GASTRIC LESIONS

Groups	Number of rats	Ulcer index (%) (mean ± SD)	Gastro-protection (%)	ED_{50} (95 % CL) (mg/kg)
Control (ethanol only)	8	25.04 ± 10.60	–	–
250 mg/kg PQEG + ethanol	8	16.77 ± 10.83[a]	33.0	588.7 (426.8 – 850.3)
500 mg/kg PQEG + ethanol	8	12.79 ± 6.61[b]	48.9	
1250 mg/kg PQEG + ethanol	8	9.22 ± 6.30[c]	63.2	

The values are presented as mean ± SD; PQEG = *Panax quinquefolium* extract granule; [a]$p < 0.05$, [b]$p < 0.005$, [c]$p < 0.001$, *vs.* control group.

Effect of *Panax quinquefolium* extract granule on gastric mucosal lesions induced by ethanol: Fig. 1A shows profile ranges of moderate (grade 3) to severe (grade 5) erosion by 70 % ethanol along-induced gastric mucosal injury. In Fig. 1B, the low-dosed group (70 % ethanol + *Panax quinquefolium* extract granule 250 mg/kg) reveals mild (grade 2) to moderate-severe (grade 4) gastric lesions. The Fig. 1C discloses the median-dosed group (70 % ethanol + *Panax quinquefolium* extract granule 500 mg/kg) is in mild (grade 2) to moderate-severe (grade 4). Finally, the high-dosed group (70 % ethanol + *Panax quinquefolium* extract granule 1250 mg/kg) demonstrates ranges of minimal (grade 1) to moderate (grade 3) erosion (Fig. 1D). Table-2 shows pretreatment with *Panax quinquefolium* extract granule (250, 500, 1250 mg/kg orally; n = 8 for each dose) caused a significant and dose-dependent inhibition of ethanol-induced damage of gastric mucosa, with an ED_{50} of 588.7 mg/kg (95 % CL: 426.8 - 850.3).

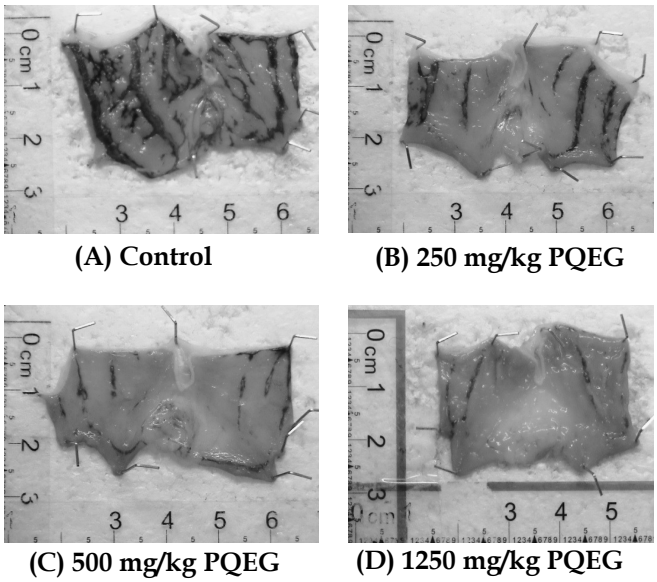

Fig. 1. Macroscopic findings of ethanol-induced gastric mucosal lesion. Gastric injuries were clearly inhibited by pretreatment with *Panax quinquefolium* extract granule (PQEG)

The results for the first time show that the *Panax quinquefolium* extract granule possesses gastro-protection and cyto-protection properties induced by chemicals in rats. Pre-treatment with *Panax quinquefolium* extract granule increases cell viability in indomethacin-induced gastric mucosa cell injury *in vitro* and decreases ethanol-induced gastric mucosa lesions *in vivo* in a dose-dependent manner. There are various plant extracts with different compositions that have been reported in gastro-protective effects due to their beneficial effects on the mucosa of gastrointestinal tract[19]. Asian ginseng (*Panax ginseng* C.A. Meyer), a species of genus *Panax*, with antiulcer activity has been reported[14,20]. The results obtained in this study show that the gastro-protective effect of *Panax quinquefolium*, another species of genus *Panax*, was similar to the results observed in the studies undertaken with Asian ginseng.

In vitro study of this research, cell death increased when RGM1 gastric mucosal cells were treated with indomethacin. Incubated gastric mucosal cells with *Panax quinquefolium* extract granule caused a concentration-dependent decrease in gastric mucosal cell death. Indomethacin increased cell death may result from caspase activation[21]. We acknowledged that our research is exploratory and the mechanisms of protective effects of *Panax quinquefolium* extract granule are not well understood. However, *Panax quinquefolium* has been previously shown to down-regulate caspase generation and reduce apoptosis of rat pancreatic β cells *in vitro*[22]. Therefore, the gastro-protective effect of *Panax quinquefolium* on indomethacin-induced gastric cellular injury is probably due to down-regulation of caspases activity.

Gastric mucous damage caused by ethanol is a commonly utilized experimental model in the evaluation of anti-ulcerogenic activity in rats. Several mechanisms have been suggested to cause ethanol-induced gastric damage, including weakening of the mucosal barrier function[23]. Additionally, the damage is associated with decreased blood flow that provoked ischemia-reperfusion injury[24]. Moreover, some studies have indicated that overproduction of oxygen-derived free radicals and peroxidation of biological membrane is implicated as possible pathogenesis of ethanol-induced gastric damage[25].

It has been suggested that upregulation of nitric oxide (NO) and prostaglandins by decreased cyclooxygenase can reduce the severity of damage to the gastric mucosa induced by ethanol[26,27]. Nitric oxide and prostaglandins participate in the gastric defense mechanisms by strengthening gastric mucosal barrier and increasing mucosal blood flow[27,28]. Besides, some antioxidant chemicals have protective effect on ethanol-induced acute gastric damage[29,30]. *In vivo* study of the present research, *Panax quinquefolium* extract granule produced a dose-dependent gastro-protective effect in ethanol-induced gastric lesions in rats. The mechanism whereby *Panax quinquefolium* extract granule protect the gastric mucosa against injury is not elucidated. However, *Panax quinquefolium* have been shown to possess antioxidant capacity[31,32] and down regulate cyclooxygenase activity[33].

This study indicates that *Panax quinquefolium* extract can decrease cell death in indomethacin-induced gastric mucosa cell injury and decrease in ethanol-induced gastric mucosa lesions. The results suggest that *Panax quinquefolium* treatment shows protective role in indomethacin-induced gastric mucosa cell injury *in vitro* and ethanol-induced gastric mucosa lesions *in vivo*. The possible mechanism by which *Panax quinquefolium* combats gastric mucosal injury was not assessed in this study and remains for future investigations.

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