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RESEARCH NOTE

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Anti-*Helicobacter pylori*, Cytotoxic, and Anti-inflammatory Activities of White Ginseng Extract

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Abstract The anti-*Helicobacter pylori* activity, cytotoxicity, and anti-inflammatory activity of white ginseng extract (WGE) were investigated *in vitro* in this study. The antimicrobial effects of WGE toward *H. pylori* strains 52 J99, SSI, and 51 were tested using the disk diffusion method. Among these *H. pylori* strains, *H. pylori* 52 was the most sensitive, having the largest inhibition zone (19 mm), followed by J99, SSI, and 51. The zone of inhibition due to WGE increased significantly with increasing dosage. The cytotoxicity of WGE toward the human cancer cell lines A-549 (human lung carcinoma), HEC-1-B (human endometrial adenocarcinoma), HeLa (human uterin adenocarcinoma), and SW-156 (human kidney carcinoma) was measured using the 3-(4,5-dimethylthizol-2-yl)-2,5-diphenylate-tetrazolium bromide (MTT) assay. WGE exhibited an inhibitory effect on cell growth at 2.0 mg/mL for all tumor cell lines. An analysis of anti-inflammatory activity using the RAW 264.7 cell line showed that the inhibition of nitric oxide (NO) production increased as the WGE content increased. These results demonstrate the potential of WGE to be used as a health-promoting substance.

Keywords: white ginseng extract, *Helicobacter pylori,* anti-*Helicobacter pylori* activity, cytotoxicity, anti-inflammatory activity

Introduction

Ginseng (*Panax* spp.; Araliaceae) has been widely used in traditional oriental medicine for its wide spectrum of medicinal effects including anti-aging (1), anti-diabetic (2), anti-tumor (3), anti-stress (4), anti-fatigue (5), and memory enhancing (6) effects, as well as the promotion of some types of protein synthesis (7). Commercially available ginseng is classified into fresh, white, and red ginseng. White ginseng is made by peeling the fresh ginseng roots and drying them without steaming.

Many of its medicinal effects are attributed to the triterpene glycosides known as ginsenosides (saponin) (8). Ginseng saponin (ginsenosides) is one of the most important secondary metabolites in ginseng and has various pharmacological activities. Beginning in the late 1960's, a variety of studies has been conducted concerning its constituents. Pharmacological studies show that the bioactivities of the different ginsenosides vary, depending on the extraction method. Most extraction procedures utilize methanol or ethanol (9) as a solvent in pure form or as an aqueous solution (10), and often couple with heat, refluxing, or sonication. So far, approximately 200 substances have been isolated and characterized from *Panax ginseng* including ginsenosides, polyacetylene, alkaloid, polysaccharides, oligosaccharides, oligopeptides, flavonoids, lipids, vitamins, and minerals (11).

Previous studies have found that various components of ginseng extract can inhibit microbial growth. For example, acidic polysaccharide from *P. ginseng*, PG-F2, were shown to have anti-adhesive effects toward *Actinobacillus actinomycetemcomitans*, *Propionibacterium acnes*, and *Staphylococcus aureus* but not toward *Lactobacillus acidophilus, Escherichia coli,* or *Staphylococcus epidermidis* (12). Acidic polysaccharides have also been shown to inhibit *Helicobacter pylori*. Other proteins isolated from the *Panax* family *(panaxagin* and *quinqueginsin)* also exhibit antifungal activity (13).

H. pylori is a motile, microaerophilic Gram-negative bacterium that is a pathogenic agent which colonizes the gastrointestinal tract. For many years, natural compounds such as propolis, herbs, and other plants have been tested for inhibitory effects on *H. pylori* (14). These include the protective roles of red ginseng extract (RGE) from *H. pylori*-associated cytotoxicity through the inhibitory actions of both mitogen-activated-proteinkinase (MAPK) and redox sensitive transcriptional activity (15). The influence of *H. pylori* infection on the lipooxygenase (LOX) pathway and the efficacy of RGE modulating the arachidonate-5-LOX-inflammatory link in gastric epithelium after *H. pylori* infection have also been reported (16).

The purpose of this study was to test the anti-*H. pylori* activity, cytotoxicity toward human cancer cells, and anti-inflammatory activity of white ginseng extract (WGE).

Materials and Methods

Materials White ginseng extract (WGE) was provided by the Dongwon Korean Ginseng Co., Ltd. (Seoul, Korea). Dried white ginseng was extracted 4 times using 70% aqueous ethanol for 4 hr at 95°C. The 4 extracts were combined, and the solvent was evaporated until 60% solid contents remained by rotary evaporator in vacuum. The WGE was then precipitated 3 times with 80% alcohol. And supernatant was obtained from former treatment and the 2nd supernatant was obtained that 3 times ethyl alcohol added to former residue. Purified ginseng extracts were

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obtained by the filtration and vacuum evaporation of supernatants. Acetonitrile and distilled water for high performance liquid chromatography (HPLC) were purchased from J. T. Baker SOLUSORB (Phillipsburg, NJ, USA). 20(*S*)- and 20(*R*)-ginsenoside Rg_3 standards from *P. ginseng* were purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). The composition of WGE is as follows: water solid contents 89.1%, crude protein 13.16%, crude lipid 2.98%, crude fiber 6.47%, ash 4.65%, total saponin contents 12-14%, total ginsenosides 47.9 mg/g, ginsenoside Rb_1 12.3 mg/g, Rb_2 10.5 mg/g, Rc 11.29 mg/g, Rd 4.23 mg/g, Re 5.6 mg/g, Rg_1 3.8 mg/g, and Rg_3 3.7 mg/g.

Bacterial strains *H. pylori* strains 51, 52, J99, and SSI were kindly provided by the *H. pylori* Korean Type Culture Collection (HpKTCC; Gyeongsang National University, Jinju, Korea). The 4 strains were incubated on Brucella agar (Difco Laboratories, Detroit, MI, USA) supplemented with 5% horse serum at 37°C for 3 days under micro-aerophilic conditions (10% CO_2 atmosphere).

Anti-*H. pylori* activity The anti-*H. pylori* activity of WGE toward 4 different strains was tested by the disk diffusion method. Briefly, 10^7 CFU/mL of *H. pylori* were inoculated on Brucella agar in petri dishes. Sterilized filter paper disks were placed on the surface of Brucella agar. Test samples were diluted in sterilized distilled water at concentrations of 2.5, 5.0, 10.0, 20.0, and 40.0 mg/mL, and 50 μL of each dilution was added to the filter paper disks. The lowest concentration of WGE tested for which an inhibitory zone was observed at the points of inoculation was after incubation at 37°C for 3 days. The inhibition zone diameter was measured (including the filter paper disk, 8 mm in diameter) and expressed in mm.

Cell lines and culture conditions Human caner cell lines and mouse macrophage cell line were purchased from the Korean Cell Line Bank (KCLB; Seoul National University, Seoul, Korea). The A549 (human lung carcinoma), HEC-1-B (human uterus adenocarcinoma), and SW-156 (human kidney carcinoma) cell lines were maintained in RPMI1640 (Gibco Laboratories, Grand Island, NY, USA) containing 10% heat-inactivated fetal bovine serum (FBS, HyClone, Logan, UT, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL). HeLa (human uterine adenocarcinoma) cells were grown in Minimum Essential medium (MEM), and RAW 264.7 cells were grown in Dublecco's modified Eagles's medium (DMEM) supplemented with 10% heat-inactivated FBS, penicillin, and streptomycin. All cell lines were cultured at 37°C in a humidified incubator containing 5% CO_2. For the testing of cytotoxic activity, cells were seeded in new dishes and grown to 80% confluency.

***In vitro* cytotoxicity test (MTT assay)** The *in vitro* cytotoxic effects of WGE were determined by 3-(4,5-dimethylthizol-2-yl)-2,5-diphenylate-tetrazolium bromide (MTT) assay as described by Park *et al.* (24). Briefly, 100 μL aliquots of cell suspension were transferred to 96-well micro plates and incubated for 24 hr. Hundred μL of WGE was added to each cell suspension, followed by incubation at 37°C for 44 hr. At the end of the incubation, MTT solution (2.5 mg MTT/mL of phosphate buffered saline, PBS) was added and the plate was further incubated for 4 hr. The supernatant was then removed from each cell suspension and 100 μL of dimethyl sulfoxide was added to dissolve the colored formazan crystals produced from reaction with MTT. The optical density (OD) values of each solution were then measured with an microplate reader at 540 nm.

Nitrite assay The accumulation of nitrite (NO_2^-) in the culture media, an indicator of NO synthase activity, was measured using the Griess reaction. RAW 264.7 cells at a density of 1×10^5 cells/well were distributed into 96-well microtiter plates and allowed to adhere overnight. Cells were then treated with the test drug at 37°C for 1 hr before stimulation with 1 μg/mL lipopolysaccharides (LPS, Sigma-Aldrich, St. Louis, MO, USA) for 24 hr in a final volume of 200 μL. Fifty μL of each culture supernatant was mixed with 50 μL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamide dihydrochloride, and 2% phosphoric acid) at room temperature. After 15 min, the absorbance was determined at 540 nm using microplate reader.

Results and Discussion

Anti-*H. pylori* activity The inhibitory effects of WGE on growth of several strains of *H. pylori* were measured using the disk diffusion method as presented in Table 1. The data obtained indicate that *H. pylori* 52 was the most sensitive strain tested, having the largest zone of inhibition (19 mm), followed by J99, SSI, and 51, all with large zones of inhibition (16-17.5 mm). The zones of inhibition were in the range of 11-17.5 mm, and the WGE inhibition zone increased in a dose-dependent manner. The negative control used in this study, did not effect growth of any strains tested.

Bae *et al.* (23) reported that the ginseng BuOH fraction, and ginsenoside Rb_1, Rb_2, Rc, Rg_3, and Rh_2 did not inhibit the growth of *H. pylori* (43504, 82548). However, ginsenoside Rg_3-enriched ginseng and fermented ginsenoside Rh_2-enriched ginseng did inhibit *H. pylori* growth. The minimum inhibitory concentration (MIC) for each were from 500-1,000 μg/mL. 20(*S*)- and 20(*R*)-protopanaxadiols also inhibited *H. pylori* growth with MIC of 50-100 μg/mL. The inhibitory effects of these compounds on the activities of *H. pylori* urease and H^+/K^+ ATPase were also measured. Most of the tested compounds did not inhibit these enzymes. However, 20(*S*)-ginsenoside Rg_3 and Rh_2 weakly inhibited H^+/K^+ ATPase from rat stomach, with IC_{50} values of 0.6 and 0.48 mg/mL, respectively. Park (16) reported that RGE at levels of 1 to 100 μg selectively suppressed *H. pylori*-stimulated C-5(*S*)-hydroxyeicosatetraenoic acid (HETE) production implying the attenuation of 5-lipoxygenase activity, which is similar to the known LOX inhibitor nordihydroguaiaretic acid (NDGA). In this paper, it has shown that WGE has an antimicrobial effect on *H. pylori* to add RGE and some ginsenoside Rg_3 and Rh_2.

Cytotoxic effects of WGE MTT is a tetrazolium salt which is reduced to formazan by living cells via the 'succinate-tetrazolium reductase' system. The formazan produced by cellular suspensions is directly correlated with the number of metabolically active cells, and thus the colorimetric MTT assay is used to assay cell proliferation

Table 1. Anti-*Helicobacter pylori* activity of white ginseng extract (WGE) (Inhibition zone diameter: mm)

Sample (mg/mL)	*H. pylori*[1)]			
	51	52	J99	SS1
WGE 40	16.0±0.000	16.5±0.707	17.5±1.414	16.5±0.707
20	13.0±0.000	14.0±0.000	13.5±0.707	13.0±0.000
10	12.5±0.707	13.0±0.000	13.0±0.000	13.0±0.000
5	11.0±0.000	11.0±0.000	12.0±1.414	11.5±0.707

[1)]Values represent the mean±SD (n=2).

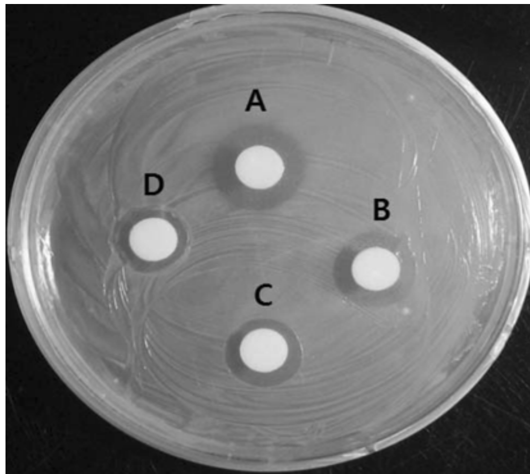

Fig. 1. Inhibitory effect of white ginseng extract on the growth of *H. pylori* 52 as measured by disc diffusion method. A, 40 mg/mL; B, 20 mg/mL; C, 10 mg/mL; D, 5 mg/mL.

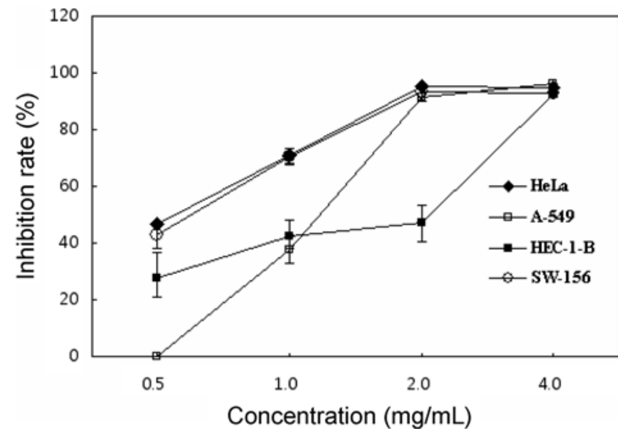

Fig. 2. Cytotoxicity of white ginseng extract (WGE) toward various tumor cell lines as a function of concentration. Values represent the mean±SE of 3 independent determinations.

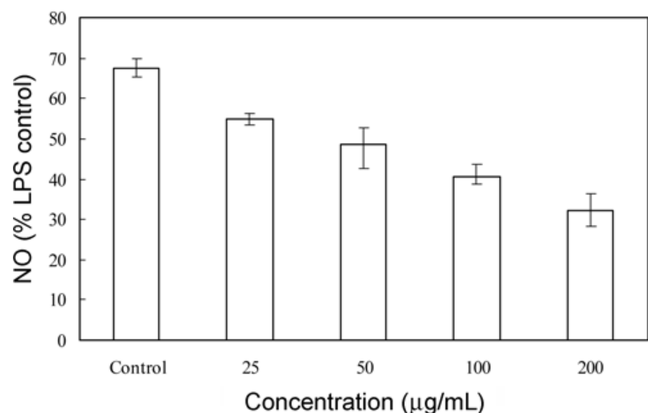

Fig. 3. The effects of white ginseng extract (WGE) on NO production induced by LPS (1 μg/mL) in RAW 264.7 cells. The results are expressed as percentages with the LPS-only values considered as 100%. Values represent the mean±SE of 3 independent determinations.

(17). To determine the cytotoxic effects of WGE on tumor cells, A-549, HEC-1-B, HeLa, and SW-156 cells were treated with a range of 0.5 to 4.0 mg/mL WGE for 48 hr. The inhibition of tumor cell proliferation by WGE at various concentrations is shown in Fig. 2. WGE demonstrated cytotoxic activity that increased steadily with increasing concentration. In particular, proliferation of the SW-156 cell line was inhibited more than 93% at a WGE concentration of 2.0 mg/mL. However, WGE did not inhibit tumor cells growth at a concentration of 0.5 mg/mL.

Jung *et al.* (18) reported that RGE and WGE had cytotoxity toward cancer cells, various mouse cancer cells and peritoneal macrophages, *in vitro,* and that red ginseng was more cytotoxic toward cancer cells than white ginseng. Our result shows that cytotoxicity of WGE was very effective on various human cancer cells when compared to mouse cancer cells. Keum *et al.* (19) reported that ginsenoside Rg_3 inhibits phorbol ester-induced cyclooxygenase-2 expression, NF-κ activation, and tumor promotion. Yun *et al.* (20) reported that Rg_3 causes a statistically significant reduction of lung tumor incidence, as does. These results strongly demonstrate that the anticancer effect of ginseng is due to the ginsenosides Rg_3, Rg_5, and Rh_2 present in Korean red ginseng.

Anti-inflammatory activity of WGE The over-production of NO by iNOS has been implicated in the pathogenesis of cancer via reactive nitrogen oxide species-mediated reactions such as the deamination of DNA bases and DNA strand breaks (21). To analyze the potential anti-inflammatory properties of WGE, we used the murine macrophage cell line RAW 264.7, which can produce NO upon stimulation with LPS, thus providing a suitable model for studying inflammatory responses in culture cells. As a first step, the cytotoxic activity of WGE on RAW 264.7 macrophages in the absence and presence of LPS was assessed using the MTT assay. Cell viability was not affected by WGE at concentrations up to 200 μg/mL (data not shown). Thus, 25-200 μg/mL concentrations of WGE were chosen in

subsequent experiments. When RAW 264.7 macrophages were treated with these concentration of WGE together with LPS (1 μg/mL) for 24 hr, a significant concentration-dependent inhibition of nitrite production was detected with more than 200 μg/mL WGE. Wu *et al.* (22) reported that ginsenoside Rg_1 (0.01-10 μM) and -Re (10, 100 μM), which belong to the protopanaxatriol group, decreased NO_2^- production. However, ginsenoside Rb_2 and -Rd, which belong to protopanaxadiol group, did not show any effects.

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References

1. Cheng Y, Shen LH, Zhang JT. Anti-amnestic and anti-aging effects of ginsenoside Rg_1 and Rb_2 and its mechanism of action. Acta Pharmacol. Sin. 2: 143-149 (2005)
2. Vuksan V, Sung MK, Sievenpiper JL, Stavro PM, Jenkins AL, Di Buono M, Lee KS, Leiter LA, Nam KY, Arnason JT, Choi M, Naeem A. Korean red ginseng (*Panax ginseng*) improves glucose and insulin regulation in well-controlled, type 2 diabetes: Results of a randomized, double-blind, placebo-controlled study of efficacy and safety. Nutr. Metab. Cardiovas. 21: 1-11 (2006)
3. Surh YJ, Na HK, Lee JY, Keum YS. Molecular mechanisms underlying anti-tumor promoting activities of heat-processed *Panax ginseng* C.A. Meyer. J. Korean Med. Sci. 16: S38-S41 (2001)
4. Rai D, Bhatia G, Sen T, Palit G. Anti-stress effects of *Ginkgo biloba* and *Panax ginseng*: A comparative study. J. Pharmacol. Sci. 4: 458-464 (2003)
5. Wang BX, Cui JC, Liu AJ, Wu SK. Studies on the anti-fatigue effect of saponins of stem and leaves of *Panax ginseng* (SSLG). J. Tradit. Chin. Med. 2: 89-94 (1983)
6. Bao HY, Zhang J, Yeo SJ, Myung CS, Kim HM, Kim JM, Park JH, Cho J, Kang JS. Memory enhancing and neiroprotective effects of selected ginsenosides. Arch. Pharm. Res. 3: 333-342 (2005)
7. Lu Zo, Dice JF. Ginseng extract inhibits protein degradation and stimulates protein synthesis in human fibroblasts. Biochem. Bioph. Res. Co. 1: 636-640 (1985)
8. Yuan CS, Wo JA. Ginsenoside variability in American ginseng samples. Am. J. Clin. Nutr. 75: 600-601 (2002)
9. Kwon JH, Belanger JMR, Pare JRJ, Yaylayan V. Application of the microwave-assisted process (MAP) to the fast extraction of ginseng saponins. Food Res. Int. 36: 491-498 (2003)
10. Court WA, Hendal JG, Elmi J. Reversed-phase high performance liquid chromatographic determination of ginsenosides of *Panax quinquefolium*. J. Chromatogr. A 755: 11-17 (1996)
11. Zhu S, Zou K, Cai S, Meselhy MR, Komatsu K. Simultaneous determination of triterpene saponins in Ginseng drugs by high performance liquid chromatography. Chem. Pharm. Bull. 52: 995-998 (2004)
12. Lee JH, Shim JS, Lee JS, Kim MK, Chung MI, Kim KH. Pectin-like acidic polysaccharide from *Panax ginseng* with selective antiadhesive activity against pathogenic bacteria. Carbohyd. Res. 341: 1154-1163 (2006)
13. Lam SK, Ng TB. Pananotin, a potent antifungal protein from roots of the traditional Chinese medicinal herb *Panax ginseng*. Planta Med. 68: 1024-1028 (2002)
14. Masuda H, Naohide K, Woo GJ, Shin IS. Inhibitory effects of *gochoonangi* (*Wasabia japonica*) against *Helicobacter pylori* and its urease activity. Food Sci. Biotechnol. 13: 191-196 (2004)
15. Park S, Choue RW, Cho Y, Ziboh VA. Regional biosynthesis of prostaglandins and hypoxyeicosatetraenoic acids from arachidonic acid in the rat stomach tissue. Prostag. Leukotr. Ess. 68: 35-42 (2003)
16. Park SJ. Inhibition of red ginseng on 5-hydroxyeicosatetraenoic acid (5-HETE) biosynthesis from arachidonic acid in *Helicobacter pylori*-infected gastric cells. Nutr. Sci. 9: 152-158 (2006)
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65: 55-63 (1983)
18. Jung NP, Song SO, Choi SU. Cytotoxicity of white and red ginseng against cancer cells and their effects on the cell cycle. J. Ginseng Res. 24: 183-187 (2000)
19. Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK, Kwon H, Surh YJ. Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett. 150: 41-48 (2000)
20. Yun TK, Lee YS, Lee YH, Yun HY. Cancer chemopreventive compounds of red ginseng produced from *Panax ginseng* C.A. Meyer. J. Ginseng Res. 25: 107-111 (2001)
21. Ohshima H. Genetic and epigenetic damage induced by reactive nitrogen species: Implications in carcinogenesis. Toxicol. Lett. 140/141: 99-104 (2003)
22. Wu CF, Bi XL, Yang JY, Zhan JY, Dong YX, Wang JH, Wang JM, Zhang R, Li X. Differential effects of ginsenosides on NO and TNF-α production by LPS-activated N9 microglia. Int. Immunopharmacol. 7: 313-320 (2007)
23. Bae EA, Han MJ, Coo MK, Park SY, Kim DH. Metabolism of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 by human intestinal bacteria and its relation to *in vitro* biological activation. Biol. Pharm. Bull. 25: 58-63 (2002)
24. Park WB, Choung By, Park SM, Kim HS, Lyu SY. Effects of drying process of mistletoes on cytotoxicities against cultured HL-60 and Molt-4 cells. Food Sci. Biotechnol. 8: 391-396 (1999)
25. Kim SO, Park CW, Moon S, Lee HA, Kim B, Lee DU, Lee JH, Park J. Effects of high-hydrostatic pressure on ginsenoside concentration in Korean red ginseng. Food Sci. Biotechnol. 16: 848-853 (2007)