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Effects of Black Ginseng, Ginseng Berry, Fermented Green Tea Extracts, and the Mixture on Oral health

Sejeong Kim[1)], Yun Ho Jo[1)], Bi-oh Park[1)], Gwang-Hui Jeong[2)], Yong Hwi Son[2)], Kyoung Hwan Cho[2)], and Jin Seong Kim[1)*]

[1)]Geumsan Ginseng and Herb Development Agency, Geumsan 32724, Korea, [2)]Institute of Hadong Green Tea, Hadong 52304, Korea

Abstract

This study aimed at investigating the effects of Black ginseng (BG), Ginseng Berry (GB), Fermented Green Tea (GT) Extracts, and their mixtures on oral health *in vitro*. The BG sample is a Black ginseng made by steaming ginseng over 5 times. Antimicrobial activity of GB and GT against oral pathogens (*Streptococcus mutans* and *Porphyromonas gingivalis* causing dental caries and periodontitis, respectively) was measured by disc diffusion assay. Antimicrobial activity of the purified ginsenoside, known as an index component of ginseng, was also examined. As the results, the antimicrobial activity was rarely observed in GB and FB extracts, but the clearzone was observed in the ginsenosides especially in Rg5 (22 mm) and Rk1 (20 mm). To induce inflammation in Raw264.7 cells (murine macrophage cell line) and YD-38 cells (oral mucosal cell line), LPS (100 ng/ml) and H_2O_2 (300 μM) were treated to those cell line, respectively. The BG, GB, GT extracts (50-500 μg/mL), and their mixtures were additionally treated to the inflammation-induced cell lines to examine the anti-inflammatory and anti-oxidative effects of those natural extracts. As the results, BG (500 μg/mL) and GT (100-200 μg/mL) extracts had anti-oxidative effects with no cytotoxicity. Furthermore the mixtures (BG:GT=2:1) were synergistic for anti-oxidation than the sole treatment of BG and GT extracts. In conclusion, BG and GT extracts, especially their mixtures can be used for protecting oral health.

Introduction

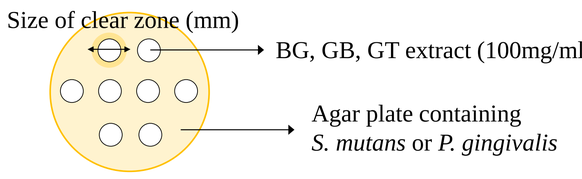
Panax ginseng C.A mayer is a perennial plant that has been traditionally used as an herbal medicine in Asian countries. The root of ginseng is widely used to prevent and treat various diseases such as diabetes, cancer, allergy and hypertension. Its main component, saponin, namely ginsenoside, are believed to have biological activity. There are many kind of ginseng such as white ginseng (WG), red ginseng (RG) and black ginseng (BG). Among them, BG requires 5 cycles of steaming that results in a distinctive black color. This process causes chemical compound change that are in the herb. But previous studies are focused on the therapeutic effect of RG.. Nitric Oxide (NO) is product of inducible NO synthase (iNOS) pathway in response to inflammatory stimuli activated by LPS. Green tea is made from the Camellia sinensis plant. The dried leaves and leaf buds of Camellia sinensis are used to produce various types of teas. Green tea is prepared by steaming and pan-frying these leaves and then drying them. Other teas such as black tea and oolong tea involve processes in which the leaves are fermented (black tea) or partially fermented (oolong tea).Green tea is one of the most popular drinks in the world and has been studied for its health-promoting properties in various diseases such as cancer, obesity, diabetes, cardiovascular disease, and neurodegenerative diseases. Many of the biological effects of green tea are believed to be mediated by its polyphenol catechins and (-)-epigallocatechin-3-gallate (EGCG), which represents 10-15% of total catechins, generally exerts the most effect.

Materials & Methods

Antimicrobial activity on oral pathogens

Disc diffusion assay

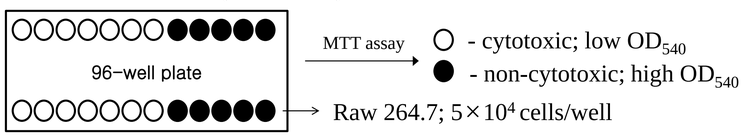
Each bacteria was activated and subcultured in BHI(*S. mutans*) and TSB(*P. gingivalis*) broth, and incubated at 37ºC (with gas pack making anaerobic condition for *P. gingivalis*) until they were grown to mid-logarithmic phase. The bacteria were mixed with 3 mL of each medium containing 0.8% agar to obtain an optical density of 0.1 at 600 nm (OD_{600}), and the mixtures were overlaid on BHI or TSA agar plates. After solidifying the agar under room temperature, the paper discs (10 mm in diameter) containing 100 μL of the extract (100 mg/mL) were placed on the agar plates. After 24 h incubation at 37°C (or with gas pack), the diameters of inhibition zones were measured.



Cytotoxicity in Raw 264.7 cells

Cell viability

RAW 264.7 cell were cultured in DMEM containing heat-inactivated 10 % FBS, 1 % penicillin and streptomycin at 37 ℃ and in 5 % CO_2. Cell viability was determined by MTT reduction assay. Cells were plated in 96 well plate (5×10^4 cells/well) for 24 hours. Cells were pretreated with BG extract and stimulated with LPS (100 ng/ml) for 1 day. Then MTT was added to cells and incubated in 37°C for 1 hour. The resulting blue reduction were resolved in DMSO. Absorbance were measured by using an ELISA kit (Minneapolis, MN, USA) at wavelength of 540 nm.



Anti-inflammatory and anti-oxidant effects

NO assay Cells were pretreated with same with cell viability. Nitric Oxide (NO) was measured in the cell supernatant by using the Griess Reagent kit (Promega). Absorbance were measured by Microplate reader at wavelength of 540 nm.

ROS assay Reactive Oxygen species (ROS) generation was determined by using fluorescence indicator DCF-DA (10 μM) for 40 min. Cells were pretreated same with those in MTT, NO assay. Fluorescence intensity was measured by using an ELISA kit (exi 455/emi 530).

References

(1) Jang, Su Kil, et al. “Double-processed ginseng berry extracts enhance learning and memory in an Aβ42-induced Alzheimer’s mouse model.” Korea J. Food Sci, Technol 51(2019): 160-168.

(2) Chung, Sook Hyun, et al. “Antimicrobial Activity of Extracts and Fraction of Green Tea Used for Coarse Tea.” J Korean Soc Food Sci Nutr 37(11)(2008): 1382-1388.

Results

Result 1. BG, GB, and GT extracts were not effective on inbition of *S. mutans*, but they had antimicrobial effect for *P. gingivalis*.

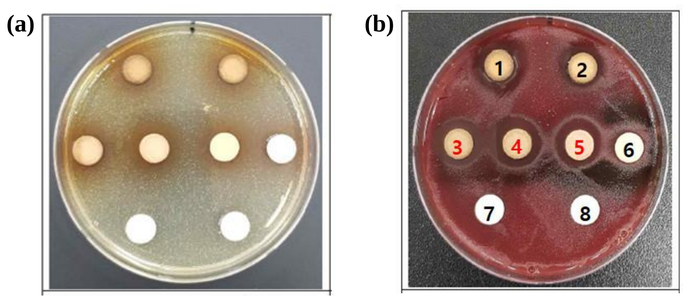


Fig 1. Disc diffusion assay. The media containing *S. mutans* (a) and *P. gingivalis* (b) was treated with disc containing BG, GB, and GT extracts.

Result 2. Ginsenoside Rk1 and Rg5, main saponins in a black ginseng, had significant antimicrobial effect on *P. gingivalis* compared to other ginsenosides.

Table 1. Antimicrobial activities (size of the inhibition zone) of Ginsenoside standards including Rg1, Re, Rb1, Rd, Rb2, Rg3, Rc, Rk1, Rg5, and Rh4 on *P. gingivalis*. The data was presented as means of the triplicated experiments.

Microorganisms	Ginsenoside STD — Inhibition zone: mm									
	Rg1	Re	Rb1	Rd	Rb2	Rg3 (s)	Rc	Rk1	Rg5	Rh4
P. gingivalis	ND	ND	11	13	14	10	12	20	22	10

Result 3. NO and ROS production induced by LPS treatment was significantly reduced by BG (>200ug/ml) and GT (>500ug/ml) extract in Raw 264.7 cells. The concentration was not cytotoxic to the cells.

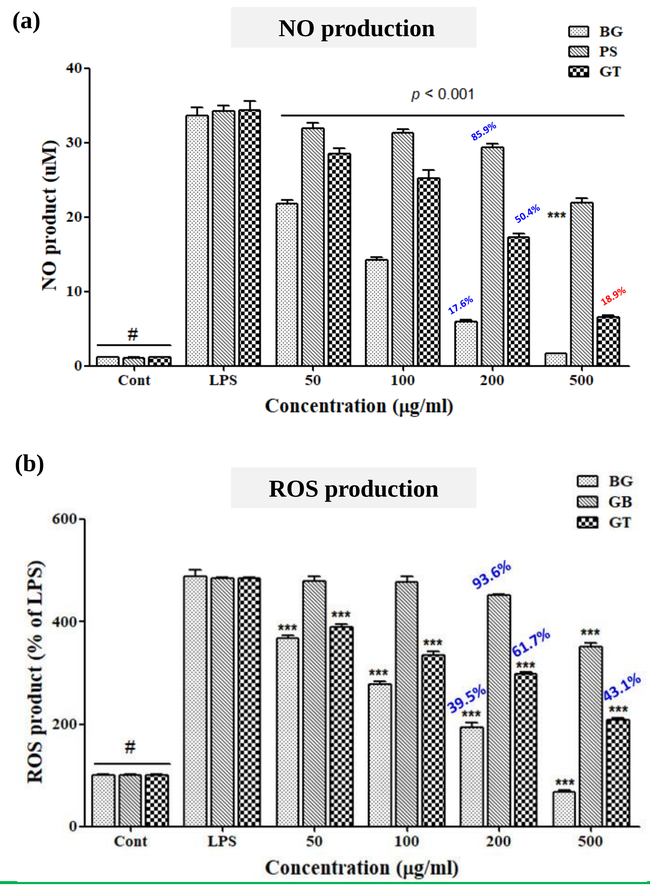


Fig 2. The effect of BG, GB and GT on inhibition of NO (a) and ROS (b) production and cytotoxicity (c) in LPS-stimulated RAW 264.7 cells. The results of MTT cell viability indicate that 200 μg/ml of BG, 500 μg/ml PS and GT have no cytotoxicity. (*p<0.05, **p<0.01 ***p<0.001). BG; Black ginseng, GB; Ginseng berry, GT; Green tea

Result 4. BG and GT had anti-oxidant effects on gingival epithelial cell line (YD-38), and the mixtures (BG:GT=2:1) were synergistic for anti-oxidation than the sole treatment of BG and GT extracts.

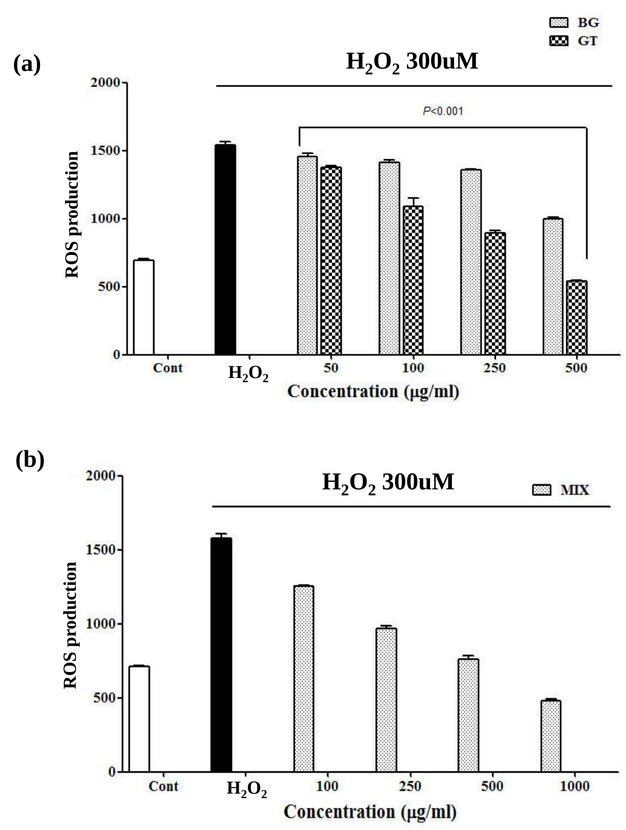


Fig 3. The effect of BG, GT and the mixture on inhibition ROS production in H_2O_2 stimulated YD-38 cells. The results denote ROS expression after 24 hr induction of inflammation. In GT treated cells, noticeable reduction of ROS was revealed. BG; Black ginseng, GT; Green tea

Conclusion

- BG and GT extracts, especially their mixtures can be used for protecting oral health.
- The mixtures can be applied to develop “complex” functional products effective on prevention of dental disease.
- Further study that observes anti-periodontitis effects of those natural products at a protein level is required.