Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20160521021323

Original Research

ScopeMed

DNA protective effect of ginseng and the antagonistic effect of Chinese turnip: A supplementation study

Yim Tong Szeto[1], Kam Shing Wong[2], Andrea Han[3], Sok Cheon Pak[4], Wouter Kalle[4]

[1]Department of Applied Science, Hong Kong Institute of Vocational Education (Shatin), Vocational Training Council, Hong Kong, [2]Department of Pathology, Yan Chai Hospital, Kowloon, Hong Kong, [3]Department of Biochemistry, Chinese University of Hong Kong, Hong Kong, [4]School of Biomedical Sciences, Charles Sturt University, NSW, Australia

Address for correspondence:
Yim Tong Szeto, Department of Applied Science, Hong Kong Institute of Vocational Education (Shatin), Vocational Training Council, Hong Kong.
E-mail: ytszeto@alumni.vtc.edu.hk

Received: February 08, 2016
Accepted: May 21, 2016
Published: June 25, 2016

ABSTRACT

Aim: The aim of this clinical study is to provide scientific evidence for supporting traditional Chinese application and usage to the patients. For this purpose, we tested the ability if *Panax ginseng* extract to lower oxidative damage to nuclear DNA in human lymphocytes by comparing the effect of cooked Chinese turnip on this effect. **Materials and Methods:** Seven healthy subjects (4 males and 3 females from 37 to 60 years) participated two occasions which were at least 2 weeks apart. About 2 mL of fasting blood sample for baseline measurement was taken on arrival. They were requested to ingest the content of 5 ginseng capsules in 200 mL water. The subject remained fasting for 2 h until the second blood sample taken. In the other occasion, the experiment was repeated except a piece of cooked turnip (10 g) was taken with the ginseng extract. The two occasions could be interchanged. Comet assay was performed on two specimens on the same day for the evaluation of lymphocytic DNA damage with or without oxidative stress. **Results:** For the group with ginseng supplementation, there was a significant decrease in comet score for hydrogen peroxide (H_2O_2) treatment over the 2-h period while no change in DNA damage for unstressed sample. For the group with ginseng together with turnip supplementation, there was no significant difference in comet score for both H_2O_2 treatment and phosphate-buffered saline treatment. Ginseng extract could reduce DNA damage mediated by H_2O_2 effectively, but this protection effect was antagonized by the ingestion of cooked turnip at the same time. **Conclusion:** In the current study, commercial ginseng extract was used for supplementing volunteers. Ginseng extract could protect DNA from oxidative stress *in vivo* while turnip diminished the protection.

KEY WORDS: Antioxidant, DNA, ginseng, protection, turnip

INTRODUCTION

DNA damage has been considered as an important cause of cancer and many diseases related to aging [1]. The main source of DNA damage is reactive oxygen species (ROS) which can be produced from endogenous sources such as mitochondria, peroxisomes, and inflammatory cell activation as well as exogenous sources including environmental pollutants, pharmaceuticals, and industrial chemicals. The oxidative stress may cause DNA damage and resulting the change in chromosome stability, genetic mutation, and altered gene expression that may lead to cancer development [2]. Reduction of DNA damage has been found to be possible by the antioxidant rich diet or supplement while negative correlation has been found between antioxidant level and DNA damage [3]. Numerous traditional Chinese medicinal herbs are recognized for their antioxidant properties [4-6]. Herbal decoctions may increase the activity of certain antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase [7]. *Panax ginseng*, which is the most famous herb, has been used for curative and restorative functions for thousands of years. It has also been used to regulate human's physical, mental, and sexual ability [8]. Its *in vivo* antioxidant activity or protecting effect on DNA has been revealed [9]. For the convenience of intake, capsules containing ginseng extract are available commercially and are widely accepted in the market. In accordance with traditional Chinese medicine beliefs, Chinese turnip counteracts the beneficial effect of ginseng. Ginseng and Chinese turnip should not be taken together. In terms of DNA protecting activity of ginseng, this phenomenon has

been shown *in vitro* [10]. To provide the scientific evidence for supporting traditional Chinese medicine application and usage, the captioned common belief would be tested *in vivo* in the current study. The ability of ginseng extract to lower oxidative damage to nuclear DNA in human lymphocytes would be compared with the presence of cooked Chinese turnip. The evaluation of DNA damage would be achieved by comet assay. Damaged DNA of individual cells would be visualized under fluorescence microscope [11].

MATERIALS AND METHODS

Ethical approval was obtained from the Human Subject Ethics Panel, Research Ethics Subcommittee, and Vocational Training Council. The trial was a cross-over study. Inclusion criteria were subjects not under long-term medication nor pregnant and at the age above 18. Physician-assessed the subjects and no acute or chronic diseases were reported. Seven healthy subjects (4 men and 3 women; 37-60 years old) entered the study with no dropout. The number of subjects tested based on the previous study which considers to detect the change of comet score of 45 (standard deviation = 15) with 80% power [9].

Written informed consent was obtained from the subject prior experiment. All subjects were requested to participate in two sampling occasions. They were requested to fast overnight before the experiment. At 09:00, 2 mL of venous blood was taken from the subject as the baseline followed by ingesting the content of 5 ginseng capsules suspended in 200 mL water. The subject remained fasting for 2 h and second blood sample was collected. Another occasion of the experiment was arranged at least 2 weeks after the first visit. The procedures of sampling were almost the same except the subject ingested a piece of cooked turnip (10 g) together with the ginseng capsules content. The two occasions could be interchanged which the subject could take turnip for the first part but no turnip intake for the second part. This could rule out the bias from carry-over effect. The comet assay was started in the afternoon of the same day of supplementation.

Harvesting of blood sample and comet assay was performed as described in the previous study in detail [12]. The gels were stained with 40 mL of ethidium bromide (2 mg/L) and then visualized at ×400 magnification using fluorescence microscope (Nikon Eclipse Ni with TRITC filter: Ex 540/25, Nikon, Tokyo, Japan). Damaged nucleus exhibited migration of the DNA toward the anode. The resulting image looked like a "comet" with the intensity of the comet tail relative to the head reflects the number of DNA breaks. The reason for this is that loops containing a break lose their supercoiling and become free to extend toward the anode. Quantification of DNA damage was done by visual scoring. Five classes (from 0 to 4) of damaged cells were classified according to different degrees of damage [13]. 100 comets per gel were counted, and two gels were prepared per slide. The score of a gel ranged from 0 to 400 in arbitrary unit which was positively associated to the DNA damage.

Equation for scoring:

Score = [(Grade 0×0) + (Grade 1×1) + (Grade 2×2) + (Grade 3×3) + (Grade 4×4)]/100

Grade 0+Grade 1+Grade 2+Grade 3+Grade 4=100

Statistical Analysis

Prism 5.0 (GraphPad software, USA) was used with Wilcoxon signed rank test to evaluate the DNA protective effect for different groups. A $P < 0.05$ was considered statistically significant.

RESULTS

The comet scores before and 2 h after ingestion were compared with ginseng group and ginseng+turnip groups. For the group with ginseng extract supplementation, there was a statistically significant decrease ($P = 0.0223$) in comet score in hydrogen peroxide (H_2O_2) treatment over the 2 h period while there was no significant difference ($P = 0.8125$) in score for unstressed phosphate-buffered saline (PBS) treatment (Figure 2a and b). There was no statistically significant change in comet score for both H_2O_2 treatment ($P = 0.2188$) and PBS treatment ($P = 0.5992$) over 2 h in ginseng+turnip group (Figure 2c and d). The results indicated that ginseng extract could lower DNA damage mediated by H_2O_2 effectively within a very short period of time (2 h). However, this protection effect was antagonized by the simultaneous ingestion of boiled turnip.

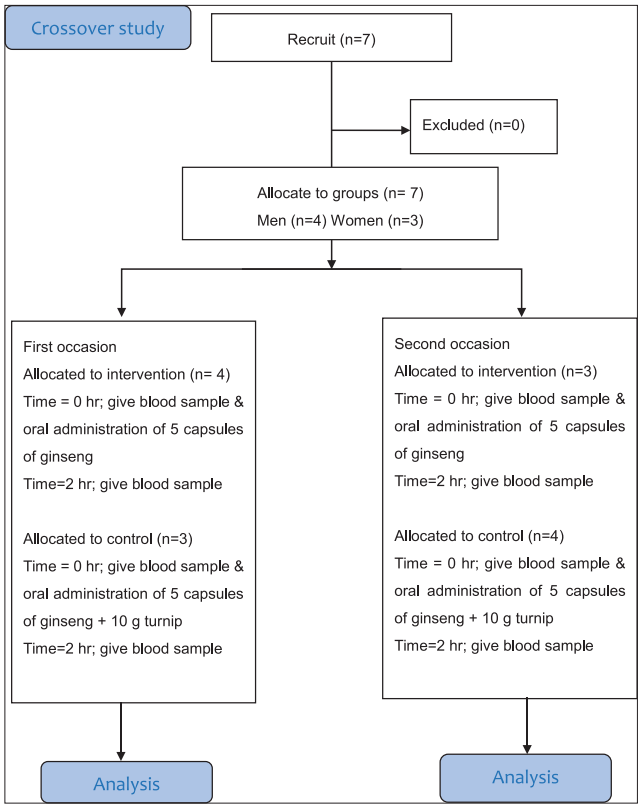

Figure 1: The flow diagram of the cross over study

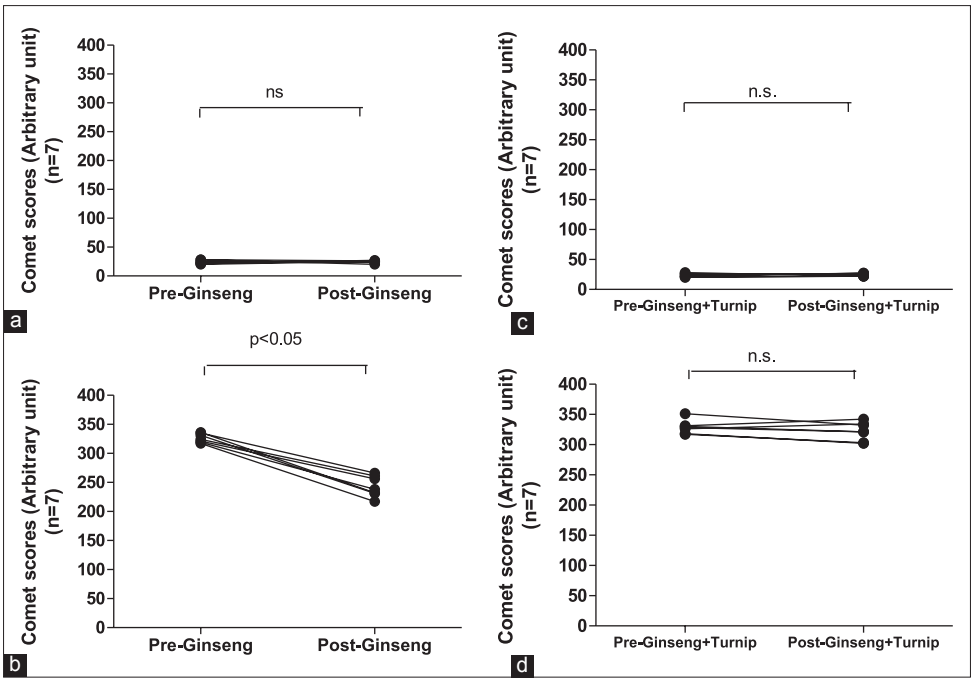

Figure 2: DNA damage before and 2 h after ginseng only supplementation. There was a significant decrease in comet score (P=0.0223) for, (a) hydrogen peroxide (H_2O_2) treated lymphocytes. No significant change in DNA damage (P=0.8125) for, (b) the control group. For DNA damage before and 2 h after ginseng and turnip supplementation. There was no significant decrease in comet score of lymphocytes for both, (c) H_2O_2 treated (P=0.2188) nor, (d) phosphate-buffered saline treated (P=0.5992)

DISCUSSION

Antioxidants are substances that neutralize oxidative species that associated with DNA damage. Natural occurring antioxidants can be found in diet and the protective effects of dietary antioxidants on DNA damage have been extensively studies [14]. DNA damage is the critical cause of aging and it directs to diseases and cancer development [1]. For preventing the development of cancer and delaying aging process, minimizing DNA damage is essential.

Traditional Chinese herb such as ginseng has been extensively used for curative and restorative functions. Commercial ginseng extract can now be obtained in capsule form for convenient intake. A number of studies have reported the antioxidant action of ginseng [6,10,15]. Their antioxidant properties have been correlated with the properties of Chinese herbs according to the yin/yang ideology of TCM although not conclusive [5,16].

Studies in animals demonstrate protection against oxidative damage from cadmium chloride [17] and carbon tetrachloride [18,19]. Ginseng has been found to decrease generation of ROS and effectively decreased serum malondialdehyde (MDA) levels in healthy subjects [20]. Hypolipidemic effect of ginseng has been observed along with increased SOD and catalase activities [21]. It also has the supportive effect on exhaustive exercise with lowering MDA level [22].

In animal model, DNA damage and reproductive toxicity were evaluated in testis of rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxinthe. A significantly decreased level of DNA damage and reduced pathological effects were observed in the ginseng extracts treated group. The effect of ginseng on DNA damage has also been shown in human in our previous study [9]. Comet assay shows decreased lymphocyte DNA damage and increased antioxidative enzymes after 8 weeks ginseng supplementation [23].

Results of this study showed that ingestion of ginseng extract could significantly increase lymphocytic DNA resistance to oxidative stress in a very short period of time as fast as 2 h. This could be reflected from the significantly reduction in comet scores for H_2O_2 treated lymphocytes in post-ginseng ingestion blood samples as compared with pre-ingestion. This agreed with our previous *in vitro* and supplementation study [6,9]. Effect of Chinese turnip on the effect of ginseng was also demonstrated in the current study. According to the rules and beliefs in the use of traditional Chinese medicine, there may be existence of incompatibility or antagonism between herbs or some foods. We have demonstrated that incubating ginseng along with turnip juice abolished the DNA protective effect of both American and Asian ginseng [10]. Both unboiled and boiled turnip juices are able to abolish protective effect offered by ginseng which implies, the effect of turnip juice is unlikely to be enzymatic [10]. No significant improvement in DNA protection 2 h after ingestion of ginseng extract together with cooked turnip confirmed turnip could diminish the protective effect of ginseng *in vivo* as well. This provided evidence to support the ideology of tradition Chinese medicine application regarding incompatibility.

In conclusion, this study showed that the ingestion of content of five capsules of commercial ginseng extract could

offer a significant decrease of DNA damage of lymphocytes demonstrated in comet assay. But with the ingestion of cooked turnip together with the ginseng extract content, the protection diminished. This supported our previous study and the traditional ideology that the incompatibility natures of ginseng and Chinese turnip in the sense of DNA protection.

ACKNOWLEDGMENTS

The authors would like to thanks the VTC Seed Funding Scheme (2014150206) for supporting this study.

REFERENCES

1. Hoeijmakers JH. DNA damage, aging, and cancer. N Engl J Med 2009;361:1475-85.
2. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 2010;38:96-109.
3. Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res 2005;570:197-203.
4. Chan SW, Li S, Kwok CY, Benzie IF, Szeto YT, Gao DJ, *et al*. Antioxidant activity of Chinese medicinal herbs. Pharm Biol 2008;46:587-95.
5. Szeto YT, Benzie IF. Is the yin-yang nature of Chinese herbal medicine equivalent to antioxidation-oxidation? J Ethnopharmacol 2006;108:361-6.
6. Szeto YT, Wong SC, Wong JW, Kalle W, Pak SC. *In vitro* antioxidation activity and genoprotective effect of selected Chinese medicinal herbs. Am J Chin Med 2011;39:827-38.
7. Guo KJ, Xu SF, Yin P, Wang W, Song XZ, Liu FH, *et al*. Active components of common traditional Chinese medicine decoctions have antioxidant functions. J Anim Sci 2011;89:3107-15.
8. Aramwit P, Wirotsaengthong S. Overview of commonly used Chinese herbs. J Med Plants Res 2012;6:4505-21.
9. Szeto YT, Ko AW. Acute genoprotective effects on lymphocytic DNA with ginseng extract supplementation. J Aging Res Clin Pract 2013;2:174-7.
10. Szeto YT, Wong JW, Wong SC, Pak SC, Benzie IF. DNA protective effect of ginseng and the antagonistic effect of Chinese turnip: A preliminary study. Plant Foods Hum Nutr 2011;66:97-100.
11. Wong VW, Szeto YT, Collins AR, Benzie IF. The comet assay: A biomonitoring tool for nutraceutical research. Curr Top Nutraceutical Res 2005;3:1-14.
12. Szeto YT, To TL, Pak SC, Kalle W. A study of DNA protective effect of orange juice supplementation. Appl Physiol Nutr Metab 2013;38:533-6.
13. Szeto YT, Lee LK. Rapid but mild genoprotective effect on lymphocytic DNA with Panax notoginseng extract supplementation. J Intercult Ethnopharmacol 2014;3:155-8.
14. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-22.
15. Ma SW, Benzie IF, Chu TT, Fok BS, Tomlinson B, Critchley LA. Effect of *Panax ginseng* supplementation on biomarkers of glucose tolerance, antioxidant status andoxidative stress in type 2 diabetic subjects: Results of a placebo-controlled human intervention trial. Diabetes Obes Metab 2008;10:1125-7.
16. Liao H, Banbury LK, Leach DN. Antioxidant activity of 45 Chinese herbs and the relationship with their TCM characteristics. Evid Based Complement Alternat Med 2008;5:429-34.
17. Shukla R, Kumar M. Role of *Panax ginseng* as an antioxidant after cadmium-induced hepatic injuries. Food Chem Toxicol 2009;47:769-73.
18. Bak MJ, Jun M, Jeong WS. Antioxidant and hepatoprotective effects of the red ginseng essential oil in H(2)O(2)-treated hepG2 cells and CCl(4)-treated mice. Int J Mol Sci 2012;13:2314-30.
19. Karadeniz A, Yildirim A, Karakoc A, Kalkan Y, Celebi F. Protective effect of *Panax ginseng* on carbon tetrachloride induced liver, heart and kidney injury in rats. Rev Méd Vét 2009;160:237-43.
20. Kim HG, Yoo SR, Park HJ, Lee NH, Shin JW, Sathyanath R, *et al*. Antioxidant effects of *Panax ginseng* C.A. Meyer in healthy subjects: A randomized, placebo-controlled clinical trial. Food Chem Toxicol 2011;49:2229-35.
21. Kim SH, Park KS. Effects of *Panax ginseng* extract on lipid metabolism in humans. Pharmacol Res 2003;48:511-3.
22. Kim SH, Park KS, Chang MJ, Sung JH. Effects of *Panax ginseng* extract on exercise-induced oxidative stress. J Sports Med Phys Fitness 2005;45:178-82.
23. Kim JY, Park JY, Kang HJ, Kim OY, Lee JH. Beneficial effects of Korean red ginseng on lymphocyte DNA damage antioxidant enzyme activity, and LDL oxidation in healthy participants: A randomized, double-blind, placebo-controlled trial. Nutr J 2012;11:47.

Source of Support: Nil, Conflict of Interest: None declared.