



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

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## 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<sub>2</sub>O<sub>2</sub>) 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<sub>2</sub>O<sub>2</sub> treatment and phosphate-buffered saline treatment. Ginseng extract could reduce DNA damage mediated by H<sub>2</sub>O<sub>2</sub> 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

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## 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:

$$\text{Score} = [( \text{Grade } 0 \times 0 ) + ( \text{Grade } 1 \times 1 ) + ( \text{Grade } 2 \times 2 ) + ( \text{Grade } 3 \times 3 ) + ( \text{Grade } 4 \times 4 )] / 100$$

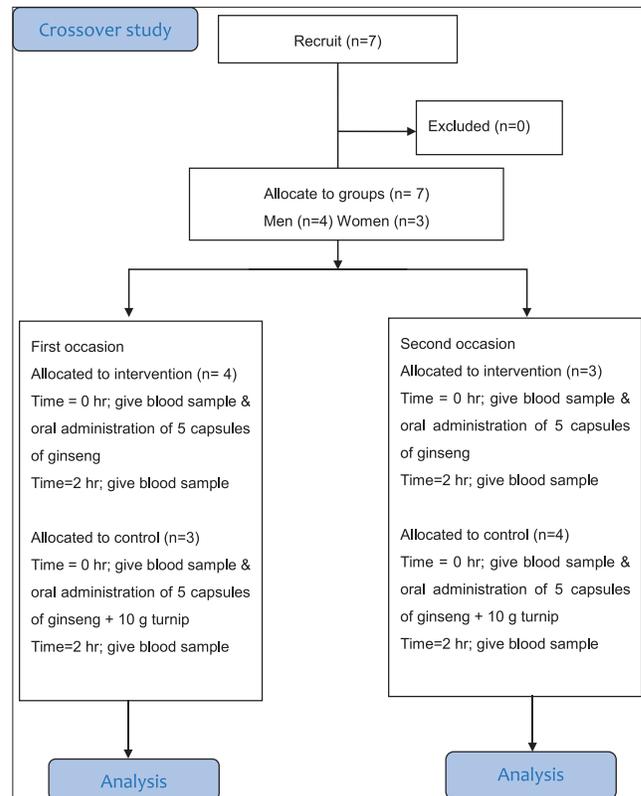
$$\text{Grade } 0 + \text{Grade } 1 + \text{Grade } 2 + \text{Grade } 3 + \text{Grade } 4 = 100$$

**Statistical Analysis**

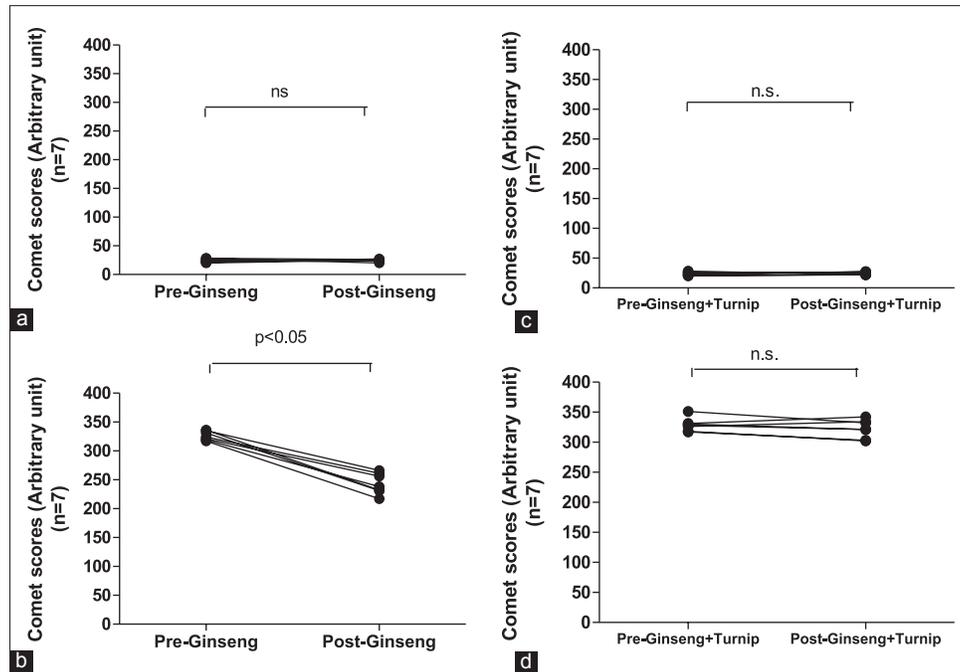
Prism 5.0 (GraphPad software, USA) was used with Wilcoxon signed rank test to evaluate the DNA protective effect for different groups. A *P* < 0.05 was considered statistically significant.

**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<sub>2</sub>O<sub>2</sub>) 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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 studied [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-dioxin. 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.

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