



# Rapid but mild genoprotective effect on lymphocytic DNA with *Panax notoginseng* extract supplementation

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## ABSTRACT

**Background:** *Panax notoginseng* (PN) is a well-known Chinese medicinal herb traditionally used as a hemostatic agent that strengthens and builds blood. The free radicals scavenging and antioxidant property of PN have been demonstrated in various studies either *in vitro* or in animal models, however, the genoprotective effect at human cellular level remains to be elucidated. **Aim:** The current supplementation study aimed to investigate the genoprotective effect of PN. The study explored the DNA protection effect after a single dose of 2500 mg commercial notoginseng extract in water. **Materials and Methods:** Six subjects, (3 males, 3 females) were recruited and each attended two trials. In the first trial, pre-ingestion and 2 hour-post-ingestion blood samples were collected and they were challenged with 50  $\mu$ M H<sub>2</sub>O<sub>2</sub>. In the second trial, water was taken instead as control. Lymphocytes with or without challenge were then subjected to comet assay. DNA damage was assessed under fluorescent microscope. **Results:** Results showed a significant ( $P < 0.05$ ) but mild decrease (3%) in the comet score after PN supplementation, indicating that PN supplementation reduces the H<sub>2</sub>O<sub>2</sub>-induced DNA damage in the lymphocytes and enhanced their resistance to oxidative damage giving a mild acute genoprotective effect against oxidant challenge. No change of comet score was observed in control trial.

**KEY WORDS:** Antioxidant, comet assay, hydrogen peroxide, pseudoginseng, supplementation

## INTRODUCTION

*Panax notoginseng* (PN) or Sanqi in Chinese, is the root of the perennial herbaceous plant PN (Burk.) F. H. Chen. The plant belongs to the araliaceae family and is mainly grown naturally in the southwest provinces of China such as Yunnan and Guangxi, though they are also found in the southwest of Nepal and Burma. The root of notoginseng is usually collected in autumn before flowering or after the seed has ripened and sun-dried for use as medicine. Notoginseng was first described in about 600 years ago in the literature of Chinese herbs, "compendium of materia medica." Notoginseng is described as "a precious Chinese herbal medicine for the blood." This traditional herbal medicine is widely used for the treatment of blood disorders such as blood stasis, bleeding and blood deficiency. It is also used to heal traumatic injuries, to alleviate inflammation, swelling and pain, to stimulate blood circulation, and to speed up healing.

Many studies have revealed the physiological functions of notoginseng on the cardiovascular system, cerebrovascular system, blood clotting system, central nervous system, and endocrine system. Studies of the functions on blood clotting system have shown the anticoagulant and the anti-platelet activation, adhesion and aggregation effects of notoginseng [1,2] and studies of the functions on central nervous system have demonstrated that notoginseng saponins enhance antioxidant activity and protect neuron from oxidative stress and apoptosis [3,4]. Notoginseng has also demonstrated the antioxidative and the free radicals scavenging effect of notoginseng saponins on reactive oxygen species (ROS) [5-7]. These studies were performed *in vitro* by adding different free radicals to the notoginseng extract, measuring the radical scavenging activities, thus the antioxidant capacity of the extract. Most commonly used radicals are peroxy radicals, hydroxyl radical, superoxide radical, and singlet oxygen because these are the most common free radical present in the human

biological system. However, *in vitro* studies could not be directly translated to *in vivo* interpretation because of the diverging test conditions [8]. Thus, the study on the genoprotective activity of notoginseng in human cells would be of research interest to provide useful information on the antioxidant property and genoprotective action of notoginseng at the human cellular level. Perhaps this may extend the pharmacological usage of notoginseng beyond the areas of cardiovascular and cerebrovascular diseases

## MATERIALS AND METHODS

### Chemicals

Sodium chloride (NaCl) and sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) for PBS were from Panreac Quimica SA (Barcelona, Spain); disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) was from Sigma; Type VII low melting point (LMP) agarose, disodium ethylenediaminetetraacetic acid (EDTA) dehydrate; Triton X-100, sodium chloride for lysis solution; ethidium bromide were from Sigma (Steinheim, Germany). standard agarose (agarose 3:1) SA from Amresco (Solon, OH, USA). Tris (hydroxymethyl) aminomethane (Tris) was from Gibco (Carlsbad, CA, USA); sodium hydroxide were from Merck (Darmstadt, Germany). Hydrogen peroxide solution was from International Laboratory (South San Francisco, CA, USA).

Commercial notoginseng vege-capsules were purchased from Watson (group) Ltd., Hong Kong. Each capsule contained 500 mg notoginseng powder and content from 5 capsules (2500 mg) was used for each dose. The PN powder inside the 5 capsules was emptied into a glass, 100 mL of hot water was added and well-mixed. Another 100 mL of cold water was added and mixed to cool down the PN solution before taken.

### Sample Collection

The study was approved by the Ethics Committee of Macao Society for the Study of Women's Health. All procedures involving human subjects complied with the Declaration of Helsinki. Six healthy subjects, 3 males and 3 females, aged from 27 to 57 (mean = 37); with no history of chronic disease and not on any medication or health supplements were recruited. A single dose of 200 mL of PN suspension was taken by subject 1 while 200 mL of water was taken by subject 2 in the same occasion. Venous blood samples were taken at 0 h and 2 h, respectively, and collected in EDTA tubes. The same procedure was repeated after 7 days with subject 1 taking water and subject 2 taking PN solution. A maximum of two subjects participated the study on a single day.

### Oxidative Challenge

Lymphocytes were harvested as previously described [9]. 1 mL of 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$  was used to challenge pre- and post-supplementation lymphocytes sample for 5 min. Stressed and unstressed cells were then subjected to comet assay.

### Comet Assay

Clean microscope slides were pre-coated with 1% SA. Two aspirations, each of 85  $\mu\text{L}$  of 1% SA, were layered onto the dried pre-coated slides. Cover-slip was used to flatten the agarose. The coverslips were removed after the agarose was set at 4°C for 5 min. 170  $\mu\text{L}$  of 1% LMP agarose was added to each tube, followed by quick layering of two aspirations, each of 85  $\mu\text{L}$  of the LMP embedded lymphocytes onto the SA platform. Cover-slip was used to flatten the agarose. The slides were then put at 4°C for 5 min to facilitate gelling. The cover-slip was removed, and the slides were put into a Coplin jar containing 40 mL cold lysis solution for 1 h at 4°C. The slides were transferred to another Coplin jar containing 40 mL cold electrophoresis solution of pH >13 at 4°C for 20 min. The slides were put under alkaline electrophoresis solution and electrophoresed 20 V for 30 min. Alkaline electrophoresis solution was removed by 3 changes of distilled water at 5 min each. The slides were left to dry at room temperature overnight.

40  $\mu\text{L}$  ethidium bromide was added to each gel for DNA staining to enable cell scoring under the fluorescence microscope (Eclipse 80i with Epi-fluorescence attachment, excitation filter G: Ex 510-560, Nikon, Tokyo, Japan). Each gel was covered with coverslip, and the slides were kept in the dark until counting. A total of 100 randomly selected cells was scored per slides (50 on each gel) using the visual scoring system from score 0 to score 4 according to the intensity of the comet head and the length of the comet tail. Score 0 indicated no tail and score 4 indicated almost all DNA is present in the tail. The degree of oxidative damage was measured by the amount of DNA liberated from the head of the comet, which meant the lower the score, the lesser damage by the hydrogen peroxide.

### Statistical Analysis

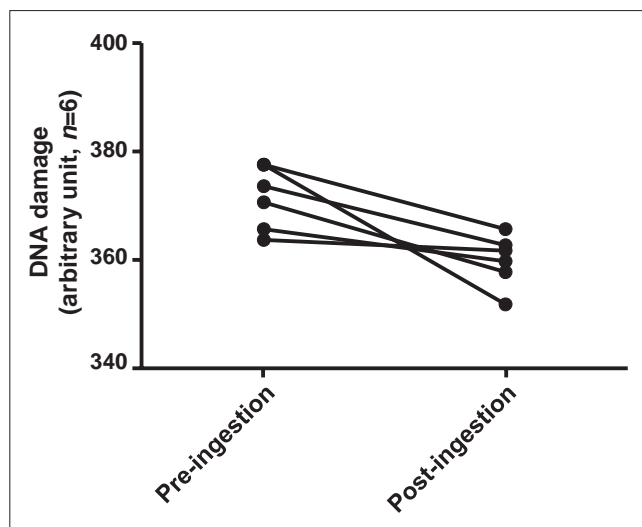
Wilcoxon signed rank test was used to investigate for any significant difference between the pre- and post-notoginseng intake score as well as pre- and post-water intake (Prism 5.0, GraphPad Software, CA, USA).

## RESULTS

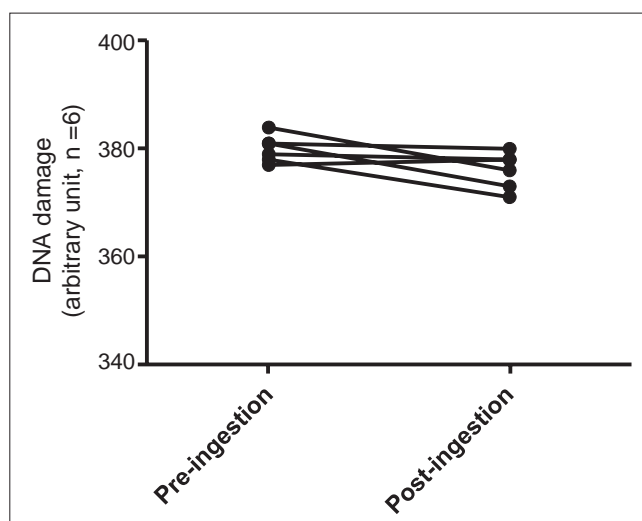
The comet scores before and after 2 h of intake of 2500 mg commercial notoginseng powder in 100 mL water were shown in Figures 1 and 2. For all of the 6 subjects, there was varying degree of increase resistance to oxidative damage (decrease DNA damage/comet score), when challenged *in vitro* by  $\text{H}_2\text{O}_2$  after notoginseng supplementation. There was a statistical significant reduction in comet score, but the effect was very mild (3%). There was no significant change in DNA damage ( $P > 0.05$ ) when challenged by  $\text{H}_2\text{O}_2$  in control (water only) trial [Figure 3].

## DISCUSSION

Being a traditional Chinese herbal medicine, PN has been used for hundreds of years for health promotion or treatment of various blood diseases, especially the cardiovascular and cardio-

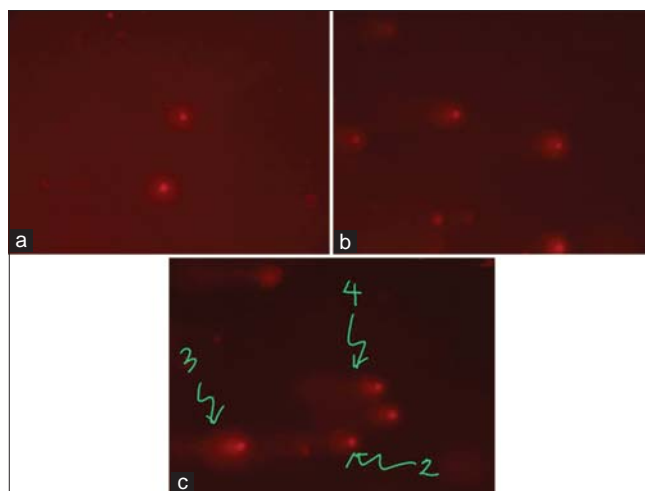


**Figure 1:** Acute effect of *Panax notoginseng* supplementation on DNA of human lymphocytes using the comet assay. Data expressed as the mean  $\pm$  standard deviation of comet scores of data obtained from 6 subjects. Lymphocytes of the subjects after ingestion of 2500 mg commercial notoginseng extract were challenged *in vitro* by 50  $\mu$ M  $H_2O_2$ . Lymphocytes of the subjects before ingestion were also challenged *in vitro* by 50  $\mu$ M  $H_2O_2$  and act as control.  $P < 0.05$ , compared to the control, the Wilcoxon signed rank test



**Figure 2:** Acute effect of water intake on DNA of human lymphocytes using the comet assay. Data expressed as the mean  $\pm$  standard deviation of comet scores of data obtained from 6 subjects. Lymphocytes of the subjects after 200 mL water intake were challenged *in vitro* by 50  $\mu$ M  $H_2O_2$ . Lymphocytes of the subjects before intake were also challenged *in vitro* by 50  $\mu$ M  $H_2O_2$  and act as a control.  $P > 0.05$ , compared to the control, the Wilcoxon signed rank test

cerebrovascular disorders. Various studies have demonstrated the effectiveness of notoginseng to treat cardiovascular diseases in humans and myocardial infarct in animal models. It is suggested that the biological functions of notoginseng are partly due to their protective effects against ROS by suppressing the attack of  $\bullet OH$  on membrane lipids, inhibiting lipid peroxidation, and thus protecting the cell membrane from oxidative injury. The inhibitory effects of notoginseng on lipid peroxidation have been



**Figure 3:** Comet images showing visual comet score (a) 0, undamaged DNA; (b) 1, mild DNA damage; (c) 2-4; moderate to severe DNA damage

reported in various animal studies [10,11]. Besides, some studies on notoginseng antioxidant activities have also demonstrated the *in vitro* antioxidative and the *in vitro* ROS scavenging effects of notoginseng saponins, showing strong scavenging activity toward  $\bullet OH$ ,  $H_2O_2$  and  $O_2^{\bullet -}$  [6,7]. Dammarane saponins are the major bioactive components of notoginseng, in which the major constituents are notoginsenoside R1, ginsenosides Rg1, Re, Rb1, Rg2, Rh1, and Rd [12].

Although the antioxidant power of notoginseng has been confirmed in various studies, the DNA protective effect of notoginseng in human cells and the effective concentrations have yet to be explored. Our previous study demonstrated *in vitro* study DNA protective effect of notoginseng extract [13]. It has been found that pre-treatment with aqueous but not ethanolic extract of notoginseng is able to diminish  $H_2O_2$ -induced DNA damage in human lymphocytes. A wide range of concentrations of notoginseng extract has been used to investigate the effective level of notoginseng for this genoprotective effect. The current supplementation study explored the genoprotective effect of notoginseng after 2 h intake. Lymphocytes of subjects after drinking water or notoginseng supplement were challenged with  $H_2O_2$  *in vitro* and DNA damage was investigated. The comet assay was used to detect DNA strand breaks, and the comet score was used to assess the DNA damage in the supplementation setting.

Results showed a statistical significant decrease ( $P < 0.05$ ) in the comet score of the lymphocytes 2 h after ingestion. These results indicated that a single dose of notoginseng supplement of 2500 mg slightly reduced the  $H_2O_2$ -induced DNA damage in the lymphocytes and enhances their resistance to oxidative damage. However, the effect was mild which led to only  $\sim 3\%$  decrease in comet score after treatment. This result contrasted the *in vitro* findings which showed an average of more than 20% reduction of the comet score, demonstrating a marked enhancement of genoprotection in the lymphocytes after notoginseng pretreatment [13]. The

difference in the extent of effectiveness between *in vitro* pretreatment and supplementation might be due to the fact that the latter involves a complicated process of absorption and metabolism before they can reach the lymphocytes in the systemic circulation to exhibit their effects. Our previous studies demonstrate supplementation with antioxidant rich dietary agents can significantly lowering of H<sub>2</sub>O<sub>2</sub>-induced DNA damage in lymphocytes within 2-2.5 h. Orange juice [14], grape seed extract, and ginseng extract (our unpublished data) are able to diminish 19-77% of comet score. Study on rat models and Caco-2 cells have proved that Rb1 and Rg1, two of the seven major saponins present in the notoginseng extract, are readily eliminated in stomach and degraded significantly in the large intestine. It is also reported that their absorption are mainly passive and the extent of absorption may be dominated by the low membrane permeability [15]. This assumption is supported by another study which reported the two main factors limiting the absorption of most ginsenosides and their deglycosylated metabolites to the systemic circulation are poor membrane permeability and active biliary excretion [16]. These studies have demonstrated low bioavailability of notoginseng in animals but that in humans remains to be elucidated. Since the bioavailability and metabolism of notoginseng have a great influence on the *ex vivo* antioxidant and genoprotective response, more data in these areas are needed before any conclusion can be drawn on the effectiveness of notoginseng as a genoprotective herb.

It is well-known that ROS damage on DNA results in single or double strand breaks; results in different forms of base damage producing products such as thymine glycol, 8-hydroxyguanosine, or abasic sites; and results in damage to deoxyribose sugar and DNA-protein cross-links. These damages, if they cannot be effectively repaired, can result in cell death or mutations and carcinogenesis. The antioxidative property of PN has already been shown in various studies. In the current study, we have further shown that this traditional herbal medicine which is intended to treat cardiovascular and cardio-cerebrovascular disorders, also possesses genoprotective effect in human lymphocytes. However, studies on the anti-cancer property of PN are scarce and whether PN behaves in the same way as the other herbs yet need to be explored. Validation of this concept requires some long-term prospective studies and clinical trials.

In conclude, the current study demonstrated that PN, which are considered to be antioxidative, also exhibited direct DNA protection against oxidant challenge in the comet assay *in vivo* with a mild effect. It could be absorbed, passed into the plasma, and offered genoprotection to the lymphocytes. Extended blood collection time point and/or supplementation may be of further research interest to explore the long-term accumulated effects and the integrated effects with other naturally produced free radicals and antioxidants in the complex “redox signaling” system in the body.

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