



Genotoxic studies on *Panax ginseng* and *Polygonum multiflorum* and their combination in mouse peripheral lymphocyte cells

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In the present studies, single cell gel electrophoresis (SCGE) was employed to assess the genotoxicities of *Panax ginseng* (*P. ginseng*) and *Polygonum multiflorum* (*P. multiflorum*), administered individually or in combination, on mouse peripheral lymphocyte DNA. *P. ginseng*, *P. multiflorum* and their combination were orally administered to mice in low, medium and high doses for seven consecutive days. Cyclophosphamide (CP) was used as a positive control. Blood samples were drawn from the vein cluster behind the eye 2 h after drug administration on the first, third and seventh day. *P. ginseng* (0.43 g/kg, 1.3 g/kg, and 3.9 g/kg, p.o.) was found to have no harmful effects on peripheral lymphocyte DNA. *P. multiflorum* (3.9 g/kg and 11.7 g/kg), on the other hand, did have harmful effects on peripheral lymphocyte DNA on the first, third and seventh day as demonstrated by changes in tail DNA, olive moment, tail length, and/or tail moment. The combination of these two herbs (5.2 g/kg and 15.6 g/kg) induced harmful effects on peripheral lymphocyte DNA on the first day, as observed in tail DNA, tail length, tail moment and/or olive moment. However, these harmful effects diminished on the third and seventh days. These results demonstrated that in vivo administered *P. ginseng*, but not *P. multiflorum*, is not genotoxic on peripheral lymphocyte DNA and that their combination may decrease potential genotoxic effects induced by *P. multiflorum*. This suggests a rationale for the use of this combination of herbs in Traditional Chinese Medicine.

Keywords: Single cell gel electrophoresis; *Panax ginseng* C. A. Meyer; *Polygonum multiflorum* Thunb; Compatibility; Cyclophosphamide

Introduction

Due to increasing incidences of adverse reactions caused by synthetic drugs, it has been suggested that Traditional Chinese Medicines might have fewer adverse effects than synthetic drugs. However, many medicines have both therapeutic and adverse actions. Traditional Chinese Medicines are not necessarily exception to this principle. The adverse reactions and toxicities of some medicinal herbs used in Traditional Chinese Medicines have been identified. These findings have engendered extensive consternation.

Both *Panax ginseng* C. A. Meyer and *Polygonum multiflorum* Thunb are medicinal herbs that have

been used in traditional medicine in China for thousands of years. They are used as general tonics for patients with chronic diseases and are frequently featured in traditional medicine prescriptions in China [1]. *P. ginseng* has a broad range of beneficial effects, including tonic, adaptogenic, immunomodulatory, anti-inflammatory, antioxidant, anti-aging, anti-diabetic, anti-mutagenic, anti-cancer and neurovascular modulatory activities, etc.[2-6]. *P. multiflorum* also has broad pharmacological activities, such as anti-inflammatory, antioxidant, anti-aging, reducing blood lipid levels, and anti-mutagenic activities [7-10]. Traditionally, *P. ginseng* and *P. multiflorum* are often used together as a so-called "drug-pair". Generally, according to the clinical experience in Traditional Chinese Medicine, use of these agents together may increase the therapeutic efficacy and/or decrease the adverse reactions induced by either herb used individually. The combination of

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P. ginseng and *P. multiflorum* has some special effects for all weaknesses in Qi and Xue according to theories in Traditional Chinese Medicine. These are often seen in treatments of chronic malaria, spermatorrhea, premature graying of the hair, and premature senility [11].

Alkaline single cell gel electrophoresis (comet assay) is a simple visual, as well as quantitative technique for measuring DNA breakage in individual cells [12]. The damage of DNA in a single cell may be observed as a tail or comet-like structure on gel electrophoresis. The tail DNA, tail length, tail moment and olive moment are related to the amount of strand breaks in the cell [13-15]. This assay has been widely used in vivo and in vitro studies to detect DNA damage and DNA repair. Furthermore, this assay has been proved to be useful in detecting organ-specific genotoxicity in mammals [16-17]. However, few studies have been carried out to detect genotoxicities of traditional herb medicines using this technique. Therefore, in this study, alkaline single cell gel electrophoresis has been used to evaluate whether or not orally administered *P. ginseng* and *P. multiflorum* individually or in combination induce any genotoxicity in peripheral lymphocyte DNA in mice.

Materials and methods

Animals

Male Swiss mice with original body weights of 18-22 g were used. The animals were supplied by Shenyang Shuangyi Company [Shenyang, China]. All animals were maintained under standard housing conditions in 12L: 12D light/dark cycles and given food and water *ad libitum*. In the present study, all animal use procedures were in accordance with the Regulations of the Experimental Animal Administration issued by State Committee of Science and Technology of the People's Republic of China on November 14th, 1988.

Drugs and chemicals

Panax ginseng C. A. Meyer and *Polygonum multiflorum* Thunb were purchased from commercial channels in China and were identified by Professor Qishi Sun. *P. ginseng*, *P. multiflorum* and their combination were separately immersed in water (water volume was 4 times that of the herb weight) for 20 min, heated to boiling for a further 20 min and then filtered. The residue was added to 3 times its weight of water, heated to boiling again for 20 min, and then filtered. The two filtrates were combined and condensed by low temperature (< 60 °C) evaporation to a particular concentration.

CP was purchased from Shanxi Taisheng Enterprise Company Limited [Shanxi, China]. Low melting agarose was purchased from Beijing Jingkehongda Biology Company [Beijing, China].

The protocol of administration of P. ginseng, P. multiflorum and their combination

According to the method described by Chuang *et al.* [18-19], whole blood was directly collected for the comet assay. Mice in the single herb experiments were divided into 5 groups: a control group treated with saline, a low-dose group, a medium-dose group, a high-dose group and a CP group. Mice in the combination experiments were divided into 5 groups: a control group treated with saline, a *P. ginseng* group, a *P. multiflorum* group, a combination group and a CP group. Each experimental group contained 4-6 mice. Each mouse, except of those in the CP group, was orally administered the herb extracts (20 mL/kg) or saline for seven consecutive days. Each mouse in the CP group was given an intraperitoneal injection of CP (100 mg/kg) on the day of performing the comet assay. Two hours after drug administration, blood was collected from the vein cluster behind the eye on the first, third and seventh days.

Alkaline Single Cell Gel Electrophoresis (Comet Assay)

The comet assay was performed under alkaline conditions, essentially according to the procedure



described by Singh *et al.* [20] with a slight modification [21-22]. Briefly, a fully frosted microscopic slide glass was coated with 1 % (w/v) normal melting-point agarose, and then covered with a coverslip and stored at 4 °C for 10 min to solidify. After removing the coverslip, a mixture of 100 µL whole blood and 100 µL of 0.5 % (w/v) low melting-point agarose was rapidly overlaid to solidify at 4 °C for 10 min. After removing the coverslip, each slide was immersed in a lysing solution containing 2.5 M NaCl, 0.1 M EDTA, 10 mM Tris-HCl buffer (pH 10.0), 1 % (w/v) TritonX-100, and 10 % (v/v) dimethylsulfoxide at 4 °C for 2 h. The glass slides were then kept at 4°C for 40 min in an electrophoresis buffer (0.3 M NaOH, 1 mM EDTA) to allow DNA unwinding and to express alkali-labile site. Electrophoresis was performed at 18 V for 30 min in the same buffer. Glass slides were neutralized by washing three times with 0.4 M Tris-HCl buffer, pH 7.5, and stained with 50 µL ethidium bromide. Then the slides were immersed in anhydrous alcohol for 1 h, then dried. To prevent additional

damage, all the steps described above were conducted in reduced ambient light. Slides were examined at 200 × magnification with a fluorescence microscope (Olympus, Tokyo, Japan) equipped with a Pulnix video camera [Sunnyvale, United States]. DNA damage was analyzed with LUCIA Comet analysis software [Praha, Czech Republic]. Fifty cells on each slide were randomly selected for analysis.

Statistical analysis

The data were analyzed with SPSS 12.0 software [Shanghai, China]. The comet assay parameters between the different groups were compared with one-way ANOVA followed by Fisher's least-significant difference (LSD). $P < 0.05$ was considered statistically significant. Values are expressed as mean ± standard error.

Results

Effects of *P. ginseng* and *P. multiflorum* on

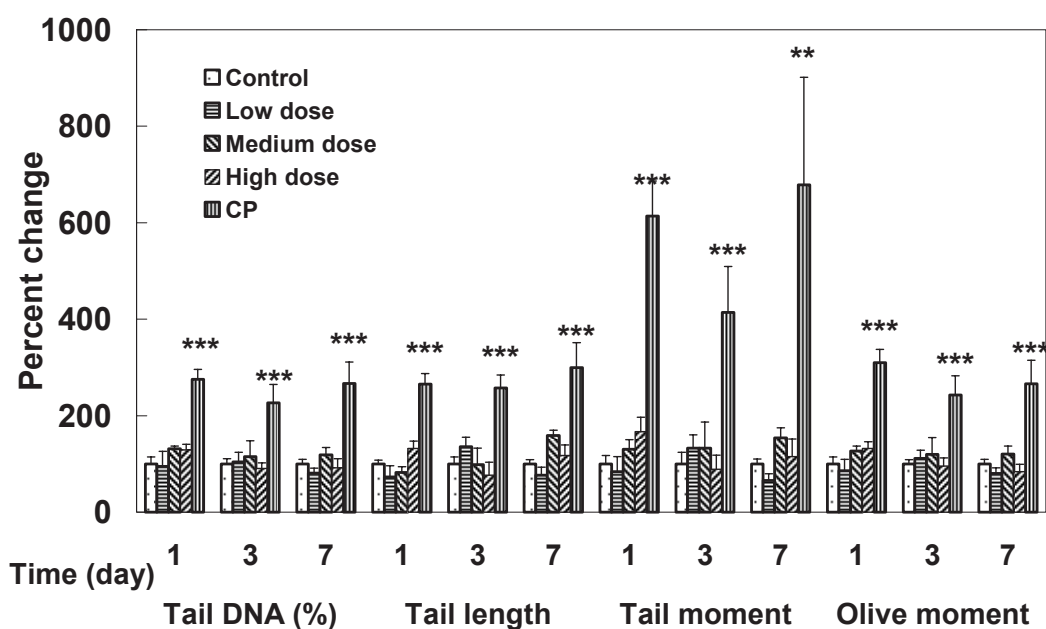


Fig. 1 Effects of *Panax ginseng* C. A. Meyer on mouse peripheral lymphocyte DNA damage. The drug was administered orally at doses of 0.43, 1.3, and 3.9 g/kg for 1, 3 and 7 days. ($n=4-6$) ** $P < 0.01$, *** $P < 0.001$ versus control group.

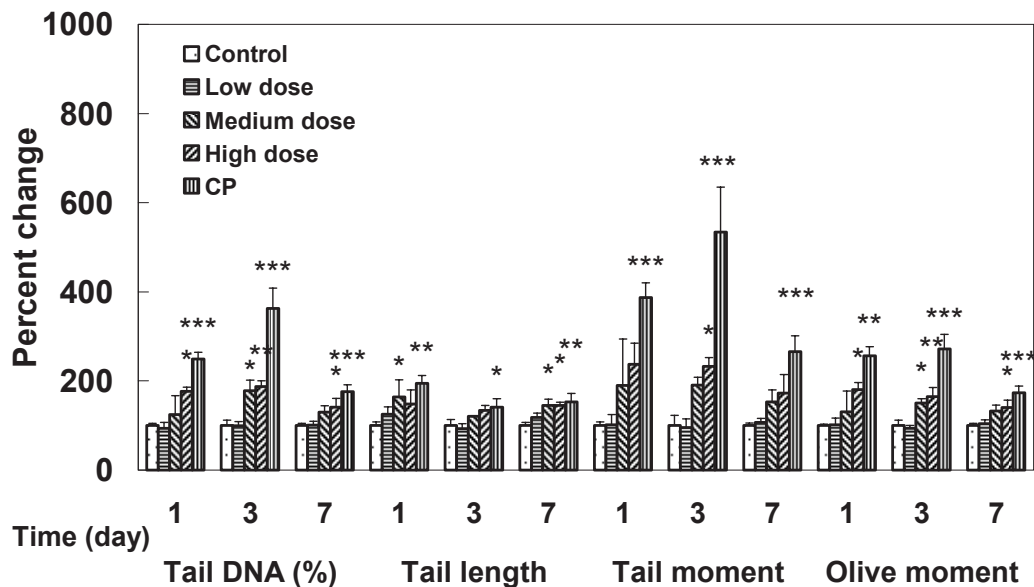


Fig. 2 Effects of *Polygonum multiflorum* Thunb on mouse peripheral lymphocyte DNA damage. The drug was administered orally at doses of 1.3, 3.9, and 11.7 g/kg for 1, 3 and 7 days. ($n=4-6$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group.

peripheral lymphocyte DNA damage

The data of the saline control group were taken as 100 % and the percentage changes were calculated for the single herb and positive control groups.

Orally administered *P. ginseng*, at the doses of 0.43, 1.3 and 3.9 g/kg, p.o., did not show any damage on peripheral lymphocyte DNA for 1, 3 and 7 days (Fig. 1).

P. multiflorum did not show any significant damage on peripheral lymphocyte DNA when administered at 1.3 g/kg for 1, 3 and 7 days. However, at the higher doses of 3.9 and 11.7 g/kg, it induced significant lesions in DNA after administered for 1, 3 and 7 days, as observed by changes in tail DNA, tail length, tail moment and/or olive moment (Fig. 2).

Effects of the combination of *P. ginseng* and *P. multiflorum* on peripheral lymphocyte DNA damage

Oral administration for 7 days of the combination of the two herbs at the lower dose of 1.73 g/kg, was

not genotoxic. At the higher doses of 5.2 and 15.6 g/kg, the combination was genotoxic after first day administration. However, at these higher doses the combination was not genotoxic after 3 and 7 days of treatment (Fig. 3-5).

Discussion

Traditional Chinese Medicine has developed for several thousand years. In addition to effectiveness, the toxicity of herbs used in the Traditional Chinese Medicine is attracting attention in clinical settings. Traditionally, acute and general toxicities of medicinal herbs are often described in ancient literature. However, little information regarding toxicities of some herbs that are used for chronic conditions is available. In particular, in depth biochemical, and/or genetic studies have not always been performed. Single cell gel electrophoresis (comet assay) is a sensitive and rapid method for the detection of DNA strand breaks at the individual cell level and is widely applied for therapy, environmental biology inspection,

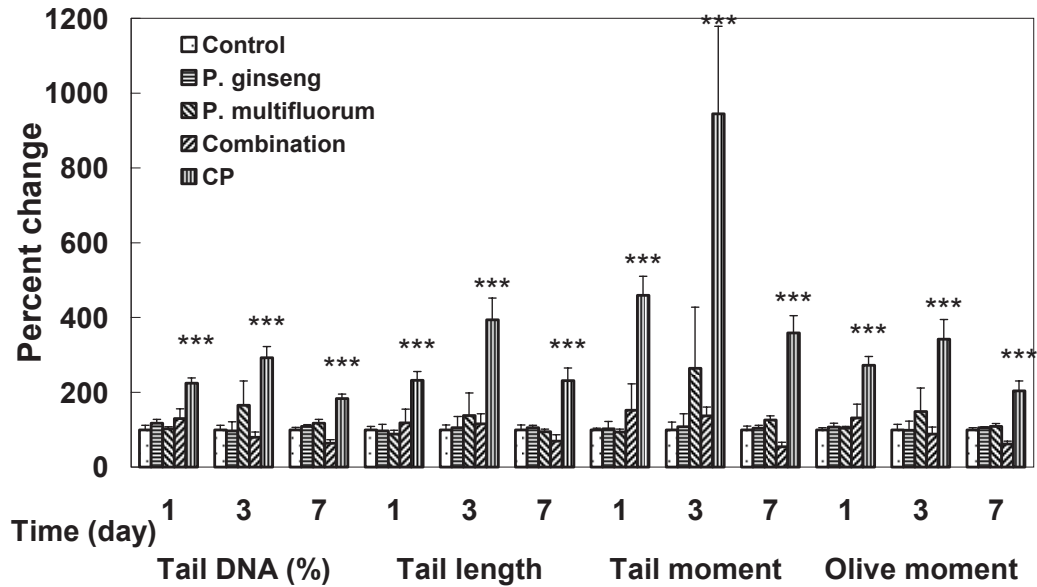


Fig. 3 Effects of low doses of *Panax ginseng* C. A. Meyer and *Polygonum multiflorum* Thunb and their combination on mouse peripheral lymphocyte DNA damage. The doses used were: *P. ginseng* 0.43 g/kg, *P. multiflorum* 1.3 g/kg, combination 1.73 g/kg for 1, 3 and 7 d, respectively. (n=4-6) *** $P < 0.001$ versus control group.

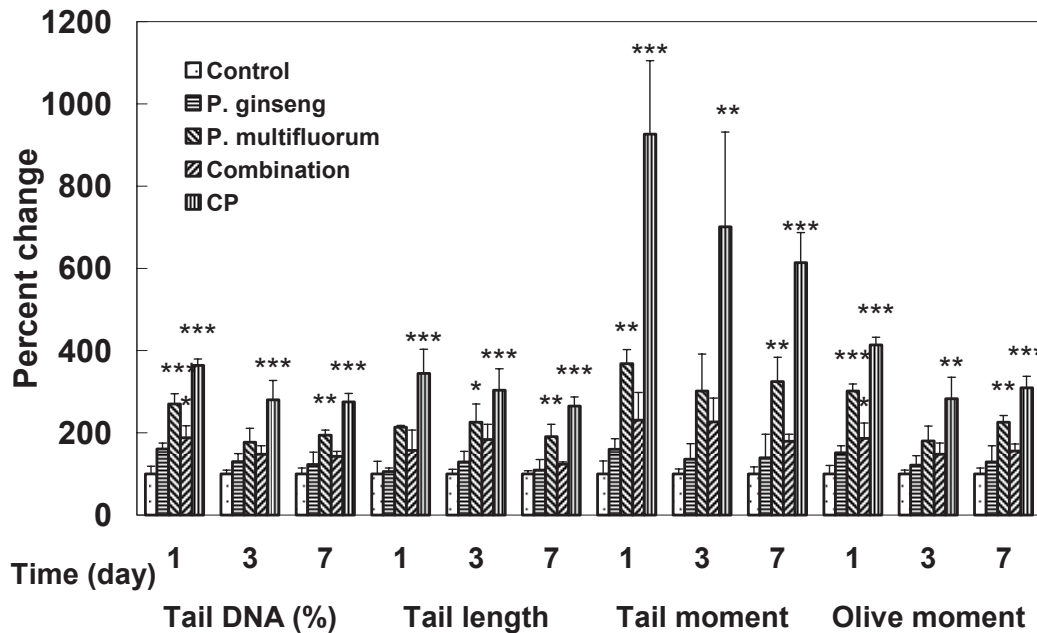


Fig. 4 Effects of medium dose of *Panax ginseng* C. A. Meyer, *Polygonum multiflorum* Thunb and their combination on mouse peripheral lymphocyte DNA damage. The doses used were: *P. ginseng* 1.3 g/kg, *P. multiflorum* 3.9 g/kg, combination 5.2 g/kg, for 1, 3 and 7 d, respectively. (n=4-6) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group.

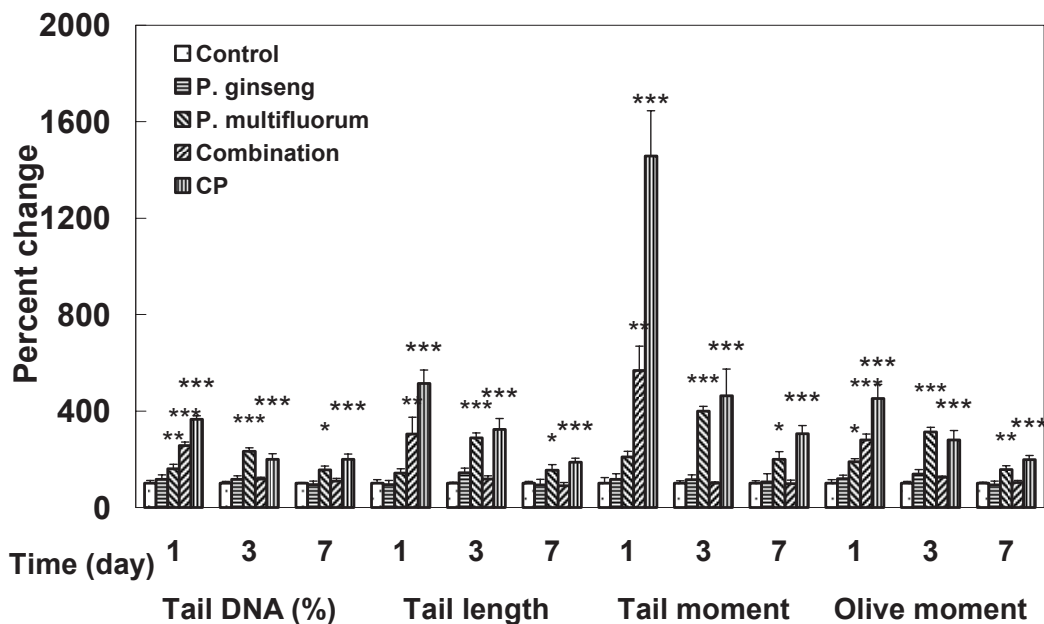


Fig. 5 Effects of high doses of *Panax ginseng* C. A. Meyer and *Polygonum multiflorum* Thunb and their combination on mouse peripheral lymphocyte DNA damage. The doses used were: *P. ginseng* 3.9 g/kg, *P. multiflorum* 11.7 g/kg, combination 15.6 g/kg for 1, 3 and 7 d, respectively. ($n=4-6$) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group.

epidemiologic studies, and studies of cell apoptosis, etc. [23-26]. Therefore, the present study by employing this method to evaluate genotoxicities of medicinal herbs often prescribed in Traditional Chinese Medicine. The results reported here provide evidence that some herb medicines could have genotoxic actions and that Traditional Chinese Medicine may have some advantages such as overcoming genotoxicity by the use of herb combinations.

P. ginseng, usually called ginseng, has been used as a tonic herb for thousands of years. Modern studies show that its active ingredients are mainly ginsenosides, which have a variety of beneficial effects similar to the natural herb, ginseng, such as anti-inflammatory, antioxidant and anticancer effects [2]. Although ginseng has some adverse reaction during clinic applications, such as inducing excitement and hypertension, usually due to an overdose or abuse, it generally has a very low incidence of toxicity in clinical trials [27]. The present results show that *P.*

ginseng, at doses equivalent to clinical dosages, does not damage mouse peripheral lymphocyte DNA, suggesting that ginseng is safe insofar as genotoxicity is concerned. In fact, studies have demonstrated that ginseng and its active components possess potent anti-oxidative activities via free radical scavenging and inducing anti-oxidant enzymes in vitro and in vivo, ranging from isolated LDL oxidation and ischemic neuron dysfunction, to heart re-perfusion injury and physical exercise [28-33]. All of these properties of ginseng might be beneficial for protecting the cell nucleus from oxidative stress and genotoxicity.

P. multiflorum is one of the tonic herbs often prescribed in Traditional Chinese Medicine. However, it was recorded to have some toxicities in the book *Bencao Huiyan* [11]. The toxicities or adverse effects include nausea, vomiting, intestinal distress, diarrhea, convulsions, breath anesthesia in heavy patient [34-37]. The present results demonstrated that *P. multiflorum*, at the lowest dose tested, did not have significant



harmful effects on mouse peripheral lymphocyte DNA. However, at higher doses it did damage mouse peripheral blood lymphocyte DNA, suggesting that *P. multiflorum* may induce genotoxicity when used in higher doses and for longer times, even at the levels of clinical prescriptions. Some studies have reported that emodin, chrysophanol, physioin and rhein are the harmful ingredients in *P. multiflorum* [38]. However, whether these compounds are those that induce the DNA lesions observed in this study is not clear. Further study will be required to investigate the possible genotoxicities of these pure compounds.

Use of two herbs in combination has been developed during the history of Traditional Chinese Medicine. The advantages of using combinations can be demonstrated by either synergistically increasing the therapeutic efficacy and/or antagonizing toxic and adverse reactions. However, few studies have been done to verify ancient clinical experience and/or theory. The first prescription that coupled *P. ginseng* with *P. multiflorum* as a combination appeared in *Jingyue Quanshu*, a famous Chinese medicinal book published in 1700 [39]. This combination has been used often in Traditional Chinese Medicines. As far as the authors know, the clinical implications of this combination are not clear in terms of modern science. The present results demonstrate that the combination of *P. ginseng* and *P. multiflorum*, at the higher doses and after seven days of administration does not damage mouse peripheral lymphocyte DNA, even though after the first day of treatment at the medium and high doses utilized, it did damage peripheral lymphocyte DNA. These results suggest that chronic usage of this combination may reduce the extent of DNA damage induced by *P. multiflorum*. The reasons for the reduction in genotoxicity by chronic administration of the combination may be due to either the presence in *P. ginseng* of antioxidant components that can prevent oxidative damage due to *P. multiflorum* or to components of *P. ginseng* and *P. multiflorum* that react with each other *in vivo* or during preparation which result in decreases in the toxic

actions of *P. multiflorum*.

In conclusion, the present study demonstrates by using the comet assay *in vivo* that *P. ginseng* has no genotoxicity and *P. multiflorum* has genotoxicity in mouse peripheral lymphocytes. The use of the combination of *P. ginseng* and *P. multiflorum* could partially eliminate the genotoxicity induced by *P. multiflorum*. Further studies are underway to elucidate the protective or genotoxic components of *P. ginseng* and *P. multiflorum* and to clarify the gene-protective mechanism(s) of their combination.

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