

# The influence of lead and arsenite on the inhibition of human breast cancer MCF-7 cell proliferation by American ginseng root (*Panax quinquefolius* L.)

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## Abstract

American ginseng root (*Panax quinquefolius*) has a number of purported therapeutic effects, including inhibition of cancer cell proliferation. The ability of environmentally relevant heavy metals to alter ginseng effects on cancer cell growth was the subject of this study. A water extract of American ginseng root was applied alone or in combination with physiologically relevant doses of either lead (Pb) or arsenite to MCF-7 breast cancer cells in vitro and effects on cell proliferation were determined. Ginseng alone produced a significant dose-dependent inhibition of MCF-7 cell proliferation starting at 0.5 mg ml<sup>-1</sup>. Treatment of MCF-7 cells with 2.5 μM arsenite significantly decreased MCF-7 cell proliferation ( $p < 0.01$ ). When cells were treated with arsenite (1.25 or 2.5 μM) in combination with ginseng extract (0.5 mg ml<sup>-1</sup>), there was an apparent synergistic inhibition of cell proliferation. Treatment of MCF-7 breast cancer cells with 50 μM Pb significantly decreased cell proliferation relative to control ( $p < 0.01$ ), and concomitant ginseng and Pb treatment did not lead to a further decrease. These results suggest that contaminant heavy metals, some of which have been detected in ginseng root extracts or commercial ginseng preparations, may alter the biological activity of ginseng.

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**Keywords:** *Panax quinquefolius*; American ginseng; Lead; Arsenite; Breast cancer; MCF-7 cell proliferation

## Introduction

For centuries, American ginseng (*Panax quinquefolius*) has been used to prevent or treat a variety of human ailments (Suits et al., 2003). Current medical and commercial interest is based upon the purported benefits of ginseng for general health, including specific beneficial effects on the endocrine, cardiovascular, immune, and central nervous systems (Bahrke and Morgan, 1994; Murphy et al., 1998; Lee et al., 2000), and for cancer prevention (Yun and Choi, 1998; Yun, 1999) and treatment (Yun and Choi, 1990; Chang et al., 2003).

Ginseng extracts have been reported to interact with some pharmacological compounds and, consequently, alter drug efficacy (Duda et al., 1999; Li et al., 2000; Kiefer and Pantuso, 2003), including warfarin, monoamine oxidase

inhibitors, chemotherapy drugs, and vitamin C. Potential interactions between ginseng and environmental elements or metals have received little attention but may also have significant pharmacological repercussions. Ginseng preparations, primarily from Asia, may contain a variety of contaminants, including heavy metals (e.g., cadmium, lead, mercury), that exceed the maximum allowed concentrations for comparable foodstuffs (Zhang et al., 1994; Khan et al., 2001; Dragun et al., 2003). The primary objective of the present research was to assess the possible interaction of ginseng extracts and two environmentally relevant heavy metals, arsenite and lead, to determine if these elements affect the biological activity (i.e., anti-cancer effects) of ginseng root extract. Lead was selected for current study because this element is a prominent metal in the environment and poses a serious human health risk. Furthermore, lead and arsenic have been detected in some ginseng preparations (Zhang et al., 1994; Khan et al., 2001). Arsenic is a metalloid element in the environment and exposure can result in adverse health effects in humans (Yoshida et al., 2004). Arsenic in drinking

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and irrigation water, present primarily as arsenite, is classified as a carcinogen, but can also inhibit the proliferation of normal and cancer cells in a time- and dose-dependent manner (Lu et al., 1999; States et al., 2002). For the study presented here, a water extract of American ginseng (*P. quinquefolius* L.), lead and arsenite were applied to in vitro cultures of MCF-7 breast cancer cells, either alone or in binary combinations, to determine if the presence of the metals altered the inhibitory action of ginseng on cancer cell proliferation.

## Materials and methods

### Plant material and ginseng extracts

American ginseng root powder was obtained from the Ginseng Board of Wisconsin (Wausau, Wisconsin, USA). A 1:9 (w/v) slurry of powdered American ginseng root and distilled water was extracted in a water bath with agitation for 1 h at 90 °C, a modification of a previous protocol (Watanabe et al., 1991). The extract was cooled for 30–45 min and then centrifuged at 1200 g for 15 min. The supernatant was saved and the pellet was resuspended in half of the original volume of distilled water and extracted a second time under the same conditions as the first extraction. After cooling and centrifugation, supernatants were combined, frozen at –70 °C, and lyophilized (LABCONCO, Freezone 4.5) for approximately 3 days. The lyophilized extract was collected and the powder stored at –4 °C. The extract was prepared for in vitro MCF-7 breast cancer cell growth assays by redissolving the desired amount of lyophilized powder in cell culture media and maintaining in a 37 °C water bath for 3 h under gentle agitation. Solutions of ginseng extract were filtered through leuer-lock syringe filters (25 mm PF, 0.2 µm cellulose acetate membrane) prior to treating cells.

### Dose–response experiments

Human breast cancer MCF-7 cells (ATCC HTB 22) were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10% fetal bovine serum (Gibco) and antibiotics (100 IU/ml penicillin G, 100 µg/ml streptomycin; Sigma). To determine the effects of ginseng extract (GE) on MCF-7 cell proliferation, cells plated in 24-well culture plates ( $5 \times 10^3$  cells/well) were treated with a wide dose range of GE (0, 0.1, 0.25, 0.5, 1.0, or 1.5 mg ml<sup>-1</sup>). Every two days, cells were treated with a dose of freshly prepared GE for 6 days ( $n=8$  wells per treatment group in duplicate experiments). For combination studies, media was replaced with 0.5 ml media containing no treatment (control), GE alone (0.5 mg ml<sup>-1</sup>), lead alone (50 µM), arsenite alone (1.25 or 2.5 µM), or combinations of GE+lead or GE+arsenite ( $n=8$  wells per treatment group in duplicate experiments) as described above. Twenty-four hours following the last treatment, cells were detached using 0.05% trypsin (Gibco) and manually counted using a hemocytometer.

### Lead and arsenite solutions

Arsenite solutions were prepared by initially dissolving sodium arsenite (Fisher Scientific, Hanover Park, IL) in distilled water and then further diluting this stock solution (5 mM) with cell culture media to working solution concentrations of 2.5 and 5 µM. Lead solutions were prepared by dissolving lead acetate trihydrate (Sigma, St. Louis, MO) in distilled water and diluting the stock solution (100 mM) with cell culture media to a working concentration of 100 µM. The arsenite and lead stock solutions were stored at 4 °C between treatments and test solutions were incubated at 37 °C just prior to use. All solutions were filtered through leuer-lock syringe filters immediately before use in cell culture experiments. For treating MCF-7 cells, working solutions were diluted with cell culture media alone or containing GE to produce final concentrations of 50 µM lead, and 1.25 or 2.5 µM arsenite, alone or in combination with 0.5 mg ml<sup>-1</sup> GE.

### Data analysis

All results are expressed as mean values and standard errors of at least 3 experiments. Data were compared between treatment groups using one-way ANOVA and Tukey's post hoc analysis. The level of significance was  $p < 0.05$ .

## Results

Treatment with GE decreased the growth of MCF-7 breast cancer cells in a dose-dependent manner between 0.1 and 1.0 mg ml<sup>-1</sup> (Fig. 1). Treatment with 0.5 mg ml<sup>-1</sup> GE or greater significantly ( $p < 0.05$ ) decreased cell number when compared to vehicle. The maximum inhibitory dose was achieved at 1.0 mg ml<sup>-1</sup>. Based upon these results, a final concentration of 0.5 mg ml<sup>-1</sup> was selected for use in the subsequent GE+heavy metal cell culture assays. This intermediate GE dose produced a 30–40% inhibition in cell number relative to vehicle.

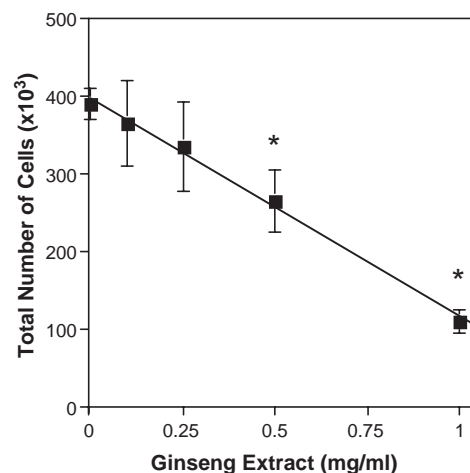


Fig. 1. Effect of American ginseng water extract on MCF-7 cell proliferation in vitro. Cells were treated with different doses of ginseng extract for 6 days (see Materials and methods). Each point represents the average of three experiments  $\pm$  standard error of the mean. \* =  $p < 0.05$  relative to untreated (0) control.

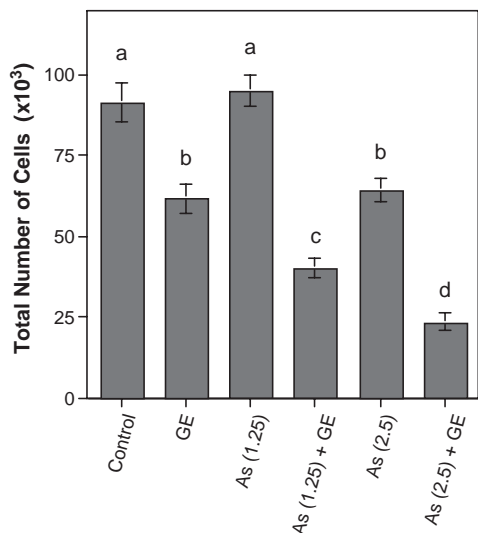


Fig. 2. Effects of American ginseng extract (GE) and arsenite (As), alone and in combination, on MCF-7 cell proliferation in vitro. Cells were treated with GE ( $0.5 \text{ mg ml}^{-1}$ ) and/or As ( $1.25$  or  $2.5 \text{ }\mu\text{M}$ ) for 6 days (see Materials and methods). Each bar represents the average of at least three experiments  $\pm$  standard error of the mean. Significance at  $p < 0.05$  was achieved when comparing bars represented by different letters; like letters indicate no significance.

Cell counts for MCF-7 cells treated with  $1.25 \text{ }\mu\text{M}$  arsenite alone were not significantly different from those of vehicle (Fig. 2). However, the  $2.5 \text{ }\mu\text{M}$  dose of arsenite alone produced a 31% decrease in cell number ( $p < 0.05$ ) and, when given in combination with GE, produced a 70% decrease ( $p < 0.01$ ). Although the lower dose of arsenite alone did not significantly alter cell proliferation, it decreased cell number by 52%, compared to vehicle ( $p < 0.01$ ), when given in combination with GE. These results are suggestive of a synergistic effect between GE and arsenite. Lead treatment ( $50 \text{ }\mu\text{M}$ ) had a potent inhibitory effect on cell number, producing a 52.8% decrease compared to vehicle ( $p < 0.01$ ; Fig. 3). When combined with GE, lead treatment produced a 60% decrease in cell number. The interaction between lead and GE was not significant.

## Discussion

The results obtained from this study suggest that toxic metals could have significant effects on the biological effects of ginseng. Earlier studies have shown potential drug–drug interactions between ginseng and MAO inhibitors, blood thinning medications such as warfarin, some anti-psychotic medications, the analgesic morphine, and some contraceptives (Kiefer and Pantuso, 2003). Moreover, ginseng in combination with chemotherapeutic agents such as tamoxifen, taxol, or fluorouracil, can result in significantly augmented chemotherapy action (Duda et al., 1999). Further, when ginseng was administered in combination with vitamin C there was a synergistic effect toward the reduction of low-density lipoprotein oxidation (Li et al., 2000). In the current study, there appears to be a synergistic interaction between ginseng and arsenite in the inhibition of MCF-7 breast cancer cell proliferation.

Ginseng extracts can show considerable variability in their bioactivity (Yuan et al., 2001). The nature of this variation and the extent to which trace heavy metals might contribute has not been considered. A difference in trace metal content of  $\geq 4$ -fold has been found between individual ginseng roots (Zhang et al., 1994). Whether contaminants in soil or contamination during product processing are responsible for the elevated and physiologically relevant concentrations of heavy metals observed in some commercial preparations of ginseng is not clear (Khan et al., 2001). Moreover, the heavy metals that interact with ginseng extracts might not necessarily be present in the ginseng preparation itself in order for an interaction to occur. Another justification for the choice of arsenite and lead in this study is that significant segments of the human population are exposed to these elements. In humans, metal exposure can occur through food and water intake (U.S. Environmental Protection Agency, 1980; Choi et al., 1995; Lasky et al., 2004; Saper et al., 2004), as well as occupational exposure (Enterline et al., 1995; Martin et al., 2003). Lead enters the body through the respiratory tract, as well as through ingestion of food, beverages, drugs, and supplements (FAO/WHO, 1989). It has been estimated that humans take in approximately  $15$ – $100 \text{ }\mu\text{g}$  per day of lead (FAO/WHO, 1989), well below the tolerable weekly intake established by the World Health Organization of approximately  $1.5 \text{ mg/week}$  (WHO, 1989). The average daily human intake of inorganic arsenic, including arsenite, is approximately  $1 \text{ }\mu\text{g/kg}$  per day, most of which is found in food and water (FAO/WHO, 1989) and is well below the WHO recommended tolerable daily intake dose of  $2.14 \text{ }\mu\text{g/kg}$  body weight per day (WHO, 1983). In the current study, the doses of lead and arsenite tested would be equivalent to approximately  $150 \text{ }\mu\text{g/kg}$  of arsenite and  $1.4 \text{ mg}$  of lead. These doses would be achievable following the ingestion of contaminated food products (Lasky et al., 2004; Saper et al., 2004). Further, ginseng/heavy metal interactions could be possible in

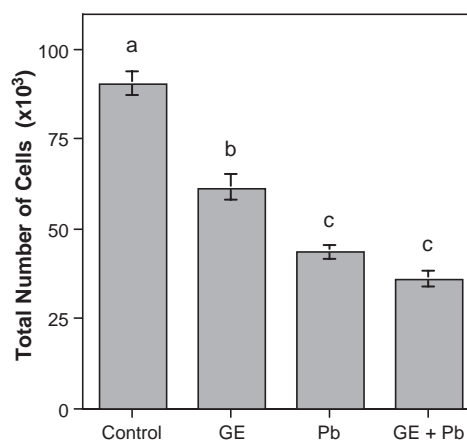


Fig. 3. Effects of American ginseng extract (GE) and lead (Pb), alone or in combination, on MCF-7 cell proliferation in vitro. Cells were treated with GE ( $0.5 \text{ mg ml}^{-1}$ ) and/or Pb ( $50 \text{ }\mu\text{M}$ ) for 6 days (see Materials and methods). Each bar represents the average of at least three experiments  $\pm$  standard error of the mean. Significance at  $p < 0.05$  was achieved when comparing bars represented by different letters; like letters indicate no significance.

populations that use ginseng extensively as an herbal supplement.

The mechanism of the interaction between ginseng, its ginsenosides, and heavy metals is unclear. Contaminating heavy metals taken up by the ginseng root have been shown to influence ginsenoside production in the root (Zhong and Wang, 1996; Li and Mazza, 1999). Furthermore, contaminants may also influence ginsenoside bioactivity. It has been shown that the biologically active ginsenosides, in particular, ginsenosides Rc, Rh2 and Rg3, have anti-cancer activity (Oh et al., 1999; Murphy et al., 2001; Kim and Jin, 2004). In cancer cells, ginsenosides inhibit cell proliferation by altering expression of proteins important in cell cycle regulation (Oh et al., 1999; Murphy et al., 2001), and apoptosis (Kim and Jin, 2004), and by exerting anti-oxidant activity (Kim et al., 1996). There is limited information regarding the effects of arsenite and lead in cancer cells. Physiologically relevant doses of lead and arsenite have by themselves been shown to alter proliferation of normal and cancer cells (Stoica et al., 2000; Vega et al., 2001; Martin et al., 2003). There is evidence that arsenite induces genotoxicity and DNA damage (Schaumloffel and Gebel, 1998; Gebel et al., 1998), as well as apoptosis (de la Fuente et al., 2002; Hayakawa and Privalsky, 2004) in normal and malignant cells. Moreover, lead can induce cytotoxicity in a dose-dependent manner by altering the physical properties of cell membranes (Adonaylo and Oteiza, 1999), signal transduction pathways (Leal et al., 2002), and inducing DNA damage (McNeill et al., 2004) and cell growth arrest (Tchounwou et al., 2004). Interestingly, both arsenite and lead have also been shown to mimic the biological actions of estradiol in MCF-7 breast cancer cells maintained on charcoal-stripped serum (Martin et al., 2003).

## Conclusion

Although the lowest dose of arsenite tested (1.25  $\mu\text{M}$ ) by itself was without effect, in combination with ginseng there was a significant interaction and decrease in cancer cell proliferation. The synergistic-like interaction between arsenite and ginseng may be due to multiple actions of arsenite and ginseng on the breast cancer cells leading to the augmented action. The lack of an additive effect of lead and ginseng could indicate a shared mechanism of action. These current findings suggest that trace heavy metals could contribute to the overall anti-cancer activity of ginseng. It would be interesting to investigate how less toxic metals, such as selenium which has both anti-oxidant and anti-cancer activities (Diwadar-Navsariwala and Diamond, 2004; El-Bayoumy and Sinha, 2004), may interact with ginseng to promote health benefits. How heavy metals and trace minerals, other than arsenite and lead, affect ginseng's bioactivity remains unknown.

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