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Research Article

Protection by *Panax ginseng* C.A. Meyer against the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*

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Abstract

Panax ginseng is one of the most widely prescribed herbal medicines for the treatment of cancer, diabetes, chronic inflammation, and neurodegenerative and cardiovascular diseases. Since the use of alternative medicines in combination with conventional therapy may increase the risk of unwanted interactions, we investigated the possible genotoxicity of a water-soluble form of the dry root of *P. ginseng* (2.5, 5.0 or 10.0 mg/mL) and its ability to protect against the genotoxicity of doxorubicin (DOX; 0.125 mg/mL) by using the *Drosophila melanogaster* wing somatic mutation and recombination test (SMART) with standard and high-bioactivation crosses of flies. *Panax ginseng* was not genotoxic at the concentrations tested, whereas DOX-induced genotoxicity in marker-heterozygous flies resulted mainly from mitotic recombination. At low concentrations, *P. ginseng* had antirecombinogenic activity that was independent of the concentration of extract used. Recombination events may promote cancer, but little is known about the ability of *P. ginseng* to inhibit such recombination or modulate DNA repair mechanisms.

Key words: antigenotoxicity, SMART, wing spot test.

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Introduction

Plant products are being increasingly used as complementary or alternative medicines for the treatment for a variety of diseases, including cancer (Meijerman *et al.*, 2006), although in many cases there is still only limited scientific evidence for their therapeutic efficacy. The root of *Panax ginseng* C. A. Meyer (Araliaceae), a common plant in eastern Asia, is widely used in Chinese natural medicine (Lee *et al.*, 2004; Yoshikawa *et al.*, 2007). *Panax ginseng* is also being increasingly prescribed in Korea, Japan and Western countries for the treatment of cancer, diabetes, chronic inflammation, and neurodegenerative and cardiovascular diseases (Yun, 1996; Radad *et al.*, 2006). Several studies have demonstrated the therapeutic potential of ginseng in the central nervous system through its ability to improve longevity (Attele *et al.*, 1999; Li *et al.*, 2000) and cognitive performance (Kennedy *et al.*, 2004; Reay *et al.*, 2005), as well as its adaptogenic properties in contributing to the equilibrium of the human body under prolonged stress (Kumar *et al.*, 1996).

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Ginseng contains many physiologically important constituents that include saponins, oils and phytosterol, carbohydrates and sugars, organic acids, nitrogenous substances, amino acids and peptides, vitamins and minerals (iron, copper, zinc), and several enzymes (Hou, 1977; Attele *et al.*, 1999). Of the various compounds isolated from ginseng roots, the ginsenosides are known to have multiple pharmacological activities (Deng and Zhang, 1991; Baek *et al.*, 2006; Wang *et al.*, 2007). At the doses commonly used, the dried roots and rhizomes of ginseng are not toxic to rats, dogs and humans (Radad *et al.*, 2006).

There is increasing interest in the identification of herbal and dietary compounds that can prevent or reduce the risks of cancer or serve as therapeutic agents (Rauscher *et al.*, 1998; Li *et al.*, 2000). One result of these efforts is that chemoprevention has emerged as a cost-effective means of preventing mutagenesis and carcinogenesis, and as a promising approach for minimizing the adverse effects of human exposure to environmental carcinogens (Pool-Zobel *et al.*, 1997; Rauscher *et al.*, 1998).

Doxorubicin (DOX), a broad-spectrum anthracycline antibiotic, is genotoxic and carcinogenic but is still widely used as an antitumor agent for the treatment of cancer (Minotti *et al.*, 2004). The potential usefulness of this drug

is limited by the development of adverse effects such as cardiotoxicity and nephrotoxicity. DOX may also be involved in secondary malignancies. The main mechanisms of action proposed for DOX include the inhibition of topoisomerase II, DNA intercalation, free radical formation, reductive bioactivation of the quinine ring to a semiquinone radical, DNA alkylation and cross-linking (Gewirtz, 1999; Ramji *et al.*, 2003; Navarro *et al.*, 2006). These mechanisms can result in the cleavage of DNA which, if not repaired, may lead to mutations and chromosomal aberrations in tumors as well as in healthy cells (Antunes and Takahashi, 1998; Gentile *et al.*, 1998; Islaih *et al.*, 2005; Antunes *et al.*, 2007; Costa and Nepomuceno, 2006; Fragiorge *et al.*, 2007; Valadares *et al.*, 2008).

We have used the *Drosophila melanogaster* (fruit fly) wing somatic mutation and recombination test (SMART) as a biological indicator of chemical genotoxicity or antigenotoxicity. This one-generation test, which is very efficient and sensitive, is based on the ability of fruit flies to metabolize certain procarcinogens to their reactive metabolites and has been used to study the genotoxicity and antigenotoxicity of various natural compounds (Idaomar *et al.*, 2002; Laohavechvanich *et al.*, 2006; Silva *et al.*, 2006; Fragiorge *et al.*, 2007; Mezzoug *et al.*, 2007; Tellez *et al.*, 2007; Valadares *et al.*, 2008). The wing SMART is based on the principle that a loss of heterozygosity leads to the expression of recessive marker genes in larval imaginal disk cells, thereby yielding clones of mutant cells that can be identified as mosaic spots on the wings (Graf *et al.*, 1984, 1998). These spots can be produced by mitotic recombination or mutations (deletions, point mutations, specific types of translocation, etc.). The analysis of two genotypes (one with structurally normal chromosomes and another with a multiply inverted balancer chromosome) allows the quantitative determination of the recombinogenic activity of genotoxic compounds (Graf *et al.*, 1998; Spanó *et al.*, 2001).

The identification of additional and more effective antigenotoxic compounds may contribute to the development of dietary supplements that could be useful in chemotherapy. Because the use of alternative medicines in combination with conventional therapy may increase the risk of unwanted interactions in cancer patients (Meijerman *et al.*, 2006), in this work we used the wing SMART to investigate the possible genotoxicity of three doses of a water-soluble form of the dry root of *P. ginseng* and its ability to protect against the genotoxicity of DOX. To our knowledge the effects of ginseng on DOX genotoxicity have not yet been studied *in vitro* or *in vivo*.

Materials and Methods

Chemical agents

The water-soluble form of the dry root of *P. ginseng* C. A. Meyer (ginseng coreano, in Portuguese) was obtained from Officinal Farmácia de Manipulação (Goiânia, GO, Brazil). Doxorubicin (DOX, Doxina® - Eurofarma Laboratórios Ltda., São Paulo, Brazil; CAS No. 23214-92-8) was obtained from Hospital de Clínicas da Universidade Federal de Uberlândia (Uberlândia, MG, Brazil) and dissolved in ultrapure water in the dark. Ultrapure water, used as a negative control, was obtained from a MilliQ system (Millipore, Vimodrone, Milan, Italy). All solutions were freshly prepared in ultrapure water immediately before use.

Strains and crosses

For the wing SMART, three strains of *D. melanogaster* [(i) the *multiple wing hairs*: *y*; *mwh j*; (2) the *flare-3*: $flr^3/In(3LR)TM3,\ ri\ p^p sep\ l(3)89Aa\ bx^{34e}\ e\ Bd^S$; and (iii) the *ORR*; *flare-3*: *ORR*; $flr^3/In(3LR)TM3,\ ri\ p^p sep\ l(3)89Aa\ bx^{34e}\ e\ Bd^S$], and two crosses were used. The two crosses consisted of a standard (ST) cross in which *flare-3* females were mated with *mwh* males (Graf *et al.*, 1989) and a high bioactivation (HB) cross in which *ORR*; *flare-3* females were mated with *mwh* males (Graf and van Schaik, 1992). The latter cross is highly sensitive to promutagens and procarcinogens because of the increased level of cytochrome P450 present in the *ORR*; *flare-3* strain. Both crosses produced experimental larval progeny that consisted of marker-heterozygous (MH) flies ($mwh\ +/+\ flr^3$) with phenotypically wild-type wings and balancer-heterozygous (BH) flies ($mwh\ +/+TM3,\ Bd^S$) with phenotypically serrate wings. Additional information about these strains and crosses is provided elsewhere (Dapkus and Merrel, 1977; Hällström and Blanck, 1985; Graf *et al.*, 1989; Graf and van Schaik, 1992; Saner *et al.*, 1996).

Larval feeding

After an 8 h mating period, the eggs were collected from the two crosses and maintained in culture flasks containing an agar-agar base (3% w/v) and a layer of fermenting live baker's yeast supplemented with sucrose. Third instar larvae from these eggs were collected and transferred to glass vials containing 1.5 g of mashed potato flakes rehydrated with 5 mL of a solution containing the water soluble form of the dry roots of *P. ginseng* (2.5, 5.0 or 10.0 mg/mL) alone or in association with DOX (0.125 mg/mL). Negative (ultrapure water) and positive (DOX 0.125 mg/mL) controls were included in these experiments. The larvae were allowed to feed on the medium until completion of their larval life (~48 h). The experiments were done at 25 °C and a relative humidity of 60%-70%.

Analysis of adult flies

Adult flies were collected and stored in 70% ethanol. The wings of MH flies were mounted on slides in Faure's solution and examined for spots by using a compound microscope at 400X magnification. The wings of BH flies were mounted and analyzed whenever a positive response was obtained in the MH progeny. Single spots resulted from point mutations, chromosomal aberrations, or recom-

bination events, whereas twin spots (*mwh* and *flr*^{3}) were produced by mitotic recombination between the proximal marker *flr*^{3} and the centromere of chromosome 3. Only *mwh* single spots were observed in the wings of BH flies. The results obtained in MH and BH flies were used to assess the recombinogenic potential of the water soluble form of the dry root of *P. ginseng* and DOX (Frei *et al.*, 1992; Graf *et al.*, 1992; Spanó *et al.*, 2001).

Data evaluation and statistical analysis

For statistical evaluation, the multiple-decision procedure of Frei and Würgler (1988) was used and allowed four diagnoses: +, positive; w+, weak positive; -, negative and i, inconclusive. The frequencies of each type of mutant clone per fly in a treated series were compared pair-wise (*i.e.*, negative control *vs. Pg*; DOX alone *vs.* DOX plus *Pg*) using the conditional binomial test described by Kastenbaun and Bowman (1970). For the final statistical analysis of all positive outcomes, the non-parametric Mann-Whitney *U*-test with $\alpha = \beta = 0.05$ was used to exclude false positive results (Frei and Würgler, 1995). The frequencies of clone induction per 10^5 cells were used to determine the recombinogenic activity based on the following parameters: mutation frequency (F_M) = frequency of clones in BH flies/frequency of clones in MH flies, recombination frequency (F_R) = 1- F_M, frequency of the total number of spots (F_T) = total number of spots in MH flies (considering *mwh* and *flr*^{3} spots)/number of flies, frequency of mutation = F_T x F_M and frequency of recombination = F_T x F_R (Santos *et al.*, 1999; Sinigaglia *et al.*, 2006). Based on the control-corrected spot frequencies per 10^5 cells the percentage of inhibition by *P. ginseng* was calculated as (DOX alone - *P. ginseng* plus DOX/DOX alone) x 100 (Abraham, 1994).

Results

Tables 1 and 2 show the wing SMART results for the chronic treatment of larvae with *P. ginseng* alone (2.5, 5.0 or 10.0 mg/mL) or in combination with DOX (0.125 mg/mL) using flies from ST and HB crosses, respectively. Larvae from both crosses were treated under identical conditions. Negative (ultrapure water) and positive (DOX 0.125 mg/mL) controls were included in each experiment. For statistical evaluation, the results from flies treated with *P. ginseng* were compared with data from the corresponding negative controls, whereas the results from flies treated with *P. ginseng* plus DOX were compared with data from the corresponding positive controls. Whenever there was a positive effect on the total number of spots in the MH progeny, the BH progeny were also analyzed.

There were no significant differences in the frequency of mutant spots between flies treated with 2.5, 5.0 or 10.0 mg of *P. ginseng*/mL and the negative control in ST cross MH flies (Table 1) and HB cross MH flies (Table 2). DOX (positive control) caused significant induction of all categories of spots in both the ST and HB crosses (Tables 1 and 2).

In ST cross MH flies, simultaneous treatment with 2.5 or 5.0 mg of *P. ginseng*/mL only weakly inhibited the increase in the total number of total spots caused by DOX whereas treatment with 10 mg of *P. ginseng*/mL did not alter the frequency of mutant spots (Table 1). In HB cross MH flies, simultaneous treatment with 2.5 or 10.0 mg of *P. ginseng*/mL reduced the total number of spots produced by DOX alone (Table 2). The frequency of mutant spots produced by DOX was not altered by simultaneous treatment with 2.5 or 5.0 mg of *P. ginseng*/mL in ST cross BH flies (Table 1), or 2.5 or 10.0 mg of *P. ginseng*/mL in HB cross BH flies (Table 2). Thus, *P. ginseng* did not interfere with the frequencies of DOX-induced spots of mutational (genic and chromosomal) origin.

The frequencies of clone induction per 10^5 cells in MH and BH flies treated with DOX alone or with *P. ginseng* plus DOX were used to assess the mutagenic and recombinogenic potential of *P. ginseng*. The genotoxicity in MH flies was attributable mainly to mitotic recombination. The dry root of *P. ginseng* had antirecombinogenic activity that was not dose-dependent.

Discussion

The wing SMART is rapid, sensitive and inexpensive assay for investigating the mutagenic and recombinogenic properties of chemicals, natural products and complex mixtures. This assay is also suitable for studying the mutagenic, antimutagenic and recombinogenic activities of drugs during multi-drug therapy (Graf *et al.*, 1984; Spanó *et al.*, 2001).

In this study, we examined the effects of three concentrations of *P. ginseng* (2.5, 5.0 or 10.0 mg/mL) in the wing SMART. *Panax ginseng* alone was not genotoxic in the ST and HB crosses. Simultaneous treatment with *P. ginseng* reduced the total number of spots produced by DOX in ST cross and HB cross MH flies, although the concentrations required for this varied between the crosses.

DOX was studied here because of its widespread use in cancer chemotherapy. A single concentration of DOX (0.125 mg/mL) was used in the wing SMART and significantly increased the number of mutant single spots and twin spots in ST and HB crosses. In addition to its mutagenic activity, DOX also has recombinogenic activity so that the frequency of twin spots reflected DOX-induced somatic recombination. These findings agree with other reports and show that DOX selectively induces homologous recombination when compared with mutational events in *D. melanogaster* somatic cells (Lehmann *et al.*, 2003; Costa and Nepomuceno, 2006; Fragiorge *et al.*, 2007; Valadares *et al.*, 2008).

The HB cross has constitutively high levels of cytochrome P450 and is characterized by a high sensitivity to promutagens and procarcinogens (Spanó *et al.*, 2001).

Table 1 - Results of the *Drosophila* wing spot test (SMART) in the marker-heterozygous (MH) and balancer-heterozygous (BH) progeny of the standard cross (ST) after chronic treatment of larvae with *P. ginseng* (*Pg*) and doxorubicin (DOX).

Genotypes and treatments		Number of flies (*N*)	Spots per fly (number of spots) and statistical diagnosis[a]				Spots with *mwh* clone[c] (*n*)	Frequency of clone formation/10^5 cells/cell division[d] (*n/NC*)[e, f]		Recombination (%)	Inhibition[g] (%)
P. ginseng (mg/mL)	DOX (mg/mL)		Small single spots (1-2 cells)[b] *m* = 2	Large single spots (>2 cells)[b] *m* = 5	Twin spots *m* = 5	Total number of spots *m* = 2		Observed	Control corrected		
mwh/flr^3											
0	0	60	0.77 (46)	0.15 (09)	0.02 (01)	0.93 (56)	55	1.87			
0	0.125	60	1.73 (104)+	1.03 (62)+	1.53 (92)+	4.29 (258)+	251	8.57	6.70	84	
2.5	0	60	0.95 (57)-	0.13 (08) i	0.10 (06) i	1.18 (71)-	68	2.32	0.45		
5.0	0	60	0.83 (50)-	0.08 (05)-	0.07 (04) i	0.98 (59)-	59	2.01	0.14		
10.0	0	60	0.70 (42)-	0.18 (11) i	0.03 (02) i	0.92 (55)-	59	2.01	0.14		
2.5	0.125	60	0.95 (57)*	0.92 (55)	1.30 (78)	3.17 (190)*	181	6.18	4.31	71	36
5.0	0.125	60	1.10 (66)*	0.95 (57)	1.30 (78)s	3.35 (201)*	199	6.79	4.92	80	27
10.0	0.125	60	1.37 (82)	1.05 (63)	1.40 (84)	3.82 (229)	222	7.58	5.71		
mwh/TM3											
0	0	60	0.20 (12)	0.03 (02)		0.23 (14)	14	0.47			
0	0.125	60	0.53 (32)+	0.15 (09)+		0.68 (41)+	41	1.40	0.93		
2.5	0.125	60	0.70 (42)	0.17 (10)		0.87 (52)	52	1.77	1.30		
5.0	0.125	60	0.53 (32)	0.13 (08)		0.66 (40)	40	1.36	0.89		

Marker-trans-heterozygous flies (*mwh/flr*^{3}) and balancer-heterozygous flies (*mwh/TM3*) were evaluated. [a]Statistical diagnoses according to Frei and Würgler [1988; 1995]. *U*-test, two-sided; probability levels: -, negative; +, positive; i, inconclusive; $p < 0.05$ *vs.* untreated control; *, $p < 0.05$ *vs.* DOX alone. [b]Including rare *flr*^{3} single spots. [c]Considering *mwh* clones from *mwh* single and twin spots. [d]Calculated according to Frei *et al.* (1992). [e]Numbers in square brackets indicate the induction frequencies corrected for spontaneous incidence estimated from the negative controls. [f]C = 48,800 (approximate number of cells examined per fly). [g]Calculated according to Abraham (1994). [h]Balancer chromosome *TM3* does not carry the *flr*^{3} mutation.

Table 2 - *Drosophila* wing spot test (SMART) results in marker-heterozygous (MH) and balancer-heterozygous (BH) progeny of the high bioactivation (HB) cross after the chronic treatment of larvae with *P. ginseng* (*Pg*) and doxorubicin (DOX).

Genotypes and treatments		Number of flies (*N*)	Spot per fly (number of spots) and statistical diagnosis[a]				Spots with *mwh* clone[c] (*n*)	Frequency of clone formation/10^5 cells/cell division[d] (*n/NC*)[e, f]		Recombination (%)	Inhibition[g] (%)
Pg (mg/mL)	DOX (mg/mL)		Small single spots (1-2 cells)[b] *m* = 2	Large single spots (>2 cells)[b] *m* = 5	Twin spots *m* = 5	Total spots *m* = 2		Observed	Control corrected		
mwh/flr^3											
0	0	60	0.47 (28)	0.13 (08)	0.05 (03)	0.65 (39)	38	1.29			
0	0.125	60	1.88 (113)+	2.45 (147)+	2.05 (123)+	6.38 (383)+	373	12.73	11.44	91.0	–
2.5	0	60	0.55 (33)-	0.05 (03)-	0.07 (04) i	0.67 (40)-	38	1.29	0.0	–	–
5.0	0	60	0.52 (31)-	0.05 (03)-	0.07 (04) i	0.63 (38)-	37	1.26	-0.03	–	–
10.0	0	60	0.50 (30)-	0.10 (06) i	0.02 (01) i	0.62 (37)-	36	1.22	-0.07	–	–
2.5	0.125	60	1.43 (86)*	1.42 (85)*	1.75 (105)	4.60 (276)*	270	9.22	7.93	90.0	31.0
5.0	0.125	60	1.73 (104)	2.22 (133)	2.62 (157)*	6.57 (394)	387	13.21	11.92		
10.0	0.125	60	1.47 (88)*	1.67 (100)*	2.12 (127)	5.25 (315)*	307	10.48	9.19	92.0	20.0
mwh/TM3											
0	0	60	0.12 (07)	0.03 (02)		0.15 (09)	9	0.3			
0	0.125	60	0.53 (32)+	0.05 (03) i		0.58 (35)+	35	1.19	0.89		
2.5	0.125	60	0.40 (24)	0.07 (04)		0.47 (28)	28	0.95	0.65		
10.0	0.125	60	0.37 (22)	0.03 (02)		0.40 (24)	24	0.81	0.51		

Marker-trans-heterozygous flies (*mwh/flr*^{3}) and balancer-heterozygous flies (*mwh/TM3*) were evaluated. [a]Statistical diagnoses according to Frei and Würgler [1988; 1995]. *U*-test, two-sided; probability levels: -, negative; +, positive; i, inconclusive; $p < 0.05$ *vs.* untreated control; *, $p < 0.05$ *vs.* DOX alone. [b]Including rare *flr*^{3} single spots. [c]Considering *mwh* clones from *mwh* single and twin spots. [d]Calculated according to Frei *et al.* (1992). [e]Numbers in square brackets indicate the induction frequencies corrected for spontaneous incidence estimated from the negative controls. [f]C = 48,800 (approximate number of cells examined per fly). [g]Calculated according to Abraham (1994). [h]Balancer chromosome *TM3* does not carry the *flr*^{3} mutation.

Comparison of the results obtained with the ST and HB crosses showed that the elevated cytochrome P450 activity in HB flies influenced the genotoxicity of DOX and that of the combined treatments, with a greater frequency of mutant spots in these flies, as also reported by Valadares *et al.* (2008). The greater genotoxicity of DOX in HB flies probably reflects the rapid one-electron reduction of this compound to its semiquinone free radical by cytochrome P450 (Goeptar *et al.*, 1993).

Many herbal and dietary products modulate cytochrome P450 activity. Gurley *et al.* (2002) used single-time point phenotypic metabolic ratios to determine whether long-term supplementation of St John's wort, garlic oil, *P. ginseng* and *Ginkgo biloba* affected CYP1A2, CYP2D6, CYP2E1 and CYP3A4 activities in humans; no significant effect was observed for *P. ginseng*. In agreement with this, Gurley *et al.* (2005) observed that the concomitant ingestion of *P. ginseng* with prescription medications in elderly patients resulted in only slight inhibition (7%) of CYP2D6 activity.

Ginseng extract significantly decreases DNA synthesis and increases the rate of DNA excision repair synthesis in V79 Chinese hamster lung cells (Rhee *et al.*, 1991), in addition to its ability to attenuate inflammation-mediated carcinogenesis (Hofseth and Wargovich, 2007). These mechanisms can reduce tumor growth and improve the prognosis in cancer patients (Wang *et al.*, 2007). The beneficial effects of ginseng and its main constituents during chemotherapy are probably related to their ability to minimize the adverse effects of antineoplastic drugs. Recent findings *in vivo* and *in vitro* have shown that ginseng partially protects against DOX-induced testicular toxicity (Kang *et al.*, 2002), significantly attenuates the effects of DOX-induced heart failure in rats (You *et al.*, 2005), and reduces cisplatin-induced nephrotoxicity in cultured renal proximal tubular epithelial cells (Baek *et al.*, 2006). In contrast, little is known about the effects of ginseng and its compounds when administered in combination with chemotherapeutic drugs.

Dietary supplementation of ginseng protects against oxidative damage *in vitro* and *in vivo*, from acute oxidative stress in cardiomyocytes to heart perfusion injury (Maffei-Facino *et al.*, 1999; Shao *et al.*, 2004). Yance and Sagar (2006) reported that *P. ginseng* has antiangiogenic activity and anticancer activities that are mediated by multiple interdependent processes, including changes in gene expression, signal processing and enzymatic activities. Although the mechanisms of action of the more than 60 ginsenosides isolated from *Panax* species remain poorly understood, studies of these compounds and their effects on tumor cells are of interest since several ginsenosides efficiently inhibit cell growth and the proliferation of human cancer cell lines (Wang *et al.*, 2007).

Total ginseng extracts or aqueous fractions of *P. ginseng* show antimutagenic effects that include a reduction in the frequency of radiation-induced DNA breaks in murine lymphocytes and protection against ^{137}Cs-induced micronuclei in human lymphocytes (Rhee *et al.*, 1991; Kim *et al.*, 1996; Lee *et al.*, 2004). Ginseng-treated Swiss white mice show a significant reduction in the frequencies of chromosomal aberrations and micronuclei induced by benzo[a]pyrene (Panwar *et al.*, 2005). Ginsan, which itself is not mutagenic, decreases the frequency of micronucleated polychromatic erythrocytes induced by gamma radiation in bone marrow cells of C57BL/6 male mice (Ivanova *et al.*, 2006). Similarly, the ginsenoside Rh_2 enhances the antitumor activity and decreases the genotoxicity of cyclophosphamide in mice (Wang *et al.*, 2006). Ginseng has powerful antioxidant (Cho *et al.*, 2008) and antimutagenic (Geetha *et al.*, 2006; Ivanova *et al.*, 2006) properties, although the mechanisms of these protective effects remain to be elucidated. Geetha *et al.* (2006) reported that ginseng extracts protected against H_2O_2-induced mutagenesis in *Salmonella typhimurium* strain TA100, and against mutagenesis produced by 4-nitroquinoline-N-oxide in *S. typhimurium* strains TA98 and TA100 in the Ames test; however, the extract was unable to inhibit the damage induced by tert-butyl hydroperoxide in strain TA102 which is highly sensitive to reactive oxygen species. The authors concluded that the protection provided by the ginseng extract against 4-nitroquinoline-N-oxide and H_2O_2-induced mutagenicity in strains TA98 and TA100 was attributable mainly to the extracts ability to promote DNA repair rather than its antioxidant effects.

Our results indicate that *P. ginseng* is not genotoxic in somatic cells of *D. melanogaster*, and that at low concentrations it protects against the genotoxicity of DOX. Inhibitors of mutagenesis often act through multiple mechanisms or can interact with other inhibitors (De Flora and Ramel, 1988). The mechanisms by which *P. ginseng* protects cells against DOC-induced genotoxicity were not examined here but could involve direct interaction of the extract constituents with DOX, resulting in an antimutagenic effect, and/or an antioxidant action through radical scavenging or the activation of intracellular antioxidant enzymes. Although homologous recombination causes rearrangements of DNA that can promote cancer, little was known about the ability of *P. ginseng* to inhibit recombination or modulate DNA repair mechanisms. More data are required on the dose-response relationship of *P. ginseng* and the potential toxicities of combinations with chemotherapeutic drugs or radiation before this product can be recommended for cancer therapy.

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