Ginseng extract exhibits antimutagenic activity against induced mutagenesis in various strains of *Salmonella typhimurium*

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Received 10 February 2005; revised 26 July 2006

Ginseng has been reported to exhibit antioxidant and antimutagenic activity. The present study was undertaken with a view to confirm whether the antioxidant activity of Ginseng is responsible for its antimutagenic action. The concentrated root extract of *Panax ginseng* (Ginseng extract I) and its lyophilized powder (Ginseng extract II) obtained from two different manufacturing houses, were tested against mutagenesis using the well-standardized Ames microsomal test system. The extracts exhibited antimutagenic effect against hydrogen peroxide induced mutagenesis in TA100 strain, and against mutagenesis produced by 4-nitroquinoline-N-oxide in both TA98 and TA100 strains of *Salmonella typhimurium*. Both the extracts failed to show any antimutagenic potential against tert-butyl hydroperoxide (an oxidative mutagen) in TA102 strain, a strain highly sensitive to active oxygen species. The extracts also indicated a weak antioxidant activity in a series of *in vitro* test systems viz., 1,1-diphenyl picryl hydrazyl (DPPH) assay, hydrogen peroxide scavenging and superoxide anion scavenging. The results indicate that the protective effects shown by ginseng extract(s) against 4-nitroquinoline-n-oxide and hydrogen peroxide induced mutagenesis in TA98 and TA100 could mainly be due to its property to initiate and promote DNA repair rather than free radical scavenging action.

**Keywords:** Ames test, Antimutagenicity, DPPH assay, Ginseng extract, *In vitro* antioxidant action

Ginseng, *Panax ginseng* C A Meyer, a native plant of Korea and China exhibits various pharmacological and clinical applications. Ginseng saponins showed hepatoprotection against CCl$_4$-induced hepatotoxicity due to the antioxidative property of ginsenoside saponins present in it. They inhibited the proliferation of cancer cells and induced their reverse transformation in cultured Morris hepatoma cells. Ginseng extract normally contains a very small percentage (about 4-5%) of ginsenosides. Red ginseng extracts were significantly chemoprotective against DMBA/Croton oil-induced skin papilloma and tumors in mice. Ginseng extract also reduced the lipid peroxidation in microsomes and mitochondria. Intravenous administration of ginsenoside increased creatinine phosphokinase and SOD activities and thus reduced lipid peroxidation. Pretreatment with ginseng extract increased survival time in irradiated Swiss albino mice. *Panax ginseng* exhibited antioxidant action *in vitro* and *in vivo*.

Ginseng extract acts as an antimutagen by increasing the rate of DNA excision repair synthesis in V79 cells in response to treatment with UV radiation or methyl methane sulphonate. There are no reports regarding the antimutagenic action of Ginseng in the Ames *Salmonella* test system. In an effort to elucidate the mechanism of the proposed antimutagenic action of Ginseng, and to check whether it is because of its oxygen radical scavenging nature, Ginseng extract has been tested in the well-standardized Ames *Salmonella typhimurium* test system using the tester strain TA102, which readily responds to active oxygen species.

In the present study, Ginseng extract I (Helios Pharmaceuticals, Ahmedabad; a concentrated aqueous extract) and Ginseng extract II (Ranbaxy Laboratories Ltd., Gurgaon; a lyophilized powder) were evaluated against tert-butylhydroperoxide induced mutagenicity in Ames tester strain TA102 and 4-nitroquinoline-N-oxide (NQNO) in TA98 and TA100 strains and hydrogen peroxide in TA 100 strain. Further, *in vitro* free radical scavenging activity of these two samples has been evaluated and compared amongst themselves and with ascorbic acid and green tea extract against a variety of free radicals to corroborate the postulation that Ginseng extract is antimutagenic because of its capacity to scavenge reactive oxygen species (ROS).

**Materials and Methods**

Microorganism—TA102, TA98 and TA100 strains of *S. typhimurium* were originally obtained as a free
gift from Dr. Bruce N. Ames (University of California, Berkeley, USA). Presently, they are being maintained in the laboratory at the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Chemicals—Ginseng extract I was gifted by Helios Pharmaceuticals, Ahmedabad, India and Ginseng extract II was gifted by Ranbaxy Laboratories Ltd., Gurgaon. All the reagents used in the study, including a 70% aqueous solution of t-butylhydroperoxide (Merck-Schuchardt, Hohenbrunn, Germany), 4-nitroquinoline-N-oxide (Sigma Chemical Co., St. Louis), hydrogen peroxide (Qualikems Fine Chemicals Pvt. Ltd., New Delhi), 1,1-diphenyl picryl hydrazyl (DPPH) (Sigma Chemical Co., St. Louis), agar (extra pure) (Hi Media Laboratories Pvt. Ltd., Mumbai), and Oxoid nutrient broth No. 2 (Oxoid Ltd., Basingstoke, Hampshire) were of AR grade.

The antimutagenicity test—The plate incorporation procedure given by Maron and Ames\textsuperscript{11} was used for antimutagenicity testing with the inclusion of pre-incubation step\textsuperscript{12}. Sterile test tubes, containing 0.5 ml phosphate buffer, pH 7.4, 0.1 ml bacterial culture (12 hr), 0.1 ml mutagen (in water or DMSO) and 0.1 ml varying concentration(s) of Ginseng extract dissolved in water, were gently vortexed and incubated at 37°C for 20 min. Then 2 ml of molten top agar containing 0.5 mM histidine-biotin, kept at 45°C was added to each tube. After thorough mixing, the contents were poured onto minimal glucose agar plates. The plates were allowed to harden horizontally and then incubated for 48 hr at 37°C before scoring the revertant colonies.

All the antimutagenicity assays were carried out in duplicate/triplicate and on at least two separate occasions. Negative and positive controls were included in each assay. The antimutagenic activity is displayed graphically as % inhibition of mutagenicity vs dose. The number of revertant colonies that appeared upon incubation with the mutagen (t-BOOH, NQNO, H\textsubscript{2}O\textsubscript{2}) alone was taken as the 100% value in terms of mutagenicity (0% inhibition). The antimutagenic effect of ginseng extract or the vitamin C and green tea extract are expressed as % inhibition of mutagenicity. Ginseng extracts I and II did not show any bactericidal/inhibitory effect on the growth of the tester strain at the tested dose levels. Analysis of variance followed by Tukey’s test showed the results to be significant and statistically significant inhibition (\(P <0.001\)) in mutagenicity with respect to the control was observed at all doses. A corresponding decrease in the His\textsuperscript{+} revertant count with an increase in dose was found to be linear (\(r = 0.92\)). The % inhibition of mutagenicity was calculated as:

\[
\left(1 - \frac{\text{His}^+ \text{ revertants induced/plate by the mutagen in the presence of antioxidant}}{\text{His}^+ \text{ revertants induced/plate by the mutagen alone}}\right) \times 100
\]

\textit{In vitro antioxidant activity}—1-1, Diphenyl picryl hydrazyl assay, superoxide anion scavenging, hydrogen peroxide scavenging, hydroxyl radical scavenging and nitric oxide scavenging were done\textsuperscript{13-18} and the percentage scavenging was determined.

\textit{Statistical analysis}—All the data were statistically analyzed by one way ANOVA followed by Tukey's test using a statistical package SIGMASTAT.

\textbf{Results}

\textit{Antimutagenic activity of ginseng extracts I and II}—In this study, Ginseng extracts I and II were examined for their antimutagenic activity against direct acting oxidative mutagens-tBOOH in \textit{S. Typhi} TA102 and alkylating agent-NQNO in TA98 and TA100 and hydrogen peroxide in TA100. Both the extracts were tested in the dose range of 500-4000 \(\mu\text{g}\). Ginseng extract I showed a significantly better (\(P <0.05\)) antimutagenic activity than Ginseng extract II in both TA 98 and TA 100 strains (Table 1, Fig. 1), while neither of the extracts showed any antimutagenic activity in TA102 strain.

\textit{Antioxidant activity of ginseng extracts I and II}—Five different test systems (DPPH assay, superoxide anion scavenging, hydrogen peroxide scavenging, hydroxyl radical scavenging, and nitric oxide scavenging) were used for determining the antioxidant activity. Ginseng extract I showed a

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Bacterial strain} & \textbf{Mutagen} & \textbf{ID}_{50} (\mu\text{g}) & \textbf{Ginseng extract I} & \textbf{Ginseng extract II} \\
\hline
\textit{S. typhi} TA 98 & NQNO & 1531.42 ± 0.87 & 2528.23 ± 0.94 \\
\textit{S. typhi} TA 100 & NQNO & 402.17 ± 1.2 & 2213.34 ± 0.54 \\
\textit{S. typhi} TA 100 & \text{H}_{2}\text{O}_{2} & 604.59 ± 0.65 & 3094.85 ± 0.43 \\
\hline
\end{tabular}
\caption{ID\textsubscript{50} values of Ginseng extract I and II in antimutagenicity assay [Values are mean ± SD of 4 replicates]}
\end{table}
significantly better (1.5-4 times) activity in all the test systems than Ginseng extract II. However, its antioxidant activity was much less than ascorbic acid and green tea extract (Table 2).

Discussion

NQNO is a DNA alkylating agent and the antimutagenic activity shown by ginseng extract(s) against it in the present study, could be due to its ability to modulate DNA replication or repair. Ginseng extract increases the rate of DNA excision repair synthesis. Hydrogen peroxide, the other mutagen used, is reported to cause DNA damage to the bacterial cells due to its involvement in the formation of hydroxyl radicals after passing through the cellular membrane by reaction with DNA-bound metal ions. This causes DNA strand breaks, which results in mutations in bacterial cells. Ginseng extracts II and I show an antioxidant and antimutagenic activity against hydrogen peroxide.

$t\text{-}BOOH$ is a direct acting oxidative mutagen that produces $t\text{-}butyl$ peroxyl radicals and induces mutations in TA102 strain of $S.\ Typhi$ in the Ames test, mainly through hydroxyl radical formation. Ginseng extract(s) did not show a significant antimutagenic activity in the TA102 strain, against $t\text{-}BOOH$.

The in vitro antioxidant studies show that ginseng extract(s) is a weak antioxidant (Table 2) in comparison to two well-established antioxidants, ascorbic acid and green tea extract. The in vitro hydroxyl scavenging activity was especially low (almost 200-500 times less than green tea extract). Earlier, good correlations between the in vitro antioxidant activity of certain natural compounds e.g. green tea catechins, sesamol and nortiodruguaic acid and their antimutagenic activity against $S.\ Typhi$ TA102 (sensitive for oxidative mutagens) have been reported. Similar correlations were also observed for other pathological conditions induced by

<table>
<thead>
<tr>
<th>Drugs</th>
<th>DPPH assay</th>
<th>$H_2O_2$ scavenging</th>
<th>Superoxide anion</th>
<th>Hydroxyl ion</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tea extract</td>
<td>$3.8 \pm 0.86$</td>
<td>$11.8 \pm 0.1$</td>
<td>$3.52 \pm 2.1$</td>
<td>$18.55 \pm 2.36$</td>
<td>$56.31 \pm 0.5$</td>
</tr>
<tr>
<td>Ginseng extract I</td>
<td>$561.89 \pm 2.65$</td>
<td>$119.41 \pm 1.8$</td>
<td>$80.19 \pm 1.2$</td>
<td>$2964.58 \pm 1.34$</td>
<td>$1224.31 \pm 0.8$</td>
</tr>
<tr>
<td>Ginseng extract II</td>
<td>$898.99 \pm 0.65$</td>
<td>$561.89 \pm 2.65$</td>
<td>$134.65 \pm 3.8$</td>
<td>$5520.0 \pm 0.74$</td>
<td>$1915.94 \pm 1.6$</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>$6.62 \pm 1.7$</td>
<td>$25.79 \pm 4.0$</td>
<td>$18.21 \pm 2.8$</td>
<td>$33.38 \pm 2.70$</td>
<td>$25.79 \pm 4.08$</td>
</tr>
</tbody>
</table>
oxidative stress. Thus, the failure of Ginseng extract to produce effect in TA102 could be due to its weak antioxidant nature. Also, the protective effects shown against NQNO and hydrogen peroxide in TA98 and TA100 could mainly be due to its property to initiate and promote DNA repair rather than free radical scavenging action. Thus, it may be classified under bioantimutagens. Interaction and permeability through the biomembranes of the tester strains could be the other parameters, which may affect the antimutagenic activity of ginseng extract. The study is a preliminary effort at elucidating the possible mechanism of action of Ginseng as an antimutagen (and an anticancer agent). The results indicate that the antimutagenic activity is not related to its antioxidant action. Latter is considered as a possible mechanism of anticancer activity because free radicals are implicated in the pathophysiology of cancer.

Acknowledgement
Thanks are due to University Grants Commission, New Delhi, India for the financial assistance.

References