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Antigenotoxic Activities of Chlorophyll-Rich Plant Extracts

Daejoong Kwon[1], Orianna Carter[2], Sun Yoon[1], George S. Bailey[2] and Roderick H. Dashwood[2]*

[1]Department of Foods and Nutrition, Yonsei University, Seoul 120-749, Korea
[2]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331-6512, USA
E-mail: rod.dashwood@oregonstate.edu

INTRODUCTION

The human diet contains a great variety of carcinogens, as well as many natural anticarcinogens. Numerous epidemiological studies have shown that intake of green-yellow vegetables rich in chlorophyll, vitamin C, vitamin E, and carotenoids reduce the risk of cancer and other chronic diseases. There is an inverse association between the consumption of vegetables and the formation of epithelial cancers, and these relationships may be stronger for raw vegetables than cooked vegetables. A growing interest exists in establishing the various health benefits of green-yellow vegetable extracts, including their potential antioxidant and antigenotoxic properties. Previous work showed the beneficial effects of methanol extracts of *Angelica keiskei*, *Oenanthe javanica*, and *Brassica oleracea* (kale) on nitrite scavenging *in vitro*, growth of cancer cells in culture, and plasma lipid and antioxidant status in smokers.

Based on these initial studies, we sought to examine the antimutagenic and antioxidant properties of methanol extracts of *A. keiskei*, *O. javanica*, and *B. oleracea*. These three plant species were chosen for study because of their widespread use in popular health drinks, such as Korean green juice, and because they are known to contain high levels of antioxidants and phytochemicals, such as chlorophyll, β-carotene, vitamin E, vitamin C, Se and Zn.

Antimutagenic Activity

Heterocyclic amines have been shown to respond *in vitro* and *in vivo* to a broad array of antimutagens and cancer chemopreventive agents, as well as various tumor promotors. In the Salmonella mutagenicity assay, *A. keiskei* caused dose-dependent inhibition against three heterocyclic amine mutagens in the presence of S9, *O. javanica* was antimutagenic only at the highest concentration in the assay (2 mg/plate), and *B. oleracea* showed no consistent inhibitory activity at non-toxic levels. None of the extracts were effective against three direct-acting mutagens in the absence of S9.

Inhibition of Cytochrome P450 Activity

Heterocyclic amines such as IQ and PhIP are metabolically activated by CYP1A2, generating *N*-OH-IQ and *N*-OH-PhIP, whereas CYP1A1 catalyzes both *N*-hydroxylation and ring hydroxylation reactions. The activities of CYP1A1 and CYP1A2 were followed in EROD and MROD assays, respectively; *A. keiskei* and *O. javanica* produced concentration dependent inhibition in both assays, whereas *B. oleracea* was the least effective of the three plant extracts tested. At the highest concentration in the assay, inhibition of both EROD and MROD activities was in the relative order *A. keiskei* > *O. javanica* >> *B. oleracea*, which agreed favorably with the order of antimutagenic activity against IQ, MeIQx, and PhIP in the Salmonella assay.

Activity of Plant Extracts in FRAP and ORAC Assays

All three plant extracts exhibited concentration-dependent ferric reducing/antioxidant power, and oxygen radical absorbance capacity. The relative order of activity in these assays was as follows: *O. javanica* > *A. keiskei* = *B. oleracea* (FRAP); *A. keiskei* = *O. javanica* > *B. oleracea* (ORAC).

Inhibition of DNA Nicking

The closed circular form of plasmid pUC19 was converted efficiently to the nicked linear form after treatment with Fe^{2+}/H_2O_2, and in some experiments the relaxed form also was detected as a minor band. Each of the plant extracts attenuated the formation of nicked DNA *in vitro*. For example, when the linear form was normalized to the corresponding control treated with Fe^{2+}/H_2O_2 but no plant extract, *A. keiskei* inhibited DNA nicking by ~50% at 20 μg/mL, *O. javanica* inhibited by >70% and *B. oleracea* inhibited by 40%. Inhibition of DNA nicking corresponded favorably with the relative order of activity in the FRAP and ORAC assays.

Antigenotoxic Effects in Human Colon Cancer Cells

Finally, since plant extracts inhibited DNA nicking induced by Fe^{2+}/H_2O_2 *in vitro*, we treated HCT116 human colon cancer cells with H_2O_2 and examined the genotoxic damage using the 'comet' assay. The median tail moment in controls treated with H_2O_2 alone (no vegetable extract) was assigned an arbitrary value of 1.0 in order to compare among the three treatment groups; extracts of *A. keiskei* and *O. javanica* produced significant, dose-dependent protection, whereas *B. oleracea* was effective only at the highest concentration tested of 20 μg/mL. Little DNA damage was detected in untreated cells.

These findings provide support for the antigenotoxic and antioxidant properties of chlorophyll-rich extracts of *A. keiskei*, *O. javanica*, and *B. oleracea*, through mechanisms that include inhibition of carcinogen activation and scavenging of reactive oxygen species.

DISCUSSION

The present investigation has demonstrated that chlorophyll-rich ethanol extracts of *A. keiskei*, *O. javanica* and *B. oleracea* exhibited antimutagenic and antioxidant activities *in vitro*. Under the present conditions, *A. keiskei* was most effective against the three indirect-acting heterocyclic amines IQ, MeIQx and PhIP in the Salmonella assay, as well as being most inhibitory in the EROD and MROD assays, but had no effect against direct-acting mutagens in the absence of S9. These findings imply that one or more constituents in *A. keiskei* interfered with the enzymes which metabolically active heterocyclic amines, without affecting the direct-acting mutagens, such as by electrophile-scavenging. Although *O. javanica* and *B. oleracea* were less effective than *A. keiskei* under the present conditions, we cannot exclude the possibility that other cultivars of these three plants might exhibit greater inhibitory activity.

Indeed, in prior studies, extract of kale (*B. oleracea*) was more effective than *A. keiskei* in preventing the oxidation of linoleic acid during storage, which is indicative of antioxidant activity, as well as exhibiting higher nitrite scavenging *in vitro*. We confirmed the high antioxidant activity of all three plant extracts in ORAC and FRAP assays, and demonstrated inhibition of H_2O_2-induced DNA damage, assessed directly using pUC19 *in vitro* and indirectly using the comet assay in HCT116 colon cancer cells. In contrast to the results obtained in the Salmonella assays, *O. javanica* was generally the most potent of the three plant extracts in the various assays used here to assess antioxidant properties.

Although, as mentioned above, different cultivars are likely to exhibit different inhibitory potencies in the various assays, in the present investigation *A. keiskei* was most effective against heterocyclic amine mutagens, whereas *O. javanica* had the highest antioxidant potential. These findings suggest that, indeed, a 'cocktail' of plant extracts is likely to prove more beneficial than an extract containing a single plant species, since this would likely provide for complementary protective mechanisms. This is one rationale used for health drinks containing a cocktail of plant species, such Korean green juice, which has high levels of phytochemicals with demonstrated cancer inhibitory activity, such as chlorophylls, indoles and isothiocyanates. Future studies should examine the possible synergistic protective effects of *A. keiskei*, *O. javanica* and *B. oleracea* in combination, and assess the optimal 'blend' of these three plant extracts with respect to antimutagenic *versus* antioxidant effects.

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