Available online at www.ehs.or.kr

ToxEHS

Preventive Effects of Korean Medicinal Herbs on the Phenanthrene-induced Oxidative DNA Damages

A-Reum Yu[1] **& Mi-Young Lee**[1]

[1]Department of Medical Biotechnology, Soonchunhyang University, Asan, Chungnam 336-600, Korea
Correspondence and requests for materials should be addressed to M.-Y. Lee (miyoung@sch.ac.kr)

Accepted 2 March 2010

Abstract

Phenanthrene-induced oxidative DNA damage in lymphocyte was evaluated using comet assay and the suppressive effects of Korean medicinal herbs on the DNA damage were examined to screen the medicinal herbs that help to detoxify environmental pollutants. Extracts of Acanthopanax, Benincasae and green tea polyphenols effectively inhibited the phenanthrene-induced oxidative DNA damage in lymphocytes among tested herbs in this study. The present study suggests that Korean medicinal herbs by virtue of their antioxidant potential may be used as effective agents to reduce the oxidative DNA damage by phenanthrene.

Keywords: Comet assay, Phenanthrene, Korean medicinal herbs

Introduction

Polycyclic aromatic hydrocarbons (PAHs), also known as polyaromatic hydrocarbons or polynuclear aromatic hydrocarbons are chemical compounds that consist of fused aromatic rings[1]. Due to their hydrophobic characters, PAHs in the environmental matrices rapidly bind to particles of sediments and soils. In addition to their presence in fossil fuels they are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat and tobacco[2].

Several particulate PAHs are classified as probable human carcinogens by the International Agency of Research on Cancer (IARC). PAHs are one of the most widespread organic pollutants in environment, and 16 PAH compounds were designated as priority pollutants by EPA[3]. The 16 EPA priority PAHs is often targeted for measurement in environmental samples. They are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]-anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*cd*]pyrene.

Phenanthrene($C_{14}H_{10}$), the simplest PAHs in structures, is a polycyclic aromatic hydrocarbon with three aromatic rings, and exists as a colorless crystalline solid. Phenanthrene occurs in various ambient sources, in particulate matter formed from combusted smoky coal, diesel emissions, cork factory and waste incinerator[4,5] Phenanthrene has been known to be implicated in the formations of tumors of the stomach, lung and skin in animals. Moreover, its damage to skin, body fluids and the immune system which help the body fight disease on the animals were known[6,7].

Phytochemicals are not necessary for life but they help to promote optimal health by lowering risk for chronic diseases and enhancing defense against environmental stressors. Some groups of phytochemicals that have been linked to decreased cancer risk and environmental toxicity risk include allyl sulfides, dithiolthiones and isothiocyanates, indoles, isoflavones, lignans, flavonoids and carotenoids[8,9]. Allyl sulfides, found in onions, scallions and leeks, may stimulate activity of enzymes that help to eliminate toxic compounds. Dithiolthiones and isothiocyanates, found in cruciferous family of vegetables, broccoli, cauliflower, cabbage and turnips may increase activity of enzymes that help to detoxify carcinogens. Indoles, which may interfere with estrogen metabolism and therefore could reduce risk for some estrogen-related cancers such as breast cancer, are also found in cruciferous vegetables. Most flavonoids are known to be excellent antioxidants and have radical scavenger properties[8]. However, information on the effect of Korean medicinal herbs on the environmental toxicants is not available, although their pharmacological properties have been known.

We examined the effects of Korean medicinal herbs on the phenanthrene-induced DNA damage in lymphocyte to find the phytochmicals that help to detoxify environmental pollutants, such as PAHs including phenanthrene.

Results and Discussion

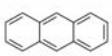
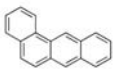
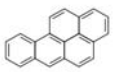
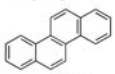
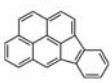
Single cell gel electrophoresis (comet assay) is a method for detecting DNA damage in individual cells[10-12]. DNA breaks at single-strand and double-strand, alkali-labile sites, incomplete repair sites, and crosslinks at the individual cells exposed to various environmental toxic chemicals can be evaluated with comet assay[13,14].

Table 1 shows the structures and toxicities of various polycyclic aromatic hydrocarbons (PAHs). PAHs may contain four-, five-, six- or seven-member rings, but those with five or six are most common. Toxicity of PAHs might be dependent on their structures, with isomers varying from being nontoxic to being extremely toxic[15].

Figure 1 indicates the effect of Acanthopanax extracts on the oxidative DNA damage of lymphocytes by phenanthrene, the simplest PAHs in structures[16]. Pretreatment with Acanthopanax extracts significantly inhibited phenanthrene-induced cellular DNA damage as revealed by decreased comet tail moment. The tail moment at 30 μM phenanthrene was about 60, whereas that at PBS-treated control was about 8%, indicating that 30 μM phenanthrene could induce approximately 6 folds more DNA damage. However, the tail moment of Acanthopanax-treated group was approximately 10, similar value to the control. This result indicates that root, stem and leaf extracts of Acanthopanax could reduce the oxidative DNA damage by phenanthrene in lymphocyte. These findings, together with our previous results[17] suggest a potential role for Acanthopanax as an anti-genotoxic, anti-oxidant and chemopreventive agent and could be used as components in chemotherapeutic applications. *Acanthopanax* sp. is used clinically as a tonic and prophylactic for chronic bronchitis, hypertension, ischemic heart disease, gastric ulcer, rheumatism, diabetes, and cirrhosis[18]. Four 3,4-seco-lupane type triterpenoids, named chiisanoside, chiisanogenin, 24-hydroxychiisanogenin, 22a-hydroxychiisanogenin have been isolated from the leaves of *A. divaricatus* var. *albeofructus* (Araliaceae)[19]. Chiisanoside has an effect on anti-cancer, rheumatoid arthritis, empyema, gatric ulcer, pharyngolaryngitis and basal metabolism action[20]. The major active constituents of the *Acanthopanax* roots are eleutheroside, acanthoside, daucosterine, β-sitosterol, sesamine and savinine, etc[21].These compounds have been also reported to have diverse effects such as anti-cancer, immune activation, anti-inflammation and anti-fever effect[22].

Benincasae hispida belongs to the family Cucurbitaceae, and it has been known to have an effect on obesity, diabetes, dropsy, liver trouble, gastric ulcer and bleeding. Moreover, *Benincasae hispida* has antioxidant effect[23], suppressive effect of vascularization[24],

Table 1. IARC classification and toxicity of polycyclic aromatic hydrocarbons[33].

Name	Structure	IARC classification
Acenaphthene		Group 3
Anthracene		Group 3
Benz[*a*]anthracene		Group 2B
Benzo[*a*]pyrene		Group 1
Benzo[*e*]pyrene		Group 3
Benzo[*ghi*]perylene		Group 3
Chrysene		Group 2B
Fluoranthene		Group 3
Fluorene		Group 3
Indeno[1,2,3-*cd*]pyrene		Group 2B
Naphthalene		Group 2B
Phenanthrene		Group 3
Pyrene		Group 3

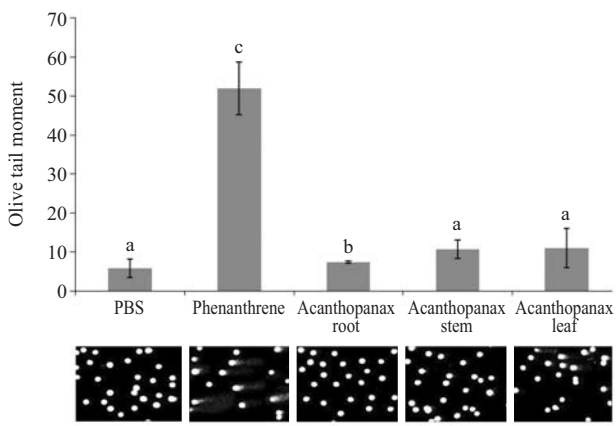


Figure 1. Inhibitory effect of *Acanthopanax* on the phenanthrene-induced oxidative DNA damage in lymphocytes. Values not sharing the same letter are significantly differnt from one another ($p < 0.05$).

protective effect on stomach and intestine[25], anti-ulcer effect[26], anti-inflammatory effect in diabetic vascular disorder[27] and allergic inflammation[28]. Red ginseng is representative tonic from ancient times and it has complex pharmacological activities. Ginsenoside, known as ginseng saponin, is major component on this effect and it has 30 different forms[28].

In Figure 2, the suppressive effects of Benincasae and red-ginseng were compared with that of Acanthopanax on the phenanthrene-induced DNA damage. Acanthopanax reduced the phenanthrene-enhanced olive tail moment the most effectively among the Korean medicinal herbs tested. Thus Acanthopanax seemed to be the most effective agent in preventing oxidative DNA damage by phenanthrene in this study. In Figure 3, it was investigated whether green tea polyphenol could ameliorate phenanthrene-induced oxidative DNA damage. Our results revealed that polyphenol provides protection against DNA oxidative damage induced by phenanthrene as measured with reduced comet tail. Green tea polyphenols exhibited antimutagenic activity in vitro, and they inhibited carcinogen-induced skin, lung, forestomach, esophagus, duodenum and colon tumors in rodents. Although several polyphenols show anticarcinogenic activity, the most active is (-)-epigallocatechin-3-gallate (EGCG), the major constituent in the green tea polyphenol fraction[29]. Several mechanisms appear to be responsible for the tumor-inhibitory properties of polyphenols, including enhancement of antioxidant enzymes and phase II (glutathione-S-transferase) enzyme activities[30,31]. The preventive effects of green tea polyphenol against DNA damage in this investigation might arise from the ability of polyphenol to effectively scavenge reactive oxygen species[16,32]. The comet assay result in this investigation elicited that the increase in genotoxicity caused by phenanthrene was counteracted by several Korean medicinal herbs. Findings from the present study suggest that dietary supplementation with medicinal herbs may be useful to protect the cells from oxidative DNA damage by phenanthrene.

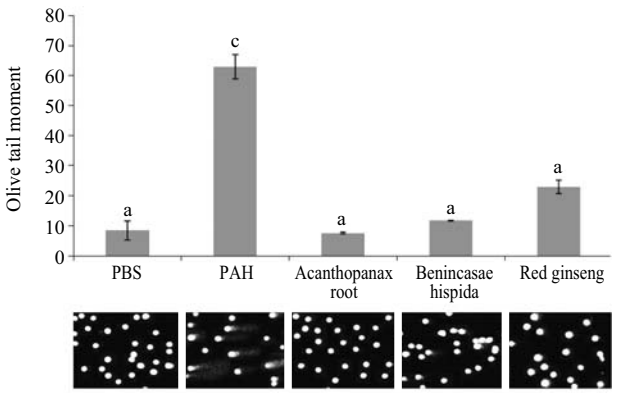


Figure 2. Inhibition of phenanthrene-induced oxidative DNA damage by Korean medicinal herbs. Values not sharing the same letter are significantly differnt from one another ($p < 0.05$).

Materials and Methods

Lymphocyte Isolation

Fresh whole blood was added to 800 μL of phosphate-buffered saline (PBS) and layered onto 200 μL of Histopaque-1077 (Sigma-Aldrich, USA). The lymphocytes were collected from the buffy coat layer, and then the lymphocytes were used in the comet assay.

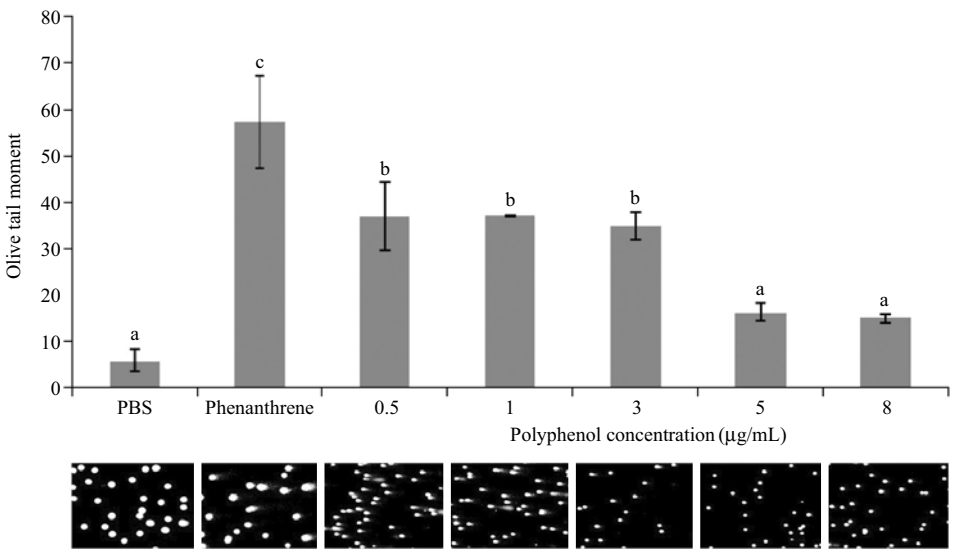


Figure 3. Inhibitory effect of green tea polyphenol on the phenanthrene-induced oxidative DNA damage in lymphocytes. Values not sharing the same letter are significantly differnt from one another ($p < 0.05$).

Medicinal Herbs

Water extracts of Acanthopanax (*Acanthopanax* sp.) and Benincasa (*B. hispida*) were provided from Susinogapy Co., Ltd (Asan City, Chungnam, Korea). Polyphenol isolated from green tea was obtained from Hansung Bio (Asan City, Chungnam, Korea). Powdered red ginseng (*Panax ginseng* C.A. Meyer) was obtained from a reginal market in Asan City. All of the herbal medicines were each dissolved in PBS at a 5 μg/mL.

Phenanthrene Treatment

Phenanthrene dissolved at a 1, 5, 20 or 30 mM was reacted with lymphocytes for 5 min at 0˚C. Lymphocytes were pretreated with herbal extracts for 30 min at 37˚C, and then 30 mM phenanthrene was added for 5 min at 0˚C.

Measurement of Oxidative DNA Damage using Comet Assay

Comet assay was performed according to Singh *et al.* (1988) for defined DNA damage by phenanthrene. Isolated lymphocytes were mixed with 75 μL of 0.7% low melting agarose (LMA) and then the drop of suspension was put on slides glass which was precoated with 1% normal melting agarose (NMA). The agarose was solidified for 30 min at 4˚C and 100 μL of 0.7% low melting agarose (LMA) was added and covered with cover glass. After solidification for 30 min at 4˚C, cover glass was removed. The slides were immersed in alkali lysis buffer(2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO and 1% laurosylsarcosinate, pH 10) for 1 hr at 4˚C in a dark condition, to remove cell proteins. After lysis, the slides were placed in an electrophoresis tank and alkaline buffer (300 mM NaOH and 10 mM Na_2EDTA, pH $>$ 13) was added for 20 min to allow the DNA to unwind. Electrophoresis was performed at 25 V, 300 mA for 20 min. After electrophoresis, the slides were washed three times and neutralized with 0.4 M Tris buffer (pH 7.0) and fixed with ethanol for 5 min.

Image Analysis

The slides were stained with 50 μM ethidium bromide and measured using fluorescence microscope (Leica DM 2000, Germany). The slides were viewed with a CCD camera (Hitachi, Japan) and each of nuclear image was analyzed using Komet 5.5 comet image analyzing system (Kinetic Imaging, UK). To quantity DNA damage in the comet assay, the olive tail moment was calculated as (Tail.mean - Head.mean) × Tail % DNA/100. Total 150 cells (75 randomly selected cells each duplicate) of lymphocytes which were duplicated each of slide were measured to evaluate the DNA damage.

Statistical Analysis

All of comet assay data were processed by SPSS package for Windows version 13 (SPSS Inc., Chicago, IL). The mean values of DNA damage (olive tail moment) for each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. $P < 0.05$ was considered significant.

Acknowledgements

This subject is supported by Ministry of Environment (Republic of Korea) as "The Eco-techonopia 21 roject" to M.Y. Lee.

References

1. Cébron, A. *et al*. Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. *Appl. Environ. Microbiol.* **75**, 6322-6330 (2009).
2. Ma, Y. G., Ying, D. L., Hang, X., Wania, F. & Wang, W. H. Critical review and recommended values for the physical-chemical property data of 15 polycyclic aromatic hydrocarbons at 25˚C. *J. Chem. Eng. Data* **55**, 819-825 (2010).
3. Valentin, L. *et al*. Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot fungus *Bjerkandera* sp. BOS55. *Process Biochem.* **42**, 641-648 (2007).
4. Fernandez-Luqueno, F. *et al*. Flocculant in wastewater affects dynamics of inorganic N and accelerates removal of phenanthrene and anthracene in soil. *J. Environ. Manage.* **90**, 2813-2818 (2009).
5. Tsai, J. C., Kumar, M., Chang, S. M. & Lin, J. G. Determination of optimal phenanthrene, sulfate and biomass concentrations for anaerobic biodegradation of phenanthrene by sulfate-reducing bacteria and elucidation of metabolic pathway. *J. Hazard Mater.* **171**, 1112-1119 (2009).
6. Mallick, S. & Dutta, T. K. Kinetics of phenanthrene degradation by *Staphylococcus* sp. strain PN/Y involving 2-hydroxy-1-naphthoic acid in a novel metabolic pathway. *Process Biochem.* **43**, 1004-1008 (2008).
7. Hannam, M. L., Bamber, S. D., Galloway, T. S., John, M. A. & Jones, M. B. Effects of the model PAH phenanthrene on immune function and oxidative stress in the haemolymph of the temperate scallop *Pecten maximus*. *Chemosphere* **78**, 779-784 (2010).
8. Messina, M. & Messina, V. Nutritional implications of dietary phytochemicals. In: Dietary phytochemicals in cancer prevention and treatment. (Plenum Press, New York, 1996).
9. Manson, M. M., Foreman, B. E., Howells, L. M. &

Moiseeva, E. P. Determining the efficacy of dietary phytochemicals in cancer prevention. *Biochem. Soc. Trans.* **35**, 1358-1363 (2007).
10. Babazadeh, Z. *et al*. Sperm DNA damage and its relation with leukocyte DNA damage. *Reprod. Toxicol.* **29**, 120-124 (2010).
11. Park, E. J. & Kang, M. H. Application of the alkaline comet assay for detecting oxidative DNA damage in human biomonitoring. *Korean J. Nutr.* **35**, 213-222 (2002).
12. Woo, S., Kim, S., Yum, S., Yim, U. H. & Lee, T. K. Comet assay for the detection of genotoxicity in blood cells of flounder (*Paralichthys olivaceus*) exposed to sediments and polycyclic aromatic hydrocarbons. *Mar. Pollut. Bull.* **52**, 1768-1775 (2006).
13. De Assis, K. R. *et al*. Genotoxicity of cigarette smoking in maternal and newborn lymphocytes. *Mutat. Re.* **679**, 72-78 (2009).
14. Park, Y. K., Jeon, E. J. & Kang, M. H. Protect effect of flavonoids on lymphocyte DNA damage using comet assay. *Korean J. Nutr.* **36**, 125-132 (2003).
15. Korchowiec, B. *et al*. Interfacial approach to polyaromatic hydrocarbon toxicity: phosphoglyceride and cholesterol monolayer response to phenantrene, anthracene, pyrene, chrysene, and benzo[a]pyrene. *J. Phys. Chem. B* **112**, 13518-13531 (2008).
16. Yoo, A. R. & Lee, M. Y. Phenanthrene-induced oxidative DNA damage of lymphocytes and the suppression by ginseng extract. *J. Ginseng Res.* **33**, 355-360 (2009).
17. Park, S. K., Kim, J. J., Jeon Y. M. & Lee., M. Y. Suppressive effects of Korean indigenous *Acanthopanax divaritacus* on the allergic inflammation. *J. Appl. Biol. Chem.* **50**, 155-159 (2007).
18. Yi, J. M. *et al*. *Acanthopanax senticosus* root inhibits mast cell dependent anaphylaxis. *Clin. Chim. Acta* **312**, 163-168 (2001).
19. Bae, E. A. *et al*. Metabolism of chiisanoside from *Acanthopanax divaricatus* var. *albeofructus* by human intestinal bacteria and its relation to some biological activities. *Biol. Pharm. Bull.* **24**, 582-585 (2001).
20. Jung, H. J., Nam, J. H., Choi, J., Lee, K. T. & Park, H. J. Antiinflammatory effects of chiisanoside and chiisanogenin obtained from the leaves of *Acanthopanax chiisanensis* in the carrageenan and Freund's complete adjuvant-induced rats. *J. Ethnopharmacol.* **97**, 359-367 (2005).
21. Shan, S. E., Yoshita, Y., Sugiura, T. & Yamashita, U. Suppressive effects of Chinese medicinal herb, *Acanthopanax gracilistylus*, extract on human lymphocytes *in vitro*. *Clin. Exp. Immunol.* **118**, 41-48 (1999).
22. Yoon, T. J. *et al*. Effect of hot water extract from *Acanthopanax senticosus* on systemic anaphylaxis. *Food Sci. Biotechnol.* **34**, 518-523 (2002).
23. Huang, H. Y., Huang, J. J., Tso, T. K., Tsai, Y. C. & Chang, K. K. Antioxidant and angiotension-converting enzyme inhibition capacities of various parts of *Benincase hispida* (wax ground). *Nahrung* **48**, 230-233 (2004).
24. Lee, K. H., Choi, H. R. & Kim, C. H. Anti-angiogenic effect of the seed extract of *Benincasa hispida* Cogniaux. *J. Ethnopharmacol.* **97**, 509-513 (2005).
25. Manish, A. R. & Sunita, M. J. Gastroprotective effect of *Benincasa hispida* fruit extract. *Indian J. Pharmacol.* **40**, 271-275 (2008).
26. Grover, J. K., Adiga, G., Vats, V. & Rathi, S. S. Extracts of *Benincasa hispida* prevent development of experimental ulcers. *J. Ethnopharmacol.* **78**, 159-164 (2001).
27. Moon, M. K., Kang, D. G., Lee, Y. J., Kim, J. S. & Lee, H. S. Effect of *Benincasa hispida* cogniaux on high glucose-induced vascular inflammation of human umbilical vein endothelial cells. *Vascul. Pharmacol.* **50**, 116-122 (2009).
28. Park, S. K., Kim, J. J., Sung, S. M. & Lee, M. Y. Suppressive effects of *Benincasae hispida* on allergic inflammation. *Mol. Cell. Toxicol.* **5**, 304-309 (2009).
29. Chen, D. & Dou, Q. P. Tea polyphenols and their roles in cancer prevention and chemotherapy. *Int. J. Mol. Sci.* **9**, 1196-1206 (2008).
30. Stoner, G. D. & Mukhtar, H. J. Polyphenols as cancer chemopreventive agents. *Cell Biochem.* Suppl. **22**, 169-180 (1995).
31. Sinha, D., Roy, S. & Roy, M. Antioxidant potential of tea reduces arsenite induced oxidative stress in Swiss albino mice. *Food Chem. Toxicol.* **48**, 1032-1039 (2010).
32. Shen, C. L. *et al*. Green tea polyphenols and Tai Chi for bone health: designing a placebo-controlled randomized trial. *BMC Musculoskelet. Disord.* **10**, 1-10 (2009).
33. Santino, O. Assessment of polycyclic aromatic hydrocarbons (PAHs) in soil of a Natural Reserve (Isola delle Femmine) (Italy) located in front of a plant for the production of cement. *J. Hazard. Mater.* **173**, 358-368 (2010).