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Antigenotoxic Effects of *Synurus deltoides* Extract on Benzo[*a*]pyrene Induced Mutagenesis

Seung-Shi Ham, Hong-Sik Han, Kun-Pyo Choi and Deog-Hwan Oh†

Division of Food Science and Biotechnology, Kangwon National University, Chuncheon 200-701, Korea

Abstract

This study investigated the antigenotoxic effects of *Synurus deltoides* extract on the mutagenesis induced by benzo[*a*]pyrene(B[*a*]P). About 80% and 90% antimutagenic effects were observed in the presence of over 200μg/plate of methanol extract of *Synurus deltoides* against *Salmonella typhimurium* TA98 and TA100 induced by B[*a*]P, respectively. The methanol extract itself did not induce an increased frequency of micronucleated polychromatic erythrocytes(MNPCE) irrespective of the sampling time(up to 72h), while the treatment with benzo[*a*]pyrene(B[*a*]P) at 150mg/kg significantly increased($p<0.05$) the incidence of MNPCE. The strongest relative frequency of MNPCE was observed at 36h after injection of B[*a*]P and the most significant reduction ($p<0.05$) in the frequencies of MNPCE was occurred at the feeding of the methanol extract to mice 12h before injection of B[*a*]P. The most significant reductions($p<0.05$) with 48% was observed in the frequencies of MNPCE when 50mg/kg of the methanol extract was given to the mice 12h before injection of B[*a*]P, while the strongest relative frequency inhibition was 54% at the multiple feeding of 5mg/kg of the methanol extract one time every day for 5 days on the frequencies of MNPCE induced by 150mg/kg of B[*a*]P. These results indicate that the methanol extract of *Synurus deltoides* have a strong modulatory effect on benzo[*a*]pyrene induced MNPCE.

Key words: antimutagenicity, antigenotoxicity, bone marrow micronucleus test, *Synurus deltoides*

INTRODUCTION

Recently, there has been increased interest in the safety of natural and processed foods as well as carcinogenic contamination of the environment, especially related to the occurrence and development of cancer(1, 2). Increasing reports on dietary carcinogens and anticarcinogens have given rise to the hope that antimutagens/anticarcinogens contained naturally in edible wild herbs might be of potential value in preventing cancer and other diseases linked with mutational occurrence. Many workers have reported the antimutagenic effect of natural food components from plants, fruits, and vegetables(3-5).

Synurus deltoides(Surichui) is wildly distributed in mountain area and is one of the edible green color plants used as natural coloring agent. Use of chemical preservatives for coloring fixation in food products has been debated due to public concerns about food safety because of toxic and carcinogenic potential(6). Thus, use of non-toxic substance as components of food has become essential. It could be desirable to use *Synurus deltoides* as natural non-toxic coloring agent for bakery or confectionary. Also, *Synurus deltoides* has been used in the folk of remedy for the cure of edema, stanching, vomiting of blood, urination, and cystitis et al.(7).

We reported the inhibitory effect on mutagenicity of 20 kinds of wild edible herbs used in traditional Korean foods, and found that most of the juices extracted from 20 kinds of edible herbs strongly inhibited the mutagenesis induced by several mutagens on *Salmonella typhimurium* TA98 or TA100(8), but no information was reported on the antigenotoxic effect of *Synurus deltoides* extract. Therefore, this study was done to investigate the antimutagenicity or antigenotoxicity of *Synurus deltoides* extract on *Salmonella typhimurium in vitro* or mammalian cell system induced by benzo[*a*]pyrene.

MATERIALS AND METHODS

Materials and chemicals

The *Synurus deltoides* obtained from Hongcheon, Kangwon province in Korea. B[*a*]P(Sigma), fetal calf

†Corresponding author

serum(GIBCO), and Giemsa staining solution(Gurr^{G} 66) were obtained from Wako Pure Chemical Industries (Japan).

Preparation of *Synurus deltoides* extract

Synurus deltoides was washed with tap water and air-dried, ground with mortar and pestle, and extracted with methanol(3x), then filtered through filter paper (Toyo No.2). The filtrate was concentrated by a rotatory evaporator and freeze-drized, and stored at 4°C prior to use.

Antimutagenic effect of methanol extract of *Synurus deltoides*

The antimutagenic effect of methanol extract of *Synurus deltoides* on benzo[*a*]pyrene induced mutagenesis was investigated with *Salmonella typhimurium* TA 100 or TA98 as described previously(9).

Animals and diets

ICR male adult mice(weighing 30 to 35g, 7 week-old) were obtained from Korea Experiment Animal Development Company(Seoul, Korea). They were maintained in the animal house at 25°C on mixed mouse diet(Samyang Oil Feed Limited) and water ad libitum.

Conduct of bone marrow micronucleus test

The animals were treated with B[*a*]P(150mg/kg per body weight) dissolved in saline. Control animals were given saline only. In this study a single or multiple feeding with designated concentrations of methanol extract of *Synurus deltoides* was given to the animals before being exposed to B[*a*]P. The genotoxin B[*a*]P was injected intraperitoneally in saline(10ml per kg body weight) a designated time after the administration of methanol extract of *Synurus deltoides*. All experiments were conducted using five mice per group.

At the 36h after treatment with a B[*a*]P, mice were killed by cervical dislocation. Both femurs were dissected, and bone marrow was flushed from the femoral cavity with fetal calf serum as described by Schmid (2). The cell suspension containing the collected marrow cells in the serum was centrifuged at ca. 1000×g for 5min. The cell pellets were resuspended and smears were air-dried, fixed in methanol, and stained with May-Grunwald Giemsa according to Schmid(2). All slides were coded and a total of 1,000 polychromatic erythrocytes(PCE) were scored for determining the frequency ratios of micronucleated polychromatic erythrocytes (MNPCE) in the bone marrow.

Statistical analysis

Analysis of variance(ANOVA) procedures using the SAS(Statistical Analyses Systems) software(10) were used to determine significant differences in the antimutagenic or antigenotoxic effects of methanol extract of *Synurus deltoides* on the frequencies of MNPCE induced by B[*a*]P and Fisher's Protected Least Significant Difference Test was used to determine significant differences($p<0.05$) between treatment means.

RESULTS AND DISCUSSION

Before testing the antimutagenicity of *Synurus deltoides* extract, the mutagenicity of the extract was tested; it was found that the extract itself at the doses added to the *Salmonella typhimurium* did not influence their spontaneous mutation frequencies(data not shown). The inhibitory effects of methanol extract of *Synurus deltoides* on benzo[*a*]pyrene-induced mutagenesis are shown in Fig. 1. It was found that each 60% inhibition was shown in the presence of 250μg/plate of methanol extract of *Synurus deltoides* against TA98 and TA100

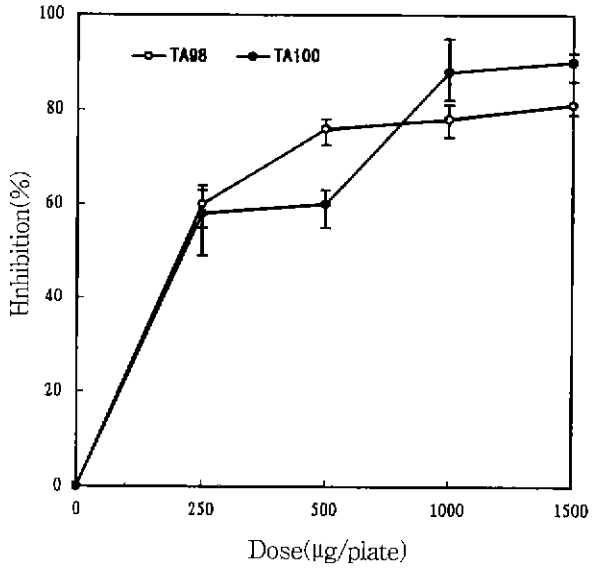

Fig. 1. Antimutagenic effects of methanol extract of *Synurus deltoides* on mutagenesis induced by benzo[*a*]pyrene in *Salmonella typhimurium* strain TA98 and TA100.

induced by B[*a*]P. However, 80% inhibition was observed at the concentration over 1000μg/plate against TA 98, whereas 90% inhibition was approximately observed against TA100.

An established antimutagenic response *in vitro* test systems should be verified in *in vivo* systems, and if not, then classification as an antigenotoxic substance may not be appropriate. Thus, we investigated the antigenotoxicity of *Synurus deltoides* extract in mammalian cell system. The effects of methanol extract of *Synurus deltoides* itself and B[*a*]P on the frequency of MNPCE based over time are shown in Fig. 2. The methanol extract itself did not induce MNPCE irrespective of the sampling time when 50mg/kg of the methanol extracts were given, while the treatment with 150mg/kg of B[*a*]P alone significantly increased($p<0.05$) the incidence of MNPCE. The result shows that the most high frequency of MNPCE was observed at 36h after injection of benzo[*a*]pyrene. Similar result was reported that the most high frequency of MNPCE was occurred at 30h and 36h after injection of benzo[*a*]pyrene in the micronucleus test(9).

The feeding time of the methanol extract before injecting B[*a*]P to determine the optimal antigenotoxicity of the methanol extract on the frequencies of MNPCE induced by B[*a*]P was investigated(Fig. 3). When the methanol extract was fed to mice 12h before injecting 150mg/kg of B[*a*]P, the most significant decrease($p<0.05$) in the frequencies of MNPCE induced by B[*a*]P was

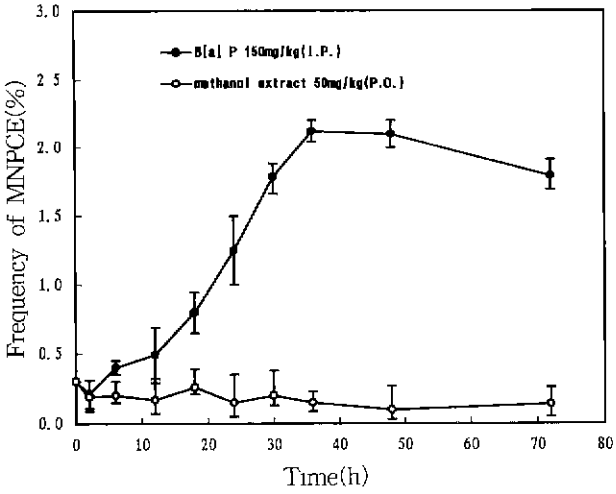

Fig. 2. The frequency of micronucleated polychromatic erythrocytes after treatment with 50mg/kg of methanol extract of *Synurus deltoides* and with 150mg/kg of benzo[*a*]pyrene.

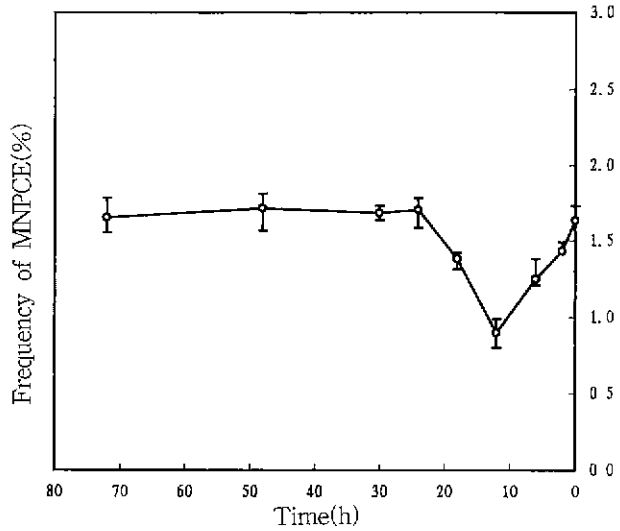

Fig. 3. The antigenotoxic effect of methanol extract of *Synurus deltoides* on the frequencies of micronucleated polychromatic erythrocytes with variation in the time interval between the metanol extract feeding and injection of benzo[*a*]pyrene.

observed, but no antigenotoxicity was observed at the feeding of 72 to 24 hour before injection of B[*a*]P. Fig. 4 shows the inhibitory effect of the methanol extract on the frequencies of MNPCE induced by B[*a*]P in the bone marrow. The appropriate amounts of the methanol extract were adminstrated to animals 12h before injecting B[*a*]P, and the exposure time was 36h. The most significant reductions($p<0.05$) were observed in the frequencies of MNPCE when 50mg/kg of the methanol

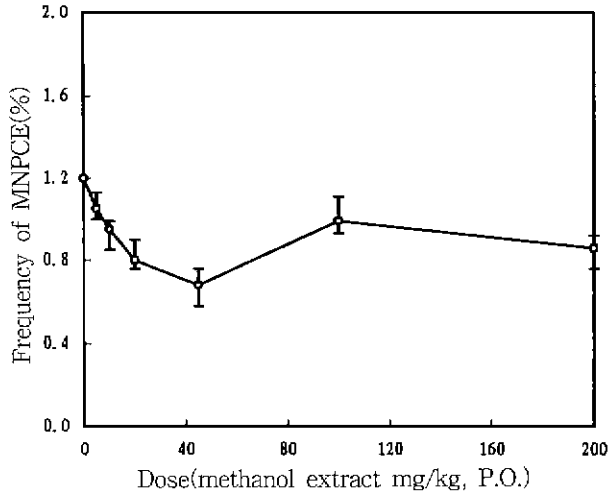

Fig. 4. The antigenotoxic effect of varying doses of methanol extract of *Synurus deltoides* on the frequencies of micronucleated polychromatic erythrocytes induced by benzo[*a*]pyrene with the methanol extract feeding 12 hour before injection of benzo[*a*]pyrene.

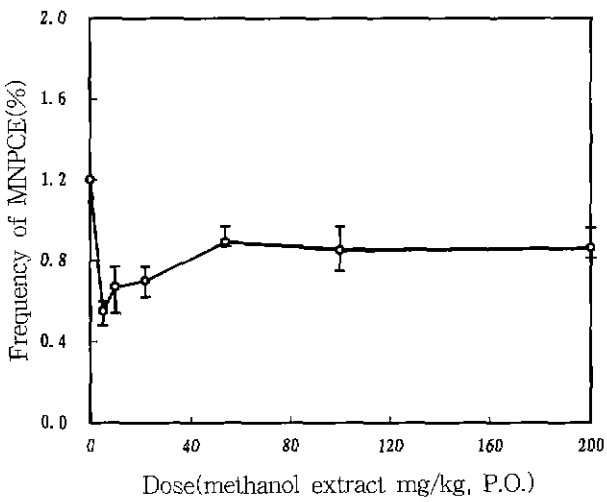

Fig. 5. The antigenotoxic effect of varing doses of methanol extract of *Synurus deltoides* on the frequencies of micronucleated polychromatic erythrocytes induced by benzo[*a*]pyrene with the methanol extract feeding once a day for 5 days before injection of benzo[*a*]pyrene.

extract was given to the mice 12h before they were exposed to 150mg/kg of B[*a*]P, and inhibitory effect was approximately 48%. Fig. 5 illustrates the effect of multiple feedings of different concentrations of the methanol extract for 5 days on the frequencies of MNPCE induced by 150mg/kg of B[*a*]P. The administration of the methanol extract once a day for 5 days was significantly effective in reducing the frequencies of MNPCE induced by B[*a*]P. The strongest relative frequency inhibition was approximately 54% when 5mg/kg of the methanol extract was given to the mice induced by B[*a*]P, and 49% and 46% inhibitions were observed at the concentration of 10 and 20mg/kg, respectively, but a limited inhibition was observed at concentration over 50mg/kg.

The fact that *Synurus deltoides* extract had strong antimutagenic effect on B[*a*]P-induced mutagenesis *in vitro* system was verified from the results of *in vivo* test. Similar results have been reported that microsome of S9 prepared from rats that received eugenol significantly decrease the mutagenic activity of B[*a*]P in the Ames test in comparison with microsomes or S9 from untreated rats(11-13).

We reported that many wild edible herbs cultivated in Korea, such as *sedum, aralia bud, mugwort, viscum coloratum(mistletoe)*, and *symphytum officinale* et al., significantly inhibited the mutagenicity induced by several mutagens such as MNNG, Trp-1, HHQ, or B[*a*]P in Ames test or spore-*rec* assay(14,15). Also, many active substances in wild edible herbs, such as β-carotene, ascorbic acid, fatty acids, edible fiber and mineral, have been identified(16). These components have been used as a food additive or medicinal purpose in preventing carcinogenesis related to active oxygen radicals (17).

The present study showed that *Synurus deltoides* extract can inhibit the mutagenesis or genotoxicity induced by B[*a*]P, but main active components have not been known. Further study should be done to elucidate mechanism of how the extract exerts strong antimutagenic or antigenotoxic effects.

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