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Desmutagenic Effect of Legumes and Plants Crude Saponins in *Salmonella typhimurium* TA 98

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Abstract

Crude saponins of soybean and Azuki bean (2.0 *mg*/plate) were most effective against Trp-p-2, and also all of legume saponins (2.0 *mg*/plate) were excellent effective against aflatoxin B_1. Crude saponins of taro, burdock and ginseng were remarkably effective at ranging from 1.0 *mg* to 2.0 *mg* per plate against MeIQ. Especially ginseng saponin was excellent effective and arrow root saponins was remarkably effective against MeIQ, respectively. Plant saponins of taro, lotus burdock, arrow root and ginseng except for dodok were most effective activities against aflatoxin B_1.

Introduction

Several epidemiology studies indicated that dietary factos are important in the incidence of cancer of the alimentary tract.[1-3]

Concern has recently focused that cooked foods could produce mutagenic or carcinogenic substances that increase the incidence of cancer in human.

Since Lijinski and Shubik[4] were first reported carcinogenic aromatic hydrocarbon on the surface of charcoal broiled beef steak, benzopyrene and other carcinogenic hydrocarbons were also produced during cooking of foods. Sugimura and Nagao[5] investigated the formation of mutagens on the surface of meat and fish subjected to naked flame broiling using a gas broiler, and found mutagenic activity which could be accounted by benzo(a) pyrene content. This led to the discovery of serveral mutagens produced during pyrolysis of amino acids and proteins. A new series of heterocylic amines as potent mutagens were isolated from pyrolysates of various amino acids.

Since 3-amino-1,4-dimethyl 5H-pyrido (4,3-b) indole (Trp-p-1), and 3-amino-1-methyl-5H-pyrido (4,3-b) indole (Trp-p-2) were first isolated from pyrolysates of tryptophane,[6,7] Yosida et al.[8] have been identified 2-amino-6-methyl-dipyrido (1,2-a; 3',2'-d) imidazole (Glu-p-1), (Glu-p-2) from pyrolysate of L-glutamic acid.[9] More recently, Kasai, *et al.*[10] were isolated from the charred parts of broiled sun-dried sardines and identified 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) and 2-amino-3,4-dimethylimidazo (4,5-f) quinoline (MeIQ). Furthermore, 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline (MeIQ) was isolated from fried beef.[11] A new series of these heterocylic amines has higher specific mutagenic activity to *Salmonella typhimurium* TA 98 in the present of S-9 mix. Morita et al.[12] and Inoue et al.[13] demonstrated that vegatables and plants has the ability to inactivate or neutralized the mutagenicity of foods.

Crude saponins were extracted from five legumes and seven plants, and examined in order to determined whether they had the capacity of elcuidate desmutagenic effect of crude saponin on Trp-p-2. MeIQ and aflatoxin B_1 using *Salmonella typhimurium* TA 98.

Materials and Methods

Materials

Samples of soybean (Glycine soja), black been (Glycine max), peanut (Arachis hypogaea), and Azuki bean (Phaseolus radiatus L.) and also plants samples of taro (Colocasia antiquorum), dodok (Codonopsis lanceolate), lotus (Nelum bonucifera), burdock (Arctium lappa), arrow root (Pueraria thumbergiana) and ginseng (Panax ginseng) used were obtained in the market of Pusan area.

Trp-p-2, and MeIQ were kindly gift from T. Sugimure, Japan National Cancer Center Research Institute,

Aflatoxin B_1, NADP and glucose 6-phosphate were purchased from Sigma.

Preparation of desmutagenic saponins from legumes and plants

Methanol extracts was prepared from legumes and plants, and dissolved in water, followed by defatting with ethyl ether. The aqueous solution were extracted with n-butanol saturated with water. The resulting butanol layer was repeatly washed with water and evaporated to dryness, which was used as a total saponin. The procedure was shown in Fig. 1.

Bacterial strains

Salmonella typhimurium TA 98 was kindly supplied by Prof. B.N. Ames, University of California.

Induction of rat liver microsomal enzymes

The rat liver enzymes were induced with a polychlorinated biphenyl (PCB) mixture (Aroclor 1254). the induction procedure was similar to the method of Czygan *et al.*[14] A single i.p. injection of Aroclor 1254 (diluted in corn oil to a concentration of 200*mg*/*ml*) at a dosage of 500*mg*/*kg* was given to rat five days before sacrifice.

Preparation of liver homogenate (S-9) and S-9 Mix

The microsomal preparation was made according to Garner et al.[15] The rat liver was washed in an equal volume of 0.15*M* KCl, minced with sterile scissors in three volumes of 0.15*M* KCl and homogenized with a potter Elvehjem apparatus. The homogenate was centrifuged at 4°C for 10 min at 8000 × *g*. The supernatant was collected and stored at -80°C. As required, sufficient S-9 fraction was thawed at room temperature and kept in ice. The S-9 mixture contained the following components per *ml*; 0.3*ml* of S-9 fraction, 8*mM* $MgCl_2$, 33*mM* KCl, 5*mM* glucose-6-phophate, 4*mM* NADP and 100*mM* sodium phophate, pH 7.4.

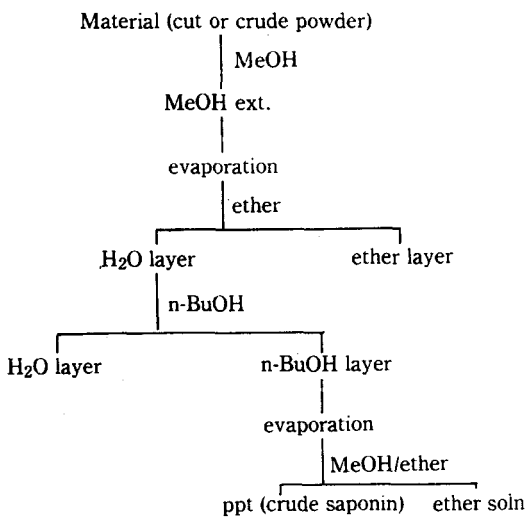

Fig. 1. Extraction procedure for crude saponin

Assay of desmutagenic test

Desmutagen assays were carried out with a Ame's strain of *Salmonella typhimurium* TA 98, and histidine reversion was measured by the standard method[16] with some modifications. The standard method of assaying the desmutagenic factors in seaweeds on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 is as follow. Mutagens were 0.005 ppm of trp-p-2, 0.00125 ppm of MeIQ, and 0.00125 ppm of aflatoxin B_1 in dimethylsulfoxide (DMSO), and were conbined with 0.5*mg*, 1.0*mg* or 2.0*mg* of each extracts of seaweeds, and then kept at 37°C for 30min. The incubated mixture was treated at 100°C for 10 min in order to stop the desmutagenic activity in seaweed extracts. Next 0.1*ml* of bacterial culture of TA 98 and 0.5*ml* of S-9 mixture were added. The reaction mixture was incubated at 37°C for 20 min, and added 2*ml* of 0.6% molten top agar containing 0.5% sodium chloride, 12.4μg of histidine and 9.6μg of biotin. The mixture was vortexed for 30 sec at low speed and poured on selective minimal glucose agar plate. All the plate was incubated at 37°C for 48 hr and then the number of His^+ revertants was counted. For each reaction mixture triplicated plate were used, and the mean value of His^+ revertants was calculated.

Results and Discussion

The desmutagenic effects of saponins extracted from legumes

Saponins extracted from legumes such as soybean, black bean, peanut, and Azuki bean were investigated for desmutagenic factor against strong mutagenic Trp-p-2, MeIQ and aflatoxin B_1 as positive controls. The induced mutation frequency on plates was compared with 0.005ppm of Trp-p-2, 0.005ppm of MeIQ and 0.0125ppm of aflatoxin B_1 per plate.

Results are presented in Figure 1-3. Crude saponins

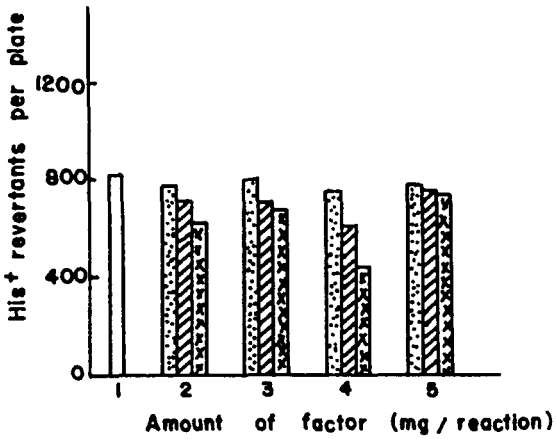

Fig. 2. Desmutagenic effects of saponins extracted from each legume on the mutagenicity of Trp-p-2 in *Salmonella typhimurium* TA 98

1. Trp-p-2 as indicator, 2. soybean,
3. black bean, 4. peanut, 5. Azuki bean
■; 0.005ppm per plate □ ; 0.5mg per plate
▨; 1.0mg per plate ▩ ; 2.0mg per plate

of legumes as these doses did not have any toxic effect on the viability of mutagenized cells. A decreases in mutation frequency were different from crude saponin concentrations of each legume. Desmutagenic saponins concentration of each sample were contained between 0.5*mg*, 1.0*mg* and 2.0*mg* per plate. No significance were desmutagenic effect of legume saponins against the mutagenesis of Trp-p-2. (Fig. 2). Peanut crude saponin were reduced about 40% in number of revertants induced against Trp-p-2 at 2.0*mg* per plate.

Legume saponins such as black soybean, peanut and Azuki bean were not effective against the mutagenesis induced by MeIQ except soybean (Fig. 3.). Saponins of soybean and Azuki bean (2.0*mg*/plate) were most effective against Trp-p-2. Aflatoxin B_1 was greatly supressed approximately 60-70% as those saponins at 2.0*mg* per plate. All samples of crude saponin containing 0.5*mg*-1.0*mg* per plate were reduced about range from 18% to 40% in number of revertants against aflatoxin B_1. (Fig. 4.)

It is reported that soja products such as Tofu (Soybean curd), soja milk, miso (Soybean paste) and Bonlact (powdered soja milk with additives) inhibit the formation of mutagenic N-nitrosodimethylamine and N-nitrosodiethylamine,[17] Kurechi and Kikigawa[18] reported that unsaturated fatty acid, linoleic acid, oleic acid and, fat emulsion with soyabean oil and yolk lecithin effectively reduced nitrite and subsequently inhibited nitrosamine formation.[19-21]

Soyabean contains many phenolic compounds, and it has been demonstrated that these effectively inhibited N-nitrosamine formation.

These were of interest to know whether aflatoxin B_1 could have effective action with desmutagenic crude saponins sample of legume than other crude saponins against Trp-p-2 and MeIQ. At present, it is not clear

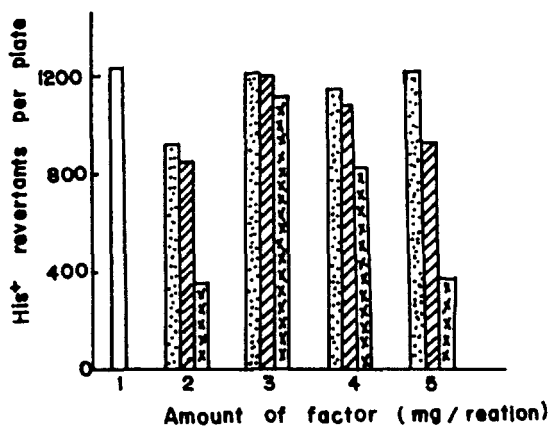

Fig. 3. Desmutagenic effect of saponins extracted from each legume on the mutagenicity of MeIQ in *Salmonella typhimurium* TA 98

1. MeIQ as indicator, 2. soybean,
3. black bean, 4. peanut, 5. Azuki bean
■ ; 0.00125ppm per plate □; 0.5mg per plate
▨ ; 1.0mg per plate ▩; 2.0mg per plate

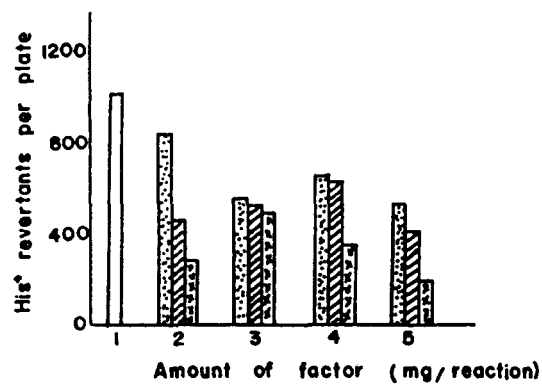

Fig. 4. Desmutagenic effects by saponins extracted from each legume on the mutagenicity of aflatoxin B_1 in *Salmonella typhimurium* TA 98

1. Aflatoxin B_1 as indicator, 2. soybean, 3. black bean, 4. peanut. 5. Azuki bean
■; 0.0125ppm per plate □; 0.5mg per plate
▨; 1.0mg per plate ▩; 2.0mg per plate

which type of mechanisms were involved.

Desmutagenic effect of crude saponin extracted from plants

In order to find systematically desmutagenic factor of plant saponins such as taro dodok, lotus, burdock, arrow root and ginseng, extracted crude saponins were collected.

Screening were made as to their mutagenic capacities against potent mutagens such as Trp-p-2, MeIQ and aflatoxin B_1. Mutation frequency induced by each mutagen on the plates were compared with that on plates containing plant saponins at ranging from 0.5*mg* to 2.0*mg* per plate. Assay was carried out at several doses giving His+ revertants per plate of each mutagens using *Salmonella typhimurium* TA 98. Plant saponins at these dose did not have any toxic effect on the viability of mutagened cells. In Fig. 5 showed the effects of treatment with crude saponin samples on the mutagenicity of Trp-p-2. Crude saponin containing 0.5*mg* per plate had no effect or moderately activities in reducing of His+ revertant against aflatoxin B_1, but taro, burdock and ginseng were at ranging from 1.0*mg* to 2.0*mg* of crude saponin per plate. In addition, lotus was moderately effective.

Fig. 6 showed the desmutagenic effects of crude saponins on the MeIQ. Taro, dodok had almost no activities in reducing the number of His+ revertants about 0.5*mg* of saponin per plate. We found that ginseng the number of His+ revertants about 0.5*mg*, 1.0*mg*, 2.0*mg* of saponin per plate saponin was most effective approximately 85% in reducing the number of His+ revertants. Burdock, and arrow root were remarkably effective approximately 60% in reducing the number of His+ revertants about crude saponin containing 1.0*mg* and 2.0*mg* per plate. Fig. 7 showed the desmutagenic effect against aflatoxin B_1. Most samples have effective activities in the reducing the number of His+ revertants, But dodok was no almost desmutagenic effect against aflatoxin B_1. The desmutagenic effect of crude saponin were ginseng, burdock, arrowroot, lotus taro and dodok in order.

Kada *et al.*[22] reported that some vegetables such as

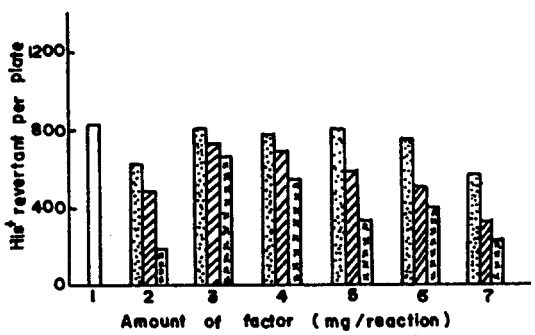

Fig. 5. Desmutagenic effects of saponins extracted from each plant on the mutagenicity of Trp-p-2 in *Salmonella typhimurium* TA 98

1. Trp-p-2 as indicator 2. Taro 3. dodok 4. lotus 5. burdock 6. arrow root 7. ginseng
■ ; 0.005ppm per plate □ ; 0.5mg per plate
▨ ; 1.0mg per plate ▦ ; 2.0mg per plate

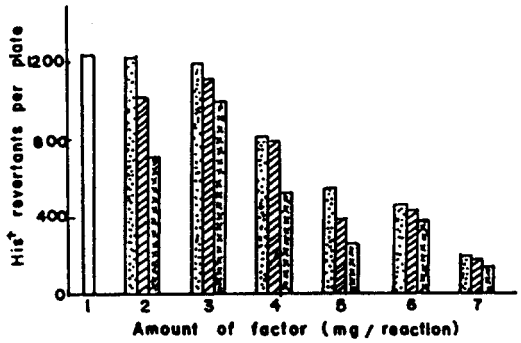

Fig. 6. Desmutagenic effects of saponins extracted from different plant on the mutagenicity of MeIQ in *Salmonella typhimurium* TA 98

1. MeIQ as indicator 2. Taro 3. dodok 4. lotus 5. burdock 6. arrow root 7. ginseng
■ ; 0.00125ppm per plate □ ; 0.5mg per plate
▨ ; 1.0mg per plate ▦ ; 2.0mg per plate

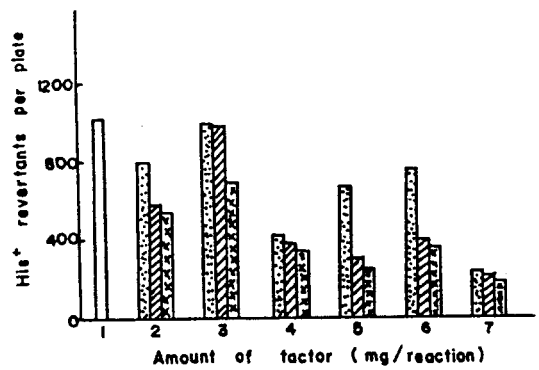

Fig. 7. Desmutagenic effects of saponins extracted from each plant on the mutagenicity of Aflatoxin B_1 in *Salmonella typhimurium* TA 98

1. aflatoxin B_1 as indicator 2. Taro 3. dodok 4. lotus 5. burdock 6. arrow root 7. ginseng
■ ; 0.0125ppm per plate □ ; 0.5mg per plate
▨ ; 1.0mg per plate ▦ ; 2.0mg per plate

cabbage, ginger and radish have desmutagenic activities on the tryptophan pyrolysate. Juice prepared from cabbage, brocoli, green paper, egg plant, apple, burdock, shallot, ginger, pineapple and mint leaf were found to posses strong capacities of inactivating the mutagenicity of tryptophan pyrolysate products. It was not clear yet the mechanism involved in the inactivation of mutagenicity principles. Inoue *et al.*[13] were checked desmutagenic factor purified from cabbage leaves having a molecular weight of 43000 and contained 45.2*mg* of sugar per *mg* of protein.

This interesting observation of the desmutagenic factors against amino acid pyrolysates products, prompted us to examine whether the factors could have a similar reducing effect against other mutagens. Further studies may figure out the reason why extracts from legumes and plants posses desmutagenic activities. These studies may be also useful to show whether taking legumes and plants in combination with cooked meat and fish might be helful to neutralized the mutagenicity of pyrolysis products amino acids, proteins and other nutrients.

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콩과 식물에서 추출한 사포닌의 돌연변이원성 억제효과

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4종과 콩과 6종의 식물에서 추출한 사포닌을 methyl-5H-pyrido[4, 3-b] indole(T-p-2), 3-amino-1-methylimidazo[4, 5-f] guinoline(MeIQ) 및 Aflatoxin B_1을 이용하여 *Salmonella typhimurium* TA 98로서 돌연변이원성 억제효과를 검토하였다. 콩과 팥의 사포닌(2.0 *mg*/plate)은 aflatoxin B_1에 좋은 효과를 나타내었고 6종의 식물의 사포닌은 대부분 돌연변이원성 억제효과가 우수하였다. 식물에서 추출한 사포닌 중 토란, 우엉 그리고 인삼사포닌은(1.0~2.0 *mg*/plate)은 MeIQ에 현저한 효과를 나타내었고, 특히 인삼과 칡의 사포닌은 우수한 돌연변이원성 억제효과를 나타내었다. Aflatoxin B_1에 대한 돌연변이원성 억제효과는 더덕을 제외한 식물성 사포닌은 좋은 효과를 나타내었다.