Antimutagenic Effect of Methanolic Extracts from Peanut Hulls

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The antimutagenic effects of methanolic extracts of peanut hulls (MEPH) were evaluated by the Ames test. MEPH inhibited the mutagenicity of 4-nitroquinoline-N-oxide (NQNO), a direct-acting mutagen. MEPH also inhibited the mutagenicity of some indirect-acting mutagens and decreased in the order of 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) > aflatoxin B1 (AFB1) > 2-amino-6-methylpyrididol(1,2-a:3', 2'd)imidazole (Glu-P-1) > 3-amino-1,4-dimethyl-5H-pyridol(4,3-b)indole (Trp-P-1) > benzo(a)pyrene (B(a)P) for S. typhimurium TA98, and IQ > Trp-P-1 > Glu-P-1 > AFB1 > B(a)P for S. typhimurium TA100.

Key words: antimutagenicity; mutagen; peanut hull

Mutagens and carcinogens of various types exist in foods, as do components acting as antimutagens or anticarcinogens such as carotenoids, fiber, flavonoids, and polyphenolic acid in some foodstuffs. Hence, the investigation of interactions between these compounds becomes increasingly important. Food components of these types are classified as chemopreventers. These are found in foods of all categories; fruits and vegetables comprise the main source. Some researchers reported that diverse compounds naturally occurring in food can exert an antimutagenic effect due to their antioxidant capacity. According to our previous works, methanolic extracts of peanut hulls (MEPH) have both strong antioxidant activity as a result of large amounts of total phenolic compounds, and properties of scavenging free-radical and active-oxygen species, but the antimutagenic activity of MEPH has not been examined. Therefore, the objective of this work was to discover the antimutagenic effects of MEPH.

Peanuts of Tainan select no. 11, Spanish type, were obtained from the Tainan District Agriculture Improvement Station, Taiwan, Republic of China. After harvest, the peanuts were hand-shelled. Peanut hulls were ground into a fine powder in a mill (Tecator Cemotec 1090 sample mill, Hoganas, Sweden). Peanut hull powder (5.0 g) was extracted with methanol (50 ml) overnight in a shaking incubator about 25°C. The extracts were filtered and the residue was reextracted under the same conditions. The combined filtrates were evaporated to dryness in vacuo. The MEPH were dissolved in sterile distilled water and used to test mutagenicity, toxicity, and antimutagenicity.

The mutagenicity of MEPH was tested according to the Ames test with a 20 min first incubation at 37°C. The histidine-requiring strains of Salmonella typhimurium TA98 and TA100 were kindly supplied by Dr. B. N. Ames (UC, Berkeley). The S9 mix (Organ Teknika Co., Switzerland) was prepared from Sprague-Dawley male rats treated with Aroclor 1254. Diluted MEPH (0.1 ml) was added to the overnight cultured S. typhimurium TA98 or TA100 (0.1 ml) and S9 mix (0.5 ml) or phosphate buffer (0.1 ml) in place of S9 mix. The entire mixture was incubated at 37°C for 20 min before molten top agar (2 ml) was added; the mixture was poured on a minimum agar plate. The his− revertant colonies were counted after incubating at 37°C for 48 h. Each sample was assayed in triplicate plates per run and data presented are means ± SD of at least two experiments. To examine the toxic effect of MEPH on S. typhimurium TA98 and TA100, the mixtures after incubation were diluted with phosphate buffer, and the diluted mixtures were poured into a nutrient agar plates. The plates were incubated at 37°C for two days and the number of colonies was counted.

The antimutagenic effect of MEPH was assayed according to the Ames method except for the addition of mutagen before incubation. The mutagens used were NQNO (1.0 μg/plate for TA98 and 0.1 μg/plate for TA100), direct-acting mutagen, IQ (0.1 μg/plate for TA98 and TA100), Glu-P-1 (5 μg/plate for TA98 and TA100), Trp-P-1 (0.5 μg/plate for TA98 and TA100), B(a)P (5 μg/plate for TA98 and TA100), and AFB1 (5 μg/plate for TA98 and TA100) which required S9 mix for metabolic activation. Mutagen (0.1 ml) was added to the mixture of a strain (TA98 or TA100) and MEPH with S9 mix for IQ, Glu-P-1, B(a)P, and AFB1 or with phosphate buffer (0.1 ml, pH 7.4) for NQNO. The mutagenicity of each mutagen in the absence of MEPH is defined as 100%. A smaller percentage of revertants of the sample to the revertants of the control means a stronger antimutagenicity of the sample.

Some phenolic compounds have been demonstrated to have strong antimicrobial activity. Yen and Liu reported that if mutagenicity occurred in a tested sample, the results of an antimutagenic assay thereafter would be influenced and confused due to increased or decreased numbers of revertants of TA98 and TA100. In our previous work, the high amounts of phenolic compounds in MEPH were evaluated. Therefore, the mutagenicity and toxicity of MEPH must be assayed before testing the antimutagenicity of MEPH. For doses at 0.05–0.5 mg/plate, no mutagenicity or toxicity (data not shown) was observed in any MEPH to S. typhimurium TA98 or TA100 either with or without S9 mix. Hence, the dose with 0.05–0.5 mg/plate was selected for the antimutagenic assay.

The antimutagenic activities of MEPH on five indirect-acting mutagens, including B(a)P, Trp-P-1, Glu-P-1, IQ, and AFB1, toward S. typhimurium TA98, are shown in Fig. 1. MEPH at 0.05–0.5 mg/plate showed a weakly inhibitory effect to the extent 19.1–28.5% on the mutagenicity of B(a)P toward S. typhimurium TA98. The inhibitory effect of MEPH on mutagenicity of Trp-P-1, Glu-P-1, IQ, and AFB1 increased with increasing amounts of MEPH. MEPH at 0.5 mg/plate showed 78.2% and 86.1% inhibitory activity to Trp-P-1 and Glu-P-1, respectively. However, MEPH at 0.25 mg/plate had 92.9% and 89.7% inhibitory activity on IQ and AFB1, respectively. According to the data presented, MEPH at 0.05–0.5 mg/plate definitely inhibited the mutagenicity of Trp-P-1, Glu-P-1, IQ, and AFB1, but not B(a)P.

Experiments were also made on the antimutagenic activities of MEPH against five indirect-acting mutagens toward S. ty-

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Fig. 1. Inhibitory Effects of Methanolic Extracts from Peanut Hulls (MEPH) on the Mutagenicity of Various Mutagens toward *Salmonella typhimurium* TA98 in the Presence of S9 Mix.

Fig. 2. Inhibitory Effects of Methanolic Extracts from Peanut Hulls (MEPH) on the Mutagenicity of Various Mutagens toward *Salmonella typhimurium* TA100 in the Presence of S9 Mix.

Fig. 3. Inhibitory Effects of Methanolic Extracts from Peanut Hulls (MEPH) on the Mutagenicity of NQNO toward *Salmonella typhimurium* TA98 and TA100 in the Absence of S9 Mix.

The antimutagenic activity of MEPH decreases in the order IQ > AFB₁ > Glu-P-1 > Trp-P-1 > B(a)P, and IQ > Trp-P-1 > Glu-P-1 > AFB₁ > B(a)P for *S. typhimurium* TA98 and TA100. These results indicate that antimutagenic compounds(s) in MEPH have bifunctional properties of inhibition of both frameshift and base-pair substitution mutations of B(a)P, Trp-P-1, Glu-P-1, IQ, and AFB₁, respectively. NQNO is a direct-acting mutagen, however, not all antimutagens naturally occurring in food can inhibit the mutagenicity of NQNO. For TA98 and TA100, the inhibitory effect of MEPH on mutagenicity of NQNO increased with increasing amounts of MEPH. According to the results showed in Figs. 1, 2, and 3, MEPH tended to inhibit the mutagenicity of indirect-acting mutagens that required metabolic activation by S9 mix (i.e., Trp-P-1, Glu-P-1, IQ, and AFB₁) more markedly than that of NQNO, a direct-acting mutagen.

Huang et al. cited how some naturally occurring plant flavonoids such as myricetin, robinetin and luteolin and related derivatives with phenolic hydroxyl groups inhibited the mutagenic activity of B(a)P. There was a positive correlation between antimutagenic activity and polyphenol content of dialyzates of vegetables and fruits. These investigations indicate that both a large polyphenol content and flavonoids with hydroxyl groups are closely related to the antimutagenic activity. Additionally, Samejima et al. also reported that luteolin isolated from peppermint, sage, and thyme was a desmutagen against Trp-P-2. MEPH contains a large amount of total phenols, and luteolin was identified as an antioxidative component in MEPH. Therefore, the large content of phenolic compounds and luteolin found in MEPH may contribute to the antimutagenic effect against mutagens. Antioxidants, therefore, should have some anti-carcinogenic activity. MEPH has been demonstrated to have a strongly hydrogen-donating ability and is a good scavenger of active oxygen species, which seems to relate directly to the antimutagenic effect of MEPH.

In conclusion, MEPH is non-mutagenic and inhibits the mutagenicity of standard mutagens/carcinogens such as B(a)P, Trp-P-1, Glu-P-1, IQ, and AFB₁. Further research on their mechanism and the isolation of the antimutagenic components in MEPH is in progress.
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References