Comparison of Antimutagenic Effect of Various Tea Extracts
(Green, Oolong, Pouchong, and Black Tea)

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ABSTRACT

The antimutagenic effects of various tea extracts prepared from nonfermented tea (green tea), semifermented tea (oolong tea and pouchong tea), and fermented tea (black tea) were investigated by Salmonella/microsome assay. No mutagenicity or toxicity in Salmonella typhimurium TA98 and TA100 was observed with any tea extract. The tea extracts markedly inhibited the mutagenicity of 2-amino-3-methylimidazo(4,5-f)quinoline, 3-amino-1,4-dimethyl-5H-pyridol(4,3-b)indole, 2-amino-6-methylpyridido(1,2-a;3,2-d)imidazole, benzo(a)pyrene, and aflatoxin B1 toward S. typhimurium TA98 and TA100 in the presence of S9 mixture, especially those of oolong and pouchong teas inhibited over 90% mutagenicity of these five mutagens at the dosage of 1 mg per plate. Among four tea extracts, black tea exhibited the weakest inhibitory effect on mutagenicity of these five mutagens. The mutagenicity of 4-nitroquinoline-N-oxide, a direct mutagen, was not inhibited by black and oolong tea extracts to S. typhimurium TA98 in the absence of S9 mixture but was increased by the tea extracts at the dose of 1 mg per plate to S. typhimurium TA100. As the antimutagenic effect of semifermented tea was stronger than nonfermented and fermented teas, some antimutagenic substances might be formed during manufacturing processes of tea.

Tea, originating in China, is one of the world's oldest known prepared beverages. Various processing methods have been developed for tea of diverse types, each with its unique composition and characteristic flavor. All teas can be roughly classified into three categories: nonfermented, semifermented, and fermented teas according to the extent of fermentation during the manufacturing process (26). Tea was used as a crude medicine in China for thousands of years. Tea possesses antipyretic, diuretic effects, and other effects. The pharmacological effects of tea have been reviewed by many researchers, including the effects of reduction of cholesterol (7), depression of hypertension (7), antioxidant (15,21), antimicrobial (5,14), antimitogenic (8,9,11,24), antitumor (6), and retardant of aging (19).

There have also been reported that tea has antimitogenic activity, Stich et al. (22) reported that polyphenols in tea inhibited the mutagenicity of nitrosated methylurea. Kada et al. (9) reported that green tea possessed antimitogenic activity; the active component was identified to be epigallocatechin gallate. The green tea extract exhibited significant inhibitory effects on mutagenicity induced by benzo(a)pyrene, aflatoxin B1, 2-amino-3,4-dimethylimidazo(4,5-f) quinoline (IQ), or extracts of fried fish in the Salmonella typhimurium TA98 system (9). The mutagenicity induced by automobile exhaust air, heated salmon extract, 3-amino-1-methyl-5H-pyridol(4,3-b)indole, IQ, benzo(a)pyrene, and 1,6-dinitropropene were reported to be reduced by extract of oolong tea (11). Liu and Cheng (12) indicated that the antimutagenic activity of polyphenols in green tea exceeded that of ellagic acid, β-carotene, ascorbic acid, butylated hydroxyanisole, and tocopherol.

The anticarcinogenic effect of green tea has been reported by many researchers (7,24,27); however, information concerning the antimutagenicity or anticancer effect of teas other than green tea was lacking. As the components of tea products may alter during manufacturing, especially during the fermentation process, the biochemical and physiological properties of tea products could also be influenced.

The objectives of this study were to compare the antimutagenic effects of various tea extracts prepared from nonfermented tea (green tea), semifermented tea (oolong tea and pouchong tea), and fermented tea (black tea), and to investigate the effect of the degree of fermentation on the antimutagenicity of tea.

MATERIALS AND METHODS

Materials
Teas, including green tea, oolong tea, pouchong tea, and black tea, were purchased from a local market in Taichung, Taiwan. Benzo(a)pyrene (B[a]P), aflatoxin B1 (AFB1), and 4-nitroquinoline-N-oxide (NQNO) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-Amino-3-methylimidazo(4,5-f)quinoline (IQ), 2-amino-6-methylindolo(1,2-a;3,2-d)imidazole (Glu-P-1) and 3-amino-1,4-dimethyl-5H-pyridol(4,3-b)indole (Trp-P-1) were purchased from Wako Pure Chemical Co. (Tokyo).

Preparation of tea extracts
Each tea (20 g) was extracted with boiling water (400 ml) for 5 min and the filtrate was freeze-dried. The yields of crude tea extracts for green tea, oolong tea, pouchong tea, and black tea were 4.09, 5.27, 4.11, and 3.86 g, respectively. These tea extracts were dissolved in sterile distilled water and tested for mutagenicity, toxicity, and antimutagenicity.
Mutagenicity and toxicity assays

The mutagenicity of tea extracts was assessed using the Ames test with a 20 min preincubation at 37°C (13). The histidine-requiring strains of Salmonella typhimurium TA98 and TA100 were kindly supplied by Dr. B. N. Ames (University of California, Berkeley). The S9 mix (Organ Teknika Co., Switzerland) was prepared from Sprague-Dawley male rats treated with Aroclor 1254 according to Ames et al. (1). Diluted tea extracts (0.1 ml) were 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 preincubated at 37°C for 20 min before molten top agar (2 ml) was added; the mixture was poured onto a minimal medium agar plate. The his+ revertant colonies were counted after incubating at 37°C for 48 h. Each sample was assayed using triplicate plates, and the data presented are means ± standard deviation of three determinations. The mutagenicity is expressed as the number of revertants per plate at a given concentration of each sample; in this testing, the result was recognized to be positive when the number exceeded twice the number of spontaneous revertants (1).

To examine the toxic effect of tea extracts to TA98 and TA100, the mixtures after preincubation were diluted with phosphate buffer, and the diluted mixtures were poured into nutrient agar plates. The plates were incubated at 37°C for 2 d, and the number of colonies was counted.

Antimutagenicity assay

The antimutagenic effect of each tea extract was assayed according to the Ames method except for the addition of mutagen before preincubation. The mutagens used were NQNO (1.0 µg per plate for TA98 and 0.1 µg per plate for TA100) (a direct-acting mutagen), IQ (0.1 µg per plate for TA98 and 0.5 µg per plate for TA100), and Glu-P-I (0.2 µg per plate for TA98 and TA100), Trp-P-I (0.5 µg per plate for TA98 and TA100), B(a)P (5 µg per plate for TA98 and TA100), and AFB1 (5 µg per plate for TA98 and TA100) which required S9 mix for metabolic activation. The mutagen (0.1 ml) was added to the mixture of strain and tea extracts with S9 mix for IQ, Glu-P-I, B(a)P, and AFB1 or with phosphate buffer (0.1 M, pH 7.4) for NQNO. The mutagenicity of each mutagen in the absence of tea extracts is defined as 100%. A smaller percentage of revertants of the sample relative to the revertants of the control denotes a stronger antimutagenicity of the sample (4).

Statistical analysis

The interaction between the treatments and the comparison of means obtained for each group were calculated as described by Duncan (3). Statistical differences at P < 0.05 were considered significant.

RESULTS AND DISCUSSION

Mutagenicity and toxicity of tea extracts

The mutagenicity and toxicity of tea extracts toward Salmonella typhimurium TA98 and TA100 were evaluated. For testing doses less than 5 mg per plate, no mutagenicity or toxicity was found in any tea extracts to TA98 or TA100 either with or without S9 mix (data not shown). These results are in agreement with Nagao et al. (16) who did not detect mutagenicity to S. typhimurium TA100 with black tea and green tea. The direct mutagenicity of tea was reported for the S. typhimurium strain BA 13, but it showed no mutagenicity with S. typhimurium TA104 (2). Therefore, the mutagenicity of tea may depend on the testing strain. Hara and Ishigami (5) indicated that tea polyphenols have strongly antibacterial activity against foodborne pathogenic bacteria, but they did not test S. typhimurium and Escherichia coli. Since the mutagenicity and toxicity of tea extracts were not observed under doses that have been tested, tea extracts at those dosages were used for the antimutagenic assay.

Antimutagenic effect of tea extracts to indirect mutagens

The antimutagenic activities of tea extracts on five indirect mutagens, including IQ, B(a)P, AFB1, Glu-P-I, and Trp-P-I, were evaluated. The inhibitory effect of tea extracts on the mutagenicity of IQ toward S. typhimurium TA98 and TA100 appears in Figure 1. In general, the mutagenicity of IQ was markedly reduced by any tea extract. The mutagenicity of IQ to TA100 was completely inhibited by oolong and green tea extracts at the dosage of 1 mg per plate; however, 47 and 93.7% mutagenicity of IQ were reduced by black tea extract at the dosage of 1 and 5 mg per plate, respectively. All of the tea extracts showed a similar inhibitory effect to IQ in S. typhimurium TA98 (P < 0.05). The antimutagenic activity of tea extracts increased with increasing concentration of tea extracts; 98% mutagenicity of IQ was inhibited by any tea extract at the dosage of 5 mg per plate. The antimutagenicity of oolong and green tea extracts in the TA100 system was greater than in the TA98 system at the dosage of 1 mg per plate, whereas pouichong and black tea extracts showed a greater antimutagenic effect in the TA98 system. This result may be caused by the distinct antimutagenic components occurring in various tea extracts.

Figure 1. Inhibitory effect of tea extracts on the mutagenicity of IQ to S. typhimurium TA100 and TA98.
Figure 2 shows the antimutagenic effect of tea extracts to B(a)P. For *S. typhimurium* TA100, the inhibitory effect of four tea extracts was in decreasing order oolong tea > pouchong tea > green tea > black tea. The mutagenicity of B(a)P was completely inhibited by oolong tea extract at the dosage of 1 mg per plate. For *S. typhimurium* TA98, the semifermented teas showed a stronger inhibitory effect; over 90% mutagenicity of B(a)P was inhibited by oolong and pouchong tea extracts at the dosage of 1 mg per plate. The mutagenicity of B(a)P was completely inhibited by all tea extracts at the dosage of 3 mg per plate to TA98.

![Figure 2](image)

Figure 2. Inhibitory effect of tea extracts on the mutagenicity of B(a)P to *S. typhimurium* TA100 and TA98.

The tea extract from pouchong tea showed the greatest inhibitory effect on AFB1 to either TA98 or TA100 (Figure 3). It exhibited over 90% inhibitory effect to AFB1 in *S. typhimurium* TA98 and TA100 at the dosage 1 mg per plate. However, no significant difference (P > 0.05) in inhibitory effect for any tea extract to AFB1 was found by a concentration of tea extracts increased over 3 mg per plate. At the dosage of 1 mg per plate, the antimutagenic activity of tea extracts was in decreasing order pouchong tea > oolong tea > green tea > black tea toward *S. typhimurium* TA98 and TA100.

Figure 4 shows the antimutagenic effect of tea extracts to Trp-P-1. For strain TA100, oolong and green tea extracts showed a stronger inhibitory effect, the mutagenicity of Trp-P-1 was completely inhibited by these two tea extracts at the dosage of 3 mg per plate. Black tea exhibited the weakest inhibitory activity to Trp-P-1; it showed only 70% inhibitory effect at the dosage of 5 mg per plate. For strain TA98, oolong tea showed the strongest inhibitory effect (P < 0.05). No significant difference (P > 0.05) was found for the antimutagenic activity among pouchong, green, and black teas to Trp-P-1 in strain TA98.

![Figure 3](image)

Figure 3. Inhibitory effect of tea extracts on the mutagenicity of AFB1 to *S. typhimurium* TA100 and TA98.

![Figure 4](image)

Figure 4. Inhibitory effect of tea extracts on the mutagenicity of Trp-P-1 to *S. typhimurium* TA100 and TA98.
The inhibitory effect of tea extracts against Glu-P-1 is shown in Figure 5. All tea extracts produced greater than 95% inhibitory effect to Glu-P-1 in strain TA100 at the dosage of 1 mg per plate except black tea. For strain TA98, pouchong and green tea extracts showed similar antimutagenic activity against Glu-P-1. The mutagenicity of Glu-P-1 was completely inhibited by green and pouchong tea extracts at the dosage of 3 mg per plate. Oolong tea showed 83.1% inhibitory effect to Glu-P-1 at the dosage of 1 mg per plate, but the inhibitory effect failed to increase with increasing concentration of tea extract (P > 0.05).

Figure 5. Inhibitory effect of tea extracts on the mutagenicity of Glu-P-1 to S. typhimurium TA100 and TA98.

Most tea extracts exhibited antimutagenic activity in decreasing order semifermented tea (pouchong tea and oolong tea) > nonfermented tea (green tea) > fermented tea (black tea). Kojima et al. (11) reported that the antimutagenic effect of oolong tea against B(a)P is greater than of green tea. As indicated in the literature (9,17,18,24), the components in tea, such as catechin, ascorbic acid, tocopherol, tannic acid, caffeic acid, chlorophyll, and gallic acid, were found to be antimutagenic against some mutagens. The content of these components in tea may vary with the variety, harvesting season, and processing method, which may also cause the variable antimutagenic activity of various tea products. The black tea showed the weakest antimutagenic activity among our four tea extracts; it might be due in part to the ascorbic acid and catechin of black tea that were severely destroyed during processing.

In general, the content of catechin in tea is related to the degree of fermentation of tea during manufacture; therefore, the content of catechin in various teas is in decreasing order green tea > pouchong tea > oolong tea > black tea (23). The ascorbic acid content in teas has a similar trend to that of catechin (10). Kojima et al. (11) reported that the tannin and ascorbic acid contents of green tea are about 2- to 3-fold greater than of oolong tea, and the content of caffeine in green tea is also higher. Therefore, the variable in antimutagenic effect of tea extracts of these four kinds may not be completely attributable to the variable contents of these components. We predict that some components might form during manufacture of semifermented tea (pouchong tea and oolong tea) to endow it with greater antimutagenic activity than other teas. Further research to isolate and to identify the minor antimutagenic component in semifermented tea is needed to confirm this point.

Antimutagenic effect of tea extracts to direct mutagens

The antimutagenic activity of tea extracts against direct mutagens, NQNO, was evaluated. The results (data not shown) indicated that black and oolong tea extracts showed no inhibitory effect on the mutagenicity of NQNO to TA98, whereas pouchong and green teas showed an only weakly inhibitory effect (ca. 15%) at the dose of 5 mg per plate of tea extracts. For TA100 system, the tea extracts showed slight antimutagenic activity to NQNO at the small dosages of 0.2 and 0.5 mg per plate; 20-80% mutagenicity of NQNO was enhanced by tea extracts at a dosage increased above 1 mg per plate. For the mutagenicity test, strain TA98 detected various frame-shift mutagens, but TA100 detected mutagens that cause base-pair substitutions (13). Thus, some components in tea extracts might react with NQNO and cause the base-pair substitutions but not influence the frame-shift mutations.

CONCLUSIONS

Our results clearly indicate that tea extracts show antimutagenicity against indirect mutagens (requiring S9 activation) but not to direct mutagens. Wang et al. (25) indicated that enzyme activity in cytochrome P-450 was inhibited by binding with catechin in green tea. Since cytochrome P-450 is the major enzyme in S9 mix, whether the antimutagenic effect of tea extracts operates through the inactivation of enzyme activity or through other mechanisms is unclear; further investigation is needed. The antimutagenic activity of tea extracts varied with the extent of fermentation of tea during the process of manufacturer. Moreover, the antimutagenic activity of semifermented tea was greater than those of fermented and nonfermented teas. Sanderson et al. (20) reported that the solid content in a cup of tea is about 400-700 mg; this amount is greater than the highest dose used for antimutagenic test in our study. Semifermented tea extracts (oolong and pouchong) have the higher yield and would have more antimutagenic activity than other teas on a liquid basis. Thus, tea would be useful to human for the purpose of mutation chemoprevention. Further studies on the isolation of the antimutagenic component and its antimutagenic mechanism are required and in progress.
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REFERENCES


