



Research Paper

ANTIMUTAGENIC EFFECT OF GREEN TEA EXTRACTS IN REVERSE MUTATION ASSAY

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In the present investigation, antimutagenic effect of petroleum ether, chloroform, methanol and water extracts of green tea was evaluated in *Salmonella typhimurium* TA-98 and TA-100 strains. Well known mutagens like sodium azide and daunomycin were added at a concentration of 10 µl and 6 µl per plate respectively resulted in the induction of histidine revertant colonies. However addition of 10 µl of petroleum ether, chloroform, ethanol and water extracts of green tea to 10 µl of sodium azide and 6 µl of daunomycin treated plates resulted in the inhibition in the number of histidine revertant colonies. Furthermore, supplementation with all the four extracts of green tea at a concentration of 10 µl per plate respectively in the presence of S9 fraction also led to significant inhibition in sodium azide and daunomycin induced colony formation. The antimutagenic activity of ethanolic extract of green tea was found to be higher than that of the other extracts. Hence the study revealed that green tea has protective efficacy in sodium azide and daunomycin induced mutagenicity in the test microbial system.

Keywords: Green tea, Antimutagenic activity, Ames assay, *Salmonella typhimurium*

INTRODUCTION

Damage to DNA is likely to be a major cause of cancer and other diseases (Ames, 1975). In modern times man is exposed to a multitude of environmental poisons (Renner, 1990). Repeated exposures to small doses of natural toxicants or carcinogens may lead to most hazardous situations (Iverson, 1991). It is difficult to eliminate mutagenic or carcinogenic factors present in our environment, but it is possible to diminish the risk of cancer through simple dietary and herbal recommendations (Block *et al.*, 1992).

Plant contains many natural substances that can promote health and alleviate illness. Flavonoids, a broad class of polyphenolic compounds, widely distributed among photosynthesizing cells, possess an impressive array of pharmacological activity (Hasten, 1983). Oxidation is a metabolic process that leads to energy production necessary for essential cell activities. However, metabolism of oxygen in living cells also leads to the unavoidable production of oxygen derived free radicals, commonly known as Reactive Oxygen Species (ROS) (McCord,

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1994 and Adegoke, 1998) which are involved in the onset of much disease (Ames *et al.*, 1993).

Flavonoids found in tea show 20 times more powerful antioxidant activity. Antioxidants protect the body against the damaging effects of free radicals generated in the body during the conversion of glucose and fat to energy. Oxidative stress induced by the over production of reactive oxygen species like hydroxyl radical disrupt the cellular function. Over production of free radical occurs with smoking, environmental pollution, chronic inflammation, external action of toxic substances, microbial attacks and ozone (Diplock *et al.*, 1998 and Jackson *et al.*, 1998).

The tea leaves contain four major catechins as colourless water soluble compounds namely Epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Green tea has received much attention as chemo preventer for promotion and progression of various forms of tumour and EGCG with five phenolic hydroxyl groups is claimed to be the most potent tea antioxidant (Ho *et al.*, 1996). EGCG has been shown to act as inhibitor of urokinase, the enzyme crucial for tumour growth (Lin *et al.*, 1996).

In the present study an attempt was made to evaluate the antimutagenic effect of green tea extracted using four different solvents viz., petroleum ether, chloroform, ethanol and water in *Salmonella* microsome assay.

MATERIALS AND METHODS

Soxhlet Extraction

10 g of green tea powder was weighed using an electrical balance and made into 8 packets using xerohaze filter paper. Soxhlet extraction of powdered green tea was carried out to obtain its

extract. Petroleum ether, chloroform, ethanol and water were used as solvents for soxhlet extraction in the increasing order of polarity. The distillation process was carried out at a low temperature of 40 °C. After evaporation of solvents; corresponding residues obtained were stored in the refrigerator for further use. 100 mg of soxhlet extract was dissolved in 2 ml of DMSO and then mixed with 100 ml distilled water and this formed 1000 ppm solution. From this stock solution, solutions of required concentrations were prepared and used in this study.

Bacterial Tester Strains

Salmonella typhimurium TA 98 and TA 100 were kindly provided by Professor Bruce N. Ames, Berkley, USA. The strains were checked routinely for ampicillin resistance, ultra violet sensitivity and spontaneous revertants.

Preparation of S9 Fraction

Swiss albino mice weighing about 25 g were obtained from animal laboratory, Food Science and Nutrition Department, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and kept in plastic cages with husk bedding and a stainless steel lid suitable for feeding and watering. The mice were fed on by standard rodent diet pellet. Mice were injected with phenobarbitone at a dose of 1mg/g body weight intraperitoneally for 3 consecutive days. On day six, no food was provided to the mice for fasting. The mice were sacrificed on the seventh day for preparation of liver S9 fraction. All the steps were performed at 0-4 °C with cold and sterile solutions and glass wares. The liver was excised out after dissecting the animal. The excised liver was then washed in an equal volume of 0.15 M KCl. Then it was mixed in 0.14 M KCl and homogenized with a homogenizer. The homo-

genate was centrifuged for 10 min at 9000 g and the supernatant so collected was the S9 mix fraction. The freshly prepared S9 fraction was quickly frozen in dry ice and stored at -20°C.

For plate incorporation assay, top agar (2 ml) was distributed into each small test tubes held in a water bath. In different set groups of experiments in above tubes, 10ml/plate of petroleum ether, chloroform, ethanol and water extracts of green tea plus the mutagen (daunomycin at the concentration of 6ml/plate and sodium azide at the concentration of 10ml/plate) and 10ml of metabolically activated S9 mix plus 10ml of standardized bacterial cultures of TA 98 and TA 100 strains were added to the top agar and then poured into minimal glucose agar plates. The plates were then inverted and placed in an incubator at 37°C for 48 h and counted for the number of histidine revertant colonies.

Similar experiments were carried out for positive controls (taking daunomycin and sodium azide) and negative controls (untreated groups) for identifying spontaneous culture for both the strains concurrently.

Statistical analysis

The mean values of number of histidine revertants/plate for different groups were subjected to statistical analysis using student 't' test.

RESULTS AND DISCUSSION

The present investigation depicts the antimutagenic potential of green tea extracts in *Salmonella typhimurium* reverse mutation assay (Tables 1 and 2). The number of spontaneous revertants was found to be 38 and 144 in TA 98 and TA 100 strains respectively. Addition of daunomycin and sodium azide to the minimal

glucose plates resulted in significant induction in the number of histidine revertants and was found to be 61 and 184 of TA 98 and TA 100 strains respectively. However supplementation with different extracts of green tea resulted in the inhibition of induction of histidine revertant colonies either by daunomycin or sodium azide.

The percentage inhibition of daunomycin was 26%, 28%, 33% and 19% for green tea in petroleum ether, chloroform, ethanol and water extracts respectively in TA 98 strains. The percentage inhibition of different extracts of green tea towards sodium azide induced histidine reversion was found to be 8% , 9% , 10% and 5% respectively for petroleum ether, chloroform, ethanol and water extracts in TA 100 tester strains. The number of histidine revertants per plates for TA 98 and TA 100 tester strains in green tea was found to reduce 45, 44, 41 and 50 and 171, 169, 167 and 175 respectively. In the presence of S9 fraction the number of spontaneous revertants for petroleum ether, chloroform, ethanol and water extracts of green tea were found to be 106 and 203 in TA 98 and TA 100 strains respectively. In addition of daunomycin and sodium azide to the above extracts in TA 98 and TA 100 strains the number of histidine revertant were found to be increased to 155 and 359 respectively. However in plates supplemented with petroleum ether, chloroform, ethanol and water extracts of green tea resulted in inhibition of induction of histidine revertant colonies either by daunomycin or by sodium azide. The number of histidine revertants/plate of TA 98 and TA 100 tester strains of green tea were found to be reduced to 141, 134, 127 and 136 and 316, 315, 311 and 338 respectively.

Table 1: Antimutagenic Effect of Petroleum Ether, Chloroform, Ethanol and Water Extract of Green tea in Salmonella Typhimurium TA 98 and TA 100 strains

Strains	Treatment	Petroleum ether	Chloroform	Ethanol	Water
TA 98	SR + GT	39.0 ± 3.29 ^{NS}	41.0 ± 3.23 ^{NS}	40.0 ± 2.79 ^{NS}	37.0 ± 5.19 ^{NS}
	SM + GT	45.0 ± 2.61** (26%)	44.0 ± 3.60** (28%)	41.0 ± 3.88** (33%)	50.0 ± 2.66** (19%)
TA 100	SR + GT	146.0 ± 5.65 ^{NS}	143.0 ± 9.08 ^{NS}	141.0 ± 10.53 ^{NS}	145.0 ± 8.18 ^{NS}
	SM + GT	171.0 ± 4.99** (8%)	169.0 ± 3.87** (9%)	167.0 ± 3.37** (10%)	

Note: Results are the average of two independent experiments: Spontaneous Revertant rate of TA 98 was 38 ± 4.1 and TA 100 was 144 ± 3.7; Standard Mutation rate for TA 98 was 61 ± 2.1 and TA 100 was 84.0 ± 3.2; NS – Not Significant; **: 1% Significance: per cent inhibition of revertant frequency with the addition of different extracts of green tea to standard mutagen induced plates is given in parenthesis.

Table 2: Antimutagenic Effect of Petroleum Ether, Chloroform, Ethanol And Water Extract of Green Tea in Salmonella Typhimurium TA 98 and TA 100s Strains in the Presence of S9 Fraction

Strains	Treatment	Petroleum ether	Chloroform	Ethanol	Water
TA 98	SR + GT	108.0 ± 8.08 ^{NS}	105.0 ± 6.48 ^{NS}	104.0 ± 6.55 ^{NS}	112.0 ± 8.60 ^{NS}
	SM + GT	141.0 ± 2.61** (9%)	134.0 ± 6.11** (14%)	127.0 ± 3.15** (18%)	136.0 ± 4.05** (13%)
TA 100	SR + GT	204.0 ± 8.12 ^{NS}	210.0 ± 6.84 ^{NS}	208.0 ± 5.86 ^{NS}	202.0 ± 3.15 ^{NS}
	SM + GT	316.0 ± 7.06** (12%)	315.0 ± 5.74** (13%)	311.0 ± 6.45** (14%)	338.0 ± 7.25** (6%)

Note: Results are the average of two independent experiments: Spontaneous Revertant rate of TA 98 was 106 ± 8.6 and TA 100 was 203 ± 5.8; Standard Mutation rate for TA 98 was 155 ± 4.9 and TA 100 was 359.0 ± 5.5; NS – Not Significant; **: 1% Significance: per cent inhibition of revertant frequency with the addition of different extracts of green tea to standard mutagen induced plates is given in parenthesis.

The *Salmonella typhimurium* reverse mutation assay is most commonly used method to assess mutagenic potential of test chemicals, which may cause base pair and frame shift mutations in the genome of this organism (Maron and Ames, 1983). Its applicability in screening the antimutagenic potential of green tea has been performed in the present study. Daunomycin and sodium azide are well known genotoxicants in mammalian and microbial test systems. Addition of daunomycin and sodium azide to the minimal glucose plates resulted in the significant induction of histidine revertant colonies. In the present study addition of green tea extracts to sodium azide and daunomycin treated plates resulted in the significant inhibition of number of colonies formed in TA 98 and TA 100 strains respectively.

Earlier reports states that Catechin component including epicatechin gallate (ECG) and epigallo catechin galleate (EGCG) provide a significant protection against mutagenicity of Trp – P – 2 and the N-OH-Trp – P – 2 using *Salmonella typhimurium* TA 98 and TA 100 (Hayatsu *et al.*, 1992 and Kuroda and Hara, 1999). EGCG also have been reported to provide strong inhibitory effect against mutagenicity of Ba P diol epoxide in TA 100 strain (Hour *et al.*, 1999). Using *Salmonella typhimurium* TA 98 and TA 100 strains, the tea catechins ECG and EGCG have been shown to inhibit the mutagenic activity of direct acting mutagens (Okuda *et al.*, 1984). In another study the extracts of green tea decreased the mutagenic activity on N-methyl – N – Nitro – N – Nitroso guanidine (MNNG) in *Escherichia coli*

WP2 in a dismutagenic manner (Kuroda and Hara, 1999).

Green tea extracts were also effective against the mutagenicity of PAH Benzo (a) pyrene, DMBA with S9 activation (Kuroda and Hara, 1999). The antimutagenic effect of tea involves interaction between the reactive genotoxic species of various promutagens and polyphenolic tea component present in the tea (Kuroda and Hara, 1999). The anti genotoxic properties of tea include induction of DNA repair and binding of activated carcinogens (Yang *et al.*, 2002).

CONCLUSION

Our findings point to higher antimutagenic activity of ethanolic extracts of green tea when compared to other three extracts using petroleum ether, chloroform and water. This study throws possibility of reduction of mutagenicity and there by carcinogenicity in people drinking green tea regularly.

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