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The Study of Antioxidant and Anticarcinogenic Green Tea and Black Tea

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Abstract: Tea is one of the most popular beverages consumed worldwide. The relationship between tea consumption and human cancer incidence is an important concern. The effect of tea extract and ingredients, polyphenol and caffeine on the mutagenicity of Sodium Azide was examined *in vitro* by using *Salmonella typhimurium* TA100, TA98 and TA1535 in the presence of induced rat liver S9 fractions. Experimental studies have demonstrated the significant antimutagenic and anticarcinogenic effects of both Green and Black tea and its polyphenol and caffeine multiple mutational assay. Caffeine was the less active. Tea comes in many variants. Common tea such as Black tea contains little antioxidant and the amount of caffeine. Green tea has about the caffeine, but contains a good amount of antioxidant.

Key words: Anticarcinogenic, antioxidant, Green tea, Black tea

INTRODUCTION

Black tea is the most popular, being consumed by over 90% of the tea consumed in US. Black tea has been fully oxidized or fermented and yield a hearty-flavored; amber brew. Green tea, unlike lack tea, is not oxidized. It has a more delicate taste and is light Green/golden in color. Caffeine was first discovered in 1827 and was named theine. Eventually it was shown that the theine of tea was identical with the caffeine of coffee and the term theine was dropped. The amount of caffeine in tea depends on a number of factors, including the variety of tea leaf, where it is grown particle size used, the particular tea leaf cut and the method and length of brewing or in steeping. Studies show that leaf location on the tea plant, affect content of coffee in that leaf (Mokhtar *et al*., 2000). Existing reports have suggested that the protective affects of Black tea is a good as Green tea (Shukla and Taneja, 2000). The antioxidant activity of tea extracts (and polyphenols) against various mutagens has been demonstrated in microbial systems and *in vivo* animal tests (Giri and Bonerjee, 1996; Gupta *et al*., 2001). Most experimental studies demonstrating antimutagenic/ anticarcinogenic effects have conducted in a wide variety of *in vivo* and *in vitro* systems with water extract of Green tea or a polyphenolic fraction isolated from Green tea, in contrast with Green tea, limited-work has been with Black tea till recently. It was assumed to be much less beneficial than Green tea because of its lower content of unpolymerised polyphenol. Particularly epigallocatechin-3-gallate (EGCG) (Steele and Kelloff, 2001; Yang *et al*., 2000). Only recently was work been inhibited with Black tea or its characteristic theaflavins and thearubignis that considering the wide spread reports of dietary agent as antimutagens/anticarcinogenes. The aim of this study is to understand whether both the tea has similar antioxidant anticarcinogenic effects or one of them have superiority over the other (Feng *et al*., 2002).

MATERIALS AND METHODS

For Black tea, Golestan, Shahrzad, Sedaghat and Tamasha tea and for Green tea, Numbers 24, 16, 64 and 22 teas were obtained from agricultural research North provinces in Iran. It should be mentioned that the specific numbers for Green teas are relating to above used Black teas, respectively, according to ISIRI, (1994).

First all of the Green teas and Black teas powdered separately. Then Powders were extracted with boiling water so the concentration of extracts was 25 mg mL^{-1} (Harbowy and Balentine, 1997). We did the liberation of caffeine by alkalization with heavy magnesium oxide and extraction it with chloroform and then detected the weight of caffeine. We confirmed the result of detect the extract amount of that by total nitrogen method if necessary (Harbowy and Balentine, 1997). Professor B.N. Ames (University of Berkeley, California, USA) kindly supplied *Salmonella typhimurium* strains TA100, TA98 and TA1535. The anticarcinogenicity activity extract and caffeine in Black tea and Green tea were evaluated in the presence of S9 using of Sodium Azide as promotagenes. In the antimutagenicity test, the inhibition of mutagenic activity of the Sodium Azide by the test samples was determined. The test samples (25 mg per plate) were assayed by plating with molten top agar (2 mL) containing over night culture (0.1 mL) of strains of *Salmonella typhimurium* (TA100, TA98 and TA1535). Positive and negative controls were also included in each

assay. Sodium Azide was used as a diagnostic mutagen (1.5 ug per plate) in the positive control and plates without Sodium Azide and without test samples were considered as negative controls (Negi *et al*., 2003). In all plates 0.5 mL of metabolic activity system (S9) were added fractions were prepared from livers of male Wistar rats (weighing 200-250 g). His^+ revertants were counted after incubation of the plates of 37°C for 48 h. Each sample was assayed using three-plicate plates. The mutagenicity of Sodium Azide in the absence of test samples was defined as 100 or 0% inhibition. The calculation of percent inhibition was done according to the formula given by Ong *et al*. (1986).

Each percent inhibition (%) = ((1-T/M) x 100 where is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and dominator (Ong *et al*., 1986).

RESULTS AND DISCUSSION

Results of Table 1 showed that Black tea is commonly having more caffeine than Green tea. The amount of caffeine in tea depends on a number of factors, including the variety of tea, where it is grown, particle size used, the particular tea leaf cut. Studies showed that leaf location on the tea plant, affects content of caffeine in that leaf. The longer the tea leaves have fermented, the greater their caffeine content (Hour *et al*., 1997).

Antimutagenic and anticarcinogenic of the caffeine from Green and Black tea were detected in microbial systems with *Salmonella typhimurium* TA100, TA98 and TA1535 in the presence of S9 against Sodium Azide in these microbial system (Table 2). Majority of the studies have been conducted with TA98 with S9 activation in the Ames Assay (Santana-Rios *et al*., 2001; Gupta *et al*., 2001). In this research *Salmonella* typhimurium TA100, TA98 and TA1535 assay have been used as a qualitative test for both black and Green tea extract were equally good inhibitor, (Table 3). But the caffeine Black tea appeared to be slightly better that Green tea caffeine. The extract. The purification step from Green tea extract to Green tea caffeine did not improve the strength of the inhibition (Steele *et al*., 2000). However the purification of the Black tea caffeine seems to have enriched the caffeine fraction by a factor of two the terms of compounds that inhibit mutagenicity in this assay system (Table 2 and 3). Bu-Abbas *et al*. (1996) however could not discern any difference in the antimutagenic potential of Green, black and decaffeinated black tea. Weisburger (1999) while emphasizing on four major areas has observed than in most studies Green and Black tea have similar effects. Different workers have proposed different mechanisms to explain the property of antimutagenesis by tea and their caffeine. In this research the extracts of Green tea and Black tea leaves and caffeine decreased the mutagenic activity of Sodium Azide to *Salmonella* typhimurium TA100, TA98 and TA1535 *in vitro* in a desmutagenic manner. The effects were also demonstrated in the rat liver S9 by assaying the bacterial mutagenic *in vitro* of Sodium Azide remarkably administration showed less effect. We examined the antimutagenic properties of black and Green tea components, including caffeine by the Ames test. Caffeine was less active. Hour *et al*. (1997) suggest that formation of different metabolites during various stages of tea fermentation may affect antimutagenic potential against different type of chemical mutagens.

Table 1: Comparison of caffeine content in Black tea and Green tea

Brand of tea	Caffeine content (mg)
Golestan (black tea)	240
Shahrzad (black tea)	140
Sedaghat (black tea)	140
Tamasha (black tea)	200
24 (Green tea)	175
16 (Green tea)	130
64 (Green tea)	100
22 (Green tea)	124

Table 2: Inhibitory effect of caffeine content Black and Green tea against carcinogenicity of Sodium Azide to *Salmonella typhimurium* with S9

No. of revertant	Sal. TA100	Sal. TA98	Sal. TA1535
Positive control	1620	1986	1720
Negative control	352	298	350
Golestan (black tea)	393	468	450
Shahrzad (black tea)	375	390	420
Sedaghat (black tea)	461	527	483
Tamasha (black tea)	578	630	498
24 (Green tea)	592	920	536
16 (Green tea)	568	820	687
64 (Green tea)	564	503	600
22 (Green tea)	491	630	557

Table 3: Inhibitory effect of Black and Green tea extract against mutagenicity of Sodium Azide to *Salmonella typhimurium* with S9

No. of revertant	Sal. TA100	Sal. TA98	Sal. TA1535
Positive control	1680	2105	1860
Negative control	293	386	350
Golestan (black tea)	619	830	557
Shahrzad (black tea)	764	703	800
Sedaghat (black tea)	886	1020	887
Tamasha (black tea)	892	1211	536
24 (Green tea)	478	530	398
16 (Green tea)	361	462	392
64 (Green tea)	320	356	430
22 (Green tea)	293	386	350

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