COMPARATIVE STUDY OF ANTIOXIDANT POTENTIAL OF TEA WITH AND WITHOUT ADDITIVES

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Abstract : Oxidative damage is one of the many mechanism leading to chronic diseases. Therefore interest is growing in the protection afforded by antioxidant nutrients against free radical reactions. More recently, the attention has shifted to polyphenols. Polyphenols are secondary plant metabolites occurring widely in plant food. They possess outstanding antioxidant properties, suggesting a possible protective role in man. Tea (Camellia sinensis) is a widely consumed beverage throughout the world containing polyphenols more than 35% of its dry weight.

In the present work we have investigated the effect of tea without milk, tea with milk and lemon tea on the serum lipid peroxidation level (as a parameter of free radical generation). The results show that there were significant decrease in serum lipid peroxidation (Malonaldehyde) level half hour after ingestion of lemon tea and tea without milk which tends to normalize with increase in time. This decrease is much significant in case of lemon tea than tea without milk after half hour or one hour.

Hence the interpretation is, tea without milk is a good source of antioxidant and addition of lemon to tea increases its antioxidant potential.

Key words : tea   free radicals   polyphenols
       lipid peroxidation   antioxidant potential

INTRODUCTION

Free radicals react with almost every known biological molecules in their vicinity and damage protein, causes breakdown of DNA strands and initiates peroxidation of various molecules. The hydroxyl radical is most reactive of all and may be considered the ultimate damaging species whenever superoxide is formed. The

ever growing significance of antioxidant nutrients such as alpha tocopherol, beta carotene and ascorbic acid has come to light (1).

A vast number of literature documenting the *in vitro* antioxidant property of polyphenol is available. It acts both as primary as well as secondary antioxidant,

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by sequestration of metallic ion and by scavenging active oxygen species (2). However, the mechanism through which these compounds act *in vivo* are still incompletely understood more so owing to their bioavailability and metabolic fate in man. Although *in vivo* appreciable antioxidant action following gastric digestion of condensed polyphenols have been reported (3), yet the possibility for interference with this activity of polyphenols by compound which are normal constituents of human diet cannot be ruled out. The antioxidant potential of polyphenols are closely related to number of hydroxyl group, higher the number more potent the chain breaking action of compound (4).

Tea leaves contain >35% of their dry weight as polyphenols whose nature differs depending upon manufacturing procedure (5). Three main classes of commercial tea has been found -[a] green tea, a non fermented type of tea rich in flavanols, flavandiols and simple phenolic acid. [b] black tea where fermentation is carried to optimum extent and is rich in theaflavins and thearubigens. [c] Oolong tea, a semi fermented type having intermediate characteristics (4, 6).

During the process of fermentation the simple polyphenols undergo enzymatic polymerization resulting in formation of compound of condensation. The red color of tea and its astringent property is due to these polyphenols-astringency is lessened when we add milk due to formation of complex with casein in milk (7, 8).

Consequently the antioxidant potential of milk tea is much reduced. The present study incorporates a complete profile of the antioxidant potential of tea without milk, tea with milk and lemon tea in normal healthy individual.

METHODS

In the present work we have taken four groups of young healthy subjects. Control group (taking 300 ml boiled water), tea without milk group (taking 300 ml of tea without milk), tea with milk (taking 300 ml of tea with milk) and lemon tea group (taking 300 ml of lemon tea). Samples were obtained from different groups after overnight fast, before intake of tea and 30, 60, 90 & 120 minutes after intake of tea. Estimation of serum lipid peroxidation was done in terms of "Thiobarbituric acid reactants" using 1, 1, 3, 3, tetramethoxy propane (9). To 0.5 ml of serum was added an equal volume of isotonic saline and to it was added 2 ml of 50% Trichloroacetic acid in cold to precipitate the protein then centrifuged at 500 x g for 10 min at 4°C. To 1 ml of supernatant was added 2 ml of 0.67% aqueous Thiobarbituric acid and mixture was heated in boiling water for 15 minutes. The tubes were then rapidly cooled by keeping in ice and volume reconstituted to original of 2 ml. Optical density was measured at 535 nm.

RESULTS

Table I shows serum malonaldehyde level in different groups before and after

TABLE I : Shows serum malonaldehyde level in different groups with time before and after intake of tea.

	0 min	30 min	60 min	90 min	120 min
Control group n=30 (Group I)	0.4580±0.0624	0.4650±0.0424	0.4156±0.0532	0.4156±0.0584	0.4248±0.0382
Tea with lemon n=30 (Group II)	0.4432±0.0612	0.2724±0.0058	0.0724±0.0038	0.1320±0.0458	0.4340±0.0328
Tea without milk n=30 (Group III)	0.4352±0.0528	0.1520±0.0382	0.2988±0.0324	0.4156±0.0426	0.4212±0.0388
Tea with milk n=30 (Group IV)	0.4380±0.0386	0.4280±0.0428	0.4032±0.0512	0.3968±0.0823	0.3884±0.0462

TABLE II : Shows comparison of serum malonaldehyde level with time.

Groups	0 min / 30 min	0 min / 60 min	0 min / 90 min	0 min / 120 min
Group I P value significance of difference	>0.1 NS	>0.1 NS	>0.1 NS	>0.1 NS
Group II P value significance of difference	<0.05 S	<0.05 S	<0.05 S	<0.1 S
Group III P value significance of difference	<0.001 HS	<0.01 MS	<0.05 S	<0.05 S
Group IV P value Significance of difference	>0.1 NS	>0.1 NS	>0.1 NS	>0.1 NS

intake of tea. Table II shows comparison of serum malonaldehyde level with time in different groups.

There was a significant decrease in serum lipid peroxidation half hour after intake of tea without milk which tends to normalize after one and half hour of intake of tea. A highly significant decrease in serum lipid peroxidation level in lemon tea group was obtained after half hour of ingestion of tea which tends to normalize after two hours.

However, no significant change in serum lipid peroxidation level in case of tea with milk group was seen after half hour of intake of tea.

DISCUSSION

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and in the progression of several chronic disease. Dietary factors are capable of modulating the oxidative stress through several

mechanism and might therefore play an important role in health protection.

In the present study, we have evaluated the *in vivo* anti oxidative potential of tea by measuring the plasma malonylaldehyde (end product of lipid peroxidation) after ingestion of tea. We have found that after half hour of ingestion of tea without milk, there was a significant decrease in plasma lipid peroxidation, which tends towards original value after one and half hour. After ingestion of lemon tea, there was a much more decrease in plasma lipid peroxidatin after 30, 60 and 90 minutes which tends towards original value after two hours, but there was no significant change in lipid peroxidation level of plasma in cases in ingestion of tea with milk after half and one hour.

The mechanism and site of absorption of polyphenols in human being and their biovailability in general have not been evaluated. We can therefore only speculate that modifications must have taken place after ingestion, in the molecular structure of black tea polyphenols. Green tea polyphenols have been known to influence the formation of DNA strand breaks and lipid peroxidation products in cultured human lung cells. The promptness of the antioxidant response (30–60 minutes) suggest that these modifications and the subsequent absorption of the modified polyphenols must occur in the higher tract of the gastro intestinal system probably starting from the stomach. The condensed polyphenol of black tea (theaflavins and thearubignes) are rapidly broken down in the stomach by acid gastric secretion similar to what happens when lemon juice is added into a cup of black tea. The simpler polyphenols released by the gastric hydrolysis of the theaflavins and thearubigens would thus become available for absorption and for exerting their antioxidant properties.

In vitro, milk protein combine with polyphenols to form complex which retain their antioxidant potential. This complex when ingested becomes resistant to gastric hydrolysis. Thus it becomes unavailable for absorption in upper gastro intestinal tract. So in effect there is very little available antioxidant property from this complex.

Another possible interpretation is the interference with the absorption in the stomach of simple phenolics due to the change in gastric pH following the introduction of milk. Simple phenolics are weak acid compounds and as such easily absorbable in their non ionized form (10). Even a small rise in gastric pH, such as that induced by the milk added to tea, would increase the phenols ionization, thereby reducing their passage through the gastric mucosa.

The interpretation of the present work is that polyphenols are bioactive dietary compounds capable of raising plasma antioxidant defenses in men, black tea which contains condensed polyphenols (theaflavins and thearubigens) have a good antioxidant potential *in vivo*. Furthermore addition of lemon to tea increases its antioxidant potential by breaking condensed polyphenols to simpler ones and by increasing its absorption. Addition of milk to tea forms polyphenols milk protein

complex which increases ionization of polyphenols and thus lowers its absorption through gastric mucosa.

To conclude, black tea without milk and lemon tea have good antioxidant potential *in vivo* than tea with milk. Ingestion of black tea and lemon tea might to some extent help in prevention of many chronic degenerative disease by increasing the antioxidant defence of body, so the ingestion of black tea without milk and lemon tea are much more beneficial than tea with milk.

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