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Biodistribution and Pharmacokinetics of Theaflavin-3,3'-Digallate, the Major Antioxidant of Black Tea, in Mice

[1]S. Maity, A. Ukil, [1]J.R. Vedasiromoni and P.K. Das
Molecular Cell Biology Laboratory, Indian Institute of Chemical Biology,
4, Raja S.C. Mullick Road, Calcutta 700032, India
[1]Department of Drug Development, Indian Institute of Chemical Biology,
4, Raja S.C. Mullick Road, Calcutta 700032, India

Abstract: The present study was conducted to investigate the absorption, distribution and pharmacokinetics of TFDG in mice for which it was labeled with ^{125}I. For comparison, the radiolabeled polyphenol was given either along with Black Tea Extract (BTE) or as pure TFDG. Following intravenous (5 mg kg^{-1}) or intragastric (500 mg kg^{-1}) administration, plasma and tissue levels were quantified by radioactive counting and the results were analysed by the SPSS program. Although lower than intravenous dosing, maximum plasma concentration (C_{max}) for TFDG was achieved at 6 h post-oral dosing with an $AUC_{0-\infty}$ of 504.92 g min L^{-1}, which was 20 fold higher than that for i.v. dosing. Maximum radioactivity (42%) was recovered in kidney following i.v., administration, whereas for oral administration maximum radioactivity (0.07%) was recovered in liver as revealed by tissue distribution studies. Uptake of TFDG was > 4-fold more efficient in hepatocytes than in non parenchymal cells. However, TFDG showed better absorption by various organs as well as by liver cells when given along with BTE. Moreover, a second equal administration of TFDG after 6 h interval enhanced tissue levels of radioactivity above those after a single administration. These results point towards a wide distribution of ^{125}I-TFDG in mouse organs and suggest that frequent consumption of black tea may be better for increased systemic availability of polyphenols.

Key words: Black tea, theaflavin-3,3'-digallate, tissue uptake

INTRODUCTION

Tea is consumed worldwide as black tea, oolong tea and green tea. Recently, there has been an increasing awareness of the potential health benefits of phytochemicals present in beverages and in tea in particular. However, considerable interest has been generated on green tea as a health beverage simply because lot of work has been carried out to document its beneficial effect as antimutagenic (Hung *et al*., 1992; Yen and Chen, 1996), antioxidative (Ho *et al*., 1992; Katiyar *et al*., 1993; Shiraki *et al*., 1994) and antiproliferative agent (Kuo and Lin, 2003). Although black tea is the most widely consumed (80% of the total tea consumption) beverage world-wide, the work done on black tea so far is much less compared to green tea. Tea polyphenols especially catechins of green tea, in particular epicatechins, epicatechin gallate, epigallocatechin and epigallocatechin gallate have been the primary agents responsible for the beneficial and disease-inhibitory activity. The production of black tea involves further processing, during which substantial proportions of catechins are converted to theaflavins and thearubigins by a polyphenol oxidase (Balentine *et al*., 1997). Theaflavins (about 1-2% of the total dry weight of black tea) including theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate (TFDG), possess benzotropolene rings with dihydroxy or trihydroxy systems. Only recently work has been initiated with black tea or its characteristics constituents, theaflavins and thearubigins, which have revealed diverse pharmacotherapeutic effect including hypocholesterolimic (Akinyanju and Yudkin, 1967), hypoglycemic (Gomes *et al*., 1995), anticarcinogenic (Weisburger *et al*., 1994; Schwab *et al*., 2000; Mukhtar and Ahmad, 2000; Kuroda and Hara, 1999) and antiatherosclerotic (Muramatsu *et al*., 1986) effects. It has been shown that black tea can totally protect human red blood cells against oxidative damage brought about by various inducing agents (Halder and Bhaduri, 1998).

Corresponding Author: Dr. Pijush K. Das, Molecular Cell Biology Laboratory, Indian Institute of Chemical Biology,
4 Raja S.C. Mullick Road, Jadavpur, Calcutta 700 032, India
Tel: +91-33-2414-0921 Fax: +91-33-2473-5197
*S. Maity and A. Ukil contributed equally in the manuscript

Parallely, Lin *et al.* (1999) have reported that TFDG can very efficiently down-regulate induction of nitric oxide synthase in stimulated macrophages. In a recent work, black tea has been found to be more efficient than green tea and its individual catechin constituents in proportionate amounts in abrogating production of NO and O_2^- in activated macrophages, of which theaflavin was shown to be the most powerful constituent (Sarkar and Bhaduri, 2001). A major problem in investigating the association of tea consumption with beneficial effects is the lack of quantitative data. Even in studies with animals, mechanistic understanding of the inhibitory effect of tea on various diseases is hampered by insufficient information regarding the absorption, distribution, metabolism and excretion of the effective components of tea.

The objective of the present study is to gain an understanding about the pharmacokinetic properties and bioavailability of TFDG, the major bioactive polyphenol of black tea in mice. The polyphenol was given either in the form of Black Tea Extract (BTE) or as pure TFDG. It was selected as a prototype black tea polyphenol because it possesses the most potent antioxidative activity and it is available in high purity. The *in vivo* disposition behaviour and pharmacokinetic characteristics of TFDG was assessed in this study.

MATERIALS AND METHODS

Animals and reagents: Female BALB/c mice weighing 25-30 g (obtained from National Institute of Nutrition, Hyderabad, India) were used for the experiments. The present study was conducted during October 2004 to April 2005 at Molecular Cell Biology laboratory of Indian Institute of Chemical Biology, Kolkata, India. Mice were housed under normal laboratory conditions i.e., at 21-24°C and 40-60% relative humidity, under a 12 h light/dark cycle with free access to standard rodent food and water. TFDG was isolated from black tea by ethyl acetate fractionation on HPLC according to the method described earlier (Chen and Ho, 1995). All other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of Black Tea Extract (BTE): Ten grams of processed black tea were soaked in 100 mL of boiled distilled water for 5 min and filtered. The filtrate was designated as black tea extract (BTE) (Maity *et al.*, 2001). In our estimate, almost 2.8-3.0 g of dried material is contained in 100 mL of filtered hot water extract of black tea.

Radiolabeling of theaflavin-3-3'-digallate (TFDG): TFDG was radiolabeled using chloramine T and $Na^{125}I$ according to the method described by Hunter (1978). The resulting TFDG was subsequently purified by descending paper chromatography by streaking onto Whatman 31 ET paper (2×40 cm) using 1-butanol: glacial acetic acid : water (12:3:5) as described for the iodination and purification of cAMP and cGMP (Brooker *et al.*, 1979). After chromatography, the paper strips were dried and the desired radioactive spots were eluted in 50 mM sodium acetate buffer (pH 4.75). Authenticity of radiolabeled compound was confirmed by immunoreactivity with anti-TFDG antibody raised in rabbit by conjugating TFDG with bovine serum albumin through 1-ethyl-3(3-dimethylaminopropyl-carbodimide hydrochloride) (EDC) according to Sarkar and Das (1997).

Tissue distribution studies: The mice were fasted overnight but with free access to water. A single intravenous (i.v., 5 mg kg^{-1} containing 10^6 cpm) dose (0.2 mL) of TFDG was given to a group of 6 mice. Similarly, a single oral (500 mg kg^{-1} containing 10^8 cpm) dose (0.5 mL) of TFDG was given to a group of 6 mice. In a separate experiment, a single oral dose of BTE (20 mL kg^{-1}) containing 10^8 cpm of TFDG was given to a group of 6 mice. After indicated time of interval, the liver, kidney, lung, spleen and heart were removed from the groups of mice. Each sample of tissue was washed with 0.9% saline and blotted with filter paper. Radioactivity was measured in the tissues after digestion in 30% KOH solution. After indicated time intervals, blood samples were collected in heparinized tubes from the orbital sinus and counted for blood clearance studies.

Tissue distribution of radioactivity in mice after a duplicate administration of ^{125}I-TFDG: Six hours after the first oral administration of ^{125}I-TFDG (500 mg kg^{-1} containing 10^8 cpm), a second administration of ^{125}I-TFDG with the same amount and radioactivity as the first was given by oral gavage (Sharma *et al.*, 2001). Similarly in a separate experiment, 6 h after the first oral administration of BTE (20 mL kg^{-1}) containing 10^8 cpm TFDG, a second administration of the same amount of BTE and ^{125}I-TFDG was given by oral gavage. At indicated time intervals, the radioactivity of various agents were measured as described above. Total radioactivity after two administrations were compared with that after a single administration.

Isolation of liver cell types: Parenchymal and Kupffer cells were obtained by perfusion of liver *in situ* according to the method described by Murray (1987). Radioactive counting for the cell suspensions was done in a gamma counter.

Table 1: Blood pharmacokinetic parameters of TFDG after oral (500 mg kg^{-1}) and intravenous (5 mg kg^{-1}) administration

	Oral administration		Intravenousadministration
Parameters	TFDG	BTE + TFDG	TFDG
C_{max} (μg mL^{-1})	226.61±31.2	293.01±42.3	476.53±52.2
T_{max} (h)	6±0	6±0	-
$t1/_{2\lambda z}$ (h)	23.33±2.5	22.94±2.3	3.97 ± 0.4
$AUC_{0-\infty}$ (g min L^{-1})	504.90±57.3	670.01±70.5	24.70±3.2

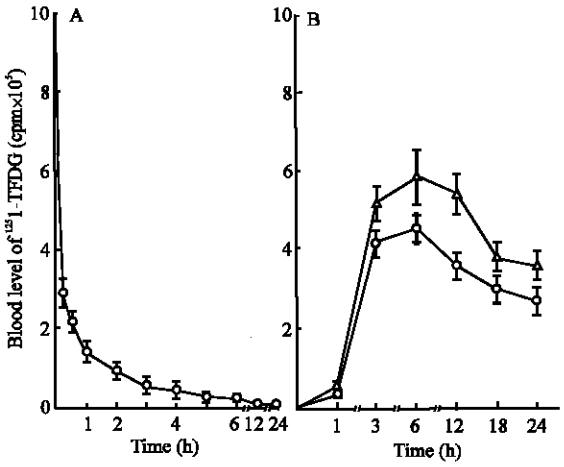

Fig. 1: A Blood levels of radioactivity versus time profile of TFDG after a single i.v. (5 mg kg^{-1} containing 10^6 cpm; 0.2 mL) administration. B: Blood levels of radioactivity versus time profile of TFDG after a single i.g. administration of BTE (20 mL kg^{-1} containing 10^8 cpm TFDG; Δ-Δ) or pure TFDG (500 mg kg^{-1} containing 10^8 cpm; o-o). Values are mean±SDof 6 mice for each group

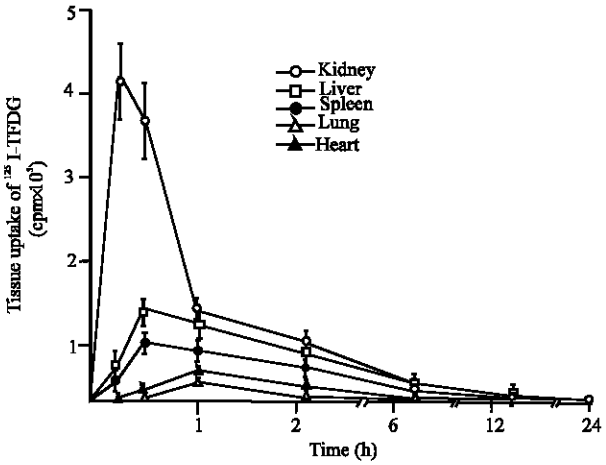

Fig. 2: Distribution of radioactivity in mice tissues after a single i.v. (5 mg kg^{-1} containing 10^6 cpm; 0.2 mL) administration. Values are mean±SD of 6 mice for each group

Statstical analysis: The significance of the data was evaluated by the two-tailed t test.

RESULTS

Blood clearance of TFDG: Figure. 1A shows the blood concentration profiles for TFDG after intravenous administration into mice. ^{125}I labeled TFDG was rapidly eliminated biexponentially from the circulating blood, with very low/undetectable levels at 12 h after dosing. The initial distribution was rapid relative to terminal elimination; elimination half-life ($t_{1/2\lambda z}$) for TFDG was almost 4 h. In clear contrast to the intravenous dose of 5 mg kg^{-1} TFDG, a much higher oral dose (500 mg kg^{-1}; 10^8 cpm) was required to achieve reasonable peak concentrations (C_{max}) (Table 1). Still the value was almost half compared to that for intravenous C_{max} (Fig. 1B). In addition, the time to reach C_{max} (T_{max}) was approximately 6 h for TFDG suggesting a slow rate of absorption.

Despite the lower C_{max} estimates observed under the two dosing conditions, systemic exposure ($AUC_{0-\infty}$) of TFDG was 20 fold higher with the oral dosing. In case of oral administration, higher C_{max} and $AUC_{0-\infty}$ were obtained when TFDG was given along with BTE suggesting better absorption by this combination.

Tissue distribution studies: The rapid clearance of ^{125}I-TFDG after intravenous administration resulted perhaps from its substantial accumulation by the kidney and to a lesser extent by the liver and spleen, as evidenced by tissue distribution studies (Fig. 2). After 30 min, 36% of the administered radioactivity was recovered in the kidney, 12% in the liver and 7.5% in the spleen. Maximum uptake of ^{125}I-TFDG was 15 min after i.v., administration for kidney, 30 min for liver and spleen and 1 h for heart and lung. However, in each case there was a subsequent gradual decrease in the amount of ^{125}I-TFDG and by 12 h the levels were almost undetectable in all the organs. In case of oral administration (Fig. 3A), maximum uptake of ^{125}I-TFDG for all the organs was 6 h showing a much slower clearance. After 6 h, 0.07% of the administered radioactivity was recovered in the liver, 0.02% in the kidney and 0.015% in the spleen. Maximum

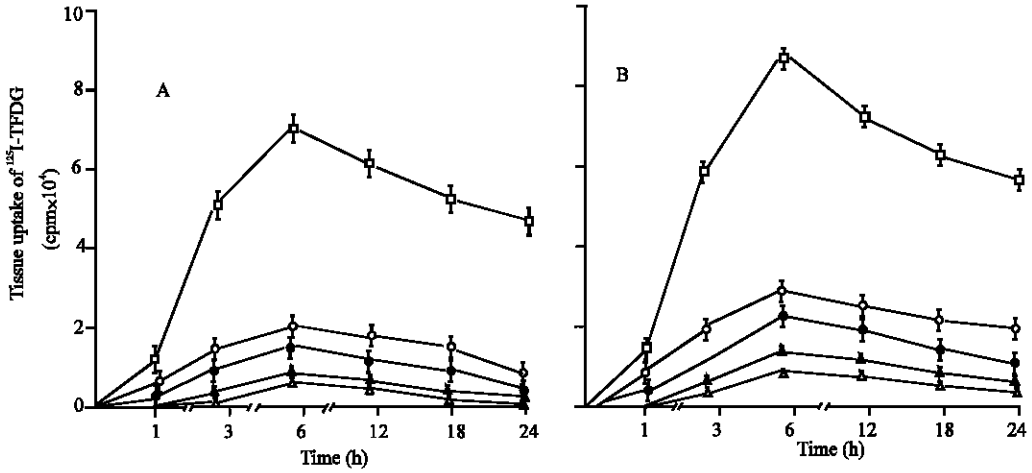

Fig. 3: Distribution of radioactivity in mice tissues after a single oral administration of (A) TFDG (500 mg kg^{-1} containing 10^8 cpm; 0.5 mL) and (B) BTE (20 mL kg^{-1} containing 10^8 cpm TFDG). Values are mean±SD of 6 mice for each group

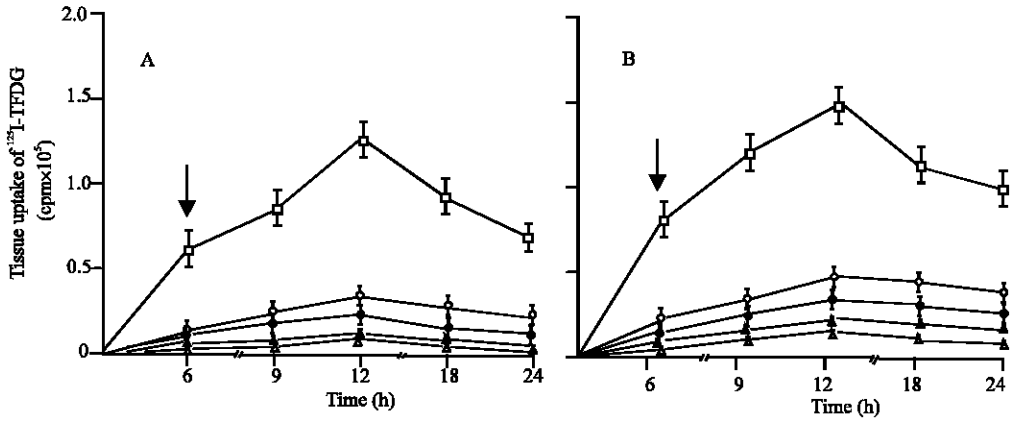

Fig. 4: Distribution of radioactivity in mice tissues after a duplicate oral administration of (A) TFDG and (B) BTE + TFDG. Each mouse was given either TFDG (500 mg kg^{-1} containing 10^8 cpm) or BTE (20 mL kg^{-1} containing 10^8 cpm TFDG). At 6 h after the first administration, a second of the same dose of either TFDG or BTE was given. Samples of each organ were taken for measurement of radioactivity. Arrow indicates the time of duplicate administration. Values are mean±SD of 6 mice for each group

uptake of ^{125}I-TFDG was observed in the liver when administered orally suggesting better systemic absorption. Moreover, unlike i.v. administration, in case of oral administration a steady detectable levels of ^{125}I-TFDG was found in all organs as measured up to 24 h.

Tissue distribution of radioactivity after a duplicate administration of ^{125}I-TFDG: Based on the fact that most individuals drink tea at least twice a day, we performed an experiment to determine whether a duplicate administration of ^{125}I-TFDG would enhance radioactivity levels in different organs. A second equal administration of ^{125}I-TFDG increased radioactivity in the liver by as much as 1.8 times over a single administration at 6 h after the first administration (Fig. 4A). In case of both the first and second administration, maximum uptake of ^{125}I-TFDG was 6 h after administration of the dose. Increases >1.5 fold were found in the spleen, kidney, lung and heart at 6 h after the second administration. In addition, increased incorporation was also observed at 24 h after a second administration compared with that at 24 h after a single administration. These results indicate that drinking black tea twice a day increases the level of tea polyphenols in various organs, suggesting that

Table 2: Hepatic cellular localization of ^{125}I-TFDG from each type of administration in mice[a]

Experiment	Radioactivity cpm/10^8 cells±SD		
	Parenchymal	Nonparenchymal	Parenchymal:Nonparenchymalcells
TFDG i.v. single administration	3300±520	480±120	7.3
TFDG i.g. single administration	600±160	120±80	6.0
TFDG + BTE i.g. single administration	920±200	200±110	4.5
TFDG i.g. duplicate administration	1200±240	280±120	4.8
TFDG + BTE i.g. duplicate administration	1520±280	640±125	5.0

[a]Radioactivity was determined 30 min after i.v. administration and 6 h after i.g. administration in parenchymal (PC) and non-parenchymal (NPC) cells. Each value represents the mean±SD of 3 separate determinations

a relatively high concentration of tea polyphenols can be maintained with the usual Indian lifestyle. Moreover, it has further been observed that TFDG displayed better uptake by tissues when given to mice along with BTE, in comparison to when it was given as pure TFDG (Fig. 3B and 4B). Based on the AUC and C_{max} values for TFDG, BTE seems to deliver TFDG more effectively than when TFDG was given as a pure compound. The molecular basis for this absorption difference is not known. It is possible that interaction between TFDG and other components in BTE may increase the absorption of TFDG.

Uptake of TFDG by mouse liver cells: To ascertain whether administered ^{125}I-TFDG is incorporated into cells of various organs, parenchymal and non-parenchymal mouse liver cells were separated after i.v., as well as i.g., administration of ^{125}I-TFDG and the counts were measured. The distribution in parenchymal and non-parenchymal cells was studied 15 min following i.v., administration and 6 h following i.g. administration. It was found that hepatocytes were 4 fold more efficient than non-parenchymal cells (Table 2) in the assimilation of ^{125}I-TFDG, as evidenced by the comparison of radioactivity per mg cell protein. This distribution pattern of TFDG indicated non-specific passive transport to various liver cells; uptake was higher in hepatocytes than in non-parenchymal cells, possibly because of the variation in their size, shape and disposition. Similar distribution ratio of TFDG in parenchymal and non-parenchymal cells was found when given either in BTE or as pure TFDG.

DISCUSSION

The leap of green tea over black tea in terms of health benefit is basically a walkover as the data on beneficial effects of black tea on health are scanty. However, in a recent study employing an *In vitro* macrophage system, it was shown that black tea is as good and if not better than green tea in scavenging superoxide ion and in abrogating NO production and that theaflavin of black tea is the most efficient component (Sarkar and Bhaduri, 2001). Clearly, the enzymatic biotransformation of monomeric catechins and their gallates to theaflavins during the processing of dried green leaves for the production of black tea does not in any way adversly affect the chemopreventive properties of black tea. The possible beneficial effects of black tea are receiving a great deal of attention, particularly in India, considering its wide consumption worldwide. Information on this bioavailability and disposition of polyphenols such as theaflavins and thearubigins is important for understnading the biological effects of black tea. To our knowledge, this is the first report on the biodistribution and pharmacokinetics of TFDG, which was shown to be the most potent antioxidant component of black tea polyphenols. Although the radioactivity distribution based on ^{125}I label does not differentiate between ^{125}I-TFDG itself or its metabolites and protein-bound forms, the experiments with ^{125}I-TFDG revealed that the major organs like liver, kidney, spleen, lung and heart showed significant incorporation of radioactivity after oral administration of ^{125}I-TFDG. The oral route of administration happened to be better than intravenous route in terms of bioavailability as judged by $AUC_{0-\infty}$ values.

Moreover, TFDG along with BTE seemed to be taken up more readily by various organs when compared with pure TFDG as evidenced from the C_{max} and $AUC_{0-\infty}$ values. Similar to green tea where catechins are known to bind with proteins tightly (Doss *et al*., 2005), it is possible that other components in BTE compete with TFDG for binding with plasma and tissue proteins, thus changing the TFDG pharmacokinetic behaviour. Other tea components in BTE may also result in different rates of TFDG glucuronidation and sulfation, which are known to be the major elimination pathways of tea polyphenols (Lee *et al*., 2002) as studied in case of green tea. The competition or interference among tea polyphenols for glucuronosyl transferase and sulfotransferase may also result in different pharmacokinetic pattern when TFDG was given along with BTE.

Studies on isolated liver cell types indicated that radioactivity was incorporated into cells of liver but that this incorporation was not equal in all cells. In fact, the increased uptake in hepatocytes compared to non-parenchymal cells indicates nonspecific passive transport as hepatocytes are much larger in shape and size than non-parenchymal cells. The extent of TFDG uptake by liver cell types was higher when given along with BTE suggesting that whole black tea may be more effective than pure TFDG. Moreover, the results of duplicate administration of TFDG suggest that frequent consumption of black tea enables the body to maintain a high level of tea polyphenols. Taken together, this study is the first pharmacolgical evidence as far as black tea is concerned, of a wide distribution of TFDG in mouse organs, indicating a similar wide range of target organs for beneficial antioxidative effects in humans.

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REFERENCES

Akinyanju, P. and J. Yudkin, 1967. Effects of coffee and tea in serum lipids in rats. Nature, 214: 570-577.

Brooker, G., J.F. Harper, W.L. Terasaki and R.D. Moylan, 1979. Radioimmunoassay of cyclic AMP and cyclic GMP. Adv. Cyclic Nucleotide Res., 10: 1-33.

Balentine, D.A., S.A. Wiseman and L.C. Bouwens, 1997. The chemistry of tea flavonoids. Crit. Rev. Food Sci. Nutr., 37: 693-704.

Chen, C.W. and C.T. Ho, 1995. Antioxidant properties of polyphenols extracted from green and black teas. J. Food. Lipids, 2: 35-46.

Doss, M.X., S.P. Potta, J. Hescheler and A. Sachinidis, 2005. Trapping of growth factors by catechins: a possible therapeutical target for prevention of proliferative diseases. J. Nutr. Biochem., 16: 259-266.

Gomes, A., J.R. Vedasiromani, M. Das and D.K. Ganguly, 1995. Antihyperglycemic effect of black tea (*Camellia sinensis*) in rat. J. Ethnopharmacol., 45: 223-226.

Hunter, W.M., 1978. Radioimmunoassay. In: Handbook of Experimental Immunology Ed.Weir, D. M., pp: 14.1-14.4. Blackwell Scientific Publication, Oxford.

Ho, C.T., Q. Chen, H. Shi, K.Q. Zhang and R.T. Rosen, 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev. Med., 21: 520-525.

Hung, M.T., C.T. Ho, Z.Y. Wang, T. Ferraro, T. Finnegan-Olive, Y.R. Lou, J.M. Mitchell, J.D. Laskin, H. Newmark, C.S. Yang and A.H. Conney, 1992. Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. Carcinogenesis, 13: 947-954.

Halder, J. and A.N. Bhaduri, 1998. Protective role of black tea against oxidative damage of human red blood cells. Biochem. Biophys. Res. Commun., 244: 903-907.

Katiyar, S.K., R. Agarwal, M.T. Zaim and H. Mukhtar, 1993. Protection against *N*-nitrosodiethylamine and benzo[*a*]pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. Carcinogenesis, 14: 849-855.

Kuroda, Y. and Y. Hara, 1999. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat. Res., 436: 69-97.

Kuo, P.L. and C.C. Lin, 2003. Green tea constituent (-)-epigallocatechin-3-gallate inhibits Hep G2 cell proliferation and induces apoptosis through p53-dependent and Fas-mediated pathways. J. Biomed. Sci., 10:219-27.

Lin, Y.L., S.H. Tsai, S.Y. LinShiau, C.T. Ho and J.K. Lin, 1999. Theaflavin-3,3'-digallate from black tea blocks the nitric oxide synthase by down-regulating the activation of NF-kappa B in macrophages. Eur. J. Pharmacol., 367: 379-388.

Lee, M.J., P. Maliakal, L. Chen, X. Meng, F.Y. Bondoc, S. Prabhu, G. Lambert, S. Mohr and C. S. Yang, 2002. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. Cancer Epidemiol. Biomarker Prevention, 11: 1025-1032.

Muramatsu, K., K. Fukuyo and Y. Hara, 1986. Effect of green tea catechins on plasma cholesterol level in cholesterol fed rat. J. Nutr. Sci. Vit., 32: 613-622.

Murray, G.J, 1987. Lectin-specific targeting of lysosomal enzymes to reticuloendothelial cells. Methods. Enzymol., 149: 25-42.

Mukhtar, H. and N. Ahmad, 2000. Tea polyphenols: prevention of cancer and optimizing health. Am. J. Clin. Nutr., 71 (Suppl. 6): 1703-1704.

Maity, S., J.R. Vedasiromoni, L. Chaudhuri and D.K. Ganguly, 2001. Role of reduced glutathione and nitric oxide in the black tea extract-mediated protection against ulcerogen-induced changes in motility and gastric emptying in rats. Jpn. J. Pharmacol., 85: 358-364.

Shiraki, M., Y. Hara, T. Osawa, H. Kumon, T. Nakauama and S. Kawakishi, 1994. Antioxidative and antimutagenic effects of theaflavins from black tea. Mutat. Res., 323: 29-34.

Sarkar, K. and P.K. Das, 1997. Protective effect of neoglycoprotein-conjugated muramyl dipeptide against *Leishmania donovani* infection. J. Immunol., 158: 5357-5365.

Schwab, C.E., W.W. Huber, W. Parzefall, G. Hietsch, F. Kassie, R. Schulte-Hermann and S. Knasmuller, 2000. Search for compounds that inhibit the genotoxic and carcinogenic effects of heterocyclic aromatic amines. Crit. Rev. Toxicol., 30: 1-69.

Sarkar, A. and A.N. Bhaduri, 2001. Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: comparison with its individual catechin constituents and green tea. Biochem. Biophys. Res. Commun., 284: 173-178.

Sharma, R.A., C.R. Ireson, R.D. Verschoyle, K.A. Hill, M.L. Williams, C. Leuratti, M.M. Manson, L.J. Marnett, W.P. Steward and A. Gescher, 2001. Effects of dietary curcumin on glutathione S-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: relationship with drug levels. Clin. Cancer Res., 7: 1452-1458.

Weisburger, J.H., M. Nagao, K. Wakabayashi and A. Oguri. 1994. Prevention of heterocyclic amine formation by tea and tea polyphenols. Cancer Lett., 83:143-147.

Yen, G.C. and H.Y. Chen, 1996. Relationship between antimutagenic activity and major components of various teas. Mutagenesis, 11: 37-41.