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PROTECTION BY BLACK TEA EXTRACT AGAINST CHROMOSOME DAMAGE INDUCED BY TWO HEAVY METALS IN MICE

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ABSTRACT

The efficacy of infusion of black tea leaf, Camellia sinensis *(Linn.) O.Kuntze (Theaceae), in reducing the cytotoxic effects of two heavy metal salts, chromium and arsenic, was tested in bone marrow cells of mice following dietary administration. Mice were given black tea infusion by gavage twice daily, in concentrations simulating human consumption, for 6 days. On day 7 after treatment with tea, separate sets of mice were given single doses of potassium dichromate and sodium arsenite, and then killed after 24 h. Control sets were treated with potassium dichromate or sodium arsenite separately and also observed after 24 hours. The concentration of each salt corresponded to 1/10th of its LD_{50} value. Chromosomes were studied from bone marrow cells following the usual colchicine-hypotonic-fixation-air-drying Giemsa schedule. Both metallic salts were highly clastogenic when given alone, inducing a high frequency of chromosomal aberrations as compared with distilled water. Tea alone, given twice daily for 6 days, was not clastogenic. When mice, given tea twice daily for previous 6 days, were treated with either of the two salts on day 7, the degree of chromosome damage induced was reduced significantly as compared with the salts given alone. This reduction was more significant for sodium arsenite as compared with potassium dichromate. Such protection against arsenic cytotoxicity by prolonged dietary administration of black tea infusion is of importance in view of the widespread exposure of human populations to arsenic damage through drinking water from tubewells in West Bengal, India and adjoining Bangladesh.*

Keywords: Tea chemoprotection, anticlastogenic effects, arsenic, chromium.

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INTRODUCTION

Tea is a well known beverage, brewed from the leaf of *Camellia sinensis* (Linn.) O.Kuntze (Theaceae) and widely consumed throughout the world. The plant types are of two main groups: China tea and Assam tea, which differ in growth pattern, leaf morphology, chemical composition and adaptation to climate. Commercial tea is available in several forms, amongst which black tea is most prevalent in India and green tea in China and Japan. Within the past decade, consumption of tea has been associated with antimutagenic and possible anti-carcinogenic effects (Blot et al., 1997; Dreosti et al., 1997; Kada, 1983; Yang, 1997; Yang & Wang, 1993). The effects have been attributed to the combined effects of a large number of antioxidants in the beverage.

Our earlier investigations have shown that black tea reduced chromosome-damaging (clastogenic) effects of chromium, a known clastogen, when given for prolonged periods, simulating human consumption in a mice model (Mukherjee et al., 1997). The present investigation was undertaken to study the effects of black tea in reducing the cytotoxic effects of inorganic arsenic in mice when given as a regular supplement. This work is of special importance in view of the widespread exposure of human populations in several districts of West Bengal, India and Bangladesh to arsenic through drinking water from deep tubewells. The problem has assumed epidemic proportions, leading to different arsenic related diseases including skin lesions and even cancer in human populations. The work was carried out on mice and a comparison was made with the degree of protection against inorganic chromium, to which human populations are exposed through effluents from a large number of open tanneries in Calcutta.

MATERIALS AND METHODS

Chemicals and Plant Products

Plant extract. Tea infusion was prepared by brewing black Tata Tea (Premium CTC leaf) in boiling distilled water in the usual method of tea preparation for human consumption. The final concentration was 1.756 mg of tea in 0.16 ml of distilled water, simulating an intake of one teaspoonful of leaves per cup, by a 60 kg person. Tea was infused for 5 min and the decanted liquid cooled and used for the experiments.

Clastogens used. (i) A hexavalent chromium compound, potassium dichromate ($K_2Cr_2O_7$; mol. wt. 294.21; Cr 35.36%, E. Merck, India), a major component of tannery effluents, was dissolved in distilled water (20 mg/10 ml) and administered at a dose of 20 mg/kg bw (corresponding to 1/10 LD_{50} of the metallic salt). Clastogenic effects of this compound and its reduction by dietary supplementation had been earlier observed by our group in a mammalian system (Sarkar et al., 1996; Singh et al,. 1990).

(ii) Sodium (III) meta arsenite ($NaAsO_2$; mol. wt. 129.92, As 57.6%, Loba Chemie, Bombay, India, CAS No. 7784-46-5). The concentration used was 2.5 mg/kg bw in distilled water (corresponding to 1/10 of the LD_{50} of the salt).

Experimental Protocol

The entire experiment was divided into six groups (I-VI) according to the schedule given in Table 1. Five mice were used for each experimental set. A negative control set (Set I) was maintained by feeding the mice distilled water for 7 consecutive days. Mice of set II were treated with tea infusion twice daily for 7 consecutive days. Tea was prepared fresh each day, cooled to room temperature and then fed to the animals by gavage. Set III mice were treated with potassium dichromate solution alone by gavage and the effects observed after 24 h. Set IV mice were given tea infusion twice daily for 6 consecutive days, gavaged with potassium dichromate on day 7, and killed after 24 h. Set V mice were given sodium arsenite solution alone and the effects observed after 24 h. Mice of Set VI were primed with tea infusion twice daily for 6 consecutive days and gavaged with sodium arsenite solution on day 7 and killed after 24 h.

Mice from all the six sets were injected (ip) with a 0.04% colchicine solution 2 h prior to killing by cervical dislocation. Bone marrow cells from the femurs were flushed out and prepared for examination of chromosomal spreads following the usual schedule of pre-treatment in hypotonic solution and fixation in acetic acid-ethanol (1:3, v/v) and air-dried (Sharma & Sharma, 1994). Slides were stained in Giemsa solution.

The slides were subsequently coded and scored blind. 100 well-scattered metaphase plates were scanned from each animal. The endpoints scored were chromosomal aberrations which included chromatid and chromosome breaks and rearrangements. For computing the chromosomal aberrations/cell (CA/cell), each chromatid break was taken as one break, while each isochromatid/chromosome break or rearrangement was taken as two breaks. Gaps were not included (Tice et al., 1987).

Statistical Analysis

The data were analysed by one-way ANOVA (with replication) (Sokal & Rohlf, 1973) followed by Duncan's multiple range test (Harter, 1960; Kotz & Johnson, 1982) to compare the significance of differences among the different experimental sets. Student's *t*-test (Fisher & Yates, 1963) was carried out to compare the effects of different treatments with their control sets.

RESULTS

The endpoints scored are tabulated in Tables 2 and 3. One way ANOVA tables followed by Duncan's multiple range test (Tables 3A, 3B) indicate the relationship between the effects of the metals given singly and protection by dietary administration of tea. Total chromosomal aberrations include both breaks and rearrangements. The data indicated that:

(i) Both distilled water (set I) and tea infusion (set II), given twice daily for 7 days by gavaging to mice in doses simulating human consumption, do not induce any chromosomal aberrations.

(ii) Both sodium arsenite (set V) and potassium dichromate (set III), when administered singly to mice and observed after 24 h, are highly clastogenic, as shown by the significantly high number of chromosomal aberrations induced.

(iii) When mice primed with the tea infusion twice daily for 6 days are then given potassium dichromate (set IV) on day 7 and observed after 24 h, the number of chromosomal aberrations is significantly reduced, as compared with the effects of potassium dichromate alone (set III). Such chemoprotection by tea against

Table 1. Experimental protocol.

Set No.	Chemicals	Followed by	Observed after
I.	Distilled water	–	7 days
II.	Tea infusion (twice daily)	–	7 days
III.	Potassium dichromate	–	24 h
IV.	Tea infusion (twice daily) for 6 days	Pot. dichromate on day 7	24 h
V.	Sodium arsenite	–	24 h
VI.	Tea infusion (twice daily) for 6 days	Sodium arsenite on day 7	24 h

Table 2. Effects of the different treatments as measured by chromosomal aberrations.

Set No.	Chemicals administered	Chromosomal aberrations in 100 cells			
		B'	B"	RR	Total
I	Distilled water	–	–	–	–
II	Tea infusion (twice daily)	–	–	–	–
III	Potassium dichromate	3.4	–	3.2	6.6
IV	Tea infusion (twice daily) followed by pot. dichromate on day 7	4.76	–	–	4.76
V	Sodium arsenite	5.8	0.53	–	6.33
VI	Tea infusion (twice daily) followed by sodium arsenite on day 7	1.1	–	–	1.1

B', B" = Chromatid and isochromatid/chromosome break, respectively.
RR = Rearrangements.

Table 3a. One-way ANOVA table for effects of sodium arsenite.

Source of variation	df	SS	MSS	F
SS between groups	3	24	8	24.24***
SS within groups	12	4	0.33	

Duncan's Multiple range test

Experimental groups	Distilled water	Tea infusion (twice daily)	Tea infusion (twice daily) →Sodium arsenite	Sodium arsenite
Sample means	0	0	1	3

Table 3b. One-way ANOVA table for effects of potassium dichromate.

Source of variation	df	SS	MSS	F
SS between groups	3	178.95	59.65	190.88***
SS within groups	16	5	0.3125	

Duncan's Multiple range test

xperimental groups	Distilled water	Tea infusion (twice daily)	Tea infusion (twice daily) →Potassium dichromate	Potassium dichromate
Sample means	0	0	5.2	6.6

Note: The straight lines denote insignificant differences between the means at $p = 0.05$ level.
*** $p = 0.001$.

potassium dichromate had been earlier observed by us (Mukherjee et al., 1997).

(iv) When mice primed with the tea infusion for 6 days are given sodium arsenite on day 7 and observed after 24 h (set VI), there is a drastic reduction in the number of chromosomal aberrations, as compared with the mice given sodium arsenite alone (set V). This reduction is significantly greater than that observed with potassium dichromate.

Therefore, administration of black tea extract twice daily for 6 days gives significantly high protection against the clastogenic (chromosome-damaging) effects of chromium and arsenic administered on day 7, the protection being much greater against arsenic.

DISCUSSION

The high arsenic content in groundwater in several districts of West Bengal, India and adjoining Bangladesh has affected millions of human beings through drinking water from deep tubewells and is described as the largest arsenic casualty in the world. The average concentration of arsenic in potable groundwater is reported to be 0.2 mg/l, reaching a maximum of 3.7 mg/l in the affected areas (Mandal et al., 1996; Chakraborty et al., 1998). These values are greatly above the permissible limit of arsenic cencentration in groundwater, given by WHO (1981) as 0.05 mg/l. Attempts are being made by our group to protect against the effects of arsenic in drinking water through dietary supplementation. Raw garlic extract has reduced the chromosome damage in mice but since garlic itself is mildly clastogenic, the effects have been reduced to the level of garlic (Das et al., 1993; Roychoudhury et al., 1997). Dietary intervention with the crude extract of *Emblica officinalis* is much more effective in counteracting clastogenic effects of arsenicals (Biswas et al., 1998, in press). In the present investigation, administration of black tea infusion daily for 6 days in doses simulating human consumption has reduced the effects of a relatively moderate concentration of sodium arsenite (1/10 of LD_{50}) in a highly significant manner.

The principal chemical constituents of tea leaf are (i) carbohydrates like sugar, starch, pectins, pectosans, crude fibre (cellulose, lignin); (ii) proteins, including the aminoacid theanine, unique to tea and others like glycine, alanine, valine, leucine, isoleucine, methionine and phenylalanine; (iii) lipids, mainly phospholipid, glycolipid, sulpholipid and triglycerides, in addition to saponins and esterified steroids and terpenoids. The unsaturated fatty acids, linoleic and linolenic acids, are derived from phospholipids; (iv) polyphenols are the most important chemical components, including epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin and gallocatechin. The relative amounts change with age. During fermentation of the tea leaves, 10% of the flavonols are converted into theaflavine, theaflavic acid and bisflavanols and 75% into thearubigens; (v) caffeine, forming 2.5 to 4.4% of dry weight of leaf, along with theobromine and theophylline; (vi) enzymes like polyphenol oxidase, peroxidase, peptidase, chlorophyllase, acid phosphatases, alcohol dehydrogenases, and others; (vii) volatile and flavour compounds (Ganguly, 1996; Graham, 1992).

The pharmacological effects of green tea have been attributed to the presence of polyphenols (Bokuchava & Skobeleva, 1980), including antimutagenesis and anticarcinogenesis (Kada, 1983; Taniguchi et al., 1992), mainly due to their anti-oxidative and free radical scavenging properties. Shiraki et al. (1994) have also suggested that the antioxidant properties of black tea can be related to the presence of theaflavins.

The present investigation has:

(i) confirmed that continued dietary administration of black tea infusion protects against the chromosome damaging effects of potassium dichromate to a statistically significant level.

(ii) continued dietary administration of black tea infusion has reduced the clastogenic effects of sodium arsenite in a highly significant manner. The protection against arsenic salt is much greater than potassium dichromate. The mode of protection may be attributed to the combined anti-oxidant and scavenging properties of the components of tea infusion as mentioned above.

The results are significant in view of the widespread effects of arsenic toxicity and to a lesser extent, of chromium toxicity, on human populations exposed to these metals through drinking water. Black tea infusion is a common everyday drink and can be utilised for protection of human populations.

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REFERENCES

Biswas S, Talukder G, Sharma A (1998): Protection against cytotoxic effects of arsenic by dietary supplementation with crude extract of *Emblica officinalis* fruit. *Phytotherapy Res*. (in press).

Blot WJ, McLaughin JK, Chow WH (1997): Cancer rates amongst drinkers of black tea. *Crit Rev Food Sci Nutr 37:* 739–760.

Bokuchava MA, Skobeleva NI (1980): CRC Critical Reviews. *Food Sci and Nutr 12:* 303–312.

Chakraborty D, Samanta G, Mandal BK, Roy Chowdhury T, Chanda CR, Biswas BK, Dhar RK, Basu GK, Saha KC (1998): Calcutta's industrial pollution: groundwater arsenic contamination – an eight year study report. *Current Sci 74:* 346–354.

Das T, Roychoudhury A, Sharma A, Talukder G (1993): Modification of cytotoxic effects of inorganic arsenic by crude extract of *Allium sativum* in mice. *Int J Pharmacog 31:* 316–320.

Dreosti IE, Wargowich MJ, Yang CS (1997): Inhibition of carcinogenesis by tea: the evidence from experimental studies. *Crit Rev Food Sci Nutr 37:* 761–770.

Fisher RA, Yates F (1963): *Statistical Table for Biological, Agricultural and Medical Research*, 6th ed., Oliver and Boyd, Edinburgh.

Ganguly SN (1996): Chemical constitutes in tea and role during tea processing. *Teage 1:* 15–16.

Graham HN (1992): Green tea composition, consumption and polyphenol chemistry. *Prev Med 21:* 334–350.

Harter HL (1960): Critical values for Duncan's new multiple range test. *Biometrics 16:* 671–685.

Kada T (1983): Environmental and biological factors suppressing induction of mutations. *Toxicol Forum 6:* 580–589.

Kotz S, Johnson NL (1982): Editors. *Encyclopedia of Statistical Sciences 2:* 424–425, Wiley, New York.

Mandal BK, Roy Chowdhury T, Samanta G, Basu GK, Chowdhury PP, Chanda CR, Lodh D, Karan NK, Dhar RK, Tamili DK, Das D, Saha KC, Chakraborti D (1996): Arsenic in groundwater in seven districts of West Bengal, India. *Current Sci 70:* 976–986.

Mukherjee P, Sarkar D, Sharma A (1997): Effect of dietary consumption of black tea infusion alone and combinations with known clastogens in mice *in vivo. Food Chem Toxicol 35:* 657–661.

Roychoudhury A, Das T, Sharma A, Talukder G (1997): Inhibition of clastagenic effects of arsenic through continued oral administration of garlic extract in mice *in vivo. Mutation Res 392*: 237–242.

Sarkar D, Sharma A, Talukder G (1996): Protection afforded by crude spinach beet leaf extract and equivalent amounts of chlorophyll and chlorophyllin against clastogenic effects of potassium dichromate in mice *in vivo*. *Int J Pharmacog 34:* 58–63.

Sharma AK, Sharma A (1994): *Chromosome Techniques: a Manual*. Harwood Academic Publishers, Basel.

Shiraki M, Hara Y, Osawa T, Kumon H, Nakayama T, Kawakishi S (1994): Antioxidative and antimutagenic effects of theaflavins from black tea. *Mutation Res 323*: 29–34.

Singh CBP, Sharma A, Talukder G (1990): Effects of chromium on cellular systems in animals. *Nucleus 33*: 84–106.

Sokal RR, Rohlf FJ (1973): Introduction to analysis of variance. In *Introduction to Biostatistics*, edited by D Kennedy and RB Park, 134–160. Freeman, San Francisco, CA.

Taniguchi S, Fujiki H, Kobayashi H, Go H, Miyado K, Sadano H, Shimokawa R (1992): Effect of (-)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. *Cancer Lett 65*: 51–54.

Tice RR, Boucher R, Luke CA, Shelby MD (1987): Comparative cytogenetic studies of bone marrow damage induced in male B6C3F mice by multiple exposures to gaseous 1,3-butadiene. *Envir Mutagenesis 9*: 235–250.

WHO, World Health Organisation (1981): Arsenic. *Environ Health Criteria 18*: Arsenic, International Programme on Clinical Safety, Geneva.

Yang CS (1997): Inhibition of carcinogenesis by tea. *Nature 383*: 134.

Yang CS, Wang ZY (1993): Tea and Cancer. *J Natl Cancer Inst 85*: 1038–1049.

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