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Chyawanprash Awaleha: A Genoprotective Agent for Bidi Smokers

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KEY WORDS Chromosomal aberrations; sister chromatid exchanges; genoprotection; *chyawanprash awaleha;* bidi smokers.

ABSTRACT Genoprotective efficacy of *Chyawanprash awaleha* (Dabur) was investigated against tobacco smoke on the somatic chromosomes of 25 male bidi smokers. 25 individuals matched with regard to age, sex and social status constituted the control. 20 gms of *Chyawanprash awaleha* (Cp) was fed to bidi smokers for 2 months, twice a day. Bidi smokers were compared with Cp-fed bidi smokers. Mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges (SCE) and satellite associations (SA) were analysed. In Cp-fed bidi smokers as compared with bidi smokers all the parameters showed a significant decrease ($P<0.01$). The frequency of CA (1.00) practically came down to the level of controls (0.88) indicating that *Chyawanprash* can minimise the genotoxic risk caused by mutagenic agents present in tobacco smoke.

INTRODUCTION

Chyawanprash awaleha, an *Ayurvedic rasayan* formulation, has been used in India as a health food continuously with the same vigour and enthusiasm for the past 4000 years (Samhita 1988). It is prepared from more than 40 herbs (Table 1). Many aspects of this formulation have been studied to corroborate its therapeutic use as described in ancient texts and traditions (Ojha et al. 1973;Verma et al. 1973; Alam et al. 1977; Ojha et al. 1988; Alam et al. 1989; Mehrotra 1995). In clinical trials it improved memory and produced significant weight gain (Ojha 1988). Above all, Chyawanprash is well recognised for alleviating geriatric complaints (Alam et al. 1977).

Vitamin C (L-ascorbic acid) has been known to have antioxidant properties (Block 1991). It has been reported to substantially reduce the risk of genetic injury caused by exposure to benzene (Aldashev et al. 1980), benzo (a) pyrene (Kallistratos and Passke 1980), halogenated ethers (Sram et al. 1982) and coal-tar (Sram et al. 1983). Because of the trend to find antimutagens and anticarcinogens in diet, *Chyawanprash awaleha* was investigated owing to its continuous use as a tonic, and primarily for its contents of *Phyllanthus emblica*, a rich source of antioxidants.

The present *in vitro* cytogenetic study has been used to investigate the genoprotective efficacy of *Chyawanprash awaleha* against tobacco smoke through sister chromatid exchanges (SCE) and chromosomal aberrations (CA) as major parameters and mitotic index (MI) and satellite associations (SA) as supporting ones.

MATERIALS AND METHODS

This study comprised 25 bidi smokers, all male, and an equal number of control individuals matched with respect to age, sex and social status. They belonged to the lower strata of the society. None of the individuals consumed alcohol or take any drugs and was X-rayed at least three months prior to when the sample was taken. Short term lymphocyte cultures were set up from heparinized blood according to the method of Moorhead et al. (1960) with minor modifications. Lymphocytes were cultured by adding 0.5 ml of blood in 5 ml of RPMI 1640 medium (Hi Media) supplemented with 20% foetal calf serum (Sigma) and 0.1 ml phytohaemagglutinin (Sigma). Colchicine (10 μg/ml,) was added to the culture two h prior to harvesting.

Lymphocytes were harvested after 48 h for chromosomal aberrations (CA). Slides were prepared and stained with 4% giemsa (E. Merck). For each person 100 well spread metaphases were analysed.

For mitotic index (MI) 5000 cells per individual were counted from giemsa stained slides. MI was calculated by using the formula

No. of dividing cells

$$MI = \frac{\text{No. of dividing cells}}{\text{Total no. of cells scored}} \times 100$$

For sister chromatid exchanges (SCE), 5-bromodeoxyuridine (BrdU, 10 ug/ml, Sigma) was added 24 h after setting up of the cultures. Harvesting was done after 72 h. Slides were prepared by air drying method and stained with Hoechst 33258 (Sigma) and 4% giemsa following the method of Perry and Wolff (1974) with slight modifications. For calculating the frequency of SCE per cell, 25 metaphases per individual were analysed as per standard international practice.

Table 1: Composition of *Chyawanprash aweleha*
Each 100 grams of Dabur Chyawanprash is prepared from:

Botanical Name	*Qty. in grams*
PART 1:	
Angel marmelos	0.398
Premma integrifolia	0.398
Oroxylum indicum	0.398
Stereospermum suaveolens	0.398
Desmodium giganticum	0.398
Teramnus labialis	0.398
Solamum indicum	0.398
Solnum xanthocarpum	0.398
Tribulux terrestris	0.398
Gmelina arborea	0.398
Sida cordifolia	0.398
Phaseolus trilabus	0.398
Teramnus labialis	0.398
Pistacia intergerima	0.398
Phyllanthus ninuri	0.398
Leptudenia reticulata	0.398
Saussurea lappa	0.398
Aquillaria agallocha	0.398
Terminalia chebula	0.398
Tinospora cordifolia	0.398
Curcuma zedoaria	0.398
Cyperus rotundus	0.398
Boerhaavia diffusa	0.398
Nymphoea stellata	0.398
Adhatoda vasica	0.398
Glycirrhiza glabra	0.398
Martynia diandra	0.398
Dioscorea bulbifera	0.796
Withamia somnifera	0.796
Asparagus racemosus	0.796
Ipomoea digitata	1.195
PART II	
Emblica oficinalis	90.000
Sesomum indicum	1.200
Santalum album	0.0092
PART III	
Bambusa arundinacea	0.800
Mesua ferrea	0.116
Piper longum	1.120
Cinnamomum zeylanicum	0.116
Cinnamomum tamala	0.116
Syzygium aromaticum	0.128
Elttaria cordamomum	0.588

For evaluating the frequency of satellite associations (SA) 100 good metaphases were scanned. The criteria described by Hansson (1970) were followed for evaluating the SA. The criteria are (1) The satellite ends of the associating chromosomes had to be directed towards each other with their longitudinal axes meeting between their short arms. (2) The distance between the centromeres of associated chromosomes should not exceed the total length of one 'G' chromosome, its satellite excluded.

After taking these observations, 25 bidi smokers were fed *Chyawanprash awaleh*a (Dabur), 20 gms twice a day, for 2 months.The smoking habit continued during the period of intake of Cp

After 2 months short term lymphocyte cultures were set up again from heparinised blood from the 25 Cp-fed bidi smokers. MI, CA, SCE and SA were again analysed.The time of collection of the blood samples was same in all the 25 volunteers before and after the intake of Cp

The data obtained were statistically analysed by applying the Students 't' test.

RESULTS

Epidemiological study revealed that due to the use of '*Chyawanprash awaleh*a' (Cp) the problem of coughing in bidi smokers almost disappeared. Also there was an increase in appetite as well as gain in weight in the smokers.

The data on various parameters obtained during the present investigation have been given in tables 2-5.

Mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges (SCE), satellite associations (SA) and micronuceli (MN) of bidi smokers were analysed before and after the administration of Cp and compared with the parameters in control individuals.

Lower values of MI were observed in Cp-fed bidi smokers (5.15) compared with bidi smokers (6.36). The values are highly significant at $P < 0.01$ (Table 2). However, MI was still significantly higher than in the control individuals (3.70).

The frequency of CA found in Cp-fed bidi smokers was 1.00, whereas in bidi smokers it was 3.52. This resulted in a significant difference at $P < .001$ (Table 4). Both chromosome type aberrations viz. dicentrics, acentric fragments, chromosome gaps and chromosome breaks and chromatid type aberrations viz. chromatid gaps, chromatid breaks and isochromatid

Table 2: Mitotic Index (MI) in control, individuals, bidi smokers and Cp-fed bidi smokers

Sample	*Number of samples*	*Number of cells observed*	*Number of meta-phases*	*MI (Mean ±SD)*
Control Individuals	25	121397	4496	3.7 ± 0.39
Bidi Smokers	25	119360	7614	6.36 ± 0.73
Cp fed Bidi Smokers	25	120771	6236	5.156 ± 0.54*

Cp - Chyawanprash
*Significant at P <0.01

Table 3: Types of chromosomal aberrations (CA) in control individuals, bidi smokers and Cp-fed bidi smokers

Group	*Control Individuals*	*Bidi Smokers*	*Cp-Fed Bidi Smokers*
No. of individuals	25	25	25
No. of metaphases	2500	2500	2500
Chromosome Type Aberrations			
Dicentrics	1(0.04)	6(0.24)	2(0.08)
Rings	0(0.00)	2(0.08)	0(0.00)
Acentric Fragments	1(0.04)	9(0.36)	1(0.04)
Chromosome Gaps	1(0.04)	2(0.08)	1(0.04)
Chromosome Breaks	1(0.04)	3(0.12)	1(0.04)
Total (without gaps)	3(0.12)	20(0.8)	4(0.25)
Chromatid Type Aberrations			
Gaps	35(1.4)	78(3.12)	51(2.0)
Breaks	19(0.76)	68(2.72)	21(0.8)
Total (without gaps)	19(0.76)	68(2.72)	21(0.8)
Total Chromosomal aberrations (without gaps)	22(0.88)	88(3.52)	25(1.0)

Values in parentheses show CA per 100 metaphases
Cp - Chyawanprash

exchanges were encountered in both the groups (Table 3). However, rings were limited only to bidi smokers. The background CA frequency was found to be 0.88.

Table 4 shows the frequency of SCE in control individuals (3.69), bidi smokers before (8.07) and after the administration of Cp (6.50). A significant decrease was observed in SCE frequency in Cp-fed smokers as compared with the smokers (P<0.005). It was, however, still more than in controls.

Table 5 shows frequency of SA in bidi smokers, Cp fed and pretreated ones. As many as seven types of satellite associations were observed viz. DD, DG, GG, 2DG, 2GD, 2D2G, and 3D. The SA showed highly increased values in bidi smokers (11.96). There was, however, a decrease in the frequency of SA in Cp-fed bidi smokers (7.12), almost to the level of control individuals (5.36). The frequency of DG type associations was maximum while 3D type was minimum.

Table 4: Frequency of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in control individuals, bidi smokers and Cp-fed bidi smokers.

Sample	*Number of samples*	*CA (Mean ± S.D.)*	*SCE (Mean ±S.D.)*
Control Individuals	25	0.88 ± 0.66	3.69 ± 0.38
Bidi Smokers	25	3.52 ± 1.82 t =6.63**	8.07 ± 1.32 t = 3.27*
Cp Fed Bidi Smokers	25	1.00 ± 0.74	6.50 ± 1.20

Cp - Chyawanprash
* Significant at P < .005
** Significant at P <.001

Table 5: Frequency of satellite association (SA) observed in control individuals, bidi smokersand Cp-fed bidi smokers

	Number of individuals		
	Control Individuals	*Bidi Smokers*	*Cp-Fed Bidi Smokers*
	25	*25*	*25*
Total cells scanned	2500	2500	2500
Types of Satellite Associations			
DD	19(0.76)	71(2.84)	43(1.72)
DG	74(2.96)	124(4.98)	70(2.8)
GG	17(0.68)	50(2.00)	40(1.8)
2DG	6(0.24)	25(1.00)	15(0.8)
2GD	6(0.24)	11(0.44)	5(0.02)
2G2D	9(0.36)	14(0.56)	3(0.12)
3D	3(0.12)	4(0.16)	2(0.08)
Total	134	299	178
Associations per cell	5.36±2.32	11.96±5.12	7.12±2.30

Values in parentheses indicate the SA per 100 metaphases
Cp - Chyawanprash

DISCUSSION

Disappearance of coughing, increase in appetite and weight gain in Cp fed smokers is in tune with the earlier reports (Ojha 1988; Singh and Behre 1991).

An increase in the frequencies of MI, CA, SCE and SA have been found to increase with

cigarette smoke(Yadav et al. 2001; Jose et al. 1995; Falk 1977; Cohen 1980; Pryor 1987) , bidi smoke (Yadav and Thakur 2000b) as well as hookah smoke (Yadav and Thakur 2000a). The present report confirms these findings

The significant decrease in various cytogenetic end points during the present investigation, particularly in the frequency of CA, has been perhaps due to the anti-oxidant property of Cp. which has been well established (Jose et al.1995). Since tobacoo smoke is known to contain thousands of potentially hazardous chemicals including radioactive agents (Falk 1977; Cohen 1980), the formation of free radicals from radioactive and non-radioactive chemicals is probably one of the major pathways by which tobacoo smoke causes genetic damage and cancer (Pryor 1987). Therefore, with supplementation of diet with substances having free radical scavenging capability is a potentially useful approach to reduce genetic damage and to minimise adverse health outcome from tobaco smoking.

Naturally occuring substances in foods have been shown in laboratory experiments to serve as dietary antimutagens. Dietary desmutagens may function as chemical inactivators, enzymatic inducers, scavengers, or antioxidants (Kohlmeier 1995).

Antioxidants provide protection to living organism from damage caused by uncontrolled production of free radicals/ reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein denaturing and DNA-strand breaking. Antioxidants exert their effect by donating electrons to unstable oxygen species generated from endogenous processes or formed as a result of radiations or chemical exposure. Pre-empting oxidative attack on chemical bonds at other points in the cell, including DNA helices, may reduce the incidence of mutations arising from oxidant-induced mutations.

Vitamin C (L-ascorbic acid) is an excellent antioxidant which can react with and scavenge out many of these free radicals and oxidative products (Shamberger 1984; Gebhart et al. 1985). In most of the previous studies (Hoda and Sinha1991 a, b) the pesticide induced genotoxicity got significantly minimised by the human therapeutic dose of vitamin C (10 mg/kg body weight/day). However, it never brought the damage frequency down to the control level.

Sram et al. (1983) reported that the frequency of CA in prophylactically administered ascorbic acid (AA) treated coaltar workers dropped from 5.07 to 1.77 and the decrease was statistically significant $P < 0.01$. However, the difference between smokers and non-smokers was not significant. The results further showed that a long-term systematic administeration of AA played a significant role in limiting the increase of abberant cells which dropped virtually to the control level, as has been described in workers occupationally exposed to haloethers (Singh and Behre 1991).

A major advantage of the antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous (Kohlmeier 1995).

Chyawanprash was found to possess significant antioxidant property (Jose et al. 1995), mainly because of its *Phyllanthus emblica* contents, which constitute 90% of its bulk (Table1), along with a large number of other secondary plant metabolites. The flarvonoid, tannid and phenolic constituents of Chyawanprash have also been reported to exhibit antioxidant properties.

While characterizing the antioxidant activity of amla Khopde et al. (2001) concluded;" amla is a more potent antioxidant than vitamin C.... ascorbic acid and other polyphenols present in the natural formultation of amla, show much superior anti-oxidant activity compared to their equivalent amounts in pure isolated from". Although one reason for the decrease could have been that a fresh wave of lymphocytes without damaged DNA was produced. Another reason could be rectification of DNA damage in circulating (G0/G1-phase) lymphocytes owing to the antioxidant property of CP. How it has happened cannot be said with certanity at present. However, Bhattacharya and Francis (1999) have detailed several possible mechanisms of action of modulators of mutagenesis.

In a study on the protective action of crude extract of *Phyllanthus emblica* fruit against known metal clastogens, Dhir et al. (1999) found that priming with *Phyllanthus* fruit crude extract afforded a stronger protection against all the metals tested, than priming with Vitamin C alone. The greater efficacy of *Phyllanthus* fruit crude extract may be due to the presence of other natural ingradients in the crude extract. Similar results were obtained by Ghosh et al. (1993) , who found protective action of orally administered *Phyllanthus* extract against damages induced by CsCl to be of considerable importance in reducing the clastogenic action *in vivo* on mice bone

marrow cells.

The significant decrease in the frequency of various parameters, especially CA, which is a major indicator of clastogenicity/genotoxicity, in Cp-fed bidi smokers during the present investigation indicates the genoprotective (bioantimutagenic) properties of Cp against bidi smoke. However, there is a need to evaluate Cp against various kinds of tobacco smoke viz. cigarette smoke, hookah smoke, chilum smoke etc. in a systematic way over a considerable period of time. Such investigations can subsequently be extended to other common environmental toxicants. The use of Cp can then be prescribed to minimise the risk caused by mutagenic agents, since exposure to several environmental pollutants cannot be completely eliminated.

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