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Antimutagenic potential of curcumin on chromosomal aberrations in *Allium cepa**

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Abstract: Turmeric has long been used as a spice and food colouring agent in Asia. In the present investigation, the antimutagenic potential of curcumin was evaluated in *Allium cepa* root meristem cells. So far there is no report on the biological properties of curcumin in plant test systems. The root tip cells were treated with sodium azide at 200 and 300 μg/ml for 3 h and curcumin was given at 5, 10 and 20 μg/ml for 16 h, prior to sodium azide treatment. The tips were squashed after colchicine treatment and the cells were analyzed for chromosome aberration and mitotic index. Curcumin induces chromosomal aberration in *Allium cepa* root tip cells in an insignificant manner, when compared with untreated control. Sodium azide alone induces chromosomal aberrations significantly with increasing concentrations. The total number of aberrations was significantly reduced in root tip cells pretreated with curcumin. The study reveals that curcumin has antimutagenic potential against sodium azide induced chromosomal aberrations in *Allium cepa* root meristem cells. In addition, it showed mild cytotoxicity by reducing the percentage of mitotic index in all curcumin treated groups, but the mechanism of action remains unknown. The antimutagenic potential of curcumin is effective at 5 μg/ml in *Allium cepa* root meristem cells.

Key words: Chromosomal aberrations, Sodium azide, Genotoxicity, *Allium cepa*
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INTRODUCTION

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. There are several ways in which the action of mutagens can be reduced or prevented. Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective antimutagens (Ferguson, 1994). Sodium azide (NaN_3 in Fig.1b) is a major environmental mutagen as it is used in medicine, agriculture, etc. (Kleinhofs and Smith, 1976) and it causes cytotoxicity in several animal and plant test systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosages (Grant and Salamone, 1994). It is mutagenic in bacteria, higher plants and human cells and has been used as positive control (Rank and Nielsen, 1997; Sandhu *et al*., 1994).

Studies suggest that turmeric, a potent antimutagen against methyl cholanthrene, 4-nitro-o-phenylene diamine (NPD), diamino fluorine, urethane (a powerful mutagen) and benzo(a)pyrene (BAP) (Polasa *et al*., 1991; Azuine *et al*., 1992; el Hamss *et al*., 1999). This beneficial effect of turmeric has been postulated to be due to (Pulla Reddy and Lokesh, 1994) curcumin (CUR), the active ingredient of turmeric plant (*Curcuma longa* Linn; Zingiberaceae). It has been shown to have a wide range of biological activities. These include its antimutagenic, anticarcinogenic, antigenotoxic, anti-inflammatory, antioxidant properties etc. in different tests systems (Chattopadhyay *et al*., 2004).

Curcumin has protective effect against cisplatin (Antunes *et al*., 2000), hydrocortisone (Ahmad *et al*., 2004), nicotine (Kalpana and Menon, 2004), lead

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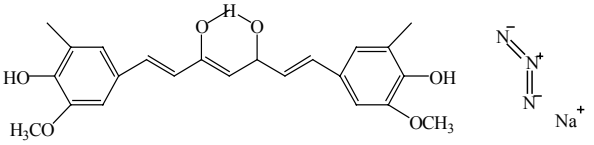
(El-Ashmawy *et al*., 2006), ethanol (Naik *et al*., 2004) and irradiation (Thresiamma *et al*., 1998) induced damage in in vivo and in vitro test systems. Furthermore it has antimutagenic potential against cyclophosphamide and BAP induced genotoxicity in microbial and mammalian test systems (Shukla *et al*., 2002; 2003).

Although the antimutagenic potential of curcumin has been extensively studied and well documented, yet there is no report on the biological effects of curcumin in plant test systems. The present study is designed to investigate the action of curcumin against sodium azide induced chromosomal aberrations in *Allium cepa* root meristem cells.



(a) (b)

Fig.1 Chemical structures of curcumin and sodium azide. (a) Curcumin; (b) Sodium azide

MATERIALS AND METHODS

Chemicals

Curcumin (Fig.1a) and colchicine were obtained from Sigma Chemical Company (Bangalore, India). Other chemicals used in this study were of analytical grade purity and obtained from Hi-media Laboratories Pvt. Limited (Mumbai, India).

Test system and treatment

Healthy onion bulbs (20~25 g) were grown in the dark in a cylindrical glass receptacle at room temperature [(28±0.5) °C] and given renewed water supply every 24 h. When the roots reached 2 to 3 cm in height, they were treated with different concentrations of 5, 10, 20 μg/ml of curcumin for 16 h. Following curcumin treatment the bulbs were washed in distilled water and then treated with 200, 300 μg/ml of sodium azide for 3 h. Later, roots are treated with 0.05% of colchicine for 3 h and then fixed in methanol-acetic acid (3:1) fixative and stored at 5 °C.

Microscopic examination

Three bulbs were used for each dosage. Three micro slides were prepared from each bulb by following Feulgen squash technique. A total of 300 well spread metaphases per bulb were analyzed for chromosomal aberrations and 3000 cells were scored for mitotic index. The mitotic index for cytotoxicity evaluation was calculated by dividing cells out of total cells counted. The suppression percentage (SP) of curcumin on chromosomal aberrations of sodium azide is calculated as (Shukla *et al*., 2003):

$$SP\ (\%)=100\%-N_1/N_2\times 100\%,$$

where, N_1 is the number of aberrations in curcumin pretreated and NaN_3 post-treated groups and N_2 is the number of aberrations in NaN_3 alone treated group.

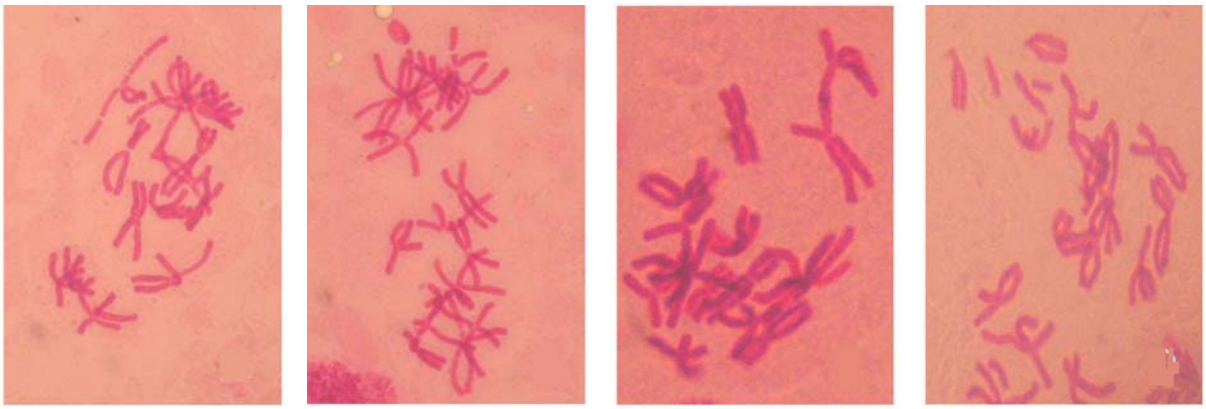
Statistical analysis

Data on total number of aberrations, mitotic index and abnormal metaphases were analyzed by analysis of variance (ANOVA), with the calculations of the *F*-statistic and respective *P* values. The *P* values were compared with calculation of the minimum significant difference for P=0.05%.

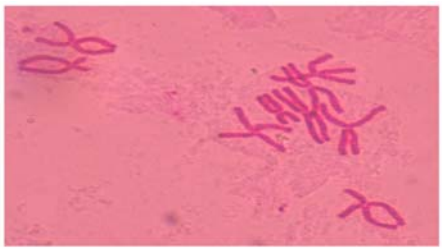
RESULTS

The sodium azide induced chromosomal aberrations such as break, gap, iso-chromatid break and exchange were analyzed in *Allium cepa* root tip cells (Fig.2). Curcumin induced chromosomal aberrations at 5, 10, 20 μg/ml were not statistically significant when compared with untreated control, which indicated its non-clastogenicity. The result showed that chromatid breaks and gaps are more common than iso-chromatid breaks and exchange. The number of aberrations and the number of abnormal metaphases induced by sodium azide increased with increasing dosages, which represented its mutagenic action in *Allium cepa* and it was statistically significant when compared with untreated control (Table 1). With increasing concentrations, the number of abnormal metaphases and the number of aberrations decreased significantly in all curcumin pretreated groups. The percentage of mitotic indexes decreased with increasing concentrations of curcumin compared with the untreated control, which explains its cytotoxicity in plant test system. Similarly the reduction of mitotic

index was also found in all curcumin pretreated groups, except 5 μg/ml. The percentage of suppression by curcumin on sodium azide induced chromosomal aberrations increased with increasing concentrations of curcumin in all the concentrations tested, indicative of its antimutagenic potential in *Allium cepa*. The effect of curcumin on the reduction of total number of aberrations induced by NaN_3 was statistically significant when compared with sodium azide control. This study implies that pretreatment of curcumin has a strong inhibitory role against the mutagenic action of sodium azide.



(a) (b) (c) (d)



(e)

Fig.2 Photographs of smear preparations of root tips of *Allium cepa* showing different kinds of chromosomal aberrations. (a) Chromosome break and gap at 200 μg/ml of NaN_3; (b) Single chromatid breaks at 20 μg/ml of curcumin and 200 μg/ml of NaN_3; (c) Single chromatid exchange at 20 μg/ml of curcumin; (d) Fragments at 300 μg/ml of NaN_3; (e) Normal metaphase at 5 μg/ml of curcumin

Table 1 Distribution of different types of chromosomal aberrations in 300 cells analyzed and mitotic index observed in *Allium cepa* after treatment with curcumin and or not sodium azide

Treatment	MI	AM	Aberrations				Total	*SP* (%)
			Bre	Gap	IsB	Exc		
Untreated control	7.06	3	3	1	−	1	3	−
CUR (μg/ml) 5	6.17	4	2	1	1	−	4	−
10	5.53	5	2	1	2	2	7	−
20	4.76	7	3	2	1	2	8	−
NaN_3 200 μg/ml	4.30	32^b	23^b	6	4	2	35^b	−
CUR 5 μg/ml+NaN_3	4.86^a	24^a	19^a	4	2	2	27^*	22.8
CUR 10 μg/ml+NaN_3	4.03^a	22^a	18^a	2	1	2	23^*	34.2
CUR 20 μg/ml+NaN_3	3.80^a	19^a	13^a	2	2	3	20^*	42.8
NaN_3 300 μg/ml	3.76	40^b	32^b	4	5	5	46^b	−
CUR 5 μg/ml+NaN_3	4.56^a	28^a	22^a	6	3	2	33^*	28.3
CUR 10 μg/ml+NaN_3	3.73^a	24^a	19^a	2	4	3	28^*	39.1
CUR 20 μg/ml+NaN_3	3.50^a	20^a	17^a	2	1	3	23^*	50.0

CUR: Curcumin; NaN_3: Sodium azide; MI: Mitotic index; AM: Abnormal metaphases; Bre: Break; IsB: Iso-chromatid break; Exc: Exchange; SP: Suppression percentage. *Statistically different when compared with sodium azide control; ^{a}Statistically different when compared with curcumin control; ^{b}Statiscally different when compared with untreated control

DISCUSSION

Historically, plants have been used as indicator organisms, in studies on mutagenesis in higher eukaryotes. Plant systems have a variety of well defined genetic endpoints including alterations in ploidy, chromosomal aberrations and sister chromatid exchanges (Grant, 1994). Dietary constituents suppress the genotoxic action or damage of xenobiotics through various intra and extra cellular mechanisms (Hayatsu *et al*., 1988; el Hamss and Idaomar, 2002).

Sodium azide is a known potent mutagen although it fails to induce chromosomal aberrations in human lymphocytes (Arenaz and Nilan, 1981; Slamenova and Gabelova, 1980; Nilan *et al*., 1973), and Owais and Kleinhofs (1988) reported that NaN_3 is a most efficient mutagen in barley, yeast and several other higher plants also. The reason behind its non genotoxicity in mammalian test system is the enzyme responsible for conversion of azide into non genotoxic azidoalanine (Arenaz *et al*., 1989) and the lack of interaction with DNA (Arenaz *et al*., 1983). But in this study it induced chromosomal aberrations in a dose dependent manner and exhibited cytotoxicity by lowering the percentage of mitotic index. When sodium azide is dissolved in water it forms a toxic hydrogen azide gas, with generation of azide ions being the possible reason for its genotoxicity and cytotoxicity in *Allium cepa* test systems. Kleinhofs and Smith (1976) reported that the possibility of azide mutagenesis was due to peroxide accumulation, which may also be the cause for its toxicity in this study.

In the present investigation curcumin exhibits antimutagenic potential against sodium azide induced damage in a dose dependent manner. Similar results were observed by Nagabhushan *et al*.(1987) and Shukla *et al*.(2003) against BAP and dimethyl benzo(a)anthracene and cyclophosphamide induced damage in Salmonella and CHO (Chinese hamster ovary) test systems. Anto *et al*.(1996) reported that natural curcuminoids from turmeric are also potent inhibitors of mutagenicity.

The protective effect of curcumin is due to its antioxidant action, trapping of free radicals, formation of complex with mutagens, modulation of mutagen metabolism by absorbing the xenobiotics (Premkumar *et al*., 2004), inhibition of SOS (super-active oxygen species) functions (Oda, 1995), a chain breaking or oxidative coupling reaction at the 3′ position of the curcumin with the lipid and a subsequent intra-molecular Diels-Alder reaction (Masuda *et al*., 2001), by altering the activation and/or detoxification of xenobiotics (Goud *et al*., 1993) and the stabilization of the formed phenoxy free radicals is responsible for its free radical scavenging activity and chemopreventive effect (Subramanian *et al*., 1994; Kunchandy and Rao, 1990; Youssef and El-Sherbeny, 2005). Interestingly, curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of other antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase (Pulla Reddy and Lokesh, 1994). Also there exists an association between agents which show antioxidant activity to act as antimutagens (Renner, 1984). The modulatory role of curcumin in inhibiting mutagenicity and carcinogenicity can also be due to its antioxidant activity (Nagabhushan and Bhide, 1987). Sreejayan and Rao (1996; 1997) reported that the phenolic hydroxyl and methoxyl groups on the phenyl ring and the 1,3-diketone systems are the two important structural features that contribute to antioxidant properties. The biological activities of curcumin are derived from the antioxidant property of the methoxy phenol group and the action of the aryl group in β-diketone (Jovanovic *et al*., 2001; Sun *et al*., 2002; Anto *et al*., 2002). The above mechanism for antimutgenic actions of curcumin was discovered in different test systems other than plants. In our studies the mechanism of action remains unknown, whether it follows any one of the above or else. The possible reason for the antimutagenic potential of curcumin in this study may be due to the following; trapping of free radicals, degeneration of toxic gas, degradation of azide ions and peroxide accumulations. Our results indicate that at 5 μg/ml of curcumin the antimutagenic potential is effective against NaN_3 induced chromosomal aberrations.

The mitotic index value of curcumin showed a reduction at high concentration; it exhibits its cytotoxic action in cell division. Similarly Adams *et al*.(2004) concluded that curcumin analogs exhibit a high degree of toxicity and Woo *et al*.(2003) reported that curcumin causes cell damage by inactivating the Akt-related cell survival pathway. But the com-

pound's cytotoxicity may arise from the presently unknown mechanism. Since mutations are induced at chromosome levels and are the probable causes of cancer related diseases, the inhibition of chromosomal damage by curcumin suggests the antimutagenic and anticarciongenic activity (Shukla *et al*., 2002).

On the basis of our results, we conclude that curcumin has antimutagenic potential against sodium azide induced clastogenic damage in *Allium cepa* in a dose dependent manner, but it is effective at low dose (5 μg/ml). At the same time it exhibits a mild cytotoxic action; similar to the earlier reports in an in vitro and in vivo test systems. However the mechanism by which it acts remains to be investigated in plant test system and further studies are necessary to clarify this point.

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