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G_2 STUDIES OF ANTIMUTAGENIC POTENTIAL OF CHEMOPREVENTIVE AGENT CURCUMIN IN ALLIUM CEPA ROOT MERISTEM CELLS

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Summary. *Turmeric has long been used as a spice and food colouring agent in Asia. Curcumin is one of the active principles in Turmeric. In this research the antimutagenic potential of curcumin in Allium cepa root meristem cells was evaluated. The root tip cells were treated with sodium azide at 200µg/ml and 300µg/ml for 1h. Curcumin was given at 5, 10 and 20 µg/ml for 18h (one cell cycle), prior to sodium azide treatment. The tips were squashed after colchicine treatment and the cells were analyzed for chromosome damage and mitotic index. Curcumin did not induce chromosomal aberration in Allium cepa root tip cell. Sodium azide alone induced chromosomal aberrations significantly with increasing concentrations. The chromosomal aberrations were significantly reduced in root tips cells pretreated with curcumin. The study reveals the antimutagenic potential of curcumin against sodium azide induced chromosomal aberrations.*

Key words: *Curcumin, chromosomal aberrations, sodium azide*

Introduction

Turmeric is a well-known spice and food colorant commonly consumed in different parts of the world. Its active principle curcumin is the powdered dry rhizome of *Curcuma longa* Linn plant of the family Zingiberaceae (1). It shows anti-mutagenic, anti-genotoxic and protective effects on various test systems (2-8). Curcumin protects cisplatin-induced clastogenesis in vitro and in vivo assays, by acting as a free radical scavenger (4). It has been identified to reduce the radiation induced DNA damage in rat lymphocytes (5), hydrocortisone induced genotoxicity in human lymphocytes (4) and nicotine induced toxicity in wistar rats (7), and lead acetate induced genotoxicity in mice (1) and reduced the frequency of micronuclei in polychromatic erythrocytes induced by gamma radiation (9). Furthermore, it showed a protection against ethanol induced cytotoxicity in liver cell culture in an in vitro condition (10). Curcumin exhibits antimutagenic effect on cyclophosphamide induced chromosomal aberration in wistar rats (2) and benzo(a)pyrene, cyclophosphamide induced genotoxicity in microbial and mammalian test systems (3). Turmeric has been found to display inhibition of genotoxic effect of urethane in Drosophila (11). The present study was designed to investigate whether or not curcumin has anti-mutagenic potential on sodium azide induced chromosomal aberrations in *Allium cepa* root meristem cells.

Materials and Methods

Chemicals

Curcumin, Colchicine, Basic Fuchsin was obtained from Sigma chemicals USA. The rest of the chemicals, potassium metabi-sulphite Hydrochloric acid, Acetic acid, Ethanol, Methanol used in this study were of analytical grade purity obtained from Hi-media chemicals India.

Test system and treatment

Healthy onion bulbs (20-25g) were grown in dark in a cylindrical glass receptacle at room temperature ($28^oC \pm 0.5^oC$). Water was changed periodically at every 24h. When the roots reached 2cm to 3cm in height, they were treated with different concentrations of 5, 10, 20 µg/ml of curcumin for 18h (one cell cycle). Following curcumin treatment, the bulbs were washed in distilled water and then treated with 200, 300 µg /ml of sodium azide for 1h. Then the roots were treated with 0.05% colchicine for 3h and then fixed in carnoy's fluid.

Chromosome analysis

Microslides were prepared from the treated root tip cells by Feulgen squash technique. A total of 300 well spread metaphases per concentration were analyzed for chromosomal aberrations and 3000 cells were scored for mitotic index. The suppressions percentage of curcumin on chromosomal aberrations of sodium azide was calculated as

$$100 - \frac{\text{Number of aberrations in curcumin Pretreated and sodium azide post Treated groups}}{\text{Number of aberrations in sodium azide alone treated group}} \times 100$$

Statistical analysis

The mean values and SD of different parameters including mitotic index and incidence of abnormal cells of all groups were subjected to statistic comparison using students t-test $p < 0.01$ considered significant.

Results

The G_2 effects of sodium azide induced chromosomal aberrations such as break, gap, iso-chromatid break, which were analyzed in *A. cepa* root tip cells pretreated with curcumin (Table 1). In this study curcumin alone did not induce any chromosomal aberrations at 5, 10, 20 μg/ml of assayed, indicate the anti-clastogenic activity. The number of abnormal cells and the number of aberrations were increased with the increasing dosage of sodium azide in 200μg/ml and 300μg/ml tested. The number of abnormal cells and the number of aberrations were decreased significantly in all curcumin-pretreated groups. The percentage of suppression by curcumin on sodium azide induced chromsosmal aberrations increased with increasing concentrations of curcumin in all the concentrations tested (Fig. 1). The results showed that chromatid breaks were more common than isochromatid breaks and gaps. This study implies that pretreatment of curcumin has inhibitory potential against the mutagenic action of sodium azide.

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	%of
			Bre	Gap	IsB	Exc		Supp
Untreated control	7.06	3	3	1	-	1	3	-
Curcumin 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	4.30	32[b]	23[b]	6	4	2	35[b]	-
Cur 5 +NaN_3	4.86[a]	24[a]	19[a]	4	2	2	27*	22.8
Cur 10+NaN_3	4.03[a]	22[a]	18[a]	2	1	2	23*	34.2
Cur 20+NaN_3	3.80[a]	19[a]	13[a]	2	2	3	20*	42.8
NaN_3 300	3.76	40[b]	32[b]	4	5	5	46[b]	-
Cur 5+NaN_3	4.56[a]	28[a]	22[a]	6	3	2	33*	28.3
Cur 10+NaN_3	3.73[a]	24[a]	19[a]	2	4	3	28*	39.1
Cur 20+NaN_3	3.50[a]	20[a]	17[a]	2	1	3	23*	50.0

Abbrevations: Cur-Curcumin; NaN_3-sodium azide; Bre-Break; IsB-Isochromatid Break; Exc- Exchange; Supp-suppression
*Statistically different when compared with sodium azide control
[a] Statistically different when compared with Curcumin control
[b] Statistically different when compared with Untreated control

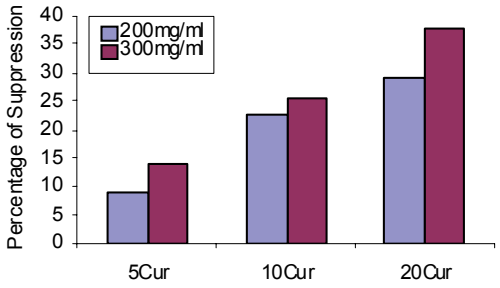

Fig. 1. Percentage of suppression on sodium azide induced chromosomal aberration.

Discussion

Considerable emphasis has been laid down on the use of dietary constituents preventing mutagen induced cytogenetic damage due to their relative non-toxic effects (12). Since oxidative damage in biological systems is considered to cause ageing, degenerative diseases and cancers, a particular attention was focused on the possibility of modulating these effects through the use of free radical scavengers to minimize cell injury. The beneficial effects of turmeric were postulated to result from curcumin (13). 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 (14,15,16). In the present study curcumin was found to inhibit the incidents of sodium azide induced chromosomal aberrations in *Allium cepa* in a dose dependent manner, suggesting its potential as an antimutagenic agent. Similar results were observed in wistar rats treated with cyclophosphamide (2) and benzo(a) pyrene and cyclophosphamide induced genotoxicity in microbial and mammalian test systems (3).

Curcumin has protective effect against radiation-induced damage in rat lymphocytes (6) hydrocortisone induced genotoxicity in human lymphocytes (Ahmed et al., 2004) and also against the cisplatin induced clastogenesis by acting free radical scavenger (5). Curcumin exerts protective effects by modulating the extent of lipid per-oxidation and enhancing the antioxidant status in wister rats (7). Curcumin significantly reduced the frequencies of micro nucleated polychromatic erythrocytes in mice exposed to gamma radiation (9) and it was also indicated as an antimutagen against environmental mutagens in vitro and antitumor drug in vivo. The protective effect of curcumin against cyclophosphamide induced genotoxicity may be due to one of the following: antioxidant action, trapping of free radicals, formation of complex with mutagens, modulation of mutagen metabolism or by absorbing the xenobiotics (17). The modulatory role of curcumin in inhibition of mutagenecity and carcinogenecity can also be implied to its antioxidant activity (18). The antioxidant property of this plant and their products may scavenge peroxides and other radical oxygen species as do natural protectors against lipid peroxidation such as retinal, ascorbic acid,

tocopherol, glutathione and antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase which have the capacity to scavenge reactive oxygen species and lipid free radicals (19,20).The antioxidant effects of these compounds mainly depend on the stabilization of the formed phenoxy free radical. The *p*-hydroxy phenyl moiety is very essential to produce the henoxy free radical responsible for free radical scavenging effect (21). Hydroxylated derivatives of curcumin are known to be antimutagenic and it has been proposed that *p*-hydroxy group is also essential for its chemo preventive-effect (22,23). Curcumin has two p-hydroxy groups and scavenges free radical DNA damage there by acting as a potent antioxidant (23,24). Since mutations induced at the cytogenetic levels are probable causes of cancer, therefore the inhibition of chromosomal aberration by curcumin suggests the antimutagenic and anticarcinogenic activity. Furthermore, curcumin acts as a potent anticarcinogenic compound through various mechanisms. The induction of apoptosis plays an important role in its anticarcinogenic effect (25) and also inhibits cancer at initiation, promotion and progression stages of development against benzo(a)pyrene induced skin tumors in female Swiss mice (22). The results of present investigation conclude a dose dependent antimutagenic potential of curcumin on sodium azide induced clastogenic damage in *A. cepa*. The molecular mechanisms of antimutagenic or antigenotoxicity of curcumin and its definite role in *Allium cepa* root meristem cells need further investigations.

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G_2 STUDIJE ANTIMUTAGENOG POTENCIJALA HEMOPREVENTIVNOG AGENSA KURKUMINA U ĆELIJAMA POKORIČNOG TKIVA KORENA VRSTE ALLIUM CEPA

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Kratak sadržaj: *Indijski šafran (turmerik) se dugo koristi kao začin i agens za bojenje hrane u Aziji. Kurkumin je jedna od aktivnih osnova šafrana. U istraživanjima je procenjen antimutageni potencijal kurkumina u ćelijama pokoričnog tkiva korena vrste Allium cepa. Ćelije vrha korena tretirane su natrijum azidom 200µg/ml i 300µg/ml sat vremena. Dodato je 5, 10 i 20 µg/ml kurkumina tokom 18 časova (jedan ćelijski ciklus), pre tretmana natrijum azidom. Vrhovi su iscedjeni posle tretmana kolcihinom i analizirano je oštećenje hromozoma i mitotični indeks ćelija. Kurkumin nije izazvao aberaciju hromozoma ćelija vrha korena Allium cepa. Samo sa povećanjem koncentracije natrijum azida povećavale su se aberacije hromozoma. Hromozomne aberacije su bile značajno manje kod ćelija vrha korena koje su prethodno bile tretirane kurkuminom. Studija je dokazala antimutageni potencijal kurkumina kod aberacija hromozoma izazvanih natrijum azidom.*

Ključne reči: *Kurkumin, aberacije hromozoma, natrijum azid*