Research Article

Antimutagenic Potential of Curcumin on Chromosomal Aberrations Induced by Sodium Azide in *Allium cepa* Root Tip Cells

Nasreen Najeeb* D. Lilly and R. Syeda Ashfianaz

Department of Biotechnology, Mohamed Sathak College of Arts and Science, Chennai, Tamilnadu, India.

ABSTRACT

The present study was conducted to evaluate the mutagenic / clastogenic potential of Sodium Azide at a concentration of 200µg / ml on Allium cepa root meristem cells and to determine the antimutagenic effect of Curcumin at doses (5µg / ml) (10µg / ml) 20µg / ml. The Allium cepa test is a cytogenetic short term bioassay that has proved to be a useful tool in basic research to evaluate the genotoxic risk of known chemicals. Sodium Azide induces chromosomal breakage, Anaphase Bridge, sticky chromosomes, but when pretreated with curcumin the chromosomal aberrations were lesser, curcumin being a dietary antioxidant has free radical scavenging activity, the effective dose was found to be 20µg / ml.

Keywords: Allium cepa root meristem, sodium azide, curcumin, chromosomal aberrations.

INTRODUCTION

Plants have been used as indicator organisms in studies on mutagenesis in higher Eucaryotes. Plant systems have a well defined genetic end point including alterations in ploidy, chromosomal aberrations and sister chromatid exchanges (Grant 1994). Among the plant systems, Allium cepa is the most commonly used species for the study of chromosomal aberrations, bulbs produce a large number of roots in a short period of time and chromosomes are relatively long. The Allium cepa test introduced by Levan (1938), is a cytogenetic short term assay that has proved to be a useful tool in basic research to evaluate the genotoxic risk of known chemicals(Fiekesjo 1998). An effective test organism for the assessment of chromosomal aberration should have chromosomes which are easy to analyse in terms of size, morphology and number (Sylvia 2006).The Allium cepa test allows the toxicity of aqueous samples to be evaluated through two cytological end points; root formation and growth restriction observable at the macroscopic level and root tip meristem chromosome aberration scored at the microscopic level.

The universality of genetic material and of the basis of genetics facilitates the use of non human test system to detect chemical mutagens and clastogens. As a rule, there appears to be good correlation between the chromosome breaking caused by chemicals in plants and cultured animal cells (Kihlman 1966).

Sodium Azide NaN3 Mol wt 65.02 is colourless odourless, crystalline solid. It is a major environmental mutagen, used in medicine, agriculture and it causes cytotoxicity in several animal and plant systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosage (Grant and Salmone 1994) .It is used in making chemicals , as a preservative in diagnostic medicines and blood tests , as a herbicide , fungicide and soil fumigant , and is the propellent used for inflating air bags (Hazardous substances fact sheet – Right to know).

Sodium Azide fails to induce chromosomal aberrations in human lymphocytes it is 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 and the lack of interaction with DNA (Arenay 1983) . Sodium Azide is a unique mutagen – because of the lack of induction of SCEs above background combined with

previous data which demonstrates the negative clastogenic but positive mutagenic activity of Sodium azide confirms the uniqueness of this mutagen (Arenay and Nilan,
1973) .The Allium cepa anaphase , telophase assay was used to show genotoxicity of N- methyl –N-Nitrosourea (MNU) , maleic hydrazide, sodium Azide NaN3 and Ethyl Methyl Sulphonate (EMS) . All agents induced chromosomal aberration at statistically significant levels (Rank and Nielson 1997)
Curcumin a polyphenolic yellow compound found in turmeric is commonly used as a colouring agent in foods, drugs and cosmetics. Turmeric has been used as a spice and food colouring agent in Asia. Curcumin (CUR), the active ingredient in turmeric plant (Cucurmalonga Linn. Zingiberaceae) have been shown to have a wide range of biological activities. These include antimutagenic, anticarcinogenic, anti genotoxic , anti inflammatory , antioxidant properties in different test systems (Chattopadhyaz et al , 2004) . Curcumin has protective effect against cisplatin (Antunes et, al, 2000) hydrocortisone (Ahmed ,et, al, 2006) , nicotine (Kalpana and Menon, 2004) , lead(El- Ashmawy et al.,2006) , ethanol(Naik et al.,2004), and irradiation (Thresiamma et al .,1998) induced damage invivo and invitro test system. Furthermore it has antimutagenic potential against cyclophosphamide and BAP induced genotoxicity in microbial and mammalian tests in a dose dependent manner. Curcumin exhibits antimutagenic potential against Sodium azide induced damage in dose dependent manner (Shukla et al., 2002; 2003).
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 xenobiotics (Premkumar et al., 2004) Interestingly, curcumin not only exhibits antioxidative and free radicals scavenging properties, but also enhances the activities of other antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase (Pulla Reddy and Lokesh, 1994).
The biologic activities of curcumin are derived from the antioxidant property of the methoxy group and the action of aryl group in B – dikitone. (Jovanovic et al., 2001; Sun et al ., 2002 ; Anto et al 2002).

EXPERIMENT

Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature and renewed water supply every 24 hrs. When the roots reached 2-3 mm in length, they were treated with different concentration 5, 10, 20µg / ml of curcumin for 16 hrs. Following curcumin
treatment the bulbs were washed in distilled water and then treated with 200 µg / ml of Sodiun Azide for 3 hrs. This formed group 1.
Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature and renewed water supply every 24 hrs. When the roots reached 2-3 mm in length, they were treated with different concentration of 200 µg / ml of Sodium Azide for 3 hrs. This formed group 2.
Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature and renewed water supply every 24 hrs. When the roots reached 2-3 mm in length , they were treated with different concentration 5, 10, 20µg / ml of curcumin for 16 hrs .This formed group 3 .
Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature and renewed water supply every 24 hrs. This formed group 4 and served as control.
After the treatment schedule the root tips were harvested and fixed in ethanol acetic acid in the ratio 3:1 and stored at 5º C.

Microscopic preparation

Hydrolysed the root tips in1N HCl at 56º C for 8 mins. The root tips were washed in distilled water and were then exposed to 4% Iron Alum solution as a mordant.The root tips were washed in distilled water.A single softened root tip was then transferred to a slide and a few drops of Haematoxylin stain was added and stained for 25 mins.The excess stain was removed with 45% Acetic acid and a cover slip was placed and squashed without any air bubbles.

Analysis

The Microscopic preparations were analysed in 40x objective lens to determine the cell division intensity by calculating the Mitotic index. This is the ratio between the number of dividing cells and the total number of cells analysed in percents.

$$\text{MITOTIC INDEX} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

Scoring of slides – Chromosomal aberrations are scored at prophase, metaphase, anaphase and telophase as fragments, disturbed chromosomes, sticky chromatin, anaphase bridge, unequal distribution of chromosomes.

Statistical Analysis of Data - The mean values were calculated for each group of concentrations and controls for the determination of the significance among the means, Independent Samples t-Test was applied ($p<0.05$).

Table 1: Comparison of Mitotic Index between different treated groups

Treated groups	Mitotic index (%)
Control	41.78
Sodium Azide(200µg / ml)	20.32
Curcumin(5µg / ml)	37.05
Curcumin(10µg / ml)	36.72
Curcumin(20µg / ml)	36.00
Curcumin(5µg /ml)+Sodium Azide (200µg / ml	26.98
Curcumin(10µg / ml)+Sodium Azide (200µg)	27.89
Curcumin (20µg / ml) + Sodium Azide (200µg	30.02

TABLE 2: ANOVA for the effect of sodium azide treated with curcumin at 5µg on chromosomal aberrations *Allium cepa*

Mitotic Stages	Different Concentration	Mean and Standard Deviation	P- Value
Prophase	Sodium Azide	43.70 ±12.23	0.000***
	Curcumin 5µg	11.42 ±3.54	
	Sodium Azide & Curcumin5µg	51.72±4.59	
Metaphase	Sodium Azide	61.61 ±7.69	0.000***
	Curcumin5µg	11.11 ±1.94	
	Sodium Azide & Curcumin5µg	52.38±5.38	
Anaphase	Sodium Azide	55.55±13.87	0.203ns
	Curcumin5µg	14.28±15.37	
	Sodium Azide & Curcumin5µg	66.66±57.37	
Telophase	Sodium Azide	56.02±15.98	0.017*
	Curcumin5µg	11.66±06.42	
	Sodium Azide & Curcumin5µg	33.78±	

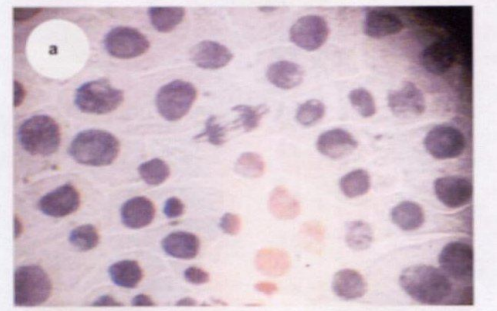
Table 3: ANOVA for the effect of sodium azide treated with curcumin at 10µg on chromosomal aberrations *Allium cepa*

Mitotic Stages	Different Concentration	Mean and Standard Deviation	P- Value
Prophase	Sodium Azide	43.70 ±12.23	0.002***
	Curcumin 10µg	10.28 ±3.69	
	Sodium Azide & Curcumin10µg	45.90±7.09	
Metaphase	Sodium Azide	61.61 ±7.69	0.530***
	Curcumin10µg	10.44 ±0.77	
	Sodium Azide & Curcumin10µg	33.33±57.73	
Anaphase	Sodium Azide	55.55±13.87	0.329ns
	Curcumin10µg	12.49±11.67	
	Sodium Azide & Curcumin10µg	31.66±27.53	
Telophase	Sodium Azide	56.02±15.98	0.005*
	Curcumin10µg	9.66±7.23	
	Sodium Azide & Curcumin10µg	34.05±1.33	

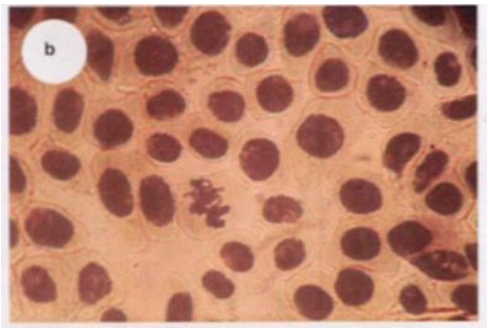
Table 4: Anova for the effect of sodium azide treated with curcumin at 20µg on chromosomal aberrations *Allium cepa*

Mitotic Stages	Different Concentration	Mean and standard Deviation	P- Value
Prophase	Sodium Azide	43.70 ±12.23	0.017*
	Curcumin 20µg	8.17 ±3.51	
	Sodium Azide & Curcumin20µg	29.49±8.72	
Metaphase	Sodium Azide	61.61 ±7.69	0.001**
	Curcumin20µg	7.85 ±1.78	
	Sodium Azide & Curcumin20µg	26.47±3.19	
Anaphase	Sodium Azide	55.55±13.87	0.090ns
	Curcumin20µg	10.27±9.30	
	Sodium Azide & Curcumin20µg	22.77±2.83	
Telophase	Sodium Azide	56.02±15.98	0.008**
	Curcumin20µg	8.17±6.72	
	Sodium Azide & Curcumin20µg	27.0±0.46	

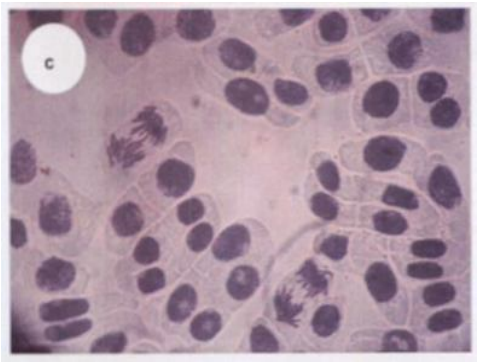
* Significant difference at p value < 0.05at 5%
** Significant difference at p value < 0.01at %
*** Significant difference at p value < 0.001at 0.001%
ns Not significant difference at p value > o.o5.



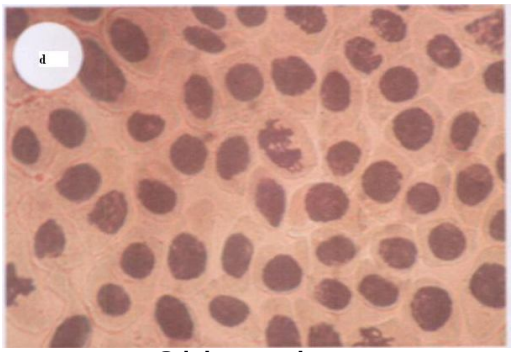
Anaphase with two bridges



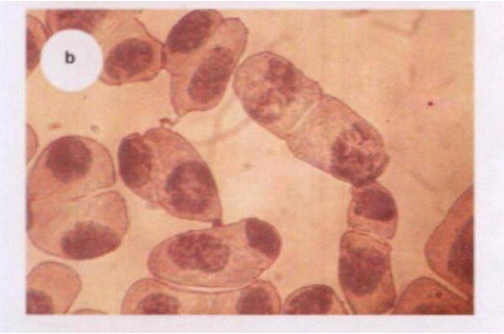
Sticky metaphase



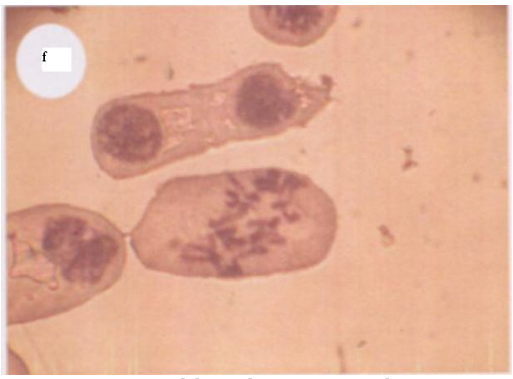
Anaphase with single bridge



Sticky anaphase



Pulverised chromosomes



Gaps and breaks at metaphase

RESULTS AND DISCUSSION

The effect of treated groups on Mitotic Index

When the root tips were exposed to Sodium Azide, the Mitotic Index was 20.32%, when compared to control 37.98%. This clearly shows the genotoxicity of Sodium Azide. When root tips were exposed to Curcumin at 5µg, 10 µg and 20 µg, the Mitotic Index was, 37.05%, 36.72%, 36.00 % .This shows that Curcumin does not reduce the Mitotic Index at this concentration, and it was only a very negligible difference when compared to control. The Mitotic Index reduced as the concentration of curcumin increased. When root tips were previously grown in curcumin at 5µg, 10 µg and 20 µg for 16 hrs and then exposed to Sodium Azide (200µg) for 3hrs , the was a significant increase in Mitotic Index at all concentrations , Curcumin at 20 µg was found to be the effective dose.

The effect of Treated groups on Chromosomal aberrations.

The data for Chromosomal aberration associated with exposure to Sodium Azide are presented in Table 2,3 & 4. The results showed that the number of aberrations induced by Sodium Azide is increased when compared to curcumin at different concentrations, curcumin was non clastogenic in plant systems.The most frequent aberrations were sticky chromosomes (Fig b&d), pulverized chromosomes,(Fig e)fragments,(Fig f) Anaphase Bridge. (Fig a&c), But when the root tips were pretreated with curcumin, the number of aberrations were significantly reduced, which indicated its antimutagenic potential.

DISCUSSION

Sodium Azide is a colourless odourless, crystalline solid. It is a major environmental mutagen , used in medicine , agriculture and it causes cytotoxicity in several animal and plant systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosage (Grant and Salmone 1994) .It is used in making chemicals , as a preservative in diagnostic medicines and blood tests , as a herbicide , fungicide and soil fumigant , and is the propellent used for inflating air bags Hazardous substances fact sheet – Right to know. When Sodium Azide is dissolved in water it forms a toxic hydrogen azide gas, with the generation of azide ions being the possible reason for its genotoxicity and cytotoxicity in plant systems. Sodium Azide induces chromosomal aberrations in Allium cepa root tip cells at statistically significant levels (Rank and Nielson 1996). It is delivered into human heteroploid HEp-2 cells via Liposomes and it produces chromosome aberrations and other major genetic damages (Raicu P , Mixich F (1992). The use of anti – mutagens in everyday life is the most effective way for preventing human cancer and genetic disease. Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective anti-mutagen (Ferguson 1994).

Curcumin, the active ingredient of Turmeric plant is anti-mutagenic and it has protective effect. Curcumin has protective

effect against cisplatin (Antunes et, al, 2000) hydrocortisone (Ahmed ,et, al, 2006) , nicotine (Kalpana and Menon, 2004) , lead(El- Ashmawy et al.,2006) , ethanol(Naik et al.,2004), and irradiation (Thresiamma et al .,1998) induced damage invivo and invitro test system. Furthermore it has antimutagenic potential against cyclophosphamide and BAP induced genotoxicity in microbial and mammalian tests in a dose dependent manner. Curcumin exhibits antimutagenic potential against Sodium azide induced damage in dose dependent manner (Shukla et al., 2002; 2003). Araujo et. al reported that curcumin was clastogenic in mammalian cell culture . Extensive gaps, chromosome fragments and exchanges appeared at doses above (20µg / ml) at all treatment hours (Loganathan Palani Kumar, Natarajan (2008.) The percentage of clastogenicity was higher at doses above 30µg / ml for curcumin. Furthermore Curcumin acts as a potent anti carcinogenic compound through induction of Apoptosis and also inhibits cancer at initiation, promotion and progression stages of development against benzo(a)pyrene induced skin tumors in female Swiss mice (Nagabhushan M , Bhinde S V). In our present study curcumin has proved to be an effective antimutagen at a particular dosage. The molecular mechanisms of its action and its interaction with xenobiotics remain to be elucidated.

CONCLUSION

Sodium Azide has wide application in various fields for human welfare . But it is mutagenic / clastogenic in plant test system . The universality of genetic code has facilitated the use of non- human assay system . Curcumin is antimutagenic and has the capacity to reduce the mutagenic potential of Sodium Azide .

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