The present work was conducted to study the genotoxic effect of Furazolidone, an anti-diarrhoeal drug. Onion root meristems were cultured and analyzed after exposure to different time periods to the drug. There was a time-dependent decrease in mitotic indices and an increase in chromosomal anomalies compared to the control. Cell division was completely arrested after 24 hours of incubation with 0.05% Furazolidone. This study proves the genotoxic effect of Furazolidone and emphasizes the prevention of the use of this drug in medicine.

Keywords: chromosomal anomaly, genotoxicity, mitotic index

INTRODUCTION

An increasing number of allopathic medications are reported to be unsafe for consumption (Howden et al. 1986). The anti-diarrhoeal drug, Furazolidone has been banned by the Food and Drug Administration (Food and Drug Administration 1976), USA but is still being prescribed by the doctors in developing countries (Machado et al. 2008). It is a stable yellow crystalline compound with the chemical name (3-(5-nitrofurfurylideneamino)-2-oxazolidinone. Furazolidone is an anti-bacterial compound commonly used in the treatment of diarrhoea caused by Escherichia coli, Salmonella spp., Shigella spp., and Vibrio cholera, Staphylococcus, Streptococcus, Giardia lamblia and Proteus infections (Sweetman 2009).

Furazolidone functions by interfering with the bacterial enzyme systems, possibly preventing the acetylation of co-enzyme A (Sweetman 2009) and also acts as a monoamine oxidase inhibitor (McCalla 1983). Currently, the adult dosage of Furazolidone is generally 100 mg to be taken four times daily. If the dosage of Furazolidone exceeds 8.8 mg/kg daily it may cause nausea, emesis, abdominal pain and diarrhoea. Other side effects that might occur to patients taking oral Furazolidone therapy include headache, malaise, hypoglycaemia, agranulocytosis, dizziness, partial deafness, polyneuritis and haemolytic anaemia (Zheng and Xing 1988; Vincentini et al. 1993).

Furazolidone is known to be genotoxic, mainly due to the products of its oxido-reductive metabolism, formation of incomplete reduction products and hence show carcinogenic effects (Sumano et al. 2004). It is known to cause inter-strand cross link in DNA (Chatterjee et al. 1989; Basak 1995), effect the tissue sulphhydryl group (Ali 1992), cause DNA damage (Chatterjee et al. 1983), and mutations (Chatterjee et al. 1983; Gao et al. 1989; Bertenyi and Lambert 1996). Recently, Furazolidone was used to eradicate Helicobacter pylori in Brazil (Eisig et al. 2008) and Iran (Ghadir and Iranikah 2009). Ali (1984) and Aila et al. (2009) showed the presence of Furazolidone in poultry meat products, which may have serious toxicological consequences (Zuidema et al. 2005). With this prospective, we evaluated the genotoxic effect of Furazolidone by determining the mitotic index and chromosomal abnormalities.

MATERIALS AND METHODS

Furazolidone (C7H6N2O3) marketed by RPG Life Sciences, D. C Estate, Ankleshwar, India, was used in this study. The root meristems of onion (Allium cepa aggregatum) (2n=16) were exposed to freshly prepared Furazolidone at 0.05% for a period of 1, 4, and 24 hours. A bioassay for monitoring the potential genotoxic effect of Furazolidone was carried out according to the classical Allium test developed by Levan (1938, 1949). The haematoxylin squash method (Mammuth and Subramanian 1960) was used in this study. Heidenhain’s stain solution was prepared by dissolving 0.5 g of haematoxylin (Sarabai Chemicals, Chennai, India) in a mixture of 95% ethanol and distilled water. The stain was filtered and allowed to ripen for 6-8 weeks before use (Sharma and Sharma 1980). Mitotic indexes were recorded from treated and control samples, examining a minimum of 2000 nuclei per root involving 6 root tips from onion bulbs (2 root tips from each bulb) according to Grant (1982). The frequency of division was calculated by following the method of Wilner and Soares (1980). The formula adopted in this method was n/N ×100, where, n is the total number of cells in division and N is the total number of cells scanned. The frequency of chromosomal aberrations in control and treated root tips were determined and classified on the basis of Buckton and Evans (1973). The mitotic irregularities like anaphasic bridge, break, lag, clamp, lag chromosome, abortive anaphase, multipolar anaphase, star anaphase, ring, metaphasic clamp were systematically screened. One way analysis of variance was used to compare the effect between test and control groups using Statistical Package for the Social Sciences (SPSS 15) for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The present study was directed to determine the genotoxic effects of the test compound on biological systems. The majority of mutagenic compounds manifest their cytogenetic effects by inhibiting cell division, generally inducing structural and numerical changes in chromosomes and their teratogenic effect by inducing anomalies in fetuses. Hence, this study was designed to evaluate the genotoxic and teratogenic potentialities of Furazolidone. Epstein (1973) emphasized the importance and need for testing chemicals in short term exposure to evaluate the accidental exposure to
Furazolidone also induced structural aberrations of chromosomes (Table 2; Fig. 1). Chromosomal anomalies such as abortive, bridged, pulverized, clumped and ringed chromosomes was significantly higher when compared to the control samples. Treatment of 0.05% Furazolidone for 1 and 4 hrs showed 29 and 36% of chromosomal anomalies, respectively proving again that the increase in the frequency of chromosomal anomalies was time-dependent. However, some of the chromosomal alterations such as fragmented chromosomes, defective anaphase and polyploidy could not be seen in any of the treatment or control groups. Chromosomal anomalies were completely absent in the control samples at all time intervals.

Similar observations were also made by Angelis (2005) on human intestinal Caco-2 cell lines. Their group also showed that Furazolidone at 5 μg/ml caused a marked decrease in cell viability and cell proliferation, inhibited DNA synthesis, decreased cell number and increased lactate dehydrogenase leakage due to membrane damage (Angelis 1994). A dose-dependent decrease in turkey, *Meleagris gallopavo*, body weight was observed due to Furazolidone (80 mg/kg body weight), along with decrease in reproductive hormone level (Hassan et al. 1986; Ali 1989) and an adverse effect on nervous system.

The ability of the drug to cause chromosomal abnormalities makes it carcinogenic. Chatterjee et al. (1983) showed a dose-dependent effect in DNA damage and mutations. Treatment of bacteria *Escherichia coli* strain TC3960 (*Azu*<sup>−</sup>, *pKM101*) cell with 10 μM Furazolidone yielded a mutation frequency 30 times higher than the spontaneous frequency; base and tandem base substitutions, frameshift and complex mutations and deletions were detected (Bertenyi and Lambert 1996). We observed that the number of dividing cells decreased as the concentration of Furazolidone increased. Cross-linking DNA strands is generally known to decrease cell division due to restricted movements of chromosomes towards the polar region. The ability of Furazolidone to cross-link DNA strands has been demonstrated (Chatterjee et al. 1989).

Furazolidone is also known to affect the reproductive system. A dose and time-dependent decrease in sperm motility was observed in semen incubated with 20 mg/ml Furazolidone for 30 min (Ali et al. 1988). They also showed a significant decrease in spermatocyte production and depression of pituitary output as an effect of Furazolidone at 20 mg/ml. (Ali 1988). A study on turkey showed that birds fed with 700 ppm of Furazolidone caused cardiotoxicity, a reduction in feed intake and growth of the birds (Czarnecki et al. 1987; Gwathmey 1991). Similarly, administration of 0.08% (w/w) of Furazolidone caused cardiotoxicity, a reduction in feed intake and growth of the birds (Czarnecki et al. 1987; Gwathmey 1991). Similarly, administration of 0.08% (w/w) of Furazolidone to chicken, *Gallus gallus domesticus*, resulted in significant decrease in the weight of the testes, wattles and combs, a reduction in the testosterone level of plasma (Ali 1984).

In conclusion, we found that Furazolidone is a genotoxic agent that causes abnormalities in onion chromosomes resulting in the failure of cell division. We also show that the effect of Furazolidone is dose- and time-dependent. Our results agree with many other published studies in other organisms. No similar research work on the effect of Furazolidone on plants was found. Therefore it is advisable to control the use of Furazolidone in developing countries.

**ACKNOWLEDGEMENTS**

The authors acknowledge Department of Plant Biology and Plant Biotechnology, Madras Christian College for providing the laboratory facilities to conduct the experiment.

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### Table 1 Mitotic indices in the root meristems of *Allium cepa* treated with Furazolidone.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>The duration of treatment (in hours)</th>
<th>Total no. of cells scored (N)</th>
<th>Total no. of cells in division (n)</th>
<th>Mitotic indices (n/N × 100 (%))</th>
<th>Prophase (n)</th>
<th>Metaphase (n)</th>
<th>Anaphase (n)</th>
<th>Telophase (n)</th>
<th>No. of cells in each stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>12240</td>
<td>852</td>
<td>6.96&lt;sup&gt;*&lt;/sup&gt;</td>
<td>528&lt;sup&gt;*&lt;/sup&gt;</td>
<td>72&lt;sup&gt;*&lt;/sup&gt;</td>
<td>84&lt;sup&gt;*&lt;/sup&gt;</td>
<td>168&lt;sup&gt;*&lt;/sup&gt;</td>
<td>30&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>13650</td>
<td>192</td>
<td>1.41&lt;sup&gt;*&lt;/sup&gt;</td>
<td>138&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>18&lt;sup&gt;*&lt;/sup&gt;</td>
<td>30&lt;sup&gt;*&lt;/sup&gt;</td>
<td>120&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>12474</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>12096</td>
<td>1284</td>
<td>10.62</td>
<td>870</td>
<td>144</td>
<td>150</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

* The mean difference is significant at P < 0.001

### Table 2 Mitotic anomalies in the root meristems of *Allium cepa* treated with Furazolidone.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>The duration of treatment (in hours)</th>
<th>Total no. of cells scored (N)</th>
<th>Total no. of cells in division (n)</th>
<th>No. of aberrant cells</th>
<th>Types of anomalies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>12240</td>
<td>852</td>
<td>214</td>
<td>C = Clump, F = Fragment, L = Lag, B = Break, TA = Tripolar Anaphase, R = Ring, MA = Multipolar Anaphase, DA = Disturbed Anaphase, Pl = Pulverized chromosomes, DP = Disturbed polarity, CM = C-Metaphase, MF = Multiple fragment, AA = Abortive Anaphase, Bg = Bridge, PO = Polyploidy, SA = Star Anaphase</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>13650</td>
<td>192</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>12474</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1 Induced chromosomal aberrations in the root meristems of *Allium cepa* treated with Furazolidone.**

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**Chemical compounds.**

Measurement of chromosomal abnormalities is an efficient, reliable and economical criterion to measure genetic toxicity (Sharma 1984) which could be indicated by alterations in structure and number. We show a significant reduction in mitotic index during all durations of treatment (Table 1). However, such adverse alterations were absent in the control group. Furazolidone had an immediate toxic effect on the dividing cells as it reduced the mitotic index after 1 hr exposure. A complete arrest of mitosis was noticed after 24 hrs (Table 1). The reduction in the frequency of division showed a time-dependent effect.

Furazolidone also induced structural aberrations of chromosomes (Table 2; Fig. 1). Chromosomal anomalies such as abortive, bridged, pulverized, clumped and ringed chromosomes was significantly higher when compared to the control samples. Treatment of 0.05% Furazolidone for 1 and 4 hrs showed 29 and 36% of chromosomal anomalies, respectively proving again that the increase in the frequency of chromosomal anomalies was time-dependent. However, some of the chromosomal alterations such as fragmented chromosomes, defective anaphase and polyploidy could not be seen in any of the treatment or control groups. Chromosomal anomalies were completely absent in the control samples at all time intervals.

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**ACKNOWLEDGEMENTS**

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