

Genotoxic Effect of Furazolidone, an Anti-Diarrhoeal Drug on the Root Meristem of *Allium cepa* L.

Anu Annamma Mammen¹ • Joyce Sudandara Priya^{1*} • Cordilea Hannah²

¹ Department of Plant Biology and Plant Biotechnology, Madras Christian College, Tambaram, Chennai 600059, India

² Department of Plant Biology and Plant Biotechnology, Women's Christian College, Chennai, India

Corresponding author: * joyce70_sam@yahoo.com

ABSTRACT

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 coenzyme 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 Iranikhah 2009). Ali (1894) 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 (C₈H₇N₅O₃) 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 (Marimuthu and Subramanian 1960) was used in this study. Heidenhan'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 indices 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 $n/N \times 100$, 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, clump, lag chromosome, abortive anaphase, multipolar anaphase, star anaphase, ring, metaphasic clump 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

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

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

*The mean difference is significant at $P < 0.001$

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

Expt. No.	The duration of treatment (in hours)	Total no. of cells scored (n)	Total no. of cells in division (n)	No. of aberrant cells	Types of anomalies															
					C	F	L	B	TA	R	MA	DA	PI	DP	CM	MF	AA	Bg	PO	SA
1	1	12240	852	214	12	-	-	-	-	1	-	-	24	108	-	-	30	66	-	-
2	4	13650	192	68	-	-	-	-	-	-	-	2	6	-	-	54	6	-	-	
3	24	12474	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

C = Clump, F = Fragment, L = Lag, B = Break, TA = Tripolar Anaphase, R = Ring, MA = Multipolar Anaphase, DA = Disturbed Anaphase, PI = Pulverized chromosomes, DP = Disturbed polarity, CM = C-Metaphase, MF = Multiple fragment, AA = Abortive Anaphase, Bg = Bridge, PO = Polyploidy, SA = Star Anaphase

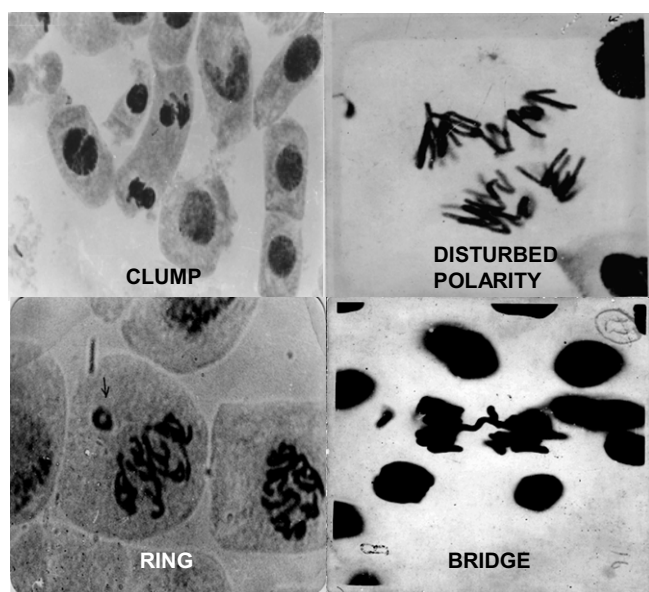


Fig. 1 Induced chromosomal aberrations in the root meristems of *Allium cepa* treated with Furazolidone.

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.

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 de-

crease 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 (Δ uvrB, 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 1986; Czarnecki and Ali 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.

REFERENCES

- Aila O, Shitandi A, Mahungu SM, Sharma HK (2009) Determination of the depletion of furazolidone residues in chicken tissues using a *Bacillus stearothermophilus* test. *Food Control* **20** (6), 543-547
- Ali BH (1989) Effect of Furazolidone and Nitrofurazone on brain gamma-amino butyric acid and glutamate concentrations in chicken. *Clinical and Experimental Pharmacology and Physiology* **16** (5), 363-366
- Ali BH (1992) Effect of furazolidone on tissue sulphhydryl groups, ascorbic acid, and lipid peroxide levels in the rat, and the influence of dimethylsulphoxide thereon. *Pharmacological Research* **25** (3), 247-254
- Ali BH, Homeida AM, Knifton A (1984) Effects of Furazolidone treatment on mature chickens. *Comparative Biochemistry and Physiology. C, Comparative Pharmacology and Toxicology* **78** (1), 43-47
- Ali BH, Silsby JL, Loseth KJ, Crabo B, Halawani ME (1988) Effects of Furazolidone treatment on male turkeys. *International Journal of Andrology* **11** (2), 141-152
- Angelis I (2005) Effects of Furazolidone on human intestinal cell lines. *International Journal of Food Microbiology* **102** (3), 343-347
- Angelis I, Hoogenboom LA (1994) A study on the toxic effect of Furazolidone on human cell lines. *Food and Chemical Toxicology* **152** (1), 119-127
- Basak J (1995) Inter-strand cross-linking of *Vibrio cholerae* DNA induced by furazolidone: a quantitative assay by four simple methods. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **327** (1-2), 5-15
- Bertenyi KKA, Lambert IB (1996) The mutational specificity of furazolidone in the *lacI* gene of *Escherichia coli*. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **357** (1-2), 199-208
- Buckton KE, Evans HJ (1973) Methods for the analysis of human chromosome aberrations. World Health Organization, Geneva. 66pp
- Chatterjee SN, Banerjee SK, Pal AK, Basak J (1983) DNA damage, prophage induction and mutation by furazolidone. *Chemico-Biological Interactions* **45** (3), 315-326
- Chatterjee SN, Basak J, Maiti M (1989) Furazolidone-induced interstrand cross-links in *Vibrio cholerae* DNA. Study of conformational change by circular dichroism. *International Journal of Biological Macromolecules* **11** (3), 172-176
- Czarnecki CM (1986) Quantitative morphological alterations during the development of furazolidone-induced cardiomyopathy in turkeys. *Journal of Comparative Pathology* **96** (1), 63-75
- Czarnecki CM, Ali BH (1987) Influence of thiamin supplements on furazolidone-induced cardiomyopathy in turkey poults. *Journal of Comparative Pathology* **97** (4), 407-413
- Eisig JN, Silva FM, Barbuti RC, Navarro-Rodriguez T, Zaterka S, Moraes-Filho JP (2008) M1070 7-Day Retreatment with Furazolidone, Levofloxacin and Lansoprazole for the eradication of *Helicobacter pylori* in São Paulo, Brazil. *Gastroenterology* **134**, A-332
- Epstein SS (1973) Environment and teratogenesis. In: Perrin EVD, Finegold MJ (Eds) *Pathobiology of Development or Ontogeny*, The Williams and Williams Press, p 105
- Food and Drug Administration (1976) Part V. New Animal Drugs. Furazolidone (NF-180), Fed. Register 41, 19906
- Gao N, Ni YC, Thornton-Manning JR, Fu PP, Heflich RH (1989) Mutagenicity of nitrofurantoin and furazolidone in Chinese hamster ovary cell strains. *Mutation Research Letters* **225** (4), 181-187
- Ghadir MR, Iranikhah A (2009) T1075. The comparison of 2 therapy regimens including Furazolidone, Amoxicillin and Omeprazole with and without Bismuth for the eradication of *H. pylori* in the province of Qum, Iran. *Gastroenterology* **136**, A-494
- Grant WF (1982) Chromosome aberration in *Allium cepa*. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research* **99** (3), 273-291
- Gwathmey JK (1991) Morphological changes associated with furazolidone-induced cardiomyopathy: Effects of digoxin and propranolol. *Journal of Comparative Pathology* **104** (1), 33-45
- Hassan T, Homeida AM, Ali BH (1986) Effects of furazolidone on some reproductive hormones during oestrous cycle in the goat. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **85** (2), 255-257
- Howden A, Boswell P, Tovey F (1986) *In vitro* sensitivity of *Campylobacter pyloridis* to furazolidone. *Lancet* **2**, 1035
- Levan A (1938) The effect of colchicine on root mitosis in *Allium*. *Hereditas* **24**, 471-475
- Levan A (1949) The influence on chromosomes and mitosis of chemicals, as studied by the *Allium* test. *Hereditas* **35**, 325-339
- Machado RS, Silva MR, Viriato A (2008) Furazolidone, tetracycline and omeprazole: a low-cost alternative for *Helicobacter pylori* eradication in children. *Jornal de Pediatria* **84** (2), 160-165
- Marimuthu KM, Subramaniam MK (1960) A haematoxylin squash method for the root tips of *Dolichos lablab* L. *Current Science* **29**, 482-493
- McCalla DR (1983) Mutagenicity of nitrofurantoin derivatives; review. *Environmental Mutagenesis* **5**, 745-765
- Sharma A (1984) Environmental chemical mutagenesis studies in India. In: *Environmental Chemical Mutagenesis Perspective Report Series 6*, Indian National Science Academy, pp 37-40
- Sharma AK, Sharma A (1980) *Chromosome Technique: Theory and Practice* (2nd Edn), Butterworth & Co. Publishers, London, 711 pp
- Sumano AH, Ocampo L, Barragan A (2004) Evaluation of the carcinogenic effects of furazolidone and its metabolites in two fish species. *Pharmacogenomics* **4**, 24-28
- Sweetman SC (2009) *Martindale: The Complete Drug Reference* (36th Edn), Pharmaceutical Press, 3335 pp
- Vincentini O, de Angelis I, Stammati A, Zucco F (1993) Functional alterations induced by the food contaminant furazolidone on the human tumoral intestinal cell line Caco-2. *Toxicology in Vitro* **7** (4), 403-406
- Wilner JL, Soares ER (1980) SCE *in vitro* in mice. *Environmental Mutagenesis* **2**, 35-42
- Zheng ZT, Xing LP (1988) Effect of Furazolidone on gut catecholamine in cysteamine-induced duodenal ulcer in the rat. *Scandinavian Journal of Gastroenterology* **23** (8), 1020-1024
- Zuidema T, Mulder J, Schat B, van Rhijn A, Heestra M, Hoogenboom P, Schat B, Kennedy DG (2005) Metabolism and depletion of nifursol in broilers. *Analytica Chimica Acta* **529**, 339-346