Chromosomal aberrations induced by Glycidol in *Allium cepa* L root meristem cells

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Abstract:

Glycidol is used as a stabilizer in the manufacture of vinyl polymers and natural oils and as an intermediate in the synthesis of glycerol, glycidyl ethers, and amines. It is also used as an alkylating agent, demulsifier, and dye-leveling agent and for sterilizing milk of magnesia. Glycidol is an alkylating agent which reacts readily with glutathione; it causes a decrease in glutathione content in rat liver, probably reflecting its binding to glutathione. It is a suspected reproductive toxicant and exposure to it has the potential to negatively affect the human reproductive system. The study investigated the cytotoxic effects of glycidol in *Allium cepa* L root meristem cells. Different concentrations of glycidol ranging 10, 20, 40 and 50µg/ml for 1, 2, 3 and 5h were treated with root meristem cells of *Allium cepa* L. Fuelgen squash technique was used to determine the aberrations in root tip cells. For each concentration 3000 well-spread cells were scored and cytological abnormalities such as break, gap, exchange, multiple breaks and chromosome fragments were observed. One way analysis of variance was used the study the significant different control and treatment exposed root tip cells. Significant difference between control and glycidol exposed cells were observed. Increasing concentrations increased the number of chromosomal aberrations.

Keywords: Glycidol, *Allium cepa* L, cytotoxicity, chromosomal aberrations, metaphase

Introduction:

It was earlier shown that carcinogenic substances were chemically reactive or were metabolised into chemically reactive intermediates (Miller and Miller, 1966). It has further been shown that these substances act through chemical reaction with DNA and, more recently, that mutations, which might be the result of DNA damage, are a key event in the process of tumor formation (Fearon and Vogelstein, 1990; McCormick and Maher, 1994; Granath et al., 1999;). The risk (probability) of cancer at exposures to genotoxic substances has been assumed to be linearly dependent on the dose (Ehrenberg et al., 1996). Because, according to this assumption, even very low exposure doses will increase the risk, it is necessary to have sensitive analytical methods to identify and quantify risk factors.

An important area of investigation in the field of experimental mutagenesis and mutational plant selection is the search for new mutagens and the study of their effects to obtain valuable mutations. The investigations by American and Japanese scientists in the field of asymmetric synthesis of molecules and mirror catalysis that were awarded with the Nobel Prize in 2001 showed that the same compounds could have different effects, depending on the spatial structure of their molecules (Morgun et al., 2011). The chirality of strereoisomers is a major criterion in the synthesis of various compounds, including plant protection means, i.e., herbicides, insecticides, and fungicides, which are widely used in agriculture (Morgun et al., 2011). Most chemical mutagens are a chiral compounds, and a small number of them have only been studied in a racemic form (±); azaridines and neuroparalytic gas sarin (Rapoport and Kostyanovskii, 1960). It should be noted that sarin and its (+) and (–) analogs considerably differ in toxicity (Rauk et al., 1995). Some nitrosoalkilureas possessing anticancer activity are chiral (Anderson et al., 1975). A high level of mutagenic activity of chiral nitrosoalkilureas with low cytotoxicity was shown for *E. coli* (Isobe and Yano, 1982). Limited studies have reported the genetic activity of (+) and (–) stereoisomers of chiral mutagens for higher plants.

Glycidol is a member of a class of chiral molecules that are important intermediates in the industrial synthesis of pharmaceutical products and other biologically active substances and in production of flavoring and sweetening agents and insecticides. It has been used in the pharmaceutical industry since the 1970s; before then, it was used solely for research purposes (Sharpless, 2001). Glycidol is used as a stabilizer in the manufacture of vinyl polymers and natural oils and as an intermediate in the synthesis of glycerol, glycidyl ethers, and amines.

It is also used as an alkylating agent, demulsifier, and dye-leveling agent and for sterilizing milk of magnesia (IARC 2000, HSDB 2009). The glycidol structure is present in two commercially important groups of derivatives, glycidyl ethers and glycidyl esters, neither of which is prepared directly from glycidol (NTP, 1990).

Glycidol was mutagenic in a variety of in vitro and in vivo short-term tests. Mutagenic activity was observed in *S.typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 exposed to glycidol with and without exogenous metabolic activation. Glycidol was positive in the absence of exogenous metabolic activation in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells; it was not tested with activation. In cytogenetic tests with CHO cells, glycidol induced both sister chromatid exchanges and chromosomal aberrations in the presence and absence of exogenous metabolic activation. Glycidol induced sex-linked recessive lethal mutations and reciprocal translocations in the germ cells of male *Drosophila melanogaster* exposed by feeding. The incidence of micronucleated polychromatic erythrocytes was increased in the bone marrow of male B6C3F1 mice administered glycidol by intraperitoneal injection (NTP, 1990). The *Allium cepa* assay is an efficient test for chemical screening and *Allium cepa* L. root tip cells were used as the test system, as the *Allium* root tip chromosome aberration assay is highly sensitive and capable of detecting mutagens, carcinogens, and clastogens from the environment, and is an effective *in situ* monitor (Palanikumar and Panneerselvam, 2007; Palanikumar et al., 2011). Studies dealing with genotoxicity testing of glycidol in plant test systems are very scarce. Therefore the present study is designed to test the genotoxic potential of glycidol in *Allium cepa* L root meristem cells. The study aimed to investigate the effects of glycidol on *A.cepa* L root meristem cells at concentrations of 10, 20, 40 and 50µg/ml for 1, 2, 3 and 5h.

Materials and methods

Glycidol (CAS code 556-52-5) (molecular weight 74.1) (Figure. 1) is obtained from Sigma Aldrich Co., USA, whereas the other chemicals used in this study were obtained from Hi-Media Laboratories, Mumbai, India. The bulbs were placed on a glass receptacle containing 5 ml of distilled water and, were then sealed and incubated at 25±1 °C in darkness for 72 h. Germinated bulbs with roots of equal length (2-4 cm) were used for experiments.

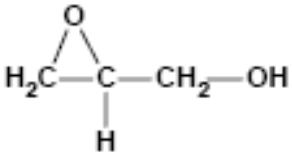

Fig. 1 Structure of Glycidol

As soon as the roots were about 2-4cm long they were treated with different concentrations of Glycidol (10, 20, 40 and 50 µg/ml) for 1, 2, 3 and 5 h. After the treatment the bulbs were washed with distilled H_2O and treated with a 0.05% solution of colchicines for 3 h, and then were fixed in methanol-acetic acid fixative. About 4 tips were hydrolyzed at 58ºC for 10 min. The Feulgen technique was used for squashing (Panneerselvam, 1993; Palanikumar et al., 2007). In the control groups *Allium cepa* L. bulbs were treated with 0.05% solution of colchicine for 3 h, and then fixed in methanol-acetic acid fixative. For each concentration 300 well-spread metaphase cells were scored for chromosomal aberrations 3000 cells were scored on the mitotic index (MI). The number of cells in mitotic division was scored and the MI was calculated as the number of dividing cells per total cells counted. Cytological abnormalities such as break, gap, exchange, multiple breaks, chromosome fragments and, were scored. The significance of the number of aberrations, abnormal metaphases, and the MI was analyzed using one way analysis of variance (ANOVA).

Results

The glycidol induced chromosomal aberrations such as break, gap, iso-chromatid break, dicentric and exchange was analyzed in *Allium cepa* L root tip cells. Glycidol induced chromosomal aberrations at 10, 20, 40 and 50µg/ml was statistically significant when compared with untreated control (Table 1). The result showed that chromatid breaks and gaps are common than iso-chromatid breaks, exchange and dicentric chromosomes. The number of aberrations and number of abnormal metaphases induced by glycidol increased with increasing dosages which represented its mutagenic action in *Allium cepa* L and it was statistically significant when compared with untreated control (Fig. 1 and 2). With increasing concentrations, the number of abnormal metaphases also increased in glycidol treated groups. The percentage of mitotic indexes decreased with

Table 1. Cytogenetic analysis of root tips of *Allium cepa* L exposed to different concentrations of glycidol for different periods

Time of treatment (h)	Concentration (µg/ml)	Mitotic Index (Mean ± SE)	NC	Break	MB	Gap	Chromatid bridge	Dicentric	Total ± SE
	Control	24.2 ± 3.2	496.0	2.45	0.00	0.00	0.00	0.00	2.45 ± 1.10
	10	20.5 ± 2.9	485.0	10.50	0.00	0.00	0.00	0.00	10.50 ± 4.70*
1h	20	18.6 ± 2.4	476.0	14.75	0.00	0.00	0.00	0.00	14.75 ± 6.60*
	40	17.3 ± 2.8	445.5	19.25	4.50	0.00	1.00	0.00	24.75 ± 8.21*
	50	16.5 ± 2.1	438.5	21.35	5.50	1.05	1.00	0.00	28.90 ± 8.96*
	Control	26.2 ± 2.8	495.5	3.55	0.00	0.00	0.00	0.00	3.55 ± 1.59
	10	18.6 ± 2.3	481.0	13.45	0.00	0.00	0.00	0.00	13.45 ± 6.02*
2h	20	17.5 ± 3.1	476.5	17.55	1.35	0.00	0.00	0.00	18.90 ± 7.72*
	40	15.8 ± 2.9	441.6	19.25	4.75	0.00	1.45	0.00	25.45 ± 8.15*
	50	14.9 ± 3.1	436.6	24.75	6.45	1.45	2.35	1.00	36.0 ± 10.04*
	Control	27.6 ± 2.4	491.6	4.55	0.00	0.00	0.00	0.00	4.55 ± 2.03
	10	17.7 ± 2.1	471.5	15.65	2.45	0.00	0.00	0.00	18.10 ± 6.81*
3h	20	15.9 ± 2.3	466.5	21.35	4.55	0.00	1.00	0.00	26.90 ± 9.12*
	40	14.2 ± 2.1	457.5	24.55	6.55	1.05	1.55	0.00	33.70 ± 10.27*
	50	13.2 ± 2.4	446.5	28.45	7.45	2.45	2.55	1.55	42.45 ± 11.40*
	Control	28.4 ± 3.1	479.5	5.75	0.00	0.00	0.00	0.00	5.75 ± 2.57
	10	17.1 ± 2.4	455.5	22.45	3.25	0.00	0.00	0.00	25.70 ± 9.78*
5h	20	15.7 ± 2.3	435.0	31.55	5.75	1.00	1.00	0.00	39.30 ± 13.43*
	40	13.2 ± 2.1	425.0	33.45	6.75	2.55	1.55	1.00	45.30 ± 13.82*
	50	11.5 ± 2.8	420.0	35.75	7.45	3.45	3.55	2.55	52.75 ± 14.21*

abbreviations: NC – normal cells; MB – Multiple break ; *p< 0.05 when compared to control

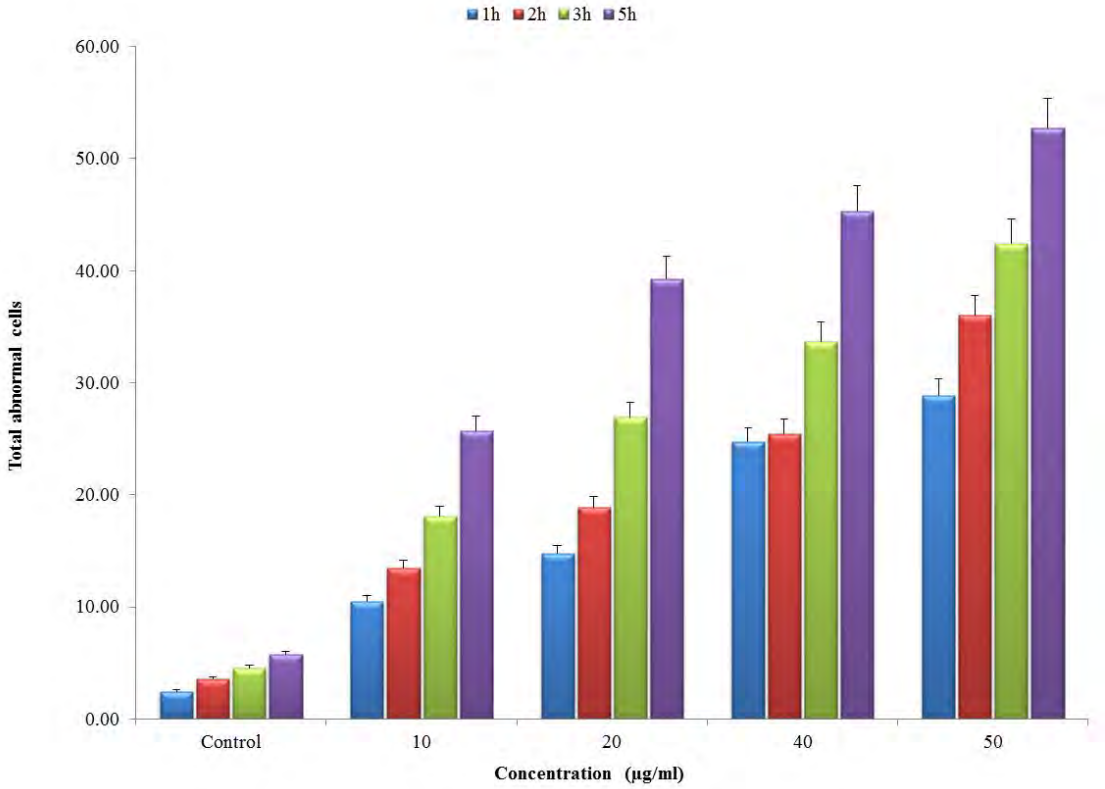

Fig 1. Cytotoxic effects of glycidol at different time points in *Allium cepa* L root tip cells

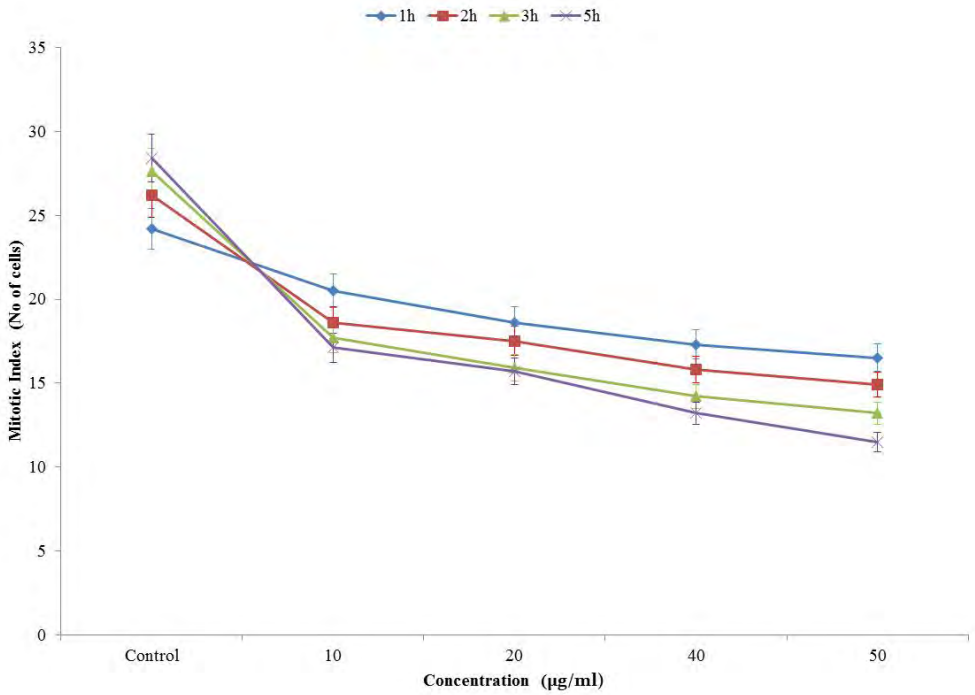

Fig 2. Reduction in Mitotic Index of *Allium cepa* L root meristem cells treated with glycidol at different time points and concentrations

increasing concentrations of glycidol compared with untreated control, which explains its cytotoxicity in plant test system.

Discussion

Numerous potentially mutagenic chemicals have been studied mainly because they can cause damaging and inheritable changes in the genetic material. Several tests are commonly used for biomonitoring pollution levels and to evaluate the effects of toxic and mutagenic agents present in the natural environment. *Allium* test has been proposed as a standard method in environmental monitoring and toxicity screening of wastewater and river water (Fiskesjo, 1993). Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division (Marcano et al., 2004). The cytotoxicity level of environmental pollutants can be determined by the decreased rate of the mitotic index (Smaka-Kincl et al., 1996). Lethal doses of 0.45 to 0.85g/kg of glycidol administred by gastric tube in rats resulted initially in nervous system depression, but surviving animals showed a reversal of the depressant effects (Hine et al., 1956). According to the 1981–83 National Occupational Exposure Survey (NOES, 1999) as many as 4900 workers in the United States were potentially exposed to glycidol.

Orally administered glycidol is absorbed from the gastrointestinal tract of male Fischer 344 rats. Seven to eight per cent of the dose remained in tissues 72 h following administration. The highest concentrations of radioactivity were observed in blood cells, thyroid, liver, kidney and spleen (Nomeir et al., 1995). Most of these aberrations are lethal which can cause genetic effects, either somatic or inherited (Swierenga et al., 1991). The severe retardation of root growth, lethal effects based on very low MI, aberrant cells, nuclear leisions and disintegration are signs of the mutagenecity of these effluents (Abu et al., 2010). Plant tests have been widely used for detecting the genotoxicity of chemical compounds and for *in-situ* monitoring of environmental genotoxic contaminants, without submitting samples to preliminary concentration or to a very long, difficult and expensive preparation phase (Grant, 1994)

The outcome of the present results showed an increasing toxicity response to different concentrations of glycidol at different time points. According to Odeigah et al., (1997), with increasing concentration and consequently, increasing toxicity, there was an inhibitory effect on cell division. This might occur in pre-prophase, where cells are prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase resulting in a high frequency of prophase cells. Mutagenic agents can be cytologically detected by the inhibition of the cell cycle, interruption induction of numerical and structural chromosomal alterations, and exchanges within sister-chromatids, among others (Vieira and Vicentini, 1997). Chromosome bridges and fragments occur as a result of chromosome breakage, vagrant chromosomes show abnormalities in the mitotic spindle (Grant, 1978). The genotoxic compounds in soil can affect human

health in various ways – inhalation of dust which contains these compounds, ingestion of plants that uptake the compounds from soil and leaching of the compounds from soil to groundwater and surface water used as drinking water (Watanabe et al., 2001). Statistical analysis showed that the genotoxic potential of glycidol showed an time and dose dependent response (Palanikumar and Panneerselvam, 2007; Palanikumar et al., 2011). Further studies are required to determine the molecular mechanisms of glycidol induced genotoxic effects.

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