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Cytotoxic effect of antidiabetic drug glibenclamide on root meristem of *Allium cepa l.*

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Abstract
Diabetes is a commonly occurring disorder in many parts of the world. It is caused by other pre-disposing factors such as obesity, ageing and antigenic/antibody reaction. The acceptable medication for new cases of Diabetes mellitus is treatment with synthetic drug Glibenclamide. It is one of the most commonly used sulphonylureas in elderly patients with Type-2 diabetes,but the risk of severe or fatal hypoglycaemia is associated with the use of this drug which in- creases exponentially with age. *Allium* test is a sensitive test that has often been used for the determination of cytotoxic and genotoxic effects of Glibenclamide. On increasing the dosage from 20% to100% the gradual increase in effect was noted.The result illustrated that the Glibenclamide,reduce the number of dividing cells in root tip of *Allium cepa*.The study states that Glibenclamide even after treatment with various time schedule remains genotoxic as it has the ability to initiate the chromosomal aberrations of various types.

Keywords: glibenclamide, diabetes mellitus, cytotoxic effect and genotoxic effect

Introduction

Diabetes is a common and a very prevalent metabolic disorder which causes diabetic complications in human and animals of developed and developing countries. It is a condition with disturbances in carbohydrate and protein metabolism resulting from de- fects in insulin secretion, insulin action or both (WHO 1999). Long term effects of dia- betes leads to progressive complications of retinopathy, renal failure and autonomic nerv- ous system dysfunction. The acceptable medication for Diabetes mellitus is treatment with Glibenclamide. It is also known as glyburidewhich is a sulphonyl urea used to treat diabetes mellitu in elderly patients of Type-2 diabetes, but the risk of se- vere or fatal hypoglycaemia is noticed more with the use of this drug. It interacts with the sulphonylurea receptor(SUR)and has been shown more recently to inhibit the cystic fi- brosis transmembrane conductance regular protein (CFTR), both protein that are mem- bers of the ABC(Adenosine 5'-triphosphate (ATP)-binding cassette) transporters.The ap- parent lack of consistency in the pharmacoki- netics of Glibenclamide, coupled with the in- complete information on the characteristics of its dose response relationship (Ferner and Chaplin,1987; Marchetti and Navalesi,1989; Nattrass,1986) [8, 22] often makes it difficult to de- termine optimum drug dosage in the individ- ual diabetic patients with the considerably in- creased potency on milligram basis of second generation sulphonylureas, as compared with older agents,there has been need for highly specific and sensitive methods of drug analy- sis in order to follow the fate and actions of *in vivo*. From different studies it has been found the increased risk of cancer in type 2 diabetes. Patients which have type 2 diabetes exposed to sulphonylureas have a significant increased risk of cancer-related mortality. It is uncertain whether this increased risk is re- lated to a deleterious effect of sulphonylurea or due to some unmeasured effect related to choose of therapy and cancer risk. The risk of mortality from solid tumors is higher in sul- phonylureas user. The long-term used of the diabetes drug sulphonylureas has been found to be associated with a lower risk of pancre- atic cancer-but only in women according to new research published in the American jour- nal of Gastroenterology. Tolbutamide and glibenclamide (glyburide) are widely used in type 2 diabetic patient because they induce insulin secretion indepentdently of the meta- bolic state of the β-cell (Aynsley *et al*., 1981; Huopio *et al*., 2002; Levetan, 2007) [2, 16, 20]. *Allium cepa* L. is a monocot plant be- longing to the family Liliaceae (Family Ama- ryllidaceae or Alliaceae. The AT tests geno- toxicity using chromosomes,hence it does not deal with simple point mutations but instead genomic mutations that cause morphological changes on the chromosomes. Moreover, it was shown that comparing the effects of cy- totoxicological agents on *A. cepa* and on ani- mal cell lines, the results obtained were simi- lar and comparable (Tedesco and Laughing- house 2012) [33]. The use of a plant, i.e. an organ- ism with relatively high level of complexity, provides data about potential damage to DNA in a multicellular context, a possibility that is not provided by cell cultures, even if the re- sults may be of importance since the genomes of cultured cells may be of mammals, as in the case of mouse bone marrow cells (Nabeel *et al*., 2008; Algarni, 2018) [24, 1] or even trans- formed lines of human cells (Li *et al*., 2015). Compared to the use of animals for testing, the AT is more cost effective (Vicentini *et al*., 2001; Tedesco and Laughinhouse, 2012) [36] and potentially provides a large amount of data with very easy cultivation techniques and without the ethical concerns that affect the use of animals in testing and that necessi- tate complex breeding guidelines. Moreover, it was shown that compar- ing the effects of cytotoxicological agents on *A. cepa* and on animal cell lines, the re- sults obtained were similar and comparable (Tedesco and Laughinghouse, 2012) [33]. Cyto- logical analysis with respect to either mitotic or neurotic behaviour is considered one of the most dependable indices to estimate the po- tency of mutagen and sensitivity of plants for different mutagens. The mitotic index (MI) is a reliable predicator of cell

proliferation in the tissue *invitro* as well as *in vivo* that is used to characterize proliferating cells in mitosis and total number of cells.The mitotic index can be worked out from a slide even with light microscopy.The mitotic index is a cyto- genetic test used as*in vivo* and *in vitro*meth- ods for the examination of genotoxic effects of a compound over a short period. In the present work five different concentrations of Glibenclamide tablet 20%, 40%, 60%, 80% and 100% were used to monitor and evaluate the physiological and genotoxicological effects in *Allium cepa* test System.

Materials and Methods Experimental Solutions

For experimental purpose five different con- centrations of Glibenclamide (5mg) viz., 20, 40, 60, 80, and 100% were prepared by using distilled water. Similarly, control was main- tained using distilled water alone. That was considered as zero percentage (0%).

Test system

A number of testing systems are now available to find out the biological effects of environmental pollutions. The use of plant material for biological testing of chemicals has gained new interest in the field of envi- ronmental monitoring. Among other test sys- tems *Allium cepa* has been listed as an exam- ple of a plant useful in screening of mutagens. The common name of *Allium cepa* is onion, which belongs to the family Lilliaceae, which is an intermediate family between the Lilliaceae and Amarillidaceae. The *Allium* test was developed by Levan (1938) [19] as an assay system for the analysis of the effect of chemicals on chromosome be- haviour. Recently Fiskesjo (1985) suggested that the *Allium* test in its present form is a val- uable tool which can be used as a standard tool in environmental monitoring.

Preparation of the test system

Healthy, i.e., disease free onion bulbs have been selected. Because, unhealthy bulbs infected by the microbes could spoil the experiments. It was necessary to scrap the base of the bulb, so that fresh root primordial would be emerged. A se- ries of cleaned small sized bulbs of on- ions, (*Alliumcepa*) were first sprouted in water as described by Fiskesjo (1987). After 12 hours, the best in terms of root growth were selected. Four each were placed on the tubes containing the differ- ent concentrations (viz.,20, 40, 60, 80, and 100%) of the experimental solutions for different exposure schedules i.e., 12,24 hours, 48 hours and 72 hours. But the control was maintained under identi- cal condition using distilled water. All the experiments were performed under the room temperature by protecting them from direct sun light. After the scheduled period, the treated and untreated (control) root tips (of 1 to2 cm length) from the bulbs were cut off and kept in Carnoy'sfluid for 15 minutes. It was done at early morning at about 4.30 to 5.30 AM. After fixation of their stages, the root tips were thoroughly washed in distilled water. Finally, they were stored in70% absolute alcohol in small labelled bottles at 4°C in a refrigerator for the fur- ther use.At the time of hydrolysis, the fixed root tips using the Farmer's fluid were taken from 70% alcohol and washed in distilled water before hydrolysis. Then they were hydrolyzed in 1N HCL by in- cubating them in a Hot Air Oven at 60°C for 1 minute. This treatment cleared the cytoplasm of the cell, leaving only the nu- cleus by changing the pH.The hydrolyzed root tips were washed with distilled water to remove the acid traces. After a clean wash, the root tips were placed individu- ally in clean dry slides and stained with acetocarmine for 8-10 minutes.

After staining, the stained root tips were squashed under the cover slip using needle base and a piece of blotting paper was used to clean the excess stain. Care was taken while doing the experiment to avoid the entry of debris and air bubbles into the cover slip. The slides thus pre- pared were viewed under the light micro- scope to study the two main aspects namely, Mitotoxicity and Karyotoxicity. The prepared slides were coded and ex- amined for chromosomal aberrations at high magnification (X1000). The mitotic index (MI) was calculated as percentage of the number of dividing cells per 500 observed cells (Fiskesjö, 1985, 1997) [10, 11] that is, 100 cells were examined per slide per concentration, including the control. The mitotic inhibition was estimated as the percentage of the difference between the mitotic indices of the control and the group, divided by the mitotic index of the control. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of the effluent (Ba- kare *et al*., 2000) [4].

Results

The cytological effects of the Glibenclamide have been tested using *Al- lium cepa* test system.It is estimated on the basis of change in mitotic index and other abnormalities (Mitotoxicity and Karyotoxicity) induced by Glibenclamide. Observations were made on mitotic indices and the percentage of prophase, metaphase,anaphase and telo- phase cells, whereas under Karyotoxicity observations were made on clastogenicity and turbagenicity. The results are pre- sented in the tables. The mitotic index and mitotic aberrations are also was de- creased with the increase in concentration of drug solution. But there were no chro- mosomal aberrations in control. The re- sults were presented in the Table1-4.

Table 1: Cytotoxic manifestation induced by Glibenclamide in the root meristem of Allium cepa after 12 hrs treatment.

Conc. of Glibencl amide (In %)	Mitotic Index (In %)	Prophase (In %)	Metaphase (In %)	Anaphase (In %)	Telophase (In %)	Mitotic Aberra- tions (In %)	Clasto- genicity	Tur- ba- genic- ity
0	90.4	85.3	32.86	1.29	0.67	0	0	0
20	77.52	75±2.16	1.31±0.25	1.10±0.10	0.56±0.16	0.43±0.20	0.17	0.26
	(-14.2)	(-12.07)	(-96.0)	(-14.7)	(-16.4)			
40	73.17	70.89±1.66	1.68±0.09	1.03±0.25	0.30±0.07	0.46±0.19	0.09	0.31
	(-19.0)	(-16.8)	(-94.8)	(-20.15)	(-55.2)			
60	72.21	69.65±3.0	1.13±1.46	1.03±0.18	0.38±0.10	1.94±0.86	1.22	0.72
	(-20.1)	(-19.05)	(-96.5)	(-20.15)	(-43.2)			
80	66.63	62.10±1.48	2.79±1.46	1.39±1.07	0.32±0.03	2.16±0.96	0.77	1.39
	(-26.3)	(-27.1)	(-91.5)	(7.75)	(-42.8)			
100	61.21	56.74±1.72	2.5±1.56	1.15±0.30	0.77±0.01	2.55±1.14	1.63	0.92
	(-32.2)	(-33.74)	(-92.3)	(-10.8)	(14.9)			

Table 2: Cytotoxic manifestation induced by Glibenclamide in the root meristem of *Allium cepa* after 24hrs treatment.

Conc.of Glibenclamide (in %)	Mitotic index (in %)	Prophase (in %)	Meta- phase (in %)	Anaphase (in %)	Telophase (in %)	Mitotic aberration (in %)	Clastoge-necity	Turba-genecity
0	75.86	66.8	1.83	1.68	0.91	0	0	0
20	73.0 (-3.77)	70.43±3.3 (5.43)	1.28±0.06 (-30.05)	1.02±0.20 (-39.2)	0.59±0.04 (-35.1)	0.12±0.07	0.07	0.05
40	71.56 (-5.6)	70.15±4.49 (5.0)	1.70±0.23 (-7.10)	1.32±0.22 (-21.4)	0.47±1.42 (-48.3)	0.13±0.06	0.092	0.040
60	66.8 (-11.9)	58.4±1.54 (-12.5)	1.32±0.17 (-27.8)	0.8±0.04 (-52.3)	0.36±0.02 (-60.4)	0.60±0.26	0.27	0.33
80	47.2 (-37.7)	43.6±1.49 (-34.7)	1.86±0.34 (1.63)	1.12±0.89 (-33.3)	0.07±0.04 (-92.3)	0.64±0.28	0.29	0.31
100	40.9 (-46.0)	37.3±1.39-(44.16)	1.23±0.56 (-32.7)	1.54±0.80 (-8.3)	0.77±0.07 (-15.3)	1.19±0.53	0.28	0.91

Table 3: Cytotoxic manifestation induced by Glibenclamide in the root meristem of *Allium cepa* after 48 hrs treatment.

Conc.of Glibenclamide (in %)	Mitotic index (in %)	Prophase (in %)	Meta- phase (in %)	Anaphase (in %)	Telophase (in %)	Mitotic Aberration (in %)	Clasto-genecity	Turba-genecity
0	90.17	86.93	1.51	1.07	0.64	0	0	0
20	78.8	75.7±1.92	1.79±0.56	1.0±0.52	0.2±0.31	0.22±0.09	0.13	0.09
	(-12.6)	(-12.9)	(18.5)	(-6.5)	(-68.7)			
40	62.9	59.84±0.78	1.63±0.05	0.8±0.03	0.63±0.08	0.35±0.15	0.14	0.21
	(-30.2)	(-31.2)	(7.94)	(-25.2)	(-1.56)			
60	69.87 (-22.5)	65.91±1.54 (-24.18)	2.34±0.88 (54)	1.05±0.16 (-1.68)	0.56±0.03 (-12.5)	0.49±0.21	0.26	0.24
80	56.7 (59.0)	52.2±1.07 (-39.9)	2.07±0.97(37.0)	1.30±0.93 (21.4)	1.09±0.25 (70.3)	1.43±0.63	0.08	1.39
100	46.75 (-48.15)	43.2±2.57 (-50.3)	1.64±0.49 (8.6)	1.14±0.68 (6.5)	0.73±0.05 (14.06)	1.52±0.69	0.09	1.41

Table 4: Cytotoxic manifestation induced by Glibenclamide in the root meristem of *Allium cepa* after 72 hrs treatment.

Conc. ofGlibencla mide (in %)	Mitotic index (in %)	Prophase (in %)	Metaphase (in %)	Anaphase (in %)	Telophase (in %)	Mitotic ab-erration (in %)	Clasto-genecity	Turba-genecity
0	83.08	80.35	1.48	0.62	0.62	0	0	0
20	80.4	76.4±1.16	1.57±0.71	0.57±0.46	0.56±0.46	0.11±0.04	0.03	0.07
	(-3.2)	(-4.8)	(6.08)	(-8.06)	(-9.6)			
40	72	68.65±2.59	1.45±0.36	1.09±0.02	0.8±0.02	0.39±0.17	0.06	0.33
	(-14.8)	(-14.5)	(-2.0)	(75.8)	(29.03)			
60	50.85	47.75±3.17	1.7±0.08	0.73±0.12	0.57±0.04	0.54±0.24	0.31	0.23
	(-38.7)	(-40.5)	(14.86)	(17.74)	(-8.06)			
80	49.5	45.3±2.74	2.08±0.19	0.31±0.05	0.76±0.30	0.59±0.26	0.2	0.39
	(-40.4)	(-43.6)	(40.5)	(-50)	(-52.8)			
100	46.9	42.8±1.07	2.36±0.18	1.13±0.20	0.96±0.27	0.64±0.28	0.13	0.48
	(-43.5)	(-46.7)	(59.4)	(82.2)	(54.8)			

Discussion

The *Allium* test has many advantages as gen- otoxicity screening assay, one being that *Al- lium* root cells possess the mixed function ox- idase system which is capable of activating genotoxic chemicals. In the *Allium* test, inhi- bition of rooting and the appearance of stunted root indicate retardation of growth and toxicity, whileroot wilting explains tox- icity (Odeigah *et al*; 1997; Grant, 1982) [26, 14].

Concentration Response

The various concentrations such as 20, 40, 60, 80, 100% and control were main- tained to expose the system for various schedules 12hrs, 24 hrs, 48 hrs, 72 hrs to com- pare the toxic effects of glibenclamide. The mitotic index is a parameter of cytotoxi- city in studies of environmental biomonitor- ing has been used to check the cytotoxicity level of test compounds (Pesnya*et al*., 2017. The cell division was inhibited at various de- grees in the four stages of mitosis in the treated root as shown in the Table 1. A strong dosage effect was obvious from declined val- ues in the mitotic indices with the increasing concentration of the Glibenclamide and ex- hibiting a marked decrease (26.3) at highest concentrations (80%) when compared to con- trol. In the presence of certain external stim- uli, the cellular progress can be blocked in one of the phases of the cell cycle or cell di- vision, and their action is called mito inhibi- tion. Mitogens act to overcome intracellular braking mechanisms that block cell cycle progression, and their action is called mi- tostimulatory. Any deviation from the orderly and directed progression of the cell cycle, and respectively, of mitosis and cytokinesis, is re- flected in a state of cytotoxicity and genotox- icity. These are evaluated by the mitotic in- dex (MI; a measurement to determine the per-centage of cells undergoing mitosis), percent- age of cells in each mitosis phase (prophase, metaphase, anaphase and telophase index), as well as a series of clastogenic, aneugenic and turbagenic changes. The result of the present study clearly indi- cate the clastogenic, turbagenic, properties of the Glibenclamide, which is evident from the lower value of Mitotic index and chromoso- mal aberrations. Mitotic index is a best bio monitor to asses the effects of various chem- icals on cells division.

Shehab (1979) [31] found similar result with the extracts of *pulicariacrispa*on the mitotic in- dex and percentage of abnormalities in onion, which increase with increase of the concen- tration and duration of treatment.The ob- served decrease in the mitotic index value and increase in the incidence of chromosomal aberrations with corresponding increase in the concentration of the extracts conformed to the findings of Bakare *et al*. (1999, 2003) [5, 3].

Allium cepa test system has been widely used in scanning for the clastogenic effects of dif- ferent chemicals due to its relative simplicity. Common clastogenic effects/aberrations in- clude chromosome and/or chromatid frag- ments, interchromatid or subchromatid con- nections, nucleoplasmic bridges, heteromor- phic chromosomes,

dicentric or ring chromo- somes, and micronuclei (MNs). Bimitosis and asynchrony of the cell cycle could also be added. Manifestations such as gaps, sticki- ness, fragments, micronuclei and bridges hasadvantages as abioassaydue to easy ma- nipulation, sensitivity,rapid analysis,low - costand an ability to be used in correlation with other models using mammalian cells Sticky chromosomes formed in onion root at be due to protein adhesion (Patil and That 1992). Mercykutty and Stephen (1980) [23] stated that stickness in the chromosome may arise

due to DNA depolymerization,nucleopro- teindissolution, breakage and exchange in basic folding fibre units of chromatids,and stripping of protein covering of the DNA in chromosomes. Later, Fiskesjo (1985) stated that a sticky chromosome has an irreversible toxic effect which may lead to cells cessa- tion.Sticky chromosomes are also found to be responsible for free anaphase separation and inversion of chromosome segments(Go- murgen 2005) [13]. The cell is Considered aber- rant even if only one chromosome deterio- rates. Interchromatid or subchromatid connections, known as chromosome bridges are chromo- somal structural changes that may result from exchanges between homologous or non-ho- mologous chromosomes, or either due to chromosome stickness producing abnormal anaphase separation or may be attribute to unequal translocation or inversion of chro- mosome segments and also due to the break- age and fusion of chromosome or chromatids and may be the consequence of dicentric chromosome formation or poor activity of replication enzymes. (Patlolla *et al*, 2012. A. cepa is suitable for the detection of genotoxi- cagents effects that are manifested in the form of clastogenic and aneugenic effects (Firbas and Amon 2014) [9].

Segregational errors lead to turbagenic ef- fects. It is related to the disturbed metaphase, anaphase, and non- orientation of chromo- somes in metaphase. Micronuclei, lagging and forward chromosomes are also included under this category. Chromosome lagging or vagrant chromosomes was one of the most frequent abnormalities observed in this ex- periment. Lagging chromosomes during ana- phase considered to be a sign of genetic ma- terial loss and closely associated with the an- euploidy formation. Similar condition was observed by Invanova *et al*., 2008 in the effect of heavy metal and cyanide- contami-nated waters. Lagging chromosomes resulted due to failure of the chromosomes to get at- tached to the spindle fiber and to move to ei- ther of the two poles and the results were cor- related with the study of Turkoglu, (2007) [35].

At the same time the literatures also illus- trates that, the regular use of these antidia- betic drugs binds to the SUR1 subunit, lead- ing to inhibition of K-ATP channel activity, membrane depolarization and insulin secre- tion, but this failure activity is not understood now, either it related disease or drug effect (Pontiroli *et al*., 1994; Genuth, 1990) [30, 12]. It is also found that the treatment with glibenclamide and tolbulatamide may induce ca2 + dependent β-cell apoptosis (Efanova *et al*., 1998) [6]. It is reported that the glibenclamide treated minimum 6 cells showed a reversible reduction in insulin con- tent and accerelerate apoptotic β-cell death (Kawaki *et al*., 1999; Takahashi *et al*., 2007) [18, 32] apoptosis is specially enhanced only by ex- pression of the receptor SUR1 but not SUR2B, in HEK 293 cells (Hambrock *et al*., 2006) [15]. The dysfunction of pancreatic β- cell causes pancreatic cancer in diabetics.

The present study was correlated with the findings of Fiskesjo a positive result in *Al- lium* indicate a potential hazard in organisms and wide variety of abnormalties is an indica- tion of the high mutagenic potentials of the Glibenclamide an antidiabetic synthetic drug tested.

Conclusion

This study was conducted to see the toxic ef- fects of Glibenclamide in the root tip cells of onion. Five different concentration of Glibenclamide (20, 40,60,80 and 100%). The control was also taken. It was observed that the Glibenclamide affected the mitotic index and caused Some anomalies on root tip cells off allium cepa. The percentage of dividing cells decreased with the increase of the con- centration of Glibenclamide. Irregularities were disturbed prophase and meta- phase, Sticky bridges at anaphase. The pre- sent investigation thus demonstrated that the Glibenclamide affects the root tip cells lead- ing to decrease in mitotic index as well as chromosomal irregularities in *Allium cepa* root cells.

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