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Chromosome Aberration Studies In *Allium Cepa* Caused By Clonazepam

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ABSTRACT:
The study is conducted to understand the genotoxic and mito-depressive potential of clonazepam. *Allium cepa* is used as the model to test the harmful effects of the drugs as they have been reported to be having long half life and thereby may cause the damage when an overdose takes place. The result of this study pointed that the concentration involved and time period included showed significant depression in the mitotic activity except at 50µg/L (48 hrs) and 150µg/L (48 & 72 hrs) when it was compared to control. The various chromosomal aberrations observed were chromosome, bridge formation, sticky chromosome and fragmentation. Vagrant chromosomes were found to be more in occurrence and fragmentation is least in the occurrence than any other types. Sticky chromosomes and fragmentation found to be highest in number at 150µg/L at 72 hrs as compared to control.

INTRODUCTION:
Benzodiazepines are neurotoxic drugs, which when ingested wrongly will cause neural alterations and also create stress [1]. The overdose of this drug affects the central nervous system and act as depressants, which act to increase toxicity [2]. These alter the chromosomal structure [3]. And hence, any overdose may affect the genetic elements of the organisms. Benzodiazepines, like alprazolam has been reported to cause varying degrees of chromosomal aberrations [4-7], nuclear alterations [4] and micronucleus formation [8]. Clonazepam is one of the benzodiazepines, is reported to cause harm and will increase the deformities when administered to a pregnant woman during gestation period [9-10].

Plant root tips are used as an easy and excellent model for understanding the mechanism of chromosomal aberrations (CA). *Allium cepa* is the universal model as it is sensitive and simple and is regarded as good indicators of contamination. *Allium cepa* is the most efficient system for Genotoxicity studies [11-14].

The intent of this study was to assess the genotoxic effect of Clonazepam by employing *Allium cepa* root tip cells.

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MATERIALS AND METHOD:

Healthy, diseased free onion bulbs (*Allium cepa*, 2n=16) were procured from the market. The dried scales were removed and are further used for the experiment. Clonazepam is a tranquilizer of class of psychoactive drug i.e benzodiazepine with molecular formula $C_{15}H_{10}ClN_3O_3$ and molecular weight 315.71 g/mol were used in the study.

Dry leaves of onion bulbs were removed to expose the root primordial and then placed in couplin jars with distilled water at 26◦C+ and in dark conditions for till the roots reach up to 2cms. These bulbs were then kept in different drug concentrations (50, 100, 150 µg/L) for three different times (24, 48, 72 hrs) to understand the dose dependant and time dependant effect of the drug. After the required time the root tips were taken and then processed for squash preparation [14] with some modifications.

Microscopic slides were prepared by using the conventional staining technique. Root tips were hydrolysed for 10 minutes in 1N HCl at 60 ^{0}C. Then roots were then treated with 2% acetocarmine in water bath at 60 ^{0}C. After staining the root tips were. The cells were observed under microscope at 1000X magnification for different types of aberrations. All the experiments were conducted in triplicate.

In the analyses, different chromosome aberrations were considered: Chromosome Bridge, sticky, vagrant chromosome, fragmentation [15]. Mitotic Index (MI) was analyzed as it is an indicator of cytotoxicity [16-17].

For the study of Mitotic Index (MI), 1000 cells were scored and for CAs 100 cells were examined. A hundred cells in metaphase were scored and the number of aberrant cells in each experimental group and is compared from with the control group.

Data has been represented as the mean, standard deviation (SD) of the means frequency of aberrant cells and MI. ANOVA was employed for statistical analysis at $p<0.05$.

RESULT AND DISCUSSION

The results showed concentration & time- dependent reduction in MI of root tip cells of *Allium cepa* (Table 1). The reduction in MI observed in the root tip cells was found to be statistically significant ($p<0.05$) after using ANVOA except at 50µg/L (48 hrs) and 150µg/L (48 & 72 hrs) when it was compared to control. The inhibition trend in MI was observed to be linear as the concentration of the drug increased with an increase in the time interval and the lowest count is found at 150µg/L at 72 hrs. Table 1 also depicts the decrement in all the

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phases of mitosis in the root tip meristems as the dose of the drug and time interval increased gradually. The least frequency observed- prophase at 150µg/L at 72 hrs, metaphase at 150µg/L at 48 hrs, anaphase at 150µg/L at 48 hrs and telophase at 100 & 150µg/L at 72 hrs.

The various chromosomal aberrations observed in the study after treating the root tip cells with clonazepam are vagrant chromosome, bridge formation, sticky chromosome and fragmentation (Table 2). Statistically significant ($p<0.05$) increase in number of CAs was observed in concentration and time dependent manner in the cells of *Allium cepa*. Among the different types, Vagrant chromosomes were found to be more in the occurrence and fragmentation is least in occurrence than any other types. However, sticky chromosomes and fragmentation found to be highest in number at 150µg/L at 72 hrs which is statistically significant when anaylsed with the ANOVA test when compared with untreated control.

Allium cepa is being accepted as a universal model for the study of chromosomal aberrations, cellular toxicity and nuclear deformities [12, 18-20] because of its direct contact with the water or soil (with substance) may be responsible for possible DNA damage or chromosomal aberrations. In this study, the damage to the genetic end resulted due to exposure to clonazepam was assessed. Clonazepam caused a significant inhibition of mitosis in root tip cells and also induced significant CAs.

The dose and time dependent increase in inhibition to MI and CAs was observed in the study, which signifies cytotoxic, genotoxic and mitodepressive function of the drug. A similar result of the dose dependant increase in CAs was also observed [5-6]. The treatment of *Allium cepa* root tip cells with alprazolam cause a significant change in the mitotic activity [5]. The analysis of chromosomal aberrations for all the dose of the drug and time provides an understanding of the clastogenic effect of clonazepam and may lead to mutation, base mismatch and CAs [21]. Different CAs was observed at different dose and time at all concentration shows a linear increase. Vagrant chromosome, sticky chromosome, bridge formation and fragmentation were found in root tip cells showing significant depression in cell division. Clonazepam cause lethal changes in the nuclear material of the *Allium cepa* root tip meristem at higher concentrations and when treated for a longer time period.

Clonazepam is benzodiazepines and having anxiolytic (anti-anxiety), sedative, anticonvulsant and muscle relaxant properties. Benzodiazepines have been reported to have mutagenic and genotoxic effect [8, 22-24]. Due to their long half life benzodiazepines get accumulated in the body which may result in overdose in the organisms and cause genotoxic or mutagenic effects [4].

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CONCLUSION:

The result of this study pointed that the concentration involved and time period included showed significant depression in the mitotic activity except at 50µg/L (48 hrs) and 150µg/L (48 & 72 hrs) when it was compared to control. The various chromosomal aberrations observed were chromosome, bridge formation, sticky chromosome and fragmentation. Vagrant chromosomes were found to be more in the occurrence and fragmentation is least in occurrence than any other types. Sticky chromosomes and fragmentation found to be highest in number at 150µg/L at 72 hrs as compared to control.

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Table 1- Mitotic index of chromosomes of *Allium cepa* found at different drug concentrations at different time intervals. (P-Pro metaphase, M-Metaphase, A- Anaphase, T-Telophase). Significance at $p<0.05$.

Concentration	Number of dividing cells	MI (%)	Pro metaphase	Metaphase	Anaphase	Telophase
Control	92±0.02	72±1.17	30±0.02	36±2.25	43±0.05	41±0.03
24 hrs						
50µg/l	69±1.73*	36.3±0.17*	16±2	18±1*	18±2.64*	19±1
100 µg/l	58±2.71*	20.6±2.2*	11±2.64*	14±1	13±2.64	12±0.2
150 µg/l	41±9.53*	13.6±0.95*	9±0.1	7±0*	7.2±1.73	4±0.2
48 hrs						
50 µg/l	40±4*	20.3±0.4	18±1.73	0±0	12±1.02	17±1
100 µg/l	19±3*	16.3±0.3*	15±1	4±2*	10±1	10±1
150 µg/l	34±3.60*	11±0.36	7±1.73*	2±1.2	7±1.01	12±1
72 hrs						
50 µg/l	36±5.29*	12±0.52*	8±2*	7±4.35*	16±3.05	9±3.60
100 µg/l	22±8.71	7.33±0.87*	3.05±1.73	5±2.64*	11±2.64	3±2.64*
150 µg/l	20±13.8*	6±1.38	2±3.60	3±2.64	8±1	3±4.58

Table 2: Number of chromosomal aberrations found in root cells of Allium cepa when treated with different drug concentration at different time interval (VC- Vagrant Chromosome, B- Bridge formation, SC- Sticky Chromosome, F-Fragmentation) Significance at $p<0.05$.

Chr. Aberrations	Control			24 hrs			48 hrs			72 hrs		
	50 µg/l	100 µg/l	150 µg/l	50 µg/l	100 µg/l	150 µg/l	50 µg/l	100 µg/l	150 µg/l	50 µg/l	100 µg/l	150 µg/l
VC	0±0	1±1.73	1±1.73	0±0	0±0	1±1	1±1	2±2.6*	2±1.73	0±0	2±1.73*	3±1.03
B	0±0	0±0	1±1.73	0±0	0.5±0.1	0.7±0.2	0±0	2±1.7*	3±0.69*	3±0.2	5±1.02*	3±0.73
SC	0±0	0±0	0±0	1±0.1	2±1.73	0±0	2±2.06*	1±0.1	1±1.73	1±1	1±0.65	9±0.21*
F	0±0	0±0	0±0	0±0	0±0	0±0	0±0	2±0.32*	4±0.91*	0±0	7±0.75*	8±0.03*

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