Chromosome aberration and irregular cell cycle in Allium cepa root cells caused by different concentrations of Alprazolam

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Abstract

Introduction: Allium cepa is a biological material of great importance for mutagenicity tests, due to the simplicity of its use in testing, the fast growth of its roots, and the response of genetic material to the presence of substances with cytotoxic and genotoxic potential.

Method: The Allium test model by using the onion seed (Alium cepa L.) without the use of pesticides or any other chemicals is used in this research. Progressive changes in Allium cepa's chromosomes caused by treating Allium cepa's roots in selected drug concentration of 1, 10, 50 and 100 μg/ml of Alprazolam were followed. For statistical analysis chi-square test with the level of significance set at $P \leq 0.05$ is used.

Results: Changes in the structure and number of chromosomes in mitosis were found.

Conclusion: Comparing the results obtained for genotoxicity of Alprazolam with those obtained in the experiments that have been performed on other preparations of this group of drugs it can be stated that Alprazolam has similar effects on the cell cycle. Because of potential risk of using Alprazolam another experiment with higher concentration of Alprazolam would give more sufficient information.

Key words: Alprazolam, Allium cepa, root, chromosome, genotoxic

Introduction

Allium cepa Linn. is a commonly used plant in mutagenicity testing.[1] Ease in handling, cost effectiveness and sensitivity of chromosomes to genotoxic materials are one the few reasons its massive use in genetic studies.[1,2] Due to the sensitivity of its genetic makeup, immediate inhibition of roots growth and deformities in the chromosomes reflect the gene-toxicity.[3] Thus confirming the

validity of *Allium cepa linn* in vitro studies providing valuable information useful for human health. This is an excelent model in vivo where roots grow in direct contact with the drug or chemical substance to predict damage to the DNA of eukaryotes.[4] Thus, there is a potential role of *Allium cepa linn* for the assessment of the genotoxicity of drugs. Particularly for some drugs that are used in medical practice like Benzodiapines (BDZs)[5,6] i.e Alprazolam limited data on genotoxicity is available. Alprazolam (Figure 1) is preferred because of its short half-life and low likelihood of its accumulation (compared with diazepam).[7,8]

Addressing the safety of Alprazolam, very limited data is available for the genotoxic and carcinogenic effects.[9] In order to test the genotoxic and carcinogenic potential of any substance both animal and non-animal based methods can be adopted. Non-animal methods include in vitro studies that provide an important tool to provide better understanding of hazardous effect of chemicals.[11] The current study also adopted a non-animal vitro using *Allium Cepa Linn* [4] with an aim to investigate the genotoxicity of Alprazolam.

Material and Method

In this study the comercial seed (onion bulbs) onion (Allium cepa L.) produced without the use of pesticides, herbicides or fungicides or any other chemicals was used. Allium cepa's chromosomes are suitable for this study due to their size and number, 2n=16.[2,4,11,17,18] The experiment was performed at constant room temperature +20°C and protected from direct sun light exposure that can affect results.[2]

Healthy bulbs of the similar size were selected. Before the experiment growth of onion's root was tested in clean tap water with no growth factor added. After this all onion bulbs were placed in a small 50 ml plastic cup.[4] containig clear tup water. Subsequently 72 to 90 hours roots from the bulbs have started to emerge. Tap (drinking) water was used in this experiment and it was used as negative control.[1,17] Once rootles have grown 2-3 cm, selected drug concentration of Alprazolam have been prepared in concentrations of 1, 10, 50 and 100 μg/ml. In this study the drug solution of Alprazolam in water has been used. Drug solutions were filled up in petri dishes (per 30 ml). The bulbs, five for each concentrations, with enough grown rootlet were transferred in to it and treated for 4, 8, 12, 24 hours for each concentration. After completing the treatment rootlet peaks, about 2 cm, were cut off and placed in fixative acetyl alcohol. Fixation lasted for 24 hours, then rootlet were hydrolyzed in 1N HCl for 5 minutes at 60°C.

After that rootlet were imported into destillated water. Next, rootlet's tops were cut (about 2mm) and placed on a glass slide in a drop of 2% aceto-orsein[13], where macerated by metal wand. Two peks were enough for one slide. Through fragmented rootlet peaks carefully placed a coverslip and over it a piece of filter paper. Kneading is done by strong measured pressure using a thumb or rubber. Waxing the subject and cover slides a temporary preparation for cytogenetic analysis is made and can be used.[12]

For each tested concentration and control five slides were made. Cytogenetic analysis and review of the preparation is the final phase of the experiment on the genotoxic effects of the selected medicament. All deviations from normal metaphase status are recorded, and observed effects are expressed per 100 analyzed cells or 1000 cells in the case of mitotic index (MI) expressed in %.

Colchicin treatmen

By using colchicine changes in the structure and number of chromosomes in mitosis in prometaphases were recorded. The bulbs were treated with drug concentrations 50 ug/ml and 100 ug/ml for 4 and 8-hours for each treatment. Two hours before the end of the treatment was added colchicine concentrations of 350 ug/ml, and then rootlet were fixed. The purpose of use of colchicine in this research was to determine the possible existence of numerical and structural changes in the chromosomal set of meristimatical cells stopping mitosis in prometaphase. For taking photos Jenaval research microscope with camera have been used. Statistical significance has been analyzed by using chi-square test with the level of significance set at $P \leq 0.05$. Also, mitotic, cytological and effects on chromosomes were analyzed. Influence of Alprazolam on mitotic activity was monitored by the mitotic index (MI), by analyzing 1000 cells for the control and each experimental treatment. The mitotic index is expressed in percentage, and the representation of Individual phases by absolute number.

Results

Mitotic index (MI) of control cultures was 8.1% (Table 1). Results showed that Alprazolam in all tested concentrations and treatment changes cell mitotic activity of merestimatical cells of onion roots. Also it is noted that Alprazolam induces genotoxical effects on onion root cells. Concentration of **1µg/ml** reduces MI in the 4-hour and 8-hour treatment while MI in 12-hour and 24-hour increases. A statistically significant difference was observed in the 8-hour treatment where MI was 5.7% ($p < 0.01$). MI of meristimatical cells treated with **10 µg/ml** increased in all treatments. It is the largest (9.3%) in 8 hour treatment. In 12-hour MI decreased to 9.1% and in 24-hour decreased to 8.5%. Concentration of **50 µg/ml** in all treatments, except in the 24-hour, reduces MI. It can be seen that shorter treatments reduce MI and by prolonging treatments MI grow. Statistically significant differences were found in 4-hour ($p < 0.01$), and the 8-hour ($p < 0.02$) treatment. Concentration of **100 µg/ml** of acts contrary concentration of **50 µg/ml, i.e.** by prolonging treatments MI decreases. The 4-hour treatment MI was 6.8%, which is lower than in the control, but not significantly. However, at other treatments the results are statistically significant different compared to control. In the 8-hour treatment MI was 5.2% ($p < 0.01$), in 12 hour MI was 5.0% ($p < 0.001$), and in 24 hour MI was 4.5% ($p < 0.001$) and it was the lowest MI in the study (Table 1). Microscopic analysis showed that Alprazolam in all tested concentrations and lengths of treatment changes the frequency of mitotic phases compared to the control. It can be seen that at concentration of 1.10 and 50 µg/ml number of prophases and telophases generally increases by prolonging treatments, and the number of metaphases and anaphases decreases. The exception was in concentration of 100 µg/ml, where the number of phases generally decreases with extending of treatment what follows and the decline of MI.

Looking at individual concentrations and phases it can be seen that at majority of treatment number of prophases increases, except for the concentration of 100 µg/ml, where decreased by prolonging treatments. In 24-hour treatment concentration of 100 µg/ml number of f prophases falls to 13 or 28.89%, and MI in this treatment significantly decreased compared to the control. Prometaphase is not observed in any of the analyzed concentrations except for one 8-hour treatment, concentration of 100 µg/ml and in the control cultures 3 prometaphases (Table 2).

Unlike of prophase number of metaphase is reduced in all experimental treatments. The exception is the 4-hour treatment concentration of 1 µg/ml. Number of anaphases is changed differently in

different concentrations and treatment. Same rule can be seen related to telophase (Table 2). Number of irregular phases of the merestimatical onion cells was determined by analyzing 100 cells in division. Number of irregular phases in the mitotic cells was relatively high in all experimental treatments ($p < 0.001$) (Table 3). The largest number of irregular phases of mitotic cells at concentrations of **1µg/ml** is found in the 8-hour treatment and was 21 aberrant cells (21.00%). By prolongation of the treatment number of irregular phase decreases (Table 3).

The most important and most frequent changes of meristimatical onion cells in this concentration was the disorder of kinetics of chromosome (Figure 2 and 3).

Very important is to say that also has been found an agglutination of chromosomes, polyploids, extended prophases, and in the 24-hour treatment disorder of spiralisation and vakuolarisation of chromosomes (Table 4) (Figures 4,5,6,7 and 8).

Concentration of 10 **µg/ml** also causes a relatively large number of irregular phasis of meristimatical cells. Number of these disorders increases parallel to prolonging treatments with 8 (38.09%) of aberrant cells in the 4-hour and 8-hour (47.05%) on 12 (50.00%) at the 12-hour, respectively 10 (62.50%) in the 24-hour treatment. Next is an agglutination of chromosomes and after that is polyploidy (Table 4). Number of irregular phasis of meristimatical cells treated with concentration of 50 µg/ml is also high but very important is that didn't find corelation between MI and number of irregular phasis (Table 3). An Agglutination was the largest changes have been found and after that was extended prophasis with relatively equable number at all treatments. Also interesting to say is that two binucleated cells have been found in 8-hour treatment (Figure 8, Table 4).

Concentration of 100 µg/ml causes increasing of the number of irregular phases in the meristematical cells by prolonging treatment. Here also can be seen that increasing in the number of irregular phase follows the decline in MI (Table 3).

Results of treatment with colchicine

For colchicine treatment selected concentrations were 50 and 100 µg/ml and treatment duration was 4 to 8-hours. A large number of microscope slides were analysed , where mitosis is stopped in prometaphase treated with Alprazolam concentration of 50 and 100 µg/ml duration of 4 and 8-hours and 2-hours with colchicine concentration approximately 350 µg/ml. For each experimental treatment 100 prometaphasis were analysed and seeked for structural and numerical chromosomal aberration. Whit this analysis it has not been found chromosomal fragments, and of the number changes were found polyploid cells in concentration of 50 µg/ml in a 4-hour treatment (3-cell), and 8-hour treatment one cell. During the microscopic examination of slides it has been seen changes in the kinetics and the agglutination of chromosomes (Figures 9,10,11 and 12), as observed in previously described disorders of genetic material that are incurred by treating onion root cells with Alprazolam without colchicine.

Disscusion

Research has shown that Alprazolam causes certain changes in the genetic material of meristematical root cells. These changes depend on the concentration and duration of treatment.

Palani Kumar and Pannerselvam (2007) have found similar results in their research. They found that the reduction of mitotic activity increases when the concentration has been incresed from 100 μg/ml to 150 μg/ml.[18] Because of similar results on Alprazolam effects this suggest that Alprazolam may cause inhibition of DNA synthesis. Alprazolam has triazolo-ring that can be cytotoxic what is very important concerning our results.[5,6] Some rule how Alprazolam affect mitotic activity can be taken for concentrations of 50 and 100 μg/ml. But what is very important is that number of irregular phasis is great for all treatments. The only regularity was observed at concentrations of 100 μg/ml, where the number of irregular phases growth by prolonging treatment. Summarizing the obtained results we can say that the drug Alprazolam cause genotoxic effects in the form of mitotic, chromosomal and cytologic changes. By analyzing Alprazolam the genetic material is evident that the majority of the changes is in the kinetics and the agglutination of chromosomes. Benzodiazepines have been extensively used since 1960. for the treatment of anxiety, as hypnotics, sedatives and for other conditions but for many of that studies do not allow the evaluation of their genotoxic effects on human or plants.[6] For this experiment and making discussion in order to compare our results we had similar problems. Kazić and Sofradžija (1985) studying the effects of Largaktil, a Nozinan and Valoron came to the conclusion that Nozinan significantly reduced mitotic activity and causes major changes in interphasic nuclei and the occurrence of abnormal metaphase and anaphase. Largaktil at the start of treatment, manifest a stimulative effects, but also the appearance of nonspecific metaphases, while very strong mutagenic effects of Valoron is manifested through inhibition of mitotic activity, the occurrence of abnormal prophase, metaphase and anaphase, and changes in the structure of chromosomes.[14] Vuković and Sofradžija (1989) examining the genotoxic action of Lorsilan, Librium, a Mepbromat and Apaurin came to the conclusion that the first three have similar effects, and is manifested by lowering the mitotic index, the advent of C-mitosis, abnormal metaphases and anaphases and polyploidy. Apaurin has the opposite effect, i.e. stimulate mitosis without involving any other visible changes in the genetic material.[15] Ibrulj (2002) studying the effects of oxazepam to cells of Allium cepa's root noted that this drug shows a number of mutagenic and cytotoxic effects manifested in the form of mitotic, cytological and chromosomal abberations. Oxazepam increased mitotic activities, accelerating meristematical cell proliferation and by this way shortening the duration and dynamics of the cell cycle. Also disrupte kinetics, organization and separation of chromosomes at all stages of cell division which leads to the conclusion that this substance disrupts the formation and function of the cell's spindle. It was also determined that Oxazepam causes an agglutination of chromosomes.[16] Comparing the results obtained for genotoxicity of Alprazolam with those obtained in the experiments that have been performed on other preparations of this group of drugs we can be stated that Alprazolam has similar effects on the cell cycle. In particular there is a similarity with Oxazepam, because both preparations interfere kinetics, organization and separation of chromosomes. However there is only limited research available on its genotoxic and/or carcinogenic potential in humans. But one effect is in all similar research is same and that is "doze-depend" increasing effects.[6,18]

Conculsions

No matter that the experiment was made on the plants, results indicate the potential risks of Alprazolam in humans. In support of this we have obtained experimental results on the slides from the same group of drugs. For safer evaluation of the degree of potential genotoxicity of Alprazolam on human cells is necessary to make more complex investigations in cultured animal and human cells. Also we suggest another experiment with higher concentration of Alprasolam.

References

1. Paiva TS, Garcias GL, Martino-Roth MG. Genet Mol Res. Increasing mutagenicity of São Gonçalo Channel waters based on the Allium cepa test. 2009;8(1):299-309.

2. Fiskesjö G, Hereditas.The Allium test as a standard in environmental monitoring. 1985;102(1):99-112.

3. Fiskesjö G. Allium test.In vitro toxicity testing protocols. Methods Mol Biol. 1995;43:119-27.

4. Solange Bosio Tedesco and Haywood Dail Laughinghouse IV (2012). Bioindicator of Genotoxicity: The Allium cepa Test, Environmental Contamination, Dr. Jatin Srivastava (Ed.), ISBN: 978-953-51-0120-8,pp.140 InTech,Available from: http://www.intechopen.com/books/environmental-contamination/bioindicator-of-genotoxicitythe-allium-cepa-test

5. Ekonomopoulou MT, Akritopoulou K, Mourelatos C, Iakovidou-Kritsi Z. A Comparative Study on the Cytogenetic Activity of Three Benzodiazepines *In Vitro.* Genet Test Mol Biomarkers. 2011 Jun;15(6):373-8. doi: 10.1089/gtmb.2010.0214. Epub 2011 Jan 25

6. Iakovidou-Kritsi Z, Akritopoulou K, Ekonomopoulou MT, Mourelatos D. In vitro genetoxicity of two widely used benzodiazepines: alprazolam and lorazepam AUMJ. 2009;36(1):39-44

7. Elmesallamy GE, Abass MA, Ahmed Refat NA, Atta AH. Interdiscip Toxicol. Differential effects of alprazolam and clonazepam on the immune system and blood vessels of non-stressed and stressed adult male albino rats, 2011.Sep;4(3):132-43. doi: 10.2478/v10102-011-0021-y

8. www.pfizer.com, access 15.03.2013.

9. Brambilla G, Carrozzino R, Martelli A. Genotoxicity and carcinogenicity studies of benzodiazepines. Pharmacol Res. 2007 Dec;56(6):443-58. Epub 2007 Sep 1.

10. http://www.newdruginfo.com/pharmacopeia/usp28/v28230/usp28nf23s0_m1640.htm

11. Cabaravdic M. Induction of Chromosome Aberrations in the Allium Cepa Test System Caused by the Exposure of Cells to Benzo(a)pyrene. Med Arh. 2010; 64(4): 215-218.

12. Sofradžija A, Hadžiselimović R, Maslić E. (1989) Genotoksičnost pesticida. Sarajevo: Svjetlost, ISBN 8601022421, 9788601022423

13. Tada I, Mimori T, Sakaguchi Y, Kusano M, Hashiguchi Y, Recinos M. The use of aceto-orcein-stained squash preparations for enumeration of nuclei in microfilariae of various filarial parasites. Am J Trop Med Hyg. 1981 May;30(3):593-7.

14. Kazić A, Sofradžija A. Mutagenic effects of some psychotropical preparations on the cells of onion root (Allium cepa).Godišnjak Biološkog instituta Univerziteta u Sarajevu. 1987;40 pp. 49-62.

15. Vuković V, Sofradžija A. The cytogenetic effects of some psychotropic preparations on the cells of onion roots (Allium cepa). Godišnjak Biološkog instituta Univerziteta u Sarajevu.1987; 40 pp. 137-153.

16. Ibrulj S, Duricić E. Genotoxicity of oxazepam--the micronucleus cytochalasin-B test. Med Arh. 2002;56(2):61-4.

17. Nielsen MH, Rank J. Screening of toxicity and genotoxicity in wastwater by the use of the Allium test. Hereditas. 1994;121(3):249-54.

18. Kumar LP, Panneerselvam N. Cytogenetic studies of food preservative in Allium cepa root meristem cells. Facta universitatis - series: Medicine and Biology 2007, vol. 14, br. 2, pp. 60-63.

Table 1: The mitotic index in cells of onion root in control cultures and in cells treated with Alprazolam concentration of 1, 10, 50 and 100 μg/ml.

Treatment duration (hour)	**Concentration μg/ml**							
	1		10		50		10	
	MI %	P	MI %	P	MI %	P	MI %	P
0 control	8.1 %							
4	7.0	-	8.4	-	5.7	p<0,01	6.8	-
8	5.7	p<0,01	9.3	-	5.9	p<0,02	5.2	p<0,01
12	9.5	-	9.1	-	7.2	-	5.0	p<0,001
24	9.4	-	8.5	-	8.6	-	4.5	p<0,001

Table 2: The mitotic index (MI) and the representation of various mitotic phases in the onion cells (Allium cepa L.) after treatment with Alprazolam concentration of 1, 10, 50 and 100 μg / ml. Analyzed per 1000 cells for each experimental treatment and control.

Concentration	Treatment duration (hour)	No.of cells In division	Prophases		Prometaphases		Mataphases		Anaphases		Telophases	
			total	%	total	%	total	%	total	%	total	%
Control	0	81	26	32.1	3	3.7	21	25.93	14	17.28	17	20.99
1 μg / ml	4	70	22	31.42	-	-	24	34.89	11	15.71	13	18.57
	8	57[a]	15	56.32	-	-	16	28.97	11	19.3	15	26.32
	12	95	38	41.75	-	-	19	20.88	12	13.19	22	24.18
	24	94	38	40.43	-	-	20	21.28	7	7.45	29	30.85
10 μg / ml	4	84	32	38.1	-	-	21	25.01	14	16.67	17	20.24
	8	93	35	37.63	-	-	19	20.43	15	16.13	24	25.81
	12	91	36	39.13	-	-	19	20.88	16	17.39	21	24.18
	24	85	36	42.35	-	-	19	22.35	7	8.24	23	27.06
50 μg / ml	4	57[a]	21	36.84	-	-	14	24.56	4	4.02	18	31.58
	8	59[b]	18	30.51	-	-	15	25.42	7	11.86	16	27.12
	12	72	34	47.22	-	-	13	18.65	11	15.28	14	19.44
	24	86	36	41.86	-	-	15	17.44	13	15.12	22	25.58
100 μg / ml	4	68	25	36.76	-	-	16	23.53	14	20.59	17	20.99
	8	52[a]	18	34.62	1	1.92	15	28.85	4	7.69	14	26.92
	12	50[c]	15	30.01	-	-	16	32.01	8	16.01	11	22.01
	24	45[c]	13	28.89	-	-	10	22.22	10	22.22	12	26.67
	Statistically significant difference compared to the control: ap<0,01; bp<0,02; cp<0,001											

Table 3: Total number of aberrant cells and MI of onion cells treated with selected concentrations of Alprazolam (1, 10, 50 and 100 µg/ml) in different treatment duration (4, 8, 12 and 24 hours).

Treatment duration (hour)	No. of analysed cells in division	Concentration µg/ml											
		1			10			50			100		
		Aberant cells		MI	Aberant cells		MI	Aberant cells		MI	Aberant cells		MI
		No.	%	%	No.	%	%	No.	%	%	No.	%	%
0		4	4	8.1	4	4	8.1	4	4	8.1	4	4	8.1
4		16	16	7.0	21	21	8.4	22	22	5.7	17	17	6.8
8	100	21	21	5.7	17	17	9.3	15	15	5.9	18	18	5.2
12		19	19	9.5	24	24	9.1	16	16	7.2	25	25	5.0
24		17	17	9.4	16	16	8.5	21	21	8.6	31	31	4.5

Table 4: Alprazolam-genotoxic effects on meristematic onion cells (analyzed 100 mitotic cells per experimental treatment)

Concentration µg/ml	Treatment Duration (hour)	Chromosome kinetics disorders		Chromosome Agglutination		Polyploidy		Disorders of Chromosome spiralisation		Extended prophase		Chromosome vacuolisation		Micronucleus		Giant cells		Binucleated cells		Total
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Control	0	2	50	1	25	1	25	-	-	-	-	-	-	-	-	-	-	-	-	4
1	4	12	75	-	0	4	25	-	-	-	-	-	-	-	-	-	-	-	-	16
	8	14	66.66	2	9.5	-	-	-	-	4	19	-	-	-	-	-	-	1	4.8	21
	12	10	52.6	4	21.1	2	10.52	2	10.5	-	-	1	5.3	-	-	-	-	-	-	19
	24	2	11.76	3	17.7	2	11.76	4	23.5	1	5.9	3	17.6	-	-	1	5.9	1	5.9	17
10	4	8	38.09	6	28.6	1	25	-	-	2	9.5	-	-	1	25	1	25	-	-	21
	8	8	47.05	7	41.2	0	-	-	-	-	-	-	-	1	5.9	1	5.9	-	-	17
	12	12	50	5	20.8	6	25	-	-	-	-	-	-	-	-	-	-	1	4.2	24
	24	10	62.5	3	18.8	2	12.5	-	-	1	6.3	-	-	-	-	-	-	-	-	16
50	4	9	40.9	6	27.3	0	-	-	-	5	22.7	-	-	-	-	-	-	-	-	22
	8	3	20	5	33.3	0	-	-	-	4	26.7	-	-	-	-	-	-	2	13.3	15
	12	1	6.25	9	56.3	1	6.3	-	-	5	31.3	-	-	-	-	-	-	-	-	16
	24	0	0	13	61.9	1	4.8	-	-	6	28.6	-	-	-	-	-	-	1	4.8	21
100	4	4	23.52	8	47.1	2	11.76	-	-	1	5.9	-	-	-	-	-	-	-	-	17
	8	9	50	7	38.9	0	-	-	-	-	0	-	-	-	-	1	5.6	1	5.6	18
	12	1	4	19	76	2	8	1	4	1	4	-	-	-	-	-	-	1	4	25
	24	8	25.8	13	41.9	2	6.5	-	-	4	12.9	-	-	1	3.2	2	6.5	1	3.2	31
Total	No.	**113**		**111**		**26**		**7**		**34**		**4**		**3**		**6**		**9**		**320**
	%		**35.31**		**34.7**		**8.12**		**2.19**		**10.6**		**1.25**		**0.95**		**1.88**		**2.81**	**100**

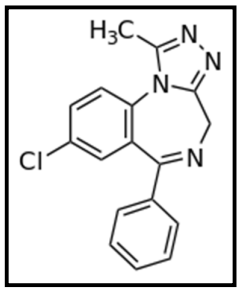
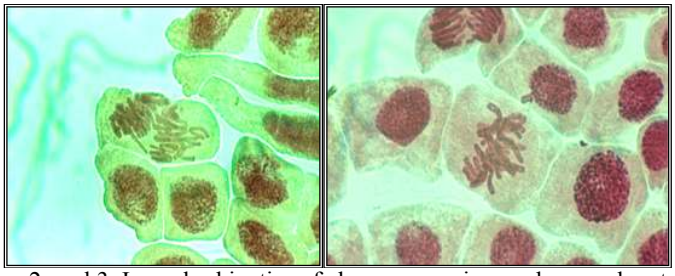


Figure 1. Alprazolam ($C_{17}H_{13}ClN_4$ Mol Wt: 308.76) (10).



Figures 2. and 3. Irregular kinetics of chromosome in anaphase and metaphase

Figure 4. Aglutination of chromosomes

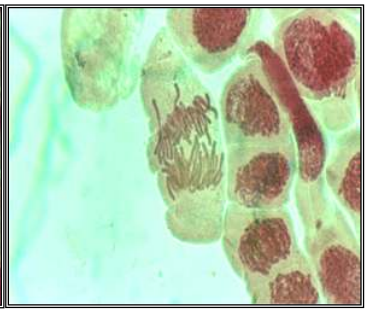


Figure 5. Poliploid cells

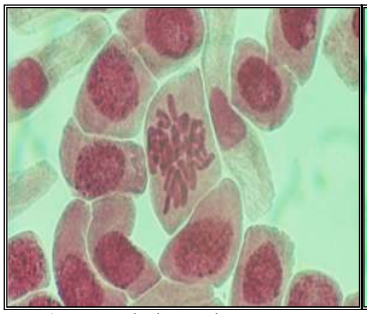


Figure 6. Extended prophase

Figure 7. Vakuolisirated chromosomes

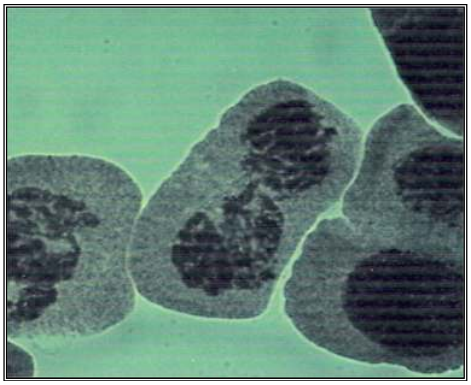


Figure 8. Binucleated cell (1000X)

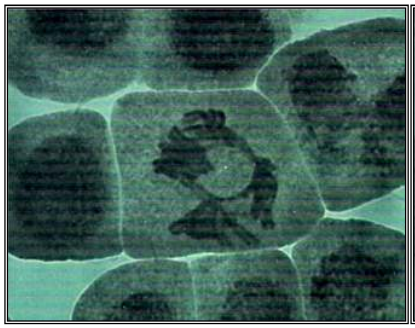


Figure. 9. Irregular kinetics of chromosome

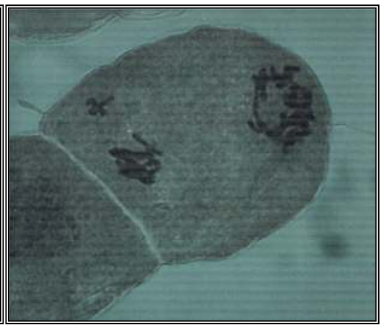


Figure. 10. Tripolar cell

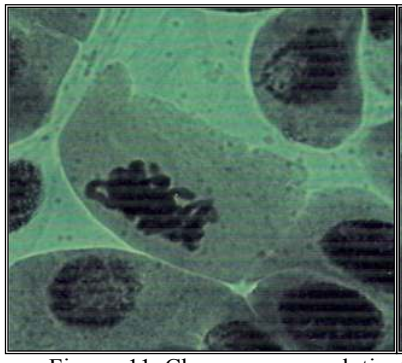


Figure. 11. Chromosome agglutination

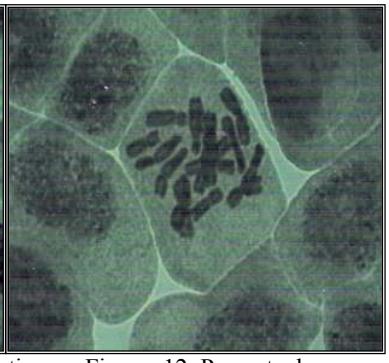


Figure. 12. Prometaphase