Effect of *Allium sativum* L. Water Extract on Mitotic Process and DNA Content in *A.cepa* Root Meristem Cells

Riad, S. A.
Department of Genetics, Faculty of Agriculture, Alexandria University,Egypt.

ADDITIONAL INDEX WORDS.garlic. mitotic index. mitotic phases. chromosomal aberrations. stickiness. recovery.

ABSTRACT

The effects of garlic water extract on the mitotic process in normal cells (*A. cepa* root meristem cells) were studied in direct and recovery experiments. An attempt was made to find out a relationship between the mitotic activity and the DNA content. The results revealed that garlic sulfer containing compounds may cause a drastic mitodepressive action. Such inhibitory effect depends on the concentration and exposure time of the direct treatment. Also, an accumulation of clumping stickimetaphase were clearly indicated. Thus, chromosomes lose the ability to undergo anaphase and telophase stages and are completly arrested at metaphase in cells treated with high doses for long exposure time. Beside stickiness, other physiological aberrations observed which can be attributed to abnormal spindle activity and inhibition of middle lamella formation. In addition, the clastogenicity of garlic sulfer components were detected during the treatments. On the other hand, the mitostatic feature may be due to the reduction in the number of cells entering mitosis and the inhibition of DNA synthesis at S phase. Recovery durations for the sublethal doses were not enough to normalize the patterns of cell division, mitotic phases frequency and the physiological effect on the spindle formation. On the contrary, the clastogenic action on the chromosomes and the physiological effects on the middle lamella formation were stopped during recovery times. Further studies are in progress to explain garlic extract action on cell cycle stages and gene expression.

INTRODUCTION

Although most of the synthetic drugs in use are in their pure and active form, many of these compounds are known to have some adverse effects on heart and many other organs of the body. Looking for an alternative therapy, natural herbs have been tried. Certain indigenous drug preparations with naturally occurring herbs have been in use for many decades for the treatment of certain diseases (Ashraf *et. al*., 1999).

Garlic (*Allium sativum* L.) has been used throughout history for the treatment of a wide variety of diseases, including high blood pressure, headache, bites, worms and tumors. During the past decade, there has been increasing awareness of the potential medicinal uses of garlic. Several reports have suggested that garlic has a protective effect against strokes, coronary thrombosis and atherosclerosis (Bordia *et. al*., 1977). These beneficial effects have been attributed to its ability to inhibit platelet aggregation and thromboxane formation (Makheja *et. al*., 1979 and Srivastava, 1984). In addition, garlic can lower LDL cholesterol (Ernst *et. al*., 1985), blood pressure (Silagy and Neil, 1994) and inhibits malignant tumors (Jill and Stansburg, 2000).

The majority of the benefical effects of garlic are attributed to its sulfur containing compounds: allicin, diallyldisulfide, diallyltrisulfide and others. Allicin is mainly responsible for the pungent odor of garlic. It is formed by the action of the enzyme alliinase on the compound alliin (Reuter, 1995). The enzyme is inactivated by heat or oxygen which account for the fact that cooked garlic as well as aged garlic preparations produce neither as strong odor as raw garlic nor nearly as powerful medicinal effects (Murray, 2001).

There have been only a few reports on the toxicity of garlic in human and animals. Stoll and Seeback (1951) observed that allicin caused diarrhea in individuals not accustomed to eating large quantities of garlic. Also, acute and prolonged feeding of large amount of raw or aqueous garlic extract to rats exhibit anemia, weight loss, failure to grow and even death (Nakagawa *et. al.*, 1984 and Joseph *et. al.*, 1989). Similarly, Alnaqeeb *et. al.* (1996) reported that high doses (500 mg/kg) of garlic administered either orally or intraperitoneally caused both lung and liver damage in rats. In contrast, low doses of it (50 mg/kg) did not reveal toxic effects.

In reality, there is no available reports concerning the cytological effect of garlic. Therefore, the present investigation was undertaken to study the action of fresh garlic aqueous extracts upon normal cells (*A. Cepa* meristem cells) in direct treatments and different recovery intervals. The essential points screened were mitotic indices, frequency of mitotic phases, and chromosome behaviour. In addition, an attempt was made to find out a relationship between the mitotic process and DNA content.

MATERIALS AND METHODS

1- Preparation of garlic extract:

50 g of garlic was homogenized in 50ml cold distilled water in the presence of some crushed ice. The homogenization was carried out in a blender at high speed for 12 minutes. Homogenized mixture was filtered three times through cheese cloth. The filterate was then centrifuged at 2000 rpm for 10 minutes and clear supernate was made up to 100 ml with distilled water (Alnaqeeb *et. al.*, 1996). Garlic extracts of lower concentrations (1, 5, 10 and 20%) were prepared by diluting this solution with distilled water

2- Samples preparation :

Roots were germinated on onion (*A. cepa*) bulbs placed on beakers containing tap water. When the roots were about 1 mm long, the bulbs were placed on beakers containing the garlic extract such that roots being immersed in the solution (Chacon *et. al.*, 1994). Four exposure times (4, 8, 12, and 24 hr.) were raised for each concentration.

Five bulbs were used for each concentration and exposure time as well as for the control grown in tap water (Williams and Omoh, 1996). Bulbs with roots treated for 8 hr. with 10 and 20% of the extract were trasferred to tap water for recovery periods of 24, 48, and 72 hr. before fixation (Lopez-Saenz *et. al.*, 1996).

3-Cytological procedure:

After treatment the root tips were cut off, washed in distilled water and fixed overnight in a mixture of ethanol: acetic acid (3:1) at 4° c. Subsequently, Feulgen-squash technique of Darlington and La cour (1976) was used: roots were hydrolyzed in 1N HCL for 50 minutes at 22° c and stained in Schiff's reagent.

For cytological determinations, at least two slides for each bulb were prepared and five high dry power microscopic fields (40 x objective) were scored for each slide noting the total number of all cells, cells in mitosis and those affected by each type of anomaly. The sum of 50 fields represents the total number of examined cells for each treatment.

4-DNA content :

For each treatment 1 g of root tips was stored at –20° c until DNA analysis. The CTAB method of Dellaporta *et.al.*(1983) was used to prepare the total DNA.The purity of DNA preparations was checked by Shimadzu UV-240 spectrophotometer using the following coefficient: $A_{280/260}$ = 0.5 - 0.6 (Fellenberg,1974) where A is the absorbance at a given wavelength. A dilution of the DNA-containing solution is scanned in an UV- spectrophotometer at 260 nm to estimate DNA concentration (OD of 1 at 260nm ≅ 50 ug/ml of DNA).

5-statistical analysis :

Spsspstt computer software was used to estimate the mean (X), standard error (SE) and t-test for significance. The level of significance in t- test was $p \leq 0.05$ and $p \leq 0.01$ (Williams and Omoh, 1996).

RESULTS

I. Direct effect:

1-Mitotic index:

As shown in Table (1) and Figure (1), fresh garlic aqueous caused mitodepressive effect in *A.cepa* root meristem cells. The mitotic indices decreased significantly below the control values for all doses and at all times of exposure. Such strong inhibitory effect increased as the concentration and the time of exposure increase.

2-Frequency of mitotic phases:

Table (1) and Figure (2-b) indicate strong accumulation of cells in metaphase. The frequency of this stage was gradually increased as the dose and the length of exposure increase. The percentages of metaphase for all concentrations and exposure times were highly significant than that of the control values, except the 1% concentration of garlic water extract at 4hr. exposure time. The rate of this accumulated effect reached 100% in the cells treated with higher doses (10 and 20%) under long exposure time (24 hr.). Consequently, percentages of cells in the remaining mitotic stages (prophase, anaphase and telophase) were decreased to 0% at the same doses and time of exposure. Also, anaphase and telophase frequency was decreased to the same

percentage (0%) in cells treated with 20% garlic extract for 12hr. (Figure 2-a and c). In addition, all control percentages were significantly higher than that of prophase, anaphase and telophase values except 4hr. treatment with 1% concentration for all these stages and 8hr. of the same dose for anaphase and telophase. It is also clear that this strong inhibitory effect increased as the concentration and the time of exposure increase.

3-percentage of abnormal mitotic phases:

Figure (3) illustrates the effect of garlic water extract upon the induction of aberrations in *A.cepa* root tip cells. C-metaphase, tetraploid cells, stickiness, laggards, fragments, binucleated cells, multipolar and micronucleated cells were observed at the level of doses tested. Total percentages of these aberrations ranged from 0.42 to 0.69% in the control group and from 2.65 to 100 % in the cells treated with high doses (10 and 20%) for 24hr. (Table (2) and Figure 4-a). Moreover, the rate of stickiness increased at the same time to 100% in the root tips treated with the same doses (Figure 4-b). Such strong induction depends on the concentration and exposure time of the direct treatment.

4-DNA content:

During the treatment with different concentrations of garlic water extract for 8hr.,a drastic inhibition of DNA synthesis was observed (table 3 and Figure 5-a). The concentrations of DNA showed gradual and significant decrease below control values.

II. Recovery effect:

In order to investigate the stability of garlic extract effects, meristem cells treated for 8hr.with higher doses (10 and 20%) were selected to study their recovery at different periods (24, 48 and 72hr.), since they revealed sublethal effects.

1-DNA concentration:

As shown in Table (3) and Figure (5 - b), there was gradual increase of DNA content during recovery periods. The values were significantly higher than that of 8hr.treatment, however they remained significantly below control samples.

2-Mitotic index:

During recovery duration the mitotic indices revealed gradual and significant increase above 8hr.treatment but these values were significantly below the control level (Table 4 and Figure 6).

3-Frequency of mitotic phases:

Table (4) and Figure (7-a) illustrate significant increasing of prophase frequency above 8hr.treatment after 72hr.of recovery for 10% garlic dose and at 48 and 72hr.for 20% treatment. Such increase depends on the period of recovery. The values of this stage were significantly below control at all recovery periods. The number of cells in metaphase showed gradual and significant decrease below 8hr.treatment after 48hr.of recovery for both doses (Figure 7-b). But, during recovery periods the values were significantly higher than control samples. After 48hr.of recovery exposure, the anaphase and telophase

frequencies were significantly higher than that of 8hr.treatment (Figure 7-c). Such increase depends on the recovery period and all obtained values were significantly below the control samples.

4-Percentage of abnormalities:

Table (5) and Figure (8) show that the total aberrations and stickiness decreased gradually and significantly below 8hr.treatment for both concentrations during recovery periods. At the same time, these values were still significantly higher than control samples. However, stickiness, bridge, multipolar and multinucleated cells were maintained during recovery periods.

DISCUSSION

The use of plant extract instead of synthetic drugs in the treatment of some diseases is in progressive increase. Many investigators made trials to test the mode of action, toxicity, physiology and cytology of some plant extract effects. Therefore, the present study was planned to investigate the action of one of the most important natural product (garlic) on the mitotic process and DNA content in normal cells (*Allium cepa* root tips). Grant (1982) and Fishesjo (1985) have shown that the cells of the meristematic region of *A.cepa* are excellent for testing the effect of the chemicals on mitosis. In addition, Khilman (1966) has noted that plant cells are 1000 times more resistant to cholchicine. Thus, it is probable that chemicals which affect plant chromosomes will also affect animals.

In the present investigation, there was a drastic reduction of mitotic activity when the roots were treated with different doses of garlic for different periods. Such mitodepressive effect depends on both concentrations and exposure time. The same action was reported for other plant extracts (Lee *et. al.*, 1999; Saenz *et. al.*, 1999; Awasthy *et. al.*, 1999 and Lee *et. al.*, 1999). In addition, Nekagawa *et.al.*(1984); Joseph *et.al.*(1989) and Alnaqeeb *et.al.*(1996) reported the cytotoxicity effect of garlic in rats. They proposed that this action is attributed to its sulfer containing ingredients. It is well known that the liver metabolizes a wide range of both exogenous and endogenous compounds, and act as a good indicator of detoxification processes taking place in the animal (Hawkins, 1988). Garlic sulfer containing compounds have been shown to affect the activity of a number of detoxification enzymes (Dalvi, 1992) and increased hepatic lactate dehydrogenase activity (Ali *et. al.*, 1991).

On the other hand, the effect of any chemical on the mitotic apparatus and on the entry into mitotic stages coud be examined by the analysis of mitotic phases (Shehab and Adam, 1983). In the healthy *untreated* roots, cells enter and leave mitosis at almost equal rates. On the contrary, the present investigation indicated that the harmful effects of garlic sulfer compounds on the cell proliferation caused an inbalance in the relative frequencies of mitotic phases. There was highly accumulation of cells in metaphase stage according to stickiness which covers the whole chromosome complement leading to clumping stickimetaphase. Thus the chromosomes lose the ability to undergo

anaphase and telophase stages and they were arrested at metaphase. This feature was gradually increased to complete metaphase arrest under certain conditions. These results coincide with those obtained by Saggoo *et.al.* (1991); Carballo *et.al.* (1992); Banerjee (1992) and Podbielkowska *et.al.* (1994), when they tested the cytological effects of *Tylophora indica* L..; *Heliotropium surassavicum* var; different types of tobacco and coumarins extracts, respectively. Darlington and Mcleish (1951) and La-Cour and Rutishauser (1954) attributed stickiness to the process of depolymerization of DNA, thus the chromosome surface becomes sticky. Also, Saggoo *et.al.*(1991) proposed that stickiness may be due to alteration in the surface nucleoprotein configuration or improper folding of chromosome fibre. As a result of the high frequency of stickiness, the rate of total mitotic abnormalities increased to 100% in cells treated with high doses for long time of exposure.

Other types of physiological aberrations were observed during garlic treatments such as c-metaphase, tetraploid, lagging chromosomes, multipolar and multinucleated cells. Therefore, the reduction of mitotic activity could be also due to the effect of garlic sulfer compounds on the spindle formation. Similar aberrations have been reported to be induced by a number of physical and chemical agents (George and Geethamma, 1990 and Williams and Omoh, 1996). In addition, garlic extract has an effect on the middle lamella activity. This type of physiological action induced the binucleated cells which were previously reported by Puerta *et.al.*(1993); Podbielkwska *et.al.*(1994) and Williams and Omoh (1996) for other plant extract effects.

In addition, the obtained data indicated that, garlic sulfer compound may be cosidered as clastogenic agents. The formation of chromosome breaks and probably chromatin bridges at anaphase were shown. Saggoo *et. al.* (1991) proposed that, the bridges observed at anaphase may be due to stickiness of chromosomes or the formation of dicentric chromosome as a result of breakage and reunion. Similar observations were noticed by many workers for some plant extract effects (Shehab *et.al.*,1978 ; Adam and Rashad, 1984 ; Ene-Obong *et.al.*,1991 and Chacon *et.al.*, 1994).

On the other hand, the mitodepressive effect may be due to a reduction in the number of cells entering mitosis. The interference of garlic sulfer containing compounds in the normal process of mitosis may caused lengthening of mitotic cycle time (Adam and Rashad, 1984) and/or inhibition of DNA and protein synthesis in G_1, S and G_2 phases (Shehata, 1993). It is of intrest to note gradual depression of DNA content in the root meristem cells of *A.cepa* during the present treatment. Thus, at least the inhibition effect on the DNA synthesis and the reduction of the number of cells in prophase exhibit the mitostatic feature.

The recovered roots revealed partial decreasing of stickiness and clumping stickimetaphase. Also, there was partial increasing in the DNA concentrations, the frequencies of the other mitotic phases and finally the mitotic indices. Therefore, recovery durations were not enough to revive the root meristem cells from the effect of garlic sublethal doses. Similar results were observed by

Saggoo *et.al.* (1991); Williams and Omoh (1996) and Gomez *et.al.* (1996) for other plant extract actions.

The physiological effects of garlic on the spindle formation were continued during the recovery periods. Sticky bridges, multipolar and multinucleated cells cytologically were observed. On the contrary, the binucleated cells and chromosomal breaks were disappeard. Thus, garlic cytological effect on the middle lamella formation and its clastogenic action on the chromosomes were stopped during recovery duration. It may be due to degradation of some active components in garlic extract or other constituents go into solutions having a neutralizing effect (Shehata, 1993).

In conclusion, the present study clearly indicated the drastic side effects of garlic sulfer containing compounds. A number of mitotic and chromosomal abnormalities were induced beside the mitodepressive and the clastogenic effects. In addition, an inhibitory action on the DNA synthesis during cell cycle S phase and reduction in the number of cells in prophase were reported. The attempt to revive the meristem cells was not effective for the sublethal doses. Therefore, it is very important to estimate the exact doses which are not harmful for human normal cells. However, the precise causation and effect at the level of cell cycle and gene expression need further clarification.

REFERENCES

Adam,Z. M. and Th.Rashad.1984. Cytological effects of medicinal plant I. Influence of *Ammi majus* extract on root tip of *Vicia faba*. Cytologia 49:265-271.

Ali, M., M. Afzal, Y.S.Saleh, C.J.Gubler, and M.S.I.Dhami.1991. Changes in the levels of lactic dehydrogenase and transketolase in liver and red cells of rats after treatment with garlic extracts. Journal of Environmental Science and Health A 26: 1-12.

Alnaqeep, M.A, M.Thomson, T.Bordia, and M.Ali.1996. Histopathological effects of garlic on liver and lung of rats. Toxicology letters 85:157-164.

Ashraf, M.Z, M.S.Y.Khan, H.A. Hameed, M.E.Hussain, and M.fahmi.1999. Endothelium modulated vasorelaxant response of a polypharmaceutical herbal drug (Lipotab) and its individual constituents. J.of Ethnopharmacol 66:97-102.

Awasthy, K.S., O.P.Chaurasia, and S.P.Sinha.1999. Prolonged murine genotoxic effects of crude extracted from neem. Phytother.Res.13 (1): 81-83.

Banerjee, A.1992. A time course study of the relative cytotoxic effects of extracts of different types of tobacco on *Allium cepa* mitosis. Cytologia 57:315-320.

Bordia, A, S.K.Verma, A.K.Vyas, B.L.Khabya, A.S.Rathore, N.Bhu, and H.K. Bedi.1977. Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. Atherosclerosis 26: 379-386.

Carballo, M., M.D.Murdry, I.B.Larripa, E.Villamil, and M. D'Aquino.1992. Genotoxic action of an aqueous extract of *Heliotropium surassavicum* var. argentium. Mutat. Res. Jun 16, 279 (4): 245-253.

Chacon, J.A., C.Martin-Cordero, and M. J. Ayuso.1994. Mitodepressive effect of *Retama sphaerocarpa* Boissier in the meristematic region of *Allium cepa* root tips. Cytobios 80: 155-159.

Dalvi,R.R. 1992. Alterations in hepatic phase I and phase II biotransformation enzymes by garlic oil in rats. Toxicology Letters 60: 299-305.

Darlington, C.D. and L.F.La-Cour.1976. The handling of chromosomes.Sixth edition George Allene and Unwin LTD London(Editor).

Darlington, C.D. and L.Mcleish.1951. Action of malic hydrazide on the cell. Cell Nature 167: 407-408.

Dellaporta, S.L., J.Wood, and J.B.Hicks.1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1(4): 19-23.

Ene-Obong,E.E., G.E.Nwofia, and C.O.Okunji. 1991. Depressive effects of five molluscicidal plants on mitosis. Fitoterapia 62 (4): 353-356.

Ernst, E., T.Weichmayr, and A.Matari.1985. Garlic and blood lipids. British Medical Journal 291:139-143.

Fellenberg, G. 1974. Chromosomal Protein. Stuttgart, Ulmer.

Fiskesjo, G.1985. The *Allium* test as a standard in environmental monitoring. Hereditas102: 99-112.

George, K. and S.Geethamma.1990. Effect of the leaf extract of *Ricinus communis* on *Allium cepa*. Cytologia 55: 391-394.

Gomez, M.A., M.T.Saenz, M.D.Garcia, R.Puerta, and M.C.Ahumada.1996. Effect of methanolic extract of parasitic *Viscum cruciatum* Sieber on mitosis in *Allium* cepa L. Cytobios 85: 59-63.

Grant, W.F., 1982. Chromosome aberration assays in *Allium* . Mutat. Res.99: 273-291.

Hawkins,D.R. 1988. Biotransformations: A survey of Biotransformations of Drugs and Chemicals in Animals. Royal Society of Chemistry. London.1-4.

Jill, E. and N.D.Stansburg.2000. Cancer prevention diet. The August issue of Nutrition science News.

Joseph, p.K., K.R. Rao, and C.S.Sundaresh.1989. Toxic effect of garlic extract and garlic oil in rat. Indian J. Exp. Biol.27: 977-979.

Khilman, B.A.1966. Action Of Chemicals On Dividing Cells.Prentice Hall Inc. Englewood cliffs New Jersey (Editor).

La-Cour, L.E. and A.Rutishauser.1954. X-ray breakage experiments with endosperm L. Subchromatid breakage. Chromosoma 6(8): 696-709.

Lee, I.S., A.Nishikawa, F.Furukawa, K.Kasahava, and S.V.Kim.1999. Effects of *Selaginella tamariscina* on *in vitro* tumor cell growth, P53 expression G_1 arrest and *in vivo* gastric cell proliferation. Cancer Lett. 20, 144 (1): 93-99.

Lee, S.K.; B.Cui, RR.Mehta, A.D.Kinghorn, and J.M.Pezzuto.1998. Cytostatic mechanism and antitumor potential of novel IH-cyclopenta (b) benzofuran lignans isolated from *Aglaia elliptica*. Chem. Biol. Interact 115 (3): 215-228.

Lopez-Saenz, J.F., G.Gimenez, and A.Gonzalez.1996. Duration of the cell division cycle and its dependence on the temperature. Cytobiologie 75: 591-600.

Makheja, A.N., J.Y.Vanderhock, and J.M.Bailey.1979. Inhibition of platelet aggregation and thromboxane synthesis by garlic.Lancet I: 781-787.

Murray, M.T.2001. Garlic: For maximum benefit use fresh garlic preparation. Doctor Murray com/articles/garlic tacts.htm.

Nakagawa, S., K.Masamoto, H.Sumiyoshi, and H.Harada .1984. Acute toxicity test of garlic extract. J.of Toxicology Scien., 9, 57-60.

Podbielkowska, M., E.Kupidlowska, M.Waleza, K.Dobrzynska, S.A.Louis, A.Keightley, and A.M.Zobel.1994. Coumarins as antimitotics. Int.J.Pharmacognosy 32(3): 262-273.

Puerta, R.,T.Saenz and M.D.Garcia. 1993. Effect of essential oil from *Helichrysum picardii* on mitosis in *Allium cepa*. Cytobios 73:165-171.

Reuter, H.D.1995. *Allium sativum* and *Allium ursinum*: part 2. Pharmacology and medicinal application, phytomed 2:73-91.

Saenz, M.T., M.D.Garcia, and M.A.Gomez.1999. Effect of hexanoic and chloroformic extracts of *Achilla ageratum* on mitosis in *Allium cepa*. Cytobios 99: 143-148.

Saggoo, M.I.S., S.Kumari, and J.Bindu.1991. Cytological effects of Indian medicinal plants: I. Mitotic effects of leaf homogenate of *Tylophora indica* L. on *Allium cepa*. Cytologia 56: 633-637

Shehab, A. S. and Z.M.Adam.1983. Cytological effects of medicinal plants in Qatar: III. Mitotic effect of water extract of *Anastatica hieszochuntica* on *Allium cepa*. Cytologia 48 (2): 343-348.

Shehab, A.S., H.A.Hakeem, and Z.Abo-El-Kheir.1978. Cytological effects of *achillea fragrantissima* extract on *Allium cepa* and *Vicia faba*. Cytologia 43: 623-629.

Shehata, M.M.1993. Effect of water extracts of some medicinal plants on cell division and nucleic acids content. Ph.D. Thesis, Ain Shams University, Faculty of Science, Botany Department, Cairo, Egypt.

Silagy, C.A. and H.A.Neil.1994. A meta-analysis of the effect of garlic on blood pressure. J. Hypertens 12:463-468.

Srivastava, K.C.1984. Effects of aqueous extracts of onion, garlic and ginger on platelet aggregation and metabolism of arachidonic acid in blood vascular system. *In vitro* study. Prostaglandins, Leukotrienes and Midicine 13: 235-277.

Stoll, A. and E.Seeback.1951. Chemical investigations of allicin, the specific principal of garlic. Advances in Enzymology 11:377-381.

Williams, G.O. and L.E.Omoh.1996. Mitotic effects of the aqueous leaf extract of *Cymbopogon citratus* in *Allium cepa* root tips. Cytobios 87: 161-168.

Table 1. Mitotic indices and percentage of mitotic phases in the root meristem cells of *A. cepa* after direct treatment by different concentrations of garlic water extract at different times of exposure.

Concentration (%)	Duration of Treatment (hr.)	Mitotic Index ±SE	Mitotic Phases Percentage of dividing cells± SE		
			Prophase	Metaphase	Anaphase + Telophase
0	4	7.47±0.12	35.43±0.38	35.06±0.04	29.51±0.34
	8	7.31±0.12	34.5±0.44	35.48±0.21	30.02±0.23
	12	7.27±0.14	33.2±0.29	35.66±0.19	31.14±0.17
	24	7.41±0.05	33.33±0.18	37.17±0.1	29.5±0.26
1	4	*6.6±0.04	35.35±0.24	35.65±0.28	29.00±0.27
	8	**4.68±0.05	**30.79±0.45	**40.62±0.24	28.59±0.5
	12	**3.79±0.06	**30.82±0.14	**50.62±0.24	**18.56±0.19
	24	**3.23±0.04	**26.77±0.15	**58.03±0.14	**15.2±0.19
5	4	**4.44±0.03	*33.99±0.28	**48.01±0.07	**18.8±0.22
	8	**4.24±0.05	**29.72±0.29	**52.25±0.33	**18.03±0.54
	12	**1.81±0.05	**27.81±0.17	**54.35±0.21	**17.84±0.17
	24	**0.26±0.01	**11.38±0.4	**85.44±0.35	**3.18±0.12
10	4	**3.54±0.02	**30.76±0.34	**59.19±0.21	**10.05±0.35
	8	**2.66±0.04	**26.39±0.42	**70.83±0.42	**2.78±0.41
	12	**1.6±0.02	**22.34±0.2	**74.83±0.7	**2.83±0.24
	24	**0.1±0.01	**0±0	**100±0	**0±0
20	4	**2.74±0.02	**19.36±0.34	**76.32±0.43	**4.32±0.12
	8	**2.12±0.02	**13.31±0.42	**84.37±0.4	**2.32±0.05
	12	**0.55±0.01	**8.13±0.12	**91.87±0.12	**0±0
	24	**0.06±0.01	**0±0	**100±0	**0±0

*Significant at 0.05 level.
**Significant at 0.01 level.

Table 2. Percentage of induced aberrations in the root meristem cells of *A. capa* after direct treatment by different concentrations of garlic water extract at different times of exposure.

Duration of treatment (hr.)	Concentration(%)	Percentage of aberrations								Total ± SE
		C	Tp	S	L	b	F	Bi	Mp	
	0	0.21	-	0.21	0.21	-	-	-	-	0.63±0.01
	1	1.84	-	0.61	-	0.2	-	-	-	**2.65±0.07
4	5	3.26	-	3.58	0.65	0.33	-	-	-	**7.82±0.02
	10	3.78	-	8.82	0.84	0.84	-	0.84	-	**15.12±0.08
	20	1.47	2.21	11.59	0.74	0.74	-	1.47	-	**18.22±0.06
	0	0.23	0.23	0.23	-	-	-	-	-	0.69±0.03
	1	1.53	2.45	1.23	0.31	0.92	0.31	-	0.31	**7.06±0.13
8	5	0.77	2.32	8.87	1.16	-	-	-	0.77	**13.89±0.04
	10	-	2.53	15.82	0.63	0.63	-	-	0.63	**20.24±0.05
	20	-	2.12	16.4	0.53	0.53	0.53	-	0.53	**20.64±0.14
	0	0.21	-	-	0.21	-	-	-	-	0.42±0.02
	1	2.21	0.74	18.38	1.47	1.47	1.47	-	0.74	**26.48±0.02
12	5	1.59	-	36.5	1.59	1.59	-	-	3.17	**44.44±0.12
	10	-	-	64.29	-	-	-	-	-	**64.29±0.11
	20	-	-	91.67	-	-	-	-	-	**91.67±0.13
	0	0.22	-	0.22	-	-	-	-	-	0.44±0.01
	1	2.59	1.72	29.31	1.72	1.72	3.45	-	-	**40.51±1.07
24	5	-	-	87.5	-	-	-	-	-	**87.5±0.15
	10	-	-	100	-	-	-	-	-	**100±0
	20	-	-	100	-	-	-	-	-	**100±0

** Significant at 0.01 level.

C = C-metaphase. Tp = Tetraploid cells.
S = Stickiness. L = Laggard.
B = Bridge. F = Fragment.
Bi = Binucleated cells. Mp = Multipolar and micronucleated cells.

Table 3. DNA concentration in the root meristem cells of *A. cepa* after direct treatment for 8 hr. by different concentrations of garlic water extract and during recovery periods for higher doses.

Concentration (%)	DNA content (ug/g tissue) ± SE			
	Recovery Period (hr.)			
	24	48	72	
0	31.5±0.87	32.0±0.78	32.0±0.84	31.7±0.77
1	**19.05±0.7			
5	**16.05±0.56			
10	**11.45±0.47	**20.45±0.62^{oo}	**23.25±0.55^{oo}	**23.9±0.65^{oo}
20	**6.05±0.25	**18.15±0.59^{oo}	**20.55±0.54^{oo}	**21.55±0.59^{oo}

** Significant to control at 0.01 level.
^{oo} Significant to 8 hr. treatment at 0.01 level.

Table 4. Mitotic indices and percentage of mitotic phases in the root meristem cells of *A. cepa* after 8 hours treatment by high doses of garlic water extract and recovery periods.

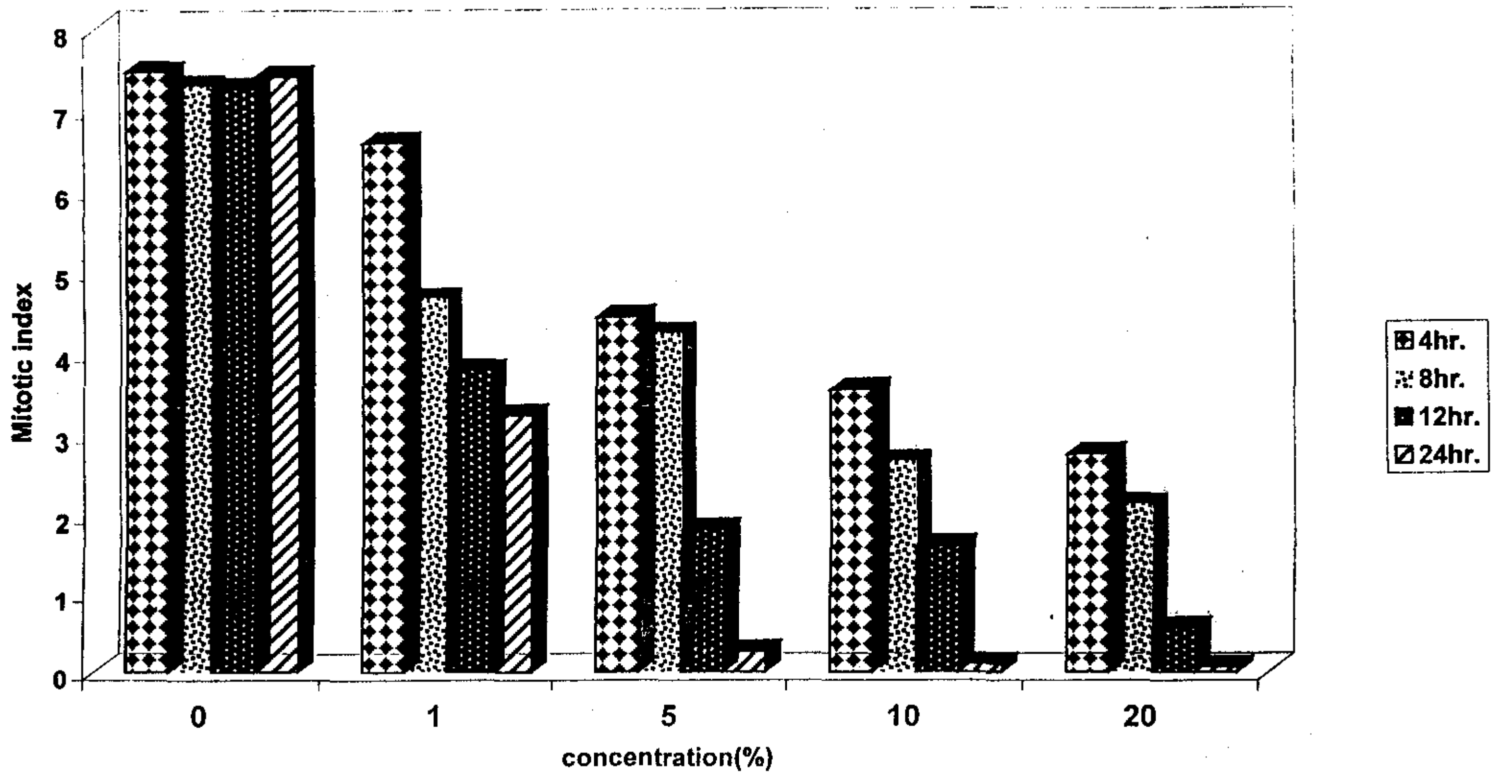
Concentration (%)	Time of Recovery (hr.)	Mitotic Index ±SE	Mitotic phases Percentage of dividing cells ± SE		
			Prophase	Metaphase	Anaphase + Telophase
0	24	7.21±0.02	36.03±0.08	33.4±0.08	30.57±0.08
	48	7.41±0.03	35.27±0.19	31.22±0.19	33.51±0.01
	72	7.55±0.17	32.11±0.04	34.23±0.06	33.66±0.08
10	24	**3.05±0.05°°	**28.13±0.2	**69.26±0.2	** 2.61±0.03
	48	**4.35±0.08	**28.98±0.09	**66.64±0.04°°	** 4.38±0.06°°
	72	**4.57±0.04°°	*31.27±0.19°°	**60.19±0.2°°	**8.54±0.11°°
20	24	**3.01±0.05°°	**13.28±0.16	**84.00±0.06	** 2.72±0.22
	48	**4.13±0.04°°	**17.12±0.06°°	**78.84±0.09°°	** 4.05±0.06°°
	72	**4.22±0.04°°	**20.47±0.14°°	**72.5±0.21°°	** 7.03±0.12°°

* Significant to control at 0.05 level.
** Significant to control at 0.01 level.
°° Significant to 8 hr. treatment at 0.01 level.

Table (5): Percentage of aberrations in the root meristem cells of *A. cepa* after 8 hours treatment by high doses of garlic water extract and recovery periods.

Concentration (%)	Time of Recovery (hr.)	Percentage of aberrations				Total ± SE
		Tp	S	B	Mp	
	24	0.62	-	0.62	-	1.24± 0.04
0	48	-	0.56	0.56	-	1.12±0.01
	72	-	0.55	0.55	-	1.0±0.01
	24	2.67	13.33	-	1.3	**17.3±0.09°°
10	48	2.86	8.57	5.71	-	**17.09±0.06°°
	72	2.5	2.5	1.25	-	**6.25±0.12°°
	24	2.67	13.33	-	1.3	**17.3±0.1°°
20	48	2.63	7.89	-	1.31	**11.83±0.05°°
	72	2.75	2.75	-	-	**5.5±0.03°°

** Significant to control at 0.01 level.
°° Significant to 8 hr. treatment at 0.01 level.
Tp = Tetraploid cells.
S = Stickiness.
B = Bridge.
Mp = Multipolar and micronucleated cells.



Figure(1): Mitotic indices in the root meristem cells of *A. cepa* after direct treatment by different concentrations of garlic water extract at different exposure times

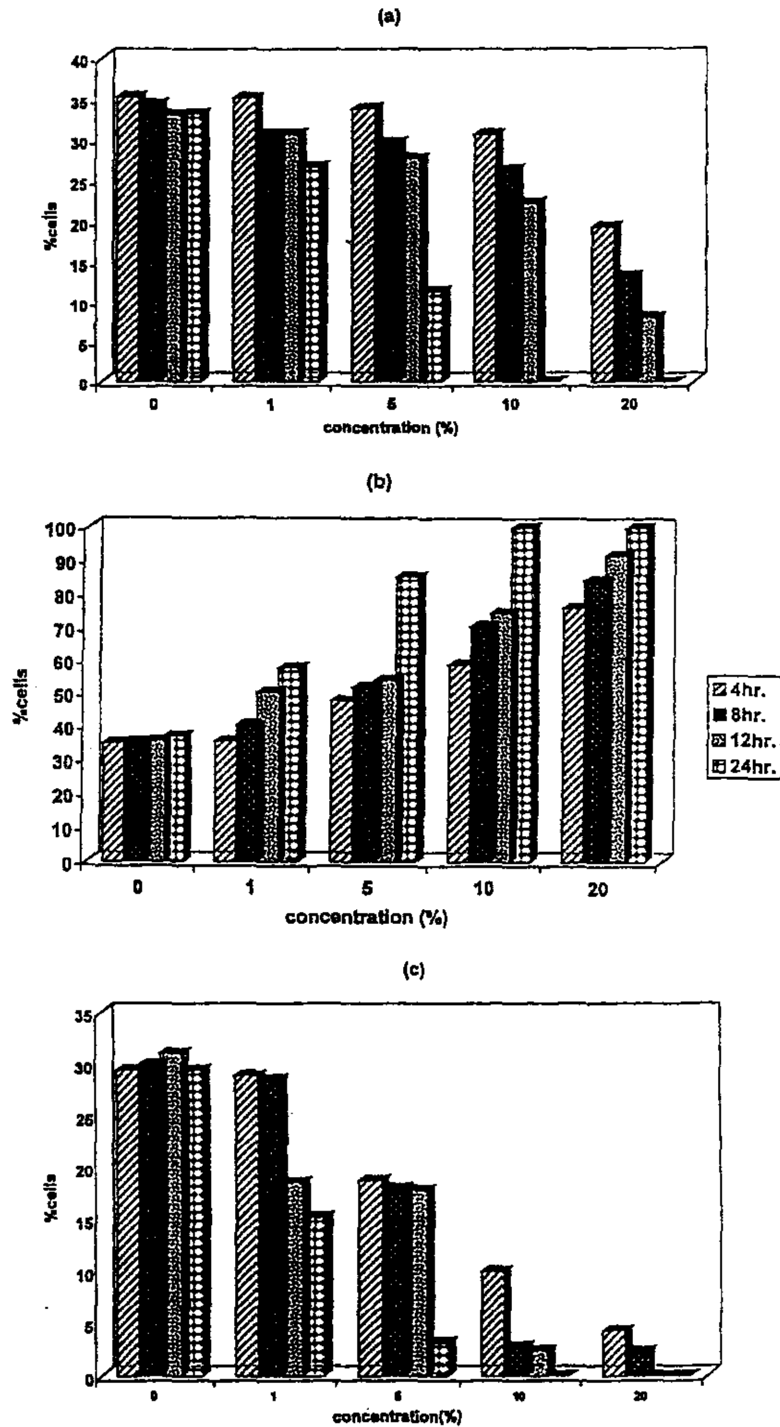


Figure (2):Percentage of mitotic phases in the root meristem cells of A. cepa after direct treatment by different concentrations of garlic water extract at different times of exposure: (a) prophase, (b) metaphase and (c) anaphase and telophase.

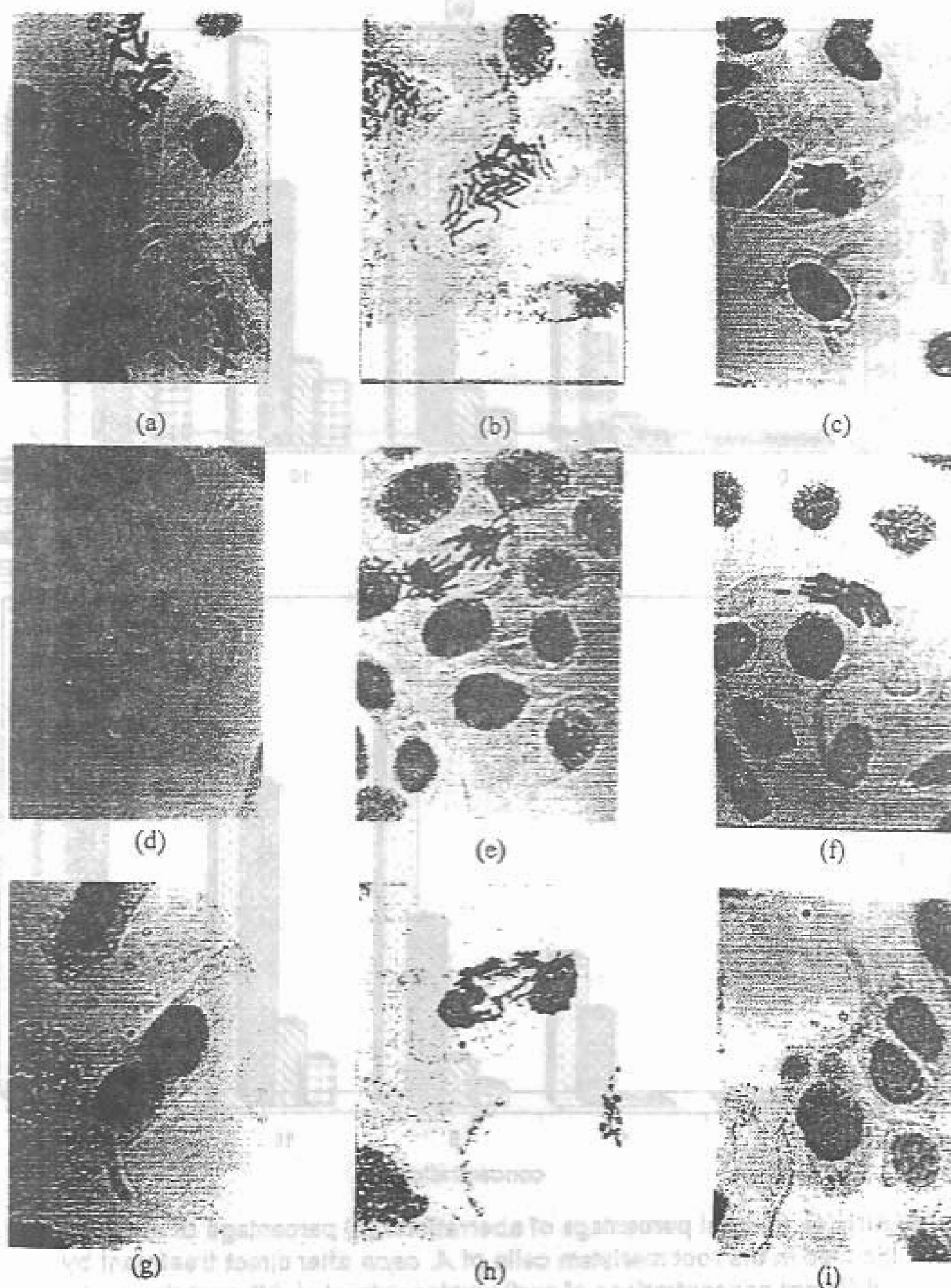


Figure (3): photomicrographs showing some different aberrations induced in the root meristem cells of *A. Cepa* after direct treatments by garlic water extract : (a) c- metaphase , (b) tetraploid cell , (c) clumping stickimetaphase , (d) laggard , (e) bridge, (f) fragment , (g) binucleated cell , (h) multipolar cell and (i) micronucleated cell.

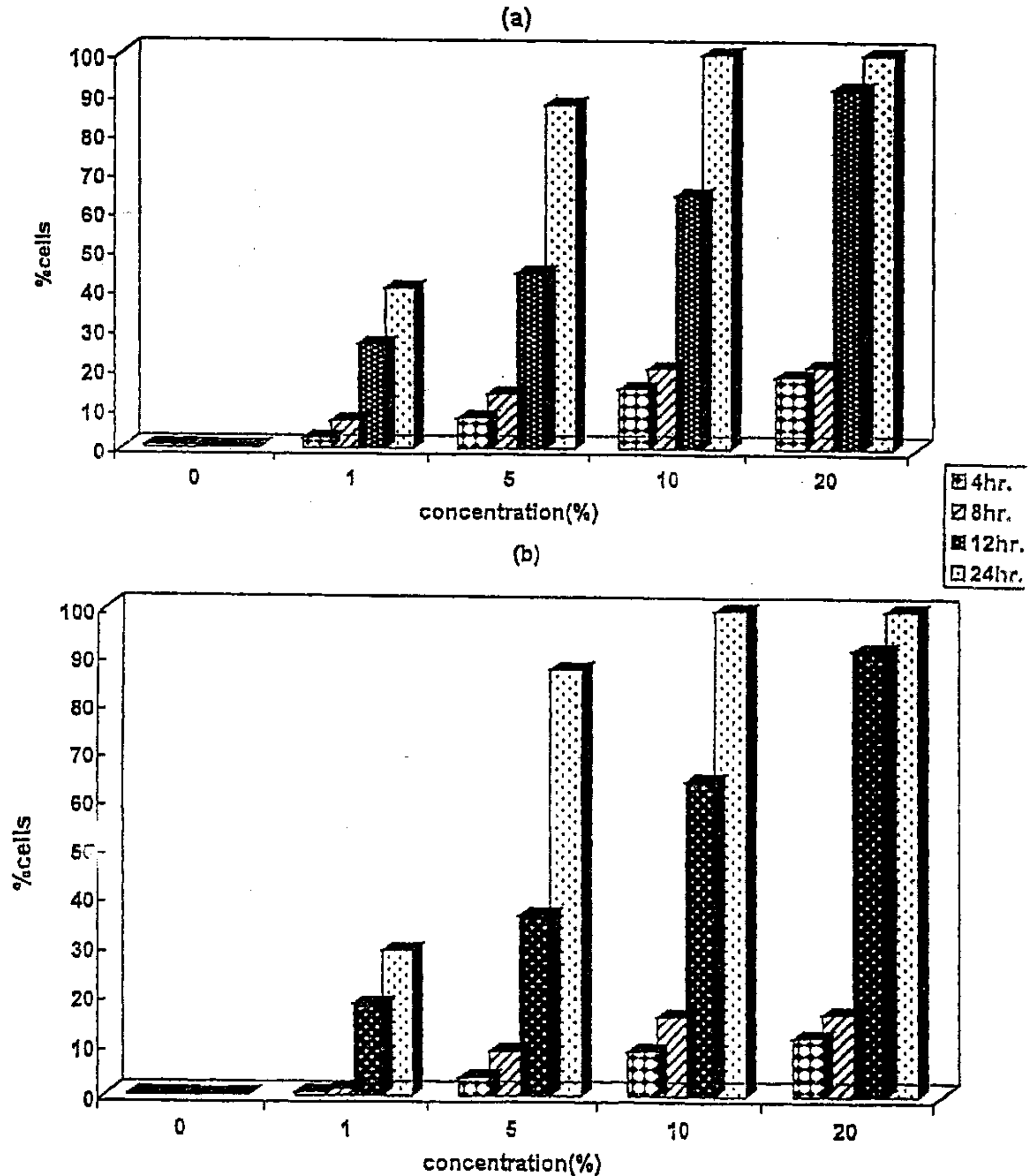
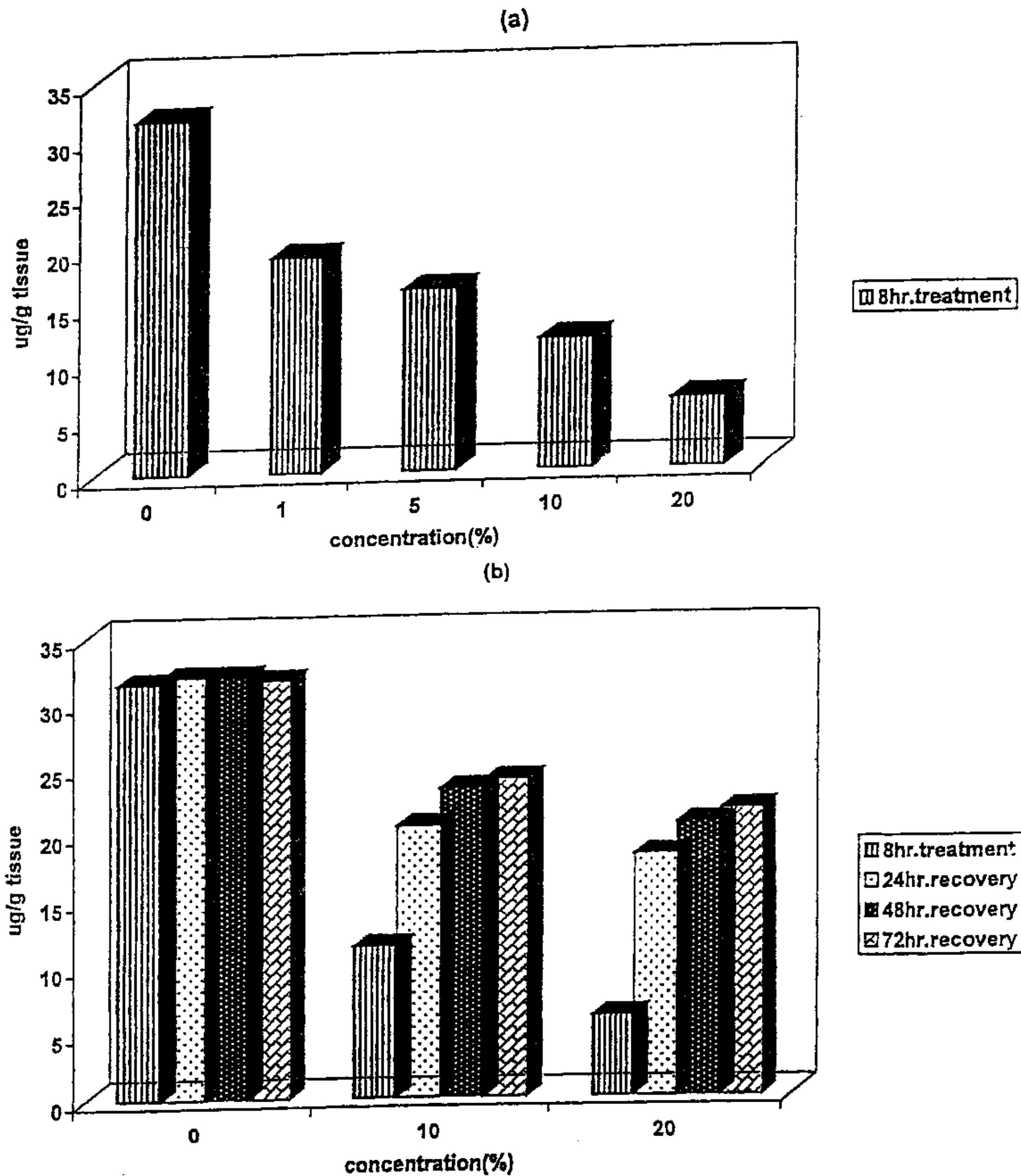
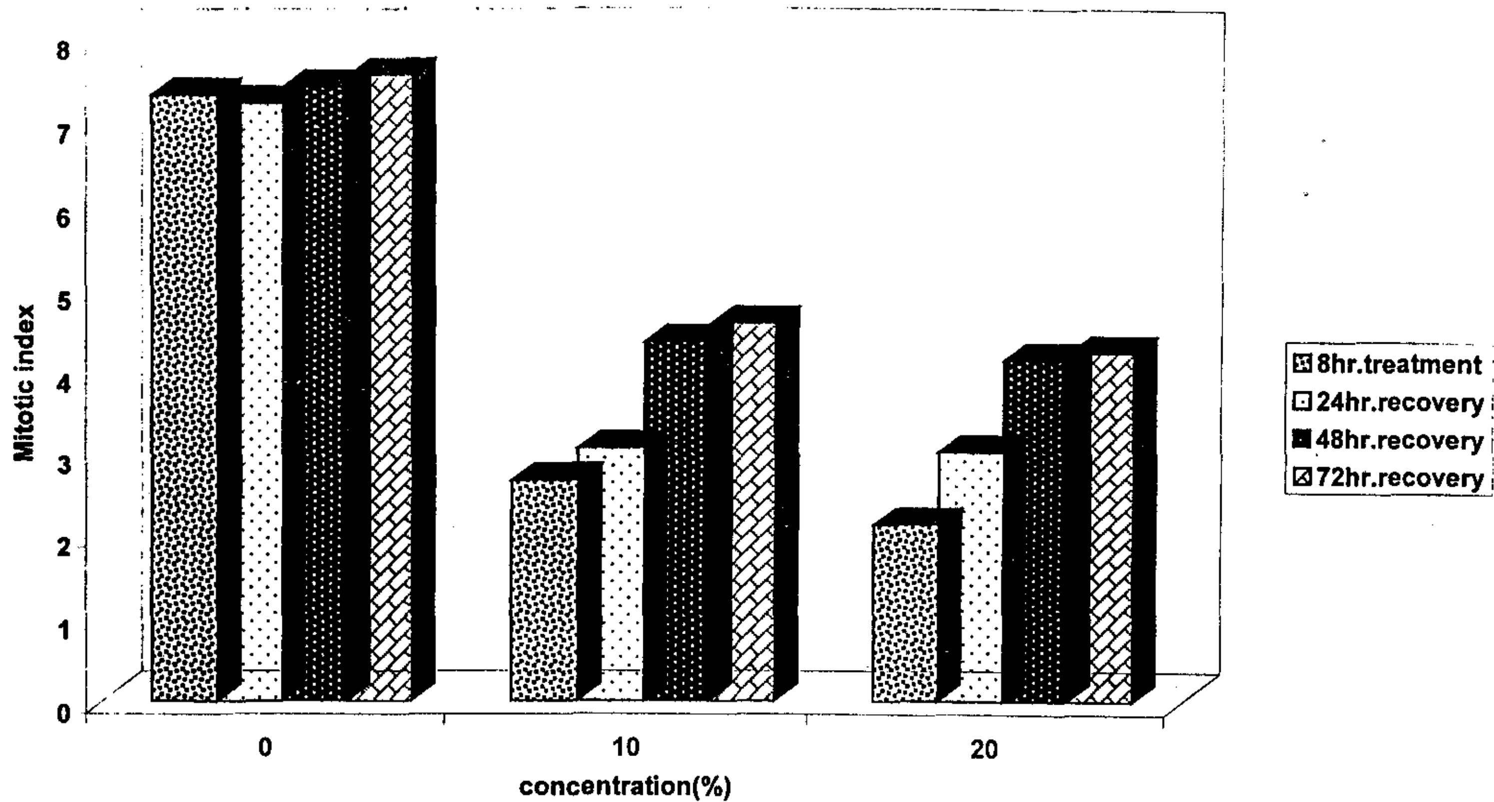


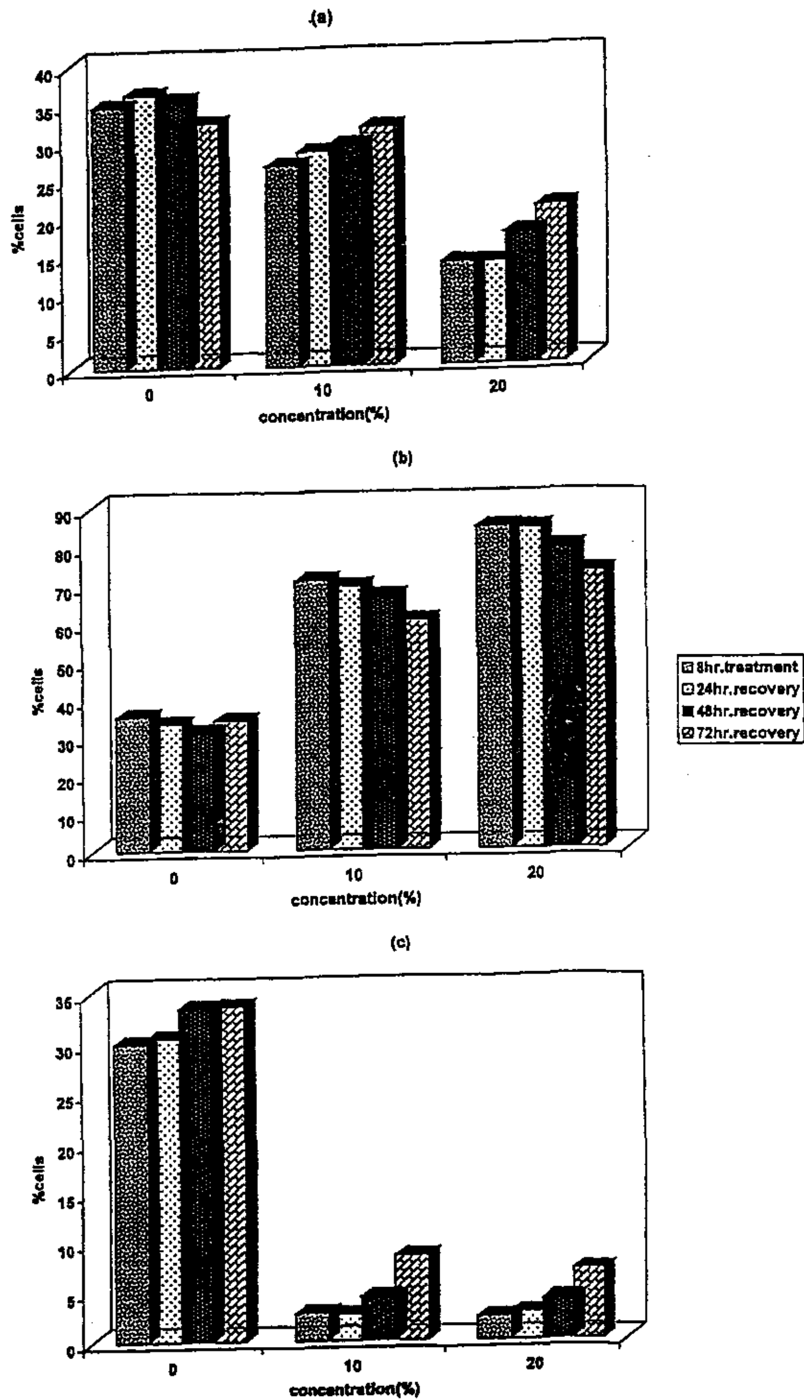
Figure (4): (a) total percentage of aberrations,(b) percentage of stickiness induced in the root meristem cells of *A. cepa* after direct treatment by different concentrations of garlic water extract at different times of exposure.



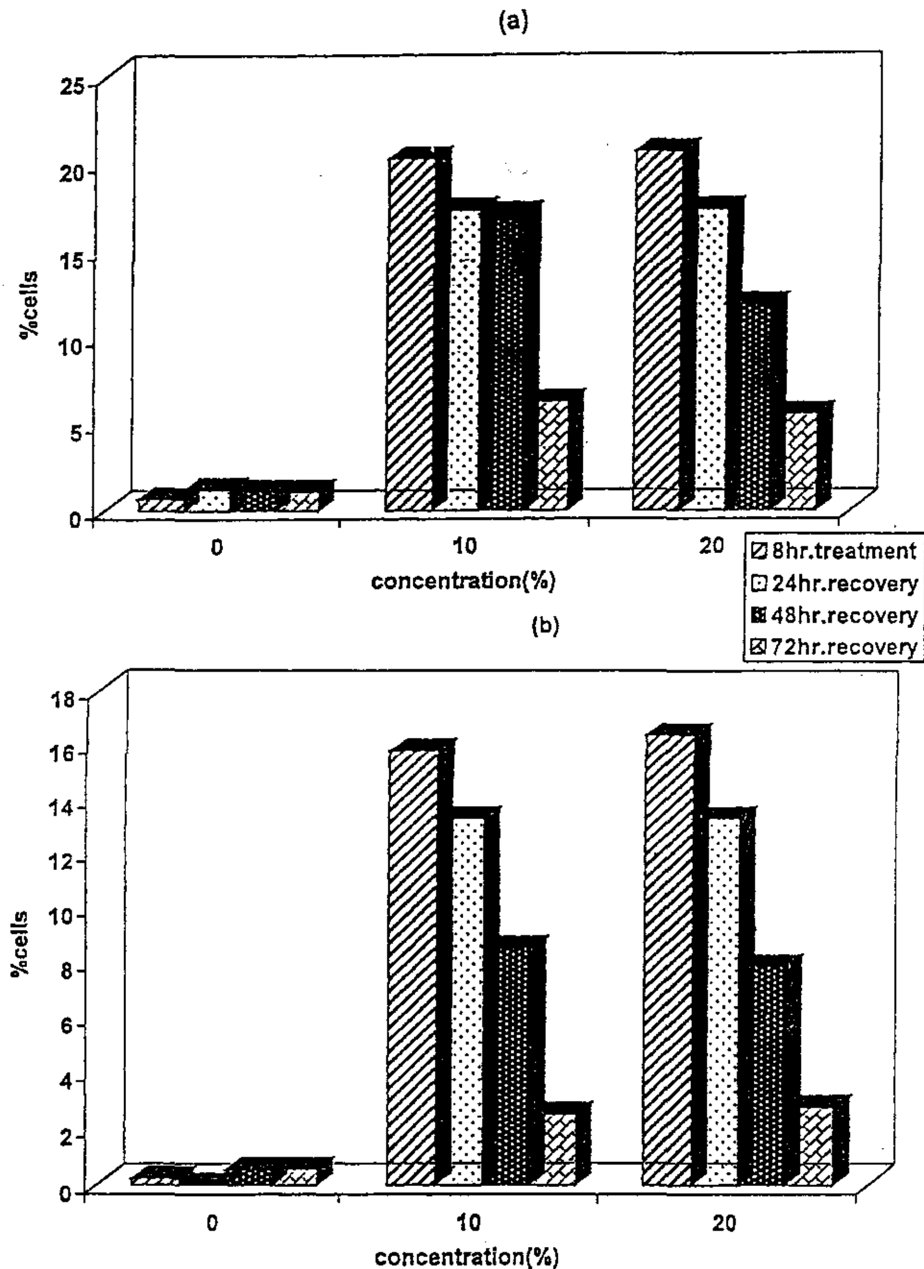
Figure(5): DNA- content in the root meristem cells of *A.cepa*, (a) after direct treatment by different concentrations of garlic water extract ,(b) after 8hr.treatment by high doses and recovery periods.



Figure(6): Mitotic indices in the root meristem cells of *A.cepa* after 8hr. treatment by high doses of garlic water extract and recovery periods.



Figure(7): Percentage of mitotic phases in the root meristem cells of *A.cepa* after 8hr. treatment by high doses of garlic water extract and recovery periods,(a) prophase, (b) metaphase,and (c) anaphase and telophase.



Figure(8): (a) total percentage of aberrations, (b) percentage of stickiness in the root meristem cells of *A.cepa* after 8hr. treatment by high doses of garlic water extract and recovery periods.

الملخص العربي

تأثير المستخلص المائي للثوم على الانقسام الميتوزي و محتوى المادة الوراثية في الخلايا الميراستيمية لجذور البصل

سناء أحمد رياض

قسم الوراثة، كلية الزراعة، جامعة الاسكندرية.

استهدف هذا البحث دراسة تأثير المستخلص المائي للثوم على الانقسام الميتوزي و محتوى المادة الوراثية في الخلايا الطبيعية(الخلايا الميراستيمية لجذور البصل) و ذلك من خلال المعاملة المباشرة للجذور بتركيزات مختلفة و لمدد زمنية مختلفة . كما تم دراسة مدى ثبات تأثير هذا المستخلص من خلال انعاش الجذور المعاملة بالتركيزات تحت المميتة لفترات مختلفة. أظهرت النتائج أن لمركبات الثوم الكبريتية تأثير مثبط للانقسام الميتوزي وأن لتلك الفاعلية علاقة طردية مع زيادة التركيز أو مدة المعاملة الزمنية. كما اتضح تراكم الخلايا في الدور الاستوائي كنتيجة للزوجة الكروموسومات الشديدة و التي أظهرتها ككتلة كروماتينية و أعاقتها من الاستمرار إلى الأدوار الميتوزية التالية خاصة في المعاملات عالية التركيزلفترات زمنية طويلة. الى جانب اللزوجة الكروموسومية ، شوهدت أيضا أنواع أخرى من الشذوذات الفسيولوجية الناتجة من التأثير على خيوط المغزل وكذلك تثبيط تكوين الصفيحة الوسطى، كما تبين استحداث تلك المركبات الكبريتية لكسور كروموسومية. من جهة أخرى قد يعزى التأثير التثبيطي للانقسام الى انخفاض أعداد الخلايا المقبلة على الانقسام وكذلك لتثبيط بناء المادة الوراثية في المرحلة الخلوية S. تبين أيضا من الدراسة عدم فاعلية فترات انعاش الجذور لاستعادة المعدلات الطبيعية للانقسام أو نسب الأدوار الميتوزية أو التأثير على خيوط المغزل. و على العكس من ذلك فان تلك الفترات كانت كافية لإنهاء التأثير الفسيولوجي على تكوين الصفيحة الوسطى و كذلك على استحداث الكسور الكروموسومية. يجرى حاليا مزيد من البحث لتفسير تأثير مستخلص الثوم على المراحل المختلفة من الدورة الخلوية وكذلك على التعبير الجيني.