Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **1(6),** 22-26, June **(2012)** *Res.J.Recent Sci.*

Antimitotic activity of a New Compound Isolated from the Flower of *Prosopis juliflora*

Singh Shachi
Center for Biotechnology, Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, INDIA

Available online at: www.isca.in
(Received 22nd March 2012, revised 26th March 2012, accepted 28th March 2012)

Abstract

A new compound was isolated from the flower of Prosopis juliflora and identified by mass, IR and NMR techniques. Its antimitotic activity was evaluated with the help of allium test. Onion root tips were subjected with 4 and 8 mg/ml concentrations of the compound, up to 48 hours for studying its effect on root mitosis. The roots were examined in permanent root tip squash preparations stained by the aceto-carmine. The results obtained confirmed that the compound have various effects on chromosomes and induced different mitotic abnormalities and structural aberration of chromosomes. Various chromosomal aberrations such as clumping and stickiness, fragmentation, C-mitotic effect, anaphase bridge were observed, which clearly showed the clastogenic, antimitotic and cytotoxic effect.

Key words: *Prosopis juliflora*, IR technique, NMR technique, antimitotic, allium test, chromosomal aberration.

Introduction

Cancer is believed to become the main cause of death in worldwide[1]. Even when the current antitumoral therapeutic strategy comprises multiple points of intervention, cytotoxic drugs remain a mainstay in cancer chemotherapy for the next future[2]. Antimitotic agents constitute a major class of cytotoxic drugs, and among them are included plant-derived compounds such as paclitaxel, vincristine, and combretastatin[3].

Plants have always been a source of natural product for the treatment of various disease[4,5]. Over the last decade, several novel highly active natural products have been described whose therapeutical potential for anti-cancer treatments are tested[6]. However, due to the great number of still non-treatable kinds of cancer and their tendency to produce resistances during anti-cancer treatment, we are faced with a current need to find new compounds and new lead structures for cancer chemotherapeutical purposes[7]. Therefore, the present research work was conducted in order to identify antimitotic activity of a compound isolated from the flower of a plant species, *Prosopis juliflora* with the help of Allium test.

Allium has proven a rapid, reliable, and inexpensive system by which the antimitotic effects of various chemical compounds may be monitored[8,9]. Characterized by rather homogenous meristematic cells, very large chromosomes and only sixteen chromosome numbers, the *Allium cepa* species (common onion) is ideal for use in bioassays[10]. It has also been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin[11]. The results obtained by Allium test could be useful in correlating the antimitotic effect of *P. juliflora* compound on *A. cepa* with that of mammalian cells as it is reported that Allium test shows good correlation with mammalian test systems[12].

Material and Methods

Plant material: Flower of *P. juliflora* was collected from their natural habitat in the Shekhawati regions of Rajasthan, India. It was washed thoroughly under tap water and then air dried under shade for one week and oven dried for 24 h at 40°C.

Extraction of compound: The dried plant material was grounded to form fine powder and filtered through sieve of 345 micron pore size. Plant sample was extracted with ethanol and then was fractionated between petroleum ether and water with the help of separating funnel. The aqueous layer was basified with ammonium hydroxide until it reached pH 11, and then was extracted with chloroform to remove basic components. The resultant aqueous layer was kept in refrigerator for one month. The pale-yellow crystals, thus formed were washed with ethanol and then filtered. It was subjected for mass, IR, ^{1}H and ^{13}C NMR analysis for further identification of the compound.

Identification techniques: Mass was recorded on a JEOL-AccuTOF JMS-T100LC Mass spectrometer having a DART (Direct Analysis n Real Time) source. The given sample was subjected to DART source and analyzed in positive ion mode. FTIR (Fourier transform infrared spectrophotometer) spectra of the compound was recorded on Shimadzu Corporation model, IR prestige 21 (200VCE) using KBr pellet method. Pellet was inserted into the FTIR sample holder and the spectrum was recorded. The spectrum was focused in the mid IR region of 400-4000 cm^{-1}. The ^{13}C (75.4 MHz) and ^{1}H (300 MHz) NMR (Nuclear magnetic resonance) spectra were recorded on a Varian XL-300 NMR spectrometer. For ^{1}H and ^{13}C NMR, 4 mg

and 30 mg of samples respectively, were dissolved in an inert solvent. The different chemical shifts of the proton according to their molecular environments within the molecule were measured in the NMR apparatus relative to a standard, tetramethyl silane (TMS).

Allium test: The Allium test was performed according to the method described by Fiskesj¨o, 1988. The roots of *A. cepa* were grown in distilled water in 200mL Erlenmeyer flasks under laboratory conditions (dark, 24^{o}C). After reaching a length of 3 cm (±0.5 cm), roots were incubated in the compound at 4 and 8 mg/ml concentrations. Pure water was used as control. The root tips were collected after 6, 12, 24 and 48h of incubation. For each concentration five root tips from three analogous onions were taken. Root tips were hydrolyzed in 1 N HCl, followed by squashing in 2% acetocarmine stain in 45% acetic acid. The squash preparation was observed under the microscope. Chromosome morphology and their changes were observed and mitotic index was calculated. Changes in cellular and chromosomal morphology were photographed under a light microscope (Olymphus BX41).

Results and Discussion

Identification of the compound: The compound was extracted from the flower as yellow crystal, soluble in more polar solvents, with $[M+H]^+$ value of 391.37319. Literature survey did not reveal any compound identified in *P.juliflora* having similar m/z value. Therefore this compound was subjected to, IR, ^{1}H and ^{13}CNMR analysis for its identification.

IR spectra showed presence of carboxylic acid and alkyl groups. A strong, wide band for the O–H stretch in the region between 3300-2500 cm^{-1}, centered at about 3000 cm^{-1} observed. This is in the same region for the C–H stretching bands of alkyl groups. Thus a carboxylic acid showed a somewhat "messy" absorption pattern in the region 3300-2500 cm^{-1}, with the broad O–H band superimposed on the sharp C–H stretching bands. The reason of broad O–H stretch band of carboxylic acids could be attributed to the presence of hydrogen-bonded dimmers. The carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 cm^{-1}. The C–O stretch appears in the region 1320-1210 cm^{-1} and the O–H bend is in the region 1440-1395 cm^{-1} and 950-910 cm^{-1}.

The compound was converted into esters by dissolving in methanol and subjected to NMR analysis, which showed the presence of an ester.[13] CNMR spectra showed the presence of carboxylic group/ester at 184.39 ppm and saturated alkane (methyl) at 26.15 ppm.

^{1}H NMR also showed the presence of a methyl ester at 3.71 ppm where a single peak was observed. Signal for alkanes were observed between 0.9-2.2 ppm. Terminal CH_3 peak appeared at 0.92 ppm indicating the presence of methyl protons in highly shielded environment and the signal was split up into triplet indicating presence of an adjacent CH_2 group. A triplet at 1.18 ppm again indicates presence of an intermediate CH_2 group. Two single peaks at 2.13 and 1.7 ppm were observed, which corresponds to the hydrogens present on alpha and beta carbons of the aliphatic acid. Two additional signal for alkanes were observed at 1.36 (triplet) and 1.53 (multiplet). A mulitiplet was also observed at 3.6 ppm and a single large peak at 1.92 ppm

Antimitotic activity: The compound isolated from flower extract was found to show antimitotic activity. Both the concentrations used in the experiment caused inhibition of mitotic activity depending on the time of incubation. Lower concentration (4 mg/ml) was found to be less effective in reducing cell division and caused significant decrease in mitotic index value only after 48h as compared to control. When the concentration was increased upto 8 mg, there was significant reduction of mitotic index values proportional to the time period, which finally decreased to 0 after 48h (table-1).

Table-1
Mean Mitotic index values (in percent) of the cells treated with 4 mg/ml and 8 mg/ml of the compound at different time period

Time	4 mg	8 mg
0 h	94±5.3	94±5.3
6 h	92±6.5	70±4.4
12 h	91±3.6	10±1.2
24 h	88±5.9	2±1.4
48 h	61±3.3	0

Changes in chromosome and cellular morphology were observed with increasing time and concentration. At lower concentration (4 mg/ml), partial c-mitosis (Colchicine like mitosis), full c-mitosis, with partially functional spindles and completely normal mitotic phases were seen in the various cells of the same root-tip between 6 to 48h time period (figure-1.). Partial c-mitosis with stickiness between chromosomes were observed at early stages (figure-1a.). A full c-mitosis with complete spindle inactivation and c-pairs in most of the cells was observed only after 12h of treatment with 4 mg/ml (figure-1c) concentration and after 6h for 8 mg/ml concentration. After 12h incubation with 4 mg/ml concentration, typical X-shaped c-pairs (c-pairs were found to generally separate and diverge widely), characteristic of colchicine treatment[13], were observed, along with all the intermediate shapes (figure-1d).

According to Steinegger and Levana[14], the elastic repulsion between the two chromatids is responsible for the peculiar X-shaped appearance of the c-pairs. When stickiness is acting in different regions of the chromosome body, the shape assumed by the c-pairs is the result of two forces, viz. elastic repulsion localized at the centromere and attraction between the chromatids at the sticky regions.

C-pairs, scattered over the cell and presenting a normal development to the division of the centromere and the

production of tetraploid cells, are regular feature in the material treated for 12h with 4 mg/ml concentration of the compound (figure-1e and 1f). After the 24h treatments this normal course of the c-mitosis was found only in a few cases. Generally new forces seem to enter into action during c-metaphase, causing distributed c-mitosis, the peculiarity of which is a partition of the c-pairs in two polar groups (figure-1i), similar pseudo-anaphases with chromosomes lacking the customary polarization and having their arms extending in all directions (figure-1g), were visible. In addition to the different distributions of c-pairs described above, interphases with c-pairs clumped in the centre of the cell (figure-1k) were also observed, together with the intermediate transitions after 48h of treatment.

Higher concentration of the compound (8 mg/ml) was found to have more damaging effect on the root tip cells. After 6h c-mitosis, distributed c-mitosis, multipolar anaphase (figure-2a), psuedoanaphase, stickeness between chromosome, clumping of c-metaphase in the center of the cell (figure-2d), misdivision during c-metaphase and c-anaphase (figure-2e) and lagging chromosomes (figure-2c) were observed. In some cells large bridges joining the two telophase nuclei appeared (figure-2b). At increased time period of 12h, breakages in the chromosomes were visible (figure-2f).

The induction of bridges between chromosomes could be attributed to chromosome breaks, stickiness and breakage and reunion of the broken ends. The stickiness prevented the separation of daughter-chromosomes and thus they remained connected by bridges[15]. Large number of vagrant chromosomes and c-anaphases indicates that compound 10 acts as a potent spindle inhibitor. In the present study, a few cells with fragments of chromosomes were observed indicating the clastogenic effect of test compound as suggested for cypermethrin compound in *A. cepa*[16].

After 24h of treatment the toxic symptom appeared in whole cell (Figure-3). Autolysis in the cytoplasm of the cell was visible, which continued with increasing time, causing complete dissolution of the cytoplasm, leaving behind only nucleus (Figure-3c and 3d). In some cells, nuclear material was found to be disintegrated with the cellular boundary still intact (Figure-3b). This disintegration of nuclear material continued with the increase in time. Within 48h nuclear material was completely lost with the cellular boundary still maintained forming a ghost cell (Figure-3e).

Conclusion

For many years, laboratories from all over the world have been working on finding effective remedy for tumors, up to now the most severe disease of our civilization[9]. Among many therapeutic plants that are objects of interest, the compounds from *P. juliflora* seemed particularly important, as shown in our results. According to the obtained results, one can conclude that the compound play a role in the antiproliferative and cytotoxic effect on *A. cepa* cells and these results could be useful in correlating with the mammalian system.

Acknowledgments

We acknowledge Birla Institute of Technology and Science for providing laboratory facilities to conduct the research work. We are also thankful to Central Drug Research Institute (CDRI), Lucknow, for performing mass, NMR and IR analysis. Funding for the research work was provided by CSIR (Council for Scientific and Industrial research).

References

1. De Flora S., Izzotti A., D'Agostini F. and Balansky R.M., Multiple points of intervention in the prevention of cancer and other mutationrelated diseases, *Mut. Res.,* **9**, 480 – 481 **(2001)**

2. Fonrose X., Ausseil F., Soleilhac E., Masson V. and David P., Parthenolide inhibits tubulin carboxypeptidase activity, *Cancer Res*., **67**, 3371 – 3378 **(2007)**

3. Iwasaki S., Antimitotic agents; Chemistry and recognition of tubulin molecule, *Med. Res. Rev.*, **13**, 183 – 198 **(1993)**

4. Mangale S.M., Chonde S.G. and Raut P.D., Use of Moringa oleifera (drumstick) seed as natural absorbent and an antimicrobial agent for ground water treatment, *Res.J.Recent Sci.,* **1(3),** 31-40 **(2012)**

5. Mondal D. and Mondal T., A review on efficacy of Azadirachta indica A. juss based biopesticides: an Indian perspective, *Res.J.Recent Sci.,* **1(3),** 94-99 **(2012)**

6. Edelman M.J., Novel cytotoxic agents for nonsmall cell lung cancer, *J. Thorac. Oncol.,* **1**, 752-755 **(2006)**

7. Latha P.G. and Panikkar K.R., Chemoprotective effect of *Ixora coccinea* L. flowers on cisplatin induced toxicity in mice, *Cancer Lett*., **130**, 197 – 202 **(1998)**

8. Andrade L.F., Campos J.M.S. and Davide L.C., Cytogenetic alterations induced by SPL (spent potliners) in meristematic cells of plant bioassays, *Ecotoxicol. Environ. Saf.,* **71**, 706–710 **(2008)**

9. Leme D.M. and Marin-Morales M.A., Chromosome aberration and micronucleus frequencies in *Allium cepa* cells exposed petroleum polluted water – a case study, *Mutat. Res*., **650,** 80–86 **(2008)**

10. Havey M.J., Rabinowitch H.D., Currah L., Genome organization in Allium. In Allium Crop Science, (Eds.). pp. 59-79. Recent Advances, CABI Publishing, United Kingdom, **(2002)**

11. Kura's M., Nowakowska J., S´ liwin´ ska E., Pilarski R., Ilasz R., Tykarska T., Zobel A. and Gulewicz K., Changes in chromosome structure, Mitotic activity and nuclear DNA content from cells of Allium test induced by bark water extract of *Uncaria tomentosa* (Willd.) DC., *J. Ethnopharmacol*., **107**, 211–221 **(2006)**

12. Fiskesjo G., Allium test for screening chemicals; evaluation of cytological parameters. In Plants for Environmental Studies, Wang W., Gorsuch J. W., Hughes J.S. (Eds.) 307-333 CRC, Lewis Publishers, New York, **(1997)**

13. Witkus and Berger C.A., Veratrine, a new polyploidy inducing agent, *J. Heredity*, **35**, 129-133 **(1944)**

14. Steineggeer E. and Levana A., Constitution and c-mitotic activity of isocolchicine, *Hereditas*, **33**, 385-396 **(1947)**

15. Badr A., Ghareeb A. and El-Din H.M., Cytotoxicity of some pesticides in mitotic cells of *V. faba* roots, *Egyptian J. App. Sci*., **7**, 457-468 **(1992)**

16. Saxena P.N., Chauhan L.K.S. and Gupta S.K., 2005, Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: Spectroscopic basis of chromosome damage, *Toxicol.*, **216,** 244-252 **(2005)**

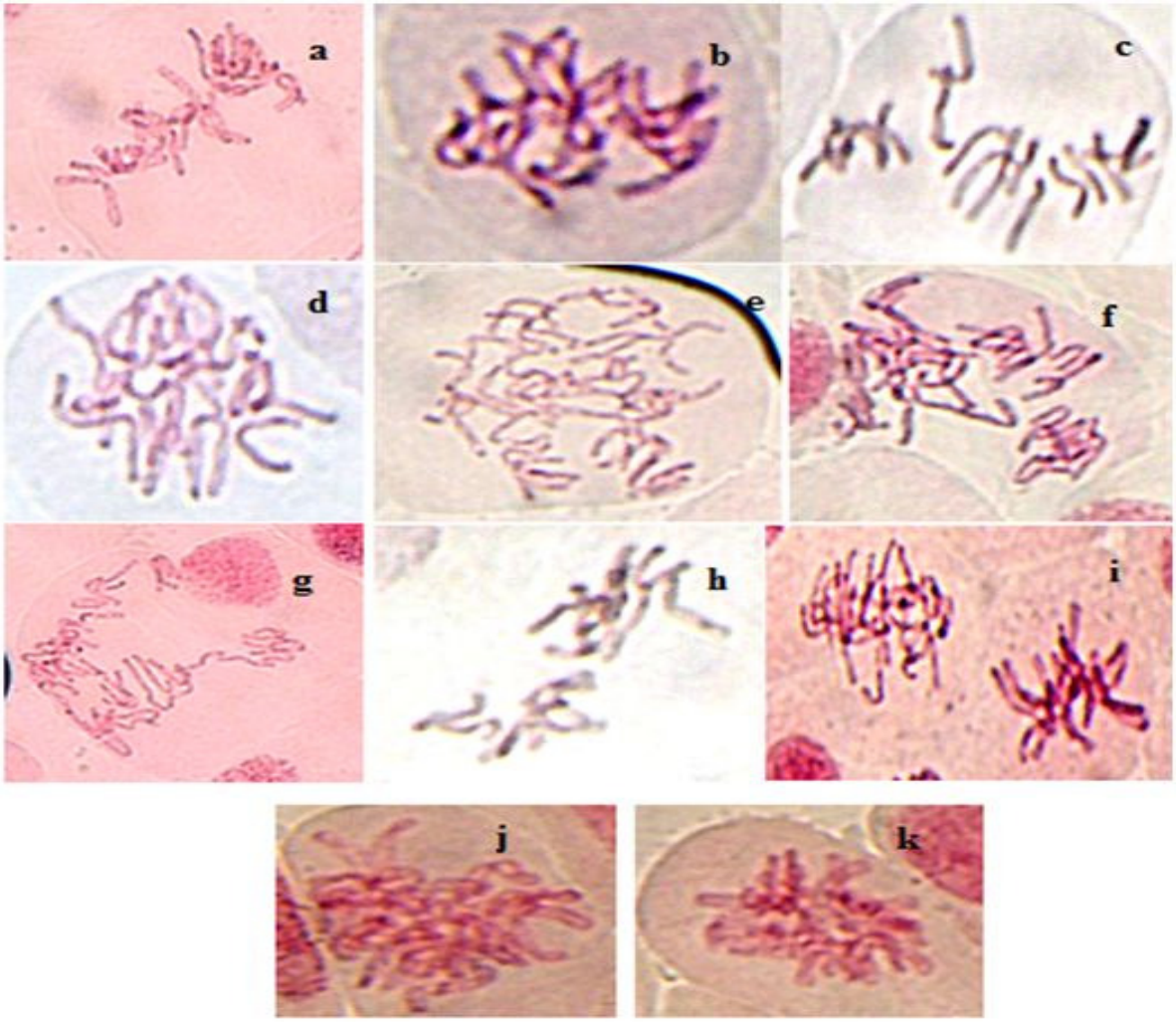

Figure-1

Chromosomal abnormalities in root cells of *Allium cepa*, seen after treatment of the Compound at concentration of 4 mg/ml within 48h - cells after 6h: a) Partial c-mitosis b) Partial c-mitosis sticking of the cell **cells after 12h:** c) C-mitosis d) Stickiness between c-mitosis X shaped **cells after 24h:** e) and f) Tetraploid g) Psuedoanaphase h) and i) Division in groups **cells after 48h:** j) and k) clumping in the center. 100x

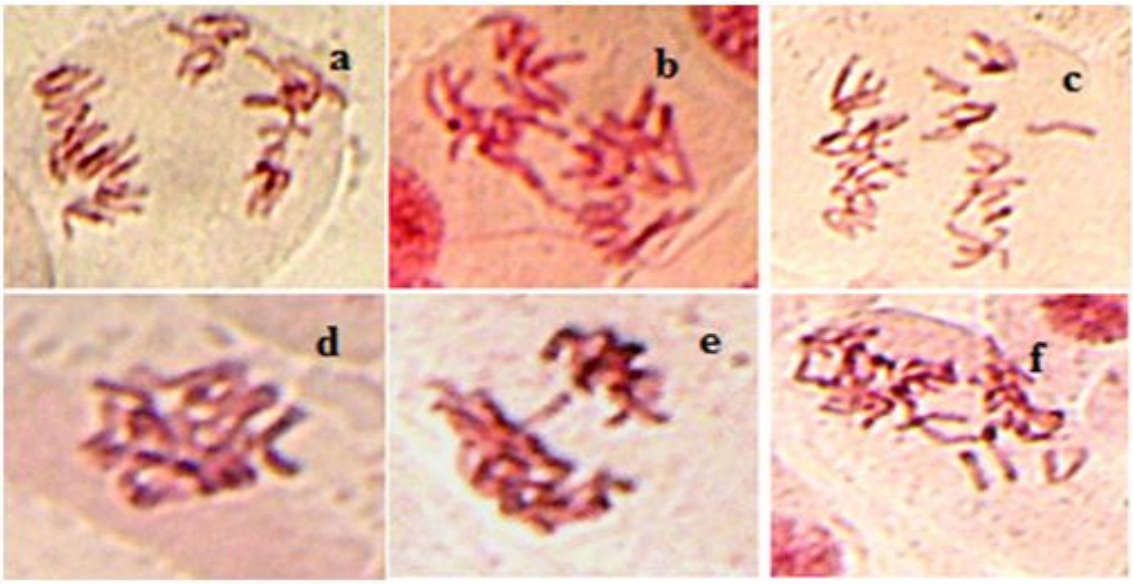

Figure-2

Chromosomal abnormalities in root cells of *Allium cepa*, seen after treatment of the compound at concentration of 8 mg/ml within 12h - cells after 6h: a)Multipolar anaphase b) chromosomal bridge c) lagging chromosome d) clumping of chromosome in the centre e) unequal division with chromosomal bridge **cells after 12h:** f) framentation of chromosomes.

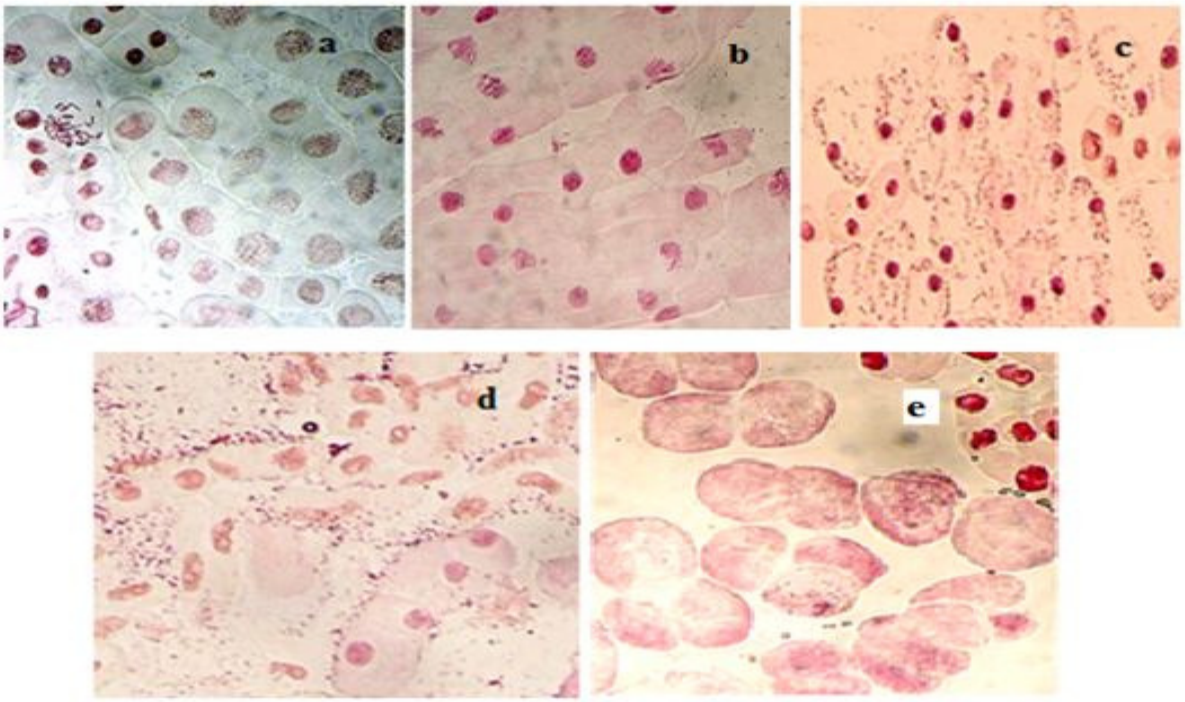

Figure-3

Cytotoxic effect of the Compound on root cells of *Allium cepa* seen after treatment with 8 mg/ml concentration within 48 h - a) cells after 6h showing chromosomal abberations b) and c) cells after 12h showing nuclear disintrigation and initiation of cellular autolysis d) cells after 24h showing incresed autolysis e) cells after 48 h showing formation of ghost cells. 40x