Influence of Cellular Phone and Tower on Mitosis in Allium Cepa Model

Devnarayan Gautam¹, Savita Marmat¹, Hemant Rathore^{1*}, Tajnudrat Qureshi¹, Archana Akhand² and Sharad Shrivastava²

¹Cell Biology, School of studies in Zoology and Biotechnology, Vikram University, Ujjain, MP, 456010, India ²Limnology Laboratory, School of studies in Zoology and Biotechnology, Vikram University, Ujjain, MP, 456010, India hrvuz2000@rediffmail.com

Available online at: www.isca.in, www.isca.me

Received 8th July 2016, revised 20th July 2016, accepted 6th August 2016

Abstract

An attempt is made to know cytological effects of a cell phone (mobile phone) and a cell phone tower on mitotically dividing root tip cells in Allium cepa protocol. Unexposed Allium cepa bulbs grown in the laboratory acted as control (Gr I). Second group of bulbs were exposed overnight to a cell phone (CP) in a room on the first floor of building (Gr II). In third group bulbs were placed on the open terrace, outside the room and exposed to cell phone tower (CPT) located on the roof of same room (Gr III). Medium for growing Allium cepa for roots was laboratory tap water. At the end of experimentations (96 hours) dissolved oxygen (DO) was measured in the medium of all groups. Every subsequent day temperature of the medium was recorded for Gr I and II. Morphology of root tips was observed, mean root length (MRL) was calculated. N-HCl acetocarmine squash preparations were made to observe mitosis, chromosomal aberration, micronuclei formation and disturbed mitosis. Mitotic index was also calculated. Results revealed no change in the temperature of the medium due to cell phone exposure. Dissolved oxygen increased following both exposures; more in tower exposure Gr III. No morphological changes could be detected in Gr I and II but in Gr III i.e tower exposure caused 'blunt ended' tips which is an unusual observation. No aberrations, no micronuclei and no abnormal mitoses were seen in any group however, mean root length and mitotic index declined in Gr II and III; more in Gr III. Unusual mitosis could be seen in Gr III as most of the cells were seen arrested in 'prophase' only; no other stages seen. At some places prophase nuclei were seen fused. It is concluded that CP and CPT lowers mitosis but CPT affects more severely. Genotoxicity not detected.

Keywords: Cellular phone (CP), Cell Phone Tower (CPT), Genotoxicity, Prophase inhibition, A.cepa.

Introduction

Global population of living beings is already constantly exposed to radiofrequency (RF) a form of electromagnetic radiations (EMR) from manmade sources (radios, wireless and related appliances) but during recent past cell phones and their towers have further increased their exposure level which affects human life too^{1,2}. Available reports are compiled as a mini review ³. Cytogenotoxicity findings are controversial ⁴ hence present study was planned to learn about cytological and genotoxicological potential of a mobile phone and a tower in *Allium cepa* protocol of Fiskesjo⁵.

Materials and Methods

Allium cepa: Locally available commercial variety of common onions (2n=16) of almost equal size (1.5 to 2.00 cm in diameter) were selected.

Medium: Laboratory tap water is used during entire experimentation. Physiochemical characteristics of tap water were determined by standard protocol of Winkler⁶.

Cell Phone (CP) and Cell Phone Tower (CPT): Approximately 150 feet high cell phone tower situated in the

corner of roof a room on the first floor of a **building**. Cell phone of manufacturing year 2013 is used.

Exposure of Cellular Phone and Tower towards Allium Cepa Bulbs: Followed standard protocol of Fiskesjo⁵ with minor changes. Pink brown dried outer scales and some of the brownish bottom plate of each onion bulb was removed carefully leaving the root primordia intact. For each experiment glass tubes of 330 ml capacity were filled with tap water. Each descaled onion was placed on the top of each tube with root primordia downwards touching the tap water.

Gr I: First set of 06 tubes with bulbs were placed in the departmental laboratory to serve as control (unexposed) against GR II and III for 4 days (96 hr).

Gr II: Second set of 06 test tubes with bulbs were placed in a rack and placed inside the room whose roof is lodging cell phone tower. Each day phone was turned on vibration mode and placed near the rack at 9.00 in the night till 9:00 of next day. Cell phone exposure duration was 12 hours per night hence for four nights total 48 hours.

Gr III: Third group of 06 tubes with bulbs were placed beneath

Vol. 5(8), 53-57, August (2016)

the cell phone tower on the roof of room of first floor for 4 days (96 hr). Lost volume of tap water was making up daily by adding tap water in each tube.

Processing of Roots: After 96 hours i.e 4 days of cultivation roots of each bulb was cut at the bases and kept in tap water. Lengths of 05 longest roots per bulb were recorded and their tips (2mm) were cut and fixed in acetoalcohol (1:3 v/v) for 24 hours then stored in 70% ethyl alcohol in refrigerator. Morphology of root tips (shape and color) of each set of onions was also recorded. Whole mount preparation of tips were made.

Squash Preparation and Observations: Routine N-HCl -2% acetocarmine squashes were made. Percentage of cells showing mitosis per field of observation is taken as mitotic index (MI). Four fields per slides were observed and twelve (two tips for each bulb) slides per group were made. Squashes were observed for aberrations; lagging chromosomes, bridges, abnormal mitosis etc and photographed. About 2000 cells per slide were analysed.

Dissolved Oxygen Determination (Do): Medium of *Allium cepa* cultivation of each group at the end of experimentation was used to find DO by Winkler's method ⁷.

Temperature Recording: Temperature of medium i.e. tap water was recorded following overnight exposure to cell phone on subsequent morning. Also, temperature of medium of control group, at laboratory was also measured every morning for comparison.

Statistics: Experiments were done three times and consistent data were used. Student's 't' test was applied to find significance.

Results and Discussion

Physicochemical Properties of Medium: Laboratory tap water used for growing Allium cepa bulbs revealed usual characteristics (Table-1).

Dissolved oxygen of the medium: Dissolved oxygen is found increased following both exposures, however, tower exposure enhanced more (Table-2).

Morphology of Root tips: No abnormality could be seen in the color and shape of root tips in control and cell phone exposed groups. Cell phone tower exposure caused 'blunt' ended tips against usual tapering ended tips (Table-3, Figure-1).

Mean Root Length (MRL) and Mitotic Index (MI): Both exposures declined both parameters i.e. lowered growth and mitosis. Mitotic Index is 13.25% more decreased after CPT exposure, similarly MRL is 21.61% more reduced due to CPT exposure (Table-4).

Table-1
Physicochemical properties of tap water (2015-16)

S. No.	Properties	Mean value	
1.	pН	7.3 ± 0.5	
2.	Colour	Clear	
3.	Total Alkanity	160± 6 mg/L	
4.	Carbonates	11 ± 2 mg/L	
5.	Bicarbonates	143± 7 mg/L	
6.	Hardness	90 ± 6 mg/L	
7.	Chlorides	17.8 ± 5 mg/L	
8.	BOD	14 ± 2	
9.	COD	42 ± 2	
10.	Fluoride	0.1 ± 0.002	
11.	Dissolved O2 6.4 ± 0.5		
12.	Turbidity NO		
13.	Nitrite	Nil	

Table-2
Dissolved oxygen of medium used to grow Allium cepa bulbs for cell phone and tower exposures.

S. No.	Groups (Treatment)	DO (mg/l) Mean ± SEM
1	Controls (Unexposed) Gr I	3.93± 0.0567
2	Cell Phone (Exposed) Gr II	$5.23 \pm 0.225*a$
3	Cell Phone Tower (Exposed) Gr III	8.41 ± 0.458*a,b

*Significant based on 't' test at 5% level of significance (p<2.201), a = control v/s both groups, b = Gr II v/s Gr III

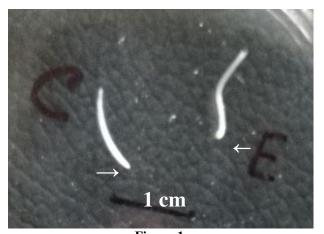


Figure-1 Showing normal tapering root tip of unexposed A. cepa bulbs $(C \rightarrow)$ while cell phone tower exposure caused thickened blunt end $(\leftarrow E)$. 10 X

Int. Res. J. Biological Sci.

Table-3
Morphological effects on root tips following cell phone and tower exposures

	00 11 02	GROUPS			
S.No.	PARAMETERS	I	II	III	
		Control	Cell Phone Exposed	Cell Phone Tower Exposed	
1.	Color of root tips	White (Usual)	White (Usual)	White (Usual)	
2.	Shape of tips	Straight & Tapering (Usual)	Straight & Tapering (Usual)	Straight but 'Blunt' ended (unusual)	
3.	Prophase Metaphase Anaphase Telophase	Usual Usual Usual Usual	Usual Usual Usual Usual	Prophase arrested NO NO NO	

Usual = Normal, NO=Not Observed, unusual=Abnormal

Effects on the temperature of growth medium: After (overnight) cell phone exposure on subsequent morning no change occurred in the temperature of medium in comparison to laboratory controls.

Mitotic Effects: Controls and cell phone exposed root tip revealed usual mitotic stages (Figure-2). Cell phone tower exposure induced very typical effect. No mitotic figures characteristics of mitosis could be seen in the tip's cells. Most of

the cell appeared in 'prophase' and a few in interphases. At places, two to three adjacent cell's nuclei are seen fused forming large 'prophase mass'. This indicates that mitosis in these cells were probably 'arrested' in prophase stage (Figure-3).

Discussion: Results indicate that cell phone and tower exposures have declined root growth and mitosis hence low mean root lengths and low mitotic indices are obtained. Cell phone tower exposures affected more severely. Also, cell phone tower exposure caused 'blunt ends' of tips, prophase is arrested in tip cells and enhanced DO (dissolved oxygen) of the medium (Gr III).

Reports do exist on the influence of cell phone exposure on mitotic cell division in plants.

Allium cepa seeds exposed to radiofrequency revealed increased mitotic index at higher dose and duration but chromosomal aberrations were observed at both low and high doses⁸. Mobile phone radiation induced root growth inhibition was attributed due to increased oxidative stress in root cells at Vigna radiata⁹. Mobile phone exposure also induced C-mitosis, lagging chromosome, vagrant chromosomes, chromosomal bridge, multipolar anaphases, multipolar telophases in germinating Lens culinaris and declined root growth¹⁰. Authors correlated low root growth with low mitotic activity in agreement with an earlier report⁸ and disturbed spindle formation with an earlier explanation for disturbed mitosis¹¹.

Mobile phone exposure induced chromosomal aberrations and mitotic index in *Allium cepa* model are on record¹². Present findings did not reveal cyto-genotoxicities i.e. aberrations, abnormal spindle formation or disturbed mitosis. Unfortunately conclusive reports have yet to come as controversial reports showing both genotoxic effects and no effects have been discussed⁴. It is of interest to mention here that results of a test substance may be model dependent in *A. cepa* models¹³.

Table-4

Mean Root Length and Mitotic Index in *Allium cepa* root tip cells following cell phone and tower exposures (Mean±SEM)

S. No.	Groups	Parameters		% change from controls	
		MRL(mm)	MI (% of dividing cells)	MRL	MI
1	Controls (Unexposed)	58.93±1.49	38.60±1.10		
2	Cell Phone (Exposed)	45.46*±1.65	28.37*±0.40	22.85% ↓	27% ↓
3	Cell Phone Tower (Exposed)	32.73*±0.60	23.06*±0.45	44.46% ↓	40.25% ↓

Low *Statistically significant based on 't' test at 5% level of significance (p<2.201)

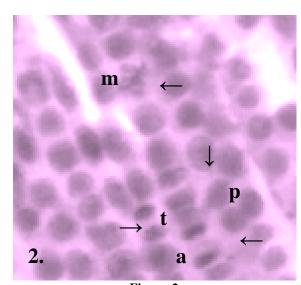


Figure-2
Squash preparations of A.cepa root tips Control Showing usual mitotic cell division: p=prophase m=metaphase, a=anaphase, t=telophase

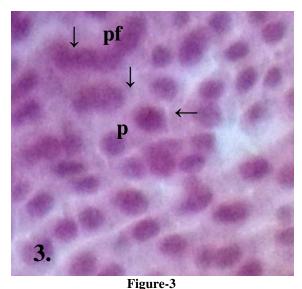
In *Allium cepa* root tip model of ¹¹ 'tumor like' root tips were seen due to C-mitosis by colchicines and some toxicants. In the present case no tumor like swelling could be seen and only 'blunt' end of tips were seen after CPT exposures. Squash preparations showed only prophases and no other mitotic stages.

This indicates that CPT exposure initially lowered mitosis and at some threshold ultimately inhibited cells at prophase stage of cell cycle. Perhaps due to this tips could not attain typical (usual) tapering shape. At places prophase nuclei are seen fused forming larger chromosomal mass.

In the present case CPT exposure caused mitotic arrest at prophase but no chromosomal aberration or abnormal mitosis could be seen. In fact reputed herbal drugs like trifla and its one component myrobalan have been found to inhibit mitosis in *Allium cepa* test at 'prophase' checking G1-S transition^{14,15} and such mitostatic (cytotoxic) property of trifla and myrobalan was held responsible for their cytotoxicities against cancer cells¹⁶⁻¹⁹.

Similarly *Cassia tora* seeds decoction caused mitostatic/mitodepression in *A. cepa* model due to prophase progression check²⁰ and this herbal substance is found cytotoxic against cancer cell lines. Increased DO i.e. dissolved oxygen has increased in the medium of Gr II & III; more in Gr III (CPT).

It can be explained up to limited extend at the moment. Electromagnetic treated water has shown diverse biological effects on both animal and plant cells probably due to an effect known as 'Memory of Water' hypothesis²¹. Electromagnetic field can affect hydrogen bonding and increased evaporation rate and DO too²².



Squash preparations of A.cepa root tips Cell phone tower exposed Shows only prophases (p). At two place nuclei have fused with prophase nuclear masses, (pf). No other stages are seen

Conclusion

Radiofrequency electromagnetic radiations emitting cellular phone (mobile phone) and its tower's exposures, both have exerted inhibitory action on the process of mitotic cell division in *Allium cepa* roots. No genotoxicity detected.

References

- 1. Jain A. and Bagai D. (2014). Effects of exposure to electromagnetic radiations on living beings and environment. *International Journal of Electrical*, *Electronics and Data Communication*., 2(7), 1-5.
- 2. Fathi E. and Farahzadi R. (2014). Interaction of mobile telephone radiation with biological systems in veterinary and medicine. *Journal of Biomedical Engineering & Technology.*, 2(1), 1-4.
- **3.** Gautam D. N. (2015). Biological effects of electromagnetic waves from cell phones and cell phone towers: A Mini Review. Submitted as a part of M. Phil. Course work to Vikram University, Ujjain, India
- **4.** Ruediger H. W. (2009). Genotoxic effects of radiofrequency electromagnetic fields. *Pathophysiology*., 16, 89.
- **5.** Fiskesjo G. (1995). Allium Test. Methods in Molecular Biology,43(14), In Vitro Toxicity Testing Protocols (Edited by O' Hare S, Atterwill C.K.). Humana Press Inc. Totowa, NJ., 119-127.
- **6.** APHA. (1998). Standard methods for examinations of water and waste water. 20th edn., Washington DC.

- 7. Winkler L.W. (1888). Die Bestimmung des in Wasser gelösten Sauerstoffen. *Berichte der Deutschen Chemischen Gesellschaft*, 21, 2843-2855.
- 8. Tkalec M., Malaric K., Pavalika M., Pevalek-Kozina B. and Vidakovic-Cifrek Z. (2009). Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of *Allium cepa L. Mutation Research/Genetic Toxicity and Environmental Mutagenesis.*, 672(2), 76.
- **9.** Sharma V. P., Singh H. P., Kohli R. K. and Batish D. R. (2009). Mobile phone radiation inhibits *Vigna radiata* (Mung bean) root growth by inducing oxidative stress. *Sci Total Environ.*, 407(21), 5543-07.
- 10. Akbal A., Kiran Y., Sachin A., Turgut-Balik D. and Balik H. H. (2012). Effects of electromagnetic waves emitted by mobile phones on germination, root growth and root tip cell mitotic division of *Lens Culinaris Medik. Pol. J. Environ. Stud.*, 21(1), 23-29.
- 11. Fiskesjo G. (1993). The *Allium* Test- A potential standard for the assessment of environmental toxicology. Environmental Toxicology and Risk Assessment, 2. ASTM STP 1216, Gorsuch JW, Dawyer FJ, Ingersoll CG, La Pount T W Eds, American Society for testing and Materials, Philadelphia, PA, 331-344.
- **12.** Pesnya D. S. and Romanovsky A. V. (2013). Comparision of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phones GSM 900 radiation in the Allium cepa test. *Mutation Research/Genetic Toxicity and Environmental Mutagenesis.*, 750(1-2), 27-33.
- **13.** Rathore H. S., Rathore M., Panchal S., Makwana M., Sharma A. and Shrivastava S. (2010). Can genotoxic effect be model dependent in *Allium* test?- An Evidence. *Environment Asia.*, 3(2), 29-33.

- **14.** Rathore H. S., Khare A., Sharma A., Shrivastava S. and Bhatnagar D. (2006). A study on the cytological effects of myrobalan (Fruit of *Terminalia chebula*) in *Allium* tests. *Ethanobotanical Leaflets.*, 10, 92-97.
- **15.** Marmat S. and Rathore H. S. (2013). Cytological effects of Triphala in *Allium cepa* models. *International Journal of Pharmacy & Technology.*, 5(2), 5602-5609.
- Kashiwada Y., Nonaka G., Nishioka I., Chang J. J. and Lee K. H. (1992). Antitumor agents, 129, Tannins and related compounds as selective cytotoxic agents. *J. Nat Prod.*, 55, 1033.
- **17.** Lee S.H., Ryn S. Y., Choi S. U., Lee C. O., No Z., Kim S. K. and Ahn J. W. (1995). Hydrolysable tannins and related compounds having cytotoxic activity from fruit of Terminalia chebula. *Arch. of Pharmacol Res.*, 18, 118.
- **18.** Kaur S., Micheal H., Arora S., Harbonen P. L. and Kumar S. (2005). The *in-vitro* cytotoxic and apoptotic activity of Triphala: an Indian drug. *J Ethnopharmacol.*, 97, 15-20.
- **19.** Jose J. K., Kuttan G. and Kuttan R. (2001). Antitumor activity of *Emblica officinalis*. *J Ethnopharmacol.*, 75, 65-69.
- 20. Solanke P., Singh M., Rathore H. S., Sharma A., Makwana M. and Shrivastava S. (2007). An evaluation of the genotoxic effects of seed decoction of *Cassia tora Linn* (Leguminosae) in *Allium cepa* model. *Ethanobotanical Leaflets.*, 11, 217-223.
- **21.** Yamabhai M., Chumseng S., Yoohat K. and Srila W. (2014). Diverse biological effects of electromagnetic treated water. *Homeopathy.*, 103(3), 186-192.
- **22.** Kitazawa K., Ikezoe Y., Uetake H. and Hirota N. (2001). Magnetic Field effects on water, air and powders. *Physica B.*, 294-295, 709-714.