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IN VITRO CYTOTOXICITY AND CYTOGENETICITY ASSAY MEASURING THE MUTAGENICITY INDUCED AS EFFECT OF CELL PHONE RADIATION

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ABSTRACT

Mutagenicity caused by mobile phone radiations were investigated in this study. Ames test determines the mutations caused due to the chemical or substance in the Salmonella with the utilization of specific bacterial strain. The bacterial reverse mutation assay detects the mutational reversion of his-dependent *Salmonella* to his-independent colonies. Mutation causing radiation increases the frequency of occurrence of his-independent colonies. MTT spectrophotometric assay determines the ability of viable cells to convert a soluble tetrazolium salts into insoluble formazan precipitate. The reaction converts yellow salts to blue formazan crystals that can be dissolved in an DMSO whose concentration can be determined at 570nm. From the Ames test , it is concluded that the dose response curve using various various concentration shows that the mobile phone head speaker radiation was partially mutagenic at the range of 313 to 5000 µg/plate. For MTT assay, a significant concentration dependent 70% inhibition of HepG2 cells were detected at 0.625 mg/ml.

KEYWORDS: ***Mobile phones, Mutagenicity, Ames test, MTT assay, Salmonella.***

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INTRODUCTION

Mobile phones have evolved in time like everything else. Named “car phones”, and bulky in nature, became cumbersome, and expensive compared to today’s smart successors. John F. Mitchelland Martin Coopermade the first working of a mobile of Motorola in 1973, that weighed a heavy 2 kilograms . In 1983, DynaTAC 8000x made its way as the first decent hand held mobile phone. GSM mobile phones work within 2 W and 1 W at 900 Hz and 1800 Hz, respectively[1]. Radio frequency induce RF electric fields in tissue a part of the radiated energy will be absorbed in tissues[2]. Mobile phones work using the spectrum of electromagnetic radiation, particularly the microwave range. In 2011, mobile phones were classified as Group 2B - possibly carcinogenic by the IARC -International Agency for Research on Cancer.Meaning, mobile phones could induce cancer due to overuse[3]. Despite proven the health problems a mobile phone could cause people oversee the defects. For instance, cell phone radiation directly affects the brain, reproductive organs specifically the production of sperm in male genitals. Using headsets does not seem like a viable alternative as it opens a direct path to bacteria in the head which would compromise the proper functioning of the body system [4]. Radiofrequency radiation and electromagnetic fields exposes to biological effects within seconds of exposure[5].A chronic exposure of the level of a base station can result in possible illness. Electromagnetic radiation and its harmful biological effects have been successfully reported through scientific research of hundreds of scientists over the last 40 years reporting genotoxicity, single and double stranded DNA damage chromatin condensation, loss of DNA repair capacity in human stem cells, abnormal gene transcription, neurotoxicity, carcinogenicity and effects of brain development[6]. Recent research shows that chemical bonds present in a DNA are not only broken by mobile phone radiation but is also carried to the next generation due to prolonged use[7-8].

Ames test

The Ames test is a widely employed method that uses bacteria to test whether a given chemical can cause mutations in the DNA of the test organism. More formally, it is a biological assay to assess the mutagenic potential of chemical compounds [9]. A positive test indicates that the chemical is mutagenic and therefore may act as a carcinogen, because cancer is often linked to mutation. The test serves as a quick and convenient assay to estimate the carcinogenic potential of a compound because standard carcinogen assays on mice and rats are time-consuming (taking two to three years to complete) and expensive [10]. However, false-positives and false-negatives are known [11].The procedure was described in a series of papers in the early 1970s by Bruce Ames and his group at the University of California, Berkeley[12-14].

Sample preparation

Solid test substances should be dissolved or suspended in appropriate solvents or vehicles and diluted if appropriate prior to treatment of the bacteria. Liquid test substances may be added directly to the test system and /or diluted prior to treatment. Fresh preparations should be employed unless stability data demonstrate the acceptability of storage. Dose formulation concentrations of 0.625, 1.25, 2.5 and 5.0 mg/ml of samples were prepared by serial dilution for the study [15].

Methodology

Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. In the plate incorporation method, these suspensions are mixed with an overlay agar and plated immediately onto minimal medium. In the preincubation method, the treatment mixture is incubated and then mixed with an overlay agar before plating onto minimal medium. For both techniques, after two or three days of incubation, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates [15]. the plate incorporation methodology, the tester strain, samples, were combined in molten top agar, which was then overlaid onto a minimal bottom agar plate. These procedures were used in the dose range-finding and mutagenicity assays. Each plate was labeled with the samples, test phase, tester strain, activation condition, and dose. Treatments in the absence of S9 were performed by adding 100 μl tester strain and 100 μl test or control article to 2.5 ml molten diluted top agar (maintained at 45 ± 2 °C). The mixtures were vortexed and overlaid onto the surface of bottom agar dishes. After the overlay has solidified the plates were inverted and incubated for 72 hrs at 37 ± 2 °C. After incubation the plates were evaluated for the condition of the background lawn for the evidence of cytotoxicity and samples precipitate in comparison with the control and the plates were evaluated for the number of revertant colonies [15]. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, first described by Mossmann in 1983, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of the cells by the addition of a detergent results in the liberation of the crystals which are solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The color can then be quantified using a simple colorimetric assay. The results can be read on a multiwell scanning spectrophotometer (ELISA reader).

MTT methodology

For cell viability quantitative MTT assay, HepG2 cells culture were plated as 1 X 10^4 cells /well in 96-well plates for 48 hours. The plate was incubated at 37° C in a humidified atmosphere for 24 hours. At the end of 24 hours, the media were removed and 200 μl of freshly prepared medium was added to blank wells (without drug), positive control wells (contains 100μl of cell suspension media and 100μl of medium without drug) negative control wells (contains 100μl of cell suspension media and 100μl of media with DMSO) and for test wells (100μl of cell suspension and 100μl of different concentrations (5,2.5,1.25,0.625 mg/ml) of mobile phone head speaker was added and diluted with medium to the desired test wells in duplicates. Then the

plate was incubated for 24 hours. 20µl of MTT was added to all the wells except for the blank wells and incubated for 4 hours at 37°C in a humidified atmosphere. Then the medium was aspirated and 150 µl of formazan crystal dissolved in DMSO was added and kept it for shaking to dissolve the crystals. The Purple color developed was read at 570 nm in an ELISA reader. The growth inhibition was determined by

Growth inhibition (%) = control OD– sample OD /control OD* 100

RESULT

Ames test - tester strain density

The optical density of the overnight cultures were determined at 650 nm in a spectrometer and found to be within the range of 0.0452. The organism *Salmonella typhimurium* used in this experiment were grown as overnight cultures for 24 hours in nutrient broth. Then its density was determined at 650 nm in a spectrometer and found to be within the range of 0.04 to 0.06, which demonstrated that the cultures were in late exponential or early stationary phase with ≥ 10^9 cells/ ml.

Counting of colony forming units (CFU) per plate

The mutagen (positive control) (Fig. 1) treated without metabolic activation system showed a 3 fold increase of average revertant colonies per plate when compared with that of concurrent vehicle control, thus exhibiting the ability to identify the mutagen by the tester strain.

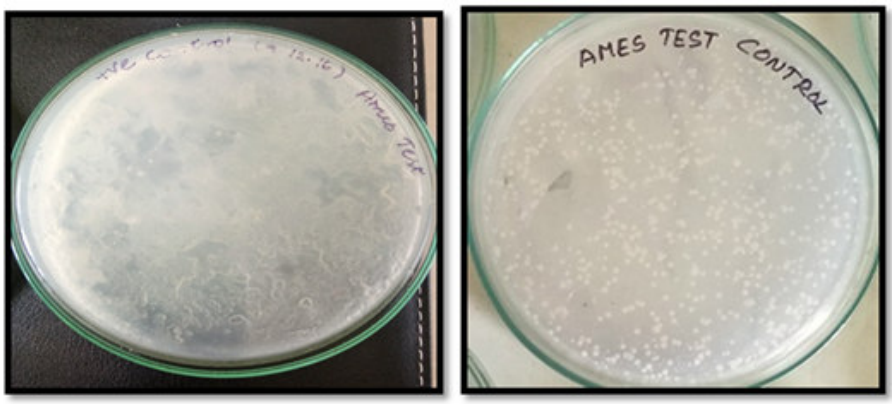

Figure 1
Normal and Positive control for TA 98

Table 1
Mean Colony Count - Strain TA 98 - spontaneous mutation

Samples	Test Concentration (µg/plate) / (mg/ml)		Histidine Revertant Colonies CFU/Plate		Mean	SD
Normal control	only culture		70	73	71.5	2.1
Positive Control	Sodium azide	(1.5 µg/plate)	1052	1065	1058.5	9.2
Test Sample	5		TNTC	TNTC	TNTC	TNTC
	2.5		254	236	245	12.7
	1.25		306	350	328	31.1
	0.625		175	150	162.5	17.7

***TNTC – Too numerous to count**

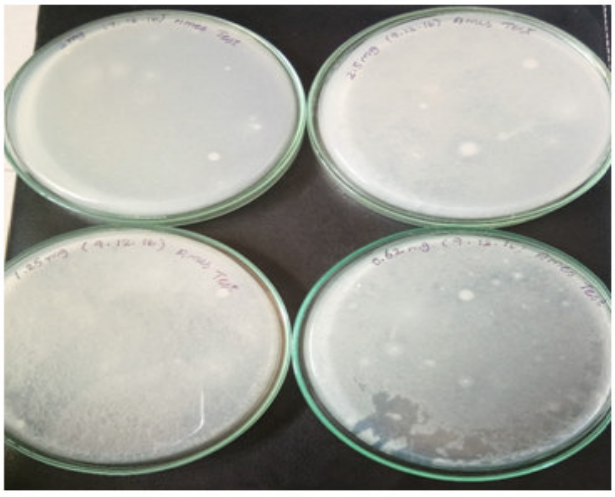

Figure 2
CFU /plate

The average revertant colonies per plate treated with the control in the absence of metabolic activation system were found to be within the acceptance limits of the spontaneous revertant control values of respective *Salmonella* strain.

MTT assay

Table 2
OD values

Concentration	OD @ 570nm		Mean	% inhibition	% cell viability
0	0.395	0.400	0.398	0.0	100.0
20	0.35	0.353	0.352	11.6	88.4
40	0.264	0.266	0.265	33.3	66.7
60	0.187	0.190	0.189	52.6	47.4
80	0.155	0.152	0.154	61.4	38.6
100	0.124	0.122	0.123	69.1	30.9
200	0.106	0.102	0.104	73.8	26.2
DMSO	0.396	0.393	0.395	0.8	99.2

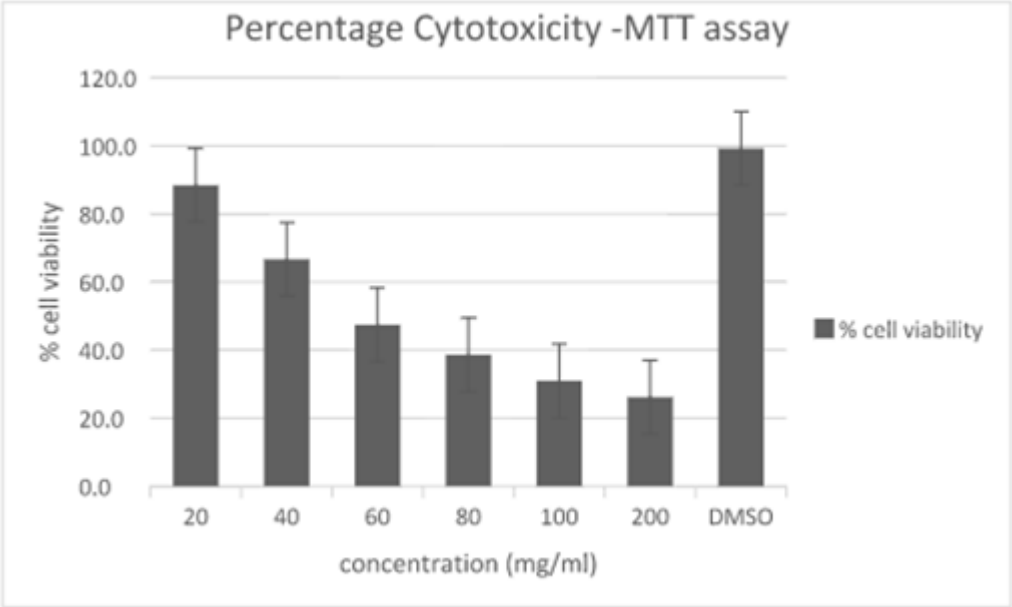

Figure 3
Percentage cytotoxicity expressed as % cell viability

DISCUSSION

Mutagens identified via Ames test are also possible carcinogens, and early studies by Ames showed that 90% of known carcinogens may be identified via this test. Later studies however showed identification of 50–70% of known carcinogens. One interesting result from the Ames test is that the dose response curve using varying concentrations of chemical is almost linear, indicating that there is no threshold concentration for mutagenesis. It therefore suggests that, as with radiations, there may be no threshold for chemical mutagens or carcinogens. However some proposed that organisms can tolerate low level of mutagens due to protective mechanisms such as DNA repair, and threshold may exist for certain chemical mutagens. The Ames test is often used as one of the initial screens for potential drugs to weed out possible carcinogens, and it is one of the eight tests required under the Pesticide Act (USA) and one of six tests required under the Toxic Substances Control Act (USA). Based on the above results, it is concluded that the sample (mobile phone head speaker) is partially mutagenic at the dose levels ranging from 313 to 5000 µg/plate by the Ames bacterial reverse mutation assay in the absence of S9 mix under the conditions of the test employed. MTT assays are well known to study chemo sensitivity or toxicity of drugs in human tumor cell lines. The assay is less common to study survival of cancer cells after irradiation, in particular when the MTT assay is performed for studying proliferation of treated cells. Based on the above results ,the cells treated with various concentrations of sample(mobile phone head speaker) (20, 40, 60, 80, 100 and 200µg/ml) for 24 hr, a significant concentration-dependent inhibition of the viability of HepG2 cells was detected. The 50% of cell death were detected at 40 (66.7%)- 60µg/ml(47.4%) and cytotoxicity of 73.78% cell death were detected at 200µg/ml (26.22%). No significant change in cell viability was observed in DMSO treated group when compared with control.

CONCLUSION

The mutagenicity assay was performed with five dose levels (0.625, 1.25, 2.5 and 5.0 mg/ml) in the absence of metabolic activation system. From the result, it is concluded that sample(mobile phone head speaker) shows partial mutagenicity via Ames test.The average

revertant colonies were found to be within the acceptance limits of the spontaneous revertant control values of respective *Salmonella* strain. For MTT assay,When cells were treated with various concentrations of sample (mobile phone head speaker) (5, 2.5, 1.25, 0.625mg/ml) for 24 hr, a significant concentration-dependent inhibition of the viability of HepG2 cells was detected. The 70% inhibition were detected at 0.625mg/ml(73.73737%).

CONFLICT OF INTEREST

Conflict of interest declared none.

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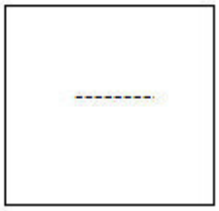

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