



Bharath

INSTITUTE OF HIGHER EDUCATION AND RESEARCH

(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)



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Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-05

Date: 29.08.2019

TO

Mr. B. Rajesh
Professor/Anatomy,
BIHER.



Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Assessment of DNA Damage in Chick Embryo Brains Exposed to 2G and 3G Cell Phone Radiation using Alkaline Comet Assay Technique

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 22.08.2019

Payment details:

Voucher No.60

Dated: 03.09.2019

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 03/09/2019

V.No. 60

Debit _____ Amount _____

Rs. 1,00,000/-

PAID TO Dr. B. Rajesh

RUPEES One Lakh Only

TOWARDS Seed Money Scheme - 2018



Authorised by [Signature]

Finance Manager

Cashier/Accountant



Payee's Signature

PROPOSAL SUBMISSION

1. Details of Principal Investigator

Name : Dr. B Rajesh
Designation : Professor
Highest Qualifications : Ph.D.
Department : Anatomy
E-mail : anat_rajesh@rediffmail.com
Contact no : 9345575143
Date of Joining : 14.06.2010

2. Details of Principal Investigator

Name : N.Anandaramajayan
Designation : Assistant Professor
Highest Qualifications : M.Sc.,
Department : Anatomy
E-mail : anandaraman2006@gmail.com
Contact no : 9894343517
Date of Joining : 01-12-2010

Technical details

1. Introduction:

The 21st century has seen tremendous development in various fields of science and technology. With new inventions and devices that produce man-made electromagnetic fields, we have interfered too much with the natural environment causing unintended and undesirable negative impacts on the environment and living beings [1]. These inventions include cellular phones/mobile phones, wireless Local Area Network (wireless LANs), Bluetooth, Digital Enhanced Cordless Telecommunication (DECT), Ultra-Wide Band technology (UWB technology) and Wireless Power Transmission (WPT) from Solar Power Satellites (SPS). Among all these devices that emit man-made electromagnetic fields, the cellular mobile phone industry has undergone tremendous growth and development since its inception in Europe in early 1980's. According to latest Groupe Speciale Mobile Association (GSMA) intelligence report, there are 5.16 billion cell phone users in the world with the number of smartphone users are increasing at a rate of 8% annually [2].

The cellular phone services require radiofrequency fields or high frequency electromagnetic fields which are a part of electromagnetic spectrum for transmitting and receiving signals. These fields cause the free radicals to stay longer within the cells and also alter the integrity of plasma membrane [3]. DNA, which is the highly stable macromolecules of the cell, is continually damaged by various endogenous factors (free radicals) and exogenous factors (UV rays, ionising and non-ionising radiation, chemicals and so on). The damaged DNA is usually repaired by DNA repair enzymes [4]. Any imbalance in DNA damage and its repair mechanisms or mistakes during repair may result in accumulation of damaged DNA resulting in cell death [5], ageing of the cell [6] or cancer [7]. The most common types of DNA damage are DNA strand breaks - Single Strand Breaks (SSB), Double Strand Breaks (DSB) and DNA cross links [4]. Most SSB's are rapidly repaired with the intact strand serving as a template to direct rejoining process. On the other hand, DSB's are more lethal and they are believed to be irreparable [8].

Various reports are available on the deleterious effect of RFR on DNA molecules on both animal models and human beings. The exposure of different animal models to RFR resulted in increased DNA strand breaks and rearrangement of DNA segments in various tissues like testis [9], brain [10], lung cells [11], embryonic stem cells [12] eyes [13] and liver [14]. Studies on various human cell cultures to RFR exposure have also shown an increased DNA damage-SSBs and DSBs. The RFR exposure ranging from 900-1800 MHz resulted in irreversible DNA damage in Human Lens Epithelial Cells (HLEC) [15,16], repairable DNA damage in HLECs [16,17], human fibroblasts [18], human lymphocytes [19], human hair root cells [20]. However, some studies have shown contradictory results in DNA damage on exposure to RFR fields in both animal models and human beings. No significant DNA damage was observed in murine C3H10T1/2 fibroblasts [21], Molt-4 cells [22], rat brain cells [23], spermatozoa of mouse caudal epididymis [24] and human lymphocyte cultures [25].

The various inconclusive controversial scientific reports and the rapid proliferation of cell phone industry going for a higher version of generation cell phones and their possible health impacts on the public has prompted us to undertake this research study. The present study was designed to evaluate the possible DNA damage to RFR exposure from 2G and 3G cell phone on developing and differentiating brain of the chick embryos using comet assay technique.

2. Review of status of Research and Development in the subject

Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. De Iuliis GN, Newey RJ, King BV, Aitken RJ PLoS One. 2009 Jul 31; 4(7):e6446.

The past decade has seen tremendous development in the wireless telecommunications industry with the introduction of cellular phones (cell phones/mobile phones). The development has revolutionized the telecom industry by making telecommunication faster, economical and more convenient [1]. With the introduction of new applications and multifunctional features in the mobile hand set, the telecom industry is wooing both young and old generation. Pulsed electromagnetic radiations are created when the cell phone is in operation [2] most of which are absorbed by body surface particularly the head region. This absorbed energy causes both thermal and non-thermal stress in the body [3, 4]. Non-thermal stress is more deleterious than thermal stress and is known to cause oxidative stress [5], production of free radicals [6], structural changes in plasma membrane [7], changes in ionic transport [8] and also increased DNA damage [9].

2.1. International Status:

Several scientific reports are available to establish the role of RFR emitted from cell phones in causing DNA damage in various biological tissues in both human and animal models [23-26]. The long term exposure resulted in increased DNA strand breaks, Single Strand Breaks (SSB's) and Double Strand Breaks (DSB's), and rearrangement of DNA segments in testis [23], brain [24] and eyes [25] of different animal models and also in human lymphocyte culture [26]. However, various authors reported no significant DNA damage in different tissues of various animal models [27] and in human lymphocyte culture [28].

2.2. National Status:

NIL

3. Progress/ achievement so far, if any

- a) Reference papers was collected.
- b) Literature survey was studied.
- c). Materials and methods were designed

4. Work plan

4.1 Methodology

The present work was an experimental study that has been carried out during the year August 2011-June 2015 and was designed according to the Ethical Guidelines for care and use of experimental animals. The protocol was approved by Institutional Animal Ethical Committee (IAEC). Fresh fertile hen eggs (*Gallus domesticus*) were procured from Rajiv Gandhi College of Veterinary and Animal Sciences, Puducherry, India. The eggs having approximately similar weight ($65-70\pm 5\text{gm}$) were selected for incubation in one particular batch. The eggs were incubated in six batches of 12 eggs each (total- 72 eggs) in a standard egg incubator at $37\pm 0.5^\circ\text{c}$ and 50-55% of humidity and ventilation. The eggs were rotated manually two times a day along the longitudinal and vertical axis and checked with a Candler for the viability of embryos. All live and healthy looking embryos showing normal curvature were included in the study. Dead embryos, embryos showing congenital anomalies and embryos without normal curvature were excluded from the study.

The first two batches of eggs ($2\times 12=24$ eggs) were grouped as sham exposed group (Group-C) and the eggs were incubated along with a popular brand cell phone with the Specific Absorption Rate (SAR) of 0.310 watts/kilogram hung from above with 5 cm distance separating the egg and kept in null status (switched off). Next two batches of fertilised eggs ($2\times 12=24$ eggs) were exposed to 2G cell phone radiation (Group-A) and last two batches of eggs ($2\times 12=24$ eggs) were exposed to 3G cell phone radiation (Group-B). They were incubated with the cell phone switched on and kept in silent mode. The head phone was plugged in to ensure the automatic activation of cell phone whenever it received a call and a radiofrequency meter (RF meter, Less EMF Inc, USA) was used to measure the intensity of radio frequency waves [Table/Fig-1]. In the present study, same cell phone hand set and service provider were used for network connection for all the three groups. The first exposure was initiated at the 12th hour of incubation at 4.30 AM for 3 minutes duration period. Thereafter, on every half an hour lapse, the cell phone was rung for duration of three minutes each till 4.30 PM. Thus the embryos were exposed for 72 minutes duration over a 12 hour period (4.30am-4.30pm) followed by 12 hour of exposure-free period in a day. This was repeated regularly on subsequent days up to 12th day of incubation.

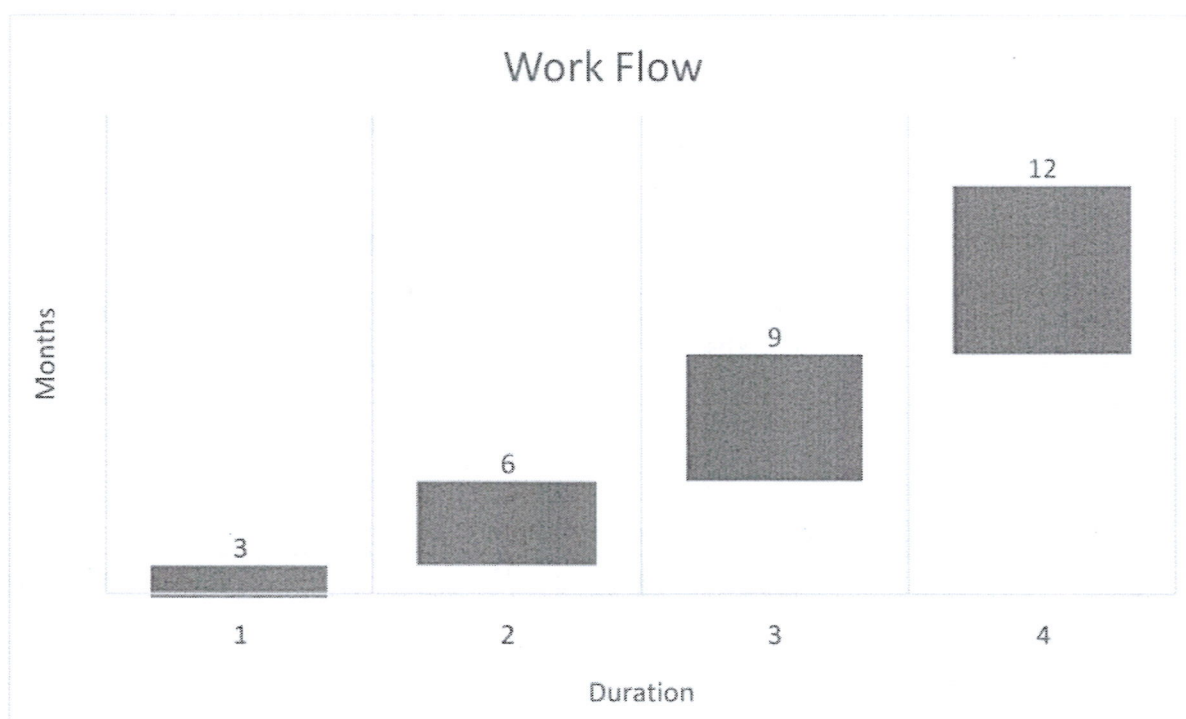
Three embryos per day were terminated from 9th day to 12th day. The brain were dissected out and minced in Hanks Balanced Salt Solution (HBSS w/ Phenol Red w/o ca and mg, Cat.No.55021C). The slides were stained with silver nitrate with modifications in staining procedure [27]. Randomly selected 100 cells from the brain were then analysed using automated comet scoring software (Comet Score IV) to assess and quantify the levels of DNA damage in control group and both the experimental groups.

STATISTICAL ANALYSIS

The mean comet length, the mean tail length, mean percent of DNA in the tail and mean tail moment of all three groups were statistically compared using one-way ANOVA with Graph Pad Instat 3 and the significance was determined using a "Tukey's post-hoc" with $p < 0.05$ for statistical significance. All the data were expressed as Mean \pm SEM (Standard Error of Mean).

Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)

S. No	Activity/ mile stolen	1 st Year			
		1-3 month	4-6month	7-9 month	10-12
1	Literature review	1-3 month			
2	Analysis of existing work	-	4-6month		
3	Designing & work initiated	-	-	7-9 month	
4	Statistics & Discussion with results	-	-	-	10-12



4.2 Expected outcome within the time period of See Money Scheme

Based on the results from present study, it is concluded that the chronic exposure of developing chick embryos to RFR emitted from 2G and 3G cell phone resulted in DNA damage in the brain of both 2G and 3G group embryos with the damage more prominent in 3G group embryos. The DNA damage observed in the current study probably would have resulted from increased free radical production or due to structural alteration in DNA repair enzymes. Thus by increasing the production of free radicals the external electromagnetic field produced from cell phones/mobile phones would have interacted with the internal biological processes of a cell, initiating a chain of reactions causing DNA damage.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography: Nil

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
	NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
	NA	NA NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (issue)	Page no.	Year
1.	M. Senthil Murugan ^{1,2*} , Rajesh Bhargavan ³	Extrinsic factors in regulation of adult hippocampal neurogenesis	Drug Invention Today	13(4)	623-627	2020
2.	Hena Chandran, Jayanthi K., Prabavathy S., Renuka K. and Rajesh Bhargavan	Effectiveness of video assisted teaching on knowledge, attitude and practice among primary caregivers of children with Autism Spectrum Disorder	Advances in Autism	8(6)	10-15	2019
3.	Arivalagan Arunkumar, Bhargavan Rajesh , V. Tamilalagan	Variations among foramen transversarium in cervical vertebrae and its clinical significance	Indian Journal of Anatomy	7(2)	144-148	2018
4.	Mary Hydrina D'Silva ¹ , Rijied Thompson Swer ² , J. Anbalagan ³ , Bhargavan Rajesh ⁴	Effect of Radiofrequency Radiation Emitted from 2G and 3G Cell Phone on Developing Liver of Chick Embryo – A Comparative Study	Journal of Clinical and Diagnostic Research	11(7)	AC05 - AC09	2017
5.	M. Senthil Murugan, ^{1,*} R. Sudha, ¹ and Rajesh Bhargavan ²	Clinical Significance of an Unusual	Sultan Qaboos	16(4)	e491–494,	2016

		Variation Anomalous additional belly of the sternothyroid muscle	University Med J,			
6.	Mary Hydrina D'Silva, ¹ Rijied Thompson Swer, ¹ J. Anbalagan, ¹ and Rajesh Bhargavan²	Effect of Ultrahigh Frequency Radiation Emitted from 2G Cell Phone on Developing Lens of Chick Embryo: A Histological Study	Advances in Anatomy	10(2)	1-9	2014
7.	Vasudev Anand Rao, Subashini Kaliaperumal, Thanikachalam Subramanyan, Kotapalli Rachandra Rao, Rajesh Bhargavan	Goldenhar's sequence with associated juvenile Glaucoma in turner's syndrome	Indian Journal Of Ophthalmology	53(4)	267-268	2005

9. Budget

SI. No	Head	Amount in INR
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50000
2	Consumables (gels bottles, cotton, spirit, testing charges, tools, etc.)	5000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Other's consumables	10000
	Total	1,00,000

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

<p>1. Dr. M. Sivakumar Professor Dept. of Anatomy JIPMER, Puducherry Mobile No: 9994264019 E-mail id: sivakumar96@yahoo.com</p>	<p>2. Dr. J Anbalagan Professor of Anatomy Mahatma Gandhi Medical College and Research Institute, Pondicherry Mobile No: 9443500366 E-mail id: jayaramanbalagan@gmail.com</p>
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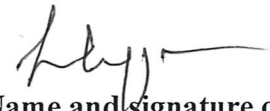
CERTIFICATE FROM THE INVESTIGATOR

Project Title: Assessment of DNA Damage in Chick Embryo Brains Exposed to 2G and 3G Cell Phone Radiation using Alkaline Comet Assay Technique

It is certified that


1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.


Name and signature of
Principal Investigator



Name and signature of
Co-Principal Investigator

Date: 29.07.2019

Place: Pondicherry

Forwarded by 
Head of the Department

Signature of the Head


DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	Dr. B Rajesh
Name of the Co-Principal Investigator	N.Anandaramajayan
Name of the Department	Anatomy
Title of project	Assessment of DNA Damage in Chick Embryo Brains Exposed to 2G and 3G Cell Phone Radiation using Alkaline Comet Assay Technique
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 1,00,000/-

SI. No.	Head	Amount
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50000
2	Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc.	5000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	1,00,000

Name and Signature of the Research Advisory Committee members with date




(Dr. G. Jayalakshmi)