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The Effect of Exposure of EMF Radiations from Cell Phones on Percentage of Glucose, Cholesterol and Protein in Developing Chick Embryos

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Abstract Electromagnetic field radiations have influenced the range of bodily functions. EMF had put a major public concern due to its widespread applications and capability of producing deleterious effects. The present study is aimed to evaluate the changes in percentage of glucose, cholesterol and protein in chick embryo tissues following exposure to EMF radiations emitted from a cell phone. Fertile hen eggs of RIR species (*Rhode Island Red)* were incubated in two groups in standard egg incubators (group A=16) (group B=16). Group A serves as control while Group B would be experimental and exposed to radiations emitted from a cell phone. On completion of 7^{th} and 14^{th} day of incubation, the embryos were sacrificed; tissues were dissected, centrifuged and samples thus obtained after centrifugation was taken and estimations of glucose, cholesterol and protein were performed. Glucose estimations had done by O-toluidine method. Cholesterol estimations were performed by Liebermann-Burchard method and protein conc. were estimated by Biuret method. Student's t-test had been applied to check the statistical significance among Group A and Group B. Exposure of EMF from cell phone didn't have considerable effect on cholesterol percentage on 7^{th} day of incubation and on glucose percentage on 7^{th} as well as 14^{th} day of incubation in chick embryo tissues. In the present study, cholesterol percentage on 14^{th} day of incubation ($P = .001$) and protein concentration on 7^{th} and 14^{th} day of incubation ($P < .001$) showed significant changes among group A and group B. Decrease in the percentage of cholesterol and protein showed that EMF radiations emitted from cell phone might affect metabolism directly or indirectly and thus it might be a factor that is responsible for increased mortality in chick embryos.

Keywords: *EMF, cell phone, exposure, chick embryo, incubation*

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1. Introduction

In day-to-day life, we use technology to make our lives simply better. This technology brings electrification with it. The electrification is based on the principle of electromagnetic radiations. There are so many electrical appliances that operate on electromagnetic radiations to perform their action. These electromagnetic radiations are of non-ionizing type and they are categorised into Extremely low frequency electromagnetic field (ELF) that covers the frequency range of 3Hz to 3 KHz and radiofrequency radiations (RFR) that covers frequency range from 10 KHz to 300 GHz. various applications uses different frequencies of EMF radiations. Cell phone is among the basic need of human's life. The cell phone technology uses frequencies of up to 800 MHz to 3 GHz [1]. The constantly increased use of cell phones from last several years put a major public concern about the potential risk associated with it.

The World Health Organisation stated that "Cell phone radiations can possibly carcinogenic to humans" as it may represent a long term health risk and classified the cell phone in category 2B which ranks it alongside coffee and other possibly carcinogenic substances [2]. Numerous studies suggests that electromagnetic field affects on biological systems [3], it is associated with increased risk of childhood leukaemia, brain tumours, neurological effects, neurodegenerative diseases, breast cancer, miscarriage and some cardiovascular effects [4]. EMF had shown to interact with the biomolecular system by amplifying initial weak signals associated with binding of antibodies, neurotransmitters and hormones to their specific binding sites [5], it might have the capacity to alter cell structure from plasma membrane to different biomolecules present within a cell that might cause genotoxicity [6]. A study on effects of EMF exposure on birds had shown that there were behavioural, physiological changes, increased oxidative stress, changes in immune and endocrine functions and had an effect on growth and development in birds that were exposed to EMF from power lines [7]. It

was found that EMF might induced stress related behaviour and it might be responsible for elevated levels of hormone ACTH in male *wistar* rats [8]. As a result of EMF exposure, there were decrease in organ weight in newly hatched chicks [9], irreversible developmental alteration and external malformations [10], as well as high mortality and developmental disorders had been seen in chick embryos [11].

Conversely no any harmful effects were reported by others. EMF found to stimulate proliferation and differentiation of embryonic cells [12]. A scientific study on cultured human lymphocytes suggests that there were no genotoxic effects had been found after exposure of lymphocytes with EMF radiations [13]. While some researches had shown that low frequency pulsed Electromagnetic field could be beneficial for the treatment of varieties of musculoskeletal disorders [14], exerts anti-inflammatory action, alleviates pain in arthritis condition and helps in bone remodelling [15].

Considering all these reviews, the present work had been taken to investigate any changes caused by EMF exposure on biochemical measures such as percentage of glucose, cholesterol, and protein in chick embryos. Glucose plays an essential role of providing energy to the body. Cholesterol is the steroid lipid found in the plasma membranes of all tissues in the body and is responsible for several vital biochemical processes such as regulating immunity and defence mechanism, transportation of lipid and other vital fats to body and provides protection to arteries, veins and muscles of the body. Proteins are building blocks of body and it plays an essential role in normal growth and development of an individual and cellular repair. All these biomolecules plays an essential role in metabolism. Hence the study had been carried out to observe changes if any, and either it would be beneficial or hazardous. For that purpose, chick embryo is used as an experimental model. Various researchers demonstrated that chick embryo as a role model for different studies as its development is external hence embryos were not compromised by changes in mother's biological systems [16,17,18].

2. Materials and Method

Two separate electrical incubators were used to kept control and experimental eggs. The incubators were sterilized with 70% ethanol before keeping eggs in incubators. The temperature 37.5□ and humidity 50±5 % was maintained till the 21 days of incubation.

Freshly laid fertile hen eggs of RIR species (*Rhode Island Red*) were obtained from Government Poultry Farm, Camp road, Amravati. The eggs were incubated in two batches. Each batch comprise of 32 eggs, out of which 16 eggs were incubated in standard egg incubator. They were treated as control group (Group A). The other 16 eggs were treated as experimental group (Group B). These experimental eggs were kept in a separate incubator where there had an arrangement of mounting cell phone on top of the egg tray. The approximate distance between the cell phone and eggs kept in the centre was 5.8 cm. Before keeping Group A and Group B eggs in incubators, they were weighed and numbered. The mean egg weight was 50.6 gm (Figure 1).

Figure 1. Showing Arrangement of eggs for Group A and Group B respectively in an incubator with normal incubating temperature and humidity

Figure 2. Showing Candling of eggs on 7th and 14th day of incubation respectively

During the period of incubation, all the eggs from both the groups were rotated manually in 45° angle at least thrice or four times a day. The egg candling takes place from 4th day of incubation so that growth of embryos within egg could be observed. The egg candling is the process of observing appearance and growth of an embryo inside the egg without breaking egg shell (Figure 2).

A popular brand cell phone with a frequency bandwidth of 900-1900 MHz and SAR value of 1.11W/kg. in head and 0.22W/kg in body measured by FCC was used for the present work to expose the experimental embryos. The SAR value (Specific Absorption Rate) is the measure at which energy is absorbed by tissues when exposed to Radiofrequency EMF radiations. The cell phone was placed in incubator continuously up to 21 days, in silent mode, rang up to four times for 15 minutes each daily from another cell phone with time interval of one hour.

On the 7thand 14th day of incubation, 6 eggs each from Group A as well as Group B were sacrificed. After breaking of egg shell, air sac had been removed and then embryo got extracted from egg. The mortality of all embryos from Group A and Group B was observed. Also morphological anomalies were seen if present. The extracted live embryos were kept in 0.9% saline solution to clean it then the embryos were transferred in a clear solution of 0.9% saline. The weight and length of all the embryos sacrificed were measured and all the embryos were photographed. All the embryos from Group A and Group B were dissected and the tissues of embryos were taken out and homogenized in mortar pestle with the addition of saline in it. The solution thus obtained after homogenization of each embryo tissue had been centrifuged in 8000 R.P.M. for 10 min. The supernatant thus obtained after centrifugation, serves as a test sample from each embryo for the estimation of glucose, cholesterol and protein concentration in the tissues.

2.1. Estimation of Glucose

Glucose estimation was done by O-Toluidine method. For glucose analysis from sample, O-Toluidine reagent had been used. In each test tube, 5 ml of O-Toluidine reagent had been taken. In the test tube marked as blank, 0.1 ml of distilled water was added. In the test tube marked as standard, 0.1 ml of glucose working standard (1 ml. of stock standard in 9 ml benzoic acid saturated.) was added which was prepared from glucose stock standard solution (glucose 1 gm., benzoic acid 250 mg. in 100 ml of distilled water) and in test tubes mark as test, 0.1 ml of test sample were added from each sample tube.

All the test tubes then kept in boiling water bath exactly for 8 minutes. After 8 min. all the test tubes were moved from water bath and kept in cold water in a beaker. Optical density was measured in 630 nm in UV spectrophotometer with blank set as zero. Calculations were done for percentage of glucose per 100 ml.

Formula used to calculate the percentage of glucose in tissues:

Optical density of test/optical density of standard × 100.

2.2. Estimation of Cholesterol

Cholesterol estimation was done by Liebermann Burchard method. For analysis of Cholesterol from tissues, glacial acetic acid Aldehyde free was used. In test tube marked as Blank, 6ml of glacial acetic acid had been added then 0.1 ml of distilled water was added followed by 4 ml colour reagent (10% FeCl3 0.5ml was taken in 50 ml measuring cylinder and volume was make up with H2SO4 to the mark).

In test tube marked as standard, 5 ml of glacial acetic acid aldehyde free was taken. 1 ml of cholesterol working standard (10 ml of cholesterol stock standard in 50 ml glacial acetic acid extra pure)was added in test tube which was prepared from cholesterol stock standard solution (1mg cholesterol in 1 ml distilled water)followed by 4 ml of colour reagent.

In test tubes marked with test, 5 ml of acetic acid aldehyde free was taken. 0.1 ml of test sample was added from each sample tube followed by 4 ml of colour reagent in each test tube. All the test tubes were allowed to cool at room temperature. Absorbance was measured in 630 nm in UV Spectrophotometer against the reagent Blank. Blank was set as zero. Calculations were done for percentage of cholesterol present per 100 ml.

Formula used to calculate the percentage of cholesterol in tissues:

Optical density of test/optical density of standard × 0.2×100/0.1.

2.3. Estimation of Protein

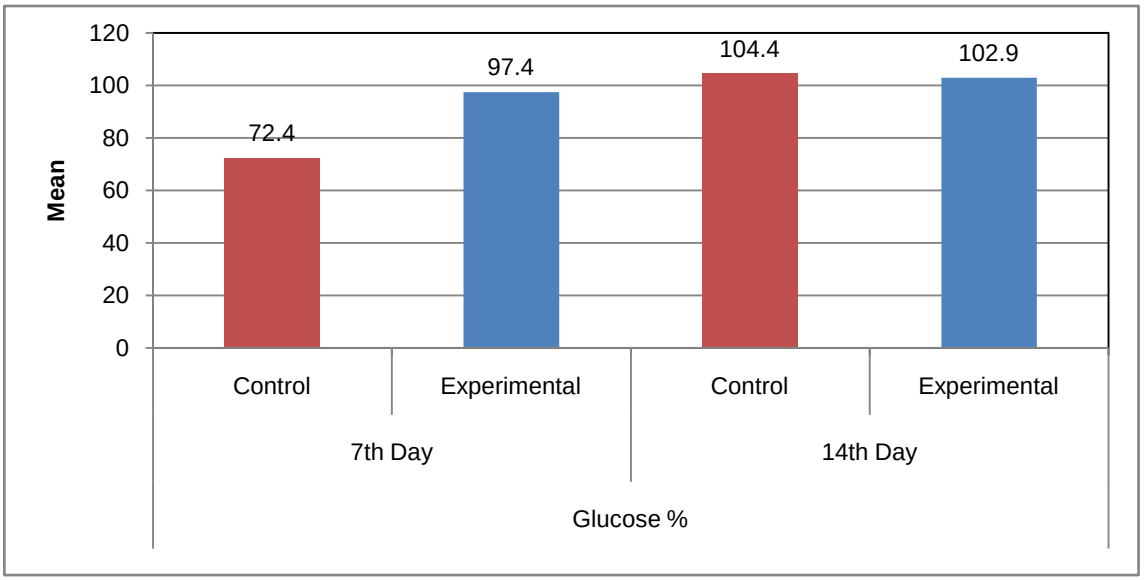
Protein estimation was done by Biuret method. To each of the test tubes, we have added 5 ml of biuret reagent. To the test tube mark as blank, 1 ml of distilled water was added. To the test tube mark as standard, 1 ml of protein reference standard was added. To each test tube marked as test, 1ml each test sample were added in biuret reagent. Absorbance was measured in 540 nm in UV Spectrophotometer with Blank set as zero. Absorbance for various samples were recorded.

2.4. Statistical Analysis

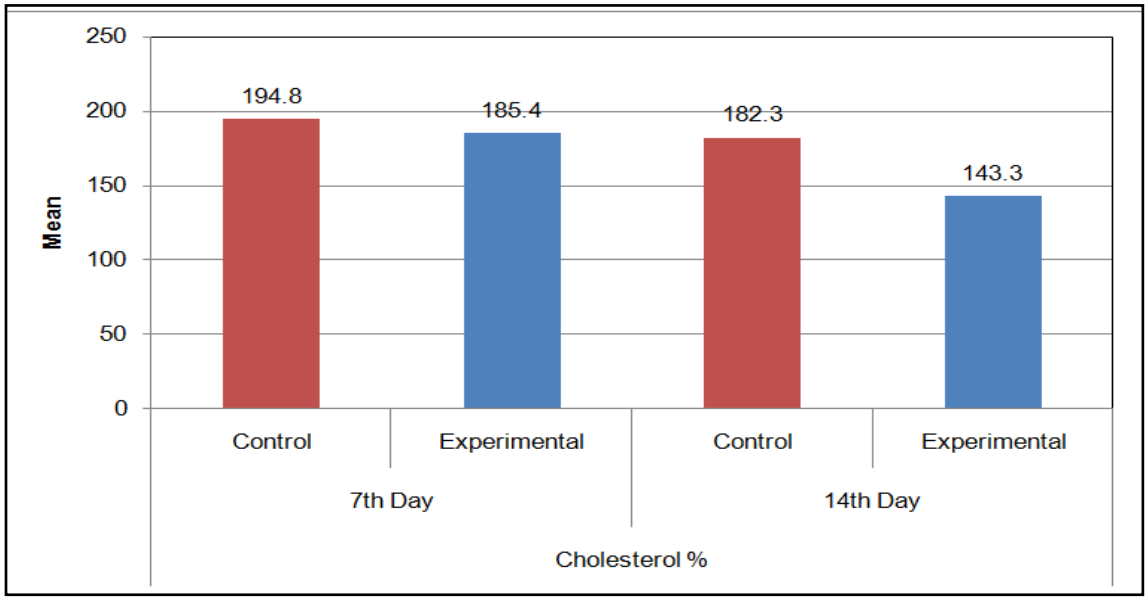
Collected data were analysed and Student's t-Test (paired) was applied to find out level of significance among means of Group A and Group B. Significance was set at $p < 0.05$.

2.5. Observations

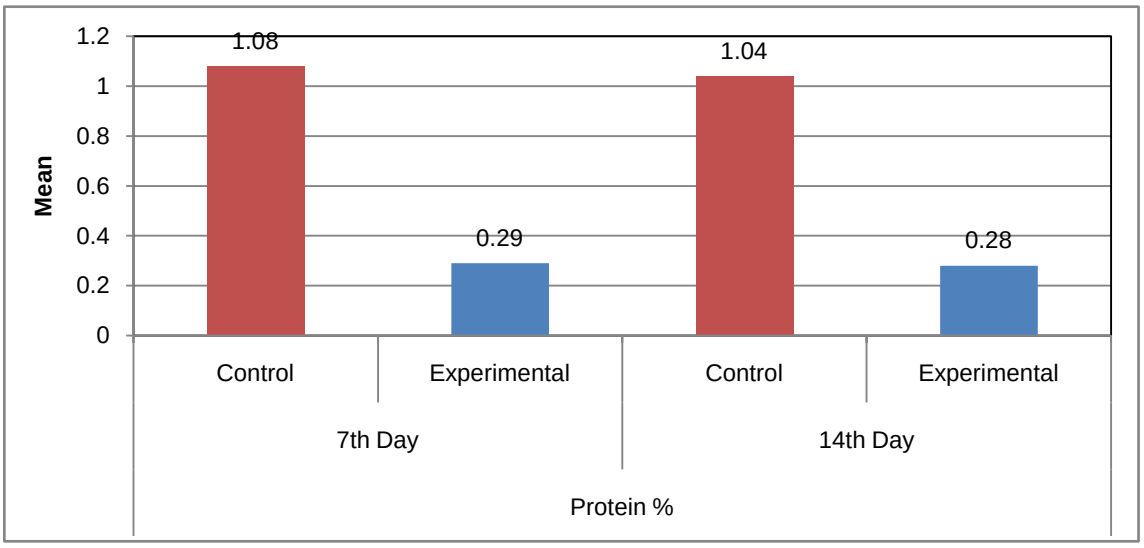
Graphs were plotted against percentage of glucose (Graph 1), cholesterol (Graph 2) and protein (Graph 3) on X-axis and calculated means for each on Y-axis.



Graph 1. Percentage of glucose on 7th day and 14th day of incubation in Control and Experimental group embryos after EMF exposure



Graph 2. Percentage of Cholesterol in tissues on 7th day and 14th day of incubation in Control and Experimental group embryos after EMF exposure



Graph 3. Concentration of protein in tissues on 7th day and 14th day of incubation in Control and Experimental group embryos after EMF exposure

Table 1. Showing t-Test, values are ± SD taken from control and experimental group, p-value of samples and interpretation of p-value indicating statistical significance

Parameter	Day	Group	Mean	±SD	Minimum	Maximum	t value	P Value	Interpretation of P Value
Glucose %	7th Day	Control	72.4	±45.4	5.9	108.0	-1.208	.294	Not Significant
		Experimental	97.4	±3.6	92.2	102.0			
	14th Day	Control	104.4	±12.2	96.1	125.5	0.890	.424	Not Significant
		Experimental	102.9	±10.0	94.1	119.6			
Cholesterol %	7th Day	Control	194.8	±16.2	173.5	213.3	0.865	.436	Not Significant
		Experimental	185.4	±12.9	165.3	200.0			
	14th Day	Control	182.3	±6.4	173.9	191.6	8.909	.001	Significant
		Experimental	143.3	±3.9	138.7	149.3			
Protein %	7th Day	Control	1.08	±0.08	1.00	1.20	23.729	.000	Significant
		Experimental	0.29	±0.05	0.20	0.35			
	14th Day	Control	1.04	±0.11	0.90	1.20	12.483	.000	Significant
		Experimental	0.28	±0.03	0.25	0.30			

3. Results

In day-to-day life, we use many electrical appliances that operate on electromagnetic radiations. Numerous studies suggests that electromagnetic field affects on biological systems [3], which is associated with increased risk of childhood leukaemia, brain tumours, neurological effects, neurodegenerative diseases, breast cancer, miscarriage and some cardiovascular effects [4]. EMF had shown to interact with the biomolecular system by amplifying initial weak signals associated with binding of antibodies, neurotransmitters and hormones to their specific binding sites [5], it might have the capacity to alter cell structure from plasma membrane to different biomolecules present within a cell that might cause genotoxicity [6]. As a result of EMF exposure, there were decrease in organ weight in newly hatched chicks [9], irreversible developmental alteration and external malformations [10], as well as high mortality and developmental disorders had been seen in chick embryos [11].

Considering all these reviews, the present work had been taken to investigate any changes caused by EMF exposure on biochemical measures such as percentage of glucose, cholesterol, and protein in developing chick embryos. All these biomolecules plays an essential role in metabolism. Hence the study had been carried out to observe changes if any, and either it would be beneficial or hazardous. For that purpose, chick embryo is used as an experimental model.

It had been found that there were increased rate of mortality in experimental embryos (Group B) as compared to control embryos (Group A). The morphological anomalies like deformation of limbs, beak were also seen in Group B. There were no significant differences found in percentage of glucose in tissues on both 7^{th} and 14^{th} day of incubation while percentage of cholesterol on 7^{th} day of incubation is statistically not significant but on 14^{th} day of incubation, there were decrease in cholesterol percentage in experimental group was found (Table 1). It means that there were significant differences had been found for cholesterol percentage in tissues amongst control and experimental group on 14^{th} day of incubation ($P = .001$). The percentage of protein in tissues had been decreased in experimental embryos on both 7^{th} and 14^{th} day of incubation and highly significant differences had been found between control and experimental group ($P < .001$).

4. Conclusion and Discussion

Electromagnetic fields were, are and will be a very essential part of our life due to modern technological advances. The hazardous or beneficial effect of EMF radiation on living being is a topic of so many earlier researches [19].

There are many studies related to electromagnetic field and its impact on biosystem and ecosystem [20,21]. The present study on exposure of EMF radiations emitted from cell phone on chick embryos showed that there were increased mortality rate in experimental group, changes in the concentration of cholesterol and protein in chick embryo tissues. Some findings from our results also observed in other studies. A study on male *Wistar* rats concluded that there was reduction in the level of lipid peroxidise, Glutathione reductase and total cholesterol in different tissues of rats exposed to EMF base station [22], this study supports our obtained results. A study by [23] on Syrian Hamsters showed that cell phone radiations might be responsible for decrease in plasma cholesterol and triglycerides concentration in rodents for long term. In our study, there was decreased protein concentration in chick embryo tissues. One of the study also showed that there was decrease in total protein concentration in Swiss albino mice after exposed to 10 GHz microwaves [24]. Exposure to ELF-EMF decreases the total cholesterol of the liver in rats [25]. A study on effects of exposure of alternating magnetic field on rats concluded that there was decrease in level of total cholesterol in blood plasma of rats and showed that magnetic field might affect on hormonal system and slowed down metabolism [26]. [27] demonstrated that total cholesterol and triacylglycerols levels had been reduced in rats due to total body exposure to radiations. It had been found that EMF radiations might have effects on cholesterol in the biological membranes and it might have significant consequences for the structural and functional properties of cells [28]. Finally,

these all observations are consistent with an idea that, EMF radiations affects many biological systems by interacting with internal electrochemical environment of body and caused harmful as well as deleterious effects.

It had been concluded that EMF exposure from cell phone on chick embryos might increase mortality rate in chick embryos during incubation as well as it might be responsible for reduced level of cholesterol and protein in chick embryos. The alteration in concentration of cholesterol and protein suggest that there might be changes in metabolism. It would be suggested that use of cell phones during pregnancy should be minimized as it could affect the developing foetus.

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