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Impact Of Cell Phone Radiations On Pituitary Gland And Biochemical Parameters In Albino Rat

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ABSTRACT

The present study was carried out to investigate the possible effects of non-ionizing electromagnetic radiations (EMR) of cell phone on hypothalamo-hypophysial axis followed by changes in reproductive parameters of albino rats. Animals were placed in polypropylene cages and exposed to EMR from cell phone at a specific absorption rate (1.25 watt/kg) for a period of 2 months (at 15 cm). Power density (PD) was detected at the distance of 15 cm during off call (6.28v/m) and on call (20.06v/m). Animals were sacrificed for histological and biochemical studies. Biochemical parameters such as total proteins, ALT, AST significantly increased in exposed rats as compared to control rats. The levels of LH and FSH decreased significantly in males. In females, the level of estrogen, LH and FSH decreased significantly however the level of progesterone increased significantly. Significant decrease in sperm concentration, sperm motility was observed in exposed male rats. Alteration in sperm morphology was also observed. Follicular atresia was higher in ovaries of exposed female rats. Histological examination revealed alteration in pituitary gland and reproductive organs of male and female rats. It was concluded that electromagnetic radiations of mobile phone have harmful effect on hypothalamus, pituitary gland and reproductive system of male and female rats. These effects increased with duration of exposure of radiations.

INTRODUCTION

Due to the development of new technologies the number of appliances,TV sets, mobile phones, computers and so on which emit electromagnetic radiations has increased.In 1995, mobile services were started in India. Mobile telephony industries are known to be fastest growing in the world. As per Telecom Regulatory Authority of India (TRAI 2012), 63.27% of urban people and 33.20% of rural peopleare using wireless forms of communication.People are exposed to continuous and low intensity radiations from the cell phone towersplaced randomly in densely populated areas. Epidemiological studies have shown that prolonged cell phone usage can cause ipsilateral brain tumour. Microwave radiation can alter brain antioxidant enzyme at 2.45GHz and 50 GHz, alteration in calcium of rat brain and increase in brain glial cells. Exposure of electromagnetic radiation to gonads leads to interference in the function of sex hormone. Studies on female rat gonads indicated that long term exposure to radiation changes reproductive endocrine systems, alters the structure of oocytes and reduces the rate of successful mating (Bahararaet al 2004).Several other studies confirmed that electromagnetic radiation increases the incidence of cancer and damage the DNA of sperm and brain cell (Paulrajet al 2006).People living within 100 meters of cell phone towers showed reduction in the production of many hormones such as ACTH from the pituitary gland and cortisol from the adrenal gland (Eskanderet al 2012). Adrenaline is a neurotransmitter, synthesized in adrenal medulla in response to signal from sympathetic nervous system. Overproduction of adrenaline can cause headache, tremors, high blood pressure, anxiety and inability to sleep. However, people living near cell phone towers had significantly increase in headache, tremors, dizziness and poor sleep (Abdel-Rassoulet al 2007).

MATERIALS AND METHODS

Procurement and maintenance of animals

Albino rats were procured from Department of Livestock Production and Management, Guru AngadDev Veterinary and Animal Sciences University (GADVASU) Ludhiana. The rats were maintained in laboratory under standard conditions providing them laboratory pelleted feed and water ad libitum. The rats were acclimatized to their new quarters for one week before starting the treatment. The experimental protocol met the national guidelines on proper care and use of animals in the laboratory research. The national Institutional Animal Ethics Committee (IAEC) approved this experimental protocol.

Exposure with electromagnetic radiations

Two groups (control and experimental groups) of rats were formed. Each group had 6 rats. Animals were housed collectively in polypropylene cages and exposed to EMR from cell phone at a specific absorption rate (SAR) 1.25 watt/kg (SAR at which energy is absorbed by the body). Power density (amount of power per unit volume) of this cell is measured by using the field strength meter (3 Axis field strength meter Waco 195). Standard pelleted diet was provided to the rats and water ad-libitum. The experimental rats were exposed to electromagnetic radiation for 2 hour in active and 30 minutes in inactive mode twice daily. Mobile phone was kept at a distance of 15 cm from the rats. The rats were exposed for a period of 60 days.

Histological Studies

Gonads (testis/ovary) and pituitary gland from brain of rats were cleared fromadhering tissues and representative samples were fixed in Bouin's fixative for 24 hours.

Tissue processing, sectioning and staining

Dehydration of the tissue was done in graded series of ethanol, cleared in xylene and embedded in paraffin wax (melting point between 58-60°C) after complete fixation. The 5-7 µm thick sections were cut for ovary, testes and pituitary gland with the help of microtomeafter usual de-waxing and rehydration in descending series of ethanol to water, the sections were stained in haematoxylin, counterstained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX.

Biochemical studies

Estimation of total soluble proteins

Total soluble proteins were estimated by method of Lowry et al (1951).

Estimation of alkaline phosphatase activity

Alkaline phosphatase (AKP) was estimated by the method of Besseyet al (1946).

Estimation of aminotransferases

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were estimated by colorimetric method of Reitman and Frankel (Bergmeyer1974).

Hormone Assay

Blood sample from each rat was collected by the use of heparinised syringe as described earlier It was transferred to heparinised vials and centrifuged at 2300 r.p.m for 15 minutes. Supernatant was obtained as plasma which was used for the determination of estrogen, progesterone, LH and FSH concentration using ELISA kits.

Statistical Analysis

Statistical comparisons for biochemical, hormones and cyclicity analysis were presented as mean ± standard error of mean. Comparisons were made between control rats, two month exposed rats using T-Test. A "P" value of 0.05 was selected as a criterion for statistically significant differences.

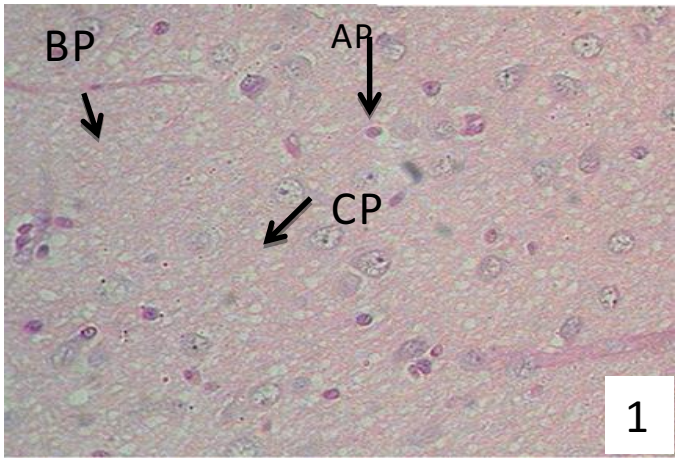
RESULTS AND DISCUSSION

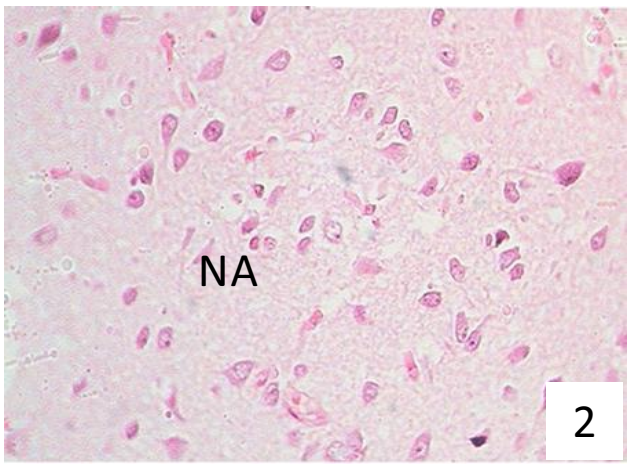
Histological studies in pituitary gland

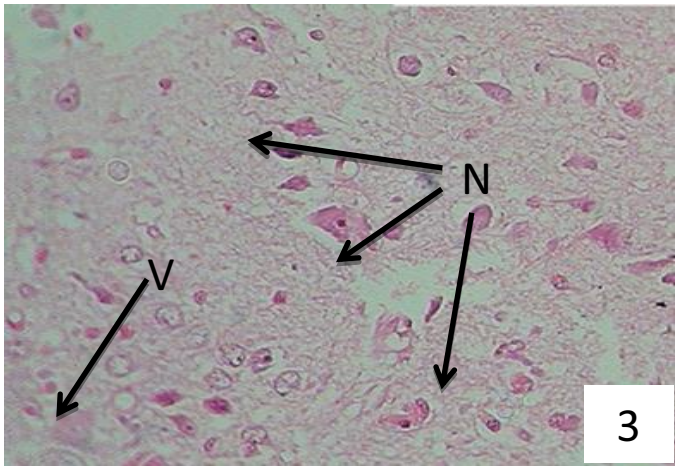
Pituitary gland is an endocrine gland, protrusion off from the hypoyhalamus. The anterior pituitary is a lobe of the gland that regulates several physiological processes, the intermediate lobe secretes and synthesizes melanocyte stimulating hormone. Hypothalamus secretes hormones which are responsible for regulation of hormone secretion from anterior pituitary. The posterior pituitary is a lobe of the gland that is functionally connected to the hypothalamus by the median eminence via a small tube called the pituitary stalk.

The micromorpholgy of anterior pituitary showed primarily three types of cells acidophils, basophilsand chromophobes. The acidophils were characterized by eosin-stain, basophils by haematoxylin and chromophobes with very little staining. The outline of the cells in pituitary of control rats depicted normal outline of the cells. However, in treated group although the majority of cells appeared to be as observed in control, some cells had an appearance of shrunken cells. In addition pyknotic nuclei were also observed in acidophils. Certain undifferentiated and unidentified cells were also observed.

Histological studies of control group of pituitary showing normal acidophils, basophils and chromophobe cells. Numerous acidophils were observed in control group of rats.Pituitary gland of exposed group of rats showing haemorrhage in acidophills, absence of chromohobe cells and necrosis.After exposure to electromagnetic radiation lipid peroxidation increased that damaged the hypothalamus and pituitary gland which leads to hypopituitarism (Brodskyet al 1996). Reduced level of FSH and LH may be due to malfunctioning of hypothalamus and pituitary gland.







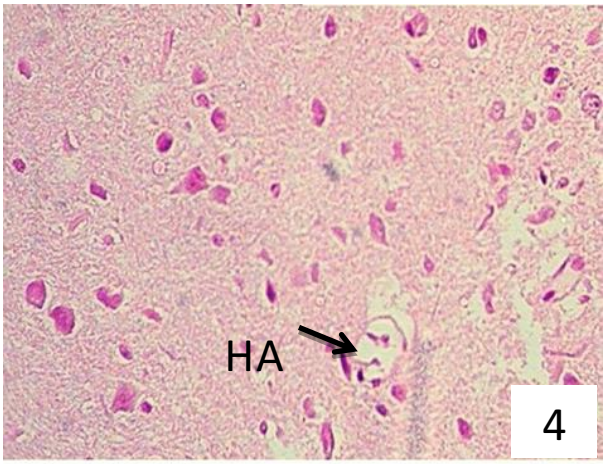


Fig 1:T.S. of pituitary gland of control rat showing numerous acidophils (AP), basophils (BP) and chromophobe (CP) (40x).
Fig 2: T.S. of pituitary gland of control rat showing numerous acidophils (NA) (40x).
Fig 3:T.S. of pituitary gland of treated rat showing necrosis (N) and vacuoles (V) has been observed (40x)
Fig 4:T.S. of pituitary gland of treated rat showing haemorrhage in acidophils (HA) and absence of chromophobe (AC) (40x).

Table 1: Effect of exposure of mobile phone radiation on concentration of proteins (g/dL of sample) in the various organs viz, plasma and testis of exposed and control male rats

Organ	Control	Exposed
Plasma	14.68±0.29	12.16±0.3*
Testes	3.49±0.01	2.61±0.06*

Values are Mean±SE
*Significance difference at ($p<0.05$) of control and treated rats

Biochemical estimation:

To observe the alterations at cellular level due to mobile phone radiations, biochemical estimation of vital molecules were done in male rats exposed to electromagnetic radiation for 60 days.

Proteins

Proteins were estimated in the plasma and testes of control and exposed male rats of EMR from mobile phones for two months and were expressed as g/dl .Results showed significant decrease in the level of protein in plasma of exposed group as compared to control group (Table 1, Fig.1)

Table 2: Effect of exposure of mobile phone radiations on concentrations of Acid phosphatise (ACP) alkaline phosphatse (AKP), Aspartate aminotransferase (AST) and Alanine amino tranferase (ALT) in the plasma of exposed and control male rat

Biochemical constituents	Control	Treatment
AST(IU/IT)	33.727±0.67	40.32±0.1*
ALT(IU/IT)	26.519±0.53	50.611±17.2*
ACPμ mole/mg protein	2.36±0.018	2.56±0.009*
AKPμ mole/mg protein	11.99±0.007	15.89±0.08*

Values are Mean±SE.*Significance difference (p<0.05) of exposed and control rats

Table 3: Effect of exposure of mobile phones on the level of FSH and LH in plasma of control and treated male rats.

Hormones	Control	Treated
LH(IU/L)	9.38±0.15	6.83±0.18*
FSH(IU/L)	9.94±0.06	7.77±0.23*

Values are MEAN±SE. *Significance difference (p<0.05) of control and treated rats.

Table 4: Effect of exposure of mobile phones on the level of LH, FSH, Estrogen, Progesterone in plasma of control and treated female rats.

Hormones	Control	Treated
LH	9.39±0.17	7.39±0.16*
FSH	9.77±0.11	7.50±0.48*
Estrogen	8.99±0.08	5.20±0.01*
Progesterone	9.99±0.05	11.21±0.2*

Values are MEAN±SE. *Significance difference (p<0.05) of control and treated rats.

Table 5: Effect of exposure of mobile phone radiations on the estrus cycle of exposed and control female rats.

Cycle(days)	Proestrus	Estrus	Metestrus	Diestrus	Diestrus index
Control	2.5±0.5	3.5±0.5	2.5±0.5	7.5±0.5	53.57
treatment	3.5±0.5	4.5±1.5	3.5±0.5	4±1	28.57

Values are MEAN±SE. *Significance difference (p<0.05) of control and treated rats.

Table 6: Effect of exposure of mobile phone radiations on concentrations of Acid phosphatase(ACP) alkaline phosphatse (AKP),Aspartate aminotransferase(AST) and Alanine amino tranferase(ALT) in the plasma of exposed and control female rat.

Biochemical constituents	Control	Treatment
AST(IU/it)	5.65±0.047	7.22±0.21*
ALT(IU/IT)	3.129±0.39	6.455±0.19*
ACP(μ mole/mg protein)	1.76±0.16	3.59±0.0019*
AKP(μ mole/mg protein)	11.27±0.03	15.67±0.30*

Values are MEAN±SE. *Significance difference (p<0.05) of control and treated rats.

Table 7: Effect of exposure of mobile phone radiations on concentration of proteins (g/dL of sample) in the Plasma and Ovary of exposed and control female rats.

Organ	Control	Treatment
Plasma	4.313±0.01	4.883±0.06*
Ovary	2.740±0.58	3.520±0.014*

Values are MEAN±SE. *Significance difference (p<0.05) of control and treated rats.

Similarly the level of protein decreases in testes of exposed group as compared and to control group. On the other hand Verma (2016) observed elevated level of proteins in plasma and testis of 2 month exposed rats. However, the present study is in agreement with the findings of Singla(2015) who observed significant decrease in the level of proteins in testis and plasma of rats exposed to cell phone radiation for 2 months.

PLASMA

Aminotransferase

The activity of aspartate aminotransferase increased significantly in plasma of exposed male rats i.e., 40.32±0.1005 as compared to control rats i.e., 33.72±0.67. The activity of alanine aminotransferase also increased significantly in plasma of exposed rats i.e., 50.61±17.2 as compared to control rats i.e., 26.51±0.53(Table 2,Fig.2)

Previous studies too, reported significant increase in AST and ALT in serum of exposed rats thus indicating the cytotoxic effect of non-ionizing radiation on hepatocyte inducing cell damage and necrosis (Boris et al 2011). Similarly, studies by Purushothamanet al (2013) revealed significant increase in AST and ALT in plasma of rats exposed to cell phone radiation indicating the involvement of radiation on liver cell membrane. During cell injury, because of higher permeability of hepatocyte membrane, enzymes like AST and ALT penetrate to sinusoids and then enter into the peripheral blood thus increase in the level of such enzymes was observed(Friedalet al 1979).

Phosphatase

The activity of acid phosphatase(ACP) increased significantly in plasma of exposed rats i.e.2.56±0.009 as compared to control rats 2.36±0.018. Similarly, the activity of alkaline phosphatase increased significantly in exposed rats i.e., as 15.89±0.089 as compared to control rats 11.99±0.007 (Table 2). Electromagnetic radiation can disrupt the cell membrane which inhibit signal transduction pathways that resulted in increase in ACP level in serum (Lahijani et al 2009).

Hormones

In males

Hormonal balance in males plays a vital role in maintaining fertility. Present studies showed significant decrease in the level of FSH and LH in male rats exposed to cell phone radiations for 60 days (Table 3). Pituitary gland produces leutinizinghormone(LH) and follicle stimulating hormone(FSH) that regulates reproduction in males. LH stimulates testosterone production from the interstitial cells of the testes (Leydig cells). FSH stimulates testicular growth and enhances the production of an androgen binding protein by sertoli cells, which are component of the testicular tubule necessary for sustaining the maturing sperm. Male rat exposed to EMR (950 MHz) provokes oxidative stress which induces significant decrease in FSH, LH compared to control group (Abd El Rahmanet al 2014). The decrease level of gonadotropin observed in the present study could be a factor of decreased in sperm numbers and associated parameters.

In females

The present study showed significant decrease in the level of LH, FSH and estrogen in the exposed rats as compared to control rats However, the level of progesterone increased in two month exposed group as compared to control group (Table 4). Singla (2015) reported significant decrease in the level of estrogen in the exposed group of rats. However, Bahararaet al (2004) reported no change in the level of estrogen in female rats exposed to cell phone radiation.

Jahromi et al., (2015) however, reported increase in the level of FSH, LH and progesterone after exposure to cell phone radiation. Radiation from cell phone affects central nervous system thus could alter the secretion of gonadotropins releasing hormone (GnRH). Studies by Huuskonen and Saastamoinen (2001) are in accordance with the present study who reported reduction in the level of LH and FSH in rats exposed to electromagnetic radiation.

Rats exposed to radiation showed non- significant increase in the level of progesterone in period of one month and two months (Singla 2015).The higher level of progesterone could be because of more follicles giving atretic or getting leutinized without ovulation during the LH surge. However, long term exposure to cell phone radiation increases serum progesterone level in female rats (Razavinia et al., 2014).

Length of cyclicity in females

No. of cycle viz. proestrus, estrus, metestrus and diestrus does not alter significantly in rats exposed to cell phone radiation as compared to control rats (Table 5). Our results are in accordance withVerma (2016) who reported non-significant changes in estrus cycle in exposed group. Similarly, rats exposedto 10 kHz, 0.2 mT sine wave EMF does not affect the estrus cycle(Dawson et al 1998).

Plasma

Phosphatase

The biochemical constituents such as ACP and AKP showed considerable alteration in plasma of rats exposed to radiationsas compared to control rats. The activity of ACP and AKP showed significant increase in plasma of exposed rats as compared to control rats (Table 6).

Aminotransferase

The activity of AST and ALT showed significant increase in plasma of rats exposed to electromagnetic radiation for 2 months as compared to control rats. Studies by Moussa(2009) revealed significant increase in AST and ALT in serum of exposed rats as compared to control rats. Elevated level of AST and ALT might cause damage to heart, skeletal muscle and liver parenchyma(Vozarova et al 2002).

Proteins

Concentration of protein was estimated in plasma and ovary of control and rats exposed to cell phone radiation. Significant increase in concentration of protein in plasma was observed in exposed group i.e., 4.88±0.065 as compared to control group i.e., 4.31±0.016. Concentration of protein increased significantly in ovaries in exposed group i.e., 3.52±0.014 as compared to control group 2.74±0.58 (Table 7). Similarly, studies by Verma (2016) reported significant increase in concentration of protein in exposed group as compared to control group.

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