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The influence of prenatal 10 GHz microwave radiation exposure on a developing mice brain

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Abstract. Our objective was to investigate alterations in the developing mice brain after intrauterine microwave exposure from different gestation days (0.25 and 11.25) till term. Pregnant mice from 0.25 and 11.25 days of gestation were isolated from an inbred colony and divided into sham-exposed (control) and microwave-exposed (10 GHz) groups. The follow-up study of mice at 3 weeks of age showed significant reduction in the brain and body weight of microwave-exposed group. Results showed an increased level of lipid peroxidation, decreased level of glutathione and protein after microwave exposure on both 0.25 and 11.25 day of gestation. Moreover, changes in cytoarchitechure of hippocampus and cerebellum of the brain and reduction in Purkinje cell number were observed statistically significant after microwave exposure from both 0.25 and 11.25 days of gestation. In conclusion, the degree of severity of damage in neonatal mice brain was much higher, when exposure started from 0.25 day of gestation compared to 11.25 days of gestation.

Key words: Microwaves — Neonates — Cerebellum — Hippocampus — Gestation

Introduction

For decades, there has been an increasing concern on the hazardous effect of microwave radiations on human health. Studies based on *in vitro* and *in vivo* model show the fact that radiofrequency electromagnetic field (RF-EMF) exposure causes neurological damage. In 2011, the International Agency for Research on Cancer (IARC) classified RF-EMF as "possibly carcinogenic to humans" (Group 2B) (Baan et al. 2011). This has also been supported by several other studies showing a correlation between long-term RF-EMF exposure and cancer risk (Hardell and Carlberg 2013; Hardell et al. 2013a, 2013b, 2013c; Coureau et al. 2014). In 1998, the International Commission on Non-Ionizing Radiation Protection released guidelines and reported that the specific absorption rate (SAR) of mobile phones could be legally limited to 2.0 W/kg (ICNIRP 1998). Later, in USA, Canada and Australia, the maximum SAR level was limited to 1.6 W/kg and 2.0 W/kg in Europe (Dahal 2013), but most have an average SAR of ~1.4 W/kg (Agarwal et al. 2011).

Serious health concern on fetus and children´s developing brain are often linked to long-term exposure of microwave radiations. Several epidemiological investigations show that the women and children (especially pregnant women or fetus) are particularly sensitive to EMF exposure (Ahlbom et al. 2001; Aldad et al. 2012). Neurobehavioral disorders in children are the main consequence attributed to these radiations (Barkley 1997; Rappley 2005). In this series, Morgane et al. (1992) has reported an early foetal period is very active phase of cortical development in rodent brain. Though, in developing nervous system, the brain tissues are more conductive in children than that of adults, because it contains higher water content and ion concentration. Children have greater possibility to absorb microwave energy deep into the brain (Kheifets et al. 2005). Therefore, microwave radiations have potential to penetrate the cranium, and nearly 40% of these can reach deeper into the brain (Kang 2001; Barnett et al. 2007), where penetration

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depth assumed to be 4–5 cm deep into the brain (Dimbylow and Mann 1994; Rothman 1996).

In view of the above, we selected 10 GHz microwave radiation as the main exposure source in the present study. Microwaves, electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz, have been widespread usage in aeronautical radio-navigation, radiolocation, medical, traffic light crossing detection and military field. This range of frequency has been considered as hazardous for human health (Walani et al. 2014). Increased usage of particularly 10 GHz microwave radiation is potentially strong to threat the human health, as also reported previously by our group (Kumar et al. 2012; Sisodia et al. 2013; Sharma et al. 2014; Zhang et al. 2014).

Certain serious health concerns are often linked to various type of RF-EMF exposure on whole brain or particular region (hippocampus, hypothalamus, cerebral cortex) (Ferreri 2006; Kumlin et al. 2007; Maskey et al. 2010a, 2010b, 2013; Kesari et al. 2013a, 2013b). The most common concerns include impact on hippocampus, impairs long-term potentiation, decreases neurotransmitter concentrations, reduces synaptic vesicles in number and results in memory impairment (Xu et al. 2006; Zhao et al. 2012; Wang et al. 2013). Yüksel et al. (2016) reported an increased oxidative uterine injury in growing rats and decreased hormone levels in maternal rats after mobile phone and Wi-Fi-induced EMF exposure. The present study aimed to observe alterations in developing brain after intrauterine microwave exposure at different gestation days. To evident the changes occurred after microwave exposure, following parameters i.e. lipid peroxidation (LPO), glutathione (GSH) and protein level were measured. Also histopathology of mice brain was observed qualitatively and quantitatively.

Materials and Methods

Experimental animals

Mice were procured from inbred colony maintained in an animal house facility of the University of Rajasthan, Jaipur, India. Pregnant female Swiss albino mice (*Mus musculus*) from 0.25 and 11.25 day of gestation were isolated after checking vaginal plug early in the morning. All animals were housed in an air-conditioned room, where the temperature was maintained at 25 ± 1.5°C, with constant humidity (40–50%) and kept on 12/12 h light/dark cycle throughout the experiment. The animals were fed on standardized normal diet (Hindustan Unilever Limited, Delhi, India) and water *ad libitum*. The Institutional Animal Ethical Committee (IAEC) approved the protocols for animal experimentation described in the present study. All subsequent animal experiments were followed as *per* the "Guidelines for Animal Experimentation" of the University.

Animal exposure

Plexiglas was used to fabricate an exposure cage of which the dimensions were 4.5 × 4.5 × 9 cm with fixed holes (1 cm diameter). This was designed so that animals could be lightly restrained in a fixed position and with proper ventilation. Exposure was carried out in an anechoic chamber. The study was carried out into two sets, where first and second set of pregnant mice were received treatment from 0.25 day and 11.25 days, respectively, of gestation till term. Total exposure period were 20 and 8 days for both 0.25 and 11.25 gestation days. Pregnant mice from the both gestations day (0.25 day and 11.25 day) were further divided into two sub groups containing 6 females in each group. Mice were mainly divided into two groups:

Sham-exposed (Group I): Pregnant mice from 0.25 and 11.25 day of gestation were served as control and kept in a Plexiglas cage. Animals were placed symmetrically towards the midline of pyramidal horn antenna aperture without energizing the system for 2 hours/day till term.

Microwaves-exposed (Group II): Pregnant mice from 0.25 and 11.25 day of gestation were exposed with 10 GHz microwave radiation for 2 hours/day till term.

All animals were kept in such position, where the head of animals facing the horn antenna. The horn antenna was kept in H (magnetic field) plane configuration. Field was almost uniform because the dimensions of the cage were of the order of wavelength. The maximum power density 0.25 mW/cm^2 (milliwatt *per* centimeter square) was recorded at the near field distance from the horn antenna. A power meter measured the emitted power of microwaves, which was a peak sensitive device (RF power sensors 6900 series and IFR 6960B RF power meter; Aeroflex, Inc., Wichita, Kansas, USA). A sketch diagram of 10 GHz microwave exposure system is represented in Figure 1. SAR was estimated to be 0.1790 W/kg following the Durney et al. (1984) method. Similar exposure system for microwave exposure was fabricated earlier by Sharma et al. (2014). Similar experiment was performed with sham-exposed animals without energizing the system.

In experimental studies, all the females were allowed to deliver normally. The following parameters were studied immediately after birth viz. average litter size, average body weight of litters and average crown-rump length of litters. Litters were then allowed to grow and attain 3 weeks of age. Thereafter, they were autopsied to study various end points, like brain weight and different enzymatic parameters (LPO, GSH and total protein). Histopathological studies were also undertaken in the hippocampal region of the brain and cerebellum. In each group, six pups brain were used

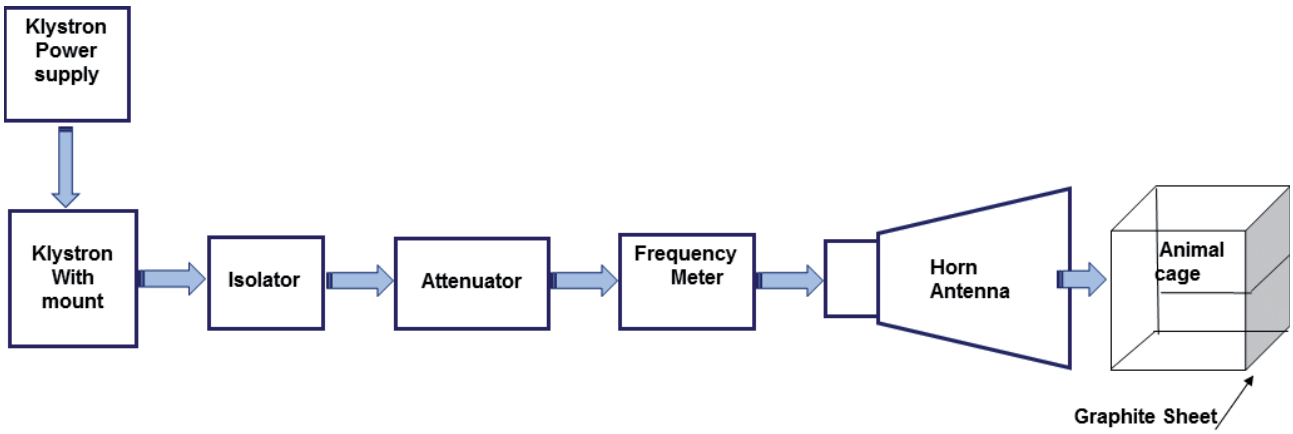

Figure 1. Schematic diagram of 10-GHz microwave source exposure setup with animal cage indicating individual animal's position.

for biochemical studies and another six were used for histopathological studies.

Sample preparation

After completion of experiment, twelve mice were sacrificed by cervical dislocation from each group and thereafter biochemical assays, and histopathological studies were performed. An incision was made at the side of the jaws to separate the upper and the lower palates. The upper palate was cut from the middle, thereafter having cleared the surrounding tissue and then the brain was excised. The intact whole brain was removed carefully and further used for histological and biochemical measurements. Six pups brain was processed for biochemical assay and another six for histopathological studies. These were selected randomly from the pups born to the six females exposed to microwaves from gestation day 0.25 and 11.25 days, including sham-exposed.

Lipid peroxidation (LPO) assay

LPO was measured by the method of Buege and Aust (1978). One gram of the tissue was homogenized in 9 ml of 1.15% KCl. The tissue homogenate (0.8 ml) was mixed with 1.2 ml of trichloroacetic acid (TCA) (15% w/v), thiobarbituric acid (TBA) (0.375% w/v) and hydrochloric acid (HCl) (0.25 N) solutions (Himedia, Mumbai, Maharashtra, India), prepared in a 1:1:1 ratio. The mixture was heated in a boiling water bath for 30 minutes. Samples were centrifuged at 1000 × *g* for 10 minutes. After centrifugation, the absorbance was recorded at 532 nm by using Ultra Violet-Vis double beam spectrophotometer (Double Beam Spectrophotometer 2203, Systronics, Ahmedabad, Gujarat, India). A standard curve was prepared by using tetra-methoxy-propane (TMP, purchased from Himedia, Mumbai, Maharashtra, India). After comparison with a standard curve, the LPO level was expressed in nmol/gm tissue.

Glutathione (GSH) assay

The reduced GSH level was determined in the brain by the method of Moron et al. (1979). Tissue samples were homogenized in the sodium phosphate-EDTA (Ethylenediaminetetraacetic acid) buffer (Himedia, Mumbai, Maharashtra, India). Thereafter, 0.6 ml of DTNB reagent (5,5'-Dithiobis-(2-nitrobenzoic acid), Himedia, Mumbai, Maharashtra, India) was added into it. On addition, the yellow colored complex was developed by the reaction of GSH and DTNB, and then the optical density was measured at 412 nm by using a UV-vis spectrophotometer (Double Beam Spectrophotometer 2203, Systronics, Ahmedabad, Gujarat, India). The results were expressed in nmol GSH/100 mg of tissue.

Protein assay

The protein concentration was estimated following the method of Bradford (1976). The procedure was based on an interaction of dye, coomassie brilliant blue with proteins. The absorbance of unbound dye was maximum at 465 nanometer (nm). After an interaction of dye with protein, it turns blue and their absorbance maxima were displaced to be 595 nm. Thus from the absorbance at 595 nm, the amount of protein in a sample was estimated in mg/g tissue. In brief, 10% homogenate of each excised brain was prepared in 0.85 M NaCl (sodium chloride, purchased from Himedia, Mumbai, Maharashtra, India) solution. Thereafter, 0.1 ml of the sample was taken for the Bradford assay. The volume in the test tube was adjusted to 1 ml with phosphate buffer saline (PBS with pH 7.4) (Sigma-Aldrich, St. Louis, MO, USA). Five milliliters of Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA)

was added to the test tube and the contents were mixed by vortexing. The sample absorbance was measured after 2 minutes at 595 nm in spectrophotometer (Double Beam Spectrophotometer 2203, Systronics, Ahmedabad, Gujarat, India). For calibration, blank was prepared from 0.1 ml of the phosphate buffer saline (pH 7.4) and 5 ml of Bradford reagent. The amount of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples.

Histopathology of brain

After completion of the experiment, brain was excised and fixed in Bouin's fluid. It was dehydrated and embedded in paraffin blocks. The transverse sections of tissues were passed through a graded series of alcohol and stained in Eosin and Harris Haematoxylin stain (Mallory et al. 1994), then used for various qualitative and quantitative histopathological studies. Qualitative studies were done in CA1 region of hippocampus and ansiform am lobule of cerebellum. Quantitative studies include, counting of Purkinje cells number, thickness of molecular layer and granular layer of cerebellum and cerebral cortex. Morphological changes in cerebral cortical cell and hippocampal pyramidal cell regions were visualized under light microscope (400×). Width or thickness of molecular layer, granular layer and cerebral cortex were recorded by using oculometer, which was calibrated with standard stage micrometer. Counting of Purkinje cell (mm^{-1}) number was carried out manually in six different randomly selected areas ($200 \times 200\ \mu m^2$) from three different slides of the ansiform lobule of cerebellum.

Statistical analysis

Data were analyzed using Student's *t*-test. Comparison was made between sham-exposed and microwave-exposed group of animals. A *p*-value of less than 0.05 was considered statistically significant where higher statistical significance was assessed ** $p < 0.001$.

Table 1. Variations in crown rump length and the body weight of mice neonates (1 day old) after 10 GHz microwave exposures of pregnant females from 0.25 day and 11.25 days of gestation till term

Groups	Gestation day	Crown rump length (cm)	Body weight (g)
Sham-exposed	0.25	2.87 ± 0.01	1.19 ± 0.02
	11.25	2.87 ± 0.02	1.19 ± 0.02
Microwave-exposed	0.25	2.86 ± 0.01	1.01 ± 0.03**
	11.25	2.87 ± 0.01	1.12 ± 0.04**

The average litter size (4.33 ± 0.21 cm) was observed similar in all the cases (sham and microwave-exposed groups for both gestation days). Statistical significances (**) were assessed to see the effects between sham-exposed and microwave-exposed groups. The values presented here are mean ± SD for $n = 6$ independent experiments.

Results

Morphometric parameters

No statistically significant changes in litter size and crown-rump length were recorded after exposure. Also body weight was recorded to see the radiation-induced growth retardation in the offspring after *in utero* exposure to 10 GHz microwave radiation (Table 1). A significant decrease ($p < 0.001$) in body and the brain weight were observed more in neonates born to mothers, those exposed throughout the pregnancy (0.25 day). Similar pattern was measured in follow-up study at 3 weeks of age (Table 2).

Lipid peroxidation (LPO), glutathione (GSH) and protein measurements

After completion of exposure to pregnant females from different gestation days, the LPO, GSH and protein concentration were measured in the follow-up study of mice at the 3 weeks of age. Statistically significant ($p < 0.001$) increase in LPO level and decrease in GSH and protein levels were measured in mice whole brain after exposure to 10 GHz microwave radiation by comparing with sham-exposed group (Table 3).

Histopathological studies

Quantitative studies. Thickness of cerebral cortex was measured and no significant changes were observed in this region. Also no significant changes were recorded in the thickness of molecular layer of cerebellum (Fig. 2). However, granular layer thickness and the number of Purkinje cells were reduced significantly in exposed group compared to the sham-exposed group (Table 4).

Table 2. Variations in an average body weight and the brain of Swiss albino mice after 10 GHz microwave exposure from 0.25 day and 11.25 days of gestation till term and follow-up at 3 weeks of age

Groups	Gestation day	Body weight (g)	Brain weight (g)
Sham-exposed	0.25	10.31 ± 0.25	304 ± 3.16
	11.25	10.26 ± 0.11	303.87 ± 3.75
Microwave-exposed	0.25	9.81 ± 0.20**	294 ± 3.96**
	11.25	9.16 ± 0.05**	302.62 ± 4.30

Statistical significances (**) were assessed to see the effect between sham-exposed and microwave-exposed groups. The values presented here are mean ± SD for $n = 6$ independent experiments.

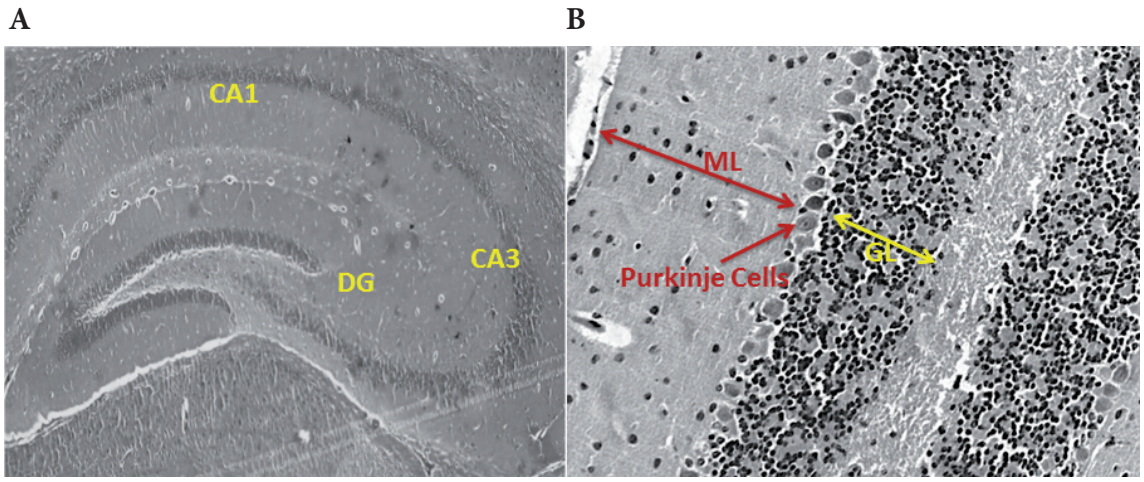

Figure 2. A. Mice hippocampus showing Cornus ammonis 1 (CA1), Cornus ammonis 3 (CA 3) and Dendate gyrus (DG) region. **B.** Purkinje cell numbers were counted and thickness of molecular layer (ML) and granular layer (GL) of ansiform lobule of cerebellum was measured in all the groups.

Qualitative studies. Histopathological alterations in mice hippocampus and cerebellum cortex region were studied in 3 weeks of aged mice after *in utero* exposure from 0.25 day and 11.25 day of gestation till term. Pups born to the sham-exposed mice showed normal cytoarchitechture of hippocampal pyramidal cells as well as normal arrangement of Purkinje cells, molecular layer and granular layer in ansiform lobule of cerebellum was observed (Fig. 3). Pups born to the mice exposed throughout gestation period (i.e. starting from 0.25 day till term), showed more pronounced effects, compared to those born to the mice, where exposure was initiated from day 11.25 of gestation till term. Relatively

Table 3. Measurement of LPO, GSH and protein levels in the whole brain of Swiss albino mice after 10 GHz microwave exposures from 0.25 day and 11.25 day of gestation (at the initiation of treatment day) till term and follow-up at 3 weeks

Groups	Gestation day	LPO levels (nmol MDA/g tissue)	GSH (nmol/100 mg)	Protein levels (mg/g tissue)
Sham-exposed	0.25	58.18 ± 1.23	9.04 ± 0.16	102.98 ± 1.74
	11.25	55.13 ± 1.35	9.07 ± 0.15	103.33 ± 0.75
Microwave-exposed	0.25	81.12 ± 1.67**	6.77 ± 0.32**	90.91 ± 1.97**
	11.25	67.87 ± 1.22**	7.39 ± 0.18**	96.76 ± 0.75**

Statistical significances (**) were assessed to see the effect between sham-exposed and microwave-exposed groups. The values presented here are mean ± SD for $n = 6$ independent experiments.

Table 4. Measuring thickness of cerebral cortex, molecular layer, granular layer and Purkinje cell numbers in cerebellum of Swiss albino mice after 10 GHz microwave exposures from 0.25 day and 11.25 days of gestation till term and follow-up at 3 weeks of age

Groups	Gestation day	Cerebral cortex (μm)	Molecular layer (μm)	Granular layer (μm)	Number of Purkinje cells (mm^{-1})
Sham-exposed	0.25	969 ± 2.1	72.2 ± 1.8	95.4 ± 2.4	24.8 ± 1.6
	11.25	968.4 ± 2.4	71.8 ± 2.3	96.2 ± 2.7	25.1 ± 2.2
Microwave-exposed	0.25	967.6 ± 3.2	71.7 ± 2.7	83.4 ± 3.2**	18.6 ± 1.2**
	11.25	998.4 ± 3.2	71.9 ± 2.4	91.7 ± 2.4**	20.7 ± 1.8**

Statistical significances (**) were assessed to see the effect between sham-exposed and microwave-exposed groups. The values presented here are mean ± SD for $n = 6$ independent experiments.

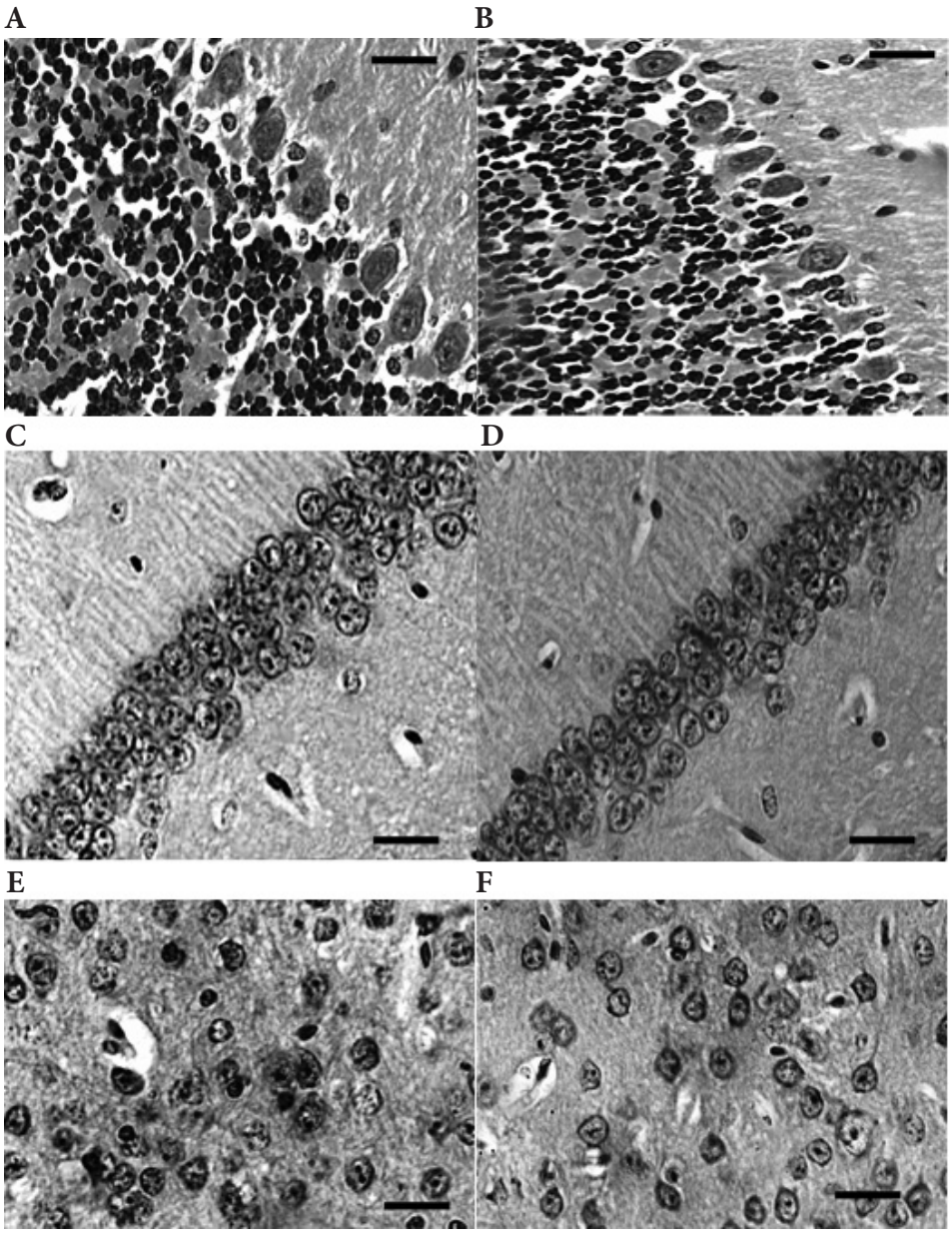

Figure 3. Effect of intrauterine sham exposure starting from 0.25 and 11.25 day of gestation till term during postnatal development. Left: Sham exposure from 0.25 day of gestation: normal alignment of Purkinje cells (**A**), normal distribution of pyramidal cell density in hippocampus (**C**), normal cyto-architecture of neuronal cells of cerebral cortex (**E**). Right: Sham exposure from 11.25 day of gestation: normal alignment of Purkinje cells with normal cyto-architechture (**B**), hippocampal pyramidal cell layer in CA1 region (**D**), neuronal cells of cerebral cortex (**F**). Calibration bars = 50 μm.

more number of necrotic Purkinje cells in cerebellum, hippocampal pyramidal cells and cortical neuronal cells were observed in mice, prenatally exposed to 10 GHz microwave exposure starting from 0.25 day (Figure 4A,C,E), by compared to those, exposed from 11.25 day of gestation till term (Figure 4B,D,F).

Discussions

The present study provides several important findings related to pathophysiology of prenatal 10 GHz microwave radiation and its effects on developing mice brain. There are many environmental factors, which behaves detrimental to the optimal development of the embryo and those that occur during the pre-and-postnatal period. The development of prenatal period was divided as: pre-implantation, organogenesis and the foetal. Whereas in postnatal period, the developmental changes took place after birth. This could be because, the development of brain continues up to 3 weeks of age, and the effect occurred on primitive brain cells gets amplified, as metabolic rates are high in the developing brain. Stringari et al. (2006) found exposure to microwaves were extremely sensitive during such developing stage of brain.

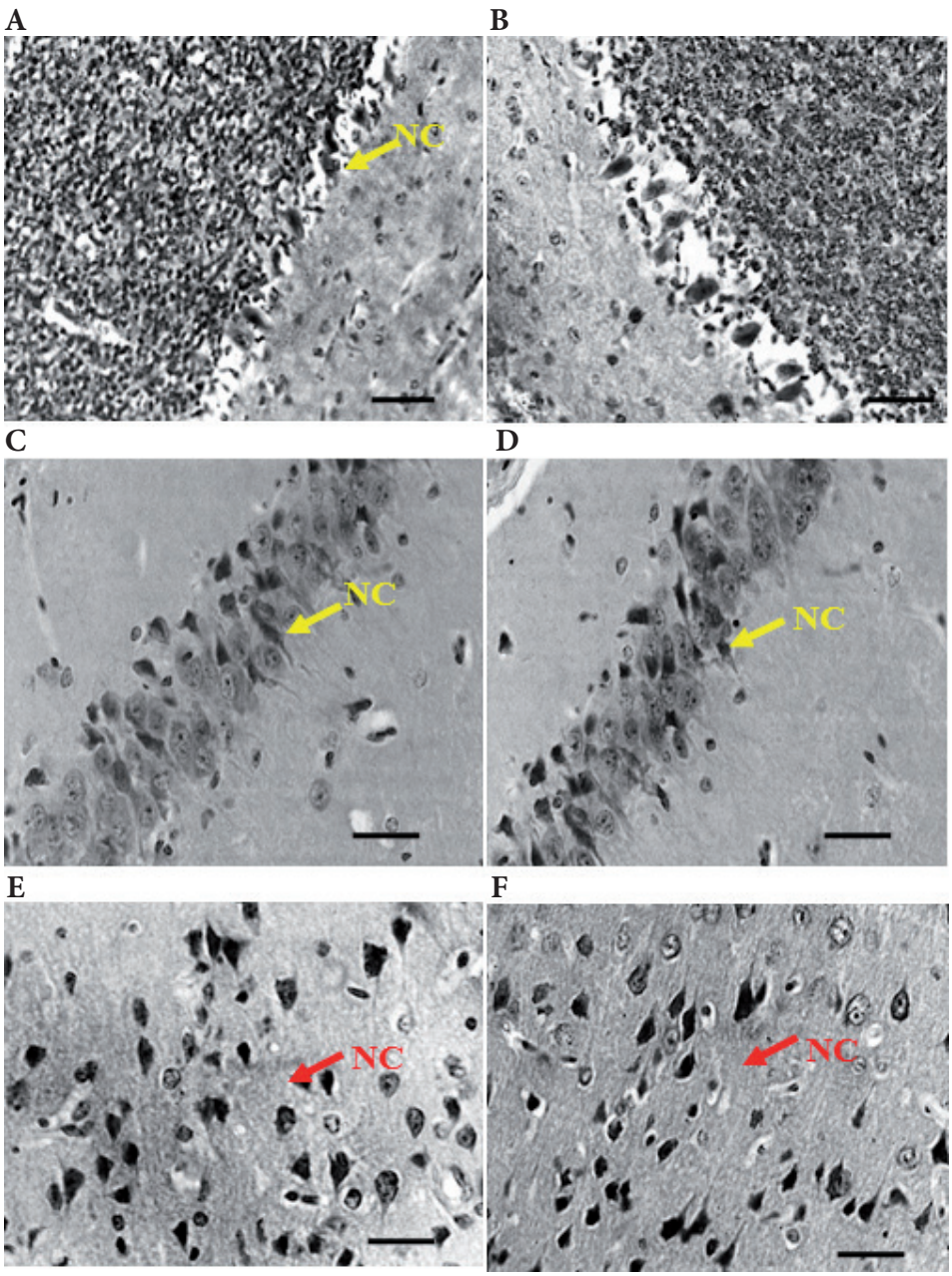

Figure 4. Effect of intrauterine microwave exposure starting from 0.25/11 day of gestation till term during postnatal development. Left: Exposure from 0.25 day of gestation: disturbance in alignment of Purkinje cells along with necrosis (NC) of most of cells (**A**), reduction of density along with necrosis (NC) of pyramidal cells (**C**), necrosis (NC) of neuronal cells of cerebral cortex (**E**). Right: Exposure from 11 day of gestation: disturbance in alignment of Purkinje cells with comparatively lesser disturbance in cytoarchitechture (**B**), necrosis (NC) of few pyramidal cells (**D**), necrosis (NC) of neuronal cells of cerebral cortex (**F**). Calibration bars = 50 μm.

Later, Encinas et al. (2008) reported that the quiescent neural stem cells found most sensitive to these radiations rather than their rapidly dividing progeny. In this study, several factors involved in exposure to microwave radiation effect on prenatal period and linkage to further development. During developmental stage, mainly three factors found to be involved: i) exposure time or duration (i.e. number of exposure days); ii) the greater number of primitive cells exposed to microwaves (greater will be the effect amplified in the daughter cells during development); iii) the greater amount of water in an organ during development (greater will be the effect of microwave exposure). Therefore, in primitive stage of gestation, when amount of water found more as compared to later stages of gestation, than the effect was more prone.

In this study, microwave exposure to female mice from 0.25 and 11.25 days of gestation period produced no appreciable changes in litter size or crown-rump length, but an appreciable decrease in body weight of neonates was observed in exposed group (Table 1). The postnatal reduction in body weight directly express the radiation-induced growth retardation of fetuses (Jensh 1997). Growth retardation in

newborn animals is common and predictable consequence of irradiation *in utero*. The degree of retardation depends on an administered dose of radiation. Therefore, the fetal body weight found to be a sensitive indicator for analyzing the growth retardation effects of intrauterine radiation. Whole gestational period was sensitive to radiations for weight loss of delivered newborns, but an early exposure to microwave radiation was (0.25 day of gestation) recorded more harmful in this study (Table 2). Decreased body weight of newborns (whose mothers were exposed to microwaves) were probably due to impairment of blood flow to the placenta and reduced uterine blood flow after microwave exposure. This may lead to reduce the transport of nutrients and oxygen to fetal circulation. Reduced weight of newborn mice may be justified with an induction of apoptosis after 10 GHz microwave-induced oxidative stress.

Shahin et al. (2013) concluded that a low level of microwave radiation-induced oxidative stress not only suppresses implantation, but also it may lead to deformity of the embryo in the case of pregnancy continues. Additionally, this microwave radiation-induced oxidative stress might increase the production of reactive oxygen species (ROS) and lead to DNA damage of brain cells, implantation failure/resorption or abnormal pregnancy in mice. Oxidative damage plays crucial role in many neurological disorders, neurodegenerative diseases and injuries of CNS (Adibhatla and Hatcher 2010). Therefore, oxidative stress-induced ROS is a well-known factor for cellular disruptions in hormonal communication between the brain, pituitary, and ovary. Although, an interaction of RF radiation with biological matter is a biochemical mechanism, which is based on responses, caused by activating secondary chemical messengers, such as ions, radicals or molecules (Belyaev et al. 2006). Due to the overproduction of free radicals and deficiency in amount of antioxidants, the balance between free radicals and antioxidants is disrupted in favor of the free radicals, and resulting in oxidative damage (Halliwell and Gutteridge 2000). Oxidative stress may result in severe metabolic dysfunctions, including peroxidation of membrane lipids, depletion of nicotine amide nucleotides, rises in intracellular free Ca^{2+} ions, cytoskeleton disruption and the DNA damage.

Microwave-induced oxidative stress plays a major role in metabolic dysfunction by affecting the level of LPO. Lipid peroxides, which are formed as a result of complex chain reactions mediated by ROS, are considered to be an important cause of damage to cell membranes. In the present study, 10 GHz microwaves radiation exposure to pregnant mice (from 0.25 and 11.25 day of gestation till term) increased the LPO level and decreased the GSH and protein levels of newborn mice in whole brain, that was estimated at the age of three weeks. Microwave-induced LPO not only damages the cell membranes, but also induces antioxidant enzymes like GSH, catalase and DNA damage (Noda et al. 1993; Kesari and Behari 2010). Based on these findings, we opine that the microwave radiation has potential to change biological lipid membranes and the outcomes of these changes can be seen in structural and functional properties of the cell (Yurekli et al. 2006). The plasticity of immature brain and nervous system toward environmental factors identified as potential target of microwave radiation induced oxidative damage. Developing brain is highly vulnerable to oxidative damage which results in production of stress proteins (HSPs) and nitrosative molecules, lead to early brain deterioration. The presence of two inducible HSPs: Hsp^{25} and Hsp^{70}, which are molecular chaperones, constitute a first line of defense in various stress conditions, including exposure to heat shock, inflammatory stimuli and oxidative stress.

Laurence et al. (2000) suggested a biological response of RF radiation could change protein denaturation against cellular stress. Later, Finnie et al. (2006) reported no field dependent changes neither in c-fos expression of the pyriform cortex, nor in basal ganglia of the brain of foetal mice, after daily exposures to pregnant animals during gestation period. Finnie et al. (2009) also reported no changes in Hsp^{32} and Hsp^{70} protein expression, using 3-Nitrotyrosine (3- NT) as a specific marker, in the brain of mice fetuses exposed to mobile phone radiation. Jing et al. (2012) exposed pregnant rat at different microwave intensities from first pregnant day for consecutively 20 days, and concluded that these radiations have certain harmful effects in foetal rat brain during pregnancy. Ait-aissa et al. (2013) investigated the bio-effects of exposure to wireless signals (2450 MHz) on developing nervous systems of young rodents. Authors suggested that repeated exposure to Wi-Fi during gestation and early life has no deleterious effects on the young rat brain, which was in contrast to outcomes of the present study undertaken.

In these consequences, Odaci et al. (2008) investigated the effects of prenatal exposure to EMF on number of granule cells in dentate gyrus of 4-week-old rats. Ragbetli et al. (2009) found no significant difference in pyramidal cell number of total cornuammonis (CA) region of hippocampus in mobile phone-exposed groups. Ragbeti et al. (2010) reported a significant decrease in number of Purkinje cells and tendency for granule cells to increase in cerebellum after exposure of pregnant animals to mobile phone radiations. Aldad et al. (2012) also reported that *in utero*, whole-body exposure by cell phone radiations to pregnant mother resulted in hyperactivity, impaired memory and behavioral changes in offspring's. Bas et al. (2013) showed that exposure to 900 MHz EMF during prenatal days (13–21) led to a significant decrease in number of pyramidal cells in CA region of exposed female rat pups. However, these changes may not be severe enough to alter the cerebellum-dependent functional tasks. Thus our results are in line with other reported studies, which confirms cytoarchitechural damage of cerebrum, and cerebellum in prenatally exposed mice to 10 GHz mi-

crowaves. Based on available evidences, it might be useful to explore the further research, which should be done to reduce the discrepancy regarding the effects of intrauterine microwaves exposure on developing brain.

Conclusions

The pregnant female mice exposed to10 GHz microwave radiation showed a greater effect by comparing to those exposed for shorter time during the entire term. The results from the present study are consistent with our previous findings that confirm an increase in LPO, GSH, protein levels and changes in histological parameters associated with overproduction of ROS, which may lead to acute or chronic pathological conditions in neonates. Lastly, the present study concludes the damages caused by RF-EMF in newborns are dose-dependent. These findings are helpful in initiating remedial measures to prevent hazardous effects of microwave radiation.

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Conflict of interest. The authors declare that there are no competing interests.

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