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ARTICLE

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Effects of radiofrequency electromagnetic radiation emitted from a mobile phone base station on the redox homeostasis in different organs of Swiss albino mice

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ABSTRACT

This study was designed to investigate the possible effects of exposure to mobile phone base station (MPBS) emits 1800-MHz RF-EMR on some oxidative stress parameters in the brain, heart, kidney and liver of Swiss albino mice under exposures below thermal levels. Mice were randomly assigned to three experimental groups which were exposed to RF-EMR for 6 hr/day, 12 hr/day and 24 hr/day for 45 consecutive days, respectively, and a control group. The glutathione (GSH) levels and activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) were significantly reduced in mice brain after exposure to RF-EMR for 12 hr and 24 hr per day. Exposure of mice to RF-EMR for 12 hr and 24 hr per day also led to a significant increase in malondialdehyde (an index of lipid peroxidation) levels in mice brain. On the contrary, exposures used in this study did not induce any significant change in various oxidative stress-related parameters in the heart, kidney and liver of mice. Our findings showed no significant variations in the activities of aspartate amino-transferase (AST), alanine amino-transferase (ALT), and on the level of creatinine (CRE) in the exposed mice. This study also revealed a decrease in RBC count with an increase in WBC count in mice subjected to 12 hr/day and 24 hr/day exposures. Exposure to RF-EMR from MPBS may cause adverse effects in mice brain by inducing oxidative stress arising from the generation of reactive oxygen species (ROS) as indicated by enhanced lipid peroxidation, and reduced levels and activities of antioxidants.

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Radiofrequency electromagnetic radiation (RF-EMR); mobile phone base station (MPBS); reactive oxygen species (ROS); antioxidants; oxidative stress; lipid peroxidation; serum toxicity

Introduction

Mobile phone base stations (MPBS) have become an integral part of telecommunication which use radiofrequency electromagnetic radiation (RF-EMR) to transmit wireless signals, generally operating in the frequency range of 900 MHz to 1.9 GHz (Levitt and Lai 2010). The ever-increasing use of mobile phones, especially for complex signals such as video streaming has led to a phenomenal increase in the number of MPBS required to cater for this increasing demand. Thus, the levels of electromagnetic pollution have rapidly escalated in the environment over the recent decades (Bandara and Carpenter 2018). Scientists have attempted to draw the attention of the scientific community and the authorities to the health-related adverse impact on biological systems (Belpomme et al. 2018; Miler et al. 2019). The delayed expression of health problems due to anthropogenic environmental agents such as RF-EMR, rapidly changing wireless technology without allowing sufficient time for research as well as the complexity of evaluation of the risks due to limitations in the research evidence such as insufficient data from 'real-life' exposures and combined toxic exposures are major obstacles in this field needing attention (Panagopoulos et al. 2015; Kostoff et al. 2020).

Biological effects of RF-EMR associated with the increased temperature of tissue are clarified by thermal mechanism, but there is no consensus on cellular effects induced by low-level RF-EMR exposure at non-thermal levels (Belpomme et al. 2018; Blettner et al. 2009; Viel et al. 2009). Within the past two decades, many studies have indicated an association between the RF-EMR exposure and several human disease conditions including diabetes, cancer, neurological and cardiovascular diseases (Bortkiewicz et al. 2004; Dasdag et al. 2020; Eger et al. 2004; Havas 2013; Lerchl et al. 2015; Wolf and Wolf 2004). One particular concern in recent years is the accumulated evidence on RF-EMR induced oxidative stress in biological systems, under low-intensity non-thermal levels of exposure (Alkis et al. 2019; Dasdag and Akdag 2016; Yakymenko et al. 2016). Exposure to RF-EMR has been reported to be associated with oxidative stress (OS) caused by enhanced reactive oxygen species (ROS) production which is known to inflict oxidative tissue damage including DNA damage

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which is now demonstrated by a large evidence base (Lai 2021). RF-EMR can alter the energy levels and spin orientation of electrons in biological molecules giving rise to free radicals that comprise most ROS generated in biological systems (Barnes and Greenebaume 2000, 2016; Murphy et al. 1993; Jajte et al. 2002; Turker et al. 2011; Naziroglu et al. 2012; Yakymenko et al. 2016). Uncontrolled generations of ROS causing disruption to redox homeostasis can lead to oxidative stress at cellular, tissue and organism level. Although cells are naturally equipped with an impressive repertoire of antioxidants, both enzymatic as well as non-enzymatic small molecule antioxidants to counterbalance ROS thereby preventing oxidative stress, over-production of ROS overwhelms the endogenous antioxidants leading to oxidative cellular damage with likely adverse health outcomes in the long-run (Butterfield and Halliwell 2019; Jagetia and Shetty 2016; Seifried et al. 2006). Detection and measurement of ROS in real-time using *in vivo* systems is difficult due to their extreme reactivity (Niki et al. 2005). Therefore, altered levels of antioxidants; enzymes such as glutathione-s-transferase (GST); superoxide dismutase (SOD); and small-molecule antioxidants such as glutathione (GSH) are used as markers of oxidative stress as well as stable by-products of oxidative damage to biomolecules such as lipids. Since lipid peroxidation (LPO) is a free-radical oxidation process of polysaturated fatty acids, detection and measurement of LPO is the evidence which is frequently cited to support the involvement of free-radical reactions in toxicity and disease progression (Gaschler and Stockwell 2017; Gutteridge 1995).

The adverse effects of RF-EMR related to oxidative stress have been supported by several human studies (Moustafa et al. 2001; Agarwal et al. 2009; Naziroglu et al. 2012; Zothansiama et al. 2017; Bektas et al. 2020), animal studies (Akbari et al. 2014; Avci et al. 2012; Chauhan et al. 2017; Djordjevic et al. 2015; Ghazizadeh and Nazıroğlu 2014; Ghoneim and Arafat 2016; Guler et al. 2010; Kesari et al. 2011; Kumari et al. 2012; Odaci and Oziylmaz 2015; Oyewopo et al. 2017) and cell culture studies (De-Iuliis et al. 2009; Kang et al. 2014; Kim et al. 2017; Kwee et al. 2001; Lu et al. 2012). However, several studies reported no significant effect of RF-EMR on oxidative stress in different study systems (Aydogan et al. 2015; Brescia et al. 2009; Cao et al. 2009; Dogan et al. 2012; Gannes et al. 2011; Hong et al. 2012; Khalil et al. 2011). Furthermore, studies on the effects of RF-EMR through direct exposure to the mobile phone base stations are very limited (Achudume et al. 2010; Marzook et al. 2014). Given the present state of the scientific evidence on biological and health effects induced by RF-EMR, with the majority of studies showing significant effects, high priority research is required to objectively assess the potential adverse effects of RF-EMR. In this pursuit, biological effects related to oxidative stress warrant investigation. Therefore, the present study aimed to obtain new insights into the potential effects of RF-EMR exposure in a typical real-life scenario in the antioxidant/oxidant status in different organs of mice.

Materials and methods

Chemicals

Bovine serum albumin (BSA), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), n-butanol, disodium hydrogen phosphate (Na_2HPO_4), nicotinamide adenine dinucleotide reduced (NADH) and reduced glutathione (GSH) were purchased from HiMedia, Lab. Pvt., Ltd. (Mumbai, India). 5, 5′ dithio 2-nitrobenzoic acid (DTNB), copper sulphate ($CuSO_4$), acetic acid, dipotassium phosphate (K_2HPO_4) and mono-potassium phosphate (KH_2PO_4) were purchased from Merck Ltd. (Mumbai, India). Folin-ciocalteau's reagent, 1-chloro-2,4 dinitrobenzene (CDNB), trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were purchased from SD fine-chem Ltd. (Mumbai, India).

Animal model and ethics statement

Swiss albino mice (10–12 weeks old) weighing 25 g to 30 g were selected from an inbred colony maintained under controlled conditions of temperature (23 ± 2°C), and light (12 h of light and dark, respectively) at the Animal Care Facility, Department of Zoology, Mizoram University. The animals were having free access to food and water. The animal care and handling were carried out according to the guidelines issued by World Health Organization, Geneva, Switzerland. All the experimental protocols employed in this study were approved by the Institutional Animal Ethical Committee, Mizoram University (No. MZU-IAEC/2020/04) and CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals), New Delhi, India (Registration No. 1999/GO/ReBi/S/18/CPCSEA).

RF-EMR exposure

Swiss albino mice were randomly assigned to four groups (n = 6). Unexposed control group was kept in an area where there is no nearby MPBS (within 550 metres), electric transformer, high voltage electric power line, radio and television transmitters or any other major source of anthropogenic electromagnetic

fields. The remaining three groups were exposed to RF-EMR by keeping them at a distance of 12 metres from a MPBS for 6 hr, 12 hr and 24 hr per day, respectively, for 45 consecutive days. Mice with 6 hr and 12 hr per day exposure were taken back to where the unexposed control group were placed when the daily exposure duration was over. The selected MPBS, erected on a rooftop, consists of 20 sectored antennas and 6 dish antennas that are arranged equilaterally providing 360° network coverage. The power output of the entire base station is 20 W, with their primary beam emitting radiation at an angle of 20°. The exposed animals were kept in a polypropylene cage on a neighbouring balcony which was horizontally in line with the MPBS. A power density measurement was done every day till completion of exposure (using HF-60105V4 spectrum analyser by Aaronia, Germany) and the average recorded power density at the site of exposure was 37.54 ± 1.01 mW/m^2 (corresponding electrical field 3.7 V/m) at the frequency of 1800 MHz. Apart from the selected MPBS, there is no other nearby MPBS (within 350 m) or any major source of anthropogenic electromagnetic fields such as electric transformer, high voltage electric power line, radio and television transmitters in the study site that could influence the results.

Measurement of the Specific Absorption Rate (SAR)

SAR, a measure of the rate of energy absorption from an electromagnetic radiation into biological tissue, was calculated using the formula; $SAR = \frac{\sigma E^2}{\rho}$ (W/kg). Where σ is the mean electrical conductivity of the tissues, E is the electric field at the exposure site (3.7 mW/m^2) and ρ is the mass density (Ferreira et al. 2006). Electrical conductivity (σ) value was obtained from http://www.fcc.gov/oet/rfsafety/dielectric.html (which is 0.94294 S/m). The calculated SAR value in all the exposed groups was 0.013 W/kg.

Processing of tissue sample for biochemical assays

At the end of 45 days of experimental period, the mice were taken back to the laboratory and all animals were euthanized through an overdose of ketamine followed by immediate excision of brain, liver, heart and kidney (Al-Batran et al. 2013). Organs were washed in ice-cold saline and blotted individually on filter paper. In total, 5% (w/v) tissue homogenate was prepared using ice-cold 1X PBS. The tissue homogenates were centrifuged at 13000 rpm for 30 min at 4°C and the supernatants were collected and stored at −80°C in aliquots until use for further biochemical analysis.

Biochemical assays

Protein contents of the brain, liver, heart and kidney were measured by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

Glutathione (GSH)

Glutathione (GSH) levels were measured using the method of Moron et al. (1979). GSH was measured by its reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in Ellman's reaction to give a compound that absorbs light at 412 nm. Briefly, 80 µl of the tissue homogenate was mixed with 900 µl of 0.02 M sodium phosphate buffer and 20 µl of 10 mM DTNB and incubated for 2 min at room temperature. The blank consisted of distilled water instead of tissue homogenate. The absorbance of the sample was read against blank at 412 nm. GSH concentration was calculated from the standard graph and expressed in µmol/mg protein.

Glutathione S-Transferase (GST)

Glutathione-s-transferase (GST) was measured using the standard method (Beutler 1984). Briefly, 50 µl of 20 mM CDNB was added to 850 µl of 0.1 M phosphate buffer (pH 6.5) and incubated for 10 min at 37°C. Then, 50 µl each of 20 mM GSH and tissue homogenate was added to the mixture. For blank, distilled water was added instead of tissue homogenate. The absorbance of the sample was measured at 1 min interval for 5 min at 340 nm. GST activity was measured as:

GST activity = (OD of test–OD of blank/9.6 × vol. of test sample) × 1000

where, 9.6 is the molar extinction coefficient for GST.

Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured by the nitroblue tetrazolium (NBT) reduction method (Fried 1975). Briefly, 100 µl each of tissue homogenate and 186 µM PMS was mixed with 300 µl of 3 mM NBT and 200 µl of 780 µM NADH. The mixture was incubated for 90 sec at 30°C and 1 ml of acetic acid and 4 ml of n-butanol were added to stop the reaction. The blank consisted of all the reagents, except the tissue homogenate. The absorbance of test and blank was measured at 560 nm and the enzyme activity was expressed in unit (1unit = 50% inhibition of NBT reduction)/mg protein.

% inhibition = (OD of blank–OD of sample/OD of blank)× 100

Lipid peroxidation (LPO)

Lipid peroxidation (LPO) was measured by the method of Beuege and Aust (1978). Malondialdehyde (MDA), one of the toxic products formed from the oxidation of

fatty acids such as polyunsaturated fatty acids (PUFA) and phospholipids, serve as a convenient index for determining the extent of peroxidation reaction of lipids. MDA derived from LPO reacts with 2-thiobarbituric acid (TBA) to give a red fluorescent adduct absorbing at 535 nm. Briefly, tissue homogenate was added to a mixture of 10% TCA, 0.8% TBA and 0.02 N HCl in 1:2 ratio. The mixture was boiled for 10 min in a boiling water bath, cooled immediately at room temperature and centrifuged at 1000 rpm for 10 min. The supernatant was collected and its absorbance was read at 535 nm against blank. The blank contained all the reagents minus the cell homogenate substituted with distilled water. The MDA concentration of the sample was calculated using the extinction coefficient of 1.56×10^6 $M^{-1}cm^{-1}$.

Haematological parameters and serum enzymes assays

Blood was collected from each animal by heart puncture using heparin-coated 1 ml syringe. Some amount of whole blood was used for the estimation of red blood cells count (RBC), white blood cells count (WBC) and haemoglobin content (Hb) according to standard protocols (Dacie and Lewis 1984). The remaining blood was centrifuged at 3000 rpm for 15 min at 4°C and serum was collected for enzyme assays. Activities of aspartate amino-transferase (AST) (EC 2.6.1.1), alanine amino-transferase (ALT) (EC 2.6.1.2) and the level of creatinine (CRE) were determined in serum using commercially available kits (Coral Clinical Systems, Uttarakhand, India).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Experimental data were analysed by one-way ANOVA followed by Tukey's test for multiple comparisons for different parameters between the groups. SPSS ver.16.0 software (SPSS Inc., Chicago, Illinois, USA) and Graph Pad Prism ver. 6.0 were used for statistical and graphical analysis. A p value of < 0.05 was considered statistically significant.

Results

Antioxidant/oxidant status

The levels and activities of the assayed antioxidants, and the quantified extent of oxidative damage to PUFA via MDA level in different tissue samples of mice were used as parameters to elucidate the possible adverse effects of RF-EMR exposure. Exposure of mice to RF-EMR from the base station for 12 hr and 24 hr per day resulted in a significant reduction of GSH content ($p < .01$), and activities of GST ($p < .001$) and SOD ($p < .001$) in the mice brain when compared to unexposed control group. Exposure of mice to RF-EMR also led to a significant ($p < .001$) rise in the MDA levels in the brain. However, exposure to RF-EMR for 6 hr/day only induced a significant change in SOD activity while there was no effect on GSH, GST or MDA level (Figure 1). In contrast to brain tissue, no significant changed was observed in the measured oxidative stress-related parameters in the heart, kidney and liver of mice after exposure to RF-EMR for 6 hr, 12 hr and 24 hr per day (Figure 2, 3 & 4). The only significant change observed was the reduced SOD activity in kidney tissue after 24 hr per day exposure (Figure 3).

Serum enzyme assays

Serum enzymes were assessed to investigate the hepatic and nephrotic toxicity induced by RF-EMR. The activities of aspartate amino-transferase (AST), alanine amino-transferase (ALT) and creatinine (CRE) level in different experimental groups are given in Table 1. There were no significant variations ($p > .05$) in the activities of AST and ALT, and on the level of CRE in the exposed mice when compared to unexposed control mice.

Haematological parameters

Peripheral blood was quantified to provide framework for understanding how RF-EMR alters the haematopoietic system. Total RBC count was decreased in 12 hr and 24 hr per day exposed groups when compared to unexposed control mice. Exposure of mice to RF-EMR for 24 hr/day led to a significant increase in total WBC count although 6 hr and 12 hr per day exposure induced no significant change. Similarly, no significant variation ($p > .05$) was observed in haemoglobin concentrations among the groups (Table 2).

Discussion

With the rapid increase in the deployment of wireless technologies, there is expressed concern from the scientific community (International EMF scientist appeal, 2015) and much public anxiety about the potential detrimental effects of exposure to RF-EMR. This is of particular concern for people residing close to MPBS as these populations are exposed to much higher ambient levels of RF-EMR continuously. The measured exposure in this study is typical near wireless transmitters and while being well within the current exposure guidelines, it is over 10^{18} times (a quintillion time) above the almost non-existent natural

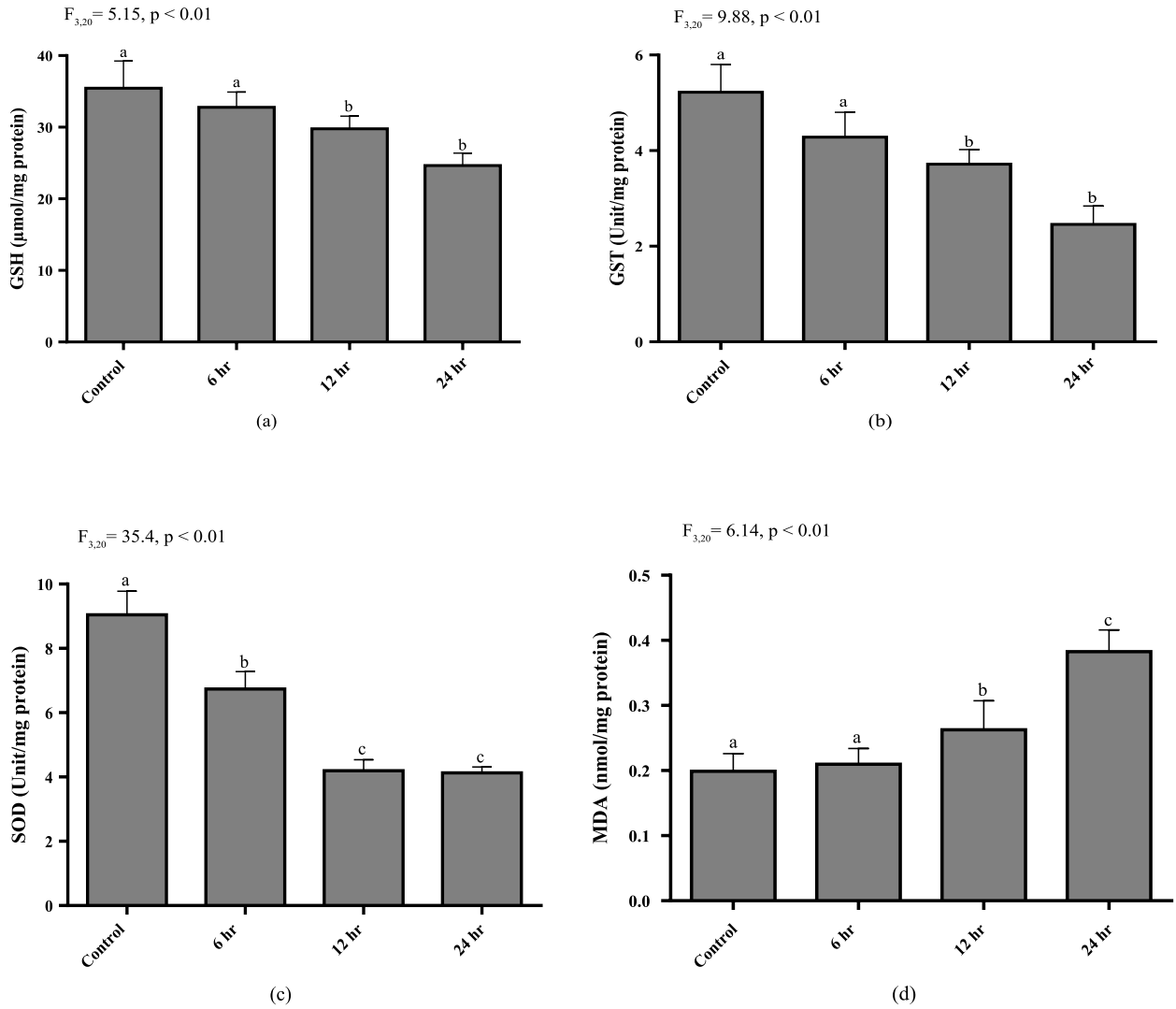


Figure 1. Effects of 1800 MHz RF-EMR exposure from a MPBS in murine brain. (A) reduced glutathione content (GSH); (B) glutathione-s-transferase activity (GST); (C) superoxide dismutase activity (SOD); and (D) MDA level, after different durations of exposure (6 hr, 12 hr and 24 hr per day). Means not sharing the same letter are significantly different.

background levels. A large number of studies have been conducted to investigate health-related effects of RF-EMR, most of which have provided evidence of biological impacts (Leach et al. 2018). However, there is still some uncertainty and no definitive conclusions have been reached by most public health authorities. Exposure to RF-EMR can lead to both thermal and non-thermal changes in living tissues. Non-thermal effects of RF-EMR have been reported to include activation of secondary chemical messengers that give rise to a range of downstream biochemical changes influencing biological functions such as growth, metabolism and apoptosis (Adair 2003; Challis 2005; Ertilav et al. 2018a). RF-EMR within living cells may cause an over-production of free radicals particularly reactive oxygen species (ROS), thereby inducing adverse effects in living cells (Yakymenko et al. 2016). Substantial evidence exist for RF-EMR induced enhanced production of ROS such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) leading to oxidative stress (Friedman et al. 2007) both *in vivo* (Meral et al. 2007; Tkalec et al. 2007) and *in vitro* (Yao et al. 2008) studies. The present study was performed to assess the potential effects of RF-EMR exposure on the redox status in different organs such as the brain, heart, kidney and liver in mice. Level and activities of serum toxicity markers including aspartate amino-transferase (AST), alanine amino-transferase (ALT) and creatinine (CRE), and haematological parameters including erythrocyte count, leucocytes count and haemoglobin content were also determined to investigate the potential effects of RF-EMR on the organ toxicity and hematopoietic system.

The levels of GSH content and enzymatic activities of GST and SOD were significantly reduced in mice brain after exposure to RF-EMR for 12 hr and 24 hr per day.

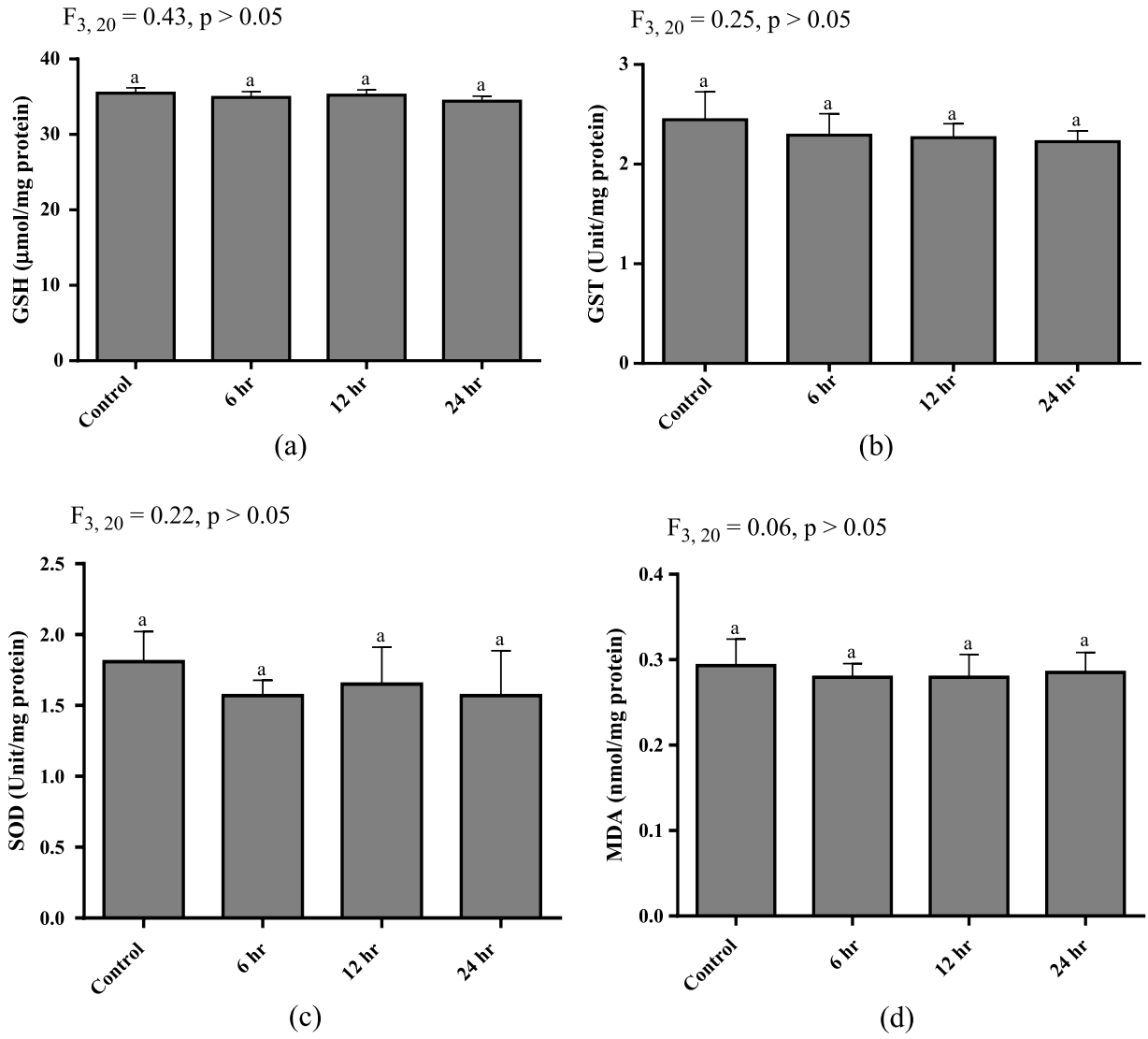


Figure 2. Effects of 1800 MHz RF-EMR exposure in murine heart. (A) reduced glutathione content (GSH); (B) glutathione-s-transferase activity (GST); (C) superoxide dismutase activity (SOD); and (D) MDA level, after different durations of exposure (6 hr, 12 hr and 24 hr per day) for 45 consecutive days. Means sharing the same letter indicates no significant difference.

Exposure of mice to RF-EMR for 12 hr and 24 hr per day also led to a significant increase in the lipid peroxidation indicated by MDA levels in mice brain. Activity of SOD was the only significant change observed in the brain after the shortest exposure (6 hr per day). Results of our study are in agreement with several recent animal studies where the activities and levels of antioxidants were significantly reduced in brain after RF-EMR exposure (Imge et al. 2010; Jing et al. 2012; Kerman and Senol 2012; Kesari and Behari 2009; Kesari et al. 2011; Kumar et al. 2010; Sokolovic et al. 2008). Male Wistar rats exposed to 50 GHz microwave radiation for 45 days continuously at a power level of 0.86 µW/cm^2 led to a reduction of GPx and SOD activity in brain cells (Kesari and Behari 2009). A similar effect has been observed earlier in the SOD and GSH-Px activities in the foetal rat brain after pregnant females were exposed to low level of RF-EMR from a cellular phone for 20 days (Jing et al. 2012). Moreover, exposure of male Wistar Albino rats to RF-EMR from cell phones with an average power density of 1.04 mW/cm^2 for 10 consecutive days led to oxidative stress and reduction in the activities of SOD, CAT, and GSH-Px in brain tissue (Kerman and Senol 2012). Sprague Dawley rats exposed to 900 MHz, 1800 MHz, and 2100 MHz microwave radiation with SAR values of 0.0845 W/kg, 0.04563 W/kg and 0.03957 W/kg, respectively, for 6 months (2 hr/day) also led to a reduction of total antioxidant status in brain tissue (Alkis et al. 2019). Furthermore, beta-amyloid protein, protein carbonyl, and malondialdehyde levels were found to be significantly

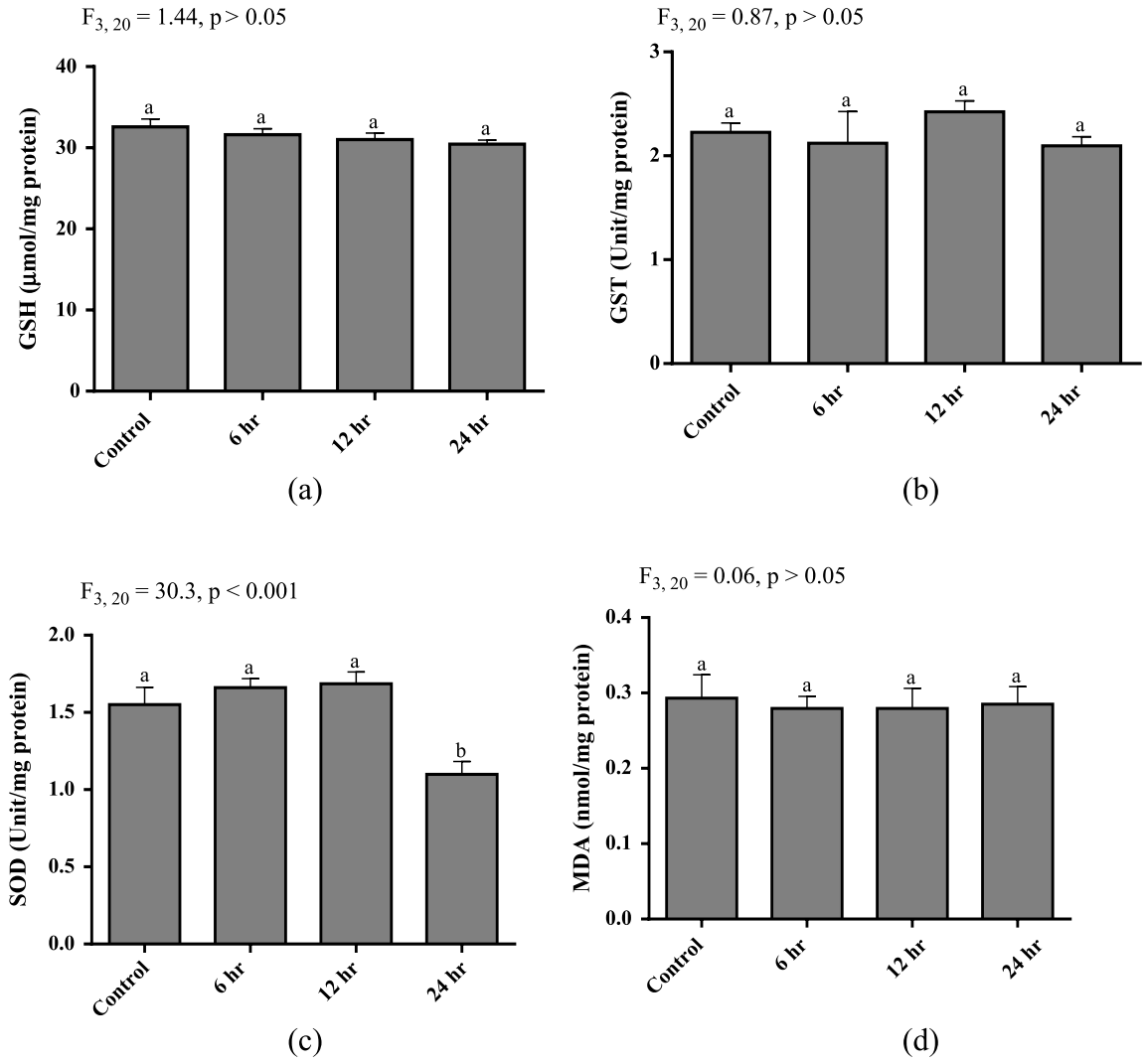


Figure 3. Effects of 1800 MHz RF-EMR exposure in murine kidney. (A) reduced glutathione content (GSH); (B) glutathione-s-transferase activity (GST); (C) superoxide dismutase activity (SOD); and (D) MDA level, after different durations of exposure (6 hr, 12 hr and 24 hr per day) for 45 consecutive days. Means not sharing the same letter are significantly different.

higher in the brain of rats exposed to 900 MHz radiofrequency radiation (Dasdag et al. 2012). However, some studies did not find a significant decrease in the activities and levels of antioxidants in brain tissue (Dogan et al. 2012; Ilhan et al. 2004; Maaroufi et al. 2011; Naziroğlu and Gümral 2009).

In the present study, the observed effects of RF-EMR in mice brain indicate interaction of RF-EMR with various cellular biochemicals leading to an increased production of ROS and resultant oxidative stress. Increased MDA from the direct oxidising chain reactions of ROS and fatty acids in the RF-EMR exposed groups serves as a marker of oxidative cellular damage. The parallel observation of reduced antioxidant activity further supports this because an overproduction of ROS would lead to an increased consumption of GSH, GST and SOD. RF-EMR has been reported to produce different free radicals earlier (Avci et al. 2009; Barcal et al. 2014; Burlaka et al. 2013; Kazemi et al. 2015). A resultant shift in the redox homeostasis in favour of oxidants which is termed oxidative stress is well-established to cause cellular dysfunction and cell death via direct oxidative modification of biomolecules and disruption of signal transduction pathways (Butterfield and Halliwell 2019; Ertilav et al. 2018a). There now exists strong experimental evidence for the ability of RF-EMR to produce highly reactive oxygen and nitrogen species (ROS/RNS) via radical pair mechanisms and thereby alter cellular biochemistry (Barnes and Greenebaume 2018). Oxidative is implicated in the pathophysiology of chronic diseases such as cancer, cardiovascular disease neurodegenerative diseases, liver cirrhosis and diabetes (Andreadis et al. 2003; Comhair et al. 2005; Kasparova et al. 2005). Brain is highly metabolically active due to the high neuronal density and as such it is a site of

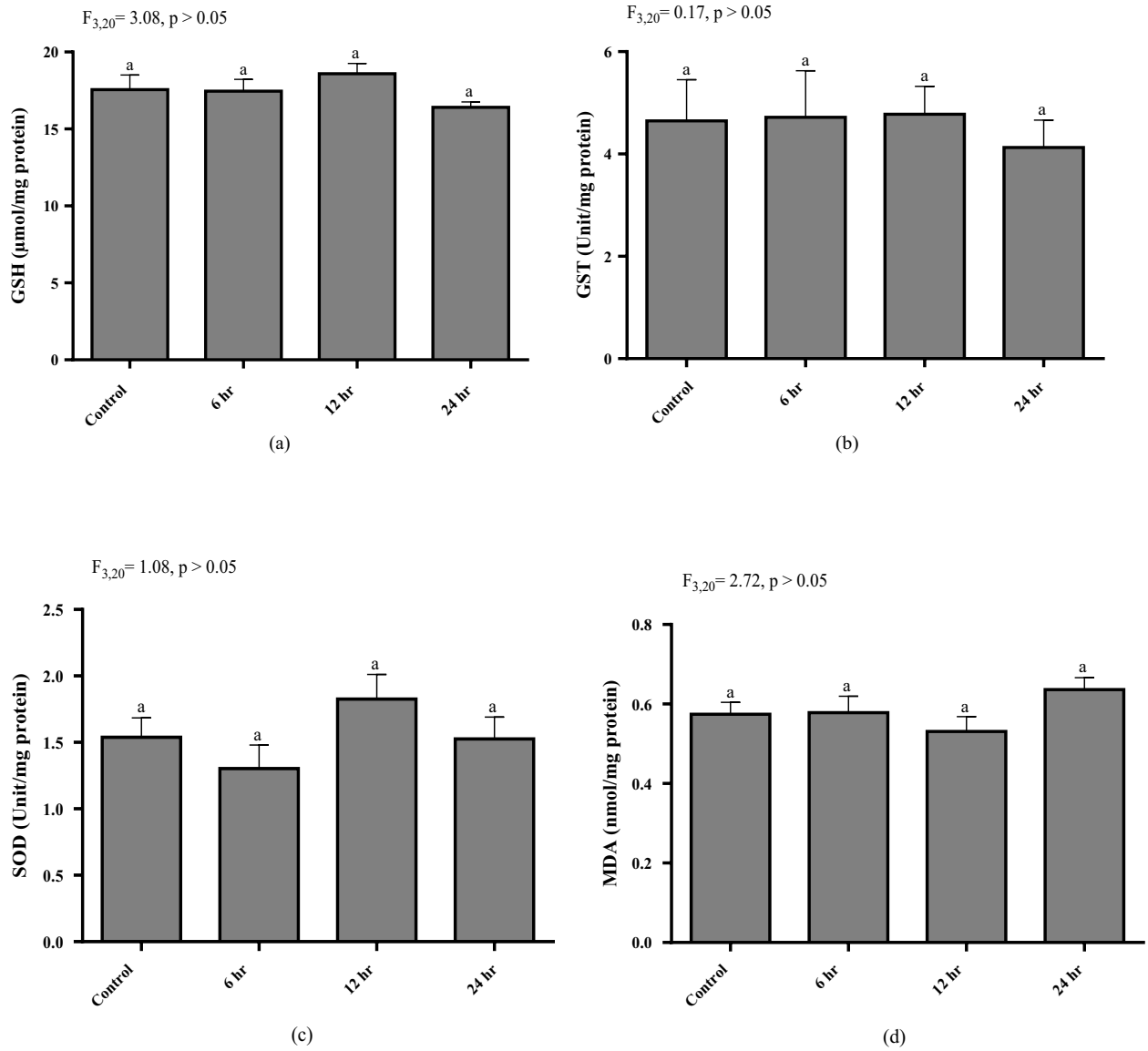


Figure 4. Effects of 1800 MHz RF-EMR exposure in murine liver. (A) reduced glutathione content (GSH); (B) glutathione-s-transferase activity (GST); (C) superoxide dismutase activity (SOD); and (D) MDA level, after different durations of exposure (6 hr, 12 hr and 24 hr per day) for 45 consecutive days. Means sharing the same letter indicates no significant difference.

Table 1. Effects of 1800 MHz RF-EMR on serum enzymes activities and CRE level.

Groups	ALT (U/L)	AST (U/L)	CRE (mg/dL)
Unexposed control	39.67 ± 2.61[a]	77.37 ± 3.92[a]	1.23 ± 0.09[a]
6 hr/day exposure	42.93 ± 2.14[a]	78.39 ± 2.59[a]	1.17 ± 0.12[a]
12 hr/day exposure	42.52 ± 2.96[a]	75.17 ± 3.40[a]	1.32 ± 0.11[a]
24 hr/day exposure	41.56 ± 0.83[a]	80.58 ± 6.59[a]	1.48 ± 0.09[a]

Values are expressed as Mean ± SEM. Means sharing the same letter are not significantly different at $p < 0.05$.

Table 2. Effects of 1800 MHz RF-EMR on different haematological parameters.

Groups	RBC (million/mm^3)	WBC (thousand/mm^3)	Haemoglobin (g%)
Unexposed control	3.96 ± 0.47[a]	1.44 ± 0.16[a]	11.33 ± 0.42[a]
6 hr/day exposure	3.53 ± 0.72[a]	1.65 ± 0.41[a]	11.37 ± 0.43[a]
12 hr/day exposure	2.03 ± 0.25[b]	2.00 ± 0.17[a]	12.25 ± 0.33[a]
24 hr/day exposure	2.35 ± 0.35[b]	3.93 ± 0.80[b]	11.50 ± 0.47[a]

Values are expressed as Mean ± SEM. Means not sharing the same letter are significantly different at $p < 0.05$.

high ROS generation as a by-product of glucose oxidation. A significant shift in redox homeostasis was observed in the brain indicating oxidative stress upon exposure of RF-EMR. This may be due to increased direct ROS production and also as a by-product of upregulated metabolic enzymes. RF-EMR has been shown to increase the glucose uptake under acute exposure conditions in human volunteers (Volkow et al. 2011). The high PUFA content in the brain makes it more sensitive to oxidative

damage mediated by ROS (Hamblin and Wood 2002). This is in fact shown by our data where increased MDA levels occurred in the brain but not in other organs tests under our experimental conditions. The first evidence for neuronal damage due to non-thermal RF-EMR exposure was reported by Salford et al. (2003). Deficient oxidant defence mechanisms and over-production of ROS have been reported to be linked with the initiation or progression of various neurodegenerative diseases such as amyotrophic lateral sclerosis (Bergeron 1995), Parkinson's disease (Fahn and Cohen 1992) and Alzheimer's disease (Benzi and Moretti 1995). Indeed, maintaining redox homeostasis is critically important for the brain to avoid neurodegeneration (Butterfield and Halliwell 2019).

It has been demonstrated that exposure of water molecules to RF-EMR led to generation of small amounts of $O_2^{\cdot-}$, H_2O_2, ozone (O_3) and atomic hydrogen indicating the ability of RF-EMR to directly induce free radical generation (Colic and Morse 1998). There are complex interactions of RF-EMR with aqueous systems such as biological matter which are not fully elucidated but profoundly change structure and reactivity of them. Radical pair mechanisms driven by electromagnetic fields provide theoretical explanations of the process of generation of ROS and other reactive species (Barnes and Greenebaume 2016). Superoxide dismutase (SOD) enzymes catalyze the dismutation of superoxide ($O_2^{\cdot-}$), the primary ROS produced during oxidative metabolism into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) thus preventing the cells from superoxide toxicity (Hayyan et al. 2016). The reduction of SOD activity in mice brain after RF-EMR suggests increased levels of $O_2^{\cdot-}$ which could be due to the high O_2 consumption (from aerobic metabolism) of the brain (Tait et al. 2008), which when exposed to RF-EMR resulted in an over-production of $O_2^{\cdot}$ GSH is a key small molecular antioxidant that serves as both scavenger of ROS as well as a co-factor in the catalytic action of antioxidant enzymes (Briviba and Sies 1994). The decreased level of GSH in mice brain after RF-EMR exposure suggests an excess utilization of GSH in ROS damaged tissue during detoxificatio of oxidised metabolites such as lipid peroxides (Aslan and Meral 2007).

In the present study, exposure of mice to RF-EMR did not induce a significant change in the assayed oxidative stress-related parameters (GSH, SOD, GST and MDA) in the heart and liver tissue of mice. Kidney exhibited signs of superoxide stress only in the highest exposure group that had 24 hr/day exposure. In contrast to our results, several studies have reported a decrease in the activities of antioxidant enzymes including CAT, SOD and GSH-Px, and increased in lipid peroxidation in myocardial tissue of animal exposed to low-intensity RF-EMR (Esmekaya et al. 2011; Kim and Rhee 2004; Ozguner et al. 2005; Ronchi et al. 2004; Turner et al. 2011). However, Türedi et al. (2014) observed an increased in SOD and CAT activity in the heart tissue of male rat pups after exposure of pregnant rats on days 13–21 of pregnancy to 900 MHz RF-EMR with power density of 0.5 W/m^2. Furthermore, the level of GSH in heart tissue of rat showed no significant changed after exposure to 2.45 GHz RF-EMR for 20 days (Turner et al. 2011). Dasdag et al. (2014) also reported no significant alterations in catalase, myeloperoxidase, total antioxidant capacity and oxidative stress index in the liver of Wistar Albino rats exposed to 900 MHz microwave radiation for 2 hours/7 days in a week during 10 months. This may be due to differences in test RF-EMR exposures, variations of genetic make-up, differences in exposure duration and interspecies differences. The majority of published studies in different animal models have reported significant increase in lipid peroxidation and a reduction in the activities of antioxidant enzymes such as SOD, CAT and GSH-Px in kidney (Kuybulu et al. 2016; Oktem et al. 2005; Özorak et al. 2013; Ragy 2015; Shahin et al. 2013) and liver (Chauhan et al. 2017; Koyu et al. 2009; Ozgur et al. 2010; Shahin et al. 2013) upon low-intensity RF-EMR exposures at different frequencies and power density. On the contrary, Fahmy et al. (2015) reported no significant change in GSH and nitric oxide (NO) levels, no histological or functional alteration in the rat kidney after exposed to 2.45 GHz for 24 hours a day over 40 days. Exposure of Wistar albino rats to 900 MHz EMR for 4 weeks caused no significant changed in the activities of SOD and GSH-Px in both kidney and liver tissues (Devrim et al. 2008). The lack of significant differences on assayed oxidative stress parameters do not mean that exposure to RF-EMR from base station is harmless for heart and liver tissue (or kidney where only SOD was affected). It can only be explained that under the experimental condition employed, those tissue did not show overt oxidative stress as exhibited by the brain with the markers used. Moreover, RF-EMR exposure might cause adverse effects to these organs through mechanism other than what was examined in our investigation which need further investigations.

AST and ALT are transaminase enzymes mostly found in the liver parenchymal cells and their levels in the serum indicate hepatic health (Lala et al. 2020). CRE is a by-product of muscle cell metabolism which is excreted by kidney through glomerular filtration. Nephrotic damage led to decreased CRE elimination resulting into their accumulation in serum (Howard 1989). Several animal studies reported increased level of CRE and activities of AST and ALT due to low-intensity RF-EMR exposure indicating the detrimental effects of RF-EMR in kidney and liver functions

(Moussa 2009; Assasa 2010; Ragy 2015; El Ghazaly et al. 2014; Kulkarni and Gandhare 2015). However, there are some studies that did not observe significant changes in the CRE level (Fahmy et al. 2015) and AST activities (Ibrahim et al. 2018) in the serum after RF-EMR exposure. Our findings did not include observable pathological damage inflicted on the liver and kidney by the RF-EMR exposure as there was no significant variations in the activities of AST and ALT, and on the level of CRE in the exposed mice when compared to unexposed control mice.

Our investigations into haematological effects of RF-EMR revealed a decrease in RBCs count with an increase in WBCs count after the longer (12 hr/day and 24 hr/day) exposures, but not the shorter (6 hr/d). These findings are in agreement with other studies where the RBCs count decreased and WBC counts increased following RF-EMR exposure (Assasa 2010; El Ghazaly et al. 2014). Usman et al. (2012) reported increase in WBCs count in 900 MHz RF-EMR exposed group and decreased WBCs count in 1.8 GMz RF-EMR exposed group irrespective of the exposure durations (4 weeks and 12 weeks) when compared to control group. However, longer exposure (12 weeks) led to decreased in RBC count in 900 MHz while it was increased in 1.8 GHz RF-EMR exposed group. On the contrary, prolong exposure (12 weeks) led to increase in Hb content in 900 MHz while it was increased in 1.8 GHz RF-EMR exposed group. The varying results in the serum toxicity markers and haematological parameters after RF-EMR exposure in different experiments can be attributed to differences in study design, durations and degrees of exposure and sources of RF-EMR (different wireless signals have different carrier frequencies as well as modulation frequencies).

In conclusion, our investigation provided evidence for induction of oxidative stress in the murine brain after prolonged exposure to a MPBS with multiple indicators of oxidative stress. Longer exposure duration caused more severe oxidative stress in the brain. Kidney tissue only exhibited reduced superoxide dismutase activity indicating superoxide stress only in the highest exposure group. No such effects were seen in the heart or the liver. In light of the findings of this study and many other studies providing such evidence of oxidative stress some of which have been referred to herein, measures aimed to reduce human exposure to RF-EMR are warranted.

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Disclosure statement

The authors report no conflict of interest.

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