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ORIGINAL ARTICLE

Exposure to mobile phone electromagnetic field radiation, ringtone and vibration affects anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats

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Abstract Research on the effects of Mobile phone radio frequency emissions on biological systems has been focused on noise and vibrations as auditory stressors. This study investigated the potential effects of exposure to mobile phone electromagnetic field radiation, ringtone and vibration on anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats. Twenty five male wistar rats were randomly divided into five groups of 5 animals each: group I: exposed to mobile phone in switched off mode (control), group II: exposed to mobile phone in silent mode, group III: exposed to mobile phone in vibration mode, group IV: exposed to mobile phone in ringtone mode, group V: exposed to mobile phone in vibration and ringtone mode. The animals in group II to V were exposed to 10 min call (30 missed calls for 20 s each) per day for 4 weeks. Neurobehavioural studies for assessing anxiety were carried out 24 h after the last exposure and the animals were sacrificed. Brain samples were collected for biochemical evaluation immediately. Results obtained showed a significant decrease ($P < 0.05$) in open arm duration in all the experimental groups when compared to the control. A significant decrease ($P < 0.05$) was also observed in catalase activity in group IV and V when compared to the control. In conclusion, the results of the present study indicates that 4 weeks exposure to electromagnetic radiation, vibration, ringtone or both produced a significant effect on anxiety-like behavior and oxidative stress in young wistar rats.

Keywords Mobile phone · Electromagnetic radiation · Vibration · Ringtone · Anxiety · Oxidative stress

Introduction

Global System for Mobile communication (GSM) network in Nigeria has replaced the services of the Nigerian Telecommunications Limited (NITEL). The current estimate in Nigeria is about 113 million connected GSM lines as at January 2012, with most people having more than one line and mobile phone (NCC 2012). In USA, an estimated 285 million people subscribe to mobile phone service while in most parts of Europe, developed parts of Asia, and Australasia, mobile phones are now virtually universal (Eyad 2012). As of 2011, there were more than 5.6 billion users worldwide (Brookes 2012). The use of mobile phone has become indispensable in our daily activities which attracts serious concern surrounding its possible health effects. This is because cell phones emit electromagnetic radiation (EMR) within the radio frequency (RF) range of the electromagnetic spectrum, which encompasses radio waves and microwaves. Mobile phones also use sounds and vibrations as alerting systems (Ahlbom et al. 2004).

In addition to limited studies on mobile phone effects in Nigeria and poor regulative capacity of its importation, there is increased importation of mobile phones that may be emitting more than standard level of electromagnetic radiation (EMR) as well as higher intensity of vibration and sound. Most of the cell phones safety recommendations are based on the basic sinusoidal wave, known as the carrier signal. However the danger does not come from this, but from abrupt

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ringtone, vibration and the modulated signal that actually carries the data or voice information that operates at higher frequency. The cell receptors respond to these signals, and get excited resulting in the impairment of delicate microtubular connections between the cells (Sareesh et al. 2009). However, more people now go for dual SIM phones due to their cheaper rates and additional SIM capacity. Unfortunately such phones have two transceivers built-in one and therefore emit more electromagnetic radiations, sounds and stronger vibrations than single SIM phones (Janet 2011). The radio waves emitted by a GSM handset can have a peak power of 2 watts, and a US analogue phone has a maximum transmitting power of 3.6 watts. Dual SIM phones may have up to 5 watts. The maximum power output is regulated by the mobile phone standard and by the regulatory agencies in each country (ICNRP 1998).

Studies investigating the effects of Mobile phone radio frequency emissions on biological systems reveal that Mobile phone induced oxidative stress in myocardium, endometrium, semen and semen fructose level in rats (Guney et al. 2007; Agarwal et al. 2009; De Iuliis et al. 2009; Salama et al. 2009). Vibrations of 20 Hz was also found to elevate the levels of plasma corticosterone, brain 5-hydroxy-tryptamine (5-HT) and 5-hydroxy-indole-acetic-acid (5-HIAA) in rats (Makoto and Akira 1983). While some investigators observed altered regional cerebral blood flow and increased permeability of Blood Brain Barrier of subjects exposed to electromagnetic fields from mobile phones (Nittby et al. 2008); others reported the deleterious effects on sleep, short term and spatial memory performances in wistar rats and humans (Hung et al. 2007; Sareesh et al. 2009).

Research has over the years focused on noise and vibrations as auditory stressors that can produce both direct and indirect health effects such as hearing loss with noise exposure higher than 90 decibels and other physiological reactions (Talbott and Thompson 1995). The presence of rattle sound and vibration has been found to increase the human body's reaction to noise, hence increasing the level of annoyance (Berglund and Hassmén 1996). At higher frequencies, the effects of individual photons of the radiation may become important, as these now have enough energy individually to damage biological molecules (Liebel et al. 2012). Children exposed to road or aircraft noise level were reported to have significantly decreased performances on complex tasks (van Kempen et al. 2009).

Reactive oxygen species (ROS) has been reported to play a crucial role in noise and vibration-induced tissue damage. Over the years, only few studies addressed the combine effects of noise and vibrations on behavioral outcomes and biomarkers of oxidative stress (Makoto and Akira 1983; Meral et al. 2007). Most of the studies carried out on sound and vibration focused on chronic exposure of the whole body systems on vibration or sounds produced by vehicles and machines (Marco 2002; Clark and Sörqvist 2012). The possible effects of vibration and sound produced by smaller machines like mobile phones have over the years received very little attention. Hence, a clear picture on whether EMR alone or in combination with vibration and sound can alter neurobehaviour and oxidative stress biomarkers will unfold more answers to how possibly mobile phone may affect the brain. Therefore, we investigated the anxiety-like behaviour and oxidative stress effects of exposure to Mobile phone electromagnetic field radiation, ringtone and vibration in young wistar albino rats for the period of four weeks.

Materials and methods

Equipment

Five (5) TV 20, Tecno Mobile phones; Portable animal cages; Elevated plus maze; A-weighed sound level meter (Version 2.0, NoiseWatch); Accelerometer (Version 1.5, VR9500. Vibration Research Corporation); Glass mortar; Dissecting sets, Syringes and needles, digital weighing balance.

Animals housing and management

Twenty five (25) Male Wistar albino rats weighing 180–200 g were used for this study. The animals were obtained from the Animal house of Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The animals were housed in standard polypropylene cages in groups of five and allowed free access to feed and water. The rats were allowed to acclimatize to the environment of the behavioural laboratory between 0900 to 1200 h for the period of one week before commencement of the experiment. The mobile phones were introduced to the animal cages during the acclimatization period to exclude the effect of handling, anxiety and novelty on behaviour. All experimental protocols were in accordance with the Ahmadu Bello University Research policy; and ethic and regulations governing the care and use of experimental animals (NIH Publication no. 85–23, revised 1996). The experiments were conducted in a quiet laboratory between hours of 900 h to 1600 h.

Animal groupings

The animals were randomly divided into 5 groups containing 5 rats each.

Group I animals were exposed to mobile phone in switched off mode (Control). Group II were exposed to mobile phone in silent mode (EMR). Group III were exposed to mobile phone in vibration (V) mode. Group IV were exposed to mobile phone in ringtone (R) mode. Group V were exposed to mobile phone in Electromagnetic Radiation, Ringtone and Vibration (EMR + R + V).

The animals in group II to V were exposed to 10 min call (30 missed calls for 20 s each) per day for 4 weeks, keeping a GSM (900/1800/MHz) mobile phone in the cage between 0900 to 1200 h. Animals were allowed to move freely in the cage (21.6″ × 13″ × 7.5″) and the phones were kept in a 4″ × 2″ × 1″ wood-bottom boxes throughout the study period avoiding animal's contact with phones (Raju et al. 2009; Sareesh et al. 2009).

A test cell phone GSM 900/1800 (model TV 20, Tecno) was used as the source of EMR. The same default ringtone was used in all the phones and using A-weighed sound level meter, the default ringtone was measured to be 76 dB. Vibration was also measured using accelerometer to be 0.47 m/s^2. However, we could not regulate interference on signal reception by environmental factors and signals from other vibrating bodies in the experimental area which could rise and fall and consequently affect electromagnetic radiation in our experiment.

Neurobehavioral study

The following anxiogenic studies were carried out for twenty four hours after the last exposure.

Assessment of anxiety-like behaviour using elevated plus maze (EPM)

The elevated plus maze (Lister 1987) was made with black painted woods. It has two closed and two open arms (50 × 10 cm) crossed at an open centre (5 × 5 cm) in plus shape. The closed arms were surrounded by high walls (40 × 10 cm) and the whole apparatus was raised to a height of 50 cm above the floor. The behavioural assessment was conducted in a red illuminated room (60 lx). Throughout the experiment, the observer stayed in the same room 1 m away from the maze. At the start of each trial, animal was placed on the central platform facing the open arm. A digital camera was mounted and the frequency and duration of 8 behavioural parameters were scored during a 5-min test for each rat after which the rat was removed from the maze to its cage. The maze was then cleaned with a solution of 70 % ethyl alcohol and permitted to dry between tests to avoid any olfactory cue.

The following behaviors were recorded:

1. Open arms entries: Frequency with which the animal entered the open arms. All four of the rat's paws were required to be in the arm to be counted as an entry.
2. Closed arm entries: Frequency with which the animal entered the closed arms. All four of the rat's paws were required to be in the arm to be counted as an entry.
3. Open arm duration: Length of time the animal spent in the open arms.
4. Closed arm duration: Length of time the animal spent in the closed arms.
5. Head dipping: Frequency with which the animal lowered its head over the sides of the open arm toward the floor.
6. Rearing: Frequency with which the animal stands on hind legs or leans against walls of the maze with front paws.
7. Defecation: Number of fecal boli produced.

Index of open arm avoidance

The Index of open arm avoidance could be drawn from the Trullas and Skolnick, (1993) formula:

$$\text{Index} = 100 - \frac{(\%\ \text{time on open arms} + \%\text{entries into the open arms})}{2}$$

Biochemical assessments

Assessment of lipid peroxidation

Samples of the brain were grinded in a cold glass mortar and homogenized (1 g of tissue/9 ml) in 100 mM phosphate buffer (pH 7.4). Malondialdehyde which is a measure of lipid peroxidation was measured quantitatively (Okhawa et al. 1979) using NWLSS™ MDA assay kit (Northwest Life Sciences Specialities, Product NWK-MDA01, Vancouver WA, Specificity: Malondialdihyde, Sensitivity: 0.08 μM). The level of thiobarbituric-acid reactive substance was determined based on the principle of its reaction with MDA to form MDA-TBA2 adduct that absorbs strongly at 532 nm (Janero 1990).

Assessment of antioxidant enzymes

Superoxide dismutase activity Samples of the brain were grinded in a cold glass mortar and homogenized (1 g of tissue/9 ml) in 100 mM phosphate buffer (pH 7.4). Activity of SOD was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The auto oxidation reaction was started by addition of freshly prepared pyrogallol solution to Tris– Hcl buffer at pH 8.5. The 50 % inhibition of hematoxylin by SOD was measured by spectrophotometer at 420 nm (Martin et al. 1987).

Catalase activity Catalase activity in brain homogenate was determined according to method of Beers and Sizer (1952), which is based on the principle of catalase consumption of H_2O_2 substrate at 240 nm. CAT activity was assessed using NWLSS™ CAT activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL).

Glutathione peroxidase activity GPx activity was assessed using NWLSS™ cGPx (GPx1) ELISA assay kit (Product NWK-GPX02, Specificity: Glutathione peroxidase, Sensitivity: 12.5 pg/ml). The NWLSS™ cGPx assay is based on a sandwich Enzyme-Linked Immunosorbent assay (ELISA), where sample GPx concentration is determined by comparing the 450 nm absorbance of sample wells to the absorbance of known standards (Tekebe 2002).

Statistical analysis

Data obtained were expressed as Mean ± SEM. Statistical analysis was carried out using SPSS version 22 and all analysis was done using one way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Values of $P < 0.05$ were considered significant.

Results

Neurobehavioral assessment

Table 1 represents the results observed in the elevated plus maze test. A significant decrease ($P < 0.05$) in open arm duration was observed [F (4, 18) = 3.514, $P = 0.027$)] in EMR (14.00 ± 5.03), EMR + V (13.50 ± 8.17), EMR + R (2.80 ± 2.56) and EMR + V + R (8.80 ± 4.07) exposed groups when compared to the control (43.50 ± 11.39).

Index of open arm avoidance

Figure 1 shows the Index of open arm avoidance. The control group shows lower value (27.88 ± 3.171 when compared to EMR (39.66 ± 2.789), EMR + V (42.00 ± 4.900), EMR + R (43.02 ± 4.531), and EMR + V + R (41.74 ± 3.540) respectively. Although there was no significant statistical difference observed.

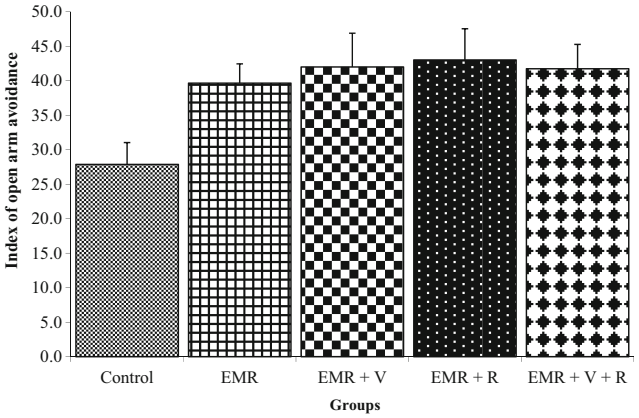

Fig. 1 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on Index of open arm avoidances in the study. Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$

Assessment of lipid peroxidation

The concentration of MDA in the various treatment groups and the control was presented in Fig. 2. There was no any significant statistical difference observed in MDA concentration in the various treatment groups when compared to the control group.

Assessment of superoxide dismutase activity

The activity of Superoxide Dismutase enzyme (SOD) in the treatment and the control groups was presented in Fig. 3. There was no any significant statistical difference observed in SOD activities in the various treatment groups when compared to the control group.

Assessment of catalase activity

The activity of Catalase (CAT) in the treatment and the control group was presented in Fig. 4. A significant decrease ($P < 0.05$) in the activities of CAT was observed

Table 1 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on neurobehaviour using elevated plus maze in the study

Parameter	Control	EMR	EMR + V	EMR + R	EMR + V + R
% Open arm entry	29.75 ± 2.81	16.00 ± 4.23	11.50 ± 7.79	13.00 ± 8.30	13.60 ± 5.91
% Closed arm entry	70.00 ± 3.000	84.00 ± 4.23	88.50 ± 7.79	87.00 ± 8.30	86.40 ± 5.91
Open arm duration	43.50 ± 11.39	14.00 ± 5.03*	13.50 ± 8.17*	2.80 ± 2.56*	8.80 ± 4.07*
Closed arm duration	221.50 ± 21.15	248.40 ± 15.43	237.50 ± 33.23	264.40 ± 10.84	241.20 ± 18.94
Head dipping	6.50 ± 1.44	5.40 ± 1.63	5.50 ± 1.44	3.40 ± 0.87	6.00 ± 1.67
Rearing	9.75 ± 2.25	7.80 ± 1.98	3.50 ± 0.65	6.60 ± 1.36	5.60 ± 1.29
Defaecation	2.25 ± 0.25	0.80 ± 0.80	1.00 ± 0.41	1.40 ± 0.87	2.40 ± 1.36

Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$; * Significance ($P < 0.05$)

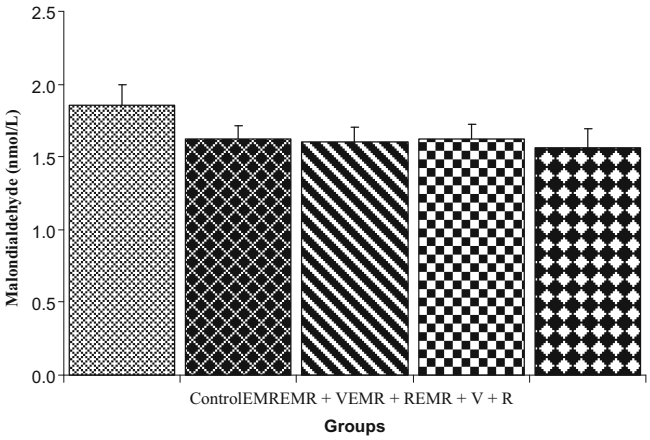

Fig. 2 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on Malondialdehyde concentration in the study. Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$

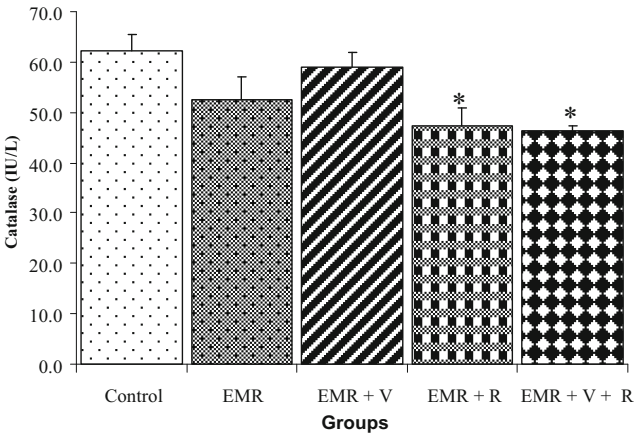

Fig. 4 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on Catalase activity in the study. Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$; * Significance ($P < 0.05$)

[F (4, 18) = 4.373, $P = 0.012$)] in EMR + R (52.60 ± 4.366 IU/L) and EMR + V + R (59.00 ± 2.944 IU/L) treatment groups when compared to the control (62.25 ± 3.301 IU/L).

Assessment of glutathione peroxidase activity

The activity of Glutathione Peroxidase (GPx) in the treatment and the control group was presented in Fig. 5. There was no significant statistical difference observed in the GPx activities in the various treatment groups when compared to the control group.

Discussion

This present study evaluated the combined effects of exposure to mobile phone electromagnetic field radiation, ringtone and vibration on anxiety-like behaviour and oxidative stress biomarkers in young albino wistar rats. Exploratory behaviours include open arm activity and head dipping a greater number of which indicate a greater level of exploration (Brown et al. 1999). In this study, a significant decreased in exploratory activities as measured by decreased time spent in open arm was observed in all the treatment groups when compared to the control. This corroborate with previous studies by some investigators who reported decreased exploratory activity and increase anxiety in EMR, vibration and noise exposed animals (Abbate et al. 2004; Khirazova et al. 2012). The statistical significance also seen between groups and the control confirmed the combined effects of EMR and other stressors (Raju et al. 2009), vibration and other stressors (Dormolen and Hertog 1994) as well as noise and other stressors (Smith et al. 1993). A decrease in percentage open arm entries, head dipping frequency, rearing and, defecation was also observed although not statistically significant. The index of open arm avoidance which is a measure of anxiety (Trullas and Skolnick

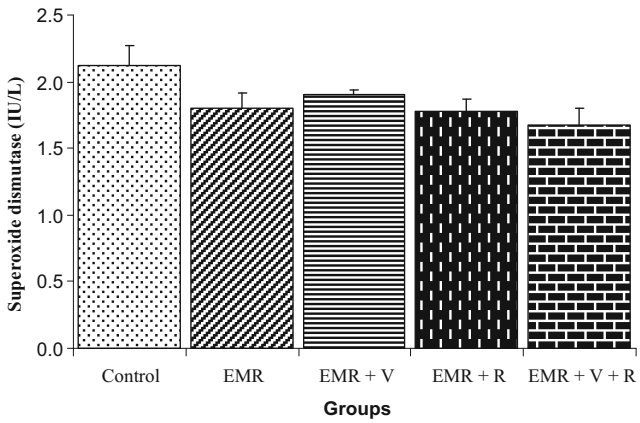

Fig. 3 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on Superoxide dismutase activity in the study. Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$

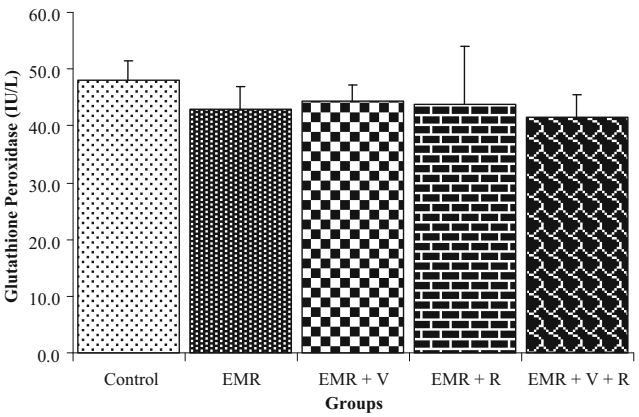

Fig. 5 Effect of exposure to mobile phone electromagnetic radiation, ringtone and vibration on Glutathione peroxidase activity in the study. Results presented as Mean ± SEM; EMR: Electromagnetic Radiation; V: Vibration; R: Ringtone; $n = 5$

1993) was also not statistically significant even though there was a general decrease in all the treatment groups when compared to the control.

The observed effects of EMR on anxiety-like behavioral parameters can be stipulated by changes in activity of dopaminergic, glutamatergic and GABAnergic brain structures described previously in rats exposed to GSM-range EMR (Mausset-Bonnefont 2004). The dysfunction of GABA signaling system has long been associated with behaviour and anxiety like related disorders. Both Gamma-Aminobutyric Acid (GABA) and glutamate are involved in regulation of the hypothalamic pituitary adrenal (HPA) axis (Di et al. 2009). While the GABAergic neurons in the hypothalamus can directly inhibit HPA axis via the hypothalamus and ultimately reduce cortisol secretion, glutamate activates the HPA axis by way of hypothalamus (Herman et al. 2004), hence increasing cortisol secretion as observed in our studies probably due to hyper-activation of HPA axis induced by electromagnetic radiation, vibration and ringtone.

The differences in the brain level of malondialdehyde (MDA) observed in the present study were not statistically significant in all the treatment groups when compared to the control even though slight decrease was observed in all the treatment groups when compared to the control. Similar results were obtained by Dasdag et al. (2003) and Aydogan et al. (2015) on the effects of EMR exposure to rats' testes and parotid gland respectively. However, other studies revealed increased in MDA concentration in the brain and other tissues on exposure to EMR and vibration produced by larger machines and cellular phone (Meral et al. 2007; Polat et al. 2013). The results obtained for the antioxidant enzymes in this study indicates no significant statistical difference in the level of superoxidase dismutase (SOD) and Glutathione peroxidase in all the treatment groups when compared to the control. This is in agreement with findings of Hirose et al. (2006) and that of Aydogan et al. (2015) who reported no significant changes in SOD, GPx and Catalase when compared to the control.

The involvement of oxidative stress in the pathophysiology of anxiety has attracted much attention over the years a causal link between the antioxidant status of the brain and anxiety-related behaviour has been hypothesized by group of investigators implicating two antioxidant enzymes; glyoxalase 1 and glutathione reductase 1 as key regulators of anxiety in mice and neuronal cell culture model of oxidative stress (Hovatta et al. 2005; Salim 2011). Others demonstrated that oxidative stress-related anxiety can be reversed in mice upon inhibition of NADPH oxidase or phosphodiesterase- 2, enzyme that is indirectly implicated in oxidative stress mechanisms (Masood et al. 2008). Results from our findings showed a significant decrease in Catalase activity in group IV (EMR + R) and V (EMR + R + V) when compared to the control. This conflicts the findings of Yurekli et al. (2006) and that of Khirazova et al. (2012) who reported increased in SOD, GPx and Catalase activity in EMR exposed animals when compared to control. Numerous investigators have reported contradictory findings on the effect of radiofrequency radiation on antioxidant enzymes and biomarkers of oxidative stress in different tissues which may be attributed to different biochemical, anatomical and physiological properties of the tissues (Devrim et al. 2008; Guney et al. 2007; Imge et al. 2010; Meral et al. 2007). While some animal studies have shown that oxidative stress develops in response to cell phone radiation by increasing production of reactive oxygen species or decreasing antioxidant enzyme activity to scavenge the free radicals produced which is similar to our findings (Oktem et al. 2005; Oral et al. 2006; Balci et al. 2007; Narayanan et al. 2014), others reported the up-regulation of the cells natural antioxidant enzymes (Yurekli et al. 2006; Khirazova et al. 2012).

The absence of significant effects on MDA, SOD and GPx seen in the present study may be compensatory in nature and postulated by mobilization of other endogenous antioxidants such as nitric oxide (NO), by other antioxidant defense system in response to lesser lipid peroxidation and generation of reactive oxygen species during the exposure. The affinity of NO for O_2^- was observed to be far greater than the affinity of superoxide dismutase (SOD) for O_2^-, hence, NO may compete with SOD for O_2^-, thereby removing O_2^- and sparing SOD and other endogenous or primary antioxidant enzymes for other scavenging duties (Das and Maulik 2006). This could also be a possible reason why exposure to EMR fails to up-regulate the activities of the indigenous antioxidant enzymes as observed in this study.

Another possible cause of the behavioral changes could be attributed to the increased activity of the HPA axis in exposed animals. Both physical and psychological stress experiences activate the HPA axis through the secretion of corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) (de Kloet et al. 2005). These neuropeptides activate the synthesis and the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which successively stimulates the adrenal cortex to synthesize cortisol and corticosterone in humans and rats respectively (Faravelli et al. 2012). These hormones influence several physiological processes and the synthesis of neurotrophic factors, with effects on mood and anxiety-like behaviours (Sapolsky et al. 2000; de Kloet 2003). However, whole-body vibration occurs when the body is supported by a vibrating surface (Griffin 1990). The vibration exposed groups in this experiment reflect a whole body vibration. Vibration produce by mobile phones on human is segmental rather than whole. Therefore, the result here cannot be translated into the effect of mobile phone vibration on human. However, it may slightly reflect the vibration caused by vehicles on man.

Conclusion

In conclusion, the result of the present study indicates that four weeks exposure to electromagnetic radiation or in combination with either vibration, ringtone or both produced significant effect on anxiety-like behaviours probably due to oxidative stress that could lead to the activation of HPA axis in young wistar rats. More studies are warranted to unveil the mechanistic pathways via which EMR, vibration and noise exert their biological effects especially in the brain.

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Compliance with ethical standards All experimental protocols were in accordance with the Ahmadu Bello University Research policy; and ethic and regulations governing the care and use of experimental animals (NIH Publication no. 85–23, revised 1996).

Conflict of interests The authors declare that there is no conflict of interests regarding the publication of this paper.

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