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Assessment of electromagnetic fields, vibration and sound exposure effects from multiple transceiver mobile phones on oxidative stress levels in serum, brain and heart tissue

Jamil Dauda Usman[a,d,*], Umar Mikail Isyaku[b], Rabiu AbduSSALAM Magaji[c], Adesoji Adedipe Fasanmade[d]

[a] Human Physiology Department, Faculty of Basic Medical Science, Federal University Dutse, Jigawa, Nigeria
[b] Human Anatomy Department, Faculty of Basic Medical Science, Federal University Dutse, Jigawa, Nigeria
[c] Human Physiology Department, Faculty of Basic Medical Science, Ahmadu Bello University, Zaria, Nigeria
[d] Physiology Department, Faculty of Basic Medical Science, University of Ibadan, Nigeria

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ABSTRACT

The present study evaluates oxidative stress markers in serum, brain and heart of albino Wistar rats after exposure to multiple transceiver mobile phone electromagnetic radiation, vibration and sound. Forty five (45) male albino Wistar rats weighing 140–180 g were randomly selected into nine groups ($n = 5$) viz; A serving as the control without mobile phone; groups B_1, B_2…E_1, E_2 were continuously exposed to electromagnetic field from double (1) and triple (2) transceiver mobile phone kept in various modalities with at least 10 min call per day within 9:00–21:00 h. Groups B_1 and B_2 were in silence mode, C_1 and C_2 in vibration, D_1 and D_2 in ring tone (sound), while E_1 and E_2 were in sound and vibration mode. After six weeks of exposure, the animals were sacrificed; serum, brain and heart were taken for oxidative stress assessment. Results showed that electromagnetic field radiation, vibration and sound from double and triple transceiver mobile phone cause decrease in the levels of glutathione peroxidase, superoxide dismutase, and malondialdehyde in the serum, heart and the brain. Catalase was observed to decrease only in the brain. Nitric oxide activities increased in the brain and heart of the treatment groups exposed to both phone types. Exposure to double or triple transceiver mobile phone radiation, vibration and sound causes oxidative stress to serum, brain and the heart.

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Introduction

Mobile phones are widely used in our routine day to day activities with subscription rate of more than 7 billion worldwide [1]. Increased mobile phone usage has recently led to rise in concerns about its potential health effects due to emission of high radiofrequency electromagnetic fields energies [2]. Mobile phones come with multimodal feedback system such as

* Corresponding author at: Human Physiology Department, Faculty of Basic Medical Science, Federal University Dutse, Jigawa, Nigeria.
E-mail address: jamso85@yahoo.com (J. Dauda Usman).

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visual, auditory and vibration [3]. In a very quiet environment or during formal or confidential meetings where distraction is not allowed, the visual feedback or alert system which has to do with silence mode is most commonly used. The auditory or sound feedback usage is mostly in a less busy or noisy environment, while the vibrotactile (vibration) feedback is suitable in lousy and noisy or public places [3,4]. The vibration frequency ranges from 130 to 180 Hz with an average of 151 Hz by most manufacturing companies. The average of 140–160 Hz is known to be sufficient enough for psychological recognition [4,5]. Report by Bliss et al., indicate that the human skin is sensitive to vibration frequency of about 100–600 Hz [6]. Previous findings have highlighted that radiofrequency energies emitted from mobile phones could cause some range of adverse biological effects, putting more preference to it oxidative stress effects [7–10]. The sounds from mobile phone ringtones at unwanted levels could result into noise. Epidemiological studies have revealed that noise is a nonspecific stressor, and chronic exposure to low level of noise like the once produce by mobile phone ringtone could cause non-auditory effects like disturbance of sleep, sleep fragmentation and communication, which results into emotional response such as annoyance and ensuing into oxidative stress [11,12].

Nowadays' people use phones that support multiple subscriber identification modules widely known as multiple SIM card phones [13]. Each SIM card is supported by an individual transceiver which aid in connecting or transmission of signal for communication [9]. The double and triple SIM/transceiver phones uses two and three transceivers respectively, for maintaining different network connectivity which allows user to have access to various network providers at once [9]. These double or triple transceivers mobile phones may emit more than the required minimum specific absorbance rate (SAR) values. Studies have revealed that frequencies within the range use of global system for mobile communication (GSM) could lead to imbalance in the reactive oxygen species (ROS) generation and antioxidant activity [13].

Antioxidants enzymes are known to provide defence system against this ROS generation, and reduced levels of some of this enzyme occurs as a result of increased level of ROS generation leading to alteration in the antioxidant defence system. Alterations in this defence system thus, can lead to pathophysiological mechanism in the biological system. Irregular rattle sound and vibration have been reported to increase level of annoyance in human [14]. Narayanan et al. [15] found that cell receptors are excited by these stimuli resulting to impairment in the microtubule connecting the cells.

Other authors revealed that radio frequency emissions in the range of mobile phone induce oxidative stress in endometrium, semen and semen fructose level in rats [16–18]. 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 [19]. Therefore, assessment of some of this antioxidant enzyme can act as an important biomarker to assess the level of oxidative stress to biological system due to mobile phone radiation exposure.

Most of the previous studies have based their research on machine simulations rather than the use of real time mobile phone, and none have assessed the level of oxidative stress damage sound or vibration from mobile phone could cause to the biological system except, Shehu et al. [9] that recently assessed vibration and sound on anxiety-like behavior and brain oxidative stress by double transceiver mobile phone. However, studies have not been done on the effects of double or triple transceiver mobile phone on oxidative stress in the heart or serum. The blood, heart and brain can be vulnerable to stress from mobile phone radiation exposure, as phones are kept in proximity to this tissue and organs whether in use or not. Therefore, it is important to assess the potential effects multiple transceiver mobile phones could cause to these organs and tissues. The aim of this work is to study and compare the effect of double and triple transceiver mobile phone electromagnetic radiation, its vibration and sound waves on the oxidative stress markers in the serum, brain and heart tissues.

Materials and methods

Apparatus

Standard plastic cages, Tecno Mobile Triple transceivers Phone (model: T312, Processor: MT6261D, with four Bands: GSM 850/900 MHz; DCS 1800/1900 MHz, CE0197), Tecno Mobile Dual transceivers Phone (model: T401, Dual Bands: GSM 900 MHz; DCS 1800 MHz CE0197), digital sound meter, accelerometer and fitted size mobile phone wooden box.

Animals care and grouping

Forty five (45) male albino Wistar rats were obtained from central animal house, College of Medicine, University of Ibadan. They were kept in standard laboratory conditions with 12 h light/dark cycle at room temperature of 25 °C with water and food *ad libitum* in the general animal house, Physiology Department, University of Ibadan. The animals were allowed to acclimatize for 1 week with the mobile phones switched off concealed in wooden boxes and placed into their cages in order to avoid novel behavior and effect of handling.

Experimental protocol

Using the modified method used by Shehu et al. [9], the Wistar rats were randomly selected into nine groups A, B_1, B_2 … E_1, and E_2 with five animals in each group as highlighted in Fig. 2.1 below. A served as the control without mobile phone, groups B_1, B_2, C_1, C_2, D_1, D_2, E_1 and E_2 were continuously exposed to electromagnetic field from double (1) and triple (2)

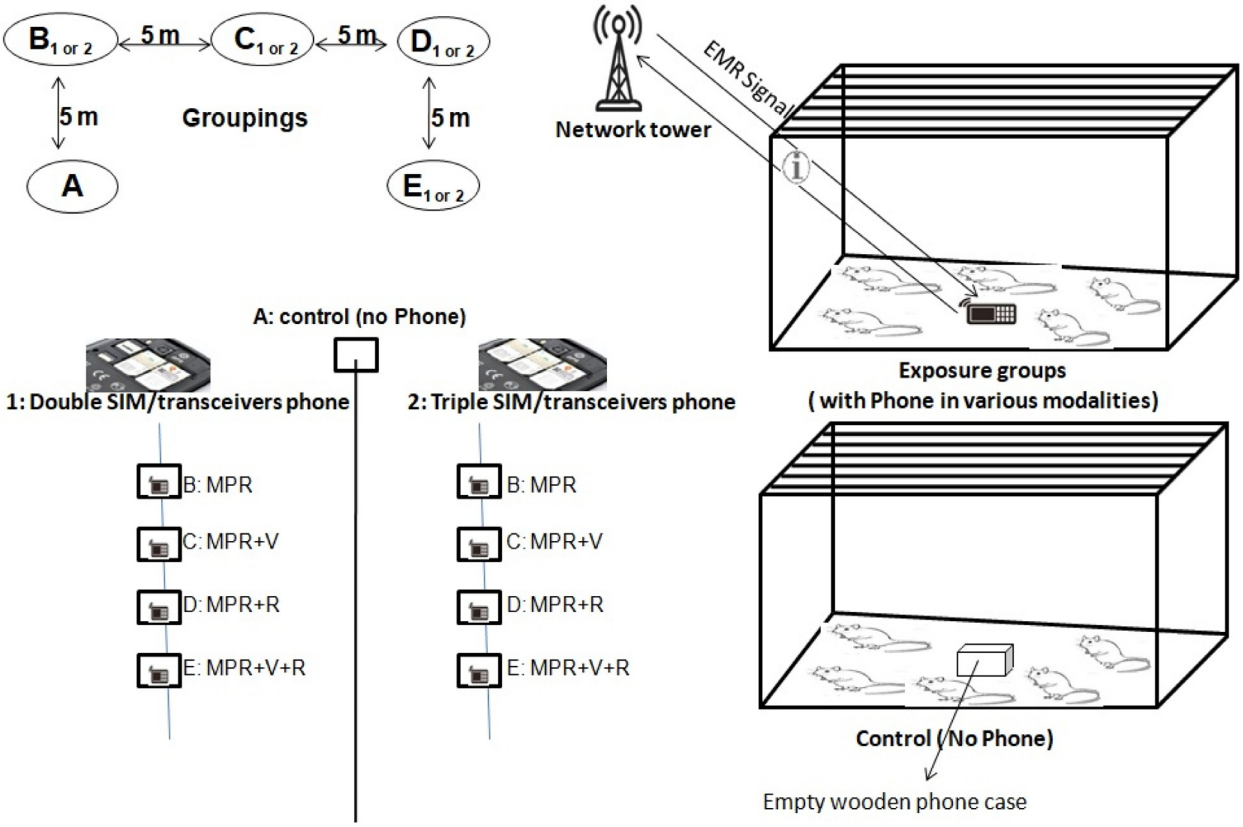

Fig. 2.1. Experimental Protocol; 1: double transceivers mobile phone; 2: triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V + R; $n = 5$.

transceiver mobile phones kept in various modalities with at least 10 min call per day within 9:00–21:00 h. Groups B_1 and B_2 were in silence mode, C_1 and C_2 in vibration, D_1 and D_2 in ring tone (sound), while E_1 and E_2 were in both sound and vibration mode. The default vibration level measured using accelerometer was approximately 0.45 m/s^2 and 0.46 m/s^2 for double and triple transceiver mobile phone, respectively. While the default sound level measured using digital sound meter was 72 dB for both mobile phone types. After six weeks of exposure, all the animals were sacrificed and their serum, brain and heart were taken for oxidative stress assessment. All experimental procedures and handling of animal were in accordance to University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) guidelines (approval no: UI-ACUREC/18/0041) and regulations governing care and use of experimental animals (NIH Pub. No. 85-23 revised 1999).

Serum and tissue homogenates preparation for biochemical studies

The animals were anesthetized with 0.1 ml per 100 g body weight of solution mixture of xylazine (40 mg/ml) and ketamine (25 mg/ml) (1:1), and their blood were collected in a plain sample bottles through intracardiac puncture and centrifuged using at 5000 rpm for ten minutes. Animals were later decapitated and their brains were immediately dissected out carefully on a dry ice. The harvested heart and the brain were weighed and homogenized using Teflon homogenizer machine (1 g of tissue per 4 ml) in an ice cold 100 mM phosphate buffer of pH 7.4. The heart and brain tissue homogenates were centrifuged at 10,000 rpm to get a clear tissue fluid for the oxidative stress biochemical assay.

Biochemical oxidative stress studies

Glutathione Peroxidase (GPx) assay

Glutathione Peroxidase (GPx) activity in the brain, heart and plasma was assessed using GPx assay kit obtain from Fortress diagnostics limited (Antrin, UK). The oxidized glutathione (by cumene hydroperoxide) is converted to reduced form in the presence of glutathione reductase and NADPH, with the NADPH simultaneously been oxidized to $NADP^+$. The total glutathione peroxidase concentration was quantitatively determined at absorbance of 340 nm.

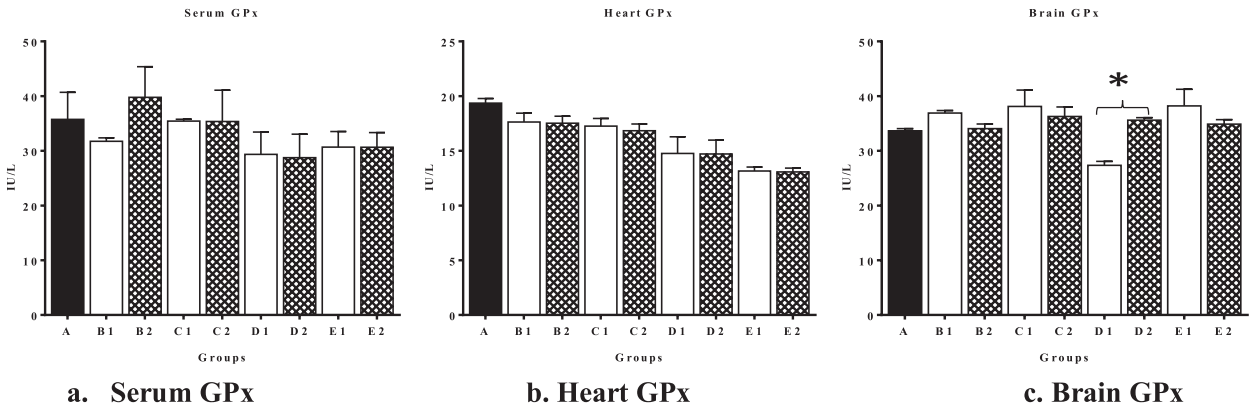

Fig. 3.1. Effect of multiple transceiver mobile phone on serum (a) Heart (b) and brain (c) tissue glutathione peroxidase after six exposure to mobile phone in various modality. Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V + R; $n = 5$; Significant at $^{*}P < 0.05$.

Superoxide dismutase (SOD) assay

The activity of SOD in the brain, heart and plasma was determined using SOD assay kits obtained from Northwest life science specialities (Vancouver WA 98662, USA). Samples from the serum, brain and heart were auto-oxidized using a freshly prepared pyrogallol solution with tris hydrochloric acid buffer of pH 8.5, while the 50% inhibition of haematoxylin by SOD was quantified at 420 nm using spectrophotometer [9].

Malondialdehyde (MDA) assay for assessment of lipid peroxidation

Using MDA assay Kit obtained from Oxford Biomedical Research (Vancouver WA 98662, USA), the MDA in serum, brain and heart tissues were quantitatively assayed. The thiobarbituric acid reactive levels were measured using the principle of its reaction with MDA to form MDA-TBA2 complex at 532 nm absorbance [9].

Catalase (CAT) assay

The activity of catalase in the heart, brain and plasma was quantified using the catalase assay kits obtained from Northwest life science specialities (Vancouver WA 98662, USA), and done based on the principle of H_2O_2 substrate catalase consumption at 240 nm absorbance.

Nitric oxide (NO) assay

Using NO colorimetric assay kit obtained from Oxford Biomedical Research (MI 48 371, USA), the nitrites/nitrates activities in the brain and heart tissue were quantified using the Griess reagent method [20]. The principle is based on reducing NO^{3-} to NO^{2-} in the presence of nitrate reductase enzyme. This reaction lead to diazotization of sulfanilic acid thereby forming NO^{2-} which then couple with N-(1-naphthyl)-ethylene diamine (azo dye) to form an optically visible medium at 540 nm absorbance.

Statistical analysis

Data were analyzed using GraphPad prism version 7 software. ANOVA and student *t*-test were done to compare means and the results were expressed as Mean ± SEM at *P*-value $P<0.05$

Results

Glutathione Peroxidase (GPx)

Glutathione Peroxidase (GPx) concentration decreased in the serum and heart in both phone types, and a significant differences was observed in the heart ($p<0.05$) of animal from groups D_1, D_2, E_1 and E_2 when compared to the control. When the corresponding treatment groups were compared, a significant decrease was observed in the brain GPx ($P = 0.001$) of group D_1 animals when compared with D_2 (Fig. 3.1(a–c)).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) concentration decreases in the serum and brain. Significant differences were observed in the serum of animals in groups B_1 and B_2 ($p<0.05$) [$F(8, 36) = 12.87$, $P = 0.00$]. The levels of heart SOD were not statistically

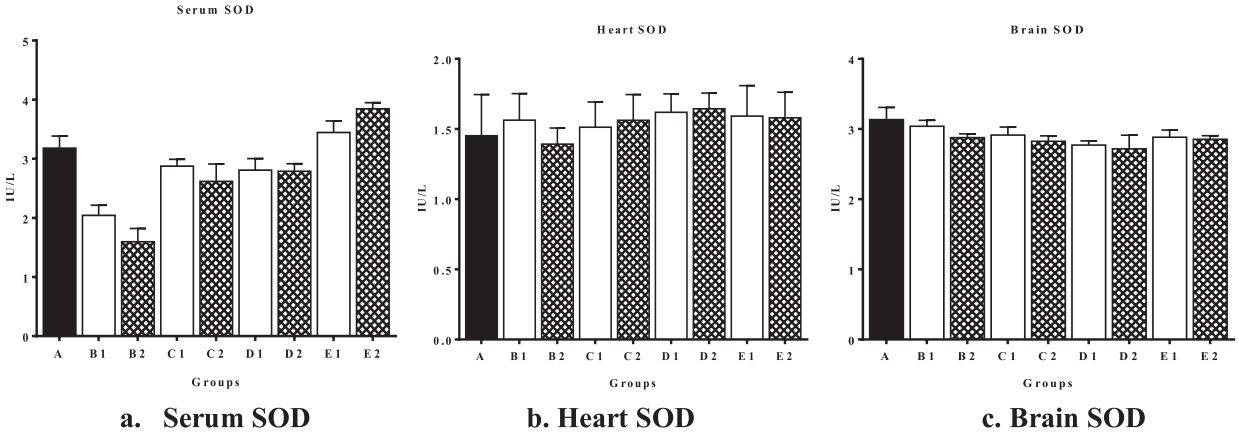

Fig. 3.2. Effect of multiple transceiver mobile phone on serum (a) Heart (b) and brain (c) tissue superoxide dismutase after six exposure to mobile phone in various modality. Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V + R; $n = 5$; Significant at $^{*}P < 0.05$.

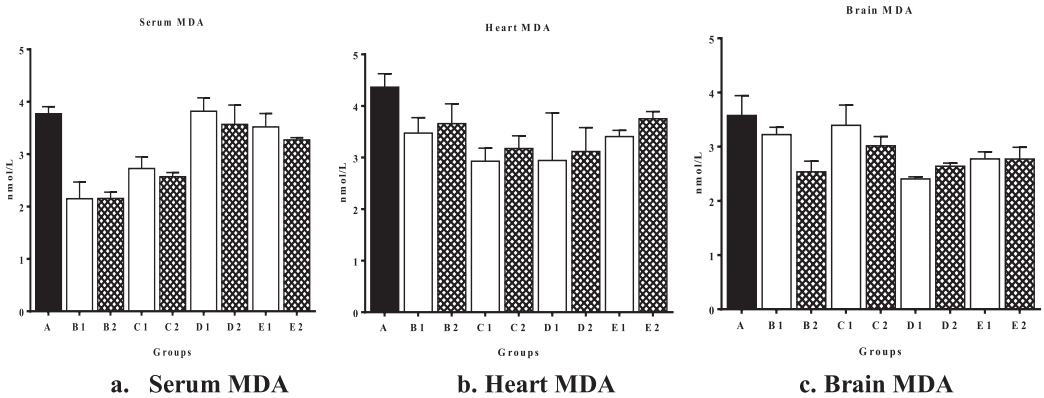

Fig. 3.3. Effect of multiple transceiver mobile phone on serum (a) Heart (b) and brain (c) tissue Malondialdehyde after six exposure to mobile phone in various modality. Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V + R; $n = 5$; Significant at $^{*}P < 0.05$.

different from the control values. When corresponding treatment groups were compared, no significant differences was observed (Fig. 3.2(a–c)).

Malondialdehyde (MDA)

Malondialdehyde (MDA) level decreased in the serum and brain and significant differences were observed in the serum of animals in groups B_1, B_2 and C_2 ($p<0.05$) [F (8, 36) = 8.84, $P = 0.00$] and brain of animals in groups B_2 and C_1 ($p<0.05$) [F(8, 36) = 3.38, $P = 0.005$]. The levels of heart MDA were not different from the control values statistically. When corresponding treatment groups were compared, no significant differences were observed (Fig. 3.3(a–c)).

Catalase (CAT)

Serum and heart CAT in the treatment groups were not different from the values of their controls. The brain CAT decreased significantly ($p<0.05$) [F (8, 36) = 10.58, $P = 0.00$] in animals of group B_2, C_2 and D_1 when compared to the control and their corresponding treatment groups [B_1 vs. B_2, C_1 vs. C_2, D_1 vs. D_2] (Fig. 3.4(a–c)).

Nitric oxide activities

No significant differences were observed in the nitric oxide activities in both brain and the heart across all treatment groups (Table 1).

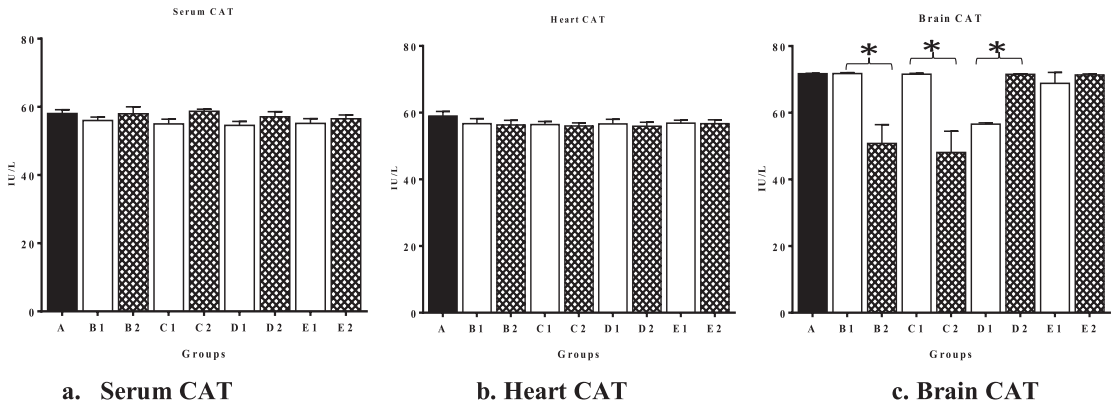

Fig. 3.4. Effect of multiple transceivers mobile phone on serum (a) Heart (b) and brain (c) tissue catalase (CAT) after six exposure to mobile phone in various modality. Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V+ R; $n = 5$; Significant at $^*P < 0.05$.

Table 1
Nitric oxide (μM) activities of the heart and brain after six exposure to mobile phone in various modality.

	Control	MPR		MPR ± V		MPR ± R		MPR ± V ±R	
	A	B_1	B_2	C_1	C_2	D_1	D_2	E_1	E_2
Heart	14.27 ± 0.51	15.74 ± 0.27	15.23 ± 0.61	15.71 ± 0.38	15.68 ± 0.82	15.92 ± 0.02	15.31 ± 0.64	16.39 ± 0.62	16.31 ± 1.10
Brain	47.06 ± 3.64	57.88 ± 3.21	55.88 ± 3.33	47.95 ± 4.89	48.97 ± 2.38	51.95 ± 1.42	48.02 ± 3.49	51.25 ± 1.12	49.34 ± 1.90

Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: Triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V +R; $n = 5$; Significant at $^*P<0.05$.

Table 2
Relative change in weight of the heart and brain after six exposure to mobile phone in various modality.

	Control	MPR		MPR ± V		MPR ± R		MPR ± V ± R	
	A	B_1	B_2	C_1	C_2	D_1	D_2	E_1	E_2
Heart	0.39 ± 0.01	0.43 ± 0.03	0.38 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.38 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.38 ± 0.02
Brain	1.05 ± 0.05	0.98 ± 0.03	0.96 ± 0.05	0.87 ± 0.09	0.86 ± 0.09	1.02 ± 0.02	0.83 ± 0.5	0.94 ± 0.02	0.84 ± 0.05

Data were expressed as mean ± SEM. 1: double transceivers mobile phone; 2: Triple transceivers mobile phone; MPR: mobile phone electromagnetic radiation; V: vibration mode; R: ring tone (sound) mode; A: control; B: MPR only (silence mode); C: MPR+V; D: MPR+R; E: MPR+V +R; $n = 5$; Significant at $^*P<0.05$.

Relative change of the brain and heart weight

Relative change in the brain weight decreased non-significantly across the entire treatment groups, while relative change in heart weight were not different from the control values (Table 2).

Discussion

The present study evaluates oxidative stress biomarker in the serum, heart and brain of Wistar rat exposed to electromagnetic radiation, sound and vibration from multiple transceiver mobile phones. Comparisons were also made to differentiate between the levels of oxidative stress in double and triple transceiver mobile phone. Oxidative stress is known to play major role in some pathophysiological conditions, and alteration in oxidative stress biomarkers (i.e. antioxidant enzymes) in tissues can give a very important clue about its relevance to oxidation stress in the biological system.

Exposure of Wistar rats to electromagnetic radiation (EMR) from mobile phone reduced the levels of some of the studied antioxidant biomarkers in the serum, heart and the brain. Serum glutathione peroxidase (GPx) decreased only in animals exposed to double transceivers' phones, while decreased heart tissue GPx was observe in animals that were exposed to either double or triple transceivers' phones in silence mode. Superoxide dismutase (SOD) levels in the serum, heart and brain decreased more in animals exposed to triple than the double transceivers' phones, while malondialdehyde (MDA) decreased more in those exposed to double than triple transceiver mobile phone. Catalase (CAT) decreased only in the brain especially in animals which were exposed to triple transceivers' phones. The above result suggests that electromagnetic radiation from multiple SIM/transceiver mobile phone cause increase in the levels of oxidative stress in serum, heart and the brain. However, triple transceiver mobile phone exposure gave more oxidative stress effects when compared to its double

transceiver counterpart. These differences were observed due to decrease in the antioxidant enzyme that is supposed to reduce the levels of reactive oxygen species (ROS) by scavenging free radicals from the biological system. More so, the triple transceiver mobile phone may also be emitting more radiofrequency electromagnetic energies than the minimum required specific absorption rate (SAR) registered with phone producer. Increase in SAR values can potentiate increase in the free radical formation. This agrees with the report of Ammari et al., [21] studies where model of rats' brain locally exposed to GSM-900 MHz signal by a head loop antenna with SAR of 1.5 W Kg^{-1} and 6 W Kg^{-1}, shows a compromised function in the activity of the specific redox-sensitive enzyme especially in the brain at the higher SAR values [21]. Shehu et al. [9] have also reported similar observation to the present work, which includes reduced level of catalase, GPx, SOD and MDA in brain exposed to phone electromagnetic energies. The altered level of these antioxidant enzymes were due to the over-production of ROS in brain and heart cells [22]. An increased amount of the ROS can cause cell death by apoptosis or by necrosis [23], which could be the possible reason for the reduction in weight of the heart and brain (Table 2). The activity of catalase and GPx has been shown to reduced in higher frequency radiofrequency electromagnetic radiation exposure compared to the control or normal individuals due to the impaired antioxidant status in serum or tissue homogenate [24]. Brain high levels of oxidative metabolism and heart mechanical activities have been reported to increase their metabolic needs, which make them vulnerable and sensitive to reactive oxygen species target [25]. The oxygen needed during their metabolic activities is converted into CO_2, water and some amount of O_2 forms ROS that reduces the level of the antioxidant enzymes [26]. The current work also agrees with Ilhan et al. [27] who have also reported a marked oxidative damage in brain tissues of rats that are exposed to 900 MHz signal for GSM (SAR of 2 W kg^{-1} in the brain) for 7 days. Experimental model of guinea pigs that was done by Meral et al. [28] also support the evidence of decrease in SOD, Catalase and GPx in blood, tissue homogenate of heart and brain after exposure of GSM signal of 890–915 MHz and SAR 0.95 W kg^{-1}, for 12 h/day for 30 days, even though an increase of MDA in present results contradicts the observation of this study.

Ringtone or sound treatment increased oxidative stress as evidenced by a decrease in the level of antioxidant enzymes in the brain and heart. This agrees with the recent finding on environmental noise exposure that suggests noise could potentially lead to oxidative stress [29]. Decrease in brain SOD observed here agrees with the results of Nair et al., [30]; Wilking et al., [31]; Roy et al., [32] and Kan et al., [33] who reported that unwanted sound can activate NADPH oxidase pathway in the brain thereby leading to NADPH oxidase subunit NOX2 to increase oxidative stress by increasing ROS production. Unwanted sound also mediates increase NADPH oxidase dependent superoxide production in the brain [33]. Increased level of Nitric oxide (NO) in the brain and heart was observed in the sound and vibration exposed group. The increased NO has been reported in the heart to be a biomarker that indicates increase oxidative stress in the myocadiac tissue [34,35]. The NO present could have reacted with super oxide in the serum, brain and the heart [35] to give peroxynitrite intermediate (ONOO-) which is a potent biological oxidant [35] that are cytotoxic which can induce oxidation modification of proteins [36]. Observation on sound contradict the report of Gannouni et al. [37] that rats expose to 70 or 85 dB at 8–16 kHz could cause an increase lipid peroxidation. They also observed upregulated level of antioxidant enzyme catalase and SOD.

Vibration was found not to cause any serious oxidative damage to the brain, serum or heart. This observation corroborates the findings of Hughes et al., [38] that CAT activities did not affect level ROS area without direct contact to vibration. They further explain that the induced soft tissue damage is depended on tissue stress and strain.

Changes observe in GPx and SOD caused by vibration lead to oxidative stress alternatively through an indirect cellular action of adrenocortical hormone in response to stress from sound and vibration [9]. Studies done by O'Brien shows that mechanical vibration induces a similar oscillatory displacement of counter ions outside the cell as elicited by electromagnetic fields exposure known as acousto-electric effect [39]. Di Carlo et al., [40] have also reports that mechanical vibration and electromagnetic fields simultaneously induce stress proteins, through counter-ion displacement, thus leading to oxidative stress that can alter biological function in the heart and brain. The effect of vibration was more pronounced when combined with the sound exposure. This implies that mobile phone usage in both sound and vibration mode can be more detrimental and potentiate more oxidative stress to the biological system.

Conclusion

Reduced level of some antioxidant enzymes in the serum, brain and heart indicates that exposure to radiation, vibration and sound from double or triple SIM GSM phone could cause oxidative damage to biological system.

Declaration of Competing Interest

None

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