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C-glycosyl flavonoid orientin alleviates learning and memory impairment by radiofrequency electromagnetic radiation in mice *via* improving antioxidant defence mechanism

Isaac O. Akefe[1]✉, Ibrahim L. Yusuf[2], Victoria A. Adegoke[3]

[1]*Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Jos, Nigeria*

[2]*Department of Veterinary Pharmacology and Toxicology, University of Maiduguri, Nigeria*

[3]*Department of Science and Laboratory Technology, Ekiti State University, Nigeria*

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ABSTRACT

Objective: To investigate the antioxidant efficacy of oral orientin on 900 MHz radiofrequency-electromagnetic radiation-induced oxidative stress in mice.

Methods: The mice were randomly allotted into 5 groups consisting of 7 mice each. The deionised water and radiofrequency electromagnetic radiations (RF-EMR) groups were administered with deionised water while orientin was administered to the RF-EMR + low dose of orientin group (10 mg/kg), RF-EMR + high dose of orientin group (20 mg/kg) and high dose of orientin group (20 mg/kg). All the groups except deionised water and high dose groups were exposed to 900 MHz radiofrequency-electromagnetic radiation for 28 consecutive days (1 h/day). Learning and memory was assessed *via* the step-down inhibitory avoidance task. Activities of lipid prexidation and antioxidant enzymes were measured using kits.

Results: Radiofrequency electromagnetic radiation caused impairment in learning and memory and reduced activities of brain antioxidant enzymes, increased lipoperoxidation and corticosterone concentration as well as histopathological aberrations in the hippocampal tissues. Conversely, orientin alleviated learning and memory deficit, improved the activities of endogenous antioxidant enzymes and mitigated brain lipoperoxidation and neuronal degeneration in mice exposed to radiofrequency electromagnetic radiation.

Conclusions: Orientin alleviates learning and memory impairment due to radiofrequency electromagnetic radiation in mice by improving antioxidant defence mechanism and may be considered as a promising therapeutic agent for improving the antioxidant system of people living in radiofrequency electromagnetic radiation-prone environment.

1. Introduction

Radiofrequency electromagnetic radiations (RF-EMR) originate from a wide range of natural and anthropogenic sources. However, the recent upsurge in technological advances has resulted in a remarkable rise in the magnitude of electromagnetic radiation with a corresponding proliferation of concomitant deleterious alterations in the central nervous system and other systems[1,2]. Non-ionizing RF-EMR emanating from 4G cell phones and Wi-Fi is currently an inexorable part of our existence. During communication, cell phones and their antennae engender RF-EMR ranging between 900-1 800 MHz. In addition, 4G mobile phones and wireless local area network

✉Corresponding author: Akefe O. Isaac, Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Jos, Nigeria.
Tel: +61 410546651
E-mail: akefei@unijos.edu.ng

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systems utilize radiations of 2 450 MHz[3]. Studies prove that RF-EMR exposure potentiates the risk of neuropsychological anxiety disorders[4,5]. van Bodegom *et al*[6] also reported that 2 450 MHz EMR exposure for 45 min activated the neuroendocrine system to secrete dynorphin, endorphins, and enkephalins which activate the hypothalamic-pituitary adrenal (HPA) axis and may be liable for anxiety. The most fundamental biological detriments of RF-EMR is oxidative stress, which arises from amplified assemblage of reactive nitrogen and/or oxygen species (RNS and/or ROS). These reactive species induce peroxidation of cellular phospholipids and loss of tissue integrity sequel to altered conductivity[7]. Sustenance of wellbeing depends on the balance in the execution of complex physiological radical reactions and disruption of this balance leads to cellular injury. Oxidative stress is a common disruptor of antioxidant homeostasis and is particularly important because a number of studies have found that it was correlated with several pathological disorders[8]. Biological organisms possess endogenous antioxidative machinery, which mitigates damages initiated by free radicals and the by-products[9]. This defence system acts by scavenging free radicals and impairing ROS triggered reactions[10].

However, following prolonged subjection to high doses of EMR, the antioxidant system becomes overwhelmed, subsequently, oxidative stress ensues[2]. Hussein *et al*[11] reported that three months of exposure to EMR induced a notable reduction in brain levels of antioxidant enzymes and increased malondialdehyde (MDA) levels in rats. Furthermore, neuronal degeneration correlating with excess expression of cyclooxygenase-2 gene for apoptosis and DNA fragmentation was seen in the hippocampus and cerebellum. In addition, the sympatho-adreno-medullary system and HPA axis are usually activated in chronic stress culminating in altered levels of corticosterone (CORT) and catecholamine[12]. Luteolin-8-*C*-glucoside (orientin) is a water-soluble flavonoid *C*-glycoside abundant in diverse medicinal plants including passion fruit, bamboo leaves and millet[13,14]. Several studies prove that orientin possesses several physiological properties, including antioxidant, neuroprotective and anti-inflammatory properties[8,14,15]. In spite of this, the protecting effect of this compound on RF-EMR-induced oxidative stress and the recovery mechanism from oxidative stress has not been demonstrated. This study used mice as models and was aimed to evaluate the protecting potential of orientin on EMR-induced oxidative stress changes by scrutinizing behaviour, neuroendocrine changes (CORT), alterations in anti-oxidative status as well as histopathological alterations in the brain.

2. Materials and methods

2.1. Experimental animals

Thirty-five male mice (13 weeks) weighing 25 to 30 g each served as models for this study. They were accommodated in groups of 5 in cages and kept under 25 ℃, 12 h light/dark cycles; 6 am-6 pm. All animals were pre-conditioned for two weeks and deliberate efforts were made to minimize stressing the mice during the experiment by ensuring only requisite handling. The mice were fed with commercially-formulated pelletized feed (Vital Feeds, Jos, Nigeria) and water *ad libitum*. This study was approved by the Animal Welfare and Central Research Committee of the Faculty of Veterinary Medicine, University of Maiduguri (AWCRC/2017/141), and all animals were treated in accordance with the National Institute of Health Guide for Care and Use of Laboratory animals[16].

2.2. Exposure and measurement of RF-EMR

Mice were exposed to 900 MHz electromagnetic radiation *via* cell phones having ceiling power output intensity of 2 W/kg [specific absorption rate (SAR); 1.25 W/kg]. The cell phones were fitted in a small wooden base wire-mesh cage, at the centre of the home-cage to prevent mice from having contact with any cell phones. Animals were subjected to radiation for 28 consecutive days (1 h/day). Each phone fitted in the cage was repeatedly activated by triggering 50 unattended calls per hour. All cell phones were purchased from one manufacturer with uniform SAR specifications. Power density measurement was verified using an Electro-smogmeter (9720, Meco Meters Ltd, India). The peak power density was recorded as 145.10 $\mu W/cm^2$ at about 2.9 cm away from the phones while they rang[17].

2.3. Drug and treatments

Orientin was procured from Sigma-Aldrich Chemical Company, USA (Cat No: 09765). It was reconstituted in 0.01% of dimethyl sulfoxide and administered by oral gavage at a daily dose of 10 mg/kg or 20 mg/kg body weight[8,15] for three consecutive weeks. Without discrimination, the mice were grouped into five groups (7 mice in each group): Deionised water (DW) group (unexposed to RF-EMR); RF-EMR group (exposed to EMR procedure); RF-EMR + low dose group (exposed to RF-EMR and low dose of orientin, 10 mg/kg body weight); RF-EMR + high dose group (exposed to RF-EMR and high dose of orientin, 20 mg/kg body weight); high dose group (with no RF-EMR exposure and high dose of orientin 20 mg/kg body weight). Both DW and RF-EMR groups were pre-treated with equal volume of deionised water and orientin, respectively for two weeks prior to EMR exposure and throughout the period of the study.

2.4. Evaluation of learning and short-term memory

The effect of the different regimens on learning was assessed *via* the step-down inhibitory avoidance task as previously described by Zhu *et al*[18]. The effect was appraised at 48 hours prior to the termination of all experiments. A 40 cm×25 cm×25 cm acrylic chamber was used with a base structured from stainless steel parallel bars of about 2-millimeter calibre, spaced a single centimetre apart. An 80 volts electric current was consigned *via* the stainless steel bars and a 2.5-centimeter high, 8 cm×25 cm platform made from wood was positioned in the left chamber boundary. The individual mouse was delicately transferred onto the platform. After a foot-shock was received, the ability of the mouse to linger on the platform before it steps down from the platform for the first time was the basis of this assessment. The index of learning was ascertained by how many times the mouse treaded down using both fore and hind limbs after receiving an electric shock. The highest ability of learning was recorded once a mouse lingered on the platform for 2 min without stepping down.

Spatial memory was estimated 24 hours later using constant procedure. In this instance, the individual mouse was once again carefully transferred onto the platform, and the time duration it lingered on the platform without stepping down was noted as an index of memory retainment. The highest memory was observed once the mouse persisted on the platform for 2 min.

2.5. Sample collection

At the termination of the study, all mice were sacrificed under anaesthesia with the jugular severed. Blood was retrieved and maintained at 4 ℃ throughout centrifugation at 4 000 rpm for 15 min. The brain tissues were excised, rinsed with normal saline and carefully dried. Afterward, the brain samples were weighed and homogenized in phosphate-buffered saline using a sonicator. The brain homogenates were maintained at 4 ℃ throughout centrifugation at 3 000 rpm for 15 min. The supernatants were retrieved to evaluate MDA levels and antioxidant enzymes activities.

2.6. Determination of MDA

To determine the concentration of MDA, we used the method described by Draper and Hadley[19]. The spectrophotometric evaluation of colour change during the reaction between thiobarbituric acid and MDA was performed. The level of MDA was assessed *via* the absorbance coefficient of MDA-TBA complex 1.56 $\times 10^5$/cm, calculated in nmol/mg protein.

2.7. Evaluation of activities of antioxidant enzymes

The activities of the brain antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)] were assessed using kits.

The SOD activity was determined by the North West Life Science Specialties (NWLSS™) kit *via* detecting the inhibition rate of hematoxylin auto-oxidation by superoxide. Briefly, 40 μL of assay buffer was added to each cuvette followed by 40 μL of individual samples. The mixture was incubated for 120 s. Thereafter, we added 40 μL of hematoxylin reagent and stirred immediately to begin the auto-oxidation response. Absorbance was measured at 560 nm every 10 s for 5 min.

The CAT activity was determined by the North West Life Science Specialties (NWLSS™) kits based on the depletion of hydrogen peroxide substrate at 240 nm. Concisely, 1 000 μL of sample dilution buffer was added to a dirt-free cuvette and fitted in the reference cuvette holder. Thereafter, we added 950 μL of working assay buffer to a dirt-free semi-micro UV cuvette. Then 50 μL of dilute sample or standard was transferred into the cuvette using a pipette and stirred immediately. The absorbance was measured at 240 nm.

The GPx activity was determined by the North West Life Science Specialties (NWLSS™) kit. The GPx level was determined by contrasting the absorbance of sample wells with the standard wells at 450 nm.

2.8. Measurement of CORT levels

Serum CORT levels were assayed using a commercial ELISA kit in line with instructions from the manufacturers (Adliteram Diagnostic Laboratories, USA).

2.9. Histopathological assessment

The histopathological assessment of hippocampal tissues was carried out as Drury *et al*[20] previously described. Concisely, the brains were cautiously isolated, then the hippocampus was dissected and fixed in Bouin's solution. The tissues were dehydrated by graded concentrations of ethanol and cleared using xylene before embedded in paraffin wax. Sections of individual brain samples at five micrometre thick were stained using haematoxylin and eosin and observed under a light microscope at ×400 magnification. The severity of histopathological aberrations was classed into 3: severe (+++), mild (+) and none (-).

2.10. Statistical analyses

Data were expressed as mean ± standard error of mean (mean ± SEM). Values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. GraphPad Prism, version 6.0 was used. Values of $P<0.05$ were considered significant.

3. Results

3.1. Effects of orientin on cognition

3.1.1. Effects of orientin on learning

The effects of low and high doses of orientin on learning are presented in Figure 1. The foot-shock count in the RF-EMR group was significantly higher than the DW group ($P<0.01$). Administration of orientin at doses of 10 mg/kg and 20 mg/kg significantly decreased number of foot-shocks ($P<0.05$ and $P<0.01$, respectively). The mice in the high dose group had the lowest count of foot-shocks.

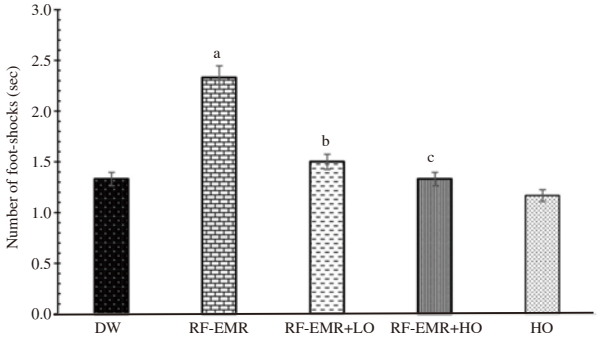

Figure 1. Ameliorative effect of orientin on learning of mice exposed to RF-EMR (Mean ± SEM, $n = 7$). [a]$P<0.01$ *vs*. DW group, [b]$P<0.05$, [c]$P<0.01$ *vs*. RF-EMR group. DW = Deionised water; RF-EMR = Radiofrequency-electromagnetic radiation; RF-EMR + LO = Radiofrequency-electromagnetic radiation + Low dose orientin; RF-EMR + HO = Radiofrequency-electromagnetic radiation + High dose orientin; HO = High dose orientin.

3.1.2. Effects of orientin on memory

The effect of orientin on memory is presented in Figure 2. Latency on the platform was significantly lower in RF-EMR group compared to the DW group (P<0.05); while the latency was significantly prolonged in RF-EMR + low dose group, RF-EMR + high dose group and high dose group (P<0.05).

3.2. Effects of orientin on serum CORT concentration

There was a significant rise (P<0.01) in the serum CORT levels of mice in the RF-EMR group compared to the DW group. The CORT concentration was significantly decreased in the RF-EMR + low dose group (P<0.05), RF-EMR + high dose group (P<0.01) and high dose group (P<0.01) (Figure 3).

3.3. Effects of orientin on activities of antioxidant enzymes

There was a significant decrease in activities of SOD, CAT, and GPx of the RF-EMR group compared with the DW group (P<0.05). The levels of SOD and CAT in the RF-EMR + high dose group rose significantly compared to the RF-EMR group (P<0.05). The increases in activities of GPx of the RF-EMR+ low dose group and the RF-EMR + high dose group were not significant (Table 1).

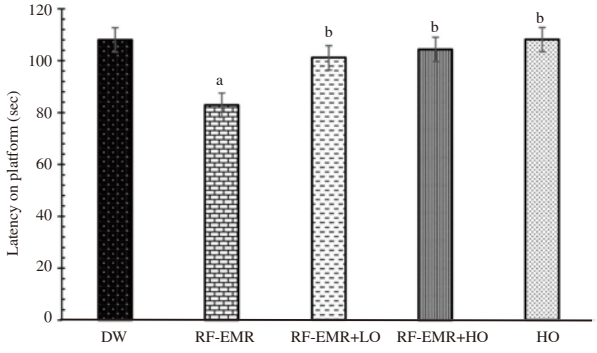

Figure 2. Ameliorative effect of orientin on memory of mice exposed to RF-EMR (Mean ± SEM, n = 7). [a]P<0.05 *vs*. DW group, [b]P<0.05 *vs*. RF-EMR group. DW = Deionised water; RF-EMR = Radiofrequency-electromagnetic radiation; RF-EMR + LO = Radiofrequency-electromagnetic radiation + Low dose orientin; RF-EMR + HO = Radiofrequency-electromagnetic radiation + High dose orientin; HO = High dose orientin.

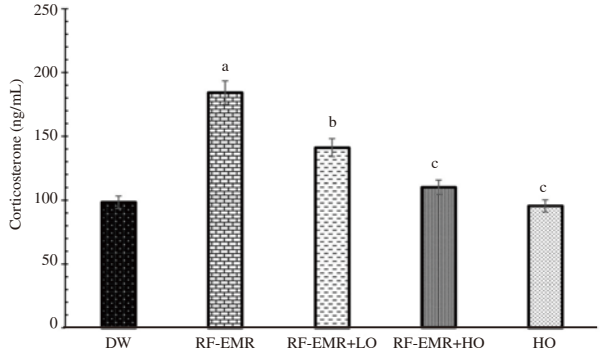

Figure 3. Ameliorative effect of orientin on serum corticosterone of mice exposed to RF-EMR (Mean ± SEM, n = 7). [a]P<0.01 *vs*. DW group, [b]P<0.05, [c]P<0.01 *vs*. RF-EMR group. DW = Deionised water; RF-EMR = Radiofrequency-electromagnetic radiation; RF-EMR + LO = Radiofrequency-electromagnetic radiation + Low dose orientin; RF-EMR + HO = Radiofrequency-electromagnetic radiation + High dose orientin; HO = High dose orientin.

3.4. Effects of orientin on MDA levels

MDA level of the RF-EMR group was significantly increased in comparison to the DW group (P<0.01), while its levels were significantly decreased in the RF-EMR + low dose group (P<0.05), RF-EMR + high dose group (P<0.01) and high dose group (P<0.01) (Figure 4).

3.5. Effects of orientin on hippocampal histology

Apparently unchanged architecture was observed in the hippocampus of mice in the DW group (Figure 5A), RF-EMR + high dose group (Figure 5D) and high dose group (Figure 5E). Moreover, the RF-EMR + low dose group revealed a slight distortion in the cytoplasm and architecture along with few Nissl substance (Figure 5C). Conversely, the hippocampus of RF-EMR group exhibited distorted neuronal architecture, degenerated neurons, disrupted neuronal cytoplasm and absence of Nissl substance (Figure 5B). Pretreatment of the mice with high dose of orientin (20 mg/kg) mitigated neuronal degeneration in the RF-EMR + high dose group (Figure 5D).

Table 1. Effect of orientin on the activity of antioxidant enzymes (Mean ± SEM, n=7).

Groups	SOD (IU/L)	CAT (IU/L)	GPx (IU/L)
DW	2.21±0.11	38.19±0.33	30.53±0.2 1
RF-EMR	1.72±0.30[a]	33.10±0.21[a]	26.10±0.10[a]
RF-EMR + LO	2.04±0.32[b]	37.12±0.24	27.81±0.13
RF-EMR +HO	2.19±0.10[b]	39.20±0.10[b]	29.51±0.21
HO	2.47±0.20[b]	43.92±0.11[b]	32.02±0.10[b]

[a]P<0.05 *vs*. DW group, [b]P<0.05 *vs*. RF-EMR group. DW = Deionised water; RF-EMR = Radiofrequency-electromagnetic radiation; RF-EMR + LO = Radiofrequency-electromagnetic radiation + Low dose orientin; RF-EMR + HO = Radiofrequency-electromagnetic radiation + High dose orientin; HO = High dose orientin.

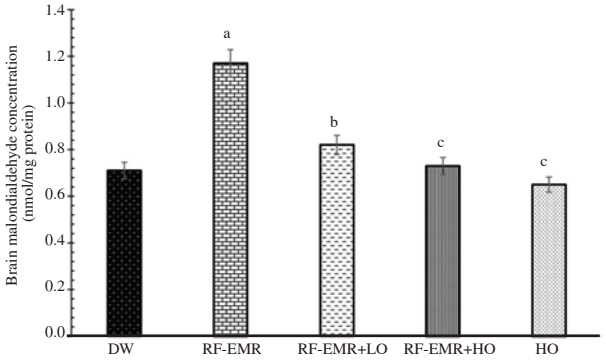

Figure 4. Ameliorative effect of orientin on brain malondialdehyde concentration of mice exposed to RF-EMR (Mean ± SEM, n = 7). [a]P<0.01 *vs*. DW group, [b]P<0.05, [c]P<0.01 *vs*. RF-EMR group. DW = Deionised water; RF-EMR = Radiofrequency-electromagnetic radiation; RF-EMR + LO = Radiofrequency-electromagnetic radiation + Low dose orientin; RF-EMR + HO = Radiofrequency-electromagnetic radiation + High dose orientin; HO = High dose orientin.

4. Discussion

The deleterious effects induced by RF-EMR emitted by cell phones and wireless local area network are increasingly investigated by scientists using various methods[5]. Our study showed that orientin, which is a *C*-glycosyl flavonoid and is abundant in bamboo leaves, millet and passion fruit, can serve as a potential agent to enhance endogenous antioxidant defence in mice exposed to RF-EMR. The rise in the number of foot-shocks and decline in latency on the platform in the RF-EMR group revealed that EMR impaired learning and acquisition of spatial memory in mice. Previous reports affirmed that EMR induce impairment in memory acquisition partly by triggering lipoperoxidation and oxidative stress[5,7,17]. Oxidative stress leads to abnormally increased production of free radicals, which overwhelms the scavenging capacity of the endogenous antioxidant system. This results in injury to vital biomolecules including proteins, lipids, and DNA[21]. It is worthy of note that the nervous system is most prone to oxidative injury owing to its higher requirement of oxygen, lower antioxidant activity rate and abundant concentration of poly-unsaturated fatty acids[22]. Consequently, the deterioration of neurons in the RF-EMR group can be attributed to oxidative damage due to EMR in mice hippocampus. Administration of orientin at low and high doses decreased the number of foot-shocks and increased latency on the platform, indicating that this antioxidant may be beneficial in mitigating impairment in cognitive function.

In addition, CORT level significantly increased after RF-EMR exposure, while orientin treatment markedly decreased serum CORT level. Functional responses to stress involve triggering the sympatho-adreno-medullary system and the HPA which is responsible for alteration in CORT levels[12]. Results from this experiment indicate that exposure to EMR induced hyperactivity of the HPA axis and triggered increased CORT secretion which is likewise implicated in learning and memory deficit.

Furthermore, it is reported that EMR could engender free radicals and modify endogenous antioxidant defence system[23]. Similarly, the subjection to EMR induced marked diminutions in endogenous antioxidant enzyme activities[24]. In contrast, orientin ameliorated cognitive decline in the RF-EMR + high dose group suggesting the role of oxidative stress in EMR-induced cognitive impairment. Moreover, orientin assuaged neuronal deterioration in RF-EMR + high dose group, and intact neurons were seen in high dose group, which was similar to the DW group. Orientin could exert its antioxidant function by upregulating the antioxidant system, and replenishing of glutathione in a dose-dependent manner during oxidative stress[8]. In line with previous studies, our findings demonstrated that orientin promotes the dismutation of superoxide anions to hydrogen peroxide and the catalysis of H_2O_2 into H_2O and O_2; thereby shields cellular constituents from the hydroxyl radicals-induced oxidative damage[12,25]. It is plausible to ascribe the elevation in antioxidant enzyme activities alongside the decrease in MDA of RF-EMR + LO, RF-EMR + HO and HO groups to orientin's potential to enhance the endogenous antioxidant system.

Furthermore, this study demonstrated that mice exposed to RF-EMR exhibit higher levels of hippocampal MDA indicating the elicitation of lipoperoxidation. Lipoperoxidation involves oxidative destruction of polyunsaturated fatty acids and consequently, diminishes fluidity of membranes and deactivation of enzymes bound to membranes[26]. EMR triggers lipid peroxidative alterations in systems by elevated levels of MDA[8] which is a major product of peroxidized polyunsaturated fatty acids and an indicator of oxidative stress[24]. In the current study, orientin inhibited lipid peroxidation in a dose-dependent manner. Previous reports indicated that orientin attenuated peroxidation of lipids by enhancing the

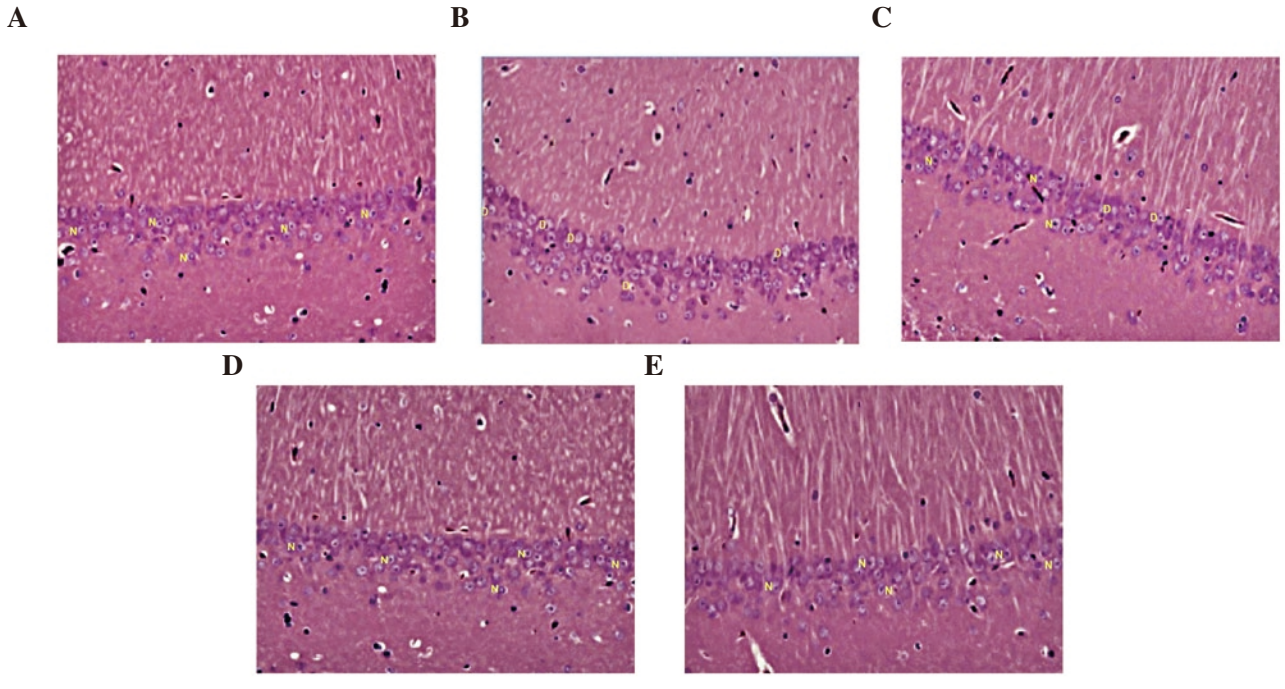

Figure 5. Photomicrograph of a section of the hippocampus of group DW (A), group RF-EMR (B), group RF-EMR + LO (C), group RF-EMR + HO (D), group HO (E). Normal neurons (N); degenerated neurons (D). H and E stain (×400).

activities of endogenous antioxidant enzymes and by scavenging free radicals[8,25].

Our results indicate that exposure to RF-EMR induced oxidative stress is characterized by impairment in cognition, increase in concentration of CORT, diminution in antioxidant enzyme activities, lipoperoxidation, and degeneration of neurons. Our results prove that 20 mg/kg body weight of orientin was more potent than 10 mg/kg body weight of orientin in mitigating cognitive impairment and neurodegeneration in mice, by enhancing antioxidant enzyme activities and inhibiting lipid peroxidation. This improvement in antioxidant defence mechanism suggests that supplementation with orientin-enriched foods might be a promising novel prophylactic therapy to mitigate oxidative stress induced by RF-EMR.

Conflict of interest statement

Authors declare that there are no competing interests.

Authors' contributions

Both YIL and AOI conceptualized this study and developed the theoretical formalism and methodology. AIO carried out formal data analysis. AAV performed the investigation, collected the data and drafted the initial manuscript. Both YIL and AIO contributed to the final version of the manuscript and supervised the project.

References

[1] Kaplan S, Deniz OG, Onger ME, Turkmen AP, Yurt KK, Aydin I, et al. Electromagnetic field and brain development. *J Chem Neuroanat* 2016; **75**(Pt B): 52-61.

[2] Kim JH, Yu DH, Huh YH, Lee EH, Kim HG, Kim HR. Long-term exposure to 835 MHz RF-EMF induces hyperactivity, autophagy and demyelination in the cortical neurons of mice. *Sci Rep* 2017; **7**: 411-429.

[3] Çiğ B, Nazıro lu M. Investigation of the effects of distance from sources on apoptosis, oxidative stress and cytosolic calcium accumulation *via* TRPV1 channels induced by mobile phones and Wi-Fi in breast cancer cells. *Biochim Biophys Acta Biomembr* 2015; **1848**: 2756-2765.

[4] Mohammed HS, Fahmy HM, Radwan NM, Elsayed AA. Non-thermal continuous and modulated electromagnetic radiation fields effects on sleep EEG of rats. *J Adv Res* 2013; **4**: 181-187.

[5] Gupta SK, Patel SK, Tomar MS, Singh SK, Mesharam MK, Krishnamurthy S. Long-term exposure of 2450 MHz electromagnetic radiation induces stress and anxiety like behavior in rats. *Neurochem Int* 2019; **128**: 1-13.

[6] van Bodegom M, Homberg JR, Henckens MJ. Modulation of the hypothalamicpituitary-adrenal axis by early life stress exposure. *Front Cell Neurosci* 2017; **11**: 87.

[7] Calcabrini C, Mancini U, De Bellis R, Diaz AR, Martinelli M, Cucchiarini L, et al. Effect of extremely low-frequency electromagnetic fields on antioxidant activity in the human keratinocyte cell line NCTC 2544. *Biotechnol Appl Biochem* 2017; **64**: 415-422.

[8] Thangaraj K, Natesan K, Settu K, Palani M, Govindarasu M, Subborayan V, et al. Orientin mitigates 1, 2-dimethylhydrazine induced lipid peroxidation, antioxidant and biotransforming bacterial enzyme alterations in experimental rats. *J Can Res Ther* 2018; **14**: 1379-1388.

[9] Santini SJ, Cordone V, Falone S, Mijit M, Tatone C, Amicarelli F, et al. Role of mitochondria in the oxidative stress induced by electromagnetic fields: Focus on reproductive systems. *Oxid Med Cell Longev* 2018; **2018**: 1-18.

[10] Kivrak EG, Yurt KK, Kaplan AA, Alkan I, Altun G. Effects of electromagnetic fields exposure on the antioxidant defense system. *J Microsc Ultrastruct* 2017; **5**: 167-176.

[11] Hussein S, El-Saba AA, Galal MK. Biochemical and histological studies on adverse effects of mobile phone radiation on rat's brain. *J Chem Neuroanat* 2016; **78**: 10-19.

[12] Liu Y, Gou LS, Tian X, Fu XB, Ling X, Sun LY. Protective effects of luteolin on cognitive impairments induced by psychological stress in mice. *Exp Boil Med (Maywood, NJ)* 2013; **238**: 418-425.

[13] Simirgiotis MJ, Schmeda-Hirschmann G, Borquez J, Kennelly EJ. The *Passiflora tripartita* (Banana Passion) fruit: A source of bioactive flavonoid *C*-glycosides isolated by HSCCC and characterized by HPLC-DAD-ESI/MS/MS. *Molecules (Basel, Switzerland)* 2013; **18**: 1672-1692.

[14] Lam KY, Ling APK, Koh RY, Wong YP, Say YS. A review on medicinal properties of orientin. *Adv Pharmacol Sci* 2016; **2016**: 1-9.

[15] Yu LJ, Wang SL, Chen X, Yang H, Li XX, Xu Y, et al. Orientin alleviates cognitive deficits and oxidative stress in Aβ_{1-42}-induced mouse model of Alzheimer's disease. *Life Sci* 2015; **121**: 104-109.

[16] Garber JC, Barbee RW, Bielitzki JT, Clayton LA, Donovan JC, Kohn DF, et al. *Guide for the care and use of laboratory animals.* 8th ed. Washington, DC: National Academies Press; 2011.

[17] Narayanan SN, Kumar RS, Paval J, Kedage V, Bhat MS, Nayak S, et al. Analysis of emotionality and locomotion in radio-frequency electromagnetic radiation exposed rats. *Neurol Sci* 2013; **34**: 1117-1124.

[18] Zhu HW, Rockhold RW, Baker RC, Kramer RE, Ho IK. Effects of single or repeated dermal exposure to methyl parathion on behaviour and blood cholinesterase activity in rats. *J Biomed Sci* 2001; **8**: 467-474.

[19] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 1990; **186**: 421-431.

[20] Drury RA, Wallington EA, Cancerson R. *Carlton's histopathological techniques*. 4th ed. New York: Oxford University Press; 1976.

[21] Halliwell B, Gutteridge J. *Free radicals in biology and medicine.* 4th ed. New York: Oxford University Press; 2007, p. 41-50.

[22] Moreira PI, Honda K, Liu Q, Santos MS, Oliveira CR, Aliev G, et al. Oxidative stress: The old enemy in Alzheimer's disease pathophysiology. *Curr Alzheimer Res* 2005; **2**(4): 403-408.

[23] Sepehrimanesh M, Nazifi S, Saeb M, Kazemipour N. Effect of 900 MHz radiofrequency electromagnetic field exposure on serum and testicular tissue antioxidant enzymes of rat. *Online J Vet Res* 2016; **20**: 617-624.

[24] Shahin NN, El-Nabarawy NA, Gouda AS, Mégarban B. The protective role of spermine against male reproductive aberrations induced by exposure to electromagnetic field-An experimental investigation in the rat. *Toxicol Appl Pharmacol* 2019; **370**: 117-130.

[25] Law BN, Ling AP, Koh RY, Chye SM, Wong YP. Neuroprotective effects of orientin on hydrogen peroxide–induced apoptosis in SH–SY5Y cells. *Mol Med Rep* 2014; **9**: 947-954.

[26] Goel A, Dani V, Dhawan DK. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos induced toxicity. *Chem–Biol Interact* 2005; **156**: 131-140.