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Myrtenal improves memory deficits in mice exposed to radiofrequency-electromagnetic radiation during gestational and neonatal development *via* enhancing oxido-inflammatory, and neurotransmitter functions

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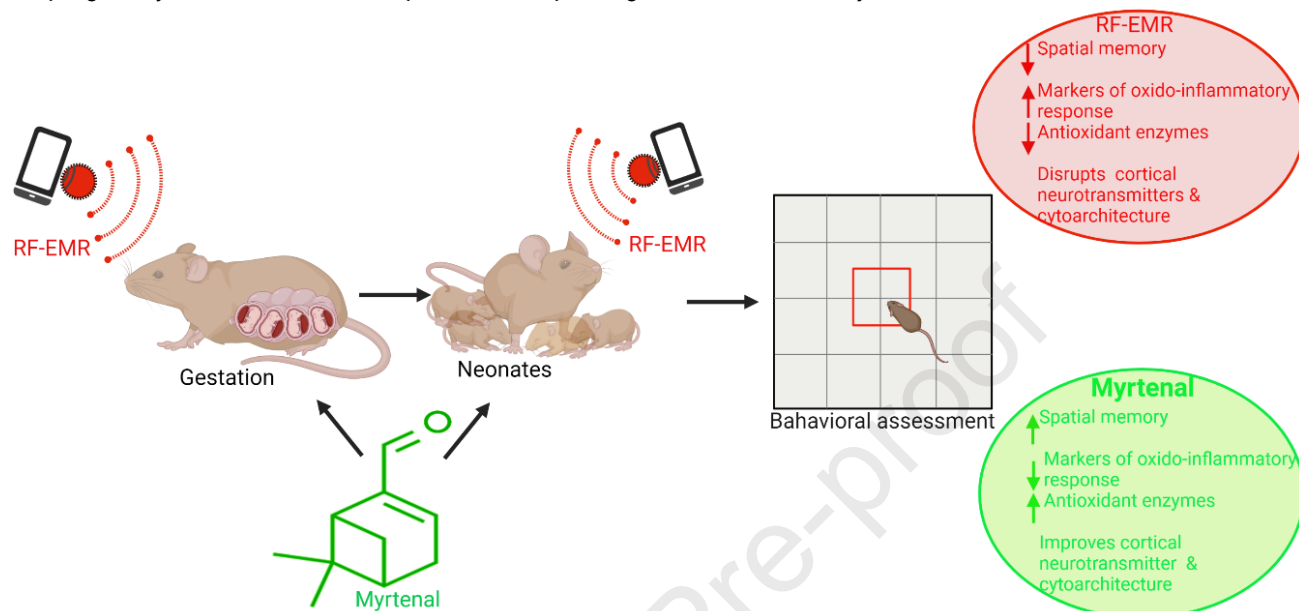
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Graphical Abstract

Myrtenal improves memory deficits in mice exposed to radiofrequency-electromagnetic radiation during pregnancy and neonatal development via improving oxido-inflammatory, and neurotransmitter functions



Abstract

Objective

Radiofrequency-electromagnetic radiation (RF-EMR) exposure during gestational and neonatal development may interact with the foetus and neonate considered hypersensitive to RF-EMR, consequently resulting in developmental defects associated with neuropsychological and neurobehavioral disorders, including learning and memory impairment. This study assessed the potential of Myrtenal (Myrt) to improve memory deficits in C57BL/6 mice exposed to RF-EMR during gestational and neonatal development.

Method

Thirty-five male mice were randomly allocated into 5 cohorts, each comprising of 7 mice. Group I was administered vehicle, Group II: RF-EMR (900 MHz); Group III: RF-EMR (900 MHz) + 100 mg/kg Myrt; Group IV: RF-EMR (900 MHz) + 200 mg/kg Myrt; and Group V: RF-EMR (900 MHz) + donepezil 0.5 mg/kg.

Results

Myrt treatment improved short-term memory performance in RF-EMR (900 MHz)-exposed mice by augmenting activities of endogenous antioxidant enzymes and proinflammatory cytokines, thereby protecting the brain from oxido-inflammatory stress. Additionally, Myrt restored the homeostasis of neurotransmitters in RF-EMR-exposed animals.

Conclusion

Results from the present study shows that exposure to RF-EMR impaired short-term memory in animals and altered the response of markers of oxido-inflammatory stress, and neurotransmitters. It is therefore conceivable that the recommendation of Myrt-enriched fruits may offer protective benefits for foeti and neonates prone to RF-EMR exposure.

Key Words: Myrtenal, Antioxidant, Radiofrequency-electromagnetic radiation, Memory, Neonates, Mice

Introduction

The recent surge in technological advancements has occasioned a notable rise in levels of radiofrequency electromagnetic radiation (RF-EMR) emitted by a variety of natural and anthropogenic sources [1-4]. The non-ionizing RF-EMR emitted by 5G cell phones and Wi-Fi has become an inextricable component of our daily lives [5, 6]. Notably, 2,450 MHz radiation is used by wireless local area network systems and 4G mobile phones. Most mobile phones produce RF-EMR in the 900-1,800 MHz range during communication [7]. These RF-EMR emissions levels adversely affect mammalian organ-systems and warrant research into potential mitigating alternatives.

Preceding studies indicated that the central nervous system, alongside other biological systems, is sensitive to RF-EMR with concomitant adverse effects [1, 8], particularly in pregnant females and offspring (given that they are more susceptible to the same level of environmental RF-EMR compared to the wider population) [4]. Notably, dielectric characteristics and organ conductivity influences RF-EMR absorption rates of tissues. The ability of the body to conduct electrical current rises in the course of pregnancy due to the higher fluid content, making the dam and foeti hypersensitive to RF-EMR [9]. Consequently, RF-EMR exposure during pregnancy interacts with the foetus, causing growth-related defects that could lead to neuropsychological and neurobehavioral disorders, characterised by memory impairment [1-4, 10].

Although neurobehavioral disorders are associated with neuroinflammation and disruption in neurotransmitter levels [11-13], oxidative stress ensuing from an increase in the production of reactive nitrogen and oxygen species constitutes a fundamental biological effect of RF-EMR on cognitive behaviour [14, 15]. These reactive species induce lipid peroxidation and loss of tissue integrity [16]. Hence, it is pertinent to maintain equilibrium between the endogenous

antioxidants and reactive species to minimise cellular harm and maintain optimum health [17-19].

Exogenous antioxidants has been shown to enhance the function of endogenous antioxidative machinery in biological organisms, thereby enhancing their capacity to scavenge free radicals and mitigate the associated damage [20]. However, the antioxidant potential of Myrt remains under-explored.

The bicyclic monoterpenoid compound, Myrtenal (Myrt), is commonly sourced from essential plant oils, such as rice [21], *astarte* [22], *Lavandula spp* [23], and *Cuminum cyminum* [24] making it readily available. Myrt is an isoprenoid lipid molecule with a number of biological activities, including anti-tumour and anti-apoptotic [25], antioxidant [20], anti-inflammatory [26], and regulation of mitochondrial and lysosomal enzymatic activities [27]. However, the neuroprotective function of Myrt and its beneficial role especially in mitigating RF-EMR associated cognitive dysfunction in young mice remains unclear.

In light of this, we reasoned that mitigating oxidative stress and improving the brain antioxidant status is a valid approach for minimising the pathology and expanding our current understanding of the mechanism underlying RF-EMR associated cognitive dysfunction in young mice exposed RF-EMR during prenatal stages of development. Consequently, we aimed to evaluate the potential of Myrt to improve memory deficits in subjects exposed to radiofrequency-electromagnetic radiation during pregnancy and neonatal development.

Materials and methods

Animals

This study was carried out in line with the approval of the Animal Welfare and Central Research Committee (AWCRC/2018/149), Faculty of Veterinary Medicine, University of Maiduguri. All

experimental subjects were treated in line with the National Institute of Health Guide for Care and Use of Laboratory Animals [28]. The animals were habituated in metal cages and maintained at a room temperature. They were exposed to 12 hr dark/light cycle and provided *ad libitum* feed and water. Minimal handling was ensured throughout the experimental procedure to minimise stress. Prior commencing the experiments, all subjects were pre-conditioned for one week.

Experimental design

Aside from the control animals, all the animals were exposed to RF-EMR for the duration of the pregnancy. A total of 35 newborn male mice were assigned by simple randomisation into five cohorts of seven animals each (7 days after birth). Each group consisted of seven animals treated as presented in table 1. The experimental mice were subjected to RF-EMR for 1 hr/day throughout the gestation period and from days 7-28 of neonatal development. Control pregnant mice, and their offspring were housed under the same cage condition, and for the same duration, without radiation exposure [29].

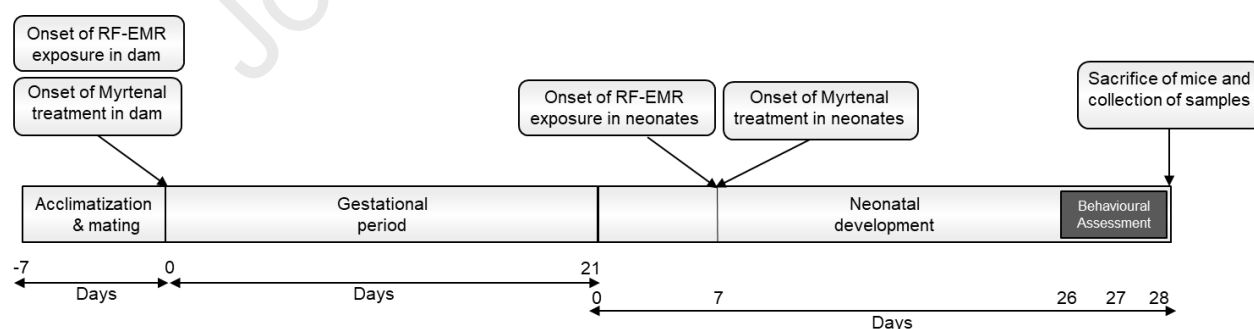


Figure 1: Diagram illustrating the experimental design

Drug Dosage and Treatment

Myrt obtained from Sigma Aldrich was reconstituted in corn oil and orally administered at 100 and 200 mg/kg body weight (low and high dose respectively) [30]. Simultaneously, the control

group was treated with 10 ml/kg of corn oil for three consecutive weeks (days 7-28). Based on a previous LD₅₀ report [31], the dosage of Myrt was selected. Similarly, donepezil (DP) powder was resuspended in 0.9 % saline and dispensed at a dose rate of 0.5 mg/kg, i.e. (0.9 % w/v) (Kumar and Singh 2017). All drugs were prepared freshly prior usage. Both control and the RF-EMR animals received an equal volume of corn oil and Myrt (10 ml/kg) throughout the period of the study.

Table 1: Experimental groups and treatment

Groups (n = 7 each)	Treatment
I	Vehicle alone
II	RF-EMR (900 MHz)
III	RF-EMR (900 MHz) + myrt (100 mg/kg)
IV	RF-EMR (900 MHz) + myrt (200 mg/kg)
V	RF-EMR (900 MHz) + donepezil 0.5 mg/kg

RF-EMR exposure and measurement

Mice used for this experiment were subjected to 900 MHz RF-EMR using cellular phones with a maximum output power of 2 W/kg [Specific Absorption Rate (SAR); 1.25 W/kg]. To avoid contact of mice with the phones, a tiny wooden foundation wire-mesh coop was placed in the centre of the cage. The mice were then subjected to radiation throughout pregnancy and for 28 days following pregnancy (1 hour every day). Each cell phone was activated 50 times per hour by automatically triggering untended calls. All the cellular phones were acquired from the same company and had the same SAR parameters. An electrosmog meter was used to verify the power density measurement (9720, Mecometrics Ltd, India). At roughly 2.9 cm distance from the phones the ringing, the highest power density was reported as 145 $\mu\text{W}/\text{cm}^2$ [32].

Behavioural assessment

Open Field Test (OFT)

The open-field apparatus is comprised of a 50 × 50 × 45 cm wooden box padded using a translucent Perspex on the inside. The base of the box was demarcated into 25 squares with of dimensions. As described previously by Zhu et al. (2001), anxiety and spontaneous locomotion was evaluated by the frequency of rearing and the number of squares traversed [33]. The intensity of light in the interior of the apparatus was maintained at 95 ± 5 lux to reduce anxiety in the experimental subjects. Locomotor activity was evaluated by placing each animal in the middle of the box and letting it to explore the chamber liberally for an initial period of 15 minutes so as to habituate to the surroundings. Following, locomotion was evaluated by the total number of squares traversed by each mouse using both fore and hind paws. Rearing was measured by vertical counts (when the forepaws were elevated freely or leant against the wall while they stood on their hindlimbs). Both parameters were assessed in the subsequent 2 minutes. Following each test session, ethanol solution was used to sterilize the apparatus to minimise residual odours. All animals were assessed on days 26, 27, and 28 (trial days 1, 2, and 3 respectively) of the treatment. The recorded parameters were analysed using a video tracking system (Bioseb, Smart 3.0).

Step-down Inhibitory Avoidance Task

Approximately 48 hours before the end of the experiment, the step-down inhibitory avoidance task was used to assess learning as previously described by Zhu et al. [33]. Basically, the apparatus comprised of a 40 x 25 x 25 cm acrylic box with a base composed of 2-mm stainless steel parallel bars separated by a distance of 1 cm. A current of 80 volts was dispensed via the bars on the base, and a 2.50 cm high, 8.0 x 25.0 cm wooden podium on the left extremity of the apparatus. Each mouse was cautiously positioned on the platform, and learning was evaluated based on the ability of each mouse to stay on the wooden platform after receiving an initial foot-

shock when it first stepped down off the platform. After receiving an electric shock, the frequency at which the animal strode down with all limbs was documented as an indicator of learning. The ceiling learning performance was obtained when the mouse persisted on the platform without striding down for 120 seconds.

A similar approach was adopted to assess short-term memory 24 hours later. Again, each mouse was gently transferred onto the platform, this time without any electric shock applied, and the total time period it persisted on the wooden platform was recorded as an indicator of memory. The maximum memory was reported when the mice continued on the wooden platform for 120 seconds.

Novel Object Recognition Test (NORT)

To determine long-term memory, the NORT was used [34]. A familiarisation phase and a test phase were included in each trial. Each animal was placed in the NORT apparatus (45 x 45 x 30 cm) throughout the familiarisation session; the intensity of light in the centre of the box was maintained at 95 ± 5 lux to lessen anxiety in the animals. For 10 minutes, the animal was allowed to freely explore the box, which included two undistinguishable objects (5 x 5 x 5 cm) arranged in symmetrically diagonal positions. One acquainted object in the box was then replaced with a new object of the same size but with distinct physical features twenty-four hours later. The animal was transferred back into the apparatus and allowed 10 minutes to examine both objects during the test session. Animals who could recall the acquainted object spent a greater amount of time investigating the unfamiliar object. A Smart 3.0 video tracking device was used to record the mice's behaviour (Bioseb).

The total time duration when the snout was in close proximity to the novel object was recorded as the novel object exploration time. The ratio of the duration of exploration of the novel object

to the time duration spent exploring both objects in the test session was defined as the discrimination index.

Novel object location (NOL) test

The NOL paradigm is a spatial alternative to the NOR test for testing spatial location memory using the animal's capacity to encode both object features and spatial position information [35]. The familiarisation session consisted of two undistinguishable and familiar objects, with either of the objects transferred to a new location 24 hours afterwards for the test phase. A Smart 3.0 video tracking device was used to record the mice's behaviour (Bioseb). The discrimination index was calculated as explained above.

Preparation of brain tissue homogenate for biochemical analysis

Following completion of the behavioural tests, all animals were anesthetized using Nembutal (i.p) administered at 200 mg/kg before humanely euthanising by cervical dislocation. Thereafter, brain was excised and dissected to separate the cortex, which was then stored at -80° for further processing. For estimation of biochemical parameters, the cortical tissues were homogenised using a sonicator and thereafter centrifuged at 9,000 rpm for 4 minutes to separate the supernatants which was then used to evaluate the activities of endogenous antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPx), and (superoxide dismutase (SOD), alongside the concentrations of nitric oxide (NO), levels of malondialdehyde (MDA; lipid peroxidation), and in addition, levels of TNF- α . Also, neurotransmitters (such as gamma-aminobutyric acid (GABA), norepinephrine (NE) serotonin (5-HT), dopamine (DA), and glutamate were carried out following an established procedure [30, 36].

Brain Oxidative Stress Markers

Measurement of brain lipid peroxidation

The level of lipid peroxidation was assessed by evaluating the concentration of MDA in cortical homogenates using a spectrophotometer as described previously by Ohkawa et al. (1979). Changes in the absorbance was recorded at 532 nm, and expressed as μ moles of MDA/ tissue weight in grams [37].

Evaluation of nitric oxide levels

The concentrations of NO in cortical homogenates were measured calorimetrically by means of the Griess reagent as previously described. The principle of this reaction is based on the formation of a reddish-pink colouration which is indicative of nitrite detected in the sample upon treatment with the Griess reagent [38]. Absorbance was measured at 548 nm.

Estimation of proinflammatory cytokines

The concentration of Tumour Necrosis Factor-alpha (TNF- α) in the cortex was assayed using a mice TNF- α commercial ELISA kit and read using spectrophotometer at 540 nm (MyBioSource, USA; MBS2507343).

Estimation of brain antioxidant capacity

Following the manufacturer's instructions, concentrations of antioxidants markers, glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were measured in cortical tissues using ELISA kits (MyBioSource, USA; MBS265946, MBS036954, and MBS726771).

Determination of neurotransmitter content

Estimation of the levels of gamma-aminobutyric acid (GABA), dopamine, glutamate, noradrenaline and serotonin, in the cortex was determined using HPLC as previously described by Pagel et al. (2000). The supernatant was further centrifuged at $10,000 \times g$, at 4 $^{\circ}C$, for 5

minutes and apportioned into 2 parts. The first part was utilised to measure dopamine, serotonin, and noradrenaline levels, while the other part was utilised to ascertain glutamate and GABA levels. The neurotransmitter levels were expressed as ng/g (for dopamine, serotonin, and noradrenaline) and ug/g (for glutamate and GABA) [39].

Data analysis

The data collected from the present study was expressed as mean \pm standard error of the mean (\pm SEM) and evaluated using analysis of variance (ANOVA), followed by Tukey's post-hoc test with the aid of GraphPad Prism, 9.0. The values of $P < 0.05$ was considered statistically significant.

Results

Myrtenal improves RF-EMR-induced deficits in open-field performance and memory

The effects of administering Myrt on locomotion of RF-EMR-exposed mouse in the open field, as indicated by the number of lines crossed (Figure 2a). There was no notable variation in locomotor activity of the different cohorts on the first day of assessment (26th day of neonatal development). However, on days 2 and 3 of the assessment (27th and 28th day of neonatal development), exposure to RF-EMR notably reduced the motor activity compared to animals in the untreated group. Notably, administration of Myrt at 100 and 200 mg/kg, p.o. considerably improved the effect ($P < 0.01$ and $P < 0.001$, respectively), compared to animals in the RF-EMR group which exhibited the lowest locomotor activity (Figure 2b). The effect of Myrt treatment on rearing activity in mice is indicated by the number of vertical counts after RF-EMR exposure, as illustrated in Figure 2c. No significant variation in the locomotor activity of the different mice groups was recorded on the first day of assessment (26th day of neonatal development).

However, on days 2 and 3 of the assessment (27th and 28th day of neonatal development), exposure to RF-EMR induced a considerable reduction in rearing compared to the untreated control group. Equally, administration of 100 and 200 mg/kg of Myrt significantly increased the vertical counts ($P < 0.05$ and $P < 0.01$, respectively) when compared to the RF-EMR-exposed mice, which recorded the lowest vertical counts (Figure 2c).

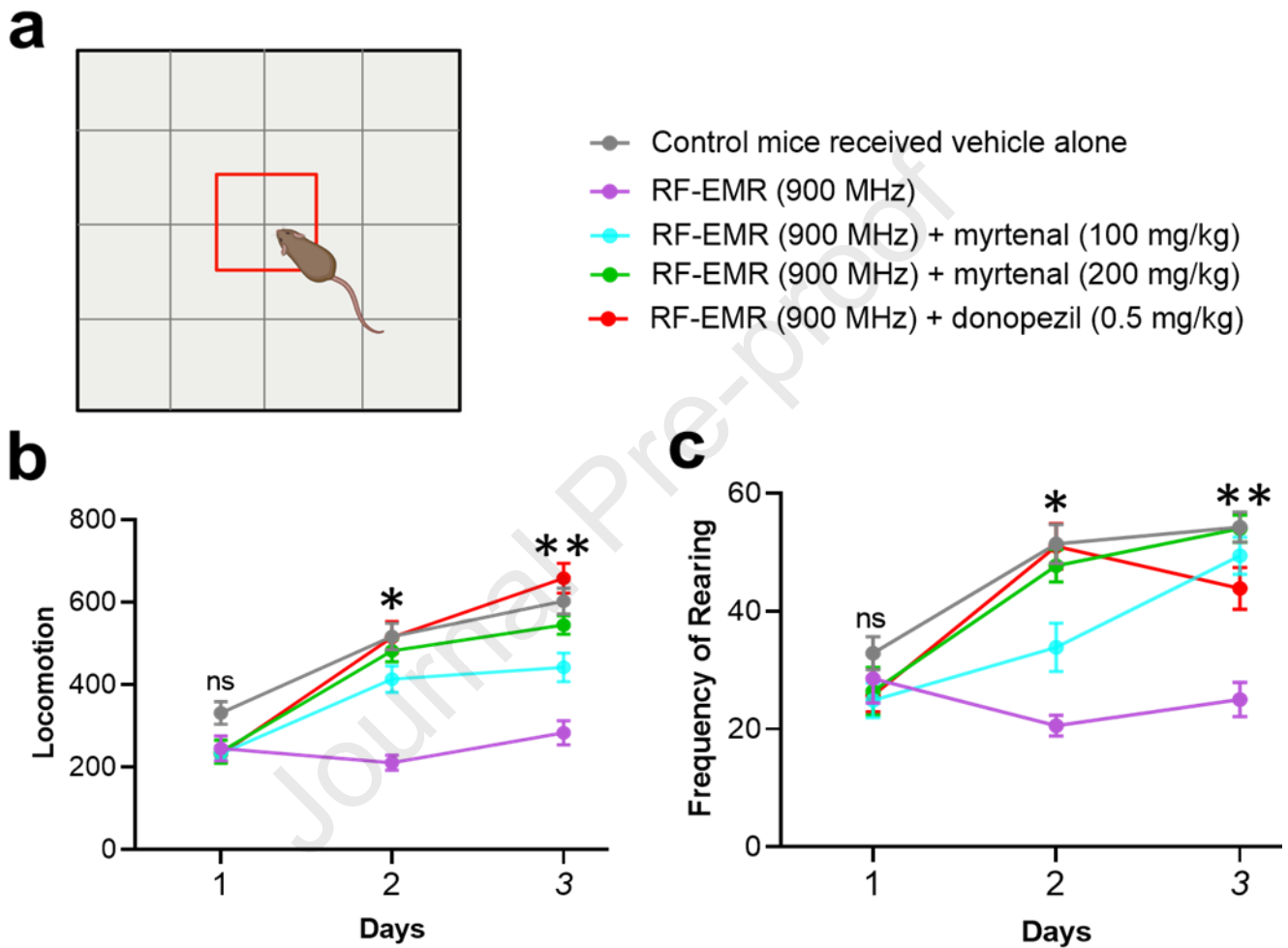


Figure 2: Assessment of effects of Myrtenal on locomotor function and rearing using open field test in mice exposed to RF-EMR (Mean ± SEM, n = 7). **a** Schematic illustrating the open field test paradigm. Effects of Myrt on **b** Locomotion and **c** Rearing. Values of * $P < 0.05$, ** $P < 0.01$, were considered significant when compared to the RF-EMR group.

Assessment of learning and memory demonstrated that RF-EMR (900 MHz) exposure induced significant deficits in learning and memory (Figure 3a). The highest foot-shocks were recorded in the RF-EMR (900 MHz) group (2.67 ± 0.34), while the lowest was reported in the untreated control group. The number of foot-shocks received by mice in the groups administered RF-EMR (900 MHz) + donepezil 0.5 mg/kg (1.17 ± 0.17), RF-EMR (900 MHz) +

Myrt (200 mg/kg) (1.20 ± 0.21) and RF-EMR (900 MHz) + Myrt (100 mg/kg) (1.44 ± 0.32) was considerably lower ($P < 0.01$, $P < 0.01$, and $P < 0.05$ respectively), compared to the animals subjected to RF-EMR (900 MHz) without additional treatment (Figure 3b).

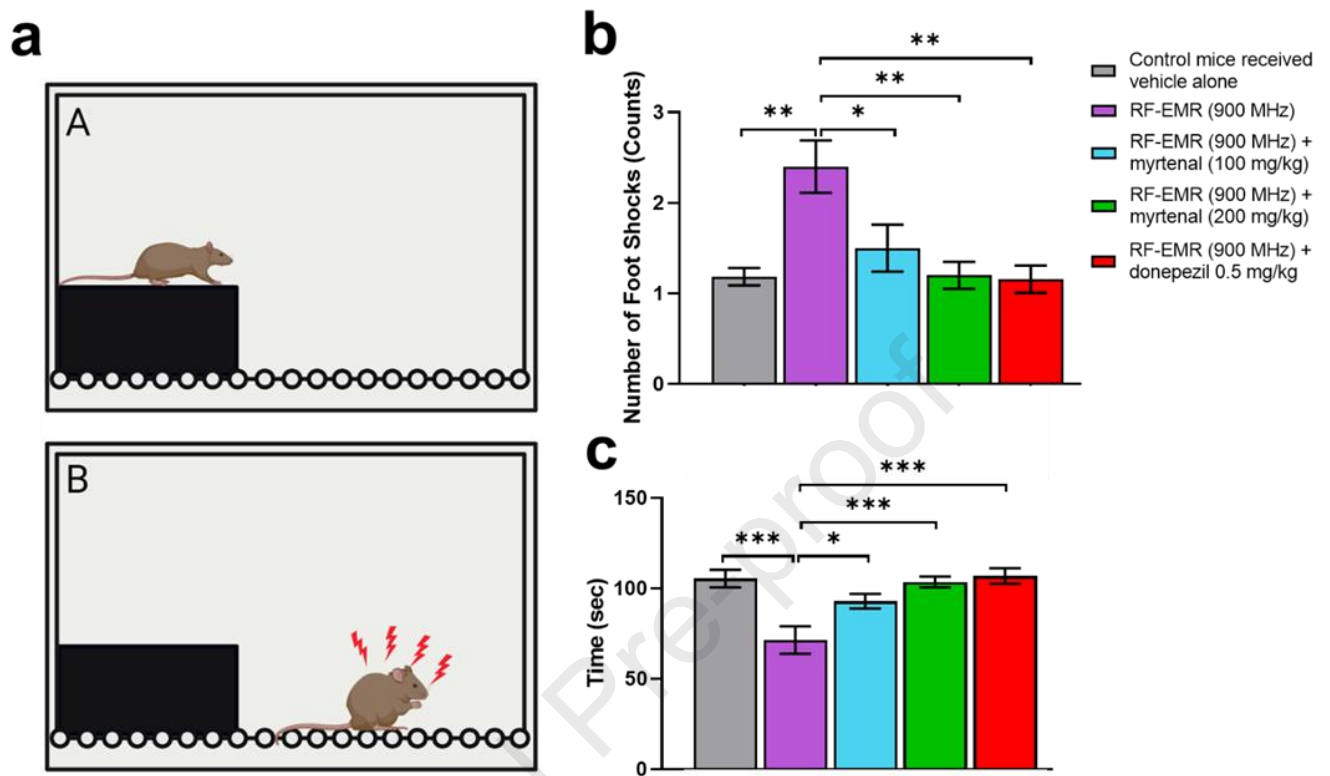


Figure 3: Memory assessment using step-down inhibitory avoidance task in mice exposed to RF-EMR (Mean \pm SEM, n = 7). **a** Schematic illustrating the step-down inhibitory avoidance test paradigm. Effect of Myrt administration on **b** Learning, and **c** Memory. Values of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, were considered significant when compared to the RF-EMR group.

A significant ($P < 0.05$) variance in memory was recorded between the different groups. The time (sec) spent on the platform by the untreated control group (107 ± 2.48 s), RF-EMR (900 MHz) + Myrt (200 mg/kg) (105.90 ± 2.84 s) and RF-EMR (900 MHz) + donepezil 0.5 mg/kg (106.70 ± 2.84 s) and was considerably higher ($P < 0.001$), compared to those observed in the groups exposed to RF-EMR (900 MHz) (70.21 ± 2.21 s) (Figure 3c). Although animals treated with RF-EMR (900 MHz) + Myrt (100 mg/kg) spent a lesser time on the platform compared to the RF-EMR (900 MHz) + Myrt (200 mg/kg) group, the difference was not significant. There was no significant variance ($P < 0.05$) in the time (seconds) spent on the platform as well as in

the total foot-shocks received by mice in the untreated control, RF-EMR (900 MHz) + Myrt (200 mg/kg), and RF-EMR (900 MHz) + donepezil 0.5 mg/kg treated groups (Fig. 3b).

In addition, spatial memory was assessed using two other complementary paradigms (NOR and NOL (Figure 4a-d). Notably, these tests examine dorsal ganglia-dependent behavioural performance in mice. For both tests, all mice groups spent a comparable time exploring both objects on the habituation day. However, on the day of the NOR test (twenty-four hours later), the group of animals exposed to RF-EMR without Myrt treatment spent significantly lesser time ($P < 0.01$) exploring the novel object location when compared to the other animal groups.

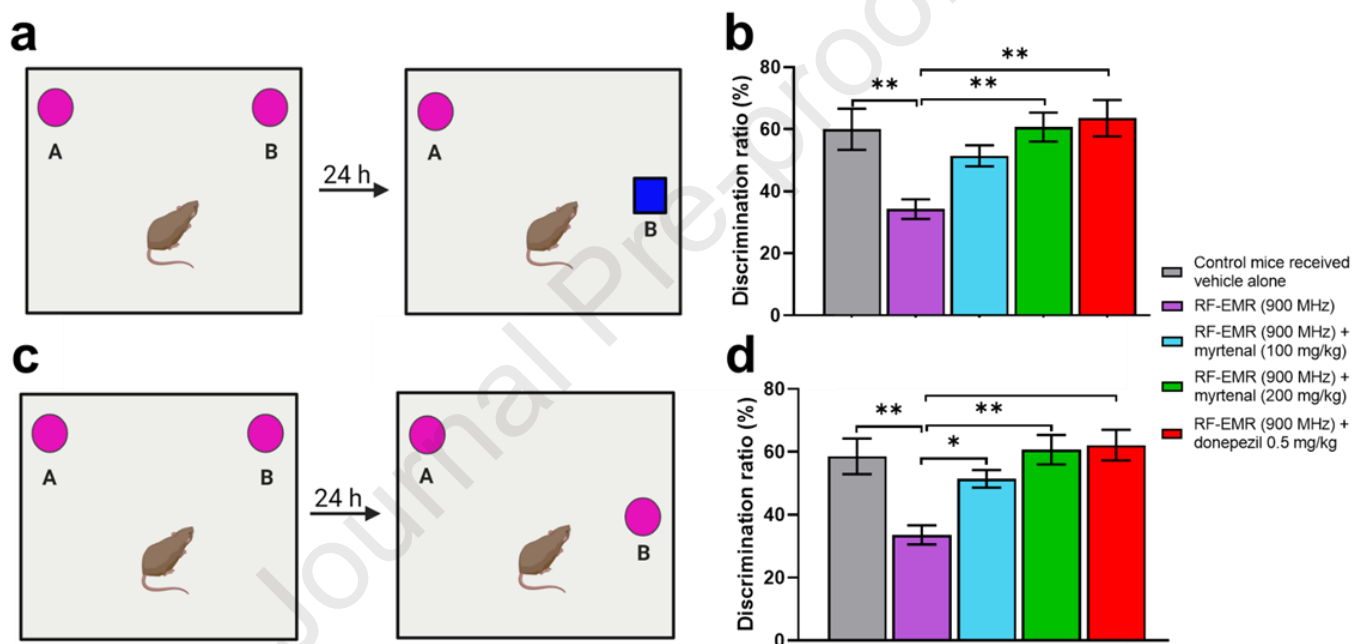


Figure 4: Assessment of the effect of Myrtenal on memory using NOR and NOL tests in mice subjected to RF-EMR (Mean \pm SEM, $n = 7$). a and c Schematic illustrating the NOR and NOL test paradigms, respectively. b and d Effect of Myrt on spatial memory. Values of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, were considered significant when compared to the RF-EMR group.

The longest time spent exploring the NO was observed in the untreated control group, while the shortest duration was reported in the RF-EMR group (Figure 4b). Corresponding to the result of the NOR test, the group of mice exposed to RF-EMR without Myrt treatment spent significantly less time ($P < 0.01$) exploring the novel object location of the compared to the other animals (Figure 4d) and treatment with Myrt significantly improved spatial memory deficits in the exposed mice.

Myrtenal mitigates RF-EMR-induced oxidative stress

The result of treatment with Myrt on NO levels in cortex of animals subjected to RF-EMR is presented in Figure 5a. Treatment RF-EMR-exposed mice with 200 mg/kg of Myrt significantly reduced the levels of cortical nitric oxide which was observed to be higher in cortex of mice exposed to RF-EMR alone. No significant variation was observed between the RF-EMR + Myrt (200 mg/kg) and RF-EMR + donepezil (0.5 mg/kg) treated groups.

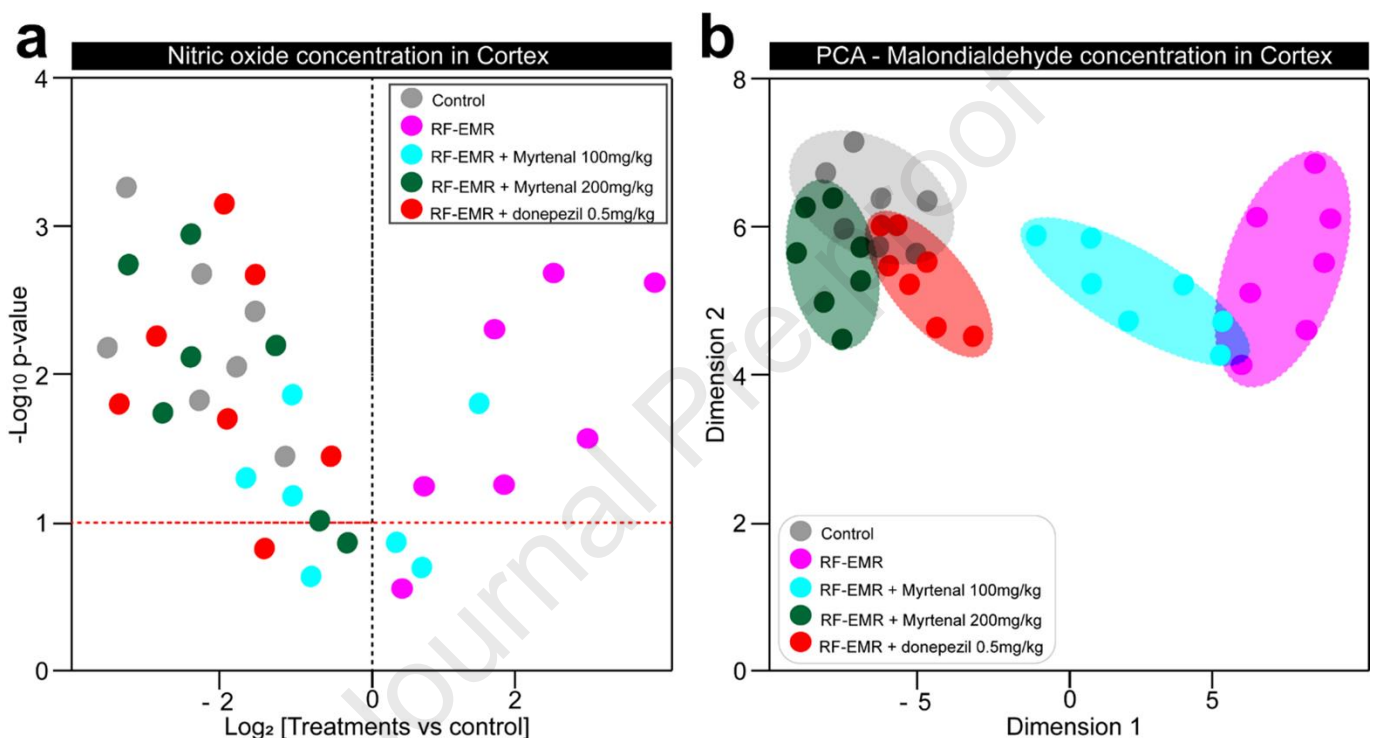


Figure 5: Assessment of markers of oxidative stress in cortex of RF-EMR-exposed mice.

Volcano plot showing the effects of treatments on levels of Nitric oxide. Each dot signifies the average response from a particular subject. Dots below the red line signifies the responses which were not statistically significant. **b** Malondialdehyde concentrations in the cortex of all experimental animals, dimensionally reduced using PCA, cluster according to their similarity, and show a clear distinction between the treated vs untreated groups.

The effects of treatment with Myrt on levels of MDA in the cortex of mice exposed to RF-EMR is presented in Figure 5b. The data obtained shows a clear distinction between the group exposed to RF-EMR with any treatment compared to the controls and the groups treated with Myrt. Principal component analysis reveals that the RF-EMR (900 MHz) group clustered separately in

comparison to the RF-EMR (900 MHz) + Myrt (200 mg/kg), RF-EMR (900 MHz) + donepezil 0.5 mg/kg, and the un-exposed control group. However, there was an overlap between the RF-EMR (900 MHz), and RF-EMR (900 MHz) + Myrt (100 mg/kg). further analysis revealed that in comparison to the control, exposure to RF-EMR alone considerably increased the levels of NO and MDA ($P < 0.01$) in cortical tissues (Supplementary figure 1). Treatment with 100 or 200 mg/kg of Myrt in RF-EMR -subjected mice notably reduced ($P < 0.05$ and $P < 0.01$, respectively) both NO levels and lipoperoxidation in comparison to mice exposed to RF-EMR alone. However, no considerable difference was recorded among the groups treated with RF-EMR + Myrt (200 mg/kg) and RF-EMR + donepezil (0.5 mg/kg). Equally, donepezil which was used as the reference drug also reduced the levels of MDA in mice brains (Figure 5b; supplementary figure 1).

Myrtenal enhances activities of endogenous antioxidant enzymes

The result of treatment with Myrt on endogenous antioxidants enzyme activities is shown in Figure 6. RF-EMR significantly decreased in the activities of all the antioxidant enzymes assayed. Equally, in RF-EMR-exposed mice, 100 and 200 mg/kg of Myrt improved the activities of SOD (3.65 ± 0.29 ; 3.87 ± 0.42 UA/min/mg protein), GPx (19.98 ± 2.11 ; 23.0 ± 0.41 UC/mg/protein), and CAT (2.60 ± 0.32 ; 3.34 ± 0.54 UB/min/mg protein), in a grade dependent manner. Likewise, donepezil (0.5 mg/kg) improved the activities of the assayed endogenous antioxidants (SOD; 4.12 ± 0.41 , GPx; 25.81 ± 2.44 ; CAT; 3.30 ± 0.40) and was similar to what was recorded in the RF-EMR + 200 mg/kg Myrt treated group ($P > 0.05$). The least antioxidant enzyme activities were reported in the RF-EMR-exposed group (2.08 ± 0.43 , 14.01 ± 2.33 , 1.59 ± 0.40 ; Figure 6a-c).

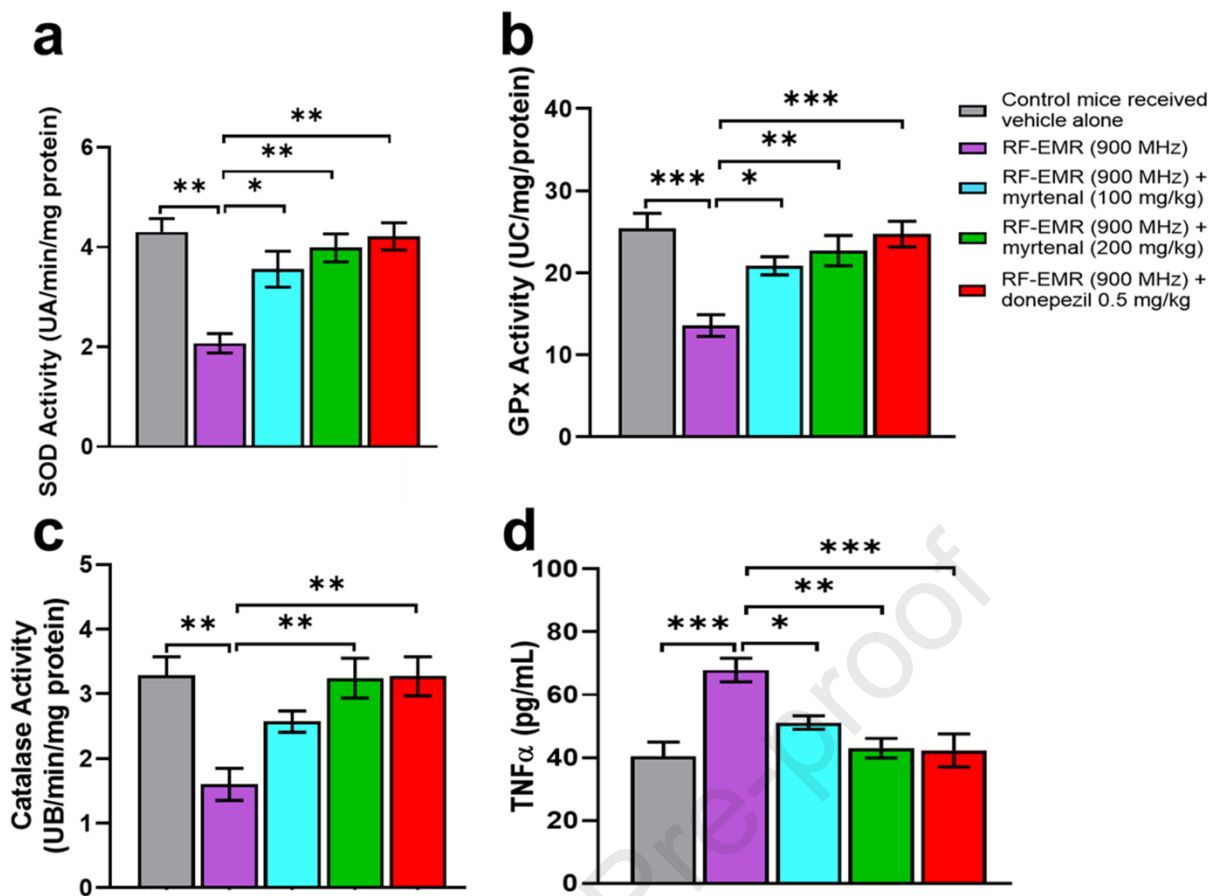


Figure 6: Effects of treatment with Myrtenal on activities of antioxidant enzymes and TNF-α levels in cortical tissues of mice exposed to RF-EMR (Mean ± SEM, n = 7). Activities of **a** Superoxide dismutase (SOD), **b** Glutathione peroxidase (GPx), and **c** catalase (CAT) were determined alongside **d** levels of TNF-α in cortical tissues. Values of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, were considered significant when compared to the RF-EMR group.

Myrtenal improves TNF-α levels in RF-EMR-exposed mice

Exposure to RF-EMR considerably increased TNF-α levels ($P < 0.01$; 68.6 ± 4.41) compared to the control. On the other hand, administration of Myrt at a dose of 100 and 200 mg/kg meaningfully decreased the concentration of TNF-α in the brains of the treated animals in comparison to the group treated with RF-EMR alone (52.04 ± 0.85 ; $P < 0.05$ and 42.6 ± 4.01 ; $P < 0.01$ respectively). Similarly, donepezil (0.5 mg/kg), decreased TNF-α activity in the cortex (42.18 ± 4.11) and did not vary from the RF-EMR + 200 mg/kg Myrt group ($P > 0.05$). The least TNF-α level was recorded in the control group (39.89 ± 5.97) (Figure 6d).

Correlation of markers of oxidative stress and object recognition memory following Myrtenal administration

A considerable correlation between memory function and activities of GPx, SOD, CAT, and MDA levels were reported with a correlation coefficient of $r = 0.69$; $P = 0.001$, $r = 0.72$; $P = 0.0016$, $r = 0.75$; $P = 0.001$, and -0.595 ; $P = 0.005$ respectively (Figure 7a-d).

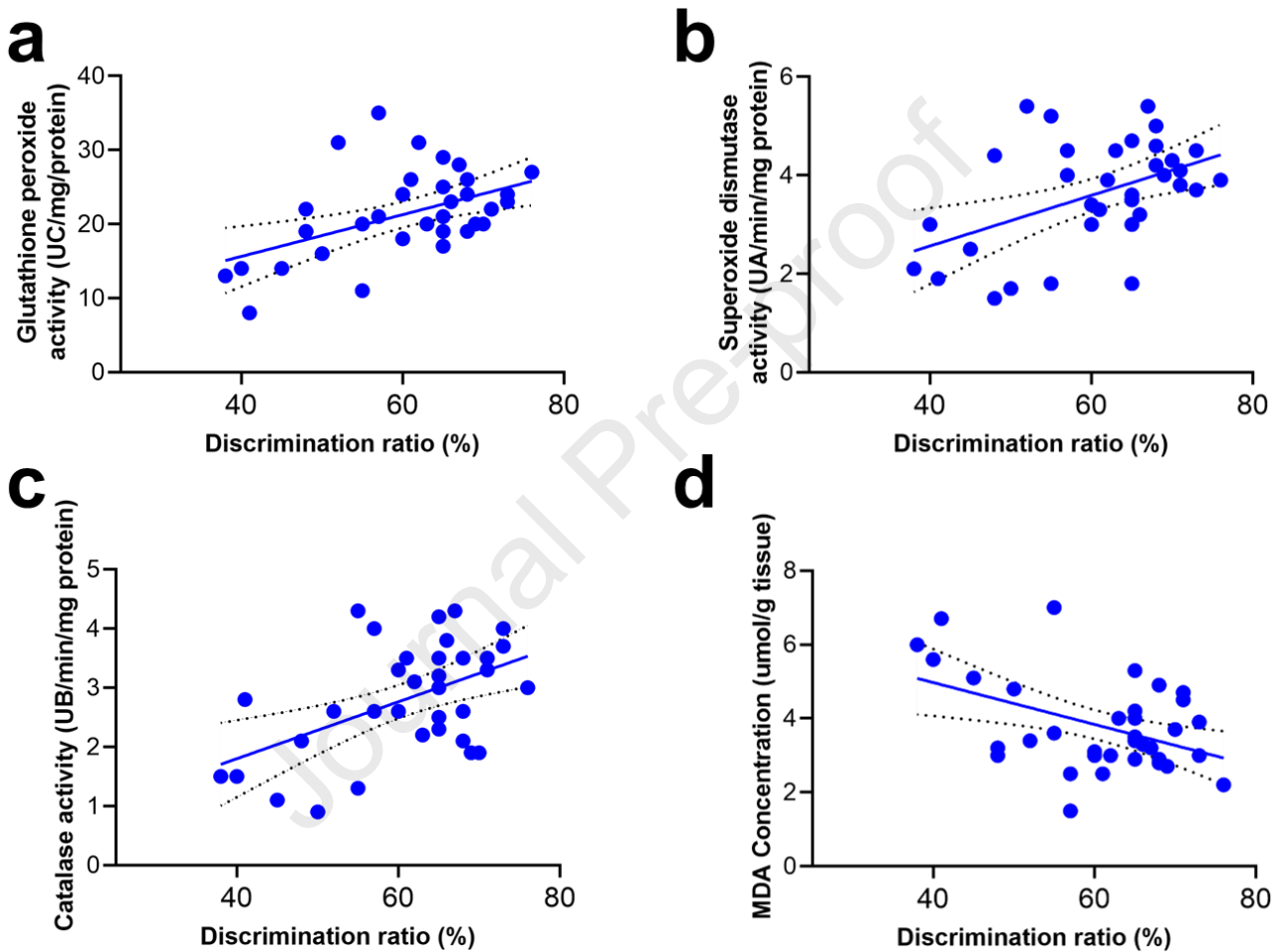


Figure 7: Correlation of memory function and activities of antioxidant enzymes, alongside lipidperoxidation in mice exposed to RF-EMR (Mean \pm SEM, $n = 7$ per group). Correlation of activities of **a Glutathione peroxidase (GPx), **b** Superoxide dismutase (SOD), and **c** Catalase (CAT) alongside **d** MDA levels in cortical tissues with object recognition memory. r = Pearson's correlation coefficient > 0.05 and P values of < 0.05 was considered significant.**

Table 2: Effects of administration of Myrtenal on concentrations of neurotransmitter levels in mice subjected to RF-EMR (Mean \pm SEM, n = 7 per group). Concentration values of * P < 0.05, ** P < 0.01, *** P < 0.001, were considered significant when compared to the RF-EMR mice groups.

	Neurotransmitters				
	Dopamine (ng/g)	Noradrenaline (ng/g)	Serotonin (ng/g)	Glutamate (ug/g)	GABA (ug/g)
Control	655.7 \pm 43.4**	743.6 \pm 67.3	440.7 \pm 41.2*	1556.0 \pm 113.4**	2604.1 \pm 102.5**
RF-EMR (900 MHz)	501.9 \pm 33.1	705.6 \pm 56.1	401.5 \pm 19.2	1101.2 \pm 141.2	2286.3 \pm 91.4
RF-EMR (900 MHz) + Myrt (100 mg/kg)	589.2 \pm 33.7*	704.2 \pm 42.3	502.2 \pm 53.8	1278.1 \pm 110.3	2462.1 \pm 123.5
RF-EMR (900 MHz) + Myrt (200 mg/kg)	680.7 \pm 33.8**	789.1 \pm 43.1	559.8 \pm 31.6**	1347.4 \pm 117.4**	2547.3 \pm 112**
RF-EMR (900 MHz) + donepezil 0.5 mg/kg	685.1 \pm 41.1**	743.5 \pm 56.1	556.4 \pm 52.3**	1480.4 \pm 187.2***	2580.2 \pm 110.2**

Myrtenal modulates levels of neurotransmitters in RF-EMR exposed mice

The exposure of animals to RF-EMR considerably perturbed the levels of neurotransmitters, including glutamate (ug/g), serotonin (ng/g), dopamine (ng/g), and GABA (ug/g). However, levels of Noradrenaline (ng/g) did not vary significantly among the various groups. Treatment with 200 mg/kg Myrt notably improved neurotransmitter levels, while the least levels were reported in the mice group treated with RF-EMR alone. The greatest level was recorded in the untreated control group (Table 2).

Discussion

Here, we sought to uncover the protective effects of Myrt in young mice exposed to RF-EMR. We leveraged on the potential of RF-EMR to impair memory through the mechanisms of modifying cellular oxidative stress and antioxidant redox systems, thereby increasing susceptibility to oxidative damage due to enhanced electrical conductivity owing to the high water content in foetus and neonates [9]. Intriguingly, data from the current study show that Myrt significantly improves memory deficits, mitigates RF-EMR-induced oxidative stress,

enhances endogenous antioxidant enzyme activities, improves TNF- α levels and modulates neurotransmitter levels in mice exposed to RF-EMR. These effects are attributed to the antioxidant properties of Myrt in the nervous system [40], and corroborate the results of Dragomanova et al. (2018) and Akefe et al. (2022), who reported significant improvement in cognitive functions upon the treatment with Myrt in animal models of memory loss and anxiety generated by recurrent injections of scopolamine or streptozotocin. Thus, the current investigation hints that Myrt may be playing an important role in mitigating processes that result in memory loss, particularly in neonates.

Myrtenal improves spatial memory and anxiolysis

Further, we investigated whether Myrt had an effect on spatial memory and anxiolysis using a battery of memory assessment tests (Step-down inhibitory avoidance, NOR, and NOL), the current study showed that exposure to RF-EMR induced spatial memory deficit and decreased the percentage of time animals spent investigating the novel object or the location of the novel object (Figure 4). We believe these findings stem from disrupted oxidative balance, consequently impeding the actual firing capacity and forming a neurotransmitter blockade that maintains metabolic homeostasis within the cortex. To further understanding, we simulated anxiogenesis by exposing the animals to RF-EMR and observing associated characteristics such as decreased exploration using open field tests. Subsequently, Myrt induced a notable reduction in the frequency of rearing, suggesting a possible anxiolytic effect associated with the modulating processes of glutamatergic and GABA-ergic neurotransmission [41]. Additionally, the antioxidant potential of Myrt on cognition significantly correlated with locomotion, as suggested previously [30, 40, 42]. The underlying mechanism of the anxiogenic effect of RF-EMR involves an increasing activity in the hypothalamic-pituitary-adrenal axis, modulation of corticotropin-releasing hormones, and alterations in dopaminergic and serotonergic neurotransmitters in the cortex [43, 44]. Thus, it is conceivable that Myrt regulates behavioural

patterns in mice by modulating oxidative processes and decreasing secretion of corticotropin-releasing hormone especially considering the fact that increased locomotion is associated with elevated levels of corticotrophin-releasing hormone and corticosterone [26].

It is noteworthy that RF-EMR-associated variations suggest a possible influence on manifold metabolic and cell-signalling associated pathways. This inference arises from the stack of consistently significant alterations recorded in both biochemical and behavioural parameters. For example, the concentrations of MDA and NO which are fundamental oxidative stress markers in the brain-signified a spike in RF-EMR subjected mice in this study (Figure 5). Taken together, these findings together with the severe alterations in $\text{TNF}\alpha$ and an imbalance in the levels of dopamine and glutamate neurotransmitters disrupted by exposing mice to RF-EMR suggest the involvement of a complex signalling mechanism [45-47].

Antioxidative and oxido-inflammatory effects of Myrtenal

The dynamics of antioxidants and oxidants in neural metabolism is well documented [14, 30, 48-52]. However, studies investigating agents that capable of disrupting these metabolic processes remain ongoing. The current study shows that exposure to RF-EMR induces a considerable decrease in antioxidant enzyme activities, and impairs the functions of the antioxidant defence system (Figure 6). Conversely, treatment with Myrt mitigated this trend and improved the endogenous antioxidant enzyme activities. These findings suggest that Myrt possesses physiologically active molecules with potential of neutralizing the activities of free radicals by donating electrons to them, consequently mitigating cellular damage. Notably, treatment with Myrt improved the activities of the endogenous antioxidant enzymes in the cortex (Figure 6). Moreover, administration of Myrt alleviated oxidative stress by equilibrating levels of MDA and NO in the cortex. It is plausible that the reduction of NO levels by Myrt may

be ascribed to its potential to downregulate NO levels and regulate expression of inducible nitric oxide synthase (iNOS) in the cortex [40, 53].

The elevated levels of TNF- α in the cortex of animals exposed to RF-EMR indicates an increase in inflammation [53]. However, treatment with Myrt considerably alleviated oxido-inflammatory stress by reducing levels of NO, TNF- α concentrations, and the degree of lipid peroxidation, via upregulating endogenous antioxidant enzymes activities in the cortex (Fig 5 and 6). This function shields the brain from oxidative injury and enhances spatial memory [54]. In line with this, we infer that Myrt may possess beneficial properties for management of neonatal pathologies linked with the nervous system. However, to further uncover these properties, the responses induced by Myrt to alter the process of neurotransmission needs to be understood.

Potential neurotransmitter mechanism engaged by Myrtenal

Dopamine is essential in mediating the process of memory [55, 56]. Specifically, the dopaminergic receptors are essentially activate nuclear factors such as CREB and CREM [57]. Suffice to say that the consolidation of memory in the NOR paradigm requires a functioning neurotransmitter mechanism to activate CREB proteins [55]. Furthermore, inflammatory markers like TNF- α inhibit CREB activation and enhances insulin resistance [58]. However, our observation that the administration of Myrt restored neurotransmitter levels, and enhanced spatial memory performance point towards the capacity of Myrt to enhance the patency of neurotransmitters in local neural circuits, consequently facilitating the activation of CREB protein, via regulating the functions of the cholinergic system, alleviating inflammation, and improving activities of antioxidant enzymes [20, 59].

Conclusion

Data from this study demonstrates that the exposure of young mice to RF-EMR during gestation and neonatal development induces spatial memory deficits in mice and alters the oxido-inflammatory response, and neurotransmitter levels in the cortex. However, treatment with Myrt improved spatial memory performance in RF-EMR-exposed animals by enhancing endogenous antioxidant enzyme activities, consequently protecting the brain from oxido-inflammatory stress. Additionally, we have demonstrated that administration of Myrt in RF-EMR exposed mice stabilizes the homeostasis of neurotransmitters. It is therefore conceivable that Myrt dynamically modulates memory functions during gestational and neonatal stages of development. Accordingly, it can be inferred that Myrt-rich fruits may confer health benefits when consumed during gestation and by neonates who are prone to RF-EMR exposure.

Author contribution statement:

Isaac Oluwatobi Akefe: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ezekiel Stephen Nyan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data, analysis tools or data.

Victoria Aderonke Adegoke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ibrahim Yusuf Lamidi, Phillip Ameh: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Uchendu Chidiebere, Simon Azubuike Ubah: Contributed reagents, materials, analysis tools or data.

Itopa Etudaye Ajayi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper

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Author contribution statement

Isaac Oluwatobi Akefe: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ezekiel Stephen Nyan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data, analysis tools or data.

Victoria Aderonke Adegoke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ibrahim Yusuf Lamidi and Phillip Ameh: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Conflict of interest

No conflicting interest has been declared by all authors.

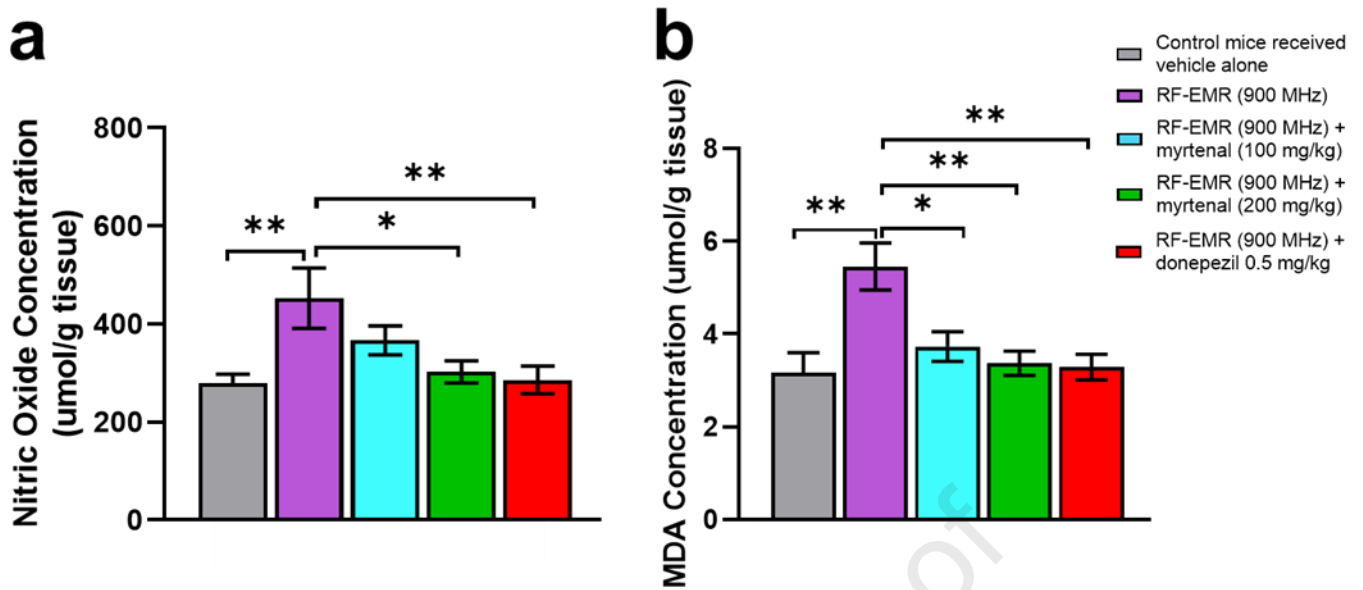
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Supplementary figure 1.



Supplementary Figure 1: Assessment of markers of oxidative stress in cortex of RF-EMR-exposed mice (Mean \pm SEM, n = 7). Effects of treatments on **a** Levels of Nitric oxide and **b** Malondialdehyde concentration. Values of * P < 0.05, ** P < 0.01, *** P < 0.001, were considered significant when compared to the RF-EMR group.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: