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Radiofrequency waves increase the brain levels of inflammatory biomarkers, neurotrophin and serotonin

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Abstract: The increase in mobile technology has raised concerns about the potential health effects of mobile phone radiation. The biological impact of exposure to radiofrequency (RF) waves emitted by electronic devices has been extensively studied and is a concern for the public, policymakers, and health researchers. The study aimed to examine the impact of 900 MHz radiofrequency waves on biomarkers such as interleukin (IL)-1α, IL-1β, tumour necrosis factor (TNF)-α, homocysteine, nerve growth factor, and serotonin in rats' serum and brain tissue. Thirty adult male Sprague Dawley rats (200 ± 20g) were randomly assigned to three groups (n=10): control (not exposed to RF), exposed I (2 hours per day), and exposed II (4 hours per day). The exposed groups were exposed to 900 MHz RFW for 30 consecutive days. The results showed that only the exposed group II significantly increased serum serotonin levels compared to the control group ($P=0.0496$). IL-1α, TNF-α, and nerve growth factor levels in brain tissue increased significantly in both exposed groups compared to the control group ($P<0.0001$). The control group had significantly lower levels of IL-1β compared to exposed groups I ($P=0.0289$) and II ($P=0.0004$). Additionally, serotonin and homocysteine levels in the brains of exposed II were significantly higher compared to the other groups ($P<0.0001$). The results showed disruptions in all biomarkers, indicating the potential impacts of daily exposure to 900 MHz radiofrequency waves from mobile phones on brain function. This suggests that mobile phone radiation may affect brain function.

Keywords: Mobile phone; Radiofrequency; Cytokines; Homocysteine; Nerve growth factor; Serotonin

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1.0 INTRODUCTION

Radiofrequency waves (RFW) are non-ionizing radiation used in mobile phones, wireless networks, and other electronic devices. The long-term effects of exposure to these electromagnetic fields (EMFs) on brain function and health are still not fully understood and are the subject of ongoing research and debate. As mobile phone usage becomes more common, it is important to understand the effects of long-term exposure to radiofrequency EMF, which falls within the frequency range of 3 kHz to 300 GHz. The Global System for Mobile Communications (GSM) uses 900 MHz radiofrequency

(RF) (The International Commission on Non-Ionizing Radiation Protection, 2020).

Research has focused on understanding potential health risks and biological mechanisms associated with EMF exposure. Studies have shown that EMF radiation can have detrimental effects on the central nervous system (CNS), including impacts on the blood-brain barrier (BBB), brain trace element balance, memory function, synaptic plasticity, neurotransmitter release, and neuronal viability (Azimzadeh & Jelodar, 2020a, 2020b; Bertagna et al., 2021; Sırav & Seyhan, 2016).

Cytokines are multifunctional proteins that immune system cells produce in response to injury or pathogens. In the brain, activated neuronal and glial cells continuously produce cytokines (Bourgognon & Cavanagh, 2020), which play a role in various functions, such as neuronal development (Monet & Quan, 2023), sleep regulation (Krueger, 2008), synaptic plasticity, neurotransmitter metabolism (Zipp et al., 2023), neuroendocrine functions, and BBB modifications (Yang et al., 2022). IL-1 is a crucial regulator of inflammation and stress in the central nervous system by controlling various innate immune responses (Park et al., 2018). TNF-α plays a vital role in different central nervous system functions, including synaptic homeostasis, transmission, and scaling. It also influences excitotoxicity, neuroinflammation, and the permeability of the BBB (Fresegna et al., 2020). Exposure to EMFs has been shown to increase levels of certain cytokines at both the protein and mRNA levels (Wu et al., 2012).

Homocysteine (Hcy) is a sulfur-containing amino acid that is derived from methionine and is known to be a potent pro-inflammatory factor, stimulating the production of pro-inflammatory cytokines (Borowska et al., 2021; Li et al., 2015). Elevated levels of Hcy are a well-established risk factor for vascular disorders, brain atrophy, and Alzheimer's disease (Smith et al., 2018).

Nerve Growth Factor (NGF) is a crucial neurotrophin that supports the development and survival of specific neurons in the CNS and peripheral nervous systems (PNS). It is mainly expressed in the hippocampus, olfactory, and cortex regions, and the sympathetic ganglia (Berry et al., 2012). Inflammatory conditions, such as cerebral ischemia and reperfusion, can trigger NGF expression in neurons (Li et al., 2022). NGF also regulates communication between the nervous and immune systems. It is produced by various brain cells, including astrocytes, glial cells, neurons, lymphocytes, and mast cells. It is found in different brain regions, such as the hippocampus, cortex, basal forebrain, cerebellum, and brainstem (Minnone et al., 2017).

Serotonin is a neurotransmitter that regulates mood, anxiety, and happiness (Hensler, 2010). It plays a crucial role in neurodevelopment, learning and memory, neuropsychiatric diseases, and autonomic regulation (Shah et al., 2018; Witteveen et al., 2013). While the majority of serotonin (90%) is produced in the gastrointestinal tract, a small percentage (1 –2%) is produced by neurons in the brain (Terry & Margolis, 2017). Serotonin in platelets affects cytokine production and is involved in inflammation (Jenne & Kubes, 2015; Li et al., 2012). Exposure to 1800 MHz for 1 and 2 months significantly increased hippocampal serotonin levels (Aboul Ezz et al., 2013). Additionally, exposure to 900 MHz RFW for 45 minutes significantly increased plasma serotonin levels (Eris et al., 2015).

Previous studies have shown that exposure to 900 MHz RFW can have harmful effects on the pancreas and testis tissue, leading to decreased testosterone levels and increased levels of specific biomarkers such as cytokines (IL-1β and TNF-α) and homocysteine (Azimzadeh & Jelodar, 2019; Jelodar et al., 2021). Additionally, exposure to RFW from mobile phones can have a greater impact on the brain due to its weak protective enzymes and high lipid content, making it susceptible to lipid peroxidation and oxidative stress (Fang et al., 2013). In this study, we investigated the effects of 900 MHz RFW on regulatory and functional biomarkers IL-1α and β, TNF-α, Hcy, NGF, and serotonin) in the brain tissue and serum of rats. Our findings demonstrated significant changes in cytokines, homocysteine, NGF, and serotonin levels in brain tissue following exposure to radiofrequency waves, while serum parameters remained essentially unchanged except for serotonin levels.

2.0 MATERIALS AND METHODS

2.1 Animals

Thirty adult male Sprague Dawley rats weighing 200 ± 20 g were obtained from the Shiraz animal house centre. The rats were housed in polycarbonate cages (42 * 26.5 * 15 cm3) with a constant temperature of 20 ± 2˚C and a 12-hour light-dark cycle. They had free access to food and water. The exposure time was set between 9 a.m. and 1 p.m. All experiments were conducted in compliance with Shiraz University's ethical guidelines (Code No.IR.AC.REC. 1398.S9530650) and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

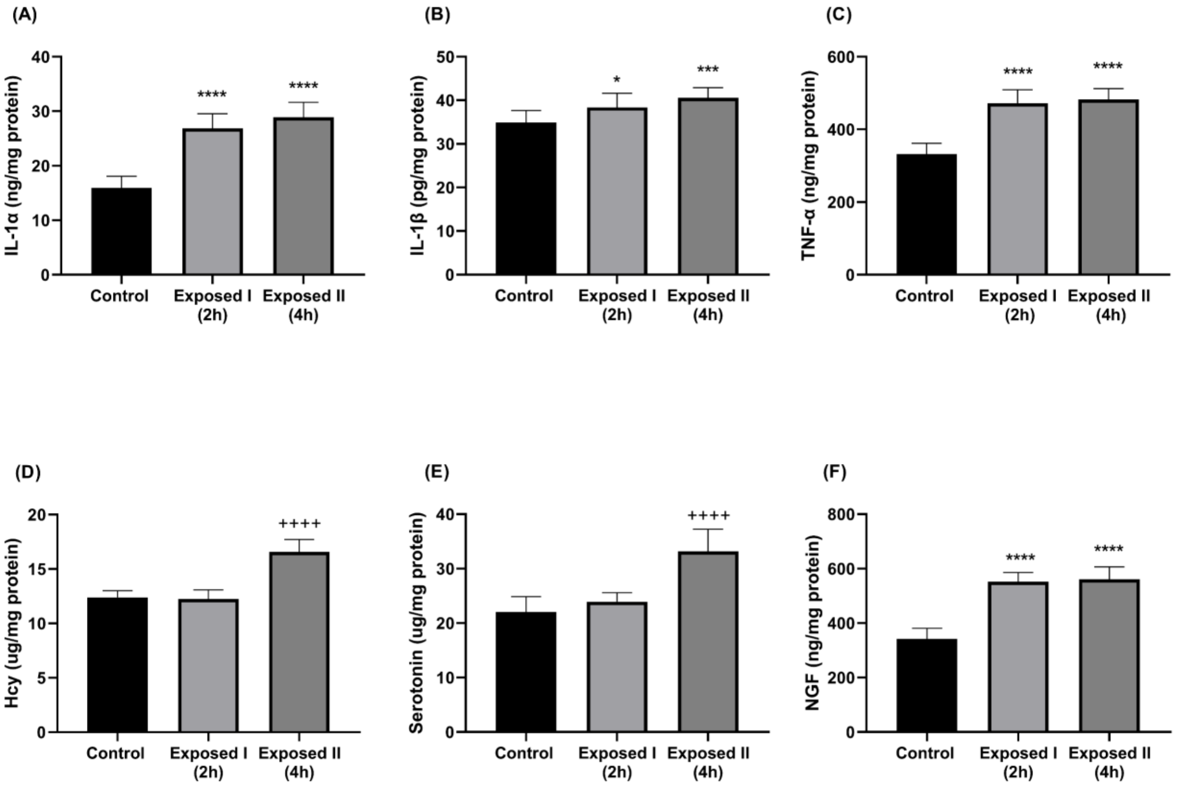

Figure 1. Effects of 900 MHz RFW exposure for 2 and 4 hours per day over 30 consecutive days on the concentrations of (**A**) IL-1α, (**B**) IL-1β, (**C**) TNF-α, (**D**) Hcy, (**E**) serotonin, and (**F**) NGF in brain tissue. The columns represent the mean ± SD, One-way ANOVA, and Tukey's multiple comparison tests. * $p < 0.05$; *** $p < 0.001$; and **** $p < 0.0001$ compared to the control group. ++++ $p < 0.0001$ compared to the control and exposed group I.

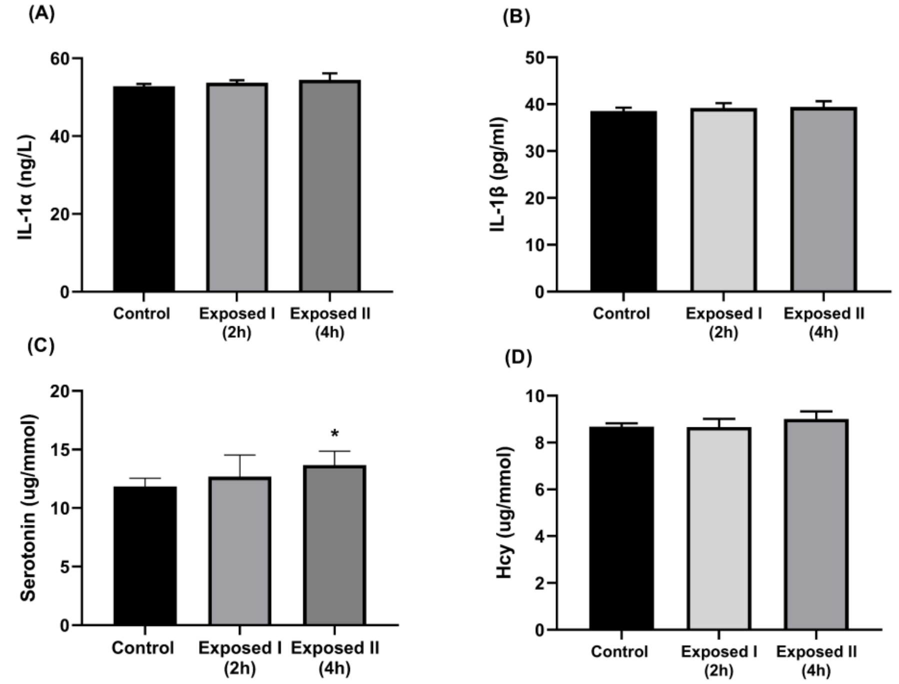

Figure 2. Effects of 900 MHz RFW exposure for 2 and 4 hours per day over 30 consecutive days on the concentrations of (**A**) IL-1α, (**B**) IL-1β, (**C**) serotonin, and (**D**) Hcy levels in the serum. The columns represent the mean ± SD, One-way ANOVA, and Tukey's multiple comparison tests. * $p < 0.05$ compared to the control group.

2.2 The radiofrequency wave exposure device and power dosimetry

We used a simulator developed by Shiraz University's Faculty of Telecommunication and Electronics Engineering to generate an electromagnetic field at a frequency of 900 MHz. The simulator has a 12 cm antenna that emits 900 MHz RFW circularly. The output power of the simulator was monitored using a spectrum analyzer (FSH6, Rohde and Schwarz, Germany), with the antenna of the spectrum analyzer placed 1 meter away from the simulator during the measurements. The MCS Real-Time Spectrum Analyzer Software recorded the real-time readings during the test. The peak power density recorded from the proximity of the simulator in the downlink band was 0.6789 mW/cm2 at 876 MHz. We also detected a specific absorption rate (SAR) value of 0.035 W/kg using a field-probe device (300 kHz -18 GHz, Wave Control, Spain) (Azimzadeh & Jelodar, 2020c).

2.3 Experimental protocol

All rats were divided into three groups of 10 each: control group (with no exposure); exposed I group (2 hours per day); and exposed II group (4 hours per day). The signal generator was placed one meter away from the cages of the exposed groups. Both exposed groups were irradiated to 900 MHz RFW for 30 consecutive days (Azimzadeh et al., 2018).

2.4 Sampling and tissue preparation

On the last day of the exposure period, all animals were anaesthetized with a 2% diethyl ether-saturated cotton ball in a chamber for 3–5 minutes and then euthanized by collecting whole blood through heart puncture. The blood was collected in glass tubes and left to clot at room temperature for thirty minutes. It was then centrifuged at 1300 g for 10 minutes. The serum was collected and stored at -70°C for later analysis. Brain tissue was rapidly removed, isolated, and stored at -70°C. The brain tissue was washed once with distilled water, homogenized using a tissue grinder on ice and centrifuged at 2000 rpm for 20 minutes at 4ºC to collect the resulting supernatant. The concentration of biomarkers (serotonin, Hcy, NGF, TNF-α, IL-1 α, and β) was measured in the serum and brain supernatant using the ELISA kits according to the manufacturer's instructions (serotonin: IBL, Hamburg, Germany; Hcy: Diazyme, Shanghai, China; NGF, TNF-α, IL-1 α, and β: Crystal Day Biotech, Shanghai, China). The total protein concentration of the brain tissue was evaluated using the Bradford method (Bradford, 1976).

2.5 Statistical analysis

The data were presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison tests with Graph Pad Prism® 8.0.1. A p-value of <0.05 was considered statistically significant in all experiments.

3.0 RESULTS

3.1 The effects of RFW exposure on the brain tissue

Figure 1 shows the concentration of all studied biomarkers in the brain. The mean levels of IL-1α, IL-1β, and TNF-α in the exposed groups (I and II) were significantly higher compared to the control group. The one-way ANOVA revealed a significant difference in the mean IL1-α levels between the studied groups (F (2, 24) = 68.12, P<0.0001). The post hoc test indicated that both exposed groups I and II had significantly higher mean IL1-α levels compared to the control group (P<0.0001) (**Figure 1A**). Similarly, there was a significant difference in the mean levels of IL-1β among the groups (F (2, 27) = 10.20, P=0.0005, one-way ANOVA). The control group had significantly lower mean levels of IL-1β compared to exposed groups I (P=0.0289) and II (P=0.0004) (**Figure 1B**). The one-way ANOVA also indicated a significant difference in the mean TNF-α concentration (F (2, 27) = 67.67, P<0.0001). The mean TNF-α levels in the control group were significantly lower than in both exposed I and II groups (P<0.0001) (**Figure 1C**).

The mean levels of Hcy, serotonin, and NGF in the brains of the studied groups showed significant differences (Hcy: F (2, 27) = 73.84, P<0.0001; serotonin: F (2, 21) = 31.27, P<0.0001; NGF: F (2, 27) = 99.02, P<0.0001, one-way ANOVA). Post hoc analysis revealed that the exposed II group had significantly higher mean Hcy and serotonin levels than the exposed I and control groups (P<0.0001, Tukey's multiple comparison tests). Additionally, both exposed groups had significantly higher mean NGF levels compared to the control group (P<0.0001, Tukey's multiple comparison tests) (**Figure 1D-F**).

3.2 The effects of RFW exposure on the serum parameters

Figure 2 shows the levels of IL-1α, IL1-β, Hcy, and serotonin in the serum of the experimental groups (**Figure 2A-D**). The only significant change observed in the serum was an increase in serotonin concentration (F (2, 26) = 4.414, P=0.0224, one-way ANOVA). Post hoc analysis revealed a significant increase in serotonin levels in group II compared to the control group (P=0.0172, Tukey's multiple comparison tests)

(**Figure 2C**). It should be noted that due to technical issues, we could not evaluate the TNF-α and NGF levels in the serum.

4.0 DISCUSSION

The study found that exposure to 900 MHz RFW had a significant impact on biomarkers in the brain, while only a few changes were observed in the serum. Inflammatory cytokines (IL-1α, IL-1β, and TNF-α) showed a significant increase in the brain tissue in both exposed groups compared to the control group. These results demonstrated an increase in the inflammatory markers assessed, reinforcing concerns about the biological effects of such radiation. The findings suggest that radiofrequency exposure may trigger inflammation in the brain, consistent with previous experiments showing elevated inflammatory cytokine levels in other tissues following exposure to 900 MHz RFW or microwave radiation (Azimzadeh & Jelodar, 2019; Jelodar et al., 2021; Wu et al., 2012). Increased levels of these mediators have been associated with diseases such as depression, Alzheimer's, and epilepsy (Bourgognon & Cavanagh, 2020). Elevated levels of IL-1α and IL-1β in brain tissue did not correlate with changes in serum levels, suggesting localized production of these cytokines.

Research on the effects of EMF on cytokine levels is limited, and the findings are conflicting. Some studies suggest that short-term exposure to EMF can increase innate immunity cytokines, while long-term exposure may decrease the adaptive immune response (Mahaki et al., 2019). Exposure to EMF has also been associated with increased production of inflammatory cytokines and reactive oxygen species (Kim et al., 2017; Patruno et al., 2018). However, other studies have found different effects (Mahaki et al., 2020), and some have reported no effects of EMF exposure on cytokine production (Fan et al., 2015; Ikeda et al., 2002). The discrepancies in findings may be due to differences in study design and methodologies.

The Hcy levels in group II brains were significantly higher than in the exposed I and control groups ($P < 0.05$). Previous research has shown that exposure to 900 MHz RFW increased Hcy levels in pancreatic tissue, with no significant change in testicular tissue (Jelodar et al., 2021). To our knowledge, there are no other published reports on the effects of EMF on Hcy levels in tissues.

The brain lacks metabolic pathways to eliminate Hcy, making neurons and glial cells more susceptible to its toxic effects, impacting neuronal survival and signalling (Boldyrev et al., 2013; Škovierová et al., 2015). Our study did not find significant changes in serum Hcy levels, supporting this hypothesis. However, high Hcy levels can adversely affect the CNS, including increased excitatory neurotransmission, neuronal damage, and compromised BBB integrity (Lehotský et al., 2016). High homocysteine (HHcy) and nitrosative stress can also compromise the integrity of the BBB, leading to cerebrovascular permeability and neuronal degeneration (Kamat et al., 2016). HHcy inhibits nitric oxide production and bioavailability, induces reactive oxygen species generation, and increases the production of inflammatory cytokines (Li et al., 2015). Our study found that long-term exposure to 900 MHz RFW led to a significant increase in Hcy levels in brain tissue, accompanied by an increase in the production of inflammatory cytokines (IL-1α, IL-1β, and TNF-α), which may have detrimental effects on the CNS.

The levels of NGF in the brain tissues of both exposed groups (I and II) were significantly higher compared to the control group. An increase in NGF may be a compensatory response to counteract the stress and potential damage caused by exposure to RFW. However, persistently elevated levels of neurotrophins may lead to dysregulation of neuronal growth and function, potentially contributing to neuropathology. Previous studies have also reported changes in NGF levels in various tissues following exposure to 900 MHz RFW or pulsed electromagnetic fields (Azimzadeh & Jelodar, 2019; Jelodar et al., 2021; Longo et al., 1999). Differences in tissue type, size, and capacity for NGF generation may explain variations in results. NGF can activate innate immune responses and regulate inflammation to prevent tissue damage (Minnone et al., 2017). It can increase inflammatory cytokines (Bayas et al., 2003; Hepburn et al., 2014) and stimulate the release of anti-inflammatory cytokines (Liew et al., 2005). The significant increase in NGF concentration in both exposed groups following exposure to RFW may modulate inflammatory cytokines.

Exposure to 900 MHz RF increased serotonin levels in both the serum and brain. This finding is particularly interesting because it suggests that exposure to RFW may affect emotional states and cognitive functions regulated by serotonin. Altered serotonin levels have complex implications. Increased serotonin may improve mood and reduce anxiety, but imbalances in serotonin levels have been linked to psychiatric disorders such as depression and schizophrenia (Celada et al., 2013a; Jenkins et al., 2016).

Previous research has also found decreased pancreatic serotonin levels following similar exposure (Jelodar et al., 2021). Studies on the effects of EMF on neurotransmitters such as serotonin, acetylcholine, and catecholamines have shown conflicting results. For example, exposure to 1800 MHz EMF increased serotonin and decreased dopamine levels in the hippocampus and hypothalamus (Aboul Ezz et al., 2013), while exposure to 900 MHz RFW increased blood serotonin levels (Eris et al., 2015). Furthermore, exposure to 800 MHz EMF decreased the release of acetylcholine, while exposure to 1800 MHz EMF had no effect (Li et al., 2017; Testylier et al., 2002). Additionally, exposure to different frequencies (900,1800,2100 MHz) reduced brain serotonin levels in newborn rats (Ismail et al., 2015).

Several potential mechanisms have been proposed for increased serotonin levels following RFW exposure. These include increased catabolism due to the heightened activity of monoamine oxidase (Said et al., 2012), EMF-induced damage to the ileal mucosa (Herrera et al., 1995), and decreased synthesis and absorption of the serotonin precursor tryptophan. Since 90% of the body's serotonin is produced in the gastrointestinal tract, the significant increase in serum serotonin concentrations in the current study is due to this source. Additionally, exposure to different EMF frequencies can modify blood-brain barrier permeability (Salford et al., 2003; Zhou et al., 2013), suggesting that the primary source of increased brain serotonin levels is the serum, originating from the gastrointestinal tract.

Serotonin-producing neurons are widely distributed in the brain, and evidence shows that several serotonin receptor subtypes are densely expressed throughout the brain (Carhart-Harris & Nutt, 2017; Celada et al., 2013b). Pro-inflammatory cytokines such as TNF-α and IL-1β have been reported to positively correlate with elevated brain serotonin levels (Masson & Hamon, 2009), indicating that serotoninergic neurons also contribute to the increase in serotonin concentration in the brain.

Further research, including replication studies and investigations into underlying mechanisms, may be needed to establish the significance and broader implications of the findings fully. Nonetheless, the findings contribute valuable insights into the potential effects of radiofrequency wave exposure on brain health, laying the groundwork for future research and discussions in the scientific community.

5.0 CONCLUSION

Our study revealed that daily exposure to 900 MHz radiofrequency waves for 30 days resulted in significant alterations in cytokines (IL-1α, IL-1β, and TNF-α), Hcy, NGF, and serotonin levels in the brain tissue with long-term exposure (4 hours). Short-term exposure also caused significant changes in cytokines (IL-1α, IL-1β, and TNF-α), and NGF levels in the brain tissue. There were no significant changes in serum parameters, except for serotonin levels, indicating that the effects of electromagnetic fields may be limited to specific tissues. These findings suggest that radiofrequency waves (900 MHz) could disrupt brain function by affecting the neuroendocrine, neurotransmitter, and immune systems.

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Author Contributions:

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; MA and FR conducted the experiments. GJ supervised, designed the study, and edited the article.

Conflicts of Interest:

The authors declared no conflict of interest.

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