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Electromagnetic Biology and Medicine

ISSN: 1536-8378 (Print) 1536-8386 (Online) Journal homepage: www.tandfonline.com/journals/iebm20

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**To cite this article:** Sinem Oruç, Sevilay Günay, Bahriye Sirav Aral, Yavuz Erden, Mahmud Mustafa Özkut, Kayhan Ateş, Gökçen Özdemir & Ersen Eraslan (2025) Combined effects of agomelatine and 2600 MHz radiofrequency field on inflammation induced by chronic lipopolysaccharide administration, Electromagnetic Biology and Medicine, 44:4, 520-529, DOI: 10.1080/15368378.2025.2545871

**To link to this article:** https://doi.org/10.1080/15368378.2025.2545871

Published online: 12 Aug 2025.

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ELECTROMAGNETIC BIOLOGY AND MEDICINE
2025, VOL. 44, NO. 4, 520–529
https://doi.org/10.1080/15368378.2025.2545871

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# Combined effects of agomelatine and 2600 MHz radiofrequency field on inflammation induced by chronic lipopolysaccharide administration

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**ABSTRACT**

The aim of this study is to investigate the potential synergistic effects of agomelatine(AGM) and 2600 MHz radiofrequency(RF) field exposure on inflammation induced by chronic lipopolysaccharide(LPS) administration in rats. A total of 49 female Wistar albino rats were randomly divided into 7 groups($n = 7$ per group): Control, Sham, LPS, LPS+AGM,LPS+RF,AGM+RF, and LPS+AGM+RF. Animals in the RF groups were exposed to a 2600 MHz field (1 h/day for 15 days). The whole-body averaged SAR was calculated to be 0.637 W/kg, while the localized SAR values were found to be 1.27 W/kg for 1 g and 0.91 W/kg for 10 g of brain tissue at a frequency of 2600 MHz. Chronic LPS administration(1 mg/kg/day for 15 days) successfully induced a systemic inflammatory state. The most significant finding was observed in plasma IL-6 levels. While AGM or RF exposure alone did not significantly alter IL-6 levels in LPS-treated animals, the combined treatment of LPS+AGM+RF resulted in a substantial and statistically significant decrease in plasma IL-6 compared to the sham group($p < 0.001$). This represents a large-magnitude effect (Cohen's d = 1.59), suggesting a potent anti-inflammatory action of the combined therapy. In contrast, plasma IL-1β and TNF-α levels showed no statistically significant differences among any of the groups. In the hypothalamus, chronic LPS exposure was associated with a downregulation of MAP kinase signaling pathways (ERK, JNK), indicative of endotoxin tolerance, and with reduced NF-κB level. This study provides novel evidence that 2600 MHz RF, when combined with AGM, may exert a powerful anti-inflammatory effect, highlighting a potential therapeutic interaction that warrants further investigation.

**PLAIN LANGUAGE SUMMARY**

This study investigates the effects of both RFF alone and its combination with AGM on intracellular signaling pathways and pro-cytokines levels in rats with chronic LPS administration. Totally 49 female wistar albino rats were randomly divided into 7 groups with 7 animals in each group. The goups were control, sham, LPS, LPS+AGM, LPS+RF, AGM+RF, LPS+AGM+RF groups. No treatment was applied to the control group, while the sham group received a daily intraperitoneal saline injection for 15 days. Then, animals were kept in the exposure setup (1 h/day, 15 days) without RFF exposure (RFF system was OFF). The RFF groups were exposed to a frequency of 2600 MHz (electric field 16.36 V/m) for 1 hour per day over 15 days. In the LPS and AGM groups, 1 mg/kg LPS was administered intraperitoneally followed by 10 mg/kg AGM intraperitoneal injection after 30 minutes. At the end of 15 days, hypothalamus tissues were analyzed by Western blot, while plasma samples were examined using spectrophotometric methods. The findings suggest that both 2600 MHz RFF and its combined application with AGM may exhibit anti-inflammatory effects and regulate intracellular signaling pathways.

**ARTICLE HISTORY**
Received 22 May 2025
Accepted 5 August 2025

**KEYWORDS**
Radiofrequency fields; agomelatine; lipopolysaccharide; cytokines; intracellular signaling pathways; electromagnetic field; mobile phone

## Introduction

Individuals living in the age of technology are increasingly exposed to various electromagnetic field EMF) sources with the rapid development of electronic information technologies; this situation leads to significant concerns and important results in terms of the biological effects that chronic exposure may cause. The possible interactions of RFF, which are in the non-ionizing class of the electromagnetic spectrum, with human organs, especially the central nervous system and the brain, are among the most focused and discussed topics of scientific research today (Hu et al.

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2021; Şenol et al. 2023). RFF applications may affect organisms through complex biological mechanisms that are highly sensitive to parameters such as the frequency, field intensity, and duration of exposure. Even minor changes in these parameters can lead to significant and markedly different biological responses (Postaci et al. 2018; Schuermann and Mevissen 2021). RFF applications have been reported to possess the potential to reduce inflammation at the cellular level through various mechanisms, including the regulation of cytokine levels, suppression of key inflammatory signaling pathways, reduction of oxidative stress, and modulation of immune cell activity (Costantini et al. 2022; Kim and Lee 2024; Mert and Yaman 2020; Patruno et al. 2020; Tang et al. 2019).

LPS injection, a commonly used immune stimulation model, induces the synthesis of various proinflammatory cytokines, including interleukin (IL)-1β and IL-6, which play a critical role in the pathophysiology of inflammation-mediated neuronal damage. These cytokines, produced in the periphery through yet unexplained molecular mechanisms, activate stress-related neuroendocrine pathways in the central nervous system (Quan et al. 1998; Sapolsky et al. 1987; Tilders et al. 1994; Turnbull and Rivier 1999) and cause stimulation of the hypothalamus -pituitary-adrenal axis (McEwen et al. 1997; Munck et al. 1984). Mitogen-activated protein kinases (MAPKs) are a family of kinases activated by various extracellular stimuli, including stress factors such as inflammatory cytokines, and consist of subgroups such as c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK) (Johnson and Lapadat 2002; Wilkinson and Millar 2000). Studies have shown that oxidative stress triggered by reactive oxygen species (ROS) can activate MAPK members through phosphorylation (Navarro et al. 2006, Pandey et al. 2017). Specifically, ERK1 and ERK2 have been shown to be activated by mitogenic stimuli and to promote cell proliferation, whereas JNK and p38 MAPKs are activated in response to environmental stressors and inflammatory signals, generally contributing to processes such as apoptosis and the inhibition of cell growth (Nebreda and Porras 2000; Takenobu et al. 2003).

The novel antidepressant AGM, classified as a melatonin receptor agonist, also exhibits anti-inflammatory and antioxidant properties (Chumboatong et al. 2017; Molteni et al. 2013). AGM has been shown to inhibit the activation of LPS-induced gene transcription, particularly involving inflammatory mediators such as IL-1β (Rossetti et al. 2018). Acute administration of AGM has been shown to play an effective role in suppressing LPS-induced neuroinflammation in various brain regions and the cerebellum. However, it has also been stated that chronic applications can lead to differential effects in brain regions (Koresh et al. 2012). The increasing level of RF-EMF exposure in daily life necessitates the investigation of the following two fundamental questions: (1) Whether exposure to high-frequency, non-thermal RF-EMF triggers an inflammatory response in the hypothalamus, a key regulator of homeostasis in the central nervous system, and (2) How RF-EMF exposure may interact with the use of agomelatine – commonly prescribed today and known for its anti-inflammatory effects – in terms of changes in inflammation levels and intracellular signaling pathways.

Therefore, this study aimed to evaluate the effects of AGM and RFF exposure, both individually and in combination, on LPS-induced inflammatory responses in rats. The objective was to investigate the potential synergistic anti-inflammatory effects of these interventions by assessing changes in both systemic and central inflammatory markers. Specifically, the study focused on examining how these treatments influence intracellular signaling pathways in the hypothalamus tissue and cytokine levels in plasma, thereby elucidating the modulatory effects of agomelatine and radiofrequency field on inflammatory responses.

## Materials and methods

### *Experimental design*

In this study, 49 female wistar albino rats weighing between 250 and 300 grams were obtained from the Gazi University Laboratory Animals Raising and Experimental Research Center (GUDAM). During the entire experimental process, all animals were maintained under standardized environmental conditions (23 ± 1°C, 12-hour light/dark cycle). The rats had unrestricted access to standard food and tap water. Blinded observations by two independent researchers conducted throughout the experimental period revealed no differences in food intake, water consumption, or behavior among groups. Additionally, body weights measured at the beginning and end of the study showed minimal (~±10%) changes within normal physiological limits, indicating stable conditions in the animals. The study was approved by the Gazi University Local Ethics Committee for Animal Experiments (Protocol No: 20.040) and conducted in a blinded manner. The experimental groups and the procedure applied are presented in Table 1.

**Table 1.** Experimental groups and procedure details.

| Groups | Procedure |
|---|---|
| Control | No procedures were applied to the experimental animals. |
| Sham | Rats were given 1 ml/kg saline by intraperitoneal injection for 15 days. After the injection, the rats were placed in Plexiglas boxes and kept for 1 hour with the device not being operated (RF OFF) (between 09:00 and 11:00 a.m.) |
| LPS | Neuroinflammation was applied by intraperitoneal injection of 1 mg/kg LPS in 1 ml/kg saline solution to rats for 15 days (between 09:00 and 11:00 a.m.) |
| LPS+AGM | Rats were given 1 mg/kg LPS in 1 ml/kg saline solution for 15 days and 30 minutes later, 10 mg/kg AGM in 1 ml/kg saline solution was given to the animals by intraperitoneal injection (between 09:00 and 11:00 a.m.) |
| LPS+RF | Neuroinflammation was induced by intraperitoneal injection of 1 mg/kg LPS in 1 ml/kg saline solution to rats for 15 days. Following the application, rats were placed in plexiglas boxes and exposed to 2600 MHz for 1 hour (between 09:00 and 11:00 a.m.) |
| AGM+RF | 10 mg/kg AGM in 1 ml/kg saline solution was administered to rats by intraperitoneal injection for 15 days. Following the administration, rats were placed in Plexiglas boxes and exposed to 2600 MHz for 1 hour (between 09:00 and 11:00 a.m.) |
| LPS+AGM+RF | For 15 days, 1 mg/kg LPS was administered to rats in 1 ml/kg saline solution and 30 minutes later, 10 mg/kg AGM was administered to the animals in 1 ml/kg saline solution by intraperitoneal injection. Following the administration, rats were placed in plexiglas boxes and exposed to 2600 MHz for 1 hour (between 09:00 and 11:00 a.m.) |

Animals ($n = 49$) were divided randomly into 7 experimental groups. There were 7 animals in each group.

### Tissue collection, processing, and analyses

At the conclusion of the experimental protocol, animals were deeply anesthetized, and blood and brain tissues were collected. Blood samples were immediately centrifuged to separate serum, which was stored at −80°C for subsequent biochemical analyses. Hypothalamic tissues were processed specifically for molecular analyses and stored at −80°C until use. Serum levels of IL-6, IL-1β, and TNF-α were quantified using ELISA kits according to the manufacturer's protocols. Each cytokine assay was performed on all experimental groups (Control, Sham, LPS, LPS+AGM, LPS+RF, AGM+RF, and LPS +AGM+RF) under the same conditions on the same day. Each cytokine analysis was conducted on consecutive days. Hypothalamic tissues were used to determine the expression levels of p38, ERK, JNK, MAPK, NF-κB, and Beclin-1 (Santa Cruz Biotechnology, Texas, USA). Each experimental group was analyzed on the same day, while different protein expression analyses were performed on consecutive days. These procedures ensured minimization of variability and maintained consistency across all experimental measurements.

### Experimental timing and blinding

To mitigate potential confounding from temporal variations, all experimental procedures, including injections, RF exposures, and subsequent sample collections, were performed concurrently for all seven groups. The animals were processed in a randomized order each day between 09:00 and 11:00 to minimize circadian effects. All biochemical and molecular analyses were conducted by researchers blinded to the experimental group assignments to prevent observer bias.

A separate group exposed solely to RF fields was not included in the study. The sham group consisted of animals subjected to mock injections while the RF device was turned off. This design reflects the primary aim of our study, which was to evaluate the synergistic effects of RF exposure combined with pharmacological intervention. Therefore, including a group exposed only to RF was beyond the scope of the current study and was excluded due to both methodological focus and ethical considerations. Additionally, the study design was planned in accordance with the ARRIVE guidelines and the 3Rs principles (Replacement, Reduction, Refinement), ensuring minimization of animal use and adherence to ethical responsibilities.

### Radiofrequency fields exposure system

In this study, electromagnetic radiation at 2600 MHz was generated using a signal generator (Rohde & Schwarz, Munich, Germany) and delivered through a standard gain horn antenna (ETS Lindgren, Cedar Park, TX, USA). Female Wistar albino rats were housed in chamber made of transparent plexiglass with dimensions of $35 \times 35 \times 15$ cm. The chamber was positioned directly beneath the horn antenna to ensure uniform exposure. Electric field strength measurements were conducted using a broadband electromagnetic field meter (Narda EMR-300) equipped with a probe (Model 8.3). To assess spatial field distribution within the chamber, measurements were taken at three predefined locations: the center, left, and right sections of the interior. Under active exposure conditions, the mean internal electric field strength at 2600 MHz was determined to be 16.36 V/m. Background measurements, recorded with the RF system turned off, showed an external ambient electric field level of 0.76 V/m.

### Specific absorption rate (SAR) calculations

AR simulations were performed using the CST Microwave Studio (3DEXPERIENCE®, Dassault Systems, Hamburg) software, which is based on the Finite Integration Technique (FIT). FIT solves Maxwell's equations in their integral form, while the grid discretization is carried out in the time domain similarly to the Finite Difference Time Domain (FDTD) method. The rat model used in the simulations is a voxel-based model with a resolution of $1.827 \times 1.827 \times 2.015\ mm^3$, available in the simulation software's library. The dielectric properties of the rat model at 2600 MHz were obtained from an open-source library in the literature. The behavior of biological tissue under electromagnetic wave exposure is influenced by its dielectric properties (Abdilla et al. 2013; Gabriel et al. 1996). The electromagnetic exposure problem space was modeled using a horn antenna operating at 2600 MHz fed with 29.55 dBm (0.9 W) power, replicating the experimental setup. SAR simulations were conducted according to the IEEE/IEC 62,704 standard.

### Biochemical analyses

At the end of the experiment, all rats were decapitated under anesthesia. Hypothalamus and blood samples were collected. Plasma was separated by centrifugation and then stored at −80°C until analysis. Cytokine levels of IL-1β [Thermo-Invitrogen Brand-BMS630, USA] and IL-6 [Thermo-Invitrogen Brand-BMS628, USA] were assessed using commercially available ELISA kits according to the manufacturer's protocols.

### Western blot analysis

The hypothalamus tissue was analyzed using Western Blot to assess the expression of p38, ERK, JNK, MAPK, NF-κB, and Beclin-1 [Santa Cruz, Biotechnology, Texas, U.S.A.]. After the experiment, the hypothalamus tissue from the rats was quickly collected, washed with physiological serum, and frozen at −80°C. Tissue samples were homogenized in lysis buffer (Tris-EDTA, NaCl, SDS, deoxycholic acid, Igepal, and protease inhibitor). The homogenate was centrifuged at 12,000 rpm for 10 minutes, and the supernatant was used for protein quantification with Bradford reagent. Proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, blocked, and incubated with primary antibodies. The membrane was then washed with washing solution. The membrane was then incubated with a secondary antibody, and bands were visualized using substrate solution. Band intensity was measured using ImageJ software and compared with β-actin (Chatterjee et al. 2012).

### Statistical analysis

Statistical analyses for quantitative comparisons between groups were performed using SPSS v20. Normality of data distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were assessed before conducting the analyses. Differences among group means were evaluated using One-Way Analysis of Variance (ANOVA). When ANOVA results indicated statistical significance ($p < 0.05$), Tukey's HSD post-hoc test was applied to identify specific group differences. Effect sizes (Cohen's d) were calculated to assess the practical significance and magnitude of observed differences. Cohen's d values, calculated using pooled standard deviation to account for unequal sample sizes, were interpreted as small ($d \approx 0.2$), medium ($d \approx 0.5$), or large ($d \geq 0.8$). Statistical significance was accepted at $p < 0.05$ for all analyses, and data are presented as mean ± standard deviation (SD).

## Results

### Specific absorption rate calculations

The SAR was calculated both as a whole-body average and over localized tissue volumes of 1 g and 10 g, in accordance with international standards (Organization 2020). Based on a total body mass of 271 g (Alkis et al. 2021), the whole-body averaged SAR was determined to be 0.637 W/kg. Furthermore, the localized SAR values were 1.27 W/kg for 1 g of brain tissue and 0.91 W/kg for 10 g of brain tissue at 2600 MHz frequency. These values represent the maximum local SAR values within the tissue. The exposure parameters were set to remain within non-thermal limits according to established guidelines (Fields 2020). The SAR distribution are shown in Figure 1

### Western-blot results

No statistically significant differences were observed in Beclin-1 protein levels among the experimental groups (ANOVA, $F = 0.54$, $p = 0.7695$). Similarly, MAPK levels did not differ significantly across groups (ANOVA, $F = 0.60$, $p = 0.7249$). Although p38 protein levels showed a near-significant variation among groups, the results were not statistically significant (ANOVA, $F = 2.66$, $p = 0.0618$). In contrast, ERK protein levels showed

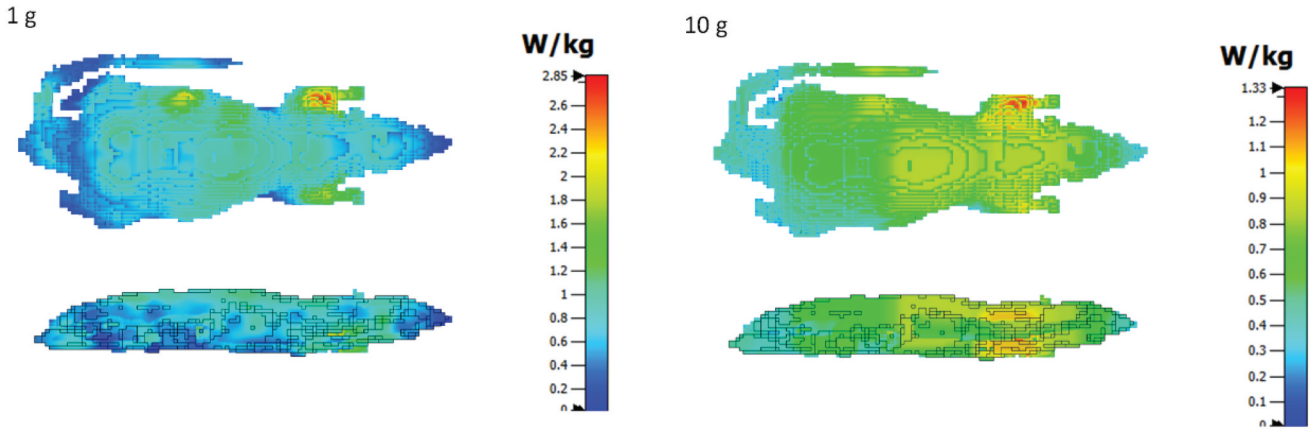



**Figure 1.** SAR distributions for 1 g and 10 g of samples at frequencies of 2600 MHz.

a significant difference among groups (ANOVA, F = 5.24, $p = 0.0088$), with the LPS group showing the most pronounced change. ERK levels in the LPS group were significantly lower than those in the Control ($p < 0.0001$; Cohen's d = −15.51), LPS+AGM ($p = 0.0142$; Cohen's d = −3.40), LPS+RF ($p = 0.01$; Cohen's d = −3.76), and LPS +AGM+RF ($p = 0.0173$; Cohen's d = −3.20) groups.

JNK protein levels also showed statistically significant differences among groups (ANOVA, F = 5.57, $p = 0.0039$). The LPS ($p = 0.0028$; Cohen's d = 5.34) and LPS +AGM ($p = 0.0207$; Cohen's d = 3.03) groups had significantly lower JNK levels compared to the Control group. Additionally, the combination treatment group (LPS+AGM+RF) exhibited significantly higher JNK levels compared to both the LPS ($p = 0.0131$; Cohen's d = 3.48) and LPS+AGM ($p = 0.0186$; Cohen's d = 3.13) groups.

NF-κB protein levels also differed significantly across groups (ANOVA, F = 3.47, p = 0.0258). The AGM+RF (p = 0.0002; Cohen's d = 10.94), LPS (p = 0.0157; Cohen's d = 3.29), and LPS+AGM (p = 0.0072; Cohen's d = 4.13) groups showed significantly lower NF-κB levels compared to the Control group (Figure 2 B-G).

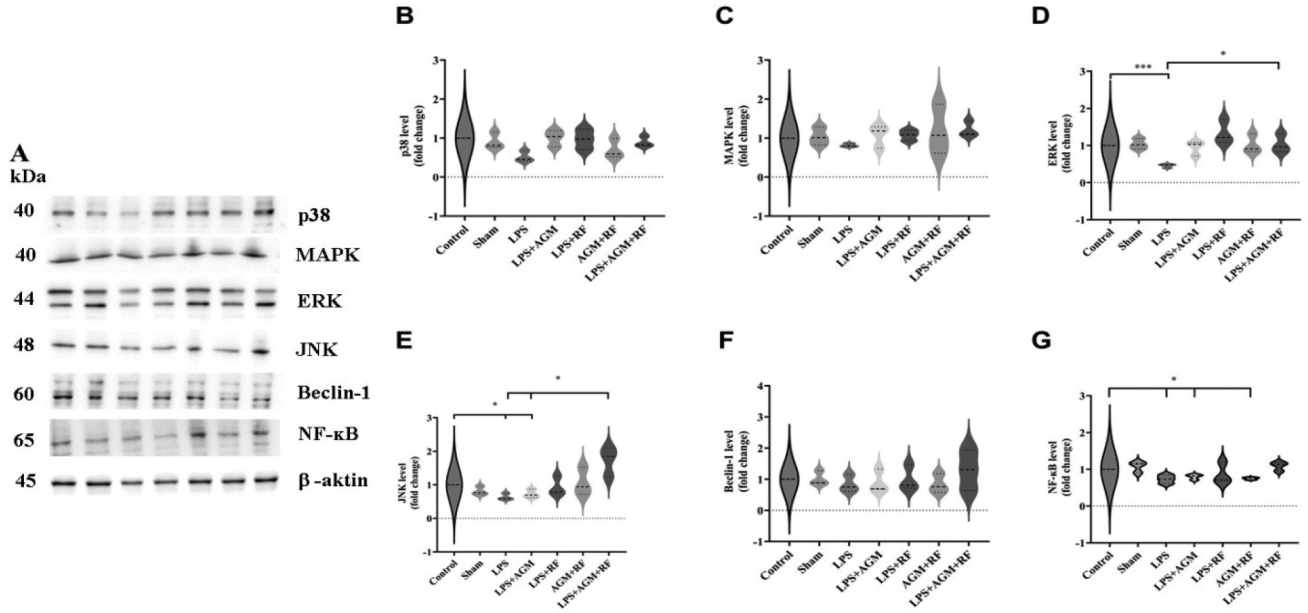



**Figure 2.** Effects on hypothalamic protein expression levels following LPS, Agomelatine, and 2600 MHz RF exposure in rats. (A) Representative gel images, and relative expression levels of (B) p38, (C) MAPK, (D) ERK, (E) JNK, (F) Beclin-1, and (G) NF-κB proteins are shown. Protein levels were analyzed by Western blot and expressed as Fold changes normalized to the Control group. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test. Significance levels are indicated as follows: *$p <$ 0.05 and ***$p < 0.001$ vs. LPS group.

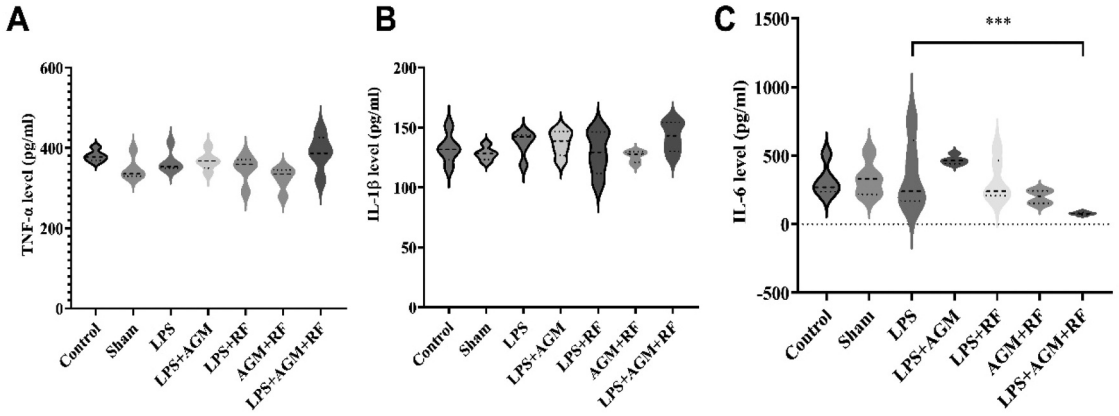



**Figure 3.** Effects on plasma cytokine levels following LPS, Agomelatine, and 2600 MHz RF exposure in rats. Graphs represent the concentrations of (A) TNF-α, (B) IL-1β, and (C) IL-6 across different treatment groups. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test for group comparisons. Significance levels are indicated as follows: ***$p < 0.001$ compared to the LPS group.

### *Cytokine results*

Serum levels of the key pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were measured across all experimental groups using ELISA (Figure 3). One-way ANOVA revealed a significant overall difference among groups for TNF-α levels ($F(6,28) = 3.89$, $p = 0.005$); however, post-hoc analyses did not identify significant pairwise differences between groups. IL-1β levels showed no significant overall difference among groups ($F(6,28) = 1.63$, $p = 0.178$), consistent with the lack of significant differences in post-hoc comparisons. In contrast, IL-6 levels demonstrated pronounced and statistically significant effects of treatments, particularly the combined therapy. One-way ANOVA showed highly significant differences across groups ($F(6,27) = 9.18$, $p < 0.0001$). Neither the LPS+AGM nor the LPS+RF groups exhibited significant reductions in IL-6 levels compared to the LPS group ($p = 0.69$ and $p = 0.95$, respectively). However, the LPS+AGM+RF group showed a markedly significant suppression of IL-6 compared to the LPS group ($p < 0.001$). The calculated Cohen's d for this comparison was 1.59, quantitatively confirming a very large effect size.

## Discussion

In this study, we investigated the combined effects of AGM and exposure to a 2600 MHz RF electromagnetic field on systemic and central inflammation in a rat model of chronic lipopolysaccharide (LPS)-induced immune activation. The most prominent and robust finding was that co-administration of AGM and RF significantly reduced plasma IL-6 levels, indicating a potent immunomodulatory or anti-inflammatory effect of the combined intervention. Concurrently, chronic LPS exposure in the hypothalamus was associated with downregulation of key MAPK signaling pathways (ERK, JNK), suggestive of an endotoxin tolerance-like state.

The central nervous system is highly sensitive to RFF exposure (Minasyan et al. 2007). Studies on the biological effects of RFF on the hypothalamus are gaining significant attention, as it connects the nervous and endocrine systems, regulating cognition, and is crucial for homeostasis. The hypothalamus PVN, plays a critical role in the adaptation and survival of all vertebrate species in response to various physical and psychological stressors (Herman and Cullinan 1997; Herman et al. 2002; Sawchenko and Swanson 1981). Dysregulation of hypothalamus function may result in symptoms like appetite loss, mood disturbances, infertility, memory impairment, and reduced serotonin and dopamine levels (Vercruysse et al. 2018). Because of the close proximity between the brain and mobile phones, the RFF emitted by these devices can be absorbed by the brain, potentially influencing its functions (Christ et al. 2010). Studies suggest that RFF exposure can result in neuronal damage, changes in neurotransmitter release, and behavioral alterations (Dubreuil et al. 2002; Leszczynski et al. 2002). The therapeutic effects of RFF on pathologies such as wound healing, musculoskeletal pain, osteoporosis, cancer, and neurodegenerative diseases have been widely studied in the literature (Asci et al. 2023; Pantelis et al. 2024). However, there is no consensus on the optimal dose and duration. Few studies have explored the effects of RFF on LPS-induced inflammation (Gevrek et al. 2023; Lee et al. 2022). Our study is the first to evaluate the use of 2600 MHz RFF both alone and in

combination with AGM in LPS-induced inflammation, demonstrating its positive impact on inflammation.

LPS, also known as endotoxins, are found in the outer layer of gram-negative bacteria and play a vital role in both structural and functional integrity. Studies have shown that LPS can induce pro-inflammatory responses and trigger serious physiological reactions, including fever and septic shock (Martich et al. 1993). This rapid innate response stimulates the release of pro-inflammatory mediators like TNF-α, IL-6, and IL-1β, which are crucial for immune system function (Erridge et al. 2002). Consistent with our findings, in macrophages pre-stimulated with LPS, phosphorylation of MAPK pathways was suppressed in response to a second LPS stimulus. This suggests that endotoxin tolerance is associated with the suppression of MAPK pathways. Additionally, repeated LPS exposures have been shown to significantly inhibit the NF-κB response (Medvedev et al. 2000). Recent studies have demonstrated downregulation of ERK, JNK, and p38 MAP kinase activation in endotoxin-tolerant mouse macrophages (Kraatz et al. 1999a, 1999b). In our study, the statistically significant decrease in ERK, JNK, and NF-κB levels following chronic LPS exposure suggests the development of endotoxin tolerance and immune system desensitization to LPS. Additionally, the hypothalamus is highly sensitive to immune system molecules such as cytokines (Dantzer et al. 2008). Proinflammatory cytokines like IL-1β, IL-6, and TNF-α regulate the hypothalamus behavioral and hormonal responses. In our study, the downregulation of MAPK pathways in the hypothalamus following repeated LPS exposure suggests disruption of the body's homeostasis. Moreover, the lack of changes in the LPS+AGM and LPS+AGM+RF groups indicates that AGM and 2600 MHz RFF regulate intracellular signaling, helping maintain hypothalamus homeostasis.

AGM, a compound derived from melatonin, possesses strong antioxidant properties (Freiesleben and Furczyk 2015) While it is known to have potent antioxidant and anti-inflammatory effects, the results across studies are inconsistent (Demirdaş et al. 2016). Some studies have shown that AGM treatment leads to an increase in cytokine levels. Although AGM application raises the levels of TNF-α, IL-1β, and IL-4, it has been reported to reduce lipid peroxidation (Demirdaş et al. 2016). Our study shows an increase in IL-6 levels in the LPS+AGM group compared to the LPS group, although this difference was not statistically significant, which is consistent with findings from previous studies. Therefore, it is hypothesized that AGM treatment regulates the balance between pro- and antioxidants in rats by downregulating oxidative stress levels while upregulating the pro-cytokine system (Demirdaş et al. 2016).

IL-6 has been reported to activate the NF-κB signaling pathway, enhancing its role in immune response and promoting pro-inflammatory cytokine production. Additionally, the JNK pathway is suggested to interact with NF-κB, modulating both the levels and effects of IL-6 (de Haij et al. 2005). Chronic LPS exposure is known to induce endotoxin tolerance and immune desensitization, characterized by the downregulation of key inflammatory signaling pathways such as ERK, JNK, and NF-κB (Biswas and Lopez-Collazo 2009; Medvedev et al. 2000). Consistent with this, our study observed a significant decrease in these markers following chronic LPS treatment. However, combined treatment with agomelatine and 2600 MHz radiofrequency field reversed this effect by upregulating JNK and NF-κB expression, suggesting a reactivation of the immune response. Notably, this immune reactivation was accompanied by a significant reduction in IL-6 levels, indicating a regulatory mechanism that may limit excessive inflammation. Similarly, previous studies have reported that radiofrequency field exposure exerts immunomodulatory effects by reducing cytokine levels (Balci et al. 2025). Together, these findings suggest that agomelatine and radiofrequency exposure may synergistically recalibrate immune signaling pathways while controlling pro-inflammatory cytokine production.

In conclusion, this study demonstrates that the combined application of agomelatine and 2600 MHz radiofrequency field exposure significantly attenuates systemic inflammation in a rat model of chronic LPS challenge. This is evidenced by a large and statistically significant reduction in plasma IL-6 levels, a key pro-inflammatory cytokine. The findings provide novel evidence for a potential synergistic, anti-inflammatory role for RF exposure when applied in a pre-existing inflammatory state, suggesting that it may act as a modulator of the immune response rather than an inflammatory trigger. This work highlights a promising avenue for future therapeutic exploration into the interactions between pharmacological agents and non-ionizing electromagnetic fields.

## Acknowledgments

We would like to also thank the Gazi University non-ionizing radiation protection application and research center (GİRKUM) for measuring the electric field values.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This study was supported by the Gazi University Scientific Research Projects Coordination Unit (BAP) [Project No: 01/2020-45].

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## Abbreviations

| | |
|---|---|
| EMF | Electromagnetic field |
| RFF | Radiofrequency field |
| RF | Radiofrequency |
| SAR | Specific absorption rate |
| LPS | Lipopolysaccharide |
| AGM | Agomelatine |
| MAPK | Mitogen-activated protein kinases |
| ERK | Extracellular signal-regulated kinase |
| JNK | c-Jun N-terminal kinase |
| NF-κB | Nuclear Factor kappa B |

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