Original Article

Comparison of the Effects of Rosmarinic Acid and Electromagnetic Radiation-Induced Cardiotoxicity on Rats

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Abstract

Background: *Electromagnetic radiation (EMR) causes stable aggregation of reactive oxygen species (ROS), producing oxidative stress. Rosmarinic acid (RA), a plant-origin antioxidant, has been proposed against the side effects of cell phone and ultrahigh-frequency waves.*

Methods: *Forty-two male Wistar rats were randomly divided into 6 groups. Group 1 (controls) received 5 mL of normal saline with the gavage method, Group 2 received 915 MHz radiation, Group 3 received 2450 MHz radiation, Group 4 received RA plus 915 MHz radiation, Group 5 received RA plus 2450MHz radiation, and Group 6 received oral RA (5 mg/kg). Treatment and radiation (1 hour per day) continued for up to 30 days.*

Results: *EMR significantly reduced the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), the content of glutathione (GSH), and the level of total antioxidant capacity (TAC) and significantly increased oxidative stress indices, such as the levels of malondialdehyde (MDA) and nitric oxide (NO), and the content of protein carbonyl (PC). In contrast, RA significantly elevated TAC level (all groups), GSH content (the RA/cell phone radiation group), GPx activity (the RA/ultrahigh-frequency radiation group), SOD activity (all groups), and CAT activity (RA/ultrahigh-frequency radiation group) and conversely reduced MDA level (all groups), NO level (all groups), and PC content (all groups) in the RA/cell phone and RA/ultrahigh-frequency radiation groups compared with the NS/cell phone and NS/ultrahigh-frequency radiation groups, respectively. The administration of RA resulted in a significant reversal of cardiac markers in EMR-intoxicated rats.*

Conclusion: *RA treatment showed a significant protective effect against EMR-induced cardiotoxicity.*

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Keywords: *Rosmarinic acid; Heart; Oxidative stress; Rats; Ultrahigh-frequency wave; Cell phone*

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Introduction

Wireless local area networks and global systems for cell phone communication are generally used in many countries. Electromagnetic radiation (EMR) produced by cell phones (915 MHz) and ultrahigh-frequency devices (2.45 GHz) is on the increase as it can be used at home or work. Recently, many investigations have reported scientific evidence regarding the health impacts of EMR. Devices that integrate wireless technology, such as laptops and cell phones, are often used near body organs, which may affect them. Radiation damages living cells extensively because of oxidative stress. The heart produces its own rhythm and regular contracts, and external stimuli can affect its contractions or rhythms.[1] Because of its characteristics, the heart has less protection against damage induced by reactive oxygen species (ROS) relative to the other organs. ROS may cause myocyte atrophy, apoptosis, and interstitial fibrosis in cardiac tissue, suppressing heart function and promoting dysfunction. Highly reactive superoxide radicals and hydrogen peroxide are toxic directly because of their assault at the molecular level and indirectly because of their production of secondary reactive species, such as hydroxyl radicals. These radicals probably cause oxidative damage to biomolecular structures. Radiation significantly decreases enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) and reversely increases oxidative stress indicators, malondialdehyde (MDA), nitric oxide (NO), and protein carbonyl (PC). Often enzymatic antioxidants fail to support cells from injury caused by ROS-induced oxidative stress. Radioprotectors suppress oxidative stress by neutralizing free radicals and ROS.

Recent years have witnessed a growth of interest in the curative abilities of natural products as antioxidants in diminishing free radical-induced tissue damage. Rosmarinic acid (RA) is isolated from many species of Lamiaceae and Boraginaceae and is one of the essential components of medicinal plants in these families (eg, Salvia officinalis, Mentha x piperita, Thymus vulgaris, Melissa officinalis, and Symphytum officinale).[2] RA has various biological activities, such as antimutagenic,[3] anticancer,[4] antimicrobial,[5] antioxidant,[6] anti-inflammatory,[7] antiangiogenic,[8] and antineurodegenerative activities.[9]

Despite the abovementioned properties of RA, the radioprotection characteristics of RA against EMR-induced oxidative stress in the hearts of rats still need elucidation. Thus the principle aim of the present study was to evaluate the radioprotective effects of RA on oxidative stress by measuring changes in TAC level and activities of antioxidant enzymes, including SOD, CAT, GPx, and GSH, and the values of stress oxidative indicators, such as MDA, NO, and PC in the hearts of Wistar rats subjected to 915 MHz and 2450 MHz radiofrequency radiations.

Methods

Phosphate buffer saline, bovine serum albumin, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 2,4-dinitrophenylhydrazine, reduced GSH, tetraethoxypropane, trichloroacetic acid, naphthylethylenediamine dihydrochloride, 5, 5-dithiobis (2-nitrobenzoic acid), thiobarbituric acid, Bradford reagent, and RA were purchased from Sigma Chemicals (St Louis, MO, USA) and Merck Company (Germany).

Forty-two adult male Wistar rats (200–250 g) were taken from the Animal House of Qom University of Medical Sciences. The rats were fed on a compact diet of granules and water and kept at a controlled temperature (22±1 °C) with a 12-hour light and 12-hour dark cycle and humidity maintained at 35% to 60%. Experiments were performed in accordance with the guidelines for the use of experimental animals set by the Animal Ethics Committee (Ethics code: IR.AJUMS.ABHC.REC.1397.013). One hour before radiation, normal saline (NS) or RA was administered as prescribed by the amount of RA in some other articles.[10, 11] The rat dose of RA (ie, 20 mg/kg) is equivalent to 2.7 mg/kg human dose of RA based on the body surface area.

The animals were randomly divided into 6 groups, each consisting of 7 rats. Group 1 (controls) received only 5mL of NS (0.9%) daily, Group 2 received 915 MHz radiation daily, Group 3 received 2450 MHz radiation daily, Group 4 received RA (5 mg/kg) plus 915 MHz radiation daily, Group 5 received RA plus 2450 MHz radiation daily, and Group 6 received only RA daily. All the drugs were administered through oral gavage, and the duration of treatment was 30 consecutive days.

Certified gigahertz transverse electromagnetic mode cells produced an electromagnetic field with a power density of 0.98 mW/cm2 for cell phones and 0.79 mW/cm^2 for Wi-Fi. During the radiation process, the incident electromagnetic field was unvarying over the entire biological object.

Twenty-four hours after the last treatment, the animals were anesthetized intraperitoneally with a combination of ketamine (50 mg/kg) and xylazine (5 mg/kg). For biochemical estimations, the rats were sacrificed by rapid decapitation, and their heart tissue was homogenized (1/10 w/v) in ice-cold Tris–HCl buffer (0.1 M, pH=7.4) with a homogenizer (WiseTis HG-150D, PMI-Labortechnik GmbH Company, Germany).[12] For the measurement of the protein content of the homogenates, the Bradford method was used, and Crystalline bovine serum albumin was employed as the standard.[13]

SOD activity was assessed by the process described by Martin,[14] CAT activity was measured by the method of Aebi,[15] GSH peroxidase activity was measured by the method described by Ellman et al,[16] and GPx activity was

determined with the GSH peroxidase Kit (Randox Labs, Crumlin, UK).

According to the method of Buege, MDA, an indicator of lipid peroxidation, was measured. NO level was measured using the Griess diazotization reaction after the conversion of nitrate to nitrite by nitrate reductase in the supernatant.[17] PC content in the rats' hearts was determined by the method of Levine et al.[18]

For the measurement of TAC level, the ferric-reducing antioxidant power (FRAP) assay as a colorimetric method was performed. In brief, 50 µL of the supernatant was blended with 1.5 mL of the fresh FRAP reagent (25 mL of 0.3M sodium acetate buffer, pH=3.6; 2.5 mL of 0.01M TPTZ in 0.04M of HCl; and 2.5 mL of 0.02M FeCl3 · 6H2O). The mixture was incubated at 37 °C for 5 minutes, and the absorbance was measured at 593 nm. For calibration, the FeSO4 solution was used, and TAC level was revealed as µmol/mg of protein.

With the aid of the Bradford method, the protein content of the heart was measured using bovine serum albumin as a standard solution.[19]

Statistical analysis was conducted using the ANOVA and t test for the comparison of data between the control group and the experimental groups. The results were expressed as mean±standard error of means (SEM). A P value of less than 0.05 was considered significant.

Results

The effects of RA on the activities of antioxidant enzymes, namely SOD, CAT, and GPx, and GSH content were revealed. GSH content and SOD, CAT, and GPx activities were significantly reduced in the cell phone and ultrahigh-frequency radiation groups compared with the control group ($^{*}P<0.05$). Treatment with RA significantly increased GSH content in the RA/cell phone radiation group relative to the cell phone radiation group ($^{\#}P<0.05$). GPx and CAT activities were significantly boosted in the RA/ultrahigh-frequency radiation group by comparison with the ultrahigh-frequency radiation group ($^{\dagger}P<0.05$). SOD activity was significantly augmented in the RA/cell phone group and the RA/ultrahigh-frequency radiation group compared with the cell phone and ultrahigh-frequency radiation group ($^{\#}P<0.05$ and $^{\dagger}P<0.05$, respectively). Additionally, the administration of RA to naïve rats did not change these antioxidant enzymes compared with the control group (Figure 1 & Figure 2).

The MDA and NO levels and the PC content of the rats' hearts were significantly increased in the EMR groups compared with the control rats (all $^{*}Ps<0.05$). Treatment with RA significantly decreased MDA and NO levels and PC content in the RA/cell phone group and the RA/ultrahigh-frequency radiation group groups compared with the cell phone and ultrahigh-frequency radiation group ($^{\#}P<0.05$ and $^{\dagger}P<0.05$, respectively). In addition, the administration of RA to naïve rats did not change these oxidative stress parameters compared with the control group (Figure 3).

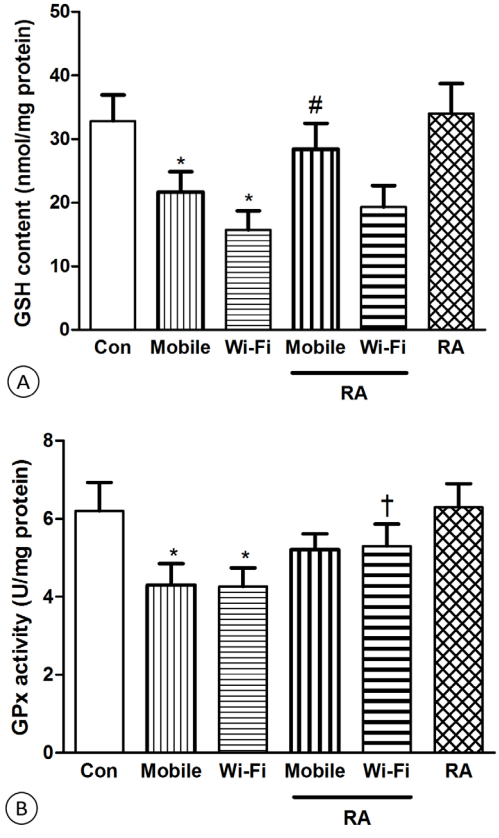


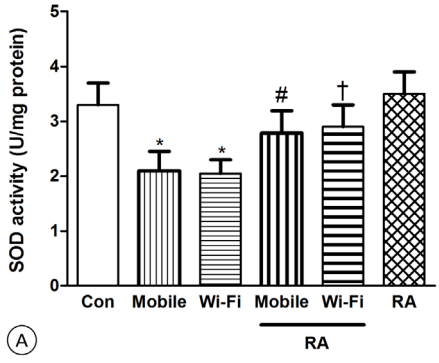
Figure 1. EMR significantly reduced GSH content and GPx activity in the EMR groups relative to the control group. RA treatment significantly increased GSH content in the RA/cell phone radiation group compared with the cell phone radiation group. Additionally, the activity of GPx was significantly increased in the RA/ultrahigh-frequency radiation group in comparison with the ultrahigh-frequency radiation groups. Values are mean±SD (n=7).

$^{*}P<0.05$ indicates a significant difference compared with the control group.

$^{\#}P<0.05$ indicates a significant difference by comparison with the cell phone radiation group.

$^{\dagger}P<0.05$ indicates a significant difference in comparison with the ultrahigh-frequency radiation group.

RA, Rosmarinic acid; EMR, Electromagnetic radiation; GSH, Glutathione; GPx, Glutathione peroxidase



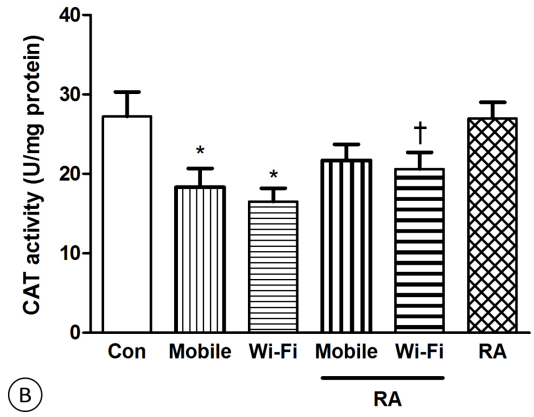


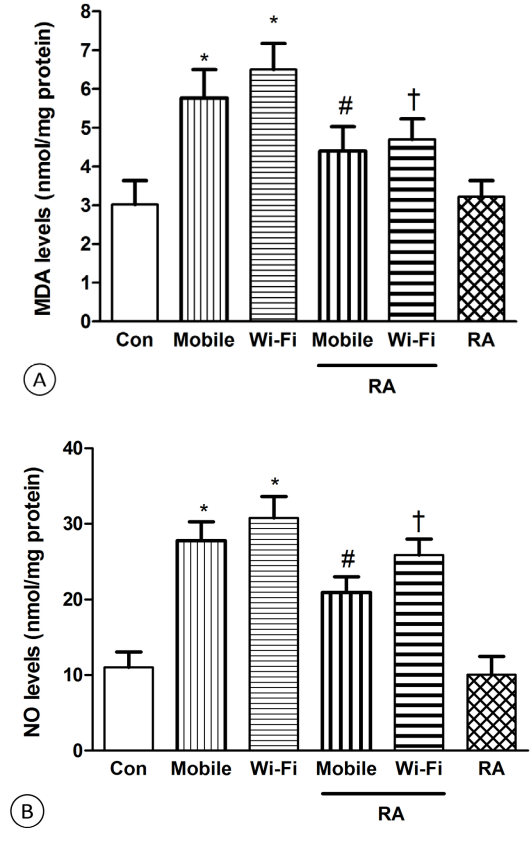
Figure 2. The images illustrate the effects of RA on SOD and CAT activity in the hearts of rats exposed to EMR. EMR significantly reduced SOD and CAT activity compared with the control group. RA treatment significantly increased the activity of SOD in the RA/cell phone and RA/ultrahigh-frequency radiation groups in comparison with the cell phone and ultrahigh-frequency radiation groups, respectively. Additionally, the activity of CAT in the RA/ultrahigh-frequency radiation group was significantly increased by comparison with the ultrahigh-frequency radiation group. Values are mean±SD (n=7).

*P<0.05 indicates a significant difference in comparison with the control group.

#P<0.05 indicates a significant difference by comparison with the cell phone radiation group.

†P<0.05 indicates a significant difference compared with the ultrahigh-frequency radiation group.

RA, Rosmarinic acid; EMR, Electromagnetic radiation; SOD, Superoxide dismutase; CAT, Catalase



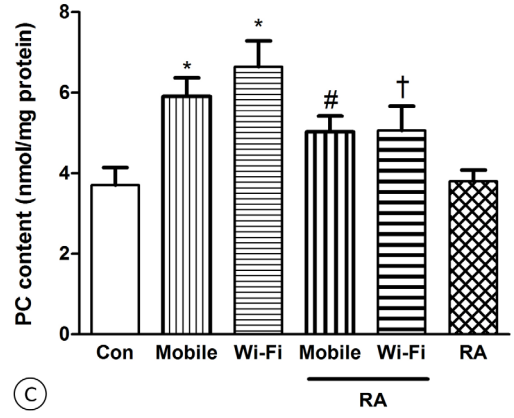


Figure 3. EMR significantly increased MDA and NO levels and PC content compared with the control group. RA treatment significantly decreased the activity of MDA and NO levels in the RA/cell phone and RA/ultrahigh-frequency radiation groups in comparison with the cell phone and ultrahigh-frequency radiation groups, respectively. Values are mean±SD (n=7).

RA, Rosmarinic acid; EMR, Electromagnetic radiation; MDA, Malondialdehyde; NO, Nitric oxide

*P<0.05 indicates a significant difference by comparison with the control group.

#P<0.05 indicates a significant difference in comparison with the cell phone radiation group.

†P<0.05 indicates a significant difference compared with the ultrahigh-frequency radiation group.

EMR significantly decreased TAC level in the cell phone and ultrahigh-frequency radiation groups compared with the control group (*P<0.05). Treatment with RA significantly reversed the level of TAC reduced by EMR. The administration of RA to naïve rats did not change TAC level compared with the control group (Figure 4).

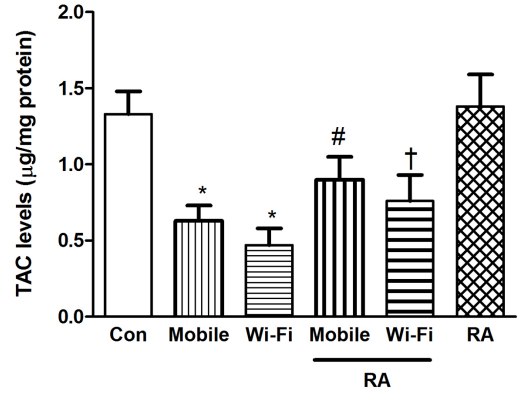


Figure 4. The image illustrates the effects of RA on TAC level in the hearts of rats exposed to EMR. EMR reduced TAC level compared with the control group. RA treatment significantly reversed the level of TAC influenced by EMR. Values are mean±SD (n=7).

RA, Rosmarinic acid; EMR, Electromagnetic radiation; TAC, Total antioxidant capacity

*P<0.05 indicates a significant difference in comparison with the control group.

#P<0.05 indicates a significant difference compared with the cell phone radiation group.

†P<0.05 indicates a significant difference by comparison with the ultrahigh-frequency radiation group.

Discussion

EMR radiation can induce oxidative stress in biological systems via free radicals by enhancing lipid peroxidation and reducing antioxidant levels. ROS is probably the principal culprit of the adverse impacts of EMR radiation related to ultrahigh-frequency and cell phone radiation.[20] Investigations have shown that oxidative stress enhances the risk of heart infection.[21] Likewise, the chronic excess production of ROS promotes the creation of plaques in cardiovascular vessels,[22] with the resultant arteriosclerosis enhancing the risk of stroke and heart failure.[23] We showed that chronic exposure to ultrahigh-frequency and cell phone radiation might cause lipid peroxidation and antioxidant suppression in cardiac tissue, which is consistent with prior studies.[24] On the other hand, we discovered the probable protective effects of RA, as a natural antioxidant, against cell phone and ultrahigh-frequency radiation-induced toxicity in the hearts of rats.

Free radical-scavenging enzymes, namely SOD, CAT, GPX, and GSH, are the first-line cellular shield against oxidative damage.[25] Any alteration in ROS-scavenging enzymes is responsible for inducing oxidative stress. SOD, an intracellular antioxidant enzyme, swiftly diminishes superoxide radicals to hydrogen peroxide.[26] CAT protects cells from oxidative damage by breaking down H2O2 into water and molecular oxygen. GSH is an antioxidant that can be found in animals and plants and is vital for combating possible injuries caused by ROS, such as lipid peroxides, peroxides, and free radicals.[27] GPx is crucial to the protection of cellular function and resistance against oxidative injuries. CAT and GPx are well-known H2O2 scavengers protecting cells against the toxic effects of H2O2. Moreover, GPX, CAT, and SOD, by changing toxic free radicals to nontoxic outputs, guard cells against oxidative stress-mediated cellular injuries.[24] TAC level is an early marker of oxidative stress used to calculate the cumulative impact of the antioxidants found in the blood and bodily fluids.[28] The biomarkers of oxidative stress are NO and MDA levels and PC content. MDA is a product of polyunsaturated fatty acid peroxidation and is used as an indicator of oxidative stress in cells and tissues. NO acts as a free radical scavenger and inactivates superoxide anions, preventing cell toxicity.[29] Protein oxidation, especially PC, can be promoted by ROS. PC level is an oxidative stress indicator in human beings and animals.[30, 31]

In the current study, we showed that chronic exposure of the heart to cell phone and ultrahigh-frequency radiation significantly reduced GSH content, TAC level, and GPx, SOD, and CAT enzymatic activities and significantly enhanced oxidative stress indices, such as MDA and NO levels and PC content, in the hearts of rats relative to the control group. These results are consistent with previous studies where GPx, SOD, and CAT activities as antioxidant enzyme activities were diminished after exposure to a low level of microwave exposure (2.45 GHz) in mice,[32] and GSH concentration was significantly decreased following EMR.[33, 34] Additionally, 945 MHz radiation elevated MDA level in the blood samples of the rats in our study. Prior investigations have shown a significant decrease in total antioxidant activities (eg, SOD and GPX) and an increase in MDA after EMR exposure.[35] Salah and Abdelmelek[33] showed that SOD activity was significantly reduced and MDA level was significantly enhanced following exposure to 2.45 GHz radiation 1 hour per day for 21 consecutive days in the livers and kidneys of rats. Ozguner et al36 indicated that GPx, SOD, and CAT activities were decreased, whereas MDA and NO levels were increased after exposure to 30 minutes per day of cell phone radiation for 90 consecutive days in rats. Acute radiation generated by cell phones could increase lipid peroxidation and diminish the activation of free radical scavengers and the activities of SOD and GPx.[37] Oktem et al[38] reported that while MDA level was increased, SOD, CAT, and GPx activities were decreased in kidney tissue after exposure to 900 MHz cell phone radiation. Ilhan et al[39] concluded that MDA and NO levels were increased in rats exposed to radiation for 1 hour per day for 7 consecutive days, and Stopczyk et al[40, 41] reported that 900 MHz radiation significantly improved MDA concentration relative to the control group.[40, 41]

Treatment with RA as an exogenous antioxidant reversed the side effects of EMR in the present study, which chimes with previous studies. Our results revealed that MDA and NO levels and PC content in the RA/cell phone and RA/ultrahigh-frequency radiation groups were significantly lowered in comparison with the cell phone and ultrahigh-frequency radiation groups in the hearts of rats, respectively. Zhang et al[42] revealed that RA increased GPx, SOD, and CAT activities and reduced MDA level in the livers and kidneys of rats. Fernando et al[43] showed that SOD and CAT activities were improved with the administration of RA by scavenging intracellular ROS induced by ultraviolet-B light. RA is known to protect dopaminergic neuronal cells by inhibiting NO production.[44, 45] Hajhosseini et al[10] showed that when rats were exposed to 50Hz EMR and then received RA (5 mg/rat) daily for 6 weeks, TAC in the serum was increased ($P<0.05$), and MDA level was reduced. In the current study, MDA and TAC levels and PC content exhibited a higher increase in the NS/ultrahigh-frequency radiation group than in the NS/cell phone radiation group, indicating that ultrahigh-frequency radiation could be more powerful and, thus, more harmful than cell phone radiation. However, this result is not confirmed by other studies.[46, 47]

RA, as a natural antioxidant, might confer protection against free radical-induced tissue damage by scavenging superoxide radicals in rats.[48] The ability of RA to reduce DNA lesions, caspase-3 and caspase-9 activities, and interleukin-6 secretion may also reflect their antioxidant

activities.[49] Within RA-related compounds, the presence of ortho-dihydroxy moiety appears to be the critical structure involved in the intrinsic antioxidant activity.[50] Fadel and Kirat[51] showed that a piece of RA could be inserted spontaneously inside membranes, and this fraction of RA could prevent lipid peroxidation. The 4'-phenolic hydrogen can adjust free radical scavenging, and catechol 2,3-dioxygenase ensures the polarity for RA to enter lipid bilayers and support them against oxidation without bothering their foundation.[51, 52] Electrochemical studies have shown that the first oxidation level is related to the caffeic acid part, while the second oxidation level refers to the oxidation of the 3,4-dihydroxyphenyl lactic acid residue.[52] Because of the combination of these basic aspects, the antioxidant ability of RA is superior to other hydroxycinnamic acid derivatives.

Conclusion

Exposure of rats to ultrahigh-frequency and cell phone radiation decreased SOD, CAT, and GPX activities, GSH content, and TAC level, while it elevated MDA and NO levels and PC content. Our experimental results revealed that RA, as a powerful antioxidant, might protect the heart and decrease the induction of ROS by enhancing antioxidant enzymes and diminishing lipid peroxidation and oxidative stress indicators. It could be concluded that nutritional support with RA before irradiation confers a protective effect against radiation in male Wistar rats. The benefits of RA supplementation in the diet as a phytogenic additive could be promising. One of the limitations and weaknesses of the current study is the lack of pathology investigation. Further studies and histopathology are suggested to shed sufficient light on this topic.

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