

## Effect of Ginseng on Calretinin Expression in Mouse Hippocampus Following Exposure to 835 MHz Radiofrequency

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Exponential rise in the use of mobile communication devices has generated health concerns due to radiofrequency (RF) exposure due to its close proximity to the head. Calcium binding proteins like calretinin regulate the levels of calcium ( $\text{Ca}^{2+}$ ) which plays an important role in biological systems. Ginseng is known for maintaining equilibrium in the human body and may play a beneficial radioprotectant role against electromagnetic field (EMF) exposure. In the present study, we evaluated the radioprotective effects of red ginseng (RG) extract in a mouse model. Calretinin (CR) expression was measured using a free-floating immunohistochemical method in the hippocampus of mice after 835 MHz EMF exposure for 5 h/d for 5 d at specific absorption rate=1.6 W/kg for the different experimental groups. The control animals were treated with NaCl while the experimental animals received 10 mg/kg ginseng, or 30 mg/kg; EMF exposed mice were also treated with NaCl, 10 mg/kg ginseng (E10), or 30 mg/kg (E30). Decreases in CR immunoreactivity (IR) along with loss of CA1 and CA3 interneurons and infragranular cells were observed in the  $\text{E}_{\text{NaCl}}$  group while such losses were not observed in the E10 and E30 groups. CR IR significantly increased in the RG-treated group compared to control and EMF-exposed groups treated with NaCl. The study demonstrates that RG extract can serve as a radioprotective agent that maintains  $\text{Ca}^{2+}$  homeostasis and prevents neuronal loss in the brain hippocampal region caused by RF exposure.

**Keywords:** *Panax ginseng*, Red ginseng, Radiofrequency, Calcium binding protein, Calretinin, Hippocampus

### INTRODUCTION

Rapid growth in the mobile communication system has been accompanied by a parallel increase in electromagnetic field (EMF) density [1]. This has generated interest as well as concern about possible health risks. Mobile phones transmit microwaves between 450 to 900 MHz in analog systems and 1.8 to 2.2 GHz in digital systems [2]. Compared to the rest of the body, the brain is subjected to a relatively higher specific absorption rate (SAR) due to the close proximity of the head to mobile telephones.

Low frequency exposure has been reported to alter ac-

tivity of the central nervous system (CNS) by influencing neuronal functions like neurotransmitter release, neuronal survival, learning, and memory [3]. Decreased neuron number and neuronal damage in the cortex, cerebellum, hippocampus, and basal ganglia have been observed in the brains of animals exposed to 900 MHz EMF [4,5]. The hippocampus is helps control behavioral and cognitive functions including learning and memory [6,7], especially the cornu ammonis (CA) which influences short term memory and learning [8].

Calcium ( $\text{Ca}^{2+}$ ) plays important biological roles in-

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involved in membrane integrity and function, and is critical for CNS activity including neurotransmitter release and action potential generation [9].  $\text{Ca}^{2+}$  mobilization in neuronal and non-neuronal cells may be affected by radiofrequency (RF) exposure but this hypothesis is controversial. It has also been observed that RF exposure induces  $\text{Ca}^{2+}$  efflux from brain tissues and neurons isolated from different species [10,11]. The possible effect of RF EMF on cellular activities may probably be due to changes in  $\text{Ca}^{2+}$  homeostasis.

Although  $\text{Ca}^{2+}$  homeostasis in the brain is regulated by influx and extrusion systems, it is also affected by calcium binding proteins (CaBPs). CaBP has been implicated as an important regulator of neuronal degeneration in pathological processes. Reduction of CaBP expression can lead to neuronal death due to failure to protect the cell against pathologically high concentrations of  $\text{Ca}^{2+}$  [12]. CR, a high affinity cytosolic CaBP that belongs to the EF-hand family of proteins, is present in a subpopulation of interneurons in all regions of the hippocampus as well as in hilar mossy and infragranular cells [13,14].

*Panax ginseng*, the best known Asian species of this plant [15,16], is an important traditional medicinal herb believed to promote longevity [17,18], enhance resistance to many diseases [19], and help in maintaining equilibrium of the human body under stressful conditions [20,21]. Ginseng products have attracted attention as a modifier of biological responses due to their proposed beneficial effects on the CNS [17,21]. Ginsenosides, principle bioactive components of ginseng, possess important pharmacological properties including antioxidant [16,17,19], anti-stress [16,17], and anti-hepatitis effects [21].

Purified ginseng components have been reported to act as radioprotective agents in irradiated rodents [20,22-24]. Water-soluble ginseng extracts have been reported to provide the best radioprotection effects in C3H mice when injected 24 h before whole-body  $^{60}\text{Co}$  gamma irradiation [25]. These extracts also reduce the frequency of radiation-induced DNA breaks in lymphocytes of C57BL/6 mice [22], and induce the expression of cytokines (IL-1, IL-6, IFN $\gamma$ , and IL-12) which are required for hematopoietic recovery in BALB/c mice after gamma irradiation [24]. Additionally, preliminary clinical observations suggest that following radiotherapy or chemotherapy, partially purified ginseng components may reduce therapy-related side effects and stimulate recovery of hematopoietic functions in cancer patients [26].

With CaBPs like calretinin (CR) implicated as important regulators of pathological neuronal degeneration

[27], these factors could be used as markers to measure brain damage due to RF exposure in the hippocampus and examine the beneficial effect of red ginseng (RG) on the CNS. Hence, the aim of the present study was to investigate the effect ginseng treatment of CR IR in the subfields of the hippocampal region of mice due following 835 MHz RF exposure at SAR 1.6 W/kg 5 h per d for 5 consecutive days.

## MATERIALS AND METHODS

### Animals

Six-week old 20-30 g ICR (Orientbio Inc., Seongnam, Korea) male mice ( $n=30$ ) were used for this experiment. Upon arrival, animals were randomly grouped and housed six per cage maintained at 20 to 25°C. The mice had access to food and water *ad libitum*. Mice were acclimated for 1 wk. All animal procedures were performed according to the National Institute of Health Guidelines for Animal Research and were approved by the Dankook University Institutional Animal Care and Use Committee. RG extract was provided by the Korea Ginseng Corporation (Daejeon, Korea). Before conducting the study, mice were divided into six groups ( $n=5$ ): control animals were treated with NaCl ( $\text{C}_{\text{NaCl}}$ ) while the experimental animals received 10 mg/kg ginseng ( $\text{C10}$ ), or 30 mg/kg ( $\text{C30}$ ); EMF exposed mice were also treated with NaCl ( $\text{E}_{\text{NaCl}}$ ), 10 mg/kg ginseng ( $\text{E10}$ ), or 30 mg/kg ( $\text{E30}$ ).

### Exposure system

The system (Wave Exposer V20) used to expose the mice to EMF has been described in detail elsewhere [28]. Briefly, a Wave Exposer V20 emitting 835 MHz equivalent to the Korean CDMA mobile phone frequency was designed by the Division of Information Technology Engineering, Soonchunhyang University [28]. SAR was set at 1.6 to 4.0 W/kg, which is the same value as electric field intensities between 59.56 and 94.18 V/m for muscles exposed to 835 MHz CDMA. Waves were generated and amplified with an electronic unit, and eventually radiated by a pyramidal rectangular horn antenna connected by a waveguide to coaxial transition. A standard mouse cage was used for the apparatus. Output power of the horn antenna was 2.5 W for SAR 1.6 W/kg and 6.3 W for SAR 4.0 W/kg.

Electric field intensities due to SAR could be calculated, and power values were determined by a computer simulation with a High Frequency Structure Simulator manufactured by Ansoft Co. (Pittsburgh, PA, USA). Five cylinder-shaped models of mice were used for the

simulation. Simulation variables were the mice location and distance from the horn aperture for freely moving mice. Power was determined by averaging the simulated peak electric field intensities for each mouse body. Wave transmission from the horn antenna to the mouse cage was provided by wave absorption material (TDK RF solutions Inc., Cedar Park, TX, USA) mimicking radiation exposure in the open environment; this limited the influence that the number of mice might have had on EMF exposure. The exposure apparatus was equipped with automatic light and air conditioning systems and a water feeder. The apparatus did not restrict movement of the mice during exposure to minimize stress during the experimental procedure.

### Experimental design and exposure conditions

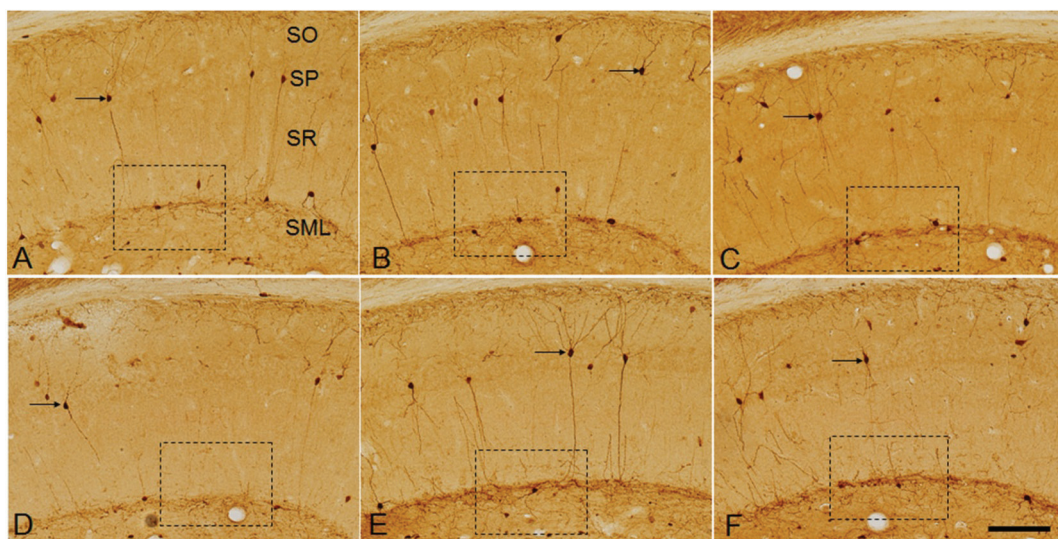
For the RF exposure experiment, the entire body of the mouse was exposed to 835 MHz radiation with an average SAR of 1.6 W/kg using the Wave Exposer V20. A solution of RG extract was prepared in 0.9% NaCl and 0.1% Tween 80 was orally administered before EMF exposure. All groups of mice were exposed to EMF for 5 h per d for 5 consecutive days. Three hr after the final exposure in the fifth day, the animals were anesthetized with diethyl ether. The brains of the mice were collected then fixed by perfusion with a 4% paraformaldehyde and phosphate buffer saline solution. Diethyl ether was used

as to prevent animal stress and avoid blood pressure elevation during the perfusion and fixation procedure.

### Immunohistochemical analysis

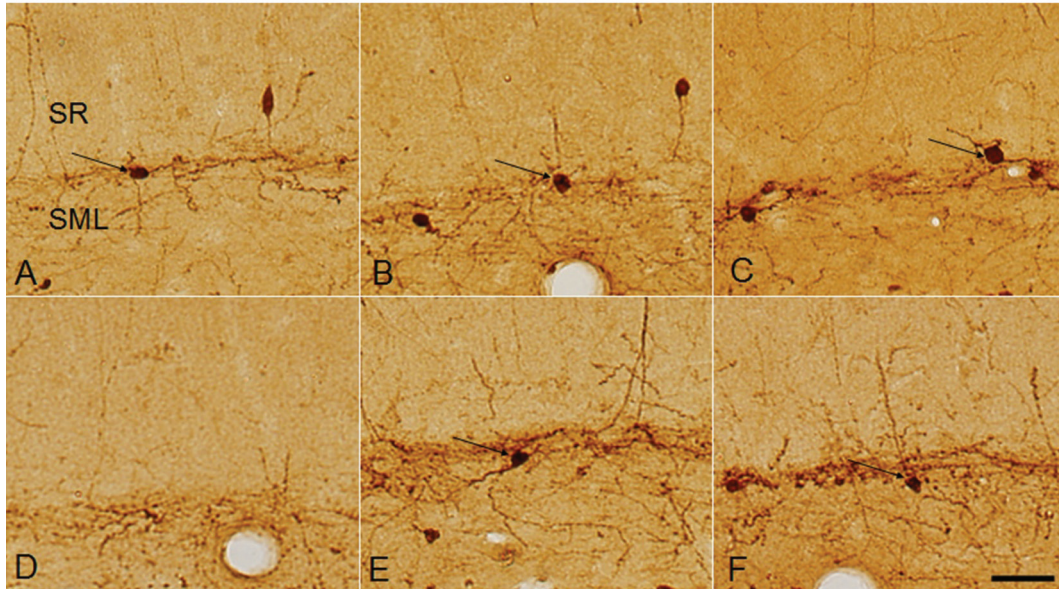
Brains were removed from the skull and were fixed in 4% paraformaldehyde for 24 h. Brain tissues were cryoprotected after being soaked in a series of sucrose solutions (10%, 20%, and 30%) at 4°C until they sank.

Serial coronal section 40  $\mu$ m thick were cut with a freezing, sliding microtome and collected in wells. Immunohistochemistry was performed with a free floating method. Polyclonal anti-goat calretinin antibody (AB1550; Millipore, Bedford, MA, USA) was applied to brain sections at a final dilution of 1:15,000 in blocking buffer containing 1% bovine serum albumin, 0.3% Triton X-100, and 1% normal horse serum in phosphate buffered saline. Sections were incubated for 48 h at 4°C. After three washes with phosphate buffer saline, the sections were incubated with biotinylated secondary antibodies at a dilution of 1:250 for 1.5 h at the room temperature. Sections from each group were stained together to eliminate variation originating from different experimental conditions. Following additional washes, section were mounted on gelatin coated slides, dehydrated in ethanol, incubated in xylene, and covered with distyrene plasticizer and xylene mountants.



**Fig. 1.** Photomicrograph of calretinin immunoreactivity (IR) in the cornu ammonis (CA)1 region of the hippocampal regions of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control animals treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract (E10), and (F) exposed group treated with 30 mg/kg RG extract (E30). Intense calretinin IR was detected in the neurons (arrows) of the CA1 area. Pyramidal cells exhibited extensive axonal and dendritic arborization. Note the loss of interneurons in the  $E_{NaCl}$  group while no such loss was observed in E10 and E30 groups. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SLM, stratum lacunosum moleculare. Scale bar=100  $\mu$ m.





**Fig. 2.** Magnified microphotograph of calretinin immunoreactivity shown in Fig. 1 at the junction of the stratum radiatum (SR) and stratum lacunosum moleculare (SML) subfield in the cornu ammonis 1 subfield of the hippocampus of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control mice treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract, and (F) exposed group treated with 30 mg/kg RG extract. Note the loss of interneurons in the  $E_{NaCl}$  group which are preserved in the exposed groups treated with RG (arrows). Scale bar = 50  $\mu$ m.

### Image and statistical analysis

The slides were analyzed with a BX 51 microscope (Olympus, Tokyo, Japan), and pictures of the sections were taken by a microscope digital camera system (DP50, Olympus). The National Institute of Health image program Scion Image was used to determine staining densities. The sum of the gray values of all pixels in selected regions was divided by the total number of pixels in the same region to determine the mean density of immunoreactivity per unit area ( $mm^2$ ). Mean density was calculated with the following formula: mean density = immunoreactivity/area ( $mm^2$ ). Data are expressed as the mean  $\pm$  SD. Comparisons of the mean density of the different subfields in the hippocampus (CA1, CA3, and dentate gyrus) between the control group and three exposed groups were performed individually using an unpaired Student's *t*-test. A *p*-value  $<0.05$  was considered to be statistically significant.

## RESULTS

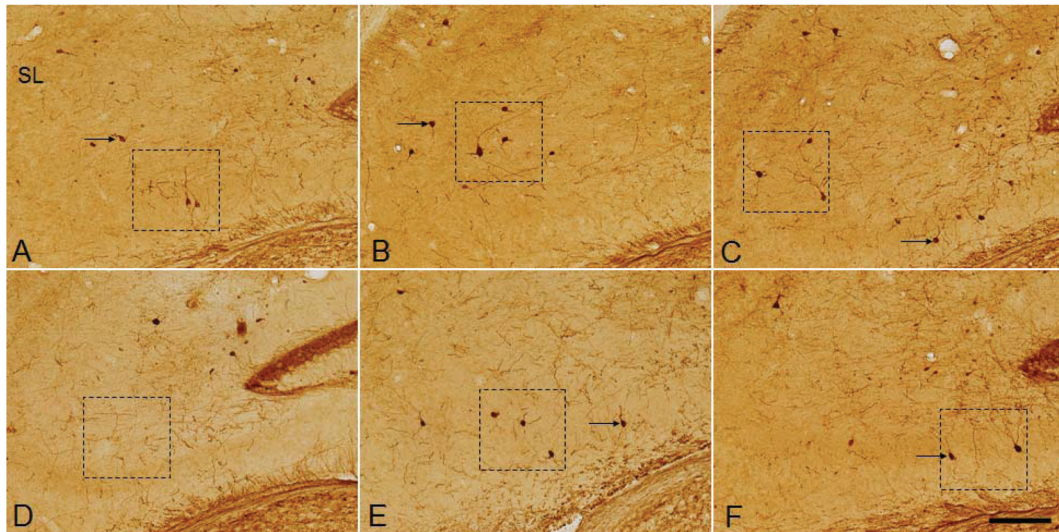
### Histopathological observations of CR IR

#### CA areas

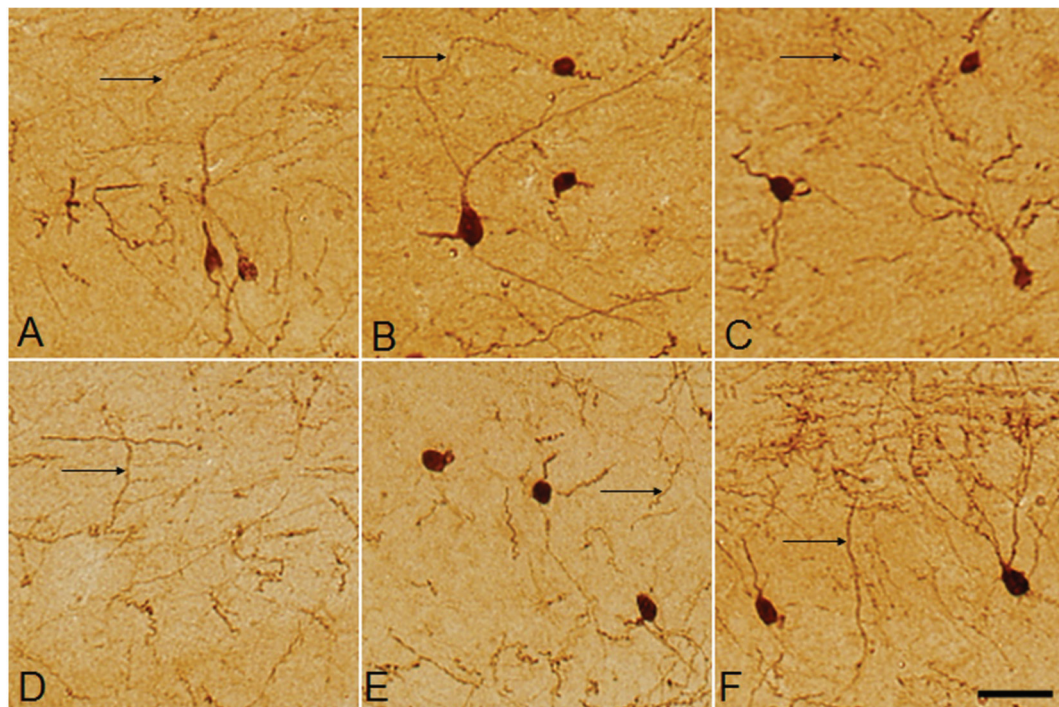
CR IR neurons were scattered throughout the CA1 in all six groups. Soma and dendrites of the principal cells and interneurons of the hippocampus contained CR IR with varying intensity. The majority of CR IR neurons in

the CA1 were identified as bipolar and multipolar neurons. Intensely stained single axons ran perpendicularly through the stratum radiatum while dendrites appeared to branch out from the CR IR cell bodies located in the stratum pyramidale (Fig. 1). Such axons appeared to be almost absent in the  $E_{NaCl}$  group while these cells were most prominent in E10 mice (Fig. 1). A dense band along the stratum lacunosum-moleculare was comprised of numerous interneurons. Such bands were more lightly stained in the  $E_{NaCl}$  group with decreased numbers of interneurons and staining intensity. The interneurons and bands of the E10 and E30 groups were darkly stained (Fig. 2).

The CA3 contained scattered single CR IR neurons with numerous dendritic arborizations as well as numerous immunoreactive fibers (Fig. 3). Compared to the control groups, loss of such CR positive neurons was noted in the  $E_{NaCl}$  group along with a lack of processes, resulting in poor dendritic arborization in the remaining neurons (Fig. 3). Prominent, intensely stained neurons were observed with numerous dendritic arborization in the E10 and E30 groups but were absent in the  $E_{NaCl}$  group (Fig. 4). Decreased IR was also observed in CR immunoreactive fibers in the  $E_{NaCl}$  group but CR IR was most prominent in E10 and E30 groups compared to the control group (Fig. 4).



**Fig. 3.** Photomicrograph of calretinin immunoreactivity in the cornu ammonis 3 region of the hippocampus of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control mice treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract, and (F) exposed group treated with 30 mg/kg RG extract. Multipolar neurons (arrows) were observed in the CA3 subfields of all the groups except for  $E_{NaCl}$  group. This illustrates the protective effect of ginseng against neuronal degeneration. SL, stratum lucidum. Scale bar=100  $\mu$ m.



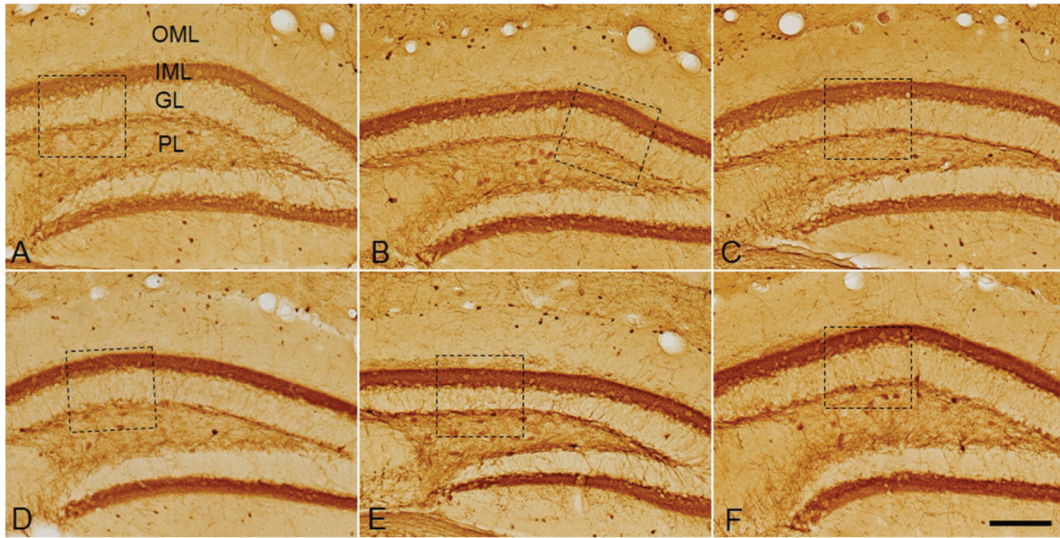
**Fig. 4.** Magnified microphotograph of calretinin immunoreactivity shown in Fig. 3 in the cornu ammonis 3 subfield of the hippocampus of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control mice treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract (E10), and (F) exposed group treated with 30 mg/kg RG extract (E30). Note the highly reactive neurons with branched dendritic arborization in the ginseng-treated groups as well as numerous immunoreactive fibers (arrows) which are less in number and have lower staining intensity in the  $E_{NaCl}$  group compared to the E10 and E30 groups. Scale bar=50  $\mu$ m.

### Dentate gyrus

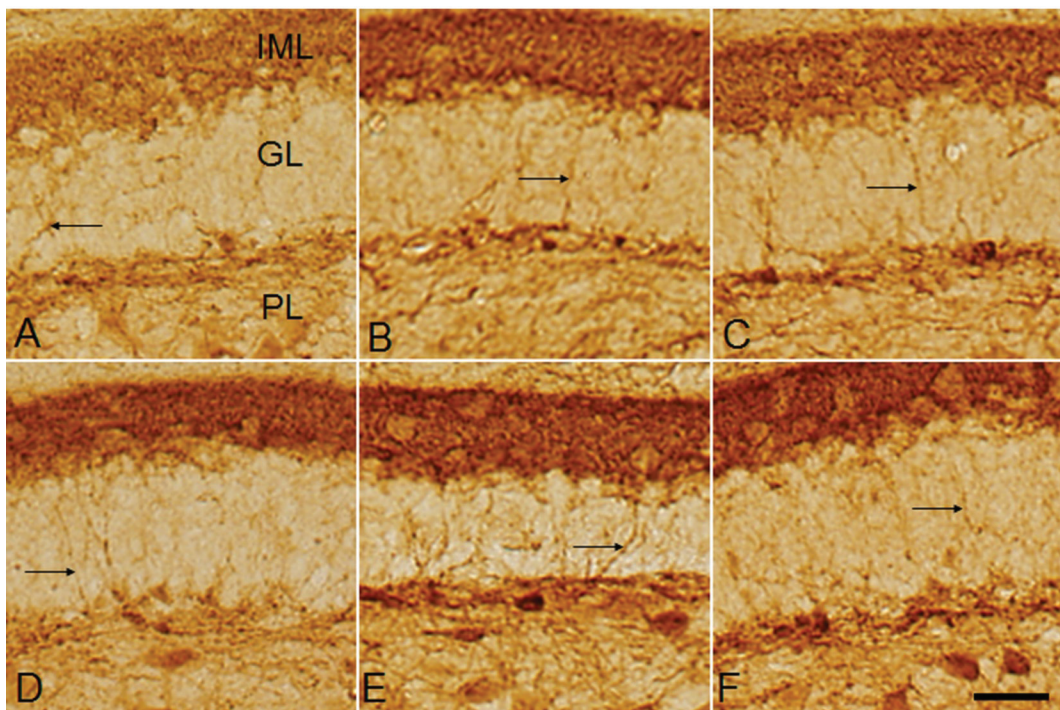
Intense CR IR was noted in the inner molecular layer

as well as the polymorphic, granular, and outer molecular layers (Fig. 5). Neurophils in the inner molecular layer





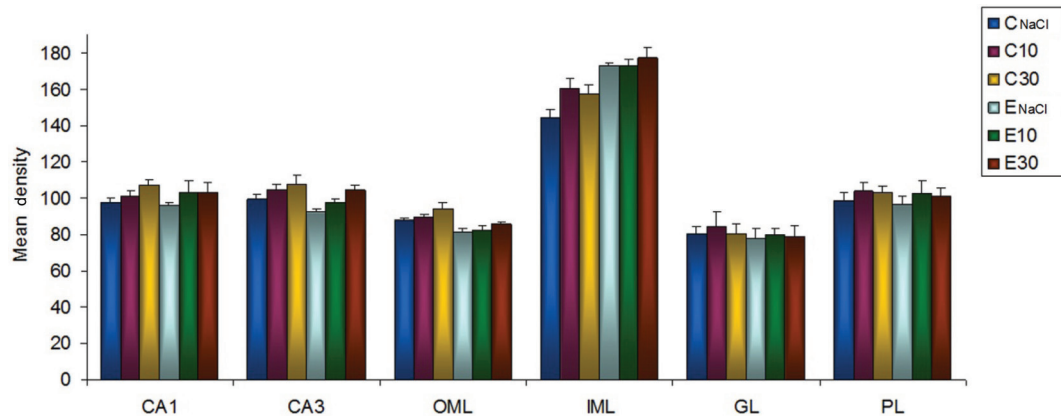
**Fig. 5.** Photomicrograph of calretinin immunoreactivity (IR) in the dentate gyrus of the hippocampus of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control mice treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract (E10), and (F) exposed group treated with 30 mg/kg RG extract (E30). Note the change in IR among the groups and the loss of infragranular cells in the  $E_{NaCl}$  group which are prevalent in the E10 and E30 groups. OML, outer molecular layer; IML, inner molecular layer; GL, granular layer; PL, polymorphous layer. Scale bar=100  $\mu$ m.



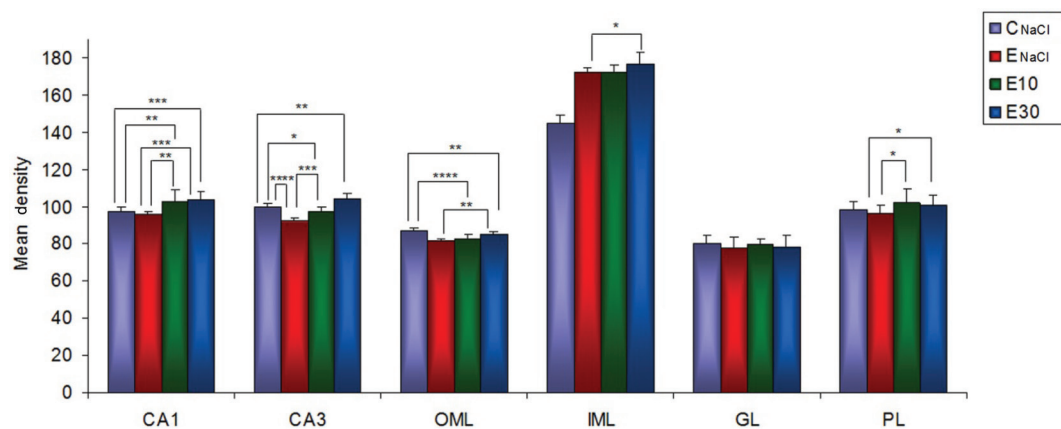
**Fig. 6.** Magnified microphotograph of calretinin immunoreactivity shown in Fig. 5 in the dentate gyrus of the hippocampus of (A) control mice treated with NaCl, (B) control animals treated with 10 mg/kg red ginseng (RG) extract, (C) control mice treated with 30 mg/kg RG extract, (D) exposed animals treated with NaCl ( $E_{NaCl}$ ), (E) exposed group treated with 10 mg/kg RG extract (E10), and (F) exposed group treated with 30 mg/kg RG extract (E30). Note the decrease of immunoreactive fiber (arrows), presumably dendrites of infragranular cells running through the granular layer, in the  $E_{NaCl}$  group compared to the other groups. The number of infragranular cells also appeared to decrease in the  $E_{NaCl}$  group but was prevalent in the E10 and E30 groups. IML, inner molecular layer; GL, granular layer; PL, polymorphous layer. Scale bar=50  $\mu$ m.

displayed most intense CR IR in all groups. Infragranular granule cells and scattered interneurons displayed faint

CR IR (Fig. 5). However, decreases in the number as well as staining intensity of infragranular cells as well



**Fig. 7.** Mean density of calretinin (CR) immunoreactivity (IR) changes in the hippocampal region after exposure to 835 MHz at specific absorption rate = 1.6 W/kg for 5 h/d for 5 days in the control groups treated with NaCl ( $C_{NaCl}$ ), 10 mg/kg red ginseng (RG) extract (C10), and 30 mg/kg RG extract (C30), and exposed animals treated with NaCl ( $E_{NaCl}$ ), 10 mg/kg RG extract (E10), and 30 mg/kg RG extract (E30). CR IR increased in the ginseng-treated mice compared to the NaCl-treated animals in both the control and exposed groups. CA, cornu ammonis; OML, outer molecular layer; IML, inner molecular layer; GL, granular layer; PL, polymorphous layer.



**Fig. 8.** Image analysis of the relative density of calretinin immunoreactivity changes in the hippocampal region of control animals treated with NaCl ( $C_{NaCl}$ ) and exposed animals treated with NaCl ( $E_{NaCl}$ ), 10 mg/kg red ginseng (RG) extract (E10), and 30 mg/kg RG extract (E30) groups. Exposed group treated with NaCl ( $E_{NaCl}$ ) were also compared to exposed groups treated with 10 mg/kg RG extract (E10), and 30 mg/kg RG extract (E30). Data are expressed as the mean  $\pm$  SD obtained from three different experiments. CA, cornu ammonis; OML, outer molecular layer; IML, inner molecular layer; GL, granular layer; PL, polymorphous layer. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

as interneurons were noted in the  $E_{NaCl}$  group. Dendrites of the immunopositive infragranular neurons were presumed to pass to the outer molecular layer through CR-immunopositive inner molecular layer of the dentate gyrus (Fig. 6). The hilus of the dentate gyrus contained a few scattered CR immunoreactive cells and extensive dendritic arborization in the control groups but these cells appeared reduced in number as well as staining intensity in the  $E_{NaCl}$  group (Fig. 5). In the E10 and E30 groups, such CR IR immunoreactive cells were present (Fig. 5).

### Calretinin immunoreactivity density

Maximum CR IR was observed in the inner molecular layer of all six groups, followed by the polymorphous, outer molecular, and granular layers (Fig. 7). CR IR was higher in the CA3 than CA1 (Fig. 7). Intergroup comparison revealed that the maximum CR IR was in the C30 group while it was lowest in the  $E_{NaCl}$  group in almost all layers excluding the inner molecular layer. The E10 and E30 groups showed marked CR IR intensity in all the layers compared to the  $E_{NaCl}$  group (Fig. 7).

Relative staining densities in the CA1 was significantly



different in the E10 ( $p < 0.01$ ) and E30 ( $p < 0.001$ ) groups compared to the  $C_{NaCl}$  and  $E_{NaCl}$  groups. All three experimental groups,  $E_{NaCl}$  ( $p < 0.0001$ ), E10 ( $p < 0.05$ ), and E30 ( $p < 0.01$ ) were significantly different when compared to the  $C_{NaCl}$  in CA3 area. E10 ( $p < 0.001$ ) mice also displayed significant difference in CR IR compared to  $E_{NaCl}$  in the CA3 (Fig. 8). Outer molecular layer analysis of relative density revealed a significant difference in E30 ( $p < 0.01$ ) animals compared to both the  $C_{NaCl}$  and  $E_{NaCl}$  groups (Fig. 8). Significant difference was also noted in the polymorphous layer when the  $E_{NaCl}$  group was compared to the E10 ( $p < 0.05$ ) and E30 ( $p < 0.05$ ) groups (Fig. 8).

## DISCUSSION

In the present study, we evaluated CR IR to observe the radioprotective effects of RG extract against radiofrequency exposure at 835 MHz at SAR of 1.6 W/kg for 5 h/d for 5 d. The mice use for our study were divided into different groups comprising of control and exposed groups treated with NaCl, 10 mg/kg of RG extract, or 30 mg/kg of RG extract. The effects of RF exposure on CR expression were studied after treatment with NaCl or RG extract.

CaBPs, such as CR, have an important role in maintaining intracellular  $Ca^{2+}$  homeostasis. Specific distribution of these proteins in the CNS suggests their involvement in important neuronal activities. Altered expression of CaBPs may lead to pathological conditions and neurodegenerative diseases due to their reduced  $Ca^{2+}$  buffering capacity which could influence diverse functions. In particular, signaling pathways could be disrupted by limiting calcium diffusion [29,30].  $Ca^{2+}$  is an important component of normal cellular function and mediates most physiological effects triggered by EMF exposure at which time ions are liberated from their intracellular stores. Interaction of  $Ca^{2+}$  at the cell membrane has been identified as the first step of RF bioeffects. Release of neurotransmitter that transfer signals between neurons requires a concerted flow of  $Ca^{2+}$  ions through membranes. Disruptions leading to  $Ca^{2+}$  leakage increase  $Ca^{2+}$  background concentration, making CNS cells hypersensitive and able to transmit spurious signals which could cloud normal mental activity, trigger random thoughts, and disrupt concentration [31].

First, comparison between the  $C_{NaCl}$  and the RG extract-treated control groups (C10 and C30) revealed an increase in CR IR in the hippocampal subfields. Second, comparison between  $E_{NaCl}$  with the RG extract-treated exposed groups (E10 and E30) also demonstrated a sig-

nificant increase in CR IR in the hippocampal subfields. Such increase in CR IR highlights the buffering capacity of intracellular  $Ca^{2+}$  that helps to protect the cells from abnormally high  $Ca^{2+}$  levels. These findings also demonstrate the radioprotective effect of ginseng for helping to maintain intracellular  $Ca^{2+}$  homeostasis. Third, comparing CR IR between the control and exposed groups treated with NaCl revealed a significant reduction of CR IR in all the subfields (CA1, CA3, molecular layer, granular layer, and polymorphous layer) of the hippocampal formation in the  $E_{NaCl}$  group. Neuronal loss could be related to decreased CR IR in the hippocampal region, indicating possible deleterious effect of RF exposure. Neuronal death has been associated with down-regulation of CR IR [32]. Neuronal loss as a contributing factor to the reduced CR IR was associated with the loss of interneurons and the immunoreactive fibers in the CA1 and CA3 subfields of the  $E_{NaCl}$  group. Disruption of granule cell alignment at the junction of granular and polymorphous layers was linked to a loss of granule cells as well as axons running through the granular layer, and may contribute to the loss of CR IR. Moreover, the RG extract-treated (E10 and E30) groups did not show any loss of interneurons, infragranular cells, or immunoreactive fibers that was observed in the  $E_{NaCl}$  group.

Further comparison of the exposed groups revealed a significant increase in CR IR in the RG extract-treated groups compared to the  $E_{NaCl}$  group. Such findings illustrate a radioprotective effect of RG extract associated with the maintenance of  $Ca^{2+}$  homeostasis. Our results are supported by other studies examining the effects of radiation. Ginseng has been reported to reduce the micronuclei yield in peripheral blood lymphocytes with 1 Gy exposure to 46% at 1500  $\mu\text{g mL}^{-1}$  and 61.5% at 2000  $\mu\text{g mL}^{-1}$ ; compared to radiation alone. These results have led researches to strongly recommend the presence of ginseng water extract inside PBL before and during the time of radiation exposure to confer radioprotection [33].

Studies have found RG to be protective in models of cell damage. Increased cell viability following glutamate and kainic acid excitotoxicity [34] as well as reduced apoptosis of PC12 cells [35] has been reported. Ginsenosides can modulate the functions of many receptors and ion channels in neurons, including N-methyl-D-aspartate (NMDA) receptors [36,37] and  $Ca^{2+}$  channels [38]. Inhibition of increased  $Ca^{2+}$  influx by RG has been observed in hippocampal neurons subjected to oxygen/glucose deprivation [39]. RG has also been suggested to act as a neuroprotectant factor by inhibiting  $Ca^{2+}$  influx through both NMDA receptor channels and L-type voltage-de-



pendent  $\text{Ca}^{2+}$  channels, leading to a reduction of intracellular free  $\text{Ca}^{2+}$  [39]. Inhibition of  $\text{Ca}^{2+}$  influx by RG has been reported in a glutamate (0.5 mM) toxicity model of cultured rat hippocampal neurons [40]. Similar findings in cultured hippocampal neurons has also been noted in multiple studies [36,37,41].

The progression of mobile communication has led to increased risk of radiofrequency exposure. Furthermore, susceptibility of the brain to EMF has been well documented. Brains of 12-26-wk old rats exposed to EMF show neuronal damage in the hippocampus, cortex, and basal ganglia [5]. Neuronal damage and cell loss in the CA subfield of the female rat hippocampus has been observed after 28 d of exposure to 900 MHz [42] as well as the loss of pyramidal cells from the CA subfields after prenatal exposure to 900 MHz EMF [43]. EMF exposure also results in reduced numbers of granule cells in the dentate gyrus of 4-wk old rats [44]. Similar studies have reported the vulnerability of granule cells in the dentate gyrus to EMF [45]. These previous reports along with the findings of our study seem to indicate the deleterious effects of RF exposure on hippocampal formation. Such effects of RF exposure were not seen in RG-extract treated groups in our study, further strengthening the view that RG acts as a radioprotective agent.

The hippocampal trisynaptic circuit is an important link that affects memory and learning [46]. Protection against loss of interneurons in CA subfields and infragranular cells in the dentate gyrus as observed in the present study is particularly important as these cells are associated with complex cognitive and emotional processes through their connections to prefrontal regions including the medial and orbital areas [47]. Potential health risks due to increased use of mobile communication devices have generated interest in identifying an effective radioprotective agent which could offer protection against exposure to mobile phone radiation and EMF. An effective radioprotector should be able to reduce the biological effects of radiation when administered before radiation exposure without any toxic side effects. Although no such ideal radioprotective agent has been identified to date, RG extract may be able to serve as a non-toxic radioprotective agent for maintaining  $\text{Ca}^{2+}$  homeostasis in the hippocampal region.

In conclusion, the present study demonstrated that RF can function as a radioprotectant that is associated with numerous beneficial properties. RG appears to assist in the protection of the hippocampus by helping to maintain CR IR that is involved in calcium buffering, without which could lead to tissue degeneration and cell

death. However, further studies with variations in RG extract dose, exposure duration, and SAR are essential to evaluate the effects of ginseng on  $\text{Ca}^{2+}$  levels following exposure of the hippocampus to EMF. Future projects should also examine the protective effects of RG against behavior, learning, and memory impairment caused by EMF exposure.

## ACKNOWLEDGEMENTS

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