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Chemoprotective action of lotus seedpod procyanidins on oxidative stress in mice induced by extremely low-frequency electromagnetic field exposure

Xiaoping Luo[a], Meng Chen[a], Yuqing Duan[a,*], Wenyi Duan[a], Haihui Zhang[a,**], Yuanqing He[a], Chunchun Yin[a], Guibo Sun[b], Xiaobo Sun[b]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Institute of Medicinal Plants, Chinese Academy of Medical Sciences, Beijing 100193, China

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ABSTRACT

With the increasing use of electromagnetic technology, the effects of extremely low-frequency electromagnetic fields (ELF-EMF) on biological systems, central neurotransmitter systems, and human health have attracted extensive attention worldwide. In this study, lotus seedpod procyanidins (LSPCs) were evaluated for their protective effects on ELF-EMF induced oxidative stress injury in mice. Sixty male ICR mice were used for the experiment. The mice were randomly divided into five equal groups. The control group did not receive LSPCs or ELF-EMF but orally received normal saline. The ELF-EMF group received ELF-EMF exposure plus normal saline orally. The other three groups received ELF-EMF exposure plus LSPCs orally (60, 90, or 120 mg kg^{-1}.bw, respectively). Each group exposed to ELF-EMF at 8 mT, 4 h day^{-1} for 28 consecutive days after administration daily of LSPCs or normal saline to mice for 15 consecutive days with the exception of the control group. Thereafter, blood and cerebral cortex of the mice were analyzed for antioxidant indices, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (GST) and malondialdehyde (MDA). LSPCs administration at different doses significantly inhibited oxidative stress damage of mice induced by ELF-EMF. LSPCs treatment augmented SOD, CAT, GSH-Px, GR and GST activity. Furthermore, administration significantly lowered MDA level in LSPCs treatment groups LSPCs. All results indicated LSPCs can effectively prevent oxidative stress injury induced by ELF-EMF exposure, which may be related to its ability of scavenging free radicals and stimulating antioxidant enzyme activity.

1. Introduction

Extremely low-frequency electromagnetic field (ELF-EMF) is an electromagnetic wave of frequency 0–300 Hz that emits a non-thermal effect of non-ionizing radiation, and it is generally produced by residential power lines, household electrical wiring, and medical devices [25]. There are several reports which indicate that ELF-EMF may act as a risk factor of the occurrence of oxidative stress in brain tissue [22]. Previous research reported that ELF-EMF-induced oxidative stress was evident by: (i) increase of malondialdehyde (MDA) and nitric oxide (NO) levels in brain tissue, (ii) decrease of brain superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and (iii) increase of brain xanthine oxidase (XO) and adenosine deaminase (ADA) activities [11]. With the increasing use of electromagnetic technique, the effects of ELF-EMF on biological systems, central neurotransmitter systems, and human health have attracted extensive attention worldwide.

Although the mechanisms of ELF-EMF giving rise to brain diseases seem to be an important factor [12], oxidative stress refers to be a serious imbalance between production of reactive oxygen species (ROS) and antioxidants that leads to potential tissue and organ damage [9]. The production of ROS in brain during ELF-EMF exposure markedly exceeds the capacity of intrinsic cellular scavengers. Excessive ROS activates antioxidant enzymes (SOD, CAT and GSH-Px enzymes activities decreased) [6], but impair mitochondrial function, and damage cellular constituents, like

* Corresponding author.
** Corresponding author.
E-mail addresses: dyq101@mail.ujs.edu.cn (Y. Duan), zhanghh@mail.ujs.edu.cn (H. Zhang).

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proteins and lipids (MDA level increased). In order to protect the brain tissue against oxidative stress injury caused by ELF-EMF, natural antioxidants to prevent reduction in the antioxidant enzyme levels and to prevent peroxidation were proposed. Previous research has proved that supplementing vitamin E, a non-specific antioxidant, can suppress the enhancement of somatic recombination which is caused by magnetic field exposure [13].

Procyanidins are polyphenol compounds which are obtained from plants and widely available in fruits, vegetables, seeds and bark. Recent studies have shown that long-term exposure to ELF-EMF cause oxidative stress injury in brain tissue. Procyanidins are known to possess better antioxidant capability than vitamins C and E [3]. Lotus seedpod procyanidins (LSPCs) are procyanidin compounds extracted from lotus seedpod. Previous research has emphasized that oxidative stress can be attenuated by LSPCs, indicating that the ability of LSPCs to stimulate antioxidant enzyme activity [8]. Our previous [6] study reported that long-term treatment for LSPCs prevented ELF-EMF related increase in oxidation products and ELF-EMF-related deficits in cognitive functions which suggested that LSPCs may have potential to stimulate antioxidant enzyme activity and to scavenge oxygen free radicals. However, relatively few studies targeting on protection against ELF-EMF-induced oxidative damage by adding LSPCs are reported. Thus, the present study tests the protective effects of LSPCs administered by oral gavage on oxidative stress injury induced by ELF-EMF exposure.

2. Materials and methods

2.1. Preparation of LSPCs

LSPCs were extracted from mature lotus seedpod (Hubei, China). Fresh plant material was ground and lyophilized. LSPCs were extracted with Me_2CO/H_2O and purified by Sephadex LH-20 column chromatography, with a purity of > 98%. In our previous research [17], we have already demonstrated the main molecular weight distribution of LSPCs (range 291.1-1155.3) by electrospray ionisation-mass (ESI–MS) analysis. LSPCs polymerization was ≤ 4 and contained monomers, dimers and tetramers in which the amounts of dimers were the greatest and catechin and epicatechin were the base components. For all experiments, final concentrations of LSPCs were prepared by dilution with normal saline.

2.2. Animals

Male ICR mice (20 ± 2 g, 3 weeks old) from the Experimental Animal Center of Jiangsu University were used. Animals were housed in $26 \times 16 \times 12$ cm clear polyethylene cages provided with a wood shavings bedding and paper nesting material with free access to food and tap water. The colony room was held under a 12 h light/dark cycle at temperature (23 ± 2 °C) and humidity ($50 \pm 5\%$). The mice were weighed everyday for seven days. Animals were treated humanely following the guidelines of the National Institutes of Health, and the protocol approved by the Institutional Animal Ethical Committee.

2.3. ELF-EMF exposure system

The electromagnetic field was generated with a pair of Helmholtz coils (1.0 mm wire diameter, 32 cm internal diameter, 36 cm external diameter) in 500 turns. The coils were connected to a 250 V adjustable transformer (Jiacheng Electric Products, Shanghai) and were fixed on a ventilated perspex box. A pair of parallel Helmholtz coils, vertical to the ground, was connected to a 220 V AC power supply via the adjustable transformer. The AC current (50 Hz) was passed through a pair of coils, producing an electromagnetic field of 0–15 mT at the center of the coils. The intensity of the electromagnetic field was measured once a week in 18 different points of ventilated perspex box by using a digital teslameter (Lz-630H, Lianzhong Technology, China) to ensure the homogeneity of the field during the course of the experiment.

The electromagnetic field generating device was placed in a room at a controlled temperature (23 ± 2 °C). Mice were placed in well ventilated perspex boxes of dimensions 14 cm × 10 cm × 12 cm in the middle of the pair of coils, but the box was not in contact with the coils. The temperature inside the ventilated perspex box was 23 ± 0.5 °C and this temperature was measured by an infrared thermometer (TM350$^+$, TECMAN, Hongkong). The background magnetic field was about 30 μT.

2.4. ELF-EMF damnification model

All of the mice in the experiments were randomly divided into six groups as follows: Group I was sham-exposed (control); Group II was ELF-EMF exposed at 2 mT; Group III was ELF-EMF exposed at 4 mT; Group IV was ELF-EMF exposed at 6 mT; Group V was ELF-EMF exposed at 8 mT; Group VI was ELF-EMF exposed at 10 mT. Each group consisted of 12 mice. Each group was exposed to the ELF-EMF under different radiation intensities, 4 h day^{-1} for 28 consecutive days. All mice had free access to standard food and tap water. SOD activity and MDA level in serum were measured daily throughout the study.

2.5. Treatment of mice with LSPCs

LSPCs dissolved in normal saline was administered once daily at doses of 60, 90, and 120 mg kg^{-1}.bw to the animals for 15 consecutive days before ELF-EMF exposure. At the beginning of ELF-EMF exposure, the mice were continued to be administered with LSPCs orally by a gavage daily for 4 weeks. All the mice in the experiments were randomly divided into five groups as follows: Group I was sham-exposed (control); Group II was ELF-EMF exposed and received normal saline orally normal saline but no LSPCs (ELF-EMF); Group III was ELF-EMF exposed and received LSPCs 60 mg kg^{-1}.bw (ELF-EMF + LSPCs60); Group IV was ELF-EMF exposed and received LSPCs 90 mg kg^{-1}.bw (ELF-EMF + LSPCs90); Group V was ELF-EMF exposed and received LSPCs 120 mg kg^{-1}.bw (ELF-EMF + LSPCs120). Each group consisted of 12 mice and exposed to ELF-EMF at 8 mT, 4 h day^{-1} for 28 consecutive days with the exception of the control group.

2.6. Blood and tissue preparation

At the end of ELF-EMF exposure, the animals were killed by decapitation. After decapitation, the blood of mice was collected and centrifuged at 3500 rpm for 10 min at 4 °C. The supernatant was stored at −80 °C until biochemical measurements. After the blood collection, the whole brain, thymus and spleen were quickly removed on ice. The left cerebral cortex was dissected from the left hemisphere and washed with cold normal saline, and stored at −80 °C for biochemical analysis. The right cerebral cortex from the right hemisphere thymus and spleen were dissected and washed with 1% ice cold saline, then fixed in neutral buffered 10% formalin for histopathological analysis.

2.7. Cerebral cortex homogenate preparation

The cerebral cortex was homogenized using the previous described method Zhong et al. [30]. Briefly, the cerebral cortex was homogenized in a glass homogenizer in ice-cold buffer (0.01 M Tris–HCl, 0.1 mM EDTA-Na_2, 0.01 M saccharose and 0.8% saline) at