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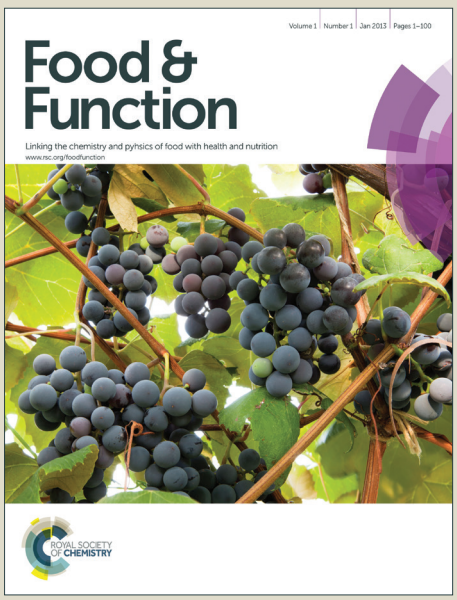

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Neuroprotective effects of dietary supplement Kang-fu-ling against high-power microwave through antioxidant action

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Kang-fu-ling (KFL) is a polybotanical dietary supplement with antioxidant property. This study aimed to evaluate the potential protective effects of KFL on cognitive deficit induced by high-power microwave (HPM) and the underlying mechanism for this neuroprotection. The electron spin resonance technique was employed to evaluate the free radical scavenging activity of KFL in vitro and KFL exhibited scavenging hydroxyl radical activity. KFL at doses of 0.75, 1.5 and 3 g/kg and vehicle were administered orally once daily for 14 days to male Wistar rats after being exposed to 30 mW/cm^2 HPM for 15 minutes. KFL reversed HPM-induced memory loss and the histopathological changes in hippocampus of rats. In addition, KFL displayed a protective effect against HPM-induced oxidative stress and activated the nuclear factor -E2-related factor 2 (Nrf2) and its target genes in the hippocampus of rats. The Nrf2-antioxidant response element signaling pathway maybe involved in the neuroprotective effects of KFL against HPM-induced oxidative stress. In summary, the dietary supplement KFL is a promising natural complex with neuroprotective effects against HPM by ameliorating oxidative stress.

Introduction

The widespread use of microwave (cellphone, microwave oven, radar, and medical exposure) has raised questions about its adverse effects on human health and the living environment. Over the past decades, considerable controversy about the impact of microwave on human health has ensued [1-3]. A definitive conclusion is that high density microwave exposure can cause detrimental effects on the central nervous system (CNS), testis, and cardiovascular system, especially the CNS. In addition, certain regions of the brain, such as the hippocampus, particularly are sensitive to microwave exposure. Studies have demonstrated that rats exposed to microwave exhibited an increase in the level of karyopyknosis in hippocampal neurons and a decline in spatial learning and memory [4-6]. At present, protection against microwave mainly relies on electromagnetic fields shielding because of the lack of effective agents. Finding an effective agent for microwave exposure is important because new, more powerful emitters are developed, and the potential for accidental exposure is increasing.

Although the mechanisms by which microwave exposure elicits diseases of the biological system are still unknown, the excessive formation of reactive oxygen species (ROS) and increased oxidative stress appears to be involved in the effects of microwave exposure[7-9]. Thus, preventing oxidative stress may be a potential therapeutic strategy for the treatment of microwave-induced disorders. The Nuclear factor -E2-related factor 2 (Nrf2)-antioxidant response element (ARE) signaling pathway plays a central role in the protection of cells against oxidative damage[10]. Under basal conditions, Nrf2 is predominantly present in the cytoplasm where it is anchored to the actin-binding protein Kelch-like ECH-associated protein 1 (Keap1) that mediates its degradation through the ubiquitin-proteasome pathway. In presence of oxidants or electrophiles, Nrf2 dissociate from Keap1 and Nrf2 degradation ceases. Stabilizes Nrf2 accumulates in nuclei, dimerizes with small Maf protein and induce expression of a set of ROS-detoxifying enzymes and other antioxidant proteins, called 'phase 2 enzymes', including heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1), and glutathione-S-thransferase (GST), the regulatory and catalytic subunits of the glutathione biosynthesis enzyme-glutamylcysteine ligase (GCLM and GCLC), peroxiredoxins (PRDX) 1 and 6, all of

which provide efficient cytoprotection by regulating the intracellular redox state[11, 12]. A series of compounds not necessarily possessing antioxidative activity themselves could nonetheless transcriptionally induce antioxidative enzymes to afford neuroprotection, many of which are plant-derived[13-16]. Studies have revealed that a number of herbal remedies (extracts of green tea, Ginkgo biloba, Astragalus mongholicus, blueberry, and a variety of other components) for treating neurological disorders have potent antioxidant properties that may explain their historical usage[17-20]. Experimental studies have demonstrated that certain natural compounds could prevent or treat microwave-induced brain injury by attenuating oxidative stress [8, 17, 18]. Some active ingredients in food, such as green tea catechins, protect rats from microwave-induced oxidative damage[17].

Kang-fu-ling (KFL), a polybotanical dietary supplement consisting of a six-ingredient comprehensive formulation (Table 1), is a new formulation we developed for the therapy of brain injury induced by HPM. Lycium barbarum L. (Gou Qi) is a Chinese plant whose fruits (Fructus Lycii) are widely used as dietary supplement for a large variety of beneficial effects including nourishing liver and kidney, improving the eyesight, improvement in immunity, antioxidation[21], and anti-aging[22]. The Fructus Lycii polysaccharides (FLPS), the active ingredients of Fructus Lycii, is reported to inhibit oxidation[23]. Astragalus membranaceus Bunge is a popular medicinal plant used as a tonic agent in traditional medicine in China, Japan, and Korea for over 2,000 years. Radix Astragali Mongolici (the root of Astragalus membranaceus Bunge) demonstrated neuroprotective and antioxidant properties in vitro and in animal experiments [24-27]. The other natural agents in the KFL also demonstrated a variety of activities against oxidative stress [19, 20, 28] and used as dietary supplement in China. As plants produce a variety of electrophilic compounds, we looked for some electrophilic compounds in KFL might protect neuron through activation of Nrf2-ARE pathway.

Table 1. Recipe of Kang-fu-ling formulation

pin yin name	English name	Latin name	Full plant name	Family	Content(%)
Gou Qi	Barbury Wolfberry Fruit	Fructus *Lycii*	*Lycium barbarum* L.	Solanaceae	11.3
Huang Qi	Mongolian Milkvetch Root	Radix *Astragali Mongolici*	*Astragalus membranaceus* Bunge	Leguminosae	29
Chi Shao	Red Peony Root	Radix *Paeoniae Rubra*	*Paeonia lactiflora* Pall.	Ranunculaceae	12.9
Dan Shen	Dan-shen Root	Radix *Salviae Miltiorrhizae*	*Salvia miltiorrhiza* Bunge	Labiatae	16.1
Lv Ping	Imbricate Mosquito Fein Herb	Herba *Azollae Imbricatae*	*Azolla imbricata (Roxb.)* Nakai	Azollaceae	22.6
Chuan Xiong	Szechwan Lovage Rhizome	Rhizoma *Ligusticum Chanxiong*	*Ligusticum chuanxiong* Hort.	Umbelliferae	8.1

In this study, KFL exhibited the scavenging hydroxyl radical activities in a cell-free system. And we evaluated the effects of KFL on cognitive defects and oxidative stress in rats caused by high-power microwave (HPM). We demonstrated that the dietary supplement KFL improved cognitive performance and suppressed the oxidative stress of rats exposed to HPM via Nrf2-ARE signaling pathway and its radical scavenging property. Our results indicate that KFL is an alternative treatment or adjuvant therapy in HPM-induced cognitive impairment.

Materials and methods

Preparation of KFL

Fructus Lycii, Radix Astragali Mongolici, Radix Paeoniae Rubra, Radix Salviae Miltiorrhizae, Herba Azollae Imbricatae, and Rhizoma Ligusticum Chanxiong were mixed in the dry weight ratio of 7:18:8:10:14: 5. The 6 raw materials were supplied by Fuzhou Adora Pharmaceutical Co., Ltd (Fuzhou, China) and authenticated by Dr. Zhao Y.M. and Dr. Ma Q.Y., both of them are botanist in Department of Phytochemistry in Beijing Institute of Pharmacology and Toxicology. The voucher specimens were deposited in Department of Phytochemistry, Beijing Institute of Pharmacology and Toxicology.

The Herba Azollae Imbricatae (140 g) was extracted for 1.5 h by refluxing in ethanol (1:10, w/v). The extract was dried using a rotary vacuum evaporator. The residue was boiled with distill water for 1.5 h, and then filtered to obtain the filtrate. The other ingredients (480g) were mixed and decocted in 5 l distilled water for 1.5 h twice, and then filtered to obtain the filtrate. All combined filtrates were concentrated. And the total extracts referred to as KFL (158.1g, 25.5%, w/w).

To confirm the quality and stability of KFL, high performance liquid chromatography (HPLC) analysis was performed with a Shimadzu LC-2010 system (Shimadzu Corp., Kyoto, Japan) with a quaternary pump and thermostat column compartment and Shimadzu LC solution software. KFL (1 mg/ml) was ultrasound dissolved in 5% acetonitrile in water, filtered using 0.45 μm filter and subjected to HPLC analysis. Separation was carried out using an Thermo Syncronis C18 column (4.6*250mm) at 25 ˚C. The mobile phase consisted of 100% acetonitrile solution in pump A, 0.1% formic acid in water in pump B, and elution was carried out using step gradients at a flow rate of 1 ml/min (Table 2). The detection wavelength was 280 nm and 10 μl of samples were injected. The HPLC profile of KFL is presented in Fig. 1.

Table 2. The composition of mobile phase for gradient elution in HPLC system

Time(min)	Mobile phase A (%)	Mobile phase B (%)
0	3	97
10	3	97
20	5	95
35	10	90
50	15	85
65	25	75
90	38	62
95	80	20

Mobile phase A, 100% acetonitrile solution; Mobile phase B, 0.1% formic acid in water.

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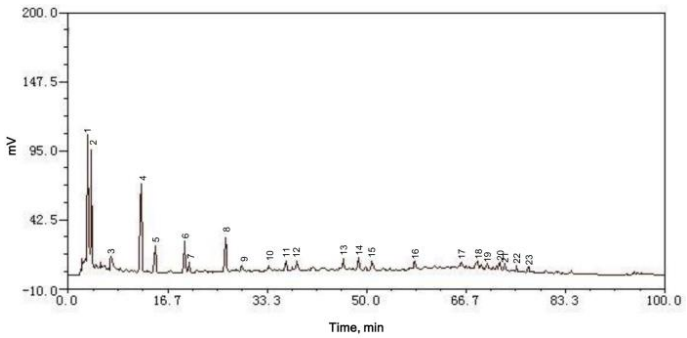

Figure 1. A representative HPLC chromatogram of KFL

Measurement of hydroxyl radical scavenging activity of KFL by ESR spectroscopy

The hydroxyl radical scavenging ability of KFL was evaluated in a cell-free, in vitro system using Bruker ESP A300 EPR spectrometer (Bruker BioSpin, Germany) as described previously[29, 30]. The parameter of ESR throughout the experiments were the following: modulation amplitude 1 G, sweep width 100G, sweep time 40.96 sec, time constant 10.24 msec, microwave power 5.13 mW. For detection of hydroxyl radical (OH·), the signal intensity was evaluated by measuring the second peak of DMPO/OH spin adduct in reaction buffer containing 5 μl $FeSO_4$ (2 mM), 5 μl DTPAC (1 mM), 5 μl DMPO (0.8 mM), 5 μl H_2O_2 (3 mM), and 30 μl sample.

Microwave exposure system and dosimetry

The microwave was generated by a klystron amplifier (model JD 2000, vacuum electronics research institute, Beijing, China). Microwave energy was transmitted by rectangular waveguide and A16-dB standard-gain horn antenna to an electromagnetic shield chamber (7 m×6.5 m×4 m). The interior walls of the chamber were covered with 500 mm and 300 mm pyramidal microwave absorber to minimize reflections (>45 dB).The horn antenna irradiates microwave from up to down on the animals. The emitted power was measured with the semiconductor detector connected to a directional coupler at one port of a circulator and displayed on an oscilloscope. The rat container was made of Plexiglas, which included 20 houses to reside in each batch. The container has a rotational symmetry around the center. The dimension of each house can be adjusted and made in such a way that the rats were comfortably placed. Fig. 2 shows the setup of the device used for exposure as previously described[31]. The average power densities were measured in the position of the rat placement using a waveguide antenna and GX12M1CHP power meter (Guanghua microelectronics instruments, Hefei, China) and with GX12M30A power heads. The average field power densities tested were 30 mW/cm^2. The SAR calculation was based on the finite difference time domain (FDTD) method as described[31]. In our study, the average SAR of brain were calculated to be 21 W/kg.

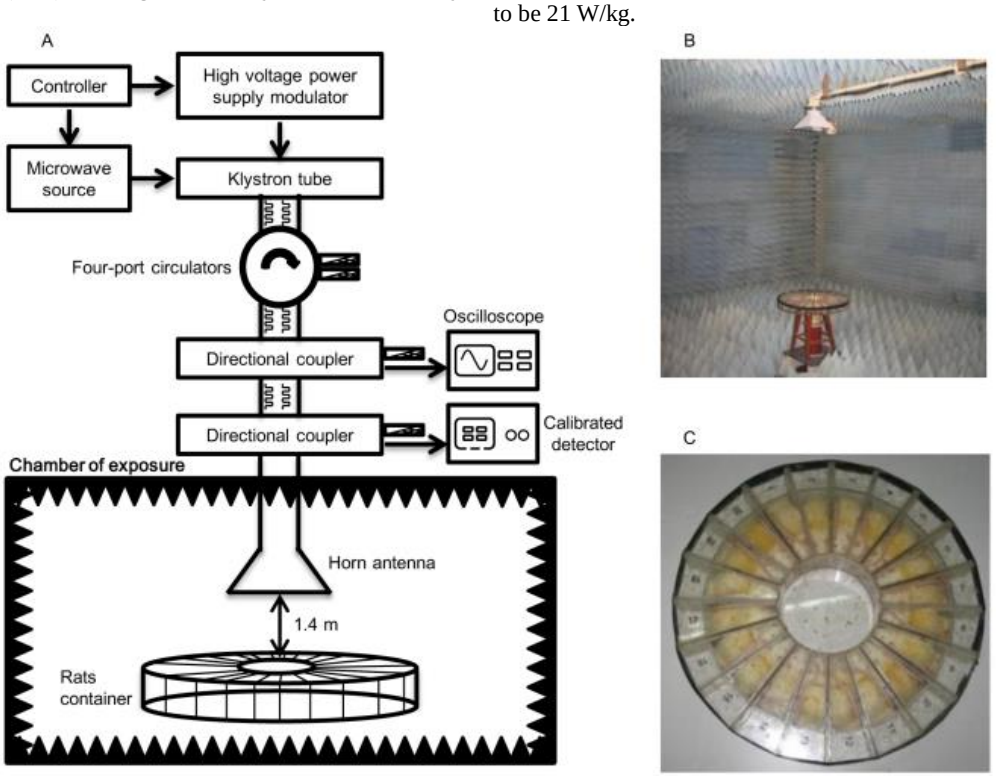

Figure 2. Experimental setup for the exposure of rats. (A) Schematic diagram of experimental setup for microwave exposure; (B) Chamber of microwave exposure; (C) Rat container.

Animals and treatments

All male Wistar rats (200±10 g, 8 weeks) were obtained from the Laboratory Animal Center (Beijing, China) and housed in a constant environment (22±2 ˚C, 55±10% humidity, 12 h light-dark cycle starting at 7:00 a.m.). Food and water were provided ad libitum. All protocols were performed in accordance with the protocol outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health and approved by Institutional Ethical Committee for Care and Use of Laboratory Animals of the

Academy of Military Medical Sciences. All experiments were performed between 8:00 a.m. and 16:00 p.m. during the light cycle. KFL at doses of 0.75, 1.5 and 3 g/kg and vehicle (distilled water) were administered orally once daily for 14 days to rats after 15 min microwave exposure.

Morris water maze test

The water maze task was measured as previously described[32]. An overhead camera and computer assisted tracking system (SLY-MWM system, Beijing Sunney Instrument Co. Ltd., Beijing, China) was used to record the track of the rats. Test was performed in a circular pool (diameter, 150 cm, height, 28 cm), filled with water (temperature maintained at 21-23 ˚C). A ferric movable escape platform (diameter, 12 cm) was placed 1.5 cm below the water level in the middle of a fixed quadrant. Before the microwave exposure, rats were pre-trained to find the hidden platform twice. The training trials were tested 4 trials per day and conducted for 4 consecutive days after the last administration. Each trial had a maximum duration of 60 sec. When a rat located the platform, it was permitted to remain on it for 15 sec. If the rat did not locate the platform within 60 sec, it was placed on the platform for 15 sec. The time taken to find the hidden platform (escape latency) was recorded. And the average escape latency was used as the final index. The probe trials were performed at the 7th day. Platform were removed, and the rats were placed in a novel start position in the maze, facing the tank wall 180 ° from the original platform position. The time for probe trials lasted 60 sec. Percentage of time in the target quadrant and the number of crossings over the point where the platform were manually placed on was calculated. The time schedule of Morris water maze test is shown in Fig. 4A.

Hematoxylin and eosin (H&E) staining

After the last administration, animals were anaesthetized with pentobarbital (45 mg/kg, intraperitoneal injection), brains were removed immediately after decapitation and sectioned coronally as 2 parts. The rear part was used for histopathological examination. The front part was washed twice with cold saline solution, separated hippocampus, and stored at -80 ˚C until processing. The brains were fixed in 10% neutral buffered formalin, dehydrated with 50–100% ethanol solutions, and then embedded in paraffin. Tissues were sectioned at thickness 4μm, followed by staining with haematoxylin-eosin dye before subjecting to photomicroscopic observation. Images were captured by the Leica microscope at ×200 magnification.

Transmission electron microscopy (TEM)

Hippocampal samples(1 mm3 cubes) were dissected from CA3 area under anatomical microscope. The samples were then fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. After dehydration with graded ethyl alcohol, the samples were embedded in EPON618. The ultrathin sections cut onto copper mesh grids were contrasted with uranyl acetate and lead citrate. Electron micrographs were taken with a HITACHI H7650 transmission electron microscope (Hitachi, Tokyo, Japan)

MDA, SOD and XO assay in hippocampus

MDA, SOD and XO analysis were spectrophotometrically measured using assay kit according to the manufacturer's protocol (Jiancheng, Nanjing, China). Hippocampal tissues were weighed and made into 10% homogenate. The homogenate was centrifuged at 3000 rpm for 10 min at 4 ℃ and the supernatant was used. The protein content was measured using the Bradford method with bovine serum albumin as the standard [33].

Preparation of cell extracts and immunoblotting analysis

Cytosolic and nuclear extracts from hippocampal tissues were prepared according to the manufacturer's instructions (BestBio, Beijing, China). Homogenised samples were examined by immunoblotting with the primary antibodies recognising Nrf2 (1:500) (Santa Cruz, USA)and appropriate secondary antibody, followed by detection with Super Signal chemiluminescence kit (Pierce, USA). Lamin B (1:500) (Santa Cruz, USA)and beta-actin (1:1000) (Santa Cruz, USA) antibodies was used as loading controls for the nuclear and cytosolic extracts, respectively.

Quantitative real-time PCR (qPCR)

Total RNA of hippocampal tissues was isolated using the TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. 1 μg of total RNA was converted to cDNA using the GoscriptTM Reverse Transcription System (Promega, USA). Quantitative real-time PCR for rat beta-actin, HO-1, GSTmu3 and NQO1 was completed using the GoTaq® qPCR Master Mix (Promega) using the following primers: beta-actin (5′-AGA TCA AGA TCA TTG CTC CTC CT-3′; 5′- ACG CAG CTC AGT AAC AGT CC-3′), HO-1 (5′-GAG TTT CCG CCT CCA ACC AG-3′; 5′- AGG AGG CCA TCA CCA GCT TA -3′), GSTmu3 (5′- TGG ACA TTC CCA ATT TGC CCT-3′; 5′- CAA AGT CAG GAC TGC AGC AA-3′) and NQO1 (5′- CAG AAA CGA CAT CAC AGG GGA-3′;5′- AGC ACT CTC TCA AAC CAG CC-3′).

Statistical analysis

Data are expressed as the mean ± sem. two-way Repeated-measures ANOVA followed by Tukey B post hoc test with the day as one variable and the treatment as a second variable was used for the analysis of escape latency in Morris water maze. Other data comparison of groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Differences were considered to be statistically significant at $P<0.05$.

Results

The free radical scavenging activity of KFL

We tested the effect of KFL on hydroxyl radical scavenging activity in a Fenton reaction system with DMPO, a spin trapping agent. The typical ESR signals of DMPO-OH adducts were concentration-dependently attenuated when addition of KFL 0.6, 3, 6, 30 or 60 mg/ml (Fig. 3). The results indicated that KFL possessed free radical scavenging abilities.

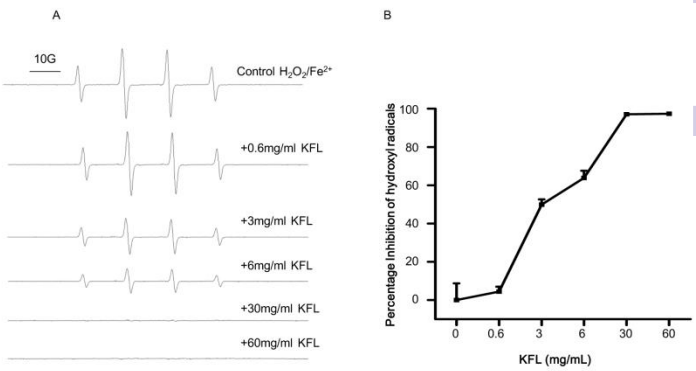

Figure 3. Scavenging hydroxyl radical activity of KFL in a cell-free chemical system was detected by ESR spectroscopy. (A) Scavenging hydroxyl radical activity. The intensity of OH• ESR signals decreased when

KFL was added. (B), Percentage inhibition of hydroxyl radicals by KFL. The results represent the means ± sem of 3 separate experiments.

KFL ameliorates the impairment of spatial memory caused by HPM

The MWM is a well-established paradigm for evaluating deficits in hippocampal-dependent memory, and the performance in learning and memory can be indicated by the escape latencies in hidden platform test, the percentage of time in target quadrant, and the number of platform crosses in the probe test. In the present study, average escape latency of control (sham exposed) rats decreased gradually during 4 days tests. Compared to control rats, the average latency of the HPM exposed rats was significantly longer. Performances of KFL treated rats were better than that of the HPM exposed rats from the third day of tests (Fig. 4B). In probe trials, percent time in the target quadrant of the HPM exposed rats was shorter than the control rats, but increased after the treatment with KFL (Fig. 4C). The number of crossings was significantly lower in the HPM exposed rats than in control rats. The number of crossings was significantly higher in KFL treated rats than that of HPM exposed rats (Fig. 4D). In contrast, the locomotory activities of rats did not differ among the groups (data not shown).

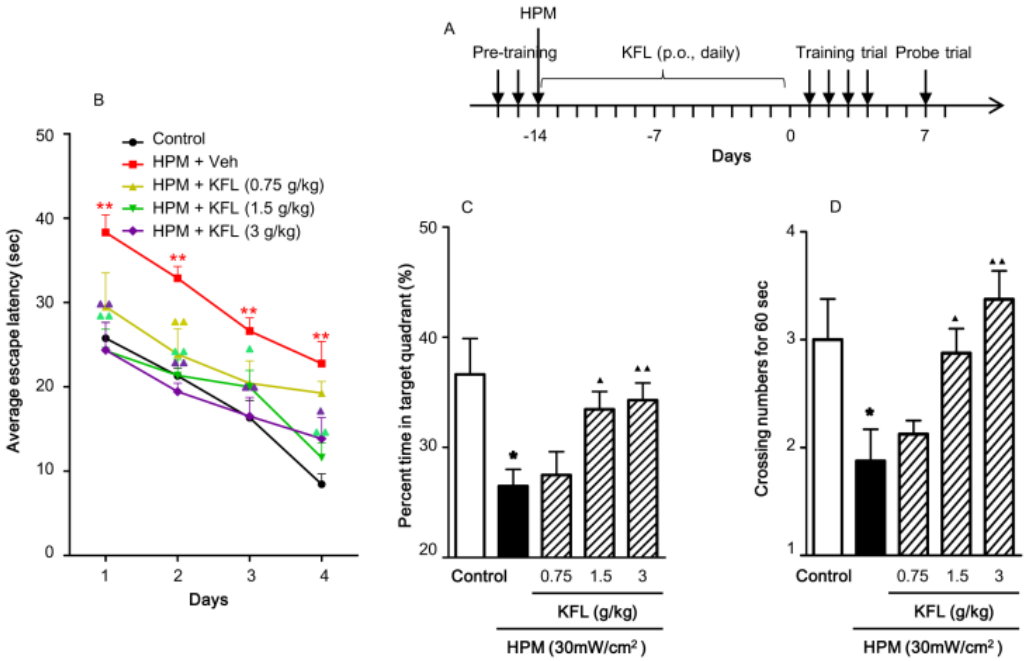

Figure 4. Performances of rats in the Morris water maze test. (A) Time schedule of experiments; (B) Average escape latency of rats in training trials; (C) Percent time in the target quadrant in probe trials; (D) Number of crossings over the position where the platform had been located in probe trials. The results represent the means ± sem of 8 rats. *p<0.05 vs. Control, **p<0.01 vs. Control; ▲p<0.05 vs. HPM + Veh, ▲▲p<0.01 vs. HPM + Veh.

Histopathological observation

Histopathological results showed that HPM at 30 mW/cm2 for 15 min caused a significant damage in hippocampus as indicated by a serious change in the tissue appearance with edema, neurons exhibited pyknosis and anachromasis (so called dark neurons) in the tissue as compared with the control group at 14 d after exposure. Treatment with KFL significantly improved the hippocampus condition in the HPM-exposed rats (Fig. 5).

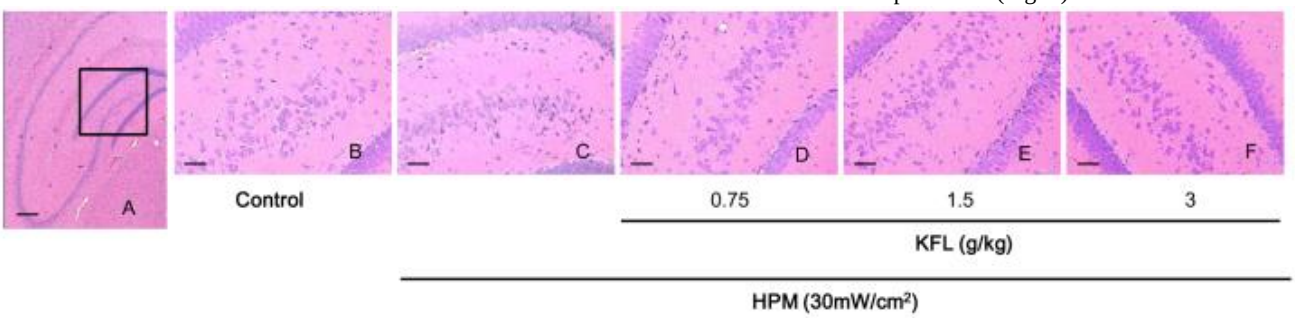

Figure 5. Effects of KFL on HPM-induced histopathologica changes in the hippocampus of rats. (A) Dentate gyrus of the hippocampus (H&E stained, scale bar = 200 μm); (B) Dentate gyrus in rat of sham exposure; (C) More dark and shrunken nerve cells (dark neurons) after microwave exposure; (D), (E), (F) Less dark neurons than microwave-exposed rats (H&E stained, scale bar = 50 μm).

Ultra-structure of hippocampus was observed by TEM. In hippocampal neurons of HPM-exposed rats, swollen mitochondria with disordered and reduced numbers of cristae, and expanded rough endoplasmic reticulum could be seen. Compared with the synapses

of hippocampal neurons in control rats, HPM-exposed rats exhibited a decrease in the quantity of synaptic vesicles in the synapse, and the synaptic clefts were widen and blurred. The treatment of KFL alleviated those changes (Fig. 6).

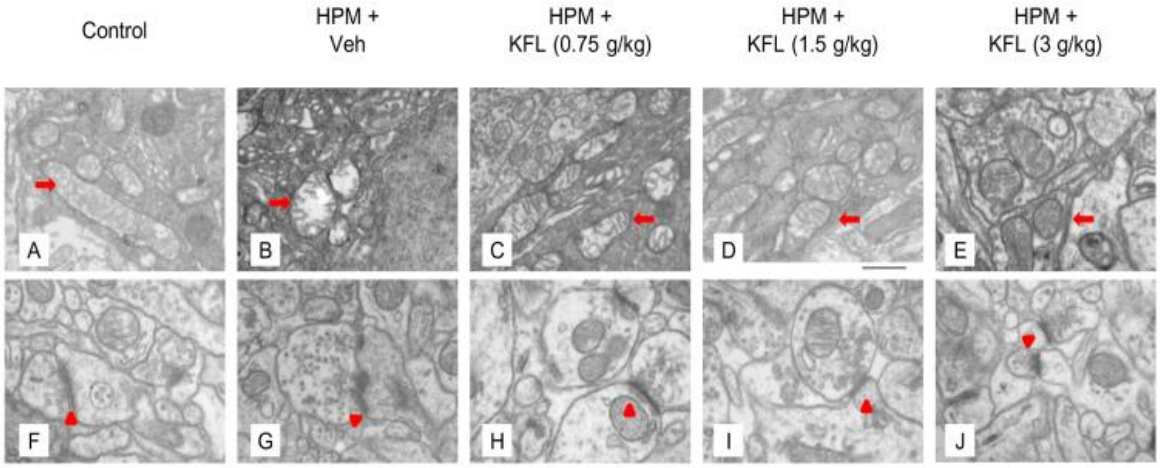

Figure 6. Effects of KFL on HPM-induced ultra-structural changes in hippocampal neurons of rats. (A) Normal mitochondria; (B) Swollen mitochondria with disordered and reduced numbers of cristae, and expanded rough endoplasmic reticulum; (C), (D), (E) Morphologically normal appearing mitochondria; (F) Normal synapse; (G) Swollen synapse with fewer vesicles, and the wider or blurred synaptic clefts; (H), (I), (J) Morphologically normal appearing synapse (TEM, scale bar = 500 nm).

Effect of KFL on HPM-induced oxidative stress in hippocampus of rats

As shown in Fig. 7, oxidative stress was assessed by measuring MDA level , XO activity, and SOD activity in hippocampal tissue after the last administration. The MDA level and XO activity in hippocampus of HPM exposed rats were significantly increased than that of controls. The SOD activity in hippocampus was significantly decreased than controls. Administration of KFL significantly enhanced the levels of SOD and decreased activity of XO in hippocampus and content of MDA in hippocampus.

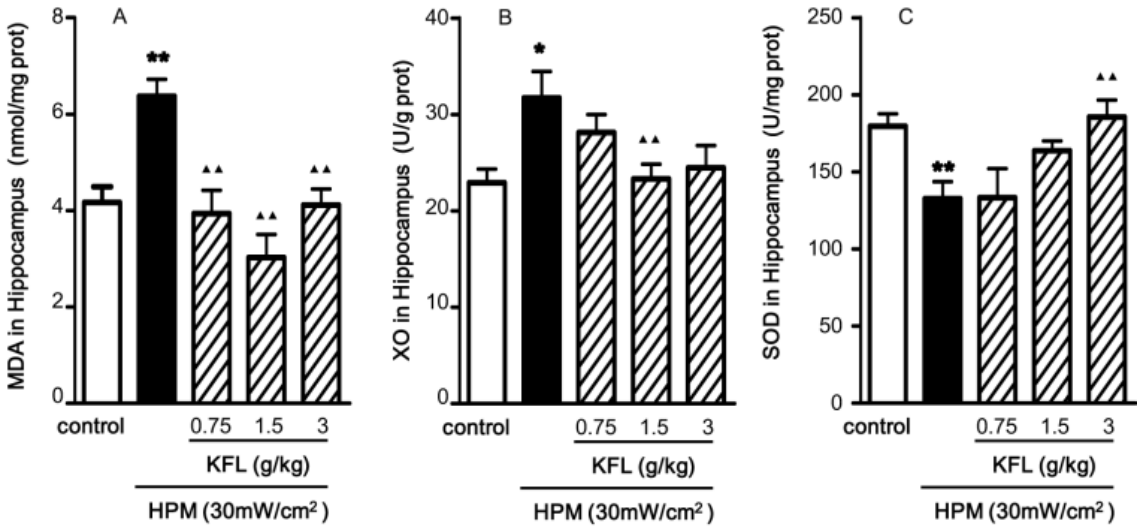

Figure 7. Effects of KFL on HPM-induced oxidative stress in the hippocampus of rats. (A) MDA level in hippocampus of rats; (B) XO activities in hippocampus; (C) SOD activities in hippocampus. The results represent the means ± sem of 5 rats. *p<0.05 vs. Control, **p<0.01 vs. Control; ▲p<0.05 vs. HPM + Veh, ▲▲p<0.01 vs. HPM + Veh.

KFL activate the Nrf2-ARE pathway in vivo

Upon activation Nrf2 is known to translocate to the nucleus. To investigate the effect of KFL on Nrf2 activation, protein levels of Nrf2 were analysed in nuclear, cytosolic lysates obtained from hippocampus of treated animals by Western blot analysis (Fig. 8A). HPM exposure slightly increased the amount of nuclear Nrf2. After treatment with KFL, the nuclear protein expression of Nrf2 significantly increased. Administration of KFL induced a nuclear accumulation of Nrf2.

Nrf2-ARE pathway activation was further supported by qPCR analysis. The expression of three Nrf2-regulated antioxidant genes (HO-1, GSTmu3, and NQO1) in the hippocampal tissues of rat is presented in Fig. 8B. As observed, exposure to HPM cause slightly

increase in phase 2 gene expression, whereas administration of KFL resulted in significant increases in HO-1, GSTmu3, and NQO1expression.

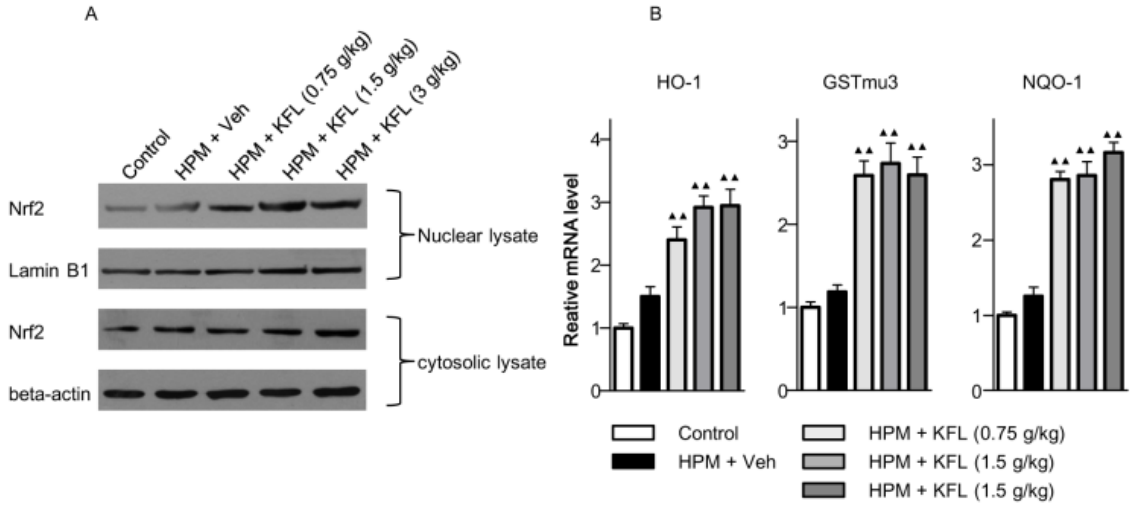

Figure 8. KFL activate Nrf2-ARE signaling pathway. (A), KFL induces nuclear accumulation of Nrf2. Representative Western blot of Nrf2. Nrf2 level was examined in nuclear, cytosolic fractions. (B), Up-regulation of Nrf2 target genes by KFL. Total RNA extracted from hippocampal tissues was used to determine mRNA expression of phase 2 genes expression by qPCR and normalized to the expression of beta-actin. Results represent the means ± sem of n=5 individuals. *p<0.05 vs. Control, **p<0.01 vs. Control; ▲p<0.05 vs. HPM + Veh, ▲▲p<0.01 vs. HPM + Veh.

Discussion and conclusion

Oxidative stress reflects to an imbalance between the intracellular production of free radicals and the cellular defence mechanisms. The CNS is particularly vulnerable to oxidative stress, as it normally produces relatively large amount of ROS for its high energy needs and O_2 consumption. The respiratory chain of mitochondria is the main source of ROS. Besides, neuronal membranes are rich in polyunsaturated fatty acids (PUFA) and antioxidant defences are modest in brain [34]. These may explain its susceptibility to microwave. The CNS, and in particular the hippocampus, has been demonstrated to be a sensitive target of microwave [35, 36]. In the present study, HPM exposure damaged the structure and function of hippocampus, consistent with previous data[4, 37], especially the mitochondrial morphology and ultrastructure of hippocampal neuron. Mitochondria with disordered and reduced numbers of cristae was seen in HPM exposed rat. Mitochondrial cristae shape determines respiratory efficiency and ATP synthesis[38]. HPM exposure may disturbance the electron transfer in the respiratory chain, and produce more ROS, and caused oxidative damage biochemically[7].

As XO can reduce molecular oxygen to superoxide and hydrogen peroxide, it is thought to be one of the key enzymes producing ROS. SOD is an important cellular antioxidant enzyme known to impact radicals involved in CNS oxidative stress, which catalyzes the dismutation of O_2- ·into oxygen and H_2O_2. MDA is a by-product of lipid peroxidation, produced under oxidative stress and used to reflects oxidative damage to the plasma membrane[34]. The MDA and XO significantly higher and the SOD marked lower further demonstrated that HPM induced oxidative stress. Then we found oral treatment with the KFL significantly prevented oxidant damage and pathological changes in the hippocampal tissue through decreasing the formation of ROS and enhancing activity of antioxidant enzymes. In present of KFL, Nrf2 accumulates in nuclei, and activates phase 2 gene containing ARE in the promoters. Our data suggested that both activation of Nrf2-ARE pathway and its direct scavenging radical activities contribute to its protective effects against HPM-induced oxidative stress. The antioxidant action via Nrf2-ARE pathway is more sustained and amplified by transcription-mediated signaling pathways.

KFL is a polybotanical dietary supplement under the guidance of "synergy" theory. Synergistic interactions suggest that the different ingredients in a formula increase the potency of each other. The whole purified extract of a formula offers advantages over a single isolated ingredient. Almost all ingredients contained in KFL except Herba Azollae Imbricatae have been studied a lot and reported exhibiting neuroprotective or antioxidative properties in vitro and in animal experiments [24, 25, 28, 39-43]. Considering these findings, we suggest that the multiple bioactive components are associated with the neuroprotective effect of KFL and some electrophilic compounds contained in KFL can activate Nrf2-ARE pathway.

Here we demonstrated that dietary supplement KFL could protect cognitive defect and oxidative stress caused by HPM by modulation of ROS formation and antioxidant enzymes. Our data also suggested that some electrophilic compounds contained in KFL protect neuron via Nrf2-ARE signaling pathway. In summary, KFL may be considered as a polybotanical dietary supplement for the therapy of brain injury induced by HPM.

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Notes and references

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