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MANDA SCAVENGES FREE RADICALS AND INHIBITS LIPID PEROXIDATION IN IRON-INDUCED EPILEPTIC FOCUS IN RATS

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1. INTRODUCTION

A number of studies have suggested that ischemia [1], trauma [2], vascular injury [3], cancer [4], aging [5] and neurological disorders such as epilepsy [6], are associated with free radicals and radical-mediated peroxidation reaction. The involvement of free radicals in such disorders opens the possibility of prevention and therapy by the use of many kind of antioxidant, including fermentation products [7] and Chinese medical herbal preparations[8]. Manda is a health food commercially sold in Japan. It is made by yeast fermentation of cane sugar, fruits, seeds, vegetables and seaweeds for more than 39 months [9].

In the present study, we examined the antioxidant effects of Manda on neural lipid peroxidation in an iron-induced epileptic focus in rats, and on reactive oxygen species *in vitro*.

2. MATERIALS AND METHODS

2.1 Chemical and Animals

Manda, a brown, sweet and sticky fermented natural food, was provided by Manda Fermentation Co., Ltd. (Hiroshima, Japan). Hypoxanthine (HPX), 1,1-diphenyl-2- picryl-hydrazyl (DPPH), diethylenetriaminepentaacetic acid (DTPA) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA), xanthine oxidase (XOD) and 5, 5′-dimethyl-

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1-pyrroline-N-oxide (DMPO) were from Boehringer Gmbh (Mannheim, Germany) and LABOTEC, Ltd. (Tokyo, Japan), respectively.

Male Sprague-Dawley rats from Clea Japan, Inc. (Tokyo, Japan) weighing 240–260 g were used in this study and housed at a constant temperature (25 ± 2°C) and a humidity (50 ± 5%). Control standard diet (MF; Oriental Yeast Co. Ltd., Japan) and water were provided freely for 7 days until the experiment.

2.2 Free Radical Analysis

Free radicals were examined by ESR spectrometry (JES-FEIXG, JEOL, Tokyo, Japan) using manganese oxide as an internal standard. Details are as follows;

1) DPPH. 50mM of DPPH was dissolved in ethyl alcohol. One hundred microliter of this solution and 100μl of sample dissolved to distilled water or distilled water as a control were mixed for 3s then placed in an ESR spectrometry flat cell. The DPPH radicals were measured exactly after 60s. The peak intensity of DPPH of a control was set as 100%. The inhibit rate of DPPH with Manda for the peak intensity of control was shown as the rate of scavenge activity.

2) Hydroxyl Radicals. 75μl of 1mM $FeSO_4$ and 1mM DTPA, 50μl of sample or distilled water as a control, 20μl of 0.092M DMPO and 75μl of 1mM H_2O_2, were mixed for 3s in the test tube, then placed in an ESR spectrometry flat cell. The DMPO-OH spin adducts were measured exactly after 50s from putting H_2O_2. The peak intensity of DMPO-OH adducts of a control was set as 100%. The inhibit rate of DMPO-OH with Manda for the peak intensity of control was shown as the rate of scavenge activity.

3) Superoxide Radicals. Fifty microliter of 2mM HPX, 35μl of 11mM DTPA, 50μl of sample or distilled water as a control, 15μl of DMPO, and 50μl of freshly prepared XOD suspension were mixed for 3s, then placed in an ESR spectrometry flat cell. The DMPO-O_2^- spin adducts were measured exactly after just 40s. The peak intensity of DMPO-OOH adducts of a control was set as 100%. The inhibit rate of DMPO-OOH with Manda for the peak intensity of control was shown as the rate of scavenge activity.

2.3 Iron-Induced Epileptic Focus in Rats

$FeCl_2$-induced epileptic foci in rats were prepared by following procedures as previously reported by Willmore [10]. Manda were given orally to rats by a canula, 1g/kg body weight, 20 min prior to cortical injection. The rats were anesthetized by pentobarbital. Then, 5 μl of 100 mM $FeCl_2$ was injected into the left cortex at a depth of 1.2 mm. Saline was injected into the left subpial cerebral cortex of control animals with the same volume and pH as of the $FeCl_2$ treated rat. Rats were killed by decapitation 45min after the iron injection. The epileptogenic focal area was rapidly excised over an ice plate, and kept at -80°C until analysis of thiobarbituric acid reactive substances (TBARS) (Ohkawa [11]).

2.4 Statistical Analysis

Statistical analysis was performed using Student's t-test.

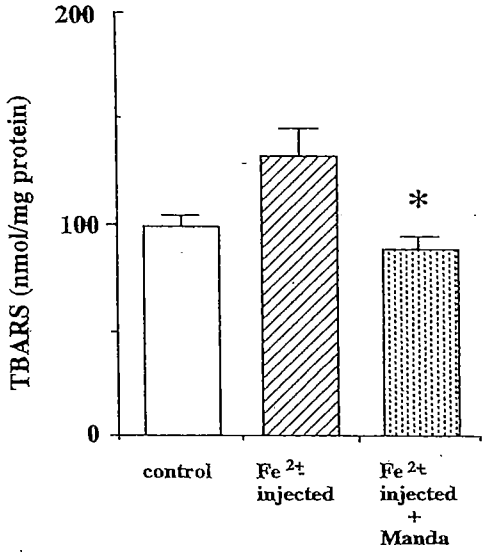

Figure 1. Effect of Manda on TBARS levels in the $FeCl_2$-induced epileptic focus of rat cerebral cortex. (means±SEM; n=5–6 determinations) * As compared with Fe^{2+}-injected group, $p<0.05$

3. RESULTS AND DISCUSSION

Oral administration of 1 g/kg body weight of Manda significantly inhibited the formation of TBARS, which were used as an indicator of lipid peroxidation in the $FeCl_2$-induced epileptic focus in rats (Figure 1).

Then, we examined scavenging action of Manda on DPPH, hydroxyl and superoxide radicals. We found that Manda scavenged these radicals especially superoxide radicals in a dose-dependent manner. Manda (22.7 g/L) decreased the signal of DMPO-OH in an ESR recording (41% of DMPO-OH spin adducts) ($20x10^{15}$ spins/ml) (Figure 3). Manda decreased also the signal of DMPO-O_2^- ($6.8x10^{15}$ spins/ml) (Figure 4). Moreover Manda (25 g/L and 5 g/L) decreased DPPH radicals, respectively (Figure 2).

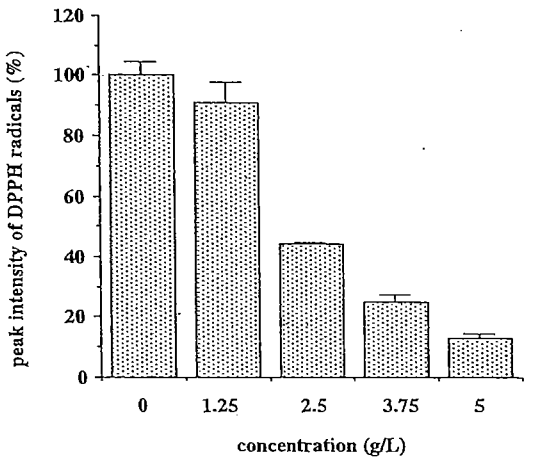

Figure 2. Effect of scavenging action of Manda on 1, 1-diphenyl-2-picrylhydrazyl. (means ± SEM of 3 determinations) ESR setting: magnetic field, 335.6 ± 10mT; response, 0.3sec; sweep time, 0.5min; amplitude, 1.6x1,000; modulation amplitude, 0.08mT; and power 8mW.

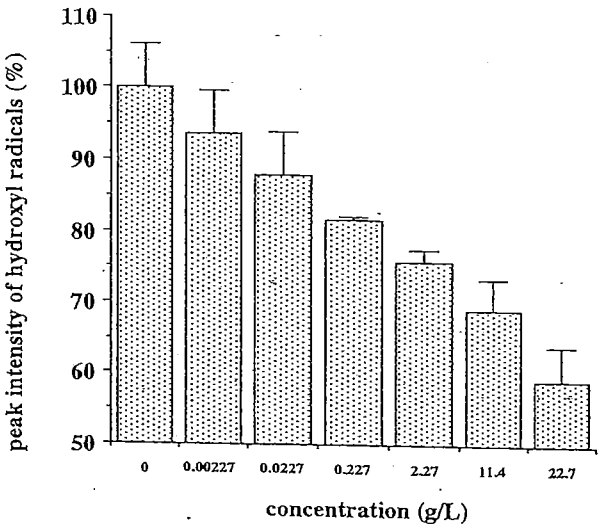

Figure 3. Effect of scavenging action of Manda on hydroxyl radicals generated from Fenton's reaction. (means±SEM of 3 determinations) ESR setting: magnetic field, 335.6±5mT; response, 0.3sec; sweeptime, 0.5min; amplitude, 1.6x1,000; modulation amplitude, 0.08mT; and power 8mW.

These results reveal that Manda is potent scavengers of superoxide radicals and have some ability in quenching hydroxyl and DPPH radicals. These radical scavenging properties may be attributed to the products by fermentation of the base plant material used in Manda preparation, though no information is available at the present.

$FeCl_2$ generates reactive oxygen radicals, in particular hydroxyl and superoxide radicals[12–15]. Both initiate and propagate peroxidation reactions [17] at the double bonds in the carbon chains of polyunsaturated fatty acids and lipids at the neuronal cell membranes. Meanwhile, antioxidants such as EPC-K1 [17, 18] and Guilingji [19] are known to inhibit

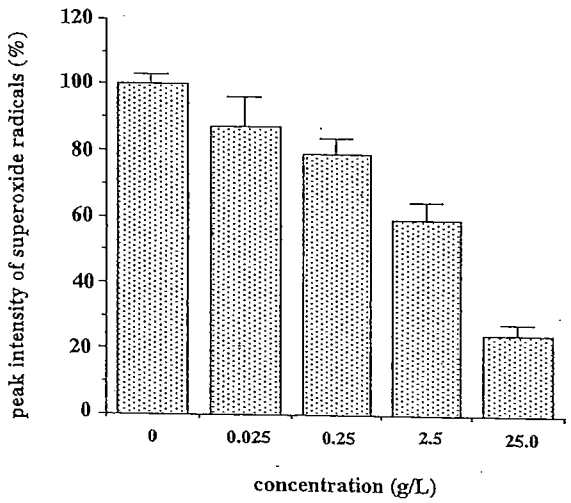

Figure 4. Effect of scavenging action of Manda on superoxide radicals generated from hypoxanthine-xanthine oxidase system. (means±SEM of 3 determinations) ESR setting: magnetic field, 335.6±5mT; response, 0.3sec; sweep time, 2min; amplitude, 1.6x1,000, modulation amplitude, 0.08mT; and power 8mW.

TBARS formation *in vivo* and prevent free radical induced cell damages. In this study, decreased TBARS formation in Manda-treated group similarly may indicate that damages by free radicals were suppressed by Manda. In conclusion, Manda scavenged free radicals, for especially superoxide radicals, and suppressed TBARS formation suggesting Manda, may be helpful to preventing neural lipid peroxidation, traumatic epilepsy, and aging [20, 21].

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