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원 저

Physiological Activity and Effects on Lipid Peroxidation of Hot Water-Extract Obtained from *Euonymus alatus* in Cultured Rat Hepatocyte

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Rat의 hepatocyte에서 amyloid-β로 유발된 세포사, 지질과산화 및 세포산화에 대한 鬼箭羽 열수 추출물의 보호효과

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목적 : 貴箭羽 *Euonymus alatus* (Thunb.) Sieb로부터 얻은 추출물에 대하여 생리활성(항산화 활성)을 검토하였고, amyloid-β (Aβ)가 지니는 Rat의 배양 hepatocyte 세포독성에 대한 세포보호효과 및 세포막지질의 과산화에 미치는 영향을 검토하였다.

방법 : 貴箭羽 추출물과 항산화제인 BHT, ascorbic acid 및 α-tocopherol을 각각 linoleic acid에 소정량 첨가하고 50℃에서 일정기간 저장하여 linoleic acid의 산화 안정성을 비교 검토하였으며, 또한 유지 자동산화의 촉진인자인 금속이온(Fe^{3+}, Zn^{2+})을 첨가하여 貴箭羽 추출물의 금속이온 봉쇄효과를 조사하였다.

결과 : 세포성지질의 과산화의 지표인 malondialdehyde (MDA) 생성이 Aβ처리로 크게 증가하였으나 세포막파괴에 의한 세포파괴의 전형적인 현상이 貴箭羽 열수 추출물의 전처리와 후처리로 크게 감소되었다. 貴箭羽 추출물의 농도에 따른 항산화 효과는 전 농도별에서 높은 항산화 효과를 나타내었다.그리고 시간 경과에 따른 항산화 효과에 있어서도 정도의 차이는 있지만 각 농도(5mg, 10mg 및 25mg)에서 유도기의 연장효과가 상당히 우수한 것으로 나타났다.貴箭羽 추출물과 항산화제와의 비교에 있어서는 합성 항산화제인 BHT 및 ascorbic acid 만큼 우수한 항산화력을 나타내었으며,천연 항산화제인 α-tocopherol보다는 아주 우수한 것으로 나타났다. 貴箭羽 추출물의 금속이온 봉쇄효과에 있어서는 linoleic acid에 貴箭羽 추출물과 Fe^{3+} 및 Zn^{2+}을 첨가한 군이 貴箭羽 추출물을 첨가하지 않은 군보다 항산화 효과가 아주 우수한 것으로 나타나 금속이온 봉쇄력이 강한 것으로 나타났다.

결론 : 貴箭羽 열수 추출물은 Aβ에 의한 세포파괴를 감소시키는 활성을 가지며 예방 및 보호효과를 나타내었고, hepatocyte에 대한 보호효과와 세포막지질 과산화의 저해 및 Aβ처리와 같은 독성에 대한 적응능력 향상을 통한 세포보호효과를 주는 것으로 간세포 보호 등의 임상적 응용에 그 효과가 기대된다. *(J Korean Oriental Med 2001;22(2):53-63)*

Key Words: *Euonymus alatus* (Thunb.) Sieb, Antioxidant Activity, hepatocyte, amyloid-β, lipid peroxidation.

INTRODUCTION

Aβ is 39-43 amino acids long and proteolytically derived from an integral membrane protein termed

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amyloid precursor protein (APP)[1], although mechanism for APP processing is not still unknown. There are many in vitro studies demonstrating that Aβ is directly toxic[1]. The toxic effect of Aβ is correlated with its ability to form aggregates[2]. Both oxygen species[3] and excessive Ca^{2+} influx[4] are also implicated in the mechanism of Aβ toxicity.

The reactivity of different free radicals varies and some cause severe damage to biological molecules, especially to DNA, lipids and proteins. In the presence of oxygen, free radicals can react with polyunsaturated fatty acids, resulting in highly reactive peroxyl free radicals. Peroxyl free radicals can further propagate the peroxidation of lipids or compromise the integrity of cell membranes, therefore this is thought to be involved toxic actions of some chemicals[5-7].

Recently, several reports presented that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis and may offer effective protection from lipid peroxidative damage in vitro and in vivo[8,9]. Therefore, much attention has been focused on natural antioxidants, in particular it was reported the hot-water extract of *Euonymus alatus* (Thunb.) Sieb (EA) may exert an anti-aging action and neuro-protective activity. Although little is yet known about the pharmacological effects or active ingredients.

Lipid peroxidation is known as one of the major factors for deterioration during the storage and processing of Bio-materials. In addition, it is considered to induce physiological obstruction causing cell aging or carcinogenesis[10-15]. For this reason, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tertary butylhydroquinone have been widely used for preventing many foods from oxidative deterioration. However, the current trend is to avoid their use as food additives because of the possibility of cell toxicity[16-19].

To resolve this problem, the application of naturally occurring safe antioxidant is being investigated. So for, natural antioxidants to have been identified include tocopherols in soybean, flavonoids such as guercetin and catechin in tea leaves, gossipol in cotton oil, seed of Plantago asiatica Linne[20], soybean isoflavones[21], sesame oil[22], rosemary and sage[18], etc. On the other hand, crude drugs prepared from plant materials are traditionally used and their pharmacolgical effect have been extensively studied from various viewpoints; however, only a few reports are available on the antioxidative activities of crude drugs[23,24].

The nitrosamines are generally produced by an electrophilic reaction of nitrite with the corresponding secondary amines and amides[25,26]. Most N-nitroso derivatives are powerful carcinogens, which can in several species. These derivatives might possibly be produced in the human stomach by the acid-catalyzed reaction between nitrite, which is present in some foods, and N-nitrosatable compounds, which may be ingested as drugs, food additives, or natural constituents of food[27,28]. N-nitrosamines are potent mutagens, many of which have been shown to cause cancer in variety of animals. It is carcinogenic in the rat, inducing tumors of liver and of the kidney[29]. It has been suggested that dimethylnitrosamine must be metabolically activated before it becomes hepatotoxic and that a metabolite or metabolites may be responsible for toxicity and carcinogenicity[30]. The biological effect which mostly concerns human is carcinogenicity, and in order to prevent this, it is very important to inhibit the formation ofnitrosamines. Much research has been done on the formation of nirosamines in food[31-33].

We have been investigated on some crude drugs of korea as a preventive for a variety of diseases including cancer. The *Euonymus alatus* (Thunb.) Sieb (EA) has long been used as an crude drug. In this paper, the authors investigated physiological activity (the antioxidative activity and the nitrate scavenging effect)

of hot-water extract obtained from EA. This study also reports the effect of EA on cytotoxicity of cultured hepatocytes and lipid peroxidation in Aβ-treated conditions. Aβ which can produce intracellular free radical was used for inducer of the peroxidation of cellular lipids.

MATERIALS AND METHODS

Materials

Linoleic acid(55%) was purchased from Fruka chemical AG. BHT (buthylated hydroxytoluene) was purchased from Ueno Co. Ltd. α-Tocopherol(95%) was purchased from Roche Co. Ltd. Ascorbic acid(99%) was purchased from Dakeda Co. Ltd. Iron standard solution($FeCl_2$: 1000 ppm) and Zinc standard solution ($ZnCl_2$: 1000ppm) were purchased from Junsei Chemical Co. Ltd. Sodium nitrite ($NaNO_2$) was purchased from Junsei Chemical Co., Ltd.

The Aβ 25-35 peptide was synthesized by Applied Biosystem' s Protein Synthesizer Model 470A (Peptron Co., LTD, Taejon, Korea). Fetal bovine serum (FBS), penicillin- streptomycin were obtained from GIBCO-BRL (Grand Island, New York, USA). Dulbecco' s Modified Eagle' s Medium (DMEM), glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide(MTT), dimethyl sulphoxide (DMSO), 2-thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane (TEP), ethylenediamine tetraacetic acid (EDTA), nitro blue tetrazolium (NBT), catalase (from bovine liver), diethylene triamine pentaacetic acid (DETAPAC), β-nicotinamide adenine dinucleotide phosphate (β-NADPH) were purchased from Sigma Chem. Co. (St. Louis, USA). 1-chloro-2,4-dinitrobenzene (CDNB), NaN3 were obtained from Aldrich Chem. Co. (Milwaukee, WI). All other chemicals used were of analytical reagent grade.

Extract from EA

EA, which has long been used as crude drug in Korea, was obtained from College of Oriental Medicine, Dongguk University,

EA (300g) was extracted with boiling water for 3 h. Then, the extract was evaporated to under reduced pressure. The last extracts diluted by 0.9% NaCl and filterated. The extract solution was stored at 4℃

Cell culture and preparation of EA

Primary hepatocyte cultures were prepared from neonatal rat (1-2 day old) pups. Cells were plated in 6-well culture plates coated with polyethylenimine (0.2 mg/ml in sodium borate buffer, pH 8.3) at a density of 40,000 cells per well. After overnight incubation in Dulbecco' s modified Eagle' s medium (DMEM; Gibco) supplemented with 20% fetal bovine serum, the medium was changed to serum-free defined medium [DMEM supplemented with 2 mM glutamine, 1 mM pyruvate, penicillin-streptomycin- amphotericin B mixture (Gibco), 5 mM HEPES, 0.5% glucose, 10 μg/ml insulin, 30 nM sodium selenite, 20 nM progesterone, 100 μM putrescine, and 20 μg/ml transferrin]. The cultures were incubated at 37℃ in an atmosphere of 5% CO_2/95% room air, and the medium was replaced every other day. Experiments were performed in 6-7-day-old culture.

Depending upon the experimental group, EA was added (at 2% volume in culture medium) to or omitted from flasks. After 16~18 h, cells were washed twice with warm phosphate-buffered saline (PBS) and serum-free medium added to the flask. Then the cells were treated with 10 μM Aβ peptide for 2 h and the content of thiobarbituric acid-reactive substance (TBARS) and enzyme activities measured. 10 μM Aβ peptide was diluted in serum-free medium and added to the cultures.

Cell viability and toxicity assay

Cell viability was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the coloured formazan product by mitochondrial enzymes in viable cells[34]. Cells were cultured in polyethylenimine-coated 24 or 96 well culture plates at a density of 10,000 cells per well for lactate dehydrogenase (LDH) assay or 40,000 cells per well for MTT reduction assay. LDH activities in the medium were measured by a Cytotox 96 nonradioactive cytotoxicity assay kit (Promega) according to the manufacturer's instructions. The results were expressed as percentages of peak LDH release on complete cell lysis (control).

The MTT reduction was measured essentially as described previously[1] with a slight modification. In brief, after incubating cells for 48 h with various samples, t-BHP and Aβ peptides, MTT solution in PBS was added to a final concentration of 0.5 mg/ml, and the cells were further incubated for 4 h at 37℃. After incubation, the plate were centrifuged at 90 x g for 10 min to obtain the resulting insoluble formazan precipitates. To dissolve the crystal precipitates, 150㎕ or 600㎕ of a 1:1 mix of ethanol and DMSO were added to each well. Each plate was gently shaken for approximately 20 min before reading on the Enzyme-Linked Immunosorbent Assay (ELISA) reader (measurement 570 nm, reference 620 nm). Absorbance of converted dye was measured. Assay values obtained on addition of vehicle were taken as 100%, and complete inhibition of MTT reduction (0%) was defined as the value obtained following addition of 0.9% Triton X-100.

To examine whether EA could attenuate the cytotoxicity of Aβ peptides, cultures were pretreated with indicated concentrations of EA for 4 h. Thereafter 10 μM Aβ 25-35 was added to cultures and incubated for 48 h. LDH activity in the culture medium was determined as described above. To investigate the effect of pretreatment with Aβ peptides on the cytotoxicity induced by hydrogen peroxide or glutamate, cells were pretreated with 10 μM Aβ peptide for 48 h, and then 100 μM hydrogen peroxide or 100 μM glutamate was added to cultures and incubated further for 4 h or 1 h, respectively. For all findings, each condition represents five separate wells pre experiment and is repeated in two or five independent experiments.

Treatment of hepatocytes with Aβ peptides

Confluent hepatocytes were trypsinized and plated into T-75 tissue culture flasks at a density of 5 x 10^6 cells/flasks (for lipid peroxidation and antioxidative enzyme activity), or into 96-well plates at a density of 5 x 10^4 cells/well (for MTT reduction assays). After 24 h, cells were washed with PBS to remove serum, and cultures were incubated in DMEM free FBS for an additional 12 h before addition of Aβ peptides or control buffer.

Lipid peroxidation assay

The release of TBARS into incubation medium was measured by the method of Glascott *et al.*[35]. Depending upon the experimental group, EA was added (at 2% volume in culture medium) to or omitted from flasks and then overnight incubation. Overnight (16~18 hr) cells were washed twice with warm PBS buffer and 10 ml of serum-free medium were added to the flask, and the cells were treated with Aβ peptide. After Aβ peptide treatment for 2 hr, the cells were removed by scraping, then trichloroacetic acid (TCA) was added to scraped cells and medium (4.5% final concentration). The scraped cells were sonicated for 20 sec and centrifuged to pellet the protein. In brief, 1 ml of TCA supernatant was added to 2 ml of TBA solution (composed of 0.45%, w/v, TBA and 7.5%, v/v, acetic acid, pH to 4.15 with 10 N NaOH). This reactive solution was placed in

a boiling water bath for 15 min, cooled to room temperature and read on a Gilford Response spectrophotometer with excitation wavelength of 532 nm. TEP was dissolved in 0.01 N HCl to produce malondialdehyde (MDA), and this was used to generate a TBARS standard curve. The data was expressed as nmol of TBARS/mg protein

Measurement of antioxidative activity

Hayase et al[36] and kirigaya et al[37] methods were used as described: one gram of linoleic acid dissolved in 20 ml of ethanol was placed in an Erlenmeyer flask with a stopper of ground glass.Twenty-five ml of 0.2 M phosphate buffer (pH 7.0) and EA extract or antioxidants were added to the above solution.The flask was tightly stoppered and stored in an incubator at 50 ℃ .Oxidized linolenic acid was extracted with chloroform by using a separatory funnel, and the peroxide value(POV) was measured by iodometry. Antioxidative activity was indicated as follows : POV (meq/kg) = POV of test samples / POV of control* 100. Accordingly, the lower the POV% is the stronger the antioxidative activity of sample.

Protein determination

Protein was determined on each sample by the method of Smith et al.[38] (using bicinchoninic acid), using bovine serum albumin as the standard.

Statistical analysis

Standard procedures were used to calculate means and standard deviation of the mean. Mean values were compared using Duncan′ s Multiple Range Test with on SAS program (SAS Institute, Cary, NC) ; $P<0.05$ was considered significant.

RESULTS

1. Effect of amyloid β on cell cytotoxicity in cultured hepatocyte cells as shown by using MTT assays

As shown in Table 1, cell cytotoxicity was significantly enhanced by addition of increasing concentrations of Aβ compared to those of untreated group. When we measured the amount of MTT reduction from cultured rat primary hepatocyte cells at 48 h after peptide treatment, the Aβ decreased MTT reduction by 55% of the control value at 0.1 μM concentration. Aβ reduced MTT reduction by 30% and 20% at 50 μM and 100 μM, respectively.

2. Effect of Aβ on cytotoxicity in cultured hepatocyte cells by LDH assays

On the other hand, the toxicities of Aβ were assessed by LDH assay. Following the appropriate incubation time. LDH activities in the medium were measured by a Cytotox 96 nonradioactive cytotoxicity assay kit (Promega) according to the manufacturer′ s guidances. The results were expressed as percentage of peak LDH release obtained on complete lysis. The Aβ increased LDH release by 48.65% of the maximal value at 100 μM concentration. Aβ induced LDH release only 11.4 and 26.2%, respectively, even at 25 and 50 μM (Table 2).

On the other hand, we measured the protective and

Table 1. Effects of Various Concentrations of Aβ on MTT Reduction in Cultured Rat Hepatocytes

Amyloid (μM)							
0	0.1	0.5	1.0	5.0	10	25	100
52.5±3.3	51.3±4.5	50.8±4.6	48.5±4.5	46.7±5.4	35.4±2.5*	29.7±2.5*	22.6±3.5*

Cell were incubated with the indicated concentrations of Aβ for 48 h. Assay values obtained on addition of vehicle were taken as 100% and complete inhibition of MTT reduction (0%) was defined as the assay value obtained following addition of 0.9% Triton X-100 to lyse the cells completely.

Table 2. LDH Activity in Cultured Rat Hepatocytes at 48 hrs after Treatment with Aβ and Protective Effects of *Euonymus alatus* (Thunb.) Sieb (EA) Treatment.

Concentration(μM)	LDH (% of maximal release)		
	Without	Pretreatment of EA (100 μg/ml)	Posttreatment of EA (100 μg/ml)
0.1	2.5±0.2	2.4±0.2	2.5±0.2
1.0	3.5±0.3	3.7±0.3	4.6±0.3
10	5.8±0.6	6.5±0.5	6.5±0.5
25	10.6±1.0	7.5±1.2*	9.3±1.3
50	26.2±2.6	20.7±3.3*	27.3±3.3#
100	48.6±4.5	38.5±3.3**	43.4±3.2

The results are expressed as percentage of maximal LDH release that was obtained on complete cell lysis. Data are mean ± SEM values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001 significant different from control. #, P<0.05 significant different from pre- and posttreatment.

Table 3. Effects of Various Concentrations of *Euonymus alatus* (Thunb.) Sieb (EA) on MTT Reduction in Cultured Rat Primary Hepatocytes.

	EA amounts treated (μg/ml)			
	1.0	5.0	10.0	20.0
MTT reduction	143.4±11.2	141.3±13.2	121.3±12.2	102.2±11.2

The results are expressed as percentage of maximal LDH release that was obtained on complete cell lysis. Data are mean ± SEM values obtained from five culture wells per experiment, determined in three to five independent experiments. *, P<0.05. **, P<0.001 significant different from control. #, P<0.05 significant different from pre- and posttreatment.

Table 4. Effects of Pretreatment of *Euonymus alatus* (Thunb.) Sieb (EA) on Aβ-Induced Cytotoxicity in Cultured Rat Primary Hepatocytes.

	EA amounts treated (μg/ml)			
	1.0	5.0	10.0	20.0
MTT reduction	45.4±5.4	43.2±3.3	38.7±6.3	37.6±6.3

Cultures were pretreated with various concentrations of EA for 24 h before application of 50μM Aβ. At 48 h after 50 μM Aβ treatments, MTT reduction was assayed.

proliferative effects of EA on LDH activity in 24 h before treatment with indicated concentrations of Aβ. The treatment of 100 μg/ml of concentrated EA solution reduced the LDH activity by 75% of control group when compared at concentration of 100 μM Aβ (48.6 vs 38.5). Also, when the protective and proliferative effects of EA on LDH activity in 48 h after treatment with indicated concentrations of Aβ, the treatment (100 μg/ml) of EA solution reduced the LDH activity by 90% of control group of 100 μM Aβ (48.4 vs 43.3). This result indicates that the pretreatment of EA is much more effective for hepatocyte protection than posttreatment of EA.

3. Effect of EA on cell cytotoxicity in cultured hepatocyte cells

We measured the protective and proliferative effects of EA on MTT reduction in cultured hepatocytes at 48 h after EA treatment (Table 3). The treatment of 100 μg/ml of concentrated EA solution increased the MTT reduction activity by 140% of control group. Interestingly, 20 μg/ml and 10 μg/ml concentrations of the EA solution resulted in by 165% and 370% increase of the control group, respectively, being maximal MTT reduction activity at 10 μg/ml. Upon further dilution of the EA solution up to 0.1 μg/ml concentration, the reduction activity was higher than that of vehicle. Thus, it was possibly concluded that the EA is highly effective

Table 5. EEffects of *Euonymus alatus* (Thunb.) Sieb (EA) on Aβ-Induced Cytotoxicity of Cultured Rat Primary Hepatocytes.

	EA amounts treated (μg/ml)				
	1.0	2.0	5.0	10.0	20.0
MTT reduction	28.6±6.5	27.7±3.6	25.9±4.3	27.5±3.4	26.4±2.1

Cultures were treated with 50 μM Aβ for 48 h before application of various concentrations of EA. At 24 h after EA treatment, MTT reduction was assayed.

Table 6. Effects of *Euonymus alatus* (Thunb.) Sieb (EA) on Lipid Peroxidation Induced by $A\beta_{25\sim35}$ Treatment in Cultured Rat Primary Hepatocytes.

	C	Aβ	EA	EA+Aβ
TBARS (nmol/mg protein)	0.63±0.1	1.2±0.1	0.4±0.1#	0.7±0.1*

Culture cell were pretreated with EA for 24 h before application of 50 μM Aβ. After 48 h, the release of TBARS into medium was measured. #, P<0.05, significantly different from control; *, P<0.05, significantly different from Aβ treatment group.

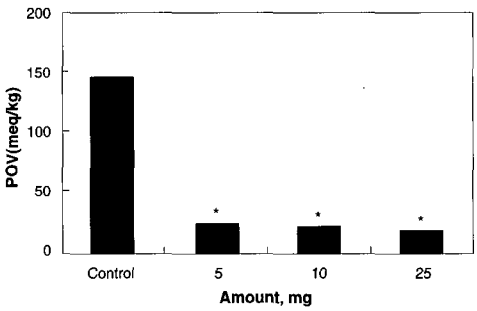

Fig. 1. Antioxidative effect of *Euonymus alatus* (Thunb.) Sieb (EA).
Each amount of EA was incubated with linoleic acid(LA) at 50℃ for 6 days.
(*, P < 0.01compared to control)

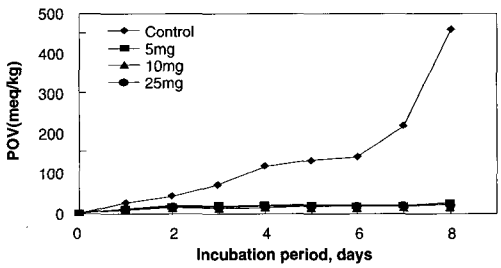

Fig. 2. Period-course in antioxidative effect of *Euonymus alatus* (Thunb.) Sieb (EA).
Five, ten and twenty-five milligrams of EA wereincubated with linoleic acid(LA) at 50℃.

for the protection and proliferation of the rat primary hepatocytes.

On the other hand,pretreatment of EA attenuated in a cell cytotoxicity enhanced by exposed to increasing concentrations of Aβ(Table 4). This indicates that cells pretreated with EA allowed its resistance against the toxic effects of increasing concentrations of Aβ peptides. However, gradual dilution of the EA concentration increased cell cytotoxicity activity of Aβ. With the pretreatment with EA, about 70% of the cells were killed within 48 h by 50 μM Aβ. In contrast, fewer than 60% of the EA pretreated cells were killed by the same concentrations of Aβ. Seemingly, post-treatment with serially diluted EA showed similar cell cytotoxicity activity in the with or without EA pretreatment (Table 5).

4. Effect of EA on lipid peroxidation induced by A β in cultured hepatocytecells

Table 6 shows the effect of EA on MDA level in cultured rat hepatocytecell exposed to Aβ. The accumulation of MDA, as measured by TBARS in the medium, is a sensitive index of the peroxidation of cellular lipids in cultured cell intoxicated with Aβ. At present assay, TBARS levels of Aβ treatment group were significantly higher than other groups. This increased level was significantly reduced by EA pretreatment.

5. Antioxidative activity of EA

The effect of the concentrations of EA extract on the antioxidative activity is shown in Fig. 1. POV of the

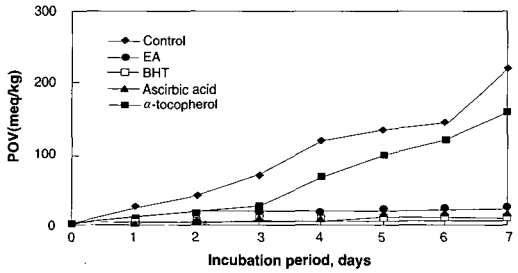

Fig. 3. Comparison of antioxidative effect of *Euonymus alatus* (Thunb.) Sieb (EA)with antioxidants in the period course.
Five milligrams of EA were incubated with linoleic acid(LA) at 50 ℃

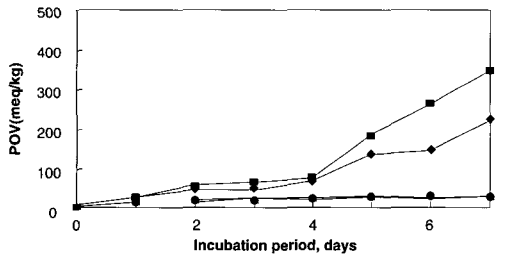

Fig. 5. Period-course in antioxidative effect *Euonymus alatus* (Thunb.) Sieb (EA) on iron ion-catalysed peroxidation of linoleic acid.
Each amount of EA and Fe^{2+}(2ppm) were incubated with linoleic acid at 50℃. linoleic acid(LA) alone, - ◆ - ; LA+Fe^{2+}, - ■ - ; LA+Fe^{2+} Euon. S 5mg, - ▲ - ; LA+Fe^{2+} Euon. S 10mg, - ● - Euon. S : EA

linoleic acid control group which was incubated at 50℃ for 6 days was 150 and POV of the linoleic acid with added EA extract(5-25mg) were about 20-25, respectively. Also, Fig. 2 shows the antioxidative effect of EA extract in the period-course at various concentrations (5 mg, 10 mg and 25 mg). POV of the linoleic acid as a control group which was incubated at 50℃ for 8 days was about 460 and POV of the linoleic acidadded with EA extract(5-25mg) were about 25-40, respectively. Accordingly, EA extract exhibited a great antioxidative activity at all concentrations tested.

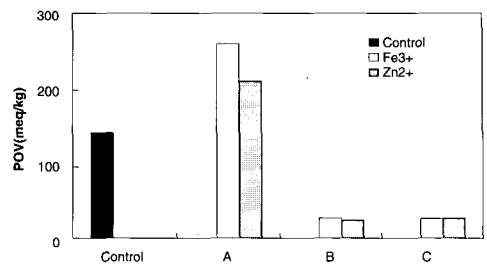

Fig. 4. Antioxdative effect of *Euonymus alatus* (Thunb.) Sieb (EA) on metal(Fe^{2+}, Zn^{2+})ion-catalysed peroxidation of linoleic acid(LA). EA and metal ion (2ppm) were incubated with linoleic acid(LA) at 50℃ for 6days.
A, linoleic acid(LA) + metal ion ; B, LA + metal ion + Euon. S 5mg ; C, LA + metal ion +Euon. S 10mg. Euon. S : EA

6. Comparison of antioxidative effect of EA extract with some synthetic antioxidants (BHT and ascorbic acid) and natural antioxidant (α-Tocopherol).

On the other hand, Fig. 3 shows the results of the comparison of antioxidative effect of EA extract, synthetic antioxidants (BHT and ascorbic acid) and natural antioxidant (α-Tocopherol). EA extract was as good as antioxidative activity of the synthetic antioxidant (BHT, ascorbic acid), and was superior than that of the natural antioxidant (α-Tocopherol).

7. Antioxidative effect of EA extract added with metal ions (Fe^{2+} and Zn^{2+}).

Fig. 4 shows the antioxidative effect of EA extract added with metal ions (Fe^{2+} and Zn^{2+}). POV of the linoleic acid as a control was about 150 and POV of the linoleic acid added with Fe^{2+} and Zn^{2+} were about 260 and 210, respectively. However, the linoleic acid group added with EA extract and metal ions (Fe^{2+} and Zn^{2+}) showed approximately about 20-25. Accordingly, in the presence of metal ions, EA extract showed strong antioxidative activity. Fig. 5 and 6 shows the period course in antioxidative effect of EA extract added with

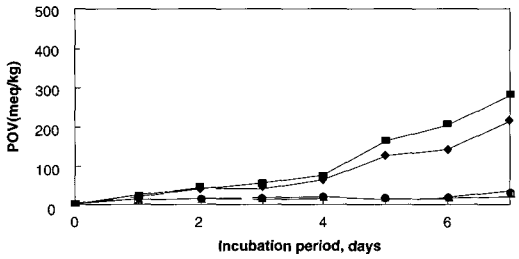

Fig. 6. Period-course in antioxidative effect *Euonymus alatus* (Thunb.) Sieb (EA) zinc ion-catalysed peroxidation of linoleic acid.
Each amount of EA and Zn^{2+}(2ppm) were incubated with linoleic acid at 50℃. linoleic acid(LA) alone, - ◆ - ; LA+Zn^{2+}, - ■ - ; LA+Zn^{2+} Euon. S 5mg, - ▲ - ; LA+Zn^{2+} Euon. S 10mg, - ● - Euon. S : EA

metal ions. The antioxidative activity of the linoleic acid added with EA extract and metal ions (Fe^{2+} and Zn^{2+}) were stronger than that of the linoleic acid added with metal ions (Fe^{2+} and Zn^{2+}).

DISCUSSION

The present study was done to investigate the effects of EA on cultured hepatocytecell system and lipid peroxidation in Aβ treatment conditions. Cell cytotoxicity was significantly enhanced by addition of increasing concentrations of Aβ. Pretreatment of EA attenuated in cell cytotoxicity enhanced by increasing concentrations of Aβ. MDA level induced by Aβ treatment was significantly increased and the level was slightly reduced by pretreatment of EA. These results of cell cytotoxicity and MDA level by Aβ treatment are in agreement with those of Glascott *et al.* [35].

The present study showed that Aβ strongly increased MDA level and the level was enhanced by addition of increasing concentrations of Aβ or by time-related exposure to Aβ.

In addition, lipid peroxidation was prevented or greatly reduced by addition of antioxidants (Vit E, Vit C, DPPD or deferoxamine)[15]. For example, addition of antioxidants in cell culture medium significantly reduced cell cytotoxicity and content of intracellular oxidants.

Zhang *et al*[39] reported that salvia miltiorrhiza Bung showed strong antioxidative activity comparable to that of BHA and BHT. Su *et al*[40] also indicated that the *Osbeckia chinensis* extract was similar to the antioxida tive activity of BHA.

In summary, it was shown that Aβ is not only potent lipid peroxide inducer, but also cause protection of neurodegeneration induced by Aβ. Thus, It can be suggested that the activation of antioxidative enzymes may be related to the inhibition of lipid peroxidative reactions. We cannot fully explain the effects of EA at present; however, the ability of EA to reduce cell cytotoxicity and MDA level induced by Aβ suggest that EA may be a protective agent against free radical generating compounds such as Aβ.

SUMMARY

This study was carried out to investigate the physiological activityof the water extract from EA. The present study was done to investigate the effects of EA on cultured hepatocyte cell system and lipid peroxidation in Aβ treatment conditions. Pretreatment of EA attenuated in cell cytotoxicity enhanced by increasing concentrations of Aβ. MDA level induced by Aβ treatment was significantly increased and the level was slightly reduced by pretreatment of EA. The ability of EA to reduce cell death and MDA level induced by Aβ suggest that EA may be a protective agent against free radical generating compounds such as Aβ. EA exhibited antioxidative activity at all concentration tested.The extract was as good as antioxidative activity of the synthetic antioxidants, butylated hydroxytoluene and ascorbic acid. Furthermore, this was superior to that

of natural antioxidant, α-tocopherol. In the presence of heavy metal ions (Fe^{2+}, Zn^{2+}), EA showed strong antioxidative activity. The extracts showed about 30-75% in the nitrite scavenging effect under pH 1.2 and 37℃ for 1 hr. There was significant difference among concentration of extracts.

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