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Effects of Ethanol Extract of *Ligularia fischeri* Leaves on Freund's Complete Adjuvant-Induced Model of Chronic Arthritis in Mice

Eun-Mi Choi

Department of Food & Nutrition, Kyung Hee University, Seoul 130-701, Korea

Abstract The aim of this study was to investigate the anti-inflammatory and anti-oxidant activity of *Ligularia fischeri* leaf extract on adjuvant induced arthritis in experimental mice. The oral administration of the *L. fischeri* leaf extract (LF), at doses of 100 and 200 mg/kg body weight once a day for 3 weeks, significantly reduced hindpaw swelling and the production of inflammatory cytokines (tumor necrosis factor(TNF)-α, interleukin(IL)-1β, and IL-6). Treatment with LF (100 mg/kg) also decreased the serum levels of triglyceride and low density lipoprotein(LDL)-cholesterol, and increased high density lipoprotein(HDL)-cholesterol contents compared with those of a control group. The induction of arthritis significantly increased oxidized proteins such as protein carbonyl, advanced oxidation protein products, and advanced glycation end-products in the lung, heart, and brain. Treatment with LF for 3 weeks reduced the levels of oxidized proteins. These results suggest that *L. fischeri* extract might be beneficial in the treatment of chronic inflammatory disorders.

Keywords: *Ligularia fischeri*, inflammation, Freund's complete adjuvant

Introduction

Inflammatory diseases are very common throughout the world. However, no substantial progress has been made in achieving a permanent cure. Arthritis-related joint problems include edema, inflammation, and damage to the cartilage and surrounding structures. Particularly, these chronic insults affect the sensory nerves innervating the arthritic joint (1). A Freund's complete adjuvant (FCA) injection results in localized monoarthritis, enabling study of arthritic lesions without the complicating factors of poor animal mobility, altered weight gain, and systemic disease. Arthritis-related syndrome is characterized by the development of chronic joint inflammation, with cell proliferation, synovium enlargement, pannus formation, and destruction of joint cartilage (2). The mouse model of adjuvant-induced arthritis has been used for many years for evaluation of anti-arthritic/anti-inflammatory agents, and has been well characterized (3). It has also been useful in the development of newer therapeutic agents (4).

Persistent local and systemic elevations of inflammatory cytokines promote lipolysis, and the systemic release of free fatty acids contributes to the dyslipidemia observed in rheumatoid arthritis (RA). Dyslipidemic patterns in RA are clearly pro-oxidative, and evidence that cytokines can directly promote the oxidative modification of low density lipoprotein (LDL) (5), and perhaps stimulate superoxide secretion from monocytes and endothelial cells, suggest high oxidized lipid levels in RA. Systemic inflammatory response in RA is central to accelerated atherogenesis due to the accentuation of established and novel risk factor pathways by the inflammatory response. Moreover, cytokines can stimulate adipocyte lipolysis, which leads to the increased release of free fatty acids (FFAs) from peripheral tissues (6).

*Corresponding author: Tel: 82-2-961-0709; Fax: 82-2-968-0260
E-mail: cheunmi@hanmail.net
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The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and the reappearance of symptoms after discontinuation. Therefore, the screening and development of agents for their anti-inflammatory activities is still in progress, with much hope being ascribed to indigenous medicinal plants (7, 8). There is a growing interest in the pharmacological evaluation of various plants used in traditional systems of medicine. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, although relatively little knowledge about their mode of action is available. Thus the present investigation was carried out to evaluate the anti-inflammatory potential of *Ligularia fischeri* leaves. *L. fischeri* (Ledebour) Turcz. var. *spiciformis* is mainly distributed in damp shady regions besides brooks and sloping fields in the eastern part of Korea. The leaves of this plant have been used to treat jaundice, scarlet-fever, rheumatoidal arthritis, and hepatic diseases (9). Monocyclosqualene, spiciformisin a and b from *L. fischeri* leaf extract (LF) were isolated (10). Pretreatment with a methanolic extract of *L. fischeri* inhibited hepatotoxicities caused by CCl_4, D-galactosamine (GalN), alpha-naphthylisothiocyanate (ANIT), and DL-ethionine in rats (11). Park *et al.* (12) reported on the isolation of a eudesmane-type sesquiterpene, (+)-intermedeol and 6-oxoeremophilenolide from the leaves of *L. fischeri* var. *spiciformis*. Moreover, intermedeol isolated from the leaves of *L. fischeri* var. *spiciformis* was reported to induce the differentiation of leukemia HL-60 cells (13). In the present study, we investigated the effects of ethanolic extract of LF, administered orally on a daily basis for 3 weeks, on inflammatory reactions in a mouse experimental model of arthritis. Lipid profiles in the serum and oxidant status in the organs were also studied.

Materials and Methods

Animal Male ICR mice (6 weeks old) were purchased

from Jungang Lab Animal Inc (Korea). These animals were maintained under constant temperature (24±2°C), with a 12hr light-dark cycle, relative humidity of 40-70%, and allowed food and water *ad libitum*. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy of Science, Bethesda, MD, USA).

Preparation of extract The dried leaves of *L. fischeri* (Ledebour) Turcz. var. *spiciformis* were collected at Kangwondo, Korea, in 2002, and identified by Dr. KT Lee, Dept. of Pharmacy, Kyunghee Univ. (Seoul, Korea). A voucher specimen (No 200202) was deposited at our institute. The dried leaves were extracted three times with 70% ethanol at room temperatures for 3 days each and the combined extracts were concentrated *in vacuo* and then freeze dried (yield: 21% w/w). Test samples were prepared by suspending them in 0.2% sodium carboxymethyl cellulose (CMC-Na) solution immediately before the start of the experiments. LF (100 and 200 mg/kg) was administered to mice orally. Control experiments were performed with the 0.2% CMC-Na. Indomethacin (10 mg/kg) was used as positive control.

Induction of arthritis by Freund's complete adjuvant (FCA) Mice were randomly divided into five groups (n =7): a normal group, a negative control group injected with FCA along with oral administration of vehicle, a positive control group injected with FCA along with oral administration of indomethacin (10 mg/kg), and experimental groups injected with FCA along with oral administration of LF (100 or 200 mg/kg) daily for 3 weeks. The body weights were measured before injection of the adjuvant (day 0). Each mouse was then injected with 0.1 mL of Freund's complete adjuvant (1 mg/mL in mineral oil; Sigma, St. Louis, MO, USA) in the right posterior plantar region (14). The pad thickness of the hind paws was measured with a Dial Thickness Gauge (Mitutoyo, Japan) before injection and at day 21 after the FCA injection, and the difference in thickness was calculated. The degree of foot-pad swelling was expressed as an increase in foot-pad thickness (mm). On day 22, after fasting overnight and an adjuvant injection, the body weights of the mice were measured before sacrifice.

Plasma and tissue homogenate preparation Twenty-two days after the initial injection, animals were sacrificed by decapitation. Blood was collected by heart puncture and placed in heparinized tubes. The blood was then centrifuged at 3,000×g for 15min, and plasma was aliquoted for the different determinations. Isolated tissues were removed and prepared as described previously (15). The chilled exsanguinated tissues were then cut into approximately 50- to 100-mg portions on ice and stored separately at -70°C in plastic vials. Homogenate from these samples was prepared after the addition of 1.0 mL phosphate buffer per 100 mg of tissue as described previously (15). Protein preparations were measured by the Bio-Rad Bradford protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Measurement of cytokines in plasma Plasma cytokine levels were determined at room temperature using ELISA kits for murine tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Determination of triglyceride and cholesterol levels in plasma The concentrations of triglyceride (TG), total cholesterol and high density lipoprotion (HDL)-cholesterol in the plasma were determined enzymatically using commercially available kit reagents (Boehringer, Mannheim, Germany). The low density lipoprotein (LDL)-cholesterol level was calculated using the Friedewald formula:

LDL cholesterol = total cholesterol - HDL cholesterol - triglyceride / 5 (16).

Protein carbonyl (PCO) assay Protein carbonyl contents were measured using a modified 2,4-dinitrophenylhydrazine (DNPH) based procedure (17). Aliquots of protein (1-4 mg/assay) were precipitated with 10 volumes of HCl-acetone (3:100) (v/v), and then washed with 5 mL of HCl-acetone to remove chromophores. The protein pellets so obtained were washed by vortexing in 5 mL 10% trichloroaceticacid (TCA) and supernatants obtained (at 800×g, 20 min) were discarded. The protein pellets were then resuspended in 500 mL of phosphate buffer, 500 mL of 10 mM DNPH (in 2M HCl) was then added, and the mix was vortexed every 5 min for 0.25 hr at room temperature. Protein blanks were prepared by adding 500 mL of 2 M HCl instead of DNPH to assay tubes containing protein samples. After mixing, 500 μL of 30% TCA was added to each tube, and samples were vortexed and placed on ice for 10 min. Following centrifugation (20 min at 800×g), supernatants were discarded and pellets were thoroughly washed with 5 mL 20% TCA followed by 3×5 mL ethanol-ethylacetate (1:1) (v/v) washes to remove any unreacted DNPH. Pellets were solubilized in 1 mL of 6M guanidine hydrochloride and 20 mM potassium dihydrogen phosphate (pH 2.3). Protein carbonyl contents were calculated by absorbance at 380 nm using an absorption coefficient (e) of 22,000 M^{-1} cm^{-1} (18).

Determination of advanced oxidation protein product (AOPP) levels Spectrophotometric determination of AOPP levels was performed by modification of Witko's method (19). Samples were prepared in the following way: Two hundred microliters of supernatant was diluted 1:3 in phosphate buffered saline (PBS), 100 μL of 1.16 mol/L potassium iodide (KI, Sigma) was then added to each tube, followed two minutes later by 200 μL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 1200 μL of PBS, 100 μL of KI, and 200 μL of acetic acid. Concentrations of AOPP were calculated by using the extinction coefficient of 26 mM^{-1} cm^{-1}.

Advanced glycation end-product (AGE) assays AGEs levels were determined spectrofluorometrically as described

by Kalousava *et al.* (20). Samples were diluted 1:50 with PBS (pH 7.4) and fluorescence intensities were recorded at 440 nm using excitation at 350 nm. Fluorescence intensities are expressed in arbitrary units as AU/mg protein.

Statistical analysis The results are expressed as the means ± SEM (n=5). Statistical significance was determined by an analysis of variance and subsequent Duncan's multiple range test ($p<0.05$). The analysis was performed using the SAS statistical software.

Results and Discussion

Body weight and organ weight of mice injected with FCA For the assessment of chronic inflammation, arthritis was induced by a FCA injection, with the LF administered orally at doses of either 100 or 200 mg/kg daily for 3 weeks. The body weights of the mice are given in Table 1. Over the study period, the body weight of normal mice steadily increased. The body weight of the adjuvant-injected mice also increased, but less than that of the normal mice. The weights of liver and spleen of the FCA-injected mice were significantly increased compared with the normal group. However, no difference was observed in the weights of the organs belonging either to the normal mice or to LF-treated mice.

FCA-induced paw edema FCA injection resulted in a significant increase in paw thickness, with a doubling of paw edema compared with the normal group after 3 weeks of monitoring. The results in Fig. 1 show that LF caused significant ($p<0.05$) inhibition of paw edema compared to the control group, with aninhibitory potency similar to that of indomethacin (10 mg/kg). Gauldie *et al.* (1) reported that seven days of treatment with either indomethacin or prednisolone caused a significant decrease in joint inflammation and associated hyperalgesia. In the case of natural products, the anti-inflammatory effects of various edible plants have been reported (7, 21). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, although relatively little knowledge is available on their mode of action.

TNF-α, IL-1β, and IL-6 levels in plasma of mice injected with FCA Typically, there is a sequential release of several inflammation-associated cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. The levels of pro-inflammatory cytokines are shown in Fig. 2. When compared with the baseline levels measured in normal mice, the FCA treatment induced pronounced changes in the plasma levels of pro-inflammatory cytokines. LF treated mice showed a significant ($p<0.05$) decrease in the levels of TNF-α, IL-1β, and IL-6 when compared to arthritic mice. The production of cytokines by leukocytes and other body cells facilitates intercellular signalling during the activation of innate and specific immunity. These cytokines regulate a wide array of physio-logical and pathophysiological processes, including the initiation and coordination of immune, acute phase and inflammatory responses, and as a consequence play important roles in human health and disease (22). Under resting conditions, serum cytokines are kept at very low levels by an intricate network of co-stimulatory and feedback loops (23). However, the concentration and/or activity of many cytokines increases dramatically in response to stressful or pathophysiological conditions (24). A growing number of reports have demonstrated that indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs) directly modulate cytokine production, both *in vivo* and *ex vivo* (25), altering the activities of certain transcription factors, including nuclear factor (NF)-κB (26).

Table 1. Body weight and organ weight of mice

Group	BW (g)	Liver (g)	Kidney (g)	Spleen (g)
Normal	30.4 ± 1.10^{a}	1.15 ± 0.08^{c}	0.37 ± 0.015^{bc}	0.12 ± 0.01^{bc}
Control	27.6 ± 1.10^{b}	1.81 ± 0.02^{a}	0.41 ± 0.02^{ab}	0.24 ± 0.04^{a}
Indo.	25.5 ± 0.35^{b}	1.65 ± 0.02^{b}	0.44 ± 0.02^{a}	0.16 ± 0.01^{b}
LF100	25.8 ± 0.74^{b}	1.04 ± 0.06^{c}	0.33 ± 0.02^{cd}	0.10 ± 0.01^{bc}
LF200	27 ± 0.50^{b}	1.01 ± 0.03^{c}	0.31 ± 0.01^{d}	0.09 ± 0.01^{c}

Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo.: arthritic animals treated with indomethacin (10 mg/kg), LF100: arthritic animals treated with *Ligularia fischeri* extract (100 mg/kg), LF200: arthritic animals treated with *L. fischeri* extract (200 mg/kg). Groups with different letters in the same column are significantly different from each other, $p<0.05$.

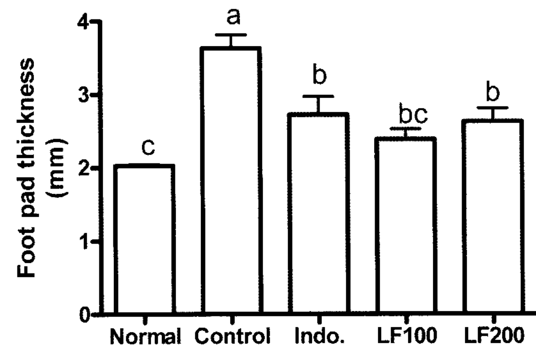

Fig. 1. Effects of *Ligularia fischeri* extract on the FCA-induced paw edema. Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo.: arthritic animals treated with indomethacin (10 mg/kg), LF100: arthritic animals treated with *L. fischeri* extract (100 mg/kg), LF200: arthritic animals treated with *L. fischeri* extract (200 mg/kg). Foot pad thickness was measured at day 22. Results are expressed as mean±SEM (n=7). Bars without a common letter differ, $p<0.05$.

Lipid profiles in plasma of mice injected with FCA Table 2 shows the results of the effects of LF on the serum lipid profiles in mice following its administration at 100 and 200 mg/kg body weight for 3 weeks. The TGs and total cholesterol contents were dose-dependently reduced in the LF-treated group when compared to arthritic mice. Moreover, a daily intake of LF (100 mg/kg body weight) for 3 weeks significantly increased HDL-cholesterol and decreased LDL-cholesterol compared with the FCA-treated control group, effects which may be beneficial in the prevention of ischemic heart disease. Lipoproteins are macromolecules of lipids and proteins that transport lipids,

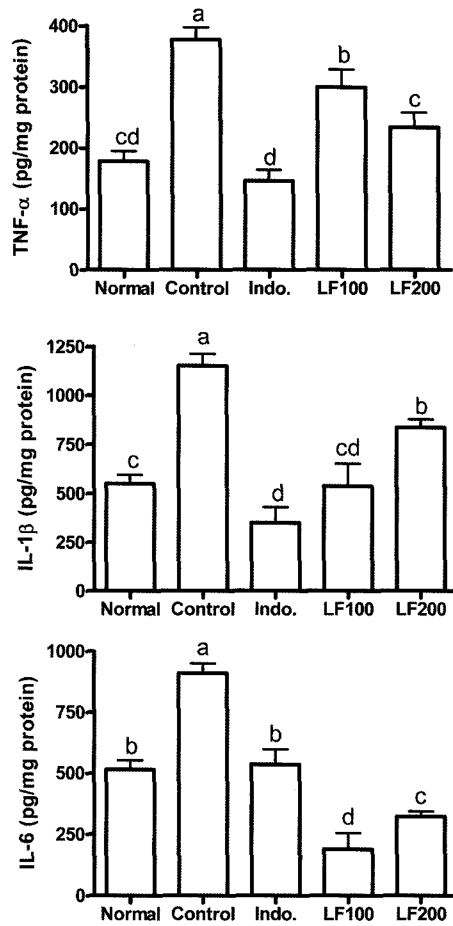

Fig. 2. Effects of *Ligularia fischeri* extract on the TNF-α, IL-1β, and IL-6 levels in plasma of mice injected with FCA. Normal: non-immunized animals, Control: arthritic animals not treated with sample, Indo.: arthritic animals treated with indomethacin (10 mg/kg), LF100: arthritic animals treated with *L. fischeri* extract (100 mg/kg), LF200: arthritic animals treated with *L. fischeri* extract (200 mg/kg). Cytokines were measured from the serum obtained at day 22. Results are expressed as mean±SEM (n =7). Bars without a common letter differ, $p<0.05$.

including cholesterol and triglycerides, through the vascular and extravascular bodily fluids, and are involved in a variety of processes, such as immune reactions, coagulation, and tissue repair. An increase in the HDL-cholesterol, and reductions in TG, total-cholesterol, and the LDL-cholesterol, is considered to minimize the potential for coronary artery disease. Numerous epidemiological studies have associated HDL with an inverse risk for coronary artery disease. This protective effect of HDL may be due, in part, to an inhibition of the oxidative modification of LDL (27). A number of studies have confirmed that HDL attenuates the cytokine-induced expression of adhesion molecules in cultured endothelial cells (28). Because TNF-α and IL-1β increase the expression of endothelial leukocyte adhesion molecules due to activation of the transcription factor NF-κB, it is hypothesized that HDL inhibits the activation of NF-κB. HDL also suppresses caspases required for apoptosis which are activated by TNF-α, and appears to modulate the permeability of the endothelium to LDL. Large doses of HDL administered to rabbits decrease the appearance of LDL particles in the intima of the aorta (29). From the above consideration, it seems that LF may take part in stimulating lipid metabolism, with the associated characteristic physiological and biochemical properties.

Oxidized protein levels in mouse organs Changes in oxidized protein levels in the lung, heart, and brain are shown in Fig. 4. We found that PCO, AOPP, and AGE levels in these organs from the arthritic animals were higher than in those of the normal mice. LF administration for 3 weeks reduced the levels of PCO, AOPP, and AGE in the lung, heart, and brain of arthritic mice. Free radical-induced damage of side chain susceptible amino acids leads to the creation of aldehyde or keto residues, with the same or decreased number of carbon atoms. These have been defined as CO products (30). Protein CO group content is currently the most general indicator and commonly used marker of protein oxidation (31). Chemically stable oxidatively modified proteins, play a significant role in the tissue damage and inflammation perpetuating process in rheumatoid synovium.

Due to the inflammatory response, a number of oxidants such as superoxide anion or HOCl are released to the extracellular space. Also, the activation of neutrophils generates HOCl/OCl- via the release of the myeloperoxidase and hydrogen peroxide (32). It has been demonstrated that HOCl/OCl- can react rapidly with amino acids and proteins to form AOPP (33). AOPP appear as novel markers of the oxidative stress associated with RA as well as being true inflammatory mediators able to amplify neutrophil activation. In fact, the AOPP, which arise from the reaction between chlorinated oxidants and proteins, constitute a new molecular basis for

Table 2. Lipid profiles in the mouse blood

Group	TG (mg/dL)	Total cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	HDL-cholesterol (mg/dL)
Normal	145.79±11.172^{ab}	465.15±11.663^{bc}	283.85±11.045^{b}	152.10±8.8754^{ab}
Control	169.38±1.9155^{a}	516.67±31.926^{ab}	352.07±25.592^{a}	130.72±9.2703^{b}
Indo.	117.98±17.255^{c}	463.64±25.813^{bc}	291.09±17.957^{b}	148.95±3.8124^{ab}
LF100	133.99±0.5959^{bc}	545.45±2.2727^{a}	281.79±15.127^{b}	175.23±11.518^{a}
LF200	126.40±8.3423^{bc}	436.36±20.243^{c}	311.48±8.8747^{ab}	151.87±13.777^{ab}

Normal: non-immunized animals, Control; arthritic animals not treated with sample; Indo, arthritic animals treated with indomethacin (10 mg/kg); LF100; arthritic animals treated with *Ligularia fischeri* extract (100 mg/kg); LF200, arthritic animals treated with *L. fischeri* extract (200 mg/kg). Groups with different letters in the same column are significantly different from each other, $p<0.05$.

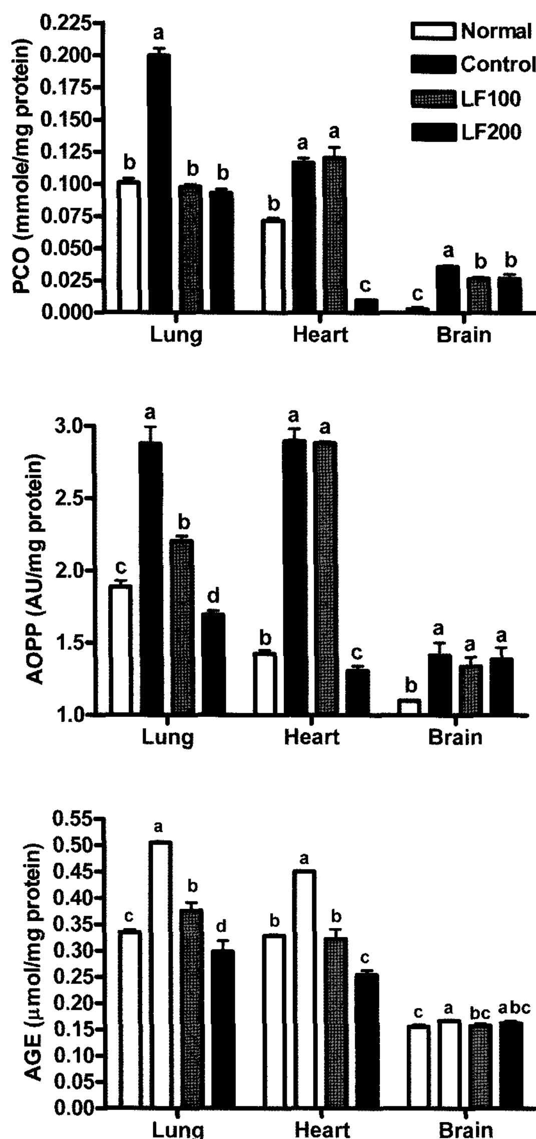

Fig. 3. Comparison of oxidized proteins in the organs of DBA/1J mice. Normal: non-immunized animals, Control, arthritic animals not treated with sample; LF100, arthritic animals treated with *Ligularia fischeri* extract (100 mg/kg); LF200, arthritic animals treated with *L. fischeri* extract (200 mg/kg). PCO, protein carbonyl; AOPP, advanced oxidation protein product; AGE, advanced glycation end-products. PCO; AOPP, and AGE levels were measured from the organs obtained at day 22. Results are expressed as mean ±SEM (n=7). Bars without a common letter differ, $p<0.05$.

the deleterious activity of oxidants, and as such might be considered as true mediators of the pro-inflammatory effects of oxidative stress (33). Witko-Sarsat *et al.* (33) investigated the relation between AOPP and chronic inflammation in patients with chronic renal failure and suggested that AOPP are more accurate markers of oxidative stress than lipid peroxidation markers. Due to their relatively early formation, greater stability and longer lifespan, AOPP have been used as markers of oxidative stress (31).

During inflammation, proteins can be damaged by nonenzymatic glyoxidation (34). Schiff bases are formed when glucose or oxidized glucose interact with surface accessible ε-amino groups. Subsequently, Amadori rearrangements occur with the formation of ketoamines and finally to the formations of advanced glycation end-products (AGEs). The generation of AGEs is an inevitable process *in vivo* and their accumulation in different tissues has been implicated in aging and in the pathogenesis of illnesses such as diabetes, atherosclerosis, Alzheimer's disease, and renal failure (35). AGE modified proteins, unlike 'normal' proteins, can activate macrophages and stimulate the secretion of IL-1, IL-6, and TNF-α (36), which accelerate bone resorption and may participate in cartilage degradation (37). From the above consideration, it seems that LF prevents the production of oxidized proteins in arthritic mice. This may be due to the presence of antioxidant polyphenolic compounds in the LF and these compounds might inhibit the formation of free radicals, which might reduce the inflammation.

In conclusion, the results of the present study clearly demonstrate that *LF* extract exerts potent anti-inflammatory actions in the FCA-induced arthritis model, and has antioxidant activity *in vivo*. Therefore, *L. fischeri* leaf extract is a promising candidate for the treatment of joint inflammation.

Acknowledgments

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References

1. Gauldie SD, McQueen DS, Clarke CJ, Chessell IP. A robust model of adjuvant-induced chronic unilateral arthritis in two mouse strains. J. Neurosci. Meth. 139: 281-291(2004)
2. Hang L, Theofilopoulus AN, Dixon FJ. A spontaneous rheumatoid arthritis-like disease in MRL/1 mice. J. Exp. Med. 155: 1690-1692 (1982)
3. Silvan AM, Abad MJ, Bermejo P, Villar AM. Adjuvant-carrageenan-induced inflammation in mice. Gen. Pharmacol. 29: 665-669 (1997)
4. Choi EM, Lee KH, Koo SJ. Effects of Ethanol Extract of Potato (Solanum tuberlosum) on Freund's Complete Adjuvant-induced Model of Chronic Arthritis in Mice. Food Sci. Biotechnol. 14: 228-232 (2005)
5. Maziere C, Auclair M, Maziere JC. Tumor necrosis factor enhances low density lipoprotein oxidative modification by monocytes and endothelial cells. FEBS Lett. 338: 43-46 (1994)
6. Chajek-Shaul T, Friedman G, Stein O. Mechanism of the hypertriglyceridemia induced by tumor necrosis factor administration to rats. Biochim. Biophys. Acta 1001: 316–324 (1989)
7. Srinivasan K, Muruganandan S, Lal J, Chandea S, Tandan SK, Prakash VR. Evaluation of anti-inflammatory activity of Pongamia pinnata leaves in rats. J. Ethnopharmacol. 78: 151-157 (2001)
8. Kim AJ, Yuh JS, Kim SY, Park SJ, Sung CJ. Anti-inflammatory Effect of the Ostrich Extract Combined with Korean Herbal Medicine. Food Sci. Biotechnol. 13: 472-475 (2004)
9. Choi OJ. Usage and constituents of medicinal plants. Seoul, Korea. Iweolseogak, p. 621 (1991)
10. Lee KT, Koo SJ, Jung SH, Choi J, Jung HJ, Park HJ. Structure of three new terpenoids, spiciformisins a and b, and monocyclosqualene, isolated from the herbs of *Ligularia fischeri* var. *spiciformis* and cytotoxicity. Arch. Pharm. Res. 25: 820-823 (2002)
11. Choi J, Park JK, Lee KT, Park KK, Kim WB, Lee JH, Jung HJ, Park HJ. In vivo antihepatotoxic effects of *Ligularia fischeri* var. *spiciformis* and the identification of the active component, 3,4-dicaffeoylquinic acid. J. Med. Food 8: 348-352 (2005)

12. Park HJ, Kwon SH, Yoo KO, Sohn IC, Lee KT. Sesquiterpenes from the leaves of *Ligularia fischeri* var. *spiciformis*. Planta Med. 66: 783-784 (2000)
13. Jeong SH, Koo SJ, Choi JH, Park JH, Ha JH, Park HJ, Lee KT. Intermedeol isolated from the leaves of *Ligularia fischeri* var. *spiciformis* induces the differentiation of human acute promyeocytic leukemia HL-60 cells. Planta Med. 68: 881-885 (2002)
14. Corsi MM, Fulgenzi A, Tiengo M, Pravettoni G, Gaja G, Ferrero ME. Effect of somatostatin on β-endorphin release in rat experimental chronic inflammation. Life Sci. 64: 2247-2254 (1999)
15. Hong H, Johnson P. Antioxidant enzyme activities and lipid peroxidation levels in exercised and hypertensive rat tissues. Int J Biochem. Cell Biol. 27: 923-931 (1995)
16. Tietz NW (ed.). Textbook of clinical chemistry. WB Com. PA, USA (1986)
17. Fagan JM, Sleczka BG, Sohar I. Quantification of oxidative damage to tissue proteins. Int. J. Biochemi. Cell Biol. 31: 751-757 (1999)
18. Johnson GD. Correlation of color and constitution. I. 2,4-dinitrophenylhydrozones. J. Am. Chem. Soc. 75: 2720-2723 (1953)
19. Witko V, Nguyen AT, Descamps-Latscha B. Microtiter plate assay for phagocyte-derived taurine-chloramines. J. Clin. Lab. Anal. 6: 47-53 (1992)
20. Kalousava M, Zima T, Tesar V, Lachmanova J. Advanced glycation end products and advanced oxidation protein products in hemodialyzed patients. Blood Purifieat. 20: 531-536 (2002)
21. Gupta M, Mazumdar UK, Sivakumar T, Vamsi ML, Karki SS, Sambathkumar R, Manikandan L. Evaluation of anti-inflammatory activity of chloroform extract of Bryonia laciniosa in experimental animal models. Biol. Pharm. Bull. 26: 1342-B44 (2003)
22. Theze J. The cytokine networks and immune functions. Oxford University Press. Oxford, UK p. 373 (1999)
23. Cannon JG. Inflammatory cytokines in nonpathological states. News Physiol. Sci. 15: 298-303 (2000)
24. Elenkov IJ, Chrousos GP. Stress, cytokine patterns and susceptibility to disease. Baillière's best practice & research. Clin. Endocrinol. Metab. 13: 583-595 (1999)
25. Sironi M, Gadina M, Kankova M, Riganti F, Mantovani A, Zandalasini M, Ghezzi P. Differential sensitivity of in vivo TNF and IL-6 production to modulation by anti-inflammatory drugs in mice. Int. J. Immunopharmacol. 14: 1045-1050 (1992)
26. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 265: 956-959 (1994)
27. Navab M, Imes SS, Hough GP. Monocyte transmigration induced by modification of LDL in cocultures of human aortic wall cells is due to induction of MCP 1 synthesis and is abolished by HDL. J. Clin. Invest. 88: 2039-2046 (1991)
28. Cockerill GW, Rye K, Gamble JR. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscl. Throm. Vas. 15: 1987-1994 (1995)
29. Klimov AN, Popov AV, Nagornev VA. Effect of high density lipoproteins on permeability of rabbit aorta to low density lipoproteins. Atherosclerosis 55: 217-223 (1985)
30. Jimenez I, Lissi E, Speisky H: Free radical-induced inactivation of lysozyme and carbonyl residue generation in protein are not necessarily associated. Arch. Biochem. Biophys. 381: 247-52 (2000)
31. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta 329: 23-38 (2003)
32. Lampert MB,Weiss SJ. The chlorinating potential of the human monocyte. Blood 62: 645-651 (1983)
33. Witko-Sarsat V, Friedlander M, Khoa NT, Blandin C, Nguyen AT, Canteloup S. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. Immunology 161: 2524-2532 (1998)
34. Singh, R., Barden, A., Mori, T., Beilin, L., Advanced glycation end-products: a review. Diabetologia 44: 129-146 (2001)
35. Miyata M, Iida Y, Horie K, Cai Z, Sugiyama S, Maeda K. Pathophysiology of advanced glycation end-products in renal failure. Nephrol. Dial. Transpl. 11: 27-80 (1996)
36. Takagi M, Kasayama S, Yamamoto T. Advanced glycation end products stimulate interleukin 6 production by human bone derived cells. J. Bone Miner. Res. 12: 439-46 (1997)
37. Isomaki P, Punnonen J. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann. Med. 29: 499-550 (1997)