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Anti-Inflammatory Activity of the Total Flavonoid Fraction from *Broussonetia papyrifera* in Combination with *Lonicera japonica*

Jeong Ho JIN[1], Hyun LIM[1], Soon Youl KWON[2], Kun Ho SON[2], and Hyun Pyo KIM[1,*]

[1]College of Pharmacy, Kangwon National University, Chunchon 200-701, [2]Department Food and Nutrition, Andong National University, Andong 760-749, Republic of Korea

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Abstract − To establish the anti-inflammatory activity of the total flavonoid fraction of the root barks of *Broussonetia papyrifera* (EBP) and a new formula, the ethanol extract of the root barks of *B. papyrifera* was fractionated with ethylacetate, yielding the hydrophobic prenylated flavonoid-enriched fraction. EBP and the ethanol extract of the whole *Lonicera japonica* (ELJ) plant were then mixed at a ratio of 1:1 (w/w) to give a new preparation (BL) in the hope of obtaining an optimal formula with a higher anti-inflammatory activity. Evaluation of the effects of these preparations on A23187-treated rat basophilic leukemia (RBL-1) cells revealed that EBP potently inhibited 5-lipoxygenase (5-LOX), while ELJ showed weak inhibition. Additionally, the mixture (BL) clearly showed stronger inhibitory effects against 5-LOX than either preparation alone. These preparations also inhibited cyclooxygenase-2-catalyzed PGE_2 and inducible nitric oxide (NO) synthase-catalyzed NO production by lipopolysaccharide-treated RAW 264.7 cells. When tested against arachidonic acid-induced mouse ear edema, EBP showed strong inhibitory activity at doses of 5-200 mg/kg when administered orally, but BL had obviously stronger inhibitory effects. When tested against λ-carrageenan-induced paw edema in mice, BL showed a potent and synergistic anti-inflammatory effect. In addition, in the acetic acid-induced writhing test, BL was found to have strong analgesic activity at 50-400 mg/kg. Taken together, these results indicate that each of these preparations exert anti-inflammatory activity in vitro and in vivo. In particular, BL showed stronger anti-inflammatory activity than EBP, and these anti-inflammatory effects were partially related to the inhibition of eicosanoid and NO production. BL may be useful for the treatment of human inflammatory disorders.

Keywords: *Broussonetia papyrifera*, *Lonicera japonica*, Flavonoid, Anti-inflammation, Lipoxygenase

INTRODUCTION

Broussonetia papyrifera (L.) Vent. (Moraceae) is widely distributed in China, Japan and Korea. The root bark of this plant has long been used as an anti-inflammatory, anti-bronchitis and antitussive agent in traditional Chinese medicine (Editorial Committee, 1999). Previously, the radix of *B. papyrifera* was shown to have PTP1B and tyrosinase inhibitory activities (Chen *et al*., 2002; Hwang and Lee, 2007). Recently, the 95% ethanol extracts of the radix, stems, leaves and fruits of *B. papyrifera* showed anti-nociceptive and anti-inflammatory activity in vivo, with the radix having the strongest activity (Lin *et al*., 2008). Moreover, it has been suggested that these anti-inflammatory effects are partially related to the inhibition of vascular permeability via autocrines and nitric oxide.

Several prenylated flavonoids including papyriflavonol A and broussochalcone A have been successfully isolated from the root barks of this plant material (Son *et al*., 2001). Among these, papyriflavonol A was found to be a relatively specific 5-lipoxygenase (5-LOX) inhibitor (Chi *et al*., 2001b). Moreover, broussochalcone A was found to suppress inducible nitric oxide synthase (iNOS) in macrophages (Cheng *et al*., 2001). In addition, papyriflavonol A has been shown to inhibit allergic response in vivo (Kwak *et al*., 2003b). Because 5-LOX inhibitors have the potential for use in the treatment of inflammatory disorders such as bronchial asthma and bronchitis, and because iNOS inhibitors may also be used as anti-inflammatory agents, the

*Corresponding author
Tel: +82-33-250-6915 Fax: +82-33-255-7865
E-mail: hpkim@kangwon.ac.kr

prenylated flavonoid-enriched fraction from the root barks of *B. papyrifera* (EBP) was prepared and its effects on several inflammatory models were examined in this study.

Lonicera japonica (Thunb.) (Caprifoliaceae) is a twining shrub that has long been used as an antidote and to treat urinary disorders, fever, and headache (Shougakukan, 1985). Additionally, *Lonicera japonica* has been used as an anti-inflammatory agent in Korea since ancient times and is widely used for the treatment of upper respiratory tract infections, diabetes mellitus, and rheumatoid arthritis (Lee *et al*., 1998). Various constituents have been isolated from the total plant materials, including lonicerosides and iridoids, and their anti-inflammatory activities have been evaluated (Lee *et al*., 1995; Kwak *et al*., 2003a; Qian *et al*., 2007). To develop a new anti-inflammatory herbal drug, the ethanol extract (ELJ) was examined for its in vitro and in vivo activity in animal models of inflammation. In addition, a new formulation (BL) composed of EBP and ELJ (1:1, w/w) was prepared and its anti-inflammatory activity was examined in the present investigation with the hope of obtaining a stronger anti-inflammatory effect.

MATERIALS AND METHODS

Chemicals

A23187, N-[2-cyclohexyloxy-4-nitrophenyl]methane sulfonamide (NS-398) was obtained from Biomol (Plymouth Meeting, PA). 2-Amino-5,6-dihydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT) was purchased from Tocris Cookson Ltd. (UK). Arachidonic acid (AA, 99%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), nordihydroguaiaretic acid (NDGA), prednisolone, indomethacin, aspirin and lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) were purchased from Sigma Chemical Co. (St. Louis, MO). DMEM and other cell culture reagents including FBS were products of Gibco BRL (Grand Island, NY). A protein assay kit was purchased from Bio-Rad (Hercules, CA).

Animals

Male Sprague-Dawley (SD) rats and ICR mice (4 weeks old, specific pathogen-free) were obtained from Orient-Bio Co. (Korea). Animals were fed with standard lab. chow and water *ad libitum*. The animals were maintained in the animal facility (KNU) at 20-22^{o}C under 40-60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation, KNU (KIACUC-09-0012). In addition, the ethical guidelines described in the KFDA Guide for the Care and Use of Laboratory Animals was followed throughout the experiments.

Plant materials

B. papyrifera was collected in the southern part (near Andong) of the Korean peninsula, authenticated by one of the authors, Dr. K. H. Son (Andong National University) and a voucher specimen was deposited in Andong National University. The dried root barks of *B. papyrifera* were chopped and extracted with 100% ethanol. The ethanol extract was then dried under vacuum and the resulting extract was dissolved in water. The solution was then fractionated with ethylacetate. The ethylacetate fraction was subsequently dried, and this dried fraction (EBP) was used throughout the experiment. The final yield for EBP was approximately 4.2% (w/w). Papyriflavonol A and broussochalcone A (Fig. 1) were isolated from this fraction as previously described (Son *et al*., 2001). The whole plant of *L. japonica* was purchased from a local herbal market, authenticated by Dr. K. H. Son and a voucher specimen was deposited in Andong National University. The dried plant was then chopped and extracted with 70% ethanol. The ethanol extract was then dried under vacuum and this dried extract (ELJ) was used throughout the experiment.

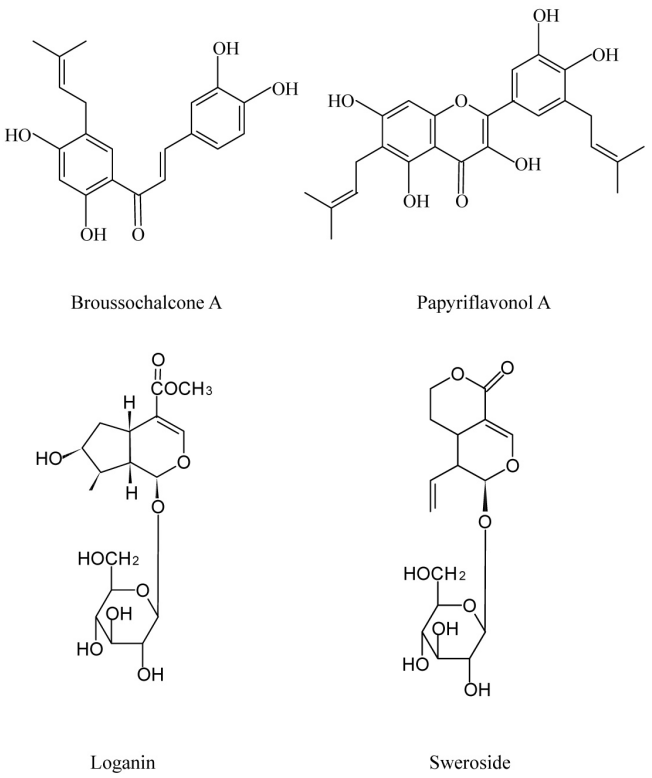

Fig. 1. Chemical structures of the major constituents of *B. papyrifera* and *L. japonica*.

The yield of ELJ was approximately 9.7% (w/w). Loganin and sweroside were isolated from this extract as previously described (Kawai *et al*., 1988). BL was a mixture of EBP and ELJ (1:1, w/w).

The contents of the prenylated flavonoids (papyriflavonol A and broussochalcone A) in EBP and the iridoids (loganin and sweroside) in ELJ were determined by HPLC analysis. EBP (10 mg) and ELJ (10 mg) were dissolved in MeOH (1 ml), filtered through a syringe filter (0.45 μm) and aliquots were injected to HPLC. The typical chromatograms are shown in Fig. 2. The contents of papyriflavonol A and broussochalcone A were found to be 0.33% and 1.03%, respectively. The contents of loganin and sweroside were found to be 4.19% and 3.30%, respectively.

Rat basophilic leukemia-1 (RBL-1) cell culture and measurement of leukotrienes (LT)

To evaluate the 5-LOX inhibitory activity, RBL-1 cells purchased from the American Type Culture Collection (ATCC, Rockville, VA) were cultured in RPMI 1640 with 10% FBS, 2 mM glutamine and 1% antibiotics under 5% CO_2 at 37°C. The cells were then incubated in 96-well plates for 2 h. The test compounds were dissolved in DMSO and diluted to appropriate concentrations with serum-free DMEM. The final concentration of DMSO was adjusted to 0.1% (v/v). The cells were then pre-incubated with the test compounds for 10 min. The cell viability was assessed using an MTT assay as previously described (Mossman, 1983). A-23187 (ionophore, 3 μM) was then added to activate the 5-LOX and the cells were incubated for 15 min as previously described, with slight modification (Tries *et al*., 2002). The media was then collected and the concentration of the 5-LOX product, cysteinyl leukotrienes ($LTC_4/D_4/E_4$), was measured using an ELISA kit (Cayman Chem, Ann Arbor, Michigan, USA) as recommended by the manufacturer.

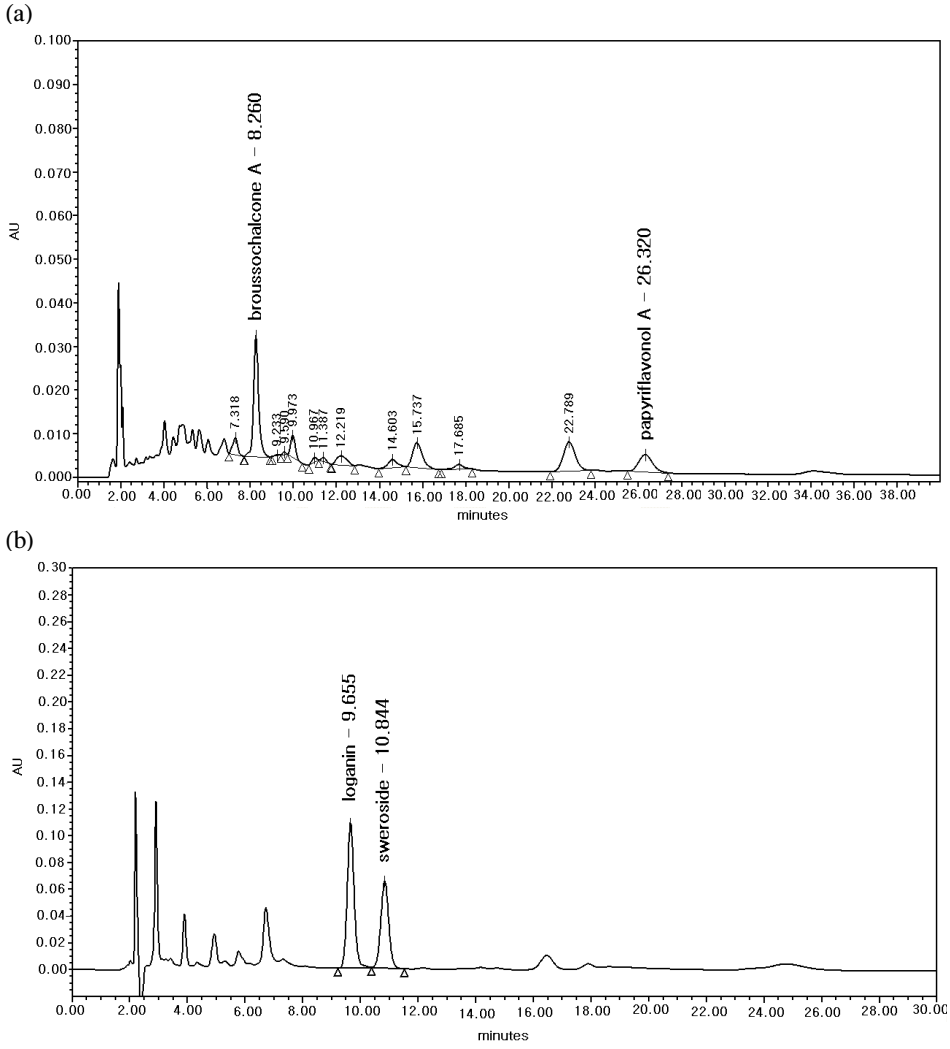

Fig. 2. Typical HPLC chromatogram of EBP and ELJ. (a) Chromatogram of EBP, (b) Chromatogram of ELJ. EBP analysis: Alliance 2695 separation module (Waters), Grom-sil C-18 column 4×150 (CROM), 5 μm, mobile phase: tetrahydrofuran: H_2O, pH 3.0 (47:53); flow rate: 0.6 ml/min, UV: 390 nm. ELJ analysis: mobile phase: acetonitrile: H_2O, pH 3.0 (13:87), UV: 239 nm.

RAW 264.7 cell culture and measurement of NO and PGE_2 concentrations

RAW 264.7 cells were obtained from ATCC and cultured in DMEM supplemented with 10% FBS and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) under 5% CO_2 at 37°C. The cells were then activated with lipopolysaccharide (LPS) as previously described (Chi *et al*., 2001a). Briefly, cells were plated in 96-well plates (2×10^5 cells/well) and then pre-incubated for 2 hr, after which the test compounds and LPS (1 μg/ml) were added and the samples were incubated for an additional 24 h unless otherwise specified. The PGE_2 concentration in the medium was then measured using an ELISA kit for PGE_2 (Cayman Chem. Co., Ann Arbor, Michigan, USA) according to the manufacturer's recommendations. To assess the NO production, the stable conversion product of NO, nitrite (NO_2^-), was measured using Griess reagent based on the optical density at 550 nm.

Western blot analysis

To measure the protein levels of cyclooxygenase-2 (COX-2) and iNOS, Western blotting technique were used (Chi *et al*., 2001a). RAW 264.7 cells were cultured in 6-well plates (5×10^6 cells/well) in the presence or absence of LPS (1 μg/ml) with/without test compounds for 16-20 h. After cell homogenates were prepared, the supernatant was obtained by centrifugation at 15,000 g for 30 min. Using Tris-glycine gels (8%), electrophoresis was carried out and bands were blotted to PVDF membranes. The membranes were incubated with COX-2 antibody (No-160116, Cayman Chem., Ann Arbor, Michigan, USA) and iNOS antibody (N32030, Transduction Lab.) and the bands were visualized by chemiluminescent reagent (Amersham, UK). Band thickness was scanned using Image J (NIH, Bethesda, Maryland, USA).

AA-induced ear edema in mice

In order to evaluate the inhibitory activity against animal model of inflammation, mouse AA-induced ear edema was employed. According to previously described procedure (Kim *et al*., 1993), 2% AA in acetone was topically applied to the ears of mice (20 μl/ear). One hour later, the ear thickness was measured using a dial thickness gauge (Mitutoyo, Japan). The test compounds dissolved in DMSO:water (1:1) were administered orally (0.1 ml/ mouse) 1 h prior to AA treatment.

λ-Carrageenan (CGN)-induced paw edema in mice

For establishing anti-inflammatory activity, mouse CGN-induced paw edema assay was also used with slight modification of Winter *et al*. (1962). Test compounds were administered orally to mice. One hour later, 1% CGN (w/v) dissolved in pyrogen-free sterile saline solution (0.05 ml/paw) was injected to right hind paw and, after 5 h, paw volume was measured using plenthysmometer (Ugo Basil, Italy). The paw volume increased from the initial non-treated paw volume was regarded as edema.

Acetic acid-induced writhing in mice

To measure the analgesic activity, 1% acetic acid (100 μl) was injected intraperitoneally to mice. At 10 min after acetic acid injection, the number of writhings were counted for 10 min. Test compounds dissolved in DMSO:water (1:1) were orally administered (0.1 ml/mouse) 1 h prior to acetic acid injection.

Statistical analysis

Experimental values were represented as arithmetic mean ± SD. One-way analysis of variance (ANOVA), followed by Dunnett's test was used to determine the statistical significance.

RESULTS

Anti-inflammatory activity in vitro

It is well known that high amounts of cysteinyl-LTs are produced by 5-LOX when RBL-1 cells were activated with

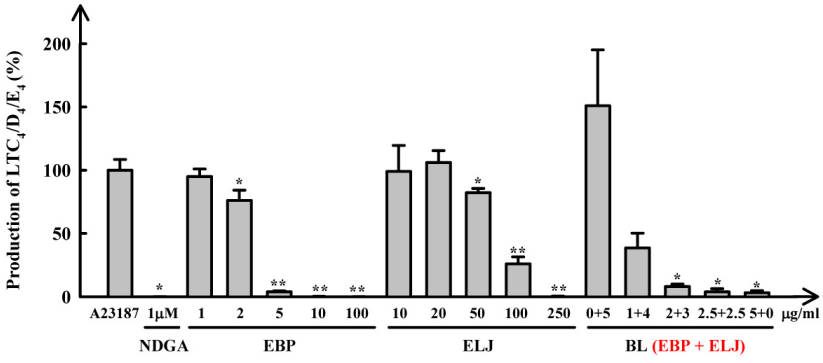

Fig. 3. Effects on 5-LOX-mediated LT production from A23187-treated RBL-1 cells. RBL-1 cells were cultured, and A23187 with/without test compounds was added to activate 5-LOX. After 15 min, cysteinyl-LTs were measured in the media. n=3, $*p<0.05$, $**p<0.01$, significantly different from the A23187-treated control group.

A23187 (calcium ionophore). In this study, RBL-1 cells produced 1,109.5 ± 93.6 pg/ml $LTC_4/D_4/E_4$ for 15 min incubation period, while the basal level was 4.2 ± 1.2 pg/ml for 15 min (n=3). Under these conditions, EBP potently and concentration-dependently inhibited 5-LOX (Fig. 3). The IC_{50} value was found to be 3.1 μg/ml. On the other hand, ELJ only weakly inhibited 5-LOX (IC_{50}=78.7 μg/ml). However, the mixture of EBP+ELJ showed potent inhibition (IC_{50} < 1 μg/ml based on the weight of EBP). When compared with the simple addition of the inhibitory activity

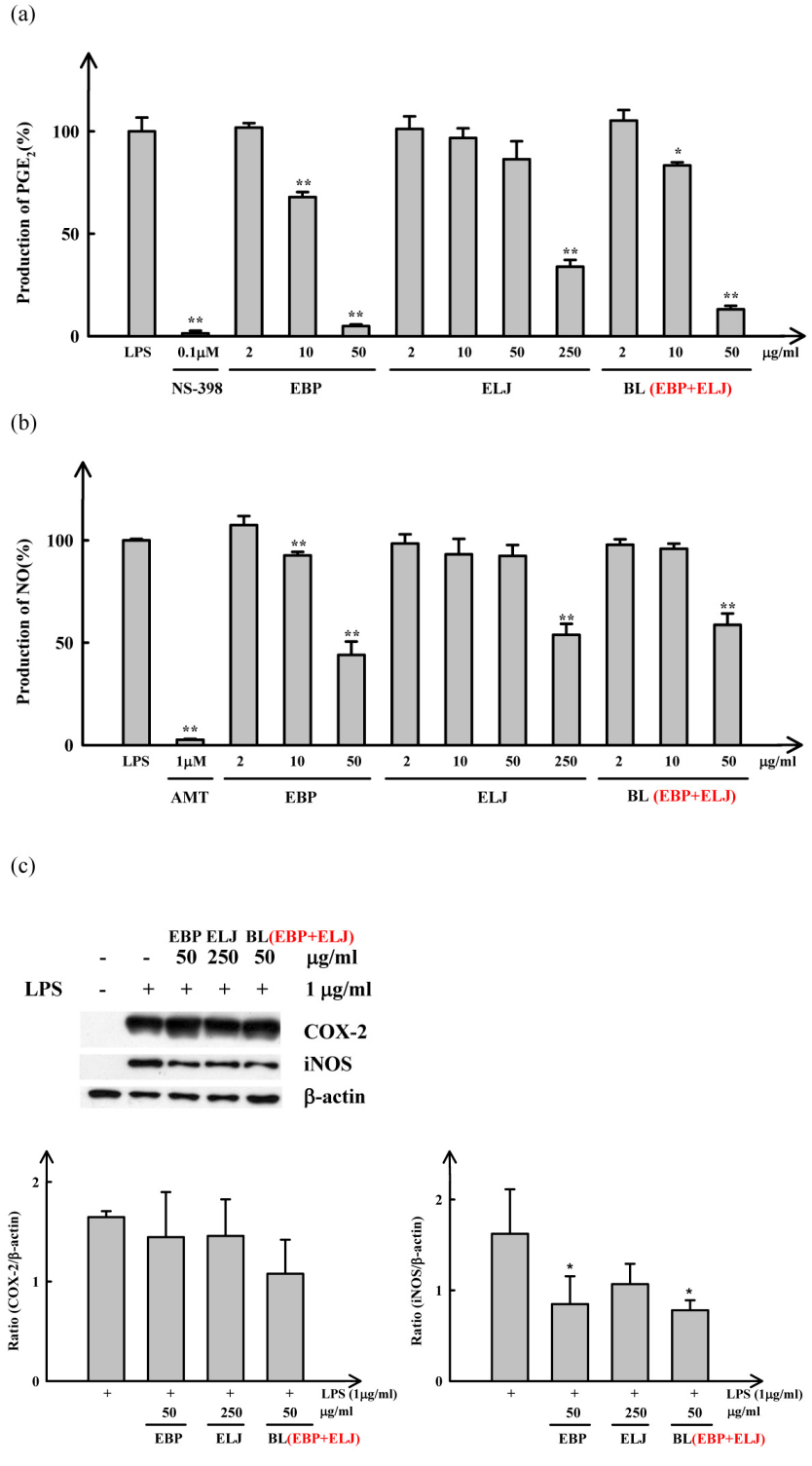

Fig. 4. Effects on PGE_2 and NO production from LPS-treated RAW 264.7 cells. (a) COX-2-catalyzed PGE_2 production, (b) iNOS-catalyzed NO production, (c) effects on COX-2 and iNOS expression (Western blot), RAW 264.7 cells were incubated with LPS in the presence or absence of test compounds. After 24 h, PGE_2 and NO concentrations were measured from the media. For the Western blot analysis, RAW 2654. 7 cells were incubated with LPS in the presence or absence of test compounds for 16-20 h. LPS-treated group produced 120.0 ± 8.0 nM PGE_2 and 32.7 ± 0.2 μM NO (Basal levels were 1.2 ± 0.4 nM and 0.8 ± 0.3 μM, respectively.). n=3, *p< 0.05, **p<0.01, significantly different from the LPS-treated control group.

of the same amounts of each material, the mixture demonstrated a stronger inhibitory effect on 5-LOX, suggesting that the mixture had a synergistic effect. Especially at 2.5 + 2.5 μg/ml of EBP + ELJ (BL, 1:1 mixture), BL showed almost complete inhibition of 5-LOX-mediated LT production (96.1%). From these results, BL among varying ratios of the mixtures was selected for further characterization. NDGA (LOX inhibitor) used as a reference compound also inhibited 5-LOX strongly (99% inhibition at 1 μM).

In addition, EBP and BL significantly inhibited COX-2-catalyzed PGE_2 and iNOS-catalyzed NO production from LPS-treated RAW 264.7 cells, a mouse macrophage cell line at concentrations of up to 50 μg/ml (Fig. 4a and 4b). The IC_{50} values of EBP and BL were 21.4 and 29.0 μg/ml for PGE_2 production, and 45.1 and 59.4 μg/ml for NO production, respectively. The effects of the preparations on the expression levels of COX-2 and iNOS were examined at concentrations showing significant inhibition of PGE_2 and NO production. As shown in Fig. 4c, EBP (50 μg/ml), ELJ (250 μg/ml) and BL (50 μg/ml) reduced iNOS induction. In contrast, EBP, ELJ and BL only weakly inhibited COX-2 expression, but not statistically significant.

Anti-inflammatory activity in vivo

For elucidating in vivo anti-inflammatory activity against animal models of inflammation, mouse AA-induced ear edema test and CGN-induced paw edema test were employed. In AA-induced ear edema, EBP and ELJ inhibited the edematic response at 50-200 mg/kg by oral administration, with EBP being more potent, as expected (Table I). In the same model, BL showed higher inhibitory activity compared to the simple addition of the percent inhibition of each extract at 100 and 400 mg/kg. Especially at 400 mg/kg, BL potently inhibited the edematic response (> 70% inhibition), which was similar to the results ob-

Table I. Inhibition of arachidonic acid (AA)-induced mouse ear edema

Compounds	Dose (mg/kg)	Ear thickness increased, mm
AA-treated	–	0.070 ± 0.010[a] (–)[b]
Indomethacin	20	0.021 ± 0.013[c] (70.0)
EBP	50	0.050 ± 0.037 (28.6)
	100	0.032 ± 0.015[c] (54.3)
	200	0.038 ± 0.023[c] (45.7)
ELJ	50	0.090 ± 0.034 (–)
	100	0.054 ± 0.017 (22.9)
	200	0.053 ± 0.025 (25.0)
BL (EBP + ELJ)	50 (25 + 25)	0.064 ± 0.029 (8.6)
	100 (50 + 50)	0.046 ± 0.015[c] (34.3)
	200 (100 + 100)	0.028 ± 0.008[c] (60.0)
	400 (200 + 200)	0.015 ± 0.013[c] (78.6)

All compounds dissolved in DMSO:water (1:1) were administered orally. Negative control group without AA treatment showed 0.003 ± 0.001 mm increase (The thickness of the untreated ears was 0.190 ± 0.011 mm). [a]n=5 (arithmetic mean ± SD), [b]% inhibition compared to the AA-treated control groupm, [c]$p < 0.05$, significantly different from the AA-treated control group.

Table II. Inhibition of carrageenan (CGN)-induced mouse paws edema

Compounds	Dose (mg/kg)	Paw volume increased, ml
CGN-treated	–	0.152 ± 0.022[a] (–)[b]
Indomethacin	20	0.116 ± 0.039 (23.7)
	100	0.084 ± 0.019[c] (44.7)
Prednisolone	20	0.076 ± 0.011[c] (50.0)
EBP	200	0.116 ± 0.045 (23.7)
ELJ	200	0.134 ± 0.047 (11.8)
BL (EBP + ELJ)	50 (25 + 25)	0.136 ± 0.030 (10.5)
	100 (50 + 50)	0.120 ± 0.047 (21.1)
	200 (100 + 100)	0.100 ± 0.020[c] (34.2)
	400 (200 + 200)	0.080 ± 0.050[c] (47.4)

All compounds dissolved in DMSO:water (1:1) were administered orally. The paw volume of negative control group without CGN treatment was 0.149 ± 0.025 ml. [a]n=5 (arithmetic mean ± SD), [b]% inhibition compared to the CGN-treated control group, [c]$p < 0.05$, significantly different from the CGN-treated control group.

Table III. Inhibition of acetic acid-induced writhings in mice

Compounds	Dose (mg/kg)	Numbers of writhings
Exp. 1		
Acetic acid-treated	–	14.5 ± 6.4[a] (–)[b]
Aspirin	100	5.7 ± 3.8[c] (60.9)
EBP	50	12.2 ± 5.2 (16.1)
	100	8.5 ± 3.6 (41.4)
	200	7.5 ± 5.2 (48.3)
ELJ	100	8.2 ± 5.1 (43.7)
	200	5.8 ± 4.4[c] (59.8)
BL (EBP + ELJ)	50 (25 + 25)	8.8 ± 5.9 (39.1)
	100 (50 + 50)	7.7 ± 4.0 (47.1)
	200 (100 + 100)	7.3 ± 5.8 (49.4)
	400 (200 + 200)	4.5 ± 3.3[c] (69.0)
Exp. 2		
Acetic acid-treated	–	17.8 ± 6.1 (–)
Aspirin	100	9.6 ± 3.2[c] (46.1)
BL (EBP + ELJ)	100 (50 + 50)	11.6 ± 6.4 (34.8)
	200 (100 + 100)	10.8 ± 5.5 (39.3)
	400 (200 + 200)	8.6 ± 4.3[c] (51.7)

All compounds dissolved in DMSO:water (1:1) were administered orally. [a]n=6 (arithmetic mean ± SD), [b]% inhibition compared to the acetic acid-treated control group, [c]$p < 0.05$, significantly different from the acetic acid-treated control group.

tained when indomethacin (20 mg/kg) was used. Against CGN-induced paw edema, BL also showed dose-dependent inhibition at 50-400 mg/kg, p.o. (Table II). BL showed 47.4% inhibition at 400 mg/kg, while EBP and ELJ showed 23.7% and 11.8% inhibition at 200 mg/kg (The sum is 35.5% inhibition at 400 mg/kg), indicating the synergistic action of BL.

When the analgesic activity was examined, BL strongly and dose-dependently inhibited acetic acid-induced writhings in mice at 50-400 mg/kg, p.o. (Table III).

DISCUSSION

The present investigation clearly demonstrated that the EBP, ELJ and a 1:1 mixture of EBP and ELJ (BL) possess anti-inflammatory and analgesic activity. Among these preparations, BL showed the strongest and synergistic inhibitory action, and its in vivo potencies were comparable to those of the currently-used potent anti-inflammatory drugs, indomethacin and prednisolone. Therefore, these results strongly suggest that BL has the potential for treating human inflammatory disorders.

Recently, several parts of *B. papyrifera* were evaluated for their analgesic and anti-inflammatory activities (Lin *et al*., 2008). They found that the ethanol extracts of the radix, leaves and fruits exerted in vivo activity at 600-2,000 mg/kg, p.o. Although these previous results also demonstrated that, among the extracts, the radix showed the most comparable activity, our preparations (EBP and BL) had much higher in vivo anti-inflammatory activities at 50-400 mg/kg. Thus, these findings may support the research rational of our study to prepare flavonoid-enriched fraction of the radix of *B. papyrifera*.

Previously, we demonstrated that the prenylated flavonoids from *B. papyrifera*, including papyriflavonol A, inhibited 5- and 12-LOX, and that these compounds were more selective for 5-LOX (Chi *et al*., 2001b). Additionally, broussochalcone A was found to down-regulate iNOS expression and inhibit respiratory bursts in neutrophils (Wang *et al*., 1997; Cheng *et al*., 2001). Based on these results, EBP and BL were prepared in the present investigation and they also potently inhibit 5-LOX. Furthermore, 5-LOX inhibitory activity of EBP was considerably enhanced by an addition of ELJ. These preparations (EBP and BL) were also found to inhibit iNOS-catalyzed NO production at least in part by iNOS down-regulation. These in vitro anti-inflammatory effects of EBP and BL were well correlated with the anti-inflammatory activities of the constituents, papyriflavonol A and broussochalcone A.

The enhancing anti-inflammatory effect of BL was also found in several in vivo experiments. Against AA-induced ear edema test, a 5-LOX inhibitor-sensitive animal model of acute inflammation (Inoue *et al*., 1988), EBP strongly inhibited edematic response, suggesting 5-LOX inhibitory action in vivo. BL clearly showed the enhancing response when compared to treatment of either EBP or ELJ alone. On CGN-induced paw edema, BL also showed the potent inhibition. The exact reason for these enhancing effects of BL is not known yet; however, it is reasonably speculated that the constituents in both extracts act synergistically. The major constituents in EBP are prenylated flavonoids and the majors of ELJ are hederagenin and iridoid derivatives (Lee *et al*., 1995; Son *et al*., 2001; Kwak *et al*., 2003a). These constituents may synergistically and cooperatively act at multiple points (5-LOX, COX-2, iNOS, etc.) in complex inflammatory pathways of in vivo experimental models tested. Lin *et al*. (2008) claimed that the major active principles of the ethanol extract of *B. papyrifera* were betulin and betulinic acid and that these compounds inhibited the vascular permeability via autocrines and nitric oxide. On the other hand, our preparation (EBP and BL) interfered with the pathways of eicosanoid and nitric oxide production. Indeed, 5-LOX products such as leukotrienes and COX products such as PGE_2 are major players that lead to the production of edematic response in AA-induced ear edema and 5 h CGN-paw edema models (Vinegar *et al*., 1969; Holsapple and Yim, 1984; Inoue *et al*., 1988; Damas *et al*., 1990). Accordingly, the strong anti-inflammatory activity of EBP and BL in these animal models of inflammation may be associated with their inhibitory effects on 5-LOX, COX and iNOS. EBP and BL showed strong activity against both models, suggesting their broad anti-inflammatory activity.

In conclusion, EBP, ELJ and BL were found to exert significant anti-inflammatory activity in vitro and in vivo. Especially, BL showed potent and enhancing effects in several animal models of inflammation. The 5-LOX, COX-2 and iNOS inhibitory actions of these preparations contributed to anti-inflammatory action in vivo. Taken together, these results suggest that EBP and BL may be safely used for the treatment of human inflammatory disorders.

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