Research Article

Anti-Inflammatory Effects and Mechanisms of Fatsia polycarpa Hayata and Its Constituents

Hsueh-Ling Cheng, Nurkholis, Shi-Yie Cheng, Shen-Da Huang, Yan-Ting Lu, Xiao-Wen Wang, Yu-Liang Liu, and Chang-Hung Chou

1 Department of Biological Science and Technology, National Pingtung University of Science and Technology, No. 1, Shuefu Road, Neipu, Pingtung 91201, Taiwan
2 Department of Agricultural Product Technology, Brawijaya University, Jalan, Veteran Malang 65145, Indonesia
3 Department of Life Sciences, National University of Kaohsiung, No. 700, Kaohsiung University Road, Nan-Tzu District, Kaohsiung 81148, Taiwan
4 Research Center for Biodiversity and Graduate Institute of Ecology and Evolutionary Biology, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan

Correspondence should be addressed to Chang-Hung Chou; choumasa@mail.cmu.edu.tw

Received 14 May 2013; Accepted 30 July 2013

Academic Editor: Jae Youl Cho

Copyright © 2013 Hsueh-Ling Cheng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

There are three species in the genus Fatsia (belonging to the Araliaceae family), that is, Fatsia polycarpa Hayata, an evergreen shrub endemic to Taiwan, Fatsia japonica, and Fatsia oligocarpa, originated from Japan and Bonin Islands, respectively [1]. F. japonica and F. polycarpa have been used as an herbal medicine in Japan and in Taiwan in treating diseases, such as coughing, ankylosing spondylitis, osteoarthritis, rheumatism, rheumatoid arthritis and tendinitis, and in blood circulation improvement [1, 2]. Nonetheless, the therapeutic activity of F. polycarpa has seldom been scientifically investigated and the bioactive constituents from these plants are not clear.

The constituents in the methanolic extract of leaves and twigs of Fatsia polycarpa were previously characterized [1]. The extract was rich in phytosterols in which brassicasterol was a major one (unpublished data). Brassicasterol is a common phytosterol present in many plants and was suggested to reduce blood cholesterol levels together with other phytosterols [3]. Triterpenoids is another type of compounds rich in the crude extract of F. polycarpa [1, 4, 5]. Seven novel structures (named fatsicarpains A–G) and two known ones (3α-hydroxyolean-11,13(18)-dien-28-oic acid (HODA) and...
(22E,24R)-Ergosta-5,22-dien-3β-ol (brassicasterol) (a)

3α-Hydroxyolean-11,13(18)-dien-28-oic acid (HODA) (b)

3α-Hydroxyolean-11-en-28,13β-olide (HOEO) (c)

3α-Hydroxyolean-9,12-dien-28-oic acid (fatsicarpain D) (d)

3α-Hydroxyolean-11-methoxy-12-en-28-oic acid (fatsicarpain F) (e)

**Figure 1: Structures of brassicasterol (a), HODA (b), HOEO (c), fatsicarpain D (d), and fatsicarpain F (e).**

3α-hydroxyolean-11-en-28,13β-olide (HOEO)) belonging to oleanane-type triterpenoids were isolated and identified from the extract [1]. Among these triterpenes, HODA, HOEO, fatsicarpain D, and fatsicarpain F are in larger amounts. Whether these molecules play roles in the medical functions of *Fatsia polycarpa* deserves to be investigated.

*Fatsia* plants exhibit pleiotropic therapeutic activities as described above. However, several of these activities, such as the treatment of ankylosing spondylitis, osteoarthritis, rheumatoid arthritis, and tendinitis, are related to anti-inflammation. Thus, in this study, the anti-inflammatory effects of the methanolic crude extract (MCE) of *F. polycarpa* and its major or feature constituents, including brassicasterol, HODA, HOEO, fatsicarpain D and fatsicarpain F (structures shown in Figure 1), were characterized.

### 2. Materials and Methods

#### 2.1. Chemicals

Specific antibodies against phosphorylated IKK-α/IKK-β, total IKK-α, total IKK-β, phosphorylated IkB-α, total IkB-α, phosphorylated JNK, total JNK, phosphorylated ERK, total ERK, phosphorylated p38, total p38, p65, and lamin B were purchased from Cell Signaling Technology (Beverly, MA, USA); antibodies against inducible nitric oxide synthase and cyclooxygenase-2 from BD Biosciences (Franklin Lakes, CA, USA); an actin-specific antibody from Chemicon (Temecula, CA, USA); all secondary antibodies from Santa Cruz Biotechnologies (Santa Cruz, CA, USA); fetal bovine serum (FBS) from Invitrogen (San Diego, CA, USA); and cell culture media, lipopolysaccharide (LPS) derived from *E. coli* 055:B5, NaF, sodium orthovanadate, sodium pyrophosphate, indomethacin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and 12-O-tetradecanoylphorbol-13 acetate (TPA) from Sigma-Aldrich (St. Louis, MO, USA) at reagent grade or cell culture grade.

#### 2.2. Isolation and Identification of MCE, Brassicasterol, HODA, HOEO, Fatsicarpain D, and Fatsicarpain F

The air-dried and powdered leaves and twigs of *F. polycarpa* (7.1 kg) were extracted with methanol (MeOH) for three days at room temperature (three times) and the combined extracts were concentrated in vacuum (under 35°C). The resulting dark green gum, that is, MCE, was suspended in H₂O and extracted sequentially with CH₂Cl₂, ethyl acetate (EtOAc), and n-butanol (n-BuOH) (saturated with H₂O). The CH₂Cl₂ extract (100 g) was subjected to column chromatography on silica gel using n-hexane-EtOAc mixtures of increasing polarity for elution to furnish 40 fractions. Fraction 20 (7.0 g) eluted with n-hexane-EtOAc (1:10) was fractionated over Sephadex LH-20 (100% acetone) to afford a major phytosterol (580 mg) that was grown by slow evaporation of the mixture CH₂Cl₂-MeOH (1:1) solution at room temperature to give a suitable colorless crystal. The chemical structure of the crystal was confirmed by comparison of its spectroscopic data with those of brassicasterol [6, 7], which was reported specifically in only one alga, an unidentified species of the order Sarcinochrysidales (Chrysophyceae).

HODA, HOEO, fatsicarpain D, and fatsicarpain F were prepared, or isolated and identified as described previously [1]. Briefly, fraction 17 (2.86 g) of the CH₂Cl₂ extract (100 g), eluted with n-hexane-EtOAc (1:4), was fractionated over...
Sephadex LH-20 (100% acetone) to afford HODA (108 mg). Fraction 20 (6.97 g), eluted with n-hexane-EtOAc (1:10), was chromatographed over silica gel, eluted in a step gradient manner with n-hexane-EtOAc-MeOH (10:1:0 to 0:0:100) to afford six subfractions. Subfraction 20–2 (3.16 g) was fractionated over Sephadex LH-20 (100% acetone) to yield a mixture (775 mg) that was subjected to RP-18 column chromatography eluting with 65% MeOH in H2O, 75% MeOH in H2O, 85% MeOH in H2O, 90% MeOH in H2O, and 100% MeOH. Five subfractions were obtained, of which subfraction 4 (258 mg) was submitted to repeated column chromatography over RP-18 using MeOH-H2O-MeCN (20:60:20 + 0.2% FA) to give a mixture (74 mg) that was further separated by RP-18 HPLC using H2O-MeCN (35:65 + 0.3% FA) to isolate fatsicarpain D (4 mg) and HOEO (40 mg). Fraction 24 (1.5 g), eluted with EtOAc-MeOH (70:1), was subjected to silica gel column chromatography (n-hexane-EtOAc-MeOH, from 10:1:0 to 0:10:1) to obtain 10 subfractions. Subfraction 24–6 (306 mg), eluted with n-hexane-EtOAc (1:3), was subsequently fractionated over Sephadex LH-20 (100% acetone) to yield a mixture that was further purified by RP-18 HPLC using MeOH-H2O-MeCN (70:25:5) to give fatsicarpain F (2 mg).

MCE was dissolved in DMSO (dimethylsulfoxide) in a concentration of 50 mg/mL as a stock solution. Brassicasterol was dissolved in 100% ethanol in a concentration of 10 mM as a stock solution. HODA, HOEO, fatsicarpain D, and fatsicarpain F were dissolved in DMSO in a concentration of 10 mM as the stock solutions.

2.3. Animal Tests. All protocols of animal tests were approved by the Committee for Animal Experiments of National Pingtung University of Science and Technology in accordance with international guidelines. Experiments were performed using groups of 8 male ICR mice (30–35 g; purchased from BioLASCO, Taipei, Taiwan) fed with a regular laboratory rodent diet and housed under a 12-hour light-dark cycle. TPA-induced ear edema of mice was performed according to previously published methods with modifications [8, 9]. In the experiment for MCE, TPA (2.5 μg/ear dissolved in 20 μL of acetone) was topically applied to the surfaces of both ears of mice, whereas in the group of control group, TPA was applied to only the right ear. Meanwhile, in the group of normal control, 20 μL of acetone was applied to both ears. Four hours later, MCE (100, 300, or 500 μg/ear) or indomethacin (500 μg/ear), both dissolved in acetone, was applied to the surface of the right ear, and acetone to the left ear, whereas for normal control and TPA control, acetone was applied to both ears. The thickness of ears was measured before and at 4 h, 16 h, and 24 h after TPA treatment using a dial thickness gauge (Peacock, Osaka, Tokyo, Japan). The experiment for HOEO was performed similarly using a different batch of mice, but each group contained 6 mice, the dosage of TPA was 3.0 μg/ear, and HOEO (300 μg/ear) or indomethacin (500 μg/ear) was applied to the ears at 1 h (instead of 4 h) after TPA treatment.

2.4. Cell Culture and Western Blot Analysis. RAW 264.7 cells (ATCC number AT TIB-71) were cultured in DMEM (Dulbecco’s modified Eagle’s medium) containing 10% fetal bovine serum. FL83B cells (ATCC number CRL-2390) were cultured at 37°C in a humidified incubator supplied with 5% CO2.

RAW 264.7 cells were seeded at a density of 1.5–2.0 × 104/35-mm plate, grown for 16 h, and treated with MCE or the natural products for 3 h. LPS was then added to the medium (final concentration 100 ng/mL) for 16 h. Subsequently, cells were washed with cold PBS (phosphate-buffered saline, pH 7.4) twice and submerged in lysis buffer (IX Cell Culture Lysis Reagent (Promega, Madison, WI, USA) containing 1 mM of phenylmethylsulfonyl fluoride, 1 μg/mL of pepstatin, 1 μg/mL of leupeptin, and 1 μg/mL of aprotinin). For the detection of phosphorylated proteins, the lysis buffer also contained 10 mM of NaF, 1 mM of sodium orthovanadate, and 10 mM of sodium pyrophosphate. Cells were scraped off the plate on ice and the suspension was centrifuged at 14,000×g for 15 min at 4°C. The supernatant was collected and the protein concentration was analyzed using the Bradford assay reagent (Bio-Rad, Hercules, CA, USA). Equal amounts of proteins were sampled and subjected to electrophoresis and Western blotting as described previously [11]. The resulting bands were scanned by a gel documentation and image analysis system (Syngene, Frederick, MD, USA), and band intensities were analyzed using the program supplied with the system. Alternatively, after hybridization with a horseradish-peroxidase-(HRP-) conjugated secondary antibody, followed by incubation with “Western HRP substrate” (Luminata Classico, Millipore, Temecula, CA, USA), immunoreactive bands were detected using a biospectrum imaging system (UVP Biospectrum, UVP, LLC, Upland, CA, USA) and band intensities were analyzed by the supplied software.

2.5. Cytotoxicity Assay. Cytotoxicity was assayed using MTT. RAW 264.7 (3×105 cell/well) or FL83B cells (2×105 cell/well) were cultured in a 96-well plate. Cells were washed twice with PBS, treated with the compounds in the indicated concentrations in serum-free medium for 24 h (HODA) or 19 h (other compounds). Cells were then washed twice with PBS and incubated in 30 μL of 5 mg/mL MTT solution at 37°C in the dark for 1 h. Subsequently, DMSO (100 μL) was added to each well for 10 minutes, withdrawn to another 96-well plate, and the absorbance at 570 nm determined using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Experiments were performed in triplicate. Average ratio ± standard deviation of viable cells was calculated against the control (cells treated by the solvent).

2.6. Semiquantitative RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction). Total cellular RNA was purified using Trizol (Invitrogen) as per the manufacturer’s instruction. The quality of RNA was checked by agarose gel electrophoresis. Sequences of the forward and reverse PCR primers of interleukin-1β (IL-1β) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as published previously [12]. RT-PCR was performed using the SuperScript One-Step
then subjected to PCR amplification in a thermal cycler by RT-PCR System kit (Invitrogen) with the following formulation: 2 μg of total RNA, 0.4 μM of forward primer and reverse primer, respectively, 12.5 μL of 2X reaction mix, and 1 μL of SuperScript RT/Platinum Taq. The final volume was adjusted to 25 μL with sterilized distilled water. It was then subjected to PCR amplification in a thermal cycler by the following program: 48 °C, 45 min (for cDNA synthesis); one cycle of 95 °C, 2 min, 35 cycles of 95 °C, 45 s, 60 °C, 45 s, and 72 °C, 1 min, and one cycle of 72 °C, 5 min. The RT-PCR products were separated electrophoretically on 1% agarose gels and visualized by ethidium bromide staining. The resulting images were recorded and band intensities were quantified as described previously [12]. Meanwhile, the RT-PCR products were purified and the sequences confirmed by DNA sequencing (Mission Biotech, Taipei, Taiwan).

2.7. Analysis of Nitric Oxide (NO) and Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}). The concentration of NO in the culture medium of cells was analyzed using a kit of Griess reagent according to the manufacturer’s protocol (Promega). That of PGE\textsubscript{2} was analyzed using an ELISA kit as per the protocol suggested by the manufacturer (R&D Systems, Minneapolis, MN, USA).

2.8. Protein Extraction from the Nuclear and Cytoplasmic Fractions of Cells. RAW 264.7 cells were seeded on 10 mm dishes until 80% confluence and treated with MCE or HOEO for 3h, followed by treating with LPS for 2h. Cells were harvested, washed with cold PBS twice, and centrifuged at 500 × g for 5 min. Nuclear proteins and cytoplasmic proteins of the cell pellet were then separated and extracted using a nuclear extract kit as per the manufacturer’s instruction (Millipore, Temecula, CA, USA). Protein concentrations in the extracts were quantified using Bradford assay reagent before being subjected to Western blot analysis.

2.9. Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe’s post hoc test and significance was considered when $P < 0.05$ and $F > 3.5546$.

3. Results

3.1. In Vivo Efficacy of MCE in TPA-Induced Mouse Ear Edema. To assess the anti-inflammatory effect of MCE in vivo, a topical inflammation model, TPA-induced mouse ear edema, was used. Both ears of mice were stimulated with TPA to induce inflammation. The right ear was treated with MCE (100, 300, or 500 μg/ear) or indomethacin (500 μg/ear, as a reference drug) [13, 14] at 4h after TPA stimulation, whereas the left ear remained unmedicated for comparison. As shown in Figure 2(a), ear edema was obvious at 4h, 16h, and 24h after TPA application without any medication (TPA control), while all three dosages of MCE significantly reduced the edema of the right ear at 16h and 24h in a time-dependent manner and in an extent comparable to the effect of indomethacin. While comparing the thickness of the right ear and that of the unmedicated left ear at 24h after TPA stimulation, the data also revealed that all three dosages of MCE effectively ameliorated the TPA-induced edema (Figure 2(b)). These results confirmed the anti-inflammatory activity of MCE in vivo. Accordingly, the anti-inflammatory effects of MCE and its characteristic components brassicasterol, HODA, HOEO, fatsicarpain D, and fatsicarpain F were further investigated.
Figure 3: Cytotoxicity assays of MCE (a), brassicasterol (b), HOEO (c), HODA (d), fatsicarpain D (e), and fatsicarpain F (f). RAW 264.7 cells and FL83B cells were treated with the selected compound in the indicated concentrations for 24 h (HODA) or 19 h (other compounds). Relative cell viability was determined against the control (0 $\mu$g/mL or 0 $\mu$M of the compound). Data represent the mean ± SD of triplicate. *$P < 0.05$ against the control.
3.2. The Anti-Inflammatory Effects of MCE and Its Feature Constituents on Macrophage Cells. The cytotoxicity of MCE and the compounds was evaluated in RAW 264.7 macrophage cell line and in FL83B normal liver cell line. MCE did not display obvious cytotoxicity in FL83B cells in concentrations of 10–400 µg/mL but did result in reduced cell survival in 400 µg/mL in RAW 264.7 cells (Figure 3(a)). Brassicasterol did not show obvious cytotoxicity in both cell lines in 10–100 µM (Figure 3(b)). HOEO did not have a significant toxic effect in FL83B cells in concentrations of 10–100 µM but did result in about 20% inhibition for the growth of RAW 264.7 cells in 100 µM (Figure 3(c)). HODA, fatsicarpain D, and fatsicarpain F on the other hand, obviously suppressed the growth of RAW 264.7 cells in concentrations of 50 µM or ≥20 µM, but they were not toxic to FL83B cells (Figures 3(d), 3(e), and 3(f), resp.). Therefore, the anti-inflammatory effects of MCE, brassicasterol, and HOEO were further characterized using RAW 264.7 cells as a model. Those of HODA, fatsicarpain D, and fatsicarpain F will be analyzed using other appropriate models.

Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are the major enzymes involved in the synthesis of the inflammatory mediators, NO, and PGE₂, respectively, and are common markers of inflammation.
Thus, the dosage-dependent effects of MCE and brassicasterol on the inhibition of LPS-induced expression of iNOS and COX-2 in RAW 264.7 cells were characterized. These enzymes were highly expressed in LPS-stimulated cells (Figures 4(a) and 4(b), lane 2 versus lane 1). MCE obviously inhibited the LPS-elevated expression of iNOS in concentrations of 50–200 μg/mL and that of COX-2 in concentrations of 100–200 μg/mL (Figure 4(a), lanes 4–6 versus lane 2). Accordingly, 100 μg/mL of MCE was considered as an effective dosage for the following experiments. In contrast, brassicasterol had no obvious inhibitory effect on the expression of iNOS and COX-2 at dosages of 10–100 μM (Figure 4(b), lanes 3–6 versus lane 2). Thus, brassicasterol was not considered as a potential anti-inflammatory agent. In Figure 4(c), HOEO markedly suppressed LPS-induced expression of iNOS and COX-2 in concentrations of 5–30 μM (lanes 4–7 versus lane 2). Thus, 20 μM of HOEO was used for the subsequent experiments.

Whether MCE and HOEO could suppress LPS-stimulated NO and PGE\textsubscript{2} secretion was also examined. Figure 4(d) showed that LPS-induced NO secretion (Group 2) was significantly reduced by the treatment of MCE or HOEO (Groups 3 and 4). Similarly, MCE or HOEO obviously inhibited LPS-induced production of PGE\textsubscript{2} in RAW 264.7 cells (Figure 4(e), Groups 3 and 4 versus Group 2). Moreover, both MCE and HOEO suppressed LPS-elevated expression of the mRNA of IL-1β (Figure 4(f), lanes 3 and 4 versus lane 2). These results further confirmed the anti-inflammatory activities of MCE and HOEO in LPS-stimulated macrophages.

Thus, the anti-inflammatory effect of HOEO was examined in animal models. As shown in Figure 5(a), the application of 300 μg/ear of HOEO on the right ear significantly reduced the extent of mouse ear edema at 16 h, 20 h, and 24 h after TPA treatment and the effect was similar with that of 500 μg/ear of indomethacin. Figure 5(b) showed the comparison of the extents of edema between the right ear and the unmedicated left ear on the same group of mice at 24 h after TPA stimulation, and the findings also supported that HOEO effectively hindered TPA-induced edema. Consequently, these results confirmed the anti-inflammatory activity of HOEO in vivo.

3.3. Interference of MCE and HOEO with the Activation of the IKK/NF-κB Pathway and MAPKs. The activation of nuclear factor-κB (NF-κB) plays a key role in mediating inflammatory responses. LPS is known to activate IKK (IκB kinase), which in turn catalyzes the phosphorylation of IκB (inhibitor of NF-κB), leading to IκB degradation and the release of NF-κB, which enters the nucleus and activates the expression of pro-inflammatory factors such as iNOS, COX-2, and IL-1β [17–19].

As revealed in Figures 6(a) and 6(b), MCE strongly inhibited LPS-induced phosphorylation of IKK (A) and IκB (B) (lanes 5 and 6) as compared with cells treated with LPS alone (lanes 3 and 4). HOEO exhibited a less potent inhibitory effect on the activation of IKK (Figure 6(a), lanes 7 and 8 versus lanes 3 and 4), but still showed a substantial suppressive effect on the phosphorylation of IκB (Figure 6(b), lanes 7 and 8 versus lanes 3 and 4). Therefore, LPS-induced IκB phosphorylation was obviously inhibited by both MCE and HOEO. Furthermore, Figure 6(c) showed that the nuclear translocation of p65, an NF-κB subunit, was apparently increased by LPS stimulation (lanes 3 and 4) as compared to untreated cells (lanes 1 and 2), whereas MCE (lanes 5 and 6) or HOEO (lanes 7 and 8) critically suppressed this LPS-induced effect, indicating that MCE...
and HOEO inhibited the shift of NF-κB to the nucleus. Overall, these data suggest that MCE and HOEO inhibit the LPS-mediated activation of IKK/NF-κB signaling.

Mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase (JNK), and p38, are also known to mediate the LPS-induced inflammatory responses [20–23]. Thus, whether MCE and HOEO could inhibit the activation of JNK, ERK, and p38 was examined. As shown in Figure 6(d), treatment of the cells with MCE (lanes 5 and 6) or HOEO (lanes 7 and 8) effectively inhibited the LPS-induced phosphorylation of ERK as compared with the LPS control (lanes 3 and 4). HOEO also markedly inhibited the phosphorylation of JNK (Figure 6(e), lanes 7 and 8 versus lanes 3 and 4), whereas MCE displayed a less strong inhibitory action on the activation of JNK (Figure 6(e), lanes 5 and 6 versus lanes 3 and 4). Figure 6(f) manifested that both MCE (lanes 5 and 6) and HOEO (lanes 7 and 8) substantially suppressed the LPS-induced phosphorylation of p38 as compared with the control (lanes 3 and 4).

4. Discussion

This study scientifically proved that the crude extract of F. polycarpa is a potential anti-inflammatory agent. Animal tests confirmed the anti-inflammatory effect of MCE in vivo.
Topical application of MCE for 12–20 h effectively ameliorated acute skin inflammation on mice ear. While compared with indomethacin, a medicine for inflammation, MCE showed a similar or even stronger effect in reducing ear edema of mice. Meanwhile, in RAW 264.7 macrophage line, MCE obviously inhibited LPS-stimulated expression or production of iNOS, COX-2, NO, PGE₂, and IL-1β. Overall, these results support the traditional use of F. polycarpa as an herbal medicine in treating inflammatory-related diseases.

MCE obviously reduced the phosphorylation of IKK and IkB and the nuclear translocation of NF-κB. These findings suggested that MCE inhibited the IKK/NF-κB pathway. MAPKs are another group of signaling pathways regulating inflammation. MCE critically suppressed LPS-induced activation of ERK and p38 and had a milder inhibitory effect on that of JNK. Generally, these results suggest that MCE interferes with the IKK/NF-κB pathway and the MAPK pathways, which likely underlies, at least in part, the mechanism of its anti-inflammatory activity.

MCE was rich in triterpenoids as well as phytosterols. It is rational to speculate that these components may contribute to the anti-inflammatory function of F. polycarpa. Thus, brassicasterol and a few major or feature triterpenes of MCE were characterized. Yet, brassicasterol did not exhibit an obvious anti-inflammatory activity, implying that brassicasterol may not contribute to the anti-inflammatory effect of MCE. Consistently, brassicasterol is known to be a phytosterol commonly present in many plants and foods [24–26], including those unknown to possess an anti-inflammatory activity. Thus, it is reasonable to infer that brassicasterol does not have an anti-inflammatory effect.

On the other hand, HOEO displayed a significant anti-inflammatory activity both in vivo and in vitro. HOEO obviously diminished TPA-induced ear edema in mice and inhibited the expression of proinflammatory markers in LPS-stimulated RAW 264.7 cells, including iNOS, COX-2, IL-1β, NO, and PGE₂. Furthermore, in accordance with the action of MCE, HOEO interfered with the activation of the IKK/NF-κB pathway and that of MAPKs ERK, JNK, and p38. Hence, HOEO likely represents one of the constituents contributing to the anti-inflammatory function of F. polycarpa. It is very likely that other components in MCE also play roles in the anti-inflammatory effect of F. polycarpa and deserve to be explored.

Interestingly, HODA, fatsicarpain D, and fatsicarpain F showed obvious inhibitory effects on the survival of RAW 264.7 cells, but they were found nontoxic to the normal liver line FL83B cell. The molecular mechanism for this effect is not clear. Nonetheless, promoting the death of macrophages without hurting other somatic cells could also be an effect of anti-inflammation. Thus, the actions, immunomodulatory effects, and molecular mechanisms of these molecules should be further investigated.

5. Conclusions

Evidence presented in this study suggests that the crude extract from the leaves and twigs of Fatsia polycarpa is a potent anti-inflammatory agent both in vitro and in vivo. The anti-inflammatory activity of MCE is probably not due to brassicasterol, a major phytosterol present in the extract, but is likely contributed by other components in the extract, such as triterpenoids like HOEO. The molecular mechanism underlying the effects of MCE and HOEO probably involves the inhibition of the IKK/NF-κB pathway and the MAPKs. To further our understanding, more studies should be conducted to continuously explore the effective constituents in the extract of F. polycarpa.

Acknowledgment

The authors are grateful to the National Science Council of Taiwan for the Research Grant funding to H.-L. Cheng (NSC 101-2621-B-020-002) and to C.-H. Chou (NSC 101-2621-B-039-002).

References

[1] S.-Y. Cheng, C.-M. Wang, Y.-M. Hsu et al., “Oleanane-type triterpenoids from the leaves and twigs of Fatsia polycarpa,” Journal of Natural Products, vol. 74, no. 8, pp. 1744–1750, 2011.
[2] ´E. P. Kemertelidze, Z. S. Kemoklidze, G. E. Dekanosidze, and A. I. Berenzyakovka, “Isolation and pharmacological characterization of triterpenoid glycosides from Fatsia japonica cultivated in Georgia,” Pharmaceutical Chemistry Journal, vol. 35, no. 8, pp. 429–432, 2001.
[3] Q. Chen, H. Gruber, C. Pakenham, W. M. N. Ratnayake, and K. A. Scoggan, “Dietary phytosterols and phytostanols alter the expression of sterol-regulatory genes in SHRSP and WKY inbred rats,” Annals of Nutrition and Metabolism, vol. 55, no. 4, pp. 341–350, 2009.
[4] S. Kitanaka, I. Yasuda, Y. Kashiwada et al., “Antitumor agents, 162. Cell-based assays for identifying novel DNA topoisomerase inhibitors: studies on the constituents of Fatsia japonica,” Journal of Natural Products, vol. 58, no. 11, pp. 1647–1654, 1995.
[5] V. I. Grishkovets, E. A. Sobolev, V. V. Kachala, A. S. Shashkov, and V. Y. Chirva, “Triterpene glycosides of Fatsia japonica. VII. Structures of glycosides D3a and D3b,” Chemistry of Natural Compounds, vol. 38, no. 3, pp. 264–267, 2002.
[6] W. C. M. C. Kokke, J. N. Shoelery, W. Fenical, and C. Djerassi, “Biosynthetic studies of marine lipids. 4. Mechanism of side chain alkylation in (E)-24-propylidenecholesterol by a chrysophyte alga,” The Journal of Organic Chemistry, vol. 49, no. 20, pp. 3742–3752, 1984.
[7] M. Rohmer, W. C. M. C. Kokke, W. Fenical, and C. Djerassi, “Isolation of two new C30 sterols, (24E)-24-N-propylidenecholesterol and 24β-N-propylcholesterol, from a cultured marine chrysophyte,” Steroids, vol. 35, no. 2, pp. 219–231, 1980.
[8] T. Ishida, Y. Mizushina, S. Yagi et al., “Inhibitory effects of glycyrrhetinic acid on DNA polymerase and inflammatory activities,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 650514, 9 pages, 2012.
[9] B. Simpson, D. Claudie, N. Smith, J. Wang, R. McKinnon, and S. Semple, “Evaluation of the anti-inflammatory properties of Dodonea polyantra, a Kainju traditional medicine,” Journal of Ethnopharmacology, vol. 132, no. 1, pp. 340–343, 2010.
Agricultural and Food Chemistry, vol. 56, no. 16, pp. 6835–6843, 2008.

[11] H.-L. Cheng, S.-M. Chang, Y.-W. Cheng, H.-J. Liu, and Y.-C. Chen, “Characterization of the activities of p21Cip1/Waf1 promoter-driven reporter systems during camptothecin-induced senescence-like state of BHK-21 cells,” Molecular and Cellular Biochemistry, vol. 291, no. 1-2, pp. 29–38, 2006.

[12] H.-L. Cheng, C.-Y. Kuo, Y.-W. Liao, and C.-C. Lin, “EMCD, a hypoglycemic triterpene isolated from Momordica charantia wild variant, attenuates TNF-alpha-induced inflammation in FL83B cells in an AMP-activated protein kinase-independent manner,” European Journal of Pharmacology, vol. 689, no. 1–3, pp. 241–248, 2012.

[13] H. Inoue, T. Mori, S. Shibata, and Y. Koshihara, “Modulation by glycyrrhetinic acid derivatives of TPA-induced mouse ear oedema,” British Journal of Pharmacology, vol. 96, no. 1, pp. 204–210, 1989.

[14] I. Andújar, M. C. Recio, T. Bacelli, R. M. Giner, and J. L. Ríos, “Shikonin reduces oedema induced by phorbol ester by interfering with IkBa degradation thus inhibiting translocation of NF-κB to the nucleus,” British Journal of Pharmacology, vol. 160, no. 2, pp. 376–388, 2010.

[15] Y. F. Chen, C. Ching, T. S. Wu et al., “Balanophora spicata and lupeol acetate possess antinociceptive and anti-inflammatory activities in vivo and in vitro,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 371273, 10 pages, 2012.

[16] E. Niederberger and G. Geisslinger, “The IKK-NF-κB pathway: a source for novel molecular drug targets in pain therapy?” The FASEB Journal, vol. 22, no. 10, pp. 3432–3442, 2008.

[17] Y. Bellik, L. Boukraa, H. A. Alzahrani et al., “Molecular mechanisms underlying anti-inflammatory and anti-allergic activities of phytochemicals: an update,” Molecules, vol. 18, no. 1, pp. 322–353, 2013.

[18] J.-C. Liao, J.-S. Deng, C.-S. Chiu et al., “Anti-inflammatory activities of Cinnamomum cassia constituents in vitro and in vivo,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 429320, 12 pages, 2012.

[19] W.-W. Chao, Y.-H. Kuo, S.-L. Hsieh, and B.-F. Lin, “Inhibitory effects of ethyl acetate extract of Andrographis paniculata on NF-κB trans-activation activity and LPS-induced acute inflammation in mice,” Evidence-Based Complementary and Alternative Medicine, vol. 2011, Article ID 254531, 9 pages, 2011.

[20] S. I. Jang, H. Jin Kim, Y.-J. Kim, S.-I. Jeong, and Y.-O. You, “Tanshinone IIA inhibits LPS-induced NF-κB activation in RAW 264.7 cells: possible involvement of the NIK-IKK, ERK1/2, p38 and JNK pathways,” European Journal of Pharmacology, vol. 542, no. 1–3, pp. 1–7, 2006.

[21] Y. Liu, E. G. Shepherd, and L. D. Nelin, “MAPK phosphatases—regulating the immune response,” Nature Reviews Immunology, vol. 7, no. 3, pp. 202–212, 2007.

[22] C. W. Lee, S. C. Kim, T. W. Kwak et al., “Anti-inflammatory effects of Bangpungtongsung-San, a traditional herbal prescription,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 892943, 12 pages, 2012.

[23] K. I. Park, S. R. Kang, H. S. Park et al., “Regulation of proinflammatory mediators via NF-kappa B and p38 MAPK-dependent mechanisms in RAW 264.7 macrophages by polyphenol components isolated from Korea Lonicera japonica THUNB,” Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 828521, 10 pages, 2012.