A Comparative Study of the Effects upon LPS Induced Macrophage RAW264.7 Inflammation in vitro of the Lipids of *Hippocampus trimaculatus* Leach

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Abstract: The present study attempts to investigate the anti-inflammatory potential of the isolated lipid extracts of three-spot seahorse which is rare marine bony fish. Petroleum ether (PE) extract was obtained from systematic solvent extraction after reflux extraction with 95% ethanol. FrIV was collected after silica gel column chromatography, and neutral lipids (NL), glycolipids (GL), phospholipids (PL) were separated from FrIV. Basic compositions were detected and analyzed via thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR). Anti-inflammatory activities of total lipids (TL), isolated NL, GL, and PL were detected by secretion of pro-inflammatory cytokines induced by lipopolysaccharide (LPS) in murine monocyte macrophage RAW264.7 cells in vitro. The results revealed that lipids of seahorse showed a positive correlation with the in vitro suppression of the release of nitric oxide (NO), interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α potently in a dose dependent manner, and showed cell compatibility. Among the fractions, GL (50 μg/mL) showed the highest capacity to attenuate the generation of pro-inflammatory cytokines which was comparable to that of the positive drug dexamethasone (DX) (20 μg/mL). Collectively, our findings indicated that the lipids from seahorse may be effective in the management of inflammation.

Key words: seahorse, lipids, glycolipids, phospholipids, anti-inflammation

1 INTRODUCTUON

Inflammation is a protective mechanism by which, upon entry of external materials such as bacteria and viruses into the body, inflammatory mediators are secreted¹. One of the most potent stimuli for macrophage is bacterial endotoxin (LPS), which activates cells via Toll-like receptor 4 (TLR4) and induces the production of large amount of pro-inflammatory cytokines such as TNF-α and IL-1β⁵⁻⁶. The inflammatory response is accompanied by the upregulation of inflammatory cytokines and the release of various inflammatory mediators including NO, TNF-α⁶.

Seahorse, a marine teleost fish, is known intimately for its peculiar medicinal composition. *Hippocampus trimaculatus* Leach is used as one of the most famous materials of traditional medicine and has been studied for many years for its diverse biological activities, including anti-tumor, appetite enhancement, antioxidant, anti-aging, anti-fatigue⁷⁻⁹. Up till now, there have been many studies on the lipids including PL, GL of sea cucumber, starfish, sea urchin and such marine aquatic products benefiting from the particular ecological habitat¹⁰. However, there has been no relevant research reported yet exploring the potential anti-inflammatory of lipids from seahorse. As a part of an ongoing research on isolating bioactive metabolites from three-spot seahorse, lipids were isolated and initially identified for composition. This study has focused on analysis and investigation of anti-inflammatory effects of lipids from seahorse.

2 EXPERIMENTAL

2.1 Materials and methods

Three-spot seahorse was purchased from Hainan Long-
Mouse mono macrophage RAW264.7 cells were obtained from Xiehe cell resource center (Beijing, CHN). Cell culture basic media DMEM was purchased from gibco life technologies (Shanghai, CHN), penicillin/streptomycin, fetal bovine serum (FBS) were purchased from gibco life technologies (AUS), Griess reagent and LPS of *Escherichia coli* O111:B4 were purchased from Sigma Chemicals, MTT reagent was purchased from Invitrogen Molecular Probes (Eugene, Oregon, USA), ELISA kits were purchased from Elabscience (Wuhan, CHN), BCA kit was purchased from Beyotime Biotechnology (Shanghai, CHN), TLC plates (Silica MF-254, Agela Technologies, CHN) were used in analytical TLC. Other chemicals and reagents used were of analytical grade available commercially.

2.2 Preparation of lipids

2.2.1 Extraction of PE extract

Three-spot seahorse was washed with tap water to remove organs and sediment, then put in the constant temperature drying box and allowed to be dried for 24 h under 60°C and through 20 mesh sieve after crushing. Analytical 95% ethanol was used for reflux extraction under 80°C by 1:10 (m/v) and lasted for 1.5 h (× 3). The obtained crude ethanol extract was filtrated through buchner funnel, the residue was re-extracted (× 2), resulted extract was concentrated by rotary evaporator at 45°C. The extract was suspended by 1:1 (m/v) of raw material and deionized water in a separating funnel. Solvent extraction with equal volume of PE, the upper organic phase (× 3) was collected and combined before concentrated by rotary evaporator at 45°C.

2.2.2 Silica gel column chromatography

PE extract was applied to column packaging silica gel, and eluted by PE and ethanol and detected and monitored under UV detector, among fractions, FrIV was mixed with chloroform/methanol/H2O in a centrifugal tube in a proportion of 8:4:3 by volume, centrifuged under 4000 rpm for 10 min after vortex mixed for 5 min, the lower layer was collected and 0.2 its volume of 0.9% (m/v) sodium chloride solution was added and vortex mixed for 1 min, and then centrifuged under 4000 rpm for 5 min, then the lower organic layer was collected and concentrated in rotary under 45°C. After the wash process, it was dissolved in small volume of chloroform and applied to the silica gel column eluting by chloroform, acetone and methanol, respectively. Crude NL, GL and PL were obtained (Fig. 1).

2.3 TLC analysis

Different elution parts were developed with a solvent system consisting of a mixture of hexane/diethyl ether/methanol/acetic acid (90:20:5:2, v/v/v/v) on TLC plate, iodine vapor was used to indicate the distribution of lipids. SubFrII was developed with CHCl3/methanol/acetic acid (65:15:2, v/v/v) on TLC plate and the plate was immersed in Molish reagent (15% α-naphthol in ethanol/sulfuric acid/ethanol/water, 10.5:6.5:40.5:4, v/v/v/v) for a few seconds to detect GL, the purple red spot could be visualized after heating in oven under 105°C for 10 min. SubFrIII was developed with CHCl3/methanol/H2O (65:25:4, v/v/v) on TLC plate and Vaskovosky reagent was sprayed to detect PL, the blue spot could be observed after heating in oven under 110°C for 5 min.

2.4 FTIR analysis of lipids

A spectral range of 4000-500 cm⁻¹ was collected using an FTIR spectrometer (Tensor27, Bruker Optics, Germany). Briefly, each around 2 mg lyophilized fraction was mixed and ground with potassium bromide, interval
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scanning was conducted with the wavenumbers ranging from 4000 to 500 cm\(^{-1}\) and determined the infrared absorption spectrum, the functional group and molecular structure were inferred in accordance to infrared absorption spectrum absorption peak and wavenumber.

2.5 Fatty acids analysis

Characterization of the fatty acids was conducted by conversion to the corresponding fatty acid methyl esters (FAME) followed by GC-MS analysis. Briefly, the lyophilized TL, NL, GL, PL (around 20 mg) was transmethylated with 2.5 mL of methanol containing 2% (v/v) sulfuric acid at 70°C for 2 h\(^{10}\). After the suspension cooled, 1 mL hexane and 1 mL saturated sodium chloride solution were added to form separated layers in the tube. The upper hexane layer containing FAME was removed for analysis by GC-MS 7890B/7000B, Agilent, US. Samples were injected into a 30 m long with 0.25 mm inner diameter and 0.25 μm film thickness HP-5MS capillary column. EI was adopted as ion source, temperature of ion source was 230°C, ion source temperature was 250°C, electron energy was 70 eV, nitrogen was used as carrier gas. The temperature of the injector and detector were set at 250°C and 280°C, respectively, the initial column temperature was maintained at 100°C for 1 min, then rose to 380°C at a rate of 5°C/min, and held at 280°C for 20 min. The individual fatty acid compositions were identified by standard mass spectrum in MastHunter Nist11, relative quality fraction for each chemical constituent was determined by peak area normalization method.

2.6 Cell viability

Murine macrophage cell line RAW264.7 were maintained in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL streptomycin-penicillin in a humidified atmosphere of 5% CO\(_2\)-95% air at 37°C. RAW264.7 cells were utilized for experimentation at 70-80% confluency. Cell viability of normal group was taken as 100\(\%\), relative cell viability of treated groups was calculated compared with untreated control group.

2.7 Measurement of NO levels

NO levels in the culture supernatant were measured by Griess reagent as described previously\(^{28}\). Briefly, RAW264.7 cells were pre-incubated overnight in 96-well plates using DMEM without phenol red at a density of 1×10\(^4\) cells per well, followed by the treatment of lipids (5, 25 and 50 μg/mL). NO production was stimulated by incubating with LPS (1 μg/mL final concentration) for 48 h. Each was carried out in quadruplicate. Fifty microliter of culture supernatant from each sample was collected and mixed with equal volume of Griess reagent and allowed to incubate for 15 min at 25°C. Absorbance values were read at 540 nm on an ELISA microplate reader (Synergy HT, Gene Company Limited). The nitrite (stable oxidation product of NO) levels of the media were calculated from regression analysis using known concentrations of sodium nitrite to deliver a standard curve \((y = 0.0124x + 0.0013, R^2 = 0.996, \text{liner range (3.12-100 μM)})\).

2.8 Enzyme immuno assay of TNF-α, IL-1β, IL-6

The inhibitory effects of lipids on the production of pro-inflammatory cytokines; TNF-α, IL-1β and IL-6 were determined by an enzyme-linked immunosorbent assay (ELISA). Cells were treated with various concentrations of lipids (5, 25 and 50 μg/mL) stimulated with LPS (1 μg/mL) for 24 h after incubated overnight. Cells per well were washed with cold PBS for two times before lysed by 200 μL 1% Triton X-100, cellular protein content was determined by BCA protein assay kit according to the manufacturer’s instructions. Collected conditioned media was analyzed per the manufacturer’s recommendations of mouse cytokine-specific ELISA kits. The concentrations of TNF-α, IL-6 and IL-1β were calculated according to the standard curves generated by each of the recombiant cytokines (IL-6: \(y = 0.0818x + 0.006, R^2 = 0.999, \text{liner range (0.31-10 ng/mL)}\); IL-1β: \(y = 0.006x + 0.0093, R^2 = 0.9921, \text{liner range (7.81-250 pg/mL)}\); TNF-α: \(y = 0.0015x + 0.0264; R^2 = 0.9902, \text{liner range (31.25-1000 pg/mL)}\)) provided with the ELISA kits.

2.9 Statistical analyses

The values were expressed as mean ± SD. The statistical significance was determined by one-way ANOVA followed by Tukey-Kramer test using SPSS Statistics 19.0. Values of *\(p < 0.05\), #\(p < 0.01\) were considered as statistically significant.

3 RESULTS

3.1 Silica gel column chromatograph

The yielding rate of fractions (Fr I, Fr II, Fr III, Fr IV) was 10.5%, 31.1%, 26.3%, 8.4%, respectively. The yielding rate of three sub fractions (SubFr I, SubFr II, SubFr III) was 65.5%, 16.0%, 17.0% respectively. Fractions were separated by polarity difference. Generally, NL has the highest yielding rate which accounted for 65.5%, and GL and PL constituted a small proportion by gravimetric
Fig. 2  (A) PE extract was applied to silica gel column installed in medium pressure purification and preparation system, and eluted according to the following procedure: 0-5 min, PE/ethanol, 95:5, v/v; 5-18.5 min, PE/ethanol, 70:30-55:45, v/v. Four fractions were obtained. (B) After deionized water wash and salt wash, FrIV was applied to silica gel column eluting by chloroform (0-15 min), acetone (15-30 min), methanol (30-45 min) respectively, three sub fractions were obtained.

3.2 TLC
TLC provides a rapid tool for qualitative analysis of lipids. (A). NL distributed along the plate, while GL migrated near the origin and PL remained at the origin. (B). The plate was immersed in Molish reagent, the purple red spot indicated that it was GL (Rf = 0.91). (C) The plate was sprayed with Vaskovsky reagent, the blue spot suggested that it was PL (Rf = 0.94) (Fig. 3).

3.3 Fatty acid analysis
Polyunsaturated fatty acids (PUFA) exist in lipids of sea-horse, especially omega-3 PUFA. The percentage content of saturated and monounsaturated fatty acid of GL containing one hydroxyl group or none is high, while percentage contents of unsaturated fatty acids, especially polyunsaturated fatty acids of NL and PL are high (Table 1).

3.4 FTIR analysis of lipids
(A) 3420 cm⁻¹ occurred hydroxyl stretching vibration, 2925, 2854 cm⁻¹ were absorption peaks of -CH₂- caused by symmetric and asymmetric stretching vibration. 1742 cm⁻¹ was the stretching vibration of -C=O, 1464 cm⁻¹ was the bending vibration of -CH₂-, 722 cm⁻¹ was the absorption peak of long chain fatty acids (Fig. 4A). Considering above, we speculate that the SubFr I was composed of glycerides plus free fatty acids¹⁹. (B) 2922, 2852 cm⁻¹ are absorption

Fig. 3  The first line is the solvent front, the second line is the origin. (A) Distribution of lipids (TL, NL, GL, PL) on TLC, developing agent: hexane/ether/methanol/acetic acid, 90:20:5:2, v/v/v/v; (B) TLC of GL, developing agent: CHCl₃/methanol/acetic acid, 65:15:2, v/v/v; (C) TLC of PL, developing agent: CHCl₃/methanol/H₂O, 65:25:4, v/v/v; All the development distances on the plates were 8 cm.
peaks of -CH₂ and -CH₃ caused by stretching vibration of lipophilic hydrocarbon chain, 1718 cm⁻¹ was stretching vibration associated with C=O, weak absorption peak of 720 cm⁻¹ showed that the existence of structure of the long aliphatic chain. 1649 cm⁻¹ was weak C=C and 1538 cm⁻¹ was weak N-H indicated that it was amide bond, 1458 cm⁻¹ was weak absorption peak of -CH₂ due to bending vibration (Fig. 4B). According to the literature data, basically infrared spectrum of GL coincided with the infrared optical character of cerebroside, regarding above, we deduce that GL obtained in the present study may be cerebroside²⁰. According to the literature data, basically infrared spectrum of GL coincided with the infrared optical character of cerebroside²⁰. (C) 3386 cm⁻¹ was the vibration frequency of hydrogen bond, 1739 cm⁻¹ was the characteristic vibration frequency of C=O from -C=O-O-, 2924 cm⁻¹ and 1462 cm⁻¹ were the characteristic vibration frequency of -CH₂C, CH₃, 1170 cm⁻¹ was the characteristic vibration frequency of -C=O-O-(sn-1), and 1236 cm⁻¹ was the characteristic vibration frequency of PO₂⁻, 1071 cm⁻¹ was the characteristic vibration frequency of C-O-PO₂⁻ (Fig. 4C), thus, SubFrIII contains PL based on the preliminary judgment²¹, ²².

3.5 Cell viability

Fractions showed no cytotoxicity at the concentrations ranging from 5 to 50 μg/mL in comparison with blank control group which may partially be explained by the fact that lipids isolated from seahorse are from the natural resource (Fig. 5). The possibility that any anti-inflammatory effects of lipids from seahorse in this study were an outcome of cellular toxicity to RAW264.7 cells was thus excluded. It suggests that concentrations up to 50 μg/mL

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<th>TL</th>
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Table 1  Fatty acid composition (%) and distribution of TL, NL, GL, PL.

Fig. 4 FTIR of NL, GL, PL with scanning wavenumber ranging from 4000 cm⁻¹ to 500 cm⁻¹.
Fig. 5 Effects of lipids from seahorse on RAW 264.7 cell viability. Cells in 96-well plate were incubated with the indicated concentrations of lipids (5, 25, 50 μg/mL) for 24 h. Each value represents mean ± SD of triplicate experiments.

Fig. 6 (A) Effects of lipid extract on LPS-induced NO formation in RAW264.7 cells. The control group was cells in media without samples containing stimuli and DMSO. Values correspond to mean ± SD. a-i letter in each sample are different significantly by Tukey-Kramer test (p < 0.05).

3.6 NO production
In RAW 264.7 cells, NO production was significantly stimulated by LPS (244.64% relative to untreated cells), TL, NL, GL, PL treatment significantly reduced the NO levels in LPS-stimulated cells in a dose dependent manner: 8.48%, 47.37%, 78.36% with 5, 25, 50 μg/mL TL, respectively; 2.63%, 22.22%, 36.84% with 5, 25, 50 μg/mL NL, respectively; 17.25%, 59.06%, 98.83% with 5, 25, 50 μg/mL GL, respectively; 2.34%, 29.82%, 59.06% with 5, 25, 50 μg/mL PL, respectively, while positive control reduced NO production by 91.73% with the addition of 20 μg/mL DX (Fig. 6).

3.7 Contents of TNF-α, IL-6, IL-1β
Exposure to LPS caused a significant accumulation of TNF-α in culture medium supernatant of RAW 264.7 cells. Simultaneously, Cells treated with lipids of seahorse were shown to reduce TNF-α expression induced by LPS. Cytokine IL-1β was upregulated in LPS-treated cells when compared to the untreated cells, while treatment with seahorse lipids was shown to reduce this LPS-induced IL-1β production. Similarly, LPS treatment was found to augment IL-6 release, which was then suppressed by seahorse lipids. Here the results showed that treatment with lipids isolated from seahorse largely decreased the secretion of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 from the LPS-stimulated murine macrophage RAW264.7 cells. Among the fractions, GL showed the highest potential to reduce the release of TNF-α, IL-6, IL-1β. For TNF-α, 8.26%, 53.82%, 78.42% at 5, 25, 50 μg/mL, respectively; for IL-6, 61.50%, 78.79%, 93.83% at 5, 25, 50 μg/mL, respectively; for IL-1β, 38.80%, 55.90%, 77.13% at 5, 25, 50 μg/mL, respectively. Furthermore, positive control DX reduced the amount of TNF-α, IL-6, IL-1β by 89.67%, 88.98%, 90.03% at 20 μg/mL, respectively (Fig. 7).

4 DISSCUSSIONS
NO has been identified as important mediator of inflammation which is produced by iNOS\(^{23}\). During inflammation some pro-inflammatory cytokines and endotoxins induce the expression of iNOS leading to generation of NO. Thus, the ability to inhibit NO production could be effectively used in screening of anti-inflammatory candidates\(^{24,25}\). And in the present study, the ability to inhibit the LPS stimulated NO production was used as the screening test for the anti-inflammatory activity of the fractions. Due to measured notable higher ability in constraining the NO amount in LPS-induced murine macrophages, FrIV was selected for further analysis of its anti-inflammatory effect. Excessive production of cytokines is induced by inflammatory stimulus such as LPS treatment in macrophages and it will increase the immune response which will result in inflammation\(^{26}\). Therefore, the inhibition of the pro-inflammatory cytokines has been identified as a target for anti-inflammatory therapies. To investigate more about the potential anti-inflammatory activities of lipids from seahorse, its effects on production of pro-inflammatory cytokines: TNF-α, IL-6, IL-1β were analyzed. These findings help to strengthen the fact that lipids from seahorse possess anti-inflammatory activity.

A result in Yoshiyuki Mizushima’s study\(^{27}\), one glucosyl compound, cerebroside from soybean showed a positive association with the in vivo inhibition of TPA (12-O tetradecanoylphorbol-13-acetate)-induced inflammation in mouse ear. A new cerebroside isolated from ethanol extract
of leaves of *Aerva sanguinolenta* showed significant inhibition of the expressions of iNOS and COX-2, and the down-regulation of the expressions of IL-1β, IL-6, IL-12 and TNF-α in LPS-stimulated macrophages via the inhibition of COX-2-mediated PGE\(_2\) release\(^{28}\). Cerebroside D\(^{29}\) reduced the levels of TNF-α, IFN-γ and IL-1β in intestinal tissue of mice with experimental colitis in a concentration-dependent manner, accompanied with markedly increased serum level of IL-10, providing a novel approach to treatment of colonic inflammation.

PL has many biological activities. In a study of Hashioka S\(^{30}\), pretreatment with PS/PC liposomes significantly reduced LPS-induced microglial NO production, indicating that PS/PC liposomes can inhibit production of both NO and superoxide anion, and thus presumably prevent a subsequent formation of peroxynitrite. Therefore, PS/PC liposomes appear to have both neuro protective and anti-oxidative properties through the inhibition of microglial activation. PL amongst the polar lipids of macroalga palmaria palmate extract could suppress LPS-induced NO production in RAW264.7 macrophage cells\(^{31}\). In addition, a research from Monika Vicenova\(^{32}\) demonstrated that phospholipids extracted from hen egg yolk exerted anti-inflammatory activity under controlled conditions of monocyte-derived macrophages in vitro induced by LPS.

As a rare marine teleost fish, Three-spot seahorse shares similar properties with fish. On the basis of investigation of fish oil upon anti-inflammation, omega-3 fatty acids are present at higher concentrations in esterified form, such as triglycerides, and GL and PL\(^{33}\). Fish oil has shown to exert anti-inflammatory effects and hence to ameliorate immune-mediated glomerulonephritis with the mechanisms underlying above effects including alterations in the production of eicosanoids, cytokines TNF-α\(^{34, 35}\).

5 CONCLUSIONS

Lipids separated from PE extract of three-spot seahorse, the resulted NL was composed of glycerol ester, free fatty acids. GL was predicted to be cerebroside, PL contained PC and phosphatidyl ethanolamine. The above biological
lipids are responsible for the anti-inflammatory activity induced by LPS in macrophage RAW264.7 cells in vitro. Lipids could suppress the production of pro-inflammatory cytokines NO, TNF-α, IL-6, IL-1β stimulated with LPS in RAW264.7 significantly. GL showed relative potent anti-inflammatory activity in comparison with NL, PL. Its anti-inflammatory efficacy may due to the specific molecular structure, the long chain base, carbonyl should be analyzed in the further study. The underlying mechanism of anti-inflammation of seahorse cerebroside also needs to be discussed in the further study in order to validate the use of seahorse lipids as effective anti-inflammatory agent.

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REFERENCES


23) Jung, W. K., Choi, I., Lee, D. Y. Caffeic acid phenethyl
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27) Mizushina, Y.; Takahashi, Y. Inhibition of DNA polymerase λ by glucosyl compounds from soybean (Glycine max L.) and their associated inflammatory activity. Food Chem. 132, 2046-2053 (2012).


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