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RESEARCH ARTICLE

Marine Microalgae

Anti-inflammatory activity of nonyl 8-acetoxy-6-methyloctanoate, isolated from the cultured marine diatom, *Phaeodactylum tricornutum* : mediated via suppression of inflammatory mediators in LPS-stimulated RAW 264.7 macrophages

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Abstract: In this study, the anti-inflammatory effects of nonyl 8-acetoxy-6-methyloctanoate (NAMO), isolated from the cultured marine diatom, *Phaeodactylum tricornutum* Bohlin, against LPS-induced RAW 264.7 macrophages were evaluated. NAMO has indicated the strongest inhibitory effects against nitric oxide (NO) and prostaglandin E_2 (PGE_2) production, dose-dependently, on lipopolysaccharide (LPS)-induced RAW 264.7 cells. The 50% NO production inhibitory concentration (IC_{50}) of NAMO was 24.8 µM with the least cytotoxic effect in both LDH and MTT assays. Inflammatory stimulators such as LPS, which induce cytokines in the process of macrophage activation and mediate tissue response in different phases of inflammation, were studied. NAMO showed significant and strong suppression of pro-inflammatory cytokine and interleukin-1β (IL-1β) production, but no significant inhibitory effect on the production of cytokines including tumor necrosis factor-α (THF-α) and interleukin-6 (IL-6) at the tested concentrations against LPS treatment on RAW macrophages. Western blot analysis was carried out to determine the protein expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins which mediate the suppression effect of NAMO on NO and PGE_2 production. The Western blot assay confirmed the suppression of iNOS and COX-2 protein expressions against LPS-stimulated RAW264.7 cells. Collectively, NAMO isolated from the cultured marine diatom, *P. tricornutum* exhibited a profound anti-inflammatory effect *in vitro*, suggesting that the compound might have a beneficial effect during the treatment of inflammatory diseases and can be used in functional food applications.

Keywords: Anti-inflammatory activity, cultured marine diatom, fatty alcohol esters, *Phaeodactylum tricornutum,* RAW 264.7 cells

INTRODUCTION

Marine microalgae, with the associated broad spectrum of secondary metabolites, have stimulated a vast interest due to biochemical and ecological diversity (Borowitzka, 1995). Exploration of new bioactive natural products from marine bio-resources like marine microalgae for pharmacological applications has been described as a promising aspect of this. The interest in the isolation of pharmacologically active metabolites from marine microalgae has increased throughout the world. Microalgae are rich sources of nutrients including a high content of protein, polyunsaturated fatty acids (PUFA), carbohydrates, minerals, vitamins, pigments, and secondary metabolites (Becker, 2007).

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Cultured marine microalgae have been widely used as a food source for aquaculture. Diatoms are abundant components of the phytoplankton, representing major nutritional sources. *Phaeodactylum tricornutum,* a marine diatom, has been characterized due to containing different forms of PUFAs and lipids, including free sterols, and esterified and glycosylated conjugates (Veron *et al*., 1996; Yang *et al*., 2019). A few reports have investigated the quantification of lipids that are available in *P. tricornutum.* The distribution of lipid constituents in marine microalgae is dependent on the growing conditions, that include different temperatures, light spectral quality, salinity, and nutrients provided in the aquaculture (Samarakoon *et al*., 2013). The possibility of changing the lipid concentration of microalgae is a very important characteristic that can be used to determine the physiological state of a microalgae population, as a population biomarker (Fabregas *et al*., 1997).

Inflammation is a physiological process initiated by pathogenic invasion or cell and tissue injury (Wadleigh *et al.,* 2000). Inflammation can be caused by the activation of various immune cells such as macrophages, lymphocytes, and neutrophils. Overproduction of pro-inflammatory cytokines including interleukin (IL) and tumor necrosis factor (TNF)-α can be stimulated in macrophages as per the inflammatory disease. In addition, inflammatory mediators such as nitric oxide (NO) and prostaglandin (PGE_2) are also activated due to inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Lee *et al*., 2006). Therefore, finding a better insight into anti-inflammatory agents is gaining interest, in order to regulate immune-related diseases, inflammatory disorders, and infections (Jung *et al*., 2009). Assessing the anti-inflammatory effect of the isolated lipid against LPS-stimulated macrophages from cultured marine microalgae was of great interest. Hence, in this study, we examined whether the cultured marine diatom, *P. tricornutum* is capable of producing novel compounds with pharmaceutical value. In our previous publication, the anticancer activity of a novel fatty alcohol ester; nonyl 8-acetoxy-6-methyl octanoate (NAMO) isolated from the cultured marine diatom *P. tricornutum* has been revealed (Samarakoon *et al*., 2014). In addition, in this study, we evaluate the *in vitro* anti-inflammatory activity of the isolated novel compound from the hexane fraction of the extract from the cultured marine diatom, *P. tricornutum,* against LPS-stimulated RAW macrophages.

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco/BRL (Burlington, ON, Canada). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulphoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in these investigations were of analytical grade.

Culturing of Phaeodactylim tricornutum

The marine diatom *Phaeodactylum tricornutum* Bohlin was kindly provided by the Korea Marine Microalgae Culture Center (KMMCC). The alga was inoculated in 30 L plastic cylinders at 20 °C after pre-cultivation in 5 L glass vessels (medium 4 L), and the air was continuously supplied at 5 L min^{-1} by air-lift. The light was provided by 60 W fluorescent lamps at an intensity of 2,500 lx (Light : Darkness = 24 : 0). The alga was cultured in Conway medium (Walne, 1966) prepared from filter-sterilized seawater, and the cultures were continuously active during the 8-10 ds after inoculation. The cells were flocculated with 200 ppm $Al_2(SO_4)_3$ (v/v) (Ilshin biochemical, Magicpool-99) and then recovered with centrifugation at 2,000 rpm using a basket centrifuge (Hanseong Co., Ansan, South Korea). The harvested diatom biomass was frozen at −25 °C and preserved until freeze drying.

Extraction and isolation

The material (38 g) was extracted three times with 80% methanol by sonication for 90 min each time, at 25 ˚C. After being concentrated by evaporating off the solvent under reduced pressure using a rotary evaporator, the crude methanol extract was further subjected to solvent-solvent partition chromatography. Then, four different fractions with varying polarity, namely, *n*-hexane, chloroform, ethyl acetate, and aqueous extracts were separated. The *n*-hexane fraction (550 mg) was found to be active and subjected to fractionation using a solid-liquid phase chromatography (normal phase silica) column (3 cm × 22 cm) using hexane and ethyl acetate as an increasing hydrophilic solvent system to

give 14 fractions, labelled F1~F14. Using the thin layer chromatography (TLC) results, visualized by heating after spraying with ethanol (90%) - sulphuric acid (10%) staining system, the desired fraction was identified as eluting with hexane (10%): ethyl acetate (90%), (F7: 19 mg), with enough purity to determine the molecular structure. Therefore, the molecular mass was measured using a high-performance liquid chromatography - diode array detector coupled with an electrospray ionization mass spectrometer (HPLC-DAD-ESI/MS) (Hewlett-Packard, Waldbronn, Germany), and NMR studies using a JEOL JNM-ECX400 spectrometer (^{1}H-NMR/400 MHz and ^{13}C-NMR/100 MHz) used for structure elucidation.

Nonyl 8-acetoxy-6-methyloctanoate (NAMO)

Liquid ($CHCL_3$): $C_{20}H_{38}O_4$ in the ESI (negative mode) as (M-H) 341.14 m/z. The calculated molecular mass is 342.28 m/z (M). ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ ppm: 0.87 (3H, t, J = 6.72 Hz, H-9'), 0.96 (3H, s, H-11), 1.25 (2H, m, H-4, 5, 5', 6'), 1.29 (2H, m, H-4', 7'), 1.31 (2H, m, H-8'), 1.43 (2H, m, H-3'), 1.50 (2H, m, H-2'), 1.53 (2H, m, H-7), 1.65 (H, m, H-6), 1.68 (2H, m, H-3), 2.21 (3H, s, H-10), 2.32 (2H, t, J = 7.53 Hz, H-2), 4.08 (2H, dd, J = 7.4,14.6 Hz, H-8), 4.13 (2H, dd, J = -7.4,14.6 Hz, H-1'). ^{13}CNMR (100 MHz, $CDCl_3$): δ 14.3 (q, C-9'), 21.2 (q, C-10), 22.8 (q, C-11), 22.9 (t, C-8'), 24.6 (t, C-3'), 24.7 (t, C-4), 24.9 (t, C-3), 29.2 (t, C-2'), 29.4 (t, C-7), 29.6 (t, C-4'), 29.9 (t, C-5'), 29.9 (t, C-6'), 32.1 (t, C-7'), 32.9 (d, C-6), 34.1 (t, C-2), 37.5 (t, C-5), 60.6 (t, C-1'), 63.3 (t, C-8), 171.3 (s, C-9), 179.2 (s, C-1).

Cell culture

The murine macrophage cell line RAW 264.7 was purchased from the Korean Cell Line Bank (KCLB, Seoul, South Korea). RAW 264.7 cell line was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 100 U mL^{-1} of penicillin, 100 µg mL^{-1} of streptomycin, and 10% fetal bovine serum (FBS). The cells were incubated and maintained in an atmosphere of 5% CO_2 at 37 °C. The cells were sub-cultured every 2 days and exponential phase cells were used throughout the experiments (Kim *et al*., 2013).

Determination of nitric oxide (NO) production

RAW 264.7 cells (1×10^5 cell mL^{-1}) were placed in a 24-well plate and after 24 h the cells were pre-incubated with various concentrations of the sample at 37 °C for 1 h. Then further incubation was done for another 24 h with LPS (1 µg mL^{-1}) at the same temperature. After the incubation, the quantity of nitrite accumulated in the culture medium was measured as an indicator of NO production (Lee *et al.,* 2006). Briefly, 100 µL of cell culture medium were mixed with 100 µL of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid), and the mixture was incubated at room temperature for 10 min, and the optical density at 540 nm was measured using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Sunrise, Tecan Co. Ltd., Australia). The fresh culture medium was used as a blank in every experiment (Kim *et al*., 2013).

Lactate dehydrogenase (LDH) cytotoxicity assay

RAW 264.7 cells (1.5×10^5 cells mL^{-1}) were plated in a 96-well plate and after 16 h the cells were pre-incubated with various concentrations of the sample for 1 h at 37 °C. Then the cells were further incubated for another 24 h with LPS (1 µg mL^{-1}) at the same temperature. After the incubation, the LDH level in the culture medium was determined using an LDH cytotoxicity detection kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, 50 µL of the reaction mixture were added to each well, and the incubation was done for 30 min at room temperature in the dark. Then, 50 µL of stop solution were added to each well, and absorbance was measured at 490 nm using a microplate reader (Sunrise, Tecan Co. Ltd., Australia) (Kim *et al*., 2013).

Cytotoxicity assessment using MTT assay

The cytotoxicity of NAMO against the RAW 264.7 cells was determined using the colorimetric MTT assay. Cells were seeded in a 24-well plate at a concentration of 1×10^5 cells/mL^{-1}. After 24 h, the seeded cells were treated with sample. Then, all of the cells were incubated for an additional 24 h at 37 °C. MTT stock solution (50 µL; 2 mg/mL in PBS) was added to each well to a total reaction volume of 250 µL. After incubating for 3 h, the supernatants were aspirated. The formazan crystals in each well were dissolved in 200 µL of dimethylsulphoxide (DMSO). The resulting absorbance was measured with an ELISA plate reader set at 540 nm (Samarakoon *et al*., 2014).

Determination of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) production.

The inhibitory effect of the sample on the production of pro-inflammatory cytokines from LPS-stimulated RAW 264.7 cells was determined according to a previously described method (Cho *et al*. 2000). Briefly, RAW 264.7 cells (1×10^5 cells mL^{-1}) were pretreated with the sample for 2 h and then treated with LPS (1μg mL^{-1}) to allow the production of pro-inflammatory cytokines for 24 h. Supernatants were used for the assay using an ELISA kit (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Determination of prostaglandin-E_2 (PGE_2) production

RAW 264.7 cells (1×10^5 cells mL^{-1}) were pretreated with the sample for 2 h and then treated with LPS (1 μg mL^{-1}) to allow the production of pro-inflammatory cytokines for 24 h. The PGE_2 levels in the culture medium were quantified using a competitive enzyme immunoassay kit (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The release of PGE_2 was measured relative to that of the control value (Cho *et al*., 2000).

Western blot analysis

RAW 264.7 cells (1×10^5 cells mL^{-1}) were pretreated for 16 h and then treated with LPS (1 μg mL^{-1}) in the presence or absence of the sample. After incubation for 24 h, the cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), and the cell lysates were prepared with lysis buffer (50 mmol L^{-1} Tris–HCl (pH 7.4), 150 mmol L^{-1} NaCl, 1% Triton X-100, 0.1% SDS and 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA)) for 20 min on ice. Cell lysates were centrifuged at 14,000 x g for 20 min at 4°C. Then the protein concentrations in the supernatants were measured using a BCA™ protein assay kit. Cell lysates containing 30 μg of protein were subjected to electrophoresis using sodium dodecyl sulphate–polyacrylamide gels (SDS-PAGE) at 12%, and the separated proteins were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were incubated with a blocking solution (5% skim milk in Tris-buffered saline containing Tween -20) for 90 min at room temperature. Then the membrane was incubated with anti-mouse iNOS (1:1000; Calbiochem, La Jolla, CA, USA) and anti-mouse COX-2 (1:1000; BD BiosciencPharmingen, San Jose, CA, USA) overnight at room temperature. After washing, the blots were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (1: 5000; Amersham Pharmacia Biotech, Little Chalfont, UK) for 90 min at the ambient temperature. Signals were developed by exposure to X-ray films and visualized according to the described procedure, using a Western Lightning-ECL detection kit (PerkinElmer, MA, USA).

Statistical analysis

All the data are expressed as mean ± standard deviation of three determinations. Statistical comparison was performed via a one-way analysis of variance followed by Duncan's multiple range test (DMRT). P values of less than 0.05 ($p < 0.05$) were considered as significant.

RESULTS AND DISCUSSION

Inhibitory effect of 8-acetoxy-6-methyloctanoate (NAMO) from P. tricornutum against LPS-induced nitric oxide production and cytotoxicity on RAW macrophages

The isolated compound (Figure 1), NAMO from the hexane fraction of the cultured marine microalga, *P. tricornutum*, was studied to assess the anti-inflammatory activity using LPS-induced RAW macrophages, and the NO production inhibitory and cytotoxic effects were determined. Five different concentrations (0, 5, 12.5, 25, and 50 μg mL^{-1}) of NAMO were pre-incubated with LPS for 24 h and the significant inhibitory effect on NO production level was observed dose-dependently. The 50% inhibitory concentration (IC_{50}) value of 8.50 μg mL^{-1} (24.8 μM) was determined. Nitric oxide (NO) is a signaling molecule. The inflammatory mediator, which is stimulated by iNOS. The effects of various

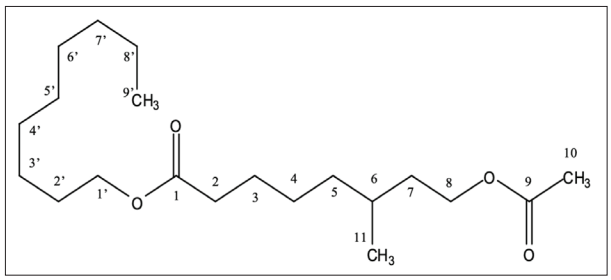

Figure 1: Structure elucidation of nonyl 8-acetoxy-6-methyloctanoate (NAMO) isolated from *Phaeodactylum tricornutum*

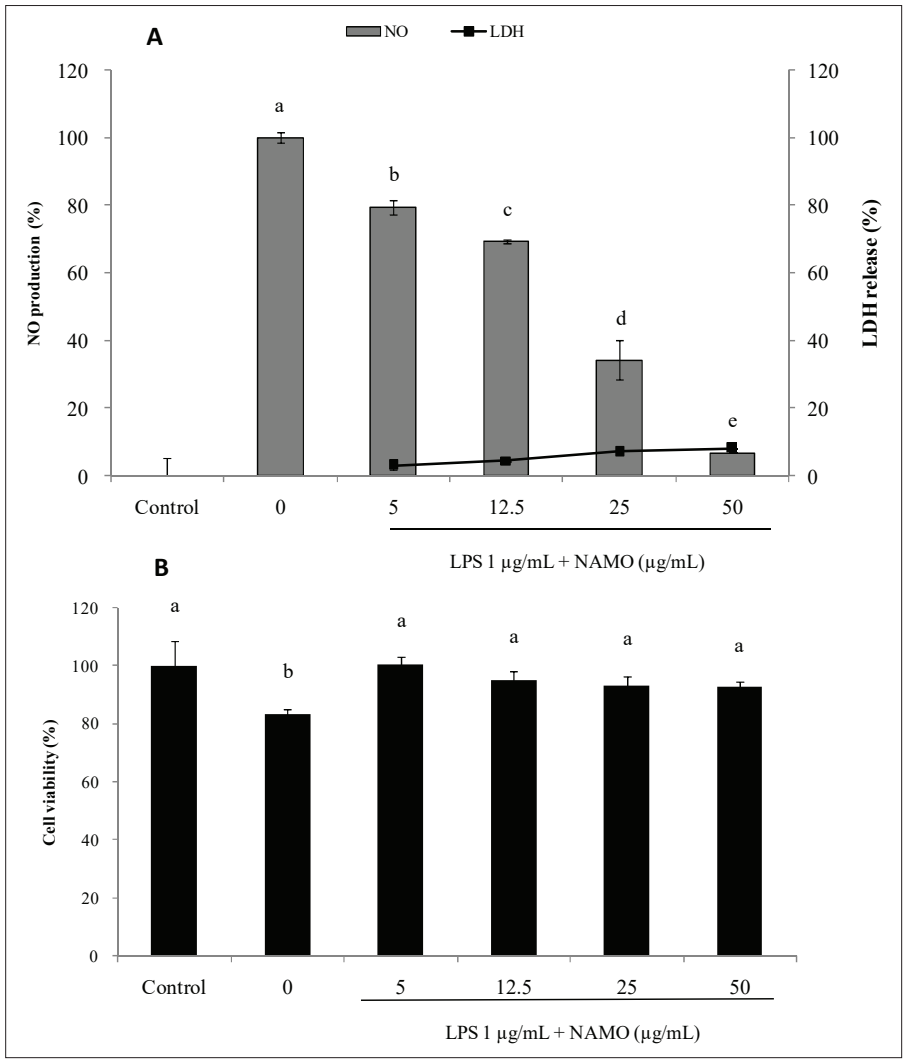

Figure 2: Inhibitory effect of the isolated NAMO compound from cultured marine diatom *Phaeodactylum tricornutum* against LPS-induced NO production (%) and cell viability (%) by LDH (A) and MTT assay in RAW 264.7 macrophages (B). Values are expressed as mean ± SD in triplicate experiments. Values with different letters are significantly different at $p < 0.05$ as analyzed by DMRT.

concentrations of effect of NAMO isolated from the cultured marine diatom *P. tricornutum* on NO production in LPS-activated RAW 264.7 cells was evaluated *in vitro*. LPS stimulates the cells to form NO molecules and causes inflammation. The increase of NO concentration in the medium was dose-dependently suppressed with the incubation of NAMO (Figure 2). Moreover, the cell viability assay indicated that NAMO did not show a cytotoxic effect on RAW 264.7 cells at all the concentrations. The cytotoxic effects on RAW 264.7 cells with the tested concentrations of NAMO were determined by MTT assay, and a 5.0 µg/mL concentration of NAMO gave the highest cell viability, 100%, compared to the negative control (Figure 2B). It is also confirmed that the LDH release assay indicated little or no cytotoxicity on macrophages at the tested concentrations (Figure 2A). Therefore, NAMO is marked as a potential agent for attenuating NO production without cytotoxic effects.

Inhibitory effect of NAMO on LPS-induced PGE_2 production

In this study, NAMO inhibited the LPS-induced PGE_2 production in a dose-dependent manner. PGE_2 production was 30% of normal at 25 µg mL^{-1} concentration of NAMO, and 100 % without NAMO treatment. Therefore, NAMO might induce anti-inflammatory effects by strongly inhibiting PGE_2 production when incubated with RAW macrophages (Figure 3 A).

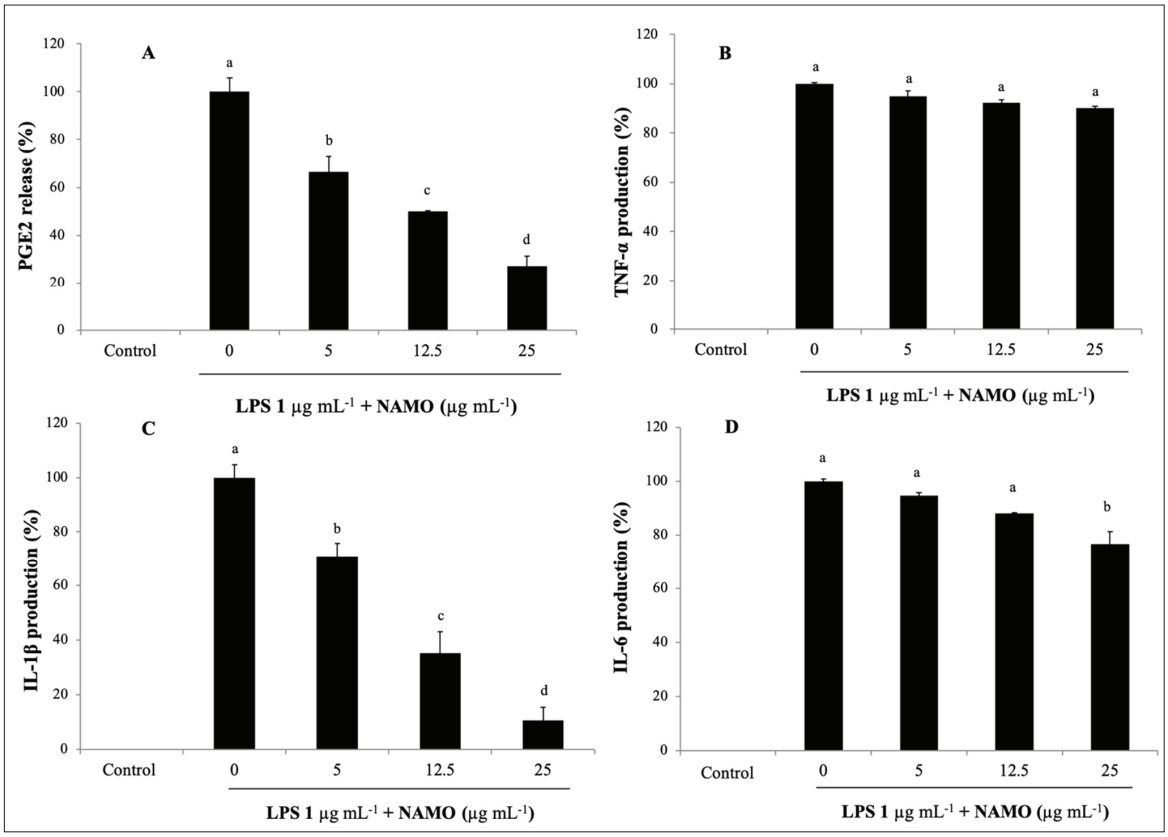

Figure 3: Inhibitory effect of the isolated NAMO compound from cultured marine diatom *Phaeodactylum tricornutum* on LPS-induced PGE_2 (A), TNF-α (B), 1L-1β (C) and 1L-6 (D) production in RAW 264.7 macrophages, after the incubation of cells with LPS for 24 h in the presence or absence of NAMO. Values are expressed as mean ± SD in triplicate experiments. Values with different letters are significantly different at $p < 0.05$ as analyzed by DMRT.

Inhibitory effect of NAMO on LPS-induced pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) Production

The inhibition of cytokine production or regulation is a key mechanism in the mediation of inflammation. Inflammatory stimulators such as LPS induce cytokines in the process of macrophage activation and mediate tissue response in different phases of inflammation. In this assay, the inhibitory effect of NAMO on the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in LPS-stimulated RAW 264.7 macrophages was studied. The NAMO did not show significant inhibitory activities on the production of cytokine TNF-α and IL-6 at the tested concentrations (Figure 3B, D). However, the strong suppression of the production of cytokine IL-1β was observed after the pretreatment of NAMO with RAW macrophages (Figure 3C). Therefore, the inhibition pathway might be mediated by the association of IL-1β formation.

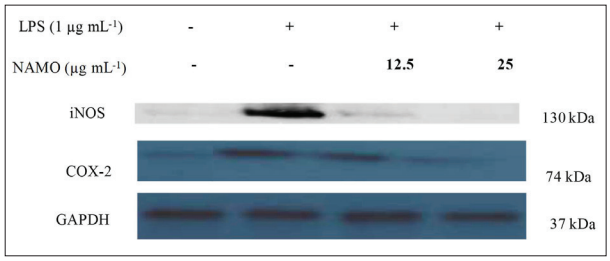

Figure 4: Inhibitory effect of the isolated NAMO compound from cultured marine diatom *Phaeodactylum tricornutum* on LPS-induced iNOS and COX-2 protein expression in RAW 264.7 macrophages. The cells were incubated with LPS for 24 h in the presence or absence of the NAMO. Then, cell lysates were subjected to electrophoresis and the expression levels of iNOS and COX-2 were detected with specific antibodies.

Inhibitory effect of NAMO on LPS-induced iNOS and COX-2 protein expression

Western blot analysis was carried out to determine the protein expressions of iNOS and COX-2 proteins which mediate the suppression effect of NAMO on NO and PGE_2 production (Figure 4).

According to the results, iNOS and COX-2 protein expressions were markedly induced when the macrophages were treated with LPS compared to the control. However, upon incubation with NAMO followed by LPS treatment, both iNOS, and COX-2 protein expression was downregulated, and at the dose of 25 μg mL^{-1}, the COX-2 protein expression was completely suppressed. Overall, it was suggested that the strong inhibitory effect on PGE2 production by NAMO was correlated with the down-regulation pattern of COX-2 protein expression. In addition, NAMO inhibited the production of NO levels by suppressing iNOS proteins.

P. tricornutum is a well-known marine diatom that accumulates fatty acids with varying degrees of unsaturation. In particular, the compositions of the sterols, PUFA, and fatty acids which are associated with *P. tricornutum* biomasses as lipids have different functional values that are of significant importance for the advancement of marine biotechnology (Radakovits *et al*., 2011). The cultivation of marine microalgae in an aquaculture system has been practised for a few decades. Open or closed (photobioreactor) microalgae culture systems are applicable based on the energy source and type of culture. Each one of the culturing systems has either desirable or undesirable effects, since the photosynthetic organisms and their mass production may directly correlate with the source of energy (Hong *et al*., 2012). The controlled optimum conditions with operational inputs such as salt, dissolved CO_2, water, nutrients, pH, and O_2 provides a great opportunity for a steady environment without being contaminated by photo-bioreactors. Researchers and technologists have been attracted to cultured marine microalgae to disclose the biochemical constituents of the crude extracts and to find out which components have pharmacological effects (Plaza *et al*., 2009). Based on the major ingredients available in cultured marine microalgae, it is a promising source for the functional food industry (Garcia *et al*., 2006; Samarakoon & Jeon, 2012;).

The results of our previous work, culturing and harvesting the biomass of the marine diatom, *P. tricornutum,* were discussed (Samarakoon *et al*., 2013). In addition, biological activity-guided fractionation led to the isolation of five different compounds from the hexane fraction of cultured *P. tricornutum*. The isolated novel fatty alcohol ester; NAMO was evaluated for inhibiting the growth of human leukemia cancer cells significantly through the p53 and caspase-3 mediated cell apoptotic pathway (Samarakoon *et al*., 2014). In the present study, the same isolated compound from P. *tricornutum* was evaluated for anti-inflammatory effects *in vitro*. Nitric oxide (NO) acts as an intercellular and intracellular signal molecule and plays crucial roles in many cellular functions in the nervous, cardiac, vascular, and immune systems (Wichard & Pohnert, 2006). However, the low levels of NO production are important in protecting organs such as the liver from ischaemic damage but the chronic expression of NO is associated with various inflammatory conditions (Kassim *et al*., 2010). Over the pathological conditions, NO production is increased by iNOS (Kim *et al*., 1999; Sahu *et al*., 2013). In terms of the potential therapeutic value when related to inflammation, inhibition of NO production by suppression of iNOS expression can be considered. A few recent publications have confirmed that the organic solvent extractions and compounds isolated from plant-based materials attenuated the inflammations caused by LPS-induced RAW macrophages via key signaling pathways such as the NF-kB (Ahujaa *et al*., 2019; Hao *et al*., 2019), and the iNOS pathway (Bharadwaj *et al*., 2020). The ethanol extract of a new microalgal strain, *Aurantiochytrium limacine* showed the significant suppression of genes for pro-inflammatory cytokines mediated by the NF-kB pathway (Takahashi *et al*., 2019). With the profound inhibitory effect on NO production evidenced by NAMO, it was further evaluated for its ability to inhibit the LPS-induced PGE2 production in RAW264.7 macrophages.

The present findings indicated that NAMO has anti-inflammatory properties, and the effects might be caused by inhibiting iNOS, COX-2, TNF-α, IL-1β, and IL-6 expression via affecting the NF-κB pathway. The accessible nature of the cultured marine microalgae is useful not only for the development of pharmacological prospects, but also to meet an increasing demand for nutraceuticals and food supplements. Exploring the sustainable use of cultured marine diatoms is considered important in future industrial and viable applications, and for bioprospecting for the novel bioactive metabolites. Developing new and safer treatment strategies using biomaterials extracted from marine microalgae to prevent and treat inflammatory diseases could be possible (Morais *et al*., 2015). Therefore, we have evidence that the isolated NAMO from the cultured marine diatom *P. tricornutum* is a promising source for both *in vitro* anticancer and anti-inflammatory effects. The findings of the study strongly emphasize the clinical and biotechnological potential of the isolated novel fatty alcohol ester from the cultured marine diatom *P. tricornutum* for further developments.

CONCLUSION

NAMO, isolated from the cultured marine diatom *P. tricornutum,* was identified as an anti-inflammatory active compound and showed promising inhibitory effects against cytokine production or regulation, which is a key mechanism in the mediation of inflammation against 264.7 RAW macrophages. NAMO demonstrated an inhibitory effect against NO with the 50% inhibitory concentration (IC_{50}) value of 8.50 µg mL^{-1} (24.8 µM). In addition, NAMO attenuated PGE_2 and 1L-1β production significantly in LPS-induced RAW cells. Moreover, the protein expression of iNOS and COX-2 confirmed the down-regulatory effect in a dose-dependent manner against RAW macrophages. Hence, the isolated NAMO can be a novel alternative with anti-inflammatory efficacy for future lead compounds in pharmacological applications.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The author(s) declare(s) that there are no conflicts of interest regarding the publication of this paper.

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