The Food and Life has published all type articles such as research articles, review articles, survey articles, research note, short communication or editorial since 2020. It covers the all scientific and technological aspects of food and life science.

https://www.foodnlife.org
In vitro induction effects of Commiphora molmo (Myrrh) extracts on cell migration through anti-inflammatory activity

Hyun Joo Ha1,†, Seung-Jae Lee2,†, Seon Gyeong Bak2,3, Seung Woong Lee2,* and Sun Hee Cheong3,∗
1Department of Food Science & Nutrition, Dong-A University, Busan, Korea
2Immunoregulatory Material Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup, Korea
3Department of Marine Bio Food Science, Chonnam National University, Yeosu, Korea

Abstract

Commiphora molmo (Myrrh) contains various biological activities. However, the underlying molecular mechanisms in cells remain unknown. The purpose of this study was to confirm the efficacy of inhibiting nitric oxide (NO) production induced by lipopolysaccharides (LPS) on J774 macrophage cells of myrrh, and additionally regulatory effects of perform wound healing. Further, vascular endothelial growth factor (VEGF), phosphorylation of signal transducer and activator of transcription 3 (p-STAT3) and Janus kinase 2 (p-JAK2) levels in macrophage cells were regulated in the presence of myrrh extract. Based on the above results, myrrh is thought to provide basic data on anti-inflammatory properties through interleukin 6 (IL–6) inhibitory activity and regulatory effects of cell migration.

Keywords: Commiphora molmo (Myrrh), anti-inflammation, cell migration, wound healing

Introduction

Cell migration and invasion are critical parameters in the metastatic dissemination of macrophage and cancer cells, the major cause of death in some diseases patients include cancer and tumor (Darvin et al., 2015). Wound healing is a complex processes that inflammation, re-epithelialization, tissue and matrix formation (Shan et al., 2014). Signal transducers and activators of transcription (STAT) are a family of transcription factors activated by several inflammatory cytokines and growth factors (Darvin et al., 2015). STAT3 plays a role in response to growth factors and cytokines such as interleukin 6 (IL-6) (Geiger et al., 2007). Also, STAT3 is phosphorylated by receptor-associated Janus kinases (JAK), form homo- or heterodimers, and translocate to the cell nucleus where they act as transcription activators (Kuchipudi, 2015). Recent research have reported the potential of several extract and/or compounds from natural products in inflammation treatment by targeting STAT3.

Commiphora molmo (Myrrh) is a yellow aromatic oleo-gum resin obtained from the stems of plants of the genus Burseraceae family included Commiphora abyssinica and Commiphora myrrha (Hanus et al., 2005). These myrrh is already known a noble plant in many country of Asian and Europe, furthermore, it has been used as a traditional medicine that biological activities include antioxidant, antibacterial, anti-nociceptive, and anti-ulcer (Dolara et al., 2000). However, anti-inflammatory activity pertinent to migration of myrrh has little information until now. Therefore, we investigated the suitability of extract of processed myrrh as a functional agent for suppressing the expression of LPS-induced pro-inflammatory mediators. As a result, the myrrh extract has strong biological activity, so it is considered that it can be used as a potential bio-activities material in the industry.

Materials and Methods

Materials

Myrrh obtained from Myrrh Molyac Institute Co. Ltd., (Daejeon, Korea). Insert with μ-dish for cell migration were obtained
from ibidi GmbH (Martinsried, Germany). Antibodies for JAK2, STAT3, VEGF and actin were purchased from Santa Cruz Biotechnology (Delaware, CA, USA). LPS (serotype 0127:B8) were procured from Sigma-Aldrich (St. Louis, MO, USA). Also, the enhanced chemiluminescence (ECL) advanced detection kit was purchased from Amersham Bioscience (Uppsala, Sweden).

**Cell culture**

J774 cells were obtained from the ATCC (Rockville, MD, USA). In brief, the cells were grown in Dulbecco’s modified eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 U/ml of penicillin, 100 μg/mL of streptomycin). Cells were maintained in 5% CO₂, 95% air and humidified atmosphere at 37°C.

**MTT assay for cell viability**

Briefly, the cells were seeded in well plates at 2×10⁵ in 100 μL/well. After 24 h, these cells were treated with 0, 1, 5, and 10 μg/mL myrrh extracts for 24 h. Afterwards, the OD values were detected at 570 nm under a microplate reader.

**Measurement of NO production**

The J774 cells (2×10⁵ cells/ml) were grown in a 24 well-plate and incubated with sample at various concentrations (5 and 10 μg/mL) in the presence or absence of LPS (50 ng/mL) for 18 h. The obtained culture media at designated time points were mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylendiamine dihydrochloride and 2% phosphoric acid) in a 96-well plate. The optical density at 540 nm was determined with microplate reader (Thermo Electron Corporation, Marietta, OH, USA). Nitrite production was determined by comparing the optical density with the standard curve obtained with NaNO₂.

**Wound healing assay**

In vitro wound healing modeling was performed using culture-insert in μ-dish (35 mm) assay (Ibidi #80206, Martinsried, Germany). When cells reached confluence, culture inserts were removed and a 500 μm cell-free area was created. After remove of DMEM was added extract of myrrh extracts (10 μg/mL), and cells were incubated for 24 h in a CO₂ incubator. In addition, the closing of the wound induced-insert was measured at 0 to 20 h using optical microscope (GX51, Olympus, Tokyo, Japan).

**Western blotting analysis**

Total protein from J774 cell lysates was denatured at 95°C for 5 min. Equal amount (20 μg) of denatured proteins was loaded in SDS-PAGE (12%), transferred onto PVDF membranes and blocked with skimmed milk (5%) for 1 h. Ultimately, these PVDF membranes were incubated with specific primary antibodies at 4°C overnight, followed by incubation with secondary antibody at room temperature for 1 h. Finally, target-specific signals were detected using an enhanced chemiluminescence solution (Intron Biotechnology, Seongnam, Korea) (Lee et al., 2012).

**Statistical analyses**

Data were reported as mean±SD for triplicate determinations. Analysis of variance (ANOVA), accompanied with Tukey’s tests (GraphPad Prism 5), were conducted to identify the significant differences between samples (p<0.05).

**Results and Discussion**

In the present study, the myrrh was selected to investigate anti-inflammatory effects on LPS-induced stimulation, as the myrrh had higher induced migration on macrophage cells. The cells were treated with the myrrh (1, 5, and 10 μg/mL) prior to LPS. All extract fraction of myrrh has no cytoxicity (Fig. 1). In addition, all extract fraction of myrrh inhibited nitrite contents, ethanol and ethyl acetate extracts which were the highest activity (Fig. 1). To investigate whether some extract fraction such as water, ethanol, ethyl acetate, hexane, and butanol could induce macrophage cell migration, we examined the effects of myrrh extracts on the migration of macrophage cells by wound healing assays. As shown in Fig. 2A and B, myrrh extracts induced the migration of J774 cells. Western blot analysis showed that the LPS induced STAT3 is reduced in myrrh ethanolic extract treated with 1, 5, and 10 μg/mL on J774 cells (Fig. 2C). Further, we measured the levels of JAK2 and VEGF (Fig. 2C). The phosphorylated STAT3 by IL-6 translocate into the nucleus, where these participate in the expression of pro-inflammatory cytokines such as interferon gamma (IFN-γ), interleukin-17 (IL-17), and interleukin 1 beta (IL-1β) (Kuchipudi, 2015; Darvin et al., 2015). IL-6 is well-known for activating immunity and inflammation through the binding to IL-6 receptor and dimeric gp130, which subsequently phosphorylates JAK2, STAT3, and extracellular-signal-regulated kinase (ERK) (Oh et al., 2014; Neurath and Finotto,
Regulatory effect of Myrrh in macrophage cells

Based on these data, we inhibitory effect of some protein by inflammatory disease from myrrh, its induced cell migration. Also, other downstream signaling molecules involved in the LPS pathway may be inhibited by suppression of phosphorylated JAK2, STAT3, and VEGF by myrrh may be responsible.
Fig. 2. Effects of myrrh on macrophage cell migration measured by wound healing assay. (A) Cells were plated in μ-dish with insert, and the cell monolayer was then insert removed the next day, a single scratch was made in the confluent monolayer followed by 10 μg/mL myrrh–treatment for 24 h, each scratch was photographed at 2, 8, and 16 h. There were highly significant differences between non-treated group and each soluble fraction of myrrh–treated groups. (B) Effect of STAT3, JAK2, and VEGF activation on LPS–stimulated by myrrh. Cells were pre-incubated with myrrh for 1 h before stimulation by LPS (50 ng/mL) using western blot. (C) The total amount of the corresponding non-phosphorylated protein was used as a loading control for phosphorylated proteins. STAT, signal transducers and activators of transcription; JAK, Janus kinases; VEGF, vascular endothelial growth factor; LPS, lipopolysaccharides.
However further studies are needed to elucidate the precise mechanism in vitro and in vivo.

**Conflicts of Interest**

The authors declare no potential conflict of interest.

**Acknowledgments**

This research was financially supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2019R1A2C2087608).

**Ethics Approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.

**Author Contributions**

Conceptualization: Cheong SH.
Formal analysis and measurement: Ha HJ, Lee SJ.
Investigation: Lee SJ, Lee SW.
Writing - original draft: Lee SW, Cheong SH.
Writing - review & editing: Ha HJ, Lee SJ, Bak SG, Lee SW, Cheong SH.

**Author Information**

Hyun Joo Ha (Professor, Dong-A University)
https://orcid.org/0000-0001-8515-9528
Seung-Jae Lee (Senior researcher, KRIBB)
https://orcid.org/0000-0002-1908-7666
Seon Gyeong Bak (Doctor’s student, Chonnam National University)
https://orcid.org/0000-0002-9305-5002
Seung Woong Lee (Senior researcher, KRIBB)
https://orcid.org/0000-0003-1025-7363
Sun Hee Cheong (Professor, Chonnam National University)
https://orcid.org/0000-0001-9120-1892

**References**

Akram M, Syed AS, Kim KA, Lee JS, Chang SY, Kim CY, Bae OK. 2015. Heme oxygenase 1-mediated novel anti-inflammatory activities of *Salvia plebeian* and its active components. J Ethnopharmacol 174: 322-330.
Darvin P, Joung YH, Nipin SP, Kang DY, Byun HJ, Hwang DY, Cho KH, Park KD, Lee HK, Yang KH. 2015. Sorghum polyphenol suppresses the growth as well as metastasis of colon cancer xenografts through co-targeting jak2/STAT3 and PI3K/Akt/ mTOR pathways. J Funct Foods 15:193-206.
Dolara P, Corte B, Ghelardini C, Pugliese AM, Cerbai E, Menichetti S, Lo Nostro A. 2000. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med 66:356-358.
Fujiwara Y, Komohara Y, Ikeda T, Takeya M 2011. Corosolic acid inhibits glioblastoma cell proliferation by suppressing the activation of signal transducer and activator of transcription 3 and nuclear factor-kappa B in tumor cells and tumor-associated macrophages, Cancer Sci 102:206-211.
Geiger PC, Hancock C, Wright DC, Han DH, Holloszy JO. 2007. IL-6 increases muscle insulin sensitivity only at super-physiological levels. Am J Physiol Endocrinol Metab 2921842-1846.
Hanus LO, Rezanka T, Dembitsky VM, Moussaieff A. 2005. *Myrrh*-commiphora chemistry. Biomedical papers 149:3-28.
Kuchipudi SV. 2015. The complex role of STAT3 in viral infections. J Immunol Res 272359:1-9.
Lee SJ, Kim YS, Hwang JW, Kim EK, Moon SH, Jeon BT, Jeon YJ, Kim JM, Park PJ. 2012. Purification and characterization of a novel antioxidative peptide from duck skin by-products that protects liver against oxidative damage. Food Res Int 49: 285-295.
Neurath MF, Finotto S. 2011. IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. Cytokine Growth Factor Rev 22:83-89.
Nizamuddinova IT, Kim YM, Chung Ji, Shin SC, Jeong YK, Seo HG, Lee JH, Chang KC, Kim HJ. 2009. Anthocyanins from black soybean seed coats stimulate wound healing in fibroblasts and keratinocytes and prevent inflammation in endothelial cells. Food Chem Toxicol 47: 2806-2812.
Oh HM, Lee SW, Yun BR, Hwang BS, Kim SN, Park CS, Jeong SH, Kim HK, Lee KS, Rho MC. 2014. *Vigna angularis* inhibits IL-6-induced cellular signalling and ameliorates collagen-induced arthritis. Rheumatology 53:56-64.
Shan S, Li Z, Guo S, Li Z, Shi, T, Shi J. 2014. A millet bran-derived peroxidase inhibits cell migration by antagonizing STAT3-mediated epithelial-mesenchymal
transition in human colon cancer. J Funct Foods 10: 444-455.