

Inhibition of Lipopolysaccharide (LPS)-Induced Inflammatory Responses by *Sargassum hemiphyllum* Sulfated Polysaccharide Extract in RAW 264.7 Macrophage Cells

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ABSTRACT: *Sargassum hemiphyllum*, a kind of brown seaweed generally found along coastlines in East Asia, has long served as a traditional Chinese medicine. *S. hemiphyllum* has shown an anti-inflammatory effect; however, its mechanism has not been elucidated clearly. This study explored *S. hemiphyllum* for its biomedical effects. *S. hemiphyllum* sulfated polysaccharide extract (SHSP) was first prepared; the mouse macrophage cell line (RAW 264.7) activated by lipopolysaccharide (LPS) was used as a model system. The secretion profiles of pro-inflammatory cytokines, including IL-1 β , IL-6, TNF- α , and NO, were found significantly to be reduced in 1–5 mg/mL dose ranges of SHSP treatments. RT-PCR analysis suggested SHSP inhibits the LPS-induced mRNA expressions of IL- β , iNOS, and COX-2 in a dose-dependent manner. At protein levels, Western blot analysis demonstrated a similar result for NF- κ B (p65) in cytosol/nuclear. Taken together, the anti-inflammatory properties of SHSP may be attributed to the down-regulation of NF- κ B in nucleus.

KEYWORDS: brown seaweed, *Sargassum hemiphyllum*, sulfated polysaccharide extract, anti-inflammation, macrophage, cytokine, NF- κ B

INTRODUCTION

Inflammation, a complicated physiological phenomenon, is a response to injury, infection, and stress. Cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , have been known to play important roles in pro-inflammatory responses. Cytokines are released from activated macrophages, which result in the expressions of adhesion molecules out of vascular endothelial cells to recruit neutrophils, monocytes, and lymphocytes, and then move out of the vessel to an injured tissue.¹ Nitric oxide (NO), a product of inflammation, is synthesized by nitric oxide synthases (NOS), which is found in various isoforms, such as neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). iNOS is expressed in response to activation of nuclear factor κ B (NF- κ B) that is otherwise induced by various effectors, such as lipopolysaccharide (LPS).^{2,3} Cyclooxygenase-2 (COX-2) synthesized in macrophages is inducible in response to infections, injuries, and stresses, which are considered to relate to inflammatory states.⁴ NF- κ B exists as an inactive heterotrimer including p50, p65 (Rel A), and I κ B in cytoplasm. Once I κ B is phosphorylated, NF- κ B is activated and then translocated to the nucleus, where it initiates transcriptions of proinflammatory cytokines.⁵ NF- κ B dysrhythmia results in constitutive pro-inflammatory disorders, including rheumatoid arthritis and Crohn's syndrome.^{6,7} Persistent NF- κ B activation also causes chronic inflammation, which has long been related to certain types of cancers.^{8,9}

On the other hand, natural substances, such as orange *Poncirus trifoliata*,¹⁰ curcumin, and capsaicin,¹¹ have been reported to

possess some biological effects to inactivate NF- κ B. Such substances may have potential in treating inflammatory disorders. *Sargassum hemiphyllum* is a kind of brown seaweed, which grows around seashores of the Far East, including Taiwan, Korea, Japan, Hong Kong, and East China. The nonstarch polysaccharide of *S. hemiphyllum* is known to consist of xylose and glucose,¹² which are soluble in water.¹³ The structure of sulfoglycolipid from *S. hemiphyllum* has also been determined to be 1-*O*-acyl-3-*O*-(6'-sulfo- α -D-quinovopyranosyl) glycerol.¹⁴ *S. hemiphyllum* was reported to be able to affect protein bioavailability in growing rats¹⁵ as well as inhibit an atopic allergic reaction.¹⁶ Other seaweed extracts, such as *Sargassum fulvellum* and *Sargassum thunbergii*, have been known to have antipyretic, analgesic, and anti-inflammatory activities.¹⁷ They suppress TNF- α -induced NF- κ B expression in human embryonic kidney cells (HEK-293). Methanol extracts of *S. hemiphyllum* have been reported to inhibit the releases of IL-8 and TNF- α ;¹⁶ however, the detailed mechanisms in anti-inflammation are still unknown.

In this study, we profiled cytokine contents, inflammatory products, and mRNA expressions of inflammatory mediators in an activated mouse macrophage cell line (RAW 264.7). We evaluated the anti-inflammatory effects of the sulfated polysaccharide extract of *S. hemiphyllum* by determining the inhibitory levels of cytosol/nuclear NF- κ B (p65).

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