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論文審査委員	主査　教　授　新井　博文 教　授　佐藤　利次 准教授　霜鳥　慈岳 教　授　菅野　亨 准教授　宮﨑　健輔

学位論文内容の要旨

Allergy is an overreaction of the immune system to normally harmless substances. It is divided into four types based on the underlying mechanisms. Type I allergies, also known as immediate hypersensitivity reactions, are triggered by antigens such as pollen and certain foods. Basophilic leukocytes and mast cells are involved, with antigens binding to IgE antibodies on FcεRI receptors, causing IgE cross-linking. This activates intracellular signaling pathways, including protein phosphorylation and calcium influx, leading to mast cell degranulation. Chemical mediators such as histamine and leukotrienes (LTs) such as LTB4 are released, causing allergic symptoms through vasodilation, increased vascular permeability, smooth muscle contraction, and leukocyte chemotaxis.

Inflammation is a defense mechanism triggered by tissue damage caused by infection, allergies, or other stimuli. Macrophages play a central role by releasing pro-inflammatory mediators and cytokines. For example, lipopolysaccharide (LPS), a bacterial component, activates macrophages via Toll-like receptors (TLRs) and stimulates the production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) and prostaglandin E2 (PGE2) by microsomal PGE2 synthase-1 (mPGES-1). Cytokines such as IL-1β and IL-6 further amplify and sustain the inflammatory response.

Allergy and inflammation are interrelated processes, as allergic reactions often trigger inflammation through the release of chemical mediators such as histamine, leukotrienes, and pro-inflammatory cytokines. In Type-I allergy, mast cell degranulation of mast cells releases mediators that promote vascular changes and immune cell recruitment, initiating an inflammatory response. Similarly, macrophages activated during inflammation amplify allergic responses by releasing cytokines such as IL-1β and IL-6. This interplay creates a feedback loop where allergic responses induce inflammation, and inflammation exacerbates allergic symptoms, contributing to the persistence and severity of immune-mediated disorders.

Astragalus membranaceus (AM), a perennial herb of the Fabaceae family, is mainly distributed in East Asia. AM root has been used as a pharmaceutical ingredient, particularly in traditional Chinese medicine. The pharmacological effects of AM roots, which contain astragalosides, have been studied for their potential to enhance immune function and exhibit antioxidant properties. In contrast, the aerial parts of AM have only been used as tea in China, and their physiological activities have not been well studied. This study aims to elucidate the anti-allergic and anti-inflammatory effects of AM leaf extract (AMLE) in mast cells and macrophages.

Leaf and root extracts of AM were prepared by hot water extraction. Total polyphenol content was measured by Folin-Ciocalteu method, and radical scavenging activity was assessed by DPPH. Histamine and LTB4 release assays were performed using RBL-2H3 and PB-3c cell lines, respectively, with chemical mediators analyzed by HPLC. Calcium levels in RBL-2H3 cells were measured using a fluorescent probe. RAW 264 macrophage cells were used to evaluate the effects of AM extracts on inflammatory mediators. NO release was measured using 2,3-diaminonaphthalene, and cytokine production (IL-1β, IL-6, and PGE2) was assessed by ELISA. iNOS and mPGES-1 expression were analyzed by qPCR and Western blot.

The results of this study show that AMLE effectively inhibits allergic and inflammatory responses.

AMLE contains a high concentration of polyphenols known for their antioxidant properties. These polyphenols, including kaempferol and quercetin, exhibited potent radical scavenging activity. AMLE significantly inhibited the release of chemical mediators such as histamine and LTB4 from stimulated mast cells, suggesting its anti-allergic effects. In addition, AMLE suppressed changes in cytoplasmic calcium concentration and inhibited 5-LOX mRNA expression in RBL-2H3 cells, suggesting its role in modulating cell signaling pathways involved in allergic reactions. In addition to its anti-allergic effects, AMLE also demonstrated potent anti-inflammatory activity in RAW 264 macrophage cells. AMLE significantly inhibited the release of NO and PGE2 in LPS-stimulated RAW 264 cells in a dose-dependent manner, while no effect was observed for AMRE. AMLE also reduced the mRNA and protein expression of iNOS and mPGES-1, enzymes responsible for NO and PGE2 synthesis, respectively. In addition, AMLE inhibited the release of the pro-inflammatory cytokines IL-1β and IL-6 and suppressed NF-κB nuclear translocation in LPS-stimulated cells. These results suggest that the anti-allergic and anti-inflammatory effects of AMLE may be due to its high polyphenol content, particularly kaempferol and quercetin, which have been shown to inhibit the release of chemical mediators in both allergic and inflammatory cell responses.

These results suggest that AMLE inhibits inflammatory and allergic responses, and the mechanism may be the inhibition of intracellular signaling in immune cells by kaempferol and quercetin, the major antioxidant polyphenols in AM leaves.

審査結果の要旨

花粉症等のⅠ型アレルギーでは、抗原抗体反応によって肥満細胞等が刺激され、細胞内シグナル伝達を経てケミカルメディエーターが細胞から放出されアレルギー症状を呈する。アレルギー反応や感染によって誘導される炎症では、マクロファージ等から一酸化窒素およびサイトカインが放出されることで炎症症状を呈する。現在、アレルギーおよび炎症症状の抑制は薬剤による対症療法に依存しているが、副作用が少ない食品の摂取による症状緩和が期待されている。キバナオウギ（*Astragalus membranaceus*）は、その根が生薬として利用さている一方、地上部の生理作用は明らかではない。

本研究は、キバナオウギ葉抽出物の抗アレルギーおよび抗炎症効果を培養細胞を用いて調べ、その作用機序の解明を試みたものである。その結果、キバナオウギ葉抽出物に抗アレルギー効果（ケミカルメディエーター放出および細胞内 Ca2+濃度上昇抑制活性）および抗炎症効果（一酸化窒素およびサイトカイン放出抑制活性）が認められた。

これらの結果は、キバナオウギ葉の摂取がアレルギーおよび炎症の予防や緩和に寄与することを示唆するものである。本研究の成果は新知見であり、査読付国際英文誌（*Preventive Nutrition and Food Science*）への掲載が承認されている。

以上より、Perleidulam Bunddulam 氏は北見工業大学　博士（工学）の学位を授与される資格があるものと認められる。