

氏名	ANUU ZORIG
授与学位	博士(工学)
学位記番号	博甲第188号
学位授与年月日	令和3年3月19日
学位授与の要件	学位規則第4条第1項
学位論文題目	Study on biological functions of Echinacea purpurea extracts (エキナセア抽出物の生理機能に関する研究)
論文審査委員	主査 教授 新井 博文 教授 星 雅之 准教授 佐藤 利次 教授 吉田 孝亨 准教授 菅野 亨

## 学位論文内容の要旨

Allergy is a disorder of immune response that is classified into several types according to the mechanism involved. Immediate hypersensitivities such as hay fever and food allergies are categorized as type-I allergies, in which mast cells and basophilic leukocytes play an important role in the allergic reaction. The specific binding of antigens to immunoglobulin E (IgE) antibodies bound to the receptor on the cell membrane induces cell stimulation by cross-linking of IgEs. This triggers the intracellular signal transduction, such as the phosphorylation of proteins, followed by  $Ca^{2+}$  influx into the cytoplasm, which induces the release of chemical mediators such as histamine and leukotrienes (LTs). Histamine and LTs are released from mast cell, cause mucous hypersecretion and smooth muscle contraction.

Inflammation is a response to tissue abnormalities caused by trauma, bruising, pathogen invasion, or chemical stimuli. The inflammatory response produces signs of rubor, throbbing pain, fever, and tumefaction with functional impairment. Histamine and leukotrienes released by mast cells and basophils dilate blood vessels and increase capillary permeability, which increase blood flow to the tissue. Macrophages migrate into the tissue and release inflammatory mediators such as prostaglandins, interleukins, and  $TNF-\alpha$ , resulting in pain and fever.

Echinacea purpurea (EP) is a perennial flowering plant of the Asteraceae family native to North America and is commonly called purple coneflower. Native Americans have used Echinacea as a traditional medicine for various diseases such as colds. The functions are polyphenols such as caffeic acid derivatives including chicoric acid, and hydrophobic alkylamides including isobutylamides which are abundant in the roots. The objectives of this study are to elucidate the effects of anti-allergic and anti-inflammation of Echinacea purpurea extracts in mast cells and macrophages.

Extracts of petal, leaf, and stem of EP were prepared using hot water. Rat basophilic leukemia cell line (RBL-2H3) and mouse bone marrow derived mast cell line (PB-3c) were used for histamine and LTB<sub>4</sub> release assay, respectively. After the cell stimulation, the chemical mediators released from the cells were determined by HPLC. Total polyphenol content in EP extracts was determined by the Folin-Ciocalteu method. The radical scavenging activity of EP in extracts was measured using the DPPH method. The effect of the petal extract on the cell signaling after the stimulation was analyzed by SDS-PAGE and Western blotting using anti-phosphorylated tyrosine and anti-spleen tyrosine kinase (Syk). Ca<sup>2+</sup> concentration in the cytoplasm of RBL-2H3 cells was measured using a fluorescent probe. Murine Leukemia macrophage cell line (RAW 264.7) was used to observe effect of EP extracts on inflammatory mediators and the underlying mechanisms. NO release from RAW 264.7 after the stimulation with lipopolysaccharide was measured by the method using 2,3-diaminonaphthalene. The production of inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, in the LPS-stimulated cells was determined by ELISA. Expression level of inducible nitric oxide synthase (iNOS) in the LPS-stimulated cells was analyzed by Western Blotting using a monoclonal antibody.

Petal and leaf extracts exhibited a significant inhibitory effect on histamine release from the stimulated RBL-2H3 cells, in which the activity of the petal extract was much stronger than that of the leaf extract, while the stem extract did not exert any effect. All the extracts significantly suppressed LTB<sub>4</sub> production in the stimulated PB-3c cells and exerted similar activities. The total polyphenol content and DPPH scavenging ability of the petal extract was higher than those of the leaf and stem extracts. The petal extracts dose-dependently decreased total tyrosine phosphorylation and Syk phosphorylation, and Ca<sup>2+</sup> influx associated with signal transduction in the stimulated cells. Petal extract significantly suppressed NO release from the LPS-stimulated RAW 264.7 cells. Petal extract inhibited the production of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. In addition, petal extract suppressed iNOS expression.

In this study, we evaluated the effect of EP extracts on major mediators of allergic and inflammatory responses in mast cells, basophil cells, macrophages. These results suggest that the petal extract may alleviate symptoms of allergy and inflammation.

## 論文審査結果の要旨

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処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。エキナセア (*Echinacea purpurea*) は、伝統的にハーブティーに用いられており、様々な薬理効果が示唆されている。本研究は、エキナセア地上部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、エキナセア花卉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  $\text{Ca}^{2+}$  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、エキナセアの摂取がアレルギーおよび炎症の予防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、日本農芸化学会の英文誌への掲載が認められている。よって、ANUU ZORIG 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。