Title of dissertation				
Identification of anti-inflammatory agents from natural products by targeting NF-kappa B activity and their application to inflammation-associated diseases				
RONPAKU Fellow				
Name	**Besse Hardianti**			
Position	Lecturer / Researcher		ID No.	R11815
Department	Makassar School of Pharmacy			
Institution	Sekolah Tinggi Ilmu Farmasi Makassar (STIFA)		Nationality	Indonesia
Japanese Advisor				
Name	Yoshihiro Hayakawa			
Position	Professor	Institution	University of Toyama	

Increasing evidence links inflammation to various pathologies and causes cellular and molecular damage by activating inflammatory signaling pathways, such as the NF-κB pathway. The study aimed to find natural anti-inflammatory agents that target NF-κB activity and apply them to inflammation-related diseases. Natural products appear to regulate inflammatory response pathways. NF-κB is a key mediator of pro-inflammatory gene induction and functions in both innate and adaptive immune cells, making its anti-inflammatory regulation crucial. NF-κB activity was targeted in extensive pharmacological studies of natural products to find a novel anti-inflammatory agent for inflammatory-induced pathogenic disease. In order to establish a novel therapeutic approach for inflammatory-induced pathogenic disease, the extensive pharmacological investigations of natural products were conducted to identify a potential anti-inflammatory agent by targeting NF-κB activity.

1. NF-κB -targeted functional screening of natural products for anti-inflammatory agents

NF-κB -targeted functional screening of 112 natural products was performed to determine anti-inflammatory properties. Using a 4T1 breast cancer cell line, Sohakuhi (Morus alba Linn. bark) extract inhibited NF-κB activity without affecting cell viability in NF-κB -targeted functional screening. Additionally, NF-κB -targeted functional screening was conducted on 35 Indonesian medicinal plants used to treat skin disease symptoms. Two K. galanga extracts from various rhizome types inhibited NF-κB activity without affecting cell viability.

2. *Morus alba* Linn (Sohakuhi) anti-inflammatory compounds, moracin O and P, target the NF-κB pathway.

We examined TRAIL-induced cellular damage of HaCaT human keratinocytes to test Sohakuhi extract's anti-inflammatory effects. TRAIL causes cellular damage in HaCaT cells by phosphorylating p65, a subunit of NF-κB. Treatment with Sohakuhi extract protects against TRAIL-induced damage, suppresses NF-κB activation, and increases anti-apoptotic BCL-XL and BCL-2 expressions. Importantly, chemical fractionation of Sohakuhi extract revealed that Moracin O and P cause its anti-inflammatory effect. These findings suggest that Sohakuhi and Moracin

compounds could be used to develop new anti-inflammatory drugs.

3. Anti-inflammatory and cytoprotective effects of Kaempferia galanga extracts via NF-κB targeting.

After screening 12 natural medicinal plants, we found that two K. galanga extracts (29 and 35) from different rhizome types significantly inhibited NF-κB activity without affecting cell viability. The biological activity of K. galanga extracts 29 and 35 was assessed by incubating HaCaT cells with rTRAIL, a mediator of inflammatory stimuli that activate NF-κB activity and cytotoxicity. Pretreatment with extracts 29 and 35 did not harm HaCaT cells, but rTRAIL significantly reduced cell viability. Additionally, pretreatment with extract 29 and 35 at 16 and 32 g mL–1 protected cell growth from rTRAIL-induced damage. Extracts 29 and 35 protect human keratinocytes from rTRAIL-induced cellular damage. The identified K. galanga extracts appear promising for developing novel anti-inflammatory natural medicines.

4. Ethyl P-methoxycinnamate: a potent anti-metastasis and chemosensitizer for melanoma cells targeting NF-κB from Kaempferia galanga

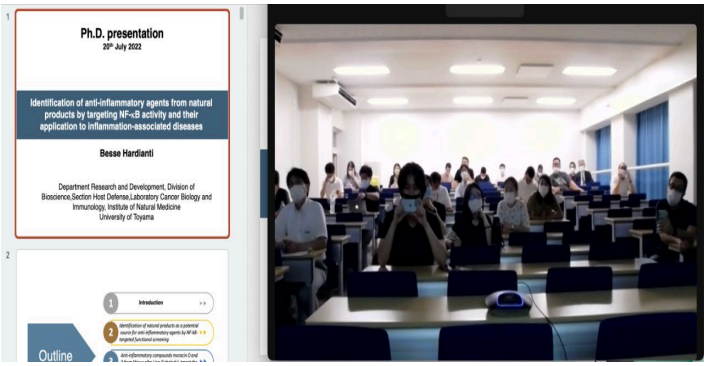
The active compound in K. galanga was isolated and identified using chromatography and spectroscopy, yielding six compounds. Inhibitory activity on NF-κB activation and cell viability was assessed using reporter assays. EPMC, a compound isolated, showed strong NF-κB inhibition against melanoma cell B16F10-NF-κB Luc2 with an IC50 of 88.7 μM. EPMC's anti-metastasis effect was tested in vitro using wound-healing, invasion, and molecular mechanism assays with Western blotting. NF-κB is linked to tumorigenesis via the PI3K/Akt/ NF-κB pathway. The study found that EPMCs inhibit NF-κB -dependent transcription by inhibiting p38 and Akt phosphorylation at serine 473. Further analysis with paclitaxel showed that the combinations could sensitize to apoptosis in response to well-known chemotherapy agents. Additional studies were done using SK-Mel 28 human melanoma cancer cells. Paclitaxel and EPMC treatment led to an increase in p-γH2AX expression, a marker for DNA damage, along with apoptosis. EPMC showed promise as an adjuvant for improving anti-metastatic and cancer chemotherapy.

5. Anti-inflammatory properties of isopimara-8(9),15-diene diterpenoids and kaempfulchraols from Kaempferia pulchra rhizomes

Kaempfulchraols, isopimara-8(9),15-diene diterpenoids from Myanmar-collected Kaempferia pulchra rhizomes, were tested for anti-inflammatory activity. Kaempulchraols P and Q, the most potent of 21 isopimara-8,14(15)-diene diterpenoids, inhibited NF-κB -mediated transactivation of a reporter gene, IL-6 production, and COX-2 expression at 25 μM doses (IC50 values: 39.88 and 36.05 μM, respectively). In addition, Kaempulchraols B-D, isolated from K.pulchra, inhibited NF-B-mediated transactivation of a reporter gene, IL-6 production, and COX-2 expression at a dose of 25 μM. Isopimarane diterpenoids may be effective NF-κB pathway inhibitors and could be explored as anti-inflammatory lead compounds.

These studies highlight the significance of natural products as anti-inflammatory agents, focusing on the NF-κB signal pathway. Therapeutic use of natural anti-inflammatory agents would benefit inflammation-related diseases.

Photos



Online Defense for Ph.D Examination

Graduation Day in University of Toyama