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Kanuka (Kunzea ericoides) Leaf Extract suppresses the activity of caspase 1 by NLRP3 inflammasome formation, and is expected to show an effective anti-aging effect.

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Introduction:

The skin is constantly exposed to ultraviolet rays (UV-B) which is known as non-pathogenic NLRP3 inflammasome activated factor. Recently, it has been reported that inflammasome is activated by UV-B irradiation and is secreted IL-1β in keratinocyte [1]. Furthermore, the downstream of IL-1α/β receptor has the secretion of the cytokines, such as IL-6, IL-8. IL6/8 is well-known as SASP (Senescence-Associated Secretory Phenotype), which promote cellular senescence [2]. Typically, the aging arrests the cell proliferation and decrease various metabolic functions. Because of this, the activation of NLRP3 inflammasome accelerates cellular senescence in the skin. In addition, the secreted IL-1β by NLRP3 inflammasome in skin causes the decreasing of fillagrin-2 (FLG-2), which moisturize the skin [3]. From these reports, it is suggested that the activation of NLRP3 inflammasome by UV-B exposure accelerates the skin aging and skin dryness.
Our reports show that UV-B irradiation induced the activation of inflammasome in human dermal fibroblasts. Surprisingly, Kanuka Leaf Extract can suppress caspase-1 activity by UV-B induced NLRP3 inflammasome activation in fibroblasts.

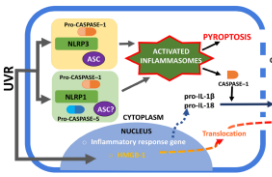

Fig.1 Activation of NLRP1 and NLRP3 inflammasomes [4]

Materials & Methods:

About Kanuka

Kanuka (Kunzea ericoides), commonly known as "white tea tree", is a traditional medicinal plant endemic to New Zealand (Fig.2). Although it is a low tree, sometimes it can grow up to a height of 20 to 30 m, and small white flowers blooming on the top of branches like a piece of brocade from September to the following February. The Maori, New Zealand's indigenous, use its leaves as medicine, and kanuka honey is also a popular health food.

Fig.2 The photograph of Kanuka Leaf

Preparation of Kanuka Leaf Extract

Extraction was prepared from dried Kanuka leaves using a polar solvent, and then the mixture was filtered and purified to prepare Kanuka Leaf Extract.
INCI name: Kunzea Ericoides Leaf Extract

DPPH radical scavenging assay

A mixture of DPPH solution, ethanol and acetic acid-sodium acetate buffer (pH5.5) was added to each extract. After standing at 37℃ for 20 minutes, the absorbance at 550 nm of the reaction solution was measured to calculate the residual amount of DPPH radicals.

Caspase-1 assay

Normal human dermal fibroblasts were seeded on 24-well plates, and each extract were added after culturing for 1 day. After further culturing for 1 day, culture medium was replaced to the medium containing LPS at 1.0 μg/mL without phenol red and cells were cultured for 1 hour. Afterwards, cells were irradiated UV-B of 100 mJ/cm2 for caspase-1 induction. After 4 hours, the cells were stained with Pyroptosis/Caspase-1 Assay Green (Immunochemistry Technologies, LLC, USA) to detect the caspase-1 activity in the cells. The cells were detached with trypsin, and then inactivated trypsin with ice-cold 5% FBS-containing PBS. After cells were rinsed twice with ice-cold PBS containing 0.1% BSA, analyzed by flow cytometer (BD Accuri™ C6 Plus, BD Biosciences) to calculate the average of fluorescence intensity (Ex. = 488nm, Em. = 538nm).

IL-1β, IL-18 assay

Normal human dermal fibroblasts were cultured and irradiated as discribed above, and after 48 hours, IL-1β and IL-18 in culture supernatant were measured by each ELISA kit.

FLG-2 Expression assay

Normal human epidermal cells were seeded on 24-well plates, and the same culture supernatant as above was added after culturing for 1 day. After further culturing for 24 hours, total RNA was extracted by a conventional method and RT-PCR was performed to evaluate the relative expression level of FLG-2.

Suppression test for erythema formation by ultraviolet rays(*in vivo*)

The lotion containing 0.5% Kanuka Leaf Extract and control lotion were applied to each test area in forearm of 6 subjects twice a day for a week.
After the application is finished, the erythema index of each test area was measured (initial value) by MEXAMETER (Courage + Khazaka electronic GmbH). After that, UV-B corresponding to 1 MED of each subject was irradiated to each test area.
The next day, each test area was photographed and the erythema index was measured same as measuring initial value.

Results & Discussion:

Component analysis of Kanuka Leaf Extract

Kanuka Leaf Extract was analyzed by using HPLC. The conditions are shown in Fig. 3. Many peaks that seemed to be polyphenols could be confirmed. A particularly large peak was found to be chlorogenic acid (Fig.3).

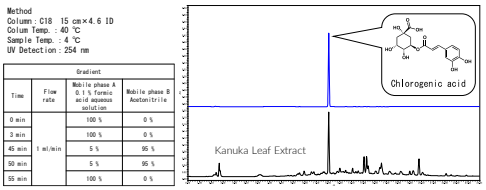

Fig.3 Component analysis of Kanuka Leaf Extract

DPPH radical scavenging assay

It was confirmed that Kanuka Leaf Extract can eliminate effectively DPPH radical in a concentration-dependent manner(Fig.4).

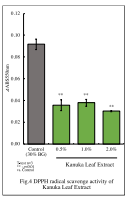

Fig.4 DPPH radical scavenge activity of Kanuka Leaf Extract

Evaluation of activity of caspase-1

The activity of caspase 1 through inflammasome formation in dermal fibroblasts was enhanced by UV-B irradiation. Compared with the UV-B Irradiation Control, the suppression was observed in Kanuka Leaf Extract added group in a concentration-dependent manner. And a significant inhibitory effect was especially confirmed in the group added with 2% Kanuka Leaf Extract(Fig.5).

Efficasy on IL-1β and IL-18 secretion

Similar to the above, Kanuka Leaf Extract showed a concentration-dependent inhibitory effect on the secretion of IL-1β and IL-18 induced by UV-B irradiation(Fig.6,7).

Efficasy on FLG-2 expression

Expression of FLG-2 in normal human epidermal cells cultured with UV-irradiated culture supernatant was significantly reduced. However, Kanuka Leaf Extract inhibited the downregulation of FLG-2 expression in a concentration-dependent manner(Fig.8).

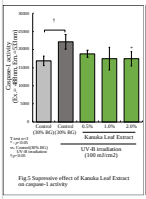

Fig.5 Suppressive effect of Kanuka Leaf Extract on caspase-1 activity

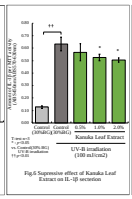

Fig.6 Suppressive effect of Kanuka Leaf Extract on IL-1β secretion

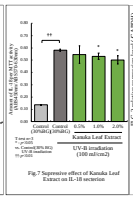

Fig.7 Suppressive effect of Kanuka Leaf Extract on IL-18 secretion

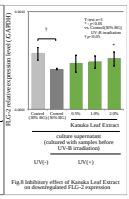

Fig.8 Inhibitory effect of Kanuka Leaf Extract on downregulated FLG-2 expression

Suppression test for erythema formation by ultraviolet rays(*in vivo*)

It was found that the value of the lotion-applied area containing 0.5% Kanuka Leaf Extract was statistically significantly lower than the increase ratio of the erythema of the control lotion-applied area (Fig. 9).
Figure 10 shows a typical test area. It was shown that the degree of erythema in the lotion-applied area containing 0.5% Kanuka Leaf Extract was low compared with control lotion applied area.

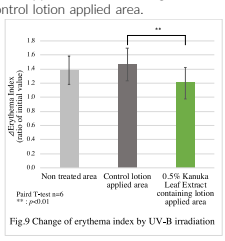

Fig.9 Change of erythema index by UV-B irradiation

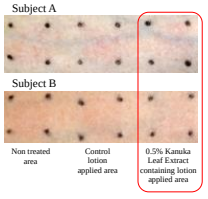

Fig.10 The photograph of typical test area 1 day after UV-B irradiation

Conclusions:

We have confirmed that there is inflammasome-related inflammation in dermal fibroblasts as well. Then, we found Kanuka Leaf Extract as a natural functional ingredient that can suppress it and prevent skin aging(inflammaging).

References:

[1] Laurence Feldmeyer, Martin Keller, Gisela Niklaus, Daniel Hohl, Sabine Werner, and Hans-Dietmar Beer; The Inflammasome Mediates UVB-Induced Activation and Secretion of Interleukin-1β by Keratinocytes; Current Biology 17, 1140–1145, July 3, 2007
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[3] Maedeh Akhlaghi, Maryam Karrabi, Hadi Atabti, Amir Raoofi & Amin Mousavi Khaneghah (2022) Investigation of the role of IL18, IL-1β and NLRP3 inflammasome in reducing expression of FLG-2 protein in Psoriasis vulgaris skin lesions, Biotechnic & Histochemistry, 97:4, 277-283
[4] Magdalena Ciążyńska, Igor A. Bednarski, Karolina Wódz, Joanna Narbutt, Aleksandra Lesiak (2020) NLRP1 and NLRP3 inflammasomes as a new approach to skin carcinogenesis, Oncology Leters 19, 1649-1656.