Investigating the cellular and molecular cascade associated with anti-inflammatory and anti-melanogenic potential of *Eucalyptus globulus* leaf extract in vitro

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Introduction:

The increasing demand for natural compounds has provoked researchers to conduct further explorations of renewable natural resources that possess biologically active substances. Furthermore, eco-friendly extraction methodologies, and modern explorative analyses are a major concern to explore new target molecules in active substances discovery.

As a plant widely cultivated in China, *Eucalyptus globulus* is widely used in traditional Chinese medicine (TCM). Eucalyptus is mainly used for the production of cellulose pulp and secondly for the production of paperboard. In both cases, eucalyptus bark is separated as waste and used as fuel. Yazaki and Hillis detected ellagitannin, methyl and glycosyl derivatives of ellagic acid, and free ellagic acid and gallic acid in methanol extract of various eucalyptus bark(Yazaki & Hillis, 1976). Gallic tannins and catechins were found in tannin extract obtained after acid hydrolysis of eucalyptus bark(Fechtal & Riedl, 1991). *E. globulus* bark methanol extract is characterized by being rich in total phenols, polymeric proanthocyanidins and ellagitannins(Conde, Cadahia, Diez-Barra, & García-Vallejo, 1996)(Cadahía, Conde, de Simón, & García-Vallejo, 1997).

In the present study, a mixture of *Eucalyptus globulus* leaf (EGL) extract was evaluated in B16F10 and Raw 264.7 cells to further elucidate the anti-inflammatory and anti-melanogenic potential2 (in vitro). Our primary objective is to broaden our understanding of *Eucalyptus globulus* leaf extract at the cellular level using established in vitro models. This will not only strengthen our knowledge but will further explore horizons in the research and development of new cosmetics.

Materials & Methods:

- **Extraction Process**

Eucalyptus globulus leaves were extracted ultrasonically by 75% ethanol and the obtained extract was eluted by 90% ethanol with AD-8 macroporous material and diluted to 10 mg/mL concentration.

- **Melanin Inhibitory Effect**

B16F10 cells were seeded at a density of 4x10^4 cells/mL into a 24-well culture plate and left to grow overnight. The old media was substituted with fresh media and treated with various concentrations of EGL extract and IBMX. After incubation for 72 hours, cells were rewashed with PBS, lysed using 1 N NaOH. The absorbance of the sample at 405 nm was measured with a microplate reader.

- **NO Inhibitory Effect**

Raw 264.7 cells were seeded at a density of 4x10^4 cells/mL into a 24-well culture plate and left to grow overnight. The old media was substituted with fresh media and treated with various concentrations of EGL extract *and LPS. After incubation for 24 hours, the amount of NO in the supernatant was detected using a commercially available NO detection kit.*

- **Gene Expression Analysis**

Cells were seeded at a density of 4x10^5 cells /mL into a 6-well culture plate and left grow overnight. The old media was substituted with fresh media with EGL extract for 24 h. Trizol method was employed to harvest total RNA from cells. Reverse transcription was performed by using PrimeScriptII1st Stand cDNA Synthesis Kit (Takara), while qPCR was done in ABI QuantStudio 3 thermal cycler using TB Green® Fast qPCR Mix (Takara). All acquired data were obtained using ABI QuantStudio 3 thermal cycler and analyzed with QuantStudio™ Design & Analysis software version 1.4.3 based on the cycle threshold (DDCT) method(Livak & Schmittgen, 2001).

Results & Discussion:

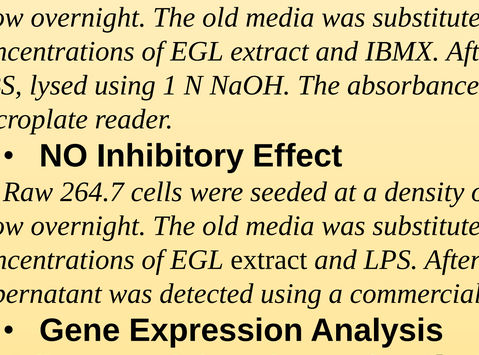

Figure 1. The effect of EGL extract of NO content on RAW264.7 cells induced by LPS, compared with LPS group. Inhibition ratio: 250 μg/mL, 75.32%; 125 μg/mL, 52.17%; 62.5 μg/mL, 25.14%; DXMS, 200μg/mL, 89.67%.

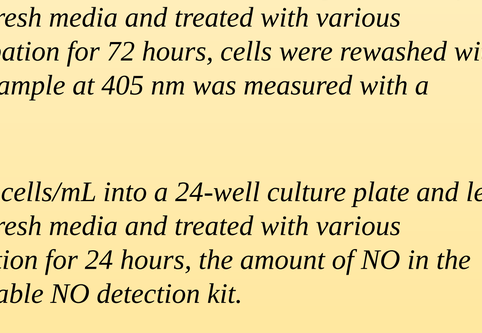

Figure 2. The effect of EGL extract of Melanin inhibitory rate, compared with IBMX group. Inhibition ratio: 125 μg/mL, 84.52%; 62.5 μg/mL, 78.38%; 31.25 μg/mL, 64.55%; arbutin, 300 μg/mL, 44.85%.(*P<0.05)

Results & Discussion:

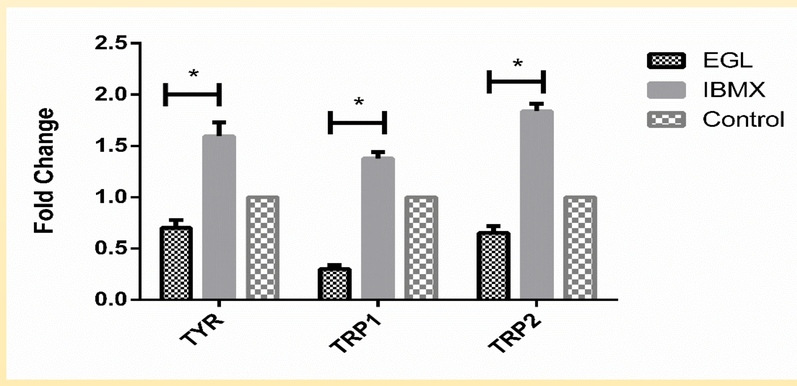

Figure 3. Relative expression of TYR. TRP-1 and TRP-2, compared with IBMX group.

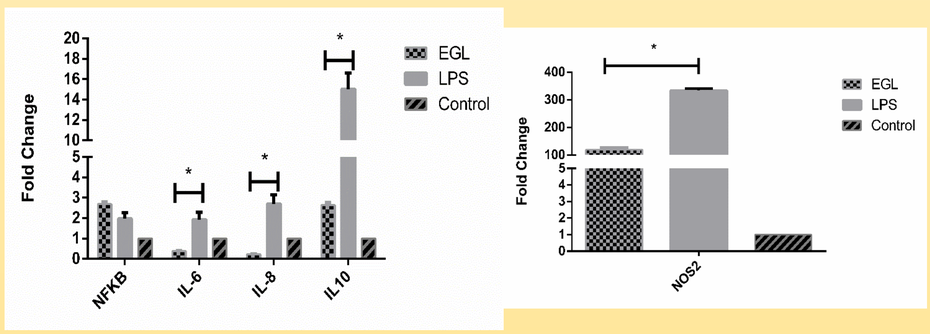

Figure 4. The effect of EGL extract of NFkB,IL-6, IL-8, IL-10 and NOS2. Compared with LPS group.

The anti-melanogenesis effect of EGL extract on IBMX-induced melanogenesis was evaluated in B16/F10 melanoma cells, and the results showed that EGL extract inhibited IBMX-induced melanogenesis in B16/F10 melanoma cells. In the melanin production induced by IBMX, the down-regulation of the expression of tyrosinase, tyrosinase-related proteins TRP-1 and TRP-2 can inhibit the accumulation of melanin. Therefore, EGL extract shows in vitro anti-melanogenesis effect in B16/F10 melanoma cells, so it can be used as a skin whitening agent in the cosmetics market. (*P<0.05)

Conclusions:

Eucalyptus globulus leaf extract have potent anti-oxidant, antimicrobial, immunoregulatory, analgesic and anti-inflammatory properties. Here, we investigated its anti-inflammatory and Anti-melanogenic activity in LPS-activated Raw 264.7 macrophages and IBMX-treated melanoma cells (in vitro). We further demonstrated that the extract inhibited the expression levels of IL-6，IL-8，NO，iNOS and IL-10 by reducing inflammatory pathways in the LPS-activated Raw 264.7 macrophages and TYR, TRP1 and TRP2 via reducing melanin synthesis signaling pathways in melanoma cells. These results further suggested that the leaf extract from *Eucalyptus globulus* have anti-inflammatory and anti-melanogenic properties and in near future, can be utilized as a natural raw material in the cosmetic industry.

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