

## A phloroglucinol from *Melicope ptelefolia* preserves barrier integrity of lipopolysaccharide-induced endothelial cells: study on junctional proteins and cytoskeleton remodeling

### ABSTRACT

Under normal condition, the endothelial cells form a compact monolayer to line the blood vessel. The integrity of this monolayer can be disrupted by lipopolysaccharide (LPS), where LPS could increase the permeability of the endothelium via dissociation of cell-to-cell junctions and rearrangement of cytoskeleton. Prolonged LPS-induced inflammation may lead to excessive plasma leakage and leukocyte migration, leading to fatal outcomes such as shock and multiple organ dysfunctions. Control on this hallmark of inflammation was suggested as a mean of therapeutic interference to improve inflammation. In this study, we investigated the capability of tHGA on maintaining barrier integrity of LPS-induced endothelial cells. tHGA is a phloroglucinol found in *Melicope ptelefolia*. The compound was previously proven to have strong therapeutic potential with anti-asthma, anti cancer and anti-inflammatory properties. In murine experimental asthma model, tHGA has been shown to inhibit cytokine secretion and leukocyte infiltration, both of which are hallmarks of LPS-induced inflammation. Therefore, it is highly probable that tHGA may have anti-inflammatory effects on LPS-induced inflammation, Human Umbilical Vein Endothelial Cells (HUVECs) were used as an in vitro representative of endothelial cells. HUVECs were seeded to confluence in cell culture insert, where the cells were pretreated with tHGA and induced with LPS. Tracer dyes or monocytes were then allowed to pass the endothelium to examine severity of vascular leakage. To examine F-actin rearrangement, pretreated HUVECs were stained with Alexa-Fluor 488 Phalloidin prior to microscopic examination. The effect of tHGA on preserving junctional protein expression were examined via Western Blot. tHGA reduced permeability and monocyte transendothelial migration at 5 $\mu$ M and 20 $\mu$ M. Furthermore, tHGA inhibited LPS-induced cytoskeletal rearrangement of HUVECs. Additionally, tHGA is able to preserve the protein expression of F-cadherin and Occludin. tHGA is able to preserve barrier integrity of LPS-induced endothelial cells via preservation of actin cytoskeleton and junctional proteins.

**Keyword:** *Melicope ptelefolia*; Phloroglucinol; Junctional protein; Cytoskeleton remodeling; Barrier integrity; Inflammation