"*In Vitro* Reversal Effect of Zerumbone on Allergen-Induced Airway Epithelial Disruption in Asthma"

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Human airway system is lined by epithelial cells attached to each other by junctional system. This epithelial barrier can be disrupted by common allergen such as house dust mites (HDM) and consequently resulting in airway diseases such as asthma. Zerumbone was found to possess anti-asthmatic effect by modulating Th1/Th2 cytokines. However, there is yet study done to assess whether Zerumbone protects the epithelial barrier from junctional disruption before the allergen invades into the immunological barrier of the airway system. This study aims to investigate the effect of Zerumbone on HDM-induced airway epithelial barrier disruption. A human bronchial epithelium 16HBE14o- cell line, which possesses characteristics of *in vivo* human airway lining, was treated with a range of concentrations of Zerumbone (6.25 μM-200 μM) for 24 hours to investigate the non-cytotoxic concentrations of the compound on 16HBE14o- cells. As a result of the cytotoxicity assay, the cells were then co-treated with 100 μg/mL HDM and three selected concentrations of Zerumbone (6.25 μM, 12.5 μM and 25 μM) for 24 hours in subsequent experiments. Transepithelial electrical resistance (TEER) assay and FITC-Dextran permeability assay were carried out to study the effect of Zerumbone on HDM-induced junctional integrity and permeability of the epithelial monolayer respectively. Zerumbone has shown protective effect on the junctional integrity as all three concentrations possess significantly lower TEER change by suppressing the change as much as 27%, 48.8% and 49.7% respectively, compared to the HDM group. The permeability assay results complement the results of the TEER assay as 6.25 μM, 12.5 μM and 25 μM of Zerumbone showed reduction in the ratio of flux of FITC-Dextran through the epithelial monolayer by 37.8%, 58.6% and 62.2% respectively, compared to the HDM group. The localization of junctional proteins, occludin and ZO-1, was studied by using immunofluorescence (IF) while the protein and gene expression were studied by immunoblotting and Real Time-Polymerase Chain Reaction (qPCR) respectively. However, no significant changes could be seen in both protein and gene expression of occludin and ZO-1 as compared to the HDM group. This study has proven that Zerumbone preserves both HDM-induced junctional integrity and permeability by maintaining the localization of occludin and ZO-1 without affecting both proteins and gene expression. As a conclusion, Zerumbone possesses protective effect on HDM-induced airway epithelial barrier disruption by preserving the junctional permeability and localization without affecting the junctional protein and mRNA expression. This study has proven that Zerumbone possess protective effect on the allergen-induced airway barrier disruption therefore enabling it to be further studied as a potential drug for epithelial barrier-related airway inflammatory diseases.

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