4th Wael Almahmeed and International Atherosclerosis Society Research Training Fellowship Grant for the Middle East and Africa

Title of the Project: Evaluation of the effect of *Plectranthus glandulosus* leaf extracts, on ELOVL Fatty Acid Elongase 6 overexpression in mouse embryonic fibroblast (MEF) mediated by adenovirus in the treatment of atherosclerosis

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Dates of Activity: From June 15 to December 5

Introduction

Cardiovascular diseases are the wold's largest killers. The known synthetic drugs which are used in the treatment of atherosclerosis such as statins and fibrates have many side effects. These have made scientists look towards for new target and new anti-atherosclerotic agents derived from plants. Our preliminary studies on *Plecthrantus glandulosus* Hook. F. (Lamiaceae) has shown that the plant exhibited antioxidant and anti-inflammatory activities (Zouheira et al., 2020); *in vitro* antioxidant properties and inhibitory effect on copper sulfate (CuSO4)-induced oxidation in human low-density lipoprotein (Zouheira et al., 2020); *In Vitro* Antilipidic and Antithrombotic activities (Zouheira et al., 2022).

Therefore, more information about *P.glandulosus* mechanism of actions related to the effect of this plant on the elongation of long-chain fatty acid family member 6 (Elovl6)-associated foam cell formation in macrophages are necessary to be evaluated.

In this project, we proposed to overexpress ELOVL6 in mouse embryonic fibroblast (MEF) mediated by adenovirus. To investigate it mechanistically, we

decided to use differentiated THP1 cells and RAW cells (macrophage**)** instead of MEF. We also use 3XFLAG-TAG huELOVL6 plasmid instead of adenovirus to overexpress Elovl6 in THP1 cells. Furthermore, the RAW cells LPS was used to induce the inflammation.

Methods and Execution

1. Raw cells were seeded in 24 well plate and further incubated for 24 h. Then, the cells were treated with extracts and subsequently incubated for 2 h. After pre-incubation, LPS was added to the wells and followed by the 16 h incubation. Then, the cells were collected and further subjected to RNA extraction and gene expression analysis by RT-PCR. Additionally, protein extraction, western blot analysis, and Immunofluorescence were conducted.
2. Differentiated THP1 cells were seeded in the 6 well plate. After 24 hours incubation, the cells were transfected with 3XFLAG-TAG huELOVL6 plasmid to overexpress ELOVL6 for another 24 h incubation time. Then, the extracts were added and followed by 24 h incubation. RNA was collected for gene expression analysis by RT-PCR. Additionally, immunofluorescence was also conducted.

Results

Inflammation and lipid toxicity are the most common characteristics of atherosclerosis. Our results confirmed that ethyl acetate fraction and hydroethanolic extract of *P.glandulosus* (PG) exhibited the favorable effects as anti-inflammatory and anti-lipogenic than that in its aqueous extract. The results of gene expression analysis showed that ethyl acetate fraction of PG possessed the potential anti-lipogenic activity by reducing SREBP1 mRNA expression. This could be the main point of the mechanism of action of this fraction to further downregulate the gene expression of Elovl6, SCD, FACD3, and HMGCR mRNA expression as lipogenic markers. Surprisingly, this fraction also exhibited the reduction in pro-inflammatory genes. As obtained in immunofluorescence analysis, this fraction showed the markedly reduction of NF-kB as the main signaling pathway to amplify the production of pro-inflammatory agents. This might be the main cornerstone of this fraction to downregulate the IL-1β and IL-6 mRNA expression in macrophage cell line mechanistically.

The results of hydroethanolic extract of PG showed the promising effect as anti-lipogenic effect by downregulating the mRNA expression of Elovl6 and HMGCR. Besides, this extract also possessed its anti-inflammatory activity by downregulating TNF-α and IL-6 mRNA expression in macrophage cell line.

Difficulties

We did not obtain any result from Western Blot analysis. This could be due to some mistakes during the processing of the western blot.

Personal impact

This fellowship allowed me to learn many techniques and meet Lab members that I can collaborate with, in the future. I started from zero, without knowing anything in cell culture as well as in all methods I used. All these will impact positively on my research and upcoming career.

Acknowledgements

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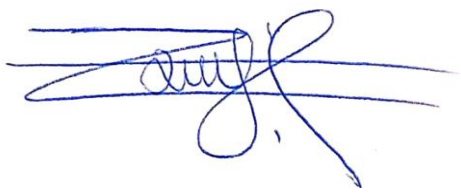
Applicant,

Supervisor, Hitoshi SHIMANO



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