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P-23**Hesperidin alleviates Experimental autoimmune encephalomyelitis in C57BL/6 mice via regulating Treg and T helper 2 polarizations**

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Abstract

Introduction: Multiple sclerosis (MS) is the most abundant central nervous system inflammatory disease, which is due to the reaction of auto reactive T cells with own myelin proteins, leading to physical disorder and paralysis among people suffering from disease. Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of this disease, causing demyelination and inflammatory disease in CNS of the animal. Hesperidin acts as an anti oxidant via inhibitory effect on HMG-CoA reductase and Cyclooxygenase and through increasing the expression of apoptotic genes. We studied effects of Hesperidin on immune responses in EAE mice.

Materials and Methods: 24 C57/BL6 female mice, aging 8-10 weeks and weighting 20 gr were used in this project. The mice were divided into 3 groups as follows: 1. Normal group 2. Control group 3. Treatment group. EAE was induced in group 2 and 3. A mixture of Myelin Oligodendrocyte Glycoprotein peptide (MOG) and freund's complete adjuvant (FCA) were injected subcutaneously; an intraperitoneal injection of pertussis toxin were followed, after 48 hours the injection of pertussis toxin were repeated to complete EAE induction. The treatment group was treated with 200 mg/kg Hesperidin using IP injection every day. The disease was scored and the mice were examined for the disease symptoms and their weight was recorded till the twenty first day, on day 21 they were anesthetized and then sacrificed. Histological study was performed by staining the brain sections using hematoxylin eosin. FoxP3, CD4, IL-4, IL-10, IL17, IL23 and IFN- γ expression were analyzed in splenocytes using real-time PCR. The validation of their protein production was measured using flow cytometry, and the secretion of IL-4, IL-10, IL-17, IL-23 and IFN- γ were validated using ELISA of the cultured splenocyte's supernatant.

Results: Our results showed mean weight on day21 had an increase ($p < 0.05$) in group 3 mice (19.01 ± 1.63 g) compared to mice in group 2 (17.67 ± 0.51 g). Clinical scores showed a decrease ($p < 0.01$) in group 3 (1.86 ± 0.9) in comparison to group 2 (3.71 ± 0.5). Histological studies revealed significant lower lymphocytic infiltration and demyelination in group3 compared to group2. Splenocytes proliferation showed reduction in group3 in comparison to group 2 ($p < 0.1$). Cytokines and transcription factors related to Th2 and Treg showed significant increase, while cytokines related to th1 showed significant decrease in all levels. On the other hand cytokines of Th17 did not show significant change.

Conclusion: Our results indicate that Hesperidin can alleviate Experimental autoimmune Encephalomyelitis, which acts at least in part through driving the polarization of Treg, and also triggering a shift from Th1 to Th2 subsets. These findings introduce Hesperidin as a potential compound for future studies on Multiple Sclerosis and its animal models.

Key words: EAE, Hesperidin, Th1, TH2, Th17, Treg.

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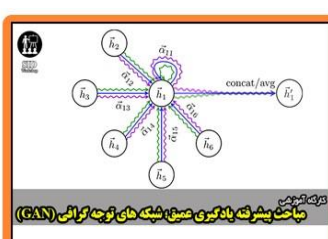


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