The Anti-inflammatory Effect of Harmonia axyridis on Lipopolysaccharide -induced inflammatory Response in RAW 264.7 Cells

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Abstract

Harmonia axyridis is known to display diverse biological activities such as growth promotion. However, few studies have been directed on the

effect of *Harmonia axyridis* on skin inflammatory function. In this study, we aimed to investigate the anti-inflammatory effect of Harmoniasin gene fragment (HaGF) peptide from *Harmonia axyridis* on macrophage cell. During the entire experimental period, 5, 25, 50, 100 µg/ml of HaGF had no cytotoxicity. In these concentrations, HaGF was showed that iNOS and COX-2 inhibition activity 51% and 49%. In addition, HaGFE reduced the release of inflammatory cytokines including TNF- α , IL-1 β , IL-6. The results above indicate that HaGF significantly reduces the effect of oxidative and inflammatory cytokines.

Materials&Methods

1. Cell viability

: measured by Carmichael¹⁾ method. 2. NO assay

3. Cytokine assay (ELISA) kit

4. Western blot analysis

Results











Fig. 1. Cell viability of HaGF on Raw 264.7 cell. Raw 264.7 cells were treated with 5, 10, 25, 50, 100, 500 µg/ml of HaGF dissolved in media for 1 h prior to the addition of LPS (1 μ g/ml), and the cells were further incubated for 24 h. Data represent the mean \pm S.D. with eight separate experiments.



Fig. 2. Inhibitory effects of HaGF on the production of nitric oxide Raw 264.7 cells. Raw 264.7 cells were cultured with LPS (1 μ g/ml) in the presence or absence of HaGF for 24 h to determine the level of NO. (Nor : LPS not induced group, Con : LPS induced group) The data represent the mean \pm SD of three separate experiments (significant as compared to control *p < 0.05).



LPS

of

%)

NO



Fig. 3. Effect of HaGF on the production of cytokines stimulated by LPS. Production of TNF- α (a), IL-1 β (b), IL-6 (c) were measured in the medium of Raw 264.7 cells cultured with LPS (1 μ g/ml) in the presence or absence of HaGF for 24 h. The amount of TNF- α was measured by immunoassay as described in materials and methods. (Nor : LPS not induced group, Con : LPS induced group) Data represent the mean \pm S.D. with three separate experiments. Oneway ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control *p < 0.05, **p< 0.01).

Conclusion

Our results suggest that Harmoniasin gene fragment may be a significant factor and can be used a therapeutic modality in managing chronic inflammatory diseases.

Reference

1) Carmichael J, DGraff WG, Gazdar AF, Minna JD and Mitchell JB (1987) Evaluation of a tetrazolium based semiautomated colorimetric assay; assessment of chemosensitivity testing. Cancer Res. 47(4), 936-942.

Fig. 4. Inhibitory effects of HaGF on the protein levels of iNOS and COX-2 in RAW 264.7 cells. Raw 264.7 cells (5 X 10⁵) cells/ml) were pre-incubated for 24 hr, and the cells were stimulated with lipopolysaccharide (1 µg/ml) in the presence of complex extracts sample (5, 25, 50, 100 µg/ml) for 24 hr. (Nor : LPS not induced group, Con : LPS induced group) Data represent the mean \pm S.D. with three separate experiments. One-way ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control p < 0.05, p < 0.01).