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Inhibitory effect of Butanol fraction of Ecklonia cava on inflammatory mediators in RAW 264.7 cells

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ABSTRACT

Objectives : The purpose of this study was to investigate the anti-inflammatory effects of Ecklonia cava butanol extract (BFEC) on RAW 264.7 cells.

Method : To evaluate of anti-inflammatory of BFEC, We examined cytokine and Nitric oxide(NO) production in lipopolysacchride (LPS)-induced RAW 264.7 cell

Result : Extract of BFEC inhibit LPS-induced interleukin (IL)-6, NO production in human monocyte RAW 264.7 cells.

Conclusion : BFEC down-regulated LPS-induced IL-6, NO production, which may be provide a clinical basis for anti-inflammatory properities of BFEC

Key words : Ecklonia cava butanol extract (BFEC), anti-inflammation, interleukin (IL)-6, Nitric oxide(NO)

Introduction

Ecklonia cava is widely used for the treatment of various diseases. Several of the pharmacological actions of Ecklonia cava have been elucidated. Ecklonia cava is known to have Anti-HIV-1[1] activity, immunomodulatory effect[2] and anti-allergic effects[3]. Recent studies have shown that materials derived from Ecklonia cava exert antioxidant and anti-inflammatory activities that are commonly found in human intestines[4]. However, no studies have been conducted to evaluate the anti-inflammatory activities of butanol fraction of Ecklonia cava (BFEC) in RAW 264.7 macrophages to date.

NO plays an important role in the regulation

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of many physiological functions[5]. NO is known to be synthesized from L-arginine by nitric oxide synthase (NOS)[6]. Thus, NO production by iNOS may reflect the degree of inflammation and provides a measure to assess the effect of drugs on the inflammatory process[7]. In this study, We investigated the cell based anti-inflammatory activity of BFEC

Materials and Methods

1. Plant material and extract preparations

Ecklonia cava collected at Jeongado Wando Korea. A voucher specimen (No. 07-32) was deposited in the Laboratory of Herbalogy, College of Pharmacy, Wonkwang university, Iksan, Korea. Ecklonia cava were extracted with 100% EtOH three times for 2 hours under heating mantle-reflux. The resultant extract was condensed to 1 L in a rotary vacuum evaporator (N-1000S, EYELA, Japan) and in succession was partitioned with n-hexane, CH_2Cl, EtOAc, n-BuOH and H_2O).

2. Drugs and Chemicals

$NaHCO_3$ and Na_2CO_3 were purchased from Daejung, Korea. RPMI 1640, penicillin and streptomycin were obtained from Hyclone (Hyclone Labs Logan, UT). Bovine serum albumin (BSA), LPS, and 3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyl-thiazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA).

3. Cell Culture

The murine macrophage cell line, RAW 264.7, was obtained from the Korea Research Institute of Bioscience and Biotechnology (South Korea) and grown in RPMI 1640 Medium containing 10% fetal bovine serum and 100 U/ml penicillin/ streptomycin sulfate. Cells were incubated in a humidified 5% CO_2 atmosphere at 37℃. To stimulate the cells, the medium was replaced with fresh RPMI 1640 and the cells were then stimulated with LPS in the presence or absence of BFEC for the indicated periods.

4. ELISA (enzyme-linked immunosorbent assay)

Cells were seeded at a density of 5 x 10^5 cells/ml in 24 well tissue culture plates and then pretreated with various concentrations of BFEC (50 and 100 μg/ml) for 30 min prior to LPS stimulation (1 μg/ml). ELISA plates (Falcon, Becton Dickinson Labware) were then coated overnight at 4°C with anti-mouse IL-6 antibody diluted in coating buffer (0.1 M carbonate, pH 9.5) and then washed four times with PBS containing 0.05% tween 20. Non-specific protein binding sites were then blocked by incubating the plates with assay diluent (PBS containing 10% FBS, pH 7.0) for at least 1 hour. Immediately following the incubation, each sample and IL-6 standard were added to the wells, after which the plates were then incubated for 2 hours. Next,working detector (biotinylated anti-mouse IL-6 monoclonal antibody and SAv-HRP reagent) was added to each well and the plates were incubated for an addtional 1 hour. Finally, substrate solution (tetramethylbenzidine: TMB) was added to the wells, after which the plates were then incubated in the dark for 30 min. Next, the reaction was stopped using stop solution (2 N H_3PO_4) and the

absorbance at 450 nm was then measured. All standards and samples were assayed in duplicate.

Result

1. The effects of BFEC on LPS Induced NO Expression

NO is known to beassociated with many conditions, including inflammation. To examine the effect of BFEC on LPS-induced NO production in RAW 264.7 cells, cells were treated with or without BFEC for 30 min and then treated with LPS (1 μg/ml) for 24 h. The cell culture medium was then harvested, after which the NO levels were determined using the Griess reaction. The LPS treated cells produced approximately 2.5-fold more NO than the control cells; however, treatment with BFEC inhibited this NO production in a concentration-dependant manner (Fig. 1.).

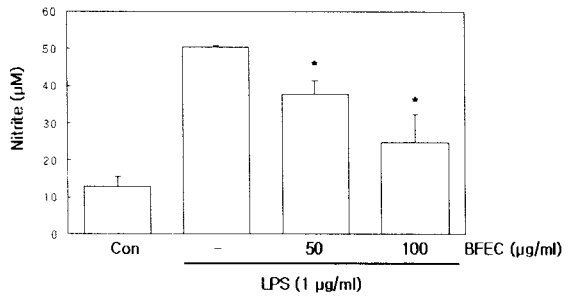

Fig. 1. Effect of BFEC on the expression of NO in LPS - stimulated RAW 264.7 cells.

RAW 264.7 cells were pretreated with the indicated concentration (50 and 100 μg/ml) of BFEC for 30 min prior to being incubated with LPS (1 μg/ml) for 24 hours. The culture supernatant was then isolated and analyzed for nitrite production.

Statistical significance: *P<0.05, as compared to the LPS treated group. Significanct differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments.

2. The effects of BFEC on LPS Induced IL-6 Expression

We investigated the effect of BFEC on the LPS-induced IL-6 release using ELISA. Pretreatment of cells with BFEC at concentrations of 50 and 100 μg/ml reduced IL-6 production (Fig. 2).

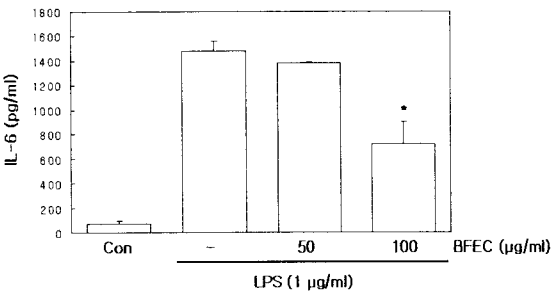

Fig. 2. Effect of BFEC on the expression of NO and IL-6 in LPS - stimulated RAW 264.7 cells.

The cells ($1x10^6$ cells/ml) were pretreated with BFEC (50 and 100 μg/ml) 30 min prior to stimulation with LPS (1 μg/ml). Twenty-four hours after LPS stimulation, the IL-6 levels of the supernatants were measured by ELISA.

Statistical significance: *P<0.05, as compared to the LPS treated group. Significanct differences between treated groups were determined using the Dunnett's t-test. Values shown are the mean ± S.E. of duplicate determinations from three separate experiments.

3. The effects of BFEC on cell viability

The cytotoxic effect of BFEC was then evaluated in RAW 264.7 cells by MTT assay (Fig. 4). Treatment with BFEC at concentrations of up to 100 μg/ml did not affect the viability of RAW 264.7 cells.

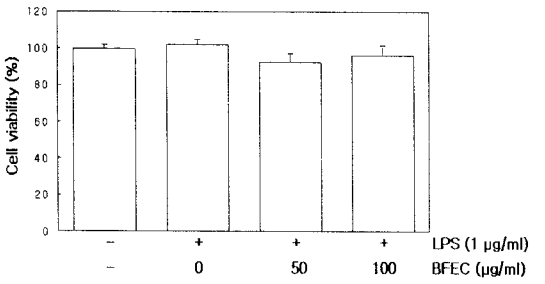

Fig. 3. The cell viability of RAW 264.7 cells assessed using an MTT assay following incubation with different doses (50 and 100 μg/ml) of BFEC for 24 h.

Discussion

Ecklonia cava is often used to treat diarrhea. To explore the mechanism underlying these beneficial effects, the effects of BFEC on macrophage functions related to inflammation were investigated. Specifically, we examined the effects of BFEC on the release of several inflammatory mediators including NO and IL-6.

Inflammation is a complex process, which involves numerous mediators. To explore the mechanism underlying these beneficial effects, the effects of BFEC on macrophage functions related to inflammation were investigated. Specifically, we examined the effects of BFEC on the release of several inflammatory mediators including NO and IL-6.

NO is known to be an important mediator of cellular communication in several systems, including macrophages of the autonomic and central nervous system[8]. However, overproduction of NO can be harmful and result in septic shock, rheumatoid arthritis, and autoimmune diseases[9]. Inhibition of NO production may have beneficial therapeutic effects in the treatment of diseases that occur due to overproduction of NO[10,11].

In this study, exposure of macrophages to LPS was associated with an accumulation of IL-6 and NO in the medium, suggesting that it enhanced NO production. BFEC down-regulated LPS-induced IL-6, NO production, which may be provide a clinical basis for anti-inflammatory properities of BFEC.

Therefore, we conclude that BFEC appears to have potential as a treatment for inflammatory disease and as an analgesic.

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