Active Concepts

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IL-6 ELISA Analysis

Tradename: AC Watercress Extract SF

Code: 11907

CAS #: 7732-18-5 & 84775-70-2

Test Request Form #: 8067

Lot #: N210513F

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **AC Watercress Extract SF** - treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of IL-6 in each sample.

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Materials

A. Kit:	Human IL-6 ELISA Kit (Invitrogen; BMS213-2)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (HDFa) (ATCC; PCS-201-121)
E. Media/Buffers:	Fibroblast Basal Media (ATCC; PCS-201-030); Fibroblast Growth Kit (ATCC; PCS-201-040); Phosphate Buffered Saline (PBS)
F. Culture Plate:	Falcon flat bottom 12-well tissue culture treated plates
G. Reagents:	Lipopolysaccharide (LPS) (1µg/mL); Dexamethasone (10µM)
H. Other:	Sterile disposable pipette tips; wash bottles

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete media. 1%, 0.1%, 0.01% concentrations of **AC Watercress Extract SF** were added to complete media containing 1µg/mL LPS and incubated with fibroblasts for 72 hours. Complete media containing 1µg/mL LPS was used to create an inflammatory environment and dexamethasone (DEX) in the presence of LPS was used as a positive control to quell inflammation.

Standards were prepared in concentrations ranging from 200pg/mL to 0pg/mL. Prior to samples being added all wells were washed with 1X wash buffer four times. 50µL of assay buffer was added to the sample and assay control wells. 50µL of the controls and samples were added to appropriate wells. 100 µL of each standard concentration was added to the appropriate well. 50 µL of Biotin-conjugate were added to all wells. After a two hour incubation at room temperature and washing, 100µL Streptavidin-HRP was added to all wells. Following an one hour incubation and washing, 100 µL of TMB substrate solution was added for the colorimetric reaction allowing to sit for 10 minutes avoiding light. 100 µL stop solution was added to stop the reaction. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **AC Watercress Extract SF** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

AC Watercress Extract SF at a concentration of 0.1% was able to decrease IL-6 production.

IL-6 production percent decrease is calculated by the following formula:

$$Percent\ (\%)\ Change = \frac{IL\ 6\ Concentration_{Sample} - IL\ 6\ Concentration_{1\mu M/mL\ LPS}}{IL\ 6\ Concentration_{1\mu M/mL\ LPS}} \times 100$$

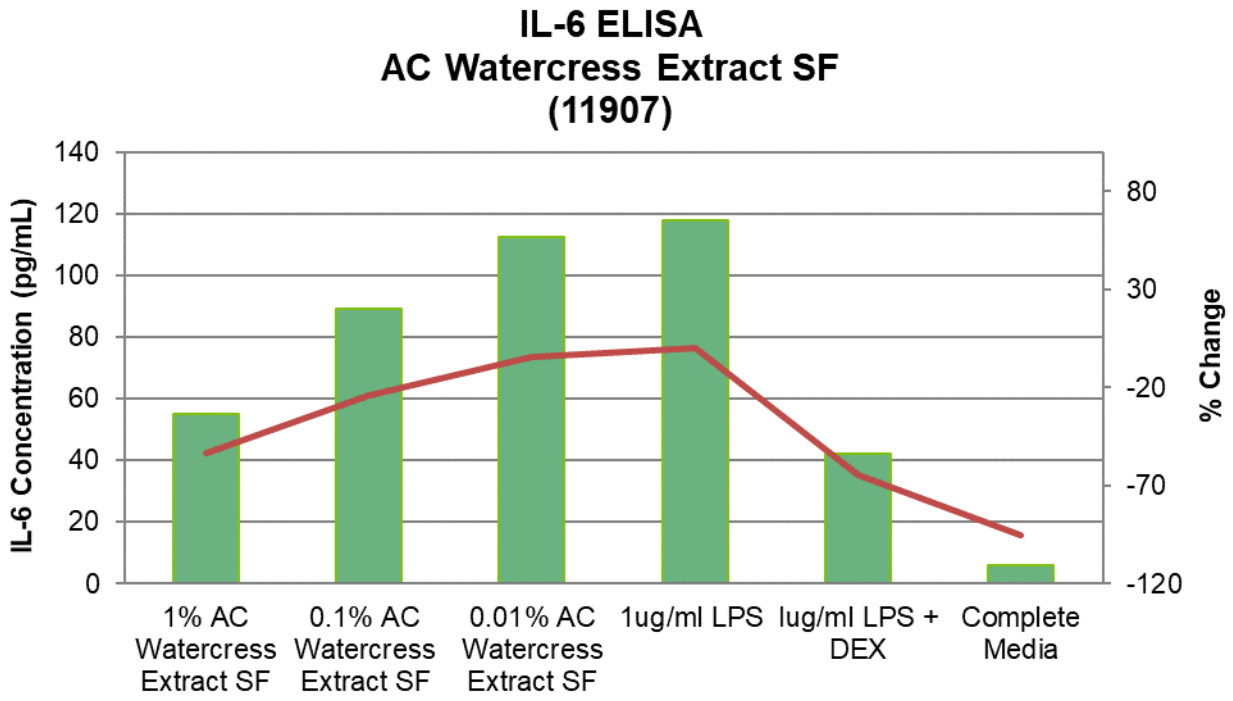

Figure 1: AC Watercress Extract SF -treated fibroblasts IL-6 concentrations and percent change

Discussion

As shown in figure 1, **AC Watercress Extract SF (11907)** exhibited anti-inflammatory effects on LPS-treated fibroblasts. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. It can therefore be concluded that at normal use concentrations **AC Watercress Extract SF** enhances soothing and anti-aging properties.