



Tradename: ABS White Willow Bark Extract Powder

Code: 10229

CAS #: 84082-82-6

Test Request Form #: 258

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

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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- κ B) 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 **ABS White Willow Bark Extract Powder**-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 collagen in each sample.

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Materials

- | | |
|----------------------------------|---|
| A. Kit: | IL-6 ELISA Kit (Biosource; KAC1261) |
| 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 (NHDF) (Lonza; CC-2511) |
| E. Media/Buffers: | Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL) |
| F. Culture Plate: | Falcon flat bottom 12-well tissue culture treated plates |
| G. Reagents: | Lipopolysaccharide (LPS) (1µg/mL); Solution A (human plasma with preservatives);
Solution B (buffer with preservative) |
| 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 DMEM. 1%, 0.1%, 0.01% concentrations of **ABS White Willow Bark Extract Powder** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used as a negative control.

Standards were prepared in concentrations ranging from 0pg/mL to 2476pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. 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 **ABS White Willow Bark Extract Powder** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

Results

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

ABS White Willow Bark Extract Powder, at a concentration of 1% was able to decrease IL-6 production compared to our positive control.

IL-6 levels are expressed by the following formula:

$$= \text{Average}_{IL-6 \text{ Concentrations}} \times \text{Dilution Factor}$$

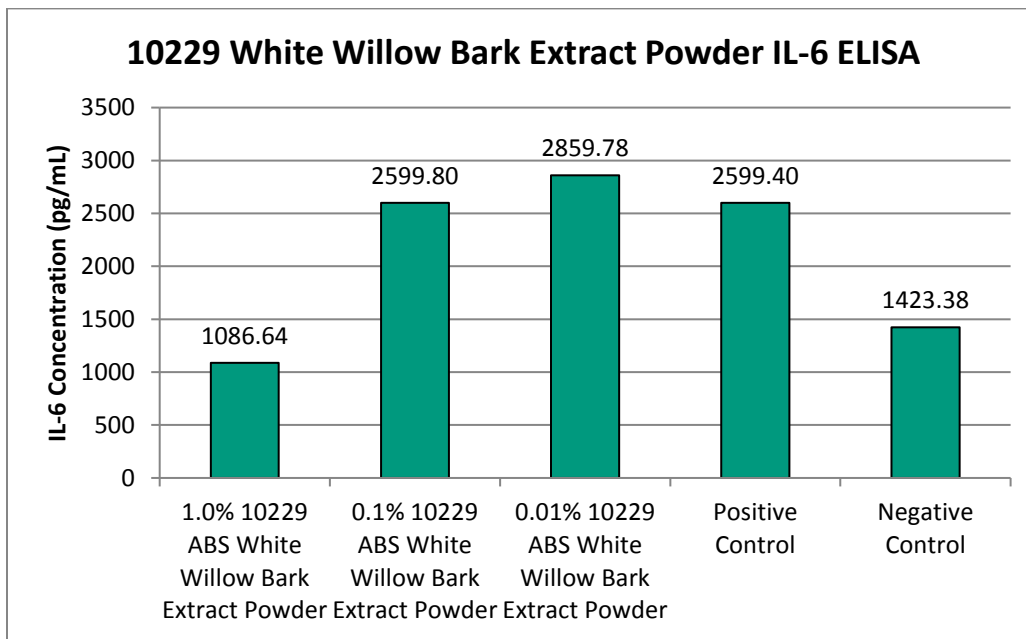


Figure 1: ABS White Willow Bark Extract Powder-treated fibroblasts IL-6 concentrations

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

$$= \frac{\text{Positive Control}_{Avg.Concentration} - \text{Sample}_{Avg.Concentration}}{\text{Positive Control}_{Avg.Concentration}} \times 100$$

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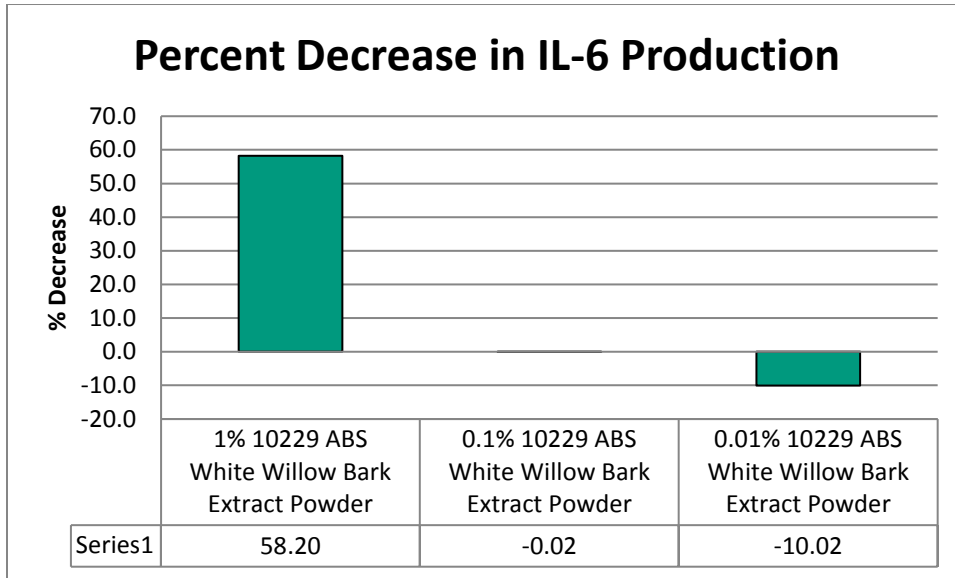


Figure 2: Percent decrease in IL-6 production compared to positive control

Discussion

As shown in figure 1, **ABS White Willow Bark Extract Powder** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **ABS White Willow Bark Extract Powder** appears to be dose dependent. 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. For these reasons, we can assume **ABS White Willow Bark Extract Powder** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.