Active Concepts

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

IL-6 ELISA Analysis

Tradename: AC Melatonin Liposome SF

Code: 61012

CAS #: 7732-18-5 & 123465-35-0 & 73-31-4

Test Request Form #: 5982

Lot #: N200114B

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

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 Melatonin Liposome 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.

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Materials

A. Kit:	Human IL-6 ELISA Kit (Thermo Scientific; EH2IL6)
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:	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); 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 DMEM. 1%, 0.1%, 0.01% concentrations of **AC Melatonin Liposome SF** were added to complete DMEM 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 2476pg/mL to 0pg/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 **AC Melatonin Liposome SF** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

Results

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

AC Melatonin Liposome SF at a concentration of 0.01% was able to decrease IL-6 production.

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

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

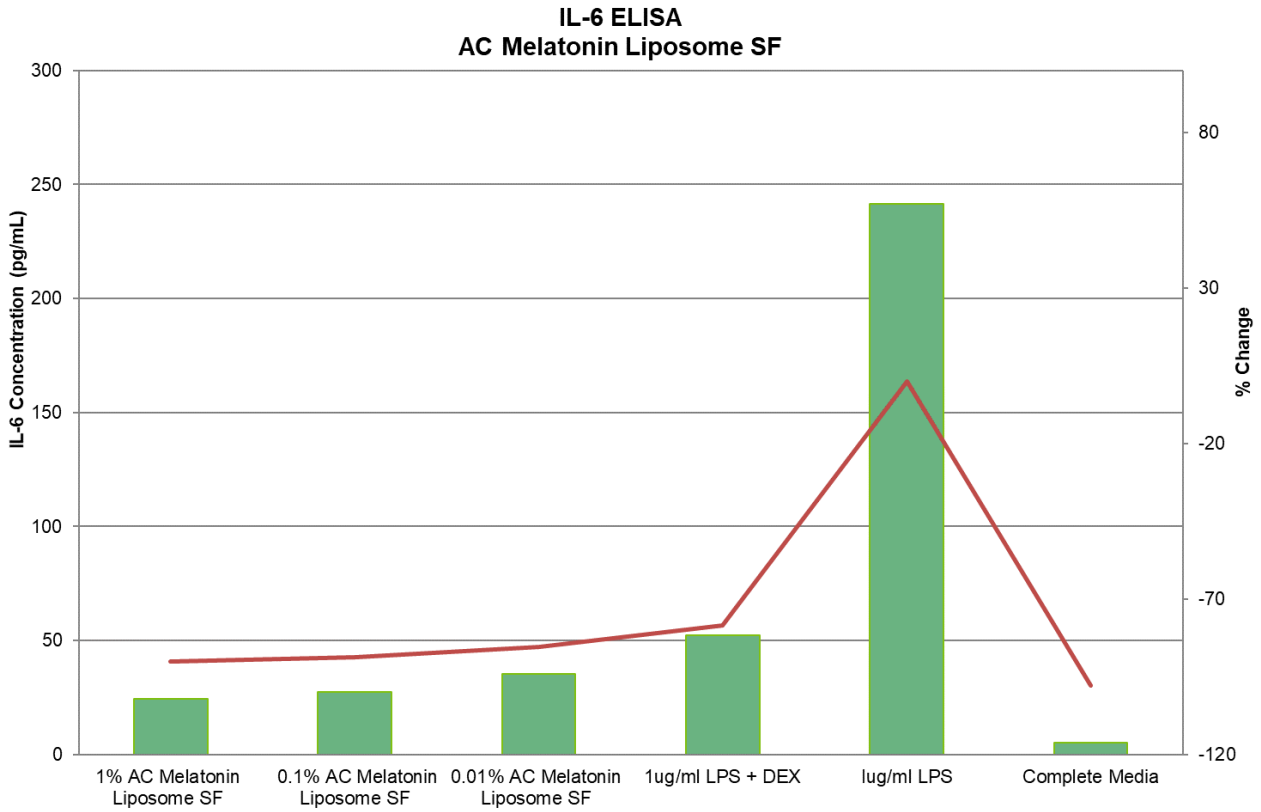

Figure 1: AC Melatonin Liposome SF-treated fibroblasts IL-6 concentrations and percent change

Discussion

As shown in figure 1, **AC Melatonin Liposome SF (61012)** 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 Melatonin Liposome SF** enhances soothing and anti-aging properties.