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Research Article

Anti-inflammatory activity of Mi LABS Joint Support supplement (MLJS-01) in RAW 264.7 cell lines

T. K. Renish*[1]

[1]Maven iLab Pvt. Ltd., No 1, First Floor, 10th Main 2nd Cross, Nandanam Colony, Horamavu Main Road, Bangalore - 560 043. India.

Abstract

The aim of this study was to investigate the effect of Mi LABS Joint Support supplement (MLJS-01) on the production of TNF-α, an important inflammatory mediator, in RAW 264.7 cell lines. Isolated RAW 264.7 cells were activated with 1 µg/ml lipopolysaccharide in the presence of MLJS-01 (25 and 50 µg/ml) and Dexamethasone (200 µM). Tumor necrosis factor-α secretion was quantified after 4h by L-929 bioassay. MLJS-01 showed dose dependent inhibition (31.19±3.40 and 24.75±4.82% at 50 and 25µg/ml, respectively) of LPS-induced TNF-α production in RAW 264.7 cell lines. The reduced production of TNF-α in RAW 264.7 cell lines may be involved in anti-inflammatory activity conveyed by MLJS-01.

Key words: Anti-inflammatory, Joint Support, TNF-α, Macrophages, Lipopolysaccharide

***Corresponding Author: T. K. Renish,** Maven iLab Pvt. Ltd., No 1, First Floor, 10th Main 2nd Cross, Nandanam Colony, Horamavu Main Road, Bangalore - 560 043. India.

1. Introduction

Arthritis, chronic inflammatory joint condition, is one of the leading causes of disability and a major cause of reduced functional mobility in the general population, particularly in older people [1]. Commonly, synthetic analgesics and anti-inflammatory agents are used in the management of arthritis but the long term use of these agents is associated with undue side effects. As an alternative, identification of suitable drugs and preparations from natural sources is gaining increasing attention in the management of inflammatory joint conditions.

Thus, the present *in vitro* anti-inflammatory study was taken up to explore one of the possible mechanisms of action of Mi LABS Joint Support supplement (MLJS-01), a scientifically formulated dietary supplement boosted with herbal blend intended for the management of inflammatory joint conditions.

2. Material and Methods

Materials

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3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide(MTT), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum (FBS), Dexamethansone, lipopolysaccharide (LPS), RAW 264.7 and L-929 cell lines and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA.

Composition of test substance

MLJS-01 contains - Glucosamine Sulfate (700mg), Chonodroitin Sulfate (100mg), Boswellia Serrata Extract (100mg), Methylsulfonylmethane (100mg) and Herbal blend consist of Turmeric Extract, Grape Extract Pineapple Extract, Ginger Extract (120mg). Developed and Manufactured by Maven iLab Private Limited, Bangalore, Karnataka, India.

Preparation of test sample

For *in vitro* anti-inflammatory study, a definite quantity of MLJS-01 was dissolved in DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial dilutions of 25 and 50µg/ml were prepared from this for carrying out study.

Induction and measurement of TNF-α accumulation in RAW cells [2]

Step I: Trypsinized RAW cells were seeded into 6 well culture dishes at a cell population of 1.5 to $2x10^5$ cells/ml in DMEM with 10% FBS. After 24h, the cells were treated with MLJS-01 (25 and 50µg/ml) or Dexamethasone (200µM) along with 1µg/ml of lipopolysaccharide (LPS) and incubated at 37^0C with 5% CO2 for 4h. After incubation, the cell supernatant was collected, centrifuged, separated and stored at -20^0C till use.

Step II*:* L-929 monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using MEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of diluted samples from the step I were added to the cells in quadruplicate wells. The cultures were then incubated at 37^0C for 24h in 5% CO2 atmosphere. After 24h, the drug solutions in the wells were discarded and 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in PBS was added to each well. The plates were gently shaken and incubated for 3h at 37^0C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm and the percentage cell viability was calculated. The cell viability is a direct indication of inhibitory properties of MLJS-01 against LPS induced TNF-α production in RAW cells.

3. Results and Discussion

Activation of macrophage and other immune-competent cells are noted to play a major role in the manifestation of inflammation [3]. Lipopolysaccharide (LPS), a potent activator of macrophages, is known to evoke a wide range of signaling pathways in macrophage and other cell types leading to the production of inflammatory mediators [4-6]. TNF-α, a proinflammatory cytokine, is one among such inflammatory mediators [7].

RAW 264.7 macrophages were stimulated with LPS (1µg/mL) for 4h to evoke TNF-α secretion. Co-treatment of cells with MLJS-01 significantly reduced accumulation of TNF-α in RAW 264.7 macrophages. It was a dose dependent inhibition of TNF-α with

31.19±3.40 and 24.75±4.82% at 50 and 25µg/ml of MLJS-01, respectively as mentioned in table 1. In this study we report that the scientifically formulated Mi Labs Joint Support supplement (MLJS-01) intended for the management of joint health, may be showing its effect by inhibiting inflammatory mediators which are responsible for causing pain and immobility in joints.

Table 1: Effect of MLJS-01 on LPS induced TNF-α production in RAW cells

Sl. No.	Samples	Concentration tested	% TNF-α inhibition
1	MLJS-01	50 µg/ml	31.19±3.40
		25 µg/ml	24.75±4.82
2	Dexamethasone	200 µM	77.12±1.93

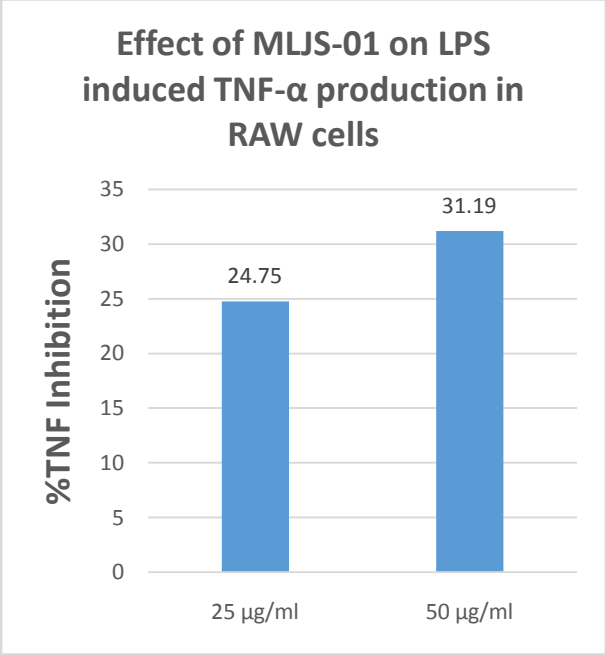


Figure 1. Effect of MLJS-01 on LPS induced TNF-α production in RAW cells

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