

The inhibition study of IL6 with the in vitro bioactivity by the product madhav rasayan

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ABSTRACT

The study focuses on In-vitro Anti-IL6 (Interleukin-6) bioactivity of Madhav Rasayan. The research highlights the Inhibition study of IL6 protein by Madhav Rasayan. The main objective is to study the effect of active molecules of MADHAV RASAYAN which can directly inhibit the IL6 and to reduce the release of IL6.

I. Inhibition of IL6 production by peripheral blood mononuclear cells (PBMC)

II. Direct inhibition of IL6 molecules

Keywords: Anti-IL6, Inhibition, Madhav Rasayan, Peripheral Blood Mononuclear Cells

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INTRODUCTION

IL6 is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. It plays a role in host defense, acute phase reactions, immune response and haemostasis. IL6 is expressed by T cells, B cells, monocytes, hepatocytes, endothelia cells and keratinocytes. It is a 21–27 kDa peptide with 184 amino acids which plays an important role in cellular and molecular mechanisms of inflammation including T and B cell activation. Human IL-6 is made up of 212 amino acids, including a 28-aminoacid signal peptide.

IL-6 is a pro-inflammatory cytokine which has many well-defined effects [1]. Its synthesis and release from mononuclear cells can be useful to test the drugs that influence the release of IL6 activity. Its release can be potentiated by the treatment with Concanavalin A (Con A). Incubation of peripheral blood mononuclear cells (PBMC) with Con A activates the release of IL6. The drugs which affect the IL6 release can be analyzed by treating the isolated PBMC cell with simultaneous treatment with test drugs & Con A. The active molecules of MADHAV RASAYAN are tested for direct inhibition of the Interleukin 6 (IL6) protein and inhibition of IL6 release from the cells. The test was performed by ELISA assay using Human IL6-ELISA kit. The release of IL6 was done by Concanavalin A (Con A). Concanavalin A, a phytoagglutinin, binds to the envelope of hemagglutinating encephalomyelitis virus, a Coronavirus [2].

EXPERIMENTAL PROCEDURE

Preparation of drug solution: Two tablets (550 mg) of MADHAV RASAYAN were dissolved and extracted with 10 ml DM water. The extraction was carried out by boiling the water at 80°C. The drug solution was prepared in water at concentration of 0.0; 0.20; 0.40 and 0.80 μ M.

Preparation of peripheral blood mononuclear cells (PBMC): The collected cells from human blood were washed twice with phosphate buffer saline (PBS) containing 2 mM of EDTA and 0.5% w/v of Bovine Serum Albumin (BSA). The cells were re-suspended in modified Dulbecco's MEM containing 100 mM NaCl, 24 mM KCl, 10 mM CaCl_2 and 10 mM MgCl_2 , and 11 mM glucose at pH: 7.2.

Treatment of PBMC cells: 450 μ l PBMC cells aliquots without any plasma or serum was treated for 20 min at 37°C with MADHAV RASAYAN drug solution or Con A (10 μ g/ml) dissolved in 50 μ l of water. The incubation was terminated by placing the tubes in cold condition of 0°C and then the liquid was centrifuged at 6000 RPM for 5 min using cold centrifuge. The water-clear supernatants were carefully removed and cytokine IL6 was determined by Human IL6 ELISA kit.

Direct treatment of drugs with IL6: Human IL6 dilution with concentration of 200 pg/ml; 100 pg/ml; 50 pg/ml; 25 pg/ml and 12.5 pg/ml was prepared with PBS buffer pH 7.2 to 100 μ l of the IL6 solution 10 μ l MADHAV RASAYAN stock solution was added and incubated for 30 min at 37°C. After that IL6 activity was tested by using Human IL6 ELISA kit.

Analysis of IL-6: Analysis of IL6 by Human IL6 ELISA kit was carried out as per the manufacture instruction (Krishgen Biosystem, Mumbai, India). For this, precoated anti IL6 plate was used. The 100 μ l of the sample per well was added, the plate was sealed and incubated for 2hr at room temperature for binding of IL6 with anti IL6. After the incubation the plate well was washed with wash buffer (1X) four times in order to remove the unbound IL6. To this, 100 μ l of the detection antibody (biotin conjugated) was added and incubated for 1hr at room temperature. The plate was further washed with wash buffer (1X) four times and streptavidin-HRP solution (100 μ l) was added and incubated for 30 min for binding of biotin to streptavidin-HRP. Again, the plate was further washed with wash buffer (1X) four

times. To this, TMB substrate was added and incubated for 20 min under dark. The reaction mixture turned blue. Next, 100 μ l of stop solution was added. The reaction mixture turned yellow. The ELISA plate was analysed at 450 nm. The absorbance was noted. Higher the OD at 450 nm higher is the IL6 activity or concentration.

The experimental work



RESULTS

Figure no 1: Standard plot of IL6

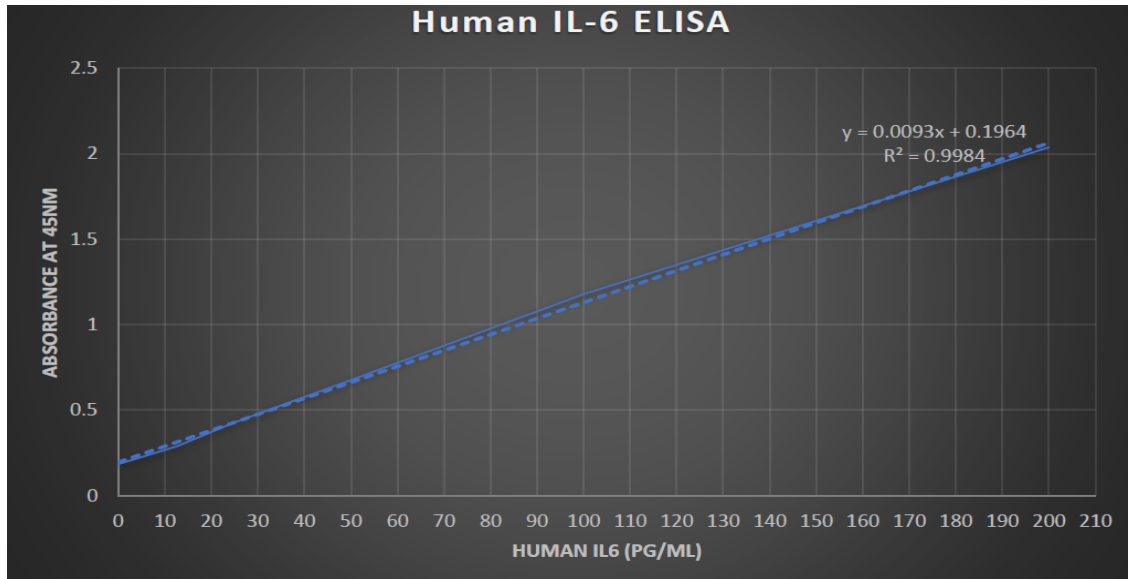
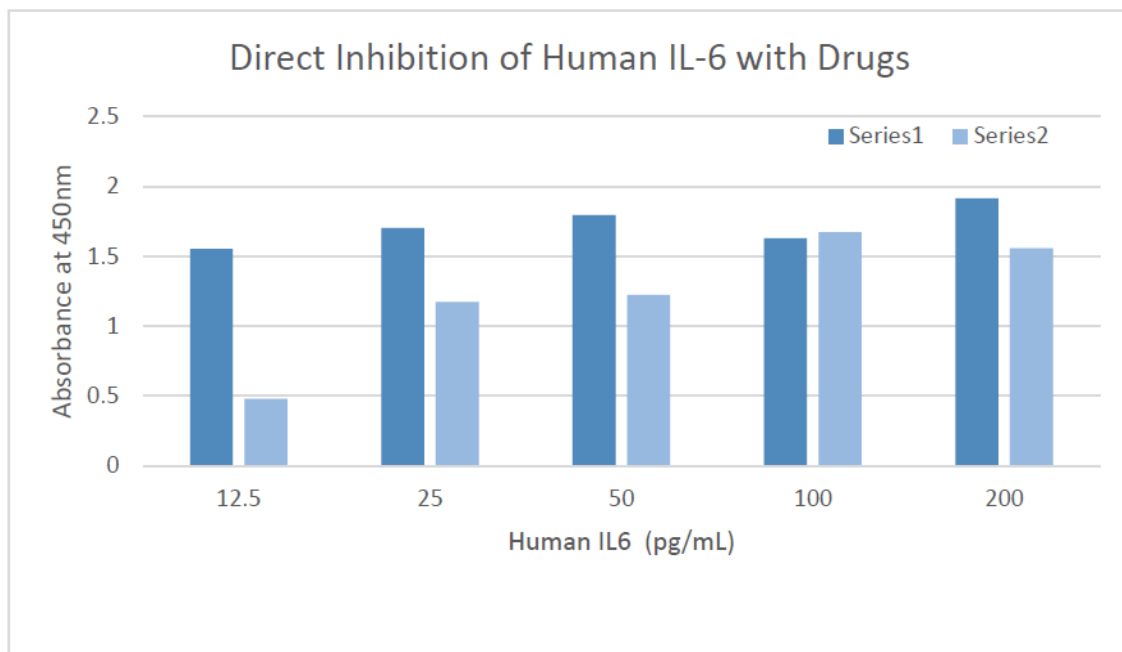


Figure no 2: Direct inhibition of IL6 by *Madhav Rasayan*

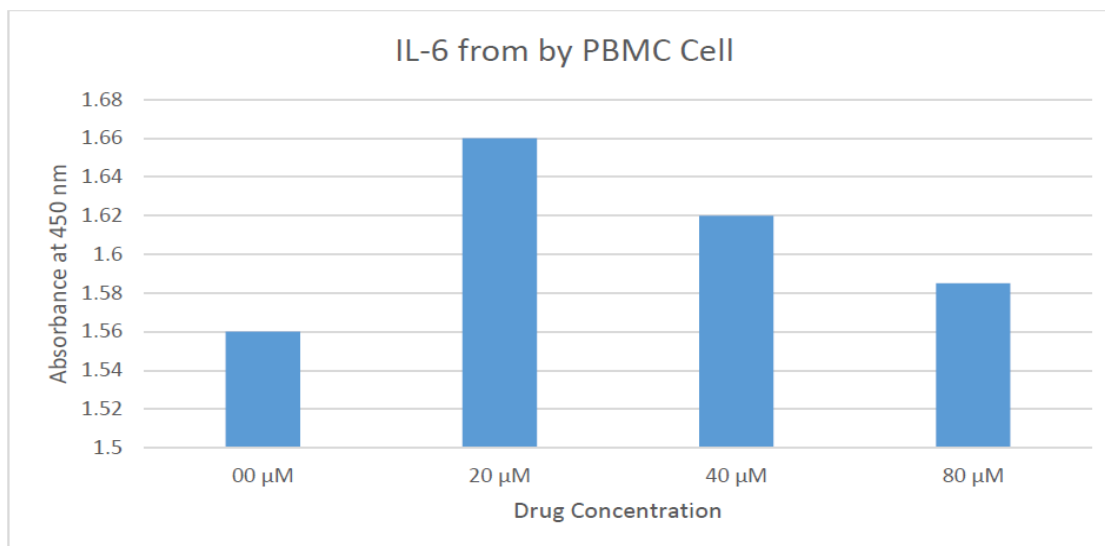


Report

The graph shows the direct inhibition of the Human IL6 by the Drug is occurring at concentration of 12.5, 25 and at 50 pg/ml however the inhibition was not observed at concentration of 100 pg/ml and at 200 pg/ml.

Table no 1: Inhibition of IL6 release from peripheral blood mononuclear cells (PBMC)

Drug concentration	Con- A (10 µg/ml)	PBMC cell	OD at 450nm
00 µM	10 µl	450 µl	1.560
20 µM	10 µl	450 µl	1.660
40 µM	10 µl	450 µl	1.620
80 µM	10 µl	450 µl	1.585

Figure no 3: Inhibition of IL6 release from peripheral blood mononuclear cells (PBMC)**Report**

The graph shows that Inhibition of IL6 release from peripheral blood mononuclear cells (PBMC) was not effective at any of the drug concentration.

CONCLUSION

The direct inhibition of the Human IL-6 by the aqueous extract of Madhav Rasayan is occurring at the concentration of 12.5, 25 and at 50 pg/ml. However, the inhibition was not observed at concentration of 100pg/ml and at 200pg/ml. The inhibition of IL-6 release from

peripheral blood mononuclear cells (PBMC) was not effective at any concentration of aqueous extract of Madhav Rasayan.

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