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IMMUNOMODULATORY ACTIVITY OF SIDDHA HERBAL FORMULATION OF KODIVELI CHOORANAM

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ABSTRACT

Siddha system of medicine is the most primitive medical system. Siddha system has popular in India especially in Tamilnadu and other countries which ensures prevention of illness, promotion of health and well being through the principle of **food is medicine and medicine is food.** Several research group have worked on the scientific basis of such immunomodulatory effects of plant products and also a result considerable data has accrued. Immunomodulation is the process that alters the immune system of the stimulation or immune suppression thus regulating or normalizing it. The present study to evaluate the immuno modulatory activity of Kodiveli chooranam using Macrophage cell line RAW264.7 using lipo polysaccharides (LPS) as a control. While the concentration level is decreased, nitrate level is increased. Hence, 25µl/ml of KC has nitrate production and thus proven to be an immunomodulator.

KEYWORDS: Kodiveli chooranam, Immunmodulator activity, RAW Cell line 264.7.

INTRODUCTION

Siddhar's classified diseases in different catagories and accounted as 4448 diseases. Medicinal plants products are known to modify different aspect of human physiology and exert an alleviating influence on several pathophysiological states and concepts of immunity and immuno modulator can be traced back several hundred years to the history of medicine. Immuno modulator can be defined as a substance, which can influence any constituent or function of the immune system in a specific or non specific manner including both innate or adaptive arms of the immune response. Hence immuno modulators referred to as biological response modifiers, improve the host defense mechanism against diseases by striking a balance between regulatory or effector cells. The active components of various medicinal plants regulate the immune system by interacting with various immuno cytes and regulating their effector mechanism for instance cytokines and their receptors. The present study to evaluate the immuno modulatory activity of KC using Macropage cell line RAW 264.7 using lipopolysccharides(LPS) as a control. While the contration level is decreased, nitrate level is increased. Hence, 25 µl/ml of KC has nitrate production and thus proven to be an immunomodulator.

MATERIALS AND METHODS

Selection and Authentication of Drug

I have selected the trial drug Kodiveli chooranam for this study from classical literature Sarabendhirar Siddha Maruthuva Sudar. The raw drugs were procured from the raw drug shop R.N. Rajan and Co, Chennai. The cost of the trial medicines are relatively economical. After proper authentication by the Botanist, Govt Siddha Medical College, Arumbakkam, Chennai, the preparation was made.

Preparation of Kodiveli Chooranam

Ingredients of Kodiveli Chooranam

1. Kodiveli (Plumbago zeylanica)
2. Adathodai (Justicia adathoda)
3. Vellarugu (Enicostemma axillare)
4. Sivanar vembu (Indigofera aspalathoides)
5. Charanai ver (Trianthema decandra)
6. Nilavarai (Cassia senna)
7. Kuppaimeni (Acalypha indica)

Procedure

All the raw drugs are powdered well and do vasthrakayam to get fine powder. Kept in a air tight container.

Determination of Invitro Immunomodulatory Effect of Extract on Cultured Raw Cell Lines

RAW 264.7 cells will be grown to 60% confluence followed by activation with 1 µL lipo polysaccharide (LPS) (1µg/mL). LPS stimulated RAW cells were exposed with different concentration (25, 50, 100 µg/mL) of sample and incubated for 24 hours. After 24 hours of incubation the cells were digested and centrifugation was done at 6000 rpm for 10 minutes.

Supernatant was discarded and cells were then resuspented in 200µl of cell lysis buffer (0.1M Tris HCl, 0.25M EDTA, 2M Nacl, 0.5 % Triton x-100). The samples were then kept at 4^0C for 20 minutes. After incubation, the immuno modulatory response was performed by estimating nitrite levels in the cell lysate.

Estimation of Cellular Nitrate Level

The level of nitrite level was estimated by the method of Lepoivre et al. (Lepoivre et. al. 1990) To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

RESULTS AND DISCUSSION

The primary target of the immuno modulatory compounds is believed to be the macrophages, which plays a major role in the generation of immune response. It is known that the activated macrophages display not only increased phagocytosis and intracellular killing of pathogens by producing effector molecules like free radicals and nitric oxide. While the concentration level is decreased, nitrate level is increased. Hence 25µg/ml of KC has nitrate production and thus proven to be an immunomodulator.

Sample Concentration (µg/ml)	OD at 540nm	Concentration (µg)
Control	0.1185	586.575
25	0.0803	397.485
50	0.0745	368.775
100	0.0701	346.995

CONCLUSION

To evaluate the immunomodulatory activity of KC using Macrophage cell line RAW264.7 using lipopolysacchrides as a control. LPS induced nitrate production used as indicator for evaluating the level of phagocytosis. The concentration was measured using the spectrophotometric technique at 540nm. While the concentration level is decreased, nitrate level is increased. Hence 25µg/ml of KC has nitrate production and thus proven to be an immunomodulator.

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