



**An Immunomodulatory Activity of Siddha Formulation *Seenthil Chooranam*
By *In Vitro* Study**

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ABSTRACT

In the Siddha system of medicine many herbs and medicinal formulations have been reported to possess immunomodulatory properties. The test drug seenthil chooranam is commonly used to treat various diseases like Diabetes, alopecia, vatha diseases, skin diseases, and respiratory infections by regulating the immune system. To investigate the immunomodulatory activity of the Siddha formulation seenthil chooranam. LPS stimulated RAW 264.7 macrophage cell line cultured in Dulbecco's Modified Eagle Medium and the immunomodulatory response was assessed by estimating nitrite level and cell viability. Seenthil chooranam had significantly reduced the nitrite level at the concentration ranges from 50 to 200µg/ml in a dose-dependent manner and the least viability of cell was observed at the concentration of 200 µg/ml shows 51.1 ± 1.801 %. The present study revealed that the Siddha formulation Seenthil chooranam had immunomodulatory activity established through in-vitro study.

KEY WORDS: *Siddha, Seenthil chooranam, Immunomodulatory activity, In vitro study, alopecia, skin diseases*

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1. Introduction

Siddha medicine or Siddhar Maruthuvam also called Tamil Maruthuvam is a system of medicine practiced in the Tamil-speaking regions of the world. It is one of the oldest systems to describe health and a healthy lifestyle and its significance in all walks of life. Generally, Siddha medicines are prepared by using herbs, metals, minerals, and animal products. Almost many Siddha medicines are used to improve the immunity level, as preventive aspect quit the disease and it had been proven during dengue and Covid-19 outbreak period. In this system of medicine seenthil chooranam is one of the 32 types of internal medicine as powder form (chooranam) [1] and it is commonly used to treat various diseases like Diabetes, alopecia, vatha diseases, skin diseases, and respiratory infections. It contains seenthil, karisalai, and poonagam and is described in a sastric Siddha textbook Agasthiyarparipooranam 400. An important objective of traditional medicine is "prevention is better than cure" which means prevention from disease is better than treating the disease. In the Siddha system of medicine, many herbs and medicinal formulations have been reported to possess immunomodulatory properties. These immunomodulatory drugs modify the immune response by acting as immunostimulators or immunosuppressives. [2] An immunomodulatory activity drug works based on stimulation of phagocytes, macrophages, lymphoid cells, increasing circulating total white cell counts and interleukin-2 levels. [3] The test drug seenthilchooranam was known to possess anti-inflammatory activity, hepatoprotective, antidiabetic activity, anti-asthmatic activity, and bronchodilator properties through previous studies. [4] It also acts as an immunostimulant and is recommended for prevention and post-viral convalescence period during covid-19 pandemic situations. In addition, raw seenthil is used as supportive medicine to manage AIDS. In routine clinical practices, Siddha physicians use seenthil chooranam to manage autoimmune disorders like rheumatoid arthritis, alopecia areata, psoriasis, and vitiligo.[5] This study is carried out to investigate and ensure the immunomodulatory activity of the seenthil chooranam through in vitro study.

2. Materials and Methods

2.1. Ingredients of Seenthilchooranam

- i. Seenthil(Tinosporacordifolia) - 350 grams

- ii. Karisalai (Ecliptaprostrata) - 350 grams

- iii. Poonagam(Eudriluseugeniae) - 105

2.2. Collection and Authentication of raw drugs

Seenthil and karisalai were purchased from Ramasamy Chettiar raw drug store at Parrys, Chennai. The Earthworm was collected from farm fields in Vedaranyam, Nagapattinam. The plant materials were identified and authenticated by the medicinal botanist and the earthworm was identified by the head of the department of Gunapadam of National Institute of Siddha, Tambaram Sanatorium and Chennai.

2.3. Method of purification

The ingredients of Seenthil chooranam were purified and prepared in Gunapadam laboratory, NIS, Chennai.

Seenthil(*Tinospora cordifolia*): The plant was purified by peeling the outer skin of the stem and then dried.

Karisalai(*Eclipta prostrata*): The plant was purified by washing the whole plant and drying it in sunshade.

Poonagam(*Eudrilus eugeniae*): The earthworm was purified by dropping the earthworms in cow's milk to make them spew out the mud. Then lime water is sprinkled to put the worms to death and it is cleaned with tap water.

2.4. Preparation of Seenthil chooranam

The required quantity of the purified drugs was taken and ground into a fine powder and sieved. Then it is stored in an airtight container.

2.5. Preparation of test solutions

For anti-proliferative studies, serial dilutions of test formulation (50, 100, and 200 µg/ml) were prepared

2.6. Cell culture, measurement of cell viability

Macrophage cell line RAW 264.7 was obtained from National Center for Cell Science (Pune, India) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (10%) containing penicillin-streptomycin (10%) at 37°C in a humidified atmosphere containing 5% CO₂. Cells were plated at a density of 1 × 10⁴

cells/well 3 in 25 or 75 cm² flasks, or in a 96-well plate overnight. RAW 264.7 were grown to 60% confluence followed by activation with 1 μ L lipopolysaccharide (LPS) (1 μ g/mL). LPS stimulated RAW cells were exposed with different concentration (50, 100, 200 μ g/mL) of the test 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 re-suspended in 200 μ L of cell lysis buffer (0.1M TrisHCl, 0.25M EDTA, 2MNaCl, 0.5 % Triton x-100). The samples were then kept at 40 Celsius for 20 minutes. After incubation, the Immunomodulatory response was performed by estimating nitrite levels in the cell lysate[6].

2.7. Estimation of Cellular Nitrite Levels

The level of nitrite level was estimated by the method of Lee 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 μ L of the supernatant, 30 μ L of 10% NaOH was added, followed by 300 μ L of Tris-HCl buffer and mixed well. To this, 530 μ L 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 [7].

3. Results and Discussion

Drugs with an immunomodulatory property are employed to modify the immune system and it acts as both immunosuppressive and immunostimulator. [8] The test with immunomodulatory property drugs also possesses antioxidant and anticancer activity in itself. [9] Such drugs widely help to treat infection, cancer, autoimmune disease, and other diseases. Numerous Siddha medicines have potent immunomodulatory property but it has not been established yet. This is the drawback of the Siddha system of medicine in the scientific world. In order to bring out hidden precious formulations in

Siddha as evidence-based medicine scientific validation is necessary. These evidence-based studies support the growth of the system and discover the latent truth of this medicine. Previously many studies were documented about immunomodulatory herbs and Siddha medicines. The test drug seenthil chooranam components are seenthil, karisalai and poonagam.

Seenthil had the chemical constituents of tinosporine, cordial, palmitine, choline, tinocordiside, octacosanol and columbin.[10] Another chemical constituents are N-formylannonain, magnoflorine, cordifolioside A, tinicordiside, berberine, saponin and tinosporic acid. These are responsible for the immunomodulatory effect of seenthil on immune system and it influences the cytokine production. [11, 12] Karisalai had the chemical constituents of oleic acid, eclipta saponin, diethylphosphate, glycine, ethinocystic acid, ecliptacystic acid, pantadecanic acid and c- sitosterol. Oleic acid, eclipta saponin components are responsible for immunomodulatory activity of the seenthil chooranam. [13] The animal drug of this chooranam is poonagam, it has rich vitamin A, carbohydrate, protein and free fatty acids. Amino acids such as histidine, serine, arginine, methionine and lysine are present in dried earth worm powder. [14] Vitamin A specially used as immunotherapy in autoimmune inflammatory condition because of their action of maintaining immunological tolerance. [15]

The seenthil chooranam has chemical constituents like alkaloid, flavanoids, steroids, triterpenoids, coumarin, phenol, tannin and betacyanin. It contains minerals such as zinc, iron, magnesium and calcium play the major role in immune cell function.

The present study is to investigate the immunomodulatory property of the Siddha formulation seenthil chooranam by using RAW macrophage cell line 264.7. An immunomodulatory property was assessed by the cellular viability and nitrite level. On observed that Lipopolysaccharide (LPS) (1 μ g/mL) treated well was served as a control with a maximum nitrite level of about 1348 \pm 36.35 μ g. The formulation Seenthil chooranam at

the dose of 50µg/ml shown a significant decrease in nitrite level of about 619.3 ± 34.59µg similarly at the concentration of 100µg/ml it shows 458.3 ± 5.132µg and the maximum percentage decrease of nitrite level of about 243.7 ± 4.041 µg were observed at 200µg/ml.

Table. 1: Effect of Siddha formulation Seenthil chooranam on Nitrite level in RAW 264.7 Cell line

Concentration (µg/ml)	Concentration of Nitrites (µg)
Control (LPS1µg/mL)	1348 ± 36.35
SEEC 50 µg	619.3 ± 34.59
SEEC100 µg	458.3 ± 5.132
SEEC 200 µg	243.7 ± 4.041

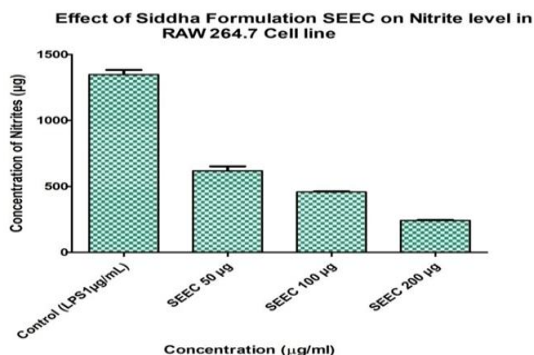


Figure 1: Effects of Siddha formulation Seenthil chooranam on Nitrite level in RAW 264.7 Cell line

The result obtained from the study reveals that the percentage of cell viability of the macrophage cell line decreases with an increase in the concentration of the test drug Seenthil chooranam. The least viability of the cell was observed at the concentration of 200µg/ml shows 51.1 ± 1.801 %.

Table. 2: Effect of Seenthil chooranam on Cell viability in RAW 264.7 Cell line

Concentration (µg/ml)	Concentration of Nitrites (µg)
Control (LPS1µg/mL)	94.32 ± 1.687
SEEC 50 µg	78.24 ± 1.121
SEEC100 µg	64.94 ± 0.5529
SEEC 200 µg	51.1 ± 1.801

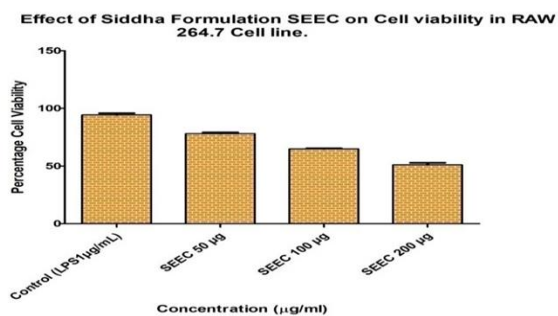


Figure 2: Effect of Siddha formulation Seenthil chooranam on cell viability in RAW 264.7 cell line

The results indicate the test drug Seenthil chooranam has significantly reduced the nitrite level at the concentration ranges from 50 to 200µg/ml in a dose-dependent manner. Hence from these data, it was concluded that the formulation Seenthil chooranam possess promising immunomodulatory property.

LPS induced proliferation in Macrophage cell line RAW 264.7

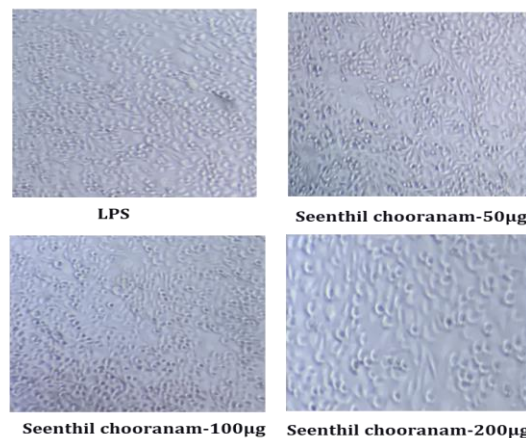


Figure 3: LPS induced proliferation in Macrophage cell line RAW 264.7

4. Conclusion

Siddha medicine is effective in the prevention of diseases and its immunomodulatory drugs are capable of improving the nonspecific immune system. The test drug seenthil chooranam is a combination of herbal and animal product. This study revealed that seenthil chooranam has immunomodulatory activity in the in-vitro study and it is used as prophylactic medicine for covid-19. Further in vivo studies can support the quality and importance of the seenthil chooranam

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