

Research Article

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***In-vitro* Evaluation of Immunomodulatory Potential of Swarna Pushpa Rasa Chendhuram Using Murine Macrophage RAW 264.7 Cell Line Model**

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Abstract

Keywords

Immunomodulatory agents,
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Siddha system,
Swarna Pushpa Rasa Chendhuram,
Macrophages,
RAW 264.7 cell line,
Lipopolysaccharide.

Immunity plays an important role in the protection of the humans and animals from foreign toxic matter. In recent years, there has been growing interest in the field of siddha medicines research and in search for promising potential compounds for investigating the immunomodulatory compounds from herbomineral products. The immune system is designed to protect the host from invading pathogens and to eliminate disease. Many formulations from siddha system of medicine have been used for the purpose of increase immunity. Immunomodulatory agents of herbomineral origin increase the immune responsiveness of the body against pathogens by activating the nonspecific immune system. However, there is a need of systemic studies on siddha preparations like *SwarnaPushpa Rasa Chendhuram* (SPRC) to substantiate the therapeutic claims made regarding their clinical utility. Macrophage cell line RAW 264.7 are the widely utilized model for evaluating the Invitro immunomodulatory efficacy of the several siddha formulations throughout the world. The main aim of the present investigation is to evaluate the immunomodulatory activity of the siddha formulation SPRC using macrophage RAW 264.7 cell line by using lipopolysaccharide (LPS) (1µg/mL) as control. LPS induced nitrite production used an indicator for evaluating the level of phagocytosis. The concentration was measure using spectrophotometric technique at 540nm. The result obtained from the present investigation indicates that the drug SPRC exhibit significant decrease in the level of nitrites in the cell line medium from 952.38 µg to 395.50 µg. It was concluded from the result obtained from the present research work that the formulation SPRC possess promising immunomodulatory and immune enhancing potential in the tested medium. Further study regarding the isolation of active moiety responsible for immunomodulatory activity present in SPRC and its mode of action need to be determined.

1. Introduction

Immune dysfunction is responsible for various diseases such as arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer, and infectious diseases [1]. The degree to which the patient becomes abnormally susceptible to infections by the microbial environment depends on the extent of immunosuppression. The suppression of the immune system is characterized by a reduction in the number and phagocytic function of the neutrophils and macrophages, as well as an impairment of the intracellular bactericidal capacity of these cells. This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infections [2]. This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs [3]. It is evident through several research that Immunomodulators improves the host defense mechanism.

Immunomodulation through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy. There is a growing interest in identifying herbal immunomodulators ever since their possible use in modern medicine has been suggested. Indian traditional systems of medicines like Siddha and Ayurveda have suggested to increase the body's natural resistance to disease [4]. Recent screening with plants has revealed many compounds (e.g. alkaloids, flavonoids, quinones, terpenoids) with pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential [5].

The immunomodulating characteristics of siddha preparations are being examined widely used to achieve the desirable effects on disease prevention. Consequently, herbal remedies have been utilized for centuries for safety, effectiveness, minor side effect and cultural acceptability. Therefore plant and its products are harmless and so, there is continuous application of plant product as an optional way to cure the patients and this approach is in practice from the ancient times [6].

Immunotherapy is the treatment of a disease by producing, improving or overcoming an immune response. Immunotherapies, produced to obtain or augment an immune response, are classified as immune stimulants. The main aim of the present investigation is to evaluate the immunomodulatory

activity of the siddha formulation *Swarna Pushpa Rasa Chendhuram* (SPRC) using macrophage RAW 264.7 cell line by using lipopolysaccharide (LPS) (1µg/mL) as control. This is a very novel attempt that was made for the first time to document immunomodulatory potential of SPRC.

2. Materials and Methods

2.1. Source of raw drugs:

The Required raw materials were procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India .All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India

2.2. Ingredients

The siddha formulation *Swarna Pushpa Rasa Chendhuram* comprises of the following ingredients

Purified Rasam (Hydragyrum)	-35 g
Purified Gandhagam (Sulphur)	- 35 g
Purified Velvangam (Stannum)	- 35g
Purified Navacharam (Ammonichloridum)	-35 g
Kalyanapoosanikai (<i>Benincasahispida</i>)	- Quantity sufficient

2.3. Purification

Rasam (Mercury)

35 gms of Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the Mercury was boiled with the juice of Kuppaimaeni (1.3 litres) until it is detoxified.

Gandhagam (Sulphur)

Sulphur was placed in an iron spoon. A small quantity of cow's butter was added and the spoon was heated till the butter melts; this mixture was immersed in inclined position in cow's milk. This procedure was repeated for 30 times to get purified Sulphur. Each time, fresh milk was used.

Velvangam (Stannum)

Velvangam was placed in iron spoon and heated. The melted velvangam was then poured into *vitexnegundo* juice and turmeric (*Curcuma longa*). The same procedure was repeated twice.

Navacharam (Ammonium Chloride)

Hot water and filtered was mixed. After self-cooling it was kept in sunlight. Followed by this the salt settles down at the bottom of the vessel.

2.4. Formulation of SwarnaPushpa Rasa Chendhuram[7]

Purified Velvengam was melted and slightly cooled to which purified rasam was added and grounded well. Purified Navacharam and Purified gandhagam are then added and grinded with lemon juice for 12 hrs. Make it into poultices (villai), followed by drying it was kept in small mud pot. Pot was then sealed with 7 layers of mud pasted cloth. New big pot was filled with sand and the small pot was then placed inside the big pot closed with suitable size mud plate. Pots were then ignited for about 12 hours. Followed by cooling the product thus formed was again grounded with *Benincasa hispida* juice for 3 hrs and dried in moon shade. The end product was powdered, weighed and preserved in an air tight container.

Dose: 130mg, twice daily

Vehicle: Honey

Duration: 48 Days

Indication: Kuttam, Megaranam, Pun puraigal.

2.5. Cell culture, measurement of cell viability [8]

Macrophage cell line RAW 264.7 was obtained from National Center for Cell Science (Pune, India) and cultured in 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 in 25 or 75 cm² flasks, or in 96-well plate overnight. RAW 264.7 were grown to 60% confluence followed by activation with 1 µL lipopolysaccharide (LPS) (1µg/mL). LPS stimulated RAW cells were

exposed with different concentration (25, 50, 100 µg/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 200µl of cell lysis buffer (0.1M TrisHCl, 0.25M EDTA, 2M NaCl, 0.5 % Triton x-100). The samples were then kept at 4°C for 20 minutes. After incubation, the Immuno modulatory response was performed by estimating nitrite levels in the cell lysate.

2.6. Estimation of Cellular Nitrite Levels [9]

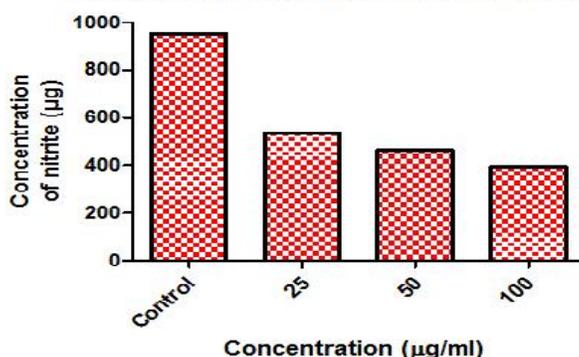
The level of nitrite level was estimated by the method described previously .To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of 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.

3. Results

264.7 medium at the concentration ranges from 25 to 100 µg/ml. Lipopolysaccharide (LPS) (1µg/mL) treated well was served as control with maximum nitrite level of about 952.38 µg. The formulation SPRC at the dose of 25 µg/ml shown significant decrease in nitrite level of about 537.57 µg similarly at the concentration of 50 µg/ml it shows 464.80 µg and the maximum percentage decrease of nitrite level of about 395.50 µg were observed at 100 µg/ml .As shown in table 1 and Figure 1.

Table 1: Effect of Siddha Formulation *SwarnaPushpa Rasa Chendhuramon* Nitrite level RAW 264.7 Cell line.

Concentration (µg/ml)	Absorbance at 540nm	Concentration of Nitrites (µg)
Control (LPS1µg/mL)	0.1924	952.38
SPRC 25	0.1086	537.57
SPRC 50	0.0939	464.805
SPRC 100	0.0799	395.505

Figure 1: Effect of Siddha Formulation *SwarnaPushpa Rasa Chendhuramon* Nitrite level in murine Macrophage RAW 264.7 Cell line**Effect of Siddha formulation Swarna Pushpa Rasa Chendhuram on Nitrite concentration in RAW 264.7 cell lines**

4. Discussion

Since ancient times siddha formulations have played a vital role in preserving human health. The use of herbal preparations has increased in rural areas and developing countries. This is due to lack of hospitals, poverty, and an increased demand of inexpensive medicines [10]. Medicinal plants continue to provide humanity with new remedies. It is therefore important to explore medicinal plants for their safety, quality, toxicity, appropriate amount of plant materials to use, and efficacy. Natural products of plants possess several biological activities including antioxidant and anti-inflammatory activity [11,12].

Antioxidants play an important role in neutralizing free radical species which are produced as end or by-products of normal biochemical reactions in normal system [13]. High amounts of free radical molecules cause oxidative stress in cells which result in damaging essential macromolecules including DNA, lipids, and proteins. The damage of macromolecules leads to inflammation and many degenerative conditions such as Parkinson's diseases, atherosclerosis, aging, immunosuppression, ischemic heart disease, diabetes, hair loss, membrane lipid peroxidation, and decreased membrane fluidity [14,15].

Macrophages have been known to play an important role in the host protection against a wide range of tumors and microorganisms. Macrophages also presents antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. When activated, macrophages increase the phagocytic activity and release various materials such as cytokines and reactive intermediates and then carry out non-specific immune responses [16].

It was observed that the formulation SPRC reveals dose dependent decrease in the nitrite level in RAW 264.7 medium at the concentration ranges from 25 to 100 µg/ml. Lipopolysaccharide (LPS) (1µg/mL) treated well was served as control with maximum nitrite level of about 952.38 µg. The formulation SPRC at the dose of 25 µg/ml shown significant decrease in nitrite level of about 537.57 µg similarly at the concentration of 50 µg/ml it shows 464.80 µg and the maximum percentage decrease of nitrite level of about 395.50 µg were observed at 100 µg/ml. Currently, worldwide, there is an increase in diseases especially infectious diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation. Malnutrition and infectious diseases have remained a challenge especially in developing nations as they greatly compromise the body's immune system responses in the affected individuals [17].

5. Conclusion

The immune system is the defense mechanism of the body and it helps to protect it from foreign bodies and infection thus playing a part in homeostasis of the body. Modulation of the immune system by way of stimulation or suppression helps in maintaining a disease-free state within an individual. Immunomodulators have therefore been used globally to control disease conditions. Based on the result of the present investigation it was concluded that the formulation *Swarna Pushpa Rasa Chendhuram* significantly decreases the level of nitrites from 952.38 µg to 395.50 µg at the concentration 100µg/ml which is considered to be one of the most important indicator of phagocytosis in the macrophage cell lines.

Hence it may conclude that the formulation *Swarna Pushpa Rasa Chendhuram* may be used as an Immunomodulators for clinical management of immune compromised diseases.

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