

Research Article



In Vitro Immunomodulatory Activity of *Kandankathari Nei* in RAW Macrophage Cell Line – A Traditional Siddha Medicine

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ABSTRACT

Siddha medicine, one of the oldest traditional medical systems in India, is predominantly practiced in Tamil Nadu. It is based on the concept that vatham, pitham, and kapam predominate during childhood, adulthood, and old age, respectively. Various herbs used in Siddha formulations are known to influence the body's immune system. In this study, the immunomodulatory potential of *Kandankathari Nei* (KKN), a Siddha herbal preparation, was assessed using the RAW264.7 macrophage cell line, with lipopolysaccharide (LPS) serving as the control. At a concentration of 200 µg/mL, KKN demonstrated the highest reduction in nitrite levels (752.7 ± 10.07 µg). The LPS-stimulated control group (1 µg/mL) showed high cell viability ($95.06 \pm 3.17\%$), confirming effective cell activation. Interestingly, treatment with KKN at 200 µg/mL maintained a cell viability of $68.34 \pm 1.74\%$. These findings, considering both nitrite levels and cell viability, indicate that KKN exhibits significant immunomodulatory activity in RAW264.7 cells.

Keywords: Siddha medicine, *Kandankathari Nei*, immunomodulatory activity, RAW264.7 macrophage cell line.

INTRODUCTION

The Siddha system of medicine is considered as ancient as mankind itself and has significantly influenced the civilization of the southern peninsula of India, particularly Tamil Nadu¹. The Siddhars classified diseases into different categories, accounting for about 4,448 types. Medicines in the Siddha system, intended for human health, are prepared based on the theory of *Panchaboothangal* and acknowledge the predominance of *Thiridhodam* (Vatham, Pitham, and Kabam), along with the concepts of the 96 *Thattuvangal* and the six *Vagai Suvaikal*.

Formulations prepared from medicinal plants are known to influence various aspects of human physiology and exert beneficial effects in alleviating several pathological conditions. The concepts of immunity and immunomodulation can be traced back several hundred years in the history of medicine. Apart from the structural and chemical barriers to pathogens, the immune system functions through two fundamental lines of defense: innate immunity and adaptive immunity. Innate immunity represents the first immunological mechanism for defending against invading pathogens. It is a rapid immune response, activated within minutes or hours following an infection or aggression, and does not involve immunologic memory. In contrast, adaptive immunity is both antigen-dependent and antigen-specific, and is characterized by its ability to retain memory, enabling the host to mount a faster and more effective immune response upon subsequent exposure to the same antigen².

Siddhars further classified medicines into three major types: metals, minerals, and herbs. The present study focuses on *Kandankathari Nei* (KKN), a polyherbal Siddha

formulation prepared as a medicated ghee. Due to its palatable nature, this formulation is particularly suitable for children's administration. The current work was undertaken to evaluate the immunomodulatory activity of KKN using the RAW 264.7 macrophage cell line, with lipopolysaccharide (LPS) serving as the control. The results revealed that at a concentration of 200 µg/mL, KKN showed the maximum percentage reduction in nitrite levels, with values of 752.7 ± 10.07 µg. The control group stimulated with LPS (1 µg/mL) demonstrated high cell viability of $95.06 \pm 3.17\%$, confirming successful cell activation. Interestingly, at the same concentration of 200 µg/mL, KKN treatment maintained a cellular viability of $68.34 \pm 1.74\%$. Taken together, these findings, based on both nitrite reduction and cellular viability in RAW 264.7 cells, strongly suggest that KKN possesses promising immunomodulatory properties.

MATERIALS AND METHODS

The formulation used in this study was referenced from the classical Siddha text *Agasthiyar Vaithiya Kaviyam 1500*. The raw drugs required for the preparation were procured from Ramaswamy Chettiyar Raw Drug Shop, Chennai. The cost of the trial medicines was found to be relatively economical. After authentication of the raw drugs by a Botanist at the National Institute of Siddha, Chennai, the formulation was prepared accordingly.

Ingredients of the formulation

1. *Kandankathari* - *Solanum surattense*
2. *Nellivatral* - *Phyllanthus emblica*
3. *Kanduparangi* - *Clerodendrum serratum*



4. *Sitrarathai - Alpinia officinarum*
5. *Vetpalai - Wrightia tinctoria*
6. *Keezhanelli - Phyllanthus amarus*
7. *Sirukanchori - Tragia cannabina Linn*
8. *Saranai - Trientema decandra*
9. *Nerunjil - Tribullus terrestris*
10. *Thaisapathiri - Taxus baccata Linn.*
11. Cow's Ghee

Determination of in vitro immunomodulatory effect of extract on cultured raw cell lines:

Preparation of test solutions:

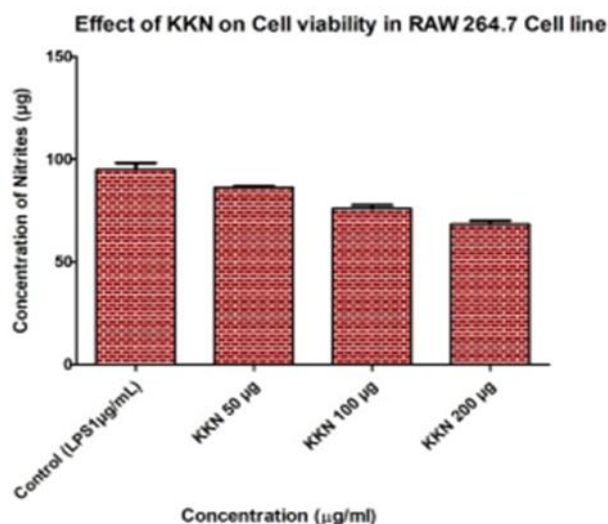
For anti-proliferative studies, serial dilutions of test formulation (50, 100 and 200 µg/ml) were prepared using DMSO.

Culture: Macrophage cell line RAW 264.7

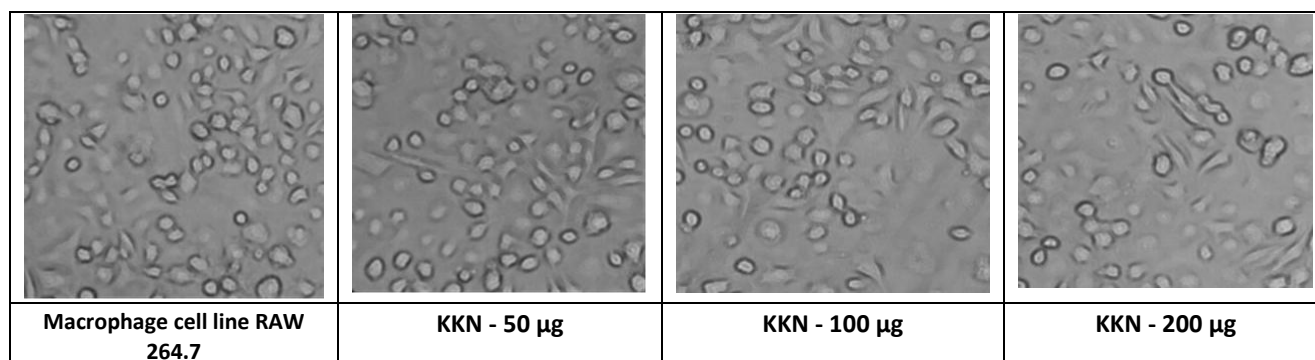
Cell culture in cell viability Measurement:

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 1×10^4 cells/well in 25 or 75 cm² flasks, or in 96-well plate overnight. RAW 264.7 grew to 60%

confluence followed by activation with 1 µL lipopolysaccharide (LPS) (1µg/mL). LPS stimulated RAW cells were exposed with different concentration (50, 100,200 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 Immunomodulatory response was performed by estimating nitrite levels in the cell lysate.

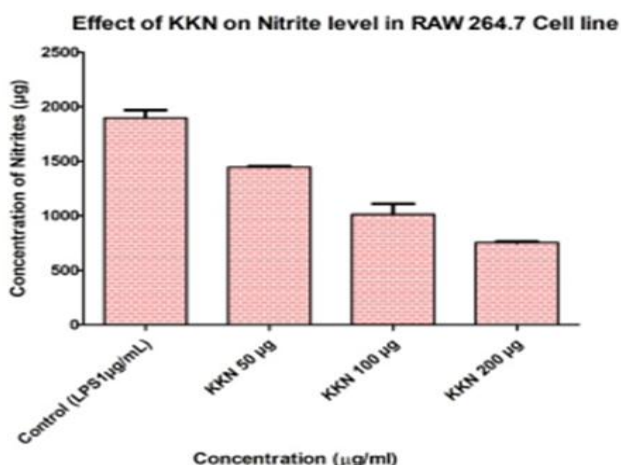


LPS induced proliferation in



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 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 targets of immunomodulatory compounds are believed to be macrophages, which play a crucial role in the initiation and regulation of immune responses. In the present study, it was observed that treatment with the test drug produced a dose-dependent decrease in nitrite levels in RAW 264.7 macrophage culture medium, at concentrations ranging from 50 to 200 µg/mL. Lipopolysaccharide (LPS) (1 µg/mL) was used as the control, which produced the maximum nitrite level of 1898 ± 70.15 µg. When treated with the formulation KKN at a concentration of 50 µg/mL, a significant decrease in nitrite level was noted, measuring 1446 ± 10.02 µg. Similarly, at 100 µg/mL the nitrite level decreased further to 1013 ± 96.47 µg, while the maximum percentage reduction was recorded at 200 µg/mL, with a nitrite level of 752.7 ± 10.07 µg.

The effect of KKN on the viability of RAW 264.7 macrophage cells was also evaluated at different concentrations (50–200 µg/mL). The LPS-stimulated control group (1 µg/mL) showed high cell viability of $95.06 \pm 3.17\%$, indicating successful macrophage activation. Treatment with KKN at 50 µg/mL resulted in a moderate reduction in cell viability to $86.37 \pm 0.56\%$. Further, at 100 µg/mL and 200 µg/mL, the formulation exhibited a dose-dependent decrease in cell viability, showing values of $76 \pm 1.62\%$ and $68.34 \pm 1.74\%$, respectively. These results collectively indicate that KKN exerts a concentration-dependent cytotoxic or suppressive effect on LPS-induced RAW 264.7 macrophage cells, correlating with its immunomodulatory potential.

Table 1: Effect of KKN on Cell viability in RAW 264.7 Cell line

S.No	Concentration in µg/ml	% cell Viability
1	Control (LPS1µg/mL)	95.06 ± 3.17
2	KKN 50 µg	86.37 ± 0.56
3	KKN 100 µg	76 ± 1.62
4	KKN 200 µg	68.34 ± 1.74

Table 2: Effect of KKN on Nitrite level in RAW 264.7 Cell line.

Concentration (µg/ml)	Concentration of Nitrites (µg)
Control (LPS1µg/mL)	1898 ± 70.15
KKN 50 µg	1446 ± 10.02
KKN 100 µg	1013 ± 96.47
KKN 200 µg	752.7 ± 10.07

CONCLUSION

The immunomodulatory activity of KKN was evaluated using RAW 264.7 macrophage cells with lipopolysaccharide as the control. KKN significantly reduced nitrite levels in a dose-dependent manner (50–200 µg/mL), indicating its promising immunomodulatory potential.

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