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IN-VITRO IMMUNOMODULATORY ACTIVITY OF SIDDHA FORMULATION ATHIMATHURA CHOORANAM (AMC) IN RAW MACROPHAGE CELL LINE

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ABSTRACT

Athimathuram is one of the most important herb *Glycyrrhiza glabra Linn.* The word Glycyrrhiza is derived from Greek term glykos (meaning sweet) and rrhiza (meaning root). *Glycyrrhiza glabra Linn* belongs to Leguminosae / Fabaceae family. Historically mulethi/liquorice has been used from more than 4000 years. Athimathura Chooranam (AMC) *Glycyrrhiza glabra Linn* Root powder is the Siddha formulation which is used for many centuries to treat various ailments such as Venkuttam(Vitiligo), Kalanjagapadai (Psoriasis) Karappan(Eczema) from time immemorial. Here we report the immunomodulatory activity of AMC *in-vitro* Raw Macrophage Cell Line. For this study, Lipopolysaccharide (LPS) stimulated RAW cells were exposed with different concentration of the AMC incubated Immunomodulatory response was performed by estimating nitrite levels in the cell lysate. The level of nitrite level was estimated by the method of Lee et al. (Lepoivre et. al. 1990). It was observed that there was dose dependent decrease in the nitrite level in RAW 264.7 medium incubated with test drug at the concentration ranges from 50 to 200 µg/ml. LPS (1µg/mL) treated well was served as a control with maximum nitrite level of about 1261 ± 52.77µg and the maximum percentage decrease of nitrite level of about 390.7 ± 78µg were observed at 200 µg/ml. In this study based on *in-vitro* experiments reveals that AMC having Immunomodulatory activity. So it can be treated for auto immune disease like Vitiligo (Venkuttam) Kalanjagapadai (Psoriasis) Karappan (Eczema) is used worthwhile.

KEYWORDS: *Athimathuram, Glycyrrhiza glabra Linn, Immunomodulatory, Cell line study, Siddha.*

INTRODUCTION

Athimathura Chooranam[1] (AMC) *Glycyrrhiza glabra L*[2] Root powder is the Siddha formulation which is used for many centuries to treat various ailments such as Venkuttam[3] (Vitiligo), Kalanjagapadai[3] (Psoriasis) Karappan(Eczema)[4] from time immemorial. Athimathuram *(Glycyrrhiza glabra Linn)* contains phytoconstituents like flavonoids, alkaloids, steroids, terpenoids, saponins, tannins and glycosides.[5] Pharmacologically possesses antibacterial[5,6] anti-inflammatory[6,7], antioxidant[5,8], antimalarial[9], and anti-hyper glycemic[10] properties. In this study based on *in-vitro* experiments on RAW macrophage cell line 264.7[11] reveals that immunomodulatory property.

MATERIALS METHODS

1. Preparation of AMC

The required raw drugs were procured from well reputed indigenous drug shop. The raw drug authenticated by the Pharmacognosist, Government Siddha Medical College, Chennai. Raw drugs are purified as mentioned in Siddha literature. Athimathuram was well grinded and made into powder & sieved in a cotton cloth. The Prepared chooranam was safely kept in the air tight container.

Fig-1.Glycyrrhiza glabra

Before Purification

Fig-2.Athimathura Chooranam

After Purification

Fig-3.Athimathura Chooranam

2. Preparation of Cell Culture

2.1 Preparation of test solutions

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

2.2 Culture

Macrophage cell line RAW 264.7.[11]

2.3 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 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 in96-well plate overnight. RAW 264.7 were grown to 60% confluence followed by activation with 1µL 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 resuspended 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 4^0C for 20 minutes. After incubation, the Immunomodulatory response was performed by estimating nitrite levels in the cell lysate.

2.4 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.

Table I: Effect of Siddha formulation AMC on nitrite level in raw 264.7 cell line.

S.No	Concentration (µg/ml)	Concentration of Nitrites (µg)
1	Control (LPS1µg/mL)	1261 ± 52.77
2	AMC 50 µg	849.3 ± 94.1
3	AMC 100 µg	491.7 ± 20.55
4	AMC 200 µg	390.7 ± 8

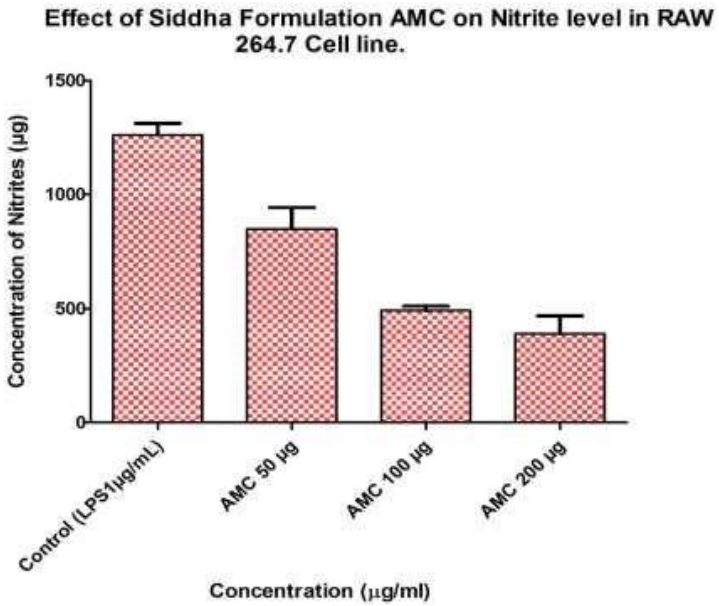
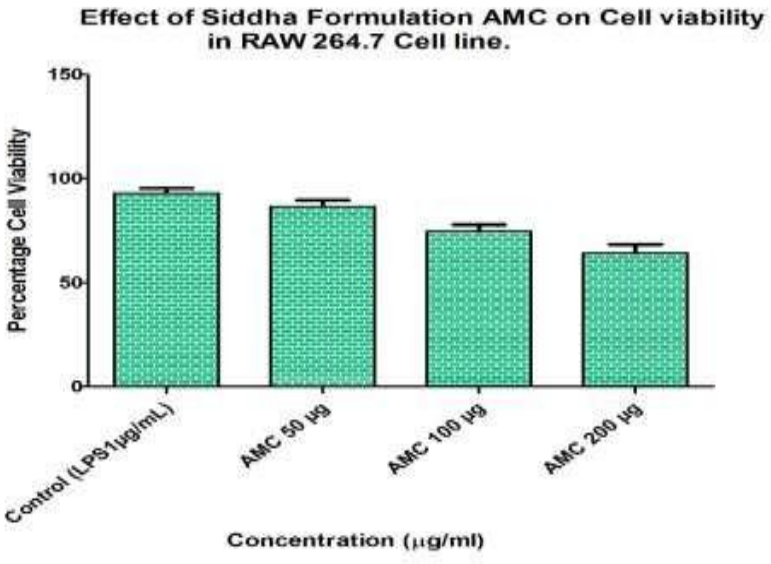


Table II: Effect of AMC on cell viability in raw 264.7 cell line.

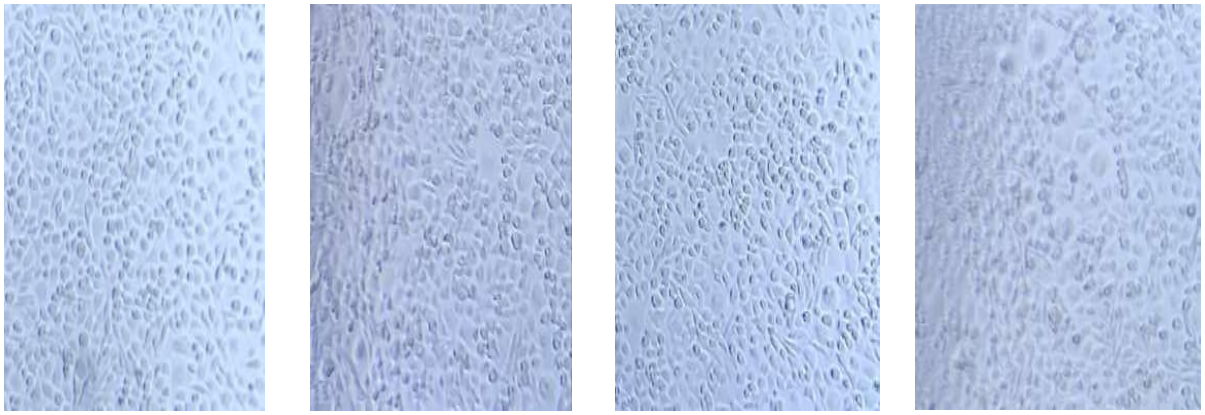
S.No	Concentration in µg/ml	% cell Viability
1	Control (LPS1µg/mL)	93.04 ± 2.392
2	AMC 50 µg	86.63 ± 3.025
3	AMC 100 µg	74.75 ± 3.182
4	AMC 200 µg	64.32 ± 4.131



LPS induced proliferation in Macrophage cell line RAW 264.7

Control – LPS

1 µg AMC - 50 µg 100 µg 200 µg



RESULT AND DISCUSSION

It was observed that there was dose dependent decrease in the nitrite level in RAW 264.7 medium incubated with test drug at the concentration ranges from 50 to 200µg/ml. Lipo polysaccharide (LPS) (1µg/ml) treated well was served as a control with maximum nitrite level of about 1261 ± 52.77µg. The formulation AMC at the dose of 50µg/ml shown a significant decrease in nitrite level of about 849.3 ± 94.1µg similarly at the concentration of 100µg/ml it shows 491.7 ± 20.55µg and the maximum percentage decrease of nitrite level of about 390.7 ± 78µg were observed at 200µg/ml. The result obtained from the study reveals that the percentage of cell viability of macrophage cell line decreases with increase in concentration of the test drug AMC. The least viability of cell was observed at the concentration of 200µg/ml shows 64.32 ± 4.131%. The test drug AMC has significantly reduced the nitrite level at the concentration ranges from 50 to 200µg/ml. Hence from the data's reveals that the formulation AMC possess remarkable immunomodulatory property.

CONCLUSION

Athimathuram[1] is one of the most important herb *Glycyrrhiza glabra Linn*[2] root powder (AMC) which has been used by traditional Siddha system for the treatment of various ailments. The modern experimental in-vitro studies confirmed as imuunomodulatory properties. From this evidence based study helpful for further clinical research are to be done and effect of AMC has to be proved scientifically and also treat the Auto immune disease like Vitiligo (Venkuttam) Kalanjagapadai (Psoriasis) Karappan (Eczema).

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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