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ANTI CANCEROUS POTENTIAL OF SIDDHA NANO MEDICINE SANGU CHUNNAM USING MTT ASSAY AGAINST HEPG2 (LIVER CANCER CELL LINE)

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ABSTRACT

Hepatic carcinoma becomes a global life threatening issue. In many developing countries like india has facing difficulty to treat the cancer patients with anti -cancer drugs associated side effects slike hair loss, mouth sores, loss of appetite, diarrhoea and fatigue. Siddha system of medicine has wide choice of therapeutic formulations which offers ailment against dreadful disease like hepatic carcinoma. Hence, from this invitro pre-clinical study of sample Sangu chunnam has proved that it is a potent anticancer drug. The siddha system has providing clinically significant results in treating hepatic carcinoma by lot of time tested medicines. From one of those medicines this sample is further manifested by proper clinical evaluation in the infected hepatic carcinoma patients.

KEYWORDS: Cancer, Siddha, Nano Medicine, Sangu, Chunnam, hepatic carcinoma.

INTRODUCTION

Liver cancer is the highly prevalenced cancer causing veryhigh range of morbidity rates among world human population. Even though various treatment modalities such as Cytotoxic

Chemotherapy, Oncolytic Virus Therapy are available we need an efficacious drug to treat hepatic carcinoma without causing any adverse effects.

METHODS

Hepg2 (Liver cancer cell line) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen). The cell lines was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, , L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extracts and compound stock

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation

After 24 hours the growth medium was removed, Venom was added to a final concentration of 50µg/ml to induce toxicity and freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology

of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\textbf{\% of Viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of control group}}$$

RESULTS AND DISCUSSION

Hepg 2 (Liver cancer cell line).

Sample volume (µl)	Average OD at 540nm	Percentage Viability
Control	1.8522	
6.25	1.8500	99.8
12.5	1.7833	96.2
25	1.6010	86.4
50	1.3219	71.3
100	0.7522	40.6

LD 50 value –**84.64 µg/ml**

Siddha medicine has various potential formulation to treat the disease cancer. Sangu chunnam is a Siddha nano drug evaluated for its anti cancerous effect against Hepg2 (Liver cancer cell line) using MTT assay.

The observed LD 50 value of Sangu Chunnam sample possessed 84.64µg/ml against Hepg2 (Liver cancer cell line). This confirms the promising anticancerous activity of siddha formulation Sangu Chunnam.

CONCLUSION

This research findings strongly confirms that Siddha nanomedicine SanguChunnam has potent anticancer effect. This therapeutic efficacy will helps to treat hepatic carcinoma worldwide. This confirms that there are many scopes in treating diseases with siddha medicinal potentialities.

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