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In Vitro Evaluation of Anticancer Activity of *Gowri Chinthamani Chendhooram*, Siddha Medicine Against *HeLa* Cells

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ABSTRACT

Cancer is one of the dangerous disease, modern science still struggle with this disease for complete cure without complications. The number of cancer patients' is increasing day by day. One of the Indian traditional system of medicine, the Siddha medicinal system plays unique role in the treatment aspects of cancer. It declares enormous medicines for curing cancer. Among them *Gowri Chinthamani Chendhooram* (GCC) is one of the drug mentioned in classical text with anticancer potential and is also considered to be a crown of Siddha system of medicine. To explore the fruits of GCC to the world, it was underwent globally accepted technique. MTT assay for GCC was done on *HeLa* cell lines. Raising modern science supports the heavy metals usage in cancer treatment. At the end of this preliminary study, GCC found as a potent anticancer medicine on human cancer cells. Further studies with this medicine will pave right way towards safe and potent anticancer medicine.

Key words: Siddha, GCC, anticancer, *HeLa* cells, heavy metals.

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INTRODUCTION

Cancer, a dangerous disease which stands tall in the research activity of many scientists giving the fact that no medicine has been found to completely cure, at least 14 million people all over the world are affected by cancer and at least 8.2 million people become victim to this disease by causing death[1]. Junk food consumption, alcohol, smoking, stress, plastic usage and tremendously adapted life are considered to be the cause for cancer.

There are more than 100 types of cancers which is still trending with new cases of cancer. Even with a remarkable advance in science and technology, there has been no medicine found forthis disease. Also, the modern medicine for cancer leads to numerous side effects and there are possibilities that the medicine itself could kill people.

Siddha medical system delivers huge line of treatments for different kinds of life threatening diseases including cancer. This unique system deals cancer and its treatment widely. In ancient Siddha literature, cancer is explained in the name of *putru* (undetermined growth) which gives the direct meaning and as *Arpudham* (spectacular tumors) and *Vanmeegam*[2] (precarious tumors). Siddha physicians consider some types of cancer growths with the symptoms of *Vippuruthi* [3](multifaceted growth) for their practice.

One of the crown of Siddha medicine, *Gowri Chinthamani Chendhooram* is mentioned for *Vippuruthi* in the classic Tamil literature " Agathiyar vaithiya kaviyam 1500" [4]. Even it is a metallic preparation[5], the safety of the drug is established already[6]. Hence this study was attempted to validate anticancer activity of Siddha mineralo metallic formulation *Gowri Chinthamani Chendhooram* through MTT assay on HeLa cells, the first immortal human cancer cell lines.

MATERIALS AND METHOD

Preparation of trial drug

Mercury, Sulphur and Borax were bought from authorized country raw drug shop at Parrys corner in Chennai, Tamilnadu. All the raw drugs were identified and authenticated by the pharmacological experts of PG department of Gunapadam, Govt. Siddha Medical College, Chennai. The samples of each raw materials were kept in PG department of Gunapadam for future reference

Procedure

The raw drugs were purified as per the methods quoted in the book 'Sigichaarathna Deepam Ennuum Vaidhya Nool' written by Vaidhya Ratnam C. Kannusami Pillai[7]. Purified Mercury and purified Sulphur were ground together in a mortar to attain the state of kajali [8] which means thick black colour compound. Then purified Borax was added with the above mixture and ground firmly. The whole mixture was separated into small equal parts. Each parts are

kept in a piece of tough cotton cloth and tied by thread carefully. Place the mixture filled pouches into the mud pot which was contained sand. This set up was sealed with proper mud plate. And the junction of two were sealed with clay smeared cotton ribbon for 7 times. Allow it to dry. The whole apparatus was kept into a pit which was already prepared as per the literature[9]. Fill the pit with cow dung cakes and furnace. This is known as sand bath incineration. After incineration allow to cool. Then the product was collected carefully and powdered in a mortar. The prepared medicine was stored in an air tight container and labelled as GCC. The trial drug underwent some special Siddha techniques to prove its perfection.

IN VITRO EVALUATION OF ANTICANCER ACTIVITY

Cell line and culture:

HeLa cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents:

MEM was purchased from Hi Media Laboratories, Fetal Bovine Serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

***In Vitro* assay for Anticancer activity (MTT assay) [10]**

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

The growth inhibitory nature of GCC against HeLa cell lines with different concentrations is

displayed in table. When the medicine increased in its concentration, there was an increase in cell growth inhibition. The 50% 0f inhibitory concentration (IC_{50}) of drug value was obtained at 31.2µg/ml. With this much lesser concentration it inhibits cell growth effectively. The percentage of cell viability to GCC in different concentration is displayed in figure 1. The images of HeLa cell lines treated with various concentration of *Gowri Chinthamani Chendhooram* are shown in figure 2

Table : 1 Anticancer effect of Sample (GCC) on *HeLa C*ell line at various concentration

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.09	16.07
2	500	1:1	0.13	23.21
3	250	1:2	0.18	32.14
4	125	1:4	0.21	37.50
5	62.5	1:8	0.24	42.85
6	31.2	1:16	0.27	48.21
7	15.6	1:32	0.32	57.14
8	7.8	1:64	0.35	62.50
9	Cell control	-	0.56	100

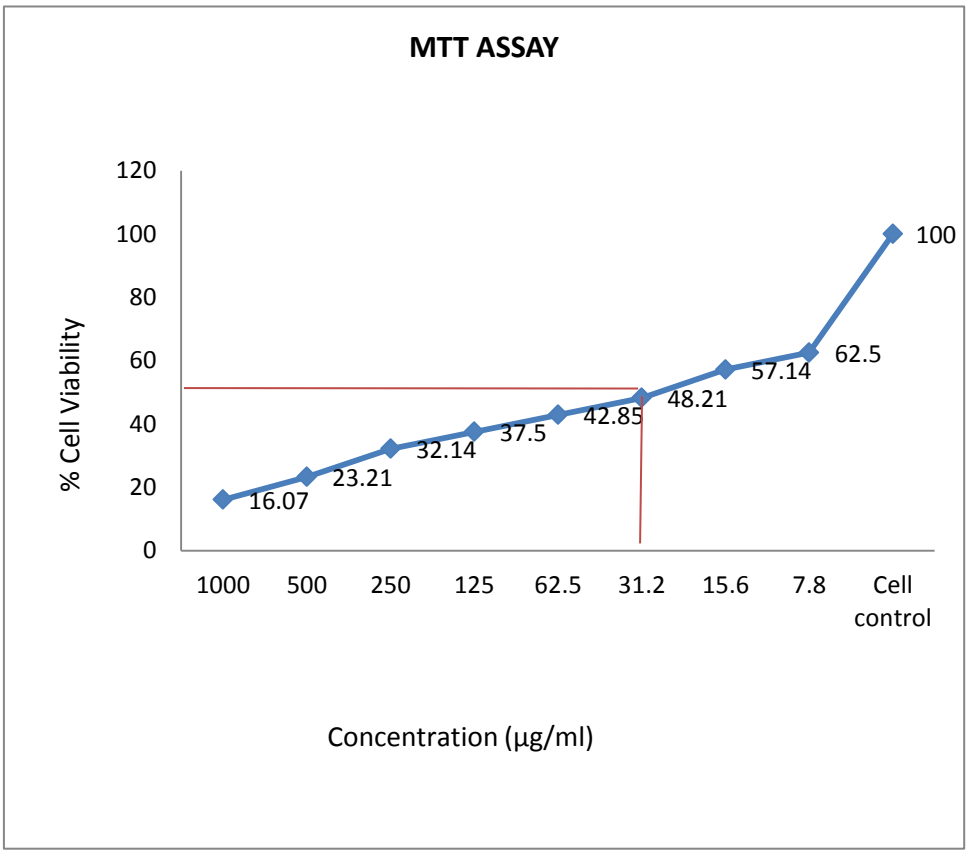

Figure: 1 Percentage of cell viability of GCC at different concentration

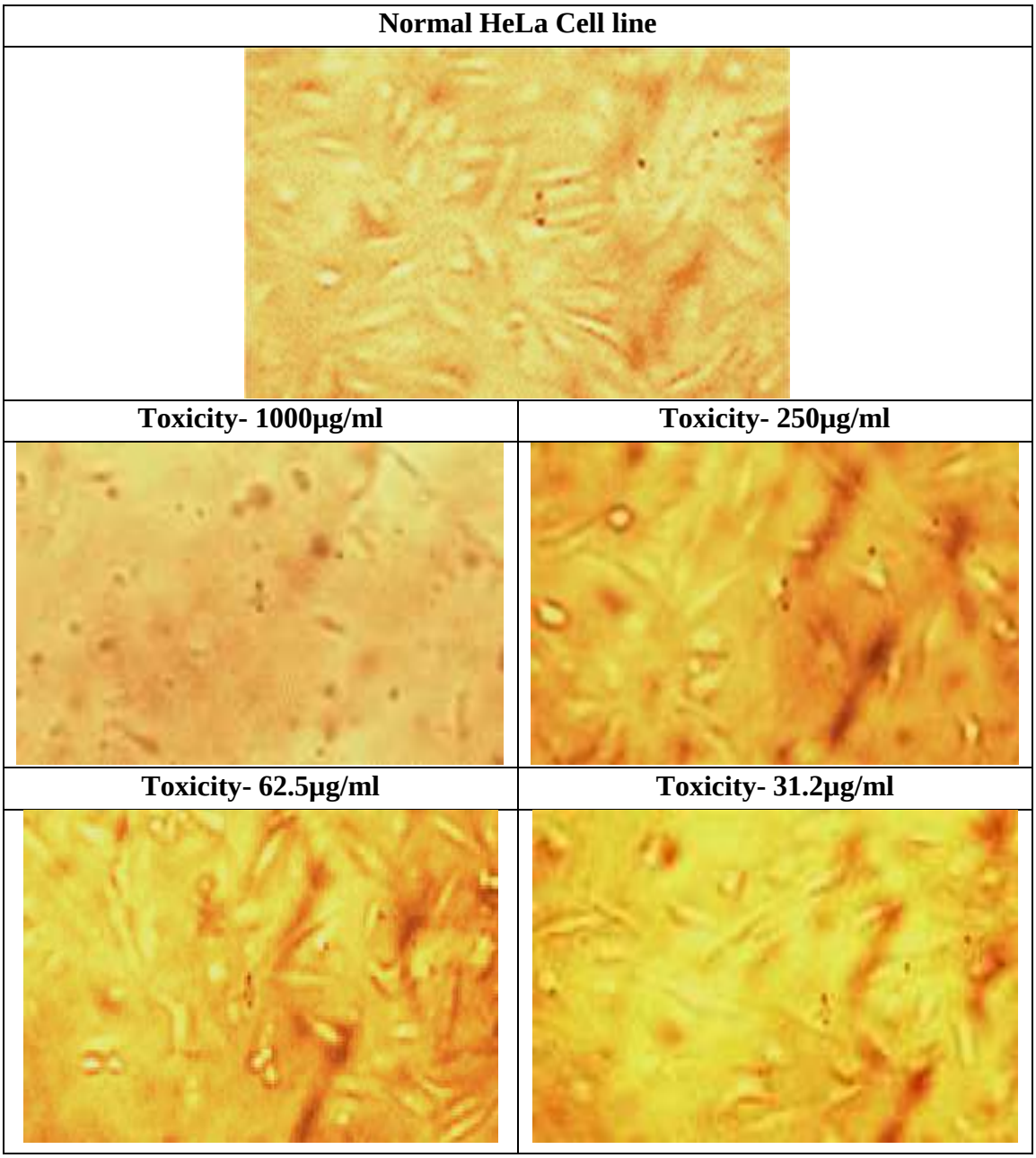

Figure: 2 Anticancer effect of Sample (GCC) on *HeLa C*ell line

The potency of anticancer activity of *Gowri Chinthamani Chendhooram* may achieved by the mixture of Mercury and Sulphur. Recent studies say that pre-administration of heavy metals induce metallothionein in target tissues which may act as a free radical scavengers [11].The biomedical organic chemistry (elemental medicine) categorized mercury as a chemotherapeutic metallic element and sulphur as a nonmetallic essential element [12].

Rasagandhi mezhugu, which is prepared by the combination of Mercury (Rasam) and sulphur (Gandhi) was clinically proved for its anticancer effect in oral cancer patients [13]. Las01 a Mercurial preparation was reported as a potent anticancer drug in human cancer cell lines which has no toxic effects on animals and human [14].

Many of the Siddha anticancer preparations contain mercury and sulphur as its major ingredients [15]. In Siddha system of medicine Mercury and Sulphur are the most important

drugs. Mercury is considered as sperm and Sulphur is considered as ovum and their combination creates power [16].

The toxic mercury and sulphur lose their toxicity during purification and preparation. After incineration process the macro particles became very smaller and this may possible for devoid of toxicity and more potent in anticancer therapeutic. Further anticancer screening methods on GCC will help the world to become a cancer free environment.

CONCLUSION

The results acquired from the *in-vitro* studies achieved via the *HeLa* cell lines reveals that the unique Siddha medicine *Gowri Chinthamani Chendhooram* has a potent anticancer activity. There was increase in the cell growth inhibition when concentration of sample was increased, the IC_{50} value was less than 50 µg/ml for the cell line studies as exposed by the MTT assay method. Hence the level of cytotoxicity of the *Gowri Chinthamani Chendhooram* can be concluded to be more effective. Further studies will help to lift the medicine globally.

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