

**EVALUATION OF ANTITUMOR ACTIVITY OF SIDDHA DRUG GOWRI  
CHINTHAMANI CHENDHOORAM (GCC) AGAINST DU 145 CELL LINES****\*Dr. S. Ramalingam<sup>1</sup>, Dr. P. Parthibhan<sup>2</sup>, Dr. K. Kanagavalli<sup>3</sup>, Dr. D. Mantela<sup>4</sup>, Dr. P. Porchelvan<sup>5</sup>**<sup>1</sup>Chief consultant, Nalam Siddha Hospital, Redhills, Chennai- 600 052, Tamilnadu, India.<sup>2</sup>Joint Director, Dept. of Indian Medicine and Homeopathy, Chennai- 600 106, Tamilnadu, India.<sup>3</sup>Principal, Govt. Siddha Medical College, Arumbakkam, Chennai – 600 106, Tamilnadu, India.<sup>4</sup>Emergency Medical Officer, National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047, Tamilnadu, India.<sup>5</sup>PG Scholar, Dept. of Pothu Maruthuvam, Govt. Siddha Medical College, Chennai – 600 106, Tamilnadu, India.**\*Corresponding Author: Dr. S. Ramalingam**

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Article Received on 23/05/2017

Article Revised on 13/06/2017

Article Accepted on 03/07/2017

**ABSTRACT**

Benign prostatic hyperplasia (BPH) is more common in men above 60yrs of age. The second most common disease in men need to go for surgery. The National Institutes of Health, reports more than 7.8 million people got diagnoses with BPH. In Siddha system, *Gowri Chinthamani Chendhooram*(GCC), a metalo-mineral preparation contains purified sulphur, purified mercury and purified borax helps to treat BPH. The symptoms of BPH are closely related with symptoms of Vatha moothira kirichuram mentioned in Siddha literatures. In pre-clinical studies, the drug shows no toxicity up to level of 200mg/kg bw and the pharmacological activity of GCC revealed by its anti-tumor activity against DU 145 cell line studies. IC<sub>50</sub> value of GCC was 15.6 µg/ml, which shows the drug works more on the tumor cell lines. From this preclinical studies, the Siddha drug, GCC should be a new line of treatment for BPH.

**KEYWORDS:** BPH, antitumor, GCC, *DU 145* cell lines, Vatha moothira kirichuram.**INTRODUCTION**

Benign prostatic hyperplasia (BPH) is a non-malignant, uncontrolled proliferation of the epithelial cells and stromal cells that occurs in the peri urethral transition zone of the prostate gland that surrounds the urethra.<sup>[1]</sup> Benign prostatic hyperplasia (BPH) is the most common condition affecting men older than 50 years of age. Associated symptoms are common from 60 years of age and some 50% of men over 80 years will have lower urinary tract symptoms (LUTS) associated with BPH.<sup>[2]</sup>

The primary symptoms of BPH are due to the prostate obstructing the urethra.

They consist of

- Hesitancy
- Poor prolonged flow
- Sensation of incomplete emptying, Secondary (irritative) symptoms,
- Comprising urinary frequency (going often),
- Urgency of micturition (going in a hurry)
- Urge incontinence (leaking if you can't get to a toilet in time).<sup>[3]</sup>

According to the National Institutes of Health, there are more than 7.8 million BPH diagnoses made. Epidemiological studies are weighted towards the

presence of BPH having a greater risk for a man to develop prostatic cancer (PCa) in his lifetime.<sup>[4]</sup> Treatment for this disease is still under research.

Siddha medical system, an ancient traditional medicinal system plays vital role in the diseases which are quit by the modern medicine. Siddha system has its own classification of disease. In that, the symptoms of BPH is similar to that of *Vatha moothira kirichuram*<sup>[5]</sup> (moothiram – urine, kirichuram – decreased) which comes under 21 types of *neerina arukkal noikal*<sup>[6]</sup> (neer – urine, arukkal – low output, noikal – diseases). Siddha system prescribes many drugs for this troubling disease. Among them *Gowri Chinthamani Chendhooram* (GCC) is considered as paramount medicine. Purified mercury, purified sulphur and purified borax are the ingredients in the preparation of *Gowri Chinthamani Chendhooram*.<sup>[7]</sup> Its safety on animals have been recognized already.<sup>[8]</sup> Nowadays water, food and also air are subjected to some scientific techniques to substantiate their properties. This article is to explore the distinctiveness uses of GCC by MTT assay on *DU 145* cell lines (brain metastatic prostate tumor cells) in the management of BPH.

## MATERIALS AND METHODS

The raw drugs mercury, sulphur and borax were procured from authorized indigenous raw drug shop in Chennai, Tamilnadu. All the raw drugs were identified and authenticated by the pharmacognosist of Central Siddha Research Institute, Chennai. The samples of each raw materials were kept in PG department of Pothu

maruthuvam, Govt. Siddha Medical College, Chennai for future reference.

### Procedure

The impurities of mercury and sulphur were removed by the suitable methods mentioned in authentic Siddha book.<sup>[9]</sup> Borax was purified by roasting.<sup>[10]</sup>

### Procedure



**Fig.no.1 preparation of GCC**

Purified mercury and purified sulphur mixed together, were ground thoroughly in a stone mortar till they get black colour. Then purified borax was added and the contents were ground firmly. The prepared mixture was subjected into sand bath incineration. After ignition the product was collected carefully and powdered in mortar then stored in an air tight container. The end product was branded as Gowri chinthamani chendhooram (GCC).

## ANTITUMOR EFFECT OF GCC ON DU-145 CELL LINE

### Cell line and culture

DU-145 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<sub>2</sub> 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 Antitumor activity (MTT assay)<sup>[11]</sup>

Cells (1 × 10<sup>5</sup>/well) were plated in 24-well plates and incubated in 37°C with 5% CO<sub>2</sub> 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<sub>50</sub>) was determined graphically.

The % cell viability was calculated using the following formula:

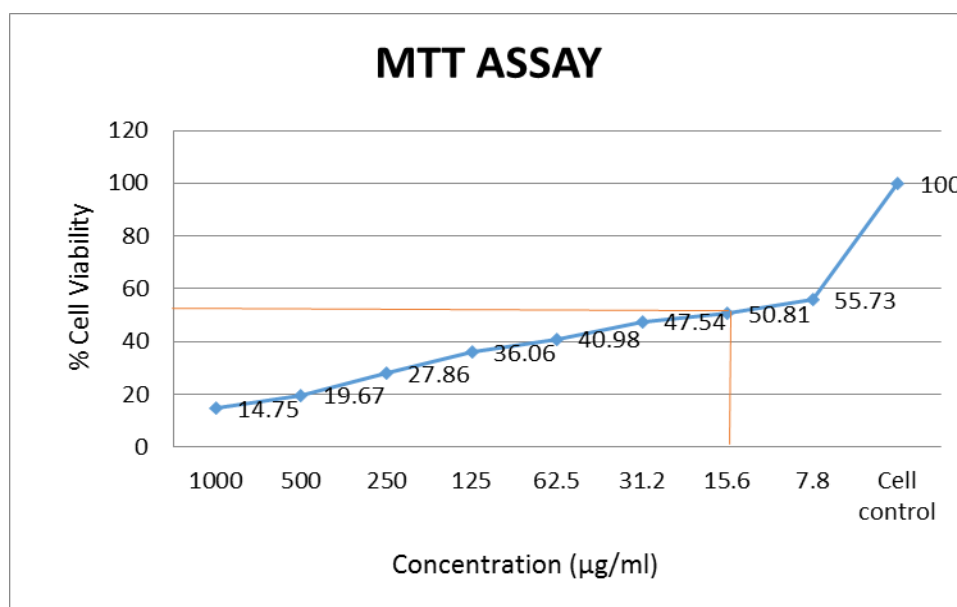
$$\% \text{ cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

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.

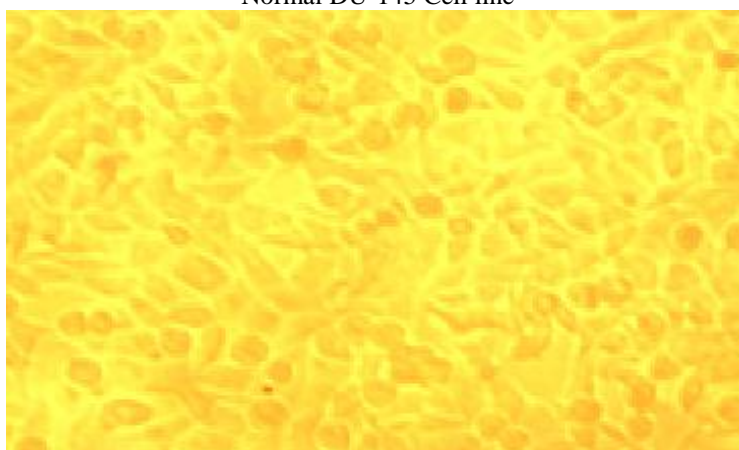
**Table no. 1 Antitumor effect of Sample (GCC) on DU-145 Cell line**

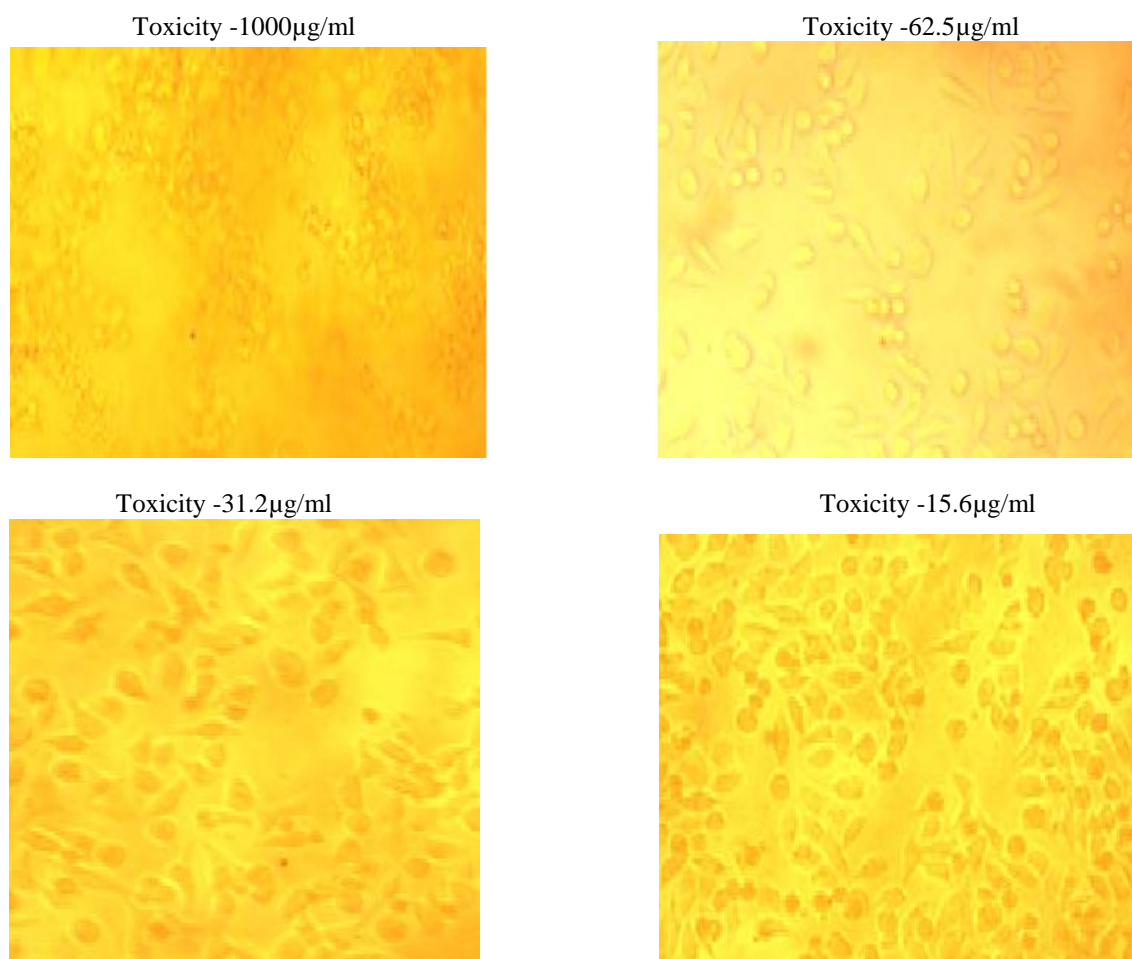
S.No	Concentration ( $\mu\text{g/ml}$ )	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.09	14.75
2	500	1:1	0.12	19.67
3	250	1:2	0.17	27.86
4	125	1:4	0.22	36.06
5	62.5	1:8	0.25	40.98
6	31.2	1:16	0.29	47.54
7	15.6	1:32	0.31	50.81
8	7.8	1:64	0.34	55.73
9	Cell control	-	0.61	100



**Fig.no.2 Percentage of cell viability of GCC**

Normal DU-145 Cell line





**Fig.no.3 Antitumor effect of GCC on DU-145 cell lines**

### RESULTS AND DISCUSSION

The antitumor effect of GCC on DU-145 cell lines with eight various concentrations are showed in the table. Viable cells decreases with rise in drug concentration. 15.6 µg/ml of drug concentration shows 50% of cell viability. This lower drug concentration causes 50% of cell death. IC<sub>50</sub> below 100 µg/ml is considered as good. So *Gowri Chinthamani Chendhooram* has antitumor effect.

Mercury and sulphur had analyzed already for its anticancer effect.<sup>[12]</sup> Their presence in GCC might be the reason for its anticancer nature.

### CONCLUSION

The in vitro evaluation of GCC on DU-145 cell lines by MTT assay tells that *Gowri Chinthamani Chendhooram* has potent antitumor effect against metastatic prostate cell lines.

Hence the sample GCC should be a best medicine to Benign Prostate Hyperplasia. Further screening methods for antitumor activity on GCC will assistance this study.

### ACKNOWLEDGEMENT

The authors thankfully acknowledge the faculties of Post Graduate department of Pothu Maruthuvam (General

Medicine), Government Siddha Medical College, Chennai for their continual support and guidance.

We should acknowledge the Lifetech Research Centre, Chennai for their procedural maintenance.

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