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ANTI-CANCER ACTIVIY OF SORGAMARA ILAI CHOORANAM (LEAVES OF *SIMAROUBA GLAUCA*) IN IN-VITRO CELL LINE MODELS AGAINST INVASIVE CERVICAL CARCINOMA

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ABSTRACT

Cancer is a class of disease characterized by out of control cell growth which tend to proliferate and in some cases to metastasize (spread), known medically as malignant neoplasm with a broad group by 100 different types. One of the Indian traditional systems of medicine, the Siddha medicinal system plays unique role in the treatment aspects of cancer. Siddha medicine, being the oldest traditional system in the world has a strong significance in detoxification, anti-oxidation, immune modulation and metabolic balance. It is a carefully guarded medical system given away incredible and rapid outcomes in various unremitting ailments. Among them *Sorgamara Ilai chooranam* (*Simarouba glauca*) is one of the herb mentioned in Siddha text with anticancer potential and is also considered being a crown of Siddha system of medicine *Simarouba glauca* also known as paradise tree has a long history of herbal medicine in many countries and belongs to the family *Simaroubaceae*. *Simarouba glauca* has 11 medicinally important quassinoids, the active principles in the tree. The Pharmacological activities are justified by anticancer effect on HeLa cell line study through in- vitro model. The main objective of this study was to evaluate the anticancer potential of *Sorgamara Ilai Chooranam* (leaves of *Simarouba glauca*) to treat cervical cancer. Anticancer activity of this Chooranam was evaluated in vitro for anticancer activity by MTT assay method in HeLa cell line. The results showed that the

studied drug possessed relevant anticancer activity and thus, therapeutic potential in the treatment of cervical cancer.

KEYWORDS: *Anti-cancer activity, cervical carcinoma, Siddha herb, Simarouba glauca.*

INTRODUCTION

Cancer is a class of disease characterized by out of control cell growth which tend to proliferate and in some cases to metastasize (spread), known medically as malignant neoplasm with a broad group by 100 different types.[1] Cervical cancer is the second largest cause of cancer mortality in India accounting for nearly 10% of all cancer related deaths in the country. It is estimated that by the year 2020 there will be almost 20 million new cases.[2] According to National Cancer Registry Program recent report of 2008, the load of breast and cervical cancer together was 23.6 to 38.7% of the total cancers. In 2009 the number of cervical cancer cases were 1, 01,938 which has increased to 1, 07,690 in 2012. Among this Tamilnadu reported 55,000 new cases per year state wide in 2012 to 2016.[3] Cancer chemotherapy strives to cause a lethal cytotoxic lesion that can arrest a tumor progression. The chemotherapeutic agents though effective against various types of tumor are not totally free from side effects.[4] Siddha medicinal system plays unique role in the treatment aspects of cancer. In Siddha literature, cancer is explained in the name of *putru* (undetermined growth) which gives the direct meaning and as *Arpudham* (spectacular tumors) and *Vanmeegam* (precarious tumors). Siddha physicians consider some types of cancer growths with the symptoms of *Vippuruthi*(multifaceted growth) for their practice.[5,6] Siddha medicine, being the oldest traditional system in the world has a strong significance in detoxification, anti-oxidation, immune modulation and metabolic balance. It is a carefully guarded medical system given away incredible and rapid outcomes in various unremitting ailments. Among them *Sorgamara ilai chooranam* (*Simarouba glauca*) is one of the herb mentioned in Siddha text with anticancer potential[7] and is also known as paradise tree has a long history of herbal medicine in many countries and belongs to the family *Simaroubaceae*. It was widely used for treatment of cancer hence it is known as tree of solace of cancer.[8] The main distribution hot spots are located at tropical areas of America, Africa, Madagascar and Australia, Cuba, Brazil, Mexico, Peru, India.[9] *Simarouba glauca* has 11 medicinally important quassinoids, the active principles in the tree.[10] The Pharmacological activities are justified by anticancer effect on HeLa cell lines through in- vitro model.

MATERIALS AND METHOD

Source of Collection

S. glauca leaves were collected from distinct region of Kolli hills.

Identification and Authentication of the drug

The leaves were identified and authenticated by the *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, Chennai – 106. The specimen sample of the herb has been preserved in PG *Gunapadam* department for future reference.

Preparation of the trial drug – *Sorgamara Ilai Chooranam*

Procedure

Fresh leaves were collected and dried at room temperature. The dried leaves were powdered by means of grinder and the powder was sieved by a cotton cloth and then bottled up. It was labeled as *Sorgamara Ilai Chooranam* (SMC).

In vitro evaluation of anticancer activity

Cell line and culture

HeLa cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 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) (Mosmann, 1983)[11]

Cells (1 × 105/well) were plated in 24-well plates and incubated in 370C with 5% CO2 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 DMEM 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 (IC50) 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

This present study showed that the first research on the potential cytotoxic and antiproliferative activity of Sorgamara ilai chooranam (Simarouba glauca) on HeLa cancer cell line at various concentrations.

The percentage of cells viability was determined by calculating the O.D of treated against the control. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540 nm. Comparison values are made on a basis of 50% inhibition of growth (IC50) in treated cells with specific agents. Results were tabulated in Table.1 and graphically represented in Fig. 1.

Table 1: Anticancer effect of SMC on *hela* cell line at various concentration.

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.209	24.61
2	500	1:1	0.276	32.50
3	250	1:2	0.346	40.75
4	125	1:4	0.415	**48.88**
5	62.5	1:8	0.468	55.12
6	31.2	1:16	0.529	62.30
7	15.6	1:32	0.590	69.49
8	7.8	1.64	0.658	77.50
9	Cell control	-	0.849	100

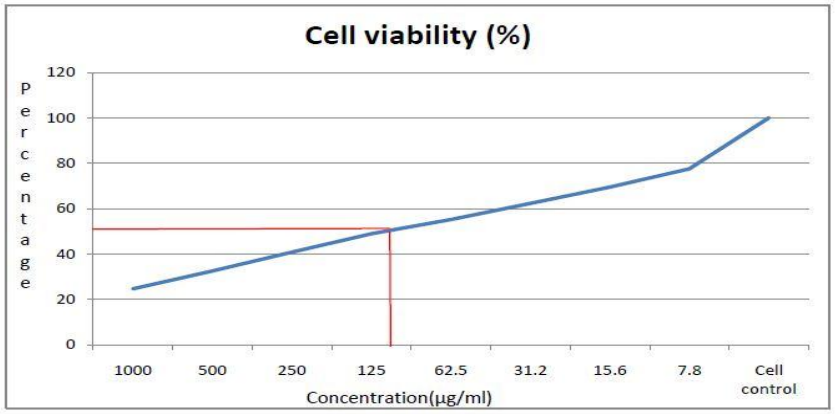
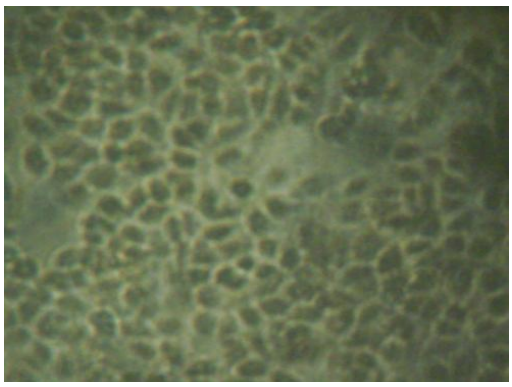
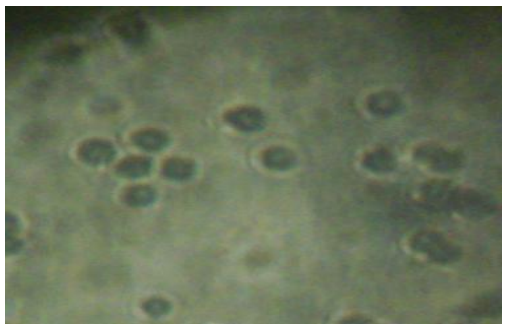


Figure: 1 Percentage of cell viability of SMC at different concentration.

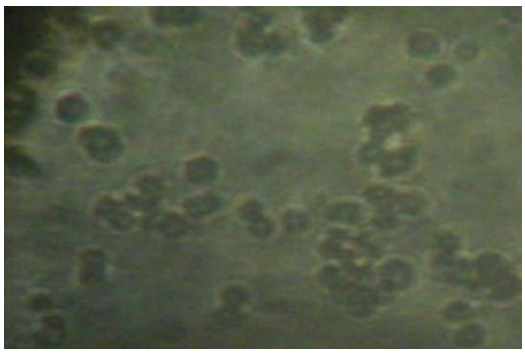
Normal HeLa Cell line



Toxicity – 1000 µg/ml



Toxicity – 125µg/ml



Toxicity – 7.8 µg/ml

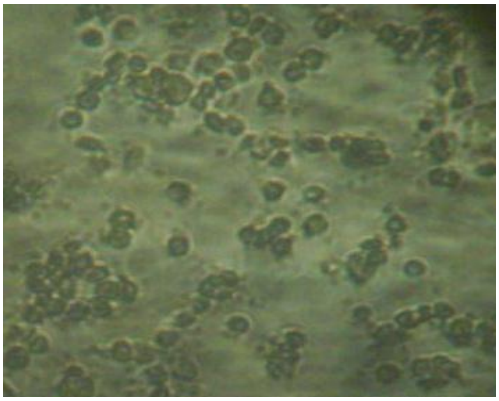


Figure 2: Anticancer effect of Sample (SMC) on *HeLa C*ell line.

The results indicated that the drug dose and % of Inhibition of HeLa cells after the Sorgamara ilai chooranam extract treatment. It can be observed by the result of MTT assay that the IC dose of Sorgamara ilai chooranam is 50µg/ml. As the dose increases the HeLa cell viability decreases and exhibited cytotoxic and antiproliferative activity providing evidence that the trial drug is a potential source of drug to be used against cervical cancer.

It was found that the % growth inhibition increasing with increasing concentration of Sorgamara ilai chooranam steadily up to 125 µg/ml on HeLa cell line (Table and Graph and that IC value on HeLa cell line was 50 and R value was 0.849.

Simaroubaceae plants have remained an interesting source of novel potential anti-neoplastic agents.[12] It contains the important chemical constituents include alkaloids, flavanoids, cardinolides, glycosides, phenolic compounds, saponins and fixed oils.[13] The main active constituents in *Simaroubaglauca* include: ailanthinone, benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, and tirucalla etc.[10-a]

Quassinoids as the main component present in Simarouba glauca. They represent a heterogeneous group of molecules with the ability to interfere with different pathways implicated in tumorigenesis. The induction of apoptosis also appears to be a common feature among quassinoids.[14] Some evidence has proposed that phenolic compounds inhibit telomerase activity in tumor cells.[15]

Alkaloids play major role as anticancer agents by inhibiting the enzyme topoisomerase which is involved in DNA replication. Their mechanisms of action in uncontrolled proliferation of

cells would help in formulating drugs.[16] Flavonoids greatly influence the cascade of immunological events associated with the development and progression of cancer. Flavonoids have the potential of modulating many biological events in cancer such as apoptosis, vascularization, cell differentiation, cell proliferation etc.[17]

From all the above facts and results suggested that Sorgamara ilai chooranam (Simarouba glauca) is emerging as a promising species that can be used as a therapeutic agent for the treatment of cervical cancer.

CONCLUSION

The present study has demonstrated with validated test in-vitro cell line model against invasive cervical carcinoma of *Sorgamara Ilai Chooranam (Simarouba glauca).* The previous phytochemical study showed the presence of alkaloids, flavanoids, cardinolides, glycosides, phenolic compounds, saponins and fixed oils but also the presence of Quassinoids predominantly in the leaves. These preliminary results could constitute a scientific basis for the search for new antitumor compounds from *Simarouba glauca.* The results acquired from the in-vitro studies achieved via the HeLa cell line reveals that the unique Siddha medicine *Sorgamara Ilai Chooranam* has a potent anticancer activity. There was increase in the cell growth inhibition when concentration of sample was increased; the IC50 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 *Sorgamara Ilai Chooranam* can be concluded to be more effective. This concludes *Sorgamara Ilai Chooranam (Simarouba glauca),* a promising anti-cancer agent for new therapeutic treatment for cervical cancer.

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