Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(10):

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Studies on the Anticancer Potential (GI50) of the Siddha Formulation, Rasagenthi Mezhugu on Human Cell Lines

J Baskaran[1], A Gnanamani[2], V.Amalan Stanley[3*] and Shaleesha A Stanley[4*]

[1]*Orchid Research Laboratories Pvt. Ltd., Chennai, India*
[2]*Biological Material Laboratory, CSIR-Central Leather Research Institute, Chennai, India*
[3]*Director and Test Facility Management, Vanta Bioscience (Kemin Industries Inc.), Gummidipundi, Chennai, India*
[4]*Department of Biotechnology, Jeppiaar Engineering College, Chennai, India*

ABSTRACT

In spite of tremendous developments in the field of Allopathy during the 20th century, plants still remain one of the major sources of drugs in the modern as well as traditional systems of medicine throughout the world, as alternative medicine. Many of the Indian medicines, after prolonged use in folk medical practices, are screened and used for treating and preventing various chronic disorders such as cardiovascular diseases and cancer. The anticancer property of one of the Siddha formulations, Rasagenthi Mezhugu (RM) was tested by standard MTT cell proliferation assay method. In MTT assay, the cytotoxicity and GI50 values were observed for three cell lines obtained from ATCC. The cell lines used are COLO205 (ATCC® CCL222) ™), (adherent, epithelial, colon derived from the lymph nodes of metastatic site), MDA-MB-231 (ATCC® HTB-26™), (adherent, epithelial, mammary gland (breast) derived from pleural effusion sites of metastasis) and HCT 116 (ATCC® CCL247™), (adherent, epithelial colon derived from colorectal carcinoma). The percentage of cytotoxicity, Growth Inhibition (GI50) and viability percentage were calculated and inferred accordingly. Results showed that the percentage cytotoxicity of RM in MDA-MB-231 cell line was found to be Maximum (93.9%) followed by COLO205 Cell line (90%) and HCT-116 Cell line (59.2%) at 2000 µg/ml (GI50) respectively. The results of cytotoxicity assay demonstrated that among all the three tested human cell lines, growth inhibition was found to be directly proportional to the concentration used. The GI50 data represent that among tested human cancer cell lines, the MDAMB-231Cell line is more receptive towards Rasagenthi Mezhugu. One of the prerequisites for an anti-cancer drug, be it an NCE or herbal alternative or a biological compound, is to be cytotoxic in order to kill the cancer cells and many anticancer products in the market are basically cytotoxic. Therefore, the results of the present study on the herbal product, RM strongly support the usefulness of Rasagenthi Mezhugu as one of the key Complementary Alternative Medicines in cancer therapy.

Keywords: Rasagenthi mezhugu; MTT assay; Anticancer; Cell lines

---

INTRODUCTION

Cancer is a major public health burden in both developed and developing countries. It's one of the major growing public health concerns that is given equally growing research efforts in discovering and developing new anti-cancer drugs. In this effort, other than new chemical entities (NCEs) support is also sought from plant derived agents for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are already in clinical use all over the world [1]. Further, a number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or preclinical development [2].

Rasagenthi Mezhugu (RM) and a combination of Siddha drugs were reported to be useful in treating HIV patients for both clinical improvements [3]. RM is one of the Siddha formulations used for the treatment of venereal diseases including diseases closely resembling HIV [4]. RM is currently prescribed by thousands of Siddha Practitioners all over India for the treatment of cancer, AIDS and skin diseases, showing considerably significant efficacy as well. Even though this drug is used by Siddha practitioners there is paucity of enough scientific evaluation available to support its efficacy against cancer [5]. Anticancer property, by *in vitro* method could be confirmed through conducting cytotoxicity tests and traditionally, cytotoxicity is primarily calculated by counting viable cells after staining with a vital dye called Trypan blue [6]. It's the simple staining method to evaluate cell membrane integrity though considered to be a less sensitive method and cannot be adapted for high throughput screening. Alternatively, measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is a reliable method but it is time-consuming and involves handling of radioactive substances. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents [7,8].

This study is carried out with the aim of evaluating the herbal drug Rasagenthi Mezhugu (RM), a new Siddha formulation primarily to confirm its anticancer activity under *in vitro* conditions, using MTT assay on cell lines.

MATERIALS AND METHODS

Cell Lines

The cell lines used for the study are COLO205 [ATCC® CCL222™ (adherent, epithelial, colon derived from the lymph nodes of metastatic site)], MDA-MB-231 [ATCC® HTB-26™ (adherent, epithelial, mammary gland (breast) derived from pleural effusion from the sites of metastasis)] and HCT 116 [ATCC® CCL247™ (adherent, epithelial colon derived from colorectal carcinoma)].

Chemicals

The chemicals, reagents and media as well as the cell lines used for the study are: Dulbecco Modified Eagle's Medium (DMEM), MCDB-105 and RPMI 1640, Dimethyl sulphoxide (DMSO), Fetal bovine serum, PBS, MTT solution (5 mg/ml) and the test drug, Rasagenthi Mezhugu (RM). MTT is prepared at concentration of 5 mg/ml in PBS. The COLO205 (ATCC® CCL222™), MDA-MB-231(ATCC® HTB-26™) and HCT 116 (ATCC® CCL247™) were sub cultured in DMEM (as per ATCC Protocol).

Method

Based on the recommendations of Thermo Fisher scientific protocol, cytotoxicity assays were carried out in 96 well sample preparations for adherent cells. After the cells reached adequate confluence levels, the cells are trypsinized, centrifuged and the pellets were re-suspended in media. These are then added to 96 well plates at 10,000 cells per well in triplicates. Different concentrations of test drug (2 mg/ml to 2 ng/ml) are added to triplicate of each cell line. One triplicate is observed with cell lines alone as control. After 72 hours of incubation 96 well plates were viewed under inverted microscope [9]. Then added 20 µl of 5 mg/ml MTT to each well and included one set of wells with MTT without cells as control. The entire experiment was carried out aseptically. The cells were incubated for 3.5 hours at 37°C in culture hood. The media was carefully removed and added with 200 µl DMSO to each well. The absorbance was read at 540 nm. The concentration of the test drug wass taken in X axis and OD in Y axis and the parameters, $GI_{50,}$ % viability and cytotoxicity were calculated.

RESULTS AND DISCUSSION

The percentage of cytotoxicity, Growth Inhibition (GI_{50}) and viability percentage observed for the three cell lines are shown as follows:

Cytotoxicity (%), Growth Inhibition (GI_{50}) and viability (%) in COLO205 (ATCC® CCL222™)

Cytotoxicity (%) of Rasagenthi Mezhugu (RM) in COLO205 (ATCC® CCL222™) Cell line was found to be a maximum of 90% and minimum of 11.8% at 2000 µg/ml and 0.002 µg/ml respectively. The GI_{50} of RM in COLO205 cell line was observed to be 1 µg/ml (Table 1).

Table 1: Cytotoxicity (%), growth inhibition (GI_{50}) and viability (%) in COLO205 (ATCC® CCL222™)

Conc.	COLO205 (ATCC® CCL222™)					
µg/ml	OD1	OD2	OD3	Mean OD	Viability (%)	Cytotoxicity (%)
2000	0.224	0.236	0.218	0.23	9.6	90.4
200	0.325	0.268	0.304	0.3	12.8	87.2
20	0.624	0.521	0.554	0.57	24.2	75.8
2	0.987	0.948	0.9057	0.95	40.4	59.6
0.2	1.326	1.254	1.026	1.2	51.3	48.7
0.02	1.687	1.813	1.915	1.81	77	23
0.002	2.036	2.15	2.015	2.07	88.2	11.8
0	2.158	2.325	2.547	2.34	100	0
GI_{50}						1 µg/ml

Cytotoxicity (%), Growth Inhibition (GI_{50}) and viability (%) in MDA-MB-231 (ATCC® HTB-26™)

Table 2: Cytotoxicity (%), growth inhibition (GI_{50}) and viability (%) in MDA-MB-231 (ATCC® HTB-26™)

Conc.	MDA-MB-231 (ATCC® HTB-26™)					
µg/ml	OD1	OD2	OD3	Mean OD	Viability (%)	Cytotoxicity (%)
2000	0.1254	0.1035	0.1689	0.13	6.1	93.9
200	0.215	0.236	0.158	0.2	9.4	90.6
20	0.427	0.357	0.358	0.38	17.6	82.4
2	0.987	0.896	0.889	0.92	42.7	57.3
0.2	1.358	1.204	1.168	1.24	57.4	42.6
0.02	1.896	1.487	1.687	1.69	78	22
0.002	2.154	2.014	1.998	2.06	94.9	5.1
0	2.354	2.158	1.987	2.17	100	0
GI_{50}						1 µg/ml

Cytotoxicity (%) of Rasagenthi Mezhugu (RM) in MDA-MB-231 (ATCC® HTB-26™) cell line was found to be maximum of 93.9% and minimum of 5.1% at 2000 µg/ml and 0.002 µg/ml respectively. The GI_{50} of RM in MDA-MB-231 Cell line was observed to be 1 µg/ml (Table 2).

Cytotoxicity (%), Growth Inhibition (GI_{50}) and viability (%) in HCT 116 (ATCC® CCL247™)

Cytotoxicity (%) of Rasagenthi Mezhugu (RM) in HCT 116 (ATCC® CCL247™) Cell line was found to be maximum of 59.2% and minimum of 5.3% at 2000 µg/ml and 0.002 µg/ml respectively. The GI_{50} of RM in HCT-116 Cell line was observed to be 1 µg/ml (Table 3). Results showed that the percentage cytotoxicity of RM in MDA-MB-231Cell line was found to be Maximum (93.9%) followed by COLO205 cell line (90%) and HCT-116 Cell line (59.2%) at 2000 µg/ml (GI_{50}) respectively. Overall, the results were promising for all the three tested human cell lines and growth inhibition was found to be directly proportional to the concentration used [10-12].

Table 3: Cytotoxicity (%), growth inhibition (GI_{50}) and viability (%) in HCT116 (ATCC® CCL247™)

Conc.	HCT116 (ATCC® CCL247™)					
µg/ml	OD1	OD2	OD3	Mean OD	Viability (%)	Cytotoxicity (%)
2000	0.879	0.821	0.615	0.77	40.8	59.2
200	1.106	0.987	0.951	1.01	53.7	46.3
20	1.236	1.325	1.354	1.31	69	31
2	1.459	1.405	1.438	1.43	75.8	24.2
0.2	1.547	1.658	1.489	1.56	82.8	17.2
0.02	1.598	1.689	1.789	1.69	89.5	10.5
0.002	1.698	1.789	1.887	1.79	94.7	5.3
0	1.987	1.896	1.789	1.89	100	0
GI 50						500 µg/ml

CONCLUSION

To conclude, being a cytotoxic compound is one of the prerequisites for an anti-cancer drug (unlike any other therapeutic drugs), be it an NCE or herbal alternative or a biological compound, in order to kill the cancer cells and many anticancer products in the market are basically cytotoxic and it is also acceptable by the regulatory authorities to approve the anticancer drugs if found clinically effective in spite of being cytotoxic. Therefore being cytotoxic, the results of this preliminary *in vitro* study on the herbal product, RM strongly support the usefulness of Rasagenthi Mezhugu as one of the key Complementary Alternative Medicines in cancer therapy. This *in vitro* study confirming the basic anticancer properties would pave scope for *in vivo* studies and support the utility of Rasagenthi Mezhugu as evidence based alternative medicines for cancer therapy [13,14].

ACKNOWLEDGEMENTS

The authors remain grateful to Dr.A. Saraswathi (late), former Director, Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Chennai for providing the test item, RM to carry out the study. Also the authors remain thankful to the R&D Management, Orchid Research Laboratories Pvt. Ltd., Chennai for providing resources to conduct the study.

REFERENCES

[1] A Riyasdeen; SV Periasamy; P Preethy; AA Alshatwi; MA Akbarsha. *Evi Based Comp Alt Med*. **2012**, 1-10.
[2] CN Deivanayagam. *Proc Nat Sem Trad Med HIV Infect AIDS*. **2012**, 67-70.
[3] CS Sharma; RK Nema; SN Meyyanathan. *Academic J Cancer Res*. **2009**, 2(1), 19-24.
[4] A Jemal; R Siegel; E Ward. *J Clin*. **2008**, 58(4), 71-96.
[5] F Yibin; W Ning; Z Meifen; F Yigang; L Hongyun; T Saiwah. *Rec Patents Food Nut Agri*. **2011**, 3(1), 30-40.
[6] K Aruna; VM Sivaramakrishnan. *Food Chem Toxicol*. **1992**, 30, 953-956.
[7] UM Acuna; DE Atha; J Ma; MH Nee; EJ Kennelly. *Phyto Ther Res*. **2002**, 16, 63-65.
[8] A Mariappan; K Manickavasakam; SMA Joseph. *J Chem Pharm Res*. **2017**, 9(4), 14-120
[9] A Mariappan; G Ganapathy; V Bhanumathi. *Int J Adv Res Biol Sci*. **2016**, 3(12), 23-31.
[10] A Phaniendra; DB Jestadi; L Periyasamy. *Ind J Clin Biochem*. **2015**, 30(1), 11-26.
[11] A Rahal; A Kumar; V Singh; B Yadav; R Tiwari; S Chakraborty; K Dhama. *BioMed Res Int*. **2014**, 1-19.
[12] A Saraswathy; AK Meena; R Shakila; KN Sunil Kumar; S Ariyanathan. *Ind Drugs*. **2010**, 2(11), 44-45.
[13] RS Ranga; R Girija; M Nur-e-Alam. *Cancer Chemo Ther Pharmacol*. **2004**, 54, 7-15.
[14] V Srivastava; AS Negi; JK Kumar; MM Gupta; SP Khanuja. *Bioorg Med Chem*. **2005**, 13(21), 5892-5908.