SIDDHA PAPERS
A PEER REVIEWED ONLINE JOURNAL
ISSN: 0974-2522

Siddha Papers 2018 (13) (1)
RESEARCH ARTICLE
https://www.siddhapapers.com

In-vitro Anti-cancer activity of a poly-herbal Siddha formulation against HCT116 Gastric cancer cell lines

Thomas M Walter[1], G. Sabari devi[2], K. Swathi[2], C. Samuel Justin Raj[2], V. S. Nandini[2], S. Merish[3]

[1]Lecturer, Government Siddha Medical College, Tirunelveli, TamilNadu, siddhawalter@gmail.com

[2]Final Year BSMS, Government Siddha Medical College, Tirunelveli, TamilNadu, samuel16jan@gmail.com

[3]PG Scholar, Government Siddha Medical College, Tirunelveli, Tamil Nadu, India, drmerishjesus@gmail.com

ABSTRACT

Siddha system of medicine is unique in its own way in treating many acute and chronic ailments. Among the various diseases mentioned, chronic diseases are mainly dealt in Siddha literatures. Many life threatening diseases are also mentioned in it which is becoming more prevalent nowadays. Carcinoma is becoming a major cause of death due to changes and modifications in lifestyle and food practices. Though there are various types of cancers, gastric cancer is becoming more prevalent worldwide and also in our nation. The most common cause for gastric cancer is Helicobacter pylori, which accounts for about 60% of the cases. Other causative factors include smoking, dietary factors etc. Since it causes much threats and drastic effects, the Quality of Life is highly affected. Hence it is the need of the hour to find a proper remedy to manage the symptoms of the disease. Siddha literatures have mentioned about many herbs and minerals which improves the quality of life, serves as a prophylactic agent and also manages the symptoms exhibited by the disease. We have made a formulation using herbal ingredients as per Siddha basic principles and subjected the same to in-vitro anticancer study and found the IC50 value as 135.62µg/ml.

KEYWORDS

Gastric Carcinoma, *Puttru*, *Vaadha kunmam*, In-vitro anticancer study, HCT 116 cell lines, Siddha Medicine.

INTRODUCTION

Gastric cancer is one of the most severe type of cancer of gastrointestinal tract. They do not show symptoms at the early stages, but they show severe symptoms in the later stages. Gastric cancer is the fourth common one worldwide. The dreadful disease can be managed with herbal preparation with reference to siddha literature. Gastric Carcinoma could be compared with that of *Vadhakunmam* among the 8 types of *KunmaNoi* as mentioned in Siddha literatures. Based on the basic principles of siddha, we have formulated a poly-herbal product and underwent in-vitro anti cancerous study for the formulation of HCT- gastric cancer cell lines. The drug showed significant inhibition on proliferation of gastric cancer cell with IC 50 value of 130.62µg/ml.

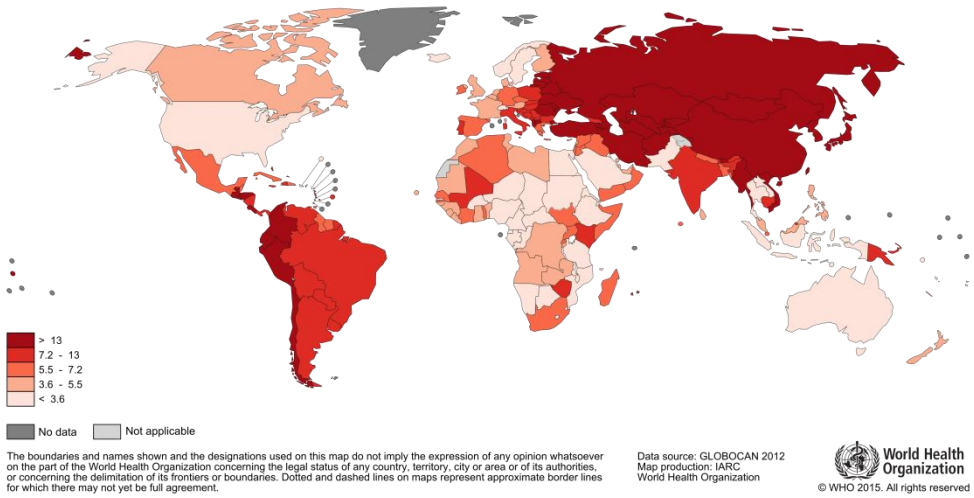
GASTRIC CANCER-CONVENTIONAL AND SIDDHA CONCEPTS

Gastric Cancer is one which develops at the inner granular tissue lining of the stomach. It then gets spread along the stomach wall and progress into cancer. The major risk factors include severe Helicobacter pylori infection, Chronic Gastritis, Polyps in stomach, EBV Infections, Smoking and other sedentary lifestyle habits. At the early stages, it may not show any prominent symptoms. However the person may exhibit some symptoms like Indigestion, Loss of appetite, bloated feeling after eating, etc.

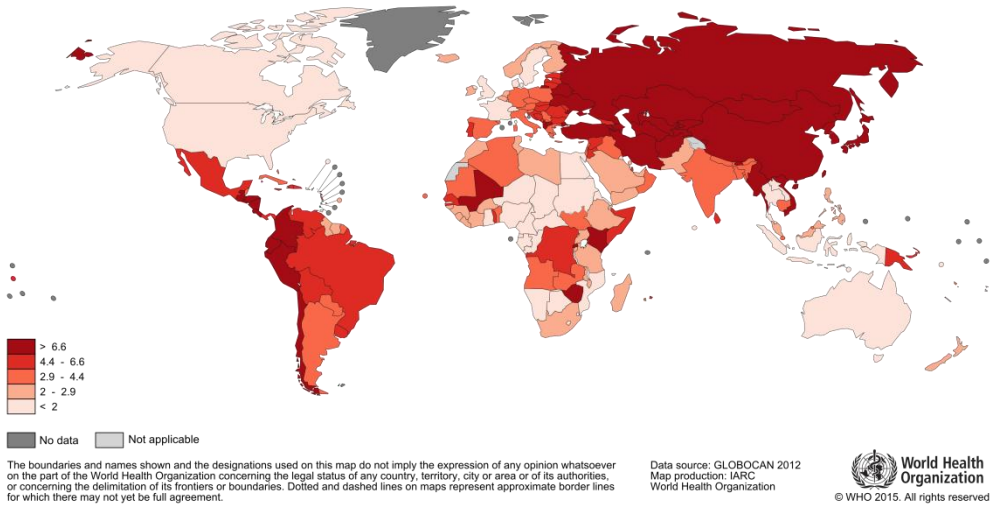
As per the Classical Siddha Literatures, Vaadha kunmam is found to be in accordance with the Symptoms of Gastric carcinoma.

MORTALITY RATES IN MEN AND WOMEN

Estimated Stomach Cancer Mortality Worldwide in 2012: Men



Estimated Stomach Cancer Mortality Worldwide in 2012: Women



IN-VITRO ASSAY

Principle

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm.

Materials

1. Cell lines:

   KB and HCT116 (From NCCS, Pune)

2. Cell culture medium:

3. DMEM- High Glucose - (#AL111, Himedia)

4. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)

5. Fetal Bovine Serum (#RM10432, Himedia)

6. MTT Reagent (5 mg/ml) (# 4060 Himedia)

7. DMSO (#PHR1309, Sigma)
8. **Camptothecin (# C9911, Sigma)**
9. D-PBS (#TL1006, Himedia)
10. 96-well plate for culturing the cells (From Corning,USA)
11. T25 flask (# 12556009, Biolite - Thermo)
12. 50 ml centrifuge tubes (# 546043 TORSON)
13. 1.5 ml centrifuge tubes (TORSON)
14. 10 ml serological pipettes (TORSON)
15. 10 to 1000 ul tips (TORSON)
16. 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (ELX-800 Biotek)
17. Inverted microscope (Biolink)
18. 37°C incubator with humidified atmosphere of 5% CO2 (Healforce, China)

Assay controls are,

(i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)
(iii) Positive control (medium with cells and with 50uM of CPT/75uM of H2O2)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

Procedure for Determining Cell Cytotoxicity

1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 12 hours.

2. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet).

3. Incubate the plate for 24 hrs at 37°C in a 5% CO2 atmosphere.

4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.

5. Wrap the plate with aluminium foil to avoid exposure to light.

6. Return the plates to the incubator and incubate for 3 hours.

7. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)

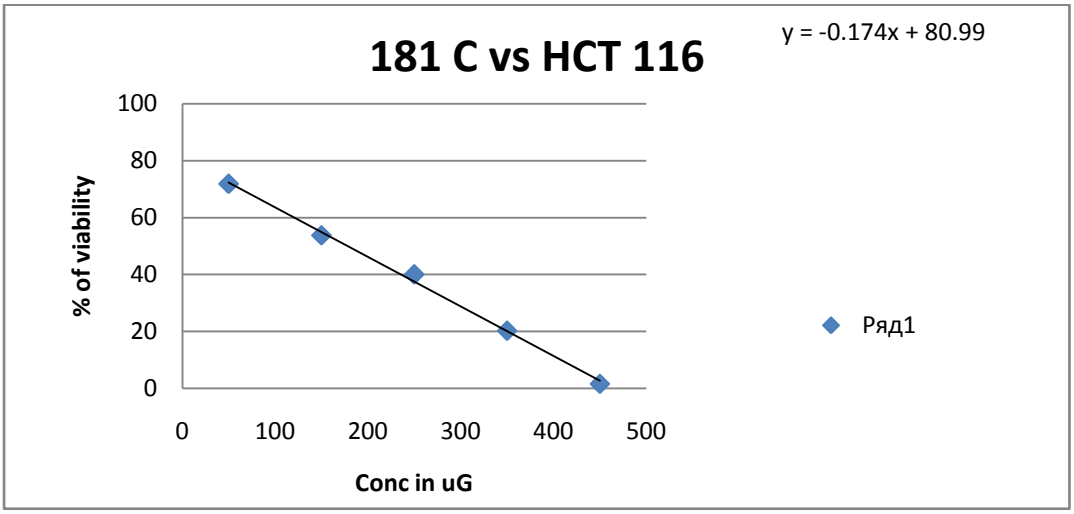
8. Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO).

9. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.

10. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.

RESULTS

Our formulation showed a good degree of inhibition on the proliferation of the HCT 116 gastric cancer cells. The inhibitory concentration (IC50) of the proposed drug is found to be 135.62µg/ml.



INFERENCE

The Ic50 value was determined by using linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph. The IC50 value is found to be 135.62µg/ml.

DISCUSSION

The outcome and the management of the dreaded disease still remain poor with available treatment modalities. Finding a novel formulation is very essential to improve the Quality of Life and prognosis of the disease. Nowadays future challenges lie with the personalized regimen of treatment modality for these kind of dreaded diseases; which is a major aspect of Siddha Basic Principle. Based on this the polyherbal drug is formulated and the IC50 value is found to be 135.62µg/ml which will manage the symptoms and also serves as a prophylactic agent.

CONCLUSION

The outbreak and mortality rate of Gastric cancer is increasing day by day. So, finding a better solution is need of the hour. Our formulation has proved its efficacy in in-vitro anticancer assay. Further extensive studies are to be done in the area of prophylactic action to rule out the mechanism of action of the drug.

REFERENCE

1. Uthamaraayan, H.P.I.M., *Thotrakirama Aaraaychiyum Siddha Maruthuva Varalaaarum*, First edition, Published by D I M & H, Chennai
2. Uthamaraayan, H.P.I.M., *Siddha Maruthuvaanga Surukkam*, First Edition, Published by D I M & H, Chennai.
3. Murugesa Muthaliar, DIM, *Gunapadam Mooligai Vaguppu* (Vegitable section), 1st Edition, Published by D I M & H,Chennai
4. Shanmughavelu, H.P.I.M., *Noi naadal Noi mudhal naadal thirattu* Part 1, First Edition, Published by D I M& H, Chennai
5. Shanmughavelu, H.P.I.M., *Noi naadal Noi mudhal naadal thirattu*Part 2, Second Edition, Published by D I M& H , Chennai.
6. Sambasivam Pillai, TVS Dictionary Part I,II,III,IV, Second Edition, Published by D I M & H ,Chennai.
7. Iarcfr. 2018. *Iarcfr*. [Online]. [10 March 2018]. Available from: http://globocan.iarc.fr/old/FactSheets/cancers/stomach-new.asp
8. Webmdcom. 2018. *WebMD*. [Online]. [10 March 2018]. Available from: https://www.webmd.com/cancer/stomach-gastric-cancer

9. Thomas M. Walter, C. Samuel Justin Raj, K. Swathi, V.S. Nandini, G. Sabari Devi, G. Sanjana, S. Merish, Siddhars' Personalised Pre-treatment Modality for Microbiomic Drug Resistance Microbiomic Drug Resistance, Volume 12, Issue 3, Siddha papers.
10. C. Suvetha, Thomas M.Walter, R. Sweety Nirmala, In –Vitro Studies Of The Cyto Toxicity Effect Of A Poly Herbal Siddha Formulation In Breast Cancer Cells, Siddha Papers; Volume 10, Issue 1.