WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 18, 964-969. Research Article ISSN 2277– 7105

INVITRO ANTI CANCER ACTIVITY OF SIDDHA HERBAL FORMULATION KODIVELI CHOORANAM AGAINST COLORECTAL CANCER

Kumudha V.[1*], Mohamed Musthafa M.[2] and Anbarasan B.[3]

[1*]PG Scholar, PG Department of Sirappu Maruthuvam, GSMC, Chennai, Tamil Nadu.

[2]Professor, PG Department of Sirappu Maruthuvam, GSMC, Chennai, Tamil Nadu.

[3]Research Associate, Traditional Knowledge Digital Library, CSIR, Chennai.

Article Received on
28 August 2018,

Revised on 18 Sept. 2018,
Accepted on 08 October 2018

DOI: 10.20959/wjpr201818-13529

***Corresponding Author**
Dr. Kumudha V.
PG Scholar, PG Department of Sirappu Maruthuvam, GSMC, Chennai, Tamil Nadu.

ABSTRACT

Cancer is one of the leading causes of death in both developed and developing countries and continues to be a major public health problem in many parts of the world. Colorectal Cancer is the 3rd leading cause of Cancer death in each sex and second overall in men and women combined. Integrated medicine would be having immense positive effect over the existing. *Kodiveli Chooranam* (KC) is one of the traditional Siddha Polyherbal formulation. The aim of the present study is to evaluate the anti-cancer activity of *Kodiveli chooranam* by MTT assay in HT-29 (Human colorectal adenocarcinoma) cell line. Results shown that the drug, *Kodiveli chooranam* reduces the cell viability to 39% at the concentration of 100 µg/mL.

KEY WORDS: Kodiveli Chooranam, In Vitro cell line study, Anticancer activity, Colorectal Cancer, Growth inhibitory activity.

INTRODUCTION

Cancer is a disease characterized by the unchecked division and survival of abnormal cells. When this type of abnormal growth occurs in the colon or rectum, it is called colorectal cancer.[1] Colorectal cancer comprises 98% of all malignant tumours of the large intestine. The incidence of carcinoma of the large intestine rises with age; average age of patients is about 60 years. Cancer in rectum is more common in males than females in the ratio 2:1.[2]

Colorectal cancer accounts for over 9% of all cancer incidence.[3,4] Several risk factors associated with colorectal cancer are increasing age, personal history of Adenomatous Polyps, Inflammatory Bowel Disease, Family history of Colorectal cancer or Adenomatous Polyps, diet high in fat esp. animal fat, Obesity, Physical inactivity, Smoking, Alcoholic consumption etc.[5]

Even with a significant improvement in science and technology, there is much difficulty in curing the disease. Treating cancer with modern medicine produces adverse effects.

Siddha system provides good line of treatment for different kinds of life-threatening diseases including cancer. In ancient Siddha literature, cancer is explained as Putru (undetermined growth), Arpudham (spectacular tumors) and Vanmeegam[6] (previous tumors). Siddha physicians consider some types of cancer growths with the symptoms of Vippuruthi[7] (multifacted growth) for their practice.[8]

MATERIALS AND METHODS

SELECTION AND AUTHENTICATION OF DRUG

The trial drug *Kodiveli chooranam* have been selected for this study from classical literature Sarabendhirar Siddha Maruthuva Sudar.[9] The raw drugs were procured from the raw drug shop R.N. Rajan and Co, Chennai. The cost of the trial medicines are relatively economical. After proper authentication by the Botanist, Govt Siddha Medical College, Arumbakkam, Chennai, the preparation was made.

Table 1. Ingredients of Kodiveli Chooranam.

S.No	Botanical name	Tamil name	Family
1	*Plumbago indica*	Kodiveli	Plumbaginaceae
2	*Adhtoda vasica*	Adathodai	Acanthaceae
3	*Enicostemma axillare*	Vellarugu	Gentianaceae
4	*Indigofera aspalathoides*	Sivanarvembu	Fabaceae
5	*Trianthema decandra*	Charanai ver	Aizoaceae
6	*Cassia senna*	Nilavarai	Fabaceae
7	*Acalypa indica*	Kuppaimeni	Euphorbiaceae

INVITRO ANTI-CANCER EFFECT DETERMINATION BY MTT ASSAY

HT-29 (Human colorectal adenocarcinoma) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen).

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

CELLS SEEDING IN 96 WELL PLATE

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

PREPARATION OF COMPOUND STOCK

The compound solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

ANTI-CANCER EVALUATION

After 24 hours the growth medium was removed, freshly prepared compound in 5% DMEM was taken from this6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml taken and make up to 250µg/ml using 5% MEM and were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

ANTI-CANCER ASSAY BY DIRECT MICROSCOPIC OBSERVATION

Entire plate was observed after 24 hours of incubation in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

ANTI-CANCER ASSAY BY MTT METHOD

15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and

100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

RESULTS AND DISCUSSION

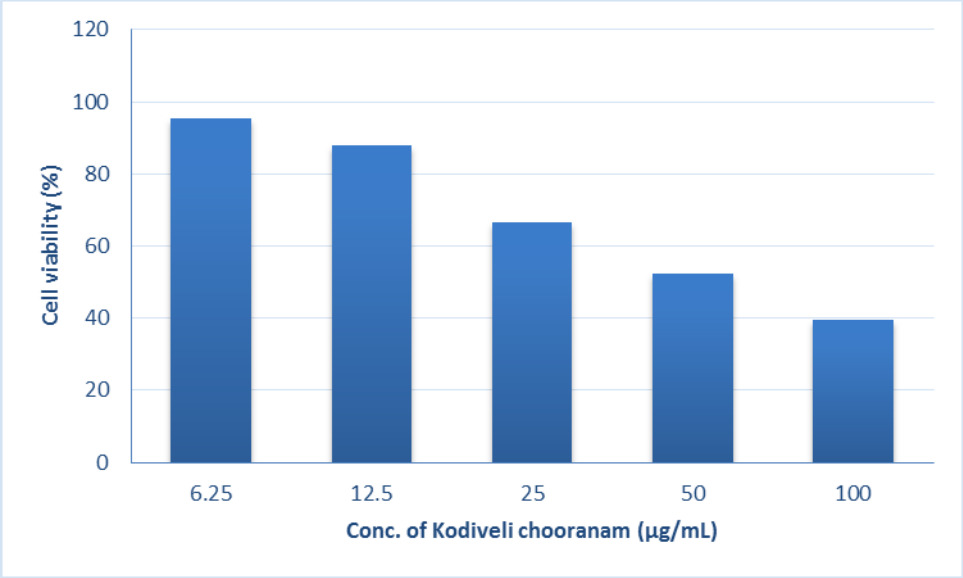

Figure 1: Shows percentage of cell viability against Kodiveli chooranam at different concentations.

Siddha classical drug *Kodiveli chooranam* (KC) was investigated to evaluate its potential therapeutic efficacy against cancer cells. *In vitro* cytotoxicity of the drug was examined by MTT cell viability against HT-29 (Human colorectal adenocarcinoma) cell line with medium used as control. Cells were treated with different concentrations (6.25, 12.5, 25, 50, 100) of KC. After treatment with drugs, KC showed lower cell viability of about 39% at higher concentration of 100 µg/mL. Cell viability decreased with increasing the concentration of KC. Search for the chemical constituents of *Plumbago indica* has resulted the isolation of plumbagin, palmitic acid and myricyl palmitate from the petrol extract.[10] The major constituent of *Plumbago indica* plumbagin has been reported to possess significant antitumor, antibacterial, antifungal and insecticidal activities.[11-13] Pallab K Haldar *etal* suggested that Methanolic extract of *Indigofera aspalathoides* another ingredients of the drug KC, has direct relationship with tumor cells. Because these tumor cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and the anticancer agent lyse the cells by direct cytotoxic mechanism.[14]

CONCLUSION

Cancer drugs have been mentioned numerous in Siddha literature. Picking from amongst them, *Kodiveli chooranam* was screened for its anti-proliferative property. With the inhibition of the cell viability to 39% at maximum concentration of 100 µg/mL, the drug exhibited good anti-proliferative property. The authors hope that further research in the drug such as with experimental animals, improving its efficacy etc will be helpful to the mankind.

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to Biogenics Research Lab, Trivandrum for the conduct of the study and all the faculties, PG Dept of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai.

CONFLICT OF INTEREST

Nil.

REFERENCES

1. Colorectal cancer Facts & Figures 2017-2019, American Cancer Society, 2017; 3.
2. Harshmohan, The Gastrointestinal Tract, Textbook of Pathology, Seventh Edition, 2015, Jaypee Brothers Medical Publishers (P) Ltd, pp586.
3. World Cancer Research Fund and Americal Institute for Cancer Research Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global perspective. Washington, DC: American Institute of Cancer Research; 2007.
4. Boyle P, Langman JS, ABC of colorectal cancer: Epidemiology, BMJ, 2000; 321(7264): 805-808.
5. Fatima A. Haggar, and Robin P. Boushey, Colorectal Cancer Epidemiology: Incidence, Mortality, Survival and Risk Factors, Clin Colon Rectal Surg., 2009 Nov; 22(4): 191-197.
6. Sathishkumar T, Putrunoyum Siddha Maruthuvamum, Thamarai noolagam publication 292, Chennai, 2006: 106.
7. Ramachandran SP, Agathiyar Vaithiya Kaaviyam 1500, Second edition, Thamarai noolagam publication, Chennai, 2001: 160.
8. Mantela D *etal*, Invitro Evaluation of Anticancer activity of Gowri Chinthamani Chendhooram, Siddha Medicine Against HeLa Cells, British Journal of Medical and Health Research, 2015.
9. Sowrirasan M, Sarabaendhirar Siddha Maruthuva Sudar, Fourth Edition, 2000, Saraswathi Mahal Library copy printers: 593.

10. B Dinda, S K Das, A K Hajra, A Bhattacharya, K De, G Chel, B Achari, Indian Journal of Chemistry, May 1999; 38 B: 577-582.
11. Krishnaswamy M and Purushothaman KK, Indian J Exp Biol., 1980; 18: 876.
12. Vijver L M, Lotter A P, Plata Med., 1917; 20: 8.
13. Kubo I, Uchida M & Kilcke J A, Agric biol chem., 1983; 47: 911.
14. Pallab K. Haldar b, Anticancer Activity of Indigofera aspalathoides and Wedelia calendulaceae in Swiss Albino Mice, Iranian Journal of Pharmaceutical Research, 2007; 6(2): 141-145.