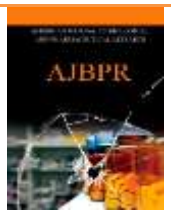
e- ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



DEVELOPMENT OF POLYHERBAL FORMULATIONS TO TREAT COLORECTAL CANCER

Srikanth Choudary Pallothu*, M.Purushothaman, Madhusudhan Malladi, Kathiravan P, Sravanthi Chirra

Department of Pharmaceutical Sciences, Scient Institute of Pharmacy, Ibrahimpatnam, Hyderabad-501506, Telangana, India.

Article Info

Received 29/08/2020
Revised 16/09/2020
Accepted 19/10/2020

Key words: -
Anticancer capsules, Piper, Clove, in vitro.

ABSTRACT

Surgery (removing the cancer in an operation) is the most common treatment for all stages of colon cancer, Cryosurgery, Radiation therapy, Chemotherapy. As a part of chemotherapy, lots of anticancer drugs are in the market, but the main problem associated with these drugs is their side effects. Because of chemotherapy treatment side effects, the patient needs secondary palliative care treatment. Plant medicines are well known for their non-toxic side effects, so the objective of the study is to develop a drug from medicinal plant against colon cancer with non-toxic side effects. It plays an important role in the discovery of lead compound for development of conventional drugs. About 60% of currently used anticancer agents are derived from natural source (i.e. plants). Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes and saponins and it is found to contain a variety of triterpenes and steroidal compounds and also to find out, a newer synthetic drug, for its anti-colon cancer potential and its toxic profile. In the present study, different formulations were prepared using herbs and the same were used to study the anticancer potential of the extract and to synthesize new anticancer formulation.

INTRODUCTION

Colorectal cancer is the second leading cause of cancer death in the United States for both men and women. The rate of colon cancer incidence was low in India but is presently increasing; out of 3.6 million cancer cases, 36,000 suffer from colon cancer. Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality. Out of all the cancers colon cancer is one of the most common cancers in the world. Every year 1.1 million patients are diagnosed for colon cancer. [1].

Corresponding Author

Srikanth Choudary Pallothu
Email:- srikanthpallothu@gmail.com

The small growths (known as polyps) in colon are often benign, although some have the potential to develop and become cancerous. It is estimated that up to two thirds of colorectal polyps are pre-malignant and associated with a risk of colorectal cancer.

However, there are often no initial symptoms and the cancer may already have spread to other parts of the body by the time the patient is diagnosed [2]. Worldwide Colorectal cancer is diagnosed in over 1.1 million people globally each year; it is the second most common cancer in women and the third most common cancer in men. The disease is responsible for approximately 610,000 deaths each year (8% of all cancer deaths) [3], making it the fourth leading cause of cancer death after lung, stomach and liver cancers. Europe Colorectal cancer is the most common cancer in Europe, with approximately 425,000 new cases each year; the highest incidence rate of colorectal cancer in

the world. It is also the second greatest cause of cancer death in Europe following lung cancer, accounting for 13% of all cancer deaths. North America There was approximately 175,000 new cases of colorectal cancer in North America in 2008, making it the second most commonly diagnosed cancer in the region. Colorectal cancer accounted for 11.5% of all cancer incidence and 9.5% of all cancer deaths in North America in the same year [3].

MATERIALS AND METHODS

Plant material

Plant parts according to table 1 are collected from local herbs store. The plant was dried under controlled temperature, powdered and passed through 40-mesh sieve. 150g of powdered plant material was packed in Soxhlet apparatus and refluxed with methanol until to get a clear solution. The extract was dried and weighed amount of the dried extract was mixed in proper proportions as per table 1 and suspended in Distilled water and was used for the present study. 2 formulations with varying concentrations were prepared and the comparisons was made in terms of anti cancer activity. they are named respectively as Formulation F10 alma (which contained amla) and Formulation F10 Oscimum (which contained oscimum).

In Vitro Cytotoxicity Studies

HT- 29 (Colon Carcinoma) cell culture was used to study the invitro cytotoxicity studies. Cell culture was procured from National Centre for Cell Sciences (NCCS), Pune. Cells were grown in Minimal essential medium supplemented with 2 mM L-glutamine, 10% Fetal Bovine Serum, Penicillin (100 µg/ml), Streptomycin (100 µg/ml) and Amphoterecin B (5 µg/ml) and The cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 and subculture twice a week [4].

Determination of Mitochondrial Synthesis by Micro culture Tetrazolium (MTT) Assay

The monolayer cell culture was trypsinized using TPVG and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100 µl of (1000 to 15.6 µg/ml) two plant extracts were added to the cells in microtitre plates. The plates were then incubated at 37^0C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT (MTT: prepared in Hank's Balanced Salt Solution without phenol red [(HBSS-PR), 2 mg/ml, Sigma Chemicals)] was added to each well. The plates were gently shaken and incubated for 3 hours at 37^oC in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a Microplate reader (ELISA Reader, Bio-rad) at a wavelength of 540nm [5]. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of Individual Test Group}}{\text{Mean OD of Control Group}}\right) X\ 100$$

RESULTS AND DISCUSSION

In this phase of study, the Formulations was evaluated for the cytotoxic activity. The cytotoxic test was carried out by using MTT method, by using different cell lines like HT-29 (colon cancer cell lines). In this study different concentration of the Formulations was treated with known quantity of cells and the % cytotoxicity in each dose level was measured by using MTT (Micro culture Tetrazolium) method. The extract shown significant % cytotoxicity in cell lines. F10 activity for oscimum shows better activity when compared to F10 activity for amla [6-11].

The CTC50 concentration shows low activity for F10 oscimum. % scavenging activity for formulation F10 amla shows the concentration (1000ug/ml) value of 95.51 and at formulation F10 oscimum , it shows the value 96.72. % scavenging activity for formulation F10 amla shows the concentration (500 ug/ml) value of 93.80 and at formulation F10 oscimum, it shows the value 92.21. % scavenging activity for formulation F10 amla shows the concentration (250 ug/ml) value of 58.07 and at formulation F10 oscimum, it shows the value 59.14. % scavenging activity for formulation F10 amla shows the concentration (125 ug/ml) value of 45.15 and at formulation F10 oscimum, it shows the value 46.83. % scavenging activity for formulation F10 amla shows the concentration (62.5 ug/ml) value of 33.18 and at formulation F10 oscimum, it shows the value 34.35. % scavenging activity for formulation F10 amla shows the concentration (31.25 ug/ml) value of 17.26 and at formulation F10 oscimum, it shows the value 18.17. % Scavenging activity for formulation F10 amla shows the concentration (15.60 ug/ml) value of 01.19 and at formulation F10 oscimum, it shows the value 02.10. % scavenging activity for formulation F10 amla shows the concentration (CTC50 ug/ml) value of 182 and at formulation F10 oscimum, it shows the value 181. value of 04.97 and at formulation PHF-ACE, it shows the value 5.70. % scavenging activity for formulation PHF-DLC shows the concentration (CTC50 ug/ml) value of 186 and at formulation PHF-ACE, it shows the value 184.

Table 1. Formulation Design of the anti-cancer formulations

Constituent	Formulation F10 Oscimum	Formulation F10 Amla
Azadirachtha indica	10mg	10mg
Catharanthus roseus	10mg	10mg
Oscimum indicum	10mg	--
Phyllanthus emblica	--	10mg
Simple syrup	Qs to make 10ml	Qs to make 10ml

Table 2. Effect of formulations on the scavenging of cancer markers

Concentration (µg/ml)	% scavenging activity	
	Formulation F10 Oscimum	Formulation F10 Amla
1000	96 .72	95.51
500	92.21	93.80
250	59.14	58.07
125	46.83	45.15
62.5	34.35	33.18
31.25	18.17	17.26
15.60	02.10	01.19
CTC_{50} (µg/ml)	181	182

CONCLUSION

With the above said findings it can be concluded that the Formulation F10 Oscimum possess anti colorectal cancer activity. Before the clinical usage of extract, thorough toxicological profile has to be determined on the crude extracts as well as on isolated compounds to confirm the safety of the formulation

REFERENCES

1. Shirikhande, S.V., Saoji, R.R., Barreto, S.G., Kakade, A.C., Waterford, S.D., Ahire, S.B., Golowale, F.M. & Shukla, P.JOutcomes of resection for rectal cancer in India: the impact of the double stapling technique. World Journal of Surgical Oncology. 2007; Vol.21, No.5, pp. 35.
2. WHO, IARC Globocan, Cancer Incidence and Mortality Worldwide in 2008 at*http://globocan.iarc.fr/*
3. Edwards BK et al. Annual Report to the Nation on the Status of Cancer, 1975-2006, FeaturingColorectal Cancer Trends and Impact of Interventions (Risk Factors, Screening, and Treatment) to Reduce Future Rates. Cancer (2009) 116(3):544-573
4. Nalini N, Manju V, Menon VP. Effect of coconut cake on the bacterial enzyme activity in 1,2-dimethylhydrazine induced colon cancer. Clin Chim Acta 2004; 342: 203–210.
5. Shiau SY and Chang GW. Effects of dietary fiber on fecal mucinase and _-glucuronidase activity in rats. J Nutr. 1983; 113: 138–144.
6. Kaplow, L.S. A histochemical procedure for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood. 1955; 10: 1023.
7. Natt, M.P and Herrick, C.A. A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poult. Sci. 1952;31: 735–738.
8. Jeremy James Johnson, Hasan Mukhtar. The role of existing and novel cardiac biomarkers for cardioprotection". Curr.Opin. Invest. Drugs PMID. 2007; 8 (9): 711–717.
9. Kaplan LA and Pesce AJ. In Clinical Chemistry Theory Analysis and Correlation. 1984; 567.
10. Symeonidisa A, Kouraklis A, Dimitris S, Evangelia A, Nikolaos A. Increased serum CA-15.3 levels in patients with megaloblastic anemia due to Vitamin B12 deficiency. *Oncology*, 2004; 67, 359–67.
11. Pedro Figueiredo & Maria Donato & Marta Urbano & Helena Goulão & Hermano Gouveia & Carlos Sofia & Maximino Leitão & Diniz Freitas. Aberrant crypt foci: endoscopic assessment and cell kinetics characterization. Int J Colorectal Dis. 2008;DOI 10.1007/s00384-008-0576-z.