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INVESTIGATION ON CAPSULES FOR ANTI-CANCER ACTIVITY

Jagadeeshwari S*, Soujanya H, Purushothaman M, Shiva Kumar K

Department of Pharmaceutics, Scient Institute of Pharmacy, Ibrahimpatnam, Hyderabad-501506, Telangana, India

ABSTRACT

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 (WHO, 2008). 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. 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. 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. In the present study, capsules were prepared by incorporating herbs into them and then studying them for their anti-cancer potential to establish a potent formulation.

KEY WORDS: Anticancer capsules, Piper, Clove, *in vitro*.

INTRODUCTION

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. 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. 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. Every year 1.5 million patients are diagnosed for colon cancer. 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 2.9 million cancer cases, 36500 suffer from colon cancer [1].

Worldwide Colorectal cancer is diagnosed in over 1.2 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 590000 deaths each year (8% of all cancer deaths) [2], 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 425000 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 12% of all cancer deaths. North America There was approximately 177,000 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% of all cancer incidence and 9% of all cancer deaths in North America in the same year. There are two pathogenetically distinct pathways for the development of colon cancer, both of which involve the stepwise accumulation of multiple mutations. However, the genes involved and the mechanisms by which the mutations accumulate are different. There are few standard ways in which the pathogenesis of colon cancer occurs. However,

Corresponding Author:- **Jagadeeshwari S** Email: jagadeeshwari.sabbu@gmail.com

there are multiple treatment ways there are four types of treatment used to treat cancers. 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, capsules were prepared by incorporating herbs into them and then studying them for their anti-cancer potential to establish a potent formulation [3].

MATERIALS AND METHODS

Plant material

Whole plant parts according to the table 1 were collected from local farm area. The plant was dried under controlled temperature, powdered and passed through 40-mesh sieve. 100g of powdered plant material was packed in Soxhlet apparatus and refluxed with Ethanol until to get a clear solution. The extract was dried and weighed amount of the dried extract was used for the present study. These extracts were weighed according to the table 1 and then filled into hard gelatin capsules of 0 size. these were then used to determine their anti-cancer potential.

Cell lines for cancer study

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].

Investigations in 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 **(**Nalini, 2004**)**. 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. The percentage growth inhibition was calculated using the formula below:

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

CTC_{50} was determined by plotting the conc Vs % growth inhibition [5-9].

RESULTS & DISCUSSION

In this phase of study, the Capsules were 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 combination of capsules were 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. % activity for capsule- clove shows better activity when compared to formulation capsule- pepper. The CTC50 concentration shows low activity for capsule clove.

Table 1. Composition of the capsules

Drug	4H capsules-cloves	5H capsules-cloves	4H capsules- pepper	5H capsules- pepper
Curcuma longa extract	150mg	150 mg	150mg	150mg
Eugenia caryophylla extract	40mg	50mg	--	--
Piper longum Extract	--	--	40mg	50mg
Additives	Qs to capsules	Qs to capsules	Qs to capsules	Qs to capsules

Table 2. Anti-cancer activity of the prepared capsules

Concentration (µg/ml)	% activity in scavenging	
	4HCapsules -Cloves	4HCapsules -Pepper
1000	97.23	95.51
500	93.31	94.10
250	60.02	59.13
125	47.18	46.27
62.5	35.11	34.52
31.25	19.01	18.19
15.60	03.43	02.84
CTC_{50} (µg/ml)	182	184

% scavenging activity for formulation capsule-pepper shows the concentration (500 ug/ml) value of 94.10 and at formulation capsule-clove, it shows the value 93.31. % scavenging activity for formulation capsule-pepper shows the concentration (250 ug/ml) value of 59.13 and at formulation capsule-clove, it shows the value 60.02. % scavenging activity for formulation capsule-pepper shows the concentration (125 ug/ml) value of 46.27 and at formulation capsule-clove, it shows the value 47.18. % scavenging activity for formulation capsule-pepper shows the concentration (62.5 ug/ml) value of 34.52 and at formulation capsule-clove, it shows the value 35.11. % scavenging activity for formulation capsule-pepper shows the concentration (31.25 ug/ml) value of 18.19 and at formulation capsule-clove, it shows the value19.01. % Scavenging activity for formulation capsule-pepper shows the concentration (15.60 ug/ml) value of 02.84 and at formulation capsule-clove, it shows the value 03.43. % scavenging activity for formulation capsule-pepper shows the concentration (1000 ug/ml) value of 95.51and at formulation capsule clove, it shows the value 97.23. % scavenging activity for formulation capsule-pepper shows the concentration (CTC50 ug/ml) value of 184 and at formulation capsule-clove , it shows the value 182.

CONCLUSION

With the above said findings it can be concluded that the Capsule prepared by incorporating the pepper extract showed highest activity because of the penetration enhancement mechanism of the extract. 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 drug.

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