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DEVELOPMENT AND INVESTIGATION OF POLYHERBAL FORMULATION FOR TUMOR

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ABSTRACT

Out of all the cancers colon cancer is one of the most common cancers in the world. Every year 1.2 million patients are diagnosed for colon cancer. The rate of colon cancer incidence was low in India but is presently increasing; out of 3.5 million cancer cases, 35,000 suffer from colon cancer. 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. Two formulations were prepared using herbs and they were compared for their anti-cancer activity. Two kinds of polyherbal formulations were prepared using different extracts and they were tested for anti-cancer activity invitro. Formulations with potent drugs like ashwagandha and colchicum showed a potent activity than other formulation. 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.

Key words: PHF-ACE, PHF-DLC, Anticancer activity.

INTRODUCTION

Out of all the cancers colon cancer is one of the most common cancers in the world. Every year 1.2 million patients are diagnosed for colon cancer. The rate of colon cancer incidence was low in India but is presently increasing; out of 3.5 million cancer cases, 35,000 suffer from colon cancer [1]. Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality. Colorectal cancer is the second leading cause of cancer death in the United States for both men and women. 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 [2]. 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 making it the fourth leading cause of cancer death after lung, stomach and liver cancers [3]. Europe Colorectal cancer is the most common cancer in Europe, with approximately 430,000 new cases each year; the highest incidence rate of colorectal cancer in the world.

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. 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. Two

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formulations were prepared using herbs and they were compared for their anti-cancer activity.

MATERIALS & METHODS

Herbs

The plant materials were collected from the herbal supplier in the market locally. They were properly authenticated. The plant was dried under controlled temperature, powdered and passed through 40-mesh sieve. 50g of powdered plant material was packed in Soxhlet apparatus and refluxed with Ethanol until to get a clear solution. The extracts were weighed and mixed according to the quantities mentioned in the table 1. The formulations were named accordingly as PHF-ACE and PHF-DLC based on the drugs that are incorporated into the formulations.

Anticancer cells

HT- 29 (Colon Carcinoma) cell culture was used to study the invitro cytotoxicity studies **(**Jeremy James Johnson, 2007**)**. 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.

Investigation of Mitochondrial Synthesis

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 [4-6]. 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. 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.

RESULTS & DISCUSSIONS

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 formulations 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 formulation PHF-ACE shows better activity when compared to formulation PHF-DLC. The CTC50 concentration shows low activity for PHF-ACE [7-9].

% scavenging activity for formulation PHF-DLC shows the concentration (1000 ug/ml) value of 97.42 and at formulation PHF-ACE, it shows the value 99.23. % scavenging activity for formulation PHF-DLC shows the concentration (500 ug/ml) value of 97.35and at formulation PHF-ACE, it shows the value 96.54. % scavenging activity for formulation PHF-DLC shows the concentration (250ug/ml) value of 61.76 and at formulation PHF-ACE, it shows the value 62.31. % scavenging activity for formulation PHF-DLC shows the concentration (125 ug/ml) value of 48.51and at formulation PHF-ACE, it shows the value 49.42.

% scavenging activity for formulation PHF-DLC shows the concentration (62.5 ug/ml) value of 36.83 and at formulation PHF-ACE, it shows the value 37.65. % scavenging activity for formulation PHF-DLC shows the concentration (31.25 ug/ml) value of 20.64 and at formulation PHF-ACE, it shows the value 21.17. % scavenging activity for formulation PHF-DLC shows the concentration (15.60 ug/ml) 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 [10,11].

Table 1: Formulation of the Polyherbal formulations ACE and DLC

PHF-ACE	Quantity	PHF-DLC	Quantity
Ashwagandha	100mg	Datura	100mg
Colchicum	100mg	Liquorice	100mg
Ephedra	100mg	Cinnamon	100mg
Additives	100mg	Additives	100mg

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Table 2: Anti-cancer activity of Polyherbal formulations ACE and DLC

Concentration (µg/ml)	% scavenging activity	
	Formulation PHF-ACE	Formulation PHF-DLC
1000	99.23	97.42
500	96.54	97.35
250	62.31	61.76
125	49.42	48.51
62.5	37.65	36.83
31.25	21.17	20.64
15.60	05.70	04.97
CTC_{50} (µg/ml)	184	186

CONCLUSION

Two kinds of polyherbal formulations were prepared using different extracts and they were tested for anti-cancer activity invitro. Formulations with potent drugs like ashwagandha and colchicum showed a potent activity than other formulation. 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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