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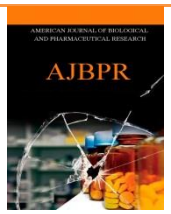

DEVELOPMENT AND INVESTIGATIONS ON HERBAL SYRUP

Mothilal K*, Mahender K, Ravi D, Chaitanya Kumar K

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

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ABSTRACT

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. 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. 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 current work, a syrup formulation with varying concentration of herbs in it and the same is tested for the anti-cancer activity. Two concentrations of the syrups were studied for the anticancer potential and its concluded to 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 drug.

INTRODUCTION

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]. 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]. 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 1.2 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.

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 [3]. 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 609,000 deaths each year (8% of all cancer deaths) [4]. However, 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

Corresponding Author

Mothilal K
Email:-k.mothilal1988@gmail.com

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 current work, a syrup formulation with varying concentration of herbs in it and the same is tested for the anti-cancer activity.

MATERIALS AND METHODS

Herbal materials

Plant parts were collected from the supplier shop in the locality and the plant parts were duly authenticated. Parts 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 Distilled water until to get a clear solution. The extracts were weighed as per proportions prescribed as per table 1. They are mixed in the quantities into the simple syrup solution. The final concentration of the extracts in the syrup will be 10%w/v.

In Vitro anti-cancer activity

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 **(**Jeremy James Johnson, 2007**)**.

Determination of 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 **[5-7]**. 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 concVs % growth inhibition.

RESULTS & DISCUSSION

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 DMP 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. % activity for syrup-300 shows better activity when compared to formulation syrup-600. The CTC50 concentration shows low activity for syrup-300.

% scavenging activity for formulation syrup-600 shows the concentration (1000 ug/ml) value of 696.23 and at formulation syrup-300, it shows the value 98.12. % scavenging activity for formulation syrup-600 shows the concentration (300 ug/ml) value of 96.19 and at formulation syrup-300, it shows the value 95.41.% scavenging activity for formulation syrup-600 shows the concentration(250 ug/ml) value of 60.45 and at formulation syrup-300, it shows the value 61.10. % scavenging activity for formulation syrup-600 shows the concentration (125 ug/ml) value of 47.36 and at formulation syrup-300, it shows the value 48.28. % scavenging activity for formulation syrup-600 shows the concentration(62.5 ug/ml) value of 35.67 and at formulation syrup-300, it shows the value 36.54.

% scavenging activity for formulation syrup-600 shows the concentration(31.25 ug/ml) value of 19.51 and at formulation syrup-300, it shows the value 20.03. % scavenging activity for formulation syrup-600 shows the concentration(15.60 ug/ml) value of 03.72 and at formulation syrup-300, it shows the value 04.63. % scavenging activity for formulation syrup-600 shows the concentration(CTC50 ug/ml) value of 185 and at formulation syrup-300, it shows the value 183 [8-11].

Table 1: Formulation of syrups

Ingredient	Dose	
	Syrup 600	**Syrup 300**
Curcuma longa extract	100mg	100mg
Withania somnifera extract	100mg	100mg
Azadirachtha indica	60mg	30mg
Oscimum sanctum	60mg	30mg
Mucuna pruriens	30mg	30mg
NaCl	--	10mg
Methyl paraben	0.5mg	0.5mg
Dextrose Sugar	1g	1g
Distilled water	Qs to make 10ml	Qs to make 10ml

Table 2: % scavenging of the prepared syrups

Concentration (µg/ml)	Anti-cancer activity in %	
	Syrup 300	**Syrup-600**
1000	98.12	96.23
300	95.41	96.19
250	61.10	60.45
125	48.28	47.36
62.5	36.54	35.67
31.25	20.03	19.51
15.60	04.63	03.72
CTC_{50} (µg/ml)	183	185

CONCLUSION

Two concentrations of the syrups were studied for the anticancer potential and its concluded to 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 drug.

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