Review Article

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IN VIVO STUDY OF COLON CANCER WITH SPECIAL REFERENCE TO INDUCER 1,2-DIMETHYLHYDRAZINE (DMH) AND STANDARD DRUG 5-FLUOROURACIL (5-FU): A REVIEW

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ABSTRACT

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal, uncontrolled cell growth. Colon cancer is the second most common cancer in females and the third most in males worldwide. The rate of colon cancer was low in India. But at present, it is increasing out 3.5 million cancer cases, 35,000 have colon cancer. Polyps, which are the small growths in the colon, are most often benign, even though some have the potential to become cancerous. For cancer treatment, 5-Fluorouracil (5-Fu) is commonly used as a chemotherapeutic drug. 1,2-Dimethylhydrazine (DMH) is a potent colon carcinogen inducer. These colorectal tumours share many resemblances to human colorectal cancer, including similar responses to promotional and preventive agents. This review article highlights various *In vivo* studies on colon cancer induced by 1,2-Dimethylhydrazine (DMH) and treated by standard drugs with the help of parameters like Aberrant crypt foci (ACF), Histopathology of the colon, Haematological parameters etc.

Keywords: Colon Cancer, *In vivo* study, 5-Fluorouracil (5-FU), 1,2-Dimethylhydrazine (DMH).

INTRODUCTION

Cancer is a multifactorial disease characterised by uncontrolled and abnormal cellular growth. Unlike normal cells, cancer cells continue to grow and divide, eventually replicating exponentially into harmful cells. Cancer is caused by external factors such as tobacco, chemicals, radiation, infectious organisms etc. and internal factors such as inherited mutations, hormones, immune conditions, mutations that occur from metabolism etc.[1] In India, the estimated number of people living with cancer is around 2.25 million. Every year, new cancer patients registered are 11 57,294, and cancer-related deaths are 7 84,821. The risk of developing cancer before 75 years is 9.81% and 9.42% in males and females, respectively.[2]

According to Globocan Data 2020, the number of new cases of colorectal cancer in both exercise 19ns, 31,590 (10%) and deaths 3, 95,173 (9.4%). Colorectal cancer is the third most common type of cancer in oncologic pathology. It is currently the most common malignant cancer in the gastrointestinal tract representing 13% of all malignant tumours. It is considered the second most common death-related cause of cancer affecting men and women. It is a prevalent disease in those aged 65-74, with a higher prevalence in women. However, this pathology is diagnosed more frequently in younger patients due to risk factors such as obesity, bad nutritional habits (high in fats and proteins), smoking and the progressive ageing of the population. The clinical presentation includes symptoms such as abdominal pain, alteration of chronic bowel habits, changes in bowel movements, involuntary weight loss, nausea, vomit, malaise, anorexia and abdominal distension etc.[3]

DMH is a potent colon carcinogen which induces colorectal tumours showing resemblances with human colorectal cancer. 5-FU is a standard drug widely used in the treatment of colorectal cancer.

Previously, various *In vitro* studies were carried out on colon cancer cell lines such as HCT-15, HCT-8 and HT-15 etc. 4, 5, 6 Various *In vivo* studies of colon cancer induced by DMH and treated by standard drug 5-Fu were reported previously. This review article emphasises the results of *In vivo* models of colorectal cancer caused by DMH and treated by 5- FU as a standard drug.

Review of Literature

Colon Cancer

Cancer is an extensive disorder that continues to be associated with high mortality rates. When this type of growth occurs in the colon or rectum, it is called colorectal cancer (CRC). CRC always begins as a polyp, a noncancerous increase in the colon or rectum's mucosal layer (inner lining). Colon cancer is the second most common cause of internal malignancy. Cancer of colon, rectum and anus are generally grouped together because of their anatomical continuity. [7, 8]

Etiological factors in Colorectal Cancer

1. Dietary factors - i) Dietary fat (especially animal fats) ii) Dietary carcinogens such as fried fish & meat, smoked foods etc. iii) Alcohol.
2. Host factors associated with increased risk of colorectal cancer- i) Familial polyposis syndrome- Familial adenomatous polyposis coli, Gardner's syndrome, Turcot's syndrome etc.

ii) Familial non-polyposis syndromes- Lynch I- familial colorectal cancer syndrome, Lynch II- familial adenocarcinomatosis syndrome.
iii) Benign colonic pathologies- Inflammatory Bowel Diseases (Ulcerative colitis, Crohn's disease), Adenomatous colorectal polyps- (Tubular, villous and tubulovillous), previous surgery, Cholecystectomy, ureterosigmoidostomy
iv) the Previous history of colorectal cancer.
v) Family history of colorectal cancer.

Clinical Features

1. Symptoms vary depending on the site of the carcinoma.

Tumours of the left colon show fresh rectal bleeding is common, and Obstruction occurs as an early feature. Tumours of the right colon show anaemia with occult bleeding, altered bowel habit and obstruction as a late feature.

2. Colicky lower abdominal pain present in 2/3rd of patients
3. Rectal bleeding occurs in 50%.
4. Carcinoma of the rectum usually causes early bleeding mucus discharge or a feeling of incomplete emptying.
5. 10-20% of patients show iron deficiency anaemia and weight loss.
6. There may be a palpable mass, signs of anaemia, or hepatomegaly from metastasis on examination. Low rectal tumours may be palpable on digital examination.

Staging of Colon Cancer

Based on pathological reporting, two staging systems (Dukes staging and TNM) are described for colorectal cancer. Out of these two Dukes staging system is widely recognised and based on histopathological reporting. TNM system describes histopathology with more detailing and is accepted as international standards. (Table 1 and 2) [9]

Dukes never described stage D, but this is often used to describe the metastatic disease.

Anticancer Drugs

Worldwide, many chemotherapeutic agents like 5-Fluorouracil (5-Fu), Cyclophosphamide, Cisplatin, Topotecan, and Methotrexate are used to treat cancer. The Classification of various anticancer drugs is given in the Table 3. [10]

5-fluorouracil (5-FU): ($C_4H_3FN_2O_2$)

5-Fluorouracil (5-FU) is widely used in the treatment of cancer. It is included in the 21st world health Organization's list of essential medicines (2019). Treatment with 5-fluorouracil (5-FU) is known to improve survival in various cancers. The largest impact of the drug has been reported in colorectal cancer. Pharmacological review of 5-Fluorouracil (5-Fu) shown in Table 4 [11, 12]

1, 2-Dimethyl hydrazine (DMH)

1,2- Dimethyl hydrazine or symmetrical dimethylhydrazine is the organic compound with the formula $(CH_3NH)_2$. It is one of the two isomers of dimethylhydrazine. Both isomers are colourless liquids at room temperature with properties similar to methylamines. It is a potent carcinogen that acts as a DNA methylating agent. It is widely used to induce colon tumours in experimental animals- mainly mice and feline cell samples. Exploration of DMH-induced colon carcinogenesis in rodent models provides the knowledge to perceive the biochemical, molecular and histological mechanisms of different stages of colon carcinogenesis. After a series of metabolic reactions finally reaches the colon, it produces the ultimate carcinogen and reactive oxygen species (ROS), which further alkylate the DNA and initiate the development of colon carcinogenesis. The preneoplastic lesions and histopathological observations of DMH-induced colon tumours may provide a typical understanding of the disease in rodents and humans. [13]

Aberrant Crypt Foci (ACF)

Aberrant crypt foci (ACF) are among the earliest neoplastic lesions of CRC and an evidential landmark during the early stage of tumour formation. Aberrant crypt foci (ACF) are clusters of abnormal tube-like glands in the lining of the colon and rectum. Aberrant crypt foci form before colorectal polyps and are one of the earliest changes seen in the colon that may lead to cancer. More ACF is detected in the distal bowel (descending and sigmoid colon and rectum) than in the proximal colon. A previous study reported that 65% of ACF were from the sigmoid colon or rectum, while the remainder were from the ascending and transverse colon. According to histopathological changes, ACF classification is of two types. In the first one, ACF is divided into 3 categories: 1) those that are almost histologically normal (Typical ACF), 2) those resembling hyperplastic polyps, and 3) those resembling microadenomas. Another classification scheme recognises 4 ACF types: normal mucosa, hyperplasia, stage I abnormality and adenoma. [14]

***In Vivo* study**

Clinical trials or medical studies may be performed either *In vivo* or *In vitro*. *In vitro* refers to medical research or experiment done in the laboratory within a test tube or laboratory dish. The term *In vivo* refers to a medical test, experiment or procedure that is done on (or in) a living organism such as laboratory animal or human. In contrast to *In vitro* studies, *In vivo* studies are needed to see how the body responds to particular substances. [15]

Table 1: Dukes' staging for colorectal cancer

A	Invasion of but not breaching the muscularis propria
B	Breaching the muscularis propria but not involving lymph nodes
C	Lymph nodes involved

Table 2: TNM classification for colonic cancer

T- Tumour stage	N- Nodal stage	M- Metastases
T1- Into submucosa	N0- No nodes involved	M0- No metastases
T2- Into muscularis propria	N1-one to three nodes involved	M1- Metastases
T3- Into pericolic fat or sub-serosa but not breaching serosa	N2- four or more nodes involved	-
T4- Breaches serosa or directly involving another organ	-	-

Table 3: Anticancer Drugs

A.Cytotoxic drugs		B.Targeted drugs		C.Hormonal drugs	
1. Alkylating agents	-Nitrogen mustards Mechlorethamine Cyclophosphamide, Ifosfamide, Chlorambucil, Melphalan-Ethylenimine Thio-TEPA-Alkyl sulfonate Busulfan-Nitrosoureas Carmustine(BCNU) Lomustine(CCNU-Triazine Dacarbazine	1.Tyrosine-protein-kinase inhibitors	Imatinib, Nilotinib	1.Glucocorticoids	Prednisolone and others
		2.EGF receptor inhibitors	Gefitinib, Erlotinib Cetuximab	2.Estrogens	Fosfestrol, Ethinyl-estradiol
2.Platinum coordination complexes	Cisplatin, Carboplatin, Oxaliplatin	3.Angiogenesis inhibitors	Bevacizumab Sunitinib	3.Selective estrogen receptor modulators	Tamoxifen, Toremifene
3.Anti-metabolites	-Folate antagonist - Methotrexate (Mtx) -Purine antagonist- 6-Mercaptopurine (6-MP) -Pyramidin antagonist- **5-Fluorouracil (5-FU)**, Capecitabine	4.Proteasome inhibitor	Bortezomib	4.Selective estrogen receptor down	Fulvestrant
4.Microtubule damaging agent	Vincristine (Oncovin), Vinblastine,Vinorelbine	5.Unarmed monoclonal antibody	Rituximab, Trastuzumab	5.Aromatase inhibitors	Letrozole, Anastrozole
5.Topo-isomerase-2 inhibitors	Etoposide	-	-	6.Anti-androgen	Flutamide, Bicalutamide
6.Topo-isomerase-1 inhibitors	Topotecan, Irrinotecan	-	-	7.5-α reductase inhibitor	Finasteride, Dutasteride
7.Antibiotics	Actinomycin-D (Dactinomycin), Doxorubicin, Mitomycin-C	-	-	8.GnRH analogues	Nafarelin, Leuprorelin
				9.Progestin	Hydroxy-progesterone acetate

Table 4: Pharmacological review of 5-Fluorouracil (5-FU)

Chemical Formula	$C_4H_3FN_2O_2$
Dose	1g taken orally on alternate days (6 doses), then 1g weekly or 12mg/kg/day i.v. for 4 days, then 6mg/kg i.v. on alternate days.
Route of Administration	Orally, Intravenous bolus, Continuous infusion, Intraperitoneal, tropical, Hepatic arterial infusion.
Indication	Early-stage colon cancer, Early-stage rectal cancer, Metastatic breast cancer, metastatic colorectal cancer, Nasopharyngeal cancer

Table 5: List of *In Vivo* studies on colon cancer induced by DMH and treated by standard drug 5-Fu

Drug Used	Standard Drug	Animal Model	Inducer	Parameters	Results
Cycas revolute (Methanolic Extract)[16]	5-FU	Male Wistar Rats	DMH	-Histopathology of colon -Biochemical parameters	In the DMH group, SOD, Catalase and GSH were decreased, while in the treated group MECR (200 & 400 mg/kg b.w.) SOD, CAT and GSH were increased.
Cuscuta reflexa (Dried Ethanolic Extract)[17]	5-FU	Male Wistar Rats	DMH	-ACF -Histopathology of colon -Haematological parameter	*C. reflexa* significantly reduced Disease activity indexing (DAI) level and ACF counting. Also, *C. reflexa* demonstrated similar activity as the standard drug 5-Fluorouracil (5-FU).
Hexahydrocurcumin (HHC) (Major metabolite of CUR)[18]	5-FU	Male Wistar Rats	DMH	-ACF	The combined effects of HHC with 5-Fu exhibit a synergistic inhibition by decreasing ACF formation mediated by down-regulation of COX-2 expression.
Annona muricata (Ethanolic Extract)[19]	5-FU	Male Wistar Rats	DMH	-ACF -Changes in body weight -Haematological Parameters -Individual organ weight -Biochemical parameters (Total cholesterol level) -Histopathology of Colon	EEAM at a dose of 300 mg/kg compared with disease control DMH significantly decreases ACF, Weight of individual organs, haematological parameters, and Total Cholesterol level. It also increases weight gain and apoptosis index.
Pumpkin Seed (Seed Extract)[20]	5-FU	Male Wistar Rats	DMH	-ACF -Haematological evaluation -Histopathology of colon -Biochemical parameters	Pre-treatment group at a dose of 200 mg/kg showed a significant decrease in the colon length/weight ratio. Pre-treatment groups showed a substantial increase in the colonic CAT, GSH and SOD levels compared to control

					and DMH control. All treatment groups demonstrated decreased hyperplasia and ACF in histology.
2- hydroxychalcones[21]	5-FU	Male Wistar Rats	DMH	-ACF -Histopathology of colon	2'-hydroxy chalcones show a significant reduction in ACF formation. Treatment with various compounds of 2'-Hydroxy chalcone derivatives showed restoration in the colon's morphology and a decrease in crypt abscess formation.
Iminoflavones[22]	5-FU	Male Wistar Rats	DMH	-ACF -Histopathology of colon -Biochemical Parameters	IMF-8 treated group significantly reduced ACF and Polyps. It greatly increased the catalase and GSH levels, reducing the TNF-α and IL-6 levels. The histopathological findings of the IMF-8 treated colon show no signs of mucosal crypt abscess.

DISCUSSION

Colon cancer is the third leading cancer in males and the second leading cancer in females in the industrialised countries. Its morbidity and mortality are increasing in developing countries. Previously, the incidence of colon cancer was low in India and underdeveloped countries, but later studies showed a drastic increase in the colon cancer incidence in Asia. Dietary habits play a crucial role in the development of colorectal cancer. Rapid urbanisation and extensive growth of economic conditions influence the people to adopt the western dietary style, which consists of high-fat, high-protein, low-carbohydrate, and low dietary fibre. This unbalanced diet is considered an important causative factor for the increased mortality in recent years.

Colon cancer usually starts as noncancerous growth known as a polyp on the inner wall of the colon or rectum. This polyp grows slowly and develops into an adenomatous polyp or adenoma for 10-20 years. Colon cancer is a multistage process which involves initiation, promotion and progression. DMH is a chemical carcinogen which is well established for inducing colon cancer *In vivo*.

Various *In vivo* studies, as described in Table 5, on colon cancer induced by DMH and treated by standard drug 5-fu with the help of parameters like ACF, Histopathology of the colon, Haematological parameters etc. can be discussed as follows-

Methanolic extracts of *C. revoluta* for anticancer activity was studied in wistar rats. In the DMH group, SOD, Catalase and GSH were decreased, while in the treated group MECR (200 & 400 mg/kg b.w.) SOD, CAT and GSH were increased. Histopathological evaluation revealed that the DMH+5FU treated group showed better results than the DMH+MECR400 group as tubular gland appeared clearly with arranged manner.

The anticancer effect of the dried ethanolic extract of *C. reflexa* induced by 1, 2 –Dimethyl hydrazine (DMH) was reported previously. It was observed that *C. reflexa* significantly reduced Disease activity indexing (DAI) level and ACF counting. Also, *C. reflexa* demonstrated similar activity as the standard drug 5-Fluorouracil (5-FU). Histopathological results revealed that the apoptotic bodies decreased in the DMH -induced group (group II) during cancer progression, while in 5-FU treated (group III) and *C. reflexa* treated (group IV and V), the apoptotic bodies were increased.

This study aimed to determine the anti-colon carcinogenic effects of HHC, a major metabolite of CUR, in combination with 5-FU. It is induced by subcutaneous injection of DMH (40 mg/kg) twice a week for two weeks. Total ACF in standard group 5-Fu was 1231, in CUR group was 1284, in HHC group was 1086, in the 5-Fu+CUR group was 880, in 5Fu+HHC group was 665. The combined effect of hexahydrocurcumin (HHC) with 5-Fu is significant and exhibits a synergistic inhibition by decreasing ACF formation mediated by down-regulation of COX-2 expression.

Anticancer activity o *Annona muricata* in DMH induced colon cancer was reported previously. The plant extract EEAM (Ethanolic extract of *Annona muricata*) at a dose of 300 mg/kg significantly decreases the aberrant crypt foci (ACF), Weight of individual organs, haematological parameters and Total cholesterol level. It also increases weight gain and apoptosis index.

This study aimed to evaluate the prophylactic and anticancer activity of pumpkin seed extract in 1,2-dimethylhydrazine (DMH) induced colon cancer in Wistar rats. Pre-treatment group at a dose of 200 mg/kg showed a significant decrease in the colon length/weight ratio. Pre-treatment groups showed a significant increase in the colonic CAT, GSH and SOD levels compared to control and DMH control. All treatment groups demonstrated decreased hyperplasia and ACF in histology.

In vivo anticancer studies of 2'-Hydroxy chalcone derivatives were reported previously. They showed a significant reduction in aberrant crypt foci formation and adenocarcinoma count and a substantial decrease in TNF-α levels compared to DMH control at a 100 mg/kg dose. Observation of the section of the colon in the normal control group displayed a typical architecture with fingers like mucosal projections called villi and the presence of crypts in between them. No sign of dysplasia or crypt abscess was observed in the normal control group. However, sections of the colon in the DMH control group had a distorted morphology with the formation of crypts abscess and ACF. Treatment with various compounds of 2'-Hydroxy chalcone derivatives showed restoration in the colon's morphology and a reduction in the shape of crypt abscess.

The antitumor potential of iminoflavones *In vivo* anticancer models was also reported. In this study, DMH control animals showed initiation of ACF. They are the focal lesions of colonic mucosa consisting of several aberrant shape crypts which can be differentiated from normal crypts. DMH treatment showed an increase in ACF count with adenoma. The standard drugs 5FU and IMF-8 showed better anticancer efficacy in maintaining fewer ACFs. Histopathological studies showed mucosal crypt abscess, a massive number of aberrant crypt foci and nuclear enlargement with adenocarcinoma in only DMH treated group. In contrast, there were fewer aberrant crypt foci with no signs of mucosal crypt abscess in the 5-FU and IMF-8 treated groups.

CONCLUSION

Various experimental drugs such as *Cycas revoluta*, *Cuscuta reflexa*, *Annona muricata*, Pumpkin seeds, Metabolite of CUR, 2'-Hydroxy chalcone derivatives, imminoflavones show anticancer activity *In vivo* model of colon cancer which DMH induces. Aberrant crypt foci (ACF) are one of the earliest neoplastic lesions. In most of the studies, Aberrant Crypt Foci are decreased.

The present study opens many new areas of research work. These experimental drugs can be continued separately or in combination with the standard drug in the future for further research with clinical trials as prophylactic and anticancerous in colon cancer.

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