Comparative study of radiolabeled ^{68}Ga DOTANOC acetate, ^{68}Ga PSMA-11 and ^{68}Ga RGD on 1, 2-dimethylhydrazine induced colon carcinoma in Sprague dawley rats

A Dissertation Submitted to
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
PHARMACOLOGY

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NOVEMBER 2019

Dedicated to Almighty,

My Beloved Parents,

Brother, Teachers &

Friends

ACKNOWLEDGEMENT

This thesis becomes a reality with the kind support and help of many individuals. First of all I would like to extend my sincere thanks to God Almighty for the wisdom he bestowed upon me, the strength, peace of my mind and good health in order to finish this research.

I would like to express my whole hearted gratitude to my father **Mr. Saseendran**, my mother **Mrs**.**Vineetha** and my brother **Mr. Nitin** for their unconditional love, affection, guidance, encouragement and motivation. They remembered me in their prayers and supported me emotionally and financially thus enabled me to concentrate fully on my studies without any worries.

I acknowledge my sincere gratitude and indebtedness to my esteemed guide **DR. G. Ariharasivakumar, M.Pharm., Ph.D., HOD,** Department of Phamacology, KMCH college of Pharmacy, for his constant insight, guidance, kindness, persistent encouragement and sharing his knowledge and expertise that helped me to learn and complete my work

I extend my heartfelt thanks to **DR. A. Rajasekaran, M.Pharm., Ph.D., Principal, KMCH College of Pharmacy**, Coimbatore,who has provided excellent facilities to do research in this institution.

I take this opportunity to convey my sincere thanks to our most beloved Managing Trustee, Hon'ble **Dr.Nalla G. Palaniswami** and respected Trustee Madam **Dr.Thavamani D. Palaniswami,** Kovai Medical Center Researchand Educational Trust, Coimbatore for all the facilities provided for me at the institution.

I owe my honest thanks to **Dr.Ajit Shinto**, MBBS, DRM, DNB, MNAMS, PGDHA/HM, chief consultant, HOD in nuclear medicine and **Dr ArunPandiyan**, Pharm.D, Clinical Pharmacist, Kovai Medical Centerand Hospital for their constant support and helpfulness and providing facilities at the hospital to carry out this project in a nice manner.

I put forth my unlimited gratitude to my all other teaching staffs for their immense support, timely help and valuable suggestion.

I express my special thanks to **Mr.Tamilarasan (Lab technician, Dept. of Pharmacology), Mr.Saravanan (Lab technician, Dept. of Pharmacology), Mrs. Muneeshwari (Lab technician, Dept. of Pharmacology),** Librarian and other Lab technicians of KMCH College of Pharmacyfor their valuable support and timely help during the course of entire work.

I have great gratitude to **Mrs.Dhanalakshmi** for helping in animal maintenance during the study.

I profusely acknowledge all my seniors **Kanupriya, Treesa** and **Srividhya**, for the advice, affection and encouragement throughout this journey.

I am indebted to my friends, **Vishali, Kavitha, Pavithra, Cuckoo, Malathi, Karthikaa, Packiyalakshmi, Nayanthara, Keerthana, Reshma, Solly, Henna, Jayanthi, Saranya, Nadhiya, Kousalya** for the dedication, love and support they gave me during the hard times. Their selfless support and motivation throughout the research is beyond words.

I wish to thank my loving juniors **Sumitha, Anitta, Jinu, Haritha, Anu**and all other juniors for the affection and encouragement throughout this journey.

Last but not least, I would like to thank each and everyone who are all part of this successful completion of my thesis.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
WHO	World Health Organisation
GLOBOCAN	Global Cancer
IARC	Institutional agency for research cancer
NICPR	National Institute of Cancer Preventive and Research
PET	Positron Emission tomography
CT	Computed tomography
DMH	Dimethylhydarzine
AOM	Azoxymethane
MAM	Methylazoxymethane
AM	Azomethane
CRC	Colorectal cancer
CIN	Chromosomal instability
MSI	Micosatellite instability
CIMP	CpG island methylator phenotype
MMR	Mismatch repair
APC	Adenosine polyposis coli
TGF β	Transforming growth factor beta
PIK3CA	Phosphatidylinositol 4,5 biphosphate 3-kinase catalytic subunit alpha
PTEN	Phosphatase and tensin homolog
CEA	Carcinoembryogenic antigen
FDG	Flurodoxyglucose
PSMA	Prostate specific membrane antigen
RGD	Arginylglycylaspartic acid
sstr	Somatostatin receptor
gm	Gram
hr	Hour

mMol/L	Millimol per litre
NaOH	Sodium hydroxide
mg/kg	Milligram per kilogram
mcg	Microgram
NH_4OAc	Sodium acetate
ITLC	Instant thin layer chromatography
cm	Centimeter
%	Percentage
µL	Microliter
µCi	Microcurie
rpm	Rotations Per Minute
min	Minute
w/v	Weight per volume
EDTA	Ethylene diamine tetraacetic acid

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ABSTRACT

Colon cancer the third most commonly diagnosed cancer and second most common cause of mortality. The survival rate can be increased by earlier diagnosis and better treatment regimen. PET/CT has recently become a vital part to achieve this goal by providing the detailed information regarding tumor lesions than other techniques. Combination of FDG PET/CT has been used initially and proven to be of great benefit for the assessment of colon cancer. Due to some limitation of FDG, a new diagnostic tracer has to be introduced. Radiolabelled peptides are widely used now a days for imaging cancer lesions. The present study aimed to compare the three radiolabelled peptides ^{68}Ga DOTA-NOC acetate, ^{68}Ga PSMA-11 and ^{68}Ga RGD efficiency on 1,2 dimethylhydrazine induced colon cancer in Sprague dawley rats by PET/CT imaging. Radiolabeling efficiency of ^{68}Ga with DOTANOC, PSMA, RGD peptides were evalauated and found to be good. PET/CT imaging were carried using three peptides in which ^{68}Ga PSMA-11 alone shows positive imaging. Other two peptides ^{68}Ga DOTA-NOC acetate and ^{68}Ga RGD were not able to image the tumor lesions. Plasma clearance were also evaluated and found higher tumor to background ratio and a low accumulation in non-target organs for ^{68}Ga PSMA. Thus on comparison, ^{68}Ga PSMA-11 peptide is more suitable for preclinical imaging of colon cancers.

Key words: PET/CT, ^{68}Ga DOTA-NOC acetate, ^{68}Ga PSMA-11, ^{68}Ga RGD, Colon cancer

1. INTRODUCTION

1.1 INTRODUCTION

This chapter present: back ground of study, statement of the problem, theoretical framework, purpose of the study, specific aim and objectives.

1.1.1 BACK GROUND OF STUDY

Cancer is a disorder that results from genetic or epigenetic changes which ultimately drive the malignant transformation of the normal cells. Cancer is most deadly and a threat to the developed and developing nation and is the second most common cause of death. By 2020, the world population is expected to have increased to 7.5 billion; of this number, approximately 15 million new cancer cases will be diagnosed, and 12 million cancer patients will die.[1]

Colon cancer is the third most common cancer which begins when healthy cells in the lining of the colon change and grow out of control, forming a mass called tumor. It is the second most common cause of death related to cancer, which overcome the mortality rate of heart diseases. It is a prevalent disease in 65-74 aged people, with a high prevalence in women. The risk can be reduced by early diagnosis and proper treatment. [2]

Many diagnostic methods are there to diagnose colon cancer. PET/CT imaging is the most advanced technique which helps to identify the location of tumor and stages of tumor. For PET/CT imaging new diagnostic tracer are developing for accurate imaging. Radio-labelled peptides are currently trending for imaging cancer as it binds to specific receptors in cancer cells due to over expression of receptors, thus provides proper imaging. [3]

1.1.2 STATEMENT OF PROBLEM

Cancer is a serious health problem worldwide and second most common cause of death globally.[4] According to GLOBOCAN 2018 database by IARC (International Agency for Research on Cancer) it is estimated that incidence rate has risen to 18.1 million cases and mortality rate to 9.6 million deaths in 2018.[5] In US nearly 1,762,450 new cases has been diagnosed and 606,880 deaths i.e, about 1,660 deaths per day due to cancer has been reported by American Cancer Society. More than 1.7 million new cases are expected to be diagnosed in

2019.[6] It is estimated that 11,57,294 new cancer cases has been diagnosed and 7,84,821 deaths and 22,58,208 people living with cancer in India.[7]

Table no. 1 Estimated new cases and mortality rate of all cancers[4]:

SITES	INCIDENCE RATE	MORTALITY RATE
Lung	228,150	142,670
Breast	271,270	42,260
Colorectal	101,420	51,020
Prostate	174,650	31,780
Skin cancer	104,350	11,650
Pancreas	56,770	45,750
Liver	42,030	31,620
Leukemia	61,780	22,840
Bladder	80,470	17,670
Kidney	73,820	14,770
All sites	1,762,450	606,880

Cancers of the lung, breast and colorectal have the highest incidence rate, whereas lung, colon, breast, liver have the highest mortality rate. Prostate remains the third most cancer in men both in terms of incidence and mortality rate.[5] The reason of these are complex but reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development. The incidence and mortality rate is higher in men than women.[8]Breast, Oral, Cervical, Gastric and lung cancers are five most cancers that affect Indian population.[7]

Colon cancer is the most common disease of the gastrointestinal tract. According to WHO it is the second and third most common cancer in women and men worldwide.[9] The incidence and mortality rate of colon cancer vary. Globally, the highest incidence rate is in countries like Australia, New Zealand, North America, Europe and in countries like South central Asia, Africa, China, India the rates are low. Lifestyle factor and environmental exposure results in variation of rates regionally.[10]

As per GLOBOCAN database 2018, it is estimated nearly 1.8 million new cases were diagnosed and 861,000 deaths due to colon cancer during 2018.[10] About 145,600 cases of colon

cancer will be diagnosed and 51,020 deaths in US in 2019. 1 in 22 for men and 1 in 24 for women has a risk for developing colon cancer. The risk is higher in men than women.[11] The incidence and mortality rates decreases slowly but steadily every year due to variations in risk factors or different effective screening and treatment method. Even though the incidence rate and mortality has been increasing in younger age groups under 50 nearly by 1.8% and 1% respectively yearly. About 1,324,922 people were living with colon cancer in US.[12]

The incidence rate of colon cancer in India is comparatively lower than western countries and it is considered as seventh leading cancer in India. As per NICPR database, about 27,605 case has been diagnosed and 19,548 deaths were estimated in India. The risk is higher in age group of 40-45 year. Nearly 53,700 people are living with disease as per 5 year prevalence for all ages estimation.[13]

1.1.3 THEORETICAL BACKGROUND

Cancer is a global epidemic causing more than 8 million annual deaths worldwide. More than 13 million new cancer diagnoses made each year. Cancers are deadliest when diagnosed at late stages, a problem that is caused by lack of early detection tests. Cancers of the colon, esophagus, liver and intra hepatic bile ducts, lung and bronchus, non-Hodgkin lymphoma, oral cavity, ovary, pancreas, and uterine cervix are diagnosed at regional or distant stages in more than 50% of cases, resulting in poor survival for many patients. Many of these diagnoses, deaths, and costs could be avoided by the development and investigation of new diagnostic modalities and innovative therapeutic tool as well as shortening the gap between pre-clinical research and regulatory approval for safe and effective therapies. Advancing cancer diagnostic and therapeutic may exceeds a decades and cost on average of $2.5B per agent. Only 10% of drugs are advances into human testing from pre-clinical study; nearly 75% of research and development are attributed to failures event at drug discovery process.[14]

Cancer research uses animal and human cancer cell lines in vitro to study biochemical pathways in cancer cells. The advantages of in vitro cancer models are highly controlled conditions, homogeneity, discovery of molecular mechanisms, and reproducibility. The main limitations of two-dimensional in vitro cell culture cancer cell lines are selection of phenotypic and genotypic cells during adaptation to in vitro conditions, accumulation of mutations in cells over time in culture, a homogeneous population of cells, and isolation of cells from the tumor

microenvironment. Mimicking the interactions between tumor cells and the cellular matrix, well defined three-dimensional in vitro cancer models and co culture systems have gained acceptance for a wide variety of diagnostic and therapeutic application. Despite all the mentioned disadvantages, cancer cell lines have been, and will continue to be, the model in vitro system for cancer studies.[15]

The developments of in-vivo animal models are valuable tools for studying the biology and genetics of human cancers as well as for pre-clinical investigation of anti-cancer therapeutics and cancer prevention. Various animal models have been generated by genetic engineering, graft transplantation, and viral/ physical/chemical induction. Accumulating data from studies using those models have enabled us to gain insight into the genetic mechanisms underlying malignant transformation and cancer progression. Studies from animal models of cancer have been utilized for preclinical investigation of therapeutic efficacy and toxicity of chemicals and biologicals. Tremendous advances have been made in the generation of animal models of cancer, which have become increasingly sophisticated by application of new technologies and integration of clinical information from patients. The goals are to faithfully recapitulate the human malignant diseases in the animal models and apply them as preclinical tools, with the hope of successfully translating the basic knowledge into treatment and prevention of cancer in humans.

The mouse has been the traditional animal model for basic and preclinical studies of cancer, and other organisms including zebra fish play important and complimentary roles as models of cancer research.[16]Rats represent another rodent commonly utilized as pre-clinical cancer models. In addition to some of the advantages of mouse models, rats have the added benefit of larger size, rendering them more amenable to interventions such as surgery and radiological imaging. Rats are commonly used to model colon and bone cancers, largely by exposure to chemical carcinogens. Various chemical agents are considered as carcinogens which helps to induce the cancer to animals as like in human and provide a better understanding about the molecular characteristics of the disease and preventive and therapeutic measures to be taken.[14]

Commonly used chemical carcinogen to induce colon cancer is 1,2dimethylhydrazine which cause cancer in colon of rats represents the human colon cancer. It is a potent carcinogen which is administered via four different routes based on the strain used. Subcutaneous administration is commonly used.[17] When this agent is administered it undergoes series of

metabolism involves multiple xenobiotic-metabolizing enzymes, which proceeds through several N-oxidation and hydroxylation steps, including the formation of MAM following hydroxylation of AOM. The reactive metabolite, MAM, readily yields a methyldiazonium ion, which can alkylate macromolecules in the liver and colon, including the addition of methyl groups at the O^6 or N^7 position of guanine (O^6-methyl-deoxyguanosineand N^7-methyl-deoxyguanosine).[18]

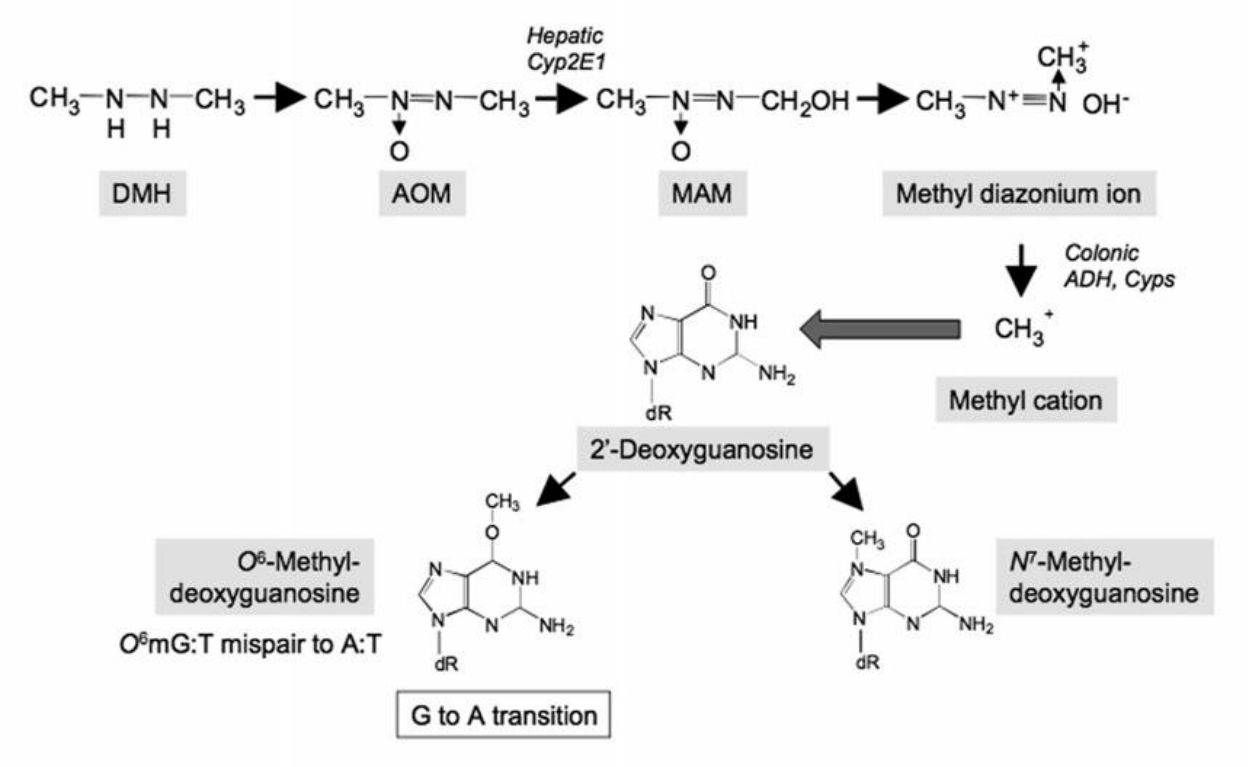


Fig no. 1 Metabolism and action of 1,2 dimethylhydrazine

MAM is a substrate of the nicotinamideadenine dinucleotide dependent dehydrogenase present in the colon and liver, suggesting that the active metabolite of MAM mightbe the corresponding aldehyde. A direct role for the alcohol inducible cytochrome P-450 isoform, CYP2E1, in activation of AOM and MAM has been developed that these metabolites ofCYP2E1 are transported to the colon via the bloodstream. The ability of AOM and DMH to target the colonic mucosa is probably a consequence of the relative stability of the hydroxylated metabolite, MAM; with a half-life of 12 h, there is sufficient time for MAM to distribute to the colon. Further activation of blood-borne metabolites may then proceed via non-P450-dependent mechanisms, including possible oxidation of MAM, directly within the colon. It was originally hypothesized

that the differential sensitivity of mouse strains to DMH may be attributed to differential activation of the procarcinogen.[19]

1.1.4 PURPOSE OF THE STUDY

PET/CT imaging is one of the advanced diagnostic method to diagnose colon cancer. Using PET/CT scan, one can correlate the morphological and functional imaging with high specificity and sensitivity.[20] ^{18}F-FDG is the most commonly used radiopharmaceutical tracer for PET imaging of various cancers. It is a glucose analogue labeled with positron emitting radionuclide ^{18}F the compound is taken into cells by glucose transporter proteins. Once internalization, ^{18}F-FDG is phosphorylated to ^{18}F-FDG-6-phosphate which cannot be further metabolized and remain trapped in the cell. Compared with normal cells, malignant cells have an increased number of cell surface glucose transporter proteins and increased intracellular glycolytic enzyme levels. Hence this compound is often used to distinguish malignant from normal tissues, to stage many types of neoplasms, and to detect recurrence after treatment.[21] But uptake of ^{18}F-FDG varies greatly for different tumor types and increased ^{18}F-FDG uptake is not necessarily specific for neoplasms. In colon cancer assessment FDG PET/CT shows false positive imaging have been reported with mucinous adenocarcinoma and also false negative findings have been reported due to inflammatory conditions such as colitis or postoperative scarring.

Radiolabelled peptides are widely used for some cancers due to over-expressions of receptors in tumor region. Various research are being conducted to understand the ability of peptide action over different tumor region. First and most successful developed imaging radiolabelled peptide is the ^{111}In-DTPA-octreotide. After intravenous injection, the radiolabelled peptide will extravacate and bind to sites with high receptor density i.e., tumor cells. In recent years, many radiolabeled peptide analogs, such as somatostatin, bombesin, vasoactive intestinal peptide, cholecysto-kinin/gastrin, neurotensin, exendin and RGD derivatives, have been developed for scintigraphic detection of different tumor types.[22]Several studies found that expression of receptors and transporters are high in density during carcinogensis phase of colon cancer. The purpose of the study is to comparing various radiolabelled peptides like ^{68}Ga DOTANOC acetate, ^{68}Ga PSMA-11, ^{68}Ga RGD expression in colon cancer cells.

1.1.5 AIM AND OBJECTIVES

AIM:

To diagnose and evaluate the radiolabeled ^{68}Ga DOTA-NOC acetate, ^{68}Ga PSMA-11 and ^{68}Ga RGD on 1,2-dimethylhydrazine induced colon carcinoma in Sprague dawley rats by using radioimaging technique.

OBJECTIVES:

- To diagnose and compare the radiolabeled ^{68}Ga DOTA-NOC acetate, ^{68}Ga PSMA-11 and ^{68}Ga RGD by radioimaging technique.
- To understand the effect of radiolabeled ^{68}Ga DOTA-NOC acetate, ^{68}Ga PSMA-11 and ^{68}Ga RGD on 1,2-Dimethylhydrazine induced colon carcinoma in Sprague dawley rats.
- To evaluate the pharmacokinetic parameters.
- To study the histopathology of colon tissues.

1.2 PLAN OF WORK

- Review of literatures
- Grouping of animals and induction of colon cancer
- Preparation of 68-gallium
- Labeling of ^{68}Ga with DOTA-NOC, PSMA-11 and RGD
- Quality control test for radiolabeled peptides
- Radio imaging
    - ❖ PET/CT scan
- Pharmacokinetic evaluation
    - ❖ Plasma clearance
- Histopathological evaluation

2. REVIEW OF LITERATURE

2.1 CANCER:

Cancer is a group of diseases in which abnormal cells divide without control and are able to invade other tissues.[23] It is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, cell division, ability to invade to distant organs through lymph or blood and metastasize.[24] Cancer is a generic term because the actual medical term for cancer is neoplasis, which from the Greek, means new formation.[25]

2.1.1 TYPES OF CANCER [23]

Most accepted classification of cancer is based on type of tissue on which it originates and the location where it developed at first.

- **Adenocarcinoma:**

  It is the type of cancer that originating in glandular tissue. This tissue is also part of a larger tissue category known as epithelial. Epithelial tissue includes, but is not limited to, skin, glands and a variety of other tissue that lines the cavities and organs of the body

- **Carcinoma:**

  Malignant out growth arising from epithelial cells is called as carcinoma. It has the property to invade adjacent tissues and organs and may metastasize, or spread, to lymph nodes and other tissues.

- **Leukemia:**

  Originates in tissues that form blood cells. It is cancer of the blood or bone marrow (which produces blood cells). A person who has leukemia suffers from an abnormal production of blood cells, generally leukocytes (white blood cells).

- **Lymphoma:**

  Lymphoma is a type of cancer that begins in immune system cells called lymphocytes. Like other cancers, lymphoma occurs when lymphocytes are in a state of uncontrolled cell growth and multiplication. Lymphoma occur when lymphocyte B or T cells transform and begin growing and multiplying uncontrollably.

- **Sarcoma:**

  A sarcoma is a type of cancer that develops from certain tissues, like bone or muscle. Sarcomas generally develop in soft tissues like fat, muscle, nerves, fibrous tissues, blood vessels, or deep skin tissues.

2.2 COLON:

Large intestine is the final region of gastrointestinal tract begins from ileoceacal region divides into caecum, colon, rectm and anus. Colon is derived from greek word *kolueinm*eans "to retard" is the largest and important region of large intestine. It is a hollow tube of about approximately 150 cm in length and 2.5 inches in diameter.[26] Its wall consists of four layers: the innermost layer mucosa is simple epithelial tissue, secretes mucus by mucousa gland to the lumen to lubricate its surface and protects from rugged food particles; the second layer submucosa surrounds the mucosa is layer of blood vessels, nerves and connective tissues to support the other layers; the third one is muscularis surrounds the submucosa is layer of visceral muscle cells which contracts, continuous contaction leads to formation of pouch like structure named haustra; the final outermost layer is serosa is layer of squamous epithelium tissues secrete water on surface to lubricate and protect from friction between abdominal organs and other nearby bones or muscles.[27]

Colon itself is divided into ascending, transverse, descending, and sigmoid colon.

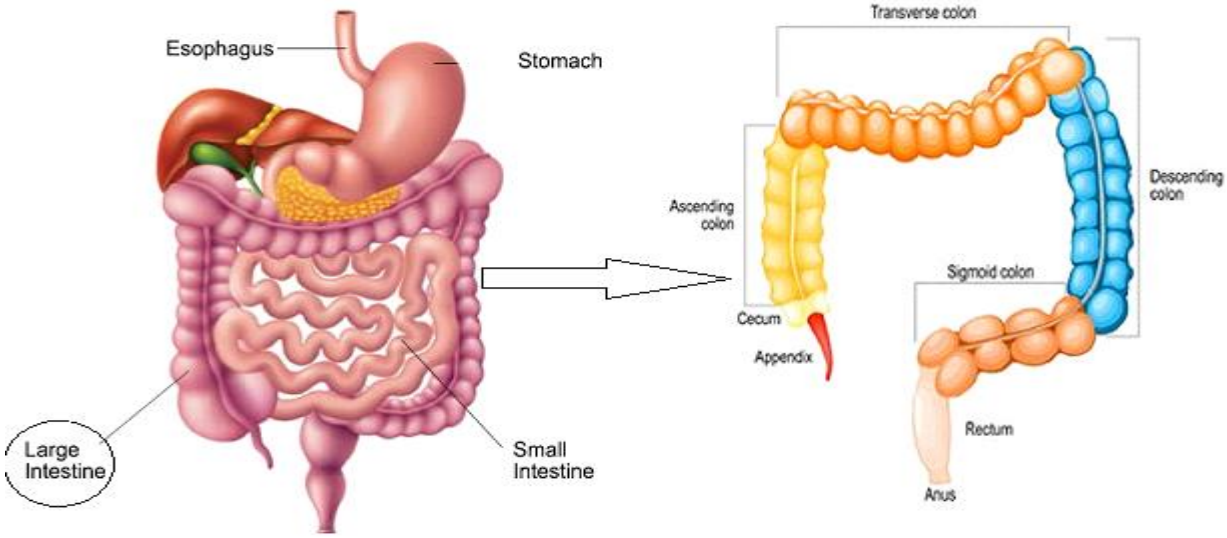


Fig no 2. Structure of colon

2.2.1 ASCENDING COLON:

Ascending colon, the superior region of colon forms a hollow tube of about 8 inches long and 2.5 inches in diameter rises from the ceacum to hepatic flexure. The inferior end of the ascending colon joins to the ceacum, from which it rises towards the inferior of the diaphragm. Before reaching liver it turns left about 90º at the hepatic flexure and continues as transverse colon. After absorption of 90% nutrients from food in small intestine it reaches to ceacum, then to ascending colon where the bacteria liberates vitamins and water by digesting unwanted material. The walls of intestine absorbs these vitamins and water.[28]

2.2.2 TRANSVERSE COLON:

Transverse colon is the longest region of colon rises from hepatic flexure to splenic flexure. It is a hollow tube of about 18 inches long and 2.5 inches in diameter. It begins at the hepatic flexure which connect at the superior end of the ascending colon and moves across the abdomen. After reaching left side it bends sharply about 90º at the splenic flexure and continues as descending colon. In this region the faeces mixes by segmentation process. As like ascending colon the bacteria liberates vitamins and water which is absorbed by colon walls.[29]

2.2.3 DESCENDING COLON:

Descending colon is the third and latter part of colon forms a hollow tube of about 9-10 inches long and 2.5 inches in diameter. The superior end of descending colon joins to the transverse colon at the splenic flexure where it moves inferiorly towards the left side of abdomen. At the end it bends about 90º to the right and continues as sigmoid colon. It absorbs remaining water and nutrients from faeces into bloodstream. The main function is to storage of faeces prior to defecation and transport it into sigmoid region.[30]

2.2.4 SIGMOID COLON:

Sigmoid colon the last part of colon is s-shaped rises from descending colon to rectum. It is a hollow tube of about 16 inches long and 2.5 inches in diameter. From the descending colon it curves medially and anteriorly towards the body mid line and turns about 90º to rectum. The faeces are accumulated here before its elimination.[31]

2.2.5 FUNCTIONS:

- **Absorption of water, potassium, bicarbonates, vitamins** especially vitamin K, B1, B6. Normally the faeces enter into colon are in fluid nature where they absorbs maximum water and convert it to solid matter. About 1.5 litres of fluids are entering into colon per day in which 100-200ml are excreted through faeces. Colon has the capacity to absorb 4.5 litres per day. Hence it prevents diarrhea. Apart from absorption of water other nutrients like vitamins, potassium and bicarbonates liberated by bacteria from fermentation are absorbed through walls of the intestine into blood stream. Synthesis of vitamin K by bacteria nurture a valuable supplement to dietary source.
- **Explusion and storage of faecal matter**: The motion of faecal matter from caecum to anus is slow portion. The right part of colon (ascending, transverse) causes contaction leads to remarkable mixing called segmentation process which helpful for the absorption of water and other nutrients whereas the left part (descending, sigmoid) causes slow propulsion thereby accumulates in this region until defaecated. After innervation of stimuli the faecal moves to rectum and excreted.
- **Provides an appropriate environment for the growth of colonic bacteria**. These bacteria are helpful for the digestion of undigested foods. It also essential for liberation of nutrients like vitamins.[32]

2.3 COLON CANCER

Colon cancer is a molecularly heterogeneous diseases results from the progressive accumulation of multiple genetic and epigenetic aberrations within cells. It begins when healthy cells in the lining of the colon change and grow out of control. As these cells grow and divide, they can lead to mass within the colon called polyps. Some types of polyps can change into cancer over time (usually many years), but not all polyps become cancer. The chance of a polyp changing into cancer depends on the type of polyp it is. The 2 main types of polyps are:

- Adenomatous polyps (adenomas): These polyps sometimes change into cancer. Because of this, adenomas are called a *pre-cancerous condition*.
- Hyperplastic polyps and inflammatory polyps: These polyps are more common, but in general they are not pre-cancerous.[33]

2.3.1 TYPES OF COLON CANCER:

CRC presents in one of three patterns: inherited, familial, and sporadic.

- **Sporadic colorectal cancers** (CRC), due to somatic mutations, account for about 60% of all CRCs, and is not associated with family history.
- **Familial CRC**, a group of diseases in which patients do not present a Mendelian inheritance patterns or genetic etiology, but only a familial predisposition (i.e, a mutated copy of the adenomatous polyposis (*APC*) gene) to develop cancer, are about 10-30%.
- **Inherited CRC** is characterized by MSI, a consequence of a defective DNA mismatch repair (MMR) system, are about 5-7%. The main inherited CRC syndromes are hereditary nonpolyposis colorectal cancer (HNPCC) and adenomatous polyposis syndrome.

The other forms of CRC include a rare syndrome called hamartomatous polyposis syndrome and the common inherited cases caused by less penetrant inherited mutations.[34]

2.3.2 RISK FACTORS:

A risk factor is anything that increases a person's chance of developing cancer. Although risk factors often influence the development of cancer, most do not directly cause cancer. Some people with several risk factors never develop cancer, while others with no known risk factors do. Often, the cause of colorectal cancer is not known. A person with an average risk of colorectal cancer has about a 5% chance of developing colon cancer overall. However, the following factors may raise a person's risk of developing colorectal cancer:

Age: The risk of colon cancer increases at older age group. It can occur in younger and teenagers, but majority occurs in people older than 50. The average age at the time of diagnosis for men is 68 and for women is 72.

Gender: Men have a slightly higher risk of developing colon cancer than women

Family history: A person's risk of colon cancer increases if any of their family members are diagnosed with colon cancer even before age 60. The risk increases if more than one relative has had colon cancer.

Rare inherited conditions: Individuals with certain inherited conditions also have a increased risk of colon cancer. Some of the inherited disorders are:

Familial adenomatous polyposis (FAP)

Attenuated familial adenomatous polyposis (AFAP)

Gardner syndrome, a variant of FAP

Lynch syndrome (hereditary nonpolyposis colorectal cancer HNPCC)

Juvenile polyposis syndrome (JPS)

Lifestyle: Some studies have shown that a sedentary lifestyle like somking, excessive consumption of alcohol, obesity, no physical activity is associated with a higher risk of colon cancer.

Diet: Researchers have proven that diet like intake of red meat and processed meat increases the risk of colon cancer. It is mostly seen in western countries where people are more addicted to red meat and processed meat, hence incidence of colon cancer is high in western countries than other countries.

Environmental factors: Some chemical exposure increases the risk of colon cancer. Apart from chemicals, radiation exposure also increases risk.

Race: Black people have the highest rates of sporadic, or non-hereditary colon cancer in the United States. Colon cancer is also a leading cause of cancer related deaths among black people. Black men are more likely to die from colon cancer than black women and other racial group.

Disease: People with inflammatory bowel disease (IBD), such as ulcerative colitis or Crohn's disease may develop chronic inflammation of the large intestine. This further increases the risk of developing colon cancer.

Drugs: Some drugs like NSAIDs increase the risk of colon cancer.[35]

2.3.3 PATHOPHYSIOLOGY:

The pathophysiology of colon cancer is quiet complex because the epithelial cells in the gastrointestinal tract undergoes series of genetic and epigenetic alterations. These alterations in the normal epithelium leads to formation of benign adenomas which then progress into carcinoma and lately metastatic tumors.

Normal gastrointestinal epithelium is structured along a crypt-villus axis. This epithelium undergoes everlasting regeneration process initiated by progenitor cells and stem cells located at the bottom of the crypt. These cells moves along the base of the crypt to top of the villus thereby differentiating colon epithelium into globlet, enterocyte, paneth and enteroendocrine cells. As it reaches the top apoptosis occurs, which takes place by 14 days. These process are organized by

several proteins like Wnt, Tp53, TGF-β. Any deviation in this pathway due to mutation or any other factor favours to development of cancerous cells.[36] The average rate of mutation per nucleotide is considered as 10^{-8} per cells approximately. In case of cancer the rate becomes high due to several mutator phenotype.[37]

Three pathways are associated with colon cancer formation i.e; CIN, MSI, CIMP.

Chromosomal Instability pathway (CIN):

CIN is the most common pathway in colon cancer which accounts for 80-85% of all cases. CIN is defines by high rate of accumulation of numerical or structural chromosomal abnormalities. This results in karyotypic variability from cell to cell, loss of heterozygosity (LOH), telomere stability and DNA damage response.

Fearon and Vogelstein in 1960 proposed a model called multistep genetic model of colon carcinoma which is widely accepted now and used as a paradigm. In this model they proposed that inactivation of APC gene occurs which leads to formation of abberant crypt foci. Then mutation in oncogene called KRAS occurs followed by mutation in Tp53, PIK3CA, PTEN, TGF-β or deletion of chromosome 18q and 17q.[38]

Single Nucleotide Polymorphism, stem cell research, array based comparative genomic hybridization techniques paved way for scientist to understand effectively about copy number variation in the human genome at high resolution. By this techniques they found during CRC the are some gain or loss of chromosomal segments. Frequently seen allelic loss are at chromosomal arm 1p, p, 8p, p, 18q, 20p, 22q and in some region of tumor suppressor gene like Apc on 5q, TP53 on 17p, DCC, SMAD2&4 on 8q. Some frequently seen gain of chromosomal material are at chromosome 7 and chromosome arm q, 8q, 12q, 13q & 20q. All those gains and loss favours for cell survival and also conversion of normal to cancerous cells.[39]

Microsatellite Instability pathway (MSI):

MSI is another form of genomic instability accounts for 15-20% of sporadic CRC and in >95% of HNPCC. It defines that there will be a high frequency of replication error due to the failure of DNA polymerase which leads to repetitive in DNA sequence. Thus the continuous insertion or deletion of nucleotides within microsatellite sequence (repetitive DNA sequence between 1 to 5 bp) results in longer or shorter allele as compared to normal cells.

In order to access MSI status of CRC tumor are classified as MSI low, MSI high, MSS. To identify this Bethesda guidelines suggested usage of five microsatellite markers consist of 2 mononucleotide (BAT26 BAT25) and 3 dinucleotide (D2S123 D5S346 D17S250). As per this guidelines patient showing ≥30% of instability with markers considered as MSI high, <30% instability as MSI low and no apparent instability as MSS. Later it is found that MSI low shows less or no prognostic value hence it comes under MSS.[40]

MSI generally associated with deficiency in DNA MMR system. DNA MMR system is one of the DNA repair system in which they detect the replication error in the new strand either by insertion or deletion of loop by proteins and repair it. The proteins responsible for detecting mismatch and directing repair are MutS (MSH2, MSH3, MSH6) and MutL (MLH, MLH2, MLH3, PSM1, PSM2). Negligence of these protein causes frameshift mutation thereby leads to nonfunctional proteins thus rises to cancer with MSI. Failure of proteins are due to either hpermethylation or mutation of genes. In many studies it is found that hypermethylation (sporadic MSI) accounts for >50% than mutation (Lynch syndrome or familial MSI) which accounts for < 15%[41]. Apart from MMR gene several other genes are found to be mutated in case of MSI namely: TGFBR2 a tumor suppressor gene, BAX a pro apoptotic gene.

CpG Island Methylator Phenotype pathway (CIMP):

CIMP is the another pathway accounts for approximately 20-30% of CRC which defines as hypermethylation at promoter region ieCpGdinucleotide (cytosine nucleotide followed by guanine nucleotide) of cancer related gene results in silencing of gene and failure in protein expression. This pathway was postulated by Toyota and colleagues in 1999. As like MSI, CIMP status can also accessed by methylation markers and categorized as $CIMP^+$ and $CIMP^-$. Generally CIMP in sporadic cases are seen as CIMP high tumors which is associated with BRAF and MLH1 methyaltion and CIMP low are associated with KRAS mutation. Especially sporadic MSI results from CIMP iehypermethylation of MLH1 leads to inactive protein.[42,43]

Molecular signaling pathway in colon cancer:

Apc (Wnt signaling pathway):

Apc (Adenomatous polyposis coli) a tumor suppressor gene located on chromosome 5q21 has a multiple function believed to be the key component of colon cancer as per multistep genetic model of colon cancer.[38] Normally Apc with multiple functional domain binds to axin

scaffold protein leads to activation of casein kinase 1 and glycogen synthase kinase-3β results in phosphorylation of β-catenin (CTNNB1). Thus the phosphorylation of CTNNB1 results in protosomeal degradation.[44] When mutation or allelic loss of Apc gene occurs, Wnt signaling pathway activates where the receptor Frizzled and low density lipoprotein receptor (LRP) gets phosphorylated by GSK-3β followed by binding of axin to disheveled prevent the phosphorylation of CTNNB1. Thus CTNNB1 gets accumulated which forms complex with TCF/LEF (T-cell factor/lymphoid enhancer family) which transcript gene like survivin responsible for anti apoptosis and C-Myc, Cyclin D_1 gene responsible for proliferation. By all these changes normal epithelium give rise to adenomatous crypt foci.[34, 45]

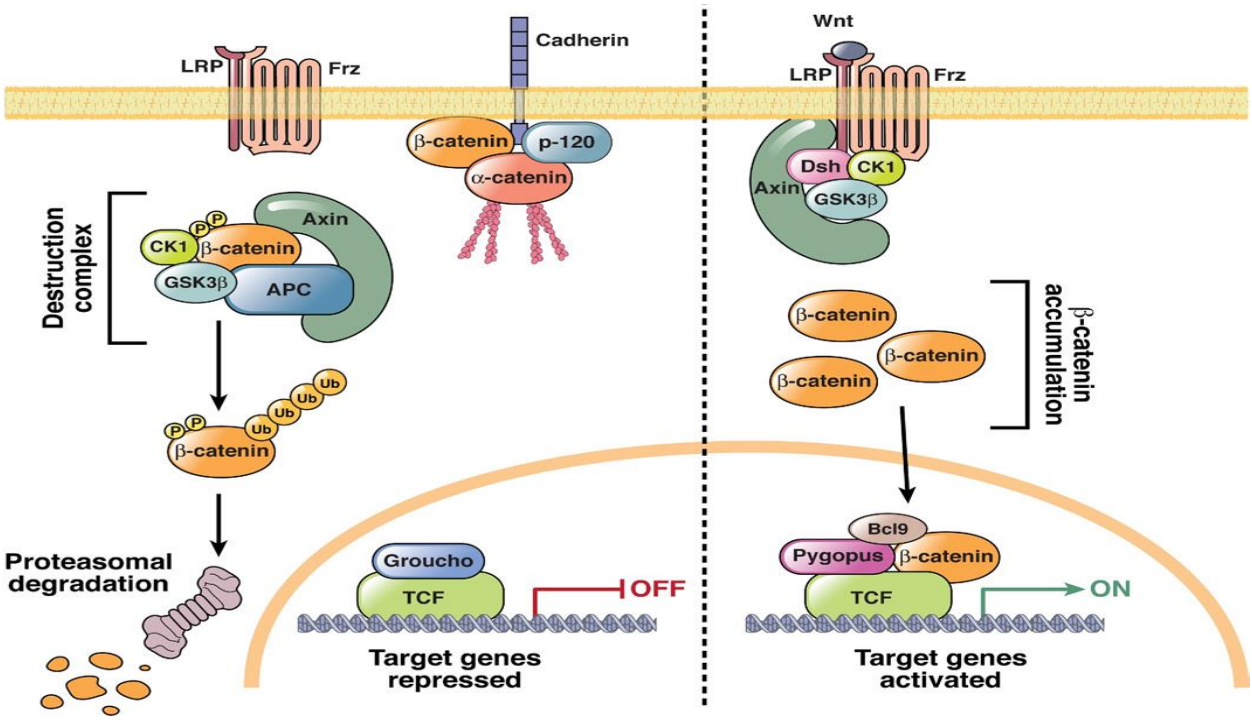


Fig no 3. Regulation of Wnt signalling

KRAS:

KRAS a oncogene belongs to the RAS family located on chromosome 12p12 is a membrane bound G-protein with intrinsic GTPase activity responsible for cell proliferation and survival. Other RAS genes are NRAS, HRAS. Normally KRAS activates Raf-MEK-ERK pathway. In this pathway Raf genes (A-Raf, B-Raf, C-Raf) phosphorylates MEK (MEK1 and 2) which leads to activation of ERK (ERK 1and 2). ERK activation leads to triggering of substrates like c-Jun, c-Fos, c-Myc which transcript gene Cyclin D1 responsible for proliferation. These

activities are controlled by GTPase which regulate the GDP/GTP activation and inactivation.[42] Any mutation in KRAS gene leads to impairment of GTPase thereby KRAS accumulates results in uncontrolled proliferation. Among RAS family mutation in KRAS found frequently than NRAS and HRAS in colon cancer.[46]

TP53:

TP53 a tumor suppressor gene located on chromosome 17q21 considered as transcription factor that regulates different genes hence called master of genome. It is also named as cell cycle checkpoint regulator. TP53 is regulated by oncogenes like c-Myc, RAS. Mutation in TP53 leads to conversion of adenoma to carcinoma. Hence TP53 is essential for diagnosis of progression of tumor.[39]

PI3K-AKT and PTEN:

PI3K is a heterodimeric lipid kinase has regulatory and catalytic subunit. It is activated by various receptor like EGFR, HER2, PDGFR. On activation it phosphorylates PIP_2 to PIP_3, PIP_3 in turn binds toAKT gene and gets activated by phosphorylating PDK1. Activated AKT phosphrylates pro apoptotic protein Bad, CASP9 and GSK 3β/β-catenin, cyclinD, c-Myc thereby leads to proliferation and survival of cell.[47] In case of colon cancer mutation in PIK3CA, a catalytic p110 α subunit of PI3K leads to upregulating the downstream of PIK3-AKT signaling pathway. Mutation in PIK3CA occurs at exon 9 and exon 20 region.[12] Apart from this PTEN, a tumor suppressor gene has a negative regulation over this pathway by dephosphorylating PIP3. Mutation in PTEN leads to loss of PTEN thereby increase the AKT activity by PIP_3 finally causes downstream of apoptosis and proliferation of cells.[48, 49]

TGFβ:

TGFβ is the important signaling pathway which controls growth and proliferation of cells by transcripting some genes. TGFβ acts on its receptor TGFβII thereby activates smad proteins by phosphorylation. Phosphorylated smad proteins translocate into nucleus thereby transcript several genes. Mutation in TGFβ leads to the development of cancer.[34]

LOH 18q:

Tumor suppressor genes like DCC, Smad2 and Smad4 are located on chromosome 18q. DCC encodes a receptor for neuronal protein netrin 1 which induces apoptosis. Smad2 and

Smad4 are the intercellular mediators of TGFβ responsible for regulation of growth and proliferation. Allelic loss of 18q leads to dysfunction of these genes which favours for the development of cancer cells.[38,39]

2.3.4 SIGNS AND SYMPTOMS

Signs and symptoms associated with colon cancer may vary with different people. The commonly seen signs and symptoms are:

- ✓ Blood in the stools and/or bleeding from the rectum
- ✓ A change in bowel habits i.e, diarrhea, constipation, or feeling that the bowel does not empty completely
- ✓ A mass or lumps in the abdomen
- ✓ Weight loss
- ✓ Weakness and tiredness
- ✓ Discomfort in the abdomen due to bloating, and cramps
- ✓ Bright red stools that look narrower or thinner than normal
- ✓ Excessive bleeding leads to anaemia.

It is also possible that these changes may be caused by a medical condition that is not cancer, especially for the general symptoms of abdominal discomfort, bloating, and irregular bowel movements.[50]

2.3.5 STAGING OF COLON CANCER:[51]

AJCC: American Joint Committee on Cancer; TNM: tumor/nodes/metastases

One tool that doctors use to describe the stage is the TNM system. Doctors use the results from diagnostic tests and scans to answer these questions:

- **Tumor (T):** Has the tumor grown into the wall of the colon or rectum? How many layers?
- **Node (N):** Has the tumor spread to the lymph nodes? If so, where and how many?
- **Metastasis (M):** Has the cancer spread to other parts of the body? If so, where and how much?

The results are combined to determine the stage of cancer for each person.

There are 5 stages: stage 0 (zero) and stages I through IV (1 through 4). The stage provides a common way of describing the cancer, so doctors can work together to plan the best treatments.

Here are more details on each part of the TNM system for colorectal cancer:

Tumor (T)

Using the TNM system, the "T" plus a letter or number (0 to 4) is used to describe how deeply the primary tumor has grown into the bowel lining. Stage may also be divided into smaller groups that help describe the tumor in even more detail. Specific tumor information is listed below.

TX: The primary tumor cannot be evaluated.

T0 (T plus zero): There is no evidence of cancer in the colon or rectum.

Tis: Refers to carcinoma in situ (also called cancer in situ). Cancer cells are found only in the epithelium or lamina propria, which are the top layers lining the inside of the colon or rectum.

T1: The tumor has grown into the submucosa, which is the layer of tissue underneath the mucosa or lining of the colon.

T2: The tumor has grown into the muscularispropria, a deeper, thick layer of muscle that contracts to force along the contents of the intestines.

T3: The tumor has grown through the muscularispropria and into the subserosa, which is a thin layer of connective tissue beneath the outer layer of some parts of the large intestine, or it has grown into tissues surrounding the colon or rectum.

T4a: The tumor has grown into the surface of the visceral peritoneum, which means it has grown through all layers of the colon.

T4b: The tumor has grown into or has attached to other organs or structures.

Node (N)

The "N" in the TNM system stands for lymph nodes. The lymph nodes are tiny, bean-shaped organs located throughout the body. Lymph nodes help the body fight infections as part of the immune system. Lymph nodes near the colon and rectum are called regional lymph nodes. All others are distant lymph nodes that are found in other parts of the body.

NX: The regional lymph nodes cannot be evaluated.

N0 (N plus zero): There is no spread to regional lymph nodes.

N1a: There are tumor cells found in 1 regional lymph node.

N1b: There are tumor cells found in 2 or 3 regional lymph nodes.

N1c: There are nodules made up of tumor cells found in the structures near the colon that do not appear to be lymph nodes.

N2a: There are tumor cells found in 4 to 6 regional lymph nodes.

N2b: There are tumor cells found in 7 or more regional lymph nodes.

Metastasis (M)

The "M" in the TNM system describes cancer that has spread to other parts of the body, such as the liver or lungs. This is called distant metastasis.

M0 (M plus zero): The disease has not spread to a distant part of the body.

M1a: The cancer has spread to 1 other part of the body beyond the colon or rectum.

M1b: The cancer has spread to more than 1 part of the body other than the colon or rectum.

M1c: The cancer has spread to the peritoneal surface.

Table no. 2 Staging of colon cancer by AJCC

Stages	T	N	M	Description
0	Tis	No	Mo	The cancer is in its earliest stage. This stage is also known as carcinoma in situ or intramucosal carcinoma (Tis). It has not grown beyond the inner layer (mucosa) of the colon or rectum
I	T1-T2	No	Mo	The cancer has grown through the muscularis mucosa into the submucosa (T1), and it may also have grown into the muscularispropria (T2). It has not spread to nearby lymph nodes (N0) or to distant sites (M0).

IIA	T3	No	Mo	The cancer has grown into the outermost layers of the colon or rectum but has not gone through them (T3). It has not reached nearby organs. It has not spread to nearby lymph nodes (N0) or to distant sites (M0).
IIB	T4a	N0	Mo	The cancer has grown through the wall of the colon or rectum but has not grown into other nearby tissues or organs (T4a). It has not yet spread to nearby lymph nodes (N0) or to distant sites (M0).
IIC	T4b	No	Mo	The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has not yet spread to nearby lymph nodes (N0) or to distant sites (M0).
IIIA	T1-T2	N1/N1c	Mo	The cancer has grown through the mucosa into the submucosa (T1), and it may also have grown into the muscularispropria (T2). It has spread to 1 to 3 nearby lymph nodes (N1) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites (M0).
	T1	N2a	Mo	The cancer has grown through the mucosa into the submucosa (T1). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0).
IIIB	T3-T4a	N1/N1c	Mo	The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 1 to 3 nearby lymph nodes (N1a or N1b) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites (M0).
	T2-T3	N2a	Mo	The cancer has grown into the muscularispropria (T2) or into the outermost layers of the colon or rectum (T3). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0).

	T1-T2	N2b	Mo	The cancer has grown through the mucosa into the submucosa (T1), and it may also have grown into the muscularispropria (T2). It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites (M0).
IIIC	T4a	N2a	Mo	The cancer has grown through the wall of the colon or rectum (including the visceral peritoneum) but has not reached nearby organs (T4a). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0).
	T3-T4	N2b	Mo	The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites (M0).
	T4b	N2	Mo	The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has spread to at least one nearby lymph node or into areas of fat near the lymph nodes (N1 or N2). It has not spread to distant sites (M0).
IVA	Any T	Any N	M1a	The cancer may or may not have grown through the wall of the colon or rectum (Any T). It might or might not have spread to nearby lymph nodes. (Any N). It has spread to 1 distant organ (such as the liver or lung) or distant set of lymph nodes, but not to distant parts of the peritoneum (the lining of the abdominal cavity) (M1a).
IVB	Any T	Any N	M1b	The cancer might or might not have grown through the wall of the colon or rectum (Any T). It might or might not have spread to nearby lymph nodes (Any N). It has spread to more than 1 distant organ (such as the liver or lung) or distant set of lymph nodes, but not to distant parts of the peritoneum (the lining of the abdominal cavity) (M1b).

IVC	Any T	Any N	M1c	The cancer might or might not have grown through the wall of the colon or rectum (Any T). It might or might not have spread to nearby lymph nodes (Any N). It has spread to distant parts of the peritoneum (the lining of the abdominal cavity), and may or may not have spread to distant organs or lymph nodes (M1c).

Dukes System (Astler-Coller modification)

Table no. 3 Staging of colon cancer by Dukes system

Stage A	Tumors invade through the muscularis mucosae into the submucosa but do not reach the muscularispropria.
Stage B1	Tumors invade into the muscularispropria.
Stage B2	Tumors completely penetrate the smooth muscle layer into the serosa.
Stage C	Tumors encompass any degree of invasion but are defined by regional lymph node involvement.
Stage C1	Tumors invade the muscularispropria with less than 4 positive nodes.
Stage C2	Tumors completely penetrate the smooth muscle layer into the serosa with 4 or more involved nodes.
Stage D	Lesions with distant metastases.

2.3.6 DIAGNOSITICS:

There are many diagnostic tests to identify the disease status of the patients. In case of colon cancer, diagnostic test information are helpful for physicians to decide the treatment planning. First of all health history ie previous health events and medications are examined. Apart from this family medical history are noted to find out any inherited cancer syndromes. There are two inherited cancer syndrome in colon cancer i.e, Lynch syndrome and FAP(Familial Adenomatous Polyps).

Physical examination like temperature, blood pressure, pulse and respiration rate and body weight will be checked. Physician also look at and feels parts of the body to diagnose any abnormalities.

Based on the above examination, some diagnostic tests are carried out. They are listed below:

Endoscopy

Endoscopy is a procedure in which equipment with a inbuilt light is used to find abnormal changes in the body by inserting it into the body. There are few types of endoscopic procedures to detect colon cancers.

Colonoscopy: A device called colonoscope is inserted into body through anus and viewed the entire part of large intestine.

Sigmoidoscopy: A small tube called sigmoidoscope is used to view the lower colon.

Double contrast barium enema-X-rays: Barium line the colon allowing to be viewed by X-ray.

Ultrasound: An ultrasound uses sound waves to create a picture of internal organs and find out the spread of cancers.

Biopsy

In this a small piece of tissue is removed during colonoscopy or sigmoidoscopy and analysis the pathology of tissue through microscopy. Biopsy makes a definite diagnosis of colon cancer. Needle biopsy is also carried out by CT or ultrasound guiding.

Blood test

The blood is collected and analysed for various parameters.

Complete blood count: In this number of cells in blood (RBC, WBC, platelets) are calculated. Colon cancer causes bleeding thus reduces the counts of blood cells mainly RBC.

CEA blood test: When colon cancer occurs certain chemical levels are increased. One among chemical is carcinoembryogenic antigen (CEA), increased amount of this chemical indicates the spread of cancer to other parts.

Imaging tests

Computed tomography: CT takes pictures of the intestine using x-rays and help to locate the tumor and its size and also during the treatment to assess the effectiveness.

Magnetic resonance imaging: MRI uses a magnetic field and radiowaves to make pictures. It helps in distinguishing normal and diseased tissues and also to pinpoint the cancerous cells. It gives more clarity and it is safer when compared to CT scan and x-rays as it does not use radiation.

PET-CT scan: It is a most advanced nuclear imaging technology which provides a more detailed picture of cancerous tissue than any other test. The images of PET-CT provides a high level of accuracy.[52, 53]

Prognostic and predictive tumor markers:

Predictive molecular markers are desirable for selecting an optimal, personalised treatment strategy for the patient. The role of genetic and epigenetic alternations is important, especially for identification and application of prognostic - and predictive markers in routine clinical use, but also to overcoming the toxicity and medical cost it entails.

Carcinoembryonic antigen

In 1965, Gold and Freedman, identified carcinoembryonic antigen (CEA) in malignant tumors of the endodermal derived epithelium from the gastrointestinal tract and pancreas, which appeared to be absent from healthy colon. CEA belongs to the immunoglobulin superfamily and is attached to the cell membrane by glycosyl phosphatidylinositol anchor and released in soluble form by phospholipase C or D. CEA is today the most important and commonly used serum tumor marker in clinical practice and is recommended for determining prognosis, surveillance followed after curative resection, and as a monitoring therapy in advanced cancer. However, because of the low sensitivity (18-69%), early detection of CEA in CRC is not recommended.

KRAS

KRAS mutations are early events in colon cancer and occur in 30-50% of all cases. KRAS mutations are more commonly seen in MSI-L and MSS tumours. The prognostic

relevance of KRAS mutations is controversial. The majority of the studies associate KRAS mutations with poor prognosis. Some associates its prognostic implications to stage of disease, specific mutation site or in combination with mutations in p53. Recently the focus on KRAS mutation status has shifted towards being a predictive marker in metastatic colon cancer with anti-EGFR target therapy. As KRAS gene mutation is associated with a negative response to anti-EGFR target therapy, patients with metastatic colon cancer and KRAS mutation in codon 12 or codon 13 are not recommended anti-EGFR therapy. Testing for KRAS mutation in patients with metastatic colon cancer before initiation of anti-EGFR therapy has been implicated in clinical practice.

BRAF

BRAF mutations are found in 4-15% of all sporadic colon cancer cases, of which 34-70% are seen in MSI-H tumors. More than 90% of all BRAF mutations involve V600E, by a substitution of valine-to-glutamic acid amino acid, resulting in abnormal activation of MEK-ERK pathway. Mutations in BRAF have been associated with poor clinical outcome. Patients with MSS tumors and BRAF mutations have shorter progression-free survival and overall survival. Patients with BRAF V600E mutations are shown to have decreased benefit of EGFR-targeted monoclonal antibody therapies, however, the predictive roll of BRAF can be of useful information in the selection of patients with metastatic colon cancer current for EGFR inhibitor therapy.

MMR deficiency

The prognostic significant of MSI tumours is their association with favourable outcome. There are two kinds of laboratory tests for this tumor marker. Depending on which method is used, if genetic defect is present the result will either be MSI-H or dMMR. Testing for this tumor marker is used to determine Lynch syndrome and to determine whether immunotherapy treatment has to be carry.[54, 55]

2.3.7 TREATMENT:

Surgery

Some colon cancer grow beyond the polyp and into the colon wall. Surgery is the most common treatment in which the tumors of the colon as well as the surrounding normal tissues as precaution are removed.

Colectomy: A surgery that removes the part of the colon with cancer. After the cancerous part is removed, the two ends of the remaining colon are often joined back together either by sewen or stapling together. It is done by creating a large cut in the abdomen and removes the cancerous part.

Laproscopic surgery: In this a small incision is madein the abdomen and tools with viewing scopes are passed to see and remove the cancerous part. It is as effective as colectomy and recovery time is often shorter.

Radiation therapy

Radiation therapy uses high energy and high focused rays to treat cancer. The rays damage DNA, thus it either kills the cancer cells or stops growth of new cancer cells.

External beam radiation: This method is used to treat colon cancer by using a machine which delivers radiation to where the cancer is located. This treatment is usually given 5 days a week for several weeks.

Stereoactive radiation therapy: This technique used if colon cancer has spread to lungs or liver. In this very precise, high dose photon beam are used.

Intraoperative radiation therapy: This uses a single high dose of radiation therapy given during surgery.

Chemotherapy

Chemotherapy is the use of drugs to kill cancerous cells by stopping the ability to grow and divide. A chemotherapy regimen or schedule, usually consists of a specific number of cycles given over a set of period of time. It is normally given after surgery to eliminate any remaining cancerous cells. Commonly used regimens for colon cancer are 5-flurouracil, capecitabine. Combination regimen are given, they are:

5-flurouracil, leucoverin

Capecitabine, oxaliplatin

5-flurouracil, leucoverin, irinotecan

5-flurouracil, leucoverin, oxaliplatin

5-flurouracil, leucoverin, oxaliplatin, irinotecan

Trifluridine, tipiracil

Targeted therapy

It is a type of cancer treatment in which drug or substance that can target and attack specific cancerous cells without affecting normal cells. It works either by stopping the growthof new blood vessels thereby cancer cells die or stops the cancer cells from receiving signals to grow. Monoclonal antibodies are generally used as a targeted therapy for colon cancers. They are usually made from a single type of immune cells. Some of the targeted therapy drugs for colon cancer are

Anti-angiogenesis therapy: bevacizumab, ramucirumab, ziv-zflibercept, regorafenib, vemurafenib

Epideramal growth factor inhibitors: cetuximab, panitumumab

Immunotherapy

Immunotherapy, also called biologic therapy is designed to boost the body's natural defenses to fight the cancer. It is made either by the body or in a laboratory to improve, target, or restore immune system function. Checkpoint inhibitors are an important type of immunotherapy used to treat colon cancer.

Pembrolizumab

Nivolumab

Nivolumab and ipilimumabcombination[52, 56]

Zhao Zuo-Quan*et al.*, (2019) prepared three new RGD peptide bioconjugates NOTA-Galacto-RGD2, NOTA-I2P-RGD2 and NOTA-4P-RGD3. ^{68}Ga radiotracer has been labelled to all three bioconjugates and purified by HPLC method. The IC50 values were calculated to be 24 ± 4, 27 ± 2, 29 ± 4 nM for NOTA-Galacto-RGD2, NOTA-I2P-RGD2, NOTA-4P-RGD3 respectively. NOTA-Galacto-RGD2 and NOTA-I2P-RGD2 almost identical αvβ3 binding affinity. It is found that the linker group between two c (RGDfK) moieties has little impact on the αvβ3 binding affinity of dimeric cyclic RGD peptides. Despite of their different peptide multiplicity, dimeric and trimeric cyclic RGD peptides shares similar identical αvβ3 binding affinity. The biodistribution properties of 68Ga radiotracers depend on cyclic RGD peptides and radiometal chelates. The radiotracer tumor uptake is RGD and αvβ3–specific. Among the ^{68}Ga radiotracers evaluated in this study, ^{68}Ga-I2P-RGD2 shows the best tumor uptake with good tumor-to-background ratios, and is a good PET radiotracer for imaging gliomas.[57]

Gutierrez-Cardo*et al.*, (2018) has assessed the ^{68}Ga-PSMA-11 PET positivity predictive factors in prostate cancer study. They done retrospective analysis included 53 studies, performed on 50 male prostate cancer patients referred due to biochemical recurrence. In all cases, previous imaging techniques were negative or inconclusive. This study confirms that ^{68}Ga-PSMA-11 PET shows a high disease detection rate in patients and modify the therapeutic approach in 54% of the patients undergoing this procedure where other techniques showed negative or doubtful images. Almost 50% of patients with prostate cancer biochemical recurrence and low PSA levels (<1 ng/ml) have active disease on ^{68}Ga-PSMA-11 PET, precisely where other radiotracers lack sensitivity.[58]

Zohar Keidar*et al.*, (2018) assessed the distribution of ^{68}Ga PSMA in prostate cancer patients and also diagnosed the metastatic patterns, incidence of benign and frequency of involved sites of prostate cancer. They had done retrospective analysis in a large group of 445 patients from two tertiary medical centres who were referred for biochemical recurrence and staging of high grade disease. ^{68}Ga PSMA avid lesions were commonly detected in the prostate, loco regional spreade, abdomino-pelvic nodes and distant metastases including bone metastases, distant lymphadenopathy and other organs. Metastases were commonly seen in patients with biochemical reccurence than referred for staging. ^{68}Ga PSMA avid lesions data are compared

with previous autopsy result and found similarity. Hence ^{68}Ga PSMA PET/CT should be included in the guidelines to evaluate disease extent in prostate cancer patients.[59]

HilalGungor*et al.*, (2018) studied the effects of nonsteroidal anti-inflammatory drugs (NSAIDs)-induced chemopreventive effects on tumor development incidence and angiogenesis in experimental CRC rats. 1,2-Dimethylhydrazine dihydrochloride (DMH) was used as cancer-inducing agent and two NSAIDs (celecoxib and diclofenac) were given orally as chemopreventive agents. It was concluded that NSAIDs, particularly cyclooxygenase-2 (COX-2) inhibitors, might have a protective effect on CRC development and slow down progression of tumor in a DMH-induced experimental cancer model. One of the possible mechanisms in the chemoprevention of colon cancer seems to be inhibition of angiogenesis by diclofenac and celecoxib. This study also support that MMP-2, MMP-9, MMP-2/TIMP-2 ratio can be used as an indicator in early diagnosis of colorectal cancer.[60]

Fitzpatrick *et al.*, (2017) diagnosed prostate cancer lesions in patients with recurrent disease by ^{68}Ga PSMA PET/CT imaging technique to review the potential of this method to improve the ability of detection with high specificity. Detection of staging and metastatic progression is an important consideration in case of tumor for future treatment and follow-up. Hence there is a need of prominent and accurate monitoring tool. Now a days^{68}Ga PSMA PET/CT imaging technique shows a prominent role in detection of prostate cancer lesions on the basis of amount of PSMA expression. For this review, retrospective analysis were carried out for last 5 years. Prostate cancer patients previously treated with radical prostatectomy (RP) and radiotherapy(RT) are considered. Totally 24 studies accounting of 2,408 patients were analysed by ^{68}Ga PSMA PET/CT imaging technique. It was found that this technique shows high sensitivity (33-93%) and specificity (99%). As detection specificity increase with increase in PSMA level, even at low levels it shows good result as compared to other tracers. Despite of some problems like affected by tracer trapping, level of PSMA etc. it act as a promising tool in the detection of cancer lesions in patients with recurrence.[61]

Sangeeta Ray Banerjee *et al.*, (2016) compared the pharmacokinetics of three PSMA-targeted radiotracers: ^{68}Ga-1, using DOTA-monoamide as the chelating agent; ^{68}Ga-2, containing the macrocyclic chelating agent p-SCN-Bn-NOTA; and ^{68}Ga-DKFZ-PSMA-11, currently in clinical trials, which uses the acyclic chelating agent, HBED-CC. The macrocyclic NOTA chelated agent

^{68}Ga-2 demonstrated the fastest rate of clearance from all tissues in this series and displayed higher uptake in PSMA+ PC3 PIP tumor compared to ^{68}Ga-1 at 1 h post-injection. There was no significant difference in PSMA+ PC3 PIP tumor uptake for the three agents at 2 and 3 h post-injection. ^{68}Ga-DKFZ-PSMA-11 demonstrated the highest uptake and retention in normal tissues, including kidney, blood, spleen, and salivary glands and PSMA-negative PC3 flu tumors up to 3 h post-injection. ^{68}Ga-2 is a clinically viable imaging agent for detecting PSMA+ lesions.[62]

Ahmed Mohamed Hussein *et al.*, (2016) assessed the diagnostic performance of ^{18}F-FDG PET/Ct scan in the detection of recurrent disease in CRC patients with suspected local and/or distant recurrent disease and compared to contrast enhanced CT scan (CECT). Pathological results, clinical or imaging follow-up, or the responsiveness of the lesion to the treatment with chemotherapy or radiation therapy were also reviewed. The specificity of ^{18}F-FDG PET/CT was statistically significantly better than that of CECT as it can reduce the false positive results of CECT in 13 patients, however regarding the sensitivity. Thus ^{18}F-FDG PET/CT showed better value but without statistical significance. ^{18}F-FDG PET/CT is a better method to evaluate postoperative CRC patients with suspected tumor recurrence or distant metastasis than CT with significantly higher specificity.[63]

Ankur Pruthi*et al.*, (2016) carried out a retrospective analysis to evaluate the efficacy of ^{68}Ga DOTANOC PET/CT imaging modality in detecting primary site in patients with metastatic neuroendocrine tumors of unknown origin and its impact on clinical decision making in such patients. For this study 263 patients underwent Ga-68 DOTANOC PET/CT imaging, out of this 68 patients has metatstatic neuroendocrine tumors with unknown primary site which is proved by histopathological analysis. Ga-68 DOTANOC PET/CT imaging identified primary site for 40 patients out of 68 patients. Identified primary sites were small intestine, rectum, pancreas, stomach, lung, and evenin kidney and prostate. Quantitative estimation of SSTR expression were analysedin the form of maximal standardized uptake value (SUVmax) of detected primary and metastases sites. From the result it is found that there is a significant positive correlation between the SUVmax of detected primary sites and histopathologically proven metastatic site. Based on this result, 3 patients underwent treatment for the detected primary tumor site; 36 patients were started on long-acting somatostatin analogues or chemotherapy or targeted therapy. Other 2

patients went for radionuclide therapy. From overall findings it is proven that Ga-68 DOTANOC PET/CT imaging is a promising modality for detecting primary site in metastatic neuroendocrine tumor of unknown origin. It also helpful to provide useful information for guiding clinical manangement for such patients.[64]

TamilaStott Reynolds *et al.*, (2015) Studied about the dual-targeting, peptide-based radiopharmaceuticals for imaging and treating prostate cancer by virtue of their ability to target the αVβ3 integrin or the gastrin releasing peptide receptor (GRPr). For this study RGD-Glu-6Ahx-RM2 was conjugated to a DOTA bifunctionalchelator (BFCA) purified via reversed-phase high-performance liquid chromatography (RP-HPLC), characterized by electrospray ionization-mass spectrometry (ESI-MS), and radiolabeled with ^{111}In or ^{177}Lu. Binding affinity was assessed for the αVβ3 integrin or GRPr in human glioblastoma U87-MG and prostate PC-3 cell lines and found to have high to moderate binding affinity for the GRPr or the αVβ3 integrin. Biodistribution studies indicated high tumor uptake in PC-3 tumor-bearing mice and prolonged retention of tracer. . Micro-single photon emission computed tomography confirmed favorable radiouptake profiles in xenografted mice at 20 h post-injection. Thus RGD-Glu-[111In-DO3A]-6-Ahx-RM2] and [RGD-Glu-[177Lu- DO3A]-6-Ahx-RM2] show favorable pharmacokinetic and radiouptake profiles.[65]

Matthias Eder *et al.*, (2014) analysed a new PET tracer [^{68}Ga]Ga-PSMA-HBED-CC for imaging of prostate cancer. In this study the radiometalchelator HBED-CC used in this molecule represents a rather rarely used acyclic complexing agent with chemical characteristics favourably influencing the biological functionality of the PSMA inhibitor. The simple replacement of HBED-CC by the prominent radiometalchelator DOTA was shown to dramatically reduce the *in vivo* imaging quality of the respective 68Ga-labelled PSMA-targeted tracer proving that HBED-CC contributes intrinsically to the PSMA binding of the Glu-urea-Lys(Ahx) pharmacophore.HBED-CC-conjugated PSMA inhibitor [^{68}Ga] Ga- PSMA-HBED-CC is suitable for being used in typical kit like radiochemical production processes for clinical use with high reproducibility and robustness.[66]

Damian Wild *et al.*, (2013) has compared of ^{68}Ga-DOTANOC and ^{68}Ga-DOTATATE PET/CT within Patients with gastroenteropancreatic neuroendocrine Tumors. ^{68}Ga-DOTANOC has high affinity for somatostatin receptor subtypes 2, 3, and 5 (sst2,3,5). It has a wider receptor binding

profile than ^{68}Ga-DOTATATE, which is sst2-selective. randomized crossover design study was conducted in eighteen patients with biopsy-proven GEP-NETs were evaluated with ^{68}Ga-DOTANOC and ^{68}Ga-DOTATATE. Histology study revealed low-grade GEP-NETs (G1) in 4 patients, intermediate grade (G2) in 7, and high grade (G3) in 7. The lesion-based sensitivity of ^{68}Ga-DOTANOC PET was 93.5%, compared with 85.5% for ^{68}Ga-DOTATATE PET. Thus sst2,3,5-specific radiotracer ^{68}Ga-DOTANOC detected significantly more lesions than did the sst2-specific radiotracer ^{68}Ga-DOTATATE in our patients with GEP-NETs.[67]

Peter Knetsch*et al.*, (2013) synthesized two RGD peptides containing alternative chelating systems, namely [^{68}Ga]NS3-RGD and [^{68}Ga]Oxo-DO3A-RGD and evaluated their properties by both invitro and invivo methods. Finally it is compared with alreadyexisting 68Ga-labeled RGD peptides[^{68}Ga]DOTA- and[^{68}Ga]NODAGA-RGD. In invitro test radiochemical purity was analysed and found good radiochemical purity for [^{68}Ga]NS3-RGD whereas [^{68}Ga]Oxo-DO3A-RGD showed two peaks with a ratio of 1:6. Both has good stability in PBS solution but failed in human serum. Protein binding was approximately 40% of the total activity for [^{68}Ga]NS3-RGD and70% for [^{68}Ga]Oxo-DO3A-RGD, respectively, resulting in high blood pool activities. Biodistribution assaysconfirmed these findings and showed an additional high uptake in liver and kidneys, especially for [^{68}Ga]NS3-RGD. [^{68}Ga]Oxo-DO3A-RGD and [^{68}Ga]NS3-RGD have inferior characteristics compared to alreadyexisting ^{68}Ga-labeled RGD peptides and thus, both are not suited to image αvβ3 integrin expression. Of all ourtested RGD peptides, [^{68}Ga]NODAGA-RGD still possesses the most favorable imaging properties.[68]

Rutao Yao (2012) reviewed the operational and technical aspects of small-animal PET and made some comparisons between small-animal PET and human PET systems, thus identify the challenges of, opportunities for, and ultimate limitations in applying small-animal PET. The application of small-animal PET has been expanded into many additional clinical indications. Its importance has been further enhanced by integration with other small-animal imaging modalities such as CT and MRI. The integration of PET and CT in both clinical and preclinical imaging settings has demonstrated the synergism of strengths achieved through the fusion of anatomic and functional imaging.[69]

Martina Perse and Anton Cerar (2011) has induced colorectal cancer in animals by 1,2 dimethyl hydrazine. The morphogenesis and genetic alterations of DMH induced colorectal

epithelial tumours in rats was analysed.Significant information on human CRC aetiology or factors influencing it has derived from studies using dimethylhydrazine (DMH) model and hence concluded it as a valuable tool for studying the molecular event of colon cancer and for developing and evaluating a variety of novel chemotherapeutic agent.[70]

Dinesh Shetty *et al.*, (2010) reviewed the clinical significances of ^{68}Ga as radionuclide for positron emission tomography and compared with [^{18}F]fluorodeoxyglucose (FDG). The use of a ^{68}Ge/^{68}Ga-generator that can consistently supply ^{68}Ga, which has a half-life of 271 days, provides a convenient way of producing ^{68}Ga for more than a year. Furthermore, the cost of the generator is comparable with those of other radionuclides used for PET. In addition, diagnostic approaches based on 68Ga-labeled agents have the additional advantage of facilitating treatment. For example, when a diagnostic scan is positive, these agents can be labeled with therapeutic radionuclides, such as, yttrium-90, lutetium-177, or rhenium-188. They concluded that, appearance of new ^{68}Ga-labeled radiopharmaceuticals with high impact are expected in the near future preclinical research and clinical studies, and may open the door to new possibilities for PET.[71]

Irene Virgolini*et al.*, (2010) fabricated the procedur guidelines for PET/CT tumor imaging with Ga-68 conjugated DOTA peptides. DOTA peptides used here are DOTANOC, DOTATOC, DOTATATE. Somatostatin receptor expression are found to be high in some cancers like gastroentero pancreatic tumor, pituitary adenoma, small cell lung carcinoma, thyroid carcinoma and also has low expression in mammary carcinoma, prostate cancer, sarcoma etc. All these peptides are analogues of octreotide which has different binding affinity for various subtypes of somatostatin receptors. DOTANOC shows good binding affinity for 3 and 5 SST receptor; DOTATOC binds to SST receptor 5; whereas DOTATATE has a affinity for receptor 2. Hence based on different studies they constructed guidelines for imaging thereby to assist nuclear medicine physician in recommending, performing, reporting, and interpreting the result of sst receptor PET/CT imaging.[72]

3. DRUG PROFILE

3.1DOTANOC acetate

- DOTANOC acetate results from the modification of the octapeptideOctreotide (D-Phe-c[Cys-Phe-D-Trp-Lys-Thr- Cys]-Thr-ol) by substitution of third Phenylalanine moiety with Naphthyl-L-Alanine.

- ^{68}Ga-DOTA NOC is a conjugate of the somatostatin analogue 1-Nal 3-octreotide (NOC) and ^{68}Ga-labeled 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA).

- The somatostatin analogue DOTANOC has a high affinity for somatostatin receptor subtypes 2, 3, and 5; these receptor subtypes have been shown to be present in large numbers on neuroendocrine tumors and their metastases, while most other normal tissues express low levels of these somatostatin receptor subtypes.

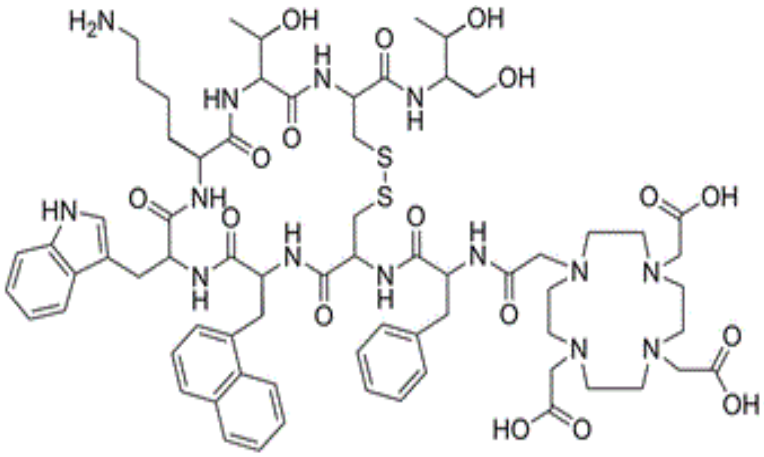
- ^{68}Ga -radiolabeled analogue of somatostatin that may be used in conjunction with positron emission tomography (PET) to image neuroendocrine tumors and metastases.[73]

Molecular formula : $C_{69}H_{94}N_{14}O_{17}S_2$

Molecular weight : 1455.7 g/mol

Chemical Name : N- [[4,7,10-Tris (carboxymethyl)–1,4,7,1tetraazacyclododec-1-yl] acetyl]-Nal=3-(1-naphthalenyl) - L- alanyl

Structure



Structure of DOTANOC

Physical properties:

Physical Description : Solid

Color : Colourless to off-white

3.2PSMA -11

- A radioconjugate composed of a human prostate specific membrane antigen (PSMA)-targeting ligand, Glu-urea-Lys(Ahx) (Glu-NH-CO-NH-Lys(Ahx)), used in diagnosing prostate cancer.

- Intravenous administration of ^{68}Ga-labeled PSMA-11, the Glu-urea-Lys(Ahx) moiety targets and binds to PSMA-expressing tumor cells. Upon internalization, PSMA expressing tumor cells can be detected during PET imaging.

- PSMA, a tumor-associated antigen and type II transmembrane protein, over expressed on prostate tumor cells.

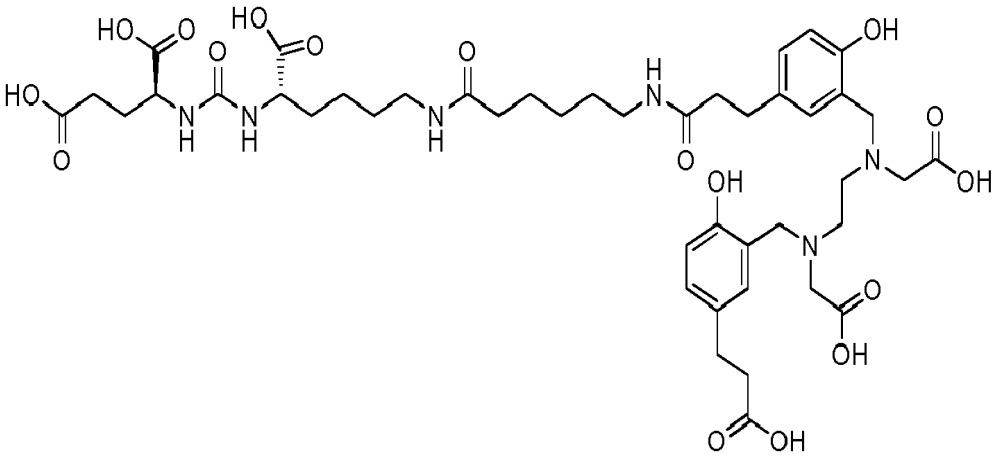
- It is also expressed in other tumors such as breast, lung, renal and colorectal cancers. Despite its presence in other tissues, PSMA-11 has been found to be an excellent agent for targeted imaging and therapy.[58]

Molecular formula : $C_{44}H_{62}N_6O_{17}$

Molecular weight : 947.0 g/mol

Chemical Name : 4,6,12,19- Tetraazadocosane-1,3,7-tricarboxylic acid,22-[3-[[[2-[[[5-(2-carboxyethyl)-2-hydroxyphenyl]methyl]methyl] (carboxymethyl)amino]ethyl](carboxymethylamino] methyl] -4-hydroxyphenyl] -5,13,20-trioxo-, (3S,7S)

Structure



Structure of PSMA-11

Physical properties:

Physical Description **:** Solid

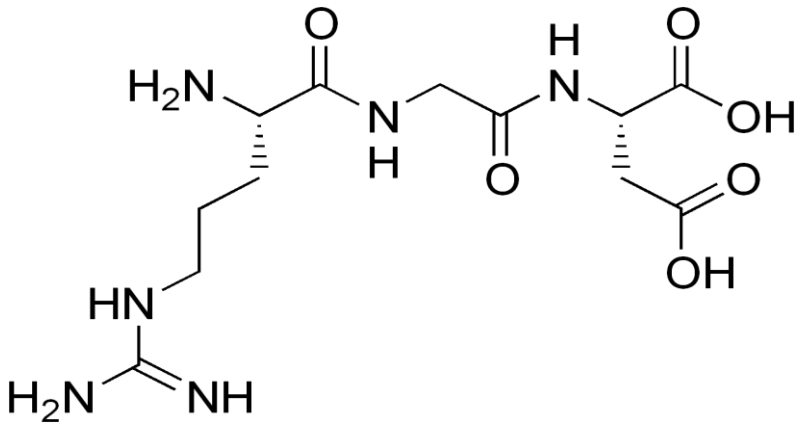
Color **:** Colourless to off-white

3.3 RGD

- Argentina-Glycine-Aspartic (RGD), is the specific recognition site of integrins with theirs ligands, and regulates cell-cell and cell-extracellular matrix interactions.
- The RGD motif can be combined with integrins overexpressed on the tumor neovasculature and tumor cells with a certain affinity, becoming the new target for imaging agents, and drugs, and gene delivery for tumor treatment.
- Further, RGD as a biomimetic peptide can also promote cell adherence to the matrix, prevent cell apoptosis and accelerate new tissue regeneration. Functionalizing material surfaces with RGD can improve cell/biomaterial interactions, which facilitates the generation of tissue-engineered constructs.[74]

Molecular Formula	**:**	$C_{12}H_{22}N_6O_6$
Molecular Weight	**:**	346.344 g/mol
Chemical Name	**:**	(2*S*)-2-[[2-[[(2*S*)-2-amino-5(diaminomethylideneamino) pentanoyl] amino] acetyl]amino]butanedioic acid

Structure:



Structure of RGD

Physical properties:

Physical Description **:** Solid

Color **:** Colourless to off-white

3.1 MATERIALS

Table No. 3 LIST OF DRUGS

SL NO.	NAME OF DRUGS	MANUFACTURER
1	1,2 dimethyl hydrazine	Sigma Chemical Co, Bangalore
2	DOTA - NOC	ABX Chemical, Germany
3	RGD	ABX Chemical, Germany
4.	PSMA	ABX Chemical, Germany
5	Ammonium acetate	Sigma Chemical Co, Bangalore
6	Sodium acetate	Sigma Chemical Co, Bangalore

Table No. 4 LIST OF INSTRUMENTS

SL.NO.	INSTRUMENTS	MANUFACTURER
1.	PET/CT	SIEMENS
2.	WELL COUNTER	CAPINTEC (CAPTUS 3000)
3.	^{68}Ge/ ^{68}Ga GENERATOR	ITG
4.	DOSE CALIBRATOR	CAPINTEC (CRC-25R)

4.2 METHODOLOGY

4.2.1 EXPERIMENTAL ANIMALS:

Male Sprague-dawley rats weighing 150-200 grams body weight were procured from the Biogen laboratory, Bangalore. All animals were kept at room temperature of 22±2ºC with relative humidity of 60-70% under 12 hr light and dark cycle respectively in the animal house. Rats were fed with modified diet and water *ad libitum* throughout the study. The animals are left for 15 days for acclimatization prior to the beginning of the experiment. Ethical clearance (registration number: 685/PO/Re/S/2002/CPCSEA) for the experimental set up was obtained from the Institutional Ethical Committee, KMCH college of pharmacy, Coimbatore. All animal procedures were performed accordance with the recommendations for the proper care and use of laboratory animals.

4.2.2 PREPARATION OF CHEMICALS:

1,2 dimethyl hydrazine (DMH) solution is freshly prepared before the induction. DMH is dissolved in 1 mMol/L EDTA-normal saline, the pH is adjusted to 6.5 with 1 Mol/L NaOH to ensure the pH suitability and the stability of the chemical.[60]

4.2.3 INDUCTION OF COLON CANCER:

DMH is being administered subcutaneously twice in a week at a dose of 20mg/kg body weight for 12 successive weeks.

4.2.4 EXPERIMENTAL DESIGN:

Rats were randomly divided into five groups, 6 animals each, placed in individual cages and classified as follow:

Group (1): Control Group:

Rats were treated with only 0.9%w/v normal saline daily throughout the experiment.

Group II :(1,2 DMH induced group):

Animals were treated with 1,2-Dimethylhydrazine 20mg/kg (body weight) dissolved in normal saline subcutaneously twice in a week for 12 successive weeks.

Group III :(1,2 DMH + ^{68}Ga DOTA NOC):

Animals were treated with 1,2-Dimethylhydrazine 20mg/kg (body weight) dissolved in normal saline subcutaneously twice in a week for 12 successive weeks followed by administration of ^{68}Ga DOTA-NOC acetate intravenously to the rats at the day of examination.

Group IV :(1,2 DMH + ^{68}Ga PSMA-11):

Animals were treated with 1,2-Dimethylhydrazine 20mg/kg (body weight) dissolved in normal saline subcutaneously twice in a week for 12 successive weeks followed by administration of ^{68}Ga PSMA-11 intravenously to the rats at the day of examination.

Group V :(1,2 DMH + ^{68}Ga RGD):

Animals were treated with 1,2-Dimethylhydrazine 20mg/kg (body weight) dissolved in normal saline subcutaneously twice in a week for 12 successive weeks followed by administration of ^{68}Ga RGD intravenously to the rats at the day of examination.

4.2.5 PROCEDURE FOR RADIOIMAGING:

4.2.5.1 PREPARATION OF ^{68}GALLIUM:

^{68}Ga was eluted from a commercially available ^{68}Ge/^{68}Ga generator with 4 ml of 0.05M HCl. The pH was adjusted to 4- 4.5 by adding sodium acetate to the preparation.[75]

4.2.5.2 RADIOLABELING OF PEPTIDES:

Allowed the peptide kit vials (DOTA-NOC, PSMA-11, RGD) to attain room temperature. 20mcg of DOTANOC acetate, 40 mcg of PSMA-11 and 50mcg of RGD was added separately to each gallium vials and gently mix it. Incubated the vials at 90^{O} C in a water bath for 10 mins and allowed to cool for few minutes and carry out the quality control test.[76]

4.2.5.3 QUALITY CONTROL TEST:

Instant Thin Layer Chromatography (ITLC):

Radiolabeling efficiency (%) of the radiolabeled conjugates was determined by ITLC using silica gel (SG) impregnated sheets. 1.0 M ammonium acetate (NH_4OAc) was prepared by dissolving 770.0 mg of NH_4OAc in 10.0 mL of distilled water. The ITLC sheets were cut into strips of dimensions 11.0 cm X 0.5 cm. The ITLC strips were marked 1.0 cm above the base as origin. 2.0 µL of the free ^{68}Ga and radiolabeled preparation was spotted at the origin, allowed to dry and placed in test tubes preloaded with mobile phase i.e.1.0 M ammonium acetate:methanol (1:1). The mobile solvent was allowed to reach the demarcation at the top of ITLC strip. The strip was taken out, dried and radioactivity counts over the entire strip were recorded using a radio chromatographic scanner. Further, the ITLC strips were also cut in two halves, the lower one-third and the upper two-third in order to calculate the radiolabeling efficiency. Both the segments were measured for radioactivity in a well counter. The counts from lower one-third segment of ITLC strip depicted (%) free ^{68}Ga whereas the (%) ^{68}Ga labeled DOTANOC acetate, PSMA-11, RGD was calculated from the upper two third segment of the ITLC strip.[77]

4.2.5.4 ADMINISTRATION OF RADIOLABELED PEPTIDES:

PET/CT scans were obtained with a PET/CT Scanner (Siemens Medical Solutions). Rats were each injected via the tail vein with 100µCi of the [^{68}Ga]DOTANOC acetate, PSMA-11, RGD respectively under ketamine and xylazine anesthesia. After the injection of radiolabeled compounds, rats were subjected to PET/CT imaging at an interval of 15, 30, 60, 90 minutes under anaesthetic condition.

For attenuation correction and anatomical reference, CT images were acquired following PET imaging. All animals were visually monitored throughout the imaging procedure.

4.2.6 COLLECTION OF BLOOD FOR PHARMACOKINETIC PARAMETERS

After the PET/CT imaging, the blood was collected from Retro-orbital sinus by using capillary tube into a centrifugation tube which contains EDTA for pharmacokinetic parameters. Plasma was separated by centrifugation at 10000 rpm for 10 min and utilized for pharmacokinetic parameters like plasma clearance were estimated.

4.2.6.1 PREPARATION OF WELL COUNTER SAMPLES

Weighed samples of 500 µL of plasma were prepared in duplicate. A volume of 50 µL 10% Triton-X (ICN Biomedicals Inc.) was added to the samples to destroy blood cells and obtain homogeneous solutions for measurement and avoid geometric effects caused by the formation of blood cell pellets.[78]

4.2.6.2 DETERMINATION OF PLASMA CLEARANCE:

Weighing method is used, transfer the syringe contents (standard) into a volumetric flask (500ml) the syringe must not be rinsed. Fill the volumetric flask with water to the 500-ml level and mix the solution thoroughly. Pipette twice 1.0ml of this new solution into counting vials. Substantial variations in counts between standard samples indicate an error, either in pipetting or in the homogeneity of the solution. New samples from the dilution of the standard should then be prepared. After selection of the adequate energy peak and window, both the blood samples and the standard are measured in a well counter. For quality control reasons,

each plasma sample and standard vial should be counted twice. A background activity should be measured in the beginning and at the end of the counting.[79]

4.2.7 HISTOLOGICAL ASSESSMENT[60]

Histopathology is the microscopical study of tissues for pathological alterations. This involves the collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of materials

After collecting the blood, the animals were sacrificed by cervical dislocation and the colon were excised immediately and washed in ice-cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. The blood, tissues and other body fluids were removed with the help of blotting paper and then washed in normal saline and transferred to ice-cold containers with 10% formalin solution. Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue.

Fixation

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,

b) Prevents autolysis,

c) Preserves the structure of the tissue, and

d) Prevents shrinkage

Common Fixatives: 10% Formalin

Haematoxylin and eosin method of staining:

Deparaffin the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX mounted and kept the slide dry and remove air bubbles.

5. RESULTS

5.1 Radiolabeling optimization

5.1.1Instant thin layer chromatography (ITLC)

Radiolabeling efficiency (%) of the final radiolabeled product [^{68}Ga] DOTANOC acetate, PSMA-11, RGD was estimated by using ITLC technique.The computer generated chromatogram depicting the movement of free ^{68}Ga and [^{68}Ga] DOTANOC acetate, PSMA-11, RGD along the ITLC strip as a function of radioactivity counts are presented in Figure (1),(2),(3) and (4)respectively.The results indicated that radiolabeling efficiency of DOTANOC acetate as 85.78%, PSMA-11 as 48.74%, RGD as 68.44% was achieved at pH 4.0.

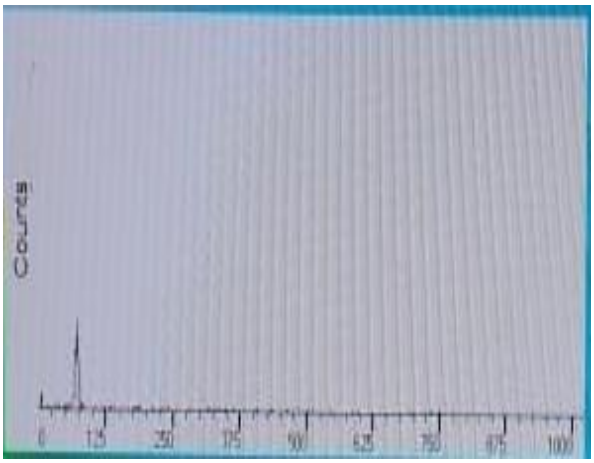


Fig no. 4 Free gallium (^{68}Ga)

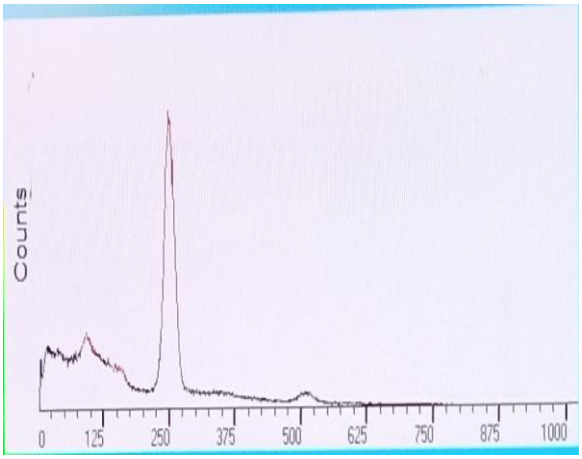


Fig no. 5^{68}Ga DOTANOC acetate

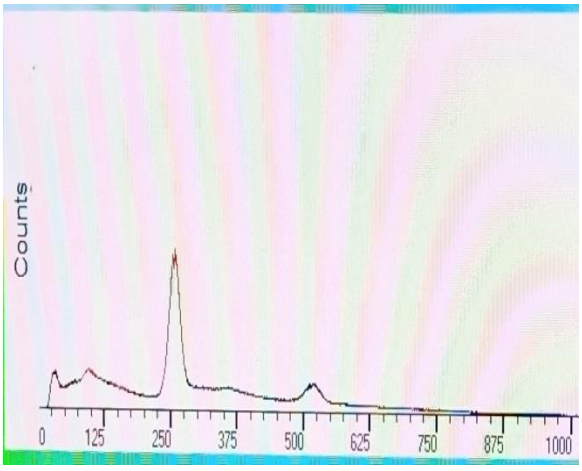


Fig no.6^{68}Ga PSMA-11

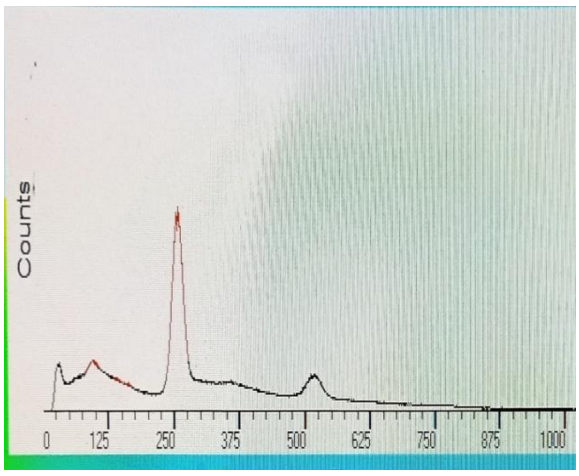


Fig no.7^{68}Ga RGD

5.2 PET/CT IMAGE:

GROUP I : Normal

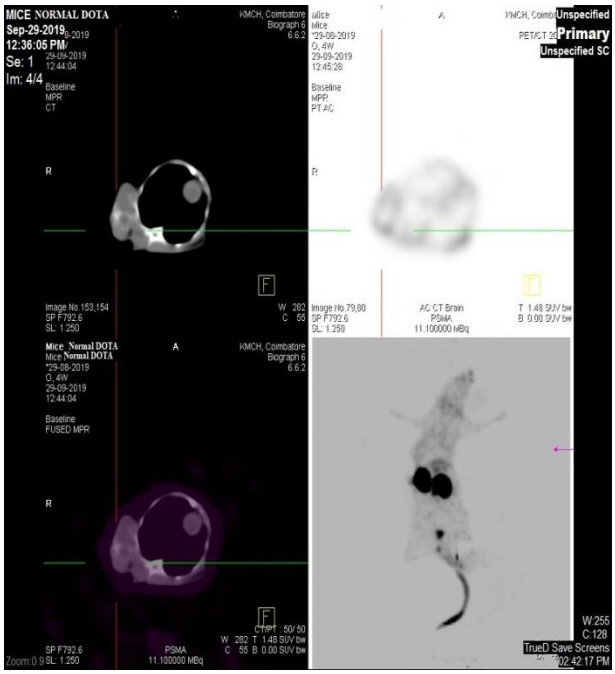


Fig no. 8 ^{68}Ga DOTANOC acetate

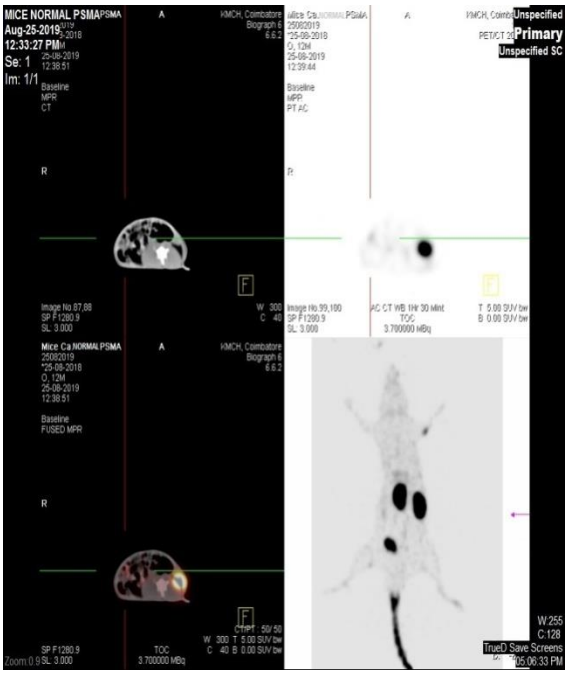


Fig no. 9 ^{68}Ga PSMA-11

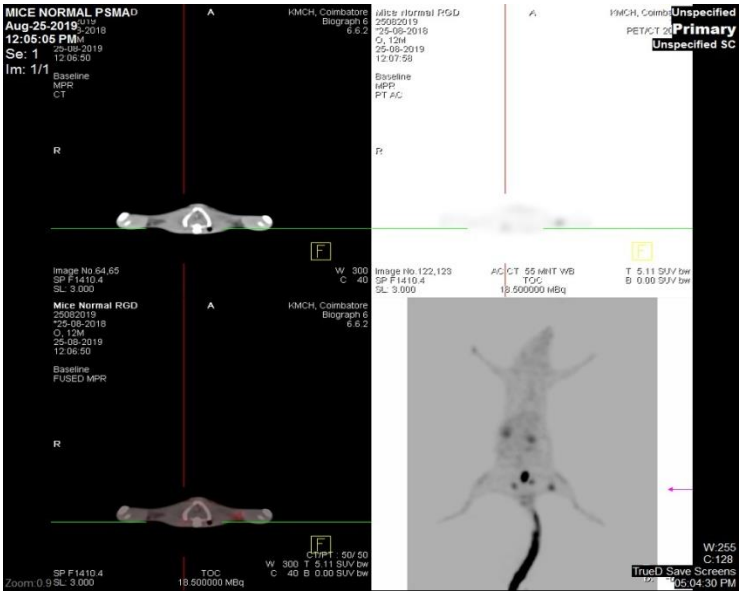
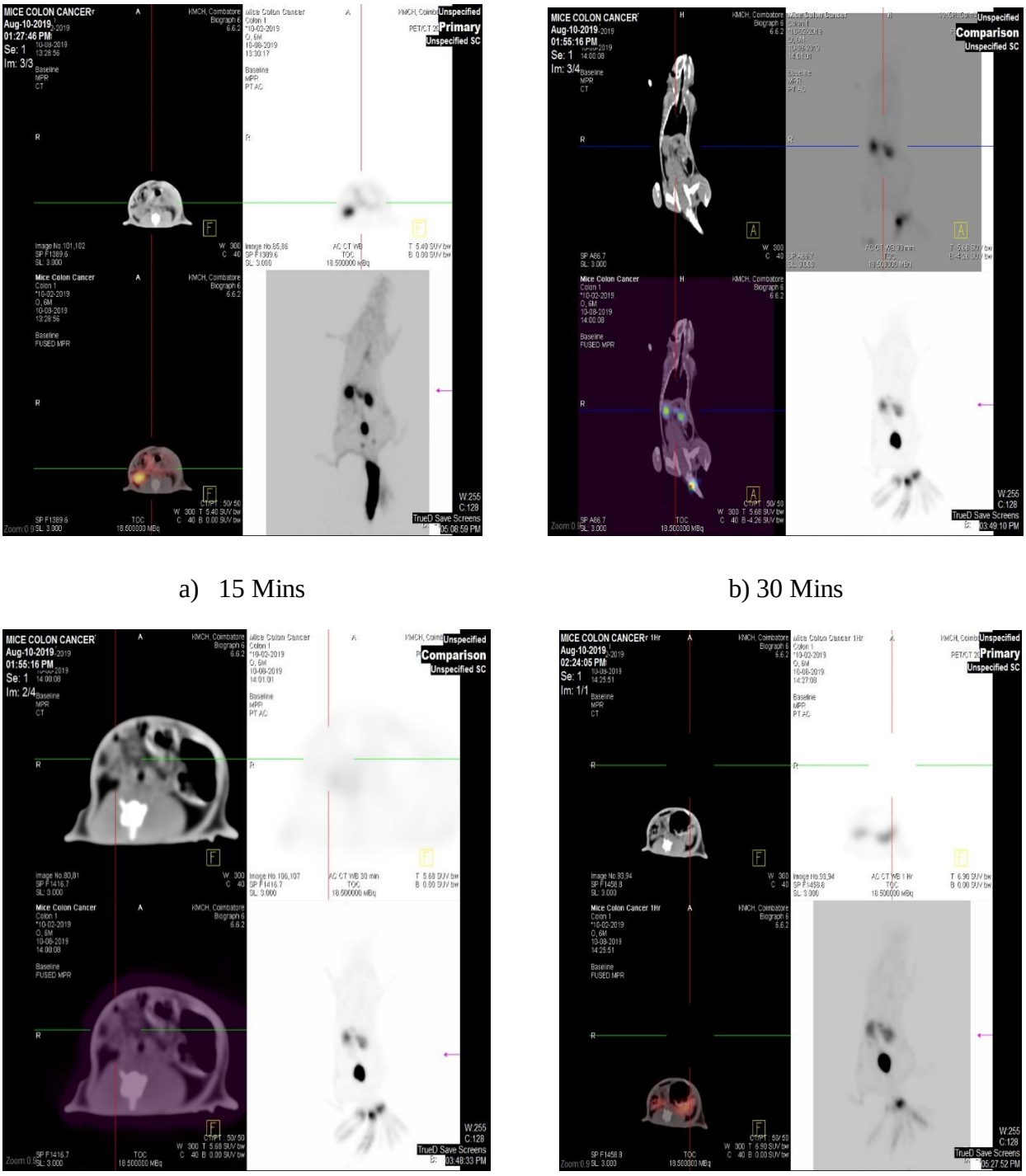


Fig no. 10 ^{68}Ga RGD

GROUP- III: ^{68}GaDOTANOC acetate

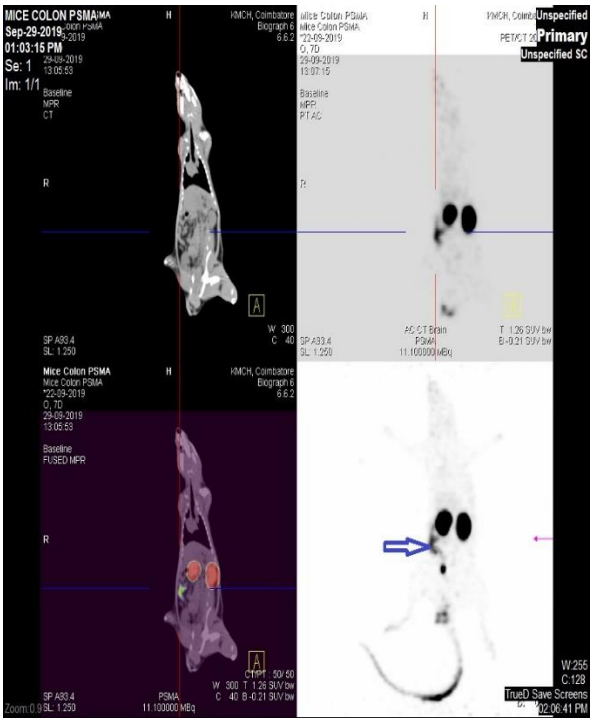


a) 15 Mins b) 30 Mins

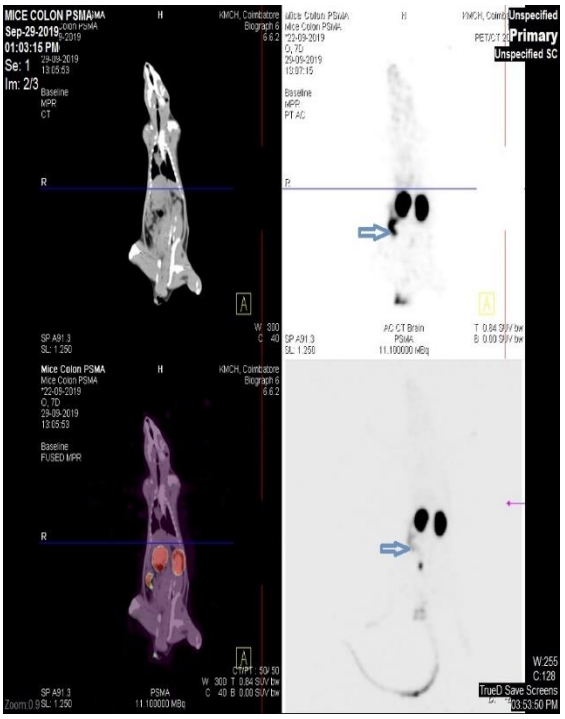
c) 60 Mins d) 90 Mins

Fig no 11. Imaging of rat with ^{68}Ga DOTANOC acetate

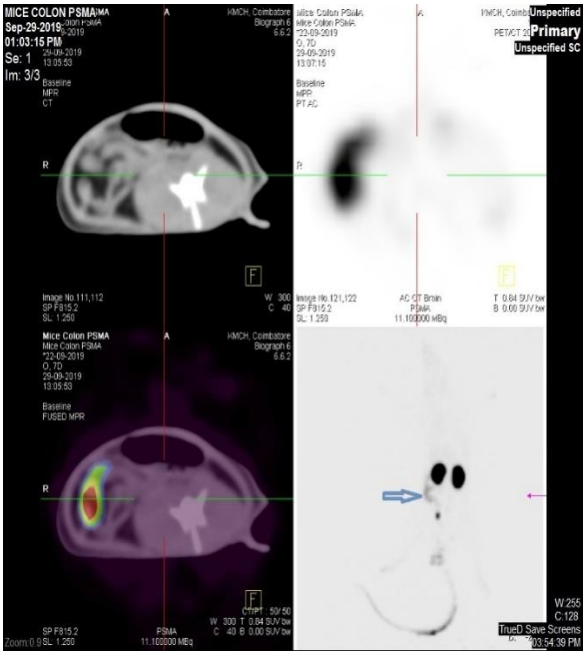
GROUP IV:^{68}Ga PSMA-11



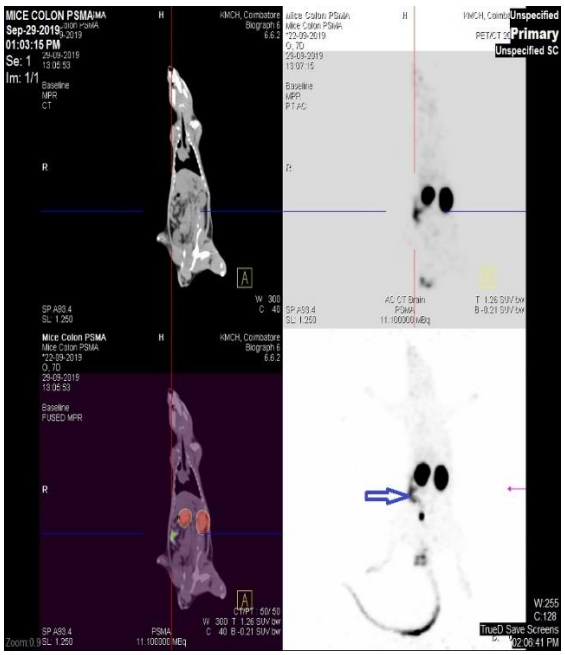
a) 15 Mins



b) 30 Mins



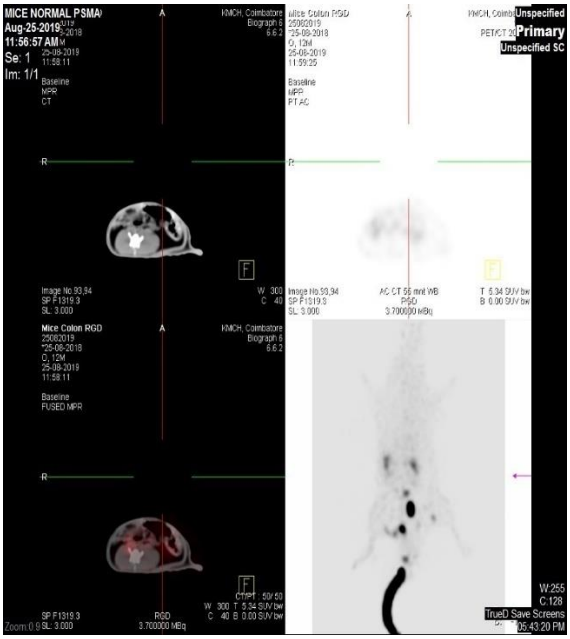
c) 60 Mins



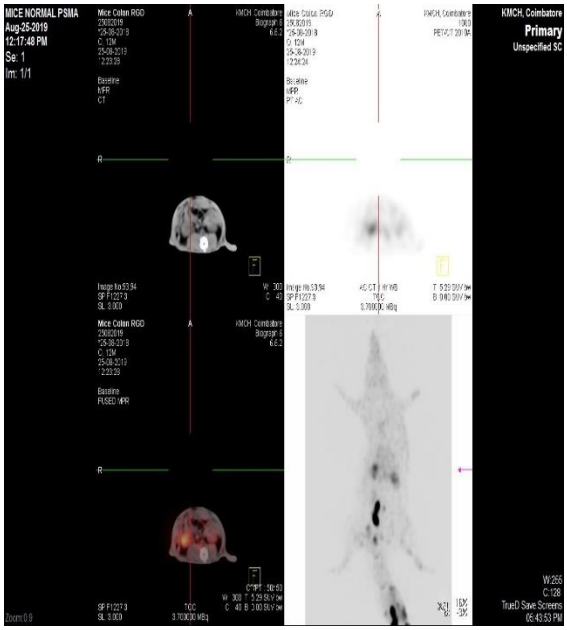
d) 90 Mins

Fig no 12. Imaging of rat with ^{68}Ga PSMA-11

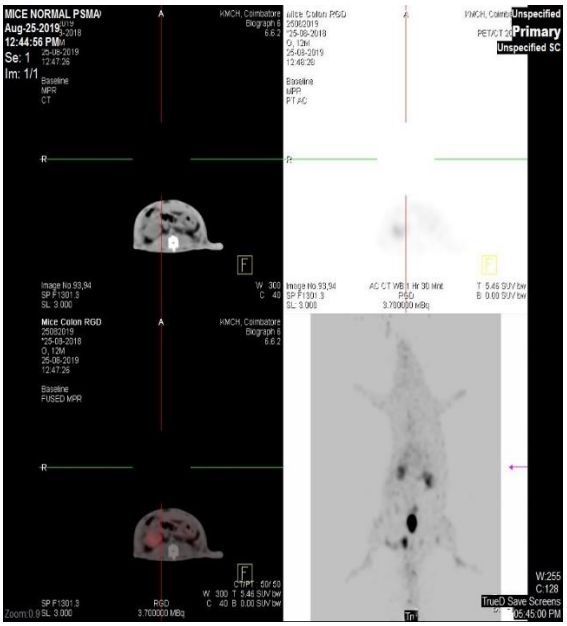
GROUP V:^{68}Ga RGD:



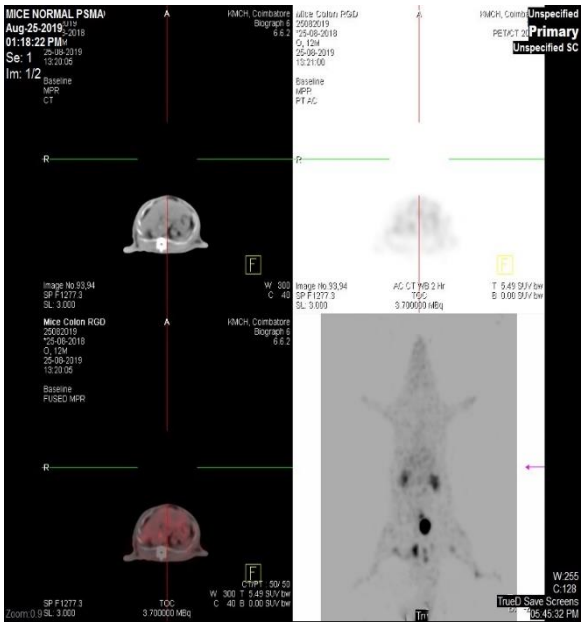
a) 15 Mins



b) 30 Mins



c) 60 Mins



d) 90 Mins

Fig no 13. Imaging of rat with ^{68}Ga RGD

5.3 GROSS NECROPSY STUDY

After PET/CT imaging animals were fasted overnight, anaesthetised and the abdomen was cut open to remove the colon to investigate the morphology, pathology (gross necropsy study). The results of the finding were depicted in Figure no: 14 and 15

Fig no 14. GROUP – I CONTROL

Animals in group - I which were treated only with normal saline showed no changes in the colon.

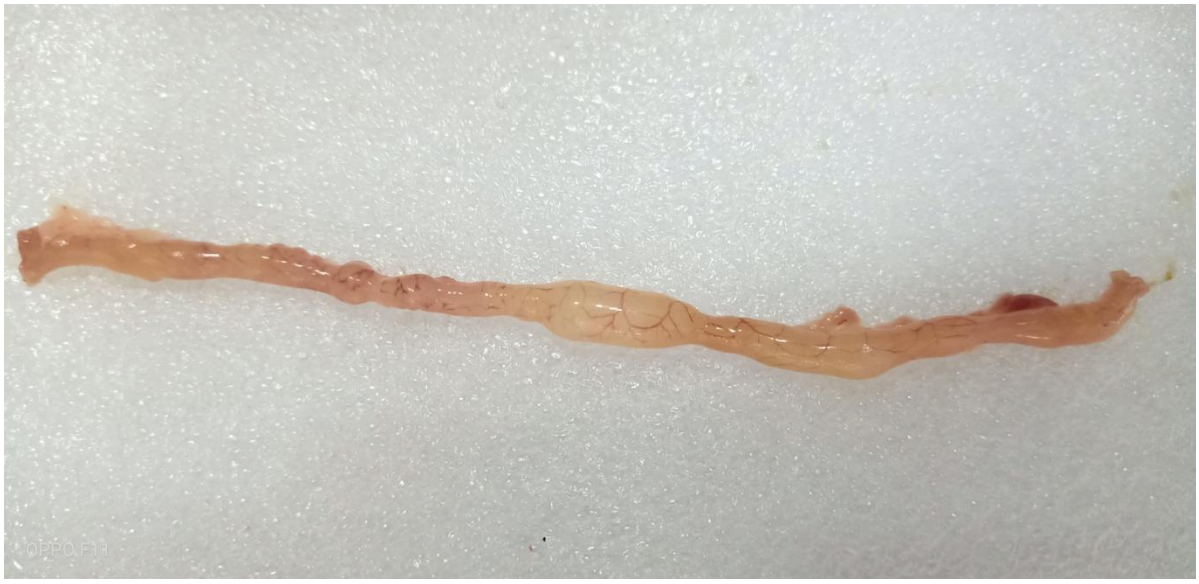


Fig no 15. GROUP – II Only 1,2 DMH (20mg/kg)

Animals in group - II which were treated with 1,2 DMH induced colon cancer showed changes like polyps formation when compared to control group

5.4 PHARMACOKINETIC EVALUATION

Blood samples were withdrawn at 15 min, 30 min, 60 min and 90 min following the intravenous administration of the radiolabeled peptides (^{68}Ga DOTANOC acetate, ^{68}Ga PSMA-11, ^{68}Ga RGD) in the mice. The blood counting of the radioactivity was evaluated by using well counter. Following table shows the counts of radiolabeled peptides at different intervals.

Table no. 6 Plasma clearance counts of radiolabeled peptides at different intervals

Time (Mins)	Counts					
	^{68}Ga DOTANOC acetate		^{68}Ga PSMA-11		^{68}Ga RGD	
	Group 1	Group 3	Group 1	Group 4	Group 1	Group 5
15	46338	48523	35208	37508	40228	41258
30	45997	47856	34726	32568	39894	40745
60	44117	47006	33991	29654	39016	39973
90	43017	46721	33007	25618	38456	39029

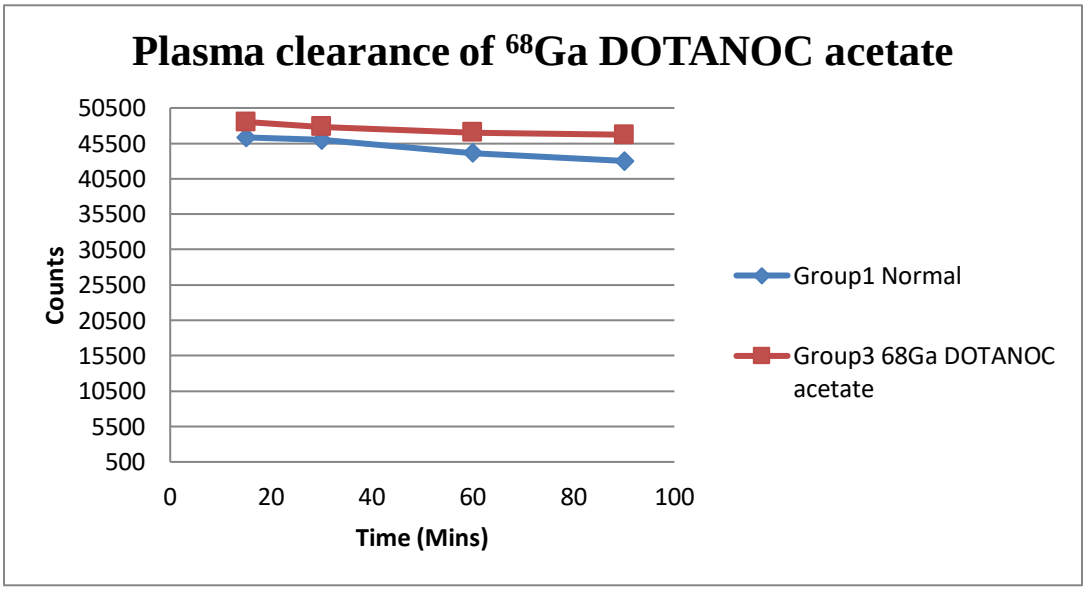


Fig no. 16 Plasma clearance of ^{68}Ga DOTANOC acetate

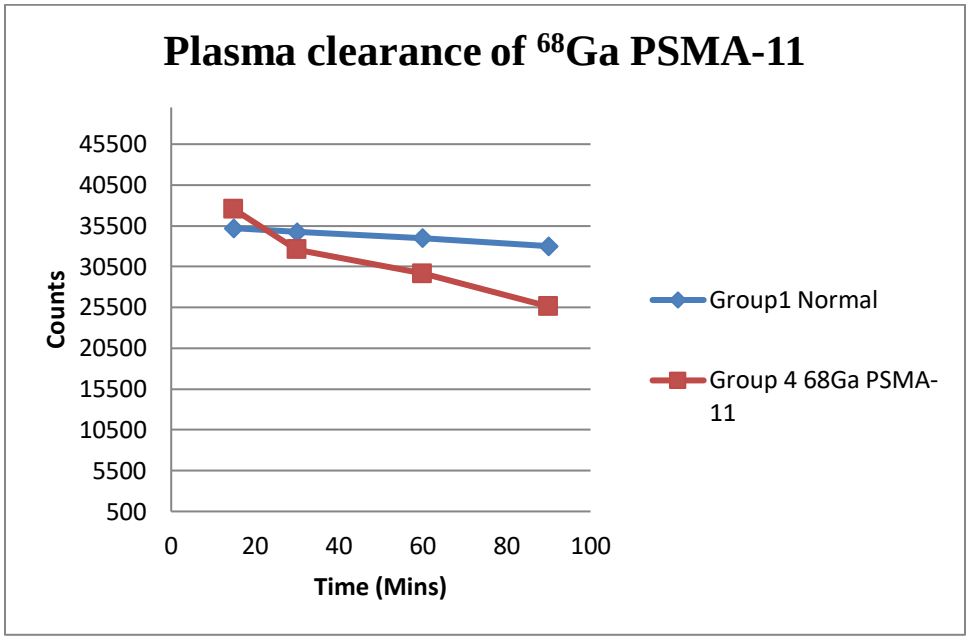


Fig no. 17 Plasma clearance of ^{68}Ga PSMA-11

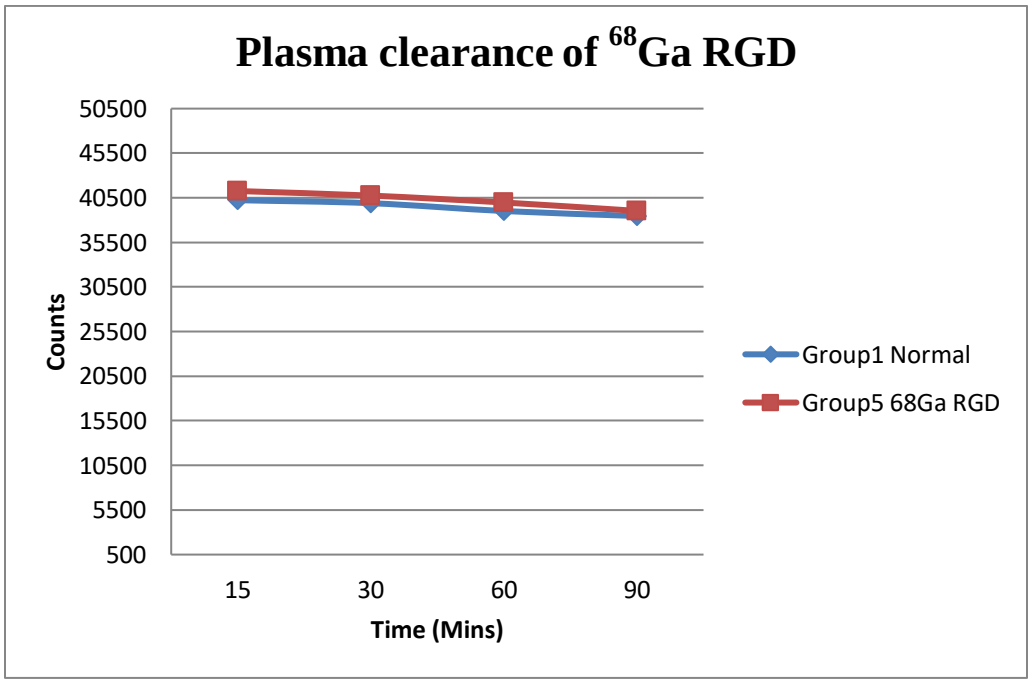
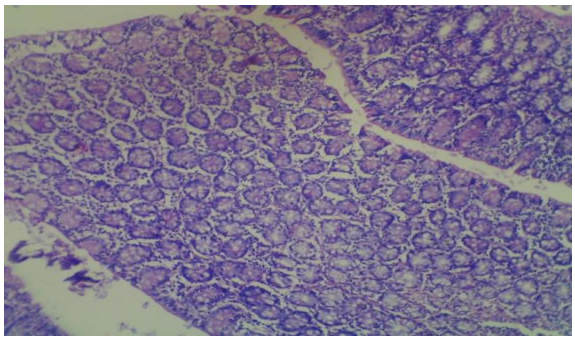


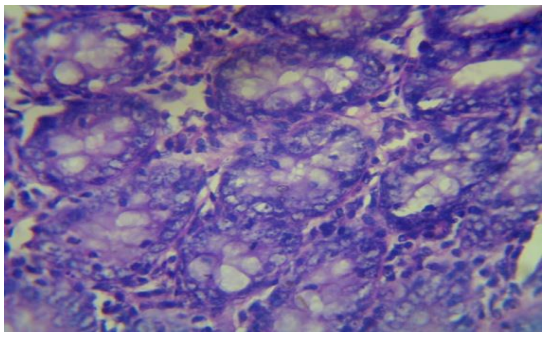
Fig no. 18 Plasma clearance of ^{68}Ga RGD

5.5 HISTOPATHOLOGY:

Group 1 Normal



a) 10X

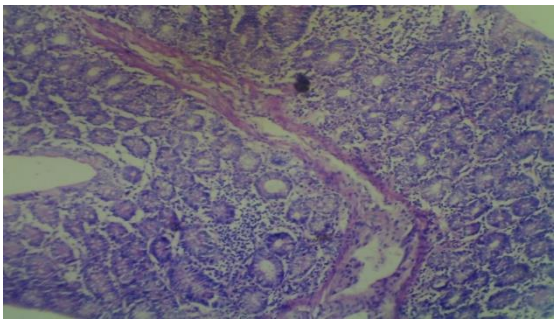


b) 40X

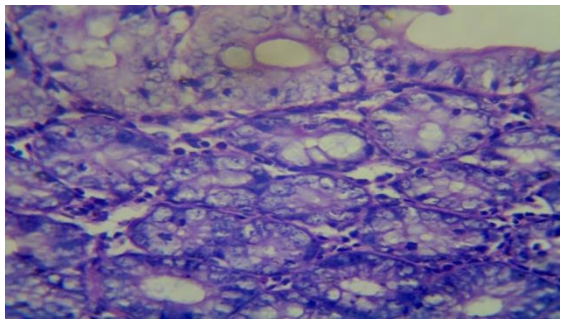
Fig no. 19 Histopathology of colon tissue in Group 1 normal

Section studied from the colon shows normal epithelium. The lamina propria shows scattered lymphocytic infiltration. Muscular layer and serosa shows no singnificant pathology. There is no evidence of dysplasia/malignancy in the section studied.

Group 2 Negative control –only 1,2 DMH



a) 10X



b) 40X

Fig no. 19 Histopathology of colon tissue in Group 2 only 1,2 DMH

Section from colon shows ulcerated epithelium. Increased number of glands with stratification and loss of polarity are also seen. Individual Cells are round to oval with moderate eosinophilic cytoplasm and vesicular nuclei showing mild dysplasia with prominent nucleoli. 6-8 mitosis /10hpf are seen. Lamina propria shows dense lymphocytic infiltrates.

6. DISCUSSION

Colon neoplasms a leading cause of cancer, characterized by excessive and uncontrolled growth of abnormal cells originating from any part of the colon. The pathology is diagnosed more frequently in younger patients, due to risk factors such as obesity, sedentarism, bad nutritional habits (high in fats and proteins), smoking, and the progressive aging of the population. Usually colon cancer begins as polyps which don't show any signs of cancer. If these polyps are screened and removed then colon cancer can be prevent.[17]

Carcinogen induced colon cancer, have proved to share many similarities with human tumors and significantly contributed to our actual understanding regarding cancer pathogenesis. 1,2 dimethylhydrazine induced colon cancer in experimental rats mimics human sporadic colon cancer and is therefore, considered as best model. After administration of 1,2 DMH it is converted to diazonium ions from intermediate metabolites AOM and MAM through series of metabolic reactions in presence of NAD^+ dependent dehydrogenase enzyme. These intermediates alkylates colonic mucosal DNA and results in oxidative stress followed by delayed repair of damaged DNA leads to the accumulation of multiple mutations such as Apc, K-ras, β-catenin and thus leads to susceptibility of specific adenocarcinoma of colon.[80] In this study, administration of 1,2 DMH to Sprague dawley rats produced significant colon tumor compared to normal group.

PET one of the molecular imaging technology are widely used in medicine to provide information regarding pharmacokinetic parameters and efficiency to reach the target site by new molecular entity which might helpful for new drug development for both therapeutic and diagnostic purposes. In case of oncology it has essential role to detect the various stages of disease progression and also to identify how well the therapy reacts with disease so that physician can decide about future treatment regimens.[81]

A variety of imaging probes have been developed for different molecular targets. Initially flurodeoxyglucose has been developed based on the principle that cancerous cells uptake glucose in high concentration than the normal cells. But targeting by this probe is not sufficient to decide the further therapy for clinical patients. Hence peptide based targeting probes has been

introduced, as receptors expression will be higher on cancerous cells than normal. This makes peptide to target on the particular receptor in higher density thereby makes better diagnosing ability.[82]

DOTANOC an octreotide analogue acts on the somatostatin receptor specifically somatostatin receptor type 2, 3, 5. The peptide is initially developed for neuroendocrine tumors localization and internal radionuclide therapy. It is found that somatostatin receptors are over expressed in several human tumors especially neuroendocrine tumors and their metastases these tumors can visualized invivo by radiometal labeled peptides. Many studies has been done to evaluate the efficiency of this peptides for both diagnosing and radiotherapy characteristics.[83]

PSMA-11 targets the transmembrane glycoprotein prostate-specific membrane antigen (PSMA). PSMA is widely expressed in normal epithelium of the prostate gland and to a lesser extent in other tissues such as the brain, salivary glands, intestines, liver and kidney. Its expression is high in benign as well as in malignant prostate tissue. PSMA is also present in the neovasculature of solid tumors including kidney, lung, stomach, colon, and breast. Based on the antigen antibody reaction, it targets specifically hence consider as better diagnostic imaging.[62]

RGD (arginine glycine aspartate) peptide has a high affinity toward $\alpha v\beta_3$ integrin receptors It is well documented that integrin $\alpha v\beta_3$ plays an important role in the regulation of tumor growth, angiogenesis, local invasiveness, and metastatic potential. Integrin $\alpha v\beta_3$ is upregulated on the activated tumor endothelial cells and also highly expressed on some tumor cells such as glioblastoma, breast and prostate tumors, malignant melanomas, and ovarian carcinomas. Radiolabeled RGD (Arg-Gly-Asp) peptides and analogs that specifically target integrin $\alpha v\beta_3$ have been widely tested for tumor imaging in pre-clinical and clinical studies.[84]

Several PET radionuclides has been introduced, amongst all ^{68}Ga has renewed interest due to its several benefits. Firstly, it has a high accessibility for users and is economically advantageous because a cyclotron is not required. Secondly, ^{68}Ga- based radiopharmaceuticals produce high spatial resolution compared with single-photon emission computed tomography (SPECT), allowing more accurate quantification. Lastly, shorter half-life of 68 min.[71]

In this study the efficiency of three radiolabeled peptides (^{68}Ga DOTANOC acetate, ^{68}Ga PSMA-11and^{68}Ga-RGD,) on colon cancer are compared under preclinical settings by PET/CT scan. The peptides are labeled with radionuclide ^{68}Ga and radiolabeling efficiency were identifed by ITLC. The ITLC paper were characterized using well counter and found the radiolabeling efficiency of DOTANOC acetate as 85.78%, PSMA-11 as 48.74%, RGD as 68.44% at pH 4.0 from the upper two third segment of the paper. The lower segment shows 2-3% of radioactivity due to free gallium. Hence the radionuclide has been well binded to the peptides.

From PET/CT imaging it is shown that ^{68}Ga PSMA alone reaches the colon cancer tissues whereas other two peptides ^{68}Ga-RGD and ^{68}Ga DOTANOC acetate shown no activity around the colon tissues. Apart from colon tissues^{68}Ga-tracers of all the peptides showed kidneys and bladder as the organs with the highest accumulation of radioactivity. It is noted from the imaging that expression of PSMA might be high in the region of colon cancer tissue hence the ^{68}Ga PSMA has a good diagnostic property for colon cancer apart from prostate tumor. However, the expression of either somatostatin or intergrin on colon tissue are documented, the expression of ^{68}Ga DOTANOC and ^{68}Ga-RGD has no radioactivity signals in the tumor region which might be because of low penetration of these peptides in the colon tissues.

Plasma clearance were estimated by well counter method for all the three radiolabeled peptides. The counts of radioactivity of peptides in the cancer induced groups were compared with normal group. It is found that all the three peptides injected in the normal group rats showed high radioactivity in plasma after 15 minutes of intravenous injection which is slightly decreases after 30, 60, 90 mins.^{68}Ga PSMA-11 showed gradual decrease in the each intervals as compared to normal group rats which might be because of the uptake of ^{68}Ga PSMA-11 by cancer cells. The other two peptides radiolabeled peptides decrease in their radioactivity in plasma, but resembles similar to the normal group counts. Hence the uptake of ^{68}Ga DOTANOC and ^{68}Ga-RGD by tissues were less.

Histopathological assessments were done on colon tissues of each group. The report showed DMH treated colon tissue has mild dysplasia cells with mitosis and high amount of infiltration when compared to the normal group.

7. CONCLUSION

The current study showed the tumor imaging capacity of ^{68}Ga DOTANOC acetate, ^{68}Ga PSMA 11 and ^{68}Ga-RGD peptides on 1,2 dimethylhydrazine induced colon cancer in sprague dawley rats. Among the three radiolabelled peptides ^{68}Ga PSMA 11 showed excellent imaging capacity; on the contrary other two peptides ^{68}Ga-DOTANOC acetate and ^{68}Ga RGD were not able to image the tumor might be because of low penetration of these peptides in colon cancer tissues. ^{68}Ga-PSMA 11 peptides also showed higher tumor to background ratio and a low accumulation in non-target organs except the excretory organs. These properties make ^{68}Ga-PSMA-11 peptides more suitable for preclinical imaging of colon cancers.

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