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Eugenol Nanoparticles as an effective treatment in Colon Cancer

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Abstract:
Colon cancer is one of the world's most common and fatal cancers, accounting for nearly 881,000 cancer-related deaths. Progression of the colorectal Carcinoma adenoma is caused by three main ways: Microsatellite instability, chromosomal instability and CpG island methylator phenotype. A growing body of scientific evidence points to the fact that colon cancer is a heterogeneous disease; The genetic traits of tumours determine their offspring. The successful treatment of colon cancer depends on an early diagnosis and efficient targeted therapies based on the most recent understanding of the molecular features of colon cancer. The main source of the musky oil eugenol (4-allyl-2-methoxyphenol) is clove. Eugenol, S. aromaticum's main active constituent, has optimistic properties including antioxidants, anti-inflammatory and anticancer activity. The review article is based on the selective delivery and release of the plant-based anticancer compound eugenol (EUG) in colorectal cancer cells.
In a variety of malignant tumours, including colon cancer, EUG is an apoptotic and anti-growth compound. Eugenol drastically reduces colon cancer stem cell population, invasion, and migration, all of which are important for tumour metastasis and medication resistance.

Key words
Eugenol, Colon cancer, Eugenol-PLGA nanoparticle , Chromosomal instability (CIN).

1. Introduction:

Eugenol is a Herbal compound present in various plants extracts including cloves and *Magnoliae flos*. Eugenol is mainly used for different therapeutic applications. Eugenol (4-allyl-2-methoxyphenol), one of the phytochemicals, is a biologically active phenolic component of *Syzigium aromaticum* (cloves). In Asian countries, Eugenol has been used traditionally mainly as an antiseptic, analgesic and antibacterial agent., Eugenol has been used as a flavouring agent in cosmetics and food products and also plays a role in dentistry as cavity filling cement. [1] It is usually acts as an antioxidant and anti-inflammatory agent at low concentrations, at higher concentration act as a pro-oxidant which causes increased generation of tissue-damaging of free radicals [**2,3**].

World Health Organization (WHO) along with Food and Agriculture Organization (FAO) accepted a daily intake of eugenol of 2.5 mg/kg body weight for humans [**5**]. It has also proclaimed by the U.S. Food and Drug Administration (FDA) that Eugenol is safe and non-carcinogenic and non-mutagenic. Current studies illustrated the anticancer activity of eugenol against various cancer cell lines and different animal models. Further more the molecular mechanism of eugenol-induced apoptosis in melanoma, skin tumors, osteosarcoma, leukemia, gastric and mast cells has been well described.

Widely, cancer is recognized as the second primary reason of decease. According to WHO Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (6). The most common in 2020 (in terms of new cases of cancer) were breast (2.26 million cases);lung (2.21 million cases);colon and rectum (1.93 million cases);prostate (1.41 million cases);skin (non-melanoma) (1.20 million cases); andstomach (1.09 million cases). The most common causes of cancer death in 2020 were,lung (1.80 million deaths);colon and rectum (916 000 deaths);liver (830 000 deaths);stomach (769 000 deaths); and breast (685 000 deaths). Colorectal cancer (CRC) is one of the most common gatrointestinal tract malignancy worldwide. The highest incidence rates being found in Eastern Asia and Central-Eastern Europe countries in

compared to Western countries, the incidence rate of CRC is low in India. According to Globocon 2020 data, India is a single country, which contributing to 6.86% of the global cancer burden. Colon cancers were the 4th most common cancers in the world and 13th most common cancer in India by the incidence, respectively. Colon cancers was the 5th most common causes of cancer-related deaths in the world and 13th most common causes of cancer-related deaths in India. In India, deaths due to Colorectal Cancer were 35,385 in 2020, which were 4.15% of total cancer deaths.[7]

Chemoprevention is described as the using of chemo agents to prevent induction, to inhibit or to decrease the progress of cancer [**4**]. Phenolic group of phytochemicals are one of the broad class of agents which are found in plants and are researched by decades.

As maximum of the drugs used in the cancer are apoptotic inducers, the apoptotic effect and anticancer mechanism of eugenol were considered against colon cancer cells. The current review will highlight the anticancer activity and molecular mechanism of the eugenol-induced apoptosis in Colon Cancer.

2. Physical Properties of Eugenol:

Eugenol is very well known compound. It was first isolated in 1929 as a volatile compound from *Eugenia caryophyllata* and commercial production began in the USA in 1940 [1-2].

Eugenol ($C_{10}H_{12}O_2$) is a phenylpropanoid [Fig: 1]- 2- methoxy-4-(2-propenyl) phenol) is an aromatic compound belonging to the group of phenols. Molar mass is 164.20gm/ml. Density-1.06gm/cc. Pka-10.19.Boiling point-25.4degree c. 1.06It is naturally obtained from the natural essential oils of the plants from the Lamiaceae, Lauraceae, Myrtaceae and Myristicaceae families and it is the most important component of clove oil (*Syzygium aromaticum*). Although it is known to occur in various concentration depending on the species(Table 1), the richest source is *S. aromaticum* , where it constitutes between 9381.7mg and 14650mg per 100gm of fresh plant material and is primarily responsible for its characteristics aroma [2,5-8].

Eugenol is a clear to pale yellow liquid with an oily consistency and spicy aroma. It is sparingly soluble in water and well soluble in organic solvents. Eugenol can be produced synthetically in two ways, one of them which involves the allylation of guaiacol with allyl chloride. The biotechnological method is based on the biotransformation of a wide range of microorganism such as *Corynebacterium sp, Streptomyces sp, and Escherichia coli* [2,6-8].

3. Chemical Properties of Eugenol:

Eugenol has low chemical stability and its sensitive to oxidation and various chemical interactions. When orally administered, it is rapidly absorbed by various organs and metabolized in the liver. Therefore, encapsulation of eugenol seems to be the best solution to prevent early absorption, improve its water solubility and thus increase its activity (e.g.-it has been reported that the amount of eugenol delivered increases at least six fold in infected cells when delivered as solid nanoparticles [10]. The eugenol inclusion complexes may be enhanced thermal stability and as a result provide slow release of eugenol. These may be micro emulsion containing eugenol prepared by simply dissolving the eugenol (0.75-1.5% w/v) essential in micelles of surfactants [2,9,15,16].

4. Mechanism of action of Eugenol:

Ali T Zari et al reported that anticancer effect of eugenol can be accomplished by various mechanism like inducing cell death, cell cycle arrest, inhibition of migration and angiogenesis on several cancer cell lines [11-12]. Besides eugenol might be given to the patients who are undergoing conventional chemotherapy. This effect gives a boosted effectiveness with reduced toxicity. There are many studies found to treat several cancer types and their possible mechanism [21-22].

Eugenol has exhibited anticancer properties against colorectal cancer cell lines. *Yoo et al* assessed that eugenol triggered cell apoptosis in these cancer cells through a process reliant on

elevated ROS production and decreased the mitochondrial membrane potential indicating that it might possess apoptosis triggering characteristics [23-24].

The effect of eugenol and gemcitabine i.e. a chemotherapeutic drug were investigated in colon cancer cells. The combination of eugenol and gemcitabine causes a significant decrease in cell viability of 84% eugenol alone to 47% combination of eugenol gemcitabine. Results showed that eugenol alone causes decrease in 84% whereas gemcitabine 51% decrease in cell viability[25].

The colon cancer cells were treated with eugenol resulted in increased lipid layer breaking. Moreover eugenol has been bound to induce apoptosis by destruction the mitochondrial membrane potential and production of reactive oxygen species [26-27].

Pingping et al reported that eugenol-PLGA nanoparticle loaded with 5-Flurouracil and perfluorocarbon having effect in in colon cancer using EGF functionalized in colon cancer[28].

Majeed et al reported that eugenol loaded with nanoemulsion(NEs) emulsified with modified starch were prepared and their apoptic potential against colon cancer cells was examined .In this study it was proved that nano emulsion loaded with eugenol having antiproliferative activity[29].

Hwang et al reported that eugenol is an essential oil and it is containing cinnamaldehyde whose study on NCM-460 cells (epithelial colon) and anticancer against colon cancer cell lines.This compound powerfully decreased the vitality colon cancer cells. Eugenol and six of its derivative were examined and their anti-proliferative action on primary melanoma cell lines. They deduced that the diphenyl eugenol derivatives enantiomer (s)-6,6-dibromo dehydrodieugenol (S7-S) posses the cell apoptosis triggering activity[30-32].

5. Eugenol in Anti cancer therapy:

Clove (Syzygium aromaticum L.) belongs to the family Myrtaceae. It is a costliest spice available in market that has been utilized for decades as a food preservative and for diverse medical uses [33-34]. It is considered as the valued sources of phenolics groups having a wide

range of medicinal values. 89% of active constituent of clove oil is eugenol. Eugenol as the principal active component of S. aromatic, has optimistic properties comprising antioxidant, anti-inflammatory, and anticancer actions[35-36]. It has broad properties like antioxidant, anticancer, anti-inflammatory, and antimicrobial activities. Ant carcinogenic properties of eugenol can have explained by different mechanisms which indues cell death, cell cycle arrest, inhibition of migration, metastasis, and angiogenesis on different cancer cell lines [38, 39,40]. It can also be used with chemotherapy who are treated with conventional chemotherapy. This combination can manage boosted effectiveness with decreased toxicity.

6. Eugenol in colon caner:

Colon cancer is third most common cancer of western countries and its percentage is increasing every year. Natural drugs are curing this degenerative disease in a broad way. Synthetic drug also in combating colon cancer. Eugenol is a natural compound found in various plants, including cloves and basil, and it has been studied for its potential anti-cancer properties. Some studies have suggested that eugenol may have anti-inflammatory, antioxidant, and anti-cancer properties, which could potentially be beneficial in the context of colon cancer. Nanoparticles are being explored as a way to improve the delivery and bioavailability of eugenol and other bioactive compounds in cancer treatment.

Here are some potential effects and mechanisms of eugenol nanoparticles in colon cancer:

Antioxidant and Anti-inflammatory Properties: Eugenol is known for its antioxidant and anti-inflammatory properties, which can help reduce oxidative stress and inflammation in the body. Chronic inflammation is linked to the development of cancer, including colon cancer.

Apoptosis Induction: Some studies have suggested that eugenol may induce apoptosis (programmed cell death) in cancer cells. Apoptosis is a natural process that helps eliminate damaged or abnormal cells, and its dysregulation is a hallmark of cancer.

Inhibition of Tumor Growth: Research in animal models and cell culture studies has indicated that eugenol may inhibit the growth of colon cancer cells. Nanoparticles may enhance the delivery of eugenol to the tumor site, potentially increasing its effectiveness.

Anti-Angiogenic Effects: Eugenol has been investigated for its ability to inhibit angiogenesis, the process by which tumors develop new blood vessels to support their growth. Inhibiting angiogenesis can potentially limit the blood supply to tumors, thereby slowing their growth. Synergy with Conventional Treatments: Eugenol nanoparticles may also be explored in combination with conventional cancer treatments, such as chemotherapy or radiation therapy, to enhance their efficacy and reduce side effects. It's important to note that while these potential effects of eugenol nanoparticles are promising, more research is needed to fully understand their safety and efficacy in treating colon cancer. Clinical trials and further preclinical studies will be necessary to determine the optimal dosage, delivery method, and potential side effects. Eugenol shows synergistic effect when used with some anticancer drugs leading to great reduction in drug toxicity on cancer cells[41,42,43] paying vital role in curing the disease but have many side effects such as decreased pathogen sensitivity due to multiple uses[44].Different studies on EUG demonstrated its strong potential *Fadialah et al* reported that eugenol and simple aromatic benzoate compound are working against Human colon HCT-116 cells and WiDr cells. The study indicated that the eugenol and SAB as phenolic compound are rich in medicinal value. Various phenolic compound contribute to their potent effects on inhibiting carcinogenesis. Extensive research has been conducted in vitro anticancer activities to colorectal cancer cell line[45].

Elhan et al reported that metabolomics investigation of eugenol on colorectal cancer cell line HT-29 by modifying the expression of APC, P53 and KRAS genes. Colorectal cancer is a multistage disease characterized by successive changes in many genes including APC, KRAS and p53. These lead to variation in several important signalling pathways of colorectal cancer

such as the Wnt/ β-Catenin pathway KRAS and p53 signalling pathways and cause several biochemical changes, including changes in in metabolism for cancer progression and greatar energy expenditure[46].

It is imperative to search for colon cancer preventive agents. The inhibitory effect of Eu and simple aromatic benzoate with halogen group in aromatic chain may be a potential chemotherapeutic or a chemo preventive agent based on its ability to induce apoptosis in cancer cell lines with relatively low toxicity[48].

Syeda et al reported that anticancer potential exhibited by eugenol is mainly attributed to its antimetastatic , antiproliferative, ant-angiogenic, anti-inflammatory, cell cycle arreset, apoptotic and autophagic effects. Hence the use of eugenol alone or along with other chemotherapeutic anticancer agents is found to be very effective in cancer therapy[49]

7. Mechanism of colon cancer:

Colon cancer, also known as colorectal cancer, develops when abnormal cells in the colon or rectum begin to grow uncontrollably. The exact cause of colon cancer is not always clear, but it is believed to result from a combination of genetic and environmental factors. Here is an overview of the mechanisms involved in the development of colon cancer:

Genetic Factors:

Family History: Individuals with a family history of colon cancer are at a higher risk. Specific genetic mutations, such as those in the APC, MLH1, MSH2, MSH6, and PMS2 genes, can increase susceptibility to the disease.

Mutations and DNA Damage:

Accumulation of Genetic Mutations: Colon cancer often develops due to the accumulation of mutations in specific genes. One well-known pathway involves the APC (adenomatous polyposis

coli) gene, which when mutated, leads to the formation of polyps in the colon. These polyps can eventually become cancerous.Microsatellite Instability (MSI): Some colon cancers are associated with defects in DNA mismatch repair genes, leading to microsatellite instability. This can result in a higher mutation rate and a greater likelihood of cancer development.

Inflammatory Bowel Disease (IBD):

Long-term inflammation of the colon, as seen in conditions like ulcerative colitis and Crohn's disease, can increase the risk of colon cancer. Chronic inflammation can lead to DNA damage and mutations over time.

Lifestyle and Environmental Factors:

Diet: A diet high in red and processed meats and low in fiber has been linked to an increased risk of colon cancer.

Sedentary Lifestyle: Lack of physical activity may contribute to colon cancer risk.

Smoking and Alcohol: Smoking and excessive alcohol consumption are associated with an increased risk of colon cancer.

Obesity: Obesity is a risk factor for various cancers, including colon cancer.

Polyp Formation:

Many colon cancers develop from adenomatous polyps, which are noncancerous growths in the colon. Over time, some of these polyps can become cancerous if not removed.

Epigenetic Changes:

Epigenetic alterations, such as DNA methylation and histone modifications, can silence tumor suppressor genes or activate oncogenes, contributing to colon cancer development.

Metastasis:

Once cancerous cells have formed in the colon or rectum, they can invade nearby tissues and spread to distant organs through the bloodstream or lymphatic system, leading to metastasis.

Early detection through regular screening, such as colonoscopies, can identify precancerous polyps or early-stage colon cancer when it is more treatable. Treatment options for colon cancer may include surgery, chemotherapy, radiation therapy, targeted therapies, and immunotherapy, depending on the stage and extent of the disease. Additionally, lifestyle modifications and risk factor management can help reduce the risk of developing colon cancer. Different molecular pathways play a significant role in colon cancer. Colon cancer arises due to mutations in tumor suppressor genes, genes linked to DNA repair and target oncogenes[50]. Occurrence and development of colon cancer depends on both genetic and epigenetic stability. Chromosomal instability (CIN), MSI and cytosine preceding guanine (CpG) island methylator phenotype (CIMP) pathways are included in colon cancer. CIN, MSI and CIMP have significant prognostic implications. In one study, MSI or MSS tumors are identified based on colon cancer cases. CIN-only, CIMP-only, CIN+CIMP, and triple negatives classify in the latter group[51]. Lowest frequency for APC and KRAS mutations, the second lowest for p53 mutations, and the highest for BRAF V600E mutations occurred in MSI tumors. Highest frequency for p53 mutations and the lowest for BRAF V600E mutations have been found in CIN-only. MSI, CIMP, BRAF-mutation, and KRAS-mutation status were identified based on patient survival outcome[53]. Highest five-year disease specific survival was found in MSI-H tumors (types 1 and 5) which was 89.5% and 93.1%. After MSI-H tumors, MSI-L/MSS tumors (type 4; 82.5%) without CIMP or BRAF and KRAS mutations, and the tumors with only KRAS mutations (type 3; 72.4%) were followed [54]. The worst survival (type 2; 49.2%) was found in tumors with CIMP and BRAF mutations. There were six types of classification of C1 to C6 tumor according to the mutational landscape. The C1, C5, and C6 tumors frequently had chromosomal instability, TP53 mutations, and were distally located lacking the mutator phenotype. Chromosomal instability and TP53 mutations were identified in the C1, C5, and C6 tumors which were distally found without the mutator phenotype [55-58]. BRAF mutations were found in C2 tumor whereas KRAS mutations

were found in C3 tumor. Now a day treatment regimen is determined by a particular mutation present in the tumor. Main line of treatment for colon cancer is a combination of either 5-fluorouracil (5-FU) or capecitabine with irinotecan or oxaliplatin. Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors have been developed as targeted therapies for both first and second lines of treatment. Recent evidence reports that tumors lacking codon 12 and 13 mutation in KRAS treatment with anti-EGFR is found to be effective. Nowadays in clinical trials patients with KRAS-mutated tumors may be found detrimental when treated with oxaliplatin. According to the guidelines of the American Society for Clinical Oncology, patients with mutant KRAS metastatic disease are used to avoid anti-EGFR inhibitors. Anti-EGFR therapies show resistance in BRAF V600E mutation correlated with poor disease specific survival[60-62]. The first line 5-fluorouracil treatment shows failure in the presence of microsatellite instability. Colon cancer treatments have been developed on the basis of genetic and epigenetic biomarkers.

8. Current status of colon cancer:

In the 21st century, colon cancer is the most popular leading cancer in men and women, around over 1.9 million new cases reported in 2020. According to GLOBOCAN 2020 data, mortality rates of colon cancer among various countries of the world [63].Many researchers have noticed that survival rates of colon cancer hangs on the stage at which it is diagnosed, with later stages having low survival rates. At early stage survival rate of colon cancer is 90 percent as compared with 13 percent diagnosed later [64]. Dying risk from colon cancer is about 0.65% among men and 0.45% among women at age 0–74. Mortality rates per 100,000 of colon cancer in both sexes is about 8.9.The incidence and mortality rates of colon cancer are significantly increasing for both male and females in high income countries.Developed countries are at the highest risk of colon cancer due to obesity, sedentary lifestyle, red meat consumption, alcohol and tobacco[65]. Colon cancer has different subtypes because it is a heterogeneous disease which may be distinguished by their specific clinical and/or molecular features. The majority of colon cancer happens due to chromosomal instability (CIN) having changes in chromosome number and structure. Chromosomal instability happens due to gain or losses of segments and

rearrangements of chromosomes, and loss of heterozygosity[67,68,69] . These chromosomal alterations affect the expression of genes located in tumors and genes that regulate cell proliferation which, in turn, may activate pathways essential for colon cancer initiation and progression.The remaining cases having high - frequency microsatellite instability (MSI) have two well described forms. One is familial adenomatous polyposis (FAP) and the other is hamartomatous polyposis syndrome. Familial adenomatous polyposis exhibit a mutated copy of the adenomatous polyposis gene which has a consequence of a defective DNA mismatch repair[70,71,72]. At their mid-teen, patients with FAP develop hundreds or even thousands of colon polyps and with high-probability, most of these colon polyps turn into cancer. All patients having earlier unrecognized and untreated FAP may be diagnosed with colon cancer before the age of 35-40. Hamartomatous polyposis syndrome is caused by less penetrant inherited mutations (32%). Many works attenuate the need to get resources for health education focused on colon cancer risk.

Incidence and Prevalence: Colon cancer is one of the most common types of cancer worldwide. Its incidence varies by region and is influenced by factors such as age, lifestyle, and genetics. Screening programs have been established in many countries to detect colon cancer at an early stage, which can improve treatment outcomes.

Risk Factors: Several risk factors are associated with an increased likelihood of developing colon cancer. These include age (risk increases with age), family history of colon cancer or polyps, a personal history of colorectal cancer or polyps, a diet high in red and processed meats, obesity, smoking, and excessive alcohol consumption.

Screening and Early Detection: Screening for colon cancer is crucial for early detection when the cancer is most treatable. Common screening methods include colonoscopy, fecal occult blood tests, sigmoidoscopy, and CT colonography. Guidelines for screening may vary by country and individual risk factors.

Advances in Treatment: Treatment options for colon cancer may include surgery, chemotherapy, radiation therapy, targeted therapies, and immunotherapy. Advances in surgical techniques and targeted therapies have improved the outcomes for many patients with colon cancer.

Survival Rates: Survival rates for colon cancer vary depending on the stage at diagnosis. Colon cancer detected at an early stage (localized) often has a high survival rate. However, late-stage colon cancer (metastatic) may be more challenging to treat and may have lower survival rates.

Research and Development: Ongoing research efforts continue to focus on improving colon cancer diagnosis and treatment. This includes the development of new drugs, immunotherapies, and personalized medicine approaches tailored to an individual's specific cancer characteristics.

Prevention: Colon cancer is preventable to some extent through lifestyle modifications such as adopting a healthy diet rich in fruits, vegetables, and fiber, maintaining a healthy weight, regular physical activity, limiting alcohol consumption, and avoiding smoking.

9. Future Prospects:

Due to its wide range of biological activities, eugenol has many applications. It has also demonstrated therapeutic potential in drugs to fight cancer. Though high concentrations of eugenol can be pro-oxidative and harmful, but doses below 2.5 mg/kg body weight are regarded as safe by the FAO. Eugenol derivatives are also having important therapeutic and pharmacological activity and a popular research object. Eugenol derivatives can appear to be promising ingredients in colon cancer along with other cancer therapy.

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Conflict of interest

I certify that no actual or potential conflict of interest in relation to this article exist.

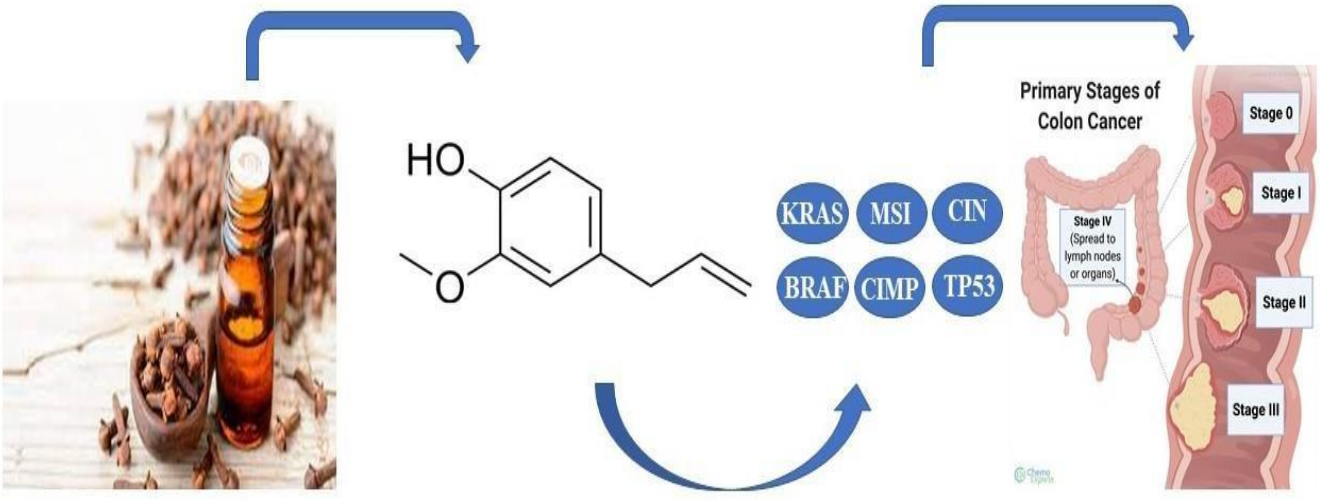

Fig: 1 **Graphical abstract**

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