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PROTEIN NANOPARTICLE MEDIATED DRUG DELIVERY USING 5-FU PRODRUGS FOR THE TREATMENT OF GASTROINTESTINAL CANCER

Arjun Sai Sreekar Aeila*, Syed Tazib Rahaman, Tirandi Manohara Sai
GITAM Institute of Pharmacy, GITAM (Deemed To Be University) Rushikonda, Visakhapatnam 530045, Andhra Pradesh, India.

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Abstract:
Gastrointestinal cancer has been a problematic disease and the efficiency of treatment of gastrointestinal tract (GIT) cancers which had always been a complication. Tumor targeting is considered to be the most desirable property for treating GIT cancer. But according to various studies most of the drugs are found to be ineffective in one or other way to produce a prolonged release in tumor targeted drug delivery. Hence produgs of 5FU were considered in treatment of git cancer. The prodrugs of 5FU are further enhanced by using protein nanoparticles as drug delivery system. In this review we discussed about the various parts that git cancer can affect and the mechanisms of 5FU prodrugs, the synthesis of protein nanoparticles and the targeting achieved by using these protein nanoparticles.

Keywords: *Cancer; Neoplastic cells; Tumor targeting; Protein.*

Corresponding author:
Arjun Sai Sreekar Aeila,*
GITAM Institute of Pharmacy,
GITAM (Deemed To Be University) Rushikonda,
Visakhapatnam 530045, Andhra Pradesh, India.
E-Mail: arjun.aeila1@gmail.com

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INTRODUCTION:

Formulating drugs to treat tumors has been of considerable interest. If proven to be highly beneficial to medications that are quickly metabolized before reaching systemic circulation, the prodrug approach is. 5FU prodrugs are of major interest in the treatment of tumours. Nanoparticles are similarly used as potential carriers of drugs that are to be delivered effectively. The 5 FU prodrugs have strong affinity to the gastrointestinal cancer targeted therapy for tumors.

Git Cancer:

Gastrointestinal cancer refers to the digestive tract (GI) and accessory organs, including the oesophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum, and anus. The signs are related to the organ affected, and may include vomiting (leading to difficulty swallowing or defecation), excessive bleeding or other related problems. The diagnosis also includes endoscopy, followed by biopsy of the infected tissue. The treatment depends on the tumor location, cancer cell type and whether other tissues have been invaded or spread elsewhere.[1] Malignancy is a major source of death worldwide: in 2008 it accounted for 7.9 million deaths (around 13 per cent of all being equal). The rising future implies an equally widening danger of creating malignant growth. Overall, deaths from malignant development are expected to continue to increase, with 12 million deaths predicted in 2030.

Gastric malignant growth is the second most fundamental disease in the world and only about 66 per cent of all cases occur in nation-building. It is the fourth most important malignant growth in men, and the fifth most common disease in ladies. Despite the fact that gastric disease frequency is declining, regardless of whether it remains a significant medical issue and a typical reason for malignancy mortality worldwide. Gastric malignant growth carcinogenesis alludes to the accumulation of genetic alteration of different qualities, e.g. oncogenes, tumor silencer, and ambiguous fixing qualities.

In the stomach, a few distinct kinds of malignant growth can occur. The most common form is called adenocarcinoma. Approximately 90 per cent of gastric malignancies is adenocarcinoma. Adenocarcinoma is known to form a solitary cell in the body. Stomach adenocarcinoma is a normal malignant development of the worldwide stomach associated tract, despite being phenomenal in the United States. It often happens in men over 40. In the United States, the pace of most types of gastric adenocarcinoma has gone down over the years. Analysts figure the decline may be on the grounds that people eat fewer salted, soothed, and smoked nourishments. There are two kinds of gastric adenocarcinoma dependent on anatomical area: cardia, or proximal, and distal, noncardiac adenocarcinomas. These ought to be considered as discrete substances on account of varying epidemiologic connections, related hazard variables, and anticipation. Truly, distal gastric carcinoma was the most incessant sort.

Cause and incidence of Gastric cancer:

The development of stomach cancer is a multifactorial process and many factors affect the probability of incidence, including family history of stomach cancer, infection with helicobacter pylori (a specific bacteria that can also cause stomach ulcers), history of an adenomatous stomach polyp larger than 2 centimeters, history of chronic atrophic gastritis, history of pernicious anemia, obesity, alcohol [1].

In 1994 Helicobacter pylori was classified as a type I carcinogen by the International Agency for Research on Cancer and the World Health Organization, the exact mechanism leading to gastric carcinoma is not clearly understood. The H-effects Due to multiple factors including host and environmental factors as well as specific bacterial strains, pylori infection with gastric cancer occurs. H. Pylori is closely associated with intestinal stomach cancers that follow a progressive yet malignant pathway similar to that of the colon.

Upper digestive tract cancer: Upper digestive tract cancer is the cancer of the upper sections of the GIT associated with the areas of the digestive sytem.

Oesophageal cancer: The esophagus is a hollow conduit of the muscle that carries food from the throat to the gastric. Oesophageal cancer can occur when a malignant tumor grows on the esophagus lining. As the tumor develops it can affect the deep tissue and muscle of the esophagus. A tumor may grow anywhere along the length of the esophagus, including where it reaches the esophagus and the stomach. There are two common types of cancer of the esophagus: A***) Squamous cell carcinoma*** occurs when cancer begins in the flat, thin cells that form the esophagus lining. This form appears most often in the top or center of the esophagus, but it can appear anywhere. B) ***Adenocarcinoma*** develops when cancer starts in the esophagus glandular cells responsible for fluid production such as mucus. In the lower portion of the esophagus, adenocarcinomas are most common.

Stomach cancer:

The stomach is just one part of the upper part of

your digestive tract (along with the esophagus). The stomach is responsible for digesting food, and then transferring nutrients to the rest of your digestive organs, the small and large intestines in particular.[2]. Stomach cancer occurs when healthy cells in the upper digestive system become cancerous and grow out of control, forming a tumor. This process is likely to happen slowly. Stomach cancer has usually tend to develop over many years. Stomach cancer treatment depends on several factors depending on cancer incidence and overall health and preferences of the patient. Treatments may include surgery, chemotherapy, radiation therapy, medications, and clinical trial participation.

Pancreatic cancer: The pancreas is a 6-inch long organ situated in the back of the abdomen behind the stomach near the gall bladder. It contains glands that produce pancreatic juices, hormones, and insulin. Cancer may affect either the pancreatic endocrine or exocrine glands. The exocrine glands produce juices which digest the proteins and fats. The endocrine glands are tiny groups of cells known as the islets of langerhans. Insulin and glucagon hormones are released into the bloodstream. There, blood sugar levels are regulated. The consequence is often diabetes when they don't work properly. Pancreatic cancer develops when, in a portion of the pancreas, unchecked cell growth begins. Tumors form and interfere with the functioning of the pancreas. Until the later stages, pancreatic cancer still has no symptoms. That's why it can be hard to manage. Approximately 3 percent of all cancers in the United States are pancreatic cancers, according to the American Cancer Society.

Liver cancer:
The liver is the body's largest glandular organ which performs many critical functions to keep the body free of toxins and harmful substances. It is situated just below the ribs in the right upper quadrant of the abdomen. The liver contains bile, a material that helps you digest fats, vitamins, and other nutrients. This vital organ also stores nutrients like insulin to keep you nourished at times when you don't feed. It breaks down drugs and toxins as well. Cancer kills liver cells as cancer grows in the liver and interferes with the liver's ability to function normally. For people over 50 years of age, liver cancer is more common.

Infection with long-term hepatitis B or C can seriously damage your liver. Hepatitis is transmitted by direct contact with an infected person's body fluids, such as their blood or semen, from person to person [3]. Through pregnancy, it can also be transferred from mother to child. By using prevention during sexual intercourse, you will reduce your risk of hepatitis B and C. A vaccination can also shield you from hepatitis B.

Over many years, drinking two or more alcoholic drinks every day increases your risk of liver cancer. Cirrhosis is a type of damage to the liver which replaces healthy tissue with scarred tissue. A scarred liver cannot function properly and can eventually lead to various complications, including hepatic cancer. The most common causes of cirrhosis in the United States are long-term alcohol abuse and hepatitis C. Once they grow liver cancer, most People with liver cancer have cirrhosis. Aflatoxin exposure is a risk factor. Aflatoxin is a toxic substance formed on peanuts, grains, and corn by a form of mold that can grow. Some risk factors are diabetes and obesity. People with diabetes appear to be overweight or obese, leading to liver cancer and other complications.

Gallbladder cancer: Gallbladder cancer is a rare disease in which the gallbladder tissues contain malignant (cancer) cells. The gallbladder is a pear-shaped organ in the upper abdomen, which sits just below the liver. Bile, a protein produced by the kidneys to absorb food, is contained in the gallbladder [4]

Once food is broken down in the stomach and intestines, bile is released through a pipe called the common bile duct from the gallbladder, which connects the gallbladder and liver to the small intestine's first portion. For the following reasons, gallbladder cancer is difficult to detect and diagnose in the early stages of gallbladder cancer, there are no signs or symptoms.

Once present, the symptoms of cancer of the gallbladder are like the symptoms of many other diseases. The gallbladder is hidden behind the liver. Gallbladder cancer is sometimes discovered when, for other reasons, the gallbladder is removed. Patients with gallstones rarely develop cancer of the gallbladder.

Gastric MALT lymphoma: The stomach is extranodal lymphoma's most common site. Gastric lymphoma from mucosa-associated lymphoid tissue (MALT) is usually a low-grade, strongly associated B-cell neoplasia with Helicobacter pylori (H. pylori) infection. Only some H. In some predisposed patients, pylori strains decide the development of lymphoma in the stomach by a complex strain-host-organ cycle. Clinical exposure is poorly descriptive, with symptoms ranging from ambiguous dyspepsia to symptoms of alarm. Similarly, various endoscopy patterns for gastric lymphoma have been identified.Early-stage H.pylori eradication is prescribed as first-line

treatment and full lymphoma recovery is achieved in 75% of cases. The stage of neoplasia, extent of infiltration in the gastric wall, presence of the translocation of API2-MALT1, position in the stomach, and ethnicity of patients were established as predictors of remission. Recent data say H. Pylori eradication therapy may also be effective in the treatment of gastric lymphoma in a small H subgroup (15%). Patients of H.pylori-negative have an average 5-year survival rates and disease-free survival rates are as high as 90% and 75% respectively [5]. Patient treatment which failed to achieve post-lymphoma remission. The eradication of H. pylori includes radiation therapy, chemotherapy and surgery in selected cases.

Gastrointestinal stromal tumours: A gastrointestinal stromal tumor (GIST) is a form of tumor in the gastrointestinal tract which occurs most often in the stomach or small intestine. It is assumed that tumors may develop from specialized cells contained in the gastrointestinal tract called Cajal interstitial cells (ICCs) or precursors to these cells. GISTs are usually found in adults between 40 and 70 years of age; these tumors are rarely formed by children and young adults. Cancerous (malignant) or non-cancerous (benign) can be the tumors. Any signs or symptoms can be caused by small tumors. Many people with GISTs, however, can experience abdominal pain or swelling, nausea, vomiting, loss of appetite, or weight loss [6]. Tumors often cause bleeding, which can lead to low levels of red blood cells (anemia) and therefore fatigue and tiredness. Bleeding in the intestinal tract can lead to black and tarry stools and bleeding in the throat or stomach can lead to blood vomiting. Individuals diagnosed with no GIST family history usually only have one tumor (Sporadic GIST). Individuals with a family history of GISTs (Family GISTs) often have numerous tumors and additional signs or symptoms, including non-cancerous overgrowth (hyperplasia) of other cells in the digestive tract and dark skin patches in various areas of the body. Many individuals affected have a skin condition called urticaria pigmentosa characterized by elevated patches of brown skin that sting or itch when touched.

Lower digestive tract cancer:

Colorectal cancer: If healthy cells in the colon or rectum lining shift and develop out of control, colorectal cancer begins to form a mass called a tumor. tumors are of two types: cancerous or benign tumor. A cancerous tumor is malignant, which means it can grow and spread to other body parts. A benign tumor means that the tumor can develop but is not going to spread. Typically, these improvements take years to develop. The variations may be triggered by both genetic and environmental factors. Nevertheless, changes can occur in months or years when a person has a rare inherited syndrome. Colorectal cancer most often begins as a polyp, a non-cancerous growth that may develop as people grow older on the inner wall of the colon or rectum. If a polyp is not treated or removed, it can turn into a life-threatening cancer. Precancerous polyps can be found and removed to avoid colorectal cancer [7].There are several polyps types. Adenomatous polyps, or adenomas, are cancerous growths. A colonoscopy can be used to identify them. During a colonoscopy, polyps are most easily found as they usually bump into the colon, creating a mound on the colon wall that the physician may identify.

Anal cancer: The anus is the end of the large intestine, under the rectum, through which the body releases the feces (solid waste). The anus is produced in part from the body's outer layers of skin and in part from the intestine. Two ring-like muscles, called muscles of the sphincter, open and close the opening of the anal and let the stool pass through the body. The anal canal is about 1-1.5 inches long, the part of the anus between the rectum and the anal opening. Anal cancer is a type of cancer developed in anus tissues. The anus is the rectum opening to the outside of the body and the Digestive tract at the middle. Anal cancer sometimes does not cause symptoms at all. Nevertheless, bleeding is often the disease's first sign. Generally the bleeding is moderate. At first, most people assume that bleeding is caused by hemorrhoids (painful, anus and rectum swollen veins that may bleed. Signs are more likely to be caused by benign (non-cancer) conditions such as hemorrhoids, anal fissures, or anal warts [8].

Gastrointestinal carcinoid tumor: Gastrointestinal carcinoid tumors are a type of cancer in the GI tract that you can get. They are most common in the large intestine appendix, small intestine, and rectum. They are the neuroendocrine tumor (NET) type most common. This means they are composed of cells that are neuroendocrine. Such cells are a cross between a nerve cell and a hormone-making cell. In your GI tract, you have a lot of them. They help control how food is broken down by the body [9]. But they can make extra hormones when they're in a tumor that you don't need. This can lead to issues such as

heart problems, flushing, and diarrhoea. These tumors are commonly caused due to zollingers syndrome (Insufficient acid production in the stomach) and Multiple endocrine neoplasia type 1.

Prodrug:
A prodrug is described as a compound that is pharmacologically inactive and converted by a metabolic biotransformation into an active agent. The goal is to change the antitumor agent chemically to make it temporarily inactive. In vivo, this prodrug decomposes through the action of enzymes, releasing the active concept. A judgmentally chosen chemical group is covalently bound to the active rule in most situations. This class must be in vivo non-toxic, inactive biologically, and labile. The solubility of the prodrug, its stability, the rate at which it releases the active principle and the specific enzymes necessary for its transformation are often regulated by this band [10].

Prodrugs of 5 FU for treatment of GIT Cancers:

A pyrimidine ring with a fluorine atom in position 5 characterizes the FU prodrugs. In a number of chemical changes, they vary from FU. The main advantage is through oral administration. These are engineered to be well absorbed intact from the digestive tract and eventually transformed into enzymatic FU in the liver or inside the tumor itself to expose the tumor to FU for a longer period of time but at lower levels than those found during an i.v. Bolus, which decreases toxicity [11].

89 percent showed a preference for oral therapy in a survey of 103 patients. Reasons for this option included comfort and less venous access issues. Nonetheless, 70% of survey respondents are unwilling to accept a lower rate of oral therapy response. Therefore, while convenience is a significant potential gain, oral agents need clinical equivalence or superiority.

That agent was designed in accordance with a specification with a well-defined mechanism to release the active principle. Some are designed to work alone, while others allow a modulator to be co-administered. The goal is to imitate the FU pharmacokinetics provided by continuous i.v infusion, not only because of its chemical structure, but also because of cautious dosage choices.

5-Flourouracil:
Fluorouracil (5-FU) is a pyrimidine analog used to treat several solid tumors, including colon, rectal, breast, thyroid, pancreatic, ovarian, lung, and liver cancer. Fluorouracil is associated during treatment with a low rate of elevations of transient serum aminotransferase and has been implicated in rare cases of medically evident acute liver injury.

Mechanism of action: 5-FU acts in a number of ways, but mainly as an inhibitor of thymidylate synthase (TS). Interrupting this enzyme's activity prevents pyrimidine thymidine synthesis, which is a nucleoside required to replicate DNA. Thymidylate synthase methylates monophosphate deoxyuridine (dUMP) to form monophosphate thymidine (dTMP). The administration of 5-FU causes scarcity in dTMP, so cancerous cells quickly divide through thymine less death. Calcium folinate provides an exogenous source of reduced folinates and thus stabilizes the complex of 5-FU-TS, thus enhancing the cytototoxicity of 5-FU [12].

1.Capecitabine:
Capecitabine is a carbamate of fluoropyrimidine recommended for the treatment of metastatic breast cancer and colon cancer with antineoplastic activity. It is a systemic prodrug administered orally that has little pharmacological activity until it is transformed to fluorouracil by enzymes produced in many tumors at higher concentrations. Fluorouracil then metabolized 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP) in both healthy and tumor cells.

Mechanism of action: Capecitabine is a prodrug that is selectively tumor-activated by thymidine phosphorylase, an enzyme present in many tumors at higher concentrations than healthy tissues or plasma, to its cytototoxic moiety, fluorouracil [13]. Fluorouracil is also metabolized within healthy and tumor cells into two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). Two different mechanisms allow these metabolites to cause cell injury. Firstly, FdUMP and folate cofactor N5-10-methylenetetrahydrofolate bind to thymidylate synthase (TS) in order to form a ternary complex that is covalently bound. This binding prevents the formation of 2'-deaxyuridylate thymidylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is important for DNA synthesis, so this compound's deficiency can inhibit cell division. Second, nuclear transcription enzymes can incorrectly incorporate FUTP during RNA synthesis instead of uridine triphosphate (UTP). By generating fraudulent RNA, this metabolic error may interfere with RNA processing and protein synthesis. Metabolized by thymidine phosphorylase to fluoruracil.

Capecitabine ⇨ 5'-Deoxy-5-fluorouridine
⇩
5-fluorouracil

2. Paclitaxel:
Paclitaxel is a chemotherapeutic agent that, among others, is sold under the brand name Taxol. Used as a medication for different cancers, paclitaxel is a mitotic blocker first isolated from the Pacific yew tree bark in 1971, that includes endophytic fungi synthesizing paclitaxel. It is available for injection as an intravenous solution and the new formulation includes albumin-bound paclitaxel sold under the Abraxane brand name. Paclitaxel is a taxoid antineoplastic agent suggested as first-line and follow-up therapy for the treatment of advanced ovarian carcinoma and other cancers including breast cancer. Paclitaxel is a novel anti-microtubule agent that promotes the assembly of microtubules from tubulin dimers and prevents depolymerization by stabilizing microtubules. This stability results in inhibition of the microtubule network's normal dynamic reorganization, which is important for crucial interphase and cellular mitotic functions. However, paclitaxel causes irregular clusters or "bundles" of microtubules during mitosis throughout the cell cycle and several microtubule asters [14].

Mechanism of action: Paclitaxel interferes with microtubule growth's normal function. Although drugs such as colchicine induce in vivo depolymerization of microtubules, paclitaxel arrests their function by having the opposite effect; their structure is hyper-stabilized. It removes the capacity of the cell to flexibly use its cytoskeleton. Paclitaxel directly binds to the tubulin β subunit. Tubulin is mictotubules ' "building block," and paclitaxel binding locks these building blocks in place [15]. There is no ability to disassemble the resulting microtubule / paclitaxel complex. This adversely affects cell function as microtubules shortening and lengthening is necessary for their role as a transport highway for the cell. For example, during mitosis, chromosomes depend on this property of microtubules. More work has shown that paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to a protein that prevents apoptosis called Bcl-2 (B-cell leukemia 2) and thus arrests its function.

3.Tegafur:
Tegafur is a Fluorouracil (5-FU) prodrug, an antineoplastic agent used to treat various cancers, including advanced stomach and colorectal cancers. It is a pyrimidine analog used as an active chemotherapeutic agent in conjunction with Gimeracil and Oteracil in combination therapies, or as Tegafur-uracil when combined with Fluorouracil [16]. Tegafur is typically administered in conjunction with other drugs that improve the bioavailability of the 5-FU by blocking the enzyme responsible for its degradation or by maintaining high levels of 5-FU at a lower dose of tegafur in order to limit the toxicity of 5-FU. Once converted and bioactivated to 5-FU, the drug mediates anticancer activity by inhibiting thymidylate synthase during the pyrimidine pathway of DNA synthesis.

Mechanism of action: The conversion of 2'-deoxyurindylate (dUMP) to 2'-deoxythymidylate (dTMP) is necessary to drive the DNA and purine synthesis in cells 4. Thymidylate synthase catalyzes the conversion of dUMP to dTMP, a thymidine triphosphate (TTP) precursor, one of the four deoxyribonucleotides required for DNA synthesis. Tegafur is transformed into the active antineoplastic metabolite, fluorouracil (5-FU), after administration into the body. 5-FU is phosphorylated in tumor cells to form the active anabolites, including 5-Fluorodeoxyuridine monophosphate (FdUMP). The FdUMP and reduced folate are bind thymidylate synthase forming a ternary complex which inhibits DNA synthesis. In addition, 5-fluorouridine-triphosphate (FUTP) is incorporated into RNA causing disruption of RNA functions [17].

4.S-1:
S-1 is a mixture of three compounds including tegafur, gimeracil and potassium oteracil (at a molar ratio of 1:0.4:1). Tegafur is a 5-fluorouracil (5-FU) prodrug, an oral fluoropyrimidine, developed as a substitute for 5-FU infusional therapy. The most reasonable first-line standards for unresectable advanced gastric cancer in Japan are S-1-based chemotherapy and the combination of S-1 and cisplatin. In Western countries, however, the application of S-1 to gastric cancer has been delayed. One reason for this delay is that tegafur's pharmacokinetics are impaired by cytochrome P-450 2A6 polymorphisms and therefore 5-FU plasma levels are more likely to increase in Western patients [18].

A nanocarrier is nanomaterial used for another substance, such as a drug, as a transportation module. Micelles, polymers, carbon-based materials, liposomes and other substances are commonly used nanocarriers. For their use in drug delivery, nanocarriers are currently being studied and their unique characteristics show potential use in chemotherapy. Cancer nanotherapy is evolving rapidly and is being used to overcome many

drawbacks of traditional drug delivery systems such as non-specific biodistribution and targeting, lack of water solubility and low oral bioavailability [19]. Advances in protein engineering and materials science have contributed to new approaches to nanoscale targeting that can bring new hope to patients with cancer. A number of therapeutic nanocarriers for clinical use have been approved. Nanoparticles have been designed to improve their biodistribution and increase their circulation time in the bloodstream to optimize the size and surface characteristics. Nanotherapeutics are able to carry loaded active drug to cancer cells by selectively using the particular pathophysiology of tumors, such as their enhanced permeability and retention effect. In contrast to this passive targeting mechanism, the specificity of these therapeutic nanoparticles is magnified by effective targeting approaches using ligands or antibodies directed against selected tumor targets. The use of nanoparticles can also overcome or reduce drug resistance, another barrier. Multifunctional and multiplex nanoparticles are now being widely studied and as the next generation of nanoparticles are on the horizon, enabling tailored and specialized cancer treatment.

Protein nanoparticles:

Ideally, anticancer agents should first be able to reach the target tumor tissues after administration by passing by different barriers in the body with minimal loss of blood flow content or activity in order to be effective in cancer treatment. Second, they should have the ability to selectively kill tumor cells after reaching the tumor sites without having an adverse effect on normal cells. Second, to have the desired therapeutic effect, they should be released in a controlled manner. Nanoparticles tend to have the ability to fulfil these criteria as effective drug carriers for cancer treatment through particle size and surface modifications. Protein-based nanoparticles are particularly interesting as they are relatively safe and easy to prepare and can be easily monitored for their size distribution. They can also be modified to include functional and targeting capabilities in various modifications. The albumin-bound nanocarrier system (130 nm) is a protein-based nanocarrier system that has had an impact on cancer therapy. To promote drug targeting ability, protein nanoparticles have been chemically modified to incorporate targeting ligands that recognize specific cells and tissues [20].

Albumin:

Albumin is a protein from a variety of sources, including egg white (ovalbumin), bovine serum albumin (BSA), and human serum albumin (HSA). Albumin is a major soluble protein in the circulation system and contributes to maintaining osmotic pressure and binding and transporting nutrients to the cells. It is understood that many medications and endogenous molecules bind to albumin [21]. This protein is easily soluble in water and diluted salt solution. Albumin is used as a storage and transporter protein. Albumin's high solubility (up to 40% w / v) at pH 7.4 makes it an appealing macromolecular carrier that can handle a wide range of drugs. It is stable in the pH range from 4 to 9 and can heat up to 10 hours at 60 C without any adverse effects. Albumin is widely used in nanospheric and nanocapsulal preparation. These albumin nanocarriers are biodegradable, easy to prepare, and have on their surface well-defined sizes and reactive functional groups (thiol, amino, and carboxyl) that can be used for ligand binding and other surface modifications. Albumin nanoparticles release of drugs can be achieved naturally by digestion of protease [22].

Elastin:

Elastin is an essential component in connective tissues that is elastic and, after stretching or contracting, helps most tissues in the body to regain their form. Elastin is produced by crosslinking of its soluble precursor tropoelastine by lysine-mediated. Tropoelastin is a protein of 60–70 kDa, the length of which depends on its alternative splicing [23,24]. Tropoelastin exists in two forms as a monomer in solution: an open globular molecule and a polypeptide distended. α-elastin and elastin-like polypeptides (ELPs) are the two types of elastin-derived polypeptides used for drug delivery applications. α-Elastin, one of the soluble elastin-related polypeptides, has a unique characteristic of being aggregated under a specified concentration and temperature condition known as the cloud point (CP). When the solution temperature rises above the CP, the complex self-assembly process that leads to aggregation begins with α-elastin. ELPs are repetitive sequence peptide polymers (Val-Pro-Gly-Xaa-Gly)n where Xaa is a guest residue and n is the number of repetitive units. These polypeptides are derived from tropoelastin and undergo a transformation in the inverse step that can be used to facilitate self-assembly depending on temperature. Such ELPs are strongly soluble under a tunable transition temperature (Tt). They co-occur in a secondary aqueous phase above Tt. This phase separation can be used in a process called reverse transition cycling to purify ELPs and their fusion proteins [25].

Gelatin:

Gelatin is one of the protein materials that can be used to make nanoparticles. It is one of the most commonly used animal proteins obtained through

controlled collagen hydrolysis, a major component of skin, bones, and connective tissues. After either acid or base hydrolysis, two different types of gelatin, A and B, can be produced, resulting in proteins with different isoelectric point (pI), molecular weight, amino acid composition, and viscosity. For example, the pI of gelatin type A is 7–9, whereas the pI of gelatin type B is 4-5. Gelatin is generally considered a safe excipient for use in pharmaceutical preparations approved by the United States FDA. Including pharmaceuticals, since the early days of drug product development, gelatin has long been recognized as a biodegradable material. It is non-toxic and easy to be chemically related or modified [26-29]. Therefore, it has enormous potential to be used to prepare drug delivery systems such as microspheres and nanoparticles Gelatin has several other advantages including: (a) it is inexpensive, (b) it can be sterilized and nonpyrogenic, and (c) it has low antigenicity. A key feature of gelatin is its high content of glycine, proline (mainly as hydroxyproline) and alanine amino acids[30-33]. Gelatin has many ionizable groups such as carboxyl, amino, phenol, guanidine, and imidazole, which may be sites for conjugation or chemical modification. Compared to unmodified gelatin, the introduction of chemical crosslinking agents such as glutaraldehyde offers in vivo strength, form, and increased circulation time. The degree of crosslinking depends on the release of drugs from gelatin nanoparticles[34]. These crosslinking increases gelatin's integrity and efficiency, such as high temperature insolubility and reduced water swelling. Noncovalent cross-linking can be accomplished by interactions with electrovalence and coordination[35].

Whey proteins:

Whey proteins are a mixture of variable composition globular proteins with functional properties. Some whey protein products such as whey protein concentrate (WPC) and whey protein isolates (WPI) are processed as ingredients for food protein[36]. The key whey protein β-Lactoglobulin(BLG) regulates the functional properties of these products[37]. The whey protein and BLG preparations were used as a medium for the delivery of medicines[38]. The use of whey proteins and specifically BLG as a drug delivery carrier is mainly based on the confinement of these molecules in hydrogels of whey protein[39]. Hydrogels are a water-swollen polymer network that, while maintaining a network structure, can withstand a large amount of water. BLG is an effective candidate for the preparation of lipophilic compound drug delivery systems due to its ability to bind hydrophobic compounds. Native BLG is stable under acidic conditions and resistant to gastric protease digestion[40,41].

Soy proteins:

At present, soybean (Glycine max L.) is one of the most abundant plant protein sources. It has been documented that the enriched form of soy protein, known as soy protein isolate (SPI), has high nutritional values and functionality of ingredients. A wide range of soy protein applications have been well documented as food ingredients[42,43]. Furthermore, SPI has a balanced composition of polar, nonpolar, and charged amino acids, enabling the incorporation of a variety of drugs. Glycinine (MW = 360,000, about 60%) and β-conglycinine (MW = 180,000, about 40%) are the major components of SPI. SPI molecules tend to aggregate and form various structures, such as microspheres, hydrogels and polymer blends, after the introduction of dissolvent or crosslinking agents. Soy protein nanoparticles can be prepared either by desolvation from a freshly prepared SPI or by using a simple method of coacervation from the glycin fraction of defeated soy flour extraction[44].

Gliadin:

Gliadin is a gluten protein found in wheat that has bioadhesive properties and has been studied for applications of oral and topical drug delivery[45-49]. Gliadin is an attractive polymer that can adhere to mucous membranes for the preparation of mucoadhesive nanoparticles. Because of its biodegradability, biocompatibility, and natural origin, it was used as a nanoproduct material[50-57]. The hydrophobicity and solubility allow nanoparticles to be designed to protect and track the release of the loaded drugs[58]. For upper gastrointestinal areas, Gliadin nanoparticles show a great tropism. The composition can explain the high ability to interact with mucosa. This protein is rich with residues of neutral and lipophilic amino acids. Neutral amino acids can facilitate hydrogen bonding with the mucosa, while lipophilic residues can interact by hydrophobic interactions with biological tissues. In addition, gliadin contains groups of amines and disulphides capable of developing mucin bonds[59].

Legumin:

Legumin is one of the key proteins used to store pea seeds. It is an albuminous material that resembles casein and acts in seed meals as a source of amino acids containing sulfur. After aggregation or chemical crosslinking with glutaraldehyde, this protein may be self-assembled to form nanoparticles[60-64].

Methods of preparation of protein nanoparticles:

1. Emulsification method:

In this method , the nanoparticles are prepared by preparing an aqueous phase of protein with distilled water and an organic phase with a plant oil. Both the phases are mixed together in a container under homogenization to from an oil in water emulsion. This emulsion is added drop wise into preheated oil which has a temperature over 120^0C. This leads to evaporation of the water part and breaking of protein leading to formation of nanoparticles which are isolated[65].

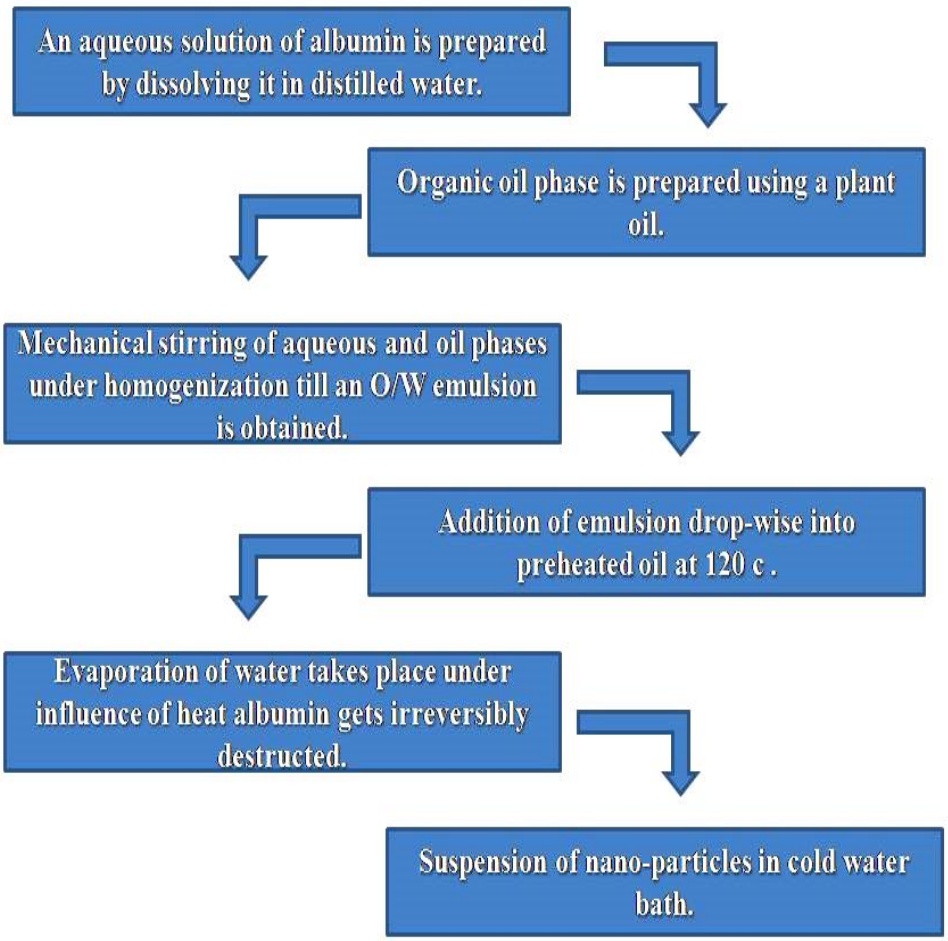

Fig 1: Emulsification method

2. Desolvation method:

In this method, the process of yielding nanoparticles is driven by using aid of specific agents called as desolvation agent such as alcohol or natural salt. An aqueous solution of protein is prepared and the desolvation agent is added to the aqueous solution pf protein. Due to the addition of these external agents, the protein deforms and changes it's structure. At a certain point, the protein forms clumps in the solution and leads to formation of nanoparticles due to the cross-linking. To separate the particles, the turbidity must be increased[66].

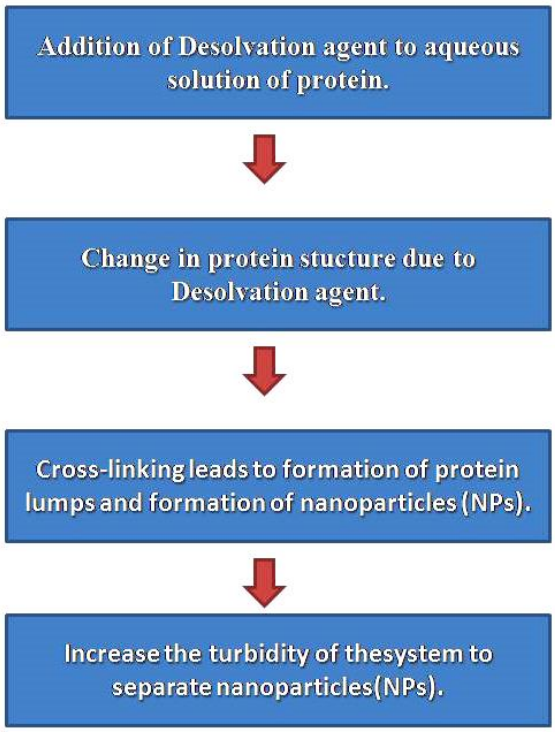

Fig2: Desolvation method

3. Complex coacervation method:

This approach uses two opposite-charge organic biodegradable polymers[67]. Alginate and gelatin are one commonly used pair of such polymers. Using this example, in order to obtain visible positive charges along its chemical structure, gelatin is first dissolved into water at an acidic pH, generally below 6. Alginate is then dissolved at a simple pH, usually above 8, in a separate water solution, in order to obtain negative charges along its chemical structure .The active compound to be encapsulated with this solution is then mixed intensively. Upon strong homogenization, under intense mixing, the alginate phase is poured into the gelatin phase and the temperature rises until the alginate-gelatin reaction occurs[68,69]. The polyanionic-polycationic insoluble polymer formed around the active compound allows the materials to be encapsulated. Because this polymer is made of ionic bonds and not covalent bonds, a further stage of reticulation is required to insolubilize the membranes completely.

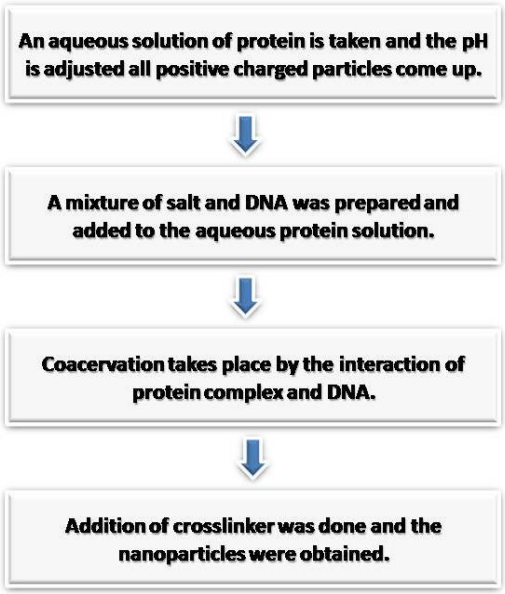

Fig3: Complex coacervation

4. Electrospray method:
The method of electrospray is one of the most effective techniques for preparing nano particles. The device contains a syringe pump with a silicone solution linked to the power supply of high voltage, which is the operational electrode. A metal foil collector as the ground electrode mounted opposite functions. The flow rate and the voltage applied are adjusted depending on the type of electro spraying solution used[70-73]. Thanks to the surface tension, the liquid flowing from the nozzle into the electric field forms tailor-made pipe. Electrosprayed nanoparticles may encapsulate drugs and could be specific drug carriers due to their active surface absorption, binding or complexation with the drug. On the other hand, nanoparticle size plays an important role in therapeutic treatment, particle size is one of the factors that determines the velocity of the drug carrier, the specificity of binding or adhesion and reactivity. Nanoparticles target the neoplastic cells by either active targeting or passive targeting which provides the insight of tumour targeted delivery of the drug.

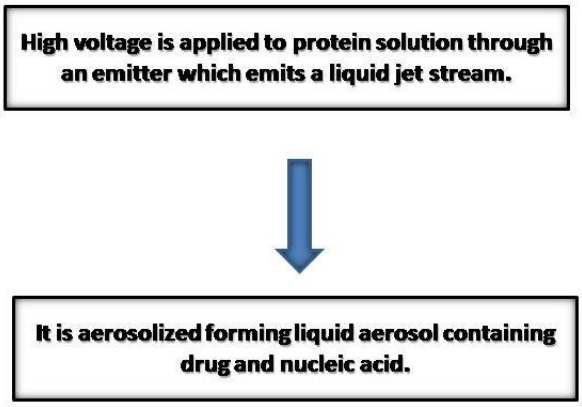

Fig4: Electrospray method

Active Targeting:
Nanoparticles containing the chemotherapeutic agents are designed to interact directly with the defective cells in the case of active targeting. Active targeting is based on the identification of molecules[74]. The nanoparticles ' surface is therefore changed to target the cancer cells. Targeting agents are usually attached to the molecular recognition surface of nanoparticles. Designed nanoparticles target cancer cells either by association with ligand receptors or by recognition of antibodies[75] .Cancer cells have certain unique properties that differentiate them at molecular level from healthy cells. Many receptors are over-expressed on their surface, allowing the distinctive feature. Attaching the complementary ligands to the nanoparticles surface makes them capable of targeting only the cancer cells. Once the nanoparticles bind to the receptors, they undergo rapid receptor-mediated endocytosis or cell phagocytosis, leading to the internalization of the encapsulated drug by cells.

Passive targeting:
Through passive targeting, nanoparticles can also target tumor. When apoptosis in cancer cells is interrupted, they continue to suck nutritional agents abnormally through the blood vessels creating large and leaky blood vessels around angiogenesis-induced cells. Due to defects in the basement membrane and reduced numbers of pericytes lining rapidly prolifying endothelial cells, leaky blood vessels are formed. This increases the permeability of molecules to pass through the wall of the vessel into the tumor cells surrounding the interstitium [76,77]. The pores range from 100 to 780 nm in leaky endothelial cells. Nanoparticles below this scale can therefore easily pass through the pores. As a result, clustering around the neoplastic cells facilitates the efflux of nanoparticles[78-80]. Nanoparticles may be directed at different capillary endothelium areas to concentrate the drug within a particular organ and perforate the tumor cells through passive diffusion or convection. Lack of lymphatic drainage facilitates the process of diffusion. The tumor interstitium consists of a network of collagen and a fluid-like gel. The fluid has strong interstitial pressures that hinder the molecules ' internal flux. Tumors often lack well-defined, leaky vasculature lymphatic networks. Therefore, in the tumor interstitium, drugs reaching the interstitial region may have prolonged storage periods. This feature is called the enhanced effect of permeability and retention and promotes the accumulation of tumor interstitial material. By through the permeability and persistence effect, nanoparticles can easily accumulate and then spread into the cells[81,82].

Rational for Developing for Protein Nanoparticles for Tumor Targeting
It is possible to achieve improved efficacy and safety of cancer therapy by rationally designing protein nanoparticles based on their behaviours in the tumor microenvironment, and based on cancer cell biology. Furthermore, multifunctional protein nanoparticles which are capable of carrying both therapeutic and diagnostic agents are now being investigated for more successful cancer treatment. Although the application of protein nanoparticles for cancer therapy has already produced some

exciting results and holds even greater promise in the future, there is still a lack of comparative data on the performance and therapeutic efficacy of protein nanoparticles and other existing delivery systems and is a much-needed area of field research.

CONCLUSION:

The utility of protein nanoparticles has been the most beneficial step which needs to be taken in order to avoid the circumstances of nanotoxicity caused due to the use of organic and inorganic nanoparticles and further protein nanoparticles could be synthesized from biological systems and they are also easy to administer without causing any adverse effects due to their presence in the body. We hypothesize that the usage of protein nanoparticles in delivering anti-cancer drugs like the 5FU drugs directly to the tumor would help in prolonged release of the drug at the target site due to its anti-neoplastic activity which decimates the cancer cells in the gastrointestinal tract.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors Contribution:

1. Arjun Sai Sreekar Aeila - Corresponding Author : Conceptualization and prodrugs and its overview, mechanisms of 5FU prodrugs.
2. Syed Tazib Rahaman : Protein nanoparticles and their use as potential carriers and synthesis of protein nanoparticles.
3. Tirandi Manohara Sai : Tumor targeting and the types of targeting drug delivery to tumors.

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