Original Article

Study of Silybinin Plant Effective Substance for use in targeted liposomal nanoparticles in the treatment of liver cancer

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Abstract

Nano-liposomes are spherical nanoscale capsules with a lipid membrane that are studied as drug carriers to improve the delivery of therapeutic agents. This research is aimed to investigate the targeting of liposomal Nano-system carriers of silybin herbal drug for delivery to liver cancer cells. Silibinin is one of the anticancer drugs that its anti-tumor property reduces N- nitrosodiethylamine in liver carcinoma cells. Small polyunsaturated silicone carriers using the lipid phase include DPPC phospholipid, phospholipid - DSPE-MPEG2000, and phosphate buffer (PBS) and HEPES as a hydrophilic phase. Encapsulating silibinin in Nanoliposomes improves its biological activity and increases silibinin stability in the blood. Particularly, the ant nociceptive potential of these drugs increases with the reduction of the size of the liposomes. The mean increase in liposomes is reduced by increasing pressure and the number of high-pressure homogenization cycles or centrifuge devices. Nylon liposomes containing cyclizine produced by targeted agents such as monoclonal antibodies HAb18 are targeted.

Keywords: Silibinin, Nano liposome, encapsulation, targeting, liver cancer

Introduction

Nano-drugs is one of the major achievements of the Nanobiotechnology field that will change the pharmaceutical industry over the next 20 years. So far, the greatest efforts have been focused on the scientific aspect and the development of nanotechnology technology and less attention has been paid to the commercialization aspect. Nanoparticle synthesis in developing and entering the semi-industrial stage European Science and Technology is Industrial. In the year of 2006, a global study by the European Technology and Observatory observes that more than 150 companies are active in the development of the field of treatment on a nanoscale scale, and 24 kinds of Nano-particle observatories (ESTOs) are used for clinical treatment [1]. Research at the New Technologies of Biological Engineering, University of Tehran, was used for the extraction of Fe3O4 iron oxide superparamagnetic nanoparticles in order to administer a dose of Doxorubicin to cancer cells [2].

Nano-liposomes in drug delivery:

Liposomes are colloidal particles in which a membrane, a double lip of a lipid composed of amphiphilic lipid self-shaping molecules, forms part of the water phase in which they are dispersed. Liposomes have a specific lipid profile and their properties are characterized by this lipid composition, particle size distribution, number of lamellae, and their internal and external watery phases. These characteristics all affect the stability and interactions of liposomes. The specific structure of the liposomes causes the loading of lipid-soluble drugs in two layers of phospholipid and water-soluble in mid-air spaces.

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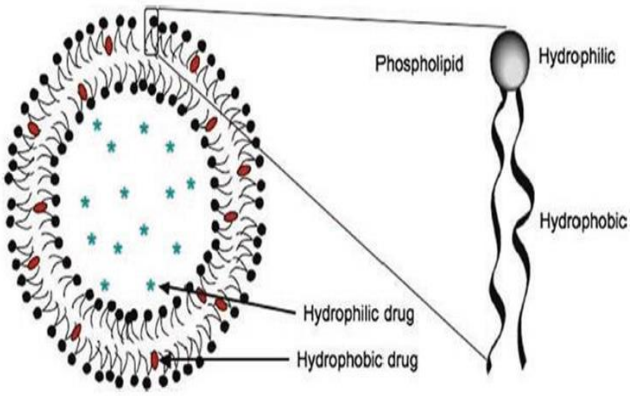
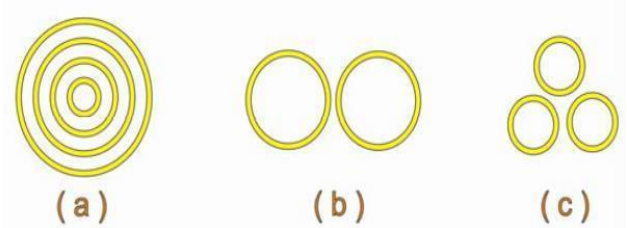


Pic. 1. A schematic illustration of the location of the loading of water-loving and fat-loving drugs inside the liposome

Types of Liposomes:

Liposomes are structurally composed of one or more concentrated concentric bivalve butter separated by aqueous or buffered portions. These spherical buildings can have a diameter of about 10 nanometers to 10 micrometers.

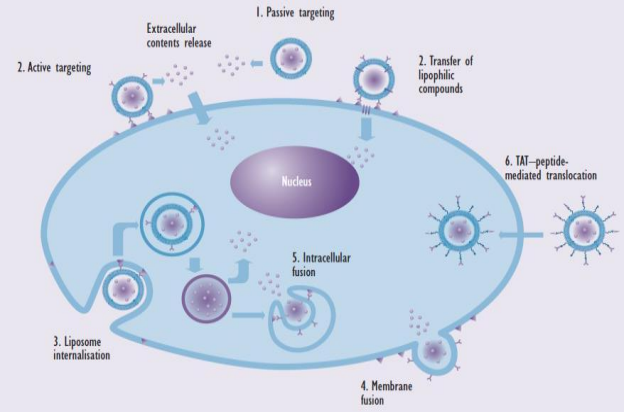
- **Multilayer vesicles (MLVs):** These liposomes have two layers of walls and were first described by Bingham, which are called multi-wall hawozykoles called MLVs.
- **Small single-walled vesicles (SUVs):** Sonication of the MLVs dispersion and reduction of their size are called vesicles with a double-headed cavity with a diameter of 25 nm to 50, which is called single-wall vesicles (SUVs).
- **Large single-walled vesicles (LUVs):** Since MLVs and SUVs have limitations as biomembrane membrane patterns or drug release systems, researchers have constructed liposomes with a double-skinned wall with a diameter of about 100 nm to 500 nm. These liposomes are called single-wall vesicles (LUVs).
- **Vesicles:** Some vesicles contain small vesicles, and these vesicles are called vesicles (MVV) to these liposomes. Figure 2 shows the types of these liposomes.



Pic. 2. Small single-layer liposomes (a). Multi-layer liposomes (b). Large single-layer liposomes (c).

Liposomes have been used extensively in the last 10 years as drug carriers, with 11 different formulations used for clinical applications. Some of their therapeutic uses include liposomal amphotericin, doxorubicin liposomal, and doxorubicin liposomal. In separate researches, researchers at the Center for the Research of New Technologies in the Life Science Engineering of the University of Tehran, pentyl nanoparticles and trained Nano-liposomes as carriers The drug doxorubicin was designed and manufactured for delivery to cancer cells. [3, 4] Liposomes are biocompatible compounds so that the encapsulation of decoxubomb in liposomes greatly reduces its side effects and its cardiovascular toxicity. Liposomes are also highly permeable biodegradable materials which, due to hydrophilic and hydrophilic parts, make liposomes a very good therapeutic carrier. Also, with the engineering of these liposomes, it is possible to carry pragmatic groups for binding to the target. Two of these are available on the US market. One of these products consists of peptidic liposomes accumulated with doxorubicin, known in the United States as Doxilles, and outside the United States are known as Kaelix. Pegylated and accumulated liposomes with Doxorubicin are also approved in Europe and are also produced and used under the name of Meish. Many of the compounds, including anticancer and antibiotics, have been used by liposomes. Widespread interest in the use of liposomes as drug carriers is due to their acceptable preparation in pharmaceuticals and drug delivery because they are both capable of increasing the scale and economically justifiable. A very promising strategy for delivering specific drugs to a wide range of sites and target organs is specific. In general, the three main ways to remove it Liposome is from the blood. First, through transplantation with plasma proteins, which is continued by the removal of liposomes by macrophages. The second case is lipid depletion or exchange during liposomal interactions with plasma proteins, which causes instability of the liposomal wall. Ultimately, direct identification and attachment to surface proteins in the cell lead to endocytosis by spleen macrophages or peptic copleptic cells. By reducing each of these three functions, it is possible to maintain the liposomal survival time Increased in the body. The spatial stability of liposomes and the absence of pregnant groups at the liposomal level also prolong their survival time in the blood. Temperature, pH, and ion strength influence the stability of liposomes. By reducing the size of the liposomes and adjusting their surface load to reduce absorption by RES, the newer liposomes of the type, with a small size of 80 to 200 nm of negative-lipid lipids, and or neutral and cholesterol is produced. Therefore, liposomes should not be swallowed by macrophages, since generally target cells are not RES cells. These systems provide the possibility of a constant and uniform plasma concentration of the drug for a specified period of time in the blood, which results in the removal of particle fluctuations and peak administration of the drug, which Lower side effects, more efficacy and patient comfort. There are some cases such as daunorubicin, which is a dinosaur liposomal drug for the treatment of cancerous tumors, or doxorubicin, which is used as a liposomal agent in the treatment of breast cancer, and other cases such as amphotericin (B) as an amniotic liposomal drug for the treatment of fungal diseases, Paclitaxel with high loading capacity in two layers of liposomal was about 3.5% in the treatment of breast cancer,

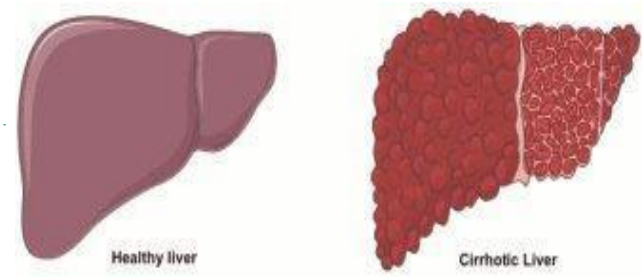
ovarian cancer and hypoxia for the treatment of hepatitis and many other drugs [5]. Liposomes are used as drug carriers in the formulation of injectable drugs, especially intravenous injection. When liposomes are supposed to be used as injectable products, if the size of the liposome is inappropriate, capillaries will be blocked. Systems that are suitable for drug delivery have a size less than 150 nm, and nanoparticles should not be smaller than 5 nm because they are excreted by the kidney system [6].



Pic. 3. Different pathways of drug delivery by liposomal systems

Liver Diseases:

- **Cirrhosis of the liver:** If the liver is worn due to chronic infection, it is called liver cirrhosis. Cirrhosis has many causes. In the United States, the most common cause is alcohol consumption and in our country, there are hepatitis B and C and D viruses, of which, of course, they end up with cirrhosis because of chronic hepatitis. Fewer are also infected with hereditary diseases such as fibrositis, an enzyme deficiency called alpha antitrypsin, galactosemia, and glycogen storage cirrhosis. Of non-common causes other reactions to some medications or long-term use of some medications and environmental toxins, as well as congestive heart failure, cause liver congestion and ultimately cirrhosis.
- **The cause of cirrhosis is:** Alcohol consumption, liver autoimmune diseases, hepatitis B or C, chronic liver inflammation, excess iron in the body (hemochromatosis).



Pic. 4. Liver with Cirrhosis (Right) Healthy Liver (left)

Patients with hepatitis B or C are at risk for liver cancer, even if they have no liver cirrhosis. For fatty liver, fatty deposits (mainly neutralized fatty acids such as triglycerides) in the liver that can be attributed to alcohol consumption, called "Alcoholic Fatty Liver," or without alcohol or in the presence of a very small amount It is called Alcoholic Non-Alcoholic Fatty Liver Disease [7]. The increase in the consumption of greasy and ready foods, and inappropriate activity or the proliferation of machine life has led to various illnesses. Liver involvement is one of the disadvantages of inappropriate food consumption. Unfortunately, fatty liver disease, which is relatively common today, is directly related to the weight of the patient and is increasing due to the increase in obesity in various societies.

liver cancer:

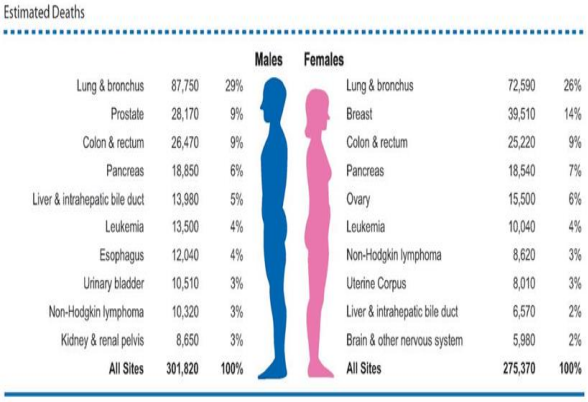
In normal mode, the cells grow and, by dividing, create similar cells. When the cells are aging, they die and young cells take their place. Sometimes this natural process is a problem, that is, new cells are produced that the body does not need to produce, or that cells that are old and should die remain active. The presence of such a condition produces a mass of cells called the tumor. Tumors may be benign or malignant. Benign tumors are not cancer and usually, doctors can remove them if necessary. In most cases, benign tumors do not recur after they are removed. The cells of a benign tumor do not spread in adjacent tissues or other parts of the body. Most importantly, benign tumors rarely endanger the patient's life. Malignant tumors are the same as cancers. Malignant cancer cells can spread to adjacent tissues or other organs. Also, cancer cells can spread to the body by bringing themselves to the blood or lymph. Actually this It is the same way that the tumor initiates its primary tumor to other organs of the body, creating another tumor (secondary tumor). This spread of cancer cells is called metastasis. Various cancers metastasize to different areas of the body. The cause of liver cancer is often due to liver cirrhosis.

Pic. 5. Cancer in the healthy liver tissue (left) and the formation of cancerous mass in the liver with cirrhosis (right)

Most primary liver cancers begin with hepatocytes (the most important liver cells). This kind of cancer is called hepatocellular carcinoma or malignant hepatoma, the most common form of liver cancer. Is cancer other than the four most common cancer-causing mortality in the world, which

is rapidly growing in the United States [8] ? Hepatitis B is 80% of the world's hepatocellular carcinoma [7]. Children can also develop hepatocellular carcinoma. One of the areas where liver cancer can spread (metastasis) is the lymph nodes of the bone and lungs. If this happens, the cells in these organs have the characteristics of the primary tumor (liver). In this case, the disease is the same as liver cancer, not bone cancer. Similarly, if cancer is transmitted from the body to the liver, we call it cancer of the liver that is different from primary cancer. The secondary liver cancer cells will be similar to the cells in the primary region. When the cells Cancer of the liver comes from other organs like the large intestine to the liver, called the tumor is a secondary liver tumor.

Table 1. Comparison of Cancer Types Based on New Patients and Death Rate by Gender, United States of America 2012.



Introducing Silybum marianum:

The herb of Silybum marianum has been used for many diseases for centuries. It is a native to the Middle East, Europe, and North America. The active ingredient of this plant is a combination of silymarin complex, which has a strong antioxidant effect that protects the body from cellular damage caused by free radicals. Medicinal plants have an important role in the pharmaceutical and health industries due to their lack of chemical compounds. In medicinal plants secondary metabolites, although essentially made by guiding genetic processes, their production is significantly influenced by environmental factors [9, 10].

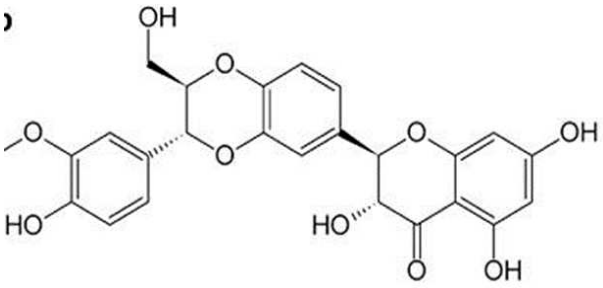
Pic. 6. a view of the Silybum marianum

The active ingredient of this plant reduces the growth of cancer cells and increases the effectiveness of chemotherapy drugs. Its active ingredients have antiviral properties that can stimulate the immune system. Reduces insulin resistance in patients who suffer from type 2 diabetes and cures gallstones and disease. The results of a study in Iran show that silymarin is beneficial in the treatment of type 2 diabetes in reducing blood glucose and HbA1c. The mechanism of the effect of silymarin is probably due to its antioxidant effects, the decrease in lipoperoxidation of the cell membrane and possibly other unknown mechanisms. Silymarin contains various compounds whose biological effects cannot be related to a single compound. Flavonoids are a group of silymarin compounds that, in addition to strong antioxidant properties, have been shown to stabilize cell membranes and increase cellular glutathione, which may be effective in reducing blood glucose and improving liver metabolism [11].

Antioxidant, anti-cancer and immune system stimulation of the active ingredients of Silybum marianum on liver cells:

The protective effect of polyphenolic extracts of mildew and sweet stem cells on rat liver cells has been investigated. The results show that these extracts have an effective protective effect on the damage caused by liver toxicity of histamine due to the antioxidant activity of polyphenolic compounds [12]. The combination of silymarin and marmosetum has anti-cancer and protective properties of the liver and has been well documented in clinical trials on various cancers such as skin cancer, prostate cancer, liver carcinoma, colon cancer, etc. [13]. This combination is divided into six other main items below:

silydianin, silychristin, silybin A, silybin B, isosilybin A and isosilybin B.



Pic. 7. Chemical structure of silibin The main substance of the silymarin complex.

Table 2. A summary of the clinical outcomes of the effects of the combination of silibinin and silymarin on organ cancers and various parts of the body.

Cancer site	Outcome with silymarin	Outcome with silibinin
Skin cancer (NMSC)	Positive	Positive
Prostate cancer	Positive	Positive
Breast cancer	Negative	ND

Colon cancer	Positive	ND
Lung cancer	ND	Positive/No effect
Bladder	Positive	ND
Ovarian cancer	ND	Positive
Hepatocellular carcinoma	Positive	ND
Pancreatic cancer	ND	ND

ND: Not determined

In the clinical study of the mechanism of the effect of silymarin on controlling liver carcinoma on male rats, silymarin has been shown to reduce the liver carcinoma by decreasing the amount of N-nitrosodiethylamine in the ongoing carcinoma, and in order to ensure more certainty, further complementary studies are needed in the near future [14]. Is a cancer-causing the agent and the liver is the main source of metabolism of this compound, the exacerbation of oxidative stress due to the metabolism of this compound and the production of reactive oxygen species (ROS) is a liver cancer agent that was found in research on rats in 2007 The silymarin combination has antioxidant ability and protective effect of the liver against the damaging effect of diethylnitrosamine on liver cells [15]. Silymarin is used as a restorer for free radicals and cellular membrane stabilization and cellular glutathione enhancer. Glutathione is responsible for toxin dehydration and radical free radical removal in the body. [16]. Silymarin can improve hepatocyte cells and rebuild liver cells by affecting the external membrane of the liver cells and preventing the penetration of the material, and also by increasing the synthesis of the ribosomal protein, thereby improving the hepatic function [17]. In 2009, an increase in anti-oxidant and anti-inflammatory effects of silymarin by selenium nanoparticles was investigated in improving the symptoms of inflammatory colitis [18]. The administration of seed extract of Silybum marianum for 60 months in chronic hepatitis B patients improved the symptoms of liver cirrhosis)13). In an article in the book Ethnomedicine in 2010, the alfalfa has been introduced as an Iranian plant species with the immune-stimulating ability [19].

Silymarin carrier delivery systems:

Paclitaxel is a micro-emulsion drug containing a combination of silymarin [20]. In 2011, a nano-carrier-based oil-based study was conducted to improve silymarin delivery with clinical and laboratory tests. Nanoparticles with a diameter of 40 to 70 nanometers had a good ability to improve the bioavailability of silymarin [21]. In another study, silymarin liposomal carriers with a mean diameter of 700 nm had better drug performance than free powder form [22].

Types of targeting of liposome nanosystems for drug delivery to the liver:

In 2010, at a University of North Saltar University School of Pharmacy Ph.D. in Boston, the United States aimed at three ways to change the level of Liposome containing deferoxamine for delivery to the liver and cardiac cells:

1. Using Polyethylene-Glycol Polymer to increase its circulation in the blood,
2. Add Aminophenyl-α-D-mannopyranoside to a solution containing deferoxamine for the purpose of monocyteing 2-layer lipid for binding to the manopes receptor of liver copleptic cells.
3. The use of DOTAP, which is a cationic lipid, is indicated for drug targeting by ion interaction with the hepatic and hepatocytes cells of the liver. Also, in order to quantitatively and qualitatively test, lipids are indicated with Rhodamine. Quantitative and qualitative studies have been performed in clinical and laboratory conditions using fluorescence microscopy [23]. In 2008, folic acid was used for treating and photographing liver cancer for binding to the cancer cell receptor [24].

Conclusion

Given that liver cancer is the second most common cancer in the world, it is important to find the appropriate treatment strategy for the most common type of liver cancer, which is hepatocellular carcinoma or malignant hepatoma. According to various studies, the combination of silibinin, an active ingredient of Silybum marianum, has anti-oxidant, anti-cancer and immune system properties, and because hepatitis viruses are one of the main causes of liver cancer, the active ingredients Silybum marianum can play an important role in treating cancer of the liver. Regarding the low stability and low silibinin content of the body, in research by the authors of the article at the Center for New Technologies of Biological Engineering, University of Tehran, nanoliposomes have been used as carriers for transferring the composition of silibinin to liver cancer cells. Purposeful Nanoliposomes are being developed using monoclonal antibodies to increase the body's complexion stability and effect on the treatment of liver cancer, which is hoping to be a valuable pathway in treating cancer of the liver in the future.

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