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Anti-Cancer Liposomes

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Abstract:- Tumor is one of the most wide-spread diseases across the planet, and along with this - the first in a row of the working-age population death causes. The pathogenesis of uncontrolled tissue growth and malignancy is still unexplored, making the tumor one of the most difficult, if not curable, diseases to treat. Tumor treatment today is carried out by extremely undesirable methods, the leading role among which belongs to chemo- and radiation therapy, the result of which is always inevitable - death. In this regard, there is an urgent need to find medicines that can save the lives hundreds of thousands of people without causing tangible harm to the body. Such drugs can be liposomes, which have long attracted the attention of scientists in the framework of the neoplasia treatment, but still remain at the research stage due to the high cost and complexity of industrial production.Using the data, accumulated to date on liposomes and their invasiveness in tumor tissue, we propose our own version of liposome production, which, due to its relatively low toxicity and ease of manufacture, has more chances of being introduced into widespread medical practice: these are liposomes based on the liquid phase from Chaga fungus and the lipid phase with natural peptides relatively easily extracted from plants and microorganisms.

Keywords:- *liposome, natural peptides, neoplasia*.

I. INTRODUCTION

An abnormally rapid growth of an abnormal cells population is called a tumor. A tumor can be benign or malignant. Cancer is a malignant neoplasm with loss of normal cell morphology. According to the type of cells affected by the process, carcinoma, sarcoma, lymphoma, germ cell tumors and blastoma can be distinquished. Carcinomas are sort into basal cell carcinoma, transitional cell carcinoma, adenocarcinoma, squamous cell carcinoma. Sarcomas are sort to bone sarcoma & soft tissue sarcoma. Lymphoma, as well as myeloma belong to leukaemia types. Cancers of brain & spinal cord are in a separate category of tumors [Alavi M. and Hamidi M., 2019]. Cancer is the world's leading cause of death, claiming thousands of lives each year, because the effectiveness of modern treatments for various types of cancer is low. Many antitumor agents are highly toxic, which limits their use in treatment. In addition, toxic drugs used in cancer therapy affect both cancer and normal cells. A number of cytotoxic chemotherapeutic agents are highly hydrophobic, which contributes to their long-term accumulation in the body with a subsequent harmful toxic side effects on normal organs and tissues, while the chemotherapeutic agents that have a short half-life, have low efficacy. The chemotherapy in tumor is also limited by the inability to reach required drug concentration in target tissue. However, the application of nanotechnology has led to the development of efficient drug delivery systems known as liposomes. In 1965, it was first demonstrated that spontaneously liquid-filled spheres of lecithin allow pass monovalent cations and anions; the process occurs similar to the diffusion of ions through a biological membrane [Bangham et al., 1965]. Then Gregoriadis et al. demonstrated the possibility of liposomes using for the selective delivery of antitumor and antimicrobial drugs to tissues [Gregoriadis, 1976a]. Liposomes, initially known as spherules, are drug delivery containers presented as vesicles filled with hydrophobic or hydrophilic reagents coated with phospholipid bilayers. Liposomes are used to deliver unstable antimicrobials, anticancer drugs protecting them from degradation. The content of the liposomes is protected from oxidation and degradation with phospholipid shield. Since they have low toxicity and are biocompatible, as well as biodegradable, they attract the attention of scientists around the world as potential anticancer agents.

Many therapeutic agents are effective only in certain concentration. For this reason, liposomal encapsulation, by reducing the clearance of drug, increases the time of its exposure to tumor. The phospholipid barrier protects the inner layer of the liposome until the contents of the liposome are delivered to the destination cell. This allows increase the therapeutic effect of the active substance without increasing the bouquet of side effects. Liposomes make it possible to vary the selectivity to certain cells of the body, provide a gradual release of the drug, the solubility of hydrophobic drugs, and reduce the toxicity of drugs in relation to normal tissues. İmportant requirements for liposomes are their biocompatibility, biodegradability, non-immunogenicity, non-toxicity and the ability to combine both hydrophilic and hydrophobic compounds. The appeal of liposomes lies in their composition making them biodegradable and biocompatible. They are mainly used as carriers of molecules that can be toxic to the body as a whole or are too labile and can be destroyed by the action of blood plasma enzymes. The duration of their circulation is provided by a change in their charge, size and lipid composition. Based on the size and the number of bilayers, liposomes can be sort into unilamellarand multilamellar; the half-life of liposomes depends on the the number of phospholipid bilayers. In unilamellar liposomes, the liposome has a single layer of phospholipid surrounding an aqueous solution. In multilamellar liposomes, several phospholipid layers separate the aqueous phases from each other. Such liposomes, when colliding with the target cell, exfoliate like cabbage. Sometimes, in multilamellar liposomes, several unilamellar vesicles are encapsulated

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within a larger liposome, forming drug-filled phospholipid spheres separated by layers of water. The number of bilayers affects the amount of drug contained in the liposomes.

Depending on the total charge, lipid composition and size, the properties of liposomes can vary significantly. The major components of liposomes are phospholipids and cholesterol, that are the major constituents of natural biomembranes. The choice of lipid composition is also critical for higher effective loading by the passive method. The chemical properties of these lipids control the behavior of liposomes. Unsaturated phosphatidylcholine forms more permeable bilayers, while dipalmitoylphosphatidylcholine forms a rigid, rather impermeable bilayer structure. Lipids can be natural or synthetic; but mostly they are biologically inert natural phospholipids with low immunogenic activity.Because of the amphiphilic properties of phospholipids, liposomes are considered as a versatile drug carrier container that encapsulate both hydrophobic and hydrophilic drugs. The hydrophobic regions of lipids are assembled into spherical bilayers called lamellae. Since lipids are amphipathic, bilayer phospholipid membrane liposomes can transport incorporated both aqueous and lipid drugs as target containers. Liposomes consist of one or more lipid bilayers that stepwise surround an aqueous units, & the polar groups are oriented to internal and external aqueous phases. Drugs are distributed heterogeneously in liposomes dependent on their different solubility: lipophilic drugs are located in the lipid bilayer, hydrophilic drugs are in the aqueous phase, and amphiphilic drugs are distributed between the lipid and aqueous phases. Encapsulation of lipophilic anticancer drugs can be achieved through hydrophobic interaction of these molecules with the bilayer of the liposomal lipid membrane [Allen, 1998] or by an active loading [Gubernator, 2011]; hydrophilic chemotherapeutic drugs can be encapsulated by trapping these drugs within the aqueous phase of the liposome.

All liposomes are prepared in 4 main steps: obtaining of lipids from an organic solvent; dispersion of lipid in an aqueous medium; purification of the resulting liposome; analysis of the final product. The methods of drug encapsulation into the liposomes can be sort into two subgroups: the passive loading, in which drug encapsulation occur during the vesicle formation process and the active loading, in which drug is entrapped after the formation of vesicles. Passive loading of the drug into liposomes consists of the encapsulation drug present in the hydrophilic phase. Passive loading methods include the mechanical dispersion of the drug, solvent dispersion method and removal of unencapsulated material. Amid mechanical dispersion methods that include sonication; french pressure cell: extrusion; freeze-thawed liposomes etc., sonication is the most extensively used method. [Akbarzadeh, A. et al., 2013]. The size and polydispersion index of nanovesiclesmay be determined by dynamic light scattering, while the morphology of nanovesicles can be assessed using scanning electron microscopy. The capture efficacy can be determined by the ratio of the amount of unencapsulatedhydrophylic compounds to the initial amount of initially loaded.

In order to reduce the cost of liposomes and make them available to the public, inexpensive, natural, readily available anti-cancer extracts should be included in their composition.This may be the **chaga extract,** which has already begun to be used as an antitumor agent. We offer chaga aqueous extract as an antitumor liposome filler, which is highly bioavailable and easy to obtain [Yakimov P.A. et al. 1961]. To prepare an easily accessible extract, Chaga fungus crushed to a diameter2-7 mm is poured with 60 ml of extractant (purified water) and extraction is carried out in a thermostat for 5 hours at a temperature of 70°C. After that, the aqueous extract is separated from the pulp by filtration, obtaining an extract from the first stage of extraction. The remaining pulp is poured with a new portion of the extractantin the amount of 40 ml and extracted for 5 hours in a thermostat at a temperature of 70°C. The extract is separated from the pulp by filtration, obtaining an extract from the second stage of extraction. The extracts obtained in the first and second stages of extraction are combined[Sbezhneva V.G. et al., 1994]. The therapeutic effect of liposomes is proportional to the hydrophylic drug volume contained in the nanoparticles. For better incorporation into liposomes, it is preferable to add maclura [Saloua F. et al., 2009] extract to Chaga fungus water extract to obtain negative charge inside liposomes; it should facilitate the combination & interaction of inner drug layer with cationic antitumor peptides. antimicrobial peptides are now being actively introduced into medicine in order to provide treatment for intractable diseases; among them there are also antitumor peptides with high activity and selective effect on the tumor, especially positively charged ones. Antitumor properties of maclura (McClure)isoflavoneshas been revealed, they are more pronounced in pomiferin[Ni Q. et al., 2013]. Active anti-neoplastic substances from maclura, p*omiferin* that is a prenylatedisoflavone and osajin, pass into tincture prepared by Sbezhneva V.G.et al. method[Sbezhneva V.G. et al., 1994], they are able to stop tumor growth. The McClure fruit is also rich in alkaloids, glucosides, lecithin, vitamin C, and flavonoid pigments [Saloua F.et al. 2009]. In addition to being anti-carcinogenic, the Mclure extract has a radioprotectiveeffect which is essential for cancer patients receiving radiation therapy. This will improve the active therapeutic load of liposomes. Hydrophilic drugs, which in our case is chaga&maclura extract, should be loaded into the inner core of liposomes by mixing with a hydrating buffer. The buffer promotes the formation of a thin hydrated lipid film. Eventually these drugs will be loaded into lipid bilayers. Chaga water extract molecules not used in the process of vesicle formation should be removed from the liposome suspension using dialysis or gel filtration chromatography. The therapeutic power of liposomes is proportional to the water volume contained in the lipid bilayer. The efficacy of drug encapsulation depends on the type and concentration of lipids, the size of liposomes, etc. [Pandey H. et al. 2016]. Since we are producing large 100-250 nm nanoparticles, we can use passive encapsulation to reduce the cost of liposomes.

Let us consider both passive and active targeting of liposomes to tumor cells. The active liposomal targeting means attaching specific ligands to the surface lipids of the nanoparticle [Federman N et al., 2010]. After active

targeting, liposomes conjugated with ligands can find their target cells. As the ligands, antibodies, antibody fragments, proteins, peptides, aptamers that can specifically bind to various motifs on cancer cell surface may be used. These liposomal-ligand complexes are distributed within the tumor tissue via receptor-mediated endocytosis. Some targeting ligands used in liposomal nanoparticles to achieve active targeting in malignancies are listed below. Inclusion of the holotransferrin on the surface of the liposome ensures its binding with transferrin receptor on the surface of hepatocellular carcinoma, small cell lung cancer, gastric cancer cells. Anti-MT1-MMP ligand may bind to MT1-matrix metalloproteinase and can deliver the drug to fibrosarcoma cells. Asn-Gly- Arg peptide, also termed NGR peptide attaches to another matrix metalloproteinase, namely aminopeptidase N in malignant cells of neuroblastoma&Kaposi sarcoma. IgG1 antihuman TfRscFv ligand binds to head and neck cancer cells, is attached by liver, lung, breast & prostate cancer cells [Yingchoncharoen, P. et al., 2016]. Folate receptor of lung multi-drug resistant carcinoma variant, oral carcinoma or squamous cell oral carcinoma, folate receptor expressing lymphoma, lung carcinoma, and mouse lymphoma cells can be attached to incorporated onto surface of liposomes folate [Tyagi N and Ghosh PC, 2011]. The CD44 receptor of Lewis lung carcinoma, adenocarcinoma, colon carcinoma, melanoma, leukemic cells can be a target for liposomes carrying hyaluronic acid on their surfaces İn breast cancer, glioblastoma are selected liposomes with Anti-EGFR-antibody that can attach to Anti-EGFR.HER2 of EGFR family is a target for Anti-HER2 scFvantibodes on surface of liposomes aimed lymphoma, gastric & breast carcinoma. VCAM-1 on the surface of ovarian cancer and multiple myeloma cells can bind to anti-VCAM monoclonal antibodies. Innovations in the production of liposomes start with the use of aptamers in the production of ligands. Thus, Sgc8 aptameris used as ligand to tyrosine kinase 7 present on the surface of ill T-cells in acute T-cell lymphoblastic leukemia & shows early onset of tumor inhibition. In liver cancer, siRNA targeting VEGF and/or siRNA targeting kinesin spindle protein binds to VEGF. $\alpha_v\beta_3$ integrin is a target for Arg-Gly-Asp ((RGD peptide) peptide in liposomal treatment ofmelanoma. [Yingchoncharoen, P. et al., 2016].

Of course, the targeted delivery of liposomes with ligands implanted in them seems more attractive, but let's not forget that today a tumor is one of the most common diseases, and we are obliged to treat both socially well-off and low-income patients. The main disadvantage of liposomes active targeting is the lack of cheap manufacturing methods leading to the high cost. Therefore, the choice of a method of passive drug delivery to target cells, based on the characteristics of malignantly modified tissues, seems more appropriate. In this work, we consider a method for manufacturing the *cheapest liposomes* using a medicinal substance obtained from a cheap available natural compounds.

The strategy for creating cheapest liposomes is based on their passive absorption by the tumor tissue. There are several reports of passive targeting by liposomes. For example, sclareol-loaded liposomes with an average particle size of 88 ± 5 nm has shown significantly higher growth inhibitor effect on A549 human lung epithelial cancer cells along with a sustained drug release after a period of 48 h compared to the free drug. In a similar study, a 50.5% inhibition of the growth of Hodgkin's lymphoma was observed with passive delivery of curcumin using liposomes. Passive delivery of temozolomidewith liposomes to glioblastoma and melanoma cells results in higher inhibition of proliferation and lower cytotoxicity than without liposomes. Passive uptake of liposomes predominantly by the tumor is provided taking into account the characteristics of feeding the tumor vascular system. The fact is that the pores in the vessels adjacent to the tumor tissue differ significantly from the endothelial pores of normal healthy endothelium. Overexpression of certain angiogenesis factors by tumor, such as vascular endothelial growth factor (VEGF), results in increased vascular permeability, resulting in increased penetration and retention, due to which there is an effect of increased permeability and retention in the tumor sites. That means significantly less fluid return to the lymphatic system, due to which liposomes up to 400 nm in size can effectively accumulate in tumor foci. Particles larger than 400 nm are characterized by difficult penetration into the vessels [BaruaS. &Mitragotri, S., 2014] and for this reason, it will be difficult to achieve an increase in their concentration in the intercellular substance surrounding the tumor, and treatment in this case will be ineffective, no matter how active the substance included in the liposome is. Maeda H. states that high molecular weight drugs accumulate in large quantities preferably in tumors. He coined the term "enhanced permeability and retention" effect [Maeda H., 2012]. Like the tumors they nourish, tumor vasculature is also immature & disorganized, and chaotic. In solid tumors, the capillary bed is built with a set of branching structures adjacent to vessels of disorganized size composed of loosely attached endothelial cells lacking pericyte support. Tumor vessels have large endothelial fenestrations ranging from 100 to 600 nm, which is why the permeability of tumor capillaries increase. There are reports, that the new tumor vessels developed during angiogenesis are irregularly shaped with discontinuous epithelium and range in size from 200 to 2000 nm [Pethe A. et al., 2018]. This in turn, leads to effusion of plasma proteins into the tumor and increased pressure in the intercellular matrix of tumor tissue. Using the "enhanced permeability and retention" effect, high molecular weight liposomes can be designed to preferentially penetrate tumors. To do this, liposomes must have an optimal diameter that allows them to overcome the vascular endothelial barrier and penetrate into the tumor interstitium. From this point of view, the size of liposomes is of great importance. We should keep in mind that fenestrated capillaries are found wherever active filtration or absorption occurs: small intestine, endocrine glands, kidney. The vessel fenestration found in muscles, skin, lungs, connective tissue and these endothelial cells contain pores between cells 50-60 nm & are more permeable than other normal vessels. Discontinuous endothelial cells found in the spleen and liver have pores up to 100 nm wide. This means that liposomes with less than 100 nm wide can cumulate in normal tissues, while those that are too large (>250 nm) will not be able to pass through the all desired fenestrations of tumor endothelial cells. Additionally,

liposomes that are too small (10 nm) will be quickly filtered out by the kidneys, there are also some reports about effective extravasation of vesicles with less than 200nm diameter. Considering all mentioned above factors we state that when creating liposomes, the diameter may be larger than 100 nm but not exceed 250 nm. This will facilitate the penetration and retention of dosage forms in the tumor tissue, while ensuring minimal invasiveness in healthy tissues.

When considering the utilization of liposomes by tumor tissue, it should be taken into account that liposomes interact with cells mainly in the following ways: formation of electrostatic bonds and hydrophobic bonds with cell membrane motifs; endocytosis by cells of the reticuloendothelial system, namely neutrophils and macrophages; fusion of the lipid bilayer of the liposome with the plasma membrane. Due to this, the fate of liposomes largely depends on the charge of its surface. Z- potential determines the total surface charge of liposomes, i.e., whether the liposome is cationic, anionic, or neutral in nature, since depending on the component used, liposomes can be neutral, negatively or positively charged. Charge is also important in the design of the tumor-targeting liposomes, because it can affect their circulation time and the potential for enhanced permeability. Anionic liposomes have the advantage of reducing self-aggregation in suspension and increasing non-specific cellular uptake. But this is true when it comes to cells without neoplasia. It has been established, that the tumor cell carries an additional negative charge on its surface due to an excess of phosphatidylserine.Normally, phosphatidylserine is located on the inner surface of the plasma membrane facing the cytoplasm [Schutters K. &Reutelingsperger C., 2010]. After the neoplastic process starts, this selectivity is lost [De M. et al., 2018], De M. etal.report that a phosphatidylcholine-stearylamine (PC-SA), induced apoptosis in majority of cancer cell lines. The change in charge on cancer cell surface allows the components of innate immunity, the antimicrobial peptides, to recognize tumor-modified cells. Such natural-derived antimicrobial peptides can replace expensive ligands and provide additional retention of drug-laden liposomes in the site of neoplasia.To neutralize negatively charged Maclura vinegar extract in the core of liposomes we need cationic compounds, which would significantly increase encapsulation efficiency due to enhanced active interaction between agents. The introduction of cationic antimicrobial peptides with antineoplastic properties, such as magainin2, should facilitate the formation of liposomes, enhance the therapeutic effect, and increase the affinity to the tumor [Boohaker R. J.. et al., 2012].It is possible to load the drug into liposomes using the active loading method, but this will increase the cost of the drug and accordingly, reduce the scope of its application. Magaininantimicrobial peptides drug development was founded by Magainin Pharmaceuticals (later called Genaera). Single magainin 2 was found to form pores ~2.8 nm in diameter in *B. megaterium* and translocated into the cytosol. The peptide significantly disrupted the cell membrane, allowing the penetration of a large molecule into the cytosol, which was accompanied by membrane budding and lipid turnover, mainly accumulating in mitochondria and nuclei [Imura Y. et al., 2008]. Such antineoplastic peptides as magainin inhibit the growth of tumor tissue in various ways [Ohsaki Y. et al., 1992]. The mechanism of action of some of them is based on the activation of immune cells in order to destroy tumor cells [Zhang, L.et al., 2019]; on the other hand, they destroy tumor cells by inducing necrosis or apoptosis in them [Kim JY et al., 2018]. Magainin also inhibits angiogenesis, prevents the formation of a vascular network that nourishes tumor tissue, and prevents metastasis [Al-Benna S. et al., 2011]. Another important mechanism of action of antineoplastic peptide magainin is based on the fact that it disrupts these processes by interfering with gene transcription and translation in the tumor cell [Huan, Y. et al., 2020].

Antimicrobial peptides directed against the tumor and naturally produced in organisms are distinguished by a high positive charge, provided by the additional arginine and lysine residues in them. Butarginine & lysine may cause hemolysis and, additionally, a large number of cationic liposomes can lead to an inflammatory tissue response because highly charged liposomes, either positively or negatively charged, can fix complement proteins [Federman N. & Denny C., 2010]. He et al. state that even when absolute zeta potential values were identical in nanoparticles, macrophages phagocytized a higher percentage of positively charged nanoparticles compared to the negatively charged [He et al. (2013]. Therefore, to prolong the action of the liposomes on the body, it is also advisable to use antimicrobial peptides with the introduced into them histidine, since it has a variable polarity and the ability to rearrange the charge depending on the charge of the molecules it interacts with. Currently, developments are also underway to obtain artificial antimicrobial peptides with the inclusion of histidine, which does not cause hemolysis, can acquire a positive charge interacting with cell membranes, has buffer properties at physiological pH, providing amphiphilicity and, therefore, a sufficiently high toxicity at low hemolytic properties of peptides, in which it is included. To reduce the high cost of liposomes, it is recommended to include highly specific and cheap antimicrobial peptides into liposomes. In the case of tumor therapy, cationic liposomes will be better absorbed, however, small cationic liposomes can be excreted by the kidneys, which have the ability to filter positively charged particles. A significantly large size of liposomes can solve this problem and prevent drug loss through the kidneys. Neutrally charged liposomes have the longest circulation time but tend to aggregation, which may limit their penetration into the tumor vasculature. This problem may be easily solved by incorporation histidine-containing antimicrobial peptides into the shell of liposomes.

The binding of charged liposomes to oppositely charged cell molecules can be controlled by changing the zeta potential of the liposomes [Smith MC et al., 2017]. Polyethylene glycol is another widely used liposome component, because it increases circulation time due to its stealth properties. But polyethylene glycol significantly affects the zeta potential of liposomes: it is –43 mV in liposomes without polyethylene glycol, but decreases with an increase in the content of this polymer, and can drop to –5 mV. In this regard, it is appropriate to recall the effect of cholesterol on the zeta potential. JovanovićA.et al. in 2017 studied the effect of different cholesterol content (0-50

mol.%) on membrane fluidity, vesicle size, and zeta potential of liposomes. They declare thatthe z-potential was negative in all cholesterol-incorporated liposome samples, and the highest absolute moduli were achieved at 50 mol % cholesterol in the sample. But Aramaki K. et al declare cationic liposomes can be prepared by mixing cationic surfactants with phospholipid liposomes, but their cytotoxicity will be high enough. Aramaki K. et al. claim that mixing quaternary ammonium monoalkyl and dialkyl chlorides or cholesterol with cationic liposomes increased the zeta potential of the liposomes from negative to positive values (more than +50 mV). Cholesterol also shifts the negative-positive transition point of the cationic fraction [Aramaki K. et al., 2016]. Optimum stability and fluidity were also achieved with 50 mole % cholesterol in the lipid phase.

Increasing of cholesterol in the liposome membrane can increase the size of liposomes. Cholesterol provides membrane fluidity, elasticity, permeability and stability to liposomes. JovanovićA.Et al. (2017) found that an increase in the cholesterol content in liposomes also reduced their fluidity and increased rigidity. At the highest cholesterol content (50 mol.%) significantly larger liposomes were obtained. Cholesterol can reduce the interaction of liposomes with certain proteins, making them less susceptible to phospholipase. This reduces the loss of phospholipids from liposomes and high density lipoproteins, and inhibits their digestion by macrophages. The cholesterol incorporation into liposomes inhibits their uptake by reticuloendothelial cells, and also reduces their interaction with certain proteins, affecting the uptake of liposomes by tissues. Freedom of rotation due to flipping motions in phospholipids creates liposomes with leaky properties. Cholesterol is the main component stabilizing the phospholipid bilayer of liposomes & preventing liposomes aggregation. Depending on the cholesterol content, the rigidity and fluidity of the phospholipid bilayer change as follows. When the mole fraction of sterol is 30-45% of the total liposome components, this provides optimal membrane fluidity and rigidity, along with good elasticity and permeability of the liposomes. However, it is too early to say that the optimal concentration of cholesterol has already been clarified. The maximum amount of cholesterol that can be introduced into the bilayers is nearly 50 mol%. Depending upon the rigidity and fluidity of bilayer, the molar percentage of cholesterol varies from 30-45% of total liposomes components [Magarkar, A. et al., 2014]. By the way, the concentration of cholesterol in the normal cell membrane is about 30–50 mol% of all lipid compounds. The most commonly used ratio of phospholipids to cholesterol is 2:1, or 1:1 ratio. When assembling liposomes, the only polar group of cholesterol (-OH group) is immersed in the polar head layer of the phospholipids in lipid bilayer. The rest cholesterol structure is the hydrophobic fused ring of cyclopentaneperhydrophenanthrene immersed in the interior of the lipid bilayers. The sterol carbohydrate tail at position C17 is also mixed with hydrophobic fatty acyl chains. Cholesterol makes the bilayer structure more compact and serves to fill the gap created by the imperfect packing of phospholipid molecules. The incorporation of cholesterol into phospholipid bilayers reduces the flip-flop and lateral movement of phospholipids in membranes.The rigidity of membraneimparted to liposomes by cholesterol reduces the leakage of encapsulated drugs.The percentage of cholesterol also affects the final phase transition temperature of the bilayer. Some studies have shown that cholesterol helps to protect the lipid bilayer from hydrolytic degradation. Sterols such as ergosterol, stigmasterol, lanosterol, β-sitosterol and cholesterol have been added to liposomes both to reduce membrane fluidity and increase the stability of the phospholipid bilayer and to reduce leakage of encapsulated active compounds.As a result of the interaction of cholesterol with membrane phospholipids, membrane adhesion increases, passive permeability for small molecules decreases, which ensures the preservation of the drug inside the liposome until the moment of interaction with the components of the tumor tissue, since cholesterol decrease the permeability of the bilayer for both non-electrolytic and dielectrolytic solvents. There is also a direct relationship between the cholesterol content of liposomes and the value of the gel-to-liquid transition temperature. It was reported, that when sterols are added, the Tm of the crystalline phase, which characterizes the transformation of the gel into a liquid, decreases. Cholesterol as a non-ionic molecule increases the highest zeta potential of cationic liposomes.

II. CONCLUSION

- The optimal liposome size for tumor targeting is 100-250;
- Chaga and Maclura extracts mixed with cationic antimicrobial peptides can promote the emergence of stable liposomes with active electrostatic interactions in the liposome core;
- Passive binding of liposomes requires the presence of a negative charge on its surface, and the inclusion of cholesterol in a ratio of 50 Mol%of all lipid compounds is the most appropriate.

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