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STUDY AND DEVELOPMENT OF TARGETED DRUG DELIVERY SYSTEMS AND DOSAGE FORMS

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ABSTRACT

Malignant cancer belongs to the number of the most widespread diseases in the world. With the purpose of increasing the efficiency of anti-cancerous medications significant attention is paid to their targeted delivery to the relevant organ, tissue and cell. High level of selectivity is reached when the active substance of the drug is transported by the liposomes. On the Black Sea coast of Adjara, Batumi, in botanical garden, grow up to 17 introduced species Magnolia. The bioactive components such as magnolol, honokiol, 4-O-metilhonakiol according to numerous pharmacological studies have shown cytotoxic, anti-stress, antidepressant, antioxidant, anti-inflammatory activity. It was found that leaves of the study objects contain liriodenins and lanuginosin, in the crust off branches was established content of liriodeninis and d-kaaverin, bark contains liriodenin, kaaverin and magnophlorine. By mean of technological and pharmacologic researches, innovative liposomal solution has been developed, which is characterized by selective cytotoxic activity and is safe for healthy – normal cells. It is offered total alkaloids obtained from Magnolia oficinalis leaves and Magnolia glauca cortex, as liposomal medication- lyophilized powder, with the average diameter of liposomes 120nm, with 62% involvement of active substance.

KEYWORDS: Targeted drug delivery, Cytotoxic, Development of dosage form.

INTRODUCTION

One of the main problems of drug therapy is selective (deliberate) drug delivery to body. Majority of the traditional drugs impact the target giving the desirable results, but they simultaneously impact on other body systems causing some complications.

Malignant cancer belongs to the number of the most widespread diseases in the world. Mortality caused by the malignant tumors is presented by disturbing statistical data. As WHO forecast notifies, number of victims of malignant cancer will be 9 million in 2015 and approximately 11 million in 2030. Among all other methods of treatment of cancer, chemotherapy tends to be one of the most widely applied means. Out of all types of chemotherapeutic drugs widely used in oncology great part comes from the compounds and derivatives obtained from medicinal plants.

With the purpose of increasing the efficiency of anti-cancerous medications significant attention is paid to their targeted delivery to the relevant organ, tissue and cell. High level of selectivity is reached when the active substance of the drug is transported by the liposomes. By means of endocytosis cytotoxic substance encapsulated liposome is absorbed by cancerous cell. The force of lysosomes will destroy the system in the cell and cytotoxic substances will be released, which further will ensure the death of cancerous cell.

On the Black Sea coast of Adjara, Batumi, in botanical garden, grow up to 17 introduced species Magnolia.

The bark and seeds of Magnolia trees were and are widely used in traditional medicine of Korea, China and Japan in the form of decoctions, tinctures, extracts of bark of the trunk and branches, roots and fruits rarely. The bioactive components such as magnolol, honokiol, 4-O-metilhonakiol according to numerous pharmacological studies have shown cytotoxic, anti-stress, antidepressant, antioxidant, anti-inflammatory activity. There is evidence of Magnolia drug treatment in progressive paralysis, and some consequences of brain inflammation. What about the one of the main types of alkaloids in Magnolia - liriodenine, it showed antimicrobial, antifungal, cytotoxic activity.

Research on the content of alkaloids in the leaves of M. obovata and M.grandiflora, introduced in Georgia, were launched in the Tbilisi State University named after I.

Javakhishvili (department of organic chemistry and natural products), together with the Institute of Plant Chemistry. SY Yunusov AN SLM.

Vegetative organs of Magnolia species introduced in Batumi botanical garden: Magnolia obovata, M. grandiflora, M. glauca, M. Stellata, M. Soulangeana, M. officinalis, M. coco, was examined by a group of aporpinis alkaloids content. Researches were held using chemical, physical and instrumental methods of analisys. It was found that leaves of the study objects contain liriodenins and lanuginosin, in the crust off branches was established content of liriodeninis and d-kaaverin, bark contains liriodenin, kaaverin and magnophlorine. The aim of the study was immobilization in liposomes of cytotoxic alkaloids medications obtained from Magnolia oficinalis leaves and Magnolia glauca cortex, and *in vitro* investigation of cytotoxic activity on cancerous and healthy cells.

The objects of research

Alkaloids obtained from Magnolia oficinalis leaves and Magnolia glauca cortex and their Liposomal Solution. The lipid - Lipoid S75 manufacturer LIPOID AG (Steinhausen, Switzerland)**.**

Methods of research

- Assays were performed on Human lung carcinoma (A-549), human colon adenocarcinoma (DLD-1) and human normal fibroblasts (WS1) cell lines, which were obtained from the American Type Culture Collection (ATCC);
- For evaluation of cytotoxicity of samples fluorescence of resasurine was measured on an automated 96-well Fluoroskan Ascent Fl^{TM} plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm;
- The size of the particles of the liposomal solution was measured by electron microscope - Jeol (jem-100SX electron microscope).
- Zeta-potential and polydispersity of the liposomal solutions were measured by Nano ZS Zen3600, Malvern Instruments, Malvern, UK on 630nm wavelength.
- Cytotoxic research was performed in laboratory of Biology, at University of Quebec in Chicoutimi (Chicoutimi, Quebec, Canada).
- In vitro cytotoxic activities of total alkaloids and were assessed against human lung cancer (A-549), human colorectal cancer (DLD-1) and normal skin fibroblasts (WS-1) using resasurin reduction test.

Cell culture

The human lung carcinoma A549 (#CCL-185), colorectal adenocarcinoma DLD-1 (#CCL-221) and skin fibroblast WS1 (#CRL-1502) cell lines were all obtained from the American Type Culture Collection (ATCC, Manassas, USA). Cell lines were grown in minimum essential medium containing Earle's salts (Mediatech Cellgro, Herdon, USA), supplemented with 10% fetal calf serum (hyclone, Logan, USA) 1* solution of vitamins, 1* sodium pyruvate, 1* nonessential amino acids, 100IU of penicillin and 100 µg/ml $^{-1}$ of streptomycin (Mediatech Cellgro). Cells were cultured at 37^{o} C in a humidified atmosphere containing 5% $CO_{2.}$

Cytotoxicity assay

Exponentially growing cells were plated at a density of 5*10^{3} cells per well in 96-well microplates (BD Falcon) in 100 µL of culture medium and were allowed to adhere for 16h before treatment. Then the cells were incubated for 48h in the presence or absence of 100 µL of increasing concentrations of extract, fraction or pure compounds dissolved in culture medium and DMSO. The final concentration of DMSO in the culture medium was maintained at o.25% (v/v) to avoid toxicity. Cytotoxicity was assessed using the resazurin reduction test. Fluorescence was measured on an automated 96-well Fluoroskan Ascent Fl plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm.

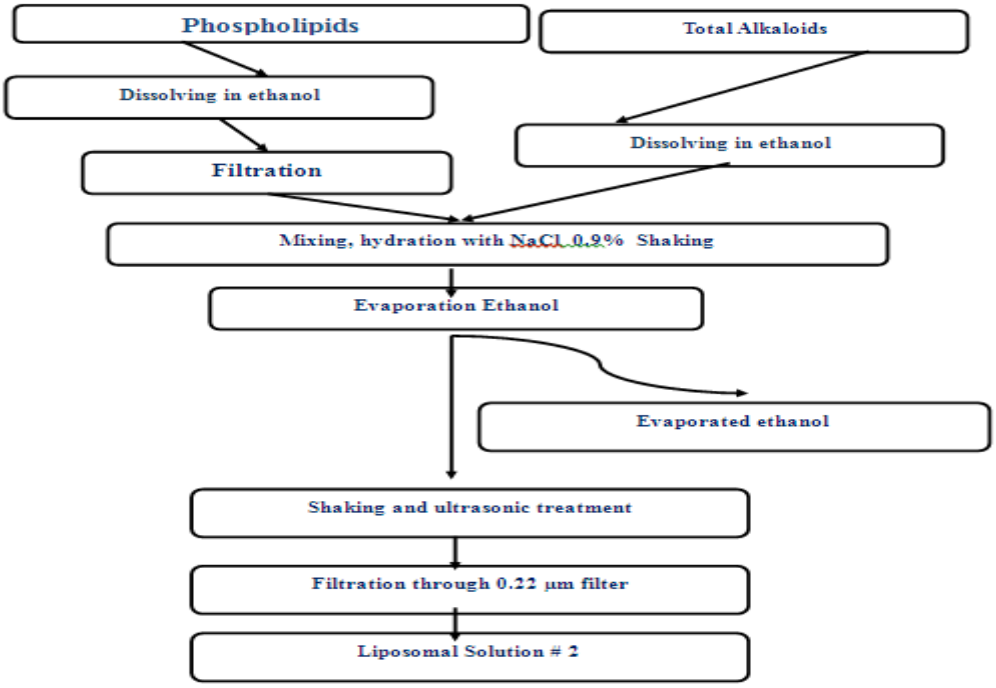
Results are given below:

The objects	Resazurine			Hoechst		
	A-549	DLD-1	WS-1	A-549	DLD-1	WS-1
Magnolia oficinalis alkaloids	16 ± 4 µg/ml	20 ± 4 µg/ml	18 ± 3 µg/ml	43 ± 3 µg/ml	54 ± 5 µg/ml	54 ± 6 µg/ml
Magnolia glauca alkaloids	29 ± 4 µg/ml	6,6 ±0,6µg/ml	7,2 ± 0,9µg/ml	27,4 ± 0,9 µg/ml	13,5 ± 0,5 µg/ml	17 ± 1 µg/ml
Etoposide	1,9 ± 0,3 µM	2,4 ± 0,4 µM	2 ± 3 µM	1,9 ± 0,3 µM	2,4 ± 0,4 µM	2 ± 3 µM

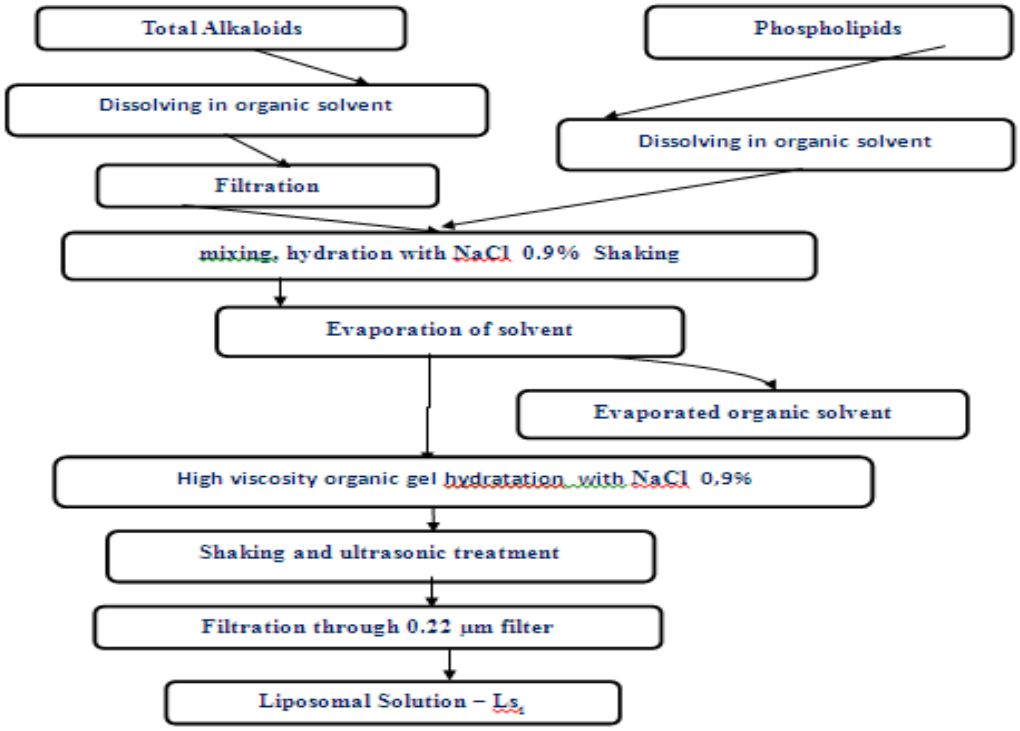
Results are expressed as the concentration of product inhibiting cell growth by 50% (IC50). Etoposide was used as positive control.

Liposomal solution was prepared by 2 methods _ 1. Modified shaking method (Ls1) and 2. Ethanol injection method (Ls2). Technological scheme of these methods are given below

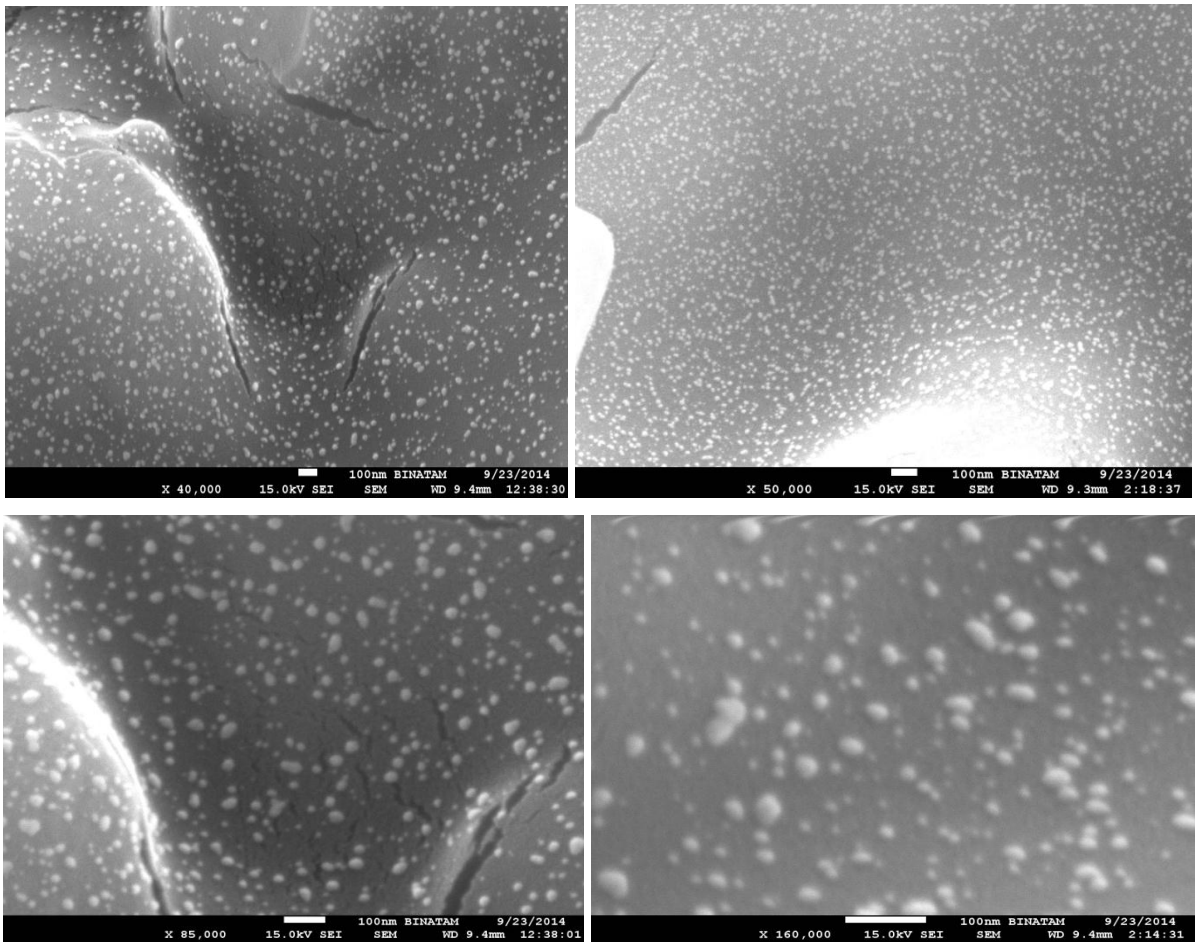
Technological scheme of preparation liposomal solution by Modified shaking method



Modified shaking method (Ls1)



Determination of Magnolia oficinalis leaves and Magnolia glauca cortex total alkaloids Liposomal Solution particles size with electronic microscope



Result of research of Liposomal solution cytotoxic activity of total alkaloids from Magnolia oficinalis leaves and Magnolia glauca cortex prepared by Modified shaking method

(Ls1)

Objects of research:	Resazurine			Hoechst		
	A-549	DLD-1	WS-1	A-549	DLD-1	WS-1
Magnolia oficinalis total alkaloids liposomal sol.	16±4 µg/ml	20±4 µg/ml	>200 µg/ml	43±3 µg/ml	54±5 µg/ml	>200 µg/ml
Magnolia glauca total alkaloids liposomal sol.	29±4 µg/ml	6,6±0,6 µg/ml	>200 µg/ml	27,4±0,9 µg/ml	13,5±0,5 µg/ml	>200 µg/ml
etoposide	1,9 ± 0,3 µM	2,4 ± 0,4 µM	2 ± 3 µM	1,9 ± 0,3 µM	2,4 ± 0,4 µM	2 ± 3 µM

Filler substances influence on alkaloids involvement in liposomes:

Filler substance	Before Lyophilization		after Lyophilization	
	Liposome sizes, nm	involvment%	Liposome sizes, nm	involvment%
Isomalt 10%	115±5	68±3	120±4	62±4
Lactosa 10%	125±4	64±4	135±5	58±5

RESULTS

By pharmacologic researches has been proved cytotoxic activity of total alkaloids obtained from Magnolia oficinalis leaves and Magnolia glauca cortex which are introduced in Georgia. Has been developed modified technologies for obtaining liposomal complex-systems.

By mean of technological and pharmacologic researches, innovative liposomal solution has been developed, which is characterized by selective cytotoxic activity and is safe for healthy – normal cells.

It is offered total alkaloids obtained from Magnolia oficinalis leaves and Magnolia glauca cortex, as liposomal medication- lyophilized powder, with the average diameter of liposomes 120nm, with 62% involvement of active substance. With adding Isomalt in dispersion area and lyophilization is obtained powder by minimum reducing of active substance involvement effectiveness and liposomes sizes.

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