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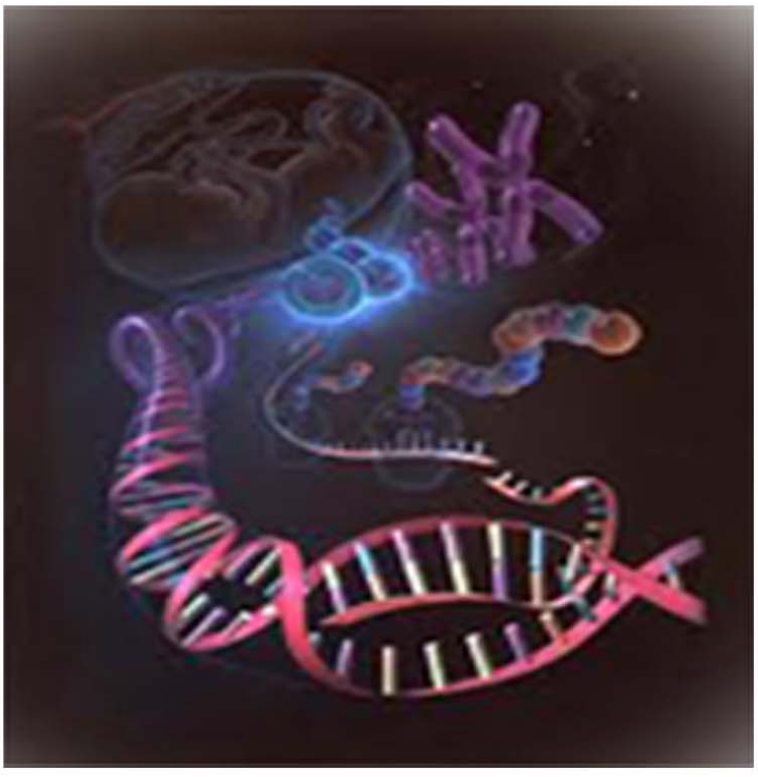

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Research Paper

ADVANTAGES OF PACLITAXEL-LOADED NANO NIOSOMES TO NANOLIPOSOMAL FORMULATION: AN *IN VITRO* STUDY

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In this study, paclitaxel was loaded into nanoniosom and liposomes employing ether injection method. The average diameters of pegylated and non pegylated nano particles of niosomes and liposomes were determined to be 146.9, 211.5, 361.3 and 410.7 nm respectively. The rates of encapsulation were considerably high in both the formulations as such the percentages of paclitaxel entrapped in pegylated and non pegylated niosomal and liposomal forms were estimated to be 96.3, 98.9, 80.4 and 84.7 respectively. The release of drug and toxicity of the formulated paclitaxel were studied by dialysis and MTT methods. The amounts of drug released from pegylated and non pegylated niosomes and liposomes were calculated to 12, 10.6, 23 and 20.2 % during 48 h respectively. The IC_{50} in niosomal and liposomal nanoparticles 2.5 and 1.5 folds were decreased in relation to free drugs respectively. This study showed that prepared niosomal paclitaxel was more effective than that of liposomal. Apart from nano carrier both the formulation can be considered for further *in vivo* studies.

Keywords: PEG-noisome, PEG-Liposome, Size distribution, Encapsulation efficiency

INTRODUCTION

Cancer is one of the greatest challenges related to human health that man is facing all over the world (Jian. 2011). Breast cancer is one of the leading cancer amongst women (Vo and Millis, 2012). Incidence of breast cancer in women over 50 years of old is about 77% (Platek, 2008). Annually; one million women are affected by breast cancer which is a leading cause of their deaths thus accounting for 18% of total cancers in women, all over the world (Key *et al*., 2001). Surgery, radio and chemotherapies are currently used to treat breast cancer (Jian, 2011). Today, new technologies are used to increase the efficacy of chemotherapeutic agents reduce their side effects. Nanotechnology in medical field is one the new technologies used for both diagnosis and treatment (Mozafari, 2006). Paclitaxel is one of the drugs used to treat breast cancer. This drug is being employed to treat ovarian, breast, head -

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neck and non small cells of lung cancers (Harirchi *et al.,* 2004, and Rezaianzadeh *et al.,* 2009). Paclitaxel belong to taxanes family. Though it is effective but clearance of drug from blood stream and resistance of tumor to the drug are considered to be major obstacles in employing paclitaxel to treat the cancers(Mozafari and Mortazavi, 2005). Nano niosomal and liposomal are two nano carriers which can be employed to improve the efficacy and reduce the side effects of paclitaxel. Injected carriers at nano scale are used in order to cross biological barriers, drug protection and optimum release of drug. Recent advances in nanotechnology have made possible the site specific treatments of animals and human diseases by lowering the drugs' adverse effects (Mozafari, 2006). Niosomes are non ionic surfactant vesicles formed by non-ionic hydration of surfactant or without incorporation of cholesterol and other fats. Their vesicular system can be used as carriers of lipophilic and amphiphilic drugs. Their non ionic nature reduces the toxicity and limits their reaction with cell. This in turn will improve the therapeutic index of drug (Pawar, 2012). Liposomes are bilayered vesicles of phospholipids and are centrally united which contain two hydrophilic and hydrophobic areas. Hydrophilic drugs in hydrophilic area and hydrophobic drugs will be in phospholipid bilayered structure (Dimitri *et al.,* 1996). In this study, it has been shown that nano-niosomal particles revealed higher rate of entrapment, more cytotoxicity, longer delayed release as compared to liposomal paclitaxel. Apart from two nano carriers' properties, it was found that their composition is suitable for paclitaxel. They showed better properties as compared to free drug. Pegylated and non pegylated niosomal and liposomal forms of paclitaxel were successfully prepared. It was found that polyethylene glycol as a part of formulations positively affects the efficacy of the drug.

MATERIALS AND METHODS

Span 20, cholesterol, phospatidyl choline, polyethylene glycol 2000 Dalton, paclitaxel and MTT reagent (0.5 mg/ml) was purchased from Sigma Chemical Company, USA. Ethanol, isopropanol and diethyl ether were obtained from Merck. RPMI 16-40 medium was bought from Invitrogen Corporation and MCF-7 cells were procured from the Cell Bank of Iran, Pasteur Institute of Iran.

Preparation of Nano Particles and Drug Encapsulation

Synthesis of Nanoniosom, Encapsulation and Pegylation Of Drug by Ether Injection Method

Niosomes containing nonionic surfactant with paclitaxel (span20) and cholesterol were prepared, the ratios of each reactant are given in Table 1. Cholesterol and surfactant were dissolved in 10 mL of diethyl ether. Then 2 ml of 98% ethanol containing 40 mg paclitaxel was added to the mixture and stirred at 300 rpm, room temperature using magnetic stirrer. Thus the mixture was dropped slowly (1ml/min) by a 14 gauge needle into 10 ml PBS solution (pH 7.4).The obtained solution was stirred on magnetic stirrer at 60-65°C. When the lipid solution is slowly introduced into the aqueous phase, the temperature difference between the phases causes rapid evaporation of ether, resulting in the spontaneous vesiculation and noisome is formed. The above procedure was followed in preparing niosomal paclitaxel where to the mentioned materials, PEG 2000 was added (Table 1).

Table 1: Composition of Surfactants, Phospatidyl Choline, Cholesterol and Polyethylene Glycol in Prepared Niosomes and Liposomes

No	Code	Drug/Surfactant/ Cholesterol ratio	Drug/ Phosphatidy-linositol choline / Cholesterol ratio	Phosphatidylinositol Choline (mg)	Drug (mg)	Surfactant (mg)	Cholesterol (mg)	PEG 2000 (mg)
1	NI-1	1:5:1	…….	…….	40	200	40	20
2	NI-2	1:5:1	…….	…….	40	200	40	. . . . .
3	LI-1	…….	1:5:1	200	40	…….	40	. . . . .
4	LI-2	…….	1:5:1	200	40	…….	40	. . . . .

Synthesis of Liposomes, Encapsulation and Pegylation of Drug by Ether Injection Method

Liposomes containing paclitaxel were prepared by phosphatidyl choline and cholesterol were prepared (at the ratio of 1:10). Cholesterol and phosphatidyl choline were dissolved in 10 mL of diethyl ether. Then 2 ml of 98% ethanol containing 40 mg paclitaxel was added to the mixture and stirred at 300 rpm, room temperature for 30 min using magnetic stirrer until yellow coloured clear suspension was obtained. Thus the mixture was dropped slowly (1ml/min) by a 14 gauge needle into 10 ml PBS solution (pH 7.4). The obtained solution was stirred on magnetic stirrer at 60-65°C. When the lipid solution is slowly introduced into the aqueous phase, the temperature difference between the phases causes rapid evaporation of ether, resulting in the spontaneous vesiculation and noisome is formed. The above procedure was followed in preparing liposomal paclitaxel where to the mentioned materials, PEG 2000 was added.

Homogenization of Vesicles

In this step, the solution was sonicated using Bandelin Sonorex Digitec (Germany), for 5 min at 60 Hz, room temperature in order to obtain smaller and uniform vesicles.

Nanoniosom Size Determination

The diameter of niosomes and liposomes were determined by Zeta sizer (Zen 3600; Malvern Instrument Ltd, Malvern Worcestershire, UK).

Encapsulation Efficiency of Niosomes and Liposomes

To check the amount of drug encapsulated, suspension containing nanoparticles was centrifuged at 13000 rpm, 4 °C for 30 min and the relevant supernatant was separated. The absorbance of supernatant was measured spectrophotometrically at ë 227 nm (SHIMADZU Model UV - 1601PC). Then following formula was used to calculate the encapsulation efficiency.

Formula 1:

$$\%\ of\ Entrapment\ Efficiency = \frac{Amount\ of\ Encapsulated\ Drug}{Total\ Drug} \times 100$$

The standard curve of paclitaxel was constructed by employing different concentration

of paclitaxel. The absorbance was read at ë 227 nm.

Drug Release Studies

The release of paclitaxel from niosomes and liposomes through the membrane was determined by diffusion technique. 8 mg of suspension of niosomal and paclitaxel was introduced into a dialysis bag (cut off 12000 Da , Sigma). Dialysis bag was floated in a container on magnetic stirrer (37ºC, 100 rpm) containing 20 ml phosphate buffer (pH 7.4). Every 5, 7, 9, 21, 24, 30 and 48 h, 2 ml of the dilaysate was removed and to the container 2 ml of fresh buffer was added. The absorbance was measured at λ 227nm against the blank containing phosphate buffer. The amount of released drug was calculated using paclitaxel standard curve.

Evaluation of Cellular Cytotoxicity

The extent of cytotoxicity was studied on MCF-7 cell line using MTT assay on 96 well plate. Cells at the dilution of $1X10^4$ at each well were cultured in DMEM medium containing 10% calf fetal serum and 1% penicillin /streptomycin under 10% CO_2 at 37ºC. After 24 h of the cell growth, The supernatant is taken away , the cells were then treated at different concentrations (1000, 500, 250, 125, 62, 31, 15, 8 µM) of different formulation prepared by two methods. After 48 h of incubation, The medium was removed, 100µl of MTT reagent was added to each well. They were further incubated for 3 h. The MTT reagent was withdrawn from each well. The formed crystalline furomozan was solubilized with 200 µl of 100% isopropanol. The absorbance was read in Elisa reader (BioTek) at λ 570 nm. The experiments were carried out three times and each time in triplicate. Cell viability of the cells were determined by the ratio of absorbance of treated cells by different drug formulation to absorbance of the control cells. The results were evaluated by Pharm program. IC_{50} of the results for each sample was reported.

RESULTS

Size Determination of Niosomes and Liposomes

The average diameters of non pegylated and pegylated niosomes, liposomes containing drug were determined to be 211.5, 146.9, 410.7 and 361.3 nm respectively.

Entrapment Efficiency

Encapsulation efficiency of paclitaxel was calculated through paclitaxel standard curve. Percentages of encapsulated drug for niosomal and liposomal of non-pegylated and pegylated paclitaxel were determined to be 96.3, 98.9, 80.4 and 84.7 respectively.

In Vitro Studies of Paclitaxel Release

The amount of paclitaxel released, from the two formulation of non pegylated and pegylated niosomes could be calculated from the constructed standard curve during 5, 7, 9, 21, 24, 27, 30 and 48 h (Figure 2). Also, the amount of drug released from the formulation obtained by non pegylated and pegylated liposomes was determined. It was found that the pattern of release was similar to that of niosomes (Figure 1).

Evaluation of Cellular Toxicity

It was found that the higher concentration of nanoparticles devoid of drugs did not affect the cell line, thereby, it was considered to be safe. It was also observed that that rate of drug's toxicity in niosomal and liposomal formulations are more than free drugs. However, increase in toxicity was more in niosomal formulation than that of liposomal formulation. The IC_{50} for simple and

Figure 1: Drug Released from Non-Pegylated and Pegylated Niosomal Paclitaxel

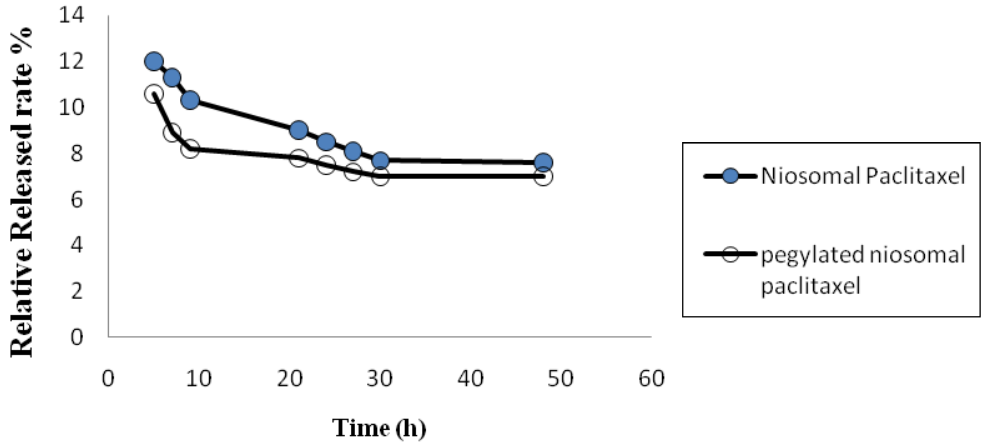
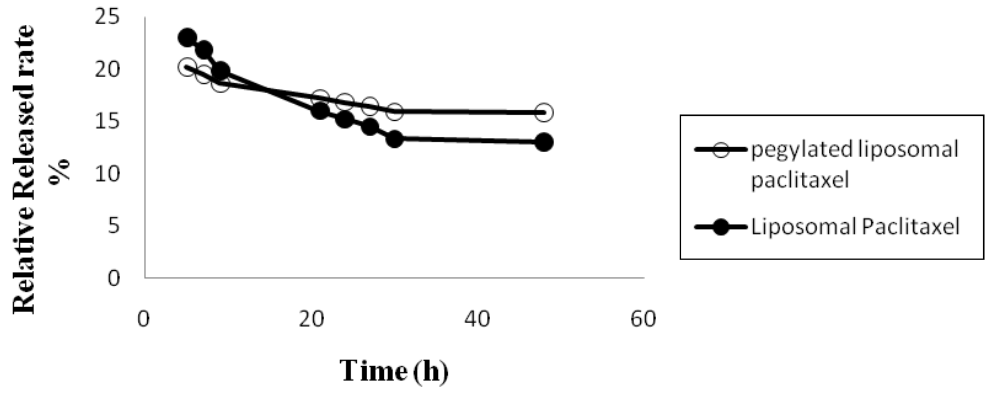


Figure 2: Drug Released from Pegylated and Non Pegylated Liposomal Paclitaxel



pegylated niosomal were estimated to be 39 and 16 µm while IC_{50} for those of liposomal formulations were calculated to be 59 and 37 µm respectively. It was noted that IC_{50} of free drug in 48 h was 96 µm.

DISCUSSION

Drug delivery is one of the main challenges in pharmaceutical biotechnology (Christiane *et al.,* 2005). It is proved that nano carriers are capable of delivering the drugs to the targeted cells. Niosome is on of the lipoid nano carriers. Fang *et al*. (2001) observed that niosomes and liposomes caused the skin permeability of enoxacin. They improved the stability of enoxacin by incorporating cholesterol. Giotall *et al*. (2007) studied the encapsulated liposome with the combination of cyclophosphstide as a first drug to treat breast cancer. The results showed that cytotoxicity of liposomated doxorubicin was more than free drug.

Mujoriya and Bodla (2012) worked on designing and preparing niosomal delivery system of ketoprofen. They concluded that design of site

specific delivery of drugs to the targeted tissue would increase the efficacy of ketoprofen and reduced the unfavorable effects on non targeted tissues. Another study by Srinivas *et al.* (2010) was conducted in order to prepare and study the niosomes containing acyclofenac. Acyclofenac is a weak therapeutic agent with short half life. They attempted to prepare and improve a formulation which could amend its bioavailability. Their study resulted that in presence of non ionic surfactants, the amount of drug released could increase in all formulations, also by increasing surfactant concentration, the efficiency of drug entrapment increased. They also studied the effects of non ionic surfactants and cholesterol on encapsulation efficiency, particles size and drug release. Their study showed that in the presence of non ionic surfactants, the amount of released drug could be increased in all formulations, also by increasing surfactant concentration, the efficiency of drug entrapment increased. Chi Chang and coworker (2012) studied the effect of liposomated curcuminoid on MCF-7, MDA-MB-435S, MDA-MB-231 cell lines. They concluded that the IC_{50} of liposomated curcuminoid was lower than non liposomated curcuminoid. Therefore, by liposomating this drug, the per cent survival of cancerous cells was decreased. In this study, paclitaxel was loaded into niosomal and liposomal nano particles through ether injection method and their properties were evaluated *in vitro*. It was found that niosomal form of paclitaxel , due to its smaller size , impart more toxicity and has high loading capacity as compared to liposomated form of paclitaxel therefore niosomal form contains better properties. Also, from the point of drug released ,it was observed that drug released in niosomal form is slower than liposomal form. Non pegylated and pegylated niosomes and liposomes forms of paclitaxel were prepared. Polyethylene glycol positively affected the properties of nano particles. Though similar studies were carried out by Cosco *et al.* (2009), in presence of polyethylene glycol increase in amount of encapsulation was observed. But size determination study showed somethingels meaning that the size of nanoparticle was considerably decreased in presence of polyethylene glycol. This could be due to hydrophilicity and high permeability properties of polyethylene glycol which is introduced into niosomes layers to compress them. Higher encapsulation efficiency of formulated pegylated niosomes as compared to other two non-pegylated formulation would confirm the above notations. Because, more compressed vesicles possibly showed that release of drug from the wall and therefore this phenomenon causes the internal encapsulation efficiency to increase.

This phenomenon was observed through the pattern of the drug released from niosomes, apart from the nanoparticles preparation methods, both the formulations containing polyethylene glycol showed lower amount of drug released. This phenomenon could be attributed to coating and inhibitory effect of polyethylene glycol on release of drug from the nano particles. The method of nano particles preparation did not considerably affect the rate of encapsulation but, however, the rate of encapsulation of drug by reverse phase evaporation was more than that of ether injection method.

This effect could be originated from (due to) decomposability of polyethylene glycol in presence of non ionic surfactant so decomposability of paclitaxel will be increased and this causes

encapsulation of drug to increase. The cytotoxicity effects of paclitaxel in non pegylated and pegylated formulations were studied by MTT assays. In this study, the effect of control nano particles (in forms, non pegylated and pegylated, at concentrations higher than nanoparticles containing drug) on the cell line was studied. The results revealed non toxicity of the control nanoparticles. Nanoparticles loaded with drugs showed the lowest IC_{50} or in other words, the highest toxicity was obtained with pegylated formulation. This effect can be attributed to the effect of polyethylene glycol in increasing encapsulation thus reducing the release.

CONCLUSION

However, both the formulations containing paclitaxel (non-pegylated and pegylated), apart from their methods of preparation, showed more toxicity than free paclitaxel resulting in improved efficacy by employing nano carriers. In this study, it was found that a suitable formulation of paclitaxel can be prepared by using nano noisome particles. This type of formulation is more effective than free paclitaxel. It results in fewer doses to be used for patients' treatments which decreases the drug's side effects. On the other hand, the treatment cost would be reduced. This formulation can also be considered as a candidate formulation to further perform *in vivo* and clinical studies

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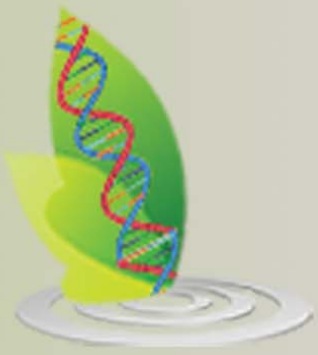

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