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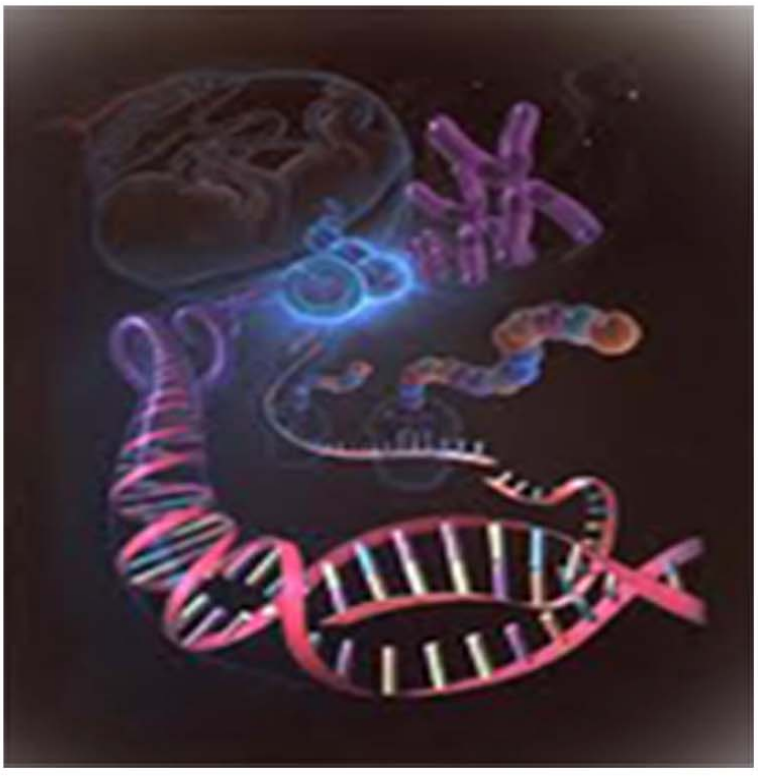

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Research Paper

EFFECT OF ARTEMISININ LIPOSOME AND ARTEMISININ LIPOSOME POLYETHYLENEGLYCOL ON MCF-7 CELL LINE

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One of the challenges in applicability of a drug is a compromise between its effectiveness and side effects. Nano liposomal drug delivery systems enable the delivery of high concentration of drug with a possible targeting of specific cells or organs. This article reports the efficacy of artemisinin nano liposome, artemisinin nano liposome polyethyleneglycol on breast cancer cell line (MCF -7 cell line). Liposomes were prepared by reverse phase evaporation method. Phophatidylcholine, cholesterol and artemisinin were combined together at certain concentrations in this method. The stability of the prepared formulation was increased by pegylattion (polyethyleneglycol 2000). The diameter of the nano liposomes were instrumentally determined by Zetasizer. The results showed that encapsulation and release of artemisinin from only liposomated formulation was more than pegylated form. This study also revealed that the cytotoxicity effect of artemisinin liposome polyethyleneglycol was more than that of artemisinin liposome.

Keywords: Artemisinin, Cytotoxicity, IC_{50}

INTRODUCTION

Today, cancer is the second cause of death in human society. If a group of cells are proliferated due to their lack of elimination, therefore, cancer can be occurred. In autoimmune diseases the immune cells fail to kill themselves while in cancer the cancerous cells are less active and neglect to sacrifice themselves in other words" cancerous cells forget the death" (Blagosklonny 1999). Breast cancer is the fifth cause of death after lung, liver, colon and gastric cancers. In 2005, 519000 deaths were occurred due to breast cancer all over the world which accounted for 70% of death caused by cancers thereby including about 1% of the total death. In USA, it is expected that 40000 people would be died of breast cancer by 2010 (Key *et al.,* 2001). In England, the annual rate of breast cancer is 2 in 1000 and it is the most common death amongst the women of 40-50 years old which stands for 1.5% of total death

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in this range of ages (Mc Pherson *et al*., 2000). Therefore, it is needed to propose new formulations of anti cancer drugs which can reduce the side effects with tumor specific target/ site. However, artemisinin is one of the promising drug that could be used to treat breast cancer (Tin *et al*., 2012). Artemisinin is obtained from *Artemisia* genera belonging to Asteraceae family. Cancer therapy by artemisinin imposes side effects on the patients, to reduce its side effects; drugs can be entrapped in nano liposomal particles. Liposomes are spherical self-closed structures, composed of curved lipid bilayers, which enclose part of the surrounding solvent into their interior. Liposomes possess unique properties owing to the amphiphilic character of the lipids, which make them suitable for drug delivery. The applicability of artemisinin is a compromise between its therapeutic effect and side effects. Nano liposomal drug delivery systems not only enable the delivery of higher drug concentrations, but also a possible targeting of specific cells or organs. Recent advances in nanotechnology have made possible the site specific treatments of animals and human diseases by lowering the drugs' adverse effects. Liposomes have been successfully used in the delivery of anti-cancer agents, and liposomal formulations of doxorubicin (Myocet, Doxil) and daunorubicin (DaunoXome) have been approved by the Food and Drug Administration (FDA) for clinical use. Developing liposomal formulations for anti-cancer agents holds great interest in research because they have certain advantages.

In this article attempts are made to encapsulate artemisinin within nano liposomal particles and then pegylate it in order to study the effect of artimisinin liposome-pegylated on MCF-7 cell.

MATERIALS AND METHODS

Lecithin granular (Acros, Orgsanics, USA), cholesterol (Sigma, USA), polyethylene-glycol 2000 (Merck, Germany) , artimisinin (USA, Sigma), dextran 7000, fetal serum albumin (Gibco), RPMI 1640 (Gibco), trypsin (Invitrogen), penicillin and streptomycin (Sigma, USA), MTT reagent (Invitrogen). All other regents used were of analytical grade.

Liposome Preparation

Lecithin granular (1.01 g) were dissolved in ethanol (120 ml). The mixture was kept on stirrer for 75 minutes at 400 rpm and room temperature. To the above mixture 0.0193 g of cholesterol and 1 mg artimisinin were added. The mixture was kept on the stirrer at 300 rpm and room temperature till they were dissolved. It was kept in the refrigerator overnight and then divided in to two equal parts (part A , B and each containing 40 ml) respectively). To part B containing 40 ml of the above solution was added 0.045 g polyethyleneglycol 2000 which was kept on magnetic stirrer at 300 rpm and room temperature for minutes. Thus two solutions of each 40 ml were obtained. They were termed as solution A (artimisinin liposomated) and solution B (artimisinin liposomated and pegylated). The alcohol contents of both the solution were eliminated using rotary evaporator separately. To each of the above containers were added 12 ml of normal saline. They were parafilmed and kept on the stirrer at 200 rpm, room temperature overnight. Finally homogenized milky solutions were obtained.

Homogenization of vesicles: Solutions A and B were sonicated for 5 min using water bath sonicator (Bandelin Sonorex Digitec) separately.

Then each sonicated container was divided into 1ml vials. The blanks were also prepared containing liposomes, pegylated liposomes named as C and D respectively .They are also divided into 1 ml vials separately .Vials A,B,C and D were kept in the freezer till they were used.

Encapsulation of artimisinin: The contents of each vial A, B, C and D (1 ml) were transferred to micro tubes carefully. The tubes were centrifuged at 50000 rpm for 30 min. The supernatants were separated and kept aside. The precipitates were washed with saline and again centrifuged at the same rpm (This step was repeated till, there was no artimisinin in the supernatant. The absorbencies of the supernatants of each A, B, C and D were read at λ 195 nm, using C and D as a respective blank. The standard curve was constructed using different concentration of artimisinin ranged from 0.03125g/ml to 0.5 g/ml.

Size Determination

The size of nano liposome (forms A and B) were determined by Zetasizer (Zen 3600; Malvern Instrument Ltd., Malvern Worcestershire, UK).

Drug Release Studies

The release of artimisinin from A and B through the membrane was determined by dynamic diffusion technique. One ml of each of A and B was introduced into a dialysis bag (cut off 12000 Da, Sigma). Dialysis bag was floated in a container on magnetic stirrer(37ºC, 50 rpm) containing 25 ml phosphate buffer (pH 7.4). Every 2,4,6,8,24 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 λ195nm against the blank containing phosphate buffer. The amount of released drug was calculated using artimisinin standard curve.

Cellular Cytotoxicity Study

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 RPMI 1640 medium containing 10% calf fetal serum and penicillin 100 units/ml, streptomycin 100µg/ml under 5% CO_2 at 37ºC. After 24 h of the cell growth, the supernatant is taken away, the cells were then treated with A, B, C, D, E and F (E, pegylated artemisinin and F, artemisinin) at different concentrations(100, 50, 25, 12.5, µg/ml). After 48 h of incubation, the medium was removed, 100 µl of MTT (at the concentration of 0.5mg/ml) 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 by SDS (12.5%) and formamid (45%). The absorbance was read in Elisa reader (BioTek) at ë540 nm. The experiments were carried out three times and each time in triplicate. Cell viability of the cells was determined by the ratio of absorbance of treated cells by different drug formulation to absorbance of the control cells.

RESULTS

Figure 1 show the percent of artimisinin entrapped into liposome (A) and liposome–polyethylene-glycol 20000 (B). As it can be seen 96.02 % of artimisinin is entrapped into liposome while for the latter formulation 91.6 % of artimisinin is entrapped.

Both the formulations (A and B) were subjected to evaluate their stabilities at refrigerated condition for 2 months. The amounts of artimisinin released from both the formulations were determined to be 25.7 and 31.5 % respectively for A and B with respect to original artimisinin contents in A and B (Figure 2). Artimisinin released from both the

Figure 1: Entrapment of Artimisinin in Liposome and Liposome-pegylated

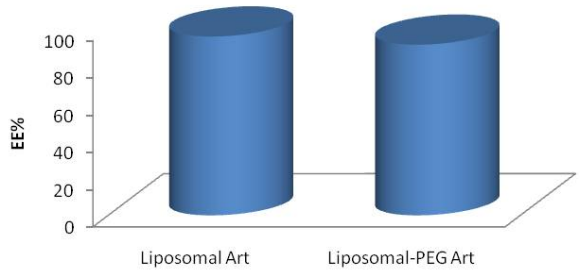
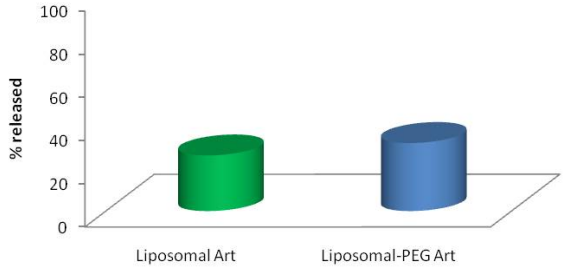


Figure 2: Artemisinin Released from A and B Formulations



formulations (A and B) were studied by dynamic diffusion technique. The study was performed for 2, 4, 6, 24 and 48 h (Figure 3). Figures 4 and 5 show the appearance of A and B formulations respectively. The average sizes for artimisinin nano- liposomated (A) and artimisinin nano-liposomated-pegylated (B) were found to be 500 and 455nm respectively

Figure 3: Percent of Artimisinin Released from Formulations A and B Through Dynamic Diffusion Technique

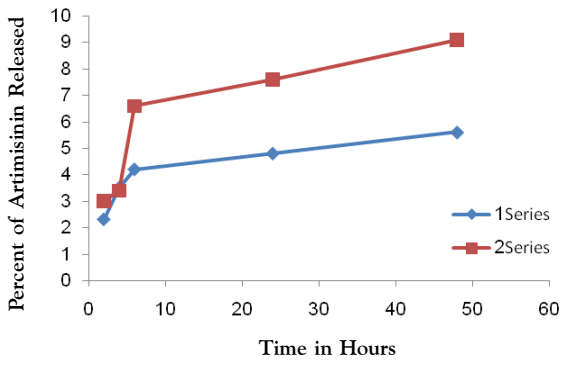


Figure 4: TEM Micrograph of Artimisinin Nano Liposome

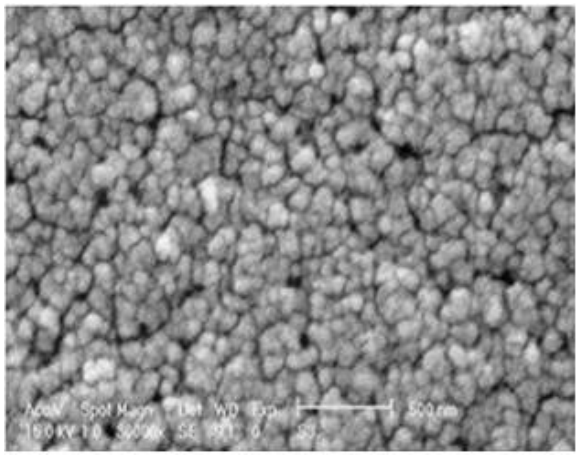
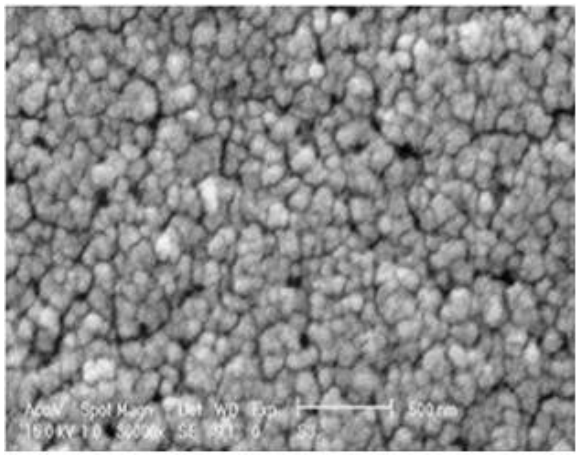


Figure 5: TEM Micrograph of Artimisinin Nano Liposome-polyethyleneglycol



Discussion

Cancer is still an important obstacle in modern medicine is the most common cause of death after heart diseases. Nowadays, cancer is known to be a genetic disease where group of cell get mutated and lose their capability in regulating their growth or repair/apoptosis thus forming malignant tumors, and invade nearby parts of the body. Amongst the cancer, breast cancer is the most common cause of death in women accounting for 1.5% of all the deaths (Murthy and Aleyamma 2004). The annual incidence of breast cancer is

17.1 to 10000 women in Iran (Sadjadi *et al* 2009).Treatment of breast cancer depending on the advancement of the disease are different. In the treatment protocol, chemotherapy is followed after surgery. Employment of different chemical agent depends on kind and state of advancement, age of patient, etc. Currently, increase in therapeutic effectiveness of an anti cancer agent with the reduction of side effect, and delivering the drug to specific site essentially employs new technology such as nano technology in the field of medicine (Brigger *et al*., 2002).Therefore, it is probably the only procedure to be used for being tumor specific action with less side effect lower harm to natural cells. Thus, such a method includes; systematic delivery, passive and active targeting, intracellular delivery, entrapment in organelles (Cegnar *et al*., 2005). Wide spectrum of nano carriers for anti cancer drug delivery are being used to overcome drug resistant (Martin 2008) such as drug loaded liposome (Sells *et al*., 1987, Cowens *et al*., 1993), polymeric nano sphere, polymeric nano capsules (Couvreur 2002), solid nano lipid and magnetite nano particles (Muller and Keck, 2004). In this article due to following reasons artemisinin was loaded into liposome (A, form) and artemisinin loaded pegylated liposome (B, form) in order to study their efficacy on breast cancer cell line namely MCF-7. Firstly, liposomes may serve as a sustained drug release system (Drummond *et al*., 2008), which results in prolonged elimination half-life of the active agent. Anti-tumor drugs are cell-cycle de-pendent and they require continuous administration to exert the maximum anti-tumor effect (Burris *et al*., 1992; Georgiadis *et al*., 1997; Gomi *et al*., 1992; Johnston *et al*., 2006). The extended elimination half-life provided by liposomal formulation may lead to a decrease in the frequency and length of drug administration (Kim *et al*., 2001; Kirpotin *et al*., 2006). Secondly, liposomes in-crease site-specific accumulation of anti-cancer agents at tumor tissues (Drummond *et al*. 2008). In this article the effects of artemisinin liposome (A) and artemisinin liposome polyethylene glycol 20000 (B) on breast cancer cell line (MCF-7) have been studied. The diameters of the both nano particles of A and B were determined by Zetasizer. The results showed that the size of particles of artemisinin liposome polyethylene glycol 20000 was smaller than that of artemisinin liposome. The difference in size of the nano particles probably could be due to the hydophilicity and high permeability of polyethyleneglycol which penetrates into the liposomal layers thus making them compressed leading to smaller size of the nano particles. The results obtained through encapsulation efficiency study state that formulation A contained 96.2% of

Table 1: IC_{50} of Different Formulations in Comparison to Standard Artimisinin

Concentration μg/ml	Formulation A	Formulation B	Free artimisinin
1.25	37.3	43.9	29.8
2.5	51.2	68.2	48.6
5	73.6	83.4	66.3
10	92.5	98.6	80.7
IC_{50}	2.1 μg/ml	1.58 μg/ml	2.7 μg/ml

artemisinin as compared to 91.6 in formulation B. This could be due to the above statements resulting in lower encapsulation of artemisinin.

Release of artemisinin from the nano particles initially followed by rapid diffusion and latter on it slowed. The release of artemisinin from the vesicles mostly occurred during first 2-4 h of the study. Decaying of A to some extent was slower than that of B, this may be because of the solubility of formulation B and permeability arising from polyethyleneglycol, thus interacting more easily with its surrounding leading to release of artemisinin from the vesicles relatively faster. The cytotoxicity effect of both A and B formulations using MTT assay was studied. In this study, liposome and liposome polyethyleneglycol devoid of artemisinin (referred as controls) showed no cytotoxicity on MCF-7 cell line. The results showed that the lowest IC_{50} could be obtained by formulation B. Also the IC_{50} of both forms (A and B) differed from the IC_{50} of standard artemisinin significantly. Therefore, artemisinin liposome and artemisinin liposome polyethyleneglycol impose more toxicity on MCF-7 cells than standard artemisinin. In this way, it can be concluded that employment of nano carriers in drug delivery such as nano liposomes play an important role in improving the therapeutic index of the drugs.

CONCLUSION

The employment of nano carriers in drug delivery such as nano- liposomes, play an important role in improving the therapeutic index of the drugs.

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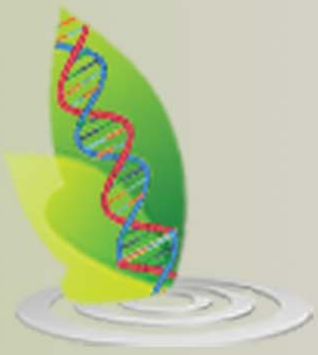

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