



In vitro investigation of anticancer efficacy of carboplatin-loaded PEGylated nanoliposome particles on brain cancer cell lines

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Abstract Recently, designing a nanocarrier for carboplatin with high retention power and entrapment efficiency and its corresponding impact has sparked a heated debate. The aim of this study was to investigate

the cytotoxic efficacy of the biodegradable nanocarrier loaded with carboplatin. This study focuses on (i) characterization, (ii) in vitro drug release evaluation, and (iii) cytotoxicity of liposome nanoparticles (NPs) loaded with carboplatin. The reverse-phase evaporation was used to synthesize nanoparticles and determine specifications including shape morphology, particle size, drug release rate, polydispersity index (PDI), stability, and zeta potential of prepared formulation. Furthermore, A172 and C6 glioblastoma cell lines were used to determine the efficacy of nanodrug using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. NPs had an average size of 240.5 nm and zeta potential of -25.8 mV. Drug loading and encapsulation efficiency of NPs were 2.65% and 71.45%, respectively. Moreover, entrapment efficiency and drug release rate increase in a time-dependent manner. The results reveal that the preparation method and PEGylation have a positive effect of the properties of NPs and the efficiency of encapsulation and loading rate. Results from stability study reported that using PEGylation helps to improve characteristics of NPs and results in minimal changes in their properties over the time. In addition, our data demonstrate that carboplatin cytotoxicity correlates with drug concentration which was considerably increased in NPs. PEGylated NPs have enhanced cytotoxicity against glioblastoma cell lines compared with free drug. Overall, our evaluation has shown that the PEGylated nanoliposome particles loaded with carboplatin hold high potential for nanoparticle-based therapy.

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Introduction

Brain cancer causes several deaths all over the world annually. Brain cancer has significantly jeopardized human well-being due to poor prognosis and rapid proliferation of malignant cells. Glioblastoma is the most common malignant primary brain and central nervous system (CNS) tumors in adults and consists 80% of malignant cerebral tumors (Dolecek et al. 2012). Treatment of glioblastoma multiform (GBM) tumors that has a survival rate of 14.6 months is a demanding problem currently (Stupp et al. 2005). Nowadays, verities of cancer treatment methods are available. The treatment that patients receive will depend on the type of cancer patient has and how advanced it is. For brain cancer, the common treatments are surgery, radiotherapy, chemotherapy, and other drug therapies. Surgery on brain and spinal cord tumors may be done to remove the tumor or as much of it as possible. Routinely, the first step in brain tumor treatment is safe removal of tumorous tissue without affecting normal brain function. Surgery may not be a good option in some situations, such as if the tumor is deep within the brain and if it is in a part of the brain that cannot be removed (Mayer 2014). Tumors that tend to spread widely into nearby brain tissue such as glioblastomas cannot be treated by surgery. Surgery is not very effective against some types of brain tumors, but surgery can reduce the amount of tumor that needs to be treated by radiation or chemotherapy.

Radiation therapy is another common treatment method which uses high-energy rays or small particles to kill cancer cells. Radiation therapy may be used in different situations such as using as the main treatment if surgery is not a good option or after surgery to try to kill any remaining tumor cells. Most often, the radiation is focused on the tumor from a source outside the body. This is called external beam radiation therapy (EBRT) (Breen et al. 2018). High doses of radiation therapy can damage normal brain tissue, so delivering the radiation to the tumor with the lowest possible dose to normal surrounding brain areas is one of the challenges of this radiation therapy (Dorsey et al. 2014). Several techniques such as three-dimensional conformal radiation therapy (3D-CRT) (Rosenzweig et al. 2001), intensity-modulated radiation therapy (IMRT) (Moreno et al.

2019), and conformal proton beam radiation therapy (DeLaney and Haas 2016) are used to focus the radiation more precisely in radiation therapy. Despite higher detrimental effects of radiation therapy on tumor tissues as compared with normal brain tissues, it is still unsafe to use due to its overall adverse effects on both normal and cancerous tissues. Possible side effects of radiation therapy are problems with thinking and memory, radiation necrosis, and increase risk of another tumor. For brain tumors that have distinct edges, this method is effective; however, it is still questionable if this technique is useful against tumor that is infiltrative or mixed with normal brain tissues such as glioblastoma.

Chemotherapy (chemo) uses anticancer drugs as a treatment agent to kill cancerous cells. Drugs enter the bloodstream and reach almost all areas of the body. Chemotherapy is typically given after surgery and possibly along with radiotherapy. The goals of chemotherapy are to destroy cancer cells remaining after surgery, slow a tumor's growth, or reduce symptoms. However, chemotherapy causes several side effects, and 80–90% of patients undergoing chemotherapy experience diarrhea, constipation, oral mucositis, nausea, and vomiting (Stojanovska et al. 2015). Due to the efficacy of chemotherapy for brain cancer and common side effects, it is important to develop new methods to enhance treatment efficiency and reduced side effects. However, there is no effective treatment for almost all brain tumors yet (Persidsky et al. 2006). The development of new therapeutic approach for brain tumor encounters with a difficult challenge. In most cases, the major cause of the ineffective treatment of brain diseases is the presence of the blood–brain barrier (BBB). This barrier delineates a unique chemical, functional, and immunologic environment in the CNS which limits the entrance of potentially neurotoxic macromolecules, bacteria, and leukocytes from the blood (Persidsky et al. 2006; Ramirez et al. 2012; Begley 2004). Similarly, the BBB affects brain cancer treatment by chemotherapy. Therefore, developing a new strategy for treatment of brain diseases that are able to pass through BBB safely is highly required. One of the most significant achievements of developing the drug delivery systems is that the functional NPs can penetrate through the BBB and can be used for drug delivery to the CNS (Begley 2004).

Nanodrug delivery is used for the ultimate goal of developing clinically useful formulations to treat various diseases as a novel method. This technique holds great potential to replace standard chemotherapy. For

the nanodrug delivery systems, the definition of NP is not really based on the size of the particles, but it rather depends on whether NPs have novel properties that non-NPs and bulk materials of the same material typically do not have. Therefore, the different sizes of NPs in the 1–1000 nm range are used for the nanodrug delivery systems. In fact, this definition is required in utilizing the NP concept in drug delivery, because it is the unique properties of NPs that are useful, instead of the size itself (Vert et al. 2012). NPs have a huge surface area as compared with microparticles or other bulk materials. Therefore, by making the drug in nanosize, surface area of particles increases and drug dissolution and the bio-availability of the poorly soluble drugs are improved (Cooper 2010). Moreover, the NP accumulation at the tumor site is greater compared with free drug delivery. Previous studies show that the concentration of nanodrugs in the tumor is 200–500% higher than that in the free form of anticancer drugs (Lee et al. 2015). This is widely accepted due to enhanced permeation and retention (EPR) effect of nanodrugs. In fact, the nanoparticulate system can radically affect drug biodistribution, hence increasing drug concentration in cancer tissues. Moreover, NPs can prolong the plasma half-life of cytotoxic drugs while keeping drug toxicity to the minimum (Wang et al. 2012; Gozde and Ufuk 2018). Therefore, designing the carrier with high retention power and entrapment efficiency is one of the main objectives for developing the nanoparticle-based drug delivery systems for treatment of brain diseases. Many types of NPs have been studied to optimize chemotherapy since NPs were nominated as a highly potential drug delivery strategy. The liposome was first developed more than 5 decades ago (Bangham and Horne 1964). Liposome NPs have suitable characteristics for drug delivery in the tissue, including the ability to alter the biodistribution of the drug in the tissue, ease of synthesis, and purification (Andrieux and Couvreur 2009; Lee et al. 2015). Furthermore, surface modification of NPs has been investigating to improve the yield of drug delivery. The addition of polyethylene glycol (PEG) to the NP surface that is named PEGylation is kind of surface modification that reduces the reticuloendothelial system (RES) uptake and increases circulation time versus uncoated counterparts (Sathyamoorthy et al. 2017). Regards to previous studies using PEG which is inexpensive, versatile, and FDA approved improves stability of NPs in the blood circulation (Kawai 2002; Veronese and Pasut 2005; Knop et al. 2010).

In this study, synthesis and characterization of carboplatin-loaded PEGylated liposome NPs were performed. Carboplatin is a platinum-loaded chemotherapeutic drug which has similar mechanism of action with cisplatin, but differs in terms of structure and toxicity (2013). Carboplatin is widely used for treatment of various cancers including brain, head, and neck. New therapeutic strategies which improve therapeutic effects of carboplatin would provide great opportunities to treat several types of cancer including brain cancer (Shahzad et al. 2009; Wernyj and Morin 2004). The aim of this study was to investigate the cytotoxic efficacy of the NPs loaded with carboplatin. In addition, we hypothesized that using PEGylate nanoliposome carrier enhances therapeutic effects of carboplatin. The reverse-phase evaporation was used to synthesize NPs and determine specifications including shape morphology, particle size, drug release rate, polydispersity index (PDI), stability, and zeta potential of prepared formulation. Furthermore, A172 and C6 glioblastoma cell lines were used to determine the efficacy of nanodrug using MTT reduction assay.

Materials and method

Carboplatin, cholesterol, lecithin, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-mPEG-2000), polyethylene glycol (PEG 4000), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT) (0.5 mg/ml), and Hoechst 33258 were purchased from Sigma-Aldrich (USA). A172 human and C6 rat glioma cell lines were provided by the American Type Culture Collection (ATCC).

NPs were prepared by the reverse-phase evaporation method. Lecithin, cholesterol, polyethylene glycol 4000, DSPE-mPEG-2000, and carboplatin were dissolved in 50 ml ethanol 96% in the molar ratio of 10:7:1:1:1. Then, rotary evaporator instrument was used to remove solvent at 40 °C and 100 rpm. After that, 25 ml phosphate-buffered saline (PBS 10X, pH = 7.4) was added to flask. The new mixture was put in an incubator at 37 °C overnight. Sonication was performed by probe sonicator (Bandelin Sonopuls HD 2070, Bandelin Elec., Germany) for 2 min with placing flask in the cold water. The mixture was maintained in the refrigerator (4 °C) for 24 h. Then, the ultracentrifuge (Beckman type 90Ti, USA) was used to separate NPs

from supernatant for 10 min at 4 °C and 10,000 rpm. The separated supernatant was removed totally, and again 5 ml PBS was added to NP mixtures.

The morphological analysis of NPs was performed by scanning electron microscopy (SEM) (XL30 scanning microscope, Philips, Netherlands). SEM produces images of a sample by scanning the surface with a focused beam electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The average size and zeta potential were determined by Zetasizer (Nano-ZS Zen 3600, Malvern Instruments Ltd., Worcestershire, UK).

In order to determine drug loading and encapsulation efficiency, the resultant formulation was centrifuged (10 min at 4 °C and 10,000 rpm and 2 iterations) by ultracentrifuge. The amount of drug in supernatant was evaluated by inductively coupled plasma optical emission spectrometry (ICP-EOS) elemental analysis (730-OES, Varian).

Equation (1) was used to calculate the encapsulation efficiency (E.E %) (all in mg/ml).

$$E.E\% = \frac{\text{The actual amount of carboplatin loaded in NPs} \left(\frac{mg}{ml}\right)}{\text{Initial drug concentration} \left(\frac{mg}{ml}\right)} \times 100 \quad (1)$$

Loading capacity helps to deal with NPs after their separation from the medium and to know their drug content. Equation (2) was used to calculate the loading amount of the drug (D.L.E %).

$$D.L.E\% = \frac{\text{The amount of drug into NPs} \left(\frac{mg}{ml}\right)}{\text{Weight of NPs} \left(\frac{mg}{ml}\right)} \times 100 \quad (2)$$

Moreover, the dialysis method was applied to study drug release behavior of NPs. One milliliter of all formulations was poured in a dialysis bag (cutoff 13,000 Da, Sigma) placed in 20 ml of PBS (pH 7.4) and placed on a magnetic stirrer (120 rpm, 48 h, 37 °C). Then, ICP-EOS was used to measure the released drug in PBS in a different time step from 1 h after stirring up to 48 h. Moreover, the stability of NPs after a 1-month storage at room temperature was investigated, and their size, zeta potential, encapsulation, and drug loading efficiency were evaluated.

Furthermore, cytotoxicity of carboplatin-loaded liposome NPs for PEGylated NPs, non-PEGylated

formulations, and free drug was determined by MTT assay using C6 rat brain cancer cell line and A172 human brain cancer cell line. Cells were seeded in 96-well plates at a density of 1×10^4 (10,000) cells. After that, they were cultured with 5% CO₂ at 37 °C in RPMI-1640 medium containing fetal calf serum (FCS) (10%), sodium pyruvate (1%), antibiotic penicillin, and glutamine (0.5%). The medium was replaced 2 times after 24 h. An MTT solution (4 mM) was added to each well for 3 h. Cells were treated with free drug and NPs containing carboplatin at different concentrations (1, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 μM). IC₅₀ of PEGylated liposome NPs, non-PEGylated, and free form of carboplatin was evaluated after 24, 48, and 72 h incubation. The absorbance (570 nm and 540 nm) was measured by a plate reader (Synergy Multi-Mode Elisa Reader, BioTek, USA) (Ebrahimifar et al. n.d.; 2017). Finally, IC₅₀ was calculated by using statistical package Pharm-PCS program.

In addition, the nuclei of healthy cells are normally spherical, and the DNA is evenly distributed. During apoptosis, the DNA becomes condensed, but this process does not occur during necrosis. Nuclear condensation can therefore be used to distinguish apoptotic cells from other cells. Hoechst 33258 binds to DNA, and it can be used to observe nuclear condensation (Crowley et al. 2016). To analyze cell death by nuclear staining with Hoechst 33258, all of medium was removed, and cells were washed twice with PBS. After that, 3.7% paraformaldehyde in PBS was added to sample and left for 20 min at room temperature. Then, the fixative was removed, and the sample was rinsed three times with PBS. A 1 μg/ml of Hoechst33258 was added, and the sample incubated the coverslip for 30 min at 37 °C. Finally, the sample was rinsed with PBS. A fluorescence microscope was used to observe sample changes.

The results were analyzed by SPSS software version 15. Statistically, *P* values less than 0.05 are considered significant. All results are expressed as a mean ± standard deviation (SD).

Results and discussion

Developing new strategy for chemotherapy to enhance treatment efficiency and reduce side effects is very important. Using an efficient carrier helps the concentration of drug at the targeted site be high enough to kill the tumor cells. Thus, developing delivery study is very

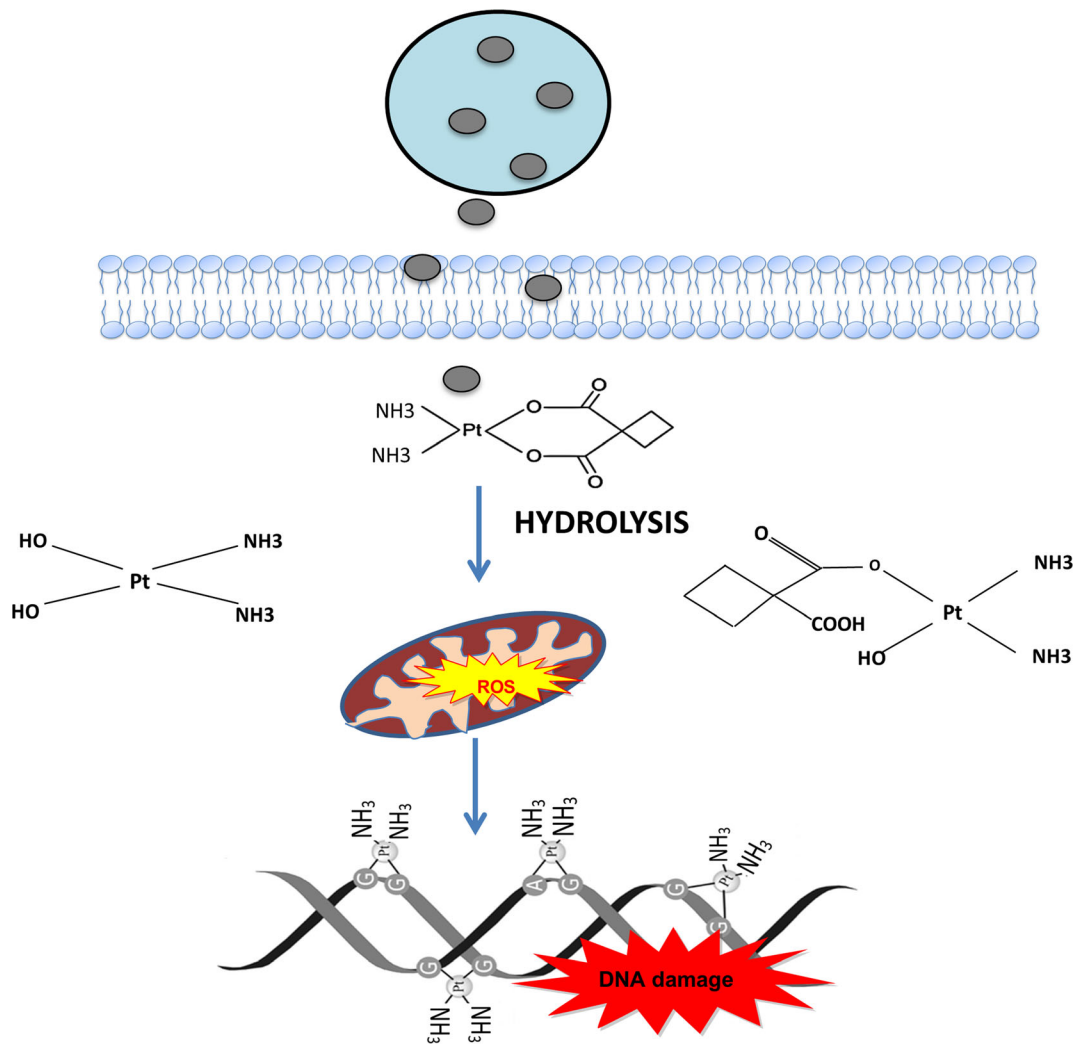


Fig. 1 The schematic action between the NPs and cancer cell

important in evaluating therapeutic efficacy for brain disease treatment (Tan et al. 2013; Sefidgar et al. 2015). In this study, liposome NPs containing high level of carboplatin have been successfully synthesized. The experiment was performed 3 times, and results showed that the preparation technique is sufficiently valid and reliable. Our results confirmed that the reverse-phase evaporation is an appropriate method for production of liposome NPs as effective carriers of chemotherapeutic drugs. In addition, PEG was used in the preparation of NPs to help increasing pharmacokinetic properties. The schematic action between the NP and cancer cell is shown in Fig. 1. The results of light microscopy evaluation confirmed preparation of NPs. NPs were spherical and unilamellar vesicles (ULVs) dispersed throughout

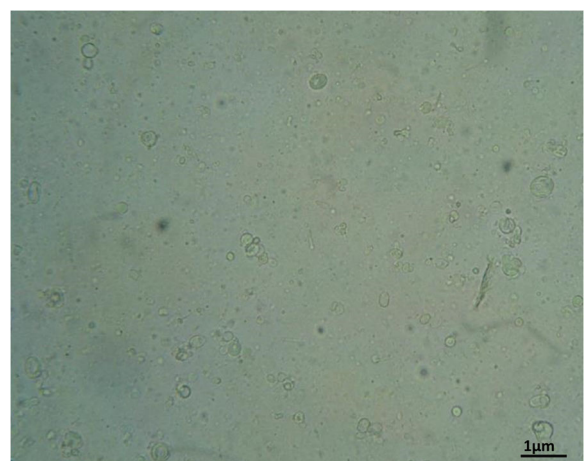


Fig. 2 Light microscopy of PEGylated liposomal NPs

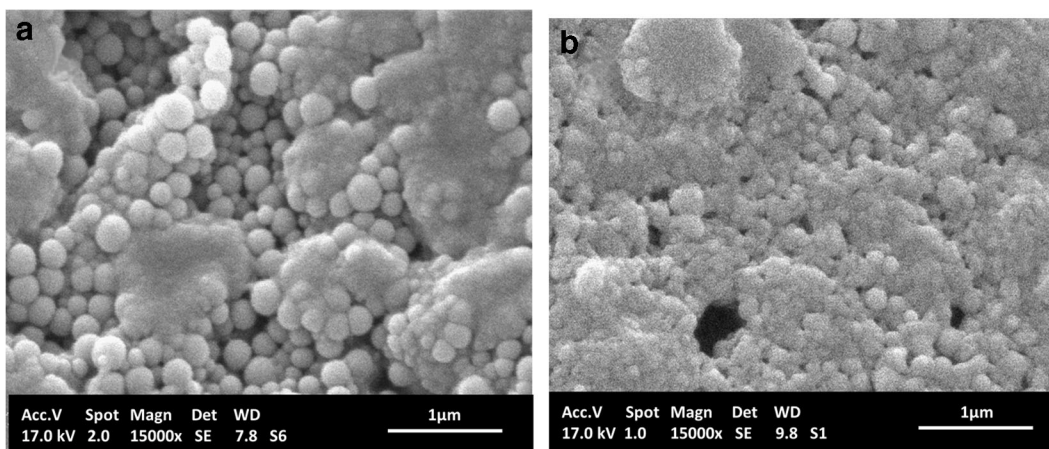


Fig. 3 Scanning electron microscope micrographs from **a** non-PEGylated liposome NPs and **b** PEGylated liposome NPs

the matrix (Fig. 2). The morphological analysis of NPs performed by SEM demonstrates that PEGylated NPs do not aggregate together, and they have a completely spherical shape compared with non-PEGylated NPs (Fig. 3).

The results of physicochemical characteristics of the NPs are summarized in Table 1. Encapsulation efficiency of NPs was reported 55.5% in non-PEGylated and 71.45% for PEGylated NPs in our study. Results illustrate adding PEG to formulations improves the D.L.E and E.E of NPs. In addition PEGylation has a pivotal effect to improving zeta potential of NPs that is known as an important parameter on NP surface modification. The previous study reported using PEG increasing drug loading efficiency (Cosco et al. 2009). It was revealed that the PEGylation has a positive effect on the properties of NPs, and using PEG enhances the stability which is important to increase drug efficacy. This was confirmed by the higher encapsulation percent and loading rate observed in PEGylated NPs compared with non-PEGylated NPs. This could be due to the drug release from the walls of tight vesicles after PEGylation becomes lower than non-PEGylated form. Therefore, retention yield and load rate increased.

In our study, we have achieved -25.8 and -19.5 mV potential for PEGylated and non-PEGylated NPs, respectively. The zeta potential of the colloidal systems and nanomedicines is an important factor exerting a major effect on the various properties of the nanodrug delivery systems. The zeta potential affects on the stability of encapsulated drugs and the release rate of drug from NPs. A nanoparticle surface is a very important consideration in drug delivery. Moreover, the NPs without surface modification and negatively charged particles can be rapidly opsonized, and they would be cleared by macrophages in the bloodstream. Therefore, zeta potential plays an important role on surface modification of the nanodrug delivery systems. Surface modification is the most common strategy to control the opsonization process and thus sustain the NPs for a longer period in the bloodstream (Honary and Zahir 2013). The physicochemical characteristics of NPs for all formulations have been evaluated after a 1-month storage at 4°C , and they are summarized in Table 2. Results illustrate that non-PEGylated NPs significantly changed their physicochemical characteristics including size, zeta potential, D.L.E, and E.E. Therefore, non-PEGylated NPs are less stable than PEGylated NPs.

Table 1 Characteristics of carboplatin loaded on NPs

Name	Size (nm)	Zeta potential (mV)	D.L.E (%)	E.E (%)
PEGylated liposome NPs	$240.5 \pm 25.28^*$	-25.8 ± 2.1	2.65 ± 0.235	$71.45 \pm 2.5^*$
Non-PEGylated liposome NPs	379.15 ± 34.22	-19.53 ± 3.18	2.14 ± 0.11	55.5 ± 1.99

E.E entrapment efficiency, D.L.E drug loading efficiency

* ($P < 0.05$) compared with non-PEGylated liposome NPs

Table 2 Characteristics of carboplatin loaded on NPs after 1 month

Name	Size (nm)	Zeta potential (mV)	D.L.E (%)	E.E (%)
PEGylated liposome NPs	294.25 ± 19.5*	-22.75 ± 0.57	2.595 ± 0.27	65.4 ± 3.1*
Non-PEGylated liposome NPs	465 ± 38.15	-17.2 ± 0.6	2.01 ± 0.14	42.9 ± 2.75

E.E entrapment efficiency, D.L.E drug loading efficiency

* ($P < 0.05$) compared with non-PEGylated liposome NPs

Moreover, results show that PEGylated NPs have a better size, zeta potential, PDI, D.L.E, and E.E than non-PEGylated NP for both formulations after this period of time.

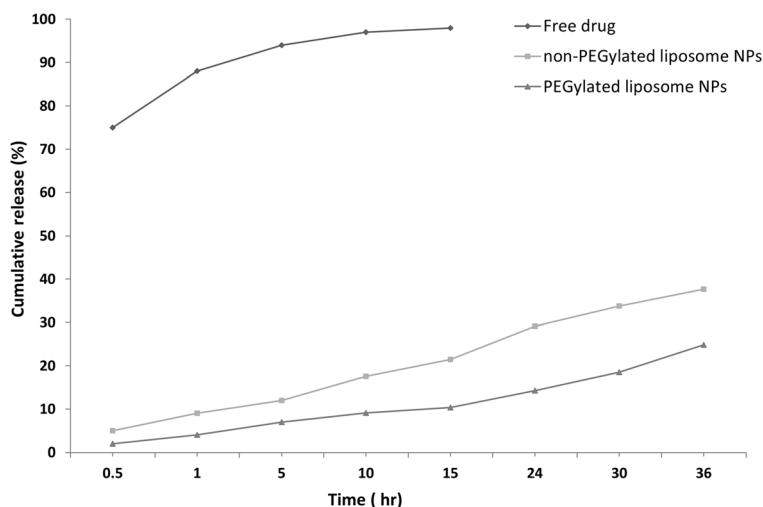
The dialysis method was applied to determine the impact of PEGylation on a drug release behavior. The cumulative release rate of carboplatin from NPs is presented in Fig. 4. Free form of carboplatin showed a sharp release of 98% of drug after 15 h. Drug release from NPs has significantly less slope in comparison with free drug. The drug release profile illustrates continuity for 36 h. Indeed, in the first 15 h of evaluation, a burst release of small amount (55% of the maximum release amount (24.8%)) of drug was observed. The release was followed by a mild ascending slope. A cumulative release of non-PEGylated NPs was 37.7% (W/W) after 36 h.

The release profile of the drug from NPs is an important factor as it determines the biological effects of the carriers (Otsuka et al. 2012; Soltani and Chen 2012). In the first hour of the study, a burst release of carboplatin was observed which is most likely due to the release of the drug adhered to the surface of NPs.

Then, a sustained release pattern with a gradual increase was prominent throughout the experiment, which confirmed the potency of the carrier to drug retention. Carboplatin release profile contains initial phase of quick-spread and slow-spread phase in PEGylated and non-PEGylated NPs. This phenomenon could be attributed to coating and inhibitory effect of PEG on the release of drug from the NPs. This phenomenon could be attributed to coating and inhibitory effect of PEG on the release of drug from NPs. Released studies demonstrated high retention capability of PEGylated nanodrug in that 24.8% of carboplatin was released from NPs in a period of 36 h. For non-PEGylated NPs, 37.7% of carboplatin was released in a same period of time. This result correlated with enhanced zeta potential property of NPs in this study.

The result of apoptosis assay is revealed in Fig. 5. The DNA of healthy cell is evenly distributed, and nuclei are spherical. Regarding the result, during apoptosis, the DNA becomes condensed, but this process does not occur during necrosis. Apoptotic cells' most significant change is chromosome condensation. DNA cracking changes the shape of the nucleus. Fluorescent dye Hoechst 33258 is a

Fig. 4 Release profile of carboplatin from liposome NPs for PEGylated NPs, non-PEGylated NPs, and free drug using the dialysis method within 40 h at 37 °C



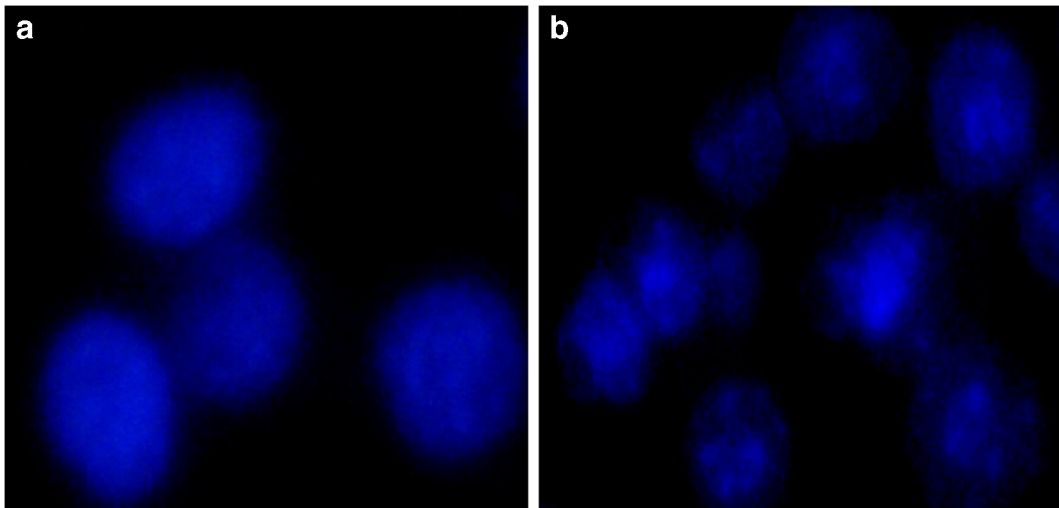


Fig. 5 Effect of PEGylated liposome NPs on cellular and nuclear morphology of A172 cells stained by Hoechst 33258 staining without treatment (a) and loaded with carboplatin (b) after 24 h

nontoxic water compound and can be used to bind to the DNA molecule as a fluorescent probe. In apoptotic cell, the

membrane's uptake in Hoechst 33258 uptake increased, and because of chromosomes' high concentration, Hoechst

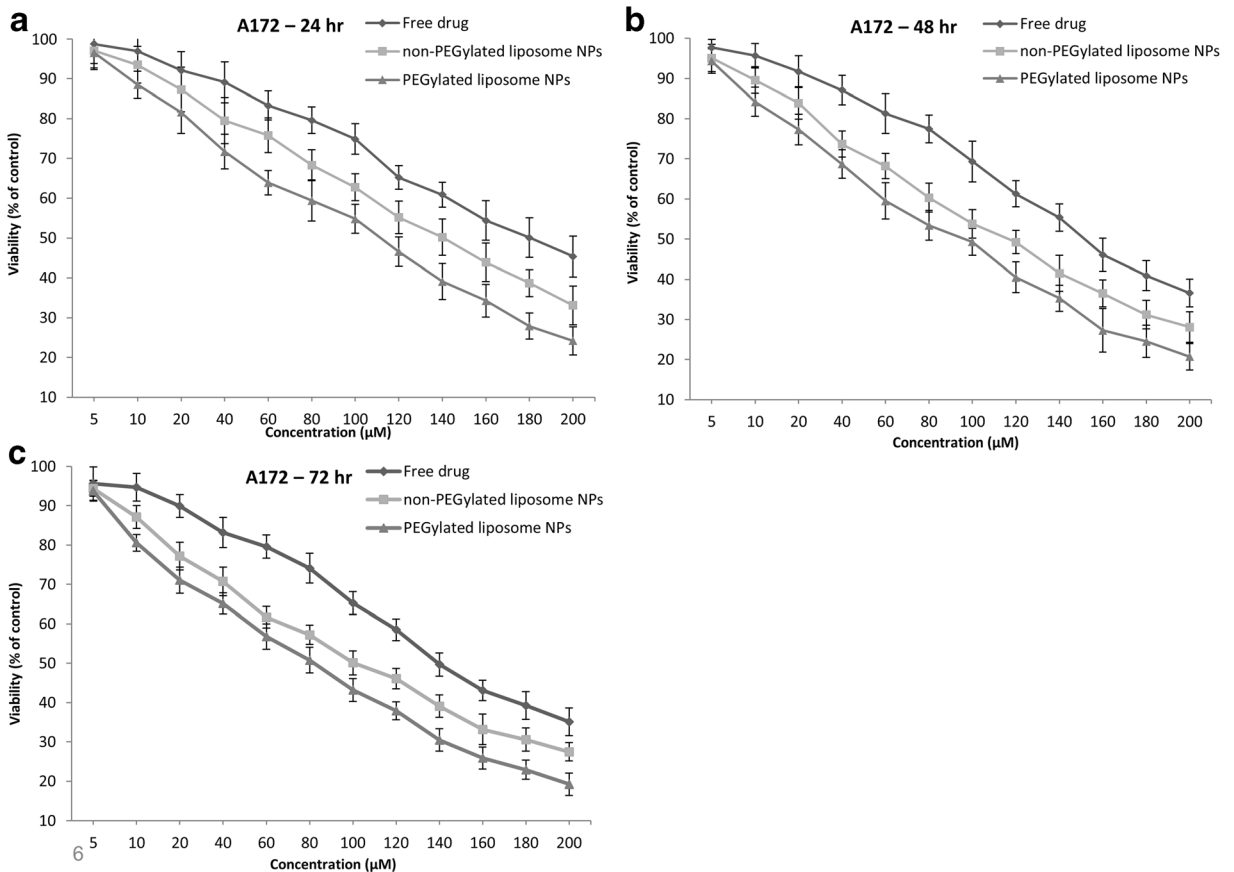


Fig. 6 Cytotoxicity effects of free carboplatin and NPs on the C6 cell line after 24, 48, and 72 h of incubation (a, b, and c, respectively). All results are expressed as a mean ± standard deviation (SD) and 3 iterations ($n = 3$)

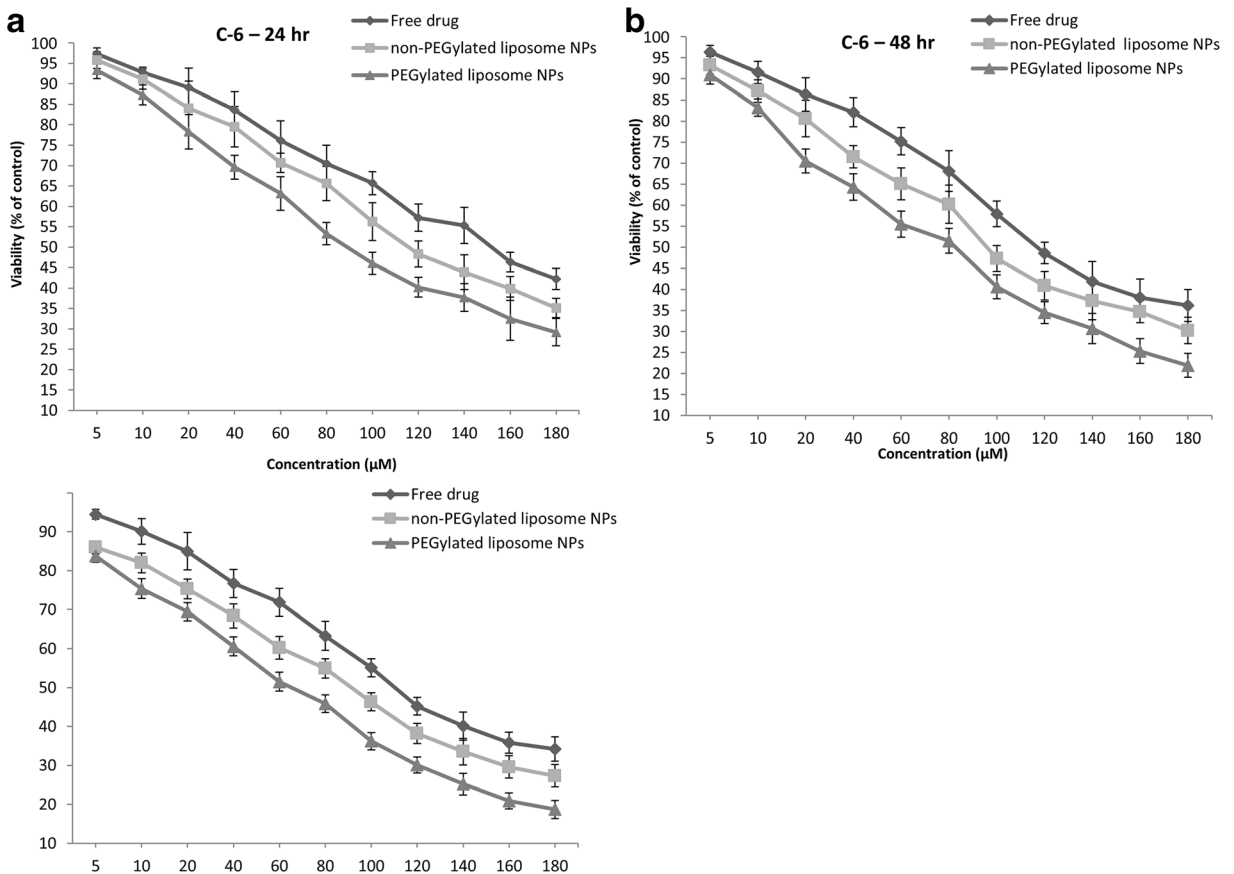


Fig. 7 Cytotoxicity effects of free carboplatin and NPs on the A172 cell line after 24, 48, and 72 h of incubation (a, b, and c, respectively). All results are expressed as a mean ± standard deviation (SD) and 3 iterations (*n* = 3)

33258 with the combination of increased staining showed strong blue fluorescence, while normal cells showed only weak fluorescence, and dead cells with disbanded/split up stained fluorescence indicates apoptosis.

Cytotoxicity of NPs and free carboplatin on C6 and A172 cell lines was tested after 24, 48, and 72 h incubation (Figs. 6 and 7). Results demonstrate that cytotoxicity of NPs is remarkably higher at all time points compared with free drug. The results demonstrate that using PEG in NPs enhances cytotoxicity compared with non-PEGylated NPs in a time-dependent manner. The results showed that the cytotoxic effect of non-PEGylated NPs was stronger than standard carboplatin. Figure 8 presents IC₅₀ of NPs and free drug on C6 and A172 cell lines. Results demonstrate that cytotoxicity of standard drug does not change significantly after 48 h. However, a significant change in cytotoxicity of non-PEGylated NPs was observed between 24 and 48 h (29%). PEGylated NPs demonstrate higher cytotoxic efficacy for carboplatin compared with other

formulation after 24, 48, and 72 h with 9–16% decrease for every 24 h. PEGylated NPs have lowest IC₅₀ and therefore have higher cytotoxicity on cancer cells compared with non-PEGylated NPs and standard carboplatin. This effect can be attributed to the effect of PEG in increasing encapsulation efficiency and as a result in reducing release. The results obtained in this study concur with the previous studies demonstrating low IC₅₀ and increased cytotoxicity indicating enhanced drug efficiency. Our cytotoxicity results are consistent with the previous publications on poly(lactic-co-glycolic acid) (PLGA) with encapsulated carboplatin. Arshad et al. encapsulated carboplatin into PLGA and evaluated the characteristics of the NPs at in vitro and in vivo studies. The authors observed that nanoencapsulation significantly increased cytotoxic effects of the drug (Arshad et al. 2015; Jafari et al. 2012). Similarly, Hamelers et al. (2006) reported strong augmented cytotoxicity effects of encapsulated carboplatin into a lipid formulation by 1000 times compared with

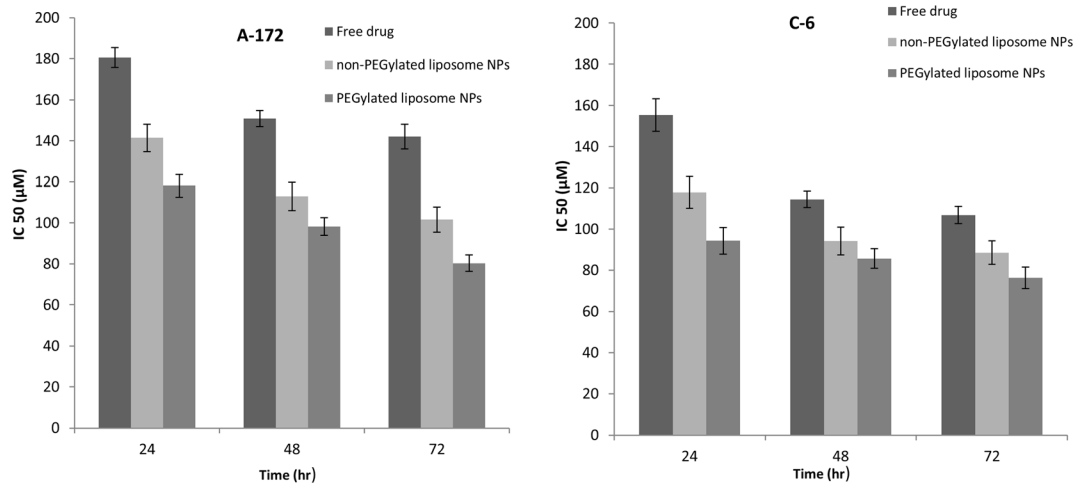


Fig. 8 Effect of IC₅₀ (µM) of PEGylated and non-PEGylated NPs synthesized by reverse-phase evaporation and free form of carboplatin on C6 and A172 cell lines in interval time (24, 48, and

72 h). All results are expressed as a mean ± standard deviation (SD) and 4 iterations ($n = 3$)

the standard drug (Hamelers et al. 2006; Müller et al. 1990; Wang et al. 2008).

Conclusion

The aim of this study was to investigate the efficacy of the liposomal nanocarrier loaded with anticancer drug carboplatin. The efficacy of a platinum-based chemotherapeutic agent, carboplatin, is limited due to the intracellular resistance. New therapeutic strategies are needed to improve therapeutic effects of carboplatin. In this study, the reverse-phase evaporation technique was demonstrated as the effective method for liposome NP preparation loaded with carboplatin. Furthermore, physicochemical characteristics of the NPs containing carboplatin were evaluated and found to be stable. The efficacy of nanodrug on A172 and C6 brain cancer cell lines was shown to enhanced cytotoxicity compared with free drug. In addition, our data demonstrate that carboplatin cytotoxicity correlates with the drug concentration. However, cytotoxicity was considerably increased for PEGylated NPs containing drug. Overall, the findings suggest that PEGylated nanoliposome carriers prepared by our method as an encapsulation system hold great potential for developing carboplatin therapy.

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Authors' contributions MH: conceptualization, data curation, formal analysis, investigation, and writing original draft; MS: formal data analysis and project supervision; AA: development of methodology, providing resources, and supervision; PC: data validation and providing resources; AH: supervision and formal data analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interest.

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