Lawsone-loaded Niosome and its antitumor activity in MCF-7 breast Cancer cell line: a Nano-herbal treatment for Cancer

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Abstract
Phytochemicals like Lawsone have some drawbacks that stem from their poor solubility. Low solubility in aqueous mediums results in low bioavailability, poor permeability and instability of phytochemical compounds in biological environments. The aim of this study was to design nanoniosomes containing Lawsone (Law) using non-ionic surfactants and cholesterol. Niosomes were prepared by thin film hydration method (TFH). Then, they were loaded with Henna extract (HLaw) and standard Lawsone (SLaw), and two resulted formulations were compared. The henna extract was analyzed by mass gas chromatography. Size, zeta potential, polydispersity index (PDI) and morphology of the loaded formulations were evaluated by dynamic light scattering (DLS) and scanning electron spectroscopy (SEM). The incorporation and release rate of Law from niosome bilayers were evaluated by UV-Vis spectroscopy. In vitro experiments were carried out to evaluate antitumor activity in MCF-7 cell line. The results showed distinct spherical shapes and particle sizes were about 250 nm in diameter and have negative zeta potentials. Niosomes were stable at 4 °C for 2 months. Entrapment efficiently of both formulations was about 70% and showed a sustained release profile. In vitro study exhibited that using of niosome to encapsulating Law can significantly increase antitumor activity of formulation in MCF-7 cell line compared to Law solution (free Law). Thus, niosomes are a promising carrier system for delivery of phytochemical compounds that have poor solubility in biological fluids.

Keywords Niosome · Lawsone · Solubility · Phytochemicals · Anticancer

Introduction
Nanotechnology approach can increase the stability and also improve the therapeutic efficiency of phytochemicals, such as polyphenols, tannins, alkaloids, terpenoids and herbal extracts. Since ancient times, compounds derived from plants have been a good source of therapeutic drugs [1]. In recent years, there has been growing interest in alternative therapies and therapeutic use of natural materials such as Taxol (a substance originated from the Pacific yew tree) and they have been approved for the clinical treatment of cancer patients [2]. Another example is Plumbagin that is a yellow dye, formally derived from naphthoquinone. Plumbagin has been shown to induce cell cycle arrest and apoptosis in numerous cancer cell lines including melanoma, lung, breast and others [3]. The advantage of using plant derivatives for cancer therapy is in their potential to be used in an edible form and lower side effects than conventional anticancer medicine [4]. However, phytochemicals have some drawbacks that decrease the patient compliance, such as penetrating smelling and nasty
taste [5]. Moreover, other factors that reduce their use in remedial are their low solubility, low dissolution rate, high doses required for an therapeutic effect and instability at excessive pH, resulting in lower or no therapeutic efficacy [6–9]. Because of these limitations, an assembled technological approach such as nanocarrier systems is necessary. These nanocarriers develop more efficient and stable formulations that are an important goal for pharmaceutical, nutraceutical, food and cosmetic industries [10].

Persian Henna (Lawsonia inermis) is a bushy and flowering tree that is found in Asia, Australia and Mediterranean coasts of Africa [11]. Crude extracts of Henna and its ingredients have many biological activities such as antibacterial, antioxidant, anti-inflammatory and anticancer properties [12]. The major constituent of Henna plant is Lawson (used in the synthesis of some anticancer drugs, like lapachol and dichloroallyl Lawson [13–15]). Moreover, there are some other chemicals like luteolin, lupeol, isopalmagbin and apigenin, the anticancer effect of which has been approved [16]. Although lawson presents many medical advantages, its delivery is a problematic challenge due to its hydrophobic nature which results in poor solubility, poor permeability, low bioavailability and instability in biological systems [17–20].

Niosomes have lamellar structures that are built by a mixture of a non-ionic surfactant and cholesterol as a helper lipid followed by hydration in aqueous media [21, 22]. In architectural point of view, niosomes are similar to liposomes but more efficient in physicochemical properties, less toxicity due to their non-ionic nature [23, 24] and a promising carrier for the delivery of active compounds [25–27]. Vesicle formulations can be variable and controllable in characteristics. Change in vesicle composition, size, lamellarity, release rate, trapped volume and surface charge can control vesicle behaviors [28]. Thanks to the special structure of niosomes, hydrophilic and hydrophobic drugs can be encapsulating simultaneously so that the hydrophilic drug is encapsulated in the vesicular aqueous core or on the surface of niosome, and the lipophilic drug is encapsulated in the lipophilic region of the vesicle. Niosomes are osmotically active, stable and can increase the solubility of hydrophobic drugs [26]. Niosomes have numerous applications in immunological adjuvants [29]. They are also anticancer and anti-infective [30], carriers of anti-inflammatory drugs [31], imaging agents for diagnostic purposes [32] and also administered in different ways [33]. Innovative nanocarriers such as niosomes are able to tune some unfavorable chemical and physical features of phytochemicals and also promote antioxidant/radical scavenging activity and bioavailability in biological fluids [34–36]. Improving in antioxidant/radical scavenging activity of the active compounds arise from increasing the resistance of phytochemicals against degradative phenomena due to their encapsulation in the niosomes [37–39].

There have been no related study about encapsulating lawson in niosome for more efficient delivery. The purpose of this study is to develop a new approach for formulating Law-entrapped niosomes by thin film hydration method. Niosomes were formulated with Henna extract (HLaw) and standard Lawson (SLaw), and their properties were compared in terms of size, morphology, stability, release profile and anticancer activity.

Materials and methods

Materials

Lawsons (2- hydroxy-1, 4-naphthoquinone), Span 60 (Sorbitan monostearate), Tween 60 (Polyoxyethylene sorbitan monostearate, 3-(4, 5-dimethy1thiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT reagent), cholesterol and dialysis bag (MWCO = 12,000 Da) were purchased from Sigma (St. Louis, MO, USA). Chloroform was obtained from Merck (Germany). All the reagents and chemicals were of analytical grade and used without further treatment.

Henna sample extraction and characterization

Fresh plant materials of the Henna were collected from Kerman district of Iran and were authenticated by a pharmacognosist. The leaves of Henna were air-dried at the room temperature. Then, 50 g of the dried and crushed leaves was extracted using alcoholic solution by soxhlet technique up to 24 h. The plant extract was filtered by membrane filters and concentrated in the rotatory evaporator at 40 °C [40].

The GC-MS analysis was performed by Varian 5300 Gas Chromatograph with a mass detector and HP-5 ms column (60 m × 0.25 mm × 0.25 μm DF). The instrument was set to an initial temperature of 60 °C and maintained at this temperature for 5 min. At the end of this period, the oven temperature was increased up to 270 °C, at the rate of an increase of 5 °C/min, and maintained for 5 min. Helium flow rate was 1 ml/min and injection port was set at 260 °C. The ionization voltage was 70 eV. The samples were injected in the split mode as 80:1. The fragmentation patterns earned from mass spectra were evaluated with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of the components was compared with relative peak area in the chromatogram.

Preparation and optimization of Lawsons encapsulated niosomes

For niosome preparation, thin film hydration method was used as reported previously [41]. Specific molar ratios of Span 60, Tween 60 and cholesterol were dissolved in 10 mL of a
chloroform solution containing 1 mg/ml of Lawsone and added to a 50 mL round bottom flask. Then, the solvent was evaporated at 60 °C under vacuum in a rotary evaporator (Eppendorf, Germany). The resulted thin lipid was hydrated with 10 ml of deionized water at 60 °C. The resulting solution was further sonicated in an ultrasonic bath for 30 min at 50 °C. Niosome purification was performed by a 0.22 um membrane filter (Sartorius AG, Göttingen, Germany).

**Morphology, size distribution and zeta potential of formulations**

The morphological analysis of niosomes was carried out by scanning electron spectroscopy (SEM) (SBC-12, KYKY, China). For SEM analysis, a drop of niosome suspension added to a lam and air dried for 24 h. DLS (dynamic light scattering) (Malvern Instruments Ltd., Worcestershire, UK) was used to evaluate size, size distribution and zeta potential of the formulations. Also, the effective formation of the vesicles was investigated by transmission electron microscopy (TEM) analysis (EM10C, Zeiss, Germany). A drop of the dispersion was directly placed onto a formvar membrane-coated grid and stained by adding a drop of 2% (w/w) sodium phosphotungstate solution, removing the excess solution using a filter paper, followed by through air-drying.

**Entrapment efficiency (EE) of lawsone into niosomes**

Lawsone was spectrophotometrically determined using a UV–Vis spectrophotometer (Agilent Technologies, Cary 50, USA) at 673 nm, at which lawsone has maximum adsorption. To obtain calibration curve absorbance versus concentration of lawsone, different concentrations of lawsone dissolved in chloroform (1, 4, 8, 10, 15, 20, 60, 80, 100 and 150 ppm) were prepared. The limit of detection for UV-Vis spectroscopy was calculated and it was 0.399 ppm. The EE (%) was evaluated by quantifying free Lawstone in the supernatant after centrifugation procedure of niosome formulations. The samples were
centrifuged at 15100 rpm for 60 min at 4 °C (5415D, Eppendorf, Germany). The free Lawsone concentration in the supernatant was evaluated by UV spectrophotometry at 673 nm. The difference between the total Lawsone content in the formulations and free Lawsone was EE (%).

### Release behavior of Lawsone from loaded formulations

First, 1 ml of each formulation was added to a dialysis bag and, then, placed in a 50 ml beaker filled with PBS (pH = 7.4). The beaker was stirred by a magnetic stirrer (150 rpm) at 37 °C for 15 h. At a specific period of time, 1 mL of the released content was withdrawn and immediately replaced with the similar content of fresh PBS solution. Then, the concentration of Lawsone was determined by a spectrophotometer at 673 nm. The percentages of the released content of Lawsone in PBS were presented as % accumulative release [42].

### Cytotoxicity assay

Cytotoxic effects of niosome formulations on MCF-7 cell line were performed by MTT assay. 7 × 10^3 MCF-7 cells were plated in 100 μL of DMEM solution and incubated for 24 h. On the next day, the incubated cells were treated with the concentration of 1.5 μM to 10 μM of niosome formulations suspended in PBS with pH = 7.4 and diluted with DMEM solution) for 24 h. After the incubation, 0.5 mg/ml of MTT was applied to the wells and incubation was carried out at 37 °C for 4 h in a dark place. Then, 100 μL of DMSO solvent was added. Finally, the absorbance was taken at 560 nm, and 630 nm was used as the reference wavelength [43].

### Statistical analysis

One-way ANOVA was employed for the statistical analysis of various experiments [44]. A p-value <0.05 was considered statistically significant. Values are reported as mean ± SD.

### Results and discussion

#### GC-MS analysis

The phytochemical compounds in the alcoholic extracts of Henna leaves were identified by the GC-MS analysis. Retention time (RT), molecular formula (MF), area and percentage of the obtained peak in the extract were also evaluated. Totally, sixteen compounds were identified from the ethanol extract of the Henna leaves (data not shown). Presence of Lawsone in the extract was 1.03%, the RT of which was in the 30th minute (Fig. 1). As expected the amount of Lawsone in Henna extract was good enough for anti-cancer study. In Henna extract, there are some active ingredients that are responsible for antioxidant and antitumor activity.

#### Mean size and physicochemical characterization of niosome formulations

The compositions and characteristics of the niosome formulations are presented in Table 1. The mean particle size of the niosomes ranged from 198 nm to 377 nm. A narrow peak distribution was obtained from DLS, which approved that the formulations had the uniform size. The average size of bare niosomes (198 ± 4.25 nm) compared to Nio/SLaw (316 ±
3.14 nm) and Nio/HLaw (377 ± 5.63) was changed significantly. Loading of HLaw and SLaw led to an increase in vesicle dimensions values (Table 1). The dimension increased when Law molecules were loaded in the vesicles, which was because of partial insertion of the guest drug in the bilayer, with a consequent decrease of the surface tension of the particles [45].

Zeta potential values were affected by the presence of Law. It has been reported that a stable nanosuspension mainly stabilized by electrostatic repulsion exhibits zeta potential values close to −30 mV [46]. In the current study, as soon as the law was loaded into niosomes, the zeta potential of these two formulations (Nio/SLaw and Nio/HLaw) moved to more negative values. Changing in zeta potential is maybe because of addition more negative charge density to the formulation bilayer that come from Lawsone. High and negative zeta potential values are an indication of stable preparations and prevent aggregation phenomena [47]. PDI parameter approved homogeneity of our suspension that values of less than 0.3 corresponded to a single, rather than a sharp peak, in the DLS profile [48]. In the case of our samples, a single and homogeneous population was detected, except for bare niosome. As we reported above, bare niosome have a low zeta potential and because of this reason the stability of formulation is slight and they coagulate. The coagulation of formulation can increases the PDI value.

The morphology and size of niosome formulations were analyzed by SEM and TEM (Fig. 2A and B). The images showed nanometer sizes, semi-spherical and homogenies form for both Nio/HLaw and Nio/SLaw formulations. Moreover, the loading of niosomes did not cause any structural deformations, which confirmed high stability of the formulations. SEM and TEM analysis gave us lower sizes than DLS. Generally, TEM gives the size of nanoparticles in dried form while DLS tells the hydrodynamic diameter that includes core plus any molecule attached or adsorbed on surface, due to which the average size would be more.

EE %

EE measurement indicated that about 70 ± 1.02% of Law was entrapped within the niosomal bilayers. As shown in Table 1, SLaw formulation showed more entrapment efficiency that might be related to more structural affinity among SLaw (as hydrophobic moiety) and the non-polar palmitate moiety of Span 60 and Tween 60. High encapsulating efficiently of Lawsone in niosomes is because of hydrophobic nature of Lawsone that results in more affinity and tus more incorporation in niosomes bilayers. Law interacts with the acyl chains of niosome bilayers and can stabilize the formulation [49].

**In vitro release study**

Drug release rate is an important parameter in the design of drug delivery systems and must be carefully analyzed. In vitro results will give an insight from in vivo performance of niosomes [50, 51]. Fig. 3 illustrates the in vitro release of free Law, Nio/HLaw, and Nio/SLaw formulations. Niosomes like other vesicular drug delivery systems showed a biphasic release, so that rapid drug release happened and, then, a steady state of release was obtained. Nio/HLaw had a less release rate than Nio/SLaw due to a complex matrix of Henna extract. Free Law releases were very fast and almost all of the Law was released within 4 h because there is not any shell for sustained release. The Law release rate in our formulation was

**Fig. 3** Sustained release (%) of free Law and Law entrapped in niosomes in PBS (pH 7.4) at 37°C. Data are expressed as the mean ± SD (n=3)

**Fig. 4** Stability measurements of the stored and freshly prepared Law-loaded niosomes for over 60 days at 4 °C by DLS. Nio/SLaw (●) and Nio/HLaw (●) prepared loaded niosomes. Data are expressed as the mean ± SD (n = 3)

**Fig. 5** Antitumor activity of loaded niosomes compare to that of free Law and bare niosomes at the concentration of 1.5 μM. * means p < 0.05
significantly slower than the free Law. These findings proved that niosomes have the capability of the sustainable and controlled release of Law. In fact, support studies working on other nanocarriers [51] have concluded that niosome formulations can be applicable for delivering phytochemicals in a sustained and controllable manner.

**Stability of the system**

Stability study showed that Nio/Hlaw have high stability. The stability of the encapsulated Law is a critical factor for the effective delivery of the formulation as reported by other authors [52]. The stability of the niosome formulations was assessed for over 60 days at 4 °C by DLS. Generally, size variation in the formulations by the lapse of time indicated that aggregation or fusion occurred and the system was destabilized [53]. The zeta potential is strongly related to the stability of the niosomes. In high zeta potential values, there is a high repulsive force between the particles that can stabilize the formulations [54]. On the other hand, close to zero or even low values lead to unstable systems with resultant aggregation or fusion processes. Our data showed that mean ± SD particle size of vesicles increased very slightly and change from 316 ± 8 nm to 374 ± 12 for Nio/Slaw and from 377 ± 10 to 402 ± 15 for Nio/Hlaw formulations (no statistically significant) which indicating good stability of both carriers. However, change in the size of Nio/HLaw was lower than Nio/SLaw formulation because of more zeta potential of Nio/HLaw formulation (Fig. 4).

**Antitumor activity of loaded niosomes**

Antitumor activity of free Law, bare niosomes (without Law) and Law-encapsulated niosomes were studied on MCF-7 cells at the concentrations 1.5 μM (based on our previous works) for 24 h. The cytotoxicity effect of Law-encapsulated formulations was significantly greater than that of the free Law. The enhanced cytotoxicity of the loaded niosomes is due to a synergistic effect of better internalizations of nanocarrier and a sustained release of Law. As expected, bare formulations did not show any cytotoxicity. Taken together, the present data indicate that loaded-niosomes have excellent cytotoxicity, thus rendering this formulation suitable for therapeutic purposes (Fig. 5).

**Conclusions**

In this study, proved that Lawsons-loaded niosomes can be used as a promising carrier for cancer therapy. The law-loaded formulation had bigger size than bare niosomes. The release profile showed similar release behaviors for both loaded formulations; however, Nio/SLaw had a faster release. Antitumor study on MCF-7 cell lines showed that, when Lawsons was encapsulated in niosome, the cytotoxicity significantly increased compared to the free Law. Finally, according to the results of this study, can conclude that niosomes can efficiently encapsulate phytochemical compounds and also improve the efficiency of nanocarrier for cancer therapy in a controlled and sustained manner.

**Compliance with ethical standards**

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

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