Indian Journal of Pharmaceutical Education and Research., 2023; 57(1):74-82.
https://www.ijper.org

Original Article

Berberine Hydrochloride-loaded Liposomes Gel: Preparation, Characterization and Antioxidant Activity

Mengmeng Wang[1], Hongmei Xia[1,*], Xinying Yang[1], Qin Zhang[1], Yufan Li[1], Yu Wang[1], Ying Xia[1], Zili Xie[2]

[1]College of Pharmacy, Anhui University of Traditional Chinese Medicine, Hefei, CHINA.
[2]Anhui Institute for Food and Drug Control, Hefei, CHINA.

ABSTRACT

Aim: Berberine hydrochloride is a Traditional Chinese Medicine component with antibacterial, antiviral, anti-inflammatory, antioxidant and lowering blood sugar effects. However, the application of berberine hydrochloride has been hindered for a long time due to its poor solubility and low bioavailability. This study aims to design a liposomes-gel to enhance the bioavailability and antioxidant activity of berberine hydrochloride. **Materials and Methods:** The thin-film dispersion hydration method was used to prepare liposomes, and liposomes-gel was prepared by the natural swelling method using sodium alginate as the hydrogel matrix. Berberine hydrochloride-loaded liposomes-gel was characterized by the encapsulation efficiency, particle size, potential, and transmission electron microscopy, and studied by *in vitro* release and antioxidant activity of the preparations. **Results:** The results revealed the encapsulation efficiency of berberine hydrochloride-loaded liposomes was 79.62±4.20% and the mean particle size was 153.7±11.2 nm. The release of berberine hydrochloride from liposomes-gel conformed to a first-order release model. The scavenging rate of DPPH free radicals and H_2O_2 by berberine hydrochloride-loaded liposomes-gel exceeds 70%. **Conclusion:** The liposomes-gel has a sustained-release effect on berberine hydrochloride. Berberine hydrochloride-loaded liposomes-gel had good antioxidant properties by measuring the scavenging ability on DPPH free radicals, H_2O_2 and lipid peroxidation resistance.

Keywords: Berberine hydrochloride, Liposomes, Sodium alginate, Hydrogel, Antioxidant.

Correspondence
Prof. Hongmei Xia
Anhui University of Traditional Chinese Medicine, Longzihu Road, Hefei City, Anhui-230011, CHINA.
Email id: sm_shine@ahtcm.edu.cn
ORCID ID 0000-0003-2011-0840

Received: 03-06-2022;
Revised: 13-09-2022;
Accepted: 11-10-2022.

INTRODUCTION

Liposomes prepared from soy lecithin and cholesterol can be used as a suitable drug carrier for the delivery of drug molecules, nucleic acids and proteins.[1] It has the advantages of drug targeting, sustained release, reduced drug toxicity and others.[2]

Sodium alginate is a natural polysaccharide derived from brown algae, which can be used in pharmaceutical preparations, food thickeners, stabilizers and emulsifiers.[3,4] Sodium alginate is non-irritating, biocompatible, and easy to form the three-dimensional cross-linked hydrogel. It can directly act on the macrophages to promote wound healing. It is suitable for various biomedical applications and provides a platform for drug delivery in different forms.[5,6]

Liposomes are similar to cell membranes in structure and have good transdermal absorption characteristics. The network structure of sodium alginate hydrogel has excellent water-locking ability, which is suitable for cell growth and improves its application in biological tissue engineering.[7] Combining liposomes with hydrogel can not only take advantage of the sustained release and high bioavailability of liposomes but also improve the disadvantages of easy leakage and difficult storage of the drug. Liposomes-gel is a good carrier for transdermal drug delivery, which can reduce the number of administrations and improve patient compliance.[8]

Berberine hydrochloride (BBH) is a kind of quaternary ammonium isoquinoline alkaloid, which is a yellow needle-shaped crystal.[9] It is a widely used natural medicine, mainly found in plants such as *Berberidaceae*, *Ranunculaceae* Juss, and *Menispermaceae* Juss.[10] The structure of berberine hydrochloride is shown in Figure 1. It has various pharmacological activities such as antibacterial, antiviral, anti-inflammatory, anti-tumor, lowering blood sugar, and regulating blood lipids.[11,12] The main clinical preparations of BBH are tablets, capsules and others. Poor intestinal absorption of BBH after oral administration results in low bioavailability, which limits its applicability.[13] Furthermore, intravenous injection of BBH can cause serious adverse reactions, such as vasodilation, cardiac depression.[14] Therefore, there is an urgent need to develop a drug delivery vehicle for improving the bioavailability of BBH and reducing toxic side effects. In this

DOI: 10.5530/001954642087

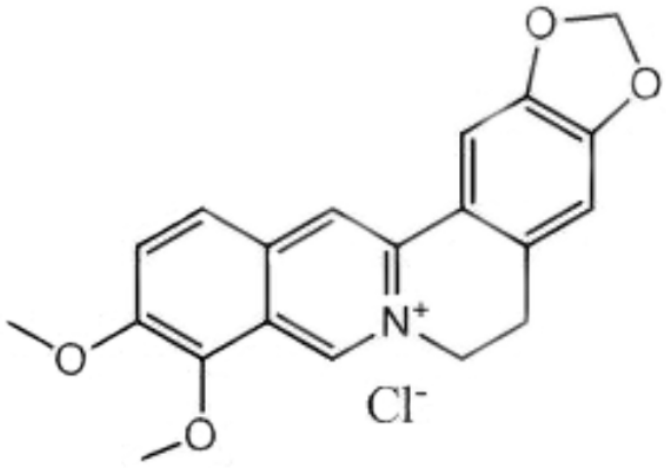

Figure 1: The structure of berberine hydrochloride.

study, we designed the liposomes-gel as the novel delivery to encapsulate BBH and investigated the release properties of the formulation *in vitro*.

Persistent inflammation in chronic wounds will increase reactive oxygen species that prevent wound healing, so antioxidants are critical in wound healing.[15,16] Freag *et al.* showed that berberine (1 mg/mL) oleate complex can effectively play an antioxidant role in the skin of psoriasis mice, reducing malondialdehyde and increasing glutathione.[17] We designed a new formulation based on liposomes and sodium alginate. The interaction of liposome and sodium alginate hydrogel changes the release characteristics. The unsaturated fatty acids contained in soy lecithin make the liposomes prepared to have good antioxidant properties and also affect the stability of the liposomes. The liposome nanoparticles evenly dispersed in sodium alginate hydrogel matrix as a novel delivery vehicle will also improve the stability and antioxidant properties of liposomes. The antioxidant activity of the BBH-loaded liposomes-gel was investigated through the inhibitory ability of DPPH free radicals, H_2O_2, and lipid peroxidation.

MATERIALS AND METHODS

Materials

Berberine hydrochloride was obtained from Xi'an Reain Biotechnology Co., Ltd. (Xi'an, China). Soy lecithin, cholesterol and sodium alginate were obtained from Tianjin Guangfu Fine Chemical Research Institut (Tianjin, China). Ethanol absolute (AR) was purchased from Shanghai RichJoint Chemical Reagents Co., Ltd. (Shanghai, China). 1,1-Diphenyl-2-trinitrophenylhydrazine (DPPH) and thiobarbituric acid (TBA) were purchased from Shanghai Yuanye Biology Science and Technology Co., Ltd. (Shanghai, China). Hydrogen peroxide (AR) was obtained from Shanghai SuYi Chemical Reagent Co., Ltd. (Shanghai, China). Iron II sulfate heptahydrate (AR) and trichloroacetic acid (TCA) (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of Berberine Hydrochloride-loaded Liposomes-gel

The preparation of liposomes refers to the thin-film dispersion hydration method.[18] Weigh 0.3 g of soy lecithin and 0.1 g of cholesterol into a round-bottom flask, add ethanol absolute to dissolve completely, spin the flask to form a lipid film, and remove absolute ethanol at room temperature. Add 10 ml of 0.4 mg/mL BBH solution into the flask, hydrate at 40℃ for 1 hr, and filter the obtained sample with 0.22μm microporous membrane for 3 times to obtain BBH-loaded liposomes (BBH-L) solution. A Sephadex G-50 column (Shanghai Blue Season Technology Development Co., Ltd. Shanghai, China) was used to remove the free berberine hydrochloride from BBH-L. Blank liposomes (B-L) were prepared in the same way.

Add 0.1 g of sodium alginate powder to 10 mL of BBH-loaded liposomes solution (sodium alginate concentration equivalent to 1%), place it on a magnetic stirrer and stir evenly, and make the hydrogel swell overnight to obtain yellow BBH-loaded liposomes-gel (BBH-L-Gel). Blank liposomes-gel (B-L-Gel) were prepared in the same way using blank liposomes. Blank hydrogel (B-Gel) and BBH-loaded hydrogel (BBH-Gel) were prepared with PBS solution and berberine hydrochloride solution in the same way. Figure 2 shows the molecular structure of BBH-loaded liposomes-gel.

Characterization of Liposomes

The encapsulated berberine hydrochloride was determined by a UV-1000 spectrophotometer (AOE Instruments, Shanghai, China) at 345nm. Calculate the concentration of encapsulated drug according to the analytical curve of berberine hydrochloride (2.0 to 10.0 μg/mL, A=0.0633*C+0.0028, R^2=0.9999). The encapsulation rate (EN%) of BBH is calculated using the following equation:

$$EN(\%) = \frac{\text{Encapsulated drug}}{\text{Total drug}} \times 100$$

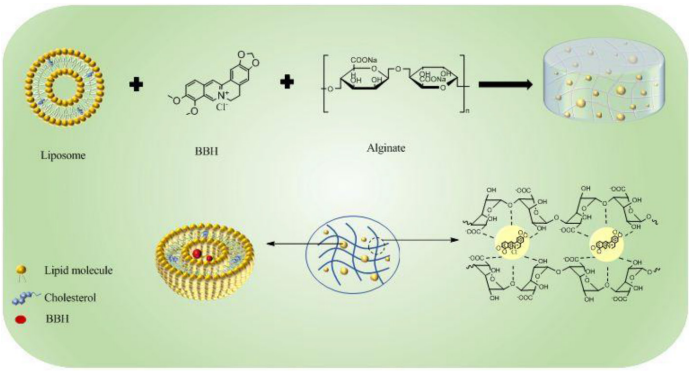

Figure 2: Molecular structure of BBH-loaded liposomes-gel.

The sample was diluted 100 times with a PBS solution, and their morphology was observed by transmission electron microscope (HITACHI-HT7700, Japan). The Malvern nano-particle size potential analyzer (ZEN3690, UK) was used to measure the particle size and potential of liposomes.

Determination of Liposomes Stability

Berberine hydrochloride-loaded liposomes solution stored at 2-8°C in the dark, and take samples for particle size and encapsulation efficiency determination after 3 months.

Viscosity and pH Measurement of Hydrogel

Check the hydrogel for characteristics such as color, pH, and viscosity. The pH meter (Leici PHSJ-4A, Shanghai, China) was used to determine pH of the hydrogel, and the average value was calculated from three measurements. The hydrogel samples were diluted 5-fold and their viscosity was measured using a capillary viscometer (Capillary inner diameter 3.00 mm, Shanghai Shenyi Glass Products Co., Ltd. Shanghai, China), recording time.

Drug Release from Liposomes-gel *in vitro*

The dialysis membrane with a molecular wight cutoff of 8000-14000 was put on Franz diffusion cell for experiments. The experiment was divided into eight groups: PBS, BBH, B-L, BBH-L, B-Gel, BBH-Gel, B-L-Gel, BBH-L-Gel. Put 1 mL of the sample into the supply tank, a small stir bar was placed in the receiving tank and fill it with PBS (pH 7.4). Put the diffusion cell device into the magnetic stirrer, and set the temperature to 37±0.5°C with moderate stirring speed. Within the first 12 hr of the experiment, 2 mL of solution was taken out of the receiving tank every 1 hr, and then take samples every 12 hr until the end of 84 hr. Add the same amount of PBS solution to the receiving tank after each sampling. The spectrophotometer measured the absorbance of various sample separately.

DPPH free Radical Scavenging Capacity Assay

The capacity of scavenging DPPH free radicals from berberine hydrochloride and its preparations was determined. Take 2 mL of the sample solution and 1 mL of 0.2 mmol/L DPPH-ethanol absolute solution in a centrifuge tube, mix well, react in the dark for 30 min, adjust to zero with ethanol absolute, and measure the absorbance at the wavelength of 517 nm. The blank group changed the sample solution into PBS solution, and the sample control group changed DPPH- absolute ethanol solution into absolute ethanol. According to the absorbance value of each group, the following formula was used to calculate the DPPH free radical scavenging rate (E%):

$$E\% = 1 - \frac{A_{sample} - A_{control}}{A_{blank}} \times 100$$

Determination of H_2O_2 Scavenging Capacity

Take 0.6 mL sample solution and 1.8 mL 0.4% H_2O_2 solution in a centrifuge tube, mix them, react for 10 min, adjust the zero with distilled water, and measure the absorbance at 230 nm. The blank group changed the sample solution into PBS solution, and the sample control group changed the H_2O_2 solution into distilled water. The H_2O_2 scavenging rate was calculated in the same way as the DPPH free radical scavenging rate.

Lipid Peroxidation Inhibition Analysis

Fe^{2+} can induce lipid peroxidation to produce malondialdehyde (MDA), and the degree of lipid peroxidation can be evaluated by the change of MDA content.[19] The product of MDA reacting with TBA at high temperature can be measured by a spectrophotometer at 532 nm.[20]

The experimental mice (Kunming female mice) were fasted for 12 hr, sacrificed, and their livers were harvested. The bloodstains were repeatedly washed with cold normal saline at 4°C, the filter paper was blotted dry, and the mass was weighed. Add 9 times the amount of m/v to cold physiological saline, and homogenize it with a glass homogenizer. The suspension was centrifuged at 4 000 r/min for 15 min to prepare 10% liver homogenate, which was stored at 4°C for later use. The experiment was divided into three groups, namely blank group, model group and sample group. First, 1 mL of liver homogenate was put in each tube, and the sample group was added to berberine hydrochloride solution with concentration of 0.4~2.0 mg/mL, blank liposomes-gel and BBH-loaded liposomes-gel 0.1 mL, respectively. The blank group and model group were replaced by the same amount of normal saline. Except for the blank group, 0.1 mL $FeSO_4$ (10 mmol/L) was added to each tube, and the blank group was replaced with the same amount of normal saline. After mixing, they were incubated for 1.5 hr in a 37°C water bath. Take out the test tubes, cool them to room temperature, then add 2 mL10% TCA, 5min later add 1 mL 0.67% TBA, 95°C water bath for 40min, take them out, cool them to room temperature with running water. Take the supernatant of the sample and measure the absorbance at the wavelength of 532 nm. According to the absorbance of blank group (A_0), model group (A_c) and sample group (A_s), the following formula was used to calculate the MDA inhibition rate (IR%).

$$IR(\%) = \frac{A_c - A_s}{A_c - A_o} \times 100$$

Statistical Analysis

SPSS statistic 23.0 software (IBM, USA) was used for one-way ANOVA to analyze the statistically significant differences of the results, which were expressed as mean ± standard deviation.

RESULTS

Encapsulation rate of liposomes

The encapsulation rate of BBH-loaded liposomes measured after centrifugation was 79.62±4.20%. The results showed that the liposomes prepared by the thin-film dispersion hydration method with soybean lecithin and cholesterol as raw materials had a good encapsulation effect on berberine hydrochloride.

Particle size, and potential of liposomes

The results of the average particle size and zeta potential of blank liposomes and BBH-loaded liposomes are shown in Table 1. The liposomes prepared by the thin-film dispersion hydration method have suitable particle size. The particle size of liposomes increases after drug encapsulation.

Transmission Electron Microscope Observation of Liposomes

Figure 3 shows the liposomes under a transmission electron microscope. The liposomes were spherical in appearance, smooth in surface, and dispersed independently of each other.

Stability Study of Liposomes

Table 2 shows the particle size and encapsulation rate of BBH-L after storage for 3 months. Compared with the initial results, there was no statistical difference in the stability of liposomes (p>0.05). The results indicated that the liposomes were stable for 3 months at 2-8°C.

Table 1: The average particle size and zeta potential of liposomes.

Group	Particle size/nm	Zeta potential/mV
B-L	134.6 ± 12.9	-30.9 ± 2.4
BBH-L	153.7 ± 11.2	-32.5 ± 2.9

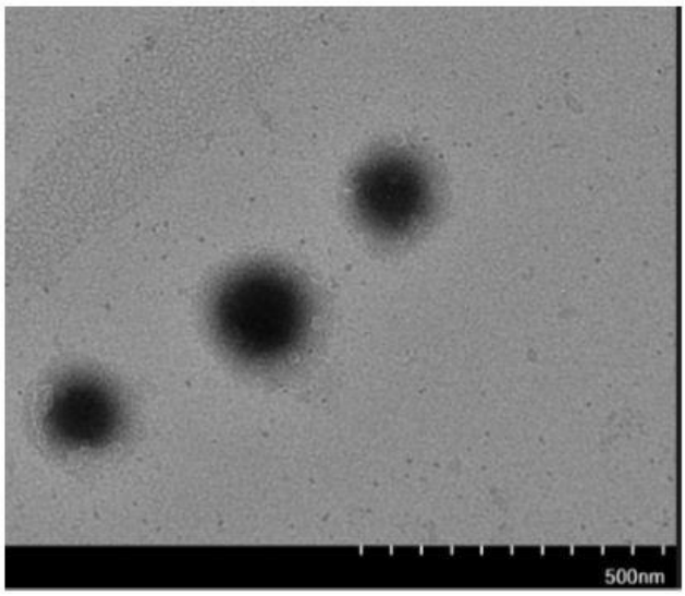

Figure 3: BBH-loaded liposomes observed under the electron microscope.

Viscosity and pH of Hydrogels

The viscosity and pH value of B-Gel, BBH-Gel, B-L-Gel, and BBH-L-Gel are shown in Table 3. The results showed that the viscosity and pH of berberine hydrochloride-loaded liposomes-gel were suitable for skin administration.

In vitro Release Study

The release curves of BBH, BBH-L, BBH-Gel and BBH-L-Gel were plotted with time as abscissa and cumulative permeation quantity (Q) per unit area as the ordinate as ordinate, as shown in Figure 4.

With the increase of diffusion time, berberine hydrochloride was released into PBS solution across the dialysis membrane. It can be seen from Figure 4 that the cumulative release quantity per unit area of BBH and BBH-Gel increased rapidly before 600 min, and then stabilized. The release quantity of BBH-L and BBH-L-Gel increased slowly and continuously within 84 hr. At 84 hr, the cumulative release per unit area of the BBH-L, BBH-Gel, and BBH-L-Gel groups was lower than that of the BBH group, indicating that both liposomes and sodium alginate hydrogel had sustained and controlled effects on berberine hydrochloride release.

The cumulative release quantity of the BBH-Gel group is lower than that of BBH, which may be due to the berberine hydrochloride being in the cross-linked structure of sodium alginate hydrogel to form interacting macromolecules, and berberine hydrochloride is difficult to release from the hydrogel.[21,22] The cumulative release amount per unit area of BBH-L increased gradually, and the final release amount was close to that of the BBH-Gel group. Due to the sustained-release effect of liposomes, the release rate of BBH-L group was slower than that of BBH-Gel group.

The release rate of the BBH-L-Gel group is the slowest because the diffusion of BBH needs to pass through the double barrier of

Table 2: The particle size and encapsulation rate of liposomes before and after 3 months.

Storage time/months	0	3
Particle size/nm	153.7 ± 11.2	147.46±10.9
Encapsulation effciency	79.62±4.20%	74.28±3.43%

Table 3: Viscosity and pH of different hydrogels.

Group	Viscosity (×10^{-3}Pa*s)	pH
B-Gel	8.23±0.83	7.12±0.06
BBH-Gel	9.44±0.70	6.69±0.05
B-L-Gel	15.67±0.76	7.27±0.06
BBH-L-Gel	17.98±7.57	7.33±..07

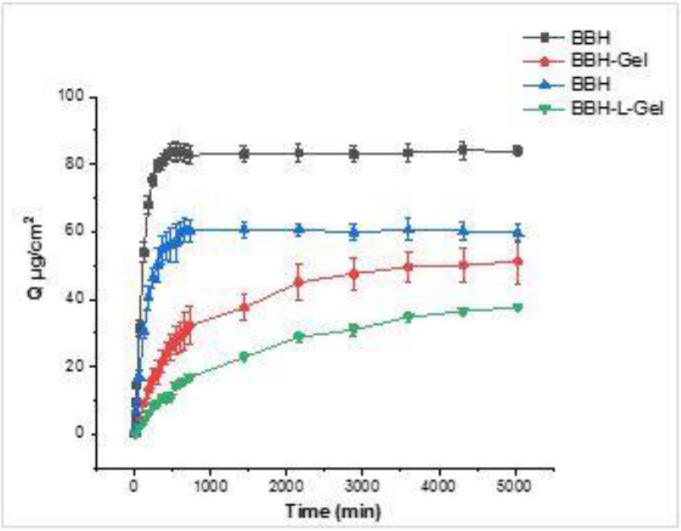

Figure 4: Cumulative release quantity per unit area of BBH and its preparations.

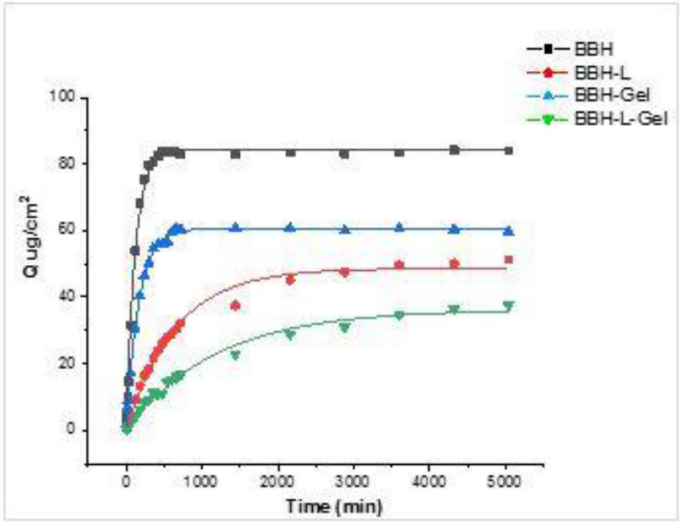

Figure 5: The fitting curve according to First-order release model.

liposomes and hydrogel, so berberine hydrochloride is difficult to release from the liposomes-gel. The viscosity of the BBH-L-Gel group is higher than that of the BBH-Gel group, which limits the molecular diffusion of berberine hydrochloride.

First-order release model is fit for the diffusion curve of BBH-loaded liposomes-gel *in vitro*, and the results are shown in Figure 5. According to curve fitting, the release of BBH from the liposomes-gel followed a first-order release model,[23] and the correlation R^2 is above 0.99 (Table 4).

Table 4: The fitting formula of the first-order release model for each group.

Group	Fitting equation	R^2
BBH	$Q=84.2110*(1-e^{(-0.00844t)})$	0.9968
BBH-L	$Q=48.6379*(1-e^{(-0.00154t)})$	0.9910
BBH-Gel	$Q=60.5666*(1-e^{(-0.00595t)})$	0.9986
BBH-L-Gel	$Q=36.2882*(1-e^{(-8.4688t)})$	0.9903

The Scavenging Effect of BBH on DPPH and H_2O_2

The mass concentration of BBH in the range of 1.00 to 5.00 mg/mL could increase the scavenging rate of DPPH free radicals from 31.70% to 94.44%, as shown in Figure 6. The curve regression was performed on the drug concentration to obtain the equation: E%=0.1469*C+0.32209 (R^2=0.973), and the half-scavenging rate (IC_{50}) of BBH on DPPH free radical was calculated to be 2.22 mg/mL.

BBH with a concentration of 0.4~2.0 mg/mL had a good scavenging ability on H_2O_2. Figure 7 shows that when the concentration of BBH reached to 1.6 mg/mL, the scavenging rate could reach over 90%. The curve regression was performed on the drug concentration to obtain the equation: E%=0.4767*C+0.0134 (R^2=0.978), and the half-scavenging rate (IC_{50}) of BBH on H_2O_2 was 0.99 mg/mL. The scavenging effect of BBH on hydrogen peroxide was higher than that of DPPH free radicals.

The Scavenging Effect of BBH-Containing Preparations on DPPH and H_2O_2

The scavenging abilities on DPPH free radicals and H_2O_2 were measured after the preparation was diluted 10 times (the content of BBH in the preparation was equivalent to 40 μg/mL). It could be seen from Figure 8 that BBH (40 μg/mL) had a low scavenging ability to DPPH free radical and H_2O_2, while the scavenging rate on DPPH free radical and H_2O_2 by the BBH-loaded preparation was higher than that of blank preparation group, which indicates that BBH and the preparation play a synergistic role in the antioxidation. The scavenging rate of the groups containing liposomes all reached more than 50%, indicating that liposomes have obvious antioxidant effects. The scavenging rate of the BBH-loaded liposomes-gel was higher than that of the BBH-loaded liposomes, which may be due to the enhanced stability of the liposomes surrounded by the sodium alginate hydrogel network and reduced the auto-oxidation of phospholipids. However, sodium alginate hydrogel group had a low scavenging rate of DPPH free radicals due to the low concentration of sodium alginate.[24] Overall, the liposomes-gel have good antioxidant properties, which improve the antioxidant application of berberine hydrochloride.

The results of MDA inhibition by BBH and BBH-L-Gel

The inhibitory rate on MDA showed an upward trend when the mass concentration of BBH was in the range of 0.4 to 2.0 mg/mL, as shown in Figure 9. Berberine hydrochloride could effectively inhibit the production of hepatic lipid peroxide MDA. The degree of lipid oxidation can also be expressed by changes in absorbance values. The larger the MDA absorbance value, the higher the degree of lipid oxidation. Fe^{2+}-induced not only

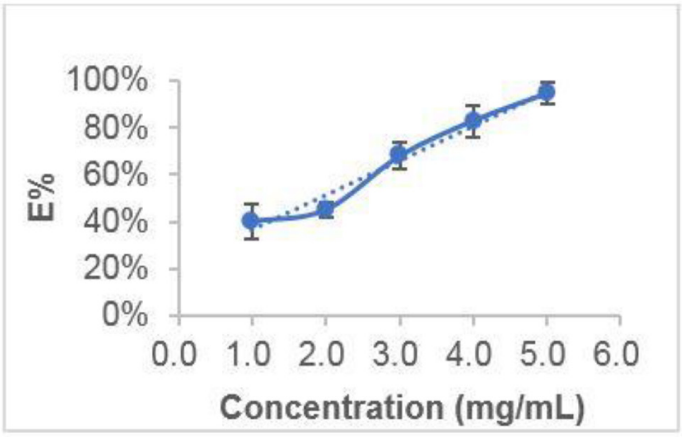

Figure 6: The scavenging rate (E%) of DPPH free radical by the different concentration of BBH.

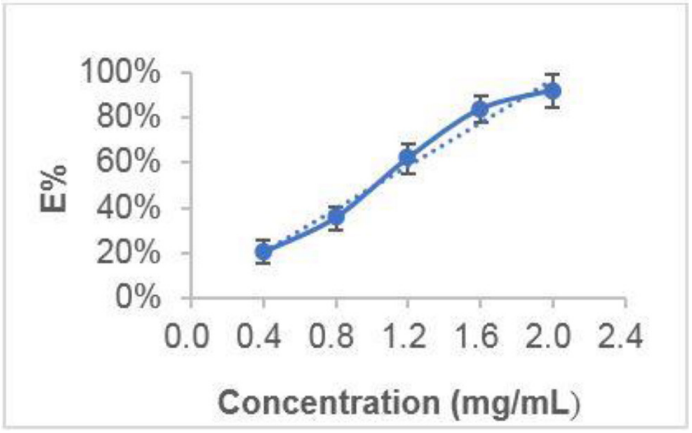

Figure 7: The scavenging rate (E%) of H_2O_2 by the different concentration of BBH.

oxidizes hepatocytes, but also leads to oxidation of liposome membranes to produce MDA. The amount of MDA produced by the blank liposomes-gel was similar to that of the model group (no difference in absorbance between the two groups, $p>0.05$), indicating that the oxidation of the liposomes-gel protects the hepatocytes from being oxidized. Figure 10 shows that the absorbance of the BBH-loaded liposomes-gel group is lower than that of the blank liposomes-gel group, indicating that the BBH-loaded liposomes-gel has a certain degree of inhibition on the production of MDA. Berberine hydrochloride is encapsulated in the liposomes-gel, in which it exerts an antioxidant effect.

DISCUSSION

The physicochemical properties of drugs and the preparation method of liposomes can affect the encapsulation rate of BBH-loaded liposomes,[25,26] and different composition of liposomes will affect their particle size and potential.[27] Liposomes prepared by thin-film dispersion method have good encapsulation efficiency and appropriate particle size. The preparation method and storage

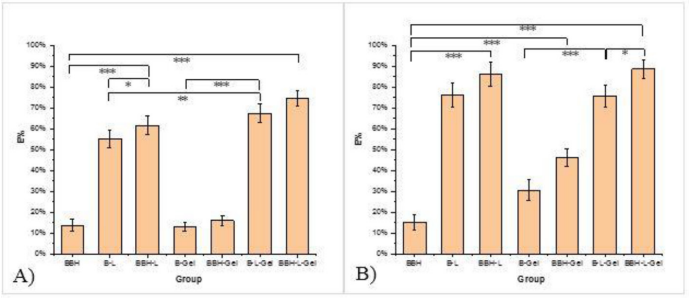

Figure 8: (A) The scavenging rate (E%) of DPPH free radical by BBH-containing preparations. (B) The scavenging rate (E%) of H_2O_2 by BBH-containing preparations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

conditions of liposomes can affect their stability.[28] Temperature of 2-8°C is a suitable storage condition for liposomes.

Sodium alginate molecule contains a large number of "-COO-" groups, and its hydrophilicity changes with the change of pH. The aqueous solution of sodium alginate has certain adhesiveness and is easy to adhere to skin and mucous membrane, so it can be used as a drug carrier for skin administration.[29] The ability of sodium alginate to readily form the hydrogel prevents inactivation of sensitive drugs, proteins and other active substances, which improves its biomedical applicability.[30] The viscosity, pH, and cross-linking form of sodium alginate will be the factors affecting drug release.[31]

Molecules of a certain size can be retained by the dialysis membrane, the smaller molecules can quickly penetrate the dialysis membrane into the release medium, while the larger molecules are intercepted outside the dialysis membrane. Compared with liposomes and sodium alginate hydrogel, berberine hydrochloride has the smallest molecular weight and is most easily permeated across dialysis membranes. This method is suitable for berberine hydrochloride release studies *in vitro*.[32,33] Through release studies *in vitro*, it was found that the liposomes-gel is suitable as a drug carrier for the release of BBH. Liposomes-gel has a sustained and controlled release effect on BBH due to the good encapsulation efficiency of liposomes and the three-dimensional network structure of hydrogel.

DPPH free radicals as organic free radicals are relatively stable, which are widely used to determine the antioxidant activity in vitro.[34] It has a strong absorption at the wavelength of 517 nm. Some antioxidants combine with DPPH free radicals to reduce their absorbance. By detecting the absorption at this wavelength, the scavenging rate of free radicals can be determined and the antioxidant capacity of the drug can be evaluated.[35] Hydrogen peroxide (H_2O_2) is a strong oxidant which can be oxidized and decomposed to generate oxygen and water. A certain amount of antioxidants can react with H_2O_2 to reduce the harm caused by it.36 Berberine hydrochloride has a much lower ability to scavenge DPPH free radicals and H_2O_2 compared with vitamin C and some natural medicines.[37] The IC_{50} of polysaccharides from

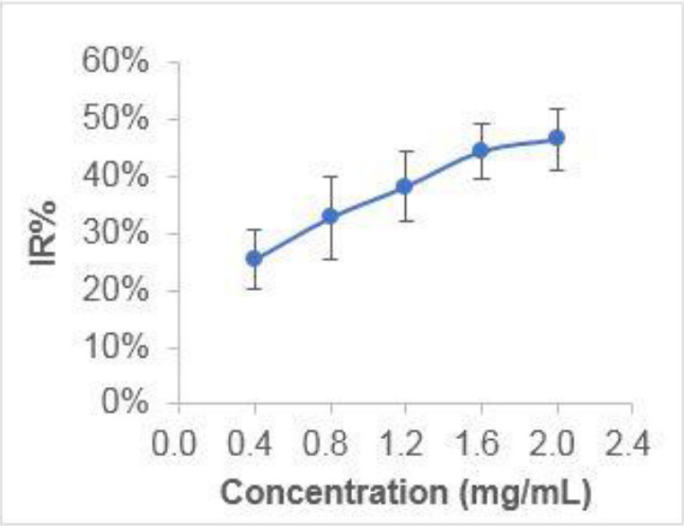

Figure 9: The inhibition rate (IR%) on MDA by the different concentration of BBH.

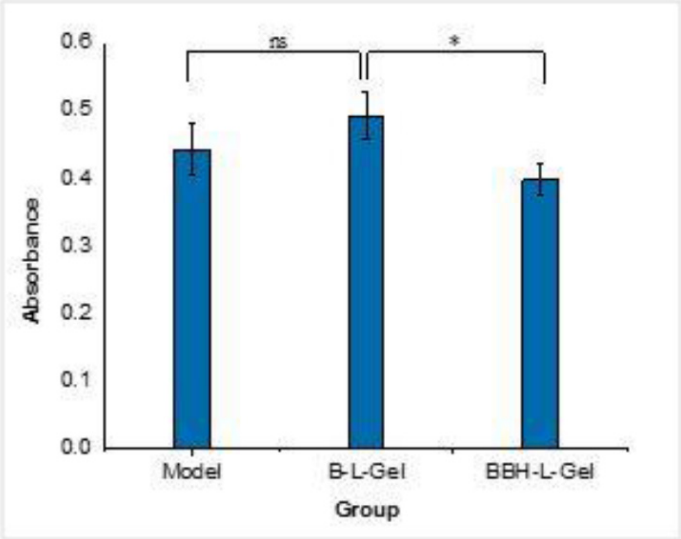

Figure 10: The absorbance of MDA in BBH-loaded liposomes-gel. (*ns*) *P*>0.05, **P*<0.05.

Semen Juglandis to DPPH scavenge rate is 0.21 mg/mL, and its antioxidant activity is obviously stronger than that of berberine hydrochloride.[38] Most natural active antioxidants contain active functional groups, such as phenolic hydroxyl groups. Derivatives containing phenolic hydroxyl groups have better direct antioxidant activity than berberine.[39]

The main components of lipids in cell membranes are phospholipids and cholesterol. The phospholipid bilayer constitutes the basic scaffold of the cell membrane. The polar top (-OH) of the cholesterol molecule is close to the hydrophilic end of the phospholipid, and the steroid ring at the non-polar end interacts with the fatty acid end of the phospholipid. The presence of cholesterol molecules weakens the dispersive force of binding between phospholipid molecules and enhances the fluidity of cell membranes.[40] Phospholipids are rich in unsaturated fatty acids, and when attacked by reactive oxygen species, peroxide products such as MDA are generated, resulting in changes in cell membrane fluidity and permeability. Therefore, the body's antioxidant is particularly important. Iron is the most important promoter of lipid peroxidation among transition metals *in vivo*.[41] We studied the anti-lipid peroxidation ability of BBH and BBH-loaded liposomes-gel with Fe^{2+} -induced lipid peroxidation of hepatocytes to produce MDA as an oxidation model. Berberine hydrochloride and liposomes-gel have synergistic antioxidant effect. Some studies have shown that berberine hydrochloride can treat diabetes, cardiovascular and cerebrovascular diseases and other diseases by reducing the oxidative stress of the body.[42,43]

CONCLUSION

Berberine hydrochloride-loaded liposomes-gel was prepared for antioxidant research in this study. The liposomes-gel showed good stability and sustained-releasing behaviors for berberine hydrochloride *in vitro*. Berberine hydrochloride-loaded liposomes-gel has good scavenging ability to DPPH free radicals and hydrogen peroxide, and berberine hydrochloride can effectively inhibit lipid peroxidation. The liposomes-gel helps to improve the bioavailability of berberine hydrochloride and its application in antioxidant.

ACKNOWLEDGEMENT

The authors are grateful to Department of Education of Anhui Province of China, Natural Science Foundation of Anhui Province of China, Anhui University of Chinese Medicine and Quality Engineering Project of Anhui Provincial Department of Education, for providing the experimental resources and financial support for the article.

Funding

This project was financially supported by Department of Education of Anhui Province of China (Grant number: KJ2019A0470), Natural Science Foundation of Anhui Province of China (Grant number: 1608085MH227), Anhui University of Chinese Medicine annual innovation training program for College Students (Grant number: 2017142, 2017171) and Quality Engineering Project of Anhui University of Chinese Medicine in 2021 (Grant number: 2021zlgc042) and Quality Engineering Project of Anhui Provincial Department of Education in 2021 (Grant number: 2021jyxm0824).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BBH: Berberine Hydrochloride**; AR:** Analytical Reagent; **PBS:** Phosphate Buffer Solution**; BBH-L:** Berberine Hydrochloride-

loaded Liposomes; **BBH-Gel:** Berberine Hydrochloride-Loaded Hydrogel; **BBH-L-Gel:** Berberine Hydrochloride-loaded Liposomes Gel; **DPPH:** 1,1-Diphenyl-2-trinitrophenylhydrazine**; TBA:** Thiobarbituric Acid**; TCA:** Trichloroacetic Acid**; MDA:** Malondialdehyde.

SUMMARY

The liposomes-gel has sustained and controlled release ability for berberine hydrochloride *in vitro* which can be used as a delivery carrier. Berberine hydrochloride-loaded liposomes-gel has good scavenging ability to DPPH free radicals and H_2O_2, and can effectively inhibit lipid peroxidation damage. Liposomes-gel helps to improve the bioavailability and antioxidant activity of berberine hydrochloride.

REFERENCES

1. Ickenstein LM, Garidel P. Lipid-based nanoparticle formulations for small molecules and RNA drugs. Expert Opin Drug Deliv. 2019;16(11):1205-26. doi: 10.1080/17425247.2019.1669558, PMID 31530041.
2. Le NTT, Cao VD, Nguyen TNQ, Le TTH, Tran TT, Hoang Thi TT. Soy lecithin-derived liposomal delivery systems: Surface modification and current applications. Int J Mol Sci. 2019;20(19):4706. doi: 10.3390/ijms20194706, PMID 31547569.
3. Kothale D, Verma U, Dewangan N, Jana P, Jain A, Jain D. Alginate as promising natural polymer for pharmaceutical, food, and biomedical applications. Curr Drug Deliv. 2020;17(9):755-75. doi: 10.2174/1567201817666200810110226, PMID 32778024.
4. Li D, Wei Z, Xue C. Alginate-based delivery systems for food bioactive ingredients: An overview of recent advances and future trends. Compr Rev Food Sci Food Saf. 2021;20(6):5345-69. doi: 10.1111/1541-4337.12840, PMID 34596328.
5. Hernández-González AC, Téllez-Jurado L, Rodríguez-Lorenzo LM. Alginate hydrogels for bone tissue engineering, from injectables to bioprinting: A review. Carbohydr Polym. 2020;229:115514. doi: 10.1016/j.carbpol.2019.115514, PMID 31826429.
6. Tønnesen HH, Karlsen J. Alginate in drug delivery systems. Drug Dev Ind Pharm. 2002;28(6):621-30. doi: 10.1081/ddc-120003853, PMID 12149954.
7. Rastogi P, Kandasubramanian B. Review of alginate-based hydrogel bioprinting for application in tissue engineering. Biofabrication. 2019;11(4):042001. doi: 10.1088/1758-5090/ab331e, PMID 31315105.
8. Yu J, Chen Z, Yin YZ, Tang C, Hu E, Zheng S, *et al*. Improving topical skin delivery of monocrotaline via liposome gel-based nanosystems. Curr Drug Deliv. 2019;16(10):940-50. doi: 10.2174/1567201816666191029125300, PMID 31660816.
9. Cicero AF, Baggioni A. Berberine and its role in chronic disease. Adv Exp Med Biol. 2016;928:27-45. doi: 10.1007/978-3-319-41334-1_2, PMID 27671811.
10. Imenshahidi M, Hosseinzadeh H. *Berberis vulgaris* and berberine: An Update Review. Phytother Res. 2016;30(11):1745-64. doi: 10.1002/ptr.5693, PMID 27528198.
11. Imenshahidi M, Hosseinzadeh H. Berberine and barberry (*Berberis vulgaris*): A clinical review. Phytother Res. 2019;33(3):504-23. doi: 10.1002/ptr.6252, PMID 30637820.
12. Warowicka A, Nawrot R, Goździcka-Józefiak A. Antiviral activity of berberine. Arch Virol. 2020;165(9):1935-45. doi: 10.1007/s00705-020-04706-3, PMID 32594322.
13. Liu CS, Zheng YR, Zhang YF, Long XY. Research progress on berberine with a special focus on its oral bioavailability. Fitoterapia. 2016;109:274-82. doi: 10.1016/j.fitote.2016.02.001, PMID 26851175.
14. Kheir MM, Wang Y, Hua L, Hu J, Li L, Lei F, *et al*. Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol. 2010;48(4):1105-10. doi: 10.1016/j.fct.2010.01.033, PMID 20138204.
15. Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, *et al*. The role of oxidative stress and antioxidants in diabetic wound healing. Oxid Med Cell Longev. 2021;2021:8852759. doi: 10.1155/2021/8852759, PMID 33628388.
16. Fitzmaurice SD, Sivamani RK, Isseroff RR. Antioxidant therapies for wound healing: A clinical guide to currently commercially available products. Skin Pharmacol Physiol. 2011;24(3):113-26. doi: 10.1159/000322643, PMID 21242718.
17. Freag MS, Torky AS, Nasra MM, Abdelmonsif DA, Abdallah OY. Liquid crystalline nanoreservoir releasing a highly skin-penetrating berberine oleate complex for psoriasis management. Nanomedicine (London, England). 2019;14(8):931-54. doi: 10.2217/nnm-2018-0345, PMID 30925102.
18. Zhang H. Thin-film hydration followed by extrusion method for liposome preparation. Methods Mol Biol. 2017;1522:17-22. doi: 10.1007/978-1-4939-6591-5_2, PMID 27837527.
19. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283(2-3):65-87. doi: 10.1016/j.tox.2011.03.001, PMID 21414382.
20. Garcia YJ, Rodríguez-Malaver AJ, Peñaloza N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. J Neurosci Methods. 2005;144(1):127-35. doi: 10.1016/j.jneumeth.2004.10.018, PMID 15848246.
21. Mourtas S, Fotopoulou S, Duraj S, Sfika V, Tsakiroglou C, Antimisiaris SG. Liposomal drugs dispersed in hydrogels. Effect of liposome, drug and gel properties on drug release kinetics. Colloids and surfaces B, Biointerfaces. 2007;55(2):212-21. doi: 10.1016/j.colsurfb.2006.12.005. PMID 17223020.
22. Takagi I, Shimizu H, Yotsuyanagi T. Application of alginate gel as a vehicle for liposomes. I. Factors affecting the loading of drug-containing liposomes and drug release. Chemical & pharmaceutical bulletin. 1996;44(10):1941-7. doi: 10.1248/cpb.44.1941. PMID 8904824.
23. Sawaftah NA, Paul V, Awad N, Husseini GA. Modeling of Anti-Cancer Drug Release Kinetics From Liposomes and Micelles: A Review. IEEE transactions on nanobioscience. 2021;20(4):565-76. doi: 10.1109/tnb.2021.3097909. PMID 34270430.
24. Elbayomi SM, Wang H, Tamer TM, You Y. Enhancement of Antioxidant and Hydrophobic Properties of Alginate via Aromatic Derivatization: Preparation, Characterization, and Evaluation. Polymers. 2021;13(15):2575. doi: 10.3390/polym13152575. PMID 34372178.
25. Nii T, Ishii F. Encapsulation efficiency of water-soluble and insoluble drugs in liposomes prepared by the microencapsulation vesicle method. Int J Pharm. 2005;298(1):198-205. doi: 10.1016/j.ijpharm.2005.04.029. PMID 15951143.
26. Has C, Sunthar P. A comprehensive review on recent preparation techniques of liposomes. Journal of liposome research. 2020;30(4):336-65. doi: 10.1080/08982104.2019.1668010. PMID 31558079.
27. Calvo A, Moreno E, Larrea E, Sanmartin C, Irache JM, Espuelas S. Berberine-Loaded Liposomes for the Treatment of *Leishmania infantum*-Infected BALB/c Mice. Pharmaceutics. 2020;12(9):858. doi: 10.3390/pharmaceutics12090858. PMID 32916948.
28. Yu JY, Chuesiang P, Shin GH, Park HJ. Post-Processing Techniques for the Improvement of Liposome Stability. Pharmaceutics. 2021;13(7):1-16. doi: 10.3390/pharmaceutics13071023. PMID 34371715.
29. Abbasi AR, Sohail M, Minhas MU, Khaliq T, Kousar M, Khan S, et al. Bioinspired sodium alginate based thermosensitive hydrogel membranes for accelerated wound healing. International journal of biological macromolecules. 2020;155:751-65. doi: 10.1016/j.ijbiomac.2020.03.248. PMID 32246960.
30. Sanchez-Ballester NM, Bataille B, Soulairol I. Sodium alginate and alginic acid as pharmaceutical excipients for tablet formulation: Structure-function relationship. Carbohydr Polym. 2021;270:118399. doi: 10.1016/j.carbpol.2021.118399. PMID 34364633.
31. Severino P, da Silva CF, Andrade LN, de Lima Oliveira D, Campos J, Souto EB. Alginate Nanoparticles for Drug Delivery and Targeting. Current pharmaceutical design. 2019;25(11):1312-34. doi: 10.2174/1381612825666190425163424. PMID 31465282.
32. Solomon D, Gupta N, Mulla NS, Shukla S, Guerrero YA, Gupta V. Role of *in vitro* Release Methods in Liposomal Formulation Development: Challenges and Regulatory Perspective. The AAPS journal. 2017;19(6):1669-81. doi: 10.1208/s12248-017-0142-0. PMID 28924630.
33. Morais JM, Burgess DJ. In vitro release testing methods for vitamin E nanoemulsions. Int J Pharm. 2014;475(1-2):393-400. doi: 10.1016/j.ijpharm.2014.08.063. PMID 25178829.
34. SIRIVIBULKOVIT K, NOUANTHAVONG S, SAMEENOI Y. Paper-based DPPH Assay for Antioxidant Activity Analysis. J Analytical Sciences. 2018;34(7):795-800. doi: 10.2116/analsci.18P014. PMID 29998961.
35. Yao J, Shahldl F. Critical Re-Evaluation of DPPH assay: Presence of Pigments Affects the Results. Journal of agricultural and food chemistry. 2019;67(26). doi: 10.1021/acs.jafc.9b02462. PMID 31184887.
36. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS letters. 2000;486(1):10-3. doi: 10.1016/s0014-5793(00)02197-9. PMID 11108833.
37. Zhang Z, Kong F, Ni H, Mo Z, Wan JB, Hua D, et al. Structural characterization, α-glucosidase inhibitory and DPPH scavenging activities of polysaccharides from guava. Carbohydrate polymers. 2016;144. doi: 10.1016/j.carbpol.2016.02.030. PMID 27083799.
38. Ren X, He L, Wang Y, Cheng J. Optimization Extraction, Preliminary Characterization and Antioxidant Activities of Polysaccharides from *Semen Juglandis*. Molecules (Basel, Switzerland). 2016;21(10):1335. doi: 10.3390/molecules21101335. PMID 27735839.
39. Rajasekhar K, Samanta S, Bagoband V, Murugan NA, Govindaraju T. Antioxidant Berberine-Derivative Inhibits Multifaceted Amyloid Toxicity. iScience. 2020;23(4):101005. doi: 10.1016/j.isci.2020.101005. PMID 32272441.

40. Schade DS, Shey L, Eaton RP. Cholesterol review: A metabolically important molecule. Endocr Pract. 2020;26(12):1514-23. doi: 10.4158/EP-2020-0347, PMID 33471744.
41. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017;482(3):419-25. doi: 10.1016/j.bbrc.2016.10.086, PMID 28212725.
42. Deng H, Jia Y, Pan D, Ma Z. Berberine alleviates rotenone-induced cytotoxicity by antioxidation and activation of PI3K/Akt signaling pathway in SH-SY5Y cells. NeuroReport. 2020;31(1):41-7. doi: 10.1097/WNR.0000000000001365, PMID 31688419.
43. Pei C, Zhang Y, Wang P, Zhang B, Fang L, Liu B, *et al*. Berberine alleviates oxidized low-density lipoprotein-induced macrophage activation by downregulating galectin-3 via the NF-κB and AMPK signaling pathways. Phytother Res. 2019;33(2):294-308. doi: 10.1002/ptr.6217, PMID 30402951.

Cite this article: Wang M, Xia H, Yang X, Zhang Q, Li Y, Wang Y, Xia Y, Xie Z. Berberine Hydrochloride-loaded Liposomes-gel: Preparation, Characterization and Antioxidant Activity. Indian J. Pharm. Edu. Res. 2023;57(1):74-82.