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Preparation and pharmaceutical/pharmacodynamic evaluation of topical brucine-loaded liposomal hydrogel

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Abstract To reduce the toxicity and enhance the therapeutic efficacy of brucine, a traditional Chinese medicine for relieving arthritic and traumatic pain, in this study, a novel brucine-loaded liposomal hydrogel (BLH) formulation, suitable for topical application, was developed. Spherical liposomes composed of lecithin and cholesterol, with brucine, was prepared by a modified ethanol-dripping method. High percentage (over 80%) of encapsulated brucine in liposomes was obtained. Topical liposomal hydrogel formulations were prepared by further incorporation of the prepared liposomes into structured carbopol 940 hydrogels with the concentration of carbopol 1.0%, the ratio of glycerol to carbopol 8:1 and the brucine content 0.1%. The liposomal hydrogel formulations provided an obvious promotion for skin permeation of bruicne while for the free brucine in hydrogels (BH), there was no detectable drug permeation through the skin. The safety evaluation showed that the prepared BLH were no irritation to both the broken and integrity skin. Pharmacodynamic evaluation revealed that the BLH showed a better therapeutic efficacy than that of the BH. So, it can be concluded that the BLH developed here could represent a safe, effective and promising transdermal formulation for local treatment of analgesic and anti-inflammatory disease.

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1 Introduction

Brucine, one of the bitter alkaloids of Chinese folk medicine, Strychnos nux-vomica L. (*Loganiaceae*), is known as an effective analgesic and anti-inflammatory agent for relieving arthritic and traumatic pain [1]. However, due to high incidence of side-effects, such as violent convulsion and even lethal poisoning, brucine, till now, has never been widely used in clinic [2]. Therefore, it is thus apparent that, for a therapeutic application of this drug, there is a need for a better brucine formulation that is less toxic while maintaining or possibly enhancing its therapeutic efficacy.

Colloidal drug delivery systems represent a mature, versatile technology with considerable potential for the entrapment of both lipophilic and hydrophilic drugs [3]. Among them, liposome, which can effectively decrease the toxic side effects and increase the bio-availability of the drug to the target tissue, thus leading to an enhanced therapeutic effect in vitro and in vivo [4], appears to be most attractive. This is especially true for the topical liposomal formulations, which are known to be the most direct and convenient administration [5, 6]. According to the previous extensive investigations, the major advantages of topical liposome formulations include the followings: (1) reduced side effects and incompatibility adsorption that may arise from undesirably high systemic absorption of drugs, (2) enhanced skin permeation and promoted dermal delivery, leading to greater dermal localization of drugs at the site of administration as a result of the high affinity of liposomes with stratum corneum [7, 8].

In recent years, either for enhanced therapeutic effectiveness or for clinical convenience, a number of modification methods for liposomal formulations have been proposed. Due to excellent biocompatibility and water permeability, polymeric hydrogels, such as drug-in-

liposome-in-gel complex formulation, are excellent candidates for long-term depot [9]. It is well-accepted that encapsulation or entrapment of drugs in liposomes and then incorporation the liposomes in hydrogels might lead to distinct changes of pharmacokinetic and pharmacodynamic properties of the drug, and in some cases, causes an apparent decrease in toxicity and/or increase in therapeutic efficacy [4, 10, 11]. In addition, the composition of the hydrogel has obvious influence on the stability as well as the rate of penetration of liposome-entrapped drugs into skin [12].

Because of long residue time at the target site, excellent skin permeation enhancing effect, controlled release properties and good buffering capacity [4, 11, 13], carbopol-based hydrogels are approved for pharmaceutical use in several different administration routes [14]. Herein, a novel carbopol-based topical liposomal hydrogels were developed to solve the problems associated with the brucine in this study. Brucine-loaded liposomes were first prepared and then formulated into a hydrogels by dispersing the liposomal solution into carbopol hydrogels matrix. In vitro release of brucine, acute dermal toxicity and skin irritation from/of the brucin-loaded liposomal hydrogel (BLH) were studied. Also, using acetic acid-induced writhing test and xylene-induced mouse ear edema models, the pharmacodynamics of the liposomal hydrogels were evaluated in vivo. For comparison, the free brucine-loaded hydrogels (BH) was also investigated. The results obtained here confirmed the feasibility of BLH for topical administration especially for analgesic and anti-inflammatory treatment.

2 Experimental

2.1 Materials

Brucine was purchased from Fluka Co. (Switzerland). Lecithin (from soy beans, ≥92%), cholesterol, sodium deoxycholate and mannitol were all purchased from Yuanju Bioech Co. (China). Sephadex G-25 was obtained from Amersham Biosciences AB (Sweden). Xylene was bought from Shanghai Chemical Reagent Co. (China). Acetic acid and Tween-80 were from Sinopharm Chemical Reagent Co. (China). All solvents used were of analytical grade.

2.2 Animals

New Zealand albino rabbits weighing 2 kg of both sexes, nude mouse (BALB/c) weighing 18–22 g of both sexes were used for the experiments. Before experiments, the animals were acclimatized for about 3 days and fed with standard diet and allowed water ad libitum, unless otherwise noted. All animal experiments were approved by the Animal Ethics Committee, Ministry of Health, Beijing, China.

2.3 Preparation of liposome brucine

A modified ethanol-dripping method was used to prepare liposomal brucine. Lipid (Lecithin:Cholesterol = 6:1,w/w), sodium deoxycholate, Tween-80, and brucine (16:4:4:1w/w) were dissolved into ethanol and subsequently sonicated to mix uniformly. After that, the solution was slowly dripped into distilled water and the concentration of the ethanolic-lipid phase in the final suspension was kept 20%. After that, the suspension was transferred into a high-pressure homogenizer (ATS Engineering Inc., Italy) and homogenized for 15 min under steady increasing pressures (20, 40, 60, and 80 MPa). Finally, the suspensions were freeze-dried for 48 h and the dry powders were stored in a desiccator. Before characterization and application, the BLH powder was rehydrated in PBS and sonicated for 3 min.

2.4 Fabrication of brucine liposomal hydrogel

2.4.1 Preparation of hydrogels for liposomal dispersion

Appropriate amount of carbopol-940 powders was weighted and added slowly into glycerin under constant stirring by a glass rod. And then specific amount of distilled water was drop-wised into the above solution. After that, the hydrogels were allowed to swell for at least 24 h until a completely swollen and transparent hydrogel was formed. Triethanolamine was then added with gentle stirring to avoid inclusion of air. The pH value of the hydrogels base thus obtained was 6.4 ~ 7. Blank hydrogels were also prepared for comparison.

2.4.2 Incorporation of liposomes into the hydrogels

Liposomal hydrogels were prepared by mixing the liposomes with the optimal hydrogels to make the final brucine content 0.1% (w/w, brucine in liposomal hydrogel). Control gels with 0.1% brucine content (w/w, free brucine/brucine hydrogel) were also made under the same conditions. Instead of liposomes, those samples contained free brucine.

2.5 Characterization of liposome

2.5.1 Measurement of the size and size distribution of liposomes

Liposomes dispersions were diluted with distillated water. The diameter and the zeta potential of the liposomes were determined using a laser light-scattering instrument

(Nicomp, 380/ZLS, Santa Barbara, USA). The measurement was performed at 25°C on the conditions of scattering angle of 90° and 12000 counts per second.

2.5.2 Morphology investigation

The morphology of the prepared liposomes was observed under a Field Emission Electron Microscope (FEEM) (JEM-2100F, JEOL, Japan). The operation parameters are listed as follows: Dot resolution rate 0.19 nm, line resolution rate 0.14 nm, accelerative electric tension 100 KV, angle of inclination 25°, STEM resolution rate 0.20 nm. The samples were diluted in distillated water and one drop of the liquid was dropped onto a copper screen and characterized.

2.5.3 Encapsulation efficiency

The encapsulation efficiency of the liposome after freeze-drying and rehydration was measured by gel filtration method using Sephadex column (Sephadex G-25 column, Sweden). The free drug was separated from the liposomes and the content of free drug was measured at λ_{max} (260 nm) with UV-1800 spectrophotometer (Japan).

The encapsulation ratio was calculated from Eq. 1:

$$EE\% = (\text{total drug} - \text{free drug})/\text{total drug} \times 100\% \quad (1)$$

2.6 In vitro drug release and skin permeation

2.6.1 In vitro release

BLH with different formulations were taken in a dialysis tube (Polycarbonate, molecular weight cut-off 3500) with the both end tied. The dialysis bags were submerged in physiological saline solution (0.9% w/v NaCl) (50 ml). The vials were placed on an orbital shaker maintained at 37°C under mild agitation (60 rpm). Aliquots of 4 ml of the physiological saline solution (PSS) were withdraw at predetermined intervals, and replaced by fresh dissolution medium. The withdrawn samples were analyzed at λ_{max} (260 nm) by a UV-1800 spectrophotometer (Japan). In order to elucidate and optimize the formulations of the hydrogel, in this work we investigated the effects of the concentration of the carbopol polymer, the ratio of glycerol to carbopol, and the concentration of brucine in hydrogels on the drug releasing rate. Also, 3 ml of liposomal brucine (drug content: 1 mg/ml) and 3 g hydrogels which contain 3 mg brucine were also studied for comparison.

2.6.2 In vivo skin permeation

In vitro skin permeation study was carried out according to the previously reported method [15]. The skin fragment used for the experiment was excised from SD mice and the adhering fat and other tissues were first removed. The skin was rinsed with PBS three times and clamped between the donor and the receptor chamber of the Franz diffusion cell with an effective permeation area of 3.14 cm^2 and a receiver cell volume 20 ml. Physiological saline solution (0.9% w/v NaCl) (PSS) was used as the receptor solution and incubated at 37 ± 0.2°C using a water bath with a magnetic stirrer at 300 rpm. An amount 2.5 g BLH and 2.5 g BH control hydrogel were all gently injected and spread onto the surface of skin in the donor chamber. The donor chamber was covered by parafilm to minimize the contamination of the gel. Prior to experiment, the Franz cell was shaken many times to ensure a full and uniform coverage of the skin. After 1, 2, 4, 6, 8 and 12 h, 2 ml samples were withdrawn from the receptor compartment and then replaced with an equal volume of fresh medium.

After skin permeation experiment, the residual formulation on skin was cleaned by distilled water, and the skin was wiped dry. Each skin were weighed, and then cut into small pieces. The tissue was further homogenized in 50% ethanol for 2 min by ultrasonication. Then the mixtures were centrifuged for 10 min at 8000 rpm and the supernatants were removed. Repeated the above extractive process for five times and the supernatants were all collected. After filtration, the drug concentration was analyzed to determine the brucine accumulation in the skin. The brucine accumulation in the skin was calculated by the following formula:

$$\text{Drug accumulation in skin} = (\text{drug residue inskin})/(\text{skin weight}).$$

The amount of brucine permeation and residue in the skin were all determined by UV-1800 spectrophotometer at 263 nm. In order to guarantee an effective and accurate determination of the brucine permeation and the brucine residue in the skin after permeation, we first performed the preliminary experiment using the brucine-free liposome. And scanned the releasing medium and the skin components with the similar pretreatment under the UV spectroscopy, and found there was no observable absorption at 260 nm nearby. That is to say, there is no interference from other releasing and skin components at 260 nm.

2.7 Acute dermal toxicity

An acute dermal toxicity study in rabbits was conducted in accordance with R.A. Isbrucker [16]. Rabbits of both sexes were divided into integrity skin group and broken skin group. There were three subgroups in each group: BH, BLH, and negative control group (blank hydrogels). One

day prior to commencing the study, the two sides of each rabbit's back were clipped with an electric clipper to expose an area about approximate 10% of the total body surface. Only those animals without skin injury or irritation were used in the test. Signs of "#" were cut on the tow depilated parts of each animal in broken skin group. The vehicle and drug were applied evenly to the exposed skin and covered with a semi-occlusive dressing. Twenty four hours later, the dressing was removed and the skin was gently cleaned. The animals were observed for mortality, signs of gross toxicity, and behavioral changes after application at least once thereafter 7 days.

2.8 Skin irritation study

A skin irritation test was also conducted on both sexes of rabbits to determine the potential irritation response based on the described previous method [17]. The groups of rabbits were divided the same as those in the acute dermal toxicity test. Each animal was treated with vehicle and drug (2 mg/g) and applied to the skin of one flank using a semi-occlusive patch. The patch was held with a bandage for 24 h. After that, the dressing and patch were removed and the skin residue was cleaned with water. Skin irritation effects were assessed at approximately 1, 24, 48 and 72 h after the removal of the dressings. Adjacent areas of untreated skin from each animal served as control. Erythema and edema were scored on a scale of 0–4, with 0 showing no effect and 4 representing severe erythema or edema (Table 1). The specific degree of irritancy was obtained by calculating the Primary Dermal Irritation Index (PDII) and classified according to the descriptive rating for mean primary dermal irritation index ($0 < \mathrm{PDII} \leq 2.0$: Slight irritation; $2.0 < \mathrm{PDII} \leq 5.0$: Moderate irritation; $\mathrm{PDII} > 5.0$: Severe irritation).

$$\mathrm{PDII} = \sum (\text{value of evaluation of dermalreactions})/4$$

2.9 Xylene-induced mouse ear edema

The assay of xylene-induced ear edema in mice was based on the reported method [18]. Male mice were divided into eight groups and each group consisted of ten mice: the positive control group received Yunnan Baiyao tincture (15 mg/kg), the negative group received blank hydrogels (15 mg/kg), three groups received BH (7.5, 15, and 30 mg/kg), three groups received BLH (7.5, 15, and 30 mg/kg), respectively. The vehicle and drug were topically administered to both faces of the right ear of the mice. 50 μl xylene was applied on the inner and outer surface of the right ear of each mouse, half an hour after the administrated of vehicle and drug. After 30 min, the animals were sacrificed by cervical dislocation. Disks of 9 mm diameter were removed from each ear and weighed in balance. The edematous was estimated as the difference in weight between the punches from right to left. The anti-inflammatory activity was expressed as percentage of the inhibition of edema (PIE). PIE was calculated using the following formula:

$$\begin{aligned}\mathrm{PIE} &= ((\text{control weight variation} - \text{treated weight variation}) \\ &\quad /\text{control weight variation}) \times 100\%.\end{aligned}$$

2.10 Acetic acid-induced writhing test

The response to intraperitoneal injection of 0.9% acetic acid was induced by the method proposed by Koster [19]. The groups of mice were the same as those in the above xylene-induced mouse ear edema test. The abdomens of mice were clipped with an electric clipper 24 h before experiments. Only those animals without injure or irritation of skin were used in the test. The abdomens of mice were treated with the vehicle and drug, and 30 min later the acetic acid (0.9%, 10 mg/kg) was injected. Immediately

Table 1 Scoring criteria for dermal reactions

Evaluation of dermal reactions			
Value	Erythema and eschar formation	Value	Edema formation
0	No erythema	0	No edema
1	Very slight erythema (barely perceptible), edges of area not well defined	1	Very slight edema (barely perceptible. Edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)	2	Slight edema (edges of area well defined by definite raising)
3	Moderate to severe erythema (defined in color and area well defined)	3	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet to crimson red) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond area of exposure)

after the injection of acetic acid, each animal was observed in an individual box within 20 min. The number of writhing and stretching response was recorded and the percentage protection (PP) was calculated using the following formula:

$$PP = ((\text{control mean} - \text{treated mean})/\text{controlmean}) \times 100\%$$

2.11 Statistical analysis

Data analysis was carried out with the SPSS software. Results are expressed as mean ± standard deviation (n = 3 independent samples). Statistically significant differences were determined with $P < 0.01$ as a minimal level of significance.

3 Results

3.1 Characterization of the prepared Brucine liposome

The particle size distribution of the prepared BLH is shown in Fig. 1. The average size of the liposomes varied between 30 and 190 nm, and the mean diameter of the liposomes was 78.6 ± 0.52 nm, a narrow population distribution. The zeta potential of BLH was −39.7 ± 1.5 eV. FEEM image of BLH prepared is presented in Fig. 2. It is clear that the particles were nearly spherical in shape and were well dispersed. The EE, determined by gel filtration, of the LB after freeze-drying and rehydration was 80.1% ± 1.2% (n = 4).

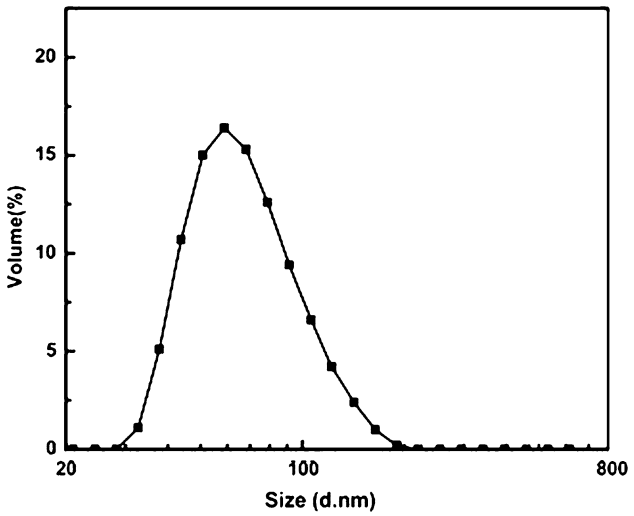

Fig. 1 Particles size distribution of the as-prepared liposomes of brucine

3.2 In vitro release study

To achieve optimal liposomal hydrogel formulation, the main experimental conditions, including concentrations of carbopol, different ratios of glycerol to carbopol and liposomal content in hydrogel, were optimized according to the drug release rate. Drug release profiles of different concentrations of carbopol in hydrogel and different ratios of glycerol to carbopol (w/w) through dialysis are showed in Figs. 3 and 4, respectively. At first glance, similar drug release patterns were observed for all liposomal hydrogel formulations. It can be seen from Fig. 3 that with the increasing of the concentration of carbopol in hydrogels in the range of 1.5–2.0%, the accumulative released of brucine from BLH were decreased slightly at the same time periods, implying that the diffusion of the brucine from these BLH was suppressed by the carbopol. As well, Fig. 4 indicated that the accumulative released of brucine from

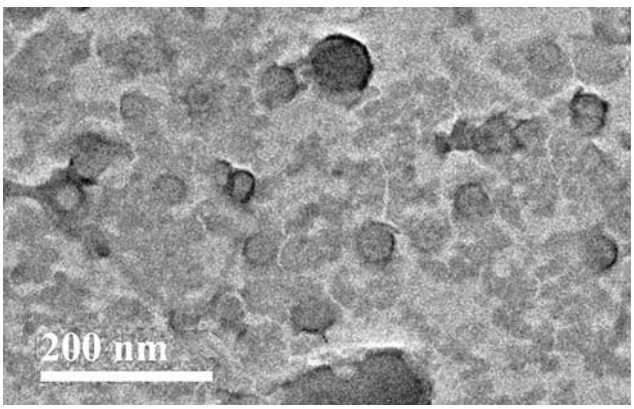

Fig. 2 FEEM photograph of liposome brucine(D-water) (×200,000)

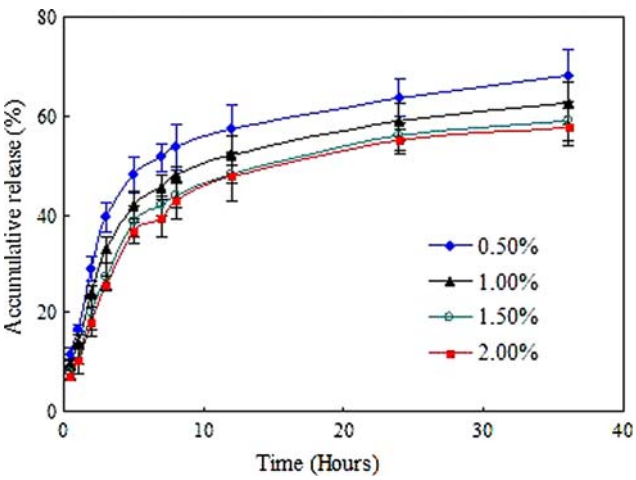

Fig. 3 Release of brucine from liposomal hydrogels with different contents of the carbopol in hydrogels (w/v). Each point represents the mean of determinations (mean ± SD, n = 3)

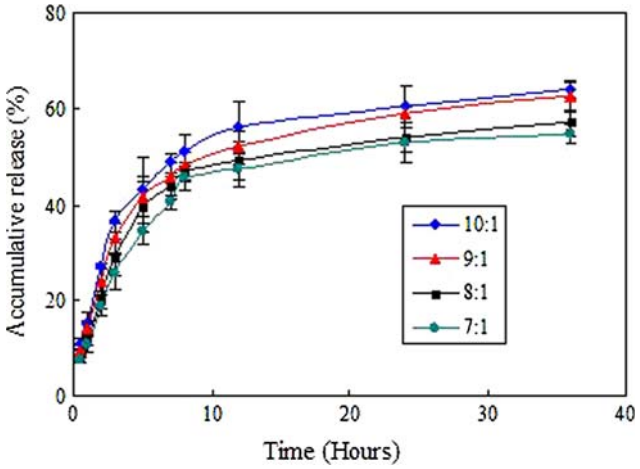

Fig. 4 Release of brucine from liposomal hydrogels with different ratios of glycerol to carbopol (w/w). Each point represents the mean of determinations (mean ± SD, n = 3)

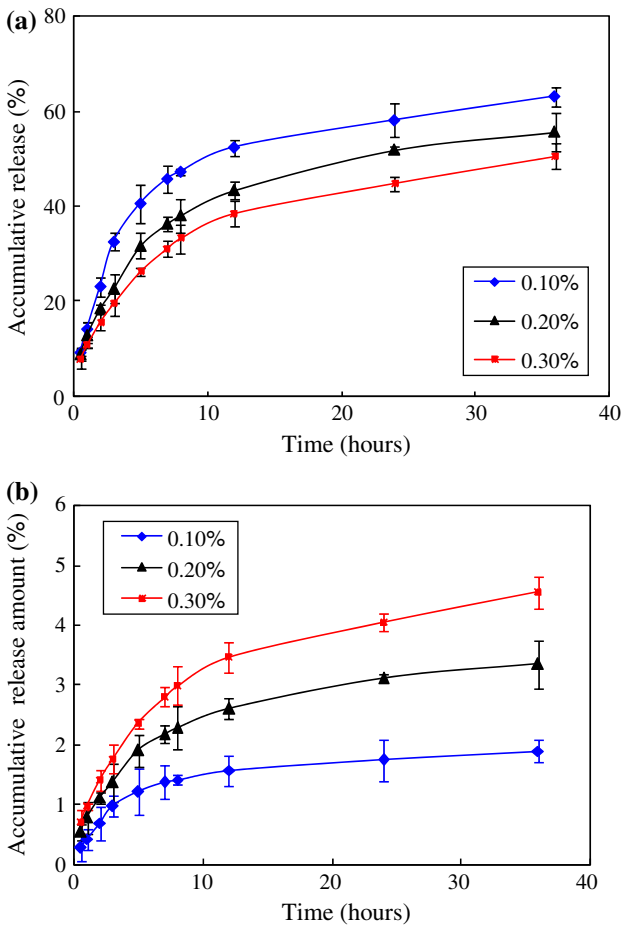

Fig. 5 Accumulative release percentage (**a**) and accumulative amount release (**b**) of brucine from liposomal hydrogels with different contents of liposomal brucine in hydrogels (0.1%, 0.2%, and 0.3%). Each point represents the mean of determinations (mean ± SD, n = 3)

the hydrogels at each time point was practically close to each other for the different ratios of glycerol to carbopol tested, proving that the ratio of glycerol to carbopol (in this range) did not obviously affect the diffusion of brucine from BLH.

The brucine amount-dependent release of brucine is presented in Fig. 5. And the Fig. 5(a and b) show the accumulative released (%) and the accumulative release amount of brucine from BLH at the specific period, respectively. It can be seen from Fig. 5a that with the increasing of the concentration of brucine in hydrogels, the drug accumulative released dramatically decreased (lower percentage of accumulative released brucine) and the lowest release of brucine was observed at 0.3% liposomal brucine loading. But Fig. 5b showed that more accumulative release amount were obtained from the higher brucine content in hydrogels.

These results led to the selection of a BLH concentration of 1.0% carbopol, a ratio of glycerol to carbopol 8:1 and a brucine amount 0.1% in the liposomal hydrogel for all subsequent experiments unless otherwise noticed.

The drug release profiles of BH, liposomal brucine and BLH prepared at the optimal conditions are compared and the results are showed in Fig. 6. It indicated that before encapsulated, brucine diffused from hydrogel quickly and the diffusion amount was up to 85% after 3 h. But after encapsulated in liposome, the release of brucine were substantially delayed, especially those loaded in the liposomal hydrogel. There was 68% of drug released into PSS receptor in liposomal brcine group after 8 h and only 42% was detected in BLH group. It is clear that encapsulation into liposomes exhibited an obvious effect of slow release, and the release rate of brucine from BLH was further decreased. We do believe that the lipid bilayer of the liposomes and the polymer chains, which provide a double barrier to drug, should be responsible for this phenomenon.

3.3 In vitro skin permeation

The skin permeation of brucine associated with BLH and BH is plotted in Fig. 7. As seen, almost no detectable brucine was permeated through skin for the BH group after 12 h. But for the BLH group, the accumulative skin permeation increased with the time increased and reached 39 μg/cm^2 after 12 h. The drug accumulation of BH and BLH in skin is compared and indicated in Fig. 8. After 12 h permeation, in the BH group, the brucine accumulation in skin reached 200 μg/g. But in the case of BLH, only 51 μg/g accumulated in the skin, an almost 3-fold decrease. These results indicated that BLH exhibited an obvious promotion of skin permeation.

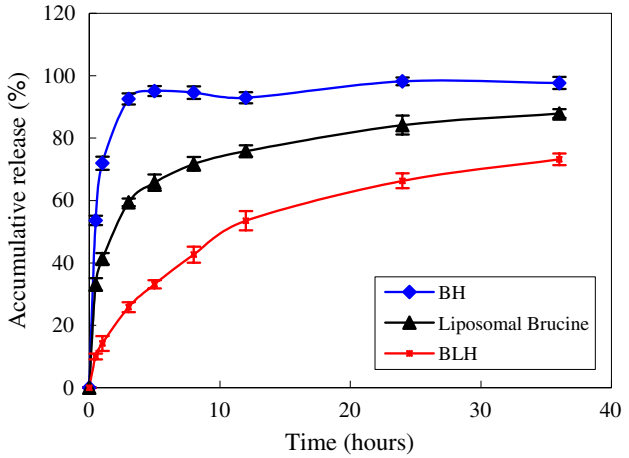

Fig. 6 Comparison of the release profiles of the brucine from different formulations. Each point represents the mean of determinations (mean ± SD, n = 3)

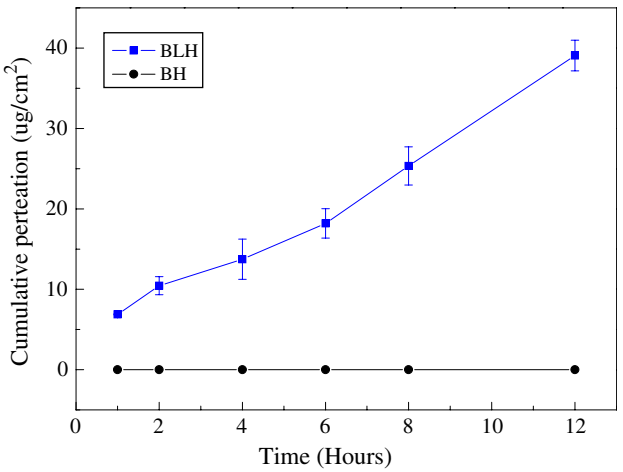

Fig. 7 In vitro permeation of brucine through skin. Each point represents the mean of determinations (mean ± SD, n = 3)

3.4 Acute dermal toxicity

No detectable systemic signs of toxicity, such as gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behaviors, were observed in the BLH and negative control group with both integrity skin and broken skin following the dermal application of liposomal gels (100 mg/kg body weight). But in the case of the BH groups, slight hyperaemia in integrity skin, and hyperaemia and cyanosisi in broken skin group were noted following the dermal application after removal of the dressing at 24 h and this persisted for up to 5 days. All animals treated by BH and BLH were survived, gained weight and appeared active and healthy. Thereby, the acute dermal toxicity of LD_{50} was greater than 100 mg/kg to both sexes of rabbits.

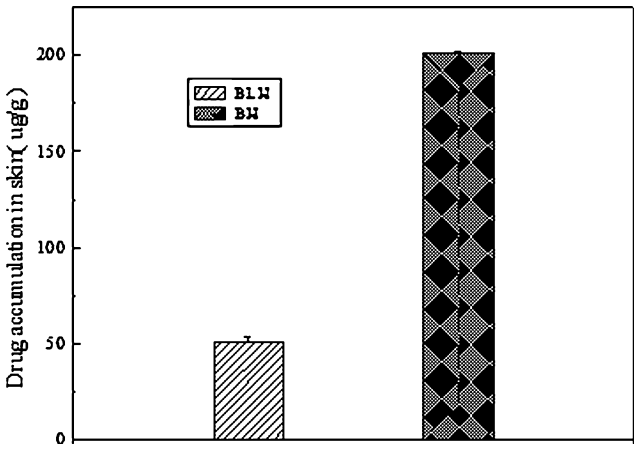

Fig. 8 Comparison of the brucine accumulations in the skin associated with the BLH and BH. Each point represents the mean of determinations (mean ± SD, n = 3)

These results showed that the BLH exhibited an obvious attenuation of the toxicity of brucine.

3.5 Skin irritation study

The objective of this study was to determine the potential irritation of BLH and BH to the integrity and broken skin of New Zealand albino rabbits. The results showed that after 1, 24, 48 and 72 h, no systemic signs of toxicity were observed in the integrity skin of the animals following the dermal application of each group. However, a slight erythema was noted in two rabbits in the broken group after 24 h application of BH, and the irritation response was improved with the increasing of the time. The specific PDII of the brucine in gels subgroup in broken skin group was 0.75, 0.25, 0.25, and 0 after 1, 24, 48, and 72 h (after removed the dressing), respectively.

3.6 Xylene-induced mouse ear edema

The anti-inflammatory responses of the BLH and BH on xylene-induced mouse ear edema are presented in Table 2. Statistical analysis demonstrated that compared with the free brucine at the same dosage, both BLH and BH showed significant anti-inflammatory response ($P < 0.05$). BLH at high dosage (30 mg/kg) showed an obvious inhibitory effect (33.0%) to the ear oedema induced by xylene ($P < 0.01$), even exceeding that of the positive control group (26.9%). Meanwhile, the BLH groups at the dosage of 7.5, 15, and 30 mg/kg caused significant stronger inhibitory effects than of the corresponding BH ($P < 0.05$). The PIE of BLH was 2-3 folds higher than that of the free BH at the same dosage, and BLH exerted its anti-inflammatory effects in a dose-dependent manner as shown in Table 2.

Table 2 Anti-inflammatory of BLH and BH on xylene-induced mouse ear edema

Groups	Dosage (mg kg^{-1})	Tumefaction percentage	PIE (%)
Negative	15	17.6 ± 6.71	/
Positive	15	12.9 ± 2.41[a]	26.9
BLH (1)	30	12.0 ± 2.35[ac]	31.8
BLH (2)	15	12.6 ± 2.18[bd]	28.2
BLH (3)	7.5	13.4 ± 2.17[bd]	23.8
BH (1)	30	15.4 ± 1.14[d]	12.5
BH (2)	15	15.7 ± 0.66[d]	10.8
BH (3)	7.5	16.1 ± 1.33[d]	8.63

[a] $P < 0.01$, [b] $P < 0.05$ *vs* negative control with the same dosage

[c] $P < 0.01$, [d] $P < 0.05$ *vs* Brucine with the same dosage

Negative group was treated with blank hydrogels

Positive group was treated with Yunnan Baiyao tincture

Data are expressed as mean ± standard deviation (n = 10)

Table 3 Analgesic effects of BLH and BH on acetic acid-induced writhing test

Groups	Dosage (mg kg^{-1})	Writhing times	PP (%)
Negative	15	74.2 ± 6.16	/
Positive	15	48.2 ± 8.56[a]	35.0
BLH (1)	30	46.0 ± 5.19[ac]	38.0
BLH (2)	15	53.6 ± 9.50[ad]	27.8
BLH (3)	7.5	63.2 ± 4.44[c]	14.8
BH (1)	30	55.6 ± 5.68[a]	25.1
BH (2)	15	59.4 ± 5.37	19.9
BH (3)	7.5	71 ± 10.2	4.31

[a] $P < 0.01$, [b] $P < 0.05$ *vs* negative control with the same dosage

[c] $P < 0.01$, [d] $P < 0.05$ *vs* Brucine with the same dosage

Negative group was treated with blank hydrogels

Positive group was treated with Yunnan Baiyao tincture

Data are expressed as mean ± standard deviation (n = 10)

3.7 Acetic acid-induced writhing test

Results obtained from acetic acid-induced writhing in mice are shown in Table 3. It was manifested that BLH and BH groups exerted significantly inhibitory effects on the writhing response induced by acetic acid with the exception of the BH at the dosage of 7.5 mg/kg. BLH inhibited writhing response induced by acetic acid in a typical dose-dependent manner. The maximal prohibition of the nociceptive response (38.0%) ($P < 0.01$) was achieved by BLH at the dosage of 30 mg/kg, greater than that of the positive control at 15 mg/kg (31.3%) ($P < 0.01$). In contrast with the free BH at the dosage of 7.5 and 30 mg/kg, more potential inhibitory effects were observed in the BLH groups. It was observed that the pain inhibitory intensity of BLH was 1.5 times higher than that of BH at the dosage of 30 mg/kg, indicating that after being encapsulated, the analgesic efficacy of brucine was pronouncedly enhanced.

4 Discussion

Brucine is one of the most effective Chinese medicines for arthritic and traumatic pain diseases. Its clinic application is limited, however, by the high incidence of side effects. So, in this study, a transdermal liposomal hydrogel system was developed to attenuate the toxicity associated with the brucine. To achieve this, a liposomal brucine was prepared by ethanol-dripping method first and the liposomes fabricated here were in the range of 30–190 nm with a mean diameter of 78.6 ± 0.52 nm (Fig. 1). The FEEM micrograph (Fig. 2) was further proof of the narrow size distribution of the as-prepared liposomes. In general, small particle sizes always result in decreased encapsulation efficiency, thereby decreasing the drug amount loaded. But in our study, high percentage of encapsulated drug in liposomes has been obtained (80.1% ± 1.2%), which was turned out to be an appropriate efficiency by the following in vitro and in vivo evaluation experiments.

For a liposomal hydrogel system, the drug release was depended on the diffusion function of both liposome and hydrogel matrix. Therefore, in this study, three sets of in vitro release experiments were carried out to optimize the formulation of BLH and the results were compared. The content of carbopol 940 in hydrogels in the range of 0.5%–2.0% had the similar drug release patterns, especially from 1.5% to 2.0%, suggesting that the carbopol content did not greatly affect the release kinetics of brucine from the BLH (Fig. 3). This is consistent with the previous report by Glavas-Dovov M, where they found that the concentration of carbopol in a range of 1.5%–2.0% slightly affected the release of lidocaine HCl [14]. From this viewpoint, the concentrations of carbopol from 0.5% to 2.0% are all suitable to prepare the BLH. But taking the appearance of the hydrogel into consideration, the ideal concentration of carbopol was 1.0% (uniformity, transparent, and no air bubbles). Glycerol, a widely used lubricant, was added into carbopol to improve the hydrogel quality in this study. The release result revealed that the amount of glycerol added into hydrogels did not significantly affect the release profile (Fig. 4). As we know, the release rate of the lipophilic drugs, such as brucine, from hydrogel matrix is predominantly determined by the drug physicochemical properties (lipophilicity and solubility). In this study, the liposomal brucine used was prepared by the same process. The increasing of the liposomal brucine

content meant the increasing of the brucine. Indeed, as drug loading increased, the amounts of accumulative released decreased (Fig. 5a). But in contrast, the accumulative amount of the drug release increased (Fig. 5b). Thus, it is proven that the drug loading (in the form of liposomes) is one of the main determining parameters of the release of brucine from BLH. In other words, liposomes are reservoirs that hold (lipophilic) drugs in gels and release them at a rate determined by the total amount of drug present in the gel.

In vitro release (Fig. 6) exhibited an obviously sustained release of brucine, in particular for the BLH. 85% of the drug was released after 3 h in BH, while, 68% brucine released from liposomal brucine group in 8 h and only 42% from BLH. The release rate of brucine from the BLH was slower than that from liposomal brucine, which could be expected with respect to the restrictions caused by the polymeric network of hydrogels [20, 21]. It is well known that controlled release is the typical property of colloidal drug delivery systems [22]. Regarding the tendency of drug release, the liposomal hydrogels formulation exerted a more prolonged release, which led to a longer pharmacodynamic action and intensive effects.

Previous studies have revealed that phospholipids may affect lipid domain of the stratum corneum, leading to an increased fluidity and thus a decreased barrier of the stratum corneum [4]. In vitro permeation test, as shown in Figs. 7 and 8, here proved that there was no detectable brucine permeation through the skin into receiver compartments and the drug accumulation in the skin was 200 μg/g after12 h for the BH group. In contrast, the accumulative drug permeation through skin in BLH group was reached 39 $\mu g/cm^2$ after 12 h while drug residue in the skin was just one-forth of that of BH. Due to the poor solubility and skin barrier, brucine can hardly permeate through skin layer and accumulate in skin. However, brucine, after loaded in liposomes and then incorporated in hydrogels, may easily penetrate through skin and sustain a long time release [23]. It was also revealed from the data of analgesic and anti-inflammatory activities (Table 2 and 3) that compared with the BH groups administration with the same dosage, the BLH groups showed an increased PIE and a significant higher PP, indicating that BLH had a better parmacodynamic action than that of BH. The results presented here suggested that a desirable and sustained delivery of brucine can be achieved by using liposomes and carbopol hydrogel vehicle.

In order to investigate the toxicity of the BLH developed here, the acute dermal toxicity and skin irritation of BLH were assessed in this study. In the present safety evaluation, the dosage was designed mainly according to the acute dermal LD_{50} of the drug. The results showed that when greater than 100 mg/kg BLH administrated, there were no irritation effects to the integrity and broken skin of New Zealand albino rabbits. However, BH exhibited acute toxicity to skin and slight irritation to broken skin. Apparently, after encapsulated in liposomes and then incorporated in hydrogels, the toxicity of brucine could be reduced dramatically. It is worthy to note that the brucine loaded in liposomal hydrogels was safe to broken skin which was common in traumatic pains. This low dermal toxicity, we think, can be attributed to the higher skin permeation and the lower skin accumulation associated with this new transdermal liposomal hydrogel system.

BLH is a safe strategy for application, but another question remains as to whether BLH retains the pharmacodynamic action after encapsulated and incorporated in liposomal gels. In order to address this question, in this study, the acetic acid-induced writhing and xylene-induced mouse ear edema models, which are the valid and reliable models of nociception and edema, were used to evaluate the therapeutic efficacies of the BLH. As shown in Table 2 and 3, whether encapsulated into liposome or not, the brucine hydrogels all exerted analgesic and anti-inflammatory efficacies. But in contrast, BLH formulations were more efficient than the corresponding BH at dosage ranging from 7.5 to 30 mg/kg. In addition, the therapeutic efficacy of the BLH exhibited a typical dose-dependent manner in the range from 7.5 to 30 mg/kg. These results confirmed that BLH not only keeps the pharmacodynamic effect of brucine, but also increases the bioavailability, which may be attributable to the controlled/sustained release and the enhanced skin penetration of liposomal hydrogels.

Brucine was mainly responsible for the analgesic and anti-inflammatory effects of the seeds of Strychnos nux-vomica. The innovative formulation of BLH system developed here can further enhance the topical and pantosomatous therapeutical efficacy of brucine while attenuating its toxicity, making it an effective and safe alternative agent for the local treatment of acute and chronic pain, such as arthritic and traumatic pain. These encouraging results represent a good basis for the further development of poisonous traditional Chinese medicine.

5 Conclusion

A novel formulation of brucine-loaded liposomal hydrogel for local pain treatment was proposed in the present study. The brucine-loaded liposome with spherical morphology and 80% encapsulation efficiency was prepared by a modified ethanol-dripping method. The brucine-loaded liposome, further incorporated into carbopol 940 hydrogels, could dramatically promote the skin permeation of bruicne and exerted negligible irritation to both broken and

integrity skin. In vivo xylene-induced mouse ear edema test and acetic acid-induced writhing test revealed that BLH showed a notable analgesic and anti-inflammatory response. Based on the above results, BLH delivery system might be a promising alternative agent for treatment of arthritic and traumatic pain diseases.

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