Original Article

Preparation of Hydroxyasiaticoside Nanoemulsions for the Therapeutic Application in Second-degree Scald Injuries in Rats

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Conflict of Interest

The authors report no conflicts of interest in this work.

Abstract

Background: Hydroxyasiaticoside attracts considerable attention due to its significant effect in the repair of scar wounds. However, its efficacy is hampered by low cell permeability and poor bioavailability, due to its high molecular weight and polarity. This limitation affects the utilization of hydroxyasiaticoside in the treatment of scalds, especially in transdermal delivery systems.

Methods: To overcome this challenge, we formulated hydroxyasiaticoside complex emulsions with improved intracellular permeability and efficacy. Firstly, the nanoemulsion formulation and process were optimized using response surface methodology to obtain a stable hydroxyasiaticoside nanoemulsion, which was then characterized for its physicochemical properties. Subsequently, a skin irritation test on rabbit skin was performed to evaluate the safety of the nanoemulsion. Finally, a second-degree scald rat model was used to evaluate the therapeutic efficacy of hydroxyasiaticoside nanoemulsions by observing changes in scalded wounds and pathologic sections in different treatment groups.

Results: The experimental results showed that the W/O/W nanoemulsions exhibited a particle size of 158.3 ± 1.07 nm, a PDI of 0.141 ± 0.020, a uniform morphology distribution, a potential of -46.1 ± 2.11 mV, and no delamination after centrifugation at 4000 r/min for 15 minutes. In addition, the hydroxyasiaticoside nanoemulsions showed superior wound-healing abilities in the rat model for second-degree scalded wounds, emphasizing their great potential for wound healing.

Conclusion: The above results provide some reference information for applying

hydroxyasiaticoside nanoemulsions in wound treatment.

Keywords: Hydroxyasiaticoside nanoemulsions, Transdermal drug delivery system, Second-degree scald, Physicochemical characterization

Introduction

As a frequent accidental injury in our daily lives, scalds are mainly caused by direct or indirect skin exposure to hot objects, resulting in varying degrees of tissue damage. It should not be overlooked that nearly half (49%) of burn cases result in disability, with 8% of them facing permanent disability.[1] Scalds not only cause immediate injuries such as redness, swelling, breakage, and localized blisters but may also leave permanent scarring or keloid scarring after healing, seriously affecting the quality of life.[2] In extreme cases, severe impairment of skin function may trigger a massive loss of body fluids, which in turn can lead to a range of life-threatening complications such as acid-base balance imbalance, shock, severe infections, and hypokalemia.[3] According to its severity, scalds can be roughly divided into three degrees and four categories,[4] of which second-degree scalds are more common in daily life. Nevertheless, its treatment and aftercare process are intricate and pose significant challenges. For second-degree scald wounds, current treatment methods mainly include physiotherapy, surgery,[5] and medication.[6] Physical therapy is usually used as an early intervention to slow the rate of wound deterioration and create favorable conditions for subsequent treatment, but its direct repair effect on the wound is limited. Surgical treatment, on the other hand,

requires a more in-depth treatment of the wound in a highly aseptic environment. However, this method not only increases the risk of wound infection and other complications but also imposes a greater financial burden on patients and families due to its high cost and the occupation of healthcare resources.[7] Therefore, surgical treatment is often not the first choice in non-essential cases. Given the above considerations, drug therapy occupies a prominent position in the treatment of scalds. By applying medications scientifically and reasonably, it can effectively control infection, promote wound healing, alleviate pain, and reduce scar formation, providing a safer, more effective, and economical treatment option for burn patients. Therefore, drug therapy should be prioritized in the treatment plan for burn patients.

In the field of drug therapy for scald treatment, the exploration of herbal monomers has increasingly emphasized its importance, attracting extensive attention and in-depth research endeavors across academia and industry alike.[8] Centella Asiatica, belonging to the genus Centella in the family Umbelliferae, as a perennial herbaceous plant, grows widely across the tropical and subtropical regions of the northern and southern hemispheres, including South Africa, India, and China, etc., demonstrating its richness as a natural resource and its wide range of applications[9]. The main active ingredients of Centella Asiatica are concentrated in pentacyclic triterpenoids,[10] especially asiaticoside, hydroxyasiaticoside (also known as Brahminoside), cumuloic acid, and hydroxycumulic acid.[10] Among them, hydroxyasiaticoside, as the key active ingredient, demonstrates remarkable efficacy in the treatment of scalded wounds through its unique bioactive mechanism. In-depth histopathological studies have revealed that its

therapeutic effects are primarily mediated through three pathways. Firstly, it exerts potent anti-inflammatory and antioxidant effects by effectively reducing the infiltration of inflammatory cells and lowering the levels of nitric oxide (NO) and malondialdehyde (MDA) in burned tissues, while simultaneously increasing the level of reduced glutathione (GSH) to enhance antioxidant capacity.[11] Secondly, it promotes tissue repair and collagen synthesis by accelerating the growth of newborn granulation tissue, promoting the proliferation of fibroblasts, and facilitating the epithelialization of the dermis. Specifically, it increases the levels of hydroxyproline—a key indicator of active collagen synthesis—and regulates the TGF-β/Smad signaling pathway while inhibiting matrix metalloproteinases, thereby preventing excessive extracellular matrix degradation and reducing scarring.[12] Finally, hydroxyasiaticoside regulates angiogenesis, ensuring adequate blood supply to the wound site, which collectively accelerates the rapid healing of the wound. However, although hydroxyasiaticoside and its derivatives show great potential in burn treatment, their highly water-soluble nature also poses challenges such as insufficient drug transdermal properties and limited bioavailability. This requires us to continuously explore the optimization of drug formulation and delivery mode in future studies to improve its therapeutic effect and ease of clinical application.

As an advanced drug delivery system, emulsions have demonstrated many significant advantages in the realm of medicine, particularly in the domain of transdermal drug delivery technology, where their application is exceptionally broad and profound.[13] The primary advantage lies in the fact that emulsions can substantially

improve the skin permeability of the drug, enabling the drug to more effectively cross the skin barrier and reach the lesion, thus realizing better therapeutic effects. In addition, the formulation design of emulsions is flexible, and the ratio and composition of the oil phase to the water phase can be precisely adjusted according to the nature of the specific drug and the therapeutic needs, to meet the diversified needs of drug delivery. Nanoemulsion is a highly dispersed and thermodynamically stable liquid dispersion system characterized by a very small particle size, and this tiny particle size gives nanoemulsion many unique physical and chemical properties, such as high surface area, excellent permeability and good stability[14]. Among many nanoemulsion systems, the dual emulsion structure of W/O/W (water-in-oil-in-water) composite nanoemulsions is particularly remarkable.[15] The outer water phase actively interacts with the moisture on the skin surface, which significantly enhances the affinity of the formulation with the skin and promotes closer contact and adhesion.[16] Meanwhile, the oil phase in the inner layer, with its excellent penetration ability, easily crosses the barrier of the stratum corneum composed of the lipid bilayer, opening up a channel for drug molecules to penetrate efficiently. More uniquely, the water-soluble drug ingredients carefully wrapped in the oil phase can penetrate deeper into the skin by this mechanism, thus significantly enhancing the transdermal absorption of the drug and further enhancing the therapeutic effect. This innovative drug delivery method undoubtedly opens up new possibilities for the development of transdermal drug delivery technology.

In summary, this study focuses on the preparation process of hydroxyasiaticoside W/O/W composite nanoemulsions and their potential application value in the field of

second-degree scald treatment. Firstly, based on the one-factor optimization strategy combined with response surface design methodology, we finely regulated the prescription composition of the nanoemulsions and successfully prepared hydroxyasiaticoside W/O/W composite nanoemulsions with stable properties and excellent performance. Subsequently, the safety and feasibility of this nanoemulsion when applied to the treatment of second-degree scalds were verified by skin irritation experiments. Finally, we implemented animal experiments to comprehensively evaluate the therapeutic efficacy of hydroxyasiaticoside W/O/W composite nanoemulsions in promoting the healing of second-degree scalds.

Materials and methods

Materials

Hydroxyasiaticoside (MA, purity≥90%) was purchased from Shanghai Macklin Biochemical Co., Ltd. Lemon oil was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Span 80, tween 80, polyvinyl alcohol, poloxamer 188 and sodium dodecyl sulfate (SDS) were purchased from Sinopharm Chemical Reagents Co., Ltd.

Preparation of hydroxyasiaticoside nanoemulsion

The hydroxyasiaticoside nanoemulsion was carefully formulated with the double emulsion method (W/O/W) employing a high-energy sonication process.[17] Drawing from the findings of one-factor experimental studies, the W/O colostrum was formulated with an aqueous solution of hydroxyasiaticoside as the internal aqueous phase, lemon oil as the oil phase, and Span80 serving as the emulsifier for the

colostrum.[18] Subsequently, the colostrum was incorporated into an aqueous solution containing SDS, the emulsifier for complex emulsions, to prepare the desired W/O/W complex emulsions.[19]

The optimal formulation process was rigorously determined using the Box–Behnken design.[20] This methodology examines the intricate correlations between variables and their responses, ultimately leading to the selection of the optimal formulation. According to the previous results of the one-factor experiment, the dosage of the W/O emulsifier (Span80) (A), the concentration of the O/W emulsifier (SDS) (B), the volume ratio between the external water phase and the oil phase (C) were selected as key factors to be further investigated, and the viscosity was selected as the response values for evaluating the performance of the different formulations.[21,22]

Characterization of the physicochemical properties of MA complex emulsion

Particle size and zeta potential

The particle size and zeta potential of nanoemulsion represent essential physicochemical attributes that fundamentally determine their behavior.[23,24] To accurately measure these properties, a Malvern laser particle size analyzer was used to provide a highly accurate assessment of particle size and zeta potential in the nanoemulsions. Additionally, to bolster the accuracy of our findings, the measurements were replicated three times, thereby ensuring the reliability and robustness of our results.

Determination of stability

A suitable quantity of the hydroxyasiaticoside nanoemulsion solution, previously refrigerated at 4°C, was retrieved to naturally warm to room temperature, and subsequently diluted with deionized water to achieve a specified concentration. An aliquot of the diluted solution was then taken to assess its average particle size and zeta potential using the Malvern laser particle size analyzer (Zetasizer Nano ZSE). Samples were analyzed on days 0, 5, 10, 15, 20, and 30. Throughout these 30 days, the variations in the average particle size and zeta potential were recorded to evaluate the stability of the nanoemulsion. In addition, accelerated experiments were also carried out by taking 1 mL of the above-prepared emulsion and centrifuging it in a centrifuge at a speed of 4000 r / min for 15 min.

Determination of viscosity

At room temperature, the prepared nanoemulsions underwent viscosity measurement employing a rotational viscometer set at 60 RPM with rotor No. 1, and the average viscosity value was obtained through triplicate testing.

Skin irritation test

Stimulation experiments were performed on the skin of individual rabbits.[25] Before drug administration, 24 hours ahead, they were shaved using an electric shaver on both sides of their spinal region on the back, covering an area of approximately 2.5 cm^2. Following the shaving, a visual inspection was conducted on the shaved skin to identify any potential injury resulting from the shaving process. If no signs of skin injury were discernible, the shaved area was sterilized, and the rabbits were then subjected to skin

irritation testing. The asiaticoside nanoemulsion was administered to the skin, and the area was monitored for the development of erythema and edema for three consecutive days.

Establishment and evaluation of animal model of second degree scald

Male SD rats (n=20), weighing about 250 g, were kept normally for 2 days to observe their survival, and then fasted for 48 hours, during which time they were fed only water. Before the experiment, rats were anesthetized by intraperitoneal injection with a 15% concentration of urethane solution according to their body weight (200 mg/kg). Then the rat's back skin was scalded with a homemade scalder (solid copper tube) for 15 seconds, i.e., a secondary scald model was prepared.

A dose of 0.2 mL was administered twice in the morning and evening, and the wound healing was photographed and recorded within 21 days, and the size of the healed wound was measured using a vernier caliper.

Four different groups of rats were treated with traumatic skin incisions for a period of 0, 7, 14, and 21 days. Subsequently, pathologic sections of the injured skin were carefully prepared and stained with hematoxylin and eosin (HE) to extensively examine the internal structural changes in the skin tissue.

Results and discussion

Preparation of MA nanoemulsion

Based on the single-factor analysis, lemon oil was chosen as the oil phase, while Span80 served as the emulsifier for preparing the crude emulsion. To evaluate the diffusion

behavior and determine the type of crude nanoemulsion containing hydroxyasiaticoside, Sudan III and methylene blue dyes were individually introduced into the system.[26] Upon addition of Sudan III to the hydroxyasiaticoside nanoemulsion, it dissolved rapidly and dispersed evenly, resulting in a homogeneous solution. Conversely, the introduction of methylene blue into the nanoemulsion led to a slower diffusion rate, poor dispersion, and observable stratification. These findings suggest that the initial nanoemulsion structure of hydroxyasiaticoside exhibits a water-in-oil (W/O) emulsion type.

The prepared primary emulsion (W/O) was added to a water tank containing an emulsifier to obtain a double emulsion. Then, using the same method, Sudan Red and methylene blue were added to identify the structure type of the double emulsion. After adding methylene blue to the primary emulsion, it could be dissolved and diffused quickly, forming a uniform solution, as shown in Figure 1(a). However, after adding Sudan III, the diffusion speed was slower, it was not easily dispersed, and there was a stratification phenomenon, as shown in Figure 1(b). Based on the above results, it can be identified that the hydroxyasiaticoside nano double emulsion is a W/O/W emulsion.

Response surface analysis

Based on a single-factor analysis, the dosage of Span 80, the concentration of SDS, and the volume ratio between the external water phase and the oil phase emerged as the pivotal factors requiring deeper scrutiny. To refine the emulsion formulation, kinematic viscosity was employed as the response variable, leveraging response surface analysis for optimization. In this context, each factor was assigned three distinct levels (-1, 0,

and 1) in the experimental design. Subsequently, the Design-Expert software was utilized to comprehensively analyze and optimize the formulation conditions, ensuring a thorough and systematic approach to the process. The specific levels of the factors in this test setup are outlined in Table 1. A total of 17 tests were conducted to analyze the response surface test results, as shown in Table 2.

Using the Box-Behnken method in Design Expert 8.0, a quadratic multiple regression analysis was performed on the data, resulting in a quadratic regression equation for the viscosity of the hydroxyasiaticoside nanoemulsion concerning A, B, and C. $R^2 = 0.9608$, indicating a good model fit for this equation. The equation was as follows:

$$Y=47.84+3*A+2.05*B-3.13*C+1.02*A*B+0.22*A*C-0.28*B*C-2.26*A^2-1.51*B^2+1.64*C^2$$

Statistical analysis results of the regression model are shown in Table 3, the model of the regression equations was significant with a *P* value of 0.0002, the *P* value of the lack of fit was 0.5657, and the coefficient of R was fine. The results of the variance analysis indicated that the volume ratio between the external water phase and the oil phase had a more significant influence on the kinematic viscosity. The kinematic viscosity gradually increases with the reduction of the volume ratio between the external water phase and the oil phase (Figure 2).

Physicochemical properties of MA nanoemulsion

The preparation of MA nanoemulsion presented a homogeneous appearance and was a white emulsion with no obvious precipitation, as shown in Figure 3(a). The average

size of the prepared nanoemulsion was 158.3 ± 1.07 nm (Figure 3(b), with a polydispersity index of 0.141 ± 0.020 and an average surface zeta potential of -46.1 ± 2.11 mV (Figure 3(c). These values demonstrate that the nanoemulsion, having undergone preparation, exhibits a comparatively narrow particle size distribution, and a relatively uniform population of nanoemulsion, signifying its exceptional stability. Furthermore, the presence of a negative charge contributes significantly to enhancing this stability, thereby preventing particle aggregation.[27]

The stability of MA nanoemulsion

An appropriate amount of MA nanoemulsion stored at 4°C was taken and naturally warmed to room temperature. Its particle size and zeta potential were measured three times using the Malvern laser particle size analyzer. With the same procedure, measurements were taken on the 0, 5, 15, 20, 25, and 30 days, and the results were plotted into a curve as shown in Figure 4. The results show that the particle size and potential of the nanoemulsion hardly changed during the observed period, indicating that the nanoemulsion was more stable when stored at 4°C and did not undergo physical changes such as aggregation. In addition, in the accelerated experiment, the nanoemulsion was centrifuged at 4000 r /min for 15 min, and the emulsion still appeared as a white homogeneous emulsion with no delamination, which also indicated that the nanoemulsion was well stabilized.

Determination of viscosity

The viscosity of the composite emulsion was measured using a rotational viscometer

(60 RPM L1), and the average value of its viscosity was 64.8 mPa·s for three times in parallel, which meets the requirement of low viscosity of nanoemulsion.

Skin irritation test

Stimulation experiments were conducted on rabbit skin to assess the biocompatibility of the nanoemulsions. After administering the drug, a comprehensive and meticulous observation was conducted on the skin of rabbits, with a particular focus on the two common skin irritation reactions of erythema and edema. Following a monitoring period, the results indicated that neither erythema nor edema was observed. The specific observations are illustrated in Figure 5. Furthermore, no other abnormal changes were noted in the rabbits' skin. Based on these experimental observations and records, it could be confidently concluded that under the given experimental conditions, the drug exhibited minimal irritation to rabbit skin, demonstrating good skin compatibility and safety. These findings hold significant implications for subsequent drug development, safety evaluation, and clinical applications.

Hydroxyasiaticoside nanoemulsions for the treatment of second-degree scald in rats

Construction of a scald model

The successful development of a second-degree burn animal model in rats was accomplished using a self-made copper scalding apparatus. During the experiments, the water bath was initially heated to 100°C, followed by applying heated, homemade iron and copper scalders onto the rat skin in a natural vertical position, with a strictly

controlled contact duration of 15 seconds. The experimental outcomes demonstrated that the homemade copper scalding instrument significantly disrupted the rat skin structure, resulting in the formation of distinct blisters.[28] In stark contrast, the iron scalding instrument, under identical conditions, merely caused skin redness and swelling, without any observable blister formation. This observation led us to conclude that the homemade copper scalding instrument, owing to its superior temperature transfer efficiency and intense heat concentration capabilities, emerged as a more suitable physical scalding tool for creating second-degree burn models in rats. The induced traumatic effects were not only pronounced but also closely aligned with the characteristic pathological manifestations of a second-degree scald model. Consequently, this customized copper scalder was adopted for rat scald modeling, thereby enhancing the accuracy, reproducibility of experimental outcomes, and biological validity of the established model.[29]

Evaluation of the efficacy of hydroxyasiaticoside nanoemulsions

Following the method above for preparing the animal model,[30] four scars were induced on the back of each rat, which were then divided into four groups: the control group (wounds treated with normal saline), the hydroxyasiaticoside nanoemulsion group, the blank nanoemulsion group (without drug), and the commercially available burn ointment control group. Each group consisted of 10 parallel samples, with each sample receiving treatment twice daily at 0.2 mL per administration. Photographs were taken to observe the changes in the wound surface on days 0, 3, 6, 9, 12, 15, 18, and 21, and the complete process for treating second-degree scalds in rats is shown in Figure 6.

Upon being scalded, the rats' wounds initially turned white and slightly elevated above the adjacent skin tissue. Following this, the wound tissue darkened in color and exuded tissue fluid. Subsequently, the wound tissue became purulent, accompanied by localized inflammatory reactions. As healing progressed, scabbing commenced, and the redness surrounding the wound edges gradually faded.[31] Ultimately, the wound underwent significant contraction, its color lightened, and it became visually similar to the surrounding normal skin tissue. Furthermore, it was evident from the figure that the four scald wounds of the same rat healed differently after different treatments. Among them, the best healing effect was observed in the hydroxyasiaticoside nanoemulsion group.

During the recovery and healing period of the rats, the transverse and longitudinal intercepts of their wounds were required to be measured using vernier calipers on days 0, 3, 6, 9, 12, and 15 until the wounds were healed. Subsequently, the wound therapeutic efficacy index (EI) was calculated using the following formula:

$$EI=\frac{S_0-S_n}{S_0}\times 100\%$$

Among them, S_0 is the area of the wound on the 0th day, and S_n is the area of the wound on the nth day.

The therapeutic efficacy index for scalded rats is shown in Figure 6 and Table 4. On the third day, the therapeutic efficacy index of the hydroxyasiaticoside nanoemulsion group and the commercially available burn ointment control group exceeded 25%, indicating that wound contraction had begun. In comparison, the therapeutic efficacy index of the saline group and the blank nanoemulsion group were both less than 25%.

On the ninth day, the therapeutic efficacy index of the hydroxyasiaticoside nanoemulsion group reached 65.1%, exceeding 60%, indicating that the wound had undergone relatively significant contraction.[32] At this time, the therapeutic efficacy index of the commercially available burn ointment control group was 58.8%, and the other two groups were both below 50%. On the 15th day of treatment, the therapeutic efficacy index of the hydroxyasiaticoside nanoemulsion group was 92.9%, indicating that the wound had healed. The therapeutic efficacy index of the commercially available burn ointment control group was 83.3%, while the other two groups had indices below 70%. After comparison, it could be seen that the wound healing effect of the hydroxyasiaticoside nanoemulsion group was the best.

In summary, the hydroxyasiaticoside nanoemulsion prepared in this experiment has a promoting effect on the healing of second-degree scald wounds in rats, and hydroxyasiaticoside nanoemulsion can accelerate the wound scab healing and leave a slightly lighter scar after the wound healing compared with the other three groups by observation, which indicates that it can promote the regeneration of fibroblasts and has a good effect in repairing the wounds.

Results of HE-stained pathology sections

The pathologic sections of each group are shown in Figure 7. After the scalding injury, the results of HE staining showed complete necrosis of the epidermis in all four groups, necrotic detachment of the epidermis and infiltration of inflammatory cells in the superficial dermis with a small amount of cellular edema, which indicated that the wounds caused by the scalding experiment were second-degree superficial scalds.[33] The

experimental results demonstrate that the method used in this experiment can ensure a regular wound gap and a consistent degree of scalding. On the 7th day of continuous drug administration after scalding, all four groups exhibited inflammatory necrosis in the wounds, accompanied by tissue edema caused by infiltrating proliferative vascular cells. On the 14th day, eschars formed at the wound sites, and pathological sections from all groups showed epidermal hyperplasia and inflammatory cell infiltration. Notably, the hydroxyasiaticoside nanoemulsion group and the commercially available burn ointment group exhibited significantly thickened epidermis, more granulation tissue hyperplasia, and a large number of newly formed capillaries. By the 21st day, in both the hydroxyasiaticoside nanoemulsion group and the commercially available burn ointment group, collagen deposition in the dermal layer of the wound had largely covered the wound surface, with significant wound repair. Meanwhile, the number of fibroblasts decreased, nuclei became elongated, and the number of proliferating capillaries gradually decreased, indicating a gradual alleviation of the inflammatory phase. Visually, the skin treated with the hydroxyasiaticoside nanoemulsion exhibited a certain degree of elasticity, essentially indistinguishable from normal skin tissue structure. In contrast, the untreated group and the blank emulsion group had larger wound defects with irregular wound margins, accompanied by infection and foreign bodies. The wounds in these groups were still in the local inflammatory phase, with the epidermis not fully covering the wound, indicating that the untreated group had evident scarring and incomplete wound scar repair. Based on the above pathological sections, it can be observed that the hydroxyasiaticoside nanoemulsion has a promotive effect on

the healing of scalded wounds, with an efficacy comparable to or even superior to that of existing commercially available burn ointment on the market.

Upon experimental observation and rigorous data analysis, it is definitively concluded that the application of hydroxyasiaticoside nanoemulsion significantly expedites the healing process of scalded wounds, markedly reducing the wound healing time and exhibiting a superior therapeutic effect compared to other administered treatment groups. The potential efficacy of hydroxyasiaticoside nanoemulsion may be attributed to its outstanding biocompatibility and bioactive ingredients, which facilitate cellular proliferation and regeneration, subsequently accelerating the wound healing process. Additionally, this nanoemulsion exhibits remarkable abilities to quell inflammatory reactions following scald injuries. Specifically, in the treatment group, there was a significant reduction in the severity of inflammation at the wound site, alongside notable decreases in inflammatory markers like white blood cell count and inflammatory cell infiltration. This favorable response may stem from the anti-inflammatory and antioxidant properties inherent in the active ingredients of hydroxyasiaticoside nanoemulsion, which mitigate the inflammatory cascade and minimize tissue damage. Furthermore, hydroxyasiaticoside nanoemulsion emerges as a potent promoter of tissue repair and regeneration post-scald injury.[34] Within the treatment group, notable enhancements in neovascularization and collagen fiber production were observed, underscoring its ability to enhance blood flow and support tissue rejuvenation. Lastly, a marked decrease in melanin pigmentation within the scalded area in the treatment group highlights the nanoemulsion's capability to mitigate

pigmentation and foster the natural recovery of the skin.

In summary, hydroxyasiaticoside nanoemulsion has multiple mechanisms of action in the treatment of scalds, including promoting wound healing, inhibiting inflammatory responses, and repairing tissue damage. These results provide a theoretical basis for hydroxyasiaticoside nanoemulsion as a potential drug for the treatment of scald wounds, and provide a reference for further research on its clinical application value.

Conclusion

This study explored the preparation of hydroxyasiaticoside nanoemulsion and its application in treating second-degree scalds in rats. Firstly, hydroxyasiaticoside nanoemulsion demonstrated significant improvements in healing time and wound healing degree when treating scalded wounds, confirming its efficacy in treating second-degree scalds. Secondly, through pathological section observation, we found that hydroxyasiaticoside nanoemulsion could significantly enhance wound regeneration capabilities, indicating that it could accelerate the regenerative repair process of wounds. These research findings provide a solid theoretical basis for further clinical applications and offer new ideas and methods for research in the field of burn treatment.

Ethical statement

All animal care and experiments were conducted in according to the Guidelines of Animal Experimentation of Hubei University of Science and Technology and the National Institute of Health. The study protocol was approved by the Institutional

Animal Care and Use Committee of Hubei University of Science and Technology (approval number: 2025-00267).

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Table 1 Box-Behnken factor lever table.

Factors	Level		
	-1	0	1
A: the dosage of Span 80	1 mL	1.5 mL	2 mL
B: the concentration of SDS	10 mg/mL	20 mg/mL	30 mg/mL
C: the volume ratio between the external water phase and the oil phase	1.5:1	2.5:1	3.5:1

Table 2 Design and results of Box-Behnken test.

Run	A: (mL)	B: (mg/mL)	C	Vicosity (mPa·s)
1	2	20	1.5	52.5
2	2	20	3.5	47.9
3	2	10	2.5	44
4	1	20	3.5	41.5
5	1.5	30	1.5	54
6	1.5	30	3.5	46
7	1.5	20	2.5	47.4
8	1.5	20	2.5	47.4
9	1.5	10	1.5	49.4
10	1	30	2.5	42.1
11	1.5	20	2.5	46.5
12	2	30	2.5	50.2
13	1	10	2.5	40
14	1.5	20	2.5	47.4
15	1.5	20	2.5	47.4
16	1.5	10	32.5	42.5
17	1	20	1.5	47

Table 3 Statistical analysis results of Box-Behnken test.

Source of variance	SS	f	MS	F	P	
Model	224.94	9	24.99	49.45	<0.0001	significant
A	72	1	72	142.45	<0.0001	
B	33.62	1	33.62	66.52	<0.0001	
C	78.13	1	78.13	154.57	<0.0001	
AB	4.20	1	4.20	8.31	0.0235	
AC	0.20	1	0.20	0.40	0.5469	
BC	0.30	1	0.30	0.60	0.4645	
A^2	15.97	1	15.97	31.60	0.0008	
B^2	6.04	1	6.04	11.95	0.0106	
C^2	16.05	1	16.05	31.76	0.0008	
Residual	3.54	7	0.51			
Lack of fit	2.89	3	0.96	5.95	0.0589	not significant
Pure error	0.65	4	0.16			
Cor total	228.48	16				

Table 4 Efficacy index values of different treatments in the rat model of second-degree scalds.

Groups	efficacy index values （%）		
	3 d	9 d	15 d
Saline	15.9±8	35.5±7	76.7±7
Scald ointment	25.8±8	58.8±9	90.9±7
MA nanoemulsion	26.7±14	65.1±8	92.3±8
Blank nanoemulsion	12.6±11	46.5±6	78.6±9

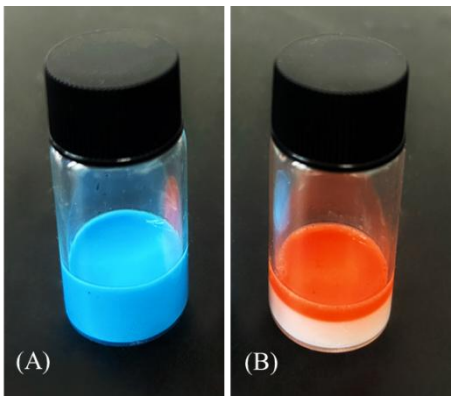


Figure 1 The results of adding methylene blue (A) and sudan red (B) to the nanoemulsion separately.

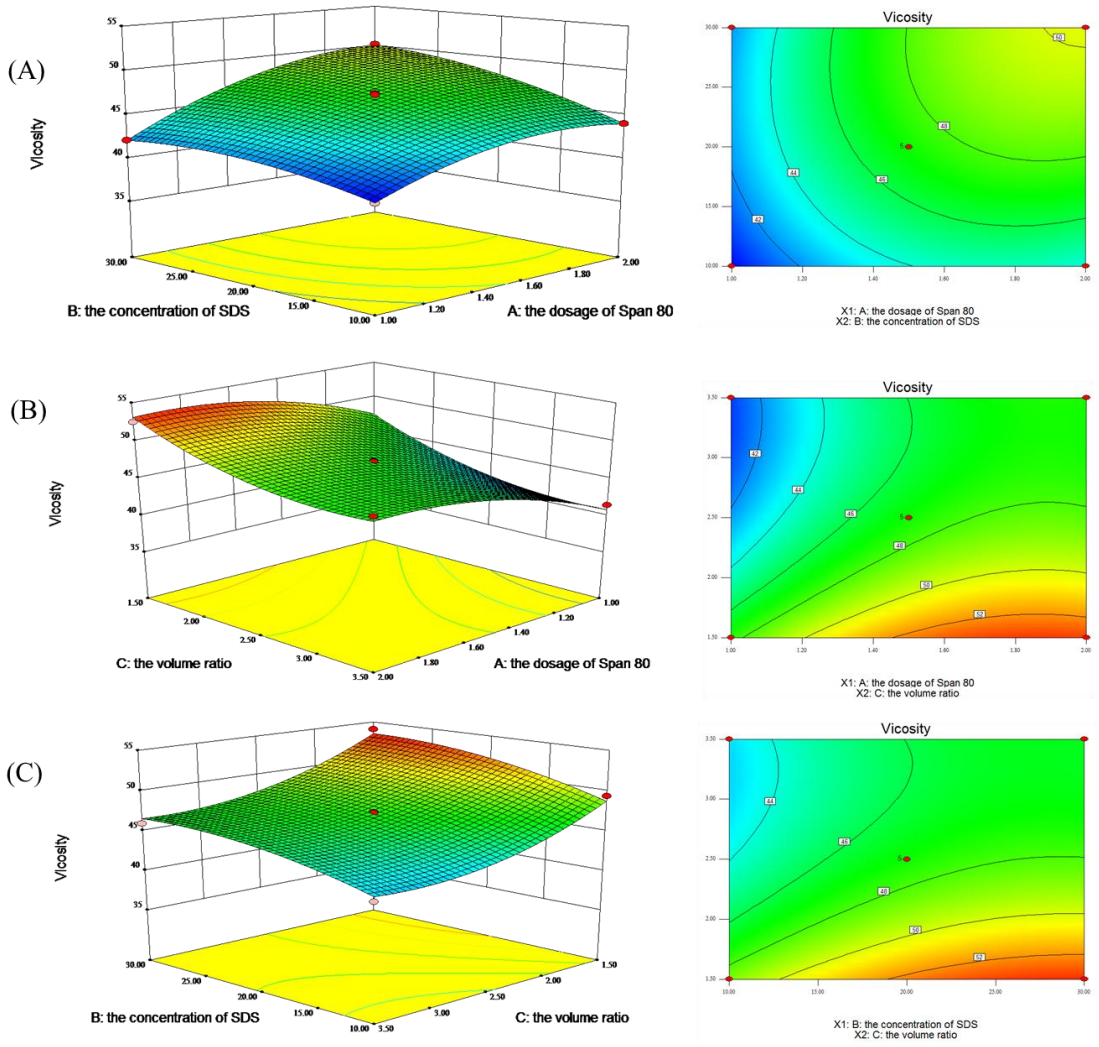


Figure 2 Exploring Response Surface Analysis with 3D Surfaces and Contour Graphs. (A): The influence of the dosage of Span 80 and the concentration of SDS on the viscosity, (B): The influence of the dosage of Span 80 and the volume ratio between the external water phase and the oil phase on the viscosity, (C): The concentration of SDS and the volume ratio between the external water phase and the oil phase on the viscosity

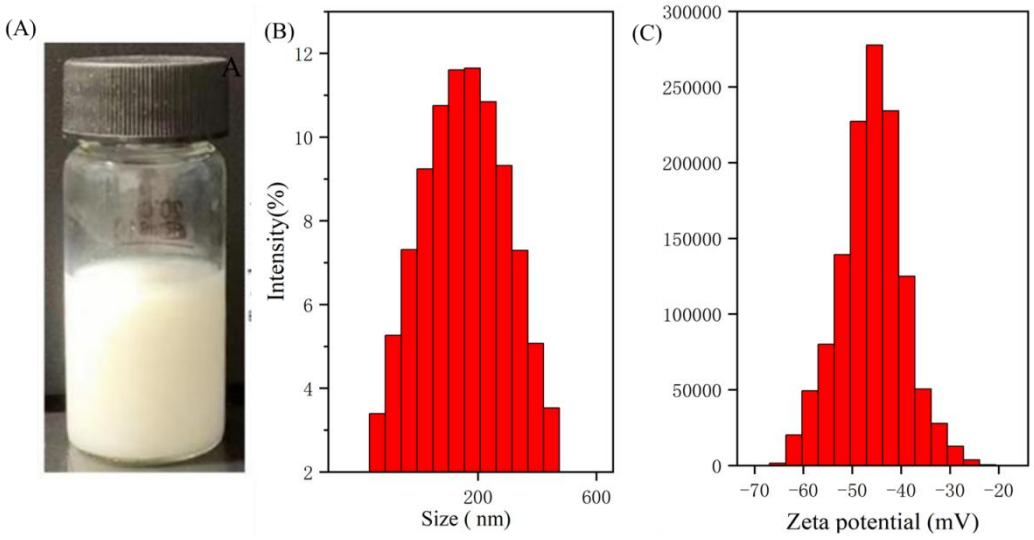


Figure 3 The appearance (A), particles size (B), and Zeta potential (C) of nanoemulsions.

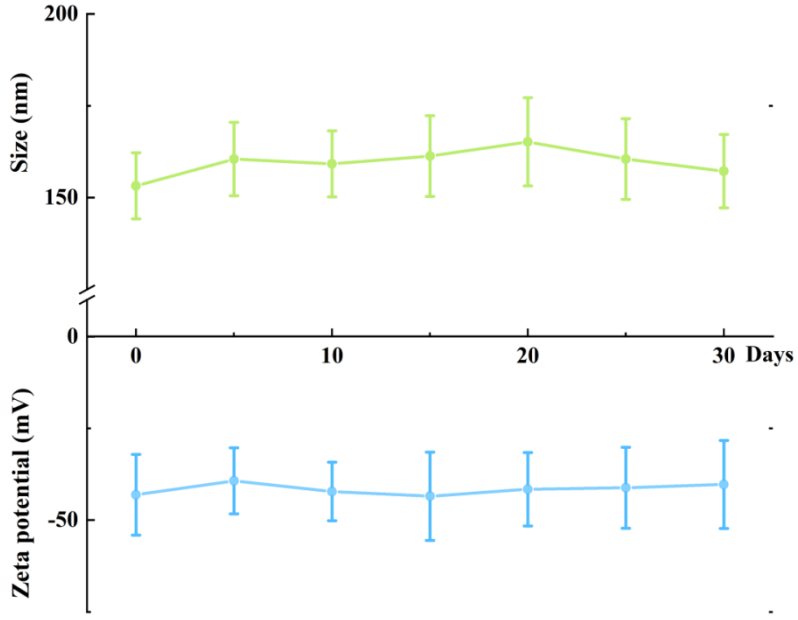


Figure 4 The changes of size and Zeta potential of nanoemulsions in 30 days.



Figure 5 Observations on the changes in the skin of rabbits before and after the administration of the hydroxyasiaticoside nanoemulsions.

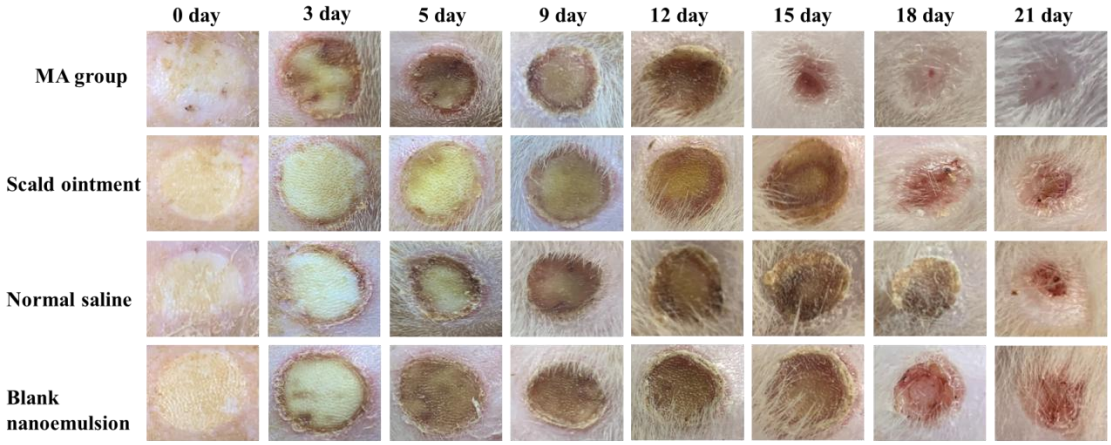


Figure 6 Evolution of the trauma surface in a rat model of second-degree scald. MA group: representing the treatment the hydroxyasiaticoside nanoemulsions. Scald ointment: serving as the control group, administered with a commercially available ointment for scalds. Normal saline group: constituting the control group receiving only normal saline. Blank nanoemulsion: denoting the blank nanoemulsion group, devoid of the active drug hydroxyasiaticoside.

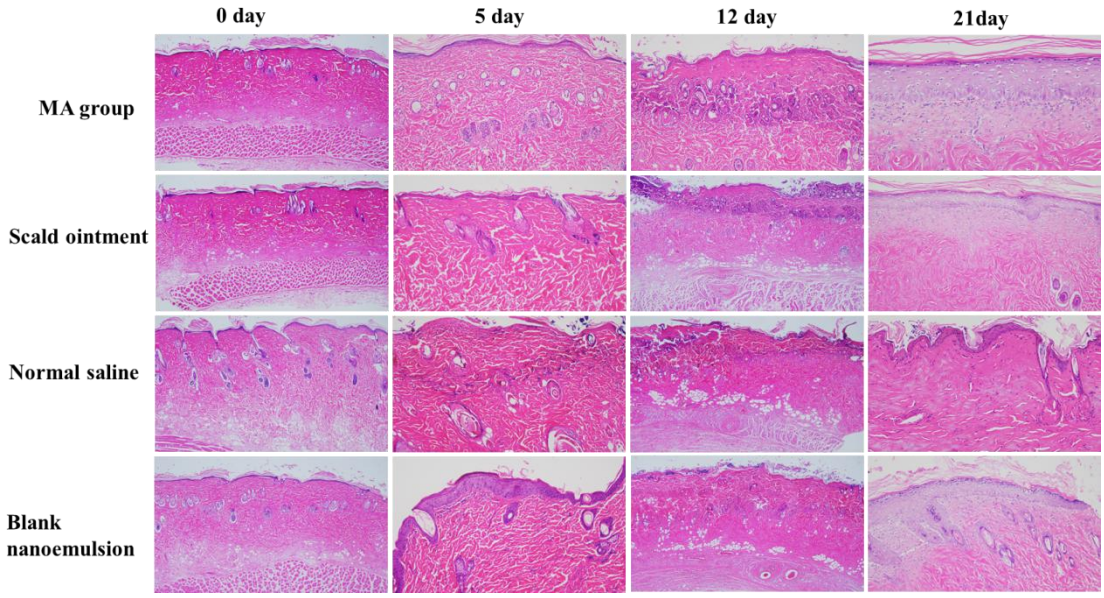


Figure 7 Observations on the changes in the skin of rabbits before and after the administration of the hydroxyasiaticoside nanoemulsions. MA group: representing the treatment the hydroxyasiaticoside nanoemulsions. Scald ointment: serving as the control group, administered with a commercially available ointment for scalds. Normal saline group: constituting the control group receiving only normal saline. Blank nanoemulsion: denoting the blank nanoemulsion group, devoid of the active drug hydroxyasiaticoside.