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Study on Preparation Technology and Safety Evaluation of Hataagqi-19 Hydrogel Patch

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Objectives: The study objective of the present study was to screen the dosage of matrix material and clarify the safety evaluation of Hataagqi-19 hydrogel patch. **Methods:** Single factor and orthogonal experiments were used to screen, and the best matrix ratio was screened out with peel strength, peel ability, viscosity, spread ability, uniformity, as evaluation indicators. Long term toxicity test was carried out in SD rats in order to test general conditions such as: body weight, food intake, electrocardiogram, blood, urine, and biochemical indicators, organ coefficients and histopathological changes. Moreover, skin allergic reaction tests were done to observe the allergic responce. **Results:** The ratio of the gel patch matrix is NP-700: aluminum glycolate: glycerol: carbomer = 6: 0.35: 35: 0.40; the ratio of azone and propylene glycol is 1: 1, and the total penetration enhancer dosage is 5%. Hataagqi-19 preparation had no obvious long-term toxicity or skin allergies. **Conclusions:** The Hataagqi-19 hydrogel patch prepared with the best ratio and transdermal agent showed good performance and safety. Furthermore, we did not observed toxic side effects, and the preparation process is feasible and reliable.

Keywords: Traditional Medicine, Dosage Forms, Methods, Toxicity

Introduction

The traditional Wuwei-Ganlu-Yaoyu bath is one of the most important therapies in Mongolian and Tibetan medicine. It is a method of decocting five medicines and soaking the whole body or part in the syrup at 38-43 ^{0}C. It was first published in the eighth century Tibetan medicine masterpiece "Angaahwuhaanidurbenwundec". In the Medical Code, it is used to treat "Xieriwusu" diseases such as stiffness of limbs, joint swelling and pain, and muscle atrophy. Pharmacological

research and network pharmacological analysis show that Wuwei-Ganlu-Yaoyu bath can treat rheumatoid by regulating the immune process and cell proliferation and differentiation [1].

Hataagqi-19 is an in-hospital preparation used in the Baotou Mongolian and Chinese Medicine Hospital for more than 30 years. It consists of 20 g of Tamarix chinensis, 30 g Juniperus formosana Hayata, 30 g of Ephedrasinica Stapf, 20 g of Artemisia frigida Willd., and 20 g of Angelica biserrata [2]. Yuan et al. used the Wuwei-Ganlu-Yaoyu bath in Mongolian medicine. On the basis of five kinds of medicinal materials, Carthamus tinctorius L. 30 g, Eucommia ulmoides Oliver 30 g, Gentiana *macrophylla* 30 g*, Scutellaria baicalensis Georgi* 20 g, *Angelica sinensis* 30 g, *Xanthoceras sorbifolia* Bunge 20 g, *Notopterygium incisum Ting ex H.T.Chang* 20 g, *Phellodendron amurense Rupr.* 20 g, *Abutilon theophrastii* 20 g, *Sophora flavescens Ait.* 20 g, *Ligusticum chuanxiong hort* 30 g, *Phryma leptostachya L.subsp.asiatica(Hara)Kitamura* 20 g, *Dipsacus asper Wall.ex Henry* 20 g and 20 g of *Avorus calamus* L. were applied in an extract and applied to gauze for external use. It has the effects of promoting blood circulation, relieving blood stasis, relieving pain, reducing swelling, and drying Xieriwusu It is used for cervical and lumbar disc herniation (LDH), knee arthritis, rheumatoid and gouty arthritis diseases.

Early animal experiments proved that the hot compress therapy of Hataagqi-19 can improve the nerve sensitivity symptoms of rats with LDH and relieve radicular pain. Moreover, it can reduce blood TNF-α, IL-6, IL-1β and other inflammatory factors in rats with LDH by promoting the aggregation and phagocytosis of macrophages to shrink and reabsorb the transplanted nucleus pulposus [2]. Clinical experiments have proved that the hot compress therapy of Hataagqi-19 can reduce the pain of the waist and legs and numbness of the lower limbs in LDH patients, and can significantly improve the self-care ability of LDH patients. It has been found through the lumbar MRI examination that the hot compress therapy of Hataagqi-19 can promote prolapsed nucleus pulposus of LDH patients [3]. Its clinical effect is good, but the previous extract easily flows when heated on gauze, contaminats clothes, and was inconvenient to clean. Therefore, in this study, we have improved the formulation of Hataagqi-19 preparation and made it into a hydrogel patch. The hydrogel patch is a transdermal drug delivery system, which is an external preparation made by adding a drug to a non-woven fabric with water-soluble polymer materials as the main matrix [4]. The matrix is a compound system with a polymer material as the skeleton, which interacts to build a three-dimensional network structure. It is insoluble in water and expands, and can maintain its mechanical properties. It has high water content, flexibility and good biocompatibility, so that the hydrogel has the advantages of high drug loading, accurate dosage, good application and moisture retention. It is a suitable improvement of the dosage formulation of traditional medicine with multiple components and large dosage [5].

Drugs should undergo strict safety evaluations before they are used in clinical practice. Hataagqi-19 preparations belong to traditional Chinese medicines and natural medicines. Before usage in China, they should be formulated in accordance with China's State Food and Drug Administration which are: Technical Guidelines for Acute Toxicity Research of Traditional Chinese medicines or natural medicines (NO. [Z]GPT2-1), Technical Guidelines for Long-term Toxicity Research of Traditional Chinese Medicine and Natural Medicines (NO. [Z].GPT3-1), Technical Guidelines for research Irritating and Hemolytic Properties of Traditional Chinese Medicine and Natural Medicines (NO. [Z] GPT4-1), Technical guidelines for research on immunotoxicity (allergic, photoallergic) of traditional Chinese medicine and natural medicine (NO. [Z]GPT5-1) for acute toxicity, long-term toxicity, skin irritation and skin allergy test. In the current study, the acute toxicity and skin irritation of the Hataagqi-19 preparation had been evaluated, and the Hataagqi-19 preparation had no obvious acute toxicity and skin irritation [6].

The purpose of this study is to investigate the best solution for the type and dosage of the matrix of the Hataagqi-19 hydrogel patch, to select the appropriate type and dosage of the skin penetration enhancer, and then to complete it through the long-term toxicity and skin allergy application in experimental animals. The safety evaluation of Hataagqi-19 preparation provides a scientific basis for its clinical application.

Materials and Methods

Hataagqi-19 extract extraction process

According to pre-experimental results, the volatile oils of Ligusticum chuanxiong hort, Angelica biserrata (Shan et Yuan) Yuan et Shan, Notopterygium incisum Ting ex H.T.Chang, Gentiana macrophylla, and Artemisia frigida Willd was used in the preparation of Hataagqi-19. Accurately weighed 1/2 of the

prescription amount of the above 5 medicinal materials were crushed and mixed. Then, 6 times the amount of water was added to soak for 2 hours and the volatile oil was extracted with a volatile oil extractor for 8 hours. Subsequently, the remaining 14 medicinal materials in 1/2 of the prescription amount was crushed and 8 times the amount of 75% ethanol was added to soak for 2 hours. The extraction was continued for 3 hours, filtered out and 5 times the amount of 75% ethanol was added for reflux extraction for 2 hours. Finally, we have combined the two medicinal liquids, and concentrated by the rotary evaporator in a water bath at 60 °C into the consistency of a semi-liquid extract.

Preparation process of Hataagqi-19 hydrogel patch

The cross-linking skeleton, cross-linking agent and thickening agent were dispersed in a beaker containing glycerin, and mixed well to form phase A. The extract made from the multi-flavored Mongolian medicine extract of Hataagqi-19 was dissolved in 20% ethanol as phase B. The cross-linking regulator was dissolved in an appropriate amount of water, stirred evenly, and used as phase C. Phase B was added to phase A, stirred magnetically and degassed ultrasonically to obtain phase D. Phase C was slowly added to phase D several times, magnetically stirred at low speed and degassed ultrasonically. Then, the paste was applied to a non-woven fabric, covered with a backing, and placed at room temperature to mold for 8 hours.

Selection of substrate types of Hataagqi-19

The screening of the matrix type of Hataagqi-19 hydrogel patch adopted a single-factor method. According to the scoring principle, the peelability, viscosity, spreadability and uniformity of the matrix were scored, and then the scores were analyzed in order to compare and screen out suitable matrix materials through comprehensive analysis (Table 1).

Screening of cross-linked framework materials: Polyvinyl pyrrolidone (PVP) (Macklin, Shanghai, China) (8 g), sodium polyacrylate NP700 (Macklin, Shanghai, China) (8 g), and other components were weighted. The gel patch blank matrix was prepared according to the above preparation process, and labeled as 1, 2, and 3 respectively. Using the peelability, viscosity, spreadability and uniformity of the substrate as indicators, the two substrates were evaluated separately.

Screening of cross-linking agent materials: Blank matrix was prepared according to the preparation process described above with other components. To produce aluminum hydroxide (Macklin, Shanghai, China), aluminum chloride with variable types of cross-linking agents (Macklin, Shanghai, China), hydroxyaluminum (Macklin, Shanghai, China) was used. Three blank substrates were marked as 1, 2, and 3 respectively for testing, based on the peelability of the substrate, viscosity, spreadability and uniformity for the observation indicators to evaluate the three substrates.

Single factor investigation of matrix material dosage of Hataagqi-19 hydrogel patch

In this experiment, a blank matrix was prepared according to the preparation process of the Hataagqi-19 hydrogel patch. Partially neutralized sodium polyacrylate NP-700 was selected as the skeleton material. Under the condition that the other components did not change, the NP-700 2, 4, 6, 10, 12, 14 g, 7 batches of different blank matrices were prepared according to the results of the preliminary experiment. The other components of the matrix were fixed, and the amount of aluminum glycolate was respectively weighed as 0.05, 0.15, 0.25, 0.35, 0.45 g, made into 5 batches of blank matrix samples. Carbomer (Macklin, Shanghai, China) was weighted into 0.15 g, 0.25 g, 0.35 g, 0.45 g, and other ingredients were prepared according to the pre-test dose preparation 1, 2, 3, 4 for four batches of blank matrices. Other ingredients were prepared according to the pre-test dose and the amount of glycerin (Macklin, Shanghai, China), of 20, 30, 40, 50 ml was prepared into 4 batches of blank matrices. The peel ability, viscosity, spread ability and uniformity of the substrate was screened as the inspection index and evaluated in each batch of substrates of sodium polyacrylate NP-700, aluminum glycolate, carbomer, and glycerin.

Orthogonal experiment method to optimize the ratio of matrix prescription

The single factor experiment showed that the dosage of NP-700, glycerol, glycerin and carbomer are the main factors which affects the performance of the hydrogel patch matrix. Therefore, an orthogonal experiment was conducted in order to determine the peel strength ($N \bullet mm^{-1}$) as an investigation indicator and to optimize the matrix ratio (Table 2). The method for measuring peel strength refers to the Chinese Pharmacopoeia (2015 edition). Before the experiment, the patch was cut into a size

of 20 mm × 100 mm, and the backing surface was fixed on the test board with double-sided tape. Using a wooden stick to roll back and forth on the sample 3 times, we have ensured that there are no bubbles in the joint. The free end of anti-sticking film was folded in half, clamped and the test plate was moved up and down on the testing machine. The peeling surface should be consistent with the test machine line. The testing machine continuously peels at a speed of 300 mm•min^{-1}, records the peel force F, and calculates the peel strength according to the formula 6 = F/B, where 6 is the peel strength (N•mm^{-1}) and F is the peel force (N), B is the sample width (mm).

Long-term toxicity test method of Hataagqi-19 preparation

Animal experiments were carried out under the control of the Animal Care and Use Committee following the University Guidelines for Animal Experiments and the Chinese Government Law Concerning the Protection and Control of Animals. 32 healthy SD rats of 3-4 months old, clean grade, half male and half female, weighing about 180-220 g (SiPeiFu, Beijing, China, Animal license number SCXK (Jing) 2019 - 0010), and raised in the Experimental Animal Center of Baotou Medical College were used in this experiment. The animals were housed in plastic cages at room temperature (21–24^{0}C) in a 12-hour light and dark cycle with free access to food and water. According to China's "Technical Guidelines for Long-term Toxicity Research of Traditional Chinese Medicines and Natural Medicines" (NO. [Z].GPT3-1), rats were randomly divided into control groups, low, medium and high dose of Hataagqi-19. Each group had 8 rats, half male and half female, and reared in separate cages. The depilation area of each back was about 4 cm × 3 cm. Hataagqi-19 extract was diluted 10 times. Each group was treated separately using 75% ethanol 5 ml/(kg to one rat) 5 ml/(kg to another rat), 10 ml/(kg to one rat), and 20 ml/(kg to yet another rat). Doses of Hataagqi-19 were applied to the back once at a day with continuous administration for 30 days.

During the administration period, the body weight and temperature were measured every 7 days. A urine test was performed 30 days after administration and abdominal anesthesia, and electrocardiogram was performed. Blood was taken from the abdominal aorta and related blood indicators was measured. Brain, heart, liver, kidney, spleen, lung, thyroid, thymus, trachea, stomach, large intestine, small intestine, uterus, ovaries, fallopian tubes, testes, and epididymis was taken, weighted, and pathological examination was done.

Test method for skin allergy of Hataagqi-19 preparation

In accordance with China's "Technical Guidelines for Research on Immunotoxicity (Allergic and Photoallergic) of Traditional Chinese Medicines and Natural Medicines" (No. [Z]GPT5-1), 18 male Cavia porcellus (SiPeiFu, Beijing, China, Animal license number SCXK (Beijing) 2016 - 0007) was randomly divided into three groups: an experimental group using Hataagqi-19), a negative control group using 75% ethanol, and a positive control group using 2, 4-dinitrochlorobenzene. Before the experiment, the back hair was removed, and the corresponding reagents were applied to each group of animals on the first 1, 7, 14 and 28 days of the experiment. After 6 hours of action on the back of the animals, reagents were washed off. On the 28th day, after the reagents were washed off, the animal skin was evaluated for erythema within 72 hours. Following the "Technical Guidelines for Research on Immunotoxicity (Allergy, Photoallergic Reaction) of Traditional Chinese Medicines and Natural Medicines" (No. [Z] GPT5-1)" evaluation standard form given in the table, erythema was graded from nothing to severe erythema by division into 0 - 4 points. Redness and swelling were graded from nothing to extremely severe redness and swelling by division into 0 - 4 points. Allergy rate (%) = number of allergic animals/total number of animals × 100%. The evaluation index of skin allergies ranged from no allergies to extremely severe allergies and was divided into 5 levels of 0% - 100%.

Statistical analysis

The mean value of continuous variables was compared using a one-way variance (ANOVA) test to analyze the levels of variance within the groups. Tukey's multiple comparison test was used to compare the difference between each pair of means with appropriate adjustment for the multiple testing. The main effects of body weights, Hataagqi-19 preparation and their interaction were determined using a mixed two-way ANOVA with a Greenhouse-Geiser adjustment for lack of sphericity. A critical p - value of < 0.05 was used. The independent measurements within groups were then compared using the unpaired t-tests. The analyses were done using SPSS 25.0.

Ethical statement

Animal study was carried out following the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. Formal approval to conduct the experiments was obtained from the Ethical Committee of the Baotou Medical College (Protocol No.BTMY201901). All efforts were made to minimize the number of animals used and their suffering.

Results

Extraction results of Hataagqi-19 extract

A total of 5.8 ml of volatile oil was extracted from 5 medicinal materials of *Ligusticum chuanxiong hort, Angelica biserrata* (Shan et Yuan) *Yuan et Shan, Notopterygium incisum Ting ex H.T.Chang, Gentiana macrophylla,* and *Artemisia frigida Willd.* in 1/2 prescription amount. The combined 19 kinds of medicinal materials were extracted twice with 75% ethanol under reflux, and the two medicinal solutions were combined and concentrated to obtain 837.2 g of extract. After mixing the volatile oil and the extract evenly, 840.8 g of Hataagqi-19 extract was prepared.

Screening results of matrix types of Hataagqi-19 hydrogel patch

Screening results of cross-linked framework materials:

Comparing PVP to NP700, PVP had poor compositional properties. Poor peelability was manifested by poor cohesion in the paste and loosening when applied to the non-woven fabric. The substrate was damaged by the slight touch of the fingers. After peeling, the paste left more residues on the hands. By comparison, the performance of the NP700 group was relatively stable and the formability was better. Therefore, we have used NP700 as the cross-linking skeleton in this experiment.

Screening results of crosslinking agent materials:

Comparing aluminum hydroxide, aluminum chloride and aluminum glycolate, the uniformity of the matrix of the aluminum hydroxide group and aluminum chloride group was significantly lower than that of the aluminum glycolate group, showing occasional agglomeration of the matrix. It did not meet the needs of experiments and industrial production, thus aluminum glycolate was selected as the crosslinking agent.

Screening of cross-linking modifiers and other materials:

By refer to a many sites in literature, it became known that the type of cross-linking modifier has far less influence on the performance of the matrix than its dosage. In this experiment, tartaric acid was selected as the cross-linking modifier. The results of preliminary experiments showed that, as a crosslinking regulator, tartaric acid can meet the requirements of laboratory preparation. Glycerin had a good moisturizing effect and was a good uniform dispersion solvent for NP700. When the amount of glycerin was less, NP700 was not dispersed well and had uneven viscosity; as the amount of glycerin increased, the dispersion came uniformly, spread ability improved, and uniformity increased. It also showed a certain dilution effect on the matrix system.

Table 1. L_9 (3^4) Orthogonal experimental design scheme and results.

Experiment number	A	B	C	D	Peel strength /N·mm^{-1}
1	1	1	1	1	3.54
2	1	2	2	2	4.02
3	1	3	3	3	3.13
4	2	1	2	3	4.86
5	2	2	3	1	5.54
6	2	3	1	2	5.41
7	3	1	3	2	4.86
8	3	2	1	3	6.44
9	3	3	2	1	3.47
K^1	10.69	13.26	15.39	12.55	
K^2	15.81	16.00	12.35	14.29	
K^3	14.77	12.01	13.53	14.43	
R	5.12	3.99	3.04	1.88	

Table 2. The results of the analysis of variance of the orthogonal experiment.

Groups				
A	B	C	D	
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p-value
4.88 ± 2.44	2.77 ± 1.38	1.56 ± 0.78	0.73 ± 0.36	0.145

Table 3. The effect of Hataagqi-19 preparation on the body weight of rats.

	Male					Female				
Body weights	Control [a, b]	Low [c]	Medium [d]	High [e]		Control [f]	Low [g, e]	Medium	High [k]	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p-value
0 d(g)	214.8 ± 5.5	213.51 ± 1.5	213.5 ± 4.2	217.3 ± 9.1	0.203	191.2 ± 8.4	178.7 ± 7.98	174.0 ± 5.7	193.8 ± 7.5	0.191
7 d(g)	225.8 ± 4.3	222.8 ± 10.8	221.3 ± 2.5	243.8 ± 10.3	0.562	218.8 ± 21.7	210.0 ± 8.2	205.0 ± 10.0	209.0 ± 9.4	0.682
14 d(g)	240.5 ± 9.9	233.8 ± 10.8	230.3 ± 4.1	257.5 ± 8.7	0.159	252.7 ± 24.8	241.2 ± 17.9	232.5 ± 8.7	228.5 ± 4.4	0.090
21 d(g)	254.8 ± 5.6	242.3 ± 8.3	249.3 ± 3.3	280.3 ± 7.8	0.366	276.8 ± 22.1	264.5 ± 23.2	247.0 ± 7.2	242.8 ± 2.9	0.748
28 d(g)	294.0 ± 11.6	291.3 ± 15.4	294.2 ± 9.5	296.8 ± 8.2	0.191	289.0 ± 20.5	284.2 ± 24.6	293.5 ± 3.9	277.3 ± 4.6	0.159

Two-way mixed ANOVA results: In male, interaction of body weights and effect of Hataagqi-19 preparation F(1.917, 338.51) = 23.195, $p < 0.001$; Main effect of body weights F (1.916, 331.49) = 331.11, $p < 0.001$; Main effect of Hataagqi-19 preparation F(1,176) = 0.667, p = 0.416; an unpaired t-test: [a]0 d(g) vs. 28 d(g), p = 0.050; [b]0 d(g) vs. 21 d(g), p = 0.045; [c]0 d(g) vs. 28 d(g), p = 0.041; [d]7d(g) vs. 28 d(g), p = 0.001; [e]0d(g) vs. 28 d(g), p = 0.001. In female, interaction of weights and effect of Hataagqi-19 preparation F (1.919, 336.59) = 27.213, $p < 0.001$; Main effect of body weight F (1.918, 337.59) = 335.31, $p < 0.001$; Main effect of Hataagqi-19 preparation F(1,185) = 0.816, p = 0.971; an unpaired: [f]0 d(g) vs. 21 d(g), p = 0.010; [g]7 d(g) vs. 14 d(g), p = 0.045; [e]0d(g) vs. 21 d(g), p = 0.051; [d]day7 vs. 28, p = 0.001; [e]day14 vs. 21 d(g), p = 0.002. [k]0 d(g) vs. 21 d(g), p = 0.045.

Table 4. The effect of Hataagqi-19 preparation on the organ coefficient of rats.

	Male					Female				
	Control	Low	Medium	High		Control	Low	Medium	High	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value
Lung (g)	1.9 ± 0.1	2.0 ± 0.0	1.9 ± 0.2	0.8 ± 0.9	0.191	2.0 ± 0.1	1.8 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	0.121
Heart (g)	0.9 ± 0.1	1.0 ± 0.3	1.2 ± 1.2	1.0 ± 0.1	0.682	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	1.0 ± 0.0	0.446
Liver (g)	6.6 ± 0.3	6.2 ± 1.0	6.1 ± 0.3	5.6 ± 0.6	0.748	6.1 ± 0.7	6.5 ± 0.5	6.0 ± 0.8	6.1 ± 0.5	0.532
Spleen (g)[a, b]	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.010	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.3	0.261
Kidney (g)	1.8 ± 0.1	2.0 ± 0.3	2.2 ± 0.2	2.0 ± 0.1	0.421	1.8 ± 0.4	1.7 ± 0.0	1.9 ± 0.2	1.9 ± 0.1	0.813

*One-way analysis of variance (ANOVA); In male, multiple comparison: [a]control vs. low, $p < 0.023$; [b]control vs. high, $p < 0.041$.

Single factor experiment method to investigate the results of matrix material dosage of Hataagqi-19 hydrogel patch

The results of our study showed that the dosage of NP-700 had a significant impact on the performance of the hydrogel patch matrix. As the dosage of NP700 increased, the viscosity of the matrix increased, but when the dosage exceeded 10g, the viscosity decreased as the dosage of NP700 increased. With the increase in the amount of aluminum glycolate, the peelability of the matrix gradually increased, the matrix was getting harder and harder, while the spreadability was reduced, it was difficult to spread, or even impossible to spread. The dosage of carbomer had a significant effect on the performance of the hydrogel patch matrix. As the dosage of carbomer increased, the matrix viscosity first increased and then decreased. The amount of glycerin mainly affected the viscosity of the hydrogel patch matrix (Table 3).

Orthogonal experimental method to optimize the matrix prescription ratio of Hataagqi-19 hydrogel patch

From the results of the 4-factor 3-level orthogonal experiment of NP-700, glycerol, glycerol and carbomer, it was found that the order of influence on the peel strength was A>B>C>D,

Table 5. Hematological changes in experimental rats.

	Male					Female				
	Control	Low	Medium	High		Control	Low	Medium	High	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value
RBC (10^{12}/L)[c]	9.33 ± 0.58	9.19 ± 0.3	10.13 ± 0.8	9.5 ± 0.43	0.482	4.78 ± 0.9	8.73 ± 0.61	8.98 ± 0.23	8.4 ± 0.16	0.042
HGB (g/L)	185.25 ± 4.19	181.25 ± 0.96	192.25 ± 18.73	186.25 ± 5.9	0.566	193.5 ± 10.54	191.0 ± 10.61	194.75 ± 4.92	168.25 ± 9.74	0.191
HCT (%)	55.4 ± 1.82	55.3 ± 1.07	58.35 ± 5.37	57.2 ± 1.86	0.154	53.1 ± 2.22	51.9 ± 3.08	53.48 ± 1.28	52.35 ± 3.55	0.082
MCV (fL)	59.78 ± 2.94	58.95 ± 2.99	57.85 ± 0.86	59.08 ± 1.24	0.101	59.63 ± 2.12	59.65 ± 1.57	59.68 ± 1.42	59.0 ± 0.77	0.748
MCH (pg)	19.83 ± 0.85	19.25 ± 0.35	19.25 ± 0.26	19.3 ± 0.42	0.795	19.55 ± 0.53	19.68 ± 0.4	19.5 ± 0.41	18.93 ± 0.1	0.103
MCHC (g/L)	331.75 ± 3.3	328.25 ± 2.87	330.5 ± 4.93	328.75 ± 3.5	0.300	327.5 ± 5.45	328.75 ± 2.75	327.0 ± 1.41	327.25 ± 0.96	0.162
WBC (10^9/L)	5.09 ± 2.44	7.79 ± 3.92	6.54 ± 3.51	6.29 ± 3.13	0.171	4.78 ± 0.9	4.61 ± 0.61	5.32 ± 1.3	5.94 ± 0.79	0.113
PLT (10^9/L) [a, b]	941.0 ± 269.2	651.0 ± 150.54	501.25 ± 138.35	612.25 ± 140.04	0.001	999.75 ± 96.72	813.5 ± 168.15	976.0 ± 95.41	868.75 ± 96.67	0.308
Ret (%)	0.12 ± 0.12	0.06 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.355	0.18 ± 0.03	0.15 ± 0.03	0.16 ± 0.03	0.18 ± 0.03	0.146
TT (s)	35.0 ± 1.41	32.25 ± 3.77	37.75 ± 4.43	38 ± 2.16	0.791	30.0 ± 3.56	33.75 ± 2.06	33.5 ± 1.73	30.0 ± 0.82	0.132
PT (s)	11.25 ± 1.26	12.5 ± 2.38	12.25 ± 1.7	11.25 ± 0.5	0.295	11.5 ± 1.91	13 ± 1.41	12.25 ± 1.5	12 ± 1.83	0.261

*One-way analysis of variance (ANOVA); Multiple comparison test results: In male, [a]control vs. high, p = 0.04; [b]control vs. medium, p = 0.05; In female; [c]medium vs. high, p = 0.001.

and the preferred level combination was $A_2B_2C_1D_3$. The results of the analysis of variance showed that the four factors had a significant impact on the comprehensive score ($p < 0.05$). The preferred prescription was $A_2 B_2 C_1 D_3$, that is, the optimal ratio of hydrogel patch was NP-700: aluminum glycolate: glycerol: carbomer = 6: 0.35: 35: 0.40 (Table 4, 5).

According to the results of orthogonal experiments, the optimal ratio of hydrogel patch NP-700: aluminum glycolate: glycerol: carbomer = 6: 0.35: 35: 0.40 was obtained. Three batches of repeated experiments were performed to verify the success of the optimization scheme. The results show that the optimization scheme is stable and feasible (Table 6).

Table 6. Blood biochemical changes in experimental rats.

	Male					Female				
	Control	Low	Medium	High		Control	Low	Medium	High	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value
GLU (mmol/L)	4.37 ± 0.36	5.11 ± 1.4	5.11 ± 1.34	4.98 ± 2.55	0.471	7.05 ± 0.43	7.24 ± 1.55	8.5 ± 1.19	10.94 ± 1.4	0.081
ALT (U/L)	36.4 ± 3.22	34.75 ± 10.33	40.33 ± 3.56	44.9 ± 5.44	0.393	22.86 ± 3.15	25.43 ± 2.85	27.65 ± 3.19	28.73 ± 3.65	0.382
AST (U/L)	137.08 ± 27.37	141.85 ± 27.07	142.2 ± 19.87	150.25 ± 14.78	0.407	137.73 ± 16.27	134.88 ± 10.28	150.95 ± 9.66	133.53 ± 4.62	0.566
ALP (U/L)	101.83 ± 8.29	103.6 ± 36.05	99.1 ± 9.09	103.7 ± 16.8	0.069	61.1 ± 14.74	65.3 ± 14.23	84.18 ± 28.59	83.65 ± 4.3	0.554
γ-GT (U/L)	1.28 ± 0.67	0.68 ± 0.05	1 ± 0.83	0.73 ± 0.24	0.098	0.55 ± 0.21	0.48 ± 0.19	0.43 ± 0.4	0.8 ± 0.08	0.301
TP (g/L)	56.6 ± 1.61	52.13 ± 8.63	55.25 ± 3.4	54.87 ± 0.52	0.386	63.45 ± 2.19	61.85 ± 1.89	63.93 ± 1.25	59.98 ± 1.71	0.695
TG (mmol/L)	0.6 ± 0.14	0.54 ± 0.08	0.5 ± 0.07	0.52 ± 0.2	0.101	0.88 ± 0.21	9.91 ± 0.21	0.69 ± 0.16	0.56 ± 0.15	0.320
TC (mmol/L)	1.35 ± 0.32	1.27 ± 0.36	1.24 ± 0.2	1.24 ± 0.29	0.121	1.47 ± 0.4	1.53 ± 0.4	1.8 ± 0.31	1.78 ± 0.27	0.791
CREA (μmol/L)	42.13 ± 12.28	37.28 ± 5.91	52.1 ± 21.42	37.15 ± 1.67	0.386	43.13 ± 5.39	49.6 ± 4.35	49.1 ± 8.24	42.9 ± 2.79	0.255
UREA (mmol/L)	5.6 ± 0.43	6.72 ± 2.63	6.57 ± 0.5	6.68 ± 1.4	0.655	6.93 ± 1.00	7.13 ± 0.86	6.82 ± 0.6	6.6 ± 0.55	0.280
UA (μmol/L)[a, b, c]	138.25 ± 7.06	140.3 ± 9.81	133.73 ± 9.13	110.85 ± 9.4	0.031	133.27 ± 6.48	136.55 ± 10.61	126.95 ± 9.49	106.0 ± 12.24	0.010
CREA[d, e]	37.55 ± 1.56	38.05 ± 1.43	32.4 ± 3.52	29.35 ± 2.45	0.004	34.77 ± 3.94	37.07 ± 1.39	31.3 ± 4.02	28.45 ± 2.39	0.004
CK (U/L)	1214 ± 556.2	1026 ± 48.36	1089 ± 150.1	1149 ± 463.6	0.072	1684 ± 211.3	1661 ± 345.9	1443 ± 437.5	1853 ± 272.7	0.875
ALB (g/L)	31.65 ± 1.1	28.58 ± 3.64	30.35 ± 1.1	30.4 ± 0.65	0.068	34.18 ± 0.95	34.05 ± 1.18	35.3 ± 0.88	32.98 ± 0.55	0.314

*One-way analysis of variance (ANOVA); Multiple comparison test results: In male, [a]low vs. high, p = 0.001; [b]control vs. high, p = 0.041; [d]control vs. high, p = 0.000; In female; [c]medium vs. high, p = 0.002; [e]low vs. high, p = 0.000.

Table 7. Test results of skin allergies of Hataagqi-19 preparations (points).

	24h total			48h total			72h total		
Group	**Erythema**	**Redness**	**Total**	**Erythema**	**Redness**	**Total**	**Erythema**	**Redness**	**Total**
Positive control	4	3	7	4	5	9	4	5	9
Negative control	-	-	-	-	-	-	-	-	-
Hataagqi-19	-	-	-	-	-	-	-	-	-

Table 8. Evaluation of skin allergies of Hatagaqi-19 preparation (%).

Group	**24h**	**48h**	**72h**	**Evaluation**
Positive control group	50	50	67	Severe allergies
Negative control group	-	-	-	Non-allergenic
Hataagqi-19 preparation group	-	-	-	Non-allergenic

Selection of the penetration enhancer of Hataagqi-19 hydrogel patch

There are many types of penetration enhancers, and the effect of promoting absorption is also strong or weak. Too much penetration enhancer will not only reach saturation [5], but also affect the performance of the cataplasm matrix. The choice of skin enhancer should follow the principle of less dosage and strong effect. According to relevant literature reports, the penetration enhancer of the dual phase is stronger than that of the monophase. The most commonly used penetration enhancers are azone and propylene glycol. Azone has a certain degree of fat solubility, while propylene glycol can increase nitrogen. The effect time and intensity of ketone on the skin stratum corneum have a synergistic effect [7], thus in this experiment, we used a combination of azone and propylene glycol binary phase penetration enhancer in a ratio of 1:1 to 5%.

Long-term toxicity test results of Hataagqi-19 preparation

The long-term toxicity test of Hataagqi-19 preparations was completed after 30 days of smearing on SD rats. Compared with the control group, the body weight, food intake, urine index, electrocardiogram and organ coefficient of the low, medium and high dose groups were compared. There was no obvious change in the pathological examination ($p > 0.05$), and there was no obvious skin irritation. Compared with the control group, the weight, organ coefficient, various hematology and blood biochemical indexes of each dose group were shown in Table 7.

Test results of skin allergy of Hataagqi-19 preparation

After the final test of the positive control group (2, 4-dinitrochlorobenzene), skin erythema, redness and swelling of the experimental animals appeared at 24, 48, and 72 hours, and the allergy score was 7, 9, 9 points. The allergy level reached 50% at 24 hours and 48 hours, and the allergy level reached 67% at 72 hours, which was evaluated as severe allergic. The animals in the negative control group and the Hataagqi-19 preparation group had no skin erythema or redness at 24, 48, and 72 hours after the final test, the allergy score was 0, and the skin allergy was evaluated as non-allergic.

Discussion

The transdermal drug delivery system has the advantages of avoiding the first pass effect, reducing systemic reactions, prolonging the efficacy of single administration and reducing toxic and side effects. However, the current external preparations of Mongolian medicine are mostly used in hospitals, and there are very few patent drugs available for community use. Systematic safety evaluation and standardization, and selecting some external preparations of Mongolian medicine with significant clinical efficacy to enrich the types of Mongolian medicine should be the future research direction of Mongolian medicine in the treatment of osteoarthritis.

The traditional Wuwei-Ganlu-Yaoyu bath is one of the most important therapies in Mongolian and Tibetan medicine, which is used to treat rheumatism, lumbar disc herniation, knee joint disease and other bone and joint diseases. The Tibetan Wuwei-Ganlu-Yaoyu bath is composed of *Ephedrasinica Stapf, Juniperus formosana Hayata, Artemisia frigida Willd*., Myricaria elegans Royle, and Folium Rhododendri Antho-pogonoidis. The traditional usage is to decoct the five medicinal materials and

soak the whole body or part with the liquid medicine [8]. In modern applications, it has been made into a granular dosage form, which eliminates the process of drug decoction. After dissolving the Wuwei-Ganlu-Yaoyu bath granules in water, the water temperature can be adjusted to perform a medicated bath treatment [9]. On the other hand, the Mongolian Wuwei-Ganlu-Yaoyu bath is composed of *Ephedrasinica Stapf, Juniperus formosana Hayata, Artemisia frigida Willd*., Myricaria elegans Royle, and *Angelica biserrata* (Shan et Yuan) *Yuan et Shan*, and its usage is the same as traditional Tibetan medicine. Hataagqi-19 is composed of 14 Mongolian medicines added to the traditional Mongolian medicine Wuwei-Ganlu-Yaoyu bath prescription. Among them, *Carthamus tinctorius* L., *Gentiana macrophylla, Scutellaria baicalensis Georgi, Notopterygium incisum Ting ex H.T.Chang, Phellodendron amurense Rupr., Ligusticum chuanxiong hort, Angelica sinensis* had the effects of clearing heat, promoting blood circulation, relieving pain and reducing swelling caused by aseptic inflammation. *Xanthoceras sorbifolia* Bunge is one of the four good medicines of Mongolian medicine "Xieriwusu", which can relieve various degenerative, rheumatism, rheumatoid and rheumatoid diseases caused. As for gouty arthritis, *Eucommia ulmoides Oliver, Abutilon theophrastii, Phryma leptostachya L.subsp.asiatica(Hara) Kitamura, Dipsacus asper Wall.ex Henry, Avorus calamus* L. are used in Mongolian medicine to fix bones and strengthen bones. Moreover, *Sophora flavescens* Ait. has heat-clearing and sweating effects in Mongolian medicine. Sophora flavescens also plays a role in other preparations. It causes sweat, opens pores, attracts "Xieriwusu" through pores, and promote the transdermal absorption of other drugs.

The factors that affect the transdermal absorption of the drug include the solubility of the drug, the oil-water partition coefficient, the molecular weight, the melting point of the drug, the characteristics of the matrix, the penetration enhancer, and skin factors. Volatile oil is an oil-soluble substance with a small molecular weight, which is easily absorbed by the skin. Some studies believe that the volatile oil of traditional Chinese medicine contains terpenoids, which mainly have the functions of regulating qi, analgesic, driving wind and resuscitation, and has a certain penetration promoting effect [7]. In this experiment, in order to make full use of the effective ingredients of the drug when extracting Hatagaqi-19 extract, the volatile oil components in the medicinal materials with higher volatile oil content were first extracted, and then the medicinal residues were combined with other medicinal materials, and then ethanol was refluxed for extraction to increase the volatile oil in the extract content.

In order to determine the performance of the matrix of Hataagqi-19 hydrogel patch, the dosage of NP-700, aluminum glycerol, glycerin, carbomer and extracts affected the quality of the hydrogel patch. The main factor in the performance of the hydrogel patch was the crosslinking agent NP-700 which was a partially neutralized polymerization product of acrylic acid. The molar ratio of acrylic acid to sodium acrylate was 1:1. The high-valent metal ions that crosslink the acrylic acid polymer usually come from aluminum salts. During the forming process of the gelling agent, the aluminum ion reacts with the carboxyl group in the polymer skeleton, which gradually increases the viscosity of the system. After the viscosity is appropriate, the aluminum ion further interacts with the polymer to form a three-dimensional network structure, so that the matrix obtains appropriate strength [10]. In the present study, the ratio of NP-700 to aluminum glycolate was 6: 0.35, which is the same as the experimental ratio that has introduced by Li et al [11]. When the amount of glycerin is too much, the substrate has insufficient adhesion and poor applicability, and when the amount is too small, the viscosity is too large and it is not easy to spread. Zhang et al. represented the percentage of the matrix formulation component part in compound Die Da Zhen Tong cataplasm as: NP-700: carbomer 980: PVP K-90: dihydroxy aluminum: tartaric: kaolinite: sorbitol: glycerin = 5: 1.2: 2.5: 0.25: 0.15: 4: 12: 5. [12]. This was similar to our results.

Different dosages will affect the forming speed of the hydrogel patch matrix, which is mainly due to the fact that when too much extract is added, it will affect the cross-linking reaction of the cross-linking system. When the drug loading is too large, the cross-linking reaction time is longer and the matrix forming is slower. When the drug loading is too small, the cross-linking reaction time is shorter and the molding speed is faster. The dosage of tartaric acid has little effect.

The long-term toxicity test results of Hataagqi-19 preparation showed that the low, middle and high doses of Hataagqi-19 preparation had no significant effect on blood routine, blood sugar, liver function, and myocardial enzymes in rats. The skin allergy test results of the Hataagqi-19 preparation showed that it had no obvious skin allergies. After consulting the relevant literature of the 19 ingredients in the Hataagqi-19 preparation

one by one, no literature supporting an allergy phenomenon was found.

In the present study, we have found that Hataagqi-19 preparation significantly reduced blood uric acid, the main cause of gout, and creatinine in male and female rats ($p < 0.05$). In clinical trials of the Wuwei-Ganlu-Yaoyu bath, the main ingredient of the Hataagqi-19 preparation, it was also revealed that the Wuwei-Ganlu-Yaoyu bath significantly reduced the blood uric acid level in patients with gout. In typical cases, gout can cause ankle joint swelling and pain. Patient who took the Wuwei-Ganlu-Yaoyu, soaked the feet for 30 minutes every night, and after 2 weeks, the blood uric acid dropped from 612 μmol/L to 412 μmol/L [13]. In the study Ying et al, it has been demonstrated that *Ephedra sinica Stapf* can reduce the blood uric acid level of hyperuricemia model rats by adjusting the urine pH value [14]. *Angelica biserrata* (Shan et Yuan) *Yuan et Shan* and *Angelica sinensis* also have the effect of lowering uric acid. The previous study of Cen et al. showed that Sanwu Duhuojietang can reduce renal tubular damage by reducing blood uric acid concentration and improving the micro-inflammatory state of the body, thereby delaying the deterioration of renal function [15]. Moreover, Li et al. showed that Danggui Buxue Decoction improves myocardial function in rats with hyperuricemia by reducing uric acid, and safflower and angelica also protect the liver. Further, in rats with hyperuricemia the contents of NO, NOS and SOD level in heart muscle tissue were lower than those in a control group, and the MDA level in a model group was higher than those in a control group. After treatment by Danggui Buxue Tang, the levels of NO, NOS and SOD in heart muscle of the treated group increased compared with the model group and MDA levels were decreased [16,17]. The ethanol extract of *Gentiana macrophylla* reduces the joint damage in rats with the gout model of sodium urate by down-regulating the expression of serum TNF-α, IL-1β, IL-6, PGE2 and MMP-3 [18]. *Phellodendron amurense Rupr.* has a protective effect on myocardial injury in hyperuricemia CKD rats, and it may be related to the reduction of blood uric acid levels and the improvement of micro-inflammatory responses [19]. *Eucommia ulmoides Oliver* can also reduce the blood uric acid level of hyperuricemia rats [20]. *Scutellaria baicalensis Georgi* prevents hyperuricemia nephropathy in mice by lowering uric acid [21]. From literature research, it can be known that *Eucommia ulmoides Oliver, Tamarix chinensis, Juniperus formosana Hayata, Ephedrasinica Stapf, Artemisia frigida Willd, Angelica biserrata* (Shan et Yuan) *Yuan et Shan, Angelica sinensis, Gentiana macrophylla, and Phellodendron amurense Rupr.* in the preparation of Hataagqi-19 have the effect of lowering blood uric acid and the compounds are mainly flavonoids, alkaloids, saponins and some polysaccharides [22]. The above-mentioned literature research also supports the reduction of blood uric acid in the high-dose group of animals in the long-toxicity experiment of the Hataagqi-19 preparation.

The limitation of this study is that the type of penetration enhancer and the amount of extract were not strictly screened, and the amount of animal samples for toxicity experiments was relatively small. In later research, the effect of different penetration enhancers on the penetration of the active ingredients of the Hataagqi-19 preparation should be investigated, and the best type and dosage of the penetration enhancer should be identified. In view of the results of lowering uric acid in the long-term toxicity experiment, an animal model of hyperuricemia should be made to study the influence of the Hataagqi-19 preparation on various parameters of the model animal, so as to facilitate the development of a wider range of clinical indications for the preparation.

Conclusion

Hataagqi-19 hydrogel patch prepared with the best ratio of NP-700: aluminum glycolate: glycerol: carbomer = 6: 0.35: 35: 0.40. the ratio of azone and propylene glycol is 1: 1. The total penetration enhancer dosage was 5%. The performance of the patch was good and the preparation process was feasible and reliable. Hatagaqi-19 preparation had no obvious long-term toxicity and skin allergy, it is a safe external preparation.

Conflict of Interest

The authors state no conflict of interest.

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