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Using Network Pharmacology to Clarify the Therapeutic Mechanism of Hataagqi-19 Hot Compress Therapy in Rats with Lumbar Disc Herniation

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Objective: This study aims to determine the primary efficacy of traditional medicine treatment of Lumbar disc herniation (LDH) and the effect of Hataagqi-19 hot compress (H19+HC) therapy on LDH rats. **Methods:** Referencing ancient books of Mongolian medicine, we studied the theoretical basis of Mongolian medicine to treat LDH. We performed operations to establish a model of SD rats with LDH treated with H19+HC. Paw withdrawal threshold (PWT) was detected, serum TNF-α, IL-6, and IL-1β were measured by ELISA, and immunohistochemical staining was used to measure IL-6, CD68, and IgG. **Results:** The medicinal properties of the drugs for the treatment of LDH are mainly dry "Xieriwusu," heat-clearing, and suppressing "Heyi" medicine. The PWT value, serum TNF-α, IL-6, IL-1β and tissue IL-6, CD68, IgG of H19+HC was lower than that of the control group. The nucleus pulposus of the H19+HC was significantly smaller than that of the control group. The positive staining result in the main inflammatory cells of the lesion was CD68. **Conclusions:** Mongolian medicine treatment of LDH is based on the dry "Xieriwusu," heat-clearing, and suppressing "Heyi" efficacy. The H19+HC therapy follows this efficacy and has the effects of improved abnormal behavior, analgesic, anti-inflammatory, and reabsorption of the nucleus pulposus.

Keywords: Intervertebral Disc Disease, Traditional Medicine, Physical Therapy

Introduction

Lumbar disc herniation is a common symptomatic clinical disease, and its main symptom is lumbar pain and sciatica caused by compression and potentially chemical irritation of spinal nerve roots by protruding disc material [1]. The treatment methods are mainly surgery and conservative treatment [2, 3]. Surgical treatment has some potential complications or sequelae, and its long-term efficacy is equivalent to conservative treatment [4, 5]. Therefore, conservative treatment is considered the best choice for adolescents or patients with mild symptom patients [6].

LDH belongs to the category of "Huyang" disease or limb white vein disease in Mongolian traditional medicine. The term "limb white vein" refers to peripheral nerves. Mongolian medicine for treating "Huyang" disease or limb white vein disease includes oral Mongolian medicine, topical medicine, acupuncture, cupping, massage, medicated bath, bloodletting, medicine fumigation, buried sand therapy, topical tea and wine therapy. Mongolian medicine mainly uses oral medications for treating "Huyang" disease or limb white vein disease. Still, oral medications often contain toxic ingredients, for instance, *Radix aconitiagrestis*, mercury, or precious drugs such as pearl, cinnabar, and coral. Although harmful medications go through a strict processing regimen, their remaining impurities may cause toxic reactions, and most precious drugs are not easy to digest. After prolonged oral administration, they tend to stimulate the gastric mucosa, cause digestive tract dysfunction, and sometimes cause liver and kidney function damage. Therefore, finding the best non-surgical treatment combination for the treatment of LDH is of great significance to the clinical treatment of this disease.

Herbal medications have anti-inflammatory, analgesic and nerve sensitivity reduction effects in sciatica caused by LDH [7]. Physical therapy can treat nerve root pain caused by herniated discs, spinal canal stenosis, lumbar spondylolisthesis, etc., and shorten the natural history of radial pain without complications [8, 9]. Traditional herbal hot compresses area treatment method that combines physical therapy and medicine. It has been applied in traditional medicine for thousands of years. During treatment, herbal compresses are applied to the diseased part to exert heat, and the effective ingredients of the herbal medicine are directly introduced into the meridians. When reaching the location of the disease, a higher concentration is formed locally, thereby achieving the effect of warming the meridians, promoting blood circulation and relieving pain [10]. However, the mechanism of herbal hot compress treatment on LDH is not clear, and there are few related studies. Moreover, LDH is a modern term for what is likely an ancient condition. There are many names for this disease in traditional Mongolian medicine. It is necessary to sort out and analyze the main effects of traditional Mongolian medicine to treat LDH. Traditional Mongolian medicine attaches great importance to a drug's properties and taste. It believes that drugs with properties cold drugs can treat heat diseases, and drugs with heat properties can treat cold diseases. The taste of the drug is related to its properties and efficacy. So knowing a drug's properties is vital to determine its impact on LDH.

Therefore, in this study, we determined the primary Mongolian medicine treatments for LDH by analyzing the Mongolian medications for "Huyang" disease or limb white vein disease treatment. From this analysis, we learned that the traditional topical preparation Hataagqi-19, combined with hot compress therapy, is used to treat LDH. Hataagqi-19 is an in-hospital preparation of Baotou Mongolian and Chinese Medicine Hospital. It is based on the dye from a safflower flower and consists of different components, namely flowers *Carthamus tinctorius L.*, *Eucommia ulmoides Oliver*, *Tamarix chinensis*, *Juniperus formosana Hayata*, *Angelica sinensis*, *Angelica biserrata (Shan et Yuan) Yuan et Shan*, *Abutilon theophrastii*, *Scutellaria baicalensis Georgi*, *Scutellaria baicalensis Georgi*, *Sophora flavescens Ait.*, *Gentiana macrophylla*, *Ephedrasinica Stapf*, *Xanthoceras sorbifolia Bunge*, *Artemisia frigida Willd.*, *Notopterygium incisum Ting ex H.T.Chang*, *Phellodendron amurense Rupr.*, *Ligusticum chuanxiong hort*, *Phryma leptostachya L. subsp. Asiatica (Hara) Kitamura*, *Dipsacus asper Wall. ex Henry*, *Avorus calamus L.* It has been used in Baotou Mongolian and Chinese Medicine Hospital for more than 30 years. It is mainly used for the treatment of LDH, gouty arthritis and knee joint disease. We sought to determine its analgesic, anti-inflammatory effects and the changes it produces to the herniated nucleus pulposus in a rat LDH model.

Materials and Methods

Data Sources

According to *Emjaslincaranhaan* (8th century, Nakancana), *Angaahwuhaannidurbenwudec* (12th

century, YutogeYundangongbu), *Hohbindoriya* (1687, DisiridSangzaizamusu), *Lhantab* (1691, Disirid Sangzaizamusu), *Durbenarshan* (1785, Yixibaljur), *Qaganbolortoli* (1786, Yixibaljur), *Obidicindalai* (1829, Jampileqoijidanzanpurile), and *Tongwagajid* (1888, Jigemuddanzanzamusu) books of Mongolian medicine, there are many medications for limb "Huyang" disease, white vein disease, "Huyang" disease, waist and leg pain, numbness, lower limb muscle atrophy, and other descriptive terms for the treatment of limb "Huyang" disease. After removing the duplicate medications, and 88 traditional Mongolian medications were identified [11].

Standardization of Mongolian medications

Chinese Pharmacopoeia (2015 edition), Inner Mongolia Mongolian Medicine Preparation Specification (2014 edition), Inner Mongolia Mongolian Medicine Preparation Specification Notes (2004 edition), Inner Mongolia Mongolian Medicine Preparation Standard (Supplementary edition) (1988 edition), Inner Mongolia Mongolian Medicine Preparation Standard (1984 edition), and Chinese Encyclopedia· Mongolian Medical Volume (1992 edition) were referred to for the medication's standard name. For the medications not included in the above books, the earliest Mongolian transliteration name was used [12].

Standardization of medicinal material names

Chinese Pharmacopoeia (2015 edition), Inner Mongolia Mongolian Medicine Standard (1986 edition) [13], Chinese Encyclopedia·Mongolian Medical Volume (1992 edition), and Mongolian Medicine Pharmacopoeia (2006 edition) [14] were referred to verify the Sanskrit, Tibetan, Mongolian and Chinese names of the Mongolian medicines. We confirmed all the 155-kinds Mongolian medicines and their standardized English medicinal names and materials in the *Chinese Pharmacopoeia* (2015 edition). Medicinal materials not included in the *Chinese Pharmacopoeia* (2015 edition) had Latin names.

Animals and anesthesia

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. A total of 48 adult male Sprague–Dawley rats (SiPeiFu, Beijing, China) weighing 200–240 gr were used. The animals were housed in plastic cages at room temperature (21–24^{0}C) in a 12h light and dark cycle with free access to food and water. A combination of 10 % chloral hydrate was prepared as an anesthetic. Before surgery, the animals were anesthetized by intraperitoneal injection of 0.1 ml/100 gr of body weight of the anesthetic.

Experimental groups

Forty-eight rats received one of two surgical treatments. One group had nucleus pulposus material transplanted from an intervertebral disc in the rat's tail and to the left L6 dorsal root ganglion to chemically irritate the nerve root (NP group, n = 36). The other group had the same operation without transplanting the nucleus pulposus (Sham group, n = 12). The NP group was then subdivided into 3 groups. One group was treated with hot compresses alone to the surgical site after the stitches were removed on postoperative day 7 (HC group, n= 12). Another group was treated Hataagqi-19 applied topically to the surgical site after the hot compresses were applied (H19+HC, n = 12). The remaining group received no treatment (Control, n = 12). In vivo noninvasive neurosenstivity testing was performed on each animal before surgery, at 1, 2, and 3 weeks after surgery, and before sacrifice at 4 weeks after surgery. After the final neurosensitivity testing was completed, 6 rats from each group were sacrificed for ELISA testing and immunohistochemical staining. The temperature of the hot compress was controlled at 39-42°C. It was applied once a day, for 1 hour for 21days.

Surgical procedure

Rats were placed in the prone position, and a posterior midline incision was made at the L4–L6 level. The fascia was incised, and multifidus muscles were elevated from the lumbar lamina and facet joints and retracted laterally to expose the facet joint between the left fifth and sixth lumbar vertebrae using an operating microscope. The left L5 dorsal root ganglion and spinal nerve were exposed by an L5/6 facetectomy. In the NP group, the nucleus pulposus harvested from the tail was placed on the surface of the dorsal root ganglion. No attempt was made to compress the dorsal root ganglion. The same surgical procedure was performed in the sham group without applying the nucleus pulposus on the dorsal root ganglion. In both groups, the deep and superficial wounds were closed with sutures.

Behavioral testing

The rats underwent mechanical pain sensitivity symptoms on the operated limbs to verify the success of the model and the efficacy of treatment. Sensitivity to non-noxious mechanical stimuli was tested by the previously established von Frey method [13, 15, 16]. Behavioral tests were performed by a physician unaware of the rats' experimental group (n = 12 in each group). Baseline testing was performed before the start of the experiment to accommodate animals with normal responses. The left hind paw withdrawal response to von Frey filament (Stoelting, USA) stimulation of the plantar footpads was determined at days 1 (baseline), 7, 14, 21, and 28. Individual rats were placed in an acrylic cage with a mesh floor and allowed to acclimate for 15 min or until cage exploration and major grooming activities ceased. The lateral plantar surface of the operated hind paw, innervated by the L5 nerve, was stimulated with 9 von Frey filaments (1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 26.0 gm) through the mesh floor. The grams force for von Frey hairs was estimated based on the manufacturer's ratings. Stimulation was initiated with the 1.0 gr filament. Progressively larger filaments were sequentially applied to the paw surface until the filament bent and were held there for approximately 3 s to see if the paw would withdraw.

Enzyme-linked immunosorbent assay (ELISA)

Six rats in each group were anesthetized with chloral hydrate (3.0-3.5 ml/kg, i.p.) on day 28 after the operation. Blood was drawn from the abdominal aorta, centrifuged, and the supernatant was taken. Rat TNF-α, IL-1β and IL-6 were measured in the supernatant using ELISA kits according to the manufacturer's instructions (Jingmei, Jiangsu, China).

Immunohistochemistry

Immunohistochemical examinations were performed on postoperative day 28 for each group (n= 6 per group). The rats were euthanized using 10% chloral hydrate ether and then perfused with fresh 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), and muscle tissue around L5 on the left was excised. A block of soft tissue incorporating the previous operative site was fixed briefly in 4 % paraformaldehyde and subsequently embedded in paraffin. Two sections (6 μm thick) were cut showing the muscle tissue and the transplanted nucleus pulposus, if present, and placed on separate slides. Sections were deparaffinized with xylene and rehydrated with 100 % ethanol followed by PBS and pretreated with Dako Target Retrieval Solution (Jingmei, Jiangsu, China) at 97°C for 20 min to enhance immunoreactivity. Samples were incubated in 2 % normal donkey or goat serum in PBS/0.3% Triton X-100 applied for 1hour at room temperature.

Goat anti-IL-6 antibody (1:100; Jingmei, Jiangsu, China), rabbit anti-CD68 antibody (1:100; Jingmei, Jiangsu, China), or rabbit anti-IgG antibody (1:100; Jingmei, Jiangsu, China) were applied, and samples were incubated overnight at 4°C, after washing with PBS. Next, the samples were incubated for 1 hour at room temperature with donkey anti-goat or goat anti-rabbit antibodies (1:200; Jingmei, Jiangsu, China).

Statistical analysis

We entered all the combinations of Mongolian medicines in 88 medications into Excel 2007 along with their properties to establish a dataset to classify and sort the drugs. Cytoscape 3.7.1 was applied to visualize the various relationships and interactions of the drugs.

The research results were analyzed using basic biostatistics; arithmetic mean (M), standard deviation (SD), confidence interval (95% CI) calculation and the statistical significance was determined by a mixed two-way ANOVA followed by Tukey tests. P-values less than 0.05 ($p < 0.05$) were considered statistically significant. The analyses were done using SPSS 25.0.

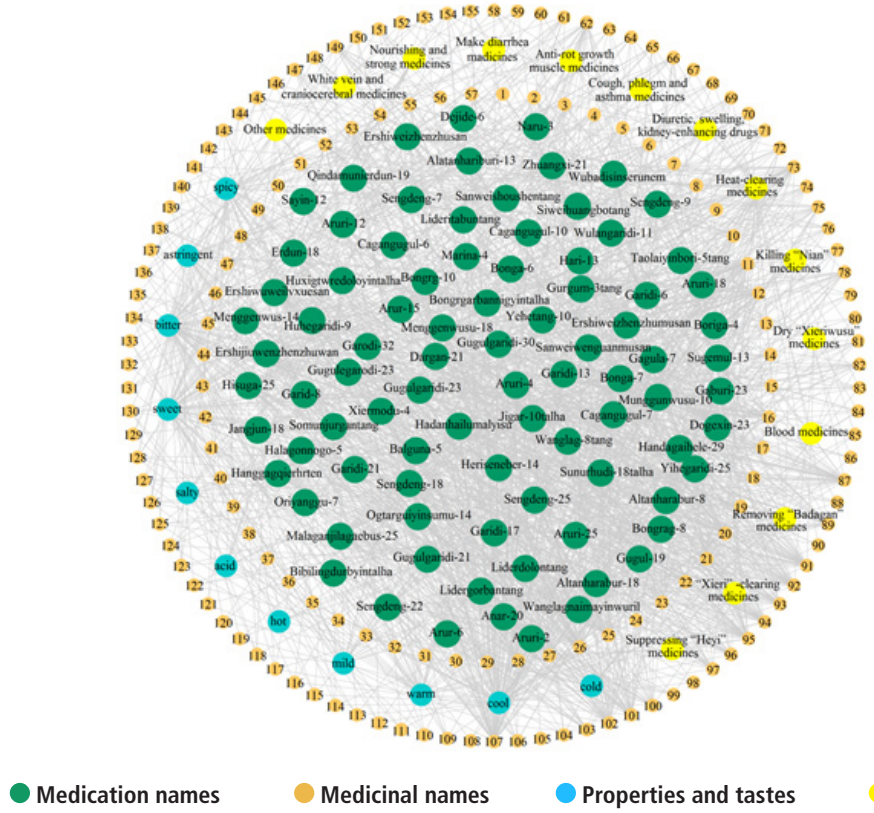
Ethical statement

Our 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

Properties, taste and efficacy of Mongolian medicine medications for LDH

We identified 88 medications and classified them according to medication name, medicinal material name, taste, and efficacy and entered them into Cytoscape 3.7.1 to visualize their drug



Medication names **Medicinal names** **Properties and tastes** **Efficacy**

1.Calcspar, 2.Aconiti Kusnezoffii Folium, 3.Caesalpinia Crista, 4.Persicae Semen, 5.Semen MangiferaeIndicae, 6.Platycladi Cacumen, 7.Canavaliae Semen, 8. Halite Violaceous, 9.Clematis, 10.Gentianae Macrophyllae Radix, 11.Liquidambaris Resina, 12.Lignum XanthoceraisSorbifoliae, 13.Vermilion, 14.Glycyrrhizae Radix Et Rhizoma, 15.Coral, 16.Aconiti Kusnezoffii Radix, 17.Inulae Radix, 18.Ferulae Resina,19.Lygodii Spora, 20.Trogopterori Faeces, 21.Akebiae Caulis, 22.Santali Albilignum, 23.Violae Herba, 24.Gossampini Flos, 25.Casslae Semen, 26.Carthami Flos, 27.Tsaoko Fructus, 28.Myristicae Semen, 29.White Sandalwood, 30. Mutton, 31.Iron Powder, 32.Artemisia Gmelinii, 33. Ligularia, 34. Asari Radix Et Rhizoma,35.Celosiae CristataeFlos, 36.Sorrel, 37. Strychni Semen, 38. Sea snail, 39. Copper Ash, 40. Rubi Fructus, 41. Mirabilis jalapa , 42. RhezomaGymnadeniae, 43. CurcumaeLongaeRhizoma, 44.Inulae Flos, 45.Bistortae Rhizoma, 46.Plantaginis Semen,47.Phytolaccae Radix,48.Granati Pericarpium,49.Rosae Rugosae Flos,50.Stalactitum,51. Turquoise, 52.Sulfur, 53. Mercury,54.Rhubarb Extract,55.Potentillae DiscolorisHerba, 56.Cinnabaris, 57.Calamina, 58.*Echinops sphaerocephalus* L., 59.Magnetitum, 60.Limonitum, 61.Margdrita, 62.Acori TatarinowiiRhizoma, 63.Halite Violaceous, 64.Rubiae Radix Rhizoma, 65.Loquat leaf, 66. Lacca, 67.Gnetum, 68.Meconopsis, 69.Euphorbiae HumifusaeHerba, 70.Nigellae Semen, 71.Fructus Cymini, 72.Malvae Fructus, 73.Aucklandiae Radix, 74.Saigae TataricaeCornu, 75.Aquilariae Lignum Resinatum, 76.Margaritifera Concha, 77.Phellodendri Chinensis Cortex, 78.Crab, 79.Black Alum, 80.Denarobii Caulis, 81.Akebiae Caulis, 82.Cinnamomi Cortex, 83.Borneolum, 84.Donkey's Blood, 85.Rhapontici Radix, 86.Entianae RhodanthaeHerba, 87.Moschus, 88.Eucommiae Cortex, 89.Sophorae Flavescentis Radix,90.Arisaematis Rhizoma,91.Olibanum,92.Abutili Semen, 93.Bambusae ConcretioSilicea, 94.Caryophylli Flos, 95.Amomi FructusRotundus, 96.Bovis Calculus, 97.Santali Albi Lignum, 98.Delphinium, 99.Piperis Fructus, 100.Piperis LongiFructus, 101.Gardeniae Fructus, 102.Toosendan Fructus, 103.Zingiberis Rhizoma, 104.Fructus Cassiae Fistulae, 105.Black False Hellebore, 106.Coptidis Rhizoma, 107.*Terminalia chebula* R., 108.Strychni Semen, 109.Camphor, 110.Mylabris, 111.Light Halitium,112.Dryopteridis CrassirhizomaRhizoma, 113.Sappan Lignum, 114.Vladimiriae Radix, 115.Arnebiae Radix,116.Glehniae Radix, 117.Cnidii Fructus, 118.Scorpio, 119.Uncariae Ramulus Cum Uncis, 120. Corydalis BungeanaeHerba, 121.Chrysanthemi Flos, 122.*Hypecoum erectum* L., 123.Rhizoma Kaempferiae, 124.Cervi Cornu, 125.Tribuli Fructus, 126. Asparagi Radix, 127.Pol YgonatiOdoratiRhizoma, 128.Rhizoma Polygonati, 129.Dalbergiae Odoriferae Lignum, 130.Tronite Trona, 131.Radix Valerianae, 132.Salviae Miltiorrhizae Radix Et Rhizoma, 133.Schisandrae ChinensisFructus, 134.Cynomorii Herba, 135.Capsici Fructus, 136.Euphorbiae Ebracteolatae Radix, 137.Crotonis Fructus, 138.Euphorbiae Pekinensis Radix, 139.Codonopsis Radix, 140.Shell, 141.Allii Sativi Bulbus, 142.*Pedicularis resupinata* L, 143.Arecae Semen, 144.Sodium Borate, 145.Crinis Carbonisatus, 146.Cinnamomi Ramulus, 147.Bear Gall, 148.Chrysosplenium Baitoshamicum, 149. Isatidis Folium, 150.Seed of Field Pennycress, 151.Herb of Cornuted Pugionium, 152.Hippophae Fructus, 153.Chaenomelis Fructus, 154.Dracocephalum moldavica L, 155.Fructus Coriandri.

Figure 1. Network diagram of 88 Mongolian traditional medicines commonly prescribed for herniated nucleus propulsus.

interactions (Figure 1).

By analyzing 88 Mongolian medications for the treatment of lumbar disc herniation, a total of 155 Mongolian medicines, with a total frequency of 1136 times. These Mongolian medicines are used 10 times or more, with a total composition using the Inner Mongolia Mongolian Medicinal Materials Standard (1986 edition). After sorting, 33 kinds of Mongolian medicines have 9 types of efficacy classifications, and the total frequency of use was 774 times. Among them, the top 5 most frequently used drugs were dry "Xieriwusu" medicine (182 times, 23.5%), heat-clearing medication (179 times, 23.1%), "Heyi"-suppressing medicine (125 times, 16.2%), "Nian"-killing medicines (104 times, 13.4%) and "Badagan"-removing medicines (67 times, 8.7%) (Table 1).

According to the Inner Mongolia Mongolian Medicinal Materials Standard (1986 edition), we statistically analyzed the medicinal properties and tastes of frequently used drugs (≥ 10 indications for usage). If the Mongolian medicine had multiple medicinal properties and tastes, all uses were included. Among the 33 Mongolian medicines, a total of 47 tastes and 33 medicinal properties were identified. Most commonly, it was bitter 19 times (40.4%), spicy 13 times (27.7%), astringent 7 times (14.9%), and the medicinal properties were cool 16 times (48.5%) and warm 9 times (27.3%) (Table 2).

Hataagqi-19 combined with hot compress therapy alleviated the behavioral changes in LDH rats

To determine the effect of hot compress therapy on the mechanical sensitivity caused by LDH, we compared the HC group with the control group. Compared to the control group, the mechanical sensitivities of the HC group were significantly higher on the 14th, 21st and 28th days after surgery ($p < 0.05$).

To determine the effect of Hataagqi-19 therapy on the hot compress therapy, we compared the H19+HC group with the HC group and control group. The difference began on the 14th day of treatment, where the H19+HC group's neurosensitivity was higher in both the HC group ($p < 0.05$) and the control

Table 1. Conditions for which drugs are commonly used in Mongolian traditional medicine.

Drug efficacy	Frequency	%
Dry "Xieriwusu medicines"	182	23.5
Heat-clearing medicines	179	23.1
Suppressing "Heyi medicines"	125	16.2
Killing "Nian medicines"	104	13.4
Removing "Badagan medicines"	67	8.7
Other medicines	55	7.1
Cough, phlegm and asthma medicines	25	3.2
"Xieri, phlegm and asthma medicines"	24	3.1
Diuretic, swelling, kidney-enhancing drugs	13	1.7

Table 2. Frequency of drug properties and tastes used to treat herniated nucleus pulposus.

Drug Property*	Frequency	%	Drug taste	Frequency	%
Cool	16	48.5	Bitter	19	40.4
Temperature	9	27.3	Spicy	13	27.7
Mild	5	12.1	Astringent	7	14.9
Cold	4	12.1	Sweet	6	12.8
Heat	-	-	Salty	1	2.1
			Acid	1	2.1

*Traditional Mongolian medicine attaches great importance to a drug's properties and taste. It believes that drugs with properties cold drugs' can treat heat diseases, and drugs with heat properties can treat cold diseases. The taste of the drug is related to the properties and efficacy of the drug.

group ($p < 0.05$). This shows that either hot compress alone or external application of Hataagqi-19 had a therapeutic effect, but Hataagqi-19 combined with hot compress resulted in the neurosensitivity of the H19+HC group to be essentially the same as the sham value (24.45 ± 1.75 vs. 23.61 ± 2.02 g, $p > 0.05$) 28 days after surgery (Table 3).

Hataagqi-19 hot compress therapy can reduce inflammation caused by LDH

To determine the anti-inflammatory effect of Hataagqi-19 hot compress therapy on LDH, we measured serum TNF-α, IL-1β and IL-6 levels in rats 28 days after surgery. The results showed that hot compress therapy alone or external application of Hataagqi-19 reduced the inflammatory response caused by LDH ($p < 0.05$), but Hataagqi-19 combined with hot compress therapy resulted in the inflammation subsiding more obviously ($p < 0.05$) (Table 4).

Hataagqi-19 hot compress therapy stimulated the phagocytic function of macrophages and shrunk the ectopic nucleus pulposus tissue

Our immunohistochemical results confirmed that hot compress therapy significantly reduced the inflammation involved in IL-6, CD68 and IgG ($p < 0.05$), and Hataagqi-19 hot compress therapy significantly reduced the inflammatory reaction ($p < 0.05$). From immunohistochemical staining, the positive staining

Table 3. Effect of Hot Compress (HC) and Hataagqi-19 Hot Compress (H-19+HC) therapies on the paw withdrawal threshold in rats.12 rats were in each group each day. All values are in gms.

	Sham	Control	HC	H19+HC
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1st day	24.28 ± 1.22	24.56 ± 1.1	24.25 ± 0.98	24.39 ± 1.25
7th day	22.69 ± 1.32	7.93 ± 1.06	8.02 ± 1.18	8.72 ± 0.62
14th day*, †, ‡	23.8 ± 1.33	9.19 ± 1.28	11.79 ± 1.61	14.26 ± 1.86
21st day*, †, ‡	24.31 ± 1.94	10.53 ± 1.07	14.75 ± 1.81	20.57 ± 2.25
28th day*, †, ‡	24.45 ± 1.75	12.74 ± 1.2	17.73 ± 2.2	23.61 ± 2.02

*Control vs. HC at $p < 0.05$; †Control vs. H19+HC at $p < 0.05$; ‡HC vs. H19+HC at $p < 0.05$ using Tukey post-hoc comparisons

Table 4. Effect of Hataagqi-19 hot compress therapy on inflammatory response in rats at 28 days after surgery. All values are in μmol/L.

Cytokine	Sham n = 6	Control n = 6	HC n = 6	H19+HC n = 6
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
TNF-α*, †, ‡	106.41 ± 16.48	341.9 ± 32.48	198.27 ± 25.58	122.79 ± 23.57
IL-1β*, †, ‡	305.46 ± 17.52	388.59 ± 20.44	357.69 ± 23.53	313.48 ± 25.62
IL-6*, †, ‡	75.56 ± 16.77	183.75 ± 19.58	114.46 ± 15.29	81.31 ± 21.69

*Control vs. HC at $p < 0.05$; †Control vs. H19+HC at $p < 0.05$; ‡HC vs. H19+HC at $p < 0.05$.

Table 5. The effect of Hataagqi-19 hot compress therapy on the ratio of IL-6, CD68 and IgG area density in the soft tissues of the left L6 dorsal root ganglia at 28 days after surgery. All values are the area density percent.

Cytokine	Sham n = 6	Control n = 6	HC n = 6	H19+HC n = 6
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
IgG*, #, Δ	0.001 ± 0.001	0.005 ± 0.002	0.003 ± 0.002	0.001 ± 0.001
CD68*, #, Δ	0.008 ± 0.003	0.016 ± 0.002	0.011 ± 0.002	0.007 ± 0.002
IL-6*, #, Δ	0.001 ± 0.001	0.005 ± 0.001	0.003 ± 0.001	0.002 ± 0.001

*Control vs. HC at $p < 0.05$; #Control vs. H19+HC at $p < 0.05$; ΔHC vs. H19+HC at $p < 0.05$.

of IL-6 and IgG was distributed in the intercellular space, and the intracellular staining was negative, while the staining results of CD68 for macrophages showed positive staining in the inflammatory cells, indicating that the nucleus pulposus was reduced. Hataagqi-19 hot compress therapy confirms the Mongolian medicine dry "Xieriwusu" theory by promoting the shrinkage and absorption of the ectopic nucleus pulposus (Table 5).

Discussion

The *Angaahwuhannidurbenwndec* records the concept of "Xieriwusu" in Mongolian medicine. "Xieriwusu" is a component of the human body. It exists between the skin and muscles, as well as the joints. Therefore, the traditional Mongolian medicine says "Xieriwusu" is similar to tissue fluid and intra-articular fluid. The nucleus pulposus is the buffering tissue between the lumbar vertebrae joints. It exists in the annulus fibrosus and acts like synovial fluid in the joints. Therefore, it is classified as "Xieriwusu" in Mongolian medicine. LDH is herniation of the nucleus pulposus following rupture of the fibrous ring of the lumbar intervertebral joint, which compresses the nerve root; the nucleus pulposus protruding from the ring is an abnormal "Xieriwusu." From the 88 medications used by traditional Mongolian medicine to treat LDH, the dry "Xieriwusu," heat-clearing, and suppressing "Heyi" medicine are the most used. "Xieriwusu" is literally translated as yellow water; the nucleus pulposus protruding from LDH belongs to the abnormal "Xieriwusu" extruded from the joint cavity. Mongolian medicine uses "Xieriwusu" drugs to dry the prolapsed nucleus pulposus. This theory is similar to the recently reported phenomenon of nucleus pulposus resorption after LDH [13].

The LDH non-compression model is the current general LDH model, which can simulate the inflammation and nerve sensitivity caused by the protrusion of the nucleus pulposus. After modeling, the animals will have the symptoms of mechanical skin sensitivity in the innervated area of the same segment. This neurosensitivity symptom is the same as the "Heyi" multi-symptom of Mongolian medicine. Relieving neurosensitivity symptoms is similar to Mongolian medicine's suppressing "Heyi" treatment. By measuring the mechanical pain threshold of the paws of the model animals, the success of the modeling can be judged, and the effect of the treatment can be observed [14]. Junichi used duloxetine to study the mechanical pain threshold in rats with lumbar disc herniation. It is shown that duloxetine can improve the nerve sensitivity symptoms caused by LDH. The nerve sensitivity increased 7 days after the operation, and the pain threshold dropped to its lowest level. The pain threshold can gradually return to a relatively high level through treatment, which is the same as our findings regarding H19+HC's pain relief and behavior improvement [15]. Jun-Nan's research shows that PDE2A inhibitors can reduce the nerve sensitivity of LDH rats, having an analgesic effect. With the decrease of nerve sensitivity, the mechanical pain threshold gradually increases. PDE2A inhibitor also reduces the nerve pain in the spinal cord of LDH rats. The TNF-a, IL-6 and IL-1B levels have an anti-inflammatory effect, which is the same finding in our research on the anti-inflammatory and analgesic effects of H19+HC [16]. In this experiment, compared with the control group, the HC group and the H19+HC group were significantly different at three treatment points ($p < 0.05$); the H19+HC group was compared with the HC group, there are significant differences in treatment points ($p < 0.05$). In addition, the paw withdrawal threshold of rats in the HC group and H19+HC group has a significant upward trend at 7, 14, and 21 days after treatment, and the threshold of the H19+HC group is close to the level of the sham group at 28 days. Indicating that Mongolian medicine combined with hot compress can reduce mechanical sensitivity more than pure hot compress and better promote the recovery of mechanical pain in the innervated segment.

In recent years, more and more inflammatory mediators have been found in the herniated disc tissues. These inflammatory factors play an important role in the pathogenesis of LDH. TNF-α is one of them, which plays a key role in the induction and development of neurogenic pain. TNF-α can induce the production of inflammatory factors such as IL-1, IL-6, IL-8 and activate MMP-2 and MMP-9. This promotes the release of PLA2 to degrade the basement membrane and damage the nerves, causing the nerve tissue to appear congestion, edema, mooring and adventitia vasodilation [11-13]. In addition, TNF-α also destroys the blood-nerve barrier together with other cytokines in the nucleus pulposus, causing macrophages to invade and release more cytokines to increase the inflammatory response [15]. IL-1 can directly stimulate the ganglia or increase the sensitivity of nerve tissue to bradykinin, and it is an important inflammatory pain-causing substance [16, 17]. Zhi-hua's research on Resolvin

D1 also showed that it could reduce the nerve sensitivity of LDH rats, reduce the levels of TNF-a and IL-1B in rat spinal cord nerve tissue, and have behavioral improvement and anti-inflammatory effects [18]. Our research results are similar. Yizhuan compared LDH rats with non-swimming rats after swimming training and concluded that swimming could reduce the level of inflammation in LDH rats, especially the levels of TNF-a and IL-6. This anti-inflammatory result is also similar to our experimental results [19]. In this study, the serum levels of TNF-α, IL-1β and IL-6 in the control group were higher than those in the HC group. The levels were higher in the HC group than the H19+HC group, indicating that that the addition of Hataagqi-19 combined with hot compress therapy is better than hot compress alone in reducing the inflammation caused by LDH. This inflammation is regarded as a manifestation of heat in traditional medicine [20]. The anti-inflammatory effect of Hataagqi-19 hot compress therapy also supports the traditional medicine theory of heat-clearing treatment.

There are many reports of the nucleus pulposus reabsorption in the natural course of LDH. It is believed that the nucleus pulposus has no blood supply in the closed fibrous annulus and is isolated from the immune system without contact with the blood circulation when it comes out of the closed fibrous annulus attacked by the immune system as a foreign body [18-20]. Many researchers have proposed that macrophages play an important role in the resorption of the nucleus pulposus [21]. Most of the previous non-surgical treatments focused on reducing nerve root pain and anti-inflammatory effects. Experimental research also focused on the anti-inflammatory and analgesic effects of drugs. The phenomenon of natural shrinkage of the prominent nucleus pulposus is observed in patients. There are few experiments on the shrinkage or resorption of the prominent nucleus pulposus. Therefore, we sought to observe the shrinkage of the nucleus pulposus and its mechanism and found that inflammation was involved. The cells are mainly macrophages, similar to previous studies on the mechanism of human nucleus pulposus resorption [22]. The removed nucleus pulposus belongs to the abnormal "Xieriwusu" of Mongolian medicine. Therefore, this shrinkage of the nucleus pulposus also supports the Mongolian medicine dry "Xieriwusu" theory.

However, our research also has some shortcomings. The size of the nucleus pulposus shrinkage was not quantified, and the mechanism of macrophage aggregation was not studied. Hataagqi-19 is a compound preparation, and the biologically active substance is anti-inflammatory and analgesic. Its mechanism in shrinking the nucleus pulposus is not clear. In a later study, the shrinking effect of Hataagqi-19 hot compress therapy on the nucleus pulposus should be quantified. In subsequent research, we will use drug screening experiments to identify bioactive substances that have anti-inflammatory, analgesic, and nucleus pulposus shrinking effects. Hopefully, we can promote the reabsorption process and thereby reduce the rate of LDH surgery.

Conclusions

The removed nucleus pulposus belongs to the abnormal "Xieriwusu" of Mongolian medicine. According to the analysis of medications in ancient books, we found that traditional Mongolian medicine treatment of LDH is based on the dry "Xieriwusu" heat-clearing and suppressing "Heyi" theory. Hataagqi-19 hot compress therapy follows this theoretical framework, which not only reduces pain and inflammation, also treats the source of the disease but promotes resorption of prominent nucleus pulposus.

Conflict of Interest

The authors state no conflict of interest.

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