A Novel Therapeutic Mechanism for Bacterial Vaginitis Based on Gentian Liver-Draining Decoction and Its Modified Prescription Combined with Sophora Flavescens Gel

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Abstract: Objective: a novel therapeutic mechanism using gentian liver-draining decoction and its modified prescription as a guide formula, combined with Sophora flavescens gel and chitosan, for the treatment of bacterial vaginitis is is proposed. Method: the variation of TNF-α and IL-1β contents and the degrees of ICAM-1 and VCAM-1 expression before and after the use of the novel therapeutic mechanism were examined by multiple bacteriostatic tests and tube double decreasing dilution method. Results: the novel therapeutic mechanism effectively reduced the content changes of TNF-α and IL-1β, and reduced the high expression of ICAM-1 and VCAM-1. It can also inhibit the reproduction of anaerobic bacteria such as staphylococcus aureus in the vagina, maintain the vaginal pH value and the stability of the ecological environment of bacteria, prevent the imbalance of the micro ecosystem in the vagina, and reduce the incidence probability of bacterial vaginitis.

Bacterial vaginitis is a kind of vaginal infectious disease, which is common in women aged 15-49. The incidence rate of vaginitis in China is as high as 75% per year. The probability of bacterial vaginitis is more than 40% of the total number of cases. Clinical treatment of vaginitis generally follows the principle of protecting lactobacillus flora and inhibiting anaerobic flora. The most commonly used clinical treatment is the use of metronidazole antibiotics for treatment, although this treatment can reduce bacterial activity, it is very easy to make changes in body function. Mild cases will have nausea, vomiting, loss of appetite, ataxia and other adverse reactions, severe cases will even lead to serious complications of liver and kidney and other systems [1].

1. Composition Materials

1.1 Gentian Liver-Draining Decoction.

The earliest record on gentian liver-draining decoction was found in Prescriptions People's Welfare Pharmacy of Song dynasty that " Clearing inflammation of the liver and gall, removing dampness-heat", which is a traditional Chinese medicine recipe, and now has a wide range of clinical usage. A large number of ancient books have documented the treatment of vaginitis by gentian liver-draining decoction. Gentian liver-draining decoction has the effect of clearing viscera heat, clearing liver and gall inflammation, and clearing liver dampness heat. Ancient doctors used this prescription to treat liver and gallbladder dampness heat syndrome, liver dampness heat syndrome and other diseases. In this scheme, poria cocos and radix bupleuri are added to the original prescription to enhance its effect of clearing damp and promoting diuresis, soothing the liver and relieving depression. Meanwhile, Akebiae Caulis, which is not suitable for pregnant women and women who are preparing for pregnancy, is deleted from the original prescription to enhance its applicability and safety [2].

Nowadays, the gynecological clinical application of gentian liver-draining decoction is wide, and the existing literature has shown that clinical comparison experiments between internal use of gentian liver-draining decoction and metronidazole, and the available clinical data have clearly shown that gentian liver-draining decoction has obvious advantages in the cure rate of vaginitis, at the same time, can effectively reduce the incidence of complications.

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1.2 Sophora Flavescens Gel.

Sophora flavescens gel is classified as a traditional Chinese medicine for external use, it is also a common gynecological clinical medicine, which belongs to a pure traditional Chinese medicine preparation, the main component is total matrine, and the content of matrine in the clinically used Sophora flavescens gel can reach more than 90%, which has the effects of antibacterial and anti-inflammatory, bactericidal and antipruritic, and expectorant tourniquet. It is used more widely and human body friendly than western drugs. At the same time, Kushen has the effects of clearing fungal infections and promoting diuresis, clearing heat and dampness, so it is quite suitable for various urinary diseases and Trichomonas infection caused by heat and dampness. “Compendium of Materia Medica” records that “radix sophorae flavescentis is bitter and cold in nature, it can tonifying the kidney, its bitter can clear dampness, and its cold in nature can clear heat. Heat can cause a class of disorders characterized by shaking and tremor, dampness can cause fungal infection, so it can cure heat and clear fungal infections.” “Materia medica of Southern Yunnan” also puts forward that radix sophorae flavescentis can "cool blood, relieve heat toxin, scabies and pyoderma. It can cure skin pruritus, tinea rubra, obstinate dandruff and hematochezia. It can clear heat, swelling and phlegm toxin”. The effects of clearing heat and dampness, clearing fungal infections and relieving itching of radix sophorae flavescentis were summarized [3].

Sophora flavescens gel is applied in modern medicine technology to combine the total matrine and matrix carbomer 940 to make brown transparent gel semisolid water-soluble gel, which is used to treat gynecologic chronic inflammation such as leucorrhea increase and bacterial vaginitis. Its safety and effectiveness are highly recognized. Clinical studies have shown that Sophora flavescens gel has significant clinical efficacy in treating bacterial vaginitis, which can promote the proliferation of lactobacillus, restore the balance of vaginal microenvironment, and reduce the relapse rate of bacterial vaginitis. Moreover, it is easy to use, easy to save, and has good patient compliance [4].

1.3 Chitosan.

Chitosan is a kind of natural polycationic polysaccharide material. In recent years, it has been widely used as the matrix of medical complex because of its inhibition on both positive and negative bacteria, strong moisturizing ability and good biocompatibility. Moreover, chitosan can be directly absorbed in human body after being decomposed by specific enzymes, penetrate human tissue, and significantly regulate physiological activity.

Because of the different molecular size of chitosan, its antibacterial mechanism is not the same. Generally speaking, the macromolecular chitosan will interact with the negative charge carried by the microbial cell membrane because of its positive charge, which will destroy the original cell wall and make the internal cell components leak, thereby exhibited antibacterial effects [5]. However, chitosan with small molecules can flocculate with biological macromolecules with anions, which can interrupt the biosynthesis of DNA, destroy its physiological function and further inhibit the reproduction and growth of bacteria. (Table 1)

Table 1. Average diameter of inhibition zone of chitosan with different molecular weight (mm, n=3)

Strains	Contrast	Viscosity-average molecular weight (ku)									
		6.9	14.9	45.6	74.4	99.2	148.8	198.4	279.6	496.1	1091.3
Escherichia coli	3.0	8.2	8.5	8.4	8.5	8.5	8.5	8.0	8.0	7.5	5.5
Proteus vulgaris	3.0	5.0	13.0	13.0	12.8	12.8	13.0	13.0	12.0	12.0	8.5
Pseudomonas aeruginosa	3.0	4.0	7.1	7.0	7.0	7.0	6.9	6.8	6.0	5.5	4.0

Based on the above reasons, under the guidance of the basic theory of traditional Chinese medicine, gentian liver-draining decoction was used as the main component to eliminate pathogenic factors. Adding Sophora flavescens gel and chitosan can enhance its efficacy of clearing away heat and dampness, and is more easily absorbed by human body. In this study, the above-mentioned materials were designed in a treatment scheme, and the treatment mechanism and antibacterial effect of the materials for bacterial vaginitis were completely characterized, and a treatment scheme for bacterial vaginitis was obtained, which is suitable for a wider range of people and has less side effects.

2. Drug Mechanism

2.1 Hemostasis Principle.

An important part of the hemostatic mechanism of chitosan is to make the red blood cells adhere and gather through its effect on the red blood cells, so as to make the blood coagulate. Chitosan can also crosslink the surface of red blood cells and make them adhere to each other; Or chitosan will polymerize in the blood to form a three-dimensional network structure, which can capture red blood cells and make them aggregate. This mechanism mainly aims at the bleeding caused by local mucosal damage in the middle and late stage of bacterial vaginitis. Many experiments show that chitosan can also be used for hemostasis in patients with platelet dysfunction. Chitosan can promote blood coagulation and can be used as hemostatic agent. It can also be used as wound filling material, with the functions of sterilization, promoting wound healing, absorbing wound exudates, not easy to dehydrate and contract, etc. It can achieve the perfect combination of physical hemostasis and promoting the hemostasis function of human body.

2.2 Absorption of Exudate and Biocompatibilit.

Chitosan can be used to solve the problem of exudation caused by vaginitis. Chitosan can accelerate the natural shedding of tissue fragments and blood clots by adsorbing the secretion on the surface of the broken skin and quickly absorbing the exudate. After the formation of blood scab, the adventitia can effectively protect the skin from external stimulation, repair the damaged vaginal mucosa, effectively alleviate inflammation and avoid secondary infection.

Chitosan also has good biocompatibility. It is insoluble in water, but has certain biodegradability. It can be more compatible with human body, reduce the foreign body feeling and improve comfort of patients.

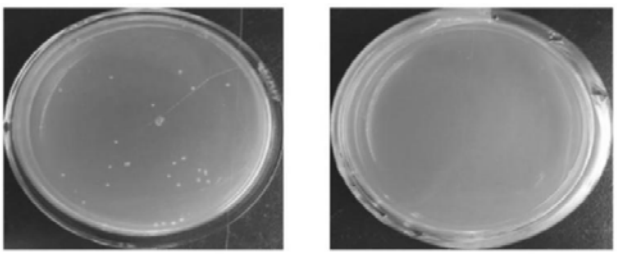
3. Experiment Results

3.1 Anti Inflammatory and Bacteriostatic Test of Sophora Flavescens Gel.

The results showed that Sophora flavescens gel could effectively inhibit the increase of TNF-α and IL-1β levels, down regulate the high expression of ICAM-1 and VCAM-1, thereby reducing the chemotaxis effect of TNF-α and IL-1β on neutrophils, macrophages and lymphocytes, thus increasing collagenase production, reducing the expression of adhesion molecules and auxiliary stimulating factors, reducing inflammatory reaction, restoring vaginal environment, and reducing the recurrence rate.

Table 2. Comparison before and after the treatment

Group	Case	TLR2(ng/L)		TNF-α		IL-1β	
		Before treatment	After treatment of two weeks	Before treatment	After treatment of two weeks	Before treatment	After treatment of two weeks
Sophora flavescens gel	70	9.93	4.61	162.38	106.68	36.79±3.78	19.54±1.82
Metronidazole	70	10.07	9.71	163.52	126.91	37.63±40.2	35.39±2.17
P value		0.454	,<0.001	0.540	<0.001	0.255	<0.001



Before treatment After treatment of two weeks

Figure 1. Schematic diagram of content change of TNF-α,IL-1β

3.2 Bacteriostatic Effect of Gentian Liver-Draining Decoction and its Modified Prescription.

According to the results of the study, antibacterial test of Staphylococcus aureus, B-hemolytic streptococcus and Escherichia coli was carried out by tube double dilution method. Diluted the tested drug solution according to a certain proportion, mixed it with the bacterial solution in groups, added 10% inactivated newborn calf serum, and the concentration of the bacterial solution was 106CFU/ml. The bacteria were cultured in 37 ℃ incubator for 18-24 hours to observe the growth of bacteria in the test tube. Another blank control tube was set up for control observation. If the tube was clear, the growth of bacteria was inhibited, and the minimum concentration (MIC) was the lowest concentration of test drug to inhibit the growth of bacteria. The growth of bacteria was observed after 18-24 hours of culture.

Table 3. Minimum inhibitory concentration and maximum bactericidal concentration of Gentian liver-draining decoction

Pathogenic bacteria	MIC	MBC
Escherichia coli	75	150
Beta hemolytic streptococcus	75	150
Escherichia coli	37.5	75
Bacteroides fragilis	75	
Comma bacillus	37.5	

The results showed that 37.5% (W/V) of gentian liver-draining decoction had inhibitory effect on Escherichia coli and arc bacillus, 75% (W/V) of gentian liver-draining decoction had bactericidal effect on Escherichia coli, Staphylococcus aureus, B-hemolytic Streptococcus and Bacteroides fragilis. When the concentration reached 150% (W/V), gentian liver-draining decoction also had bactericidal effect on Staphylococcus aureus and B-hemolytic Streptococcus.

3.3 Effect of Gentian Liver-Draining Decoction plus Sophora Flavescens Gel on the Flora.

Under normal circumstances, vaginal microenvironment is mainly composed of various lactobacilli, which can produce a large amount of acid and hydrogen peroxide gas to maintain vaginal acidic environment and inhibit the growth of harmful bacteria. After suffering from bacterial vaginitis, the normal flora in the human body will have physiological imbalance, the micro ecosystem in the vagina will be out of balance, the number of lactobacilli will decrease, and bacteria such as Gardnerella, Purple Monas and Mycoplasma Hominis will multiply in large quantities. After treatment with gentian liver-draining decoction plus Sophora flavescens gel, the pH value, bacterial density and microbial diversity of the observation group were significantly better than those of the control group ($P < 0.05$). The results showed that the external use of gentian liver-draining decoction plus Sophora flavescens gel had significant effect on vaginal flora balance in patients with bacterial vaginitis.

Table 4. Situation improvement contrast of pH value, flora density and flora diversity of Gentian liver-draining decoction plus Sophora flavescens gel

Time	Group	Case	pH value		Flora density			
			>4.5	≤4.5	Level 4	Level 3	Level 2	Level 1
Before	RS>	50	50(100.00)	0(0.00)	21(42.00)	12(24.00)	8(16.00)	9(18.00)
	Contrast>	50	50(100.00)	0(0.00)	23(46.00)	11(22.00)	6(12.00)	10(20.00)
	X^2 value		0.000		0.162	0.057	0.332	0.065
	P value		1.000		0.687	0.712	0.564	0.799
After treatment	RS>	50	19(38.00)	31(62.00)	1(2.00)	14(28.00)	28(56.00)	7(14.00)
	Contrast>	50	36(72.00)	14(28.00)	3(6.00)	6(12.00)	14(28.00)	27(54.00)
	X^2 value		11.677		0.260	4.000	8.046	17.825
	P value		0.001		0.610	0.016	0.005	<0.001

4. Discussion

Gentian liver-draining decoction and its modified prescription is a commonly used classical prescription for treating liver channel dampness heat. It also combines with Sophora flavescens gel without adding irritating chemical components, and it can evade the problem of poor compliance of Chinese medicine, poor absorption of oral administration and administration and so on. And external use of drugs can avoid the stimulation of oral capsules or tablets on the digestive tract, prevent side effects such as gastrointestinal adverse reactions, the increase of gastrointestinal burden, neurological symptoms, and even allergic reactions. Comparing with the existing treatment scheme, this scheme has a wider scope of application and higher safety.

Acknowledgement

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References

[1] Yin Xuewei, Qiu Yan, Li Zonghong, et al. Longdan Xiegan Decoction alleviates experimental autoimmune uveitis in rats by inhibiting Notch signaling pathway activation and Th17 cell differentiation[J]. Biomedicine & Pharmacotherapy, 2021,136 (1): 11291.

[2] Wang Haoran, Deng Yanquan, Li Haojing, et al. Preparation and Preperties of Chitosan Fiber/Chitosan/Alginate Hydrogel[J]. Guangdong Chemical Industry, 2021,48(02): 6-7.

[3] Chen Kai1, Liu Yunen, Tong Changci, et al. Effect of epigallocatechin gallate-carboxymethyl chitosan hydrogel on wound healing in rats[J]. Clinical Journal of Medical Officers, 2021,49(01): 15-18.

[4] Xu Yingtao, Ju Rujin, Yan Ning, et al. Systematic Evaluation of the Curative Effect of Longdan Xiegan Decoction on Vulvovaginal Candidiasis[J]. Clinical Journal of Traditional Chinese Medicine, 2020,32(10): 1884-1888.

[5] Wu Han, Ao Meiying, Chen Xueli, et al. Inhibitory effects of Longdan Xiegan Decoction on Gardnerella vaginalis in vitro [J]. Chinese Traditional Patent Medicine, 2020,42(01): 75-80.