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Comparative study of pathomorphological change of Jirug-6 suppository on the phenol-mucilage induced vaginal colpitis of rats

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SUMMARY

Introduction. Jirug-6 suppository is isolated from Mongolian traditional prescription Jirug-6, which mentioned in medicinal textbook «Four medicine code», «Uzegsdiinbayasgalan» and «Erkhemtuuver». It consists of six raw materials such as aerial part of ***Nepeta cataria*** L, ***Brassica juncea*** (L.) Czem. ***Embelia ribes*** Pall, ***Achillea asiatica*** Serge. ***Leonurus japonicus*** Houtt. and ***Polygonatum odoratum*** (Mill.) Druce.

Jirug-6 has heat clearing, wound healing, detoxifying, treating pain and antimicrobial properties. It is also clinically used to vaginosis, leukorrhea and other diseases.

Objective. To study the pathomorphological changes of Jirug-6 suppository on the vaginal tissue of a rat due to its erosion in chemical experiment.

Methods. The models are established within five days and the colpitis wasinduced with 25% phenol-mucilage by a rate of 25%. The administration was held for 11 days after the rats were sacrificed and vaginal tissues were taken. Furthermore, the Process 1, Process 2 and Process 3 were detected and pathological scores were completed on interstitial injuries of vaginal mucosa and sub-mucous under electronic microscope.

Results. The proportion of pathological changes in the three groups of histological changes: process 1> process 2 > process 3.

Conclusion. The result indicates that there is a better therapeutic efficacy of suppository in Process 1 on rat model with colpitis induced by phenol-mucilage.

Key words: suppository; colpitis, Jirug 6, erosion; pathomorphological changes

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СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ПАТОМОРФОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ ПОД ДЕЙСТВИЕМ СУППОЗИТОРИЕВ JIRUG-6 ПРИ СЛИЗИСТОМ ВАГИНАЛЬНОМ КОЛЬПИТЕ У КРЫС, ВЫЗВАННОМ ФЕНОЛОМ

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РЕЗЮМЕ

Введение. Суппозитории Jirug-6 входят в состав монгольского традиционного рецепта «Jirug-6», который упоминается в медицинских трактатах «Four medicine code», «Uzegsdiin bayasgalan» и «Erkhem tuuver». Это лекарственное средство обладает противомикробными свойствами, применяется при заживлении ран, детоксикации, лечении боли. В клинике суппозитории используются для лечения вагиноза, лейкореи и других заболеваний. Суппозитории получают из 6 видов растительного сырья: травы ***Nepeta cataria*** L., ***Brassica juncea*** (L.) Czem., ***Embelia ribes*** Pall., ***Achillea asiatica*** Serge., ***Leonurus japonicus*** Houtt. и ***Polygonatum odoratum*** (Mill.) Druce.

Цель исследования – изучить патоморфологические изменения, происходящие на вагинальной ткани при слизистом вагинальном кольпите у крыс под действием суппозиториев Jirug-6.

Материал и методы. Исследования проводили на крысах. Модель кольпита устанавливали ежедневным одноразовым введением 25% фенол-глицериновой слизи в течение 5 дней. Наблюдения проводились в течение 11 дней. Затем крыс умерщвляли и анализировали вагинальную ткань. Патоморфологическую оценку междоузельных повреждений слизистой оболочки влагалища и субсмыслины исследовали под электронным микроскопом.

Результаты. Анализ выявленных гистологических изменений в результате эрозии вагинальной ткани позволил разделить их на 3 группы: процесс 1, процесс 2 и процесс 3. По силе выраженности патологических гистологических изменений они располагались следующим образом: процесс 1> процесс 2> процесс 3. В группах, получавших лечение Jirug 6, состояние тканей было значительно лучше. Самые хорошие результаты отмечены в группе процесс 1.

Заключение. В ходе проведенного исследования показано, что суппозитории Jirug 6 оказывают существенный терапевтический эффект при лечении слизистого вагинального кольпита у животных.

Ключевые слова: Jirug 6, суппозитории, кольпит, эрозия, патоморфологические изменения.

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Introduction

Nowadays, colpitis is the most common disease of gynecology practice, many types of pathogens, easily recrudescence after treatment and serious harm to women's reproductive health [1].

In western medicine, colpitis is characterized by inflammation of the mucous membrane of the vagina, which develops the penetration from different type of infectious agents, as well as chemical or mechanical traumatic effects [2].

Pathogenesis of the disease: Occurrence of oxidative stress and vaginitis cause Th1/Th2 immune dysfunction and virulence of enhanced strains, secreted aspartic protease (SAP) of expression [3]. In Mongolian traditional medicine, colpitis is caused by non-compliance with the rules of intimate hygiene, which contributes to the development of the disease. It lives in a cool wet place for prolonged time and when getting cold after period or during pregnancy it causes uterine cold. In tools of traditional Mongolian medicine diet, eating cool or frozen foods increases inner wind and develops phlegm disorder and enhances of genital itching, leucorrhea and the smell of vaginal discharge. The principles of treatment are insecticide, reducing the wind and phlegm [4].

Jirug-6 suppository is isolated from Mongolian traditional prescription Jirug-6, which mentioned in medicinal textbook «Four medicine code», «Uzegsdiinbayasgalan» and «Erkhemtuuver». It consists of six raw materials such as aerial part of *Nepeta cataria* L, *Brassica juncea* (L) Czem., *Embelia ribes* Pall,, *Achillea asiatica* Serge., *Leonurus japonicus* Houtt. and *Polygonatum odoratum* (Mill.) Druce [5].

Jirug-6 has heat clearing, wound healing, detoxifying, treating pain and antimicrobial properties. It is also clinically used to vaginosis, leukorrhea and other diseases [6].

Objective. To study the pathomorphological changes of Jirug-6 suppository on the vaginal tissue of a rat due to its erosion in chemical experiment.

Materials

Experimental animals

Female, non-pregnant and healthy SD70 rats with weight of180-260g are used and the experiment is sponsored by Laboratory Animal Experimental Center of Inner Mongolian University.

Drugs and reagents

The Compound Jirug-6, Process 1, Process 2, Process 3 have been provided from Pharmacology

laboratory of Mongolian medicine, atInner Mongolia Medical University.Metronidazole suppository (Batch number: H14022290, Shanxi Taiyuan Pharmaceutical Co., Ltd). Reagents: Phenol (Batch number: 2011010 Tianjin Yongda Chemical Reagent Co., Ltd); Glycerol (Batch number: 20131026); West astragalus (Batch number: 20150510).

Equipment

Leica automated vacuum tissue processor ASP300; slicer: RM2235, RM2245, microscope: OLYMPUSBX53, Immuno-histochemical staining: Leica DM1000, DM2500, Leica BOND-MAX, embedding station: Hubei Xiaogan YB-6LE, Automated slide strainers: Leica Auto-Stainer XL.

Study methodology (7)

Use 1 ml of disposable syringe injection (0.1ml/100g) with a phenol-mucilage (Stylized 25% phenol-mucilage is a part of 5 ml phenol, 1 g west astragalus,4 ml glycerin and added 20 ml of distilled water). Inject slightly once a day, into the vagina1–1.5 cm deep for 5 days.

After 4 days of administration, rats had white and yellowish vaginal discharge that external genitalia were swollen and reddened and vaginal cavity were congested. Then it was repeated one more time. All animals were housed in an environmentally controlled room at 25°C in a 12h light-dark cycles and were given free access to food and water during treatment period.

Animal grouping and the treatment

70 rats were randomly divided into six groups, and then 10 rats were chosen into Non-treatment group. The Non-treatment group and modeling group received water and food. Jirug-6 (doses were previously determined by pharmacological study), suppository group: 525 $mg.kg^{-1}$; Process-1 group suppository: 107 $mg.kg^{-1}$; Process-2 group suppository: 162 $mg.kg^{-1}$; Process-3 group suppository: 167 $mg.kg^{-1}$; Control group: 420 $mg.kg^{-1}$.

In Jirug-6 suppository group, used 1ml of disposable syringe injection of drug preparation into rat's vaginal cavity and washed out. Other treatment group received suppository treatment which suppository put deep into the vaginal cavity of rat, once a day for 10 days. (Calculation of dosage: According to the weight of equivalent dose, rat dose is equivalent to human dose by 6.3 times.)

Material collection and detection

After 10 days of treatment, the rats were sacrificed. Sample tissues were taken from edge of vagina and uterus of rats, then immersion-fixed in formalin which is randomly used HE (hematoxylin-eosin staining) and is performed histopathological examination.

Evaluation score and result

Evaluation of Pathological score [8]

a) *Standards core of necrosis of mucosal epithelial layer:* Score 0: Mucosal epithelial no obvious cell shedding, ulcer and necrosis. Score 1: Mild ulcers, erosion and necrosis. Score 2: obvious ulcers, erosion and necrosis. Score 3: Extreme degenerative change, necrosis, erosion.

b) *Standard score of epithelial cell and epithelium ICI (Inflammatory cellular infiltration):* Score 0: epithelial cell and epithelium no obvious ICI. Score 1: mild inflammatory cell infiltration. Score 2: obvious ICI. Score 3: seen extremely large amountof ICI.

c) *Standards core of inflammatory exudates: Score 0:* no obvious inflammatory exudates outflow. Score 1: seen small amount of inflammatory exudates outflow. Score 2: seen large amount of inflammatory exudates outflow, no abscess formation. Score 3: seen extremely large amount of inflammatory exudates outflow and necrosis in shedding, has abscess formation.

d) *Standard score of vasodilatation and congestion:* Score 0: no obvious vasodilatation and congestion in sub-mucosal layer. Score 1: seen small amount of vasodilatation and congestion in sub-mucosal layer. Score 2: seen obvious vasodilatation and congestion in sub-mucosal layer. Score 3: seen many hyperplasias of blood vessels, obvious seen vascular dilation (Table 1).

Statistical analysis. For the statistical analysis was used Student t criteria and the standard deviation among experimental units used to the planned experimental design and specified for quantitative characters.

Study results

a) *Non-treatment group:* Histopathological changes in vaginaltissue of the rat: Vaginal mucosa is non-keratinized, flattened epithelium which epithelium has comparatively thickness of connective tissue and elastic fibers which is seen a lot of blood vessels in epithelial, myometrium consists of smooth muscle cells (Figures 1, 2).

b) *Modeling group:* hyperplasia of vaginal mucosa, cell layer has increased and surface epithelium has keratinized, epithelium-off, seen epithelial ICI in intra-epithelium, inflammatory exudates and discharge in vaginal cavity, vascular congestion in sub-epithelial, epithelial ICI, interstitial edema in layer of smooth muscle (Figure 2).

Table 1

Score of histopathology changes (n=10)

Pathological changes	Non-treatment group	Modeling group	Jirug-6	Process 1	Process 2	Process 3	Positive group
Necrosis of mucosal epithelial layer	0	3	1	0	1	0	1
Epithelial cell ICI	0	2	1	0	0	1	1
Epithelium ICI	0	3	1	1	1	1	1
Inflammatory exudate	1	2	1	1	1	0	1
Vasodilation & congestion	0	2	0	0	0	0	0
Total score	1	12	4	2	3	2	4
Statistical analysis	P≥0,005	P≥0,005	P≥0,01	P≥0,01	P≥0,01	P≥0,05	P≥0,01

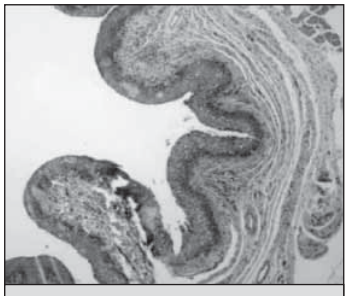

Figure 1. Non-treatment control group – The rat vaginal histology

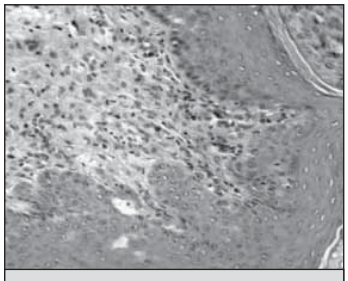

Figure 2. Modeling group – The rat vaginal histology

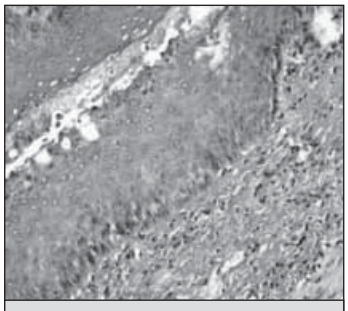

Figure 3. Process 1 group – The rat vaginal tissue

c) *Process group:* hyperplasia of vaginal mucosa repairs almost complete vaginal mucosa. Epithelium has hyperplasia, inflammatory exudates in vaginal cavity which inflammatory cells decreased to disappearing. The histogram shows that, the inflammatory cells disappearing in intra-epithelial, vascular congestions disappearing in sub-epithelial and hyperplasia of interstitial fibrous tissue and no obvious epithelial ICI. Path-morphology changes in expression of three groups by ratios with histology slices: Process 1>Process 2>Process 3 (Figures 3, 4, 5).

Jirug-6 suppository group: almost completed vaginal mucosa, thickening of epithelium persists, no obvious epithelial ICI (Inflammatory cellular infiltration) in intra-epithelial and sub-epithelial, vascular congestions disappearing, hyperplasia of interstitial fibrous tissue, inflammatory exudates in vaginal cavity, inflammatory cells have been obviously decreased (Figure 6).

Positive group (Metronidazole suppository): almost completed vaginal mucosa, thickening of epithelium persists, no obvious epithelial ICI (Inflammatory cellular infiltration) in intra-epithelial and sub-epithelial, vascular congestion disappearing hyperplasia of interstitial fibrous tissue, inflammatory exudates in vaginal cavity, inflammatory cells have been obvious decreased (Figure 7).

Discussion

After 10 days of treatment, the research results shows that, Suppository Jirug-6 group, Process 1, Process 2, Process 3 group, Control group and Positive group have the significant treatment effects to phenol-mucilage induced chemical erosion. The researchers Wanying Zhao, Shiqiong Luo were used same technological process and same chemical inducer in their experimental treatment, but had a lower treatment effect on necrosis of mucosal epithelial layer, probably with a small content of flavonoids with antiimplammatory activity [1,3].

In the Modeling group, macroscopic observation shows obvious white and yellowish vaginal discharge that the external genitalia were swollen and reddened and the vaginal cavity was congested in the vagina of rat. This result was same Zhenkun Tian and others, were used 25% phenol-mucilage as an inducer of colpitis and observed by a microscopy [3].

Process 1 suppository group and Process 3 suppository group has the results showing less vaginal discharge that the external genitalia were swollen or reddened and congestion in the vaginal cavity is better than the other groups [7].

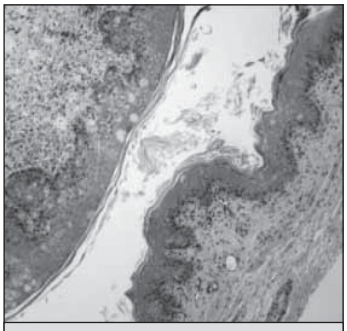

Figure 4. Process 2 group – The rat vaginal tissue

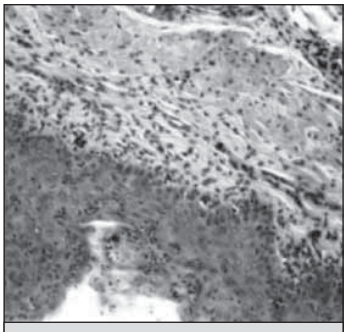

Figure 5. Process 3 group – The rat vaginal tissue

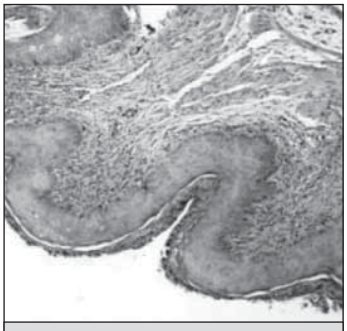

Figure 6. Jirug-6 suppository group – The rat vaginal tissue

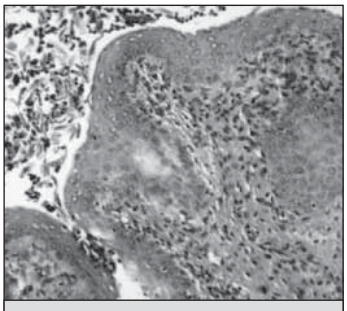

Figure 7. Positive group – The rat vaginal tissue

The results of Process 2 suppository group, Positive suppository group and Jirug-6 suppository group shows the reduced vaginal discharge, reddened external genitalia and no congestion in the vaginal cavity [8].

For the future study, we have planned to investigate the estrogen depending leucorrhea treatment activity of Jirug-6 suppository on the experimental animals.

Conclusion

Comparative analysis of pathological score shows, the Process 1 suppository group and Process 3 suppository group had same scores and have more significant effect than the other treatment group.

Histopathological comparison result shows that the administration of Process 1, Process 2, and Process 3 suppository group hassignificant therapeutic effect to phenol-mucilage induced chemical erosion of inflammation the vaginal mucosa in the pathological changes. As the analysis from pathology to histopathology, the three process groups were comparative significantly. Process 1 suppository group has higher therapeutic effect than the other Process 2 and Process 3 suppository groups. According to above mentioned results: the best optimal process condition is Process 1.

Conflict of Interest

The authors state No conflict of interest.

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