

A suppository for treating cervical erosion and its preparation method

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Summary

Objective: To study a suppository for treating cervical erosion, its preparation method, and to observe its therapeutic effect on cervical erosion in animal models. **Methods:** Twenty rats were divided into five groups: blank control group, model group, and three different therapeutic concentration groups. Phenol slurry was injected through the vagina to create cervical erosion animal models. After seven days of drug treatment, the difference of cervical histopathology was observed and compared between different groups. **Results:** Compared with the control group, the model group showed obvious cervical erosion, inflammatory cell infiltration, vascular dilatation, and congestion. Among the administration group, the high administration group had the least inflammation and cell infiltration in the mucous membrane. **Conclusions:** There is a significant effect of therapeutic drugs in rats with cervical erosion, which is related to the concentration of drugs.

Key words: Double-layer suppository; Cervical erosion; Rats; Pathological changes.

Introduction

Cervical erosion is one of the most common diseases in women, which has a certain relationship with the occurrence of cervical cancer [1]. Cervical erosion is a condition in which the endocervical columnar epithelium protrudes through the external os of the cervix and into the vaginal portion of the cervix, undergoes squamous metaplasia, and transforms into stratified squamous epithelium. Squamous epithelium is covered by columnar epithelium, resulting in a lower resistance to disease because the former is thinner [2].

The most common drug treatment for cervical erosion is a suppository. With the study of suppositories, the double-layer suppository has been shown to be helpful in the treatment of cervical erosion. The suppository consists of two layers, with each layer containing different drugs that successively take effect [3].

Basic fibroblast growth factor (bFGF) is the first clinical growth factor and it can significantly accelerate the formation of granulation tissue and re-epithelialization [4, 5]. Studies have shown that a variety of growth factors gather in local wounds [6], however they are unstable, especially in refractory ulcer wounds, where the expression of endogenous bFGF is very little. Exogenous bFGF can improve the amount of other growth factors, such as epidermal growth factor (EGF) and transforming growth factor (TGF) when it plays the role in the healing of wounds [7].

Combined with the characteristics of the suppository and drugs, curcuma, borneol, and bFGF can successively take effect with the purpose of extending the duration of action, improving therapeutic effects, and reducing side-effects.

The aim of this study was to prepare a suppository to treat cervical erosion. The three-layer suppository was applied to facilitate successful drug uptake and was used in cervical erosion animal models. The effect of the suppository was measured according to the histopathology of the model groups.

Materials and Methods

Experimental preparation

Twenty clean grade Sprague Dawley (SD) female rats purchased from the Experimental Animal department in Wenzhou Medical College were used with a weight of 150 g to 200 g. The protocols used were consistent with Ethical Principles for Animal Research adopted by the Wenzhou Medical College of Animal Experimentation.

Experimental content: preparation of suppository

This suppository is a three-layer suppository based on hollow double-layer suppository mold: the inner layer is medical absorbent cotton, the middle layer is a gelatin glycerol sustained-release layer with bFGF as the primary therapeutic drug, and the outer layer contains borneol and acts as the sterilisation layer (Figures 1 and 2).

To make the outer layer: 20 g of semi-mixed fatty acid glycerol was fragmented and melted by 70°C water bath heating, before maintaining the temperature at 50°C. Curcuma and borneol were ground into a fine powder, and one g of each was mixed with a suppository base before adding it to the suppository mold. The latter was then inserted before solidification and natural cooling.

To make the middle layer: 20 g of gelatin, glycerol, and distilled water were taken in a ratio of 6:3:1 (gelatin: glycerol: distilled water). These were melted by 75°C water bath heating, before mixing with one g of curcuma and adding them to the suppository mold. The temperature was maintained at 40°C and

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bFGF of a corresponding amount was added prior to natural cooling.

To make the inner layer: the medical absorbent cotton was formed into a cylinder and placed into the mold.

Experimental methods of animals: animal experiments

Thirty ml of liquid phenol, 40 g of arabic gum, and 50 ml of distilled water were taken to prepare a phenol slurry (w = 37.5%) [5]. Phenol slurry (0.2 ml) was injected into the rat vagina via a stomach tube (injected at different times, 0.1 ml twice), once/3d, and four times. After the creation of models, the rats were divided into four groups with four rats randomly assigned to each group, including a model group, and an administration group. The blank group was given a similar volume of distilled water. Three days after modeling, the administration groups was given the corresponding amount of drugs (divided into three groups of low grade according to the difference in the density, with a content of bFGF of 100 μ g, 10 μ g, and 1 μ g, respectively). The animals were treated daily for seven days, and were then sacrificed on day eight. The uterine tissue was dissected observed, and information was recorded. The tissue was fixed in formalin and the samples were sliced into paraffin sections, before being stained with Hematoxylin and Eosin (H&E). Light microscopic observation was then performed.

Specimen collection and processing occurred at 24 hours after the last administration when all rats were placed under intraperitoneal anesthesia using chloral hydrate (three ml/kg). The rats were then fixed and the uterine tissue was extracted after dissection. The tissue was fixed in 10% formalin for three days, then placed into an ethyl alcohol series, embedded in paraffin, sectioned at six μ m and stained with H&E, before being observed by microscopy and photographed.

From the day of the modeling, the vaginal and cervical secretions of the rats were observed, indicating whether there was less activity or piloerection [8].

Histopathological changes, namely in the squamous cells were classified as either partially thickened (more than two to three layers); obviously thickened; wildly thickened or unsmooth mucosa [9].

Columnar epithelium translocation was classified according to the following criteria: few regional squamous epithelial were erosive, adjacent columnar epithelial were proliferative; columnar epithelial were obviously proliferative and replaced the squamous epithelial; and squamous epithelial shed.

Inflammatory cell infiltration was classified according to the following criteria: a small amount of inflammatory cell infiltration in the mucous membrane; a moderate amount of inflammatory cell infiltration in the submucosal interstitium; or a vast array of inflammatory cell infiltration in the intestinal mucous membrane.

Results

The preparatory procedure finally resulted in a suppository with three layers (Figure 3). Transfiguration temperature for the outer layer was completely melted within 20 min, and the middle layer was completely melted within 50 min.

During the entire experiment, the weight variations of the animals were recorded and are shown in Table 1. After the cervical erosion animal model establishment, the conditions of the exterior entrance of the vagina were observed (Figure 4). Most of the rats had symptoms of

Table 1. — *Determination of weight variation.*

	1	2	3	4	5	6	7
Weight (g)	1.6364	1.5518	1.5949	1.6315	1.6013	1.5965	1.5805
Average weight (g)	1.5972						

vaginal swelling after model establishment and some of them showed symptoms of purulence. At the same time, they had other symptoms, such as less activity, piloerection, loss of appetite, and weight loss. After a period of therapy, the vaginal secretions of administration group gradually reduced, and four days after administration, the exterior entrance of the vagina of the administration group was basically dry (Figure 5). There was no difference seen in the appearance of the blank group.

After the uterine tissue was extracted, it was morphologically observed. Vascular congestion and edema were observed in the model group. There was some degree of improvement in the administration group (Figure 6).

By using light microscopy to observe H&E uterine tissue sections, interstitial edema, inflammatory infiltration, and squamous hyperplasia were seen in the model group. Compared with the model group, the blank group had no obvious symptoms. Compared with the high administration group, the degree of squamous epithelial thickening of the low administration group was greater. Among the administration group, the low administration group had the most inflammatory cell infiltration in the mucous membrane (Figure 7).

Discussions

Cervical erosion is a common gynecological disease which is difficult to treat. Current treatment methods are of poor efficacy, with long-lasting treatment cycles, so patients do not easily continue the medication. These factors all render cervical erosion difficult to cure, but a hollow triple-layer suppository can overcome these difficulties. The drug of the outer layer releases first, to achieve the purposes of sterilization, then the inner drug is released; therefore, this can extend the duration of its action.

This experiment used a phenol glue solution to establish the model of rat cervical erosion, in a manner that was simple and economic. After modeling, most of the rats showed symptoms of vaginal swelling, and some of them had symptoms of purulence. At the same time, they showed symptoms such as less activity, piloerection, loss of appetite, and weight loss. After a period of therapy, the vaginal secretions of the administration group gradually reduced, and after administration, the exterior entrance of the vagina of the administration group was basically dry, with no difference compared to the blank group in appearance. The clinical symptoms, physical signs and pathological changes were basically in accordance with the clinical states, therefore providing a scientific way to study drugs.

In the model group, conditions presented included interstitial edema, inflammatory infiltration, and squamous hyperplasia. Compared with the model group, the blank group

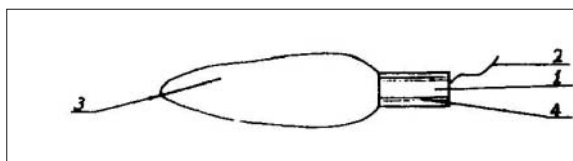


Figure 1. — Front view of the suppository.

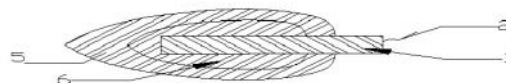


Figure 2. — Sectional view of the suppository.



Fig. 3



Fig. 4



Fig. 5

Figure 3. — The final product of the suppository.

Figure 4. — The conditions of the exterior entrance of the vagina after model establishment. The typical symptoms, such as vaginal swelling, are shown and some of the rats had symptoms of purulence.

Figure 5. — The swelling situation improved after the use of the suppository.

showed no obvious symptoms. Compared with the high administration group, the degree of squamous epithelial thickening of the low administration group was greater. In comparison with the administration group, the low admin-

istration group had the most inflammatory cell infiltration in the mucous membrane. Therefore, the suppository was useful in the treatment of cervical erosion and was related to the concentration of the drugs.

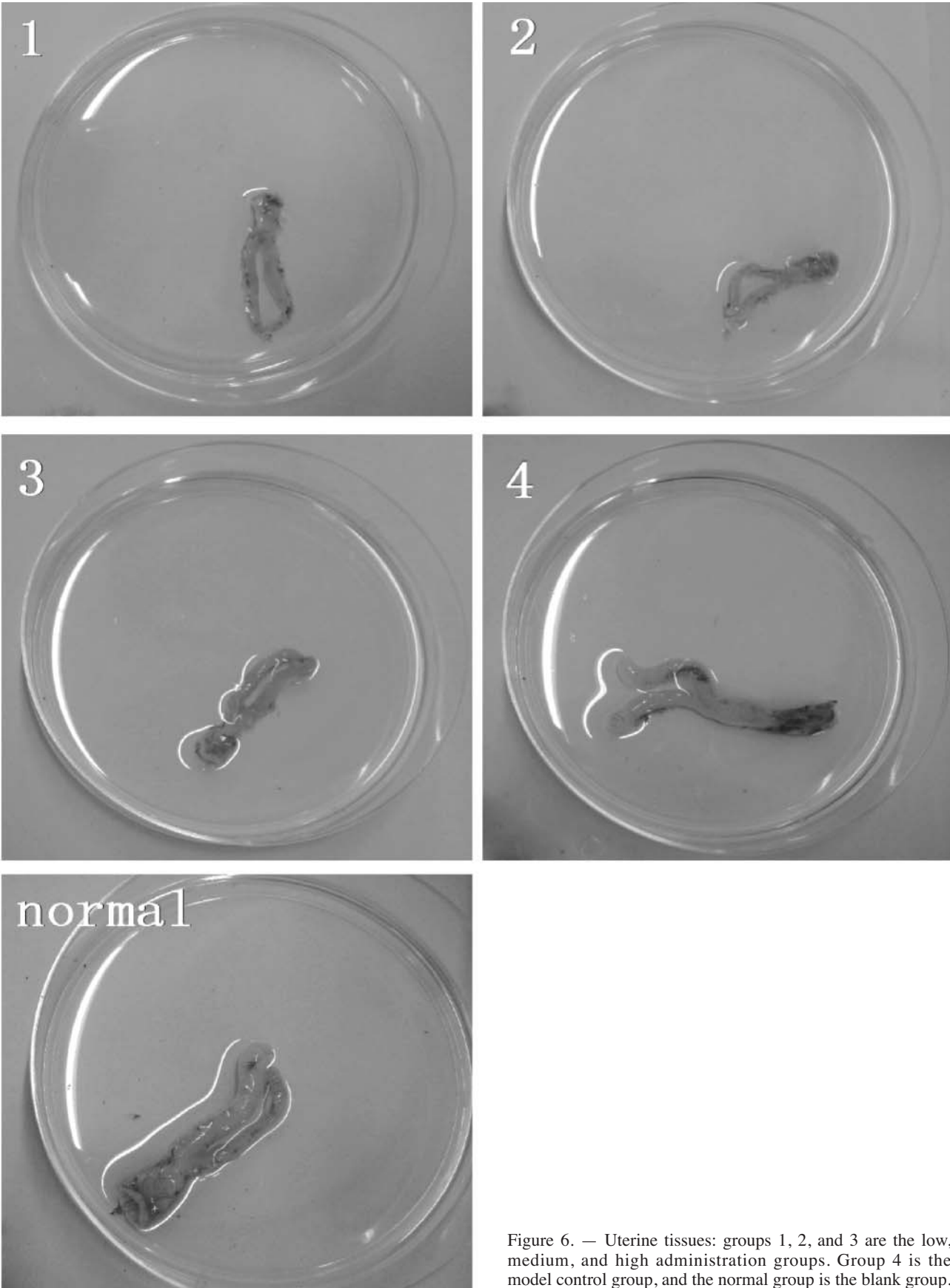


Figure 6. — Uterine tissues: groups 1, 2, and 3 are the low, medium, and high administration groups. Group 4 is the model control group, and the normal group is the blank group.

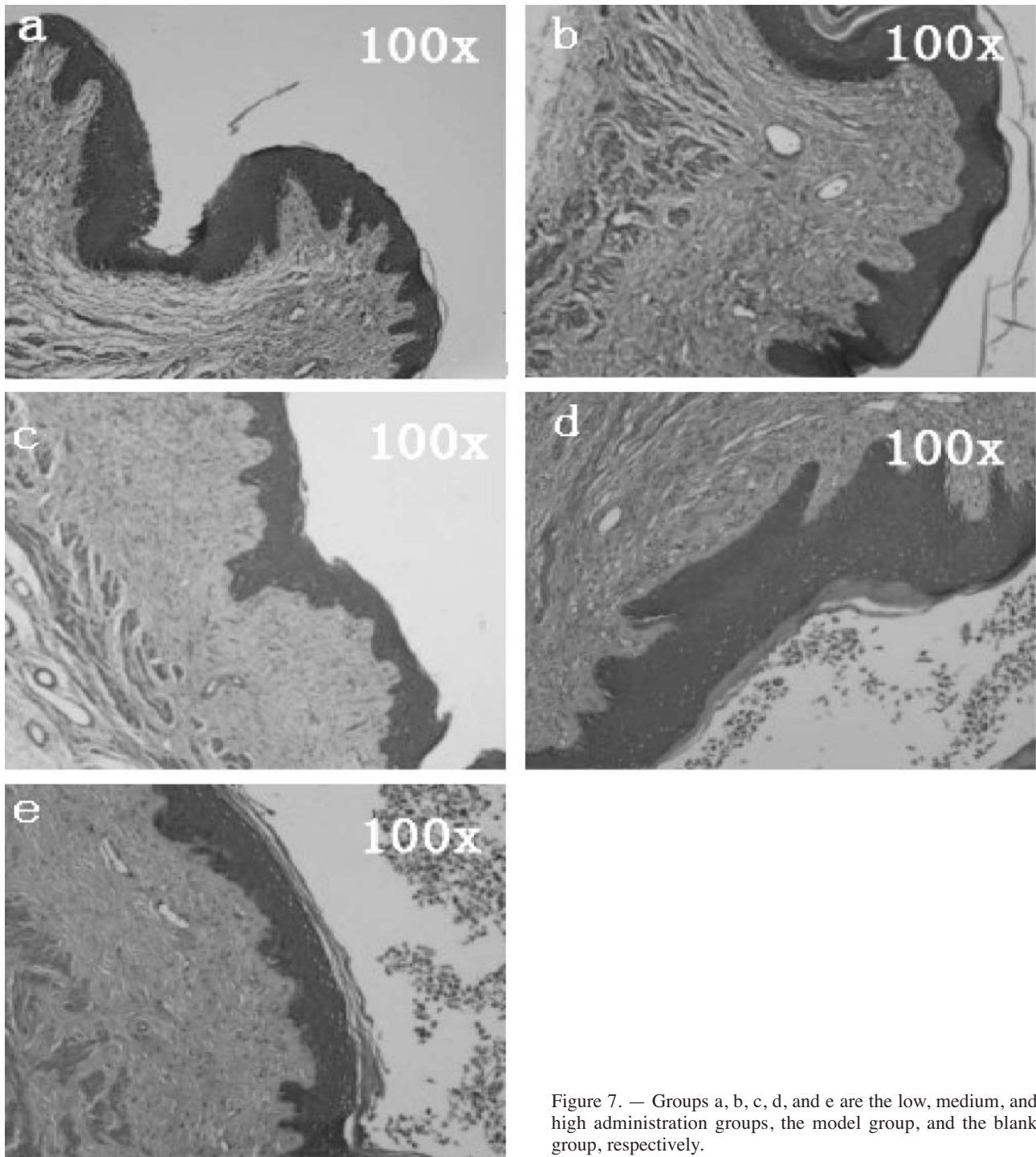


Figure 7. — Groups a, b, c, d, and e are the low, medium, and high administration groups, the model group, and the blank group, respectively.

In conclusion, the three-layer suppository had a significant therapeutic effect on rats with cervical erosion, which was related to the concentration of the drugs: the higher the concentration, the better the therapeutic effects.

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