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ORIGINAL ARTICLE

A thermosensitive gel formulation of an empirical traditional Chinese prescription for treating cervical erosion

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KEY WORDS

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Rhizoma Coptidis;
Olibanum;
Myrrha;

Abstract Cervical erosion, a pathological change associated with chronic cervicitis, is a common condition that is difficult to cure. Many patients particularly those with mild or medium infection and those preparing for pregnancy require a simple but effective medication. In this study, extracts of an empirical herbal prescription composed of six Chinese traditional drugs *viz* Cortex Phellodendri, Rhizoma Coptidis, Olibanum, Myrrha, borneol and catechu were formulated to facilitate intravaginal administration and improve efficacy. An extract of the first four components was formulated with borneol as a thermosensitive gel (TG) while an extract of catechu used to prepare a regular gel (CG) because of a chemical incompatibility. The optimized TG was prepared using poloxamer 407 and poloxamer 188. The CG was prepared using glycerin, carbopol and triethanolamine. The gels were characterized *in vitro* in terms of release of berberine (TG) and total catechins (CG) and *in vivo* in a rat model of cervical erosion. Treatment by once daily application of the TG for 7 days followed by once daily application of the CG for 3 days produced a

Abbreviations: TG, thermosensitive gel; CG, catechu gel; HPLC, high performance liquid chromatography; TCM, traditional Chinese medicine; PF-407, Poloxamer 407; PF-188, poloxamer 188

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Borneol;
catechu

restoration of normal tissues. Gel formulation of the empirical Chinese traditional remedy appears to provide a promising treatment for cervical erosion.

1. Introduction

Cervical erosion (CE), also known as cervical ectropion, is a common condition among married women and is considered to be a risk factor for cervical carcinoma[1–4]. The erosion is a pathological change associated with chronic cervicitis, the cause of which is complex and yet to be elucidated[5]. It is characterized by an epithelial defect consisting of a zone of columnar epithelium on the vaginal portion of the cervix in place of stratified squamous epithelium normally found below the external cervical os. The perception that CE reduces the ability to conceive may explain why the condition is reported in up to 52% of patients with infertility[6]. The incidence of cervical erosion is also higher in pregnant women[7]. Thus a safe and effective medication to treat CE is needed.

CE is difficult to cure and, although many treatments such as laser ablation, cryosurgery and high frequency electricity have been applied, they often lead to new scarring and a higher recurrence rate. Song and Yu[8] compared microwave tissue coagulation and CO_2 laser treatment and found that microwave tissue coagulation gave a higher cure rate with fewer complications. However, prolonged treatment for 4–8 weeks was required and an aqueous discharge occurred within 2–3 days of the operation[9]. As a result, many patients would benefit from an effective medication particularly those with mild symptomology.

In China, traditional Chinese medicine (TCM) is commonly prescribed to treat CE in both pregnant women and those with mild symptoms[10]. Many TCM preparations have been used, including vaginal suppositories of Zhimiling (catechu, Sophorae Flavescentis Radix, borneol, Calcined Alum, Cortex Phellodendri), Kushen (matrine) and Baofukang (Curcuma aromatic oils, borneol) as well as Spray of Watermelon Frost. Of these, Zhimiling Suppositories have proven to be the most effective[11]. However, an empirical formulation consisting of six Chinese herbs *viz* Cortex Phellodendri, Rhizoma Coptidis, Olibanum, Myrrha, borneol and catechu has been reported to be very effective. Of the six herbs, all but catechu have antiinflammatory properties[12–15], Cortex Phellodendri and Rhizoma Coptidis have wide spectrum antibiotic activity[16], Olibanum and Myrrha improve blood circulation and promote tissue regeneration[17] and catechin may play a protective role against cervical cancer[18] and promote tissue regeneration[19]. Borneol increases the partitioning coefficient to the *stratum corneum*[20] and play its special promotion role in the composite formulae. This mixture was chosen as the basis for a gel formulation in this study.

A thermosensitive hydrogel (TG) is in the liquid state at room temperature but converts to a gel at body temperature (37 °C). It is usually made from temperature-sensitive polymers including cellulose derivatives, polysaccharides, poly(*N*-isopropylacrylamide) and a copolymer of polyoxyethylene and polyoxypropylene. Of particular value are chitosan[21] and poloxamer[22]. In this study, TGs were prepared and evaluated in terms of their ability to release berberine, the main ingredient contained in the herbal mixture. The pharmacodynamics of the TGs was also studied in a rat model of CE.

2. Materials and methods

2.1. Materials

Berberine hydrochloride standard was purchased from the National Institute for Control of Pharmaceutical and Biological Products, China. The six TCMs (Cortex Phellodendri, Rhizoma Coptidis, Olibanum, Myrrha, catechu and borneol) were obtained from a Hangzhou pharmaceutical store. The TCMs were identified by Prof. Juanhua Xu and shown to meet the standards of the Chinese Pharmacopoeia 2010. Chitosan (91.3% deacetylation, MW 950 KDa) was obtained from Xinke Co. (Dalian, China). Poloxamer 407 (PF-407) and poloxamer 188 (PF-188) were purchased from Sigma-Aldrich Chemical Co. (USA). Acetonitrile was of HPLC grade and water was ultrapure. All other reagents were of analytical grade and supplied by Huadong Medical Company (China). Zhimiling Suppositories were obtained from Tonghua Golden-Horse Pharmaceutical Industry Co. (Jilin, China).

2.2. Optimization of extraction procedure

The traditional method of boiling with water was employed to extract the active components from the traditional drugs except for catechu and borneol. Cortex Phellodendri 10 g, Rhizoma Coptidis 20 g, Olibanum 15 g and Myrrha 15 g were placed in a beaker followed by 250 mL water. After soaking for 60 min and boiling for 15 min, the supernatant was removed after which 350 mL water was added and the mixture boiled for 30 min. The extracts were combined and a third extraction step optimized by orthogonal experimental design[23]. The extraction of berberine hydrochloride was selected as the evaluation index of the extraction procedure in terms of soaking time, volume of water and decoction time. Each factor had three levels and an L9 (3^4) orthogonal was applied to optimize the extraction process (Table 1). The supernatant obtained from 3 times boiling was combined for concentration.

2.3. Preparation of TGs

Chitosan and poloxamer were selected to prepare TGs in this study using the extract as prepared above together with borneol and the carriers. Due to chemical incompatibility, a catechu gel (CG) was prepared separately.

Table 1 Parameters and levels of the extraction condition.

Level	Parameter		
	Soaking time (A, min)	Volume of water (B, fold)	Decoction time (C, h)
1	15	10	1.5
2	30	12	2.0
3	60	14	2.5

2.3.1. Preparation of chitosan TG

Chitosan (100 mg) was dissolved in 5 g 1% acetic acid in a glass tube (diameter 1.4 cm) and 1.5 g glycerin was added. Then a 10% borax solution was added dropwise to the solution until the pH was about 7.1. The solution was then incubated in a water bath at 37 °C during which a gel formed within 1.5 min.

2.3.2. Preparation of poloxamer TG

The extract obtained in Section 2.2 was filtered and further concentrated to about 50 mL by heating at 70–80 °C. PF-407 (18 g) and PF-188 (3 g) were then added and the mixture stored overnight at 4 °C to allow the polymers to swell. Borneol (0.08 g) was dissolved in 2 mL alcohol and added slowly to the solution after which water was added with stirring to a total amount of 100 g to form the gel.

2.4. Determination of berberine content

The *in vitro* release of berberine from the gel was determined by placing it in saline and analyzing the saline for berberine content. This was done by HPLC on a C18 column (250 mm × 4.6 mm, 5 μm) using a mobile phase of acetonitrile-2% acetic acid:0.2% triethanolamine (35:65) at a flow rate of 1.0 mL/min. The detection wavelength was 347 nm, the column temperature 35 °C and the injection volume 20 μL. To analyze the berberine content of TGs, about 1.0 g TG was weighed accurately, dissolved in 100 mL distilled water and the solution diluted 100 times with mobile phase before injection into the HPLC system.

2.5. Rheological properties of TGs

The gelation temperature, sol–gel transition, viscosity and erosion of TGs made using different concentrations of PF-407 and PF-188 were investigated. The gelation temperature was determined by the tube-reverse method and erosion was determined by the membrane-free method.

2.6. Preparation of CG

An extract of catechu (20 g) was prepared by ultrasonication with 240 mL 50% alcohol for 35 min at 60 °C. After concentrating the extract to about 45 mL, glycerin (5.0 g) and carbopol (0.5 g) were added. After adequate swelling of the polymers, triethanolamine (0.5 mL) was added slowly to the solution during which the pH reached a value of 5.0. The CG was obtained by adding water to the solution to a total amount of 50 g.

2.7. In vitro release from gels

A TG solution (5 g) at 4 °C was placed in a 5 mL glass test tube and warmed to 37 °C. After the solution became a gel, 2.0 mL saline was carefully added and the tube shaken at 100 rpm. For CG, same quantity of saline was directly put into tube. The release medium was replaced every 0.5 h (TG) or 1 h (CG) and the berberine or total catechin content was analyzed.

2.8. Animal study

All animal experiments were approved by the Animal Care and Use Committee of Zhejiang University. Forty female Sprague–Dawley rats (weight 180–220 g) were obtained from the Academy of Medical Science, Zhejiang Province (certificate number SCXK (Zhejiang) 2008-0033). Animals were housed in individual cages under a 12 h light-dark cycle at 22 ± 2 °C and humidity 55–60% with free access to complete formula granulated nutrition feed and drinking water.

The rat model of CE has been previously described[24–26]. Briefly, rats were fixed upside down and 0.15 mL 25% phenol plasmagel (containing phenol and arabic gum) injected intravaginally to a depth of about 2 cm. After administration, the *ostium vaginae* was blocked with a tampon and rats kept upside down for 5 min. The treatment was repeated once a day every 3 days (total of four treatments). CE indicated by redness and swelling of the vaginal orifice and a purulent secretion was confirmed by photomicrographs of cervical sections.

Rats were randomly divided into four groups ($n=10$ per group): group A, untreated rats; group B, model control; group C, positive control treated with Zhimiling Suppositories; group D, experimental group treated with TG and CG. Treatement was applied for 10 days; Group A and B rats were given water intravaginally (0.2 mL *per* rat), group C rats were given 0.3 g Zhimiling Suppositories, and group D rats were given 0.2 mL TG for 7 days followed by 0.2 mL CG rat for the subsequent 3 days. The rats were sacrificed on the 11th day and tissues removed from the vagina to uterus subangle and preserved in 10% formaldehyde solution followed by paraffin sectioning (4 μm thick slices). Histopathology was carried out after Haematoxylin Eosin staining.

3. Results

3.1. Determination of berberine

Chromatograms of a berberine standard solution and an extract of gel are shown in Fig. 1. The assay was free of interference and completed in a run time of 8 min. The calibration curve of berberine was linear in the range 10–100 μg/mL with a regression equation $C=0.0267A-0.8801$ ($r^2=0.9997$). The average content of berberine in the TG was 10.5 mg/g.

3.2. Optimization of extraction of herbs

After the first two extractions with boiling water for 15 min and 30 min, the third extraction step was optimized by an orthogonal experimental design. The results (Table 2) of

berberine extraction efficiency show that the relative influence of each factor is in the order B > C > A and that the optimal extraction process is $A_3B_3C_2$, *i.e.*, adding a 14-fold weight of water to the herbs, soaking for 60 min and boiling for 2 h. The density of the concentrated liquid was about 1.060 g/mL and the pH of the gel solution was 5.28.

3.3. Extraction of catechu and determination of catechin

Details of the extraction of catechu and determination of catechin and epicatechin have been published[27]. In brief, the density of the concentrated liquid was 1.022 g/mL and the average content of total catechin was 49.2 mg/g. The final pH of the CG was 5.20.

3.4. Characteristics of the TG

During preliminary experiments, it was established that a chitosan gel does not form at pH < 6.2. Attempts to prepare a chitosan gel were therefore discontinued since the pH could not be adjusted to the ideal pH range for a vaginal gel (4.5–5.5).

The gelation temperature and pH of the TG were very dependent on the concentration of PF-407. A sol–gel transition was achieved at PF-407 > 17%, while the pH of gels could be adjusted to 4.55 ± 0.19, 5.16 ± 0.16 and 5.82 ± 0.18 at PF-407 concentrations of 17%, 18% and 19%, respectively.

Erosion of the TG was found to be a linear process for which weight loss was highly correlated to berberine release. At the usual shear rate of 100 s^{-1}, the viscosity of TG formulations containing different levels of PF-188 changed with increasing temperature (Fig. 2A). First, the solution viscosity positively correlated with the mass fraction of PF-188 but then increased rapidly near the critical micelle temperature of the solution (21 °C for 3% PF-188) and finally negatively correlated with the mass fraction of PF-188 after the gel formed. The shear rate (Fig. 2B) followed the same trend as the viscosity.

The concentrations of P407 and P188 were the major factors influencing gel properties including the gelation temperature, gel viscosity, erosion rate and drug release rate.

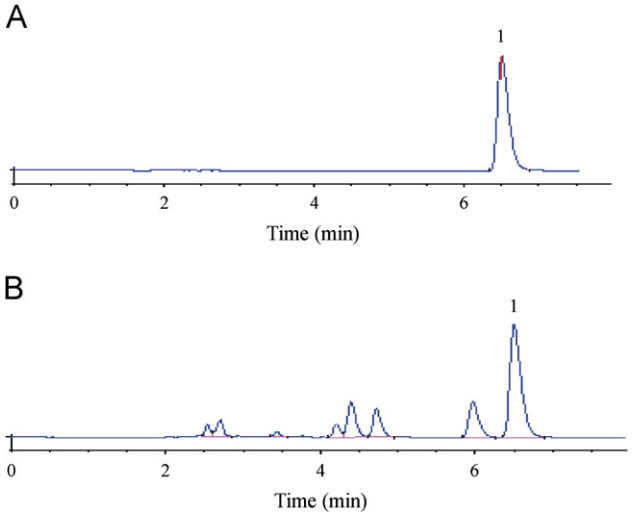

Figure 1 HPLC chromatograms of (A) a standard solution of berberine hydrochloride and (B) an extract of the thermosensitive hydrogel; Peak 1: berberine.

3.5. In vitro release

The release profiles of berberine/catechin are shown in Fig. 3. Release was retarded in the two gels being nearly complete after 5–7 h.

3.6. Animal study

Photomicrographs of the vagina and cervix of rats in the four groups are shown in Fig. 4 and the pathomorphology of formalin-fixed paraffin-embedded vaginal and cervical tissues are shown in Fig. 5. Both the vaginal and cervical tissues of untreated rats (group A) were normal. Characteristics of tissues from animals in groups B, C and D are described below:

Group B: Vagina—the pavement epithelium showed surface defects; the epithelial lamina showed neutrophil infiltration with cell congestion, necrosis and desquamation; the propria lamina showed congestion and neutrophil infiltration; there was no obvious change in the muscular layer. Cervix—squamous cells of

Table 2 Optimized results of extraction condition by orthogonal experimental design.

No.	A	B	C	D	Berberine content (mg)
1	1	1	1	1	192.09
2	1	2	2	2	210.02
3	1	3	3	3	240.23
4	2	1	2	3	237.74
5	2	2	3	1	220.55
6	2	3	1	2	236.86
7	3	1	3	2	240.55
8	3	2	1	3	200.81
9	3	3	2	1	260.66
K_1	214.11	223.46	209.92	224.43	
K_2	231.72	210.46	236.14	229.14	
K_3	234.01	245.92	233.78	226.26	
R_j	19.89	35.46	26.22	4.71	
S_j	710.87	1930.48	1262.21	33.81	
F	21.01	57.06	37.31		

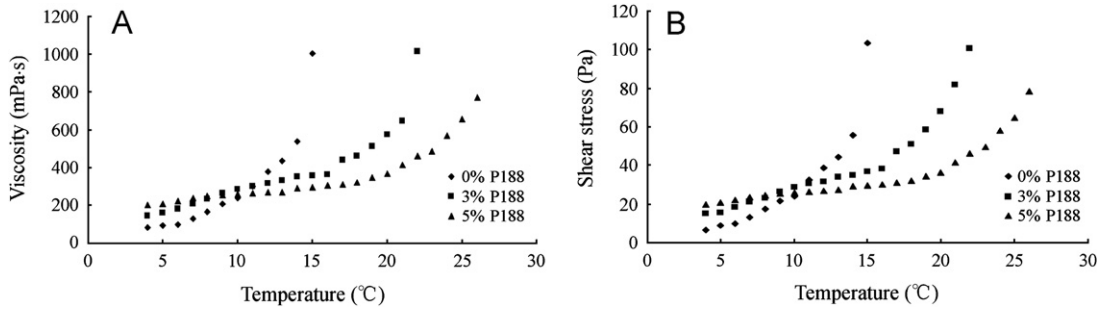

Figure 2 Profiles of (A) viscosity and (B) shear rate of thermosensitive gels made with different concentrations of PF-188 as a function of temperature.

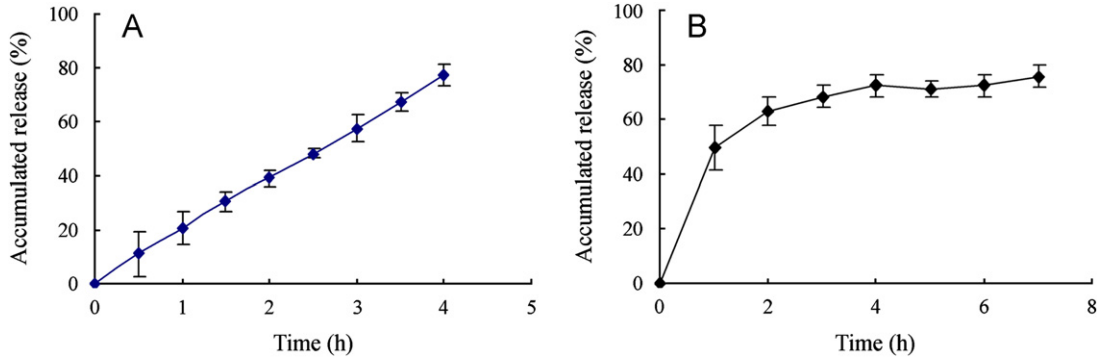

Figure 3 Cumulative release of (A) berberine from the thermosensitve poloxamer gel and (B) catechin from the catechu carbopol gel.

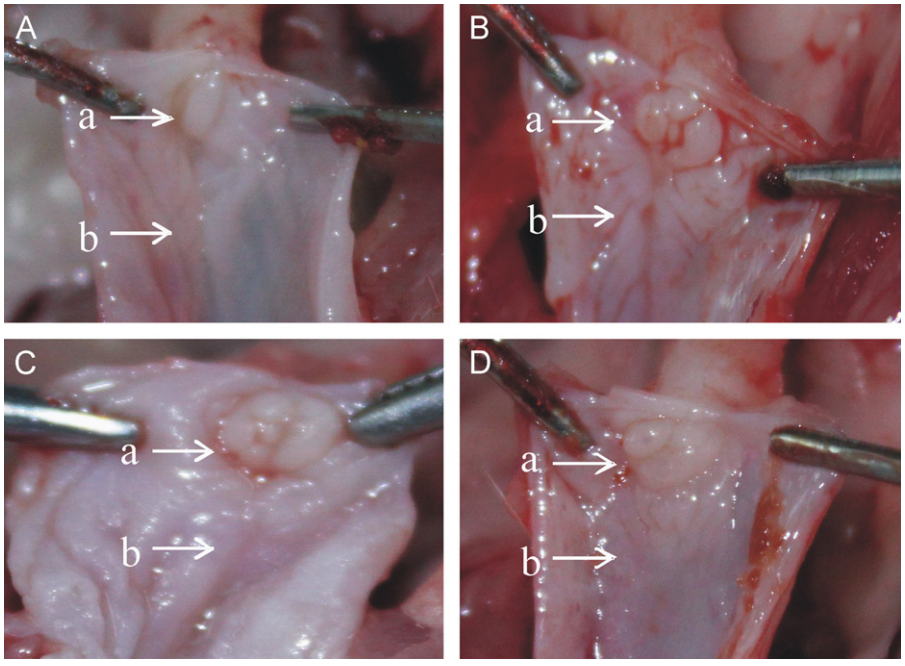

Figure 4 Appearance of (a) the cervical aperture and (b) vagina in individual Sprague–Dawley rats drawn from: (A) the untreated control group; (B) the cervical erosion control group; (C) the positive control group treated with Zhimiling vaginal suppositories; and (D) the cervical erosion group treated with the thermosensitive gel followed by the catechu gel.

cervical mucosa showed degeneration, necrosis and desquamation; the propria lamina showed congestion, dropsy and neutrophil infiltration; the vaginal canal was filled with a purulent effusion and necrotic tissue.

Group C: Vagina—the pavement epithelium was intact with a little thickening; the propria lamina showed obvious neutrophil infiltration; no obvious inflammatory exudate or necrotic tissue was found in the cavea. Cervix—some granulated tissue

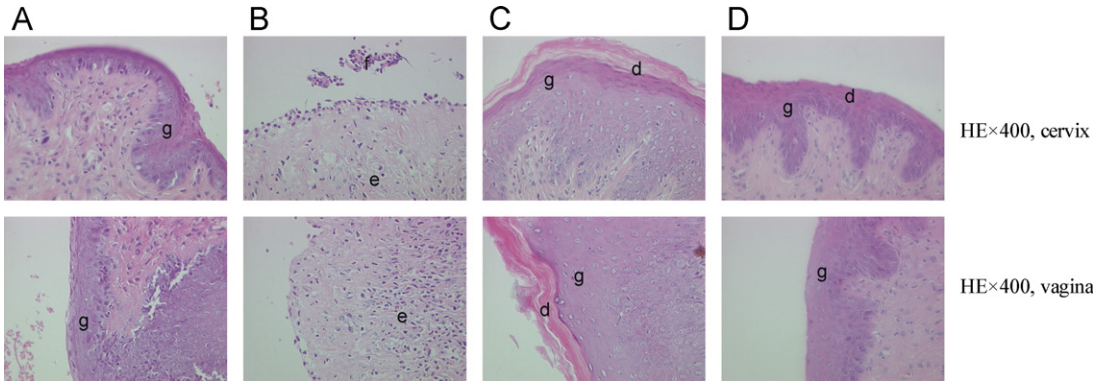

Figure 5 Pathomorphology of formalin-fixed paraffin-embedded cervical and vaginal tissues from rats representative of the four groups showing (d) epithelium cornification (e) inflammatory cells (neutrophil) (f) necrotic tissue and (g) pavement epithelium in: (A) the untreated control group; (B) the cervical erosion control group; (C) the positive control group treated with Zhimiling vaginal suppositories; and (D) the cervical erosion group treated with the thermosensitive gel followed by the catechu gel.

was evident in necrotic cervical mucosa; the mucosal epithelium was not completely repaired with hyperplasia of the propria lamina; no bulk inflammatory and necrotic tissue was found in the cervix; part of the cervical mucosa approached a normal appearance.

Group D: Vagina—the pavement epithelium was largely intact with a little thickening; no obvious neutrophil infiltration occurred in the propria lamina; no inflammatory exudate or necrotic tissue was found in the cavea; most of the vaginal mucosa appeared normal. Cervix—the pavement epithelium of the cervical mucosa was fully repaired although there was still a little thickening; no obvious neutrophil infiltration occurred in the propria lamina; no bulk inflammatory exudate or necrotic tissue was found in the cervix; the cervical mucosa appeared normal.

4. Discussion

Cortex Phellodendri and Rhizoma Coptidis are the principal herbs contributing to the extract used to make the TG and berberine is the main active ingredient. Berberine has been reported to exhibit a variety of pharmacological and biochemical effects[28–30], of which its protective effect against cervical cancer is of most relevance in this study. On this basis, berberine was selected as the index of *in vitro* release of therapeutic constituents from the TG.

Chitosan has many applications in the biomedical field due to its good biocompatibility, biodegradability, and ability to inhibit the growth of a wide variety of fungi, yeasts, and bacteria[31]. In addition, chitosan is a useful surgical hemostatic agent[32]. We wanted to incorporate drug into a chitosan–glycerol pH-dependent TG but found it does not form a gel at $pH < 6.7$[33] as required for intravaginal administration. As a result poloxamer, which forms a controlled release gel at 37 °C, was selected to prepare the TG.

For a sol-to-gel drug delivery system, the drug is incorporated into a polymer solution *in vitro* and a drug-loaded hydrogel then forms *in situ* after administration. Temperature and pH are the main physical and chemical stimuli to hydrogel formation[34]. A sol-to-gel drug delivery system has a number of advantages including ease of preparation, efficient drug encapsulation, near-linear sustained drug release and injectable formulation[35]. The TG used here was particularly useful because of its ability to cover the cervical mucosal surface and provide intimate contact with the active drugs to promote erosion cure. Considering the limited fluid in the microenvironment *in vivo*, only a small amount of liquid was used in the release studies which demonstrated a slow drug release rate. On this basis the formulation is not only useful to treat CE but may also find application as a topical pharmaceutical preparation.

The extract of catechu was chemically incompatible with PF-407 and as a result catechin was extracted individually by sonication with 50% alchohol. Carbopol was selected as the appropriate material to prepare the CG and the viscosity of the extract increased with increasing carbopol concentration (data not shown). From a practical point of view, 1% carbopol was found to be optimal.

The results of the pathomorphological study show that the animal model of CE shows clear damage to both the vagina and cervix including necrosis of the epithelium and mucosa and bulk inflammatory exudates in the cavea. It also shows that repeated treatment with the TG and subsequently the CG produces a return to nearly normal tissues. Therefore, the gel remedies and the treatment protocol used here provide a promising means for treating cervical erosion.

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References

1. Shi SE. Clinical effect of Aoping suppository on cervical erosion *J Pract Obstet Gynecol* 1999;**15**:144.
2. Oran NT, Can HO, Senuzun F, Aylaz RD. Health promotion lifestyle and cancer screening behavior: a survey among academician women. *Asian Pac J Cancer Prev* 2008;**9**:515–8.
3. Zhang JH. Study on the cervical lesion screening in pregnancy. *Chin J Misdiagn* 2008;**18**:6828–9.
4. Lin XY. Effect of infrared on cervical erosion. *Chin Trop Med* 2009;**9**:86.
5. Shao WR, Zhang XT, Li F, Wang Y. Investigation of virus interrelated with cervical erosion by PCR and Immunohistochemistry. *Chin J Lab Diagn* 2007;**11**:1034–7.

6. Feng L, Yuan DQ, Chen YJ. The correlation of female genital infection with secondary infertility. *Chin J Integr Tradit West Med* 2008;**17**:514–5.
7. Zhang CL. Clinical analysis of 60 cases of Interferon Suppository on cervical erosion. *Guide China Med* 2008;**6**:590–1.
8. Song XH, Yu XM. A comparable study of microwave tissue coagulation and CO_2 laser in the treatment of cervical erosion. *Chin J Obstet Gynecol* 1994;**29**:19–22.
9. Wang SP, Zhao T, Qiu YL. The clinical study of multiple treatment methods on the cervical erosion. *Chin Trop Med* 2007;**7**:1335–6.
10. Chen Y. Clinical analysis of 78 cases on cervical erosion treated with traditional Chinese medicine. *Chin J Matern Child Health Care* 2008;**23**:5235.
11. Zhang HP. Clinical observation of 193 cases on cervical erosion with Zhimiling Suppository. *Chin Clin New Med* 2007;**7**:351.
12. Xian YF, Mao QQ, Ip SP, Lin ZX, Che CT. Comparison on the anti-inflammatory effect of Cortex Phellodendri Chinensis and Cortex Phellodendri Amurensis in 12-*O*-tetradecanoyl-phorbol-13-acetate-induced ear edema in mice. *J Ethnopharmacol* 2011; **137**:1425–30.
13. Tjong Y, Ip S, Lao L, Fong HH, Sung JJ, Berman B, et al. Analgesic effect of Coptis chinensis rhizomes (*Coptidis rhizoma*) extract on rat model of irritable bowel syndrome. *J Ethnopharmacol* 2011;**135**:754–61.
14. Darshan S, Doreswamy R. Patented antiinflammatory plant drug development from traditional medicine. *Phytother Res* 2004;**18**: **343**–357.
15. Chen XP, Wang WL, Liu ML, Li HM. Baofukang Suppository for treating 88 cases of cervical erosion. *Shaanxi J Tradit Chin Med* 2007;**28**:792–3.
16. Xu YX, Yao DM, Pu DM, Wang LL. The clinical observation on the curative effects of Cortex Phellodendri and Rhizoma Coptidis to the cervical erosion. *J New Chin Med* 2007;**39**:47–8.
17. Ye JH. Experience on clinical application and pharmacological effects of Olibanum and Myrrha. *Clin J Anhui Tradit Chin Med* 2003;**15**:264–5.
18. Al-Hazzani AA, Alshatwi AA. Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells. *Food Chem Toxicol* 2011;**49**:3281–6.
19. Diao JH, Jin YS, Zhang SY. The content of protocatechuic acid in *Unguentum geranii* determined by HPLC method. *Chin Tradit Herb Drugs* 2007;**38**:867–8.
20. Cui Y, Li L, Zhang L, Li J, Gu J, Gong H, et al. Enhancement and mechanism of transdermal absorption of terpene-induced propranolol hydrochloride. *Arch Pharm Res* 2011;**34**:1477–85.
21. Wu YP, Huo MR, Zhou JP. Preparation of silymarin-loaded amphiphilic chitosan micelle and its *in situ* absorption in rat intestine. *Acta Pharm Sin* 2009;**44**:651–7.
22. He ZX, Wang ZH, Zhang HH, Pan X, Su WR, Liang D, et al. Doxycycline and hydroxypropyl-β-cyclodextrin complex in poloxamer thermal sensitive hydrogel for ophthalmic delivery. *Acta Pharm Sin B* 2011;**1**:254–60.
23. Shravani D, Lakshmi PK, Balasubramaniam J. Preparation and optimization of various parameters of enteric coated pellets using the Taguchi L9 orthogonal array design and their characterization. *Acta Pharm Sin B* 2011;**1**:56–63.
24. Liu YL, Li LL, Sun H, Jin W, Cao L. Comparison of the antimicrobial effect of 3 gynecological drugs *in vitro* and their anti-inflammatory effect on rat with cervical erosion. *Chin J Comparative Med* 2008;**18**:18–20.
25. Xie JD, Hong Y, Du SY. Study on protective effect of Gongyanjing to cervicitis rats and its mechanism. *Chin J Inform TCM* 2010;**17**:33–5.
26. Tian ZK, Ma YL, Wang WR. The therapeutic effect of Zhimiling effervescent capsule suppository on treating cervical erosion rats models resulted by phenol plasmagel. *Acta Chin Med Pharm* 2011;**39**:44–6.
27. Zheng XL, Zheng CH. Preparation and pharmacodynamics studies on anti-inflammatory effect of catechu gel. *China J Chin Mater Med* 2011;**36**:2493–7.
28. Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. *Phytother Res* 2008;**22**:999–1012.
29. Mahata S, Bharti AC, Shukla S, Tyagi A, Husain SA, Das BC. Berberine modulates AP-1 activity to suppress HPV transcription and downstream signaling to induce growth arrest and apoptosis in cervical cancer cells. *Mol Cancer* 2011;**10**:39–52.
30. Lu B, Zhao J, Xu L, Xu Y, Wang X, Peng J. Identification of molecular target proteins in berberine-treated cervix adenocarcinoma HeLa cells by proteomic and bioinformatic analyses. *Phytother Res* 2012;**26**:646–56.
31. Tripathi S, Mehrotra GK, Dutta PK. Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. *Int J Biol Macromol* 2009;**45**:372–6.
32. Horio T, Ishihara M, Fujita M, Kishimoto S, Kanatani Y, Ishizuka T, et al. Effect of photocrosslinkable chitosan hydrogel and its sponges to stop bleeding in a rat liver injury model. *Artif Organs* 2010;**34**:342–7.
33. Wang Y. *The application of thermo-sensitive hydrogels in drug delivery system* [Dissertation]. Hangzhou: Zhejiang University; 2006. p. 48–79.
34. He C, Kim SW, Lee DS. *In situ* gelling stimuli-sensitive block copolymer hydrogels for drug delivery. *J Control Release* 2008;**127**:189–207.
35. Peng KT, Chen CF, Chu IM, Li YM, Hsu WH, Hsu RW, et al. Treatment of osteomyelitis with teicoplanin-encapsulated biodegradable thermosensitive hydrogel nanoparticles. *Biomaterials* 2010;**31**:5227–36.