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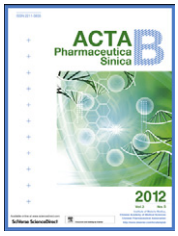

ORIGINAL ARTICLE

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.