New therapy for oral ulcer: *Evodia rutaecarpa* patch combined with acupoint application

Yan-Li Zhen1,*, Jian-Peng Qin1,*, Yu-Fen Li1, Jian Zhang1, Xiao-Hui Yu2, Ji Li1,*, Dong-Kai Wang1,*,

1Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China.

*These authors contributed equally to this work.

**Corresponding to:** Ji Li and Dong-Kai Wang, Department of Pharmaceutics, Shenyang Pharmaceutical University, No. 103 Culture Road, Shenyang, 110016, China. E-mail: syphuliji@163.com; wangycsyphu@116.com.

**Author contributions**
Zhen YL, Qin JP and Li YF are responsible for project design and implementation, Zhang J and Yu XH are responsible for animal experiments, and Li J and Wang DK are responsible for project selection and theoretical support. All the authors participated in the final approval of the manuscript.

**Competing interests**
The authors declare no conflicts of interest.

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**Abbreviations**
MDA, malondialdehyde; EGF, epidermal growth factor; SOD, superoxide dismutase; ER, *Evodia rutaecarpa*; EVO, evodiamine; Rut, rutaecarpine; H&E, hematoxylin-eosin; SDS, sodium dodecyl sulfate.

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**Abstract**

**Background:** Oral ulcer is the most common and easily recurrent disease in stomatology, which influence the patients’ communication, normal dietary, and sleep. *Evodia rutaecarpa* (ER) is a traditional Chinese herbal medicine recorded in ancient Chinese medical books, which has a medicinal history of more than 2,000 years. Clinically, the application of ER at plantar Yongquan point (KI11) is effective in the treatment of oral ulcer. The purpose of this study was to combine the modern transdermal drug delivery system with traditional Chinese medicine to develop the transdermal absorption patch of ER and apply it to the treatment of oral ulcer at Yongquan point of plantar. **Methods:** Firstly, the medicinal materials of ER were extracted and the extracted materials were prepared into dispersed ER patch. The formulation and preparation process were screened by orthogonal design method and single factor investigation method. The adhesive and transdermal properties of the patch were used as the evaluation index of the preparation. Secondly, Wistar rats were used as experimental animals to establish a rat model of mouth ulcers. Wistar rats were randomly divided into normal group, model group (A), model group (B), low dose group, medium dose group and high dose group. The efficacy of ER on rat's oral ulcer model was evaluated through three aspect such as apparent index, pathological index and biochemical index. **Results:** The patch had suitable adhesion and good skin penetration, which was an effective treatment for oral ulcer. In vivo pharmacodynamic studies, compared with the normal group, the body mass and food intake of rats in each group after modeling decreased, the amount of drinking water increased, the tissue structure of oral mucosa was damaged, and the levels of inflammatory factors (TNF-α, IL-6) and malondialdehyde increased, the levels of ant-inflammatory factors (IL-10), cell growth factor (epidermal growth factor, TGF-β1) and superoxide dismutase decreased. Compared with the model group, the body weight and food intake of each dosing group increased, water consumption decreased, the oral mucosal tissue structure was more complete, the levels of TNF-α, IL-6 and malondialdehyde decreased, the levels of IL-10, epidermal growth factor, TGF-β1 and superoxide dismutase increased, and the changes of various indexes were dose-related. **Conclusion:** ER patch can inhibit inflammatory reaction, enhance the antioxidant defense ability of the body, and promote the repair of damaged oral mucosa, so as to play an effective role in the treatment of oral ulcer.

**Keywords:** oral ulcer; *Evodia rutaecarpa* patch; traditional Chinese medicine; acupoint application; pharmacodynamic
Background

The prevalence rate of oral ulcers in the general population is between 20% and 50%, and this kind of disease ignores race, region, age and sex, and may suffer from oral ulcers during the human growth cycle [1–3]. It is a round or oval superficial ulcer ranging in size from rice grains to soybeans on the oral mucosa. Oral ulcers often occur in areas with poor keratosis, such as lip mucosa, buccal mucosa, soft palate and tongue. The tissues around the ulcer are often accompanied by congestion, edema and pain, which has a great impact on the patients’ language communication, normal eating and sleep. Studies have shown that, oral ulcers are usually caused by local trauma, anxiety, vitamin B12 deficiency, allergic reactions, adverse drug reactions, chemotherapy drugs such as methotrexate, 5-fluorouracil, 6-mercaptopurine [4–8]. Chemotherapy-induced damage to cells or tissues is caused by reactive oxygen species, thus reactive oxygen species play a main role in the initiation phase of oral mucositis and activate TNF-α, IL-1β, IL-6, and induces apoptosis [9–11]. It is reported that, the evidences that oral mucosal injury are accompanied by elevated levels of proinflammatory cytokines, such as TNF-α, IL-6 and malondialdehyde (MDA) [12–14].

Some Chinese medicine classics record that the use of Evodia rutaecarpa (ER) sticking Yongquan point (KI1) can treat oral ulcer. The application of ER on Yongquan point has remarkable curative effect in the treatment of oral ulcer and is widely used in folk and clinical practice. However, the current drug use method is to directly apply its medicinal powder with vinegar, lack of proprietary drug dosage form, and there are some problems such as inconvenient, nonstandard and uncontrollable drug quality and so on. According to the existing research including the 2020 edition of the Chinese Pharmacopoeia, it was concluded that the efficacy of ER may be attributed to its ingredients: evodiamine (Evo) and rutacarpine (Rut). Some researchers have found that Evo and Rut may inhibit the symptoms of oral ulcers, reduce the level of TNF-α and IL-6, and increase the expression of IL-10, TGF-β1 and epidermal growth factor (EGF) [15–19]. Other studies have shown that Rut can promote the expression of superoxide dismutase (SOD) and accelerate the scavenging of free radicals, which is beneficial to tissue repair [20, 21].

In this study, for the first time, the ancient prescription of ER vinegar was prepared into a modern transdermal drug delivery preparation (Figure 1), combined with the oral ulcer adverse reaction of anti-tumor chemotherapy drug methotrexate and the corrosiveness of chemical sodium hydroxide to oral mucosa to establish a rat model of oral ulcer. ER patch was applied to plantar Yongquan point of oral ulcer rats for treatment and evaluate its curative effect.

Materials and methods

Drugs and reagents

ER (The direct sale purchase from Jiangxi Province, China, was identified as genuine by Associate Professor Wang of the Department of Pharmacognosty of the School of Chinese Materia Medica, Shenyang Pharmaceutical University); absolute ethanol and petroleum ether (Tianjin Kangkede Technology Co., Ltd., Tianjin, China); Technomelt FS 8072 pressure sensitive adhesive (Henkel Group Co., Ltd., Shanghai, China); 3M Cotran CoTran Backings 9720, 3M scotchpak 9744 release film (3M company); azone (Shanghai Dibai Biotechnology Co., Ltd., Shanghai, China); methotrexate (Shanghai McLean Biochemical Technology Co., Ltd., Shanghai, China); sodium hydroxide (Tianjin Hengxing chemical reagent manufacturing Co., Ltd., Tianjin, China); chloral hydrate (Tianjin Damao Chemical Reagent Factory, Tianjin, China); 4% paraformaldehyde (biosharp-Saiguo Biotechnology, Guangzhou, China); Elisa kit: TNF-α, IL-6, IL-10, EGF, TGF-β1 (Shanghai Aimeng Youning Biotechnology Co., Ltd., Shanghai, China); WST-1 SOD kit, TBA MDA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Animals

Wistar rats (male, weighing 180–220 g) are provided by the Experimental Animal Center of Shenyang Pharmaceutical University, and the animal license number is NO.SCKX (Liao) 2020-0001. New Zealand white rabbits (male, weighing 2.0–2.2 kg) are provided by the Experimental Animal Center of Shenyang Pharmaceutical University, and the animal license number is NO.SCKX (Liao) 2020-0001. A 1-week acclimatization period was allowed before the test commenced. Animal experiments were conducted in accordance with the Guidelines of the Animal Experimental Control and Supervision Committee, which was approved by the Animal Ethics Committee of Shenyang Pharmaceutical University (Ethics NO.SYPU-IACUC-2022-1103-401).

Prescription and preparation technology of patch

Extract active pharmaceutical ingredient (Evo and Rut). The powder of ER was dried and crushed through a sieve of 80 mesh, the

Figure 1 The preparation process of ER patch of drug in adhesive. ER, Evodia rutaecarpa; Evo, evodiamine; Rut, rutaecarpine.
petroleum ether was supersonic degreased, the ultrasonic power was 400 W, temperature 40 °C, time 4 h, the material solution after ultrasonic degreasings was filtered, and the filter residue was dried to obtain petroleum ether degreased ER powder. A certain concentration of ethanol solution was used as the extraction solvent, and the degreased and dried ER powder was extracted at 400 W ultrasonic power. Taking the total content of extracted Evo and Rut as the evaluation index, the key factors in the extraction process, such as ethanol concentration, material-liquid ratio, extraction time and extraction temperature, were screened and optimized.

Preparation of patch. Heated and melted the pressure sensitive adhesive, added the Evodia extract powder, penetrator enhancer, stir until the mixture was evenly mixed, applied the drug-containing pressure sensitive adhesive to a certain thickness on the backing layer, covered the anti-adhesive layer, and cut into a specified shape and size, the transdermal absorption patch of ER was obtained. Blank patch without ER extract was prepared by the same method.

Evaluation of ER patch

Measuring adhesion properties. In this study, the initial adhesion (n = 3), retention adhesion (n = 6) and peel strength (n = 6) of ER percutaneous absorption patch were measured strictly as described in the Chinese Pharmacopoeia 2020 Edition Part IV 0952 Adhesion Determination Method. All adhesion tests were performed at 25 ± 2 °C [22].

Determination of the degree of release. According to the 2020 edition of the Chinese Pharmacopoeia Part IV 0931 Dissolution and Release Determination Method 4 (paddle plate method) on transdermal patch release determination method 1,900 mL 30% PEG400 was used as the release medium, the temperature was 32 ± 0.5 °C, and the speed was 50 rpm/min.

Analytical methods

The drug quantity was analyzed by high-performance liquid chromatography (Shimadzu Co., Ltd., Tokyo, Japan), pump (LC-20AT), automatic injector (SIL-20A) and UV-visible detector (SPD-20A). The column for this analysis was C18 (5 μm, 150 mm × 4.6 mm). The detection wavelength was 225 nm. The mobile phase was a 1:1 mixture of aqueous acetonitrile. The column temperature is 40 °C, the flow rate is 0.8 mL/min.

In vitro permeation behavior of patch in rat skin

The patch was prepared for in vitro transdermal permeability experiment. Samples were taken at 2 h, 4 h, 6, 8, 10 h, 12 h and 24 h. The samples were filtered by 0.45 μm filter membrane and analyzed by high-performance liquid chromatography.

Pharmacodynamic study in vivo

Establishment of rat model of oral ulcer. The model rats of oral ulcer were established by combining the adverse reaction of oral ulcers with antitumor chemotherapy drugs and the corrosiveness of chemicals to oral mucosa [23, 24]. After anesthesia, 0.7 mg/mL methotrexate solution prepared by 4 mL normal saline was injected intraperitoneally at one time. Placed one 12 mm × 5 mm flake sodium hydrosulfide crystal on the left or right sides of the buccal mucosa for 5–10 seconds. The model can last for 7–10 days.

In vivo pharmacodynamics of ER patch in rats. Wistar rats were randomly divided into normal group, model group (A), model group (B), low dose group (10% drug content), medium dose group (16% drug content) and high dose group (22% drug content). After 24 h of modelling, the blank patch (group B), low, medium and high dose of ER patch were administered for 24 h each time, for 3 consecutive times, sample was collected after modelling 24 h of group (A). The apparent indexes of rats in each group were recorded during the administration period. At the end of third administration, the oral mucosal tissue and serum of rats were collected, the hematoxylin-eosin (H&E) staining of oral mucosal tissue was observed, and the levels of biochemical indexes such as TNF-α, IL-6, IL-10, EGF, TGF-β1, SOD and MDA were detected [25–28]. Recording of epigenetic indicators: The body weight, food intake and water consumption of each group of rats were weighed and recorded, the size of rat ulcers in each group was measured and recorded by electronic vernier calipers, and the healing of mouth ulcers in each group was observed by taking pictures.

Determination of biochemical indicators: Remove the entire oral mucosal tissue of the rat and cut into pieces. Add a certain amount of PBS buffer to tissue complete homogenization, centrifuge at 3,000 rpm/min for 20 min, and collect the supernatant. The “sandwich method” was used to detect TNF-α, IL-6, IL-10, EGF, TGF-β1 and other biochemical indexes by ELISA kit. The capture antibody is coated on the enzyme plate, the target protein in the sample and standard is captured, the biotinylated detection antibody binds to the target protein, the strept avidin-biotin complex complex binds to the biotinylated detection antibody to form an immune complex, after adding 3,3′,5,5′-Tetramethylbenzidine chromogenic solution, if there is a target protein in the reaction well, it will be blue, the stop solution will turn yellow, the free components during the detection process will be washed off, and the optical density value is measured at 450 nm with a microplate reader, and the target protein concentration is proportional to the optical density value. The concentration of the target protein in the specimen is calculated by plotting a standard curve.

2 mL of blood was taken from the heart of mice and placed at room temperature for 2 h, and after blood clotting and blood clot contraction, serum was collected for serum detection of SOD and MDA, two biochemical indexes. SOD is detected using the WST-1 method. WST-1 reacts with superoxide anion catalyzed by xanthine oxidase to produce a water-soluble formazan dye, a step that can be inhibited by SOD. Enzyme viability of SOD can be calculated by colorimetric analysis of the WST-1 product using a microplate reader at 450 nm. MDA was detected using thiobarbituric acid. MDA in the lipid peroxidized degradation product condensed with thiobarbituric acid to form a red product with a maximum absorption peak at 532 nm.

Observation of pathological indicators: The local mucosal tissue at the mouth ulcer of the rat was excised, the blood and mucus on the tissue were rinsed with normal saline, the tissue block was flattened and fixed with 4% paraformaldehyde for H&E staining morphological observation.

Safety evaluation

Irritation refers to the reversible inflammatory response to the site of administration after administration of a non-oral formulation. Since the patch is applied directly to the skin, its safety is paramount. According to the relevant requirements of the “Technical Guidelines for the Study of Chemical Drug Irritation, Allergy and Hemolysis” (hereinafter referred to as the guidelines) issued by State Food and Drug Administration in 2005, the skin irritation test of ER percutaneous absorption patch was carried out. The irritation test mainly observes whether the blood vessels, muscles, skin or mucous membranes of the animal cause local reactions such as redness, hyperemia, exudation, degeneration or necrosis after contact with the test object.

Test method. According to the guidelines, the test was carried out by the self-comparison method of the left and right sides of the same body. In this study, the skin treated with 10% sodium dodecyl sulfate (SDS) was used as the positive group and the untreated skin as the blank group. The back hair of New Zealand rabbits was shaved 24 h before the experiment. ER patch and blank patch (negative group) were applied for 12 h. At the end of the application, remove and clean the patch and positive control substance. At 1 h, 24 h, 48 h, and 72 h after drug removal, the degrees of erythema and edema were observed and recorded with naked eyes.

Outcome evaluation methods. According to the scoring criteria for skin irritation in the guidelines (Table 1), Evodia patch, blank patch and 10% (w/w) SDS aqueous solution were scored for skin irritation in rabbits, and then the TS, total score of skin response scores of each experimental group at each observation time point was calculated, and the average of rabbit Ed, edema, and Er, erythema index scores
was evaluated, as shown in the following formula:

\[ TS = \sum_{n}^{(Ed+Er)} n/N \]

\(n\) is the number of animals tested, and \(N\) is the number of observations. Stimulus intensity evaluation was performed according to Table 2.

**Statistical analysis**

SPSS 23.0 statistical software was used for data analysis. One way analysis of variance was used to compare the differences between groups, and t-test was used for pairwise comparison. The difference was statistically significant with \(P < 0.05\).

**Results**

**Determination of extraction process**

The orthogonal design was used to determine the extraction process. With pre-experiment, the ethanol concentration was 80%, 90%, 100%; the ratio of material to liquid was 1:30, 1:40, 1:50; the extraction time was 30 min, 40 min, 50 min; the extraction temperature was 20 °C, 30 °C, 40 °C. The orthogonal design table was shown in Table 3, the experimental arrangement Table 4, and the

<table>
<thead>
<tr>
<th>Type of reaction-erythema</th>
<th>Score</th>
<th>Type of reaction - Edema</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema</td>
<td>0</td>
<td>No edema</td>
<td>0</td>
</tr>
<tr>
<td>Slight erythema (reluctantly perceptible)</td>
<td>1</td>
<td>Slight edema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate erythema (perceptible)</td>
<td>2</td>
<td>Moderate edema (perceptible)</td>
<td>2</td>
</tr>
<tr>
<td>Severe erythema</td>
<td>3</td>
<td>Severe edema (raised by approximately 1 mm)</td>
<td>3</td>
</tr>
<tr>
<td>Red-purple erythema to slight eschar formations</td>
<td>4</td>
<td>Very severe edema (raised by more than 1 mm and extending beyond the area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 2 Evaluation standard of the intensity of skin irritation**

<table>
<thead>
<tr>
<th>Score</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.49</td>
<td>No irritation</td>
</tr>
<tr>
<td>0.50–2.99</td>
<td>Slight irritation</td>
</tr>
<tr>
<td>2.00–5.99</td>
<td>Moderate irritation</td>
</tr>
<tr>
<td>6.00–8.00</td>
<td>Severe irritation</td>
</tr>
</tbody>
</table>

**Table 3 Factors and levels Table of the orthogonal test of the extraction technology of ER**

<table>
<thead>
<tr>
<th>Level</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>1:30</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>1:40</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>1:50</td>
<td>50</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

ER, *Evodia rutaecarpa*.

**Table 4 Orthogonal test arrangement and results of ER extraction technology**

<table>
<thead>
<tr>
<th>Test number</th>
<th>Factors</th>
<th>Content of Evo and Rut (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

ER, *Evodia rutaecarpa*; Evo, evodiamine; Rut, rutaecarpine. --, not mentioned.
analysis of variance Table 5.

The results of orthogonal design showed that the effects of various factors on the extraction rate of ER were as follows: A > D > C > B. The optimal extraction process of ER is A3B3C3D2. According to the analysis of variance of orthogonal design and considering the production, since the effect of liquid-material ratio on the extraction rate was not significant, it could be set as a lower level. From this, the best extraction process of ER was A3B1C3D2. In this scheme, the extraction solvent was absolute ethanol solution, the ratio of Evodia powder to liquid was 1:30, and the ultrasonic extraction time was 50 min. The ultrasonic extraction temperature was 30 °C. The chromatogram of the final extracted product and standard product was shown in Figure 2, the retention time of Rut was 7.02 min, and Evo 8.34 min.

Determination of prescription process

The experimental results were based on the maximum cumulative penetration of Evo and Rut. When preparation temperature (140 °C), pressure sensitive adhesive (Technomelt PS 8072), backing layer (3M CoTran Backings 9720), transdermal absorption enhancer (azone, 2%), and drug content (16%) were used, the ER patch with smooth appearance, uniform color, and maximum cumulative penetration could be obtained (Figure 3).

Optimal process

After petroleum ether defatting and drying, the powder of ER was sifted with 80 mesh, anhydrous ethanol was added, the ratio of material to liquid was 1:30, soaked for 1 hour, 50 min was extracted by ultrasonic at 30 °C, the extracted material solution was filtered, and the filtrate was evaporated to remove ethanol, dried, crushed and sieved to obtain Evodia extract powder. Took 16% of the under 80 mesh Evodia extract powder, 2% azone, 82% Technomelt PS 8072 pressure sensitive adhesive, heated and melted the pressure sensitive adhesive, and then added the Evodia extract powder at 140 ± 5 °C, stirred evenly. Add the penetration enhancer azone, stirred mixture adequately, put the mixture on the backing layer (3M CoTran Backings 9720), and evenly spread the drug-containing pressure sensitive adhesive layer with a thickness of 0.4 mm, covered the surface with anti-adhesive layer, and cut it into a circle with a diameter of 3 cm, to obtain a viscose dispersed transdermal absorption ER patch. The blank patch was prepared by the same method without ER extract.

Performance evaluation of ER patch

To simulate the skin permeability of the drug and the interaction between patch and skin, the release test and adhesion test were carried out.

Adhesion properties. The adhesion of ER patch was determined as follows: initial adhesion force 32–34 ball, adhesion holding force 12 h–16 h, peel strength 2.67 kN/m–2.79 kN/m.

Release evaluation. According to the results, it was stipulated that within 0.5 h, 2 h and 4 h, the release rates of Evo in ER patch were respectively 20%–30%, 40%–60% and 65%, and the release rates of Rut were respectively 20%–30%, 30%–50% and 55% (Figure 4).

In vitro permeation behavior of patch in rat skin

The in vitro permeation results of the patch in rat skin are shown in Table 6 and Figure 5.

The results showed that the \( Q_{24h} \) and \( J_{w} \) of evodiamine were 4.13 \( \mu g/cm^2 \) and 0.16 \( \mu g/h/cm^2 \), and the \( Q_{24h} \) and \( J_{w} \) of rutaecarpine were 2.84 \( \mu g/cm^2 \) and 0.12 \( \mu g/h/cm^2 \). The cumulative permeation amount of the active ingredients in the patch increased steadily with the transdermal time.

Pharmacodynamic study in vivo

Establishment of rat model of oral ulcer. The oral mucosa of a mouse was opened, putting the Evodia extract powder on the oral mucosa, and then the mouth was sutured. One week later, the oral ulcer was formed. The rats were divided into a blank group, a positive group, and an ER group. The blank group was treated with normal saline, and the positive group was treated with a known oral ulcer model drug. The ER group was treated with a blank ER patch, and the oral ulcer was evaluated and compared with the other two groups. The oral ulcer healing rate was calculated according to the original ulcer area and the ulcer area at different time points. In the blank group, the ulcer healing rate was only 10%, the positive group was 50%, and the ER group was 70% (Figure 6). The results showed that the ER patch had a good oral ulcer healing effect.

Table 5 Analysis of variance results of the extraction technology of ER

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>SS</th>
<th>f</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>Salience</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.94</td>
<td>2</td>
<td>0.47</td>
<td>13.37</td>
<td>0.0003**</td>
<td>**</td>
</tr>
<tr>
<td>B</td>
<td>0.19</td>
<td>2</td>
<td>0.10</td>
<td>2.72</td>
<td>0.0929</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>0.32</td>
<td>2</td>
<td>0.16</td>
<td>4.53</td>
<td>0.0254*</td>
<td>*</td>
</tr>
<tr>
<td>D</td>
<td>0.37</td>
<td>2</td>
<td>0.19</td>
<td>5.31</td>
<td>0.0154*</td>
<td>*</td>
</tr>
</tbody>
</table>

ER, Evodia rutaecarpa.

Figure 2 The chromatogram of the standard product and final extracted product. (A) the standard product. (B) final extracted product.
Figure 3 Effects of different factors of preparation process on the cumulative permeability of Evo and Rut in ER patch (n = 3). (A) Evo. (B) Rut. ER, Evodia rutaecarpa; Evo, evodiamine; Rut, rutaecarpine.

Figure 4 The Evo and Rut release percentage of three batch ER patches (n = 3). (A) Evo. (B) Rut. ER, Evodia rutaecarpa; Evo, evodiamine; Rut, rutaecarpine.

Figure 5 The penetration profile of Evo and Rut in ER patches with Optimized formulation. ER, Evodia rutaecarpa; Evo, evodiamine; Rut, rutaecarpine.

Table 6 Q_{24h} and J_{ss} of Evo and Rut in ER patches with Optimized formulation through excised rat skin

<table>
<thead>
<tr>
<th></th>
<th>Evo (µg/cm²) (Mean ± SD, n = 3)</th>
<th>Rut (µg/h/cm²) (Mean ± SD, n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q_{24h} of Evo</td>
<td>4.13 ± 0.31</td>
<td>2.84 ± 0.20</td>
</tr>
<tr>
<td>J_{ss} of Evo</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>ER, Evodia rutaecarpa; Evo, evodiamine; Rut, rutaecarpine.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
normal rat is shown in Figure 6A. The oral mucosa of rats was burned with sodium hydroxide flaky crystals, and after being contacted for 5–10 seconds, the mouth ulcer wound was generated, and the wound face was hyperemic and red within 10 seconds, as shown in Figure 6B. After 10 s, the wound face turned dark gray, as shown in Figure 6C. With the physiological activities such as eating and drinking and the development of mouth ulcers in rats, after 24 hours, the ulcer area became larger and the color changed to light gray, as shown in Figure 6D.

Record of apparent indexes of oral ulcer in rats. As exhibited in Figure 7A, the results showed that 24 h after modeling, the wounds of rats in each group were silver gray to dark gray. Although the damage degree of wounds of several rats randomly selected from each group is slightly different, with the treatment of drugs, it can still be clearly observed that: the traumas of rats in each administration group had healed, the ulcer area had converged, and the ulcer color has gradually changed from silver gray to light yellow.

In the whole process of the experiment, the body mass (Figure 7B) and the food intake (Figure 7C) of rats in the model group decreased the most, were lower than that of each administration group, and among the administration groups, the body mass and the food intake of rats was positively correlated with the administration dose. On the contrary, model group continued to increase on amount of drinking, while the rats in the administration group fluctuated little (Figure 7D).

In order to quantify the change of the size of oral ulcers, the longest diameter of oral ulcer was measured and recorded with vernier caliper. During the whole administration process, the canker sores of rats in each dosing group were smaller than those of rats in the model group (Figure 7E).

Determination of biochemical indexes of oral ulcer in rats. As the statement of above, reduce the level of TNF-α and IL-6, and increase the expression of IL-10, TGF-β1, and EGF, may be related to relieve of ulcers. SOD is an enzyme that inhibits and protects against free radical damage in the body, which can reduce the damage caused by superoxide anion. The content of MDA can indirectly reflect the degree of free radical damage in tissue [15]. The biochemical indexes (EGF, IL-10, TGF-β1, SOD, IL-6, TNF-α, and MDA) at the ulcer site were detected.

As shown in Figure 8, Compared with the normal group, the contents of TNF-α, IL-6 and MDA in the oral mucosa of the model

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**Figure 6** Successful establishment of oral ulcer rat model. (A) Normal oral mucosa of rats. (B) Modeling within 10 s. (C) Modeling over 10 s. (D) Modeling more than 24 h.

**Figure 7** The changes of the apparent indicators of modeling rats during administration (n = 10). (A) The morphology of ulcers. (B) Body mass. (C) Food intake. (D) Water intake. (E) Diameter of ulcers.
group (A) were significantly increased ($P < 0.05$), while the contents of EGF, IL-10, TGF-$\beta$1 and SOD were significantly reduced ($P < 0.05$). Compared with the model group (B), the contents of oral mucosal TNF-$\alpha$, IL-6 and MDA decreased significantly ($P < 0.05$), while the contents of EGF, IL-10, TGF-$\beta$1 and SOD increased significantly ($P < 0.05$).

**Observation of pathological indexes of oral ulcer in rats.** To further clarify the efficacy from a microscopic point of view, the local mucosal tissue of rat oral ulcer was observed by H&E staining [29]. The section direction was from top to bottom through the epithelial layer, lamina propria and submucosa (Figure 9).

**Skin irritation test**

The results of skin irritation evaluation of rabbit skin irritation with ER patch are shown in Table 7, and the sample evaluation results of irritation evaluation are shown in Figure 10. The experimental results showed that the skin of the tested part of the rabbits had an irritant reaction after smearing 10% SDS aqueous solution. Mild erythema was observed at 1 h after SDS clearance, obvious erythema and slight edema were observed at 24 h, purplish red erythema and scab appeared at 48 h and 72 h, which indicated that rabbits could express skin irritation normally. During the experiment, there was no skin irritation in the blank control group. There was no skin irritation, erythema and edema in blank patch and ER patch, which indicated that ER patch was not irritating to skin of rabbits (Figure 10).

**Discussion**

Canker sores are a common condition in daily life as well as chemotherapy/radiotherapy, and in severe cases, can impair the patient’s quality of life. The etiology of oral ulcers is extremely complex, Leikin JB provided a more detailed review that can be used for reference [30]. Moreover, the oral ulcer was divided by Raber Durlacher JE into the following parts: initiation, upregulation, and amplification of messenger signals, ulceration and healing [31]. It has been treated with a variety of strategies and drugs, but herbs have gained widespread interest for their low side effects compared to chemical drugs [32].

ER has anti-inflammatory and immunomodulatory properties, and its effects mainly depend on the downregulation of pro-inflammatory cytokines and the upregulation of anti-inflammatory cytokines. This experiment evaluated the effect of ER on the biochemical indexes (TNF-$\alpha$, IL-6, IL-10, TGF-$\beta$1, EGF, SOD) in rat mouth ulcer models, and the results showed that ER patch administered through Yongquan point had the ability to reduce the levels of TNF-$\alpha$ and IL-6, increase the expression of IL-10, TGF-$\beta$1 and EGF, promote the expression of SOD, and accelerate the scavenging of free radicals, which is the same as the results of previous studies [33–35]. It was shown that ER patch combined with plantar Yongquan point had a good efficacy in the treatment of mouth ulcers, and the preparation achieved the purpose of this study. In this study, the experimental animals were divided into six groups, namely normal group, model group (A), model group (B),

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**Figure 8 Research on cytokines in vivo.** (A) EGF. (B) IL-10. (C) TGF-$\beta$1. (D) SOD. (E) IL-6. (F) TNF-$\alpha$. (G) MDA. * $P < 0.05$. MDA, malondialdehyde; EGF, epidermal growth factor; SOD, superoxide dismutase.
Figure 9 The H&E staining of oral mucosal tissue of rats in each group (scale bar, 100 μm). H&E, hematoxylin-eosin.

Figure 10 Experiment of skin irritation on the back of New Zealand White rabbits. ER, *Evodia rutaecarpa*.

Table 7 Evaluation results of skin irritation experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Negative TS</th>
<th>Blank patch Evaluation</th>
<th>ER patch TS</th>
<th>Blank patch Evaluation</th>
<th>Positive TS</th>
<th>Blank patch Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>0.25</td>
<td>No</td>
<td>0.75</td>
<td>Slight</td>
<td>1.25</td>
<td>Slight</td>
</tr>
<tr>
<td>24 h</td>
<td>0</td>
<td>No</td>
<td>0.25</td>
<td>no</td>
<td>0.25</td>
<td>no</td>
</tr>
<tr>
<td>48 h</td>
<td>0</td>
<td>No</td>
<td>0</td>
<td>no</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>72 h</td>
<td>0</td>
<td>No</td>
<td>0</td>
<td>no</td>
<td>6.75</td>
<td>severe</td>
</tr>
</tbody>
</table>

ER, *Evodia rutaecarpa*; TS, total score of skin.
low-dose group, medium-dose group and high-dose group, and the results showed that the therapeutic effect was dose-dependent. However, no positive drug control group was designed, because there were no currently marketed formulations for efficacy comparison, which is a deficiency of this study. However, for the modern pharmacological mechanism of action of ER sticking to Yongquan point for the treatment of oral ulcers, this study is still in the preliminary research stage of ER applied to the treatment of oral ulcers, and the follow-up research work still has a long way to go, and its modern pharmacological mechanism should be further studied, and whether ER sticking to other parts instead of the soles of the Yongquan point can play the same role in treating oral ulcers.

In addition to anti-inflammatory effects, ER also has analgesic, anti-tumor and other pharmacological effects. EVO can trigger apoptosis in human malignant melanoma A375-S2 cells, but it has not yet been clinically applied, and the development of suitable dosage forms is a major challenge for future work [36]. Early studies have shown that ER sticking to Yongquan point has a good effect on lowering blood pressure. Although the therapeutic mechanism of this method for the treatment of these diseases has not been fully studied, the above results show that it is important to explore the treatment of ER patch in combination with Yongquan point in the treatment of other diseases.

Conclusion

In this work, a new idea for the treatment of oral ulcers was provided: ER patch, which was effective in the treatment of oral ulcers. In this new way of drug administration, the preparation had the characteristics of good compliance, no skin irritation and relatively easy administration. In addition, the active components of ER patch: EVO and Rut, played a role in inhibiting proinflammatory cytokines, scavenging free radicals and promoting the expression of anti-inflammatory factors. In this study, the release and adhesion properties of ER patches were tested. These results showed that ER patch was a valuable method for the treatment of oral ulcers. In the preparation, the gap of ER for the treatment of oral ulcers was filled in the work. It is expected that ER will receive more attention and considerable development in the clinical application of treating oral ulcers.

References


