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Wound-healing promoting effect of total tannins from *Entada phaseoloides* (L.) Merr. in rats

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ABSTRACT

The healing of wounds has always provided challenges for the medical community whether chronic or acute. Modern and traditional medicine has proved that herbal medicine shown superiority over chemical drugs. Herein, we report an *Entada phaseoloides* (L.) Merr. extract with a total tannin content of 76.18% showed wound-healing promoting effect in rat model. We found significantly accelerated wound closure already on day 7 in animals treated with total *Entada phaseoloides* (L.) Merr. tannins (TEPT) as compared to vaseline treated controls ($p < 0.05$). At day 15, histologically, the wounds in animals treated with TEPT were completely closed as compared to controls. In vitro, TEPT promotes fibroblast proliferation and migration into wounds of NIH3T3 with concentration range of 9.38–37.50 μg/ml. TEPT also had an inhibitory action against *Staphylococcus aureus* with MBC of 1.5 mg/ml and the result was further proved by transmission electron microscope. Thus, TEPT could promote wound shrinkage, improve healing rate and promote healing of infectious wounds in rats. And this effect may due to antibacterial activities and NIH3T3 cell pro-proliferative effect of the tannins compounds, which indicating that TEPT can be used as efficient treatment in traumatic injury.

1. Introduction

Wounds, particularly nonhealing wounds, become a major health care problem worldwide. They can lead to remarkable morbidity, prolonged treatment time and high costs of health care [1]. It was estimated that wounds account for nearly 4% of total health system costs, and this proportion is increasing [2]. Wound healing is a process characterized by fibroblast proliferation and migration, granulation tissue formation, collagen secretion, as well as collagen scar formation and remodeling [3,4], in which fibroblasts play a vital role and thus have become a hot topic [5,6].

Bacterial infection is another major challenge in clinical injuries, to which there is no good solution yet. Wound infection may lead to delayed wound healing or even

Abbreviations: TEPT, total *Entada phaseoloides* (L.) Merr. tannins; MBC, minimum bactericidal concentration; TEM, transmission electron microscope; bFGF, basic fibroblast growth factor.

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nonhealing, thus resulting in deterioration [7]. Microbial pathogens can produce toxins to lead to sustained production of inflammatory mediators; activated neutrophils can cause reduced number of fibroblasts and collagen regeneration and thereby delay wound healing [8–10]. Clinically, large doses of antibiotics are generally used to inhibit bacteria in wounds, but antibiotics can hardly enter and act inside wounds and are prone to drug resistance [11]. Besides, they impede the speed of wound repair as well.

Studies have shown that external application of herbal medicine can activate local macrophages, has a chemotactic effect on leukocytes, enhances local immunity, regulates matrix metabolism, promotes local microcirculation, and has anti-inflammatory, antibacterial effects, etc. [12–15]. So herbal medicine has been regarded as a cheap and green resource for wound healing chemicals. Among them, nature tannins, in plant extracts have been reported effective in antibacterial [16–18] and antioxidant activities [19,20], which can promote wound healing as well [21,22].

Guoganglong, dried rattan of leguminous plant *Entada phaseoloides*, is a common folk herbal, which is distributed in China's Fujian, Taiwan, Guangxi, Guizhou and Yunnan, generally in monsoon forests and subtropical evergreen broadleaf forests. The plant is also distributed in northeast India, Myanmar, Laos, Vietnam, etc. While pharmacological activity of *E. phaseoloides* has little been studied at present.

In this experiment, the total tannin part of *E. phaseoloides* was obtained, and then the effects on wound healing were studied using a rat model of wound infection. Following the potential mechanism was studied referring to antibacterial activities, fibroblast proliferation, migration and collagen synthesis effect. This work will provide scientific basis for the development of infectious wound healing herbal medicine.

2. Methods

2.1. Plant material

E. phaseoloides (L.) Merr. was purchased from Dalian Baidu Medicine Co., Ltd., which was identified by associate professor Diao yunpeng at the College of Pharmacy, Dalian Medical University and stored in the college's pharmacognosy laboratory with a sample number of (XT003).

2.2. Preparation of extract

100 g *E. phaseoloides* (L.) Merr. powder was added 25-fold amount of water, then extracted with reflux at 100°C for 2 h. The powder was extracted for three times and filtrates were combined, concentrated to a solid–liquid ratio of 1:5, added with a certain amount of 95% ethanol till an ethanol precipitation concentration of 60%, and stored at 4°C for 12 h, and evaporated to dryness. Afterwards, elution was performed with a 6-fold volume of 50% ethanol through AB-8 resin. Then evaporated to dryness and stored at −80°C. Total tannin content in obtained extract was 76.18% by a casein assay [23].

2.3. Wound healing assay

2.3.1. Animal model and treatment

108 healthy male SD rats, weighing 200-220 g, were provided by the Animal Laboratory of Dalian Medical University [License No.: SCXK (Liaoning) 2008-0002]. All experimental procedures were approved by the Animal Research Ethics Committee of Dalian Medical University, Dalian, China (DMU10/02/23). Regularly fed rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g), fixed with limbs and placed in the prone position. After back hair removal and alcohol disinfection, epidermis was peeled off at the same site near the neck with sterile surgical instrument to form a circular wound 1.5 cm in diameter, with depth reaching the superficial layer of deep fascia. Gauze was cut into squares larger than the wounds and covered on the wounds. 1 ml of *Staphylococcus aureus* solution was dropped on the gauze in contact with the wounds, followed by dressing of wounds were observed after one day. Wound infection model was deemed successful if yellowish white pus was present on the wounds. The model rats were randomly divided into group A (bactroban group), group B (TEPT group) and group C (vaseline group), n = 36 in each group, and fed in separate cages. Medication was given once every other day. In group A, 5 mg of bactroban ointment (SFDA approval No. H10930064, Tianjin Smith Kline & French Laboratories Ltd.) was applied on the wounds of rats at an area slightly larger than the wounds. In group B, 5 mg of drug was mixed with vaseline ointment and applied on the wounds at an area slightly larger than the wounds (the dose was calculated based on the clinical dose of *E. phaseoloides* contained formula, Luofushan Fengshi ointment). In group C, 5 mg of vaseline ointment was applied on the rat wounds.

2.3.2. Wound healing status

Wounds were observed daily for presence/absence of infection, suppuration, status of healing, etc. General conditions of rats were observed, such as food and water intake and motion.

At five time points, i.e. 3 days, 7 days, 10 days, 14 days and 21 days after treatment, six rats were randomly selected from each group and killed. Film weighing method was used, that was, a transparent film was covered on the wound, marked along the edge of the wound, cut, and weighed on an analytical balance, which was then converted into the wound area. Wound healing rates in different time periods were calculated for each group according to the following formula: wound healing rate = (original wound area − unhealed wound area)/original wound area × 100%. Average time required for complete wound healing of rats in each group was recorded.

2.3.3. Histopathological analysis

Wound tissues of 3 days, 7 days and 14 days were harvested and soaked in formalin for histological analysis. Wound tissue was harvested immediately after the rat was anesthetized for euthanasia at various time points post-wounding. These tissue samples were orderly fixed in 10% paraformaldehyde, dehydrated in ethanol, and then embedded in paraffin before sliced into 5 μm thick tissue sections. Wound tissues sections were placed under the microscope for observation of inflammation, epithelial regeneration, vascular collagen formation, etc.