

Oral Wound Healing Activities of Pistacia vera Hydroalcoholic Extract

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Information	Abstract
Article Type: Original Article	Background and objectives: <i>Pistacia vera</i> seed (pistachio) is an edible seed with various therapeutic effects, such as antimicrobial, antioxidant, and anti-inflammatory properties. This study aimed to evaluate the oral wound healing effects of <i>Pistacia vera</i> seeds extracts (PSE). Methods: The hydroalcoholic pistachio extract was prepared by the maceration method. A wound was created in the central region of the tongue on the dorsal surface in each rat. The animals were randomly allocated to four groups (n=12). The treated groups orally received 0.5 ml/day of PSE (50, 100, or 200 mg/kg), and the vehicle group orally received 0.5 ml of 10% dimethyl sulfoxide. Each group was divided into three subgroups (n=4), and the animals were sacrificed on days 3, 7, and 10 to acquire the tongue specimens. The number of arteries, as well as the mean percentage of neutrophils, lymphocytes, and fibroblasts, was determined via histopathological examination. Results: The administration of 50 mg/kg of PSE for 10 days showed a beneficial effect on the number of neutrophils and fibroblasts. Conclusion: The present study showed that the hydroalcoholic extract of pistachio could be considered a potential treatment for oral wounds.
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1. Introduction

One of the most common lesions in the oral cavity is an ulcer [1]. The prevalence rates of oral ulcers have a wide range from 25% to 50% in different populations [2]. Ulcers are characterized by the loss of epithelium and the exposure of the connective tissue [3]. Oral ulcers occur due to different reasons, such as malignancies, local trauma, infection (bacterial, viral, fungal), and systemic diseases (metabolic or immunological) [4]. They induce pain or soreness, especially when eating foods [5]. Different methods are used to increase their healing speed, such as the administration of anti-inflammatory agents and antimicrobial medications, which have various side effects [6].

Pistacia vera (Anacardiaceae) has been known for its medicinal properties since ancient times [7, 8]. The seeds of *P. vera* (pistachio) contain phytochemicals such as oleoresins, triterpenoids, β -carotenes, phytosterols, fatty acids, α -tocopherols, and lutein [9, 10]. Moreover, pistachio extract has many pharmacological effects, including antimicrobial [11], antioxidant [12, 13], and anti-inflammatory [14] properties. It is well-documented that agents with anti-inflammatory, antimicrobial, and antioxidant properties have beneficial effects on wound treatment [15, 16].

To the best of the authors' knowledge, there is no standard treatment for oral ulcers, and the current drugs have various side effects. On the other hand, there is no previous report in the field of dentistry about the evaluation of the impacts of *Pistacia vera* seed extracts (PSE) on oral wound healing. Thus, this study was designed to investigate the effects of PSE on the healing of tongue ulcers in rats at three different time points (days 3, 7, and 10 of the experiment) by using histological evaluations and measuring different cells involved in the healing.

2. Materials and methods

Ethical considerations

The study was approved by the local ethics committee of Rafsanjan University of Medical Sciences (Ethical number: IR.RUMS.REC.1398.046) and performed in compliance with the standard ethical principles (NIH, publication no. 85-23, revised 1985; European Communities Directive 2010/63/EU).

Chemicals

Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich Company (Darmstadt, Germany), and Chlorohexidine was provided by IRAN NAJO Company (Tehran, Iran).

Pistachio hydroalcoholic extract

Dried Akbari pistachios (the plant authenticated by an expert person in Pistachio Research Institute of Iran; genetic code: M30) were collected from Rafsanjan, Iran, in 2018. To prepare the extract, Pistachio nuts were pulverized (100 g) and soaked in 1 L of ethanol (80%) for 72 h. The extract was then concentrated in a rotary under low pressure and stored at -20°C .

Animals

Forty-eight rats weighing 200-220 g were procured from the Rafsanjan University of Medical Sciences Animal House. The animals were kept under standard conditions (temperature: $20-23^{\circ}\text{C}$; 12-h light/dark cycle) and had ad libitum access to food and water.

Wound induction

An intraperitoneal injection of ketamine (60 mg/kg) and xylazine (6 mg/kg) was used to anesthetize the animals. After anesthesia, a wound was inflicted in the central region of tongues, 3 mm from the tip. The wound was created by a surgical scalpel blade (Number 11), with a length of 5 mm and a depth of 1 mm. The

lesion was restricted to the mucosa without muscular involvement [17].

Experimental groups

The animals were randomly divided into four groups, including vehicles and treatments (50, 100, and 200 mg/kg). The animals in the treatment group received 50, 100, and 200 mg/kg of the extract orally on a daily basis (in a volume of 0.5 ml). The extract doses were chosen based on our prior research [18]. For administration, the frozen extract was freshly dissolved in 10% DMSO. The first dose was administered on the day after the surgery. The animals in the vehicle group received 0.5 ml of 10% DMSO. Each group was divided into three subgroups at random: four rats were sacrificed after three days, four after seven days, and four after ten days. The animals were decapitated using a guillotine. The tongues were removed to conduct pathological tests.

Pathological examinations

The tongues were fixed and stained with hematoxylin and eosin (H&E). Six slides per animal were assessed by a blind pathologist to determine the number of arteries, as well as the mean percentage of neutrophils, lymphocytes, and fibroblasts [17].

Statistical analysis

Statistical analysis was conducted using the GraphPad Prism program (version 6.01, GraphPad Software, USA). The results were presented as mean±standard error of the mean (SEM). The Shapiro-Wilk test evaluated the normality of values. The differences between the groups were assessed using one-way analysis of variance (ANOVA) followed by the Tukey post-hoc analysis for parametric data and the Kruskal-Wallis test followed by Dunn's post-hoc analysis for non-parametric data. Statistical significance was defined as $p < 0.05$.

3. Results

According to the present investigation, the mean percentage of fibroblasts only increased significantly on the tenth day ($p < 0.05$) in comparison with the vehicle group after the daily administration of 50 mg/kg of pistachio extract (Fig. 1A and 2J). Moreover, the mean percentage of neutrophils decreased on the tenth day ($p < 0.05$) after the daily administration of 50 mg/kg of pistachio extract (Fig. 1C and 2J).

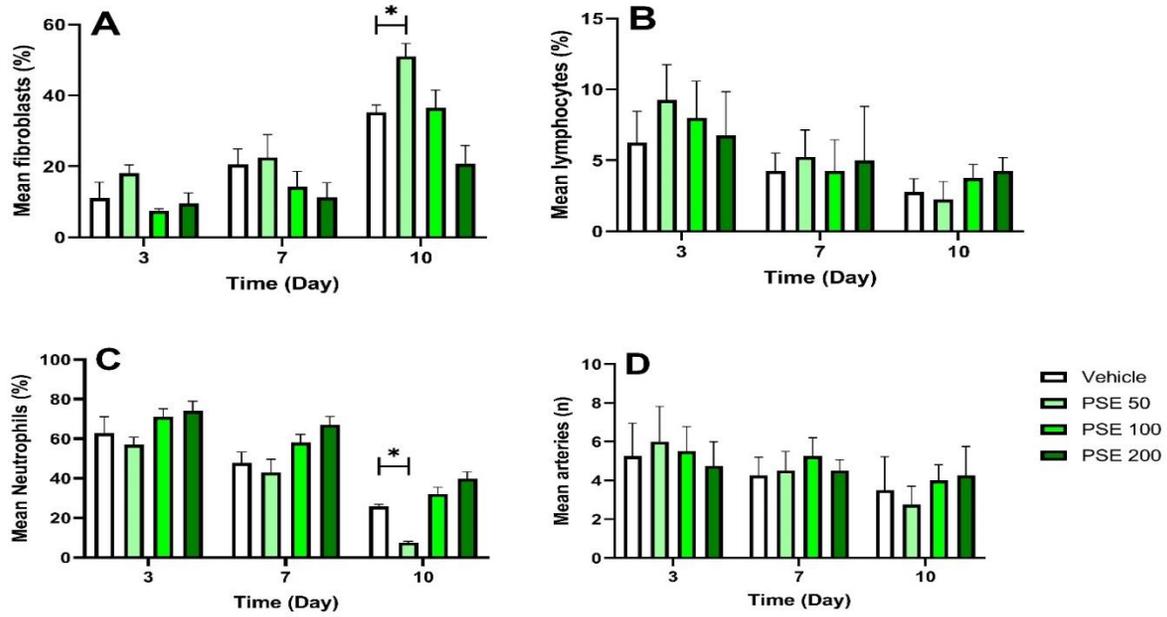


Figure 1. Effect of *Pistacia vera* seeds extracts (PSE) on the mean percent of fibroblast (A), the mean percent of lymphocyte (B), the mean percent of neutrophile (C) and the number of arteries (D) in different experimental groups. Data are expressed as mean \pm SEM. * $p < 0.05$ as compared with the vehicle group.

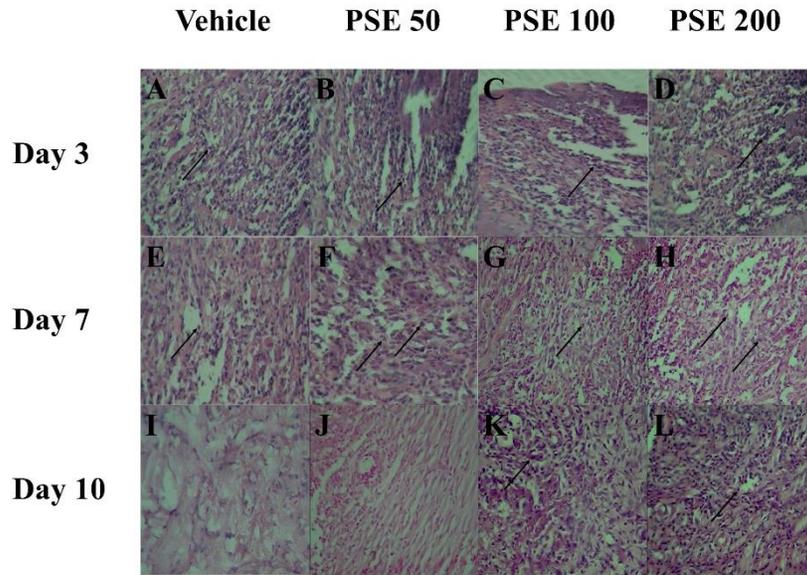


Figure 2. Histological assessments of tongue in different experimental groups (H & E, magnification X 100). PSE: *Pistacia vera* seeds extracts. Line arrows: neutrophil.

4. Discussion

Wound healing is initiated with inflammation, followed by repairing and remodeling [19]. The inflammatory phase is associated with leukocytes infiltrations such as neutrophils and lymphocytes [20]. Then, these cells release cytokines and growth factors, stimulating the endothelial cells for angiogenesis and fibroblasts for collagen deposition. Finally, the leukocytes are removed from the wound tissue, and an organized matrix is formed via collagen deposition by fibroblasts [5].

According to the present study, in all three evaluation stages, the number of fibroblasts and neutrophils in the PSE 50 group was higher and lower, respectively, than the vehicle group. The increasing process of the number of fibroblasts plays a role in collagen formation and wound healing, improving and accelerating the healing. Farahpour et al. reported an increase in fibroblasts in the wound area following the use of *Pistacia Atlantica* extract, which is consistent with the results of the present study [21]. On the other hand, neutrophils as a primary inflammatory cell are effective in the process of wound healing; however, this increase in the number of neutrophils must be replaced with macrophage and fibroblast cells in the later stages of wound healing. Our results demonstrated that this neutrophils replacement occurred only in the PSE 50 group.

In normal conditions, wound healing is a spontaneous process [22]. A variety of reasons can delay wound healing processes [23]. Therefore, early treatment of oral ulcers with various pharmacological anti-inflammatory and antimicrobial agents is essential in patients undergoing oral surgical treatments [22]. Plants have great potential for wound healing through antioxidant, anti-inflammatory, and antimicrobial mechanisms [24]. In a study,

Chamomile ointment was used to treat oral wound healing in rats. The researchers found that after 10 days of treatment, re-epithelialization and the formation of collagen fiber were stimulated; however, it did not influence the inflammation or fibroblast count [17]. Although some studies on the effects of the pistachio genus on different types of wound healing have been conducted, no research has investigated the effect of pistachio (*pistacia vera*) on oral wound healing. In line with our findings, Taghipour et al. investigated the effects of pistachio oil on burn wounds. They reported that 10% pistachio oil had beneficial effects on burn wound healing compared to dexpanthenol [25]. It has been shown that *Pistachio lentiscus* can heal stomach ulcers and reduce the size of the wound and bleeding area [26]. In another study, oleoresin, one of the active agents in *Pistacia lentiscus*, has been found to have the potential to heal episiotomy surgical wounds in women [23]. The resin of *Pistachio atlantica* has beneficial effects on burn wounds by increasing the concentration of the basic fibroblast and platelet-derived growth factors and angiogenesis [27]. Although most of these studies have been performed on other species of pistachio, they generally have the same chemical constituents. According to the present study's findings, pistachio extract did not affect the number of arteries. Considering that in the vehicle group, the number of arteries did not increase during 10 days, it can be concluded that studies with longer durations are needed to observe and evaluate the possible effects of pistachio extract or any other factors on wound angiogenesis in the animal model. In a study by Haghdoost et al., the effect of *Pistacia atlantica* resin on burn wound healing were attributed to the improvement of angiogenesis after 14 days [27].

5. Conclusion

This study demonstrated that oral consumption of PSE for 10 days at the dose of 50 mg/kg had a beneficial effect on the number of neutrophils, lymphocytes, and fibroblasts in oral ulcers in the animal model. The results also showed that administration of PSE at higher doses (100 and 200 mg/kg) had deleterious effects on the healing of oral wounds, thus requiring further study in this area to determine the exact mechanisms of this destructive phenomenon. Therefore, the current study opens new horizons in the clinical usage of PSE against oral wounds.

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Author contributions

SSS and IF conceived and designed the experiments. TE, and AM performed the experiments. SSS and MS analyzed the data. IF, and SSS contributed reagents/materials/analysis tools. IF wrote the paper. All authors read and approved the final manuscript.

conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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