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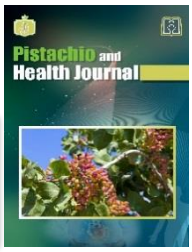

ORIGINAL ARTICLE

The effects of the topical administration of *Pistacia vera* oil on the second-degree burn model in rats

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Background: the subject of herbal remedy has attracted a lot of attention in common health fields, namely burn wounds. *Pistacia vera* (*P. vera*), being from the family of Anacardiaceae, possesses several important pharmacological properties. In this study, we aim at determining the healing effect of *P. vera* oil (topical administration) on the second-degree burn wound model of rats.

Materials and methods: After the induction of the second-degree burn wounds in the anesthetized animals using the hot plate, *P. vera* ointment was administered topically (5% and 10%) for three weeks in two groups of animals. Other groups were treated with dexpanthenol or base cream for three weeks. Macroscopic and histological assessments were made to analyze the wound healing process.

Results: data collected revealed that animals treated with *P. vera* 10% after 12 days of treatment showed significant repair in comparison with *P. vera* 5% or dexpanthenol. Moreover, after 22 days of the topical therapy, there was not any scar of wounds on animal skins which were under the treatment of *P. vera* 10%. Histological assessments showed that treatment with *P. vera* ointment is accompanied with a normal collagen deposition in the reticular layer as well as repaired epithelium in comparison with the base cream ($p<0.01$ for *P. vera* 5% and $p<0.001$ for *P. vera* 10%; in both parameters).

Conclusion: P. vera topical dosage forms may be effective treatments for the second-degree burn wounds, yet more studies are required to confirm the safety and possible repairing mechanisms.

Keywords: *Pistacia vera*; Oil; Burn wound; Healing

1. Introduction

Burn wounds are from among the common injuries all around the world [1]. It is estimated by the World Health Organization that every year there are approximately 265 thousand of death cases caused by burns to humans [2]. The half of incidents and burn-related deaths are occurred in the Southeast Asia [3]. Burn injuries are defined as destructions found in epidermal tissues, dermal tissues or deeper tissues caused by extreme heat, radiation, electricity or corrosive chemicals [4]. The optimal wound healing process occurs in different phases, including rapid hemostasis (vascular constriction and fibrin formation), inflammation (neutrophil or monocyte infiltration), proliferation, angiogenesis, prompt re-epithelialization, synthesis and the cross-linking of collagen. Wound healing and epithelization are the primary objectives pursued by burn management and therapy [5, 6].

A large body of evidence supports the use of plant extracts for the healing of burn wounds. *Pistacia vera* (*P. vera*) (family: Anacardiaceae) is a plant native to Iran and has commonly been used in traditional herbal medicine [7]. *P. vera* has a rich

nutrient profile comprised of factors such as α-tocopherol, β-carotene, lutein, selenium, flavonoids and phytoestrogens [8]. It is a well-established fact that *P. vera* has various pharmacological properties such as antioxidant (9], anti-nociceptive and anti-inflammatory effects [10, 11]. In previous studies done by the current researchers, antiepileptic, hepatoprotective and nephroprotective effects were reported for *P. vera* [12-14].

According to the literature review, the burn wound healing effect of *P. vera* has not been studied so far. The present study has been designed to examine the effects of *P. vera* oil on burn wounds in male rats.

2. Materials and Methods

2.1. Material collection, oil extraction and ointment preparation of the plant

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P. vera fruits (Pistachio) from Akbari species (genetic code of M30) were obtained from the herbal market in Rafsanjan, Iran in September 2017. In this study, a pressure system was administered (the cold extraction method) for the oil extracting process that is an easy method where the oil is preserved [15]. In order to study the wound healing effects of the pistachio ointment, two different concentrations (5% and 10%) of *P. vera* nut oil were prepared using FARABI base cream (FARABI Co., Tehran, Iran).

2.2. Animals and the experiment

All animals were collected from Rafsanjan University of Medical Sciences. A total of 28 male Wistar rats (weighing 200-250 g) was used in this study. Rats were housed in groups (four rates per cage) and maintained at a 12-hour light/dark cycle (lights on 07:00 to 19:00). Food and water were provided *ad libitum*, and the room temperature was maintained at 23±2.0 °C. Animals got habituated to the housing conditions for one week before the experiments. All experimental procedures were followed in accordance with the guidelines for the care and use of laboratory animals in Rafsanjan University of Medical Sciences as well as the Principles of Laboratory Animal Care (NIH publication #85−23, revised in 1985).

Rats were randomly assigned to four experimental groups as follows (each experimental group included seven rats):

Base group (the wounds were treated with the base cream for three weeks).

DEX group (the wounds were treated with the dexpanthenol cream for three weeks).

PIS 5% group (the wounds were treated with the pistachio ointment at the 5% concentration for three weeks).

PIS 10% group (the wounds were treated with the pistachio ointment at the 10% concentration for three weeks).

2.3. Second-degree burn wound induction

Rats were anesthetized by the intraperitoneal injection of ketamine (60 mg/kg) and xylazine (8 mg/kg). The dorsum was shaved using an electrical clipper, and 70% alcohol was used to disinfect the area. General anesthetized rats were kept in prone position. A deep second-degree burn wound was induced by a hot plate (a standard area of 3 × 3 cm) heated for 5 minutes in the boiling water and placed for 10 seconds on the skin of rats. The pressure applied to the animal skin corresponded to the mass of 51 grams of the aluminum bar used in the burn induction process [16, 17].

2.4. Histological examinations

In order to quantify the wound healing process, the histological examinations of lesions were determined on day 22 after the burn injury. All histological procedures were followed by an independent blind observer. From each sample, four slides were prepared using sterile scalpels and tissue forceps. Burned skin tissue samples were taken for histological studies with a small excision containing a part of the wound area. Tissue samples were fixed in a 10% formalin solution. The paraffin embedded tissue section of 5 μm was prepared and stained with hematoxylin and eosin (H & E). Light microscopy (Olympus BX51 microscope) was used to assess pathological changes. All specimens were examined for two pathological parameters, including epithelialization and the collagen deposition using a semiquantitative scoring system from 1 to 4 [4].

2.5. Statistical analysis

The statistical analysis was performed using the GraphPad Prism version 6.01 for Windows (GraphPad Software, USA). Data were expressed as mean ± S.E.M., and the differences among the groups were tested by the analysis of the variance (one-way ANOVA) followed by the Tukey post-hoc test. Differences among means were considered statistically significant, if $p<0.05$.

3. Results

3.1. The wound-healing effect of *P. vera* ointment based on observational documentation

All alterations in the wound healing process from day zero after the wound induction process to day 22 were recorded on a camera. Observations showed that in the group receiving only the base of *P. vera*, ointment wounds are not completely healed after 22 days. However, in animals treated with DEX, PIS 5% or 10% ointment, a general healing has occurred. The main observation indicated that 10% *P. vera* could positively affect the wounds in the middle of the treatment process (after 12 days), and it also showed a rapid pattern of healing as against other groups. Other achievements of the observational data indicated that the animals treated topically with PIS 10% had no wound scar after 22 days, yet the groups treated with DEX or PIS 5% had scars after the treatment termination period. The interpretation of such observations indicates that the use of the PIS 10% ointment may be an effective method in healing burn wounds (Fig. 1).

3.2. The histological assessments of wounds treated with *P. vera* ointment

After the H & E staining of the tissue, the wound-healing process was evaluated from histological aspects. As depicted in Fig. 2A, the epithelium layer is not completely healed in the base-treated animals, and a thin epithelium is observed (black arrows) with the burn scar being detectable on the newly formed epithelium (the white circle object). In the groups under treatment with PIS 5% ($p<0.01$) and PIS 10% or DEX ($p<0.001$), the epithelium is significantly healed compared with the base cream (Fig. 3). In the DEX group, the epithelium morphology is normal, and the PIS 10% is as effective as DEX (as depicted in Fig. 3). In animals treated with PIS 5%, epithelialization has occurred, but the related score is lower than the score related to DEX and PIS 10% ($p<0.05$, Fig. 3).

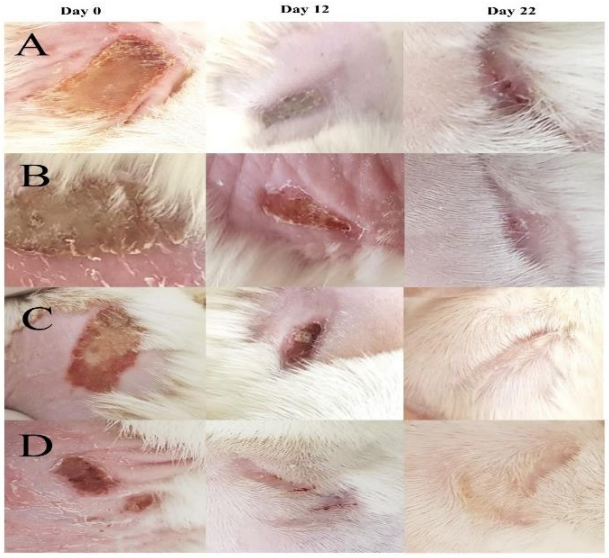

Fig. 1. The observational report of topical administration of base, dexpanthenol and *P. vera* ointment (5% and 10%) in rats with second degree burn wound (macroscopic morphology). (A) base, (B) dexpanthenol, (C) PIS 5% and (D) PIS 10% groups.

The collagen deposition has been shown using white arrows in the dermis layer (Fig. 2). In the groups treated with PIS 5% and DEX or PIS 10%, the papillary layer has been repaired, and the collagen deposition in the reticular layer is significantly higher compared with the base group (P<0.01 and P<0.001 respectively, Fig. 4). In the group treated with PIS 5%, the collagen deposition in the reticular layer is lower than the group treated with PIS 10% or DEX (p<0.05, Fig. 4).

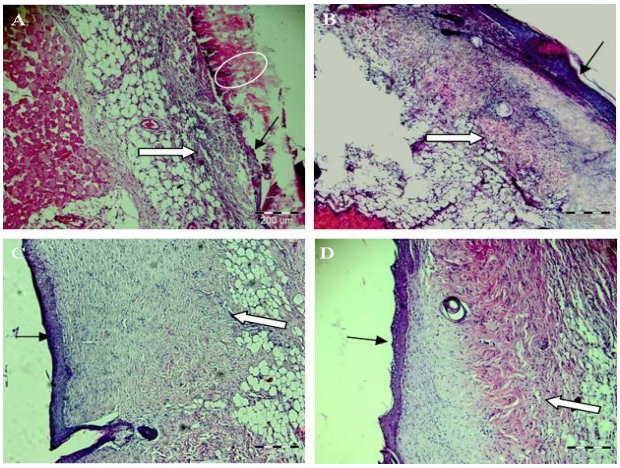

Fig. 2. The microscopic view of rat skins treated with base (A), dexpanthenol (B), PIS 5% (C) and PIS 10% (D) after hematoxylin and eosin staining (10x). Epithelium layer: black arrows. Newly formed epithelium: white circle object. Collagen deposition: white arrow.

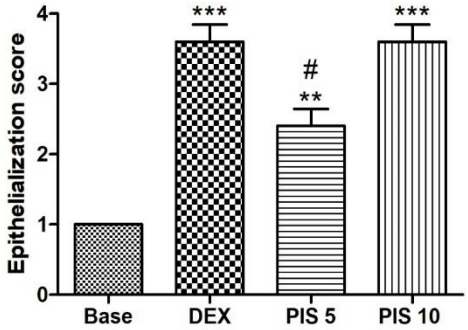

Fig. 3. The epithelialization scoring in different groups. Data has been reported as mean ± S.E.M. DEX: dexpanthenol; PIS 5 (*P. vera* ointment 5%); PIS 10 (*P. vera* ointment 10%). **p<0.01 and ***p<0.001 in comparison with base. #p<0.5 compared with DEX. Data analyzed by one-way ANOVA followed by the Tukey post-hoc test.

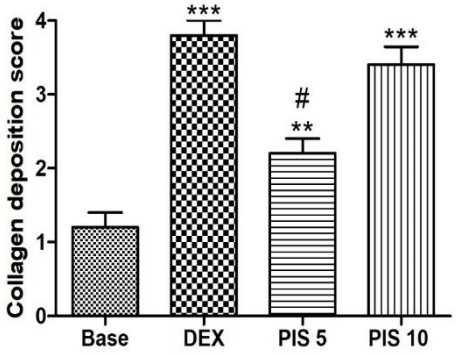

Fig. 4. The collagen deposition scoring in different groups. Data has been reported as mean ± S.E.M. DEX: dexpanthenol; PIS 5 (*P. vera* ointment 5%); PIS 10 (*P. vera* ointment 10%). **p<0.01 and ***p<0.001 in comparison with base. #p<0.5 compared with DEX. Data analyzed by one-way ANOVA followed by the Tukey post-hoc test.

4. Discussion

The study data showed that the topical treatment of animals with second-degree burn wounds using *P. vera* ointment (10%) leads to a more rapid repair of wounds, in comparison with the base group, dexpanthenol and *P. vera* (5%), and that it leaves no scar on animal skins. The histological data reported that animal skins treated with *P. vera* ointment is accompanied with a denser collagen deposition in the reticular layer as well as repaired epithelium.

Herbal therapy plays a critical role in Asian countries, specially Iran, and in the Iranian folk medicine, *Pistacia* has been used for the treatment of wound inflammation [18]. As the plants are cheap and safe, the pharmaceutical preparations of such natural materials can be of great importance in healing wounds. Previous studies explored the effects of the topical administration of different species in the genus *Pistacia* on the wound-healing process. Haghdoost et al., showed that *Pistacia atlantica* resin extracts improve the burn-wound

healing process in a rat model by enhancing the concentration of the platelet-derived growth factor and the basic fibroblast growth factor, and that it increases the angiogenesis in a concentration-dependent manner [16]. The study done by Farahpour et al., revealed that the hydroethanolic *Pistacia atlantica* hulls extract may improve the wound-healing process through several mechanisms such as affecting inflammatory phases, upregulating mast cell infiltration, accelerating the proliferation phase, lowering the RNA damage rate and upregulating the hydroxylproline content [18]. Another study demonstrated that the co-administration of flaxseed and *Pistacia atlantica* oil (in ointment formulation) improves the wound-healing process in rabbits by elevating cellularity, collagen synthesis induction and inflammation blockade [19]. There is a report on the effectiveness of the methanolic *Pistacia khinjuk* extract on the wound-healing process in rats [20]. To the knowledge of the current researchers, there is not enough documents verifying the role of *Pistacia vera* in the wound-healing process. In line with other investigations, the data gathered showed that *P. vera* topical ointment promotes the healing process in rats suffering from second-degree burn wounds by repairing epithelium, improving the collage layer in dermis and reducing the wound size. Moreover, the topical preparation provided accelerated the healing process at 10% concentration.

The species of pistachios in Iran, including *Pistacia vera*, *Pistacia khinjuk*, and *Pistacia atlantica* possess various active ingredients such as alpha-pinene, beta-pinene, alpha-tocopherol, flavonoids and phytosterol that can account for their pharmacological effects [16, 21-23]. *Pistacia* species have a vast range of pharmacological effects, including anti-inflammatory, cardioprotective, neuroprotective, hepatoprotective and nephroprotective, antibacterial and antiviral properties [7, 13, 14, 20].

The developing of efficacious and safe herbal remedies to heal burn wounds as a common health problem and accelerating the healing process as well as the infection blockade process seem to be necessary [24-26]. On one hand, as the pharmacological use of *Pistacia* is wide ranging and on the other hand, as it is mainly produced in Iran specially Kerman, it would be of high significance to develop the finished products of *P. vera* in the pharmaceutical industry (topical dosage forms or systemic products) to develop it socioeconomically and treat common diseases and adopt suitable pathologies.

5. Conclusions

To conclude, the results of the current study on the evaluation of the *P. vera* oil ointment in rats with the-second degree burn wound can support the possibility of a topical dosage form of the *P. vera* use as a new therapeutic method in healing wounds. However, more studies are required to clarify the exact mechanism of its action and effects on humans or the potential adverse reactions in the future.

6. Conflict of interest

The authors declare no conflicts of interest.

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