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Original article

Ameliorative Effects of *Pistacia atlantica* Resin Oil on Experimentally-Induced Skin Burn in Rat

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Abstract

Background and objectives: Severe burn damage and its consequences are life threatening which can complicate patients' health. Burn damage increases reactive oxygen species (ROS) generation which leads to severe damage to tissues and is implicated in burn shock. Some medicinal and traditional plants are considered as safe, natural and inexpensive sources for treatment of different of diseases. *Pistacia atlantica* has shown anti-inflammatory and anti-oxidant properties and has also been used traditionally as an ointment for wound healing in some parts of Iran. In this study, the beneficial effect of *P. atlantica* resin oil on rats' burn wound healing and its potential effects on vascular endothelial growth factor (VEGF), hydroxyproline and antioxidants in wound area was examined. **Methods:** Thirty male rats (200±10 g) were randomly and divided into three groups (n=10) as follow: Group 1: burn injury, Group 2: burn injury receiving 300 µL/kg/day *P. atlantica* resin oil topically, Group 3: burn injury receiving 300 mg/kg/day sulfadiazine cream topically. At the end of the study (day 14) the area of wounds were measured and then skins with burn damage were dissected and anti-oxidative parameters, VEGF and hydroxyproline were evaluated. **Results:** We found that *Pistacia Atlantica* oil significantly increased antioxidant defense, VEGF and hydroxyproline and reduced malondialdehyde (MDA) levels. *Pistacia atlantica* remarkably reduced wound size compared to burn the control group and showed more beneficial effects compared to sulfadiazine as the positive control. **Conclusion:** *Pistacia atlantica* resin oil could be considered as a new therapeutic agent for treatment of injuries such as burn damages.

Keywords: antioxidants; injury; malondialdehyde; *Pistacia*; vascular endothelial growth factor

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Introduction

Burn damage and its consequences lead to major problems which can complicate patients' health. Seriously burned patients need strict regular care including wound healing care and treatment, nutritional supports and control of probable infection [1]. Pathophysiological changes in burned area are caused by increased tissue temperature leading to inflammatory responses; also thermal exposure can cause necrosis in burn wound area [2]. Increased reactive oxygen

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species (ROS) lead to severe damage to the cells in the burnt area [2]. Wound healing is a dynamic process which consist three phases including inflammation, proliferation and maturation. Cytokines, reduced local ischemia and ROS have pivotal roles in this process. ROS are one of the components which participate in tissue damage. Also following thermal injury, ROS have been considered to participate in a number of pathophysiological steps including burn shock [1,2].

There are some complications which are followed by burn damages such as sepsis which is the leading cause of mortality in burn units and infection that is one of the major problems correlated with burn injuries [4]. Although antibacterial remedies are used to eliminate infection from the burn area, infection is still a problem to be solved [4]. Vascular endothelial growth factor (VEGF) is a multi-action growth factor that facilitates wound healing and helps tissue repairing [4,5]. VEGF increases inflammatory cells in the damaged area and also promotes migration and proliferation of endothelial cells [4,5]. Collagen is an extracellular matrix protein which is related to wound healing and strength. Hydroxyproline is an amino acid in collagen structure and its levels in wound parts could be a marker for wound healing rate [6].

Some medicinal and traditional plants are considered good, safe and inexpensive remedies for many diseases [7-9]; therefore, a wide range of plants are used as pharmacological agents against diseases and for improving body health [7-10]. It has been reported that Emu oil, derived from *Dromaius novaehollandiae*, It has been reported that Emu oil, derived from *Dromaius novaehollandiae*, could postpone the wound healing by affecting the inflammatory process [8]. Another study has demonstrated that *Capparis spinosa* leaves hydro-alcoholic extract attenuated inflammation and also promoted wound healing process [7].

One of these traditional plants that is commonly used is *Pistacia atlantica* which has been reported for many potential beneficial effects [10-15]. *Pistacia atlantica* is a plant which is widely distributed in Algeria, Iran, Iraq and turkey and is used in chewing gum and as mouth freshener [9,11]. *Pistacia atlantica* resin has been used as a traditional treatment for peptic ulcer disease [11]. There are some studies about wound healing and anti-inflammatory properties of *P. atlantica* resin in animal models and also as traditionally a remedy for wound healing in some parts of Iran [9,16]. Peksel et al. have shown that aqueous extract of *Pistacia atlantica* leaves have radical scavenging properties [17]. Also other studies approved the antioxidant activities of *P. atlantica* [12,14,18]. It has been documented that *P. atlantica* could be considered as a treatment for digestive diseases. It has also been traditionally used for treatment of disorders such as colitis, gastrointestinal problems, kidney, heart and liver complications [9,10]. Other studies have reported the antifungal, anti-parasite and antibacterial activities of *P. atlantica* [13-15].

In the present study, the ameliorative effects of *P. atlantica* resin oil on wound healing in rat and also its effects on VEGF, hydroxyproline, and antioxidant status in wound area were evaluated.

Material and Methods

Ethical considerations

The study was approved by the ethic committee of Kerman University of Medical Sciences, Kerman, Iran (No. IR.KMU.REC 940251; 8/16/ 2016).

Plant material

Pistacia atlantica resin oil was prepared from Hakim-Tehrani Co. (Kerman, Iran) which was traditionally prescribed for treatment of disorders such as digestive and gastrointestinal problems.

Animals

Thirty male Sprague-Dawley rats weighing 200±10 g were obtained from the animal care center of Neuroscience Research Center, Kerman, Iran. The animals were maintained at controlled condition, 25±1 ºC and 12 h light-dark cycle and had access freely to standard chow diet and water.

Burn injury

The animals were anesthetized by i.p. injection of Ketamin and Xylazine (60 and 4 mg/kg respectively). The dorsal side of the animals were shaved and then induction of burn damage was conducted by an aluminum plaque (2.5×2.5 cm) on the shaved skin of rats for 15-20 seconds which was heated to 100 ºC to create a deep dermal burn wound [20,21].

Study procedure

Duration of the study was 14 days after burn

injury and the animals were divided into three groups (n=10) randomly as follow:
Group 1: burn injury; Group 2: burn injury receiving 300 µL/kg/day *Pistacia atlantica* resin oil topically; Group 3: burn injury receiving 300 mg/kg/day sulfadiazine creams topically.
At the end of the study, the animals were deeply anesthetized by ether and sacrificed; burned skin were incised and separated afterwards. The samples were homogenized by ultrasonic processor (Hielscher, UP200H, Germany) in cold PBS (pH 7.4) and then centrifuged at 4 °C, 15000 rpm, for 15 min. Supernatants were separated into parts as aliquots, then maintained at -80 °C until further experiments. Superoxide dismutase (SOD), glutathione peroxidase (GPX), total antioxidant status (TAS), malondialdehyde (MDA), VEGF and hydroxyproline were measured in the supernatant [16].

Wound contraction assessment
The wound contractions were reported by following formula [22]:

% Wound Contraction = (wound area [day 1] - wound area [day 14] / wound area [day 1] × 100

Measurement of VEGF, hydroxyproline, MDA, TAS, SOD and GPX
Measurement of these parameters was conducted by specific kits; Hydroxyproline ELISA assay kit E0511Ra, Crystal Ray Biotech inc.; VEGF ELISA assay kit E0659Ra, Crystal Ray Biotech inc.; which is based on ELISA sandwich formation. According to the kit protocol, the first antibody is coated at wells and the VEGF or hydroxyproline is captured by this antibody (depending on kit), the second antibody forms ELISA sandwich and the yellow color formation after the addition of the stop solution to the reaction is measured at 450 nm;the color formation is proportional to the VEGF or hydroxyproline levels at the sample. SOD, GPX and TAS measurements were conducted by using specific kits supplied from RANDOX laboratories Ltd. (TAS, Cat. No. NX2332; SOD, Cat. No. SD125; and GPX, Cat. No. RS505). The quantifications of antioxidants was according to RANDOX kits protocol which is briefly stated for each parameters. For TAS measurement, the blue-green color of the radical cation ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is quantified at 600 nm. Antioxidants present in the sample result in suppression of blue-green color generation which is proportional to their concentrations. GPX measurement is based on the decrease in absorbance at 340 nm which is caused by oxidation of NADPH to $NADP^+$. SOD accelerates the dismutation of the superoxide radical (0_2) to hydrogen peroxide and molecular oxygen. In this method also, xanthine and xanthine oxidase are used to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The SOD activity was measured by the degree of inhibition of this reaction. MDA was measured as thiobarbituric acid-reactive substances (TBARS) at 534 nm and 1,1,3,3-tetramethoxypropane was used for preparation of plot calibration curve [16].

Statistical analysis
The data were expressed as mean±SEM. For comparison between groups One-way ANOVA test followed by post hoc Tukey's was performed and $p<0.05$ was considered as statistically significant.

Results and Discussion
The results of the present study showed that *P. atlantica* resin oil significantly increased antioxidant defense, VEGF and hydroxyproline levels and reduced MDA levels compared to the burn control group (table 1). Also, *P. atlantica* compared to sulfadiazine significantly increased SOD, GPX, TAS and hydroxyproline (table 1). *Pistacia atlantica* remarkably reduced wound size compared to burn control group (table1)
Bozorgi et al. reported that the gum resin of *P. atlantica* can be used for wound healing and treatment of gastrointestinal problems [9]. Previous studies have shown that *Pistacia atlantica* resin oil contained α-pinene (about 45-70%) as the major components and as the possible effective substance [9,22-24]. Memariani et al. demonstrated that 2g/kg of *P. atlantica* oil was not harmful or toxic in vivo. They also reported that *P. atlantica* oil showed protective effect against experimentally ethanol induced gastric ulcer [25]; also, α-pinene has been reported to be low in toxicity [18].
Pistacia atlantica oil anti-oxidative properties have been reported previously [12,17,26]. Rezaie et al. have reported that the oil showed remarkable antioxidant activities compared to the positive controls (ascorbic acid and α-tocopherol) [14].

Table 1. Wound size, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX) and total antioxidant status (TAS) at studied groups treated with *Pistacia atlantica*

	Wound contraction (%)	MDA (nmol/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	TAS (mmol/mg protein)	VEGF (pg/mg protein	Hydroxyproline (µg/mg protein)
Burn non-treated control	71.2± 3.4	6.1 ± 0.4	2.0 ± 0.35	7.9 ± 0.07	0.62 ± 0.04	0.56 ± 0.04	42.5 ± 1.7
Burn- treated with *Pistacia atlantica*	98.6± 2.5*	1.5 ± 0.14*	6.0 ± 0.43*#	25.1 ± 1.2*#	1.93 ± 0.1*#	3.1 ± 0.19*	53.6 ± 1.8*#
Burn- treated with Sulfadiazine	94.7± 4.1*	2.2 ± 0.22*	4.7 ± 0.35*	19.0 ± 1.18*	1.52 ± 0.07*	2.4 ± 0.22*	49.2 ± 1.46*

*significant compared to control group, # significant compared to sulfadiazine treated group, (n=10), $p<0.05$ considered as statistically significant

Koizumi et al. showed that severe burn damage caused elevation of free radicals which in turn resulted in vasodilatation and SOD could act as a protective factor against vasodilatation [27]. The present study proved that compared to the burn control group, *P. atlantica* resin oil significantly elevated SOD levels which can be considered as a protective effect against damages produced by burning. Farahpour et al. have reported that *P. atlantica* hydroalcoholic shell extract showed antioxidant activity even higher than ascorbic acid; their finding are consistent with our anti-oxidative data about *P. atlantica* resin oil.*Pistacia atlantica* resin oil has shown antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi* and *Escherichia coli* [15,26]; therefore, *Pistacia atlantica* resin oil not only increased antioxidant status but also posessed antimicrobial properties. It also reduced the rate of infections and accelerated the process of wound healing.

Galiano et al, demonstrated that topical VEGF increased growth factor in the damaged area and also recruited bone marrow derived cells which have remarkable role in wound repair. All together they showed that topical application of VEGF had wound healing properties [5]. Haghdoost et al. reported that *P. atlantica* increased bFGF and PDGF and therefore resulted in angiogenesis [20]. The present research showed that *P. atlantica* resin oil promoted VEGF levels in wound area in burn model injury in rats and this could explain its beneficial effects on wound contraction and repair. Therefore, Galiano et al. and haghdoost and colleagues reports were confirm our data regarding VEGF variations in wound area [5,20].

Collagen is an extracellular matrix protein which is related to wound contraction and strength. Collagen turnover is directly related to free hydroxyproline; thus, quantification of hydroxyproline can be considered as a good parameter to monitor collagen turnover [23,28]. Hamidi and colleagues showed that *P. atlantica* oil changed collagen pattern and caused organized collagen fiber after three weeks [29]. Farahpour et al. showed that *P. atlantica* hydroalcoholic shell extract applied as an ointment increased hydroxyproline content and histological studies showed that collagen score increased significantly. Also, they found that the extract promoted fibroblasts proliferation and therefore declined inflammation [23]. IIango et al. working on a plant extract evaluated the effects of *Limonia acidissima* methanol extract on SOD, catalase, hydroxyproline, and epithelialization. They found that methanol extract of *L. acidissima* increased SOD and also promoted hydroxyproline and epithelialization, therefore showed significant dose dependent wound healing activity [28]. The present study showed that *P. atlantica* resin oil increased hydroxyproline levels in wound area and this could be considered as a collagen turnover marker. Therefore, wound healing and contraction activity of *Pistacia atlantica* resin oil can be related to elevation of collagen turnover and consequently hydroxyproline.

Epithelialization is considered as an important factor which serves as a defining parameter of affluent wound contraction [29]. Mehrabani et al. used *P. atlantica* oil in combination with three other oils from sesame (*Sesamum indicum* L.), hemp (*Cannabis sativa* L.) and walnut (*Juglans regia* L.) in combination. The new mentioned formula increased wound contraction and accelerated epithelialization [21]. Hamidi et al. showed that *P. atlantica* oil in a gel formulation ameliorated epithelialization which is as a marker of wound contraction. Also they showed that topical *P. atlantica* oil in rat with experimental wound caused biochemical and morphological

promotion compared with the control group [29]. According to previous reports, increased epithelialization and other histological changes account for the potential mechanism of *P. atlantica* oil benefits. Elevation of VEF and antioxidant status by *P. atlantica* resin oil increase its efficacy as a good treatment for burn injuries and other damages. Finally, regarding the previous studies about beneficial properties and low toxicity of *P. atlantica* resin oil and our findings about the antioxidant effects and increase in VEGF and hydroxyproline, the oil can be considered as a safe and potent candidate in new therapeutic topical applications for wound healing.

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Author contributions

Beydolah Shahouzehi did the supervision, experimental measurements, manuscript preparation and statistical analysis; Gholamreza Sepehri was involved in supervision, study design and manuscript preparation; Sakineh Sadeghiyan designed the study and was involved in experimental measurements; Yaser Masoumi-Ardakani contributed in the experimental parts and manuscript revision.

Declaration of interest

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

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Abbreviations

ABTS: 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]; GPX: glutathione peroxidase; I.N.T: 2-(4-iodophenyl)-3-(4-nitrophenol)-5 phenyltetrazolium chloride; MDA: malondialdehyde; ROS: reactive oxygen species; SOD: superoxide dismutase; TAS: total antioxidant status; TBARS: thiobarbituric acid-reactive substances; VEGF: vascular endothelial growth factor