Effect of *Pistacia atlantica* Mastic Extract on Experimental Wound Healing and Various Biochemical Parameters of Blood Serum in Rabbit Models

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Abstract

Background: The mastic extract of *Pistacia* species can be considered as a natural herbal source in the pharmaceutical industry.

Objective: The purpose of this study was to evaluate the effectiveness of *Pistacia atlantica* mastic on wound healing, oxidative and blood biochemical parameters.

Methods: Topical cream and oral suspension was made of the mastic of *P. atlantica*, after preparing hydroalcoholic extract and essence. 40 male rabbits were selected (4 experimental groups and 4 control group). To induce oxidative stress and tissue damage the burn wound were created. Topical cream (1 gram 60% and 30%, 3 ml oral suspension 60% and 30%, respectively were used daily in treatment groups for 21 days. Inert cream and distilled water were used topically and orally in the control group, respectively. The oxidative parameters of blood serum including malondialdehyde (MDA), catalase, glutathione peroxidase, superoxide dismutase and biochemical parameters of low density and high lipoproteins, as well as glucose level were measured on day 1, 7, 14 and 21. The healing process and grading were evaluated by histological examination on day 21.

Results: Increased concentration of MDA indicated the appearance of oxidative stress. oncentrations of SOD, CAT, GPX showed significant increases as compared with the control group and in day 1 (P≤0.05). The level of LDL did not show any difference in experimental group.

Conclusion: The results show that mastic extract of *P. atlantica* has an appropriate effect on wound healing and can be used as a natural antioxidant.

Keywords: *Pistacia atlantica*, Antioxidant, Blood parameters, Hydroalcoholic extract, Wound-healing

Introduction

Herbal medicine has a long history in treatment of several diseases. Various plant parts (leaves, stems, root and bark) are used for medicine preparation, because they contain biologically active ingredients, for treating mild or chronic ailments. Alkaloids, tannins, favonoid and phenolic compounds are the most important bioactive constituents of plants [1]. The appropriate uses of botanical plants to treat various diseases are gaining new interests [2]. *P. atlantica* is a plant member of Anacaridaceae family and is native to Asia. Pistachio nut is mostly produced in Iran and some other countries [3]. *Pistacia* species have caught up the interests of researchers as study of different parts of this plant such as leaves, kernels and hulls demonstrate its various biological properties such as antioxidant, antimicrobial and anti-inflammatory potential, mainly due to the presence of various phenolic components [4]. *P. atlantica* genus and sub species of *kurdica* has wide coverage significantly in the Zagros Mountains, particularly in west and northwest of Iran, Turkey and Iraq. [5, 6]. The leaves are used for the treatment of eczema, hypoglycemia, diarrhea and throat infections coughs, asthma stomach aches, kidney stones, jaundice and as antibacterial [7]. In a study, it was shown that, total phenolic and flavonoid contents of the hull extracts were significantly higher than shell and kernel extract [8]. Antioxidants, as a defense system, are capable of protecting the body from oxidative damages caused by free radicals; in fact the antioxidants can react with free radicals and convert these compounds at least partially into the harmless substances. Free radicals are mainly oxygen molecules or atoms that have at least on unpaired electron in their outermost orbit. In the process of utilization of oxygen during normal metabolism within the cell to create energy, active free oxygen radicals are created. These free radicals essentially have an electrical charge and need an electron from any molecule or substance in the vicinity to produce reactive oxygen species (ROS). Antioxidants stop this free radical chain reaction by slowing down the other oxidation reactions and removing ROS. Body has been equipped with defense mechanisms against free radicals; of enzymes antioxidants (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic antioxidants (vitamins, A, C, E, flavonoids, uric acid, iron, copper, zinc, selenium and manganese). Imbalances between the production of free radicals and antioxidant defense mechanisms cause oxidative stress and provide the basic for pathogenic factors activity. Free radical oxidative stresses have caused a wide variety of clinical disorders [9-13]. The natural antioxidants come mainly from plants in the form of phenolic compounds (flavonoids, phenolic acids and tocopherol) [14]. Catalase is a common enzyme found in mammalian and non-mammals cells and catalyzes the decomposition of hydrogen peroxide to water and oxygen. The oxidative destruction of lipids by (ROS) is called lipid peroxidation. Lipid peroxidation takes place usually on unsaturated fatty acids and its final products are known to be active aldehydes such as malondialdehyde. Lipid peroxidation level can be revealed by measuring malondialdehyde

levels in biological samples and can be used as an indicator to measure the level of oxidative stress in a living organism. The presence and activity of superoxide dismutase enzyme is one of the most important antioxidant mechanisms of the body against invasion of reactive oxygen species. Another antioxidant factor is glutathione peroxidase that prevents the conversion of lipid peroxides to alcohol and hydrogen peroxide to water [15]. A number of studies have shown beneficial effects of *P. lentiscus*. *P. lntiscus* fatty oil has anti-lipid properties at least in reducing total cholesterol and triglycerides [16]. It is hypothesized that consumption of *P. atlantica* extract as an antioxidant increases activity of anti-oxidative enzymes and reduces cholesterol levels. Andrikopoulos et al. (2000) in a study also reports that mastic gum causes the oxidation of LDL and has a good inhibitory effect on LDL oxidation in a dose of at least 2.5 mg (5/75%) and at the maximum dose 9.99 mg (50 %) [17]. Unsaturated fatty acids have been reported to result increased glucose tolerance and insulin sensitivity and leads to in decreased serum glucose and insulin level in one day [18].

Development of an ideal wound healing herbal drug is still a challenge to the medical scientists. Earlier investigations have demonstrated that flavonoids, triterpenes and other phenolic compounds hinder free radicals formation, and hence act as antioxidants. Based on the traditional uses of the parts of *P. atlantica* (such as leaves, kernels and hulls) we selected this plant for this study [4]. However, the wound healing and antioxidant properties of *Pistacia atlantica* sub spp. *kurdica* have not been scientifically investigated already. The purpose of this study was to evaluate the effect of *P. atlantica* mastic extract in Kurdistan of Iran in activating the body's natural antioxidant system as well as its effect on the wound healing and in the case of reaching the favorable results with significant effects, this extract can be considered as a natural herbal sources in the pharmaceutical industry.

Materials and Methods

Animals

All animals used in the experiments were handled in compliance with the National institute of health guide for the use and care of laboratory animals were approved by the local ethical committee. In this study, 40 healthy inbred male albino rabbits were obtained from experimental animal house, veterinary collage, University of Sanandaj, Iran, in the same condition of weight and age (5 month age and 1.5±0.1 kg).The animals were kept in separate cages for 7 d at room condition to acclimatize.

Chemical, reagents, drugs and solvents

All chemicals were used of analytical grade and include: ethanol 96% (sigma- Aldrich, Germany), liquid nitrogen, distilled water, liquid paraffin, hematoxylin - eosin dye were purchased from Merck. Other reagents were prepared as; Acepromazine and ketamine Hcl 10% (Alfasan, JA Woerden, Netherland), MDA, SOD, CAT, GPX, LDL, HDL and glucose kits (Parsazmoon, Iran).

Equipment/ Instruments

The equipment and instruments used include: electrical animal weighting balance (B. Bran scientific and instruments Co., England), incubator, centrifuge (B. Bran scientific and instruments Co., England), UV-visible spectrophotometer (Easy – way Medical England, England), soxhlet- apparatus and rotary evaporator (B. Bran scientific and instruments Co., England), microscope (Carl Zeiss GmbH Hingen, Germany) and filter paper (Whatman No.1).

Collection and preparation of plant material

Fresh mastic gum of *P. atlantica* was collected from a forest in Marivan city of Kurdistan provinces, Iran. Mastic gum was collected as exudates after hurting the trunk of tree. Specimens were deposited and authenticated at the department of botany sciences, the Kurdistan research agriculture and natural resources center, Kurdistan, Iran (No.6673).

Extract preparation:

Mastic gum was submitted extraction with ethanol 96% (v/v) in a suxhlet apparatus. The resultant extraction was evaporated to dryness under reduce pressure in rotary vacuum evaporator 45 ºC and the following method was applied to make topical creams and oral suspension in laboratory. Mastic gum (60 g) in a 500 ml round bottom flask was set to a prescribed distillation apparatus, so boiling time and the output of the first drops of the essential oil were recorded. After 1.5 h the essential oil was obtained using hydraulic methodology, and then 150 ml ethanol 96% was added to the volumetric flask to achieve 25% hydro-alcoholic solution. This solution was placed in the apparatus for another 1 h, then was filtered through a filter paper [19, 20]. The filtrate was concentrated in rotary evaporator after cooling, at temperature of 45 ° C and to the gradual increase of 200 rpm for 1-1.5 h to yield ethanol extract, so it was incubated at 50 ° C to dry completely. On average, for each sample, 13 to 15 ml essential oil and 3.0 to 5.0 g extract was obtained. In the next step, 10 g of the obtained ethanol extract was homogenized with 30 ml liquid nitrogen, then 100 ml distilled water was added and was placed on a magnetic shaker for 2 h until completely dissolved in distilled water. To prepare the oral suspension (60%), 8.1 ml of the essential oil with 0.68 ml extract were diluted with distilled water. Distilled water 0.52 ml was used in a similar way to prepare the oral suspension (30%), 0.58 ml essential oil with 0.34 ml of the extract diluted with distilled water and 2.08 ml distilled water was used. Eucerin is merely used to prepare topical cream of the extricated essential oil and crude extracts, which in pharmacy is utilized as an inert substance in the preparation of different creams. Therefore, 32 mg eucerin with 48.3 essential oil and 18.24 ml of the crude extract diluted with distilled water was used for topical cream 60 %, and to prepare topical cream 30 %, 56 g eucerin with 9.12 ml extract and 24.15 essential oil was used [21].

Experimental design

All animals were divided in to 8 equal groups (4 treatment groups and 4 control

group, 5 animals in each group). To create the conditions of oxidative stress and tissue damage in all rabbits to examin the effect of the mastic on blood antioxidant levels and to see the histological changes, the experimental burn wounds were created in 5 groups. After induction of anesthesia with acepromazine (1 mg /kg) and ketamine Hcl 10% (45 mg /kg) animals were fixed on a surgery table. The skin of the lateral area of the hip was shaved with an electric clipper and scrubbed [21]. Following on, second degree burn wound was appeared using a metal plate (20 mm diameter) with a temperature of 100 °C, in this manner, deeper layers of the dermis will damage. The animals were grouped into 8 major groups (n=5):

Group 1: 1 g of prepared cream 60 % was applied topically to the wound daily until day 21.

Group 2: 3 ml of the oral suspension 60 % was using gavages daily until day 21.

Group 3*:* 1 g cream 30 % was applied topically to the wound daily until day 21.

Group 4*:* 3 ml of oral suspension 30 % was using gavages daily until day 21.

Control Groups 5, 6, 7, 8: placebo (eucerin) was used topically and distilled water was used orally every d. In days 1-7-14 and 21 blood samples were collected from the jugular vein [21]. Serum was separated for each sample and used for the analysis. The collected serum were used for the estimation of the MDA, CAT, GPX, SOD parameters using diagnostic kits (Pars Azmoun, Iran, Cod; 10 50 012) according to the colorimetric methods at the wavelengths of 535, 405, 412 and 420 nm respectively. In addition serum samples were analyzed spectrometrically for the level of glucose, HDL and LDL using their respective kits (Band a UV- visible spectrophotometer, Beckman DU640UV/Vis).

Histological examination

For this study, regenerated tissues were fixed in 10% neutral formalin solution for 24 h and were dehydrated with a sequence of ethanol – xylene series of solutions. The materials were infiltered and embedded at 10 µ thickness and stained with hematoxylin – eosin dye. The histological changes were observed under the 21^{th} d. The section of the tissues of 21^{th} d was evaluated for the extent of reepithelization, maturation and organization of the epidermal squamous, fibroblasts and inflammatory cells. Likewise, the thickness of the granular cell layer, the degree of tissue formation and the progress of wound healing (graded from 1 to 4) were also investigated [22, 23].

Wound healing evaluation

The sizes of the wounds were traced on a transparent paper in every 2 d intervals throughout the monitoring period; measurement was continued up to day 21. The tracing was then shifted to a graph paper, from which wound surface area was evaluated [24].The evaluated wound surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound as the first day wound size as 100% by using this equation [21, 24].

$$wound\ contraction\ (\%) = \frac{Initial\ wound\ size - specific\ day\ wound\ size\ \times 100}{Initial\ wound\ size}$$

Statistical analyses

Statistical analyses were performed using SPSS 21 software (SPSS, Chicago, IL, USA).Repeated measurements ANOVA, Mann whitney and Tukey's tests were used. Data were expressed as mean± SD, frequency and percentage. $P \leq 0.05$ was considered as statistically significant. Moreover, Bonferroni correction was performed as an appropriate form of analysis. In order to evaluate the effects of treatment type and the time of response to the treatments, repeat measures plan was used [24].

Results

Effects of treatment on histological parameters and wound healing

Histological examination showed grade 4 treatments in group with topical cream (60%) on day 21, grade 3 in the group of suspension of 60%, grade 2.5 in the group of topical cream (30%) and grad 2 in group of oral suspension 30%. Figure 1 shows the histological changes in the treated and control groups respectively, on d 21. Figure 2 exhibit the process of wound contraction in group A on d 1, 7, 14 and 21.

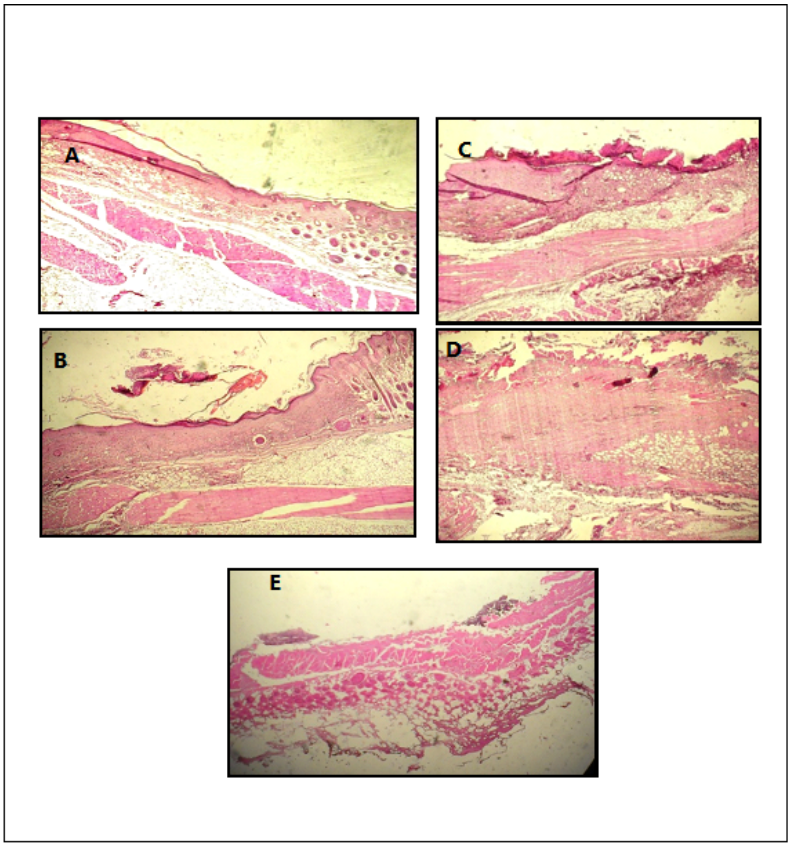

Figure 1- Histological analysis – H and E staining, X 100- 21 days after wound induction in (A) group with topical cream 60%, (B) group with suspension of 60%, (C) group with topical cream 30%, (D) group with suspension 30%, (E) control group. In group A, Complete healing, reepithelialization and dermis connective tissue has completely been renovated. Control group: Imperfect wound healing, absence of epithelium

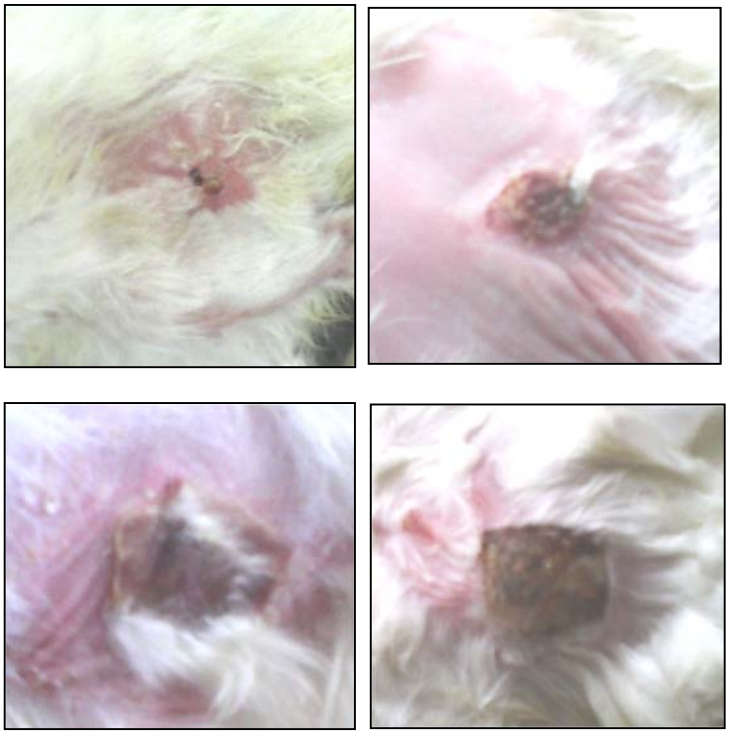

Figure 2- Process of wound contraction in group A (cream 60%), respectively, from right to left on d 0, 7, 14 and 21. Contraction and reepithelialization can be observed on d 21.

Table 1 shows the percentage of healing in the treated and control groups on d 7, 14 and 21. The all intervals showed significantly different between 7, 14 and 21days ($P \leq 0.05$). The result of Bonferroni test showed that the healing percentage was significantly different between groups in all time intervals. ($P \leq 0.05$) and Duncan's multiple range tests showed that the mean of healing percentage for all intervention groups differed from each other ($P \leq 0.05$).

Effects of treatment on biochemical parameters

Table 2 shows the concentration of GPX enzyme (mean ± SD).

GPX mean showed significant differences in different times of the measurements ($P<0.05$). Also the interaction between the time and the type of the treatment were found to be significant. ($P < 0.05$). Table 3 shows the concentration of SOD enzyme (mean ± SD).

The type of the treatment had a significant effect on mean SOD ($P < 0.05$) as well as the results showed significantly different between 7, 14 and 21days ($P < 0.05$). Data analyzed by Bonferroni test showed that in the comparison of pair wise of times, mean SOD indicated significant difference at all times couples except on d 14 and 21. Table 4 shows the concentration of CAT enzyme (mean ± SD). The all intervals (oral and topical) showed significantly different between 1, 7 and 14 days ($P \leq 0.05$). The treatment of ointment (60%) on day 21 exhibited a significant enhancement of CAT activity. Table 5 shows

the concentration of MDA enzyme (mean ± SD). The all intervals on 7 and 14 days exhibited significant decrease of MDA activity compared with control groups. Table 6 shows the concentration of glucose (mean ± SD) in blood serum. The treatments of oral (30%) and (60%) on day 21 exhibited significant decrease of glucose concentration. Table 7 shows the concentration of LDL (mean ± SD) in blood serum. The concentration of LDL did not show any significant changes in all experimental groups. Table 8 shows the concentration of HDL (mean ± SD) in blood serum. The all intervals on days 7 and 14 exhibited a significant enhancement of HDL concentration. ($P \leq 0.05$).

Table 1- Effect of *P. atlantica* mastic extract on wound healing (%) in different experimental groups

Type of treatment	Wound healing (%) 7th d	14th d	21th d
Ointment 30%	19.17±2.30[a]	39.10±1.01[b]	65.66±1.90[c]
Control	2.70±0.60	4.6±0.72	8.4±0.80
Ointment 60%	29.67±1.53[a]	62.71±3.15[b]	94.18±3.43[c]
Control	2.70±0.50	5.90±0.80	10.30±0.60
Oral suspension 30%	18.03±0.71[a]	27.06±1.88[b]	54.10±5.91[c]
Control	2.70±0.65	4.35±0.50	8.90±0.20
Oral suspension 60%	2.70±0.60	48.97±1.16[b]	54.10±5.91[c]
Control	2.69±0.60	7.70±0.60	8.90±0.20

Mean with different superscript (a, b, c) on the same row are significantly different ($P \leq 0.5$)

Table 2- Effect of *P. atlantica* mastic extract on serum concentration of GPX in different experimental groups

Type of treatment	1th d	GPX (Unit/ML) 7th d	14th	21th d
Ointment 30%	269.77±64.56[a]	372.06±11.64[b]	392.39±36.24[c]	396.81±19.54[d]
Control	295.33±10.05	312.32±5.64	315.14±7.35	347.66±40.43
Ointment 60%	344.32±15.34[a]	377.78±37.14[b]	341.88±47.87	399.40±14.50[c]
Control	290.00±50	310.50±60	310.20±5.45	335.66±40.50
Oral 30%	278.18±55.91[a]	342.21±64.66[b]	369.52±15.24[c]	417.51±9.77[d]
Control	292.30±20.05	312.00±2.60	312.00±2.50	329.66±20.44
Oral 60%	344.32±15.43[a]	377.78±37.14[b]	341.88±47.87	399.40±14.50[c]
Control	295.10±20.00	315.33±5.82	312.32±5.60	325.88±40.00

Mean with different superscript on the same row are significantly different ($P \leq 0.5$)

Table 3- Effect of *P. atlantica* mastic extract on serum concentration of SOD in different experimental groups

Type of treatment	1thd	SOD (Unit/ml) 7thd	14th d	21th d
Ointment 30%	11.67±3.36[a]	19.02±2.59[b]	21.49±1.77[c]	17.17±4.98
Control	10.37±0.64	9.0±0.53	7.67±0.65	14.92±3.92
Ointment 60%	19.14±0.31[a]	15.88±2.18	19.27±3.03[b]	21.44±2.65[c]
Control	10.50±0.60	9.0±0.85	7.56±0.75	12.84±2.82
Oral 30%	10.57±1.71[a]	14.99±3.85[b]	14.76±5.40	23.70±1.32[c]
Control	10.85±0.24	9.50±0.65	8.75±0.60	14.56±1.85
Oral 60%	12.18±2.26[a]	14.85±2.10[b]	16.80±2.25[c]	20.10±1.54[d]
Control	10.20±0.90	9.90±0.55	9.23±0.56	11.90±2.55

Mean with different superscript on the same row are significantly different ($P \leq 0.5$)

Table 4- Effect of *P. atlantica* mastic extract on serum concentration of CAT in different experimental groups

Type of treatment	1th d	CAT (Unit/ml) 7th d	14th d	21 th d
Ointment 30%	6.80±1.71[a]	10.70±4.75[b]	10.44±4.81	9.77±2.42
Control	6.90±2.14	7.07±1.56	7.33±0.25	8.92±3.41
Ointment 60%	6.05±4.45[a]	12.10±4.13[b]	10.41±4.23	17.54±8.20[c]
Control	6.84±2.30	7.1±1.90	6.30±0.86	8.25±3.95
Oral 30%	7.67±0.93[a]	23.41±2.95[b]	21.84±12.31[c]	11.52±5.33
Control	7.05±2.20	8.09±0.58	7.24±0.76	9.07±3.02
Oral 60%	11.05±6.51[a]	19.54±4.28[b]	13.95±5.00	9.11±2.26
Cotrol	6.08±2.30	6.65±1.95	6.55±0.29	7.91±3.86

Mean with different superscript on the same row are significantly different (P ≤ 0.5)

Table 5- Effect of *P. atlantica* mastic extract on serum concentration of MDA in different experimental groups

Type of treatment	1th d	MDA(Unit/ml) 7th d	14th d	21th d
Ointment 30%	24.07±3.93	28.57±2.73	29.57±6.18	26.06±6.50
Control	18.24±2.84[a]	39.23±1.96[b]	41.23±5.51[c]	21.63±1.25
Ointment 60%	27.96±5.82	27.64±4.79	29.64±4.84	35.55±4.75
Control	19.27±1.70[a]	41.25±1.58[b]	44.56±5.58[c]	19.06±1.67
Oral 30%	20.46±0.65	26.35±2.72	26.35±6.91	25.89±1.54
Control	18.68±2.35[a]	39.85±1.23[b]	42.87±5.02[c]	21.14±1.69
Oral 60%	28.68±6.64	28.19±7.08	28.19±3.69	25.50±7.15
Control	18.05±2.09[a]	41.05±2.96[b]	44.15±5.63[c]	18.68±1.45

Mean with different superscript on the same row are significantly different (P ≤ 0.5)

Table 6- Effect of *P. atlantica* mastic extract on serum concentration of glucose in different experimental groups

Type of treatment	1th d	glucose (mg/dl) 7th d	14th d	21th d
Ointment 30%	117.33±15.7[a]	109.67±40.87	165.67±66.04	104.0±634.60[b]
Control	175.0±11.0	166.33±9.61	170.67±11.59	173.14±3.68
Ointment 60%	199.0±10.15[a]	97.67±18.82[b]	91.33±10.79[c]	102.67±58.31
Control	175.0±11.0	165.44±7.87	175.66±10.88	178.55±3.23
Oral 30%	184.67±14.64[a]	134.67±17.04[b]	142.67±14.64	91.0±16.82[c]
Control	175.0±11.0	160.98±9.24	176.77±10.87	177.10±4.78
Oral 60%	90.33±17.0[a]	98.33±44.81	96.6±4.73	71.33±8.62[b]
Control	175.0±11.0	168.36±9.25	178.80±10.25	170.90±3.75

Mean with different superscript on the same row are significantly different (P ≤ 0.5)

Table7- Effect of *P. atlantica* mastic extract on serum concentration of LDL in different experimental groups

Type of treatment	1th d	LDL(Unit/l) 7th d	14th d	21th d
Ointment 30%	19.60±5.24	24.37±9.07	29.80±6.50	36.43±28.13
Control	17.33±1.15	15.50±4.36	15.10±0.87	36.96±15.58
Ointment 60%	35.50±18.08	28.00±0.85	25.67±12.90	29.47±10.21
Control	17.33±1.15	15.96±4.09	14.20±1.07	36.09±14.34
Oral 30%	22.73±1.55	34.69±3.40	37.37±17.11	20.20±9.07
Control	17.33±1.15	14.70±4.78	13.18±090	32.70±15.06
Oral 60%	26.87±1.33	27.53±4.11	38.67±9.00	49.00±17.68
Control	17.33±1.15	15.00±4.92	15.65±0.79	30.76±15.45

Mean with different superscript on the same row are significantly different (P ≤ 0.5)

Table 8- Effect of *P. atlantica* mastic extract on serum concentration of HDL in different experimental groups

Type of treatment	1th d	HDL(Unit/l) 7th d	14th d	21th d
Ointment 30%	24.33±6.81[a]	31.00±9.54[b]	43.33±11.59[c]	35.67±13.65
Control	20.00±8.72	22.00±8.66	23.67±9.61	18.06±13.32
Ointment 60%	28.00±8.72[a]	29.00±1.00[b]	38.00±3.46[c]	38.00±5.20
Control	20.00±8.72	20.68±8.15	21.06±9.54	17.85±13.74
Oral 30%	22.67±4.93[a]	29.33±6.43[b]	33.67±5.86[c]	24.00±5.20
Control	20.00±8.72	20.76±8.80	23.68±9.25	18.76±13.24
Oral 60%	22.33±2.52a	31.33±7.23[b]	46.67±8.88[c]	41.33±8.08
Control	20.00±8.72	22.08±8.55	23.05±8.66	18.44±11.89

Mean with different superscript on the same row are significantly different ($P \leq 0.5$)

Discussion

Histological process of burn healing was evaluated and scored based on the accumulation of collagen, and epithelium regeneration. The present study showed that in the groups that received ointments and syrups 60 %, respectively healing levels of 3 and 4 were obtained indicating the effect of the drug concentration on the healing process, Moreover in groups that were treated topically (60% and 30 %), a better healing progression was achieved compared with syrup treated animals (60% and 30%) by means of 2.5 and 2 healing levels. In sum, experimental *P. atlantica* treated exhibited a positive healing progression comparing to the control group (with the level 1 of healing). Wound healing is a dynamic and complex process in which the damaged tissue layers and cellular structure should be restored into the normal state as closely as possible. Plant phenolics act as primary antioxidants or free radical scavengers. Lipid peroxidation is an important process in burns, wound and skin ulcers. Collagen fibrils viability increases by inhibiting lipid peroxidation [26]. Therapeutic potentials of phenolic compounds such as anti-inflammatory and wound healing effects via the reduction of lipid peroxidation can improve vascularity, collagen synthesis and promotes cross linking of collagen [19]. There are three stages of wound healing: inflammation, proliferation and remolding. The proliferation phase includes: angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction [24], which is a multifactorial process that results in contraction and closure of the wound and restoration of the functional barrier. It is consented that ROS are deleterious to wound healing process due to the harmful effects on various cells and tissues [27, 28]. The results of the present study on the percentage of wound healing and histological examination indicated that ointment and oral suspension can reduce inflammation in initial phase. The *P. atlantica* Ointment and oral suspension by preventing secondary infections and also reducing inflammation accelerated the speed of healing, but regarding the reepithelization, specialized staining is required to have a correct judgment in this regard. The presence of phenols and flavonoids in the extract of *Pistacia* grantees the therapeutic potentials due to their anti-inflammatory, antimicrobial, antifungal, antioxidant, wound healing and astringent properties which accelerates the process of wound contraction and epithelialization [29]. The effect of *P. atlantica* mastic extract was

assessed by evaluating different parameters such as macroscopic score, and oxidative stress markers such as to MDA level. Our finding showed that the creation of burn wound leads to the oxidative stress and administration of *P. atlantica* extract caused remarkable changes in the levels of antioxidant parameters during wound healing via the scavenging the oxidative stress.

MDA is an end product of fatty acid oxidation and is often used as an indicator of lipid oxidation. Our data demonstrated that MDA levels were increased in all experimental groups; which indicates that the oxidative stress has improved in all groups compared with day 1 due to the tissue damage. Other than MDA levels were significantly decreased during extract treatment as compared to the pretreatment and control groups. SOD, GPX and CAT levels were significantly increased in treated groups indicating the enhancement of total antioxidant activity. SOD is one of the most important scavenging enzymes present in the human body. SOD catalyzes the dismutation of superoxide radicals to O2 and H2O2. GPX appears to have a major role in the prevention of oxidative stress. GSH is present in mast cells where functions as an antioxidant and protects cells from toxic effects of ROS. Histological and macroscopic data confirmed that both oral and topical administration of *P. atlantica* extract could alleviate stress oxidation and inflammation. Hatamnia et al. (2014) reported that higher antioxidant activity of hull extract may be attributed to the higher phenolic content [30]. Polyphenols are known as substances that are capable to reduce oxidation. Through the scavenging of free radicals, chelating of free metals inhibition of the enzymes responsible for the production of free radicals and the prevention of antioxidant defense system [31]. LDL levels were decreased and HDL levels were increased after the administration of *P. atlantica* extracts .A number of studies has shown the beneficial effects of *P. lentiscus*. *P. lntiscus* fatty oil possesses anti-lipid properties at least in reducing total cholesterol and triglycerides [16]. It is hypothesized that the consumption of *P. atlantica* extract as an antioxidant increases the activity of anti-oxidative enzymes and reduces cholesterol levels. The fatty acids of *P. atlantica* are mostly unsaturated, like oleic acid and linoleic acid [17]. The powder of shell and kernel of the *P. atlantica* can decrease triglyceride, cholestrol, LDL and increase HDL level [25]. The studies on phosphatidate phosphohydrolase (PAP) enzyme showed that the amount of the reduction of PAP activity is proportional to the presence of unsaturated fatty acids in *P. atlantica* and can lead to the decline of hepatic triglyceride [25]. The presence of phenolic molecules, triterpenic compounds as well as the phytosterols in mastic extract of *P. atlantica* is particularly important because of their action against the oxidation of LDL and is a significant evidence for its antioxidant effects [25]. Andrikopoulos et al. (2000) also reported that the mastic gum caused the oxidation of LDL and had a positive inhibitory effect on LDL oxidation in a dose of at least 2.5 mg (5.75%) and at the maximum of 9.99 mg (50 %) [17]. In the present study, serum level of LDL did not show any significant difference. Our finding

were approved the effect of extract of *P. atlantica* in increasing HDL that regarded useful fats. In our experiment, the concentrations of Serum glucose were monitored and showed significant increases, which in the control group was higher than the other groups. Due to the presence of antioxidants in the treated groups significant reduction was appeared. Unsaturated fatty acids have been reported to increase glucose tolerance and insulin sensitivity which leads to the in decreased serum glucose and insulin levels in first day [18].

Conclusion

The finding of this study supports the idea that the mastic extract of *P. atlantica* is a promising source of wound healing and as antioxidant. Thus it can be considered as a natural herbal source in the pharmaceutical industry. This study is the first research on the effect of the *P. atlantica* mastic extract on wound healing, antioxidant and biochemical parameters in blood serum. This therapy is potentially safe, cost effective and efficient. Further investigations of its clinical application in human model are warranted.

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