4th International Conference on Agricultural Sciences Medicinal Plants and Traditional Medicine
CINVU
Kosar University
COMSTECH Inter-Islamic Network on Virtual Universities
KOSAR UNIVERSITY
September 20, 2021 Tbilisi - Georgia

Histomorp80hological examination of skin wound healing under the effect of *avocado* oil in Wistar rats

Mohammad reza Ebad Sichani[1], Mehdi Farid[2], Parnia Pourhadi[3], Elham Moghtadaee Khorasgani[4]
1-Graduated of Veterinary Medicine Faculity, Shahrekord Branch ,Islamic Azad University ,Shahrekord, Iran
2- Assistant Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran
3- Graduated of Veterinary Medicine Faculity, Shahrekord Branch ,Islamic Azad University ,Shahrekord,Iran
4-Department of Pathobiology,Faculty of Veterinary Medicine,Shahrekord Branch, Islamic Azad University,Shahrekord,Iran

Abstract
Objective: Given the presence of most fatty acids and proven anti-inflammatory properties in *avocado oil*, the present study was an attempt to examine its effect on the skin wound healing process in rats. Aim: The effect of avocado on wound healing in rats Material and Methods: a total of 30 rats with the same weight range were randomly divided into three groups, including control, treatment with avocado oil, and treatment with phenytoin after anesthesia and creating a full-thickness skin wound with an area of 4 cm behind them. Wound healing was examined histologically and wound area was examined during the third, seventh and fourteenth days. Results: Based on results of the present study, a significant relationship was observed between the group treated with *avocado* oil and the group treated with phenytoin in terms of wound area in rats ($p < 0.05$). On the studied days, the wound area of the group treated with *avocado* oil was significantly smaller than that of the group treated with phenytoin and the control group. Conclusion: The research results revealed that *avocado* oil can increase collagen synthesis, reduce the number of inflammatory cells, accelerate the process of coagulation and accelerate the regeneration of epithelium and accelerate the healing process. Hence, it can be an appropriate option for the treatment of skin wounds.
Keywords: *Avocado*, Phenytoin, Skin Wound Healing, Rat

Introduction

Maintaining the integrity of skin in humans and animals is vital to protect against water loss, bleeding, and preventing entry of microorganisms. A wound is basically defined as a lesion on the skin surface caused by physical or thermal injuries that require medical treatment. The Wound Healing Association defines wound as "degradation of the anatomical and functional structure of the skin" for a wound. Wound healing in humans and evolved animals is done with a completely complex and advanced mechanism, as a result of passing through various stages such as inflammation, reproduction, repair and regeneration. Many factors are involved in the speed and quality of wound healing process, including wound size, local blood supply, and presence of foreign objects, microorganisms, age, health status, and nutritional status of the patient [1]. One of the therapeutic goals in medicine is wound healing in a short time and with fewer side effects and Egyptian, Greek, Indian and European physicians sought wound healing in the shortest time and with the least side effects in old days. The use of various types of herbal medications to enhance tissue regeneration and wound healing are being gradually popular. Phytomedicine as a field of medicine, uses plants either to treat disease or promote health agents. Traditional phytotherapies generally preserved the original composition and integrity of the source plant. While in new phytomedicines, the active compounds of plants such as alkaloids and flavonoids, are preserved to improve the effectiveness and decrease the harmful side effects of herbal medicines [2].

The results reported by various medical communities have been reviewed and examined clinically and in laboratory, but none of them have been recommended as an effective medicine so far . Concerning the effect of phenytoin in wound healing, a series of clinical studies have been conducted and their results indicate its positive effect in the healing of all war and non-war wounds [3].

Phenytoin increases angiogenesis and traction tolerance in wound healing. Studies suggest the effect of topical phenytoin in increasing organ transplant acceptance in rats. In a study conducted by Balbino et al on diabetic rats, the wound healing speed was faster in the group treated with topical phenytoin than in the control group (normal saline) . Their research also examined the effect of topical phenytoin on fracture healing . In a study conducted by Deodhar et al, the effect of topical phenytoin (in the form of 2% powder) on the healing process of diabetic foot wounds in patients with resistant neuropathy was investigated. They found that phenytoin accelerates wound healing process [4]. Given toxic and harmful effects of disinfectants such as betadine, acetic acid, iodophor for fibroblasts and lymphocytes, the use of medicinal plants is increasing growingly in many developed countries [5].

In recent years, physiological and pharmacological effects of plant extracts and the use of herbal medicines in the world, especially in Iran have been increasingly investigated. Factors such as fewer side effects, diversity of effective compounds in plants, lower economic costs, and development of industries related to cultivation of medicinal plants, preventing the outflow of currency from country, creating useful work and especially using medicinal plants recommended by the World Health Organization are among the reasons for the global approach to herbal medicine [6].

Studies suggest that *avocado* is effective in reducing the risk of metabolic syndrome, controlling blood sugar and improving serum lipids in patients with non-insulin dependent diabetes, lowering cholesterol and serum triglyceride levels in patients with hypercholesterolemia, and improving the symptoms of osteoarthritis [5, 7] . *Avocado* oil is natural oil derived from squeezing avocado paste. About 70% of avocado oil is made up of healthy oleic acid, which is an unsaturated omega-9 fatty acid [8]. *Avocado* oil has been examined due to its ability in treating skin wounds. Studies in rats have shown that avocado oil results in faster healing of wounds . Fatty acids and oleic acids in avocado oil can improve the synthesis of collagen, which is a process of creating new connective tissue. Fatty acids in *avocado* oil can reduce inflammation during the healing process . In addition to vitamin E, avocado oil contains potassium, lecithin and other nutrients that moisturize and nourish the skin . Epidermis can easily absorb these nutrients and use them to generate new cells. *Avocado* oil can penetrate the skin and soften the skin and prevent drying of skin from and keep it moisturized [8].

A study conducted by Camila et al under the title of “The effect of semi-solid formulation of *avocado* oil on wound healing in rats”, results of revealed the positive effect of the compounds in this fruit on the wound healing process Flores et al 2014. In a study conducted by Nayak et al, results showed that *avocado* fruit extract has a beneficial effect on experimental wound healing in rats [9].Flores et al investigated the properties and

applications of avocado oil and their research results revealed that avocado oil supplementation in Wistar rats result in an improvement in mitochondrial function, a decrease in free radical levels, lipid peroxidation, and an improvement in glutathione ratio[10]. Hence, given the beneficial effects of *avocado*, the study was conducted to evaluate the effect of *avocado* fruit oil on surgical wound healing.

Materials and Methods

Animals

To conduct this study, 30 male Wistar rats weighing 220 to 250 g were purchased from the Laboratory Animal Breeding Center of Islamic Azad University, Shahrekord Branch. Then, they were randomly divided into three groups (10 rats in each group). Standard temperature conditions of 20 to 30 ° C and light conditions were provided for 12 hours.

Wound creation

To create full-thickness skin wounds in the back of rats, they were anesthetized with a mixture of two drugs of ketamine at a dose of 50 mg / kg of body weight and xylazine at a dose of 5-8 mg / kg of body weight intraperitoneally.

Then, the hairs on the back of rats were completely shaved and after preparing the surgical site with a sterile stencil with dimensions of 2× 2 and an area of 4 cm^2, a wound was created in the back of rats. The day of surgery was considered to be day zero.

The studied groups include:

Group 1 – received no treatment (control)

Group 2- received *avocado* oil.

Group 3- received 1% phenytoin

Procedure

During the 14 days of study, the wound surface was cleaned twice a day with a tampon impregnated with physiological serum and then avocado and phenytoin oil was used to treat wounds in the study groups. Wound area was measured on days 3, 7 and 14 in the study groups by digital caliper with a precision of mm.

Preparation of tissue samples from the wound site. During days 3, 7, and 14, rats were anesthetized using anesthetics, and the restored tissue and surrounding healthy tissue were isolated for histopathological studies using a scalpel and scissors. After washing with physiological serum, the tissue sample was pinned and fixed on thick cardboard (to prevent tissue wrinkling). On the day 14, rats were euthanized by increasing the anesthesia (via ketamine and xylazine) and the wound tissues formed were carefully removed and used for further analysis.

After fixation of tissues, they were placed in 10% formalin. The mentioned tissues were transferred to histopathology laboratory of Shahrekord University for preparing pathology slides and hematoxylin Eosin staining. After examining all microscopic views, they were evaluated based on histopathological factors such as the level of collagen fibers, the order and direction of the fibers, bleeding, edema and inflammation, epithelial regeneration, superficial clots and newly-formed vessels. An attempt was made during the experiment to observe the ethical standards of the research based on the given commitment [11].Statistical analysis: The collected results were analyzed using SPSS 2016 software and ANOVA test.

Results

Results of clinical evaluation

Table (1)(Figre5) presents the mean and standard deviation of rat wound area at different times and under different treatments.

Table 1.Mean and standard deviation of rat wound area at different times and under different treatments

Time / Treatment	Day 3	Day 7	Day 14
Control	5.03^{a} ±348	5.79^{a} ±268	6.31^{a}١٦٨ ±
Avocado	5.29^{b} ± 249	4.08^{b} ±137	7.83^{b} ± 46
Phenytoin	5.30^{b} ± 252	4.10^{b} ± 142	10.12^{b} ± 54
significance level	0.001^{**}	0.001^{**}	0.001^{**}

The difference between the groups is significant (P <0.05). a, b: Treatments with different Latin letters have a statistically significant difference.

*The area of wounds is in mm^2.

The results revealed that the mean and standard deviation of the wound area in the control group on day 3 was 348 ± 5.05 mm^2, which is a larger wound area compared to the wound area in both groups treated with *avocado* oil (249 ± 5.29 mm^2) and phenytoin (252 ± 5.30 mm^2) on day 3. In fact, it can be stated that no significant relationship was observed between control group and two groups treated with *avocado* oil and phenytoin in terms of wound area on day 3. However, on day 3, there was a significant relationship between the groups treated with *avocado* oil and phenytoin in terms of wound area (P <0.05). The mean and standard deviation of the wound area in the control group on day 7 was 268 ± 5.79 mm^2, which is still a wider wound area compared to the wound area in both groups treated with *avocado* oil (137 ± 4.08 mm^2) and phenytoin (142 ± 4.10 mm^2) on day 7.

In other words, no significant relationship was observed between control group and both groups treated with *avocado* oil and phenytoin in terms of wound areas on day 7. On day 7, there was still a significant relationship between the groups treated with *avocado* oil and phenytoin in terms of wound area (P <0.05).

Mean and standard deviation of the wound area in the control group on the day 14 was 168 ± 6.31 mm^2. The wound area in this group compared to the wound areas in both groups treated with *avocado* oil (46 ± 7.83 mm^2) and phenytoin (54 54 ± 10.12 mm2) was higher on day 14. No significant relationship was found between control group and both groups treated with avocado oil and phenytoin in terms of wound area on day 14. On day 14, the relationship between the groups treated with *avocado* oil and phenytoin was still significant in terms of wound area (P <0.05). In general, based on the results of the present study, a significant relationship was observed between the group treated with *avocado* oil and the group treated with phenytoin in terms of wound area in rats. In all three days, the wound area of the group treated with avocado was slightly smaller than that of the group treated with phenytoin and it was significantly smaller than that of the control group, indicating that *avocado* oil was very effective in wound healing and was more effective than phenytoin (Figure 1,2)

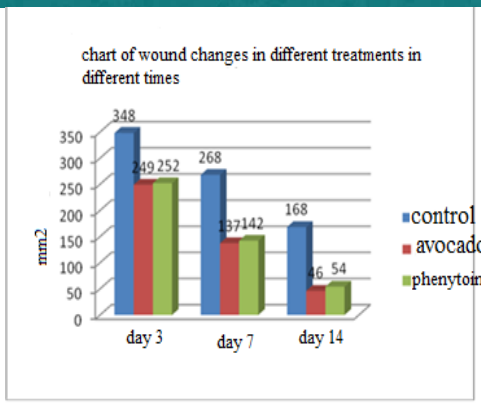

Figure 1. Wound changes in different treatments on different days

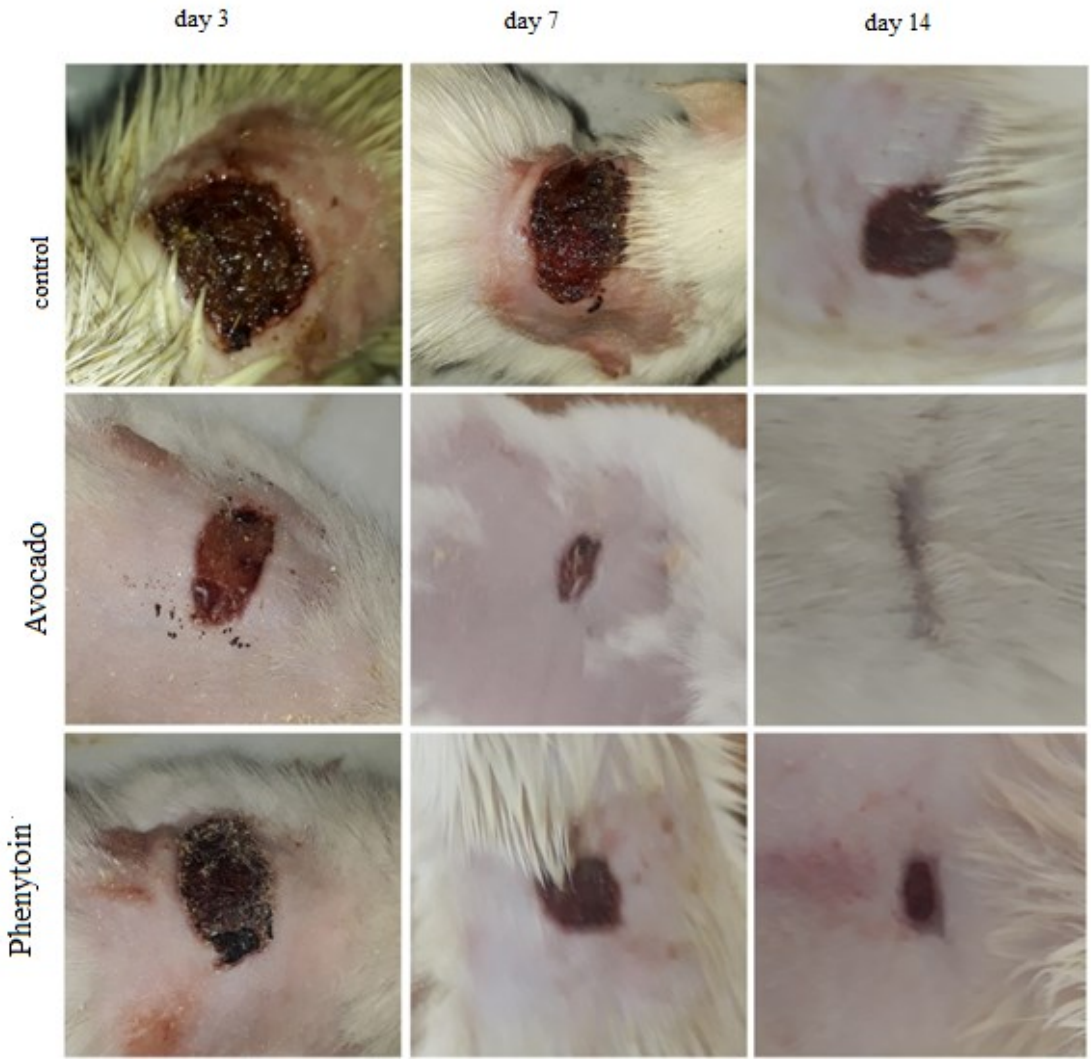

Figure 2. Appearance of wound in different treatments on days 3, 7 and 14

Histopathology Results

Histopathological examination of wounds on day 3

The results of histopathological examination of the experiment indicated that collagen fibers were relatively small on day 3 in all three groups of control, *avocado* oil, and phenytoin. histopathological examination showed that the order and orientation of fibers was observed at low levels in phenytoin group. In fact, no improvement was observed in the order and orientation of fibers in the groups treated with *avocado* oil and the control group.The highest level of bleeding was in the control group and *avocado* oil group and no bleeding was observed in the phenytoin group.

Also level of edema and inflammation in all three groups of control, *avocado* oil and phenytoin groups was moderate. epithelial regeneration did not occur in any of three control groups of control, *avocado* oil and phenytoin (Table 2).

Evaluation in terms of newly-formed vessels: The results of histopathological examinations on day 3 revealed that the rate of newly-formed vessels was small in all three groups of control, *avocado* oil and phenytoin(Figure 3)

A B C

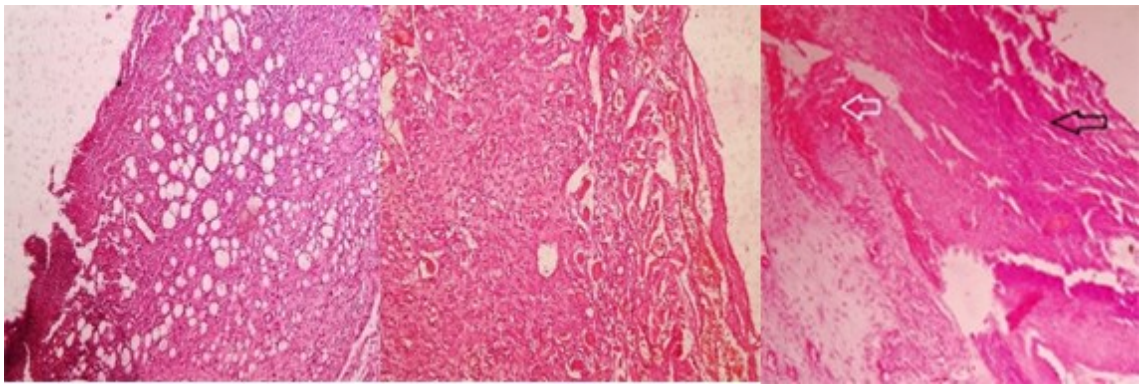

Figure 3. A) Microscopic view of the wound site 3 days after creating incision in the phenytoin group Inflammation and edema in the tissue - formation of fine collagen fibers
B) Microscopic view of the wound site 3 days after creating incision in the *avocado* group.
Edema in tissue-formation of fine collagen fibers-hyperemia
C) Microscopic view of the wound site 3 days after creating incision in the control group
Edema and bleeding (black arrow) Multicellular granular tissue with low fibers and edema Inflammatory cells (neutrophils) below the clot (white arrow) (HE 100 X)

Histopathological examination of wounds on day 7

histopathological examination of the experiment indicated that collagen fibers were moderate on day 7 in *avocado* oil and phenytoin groups and more than control group.

the highest order and orientation of fibers was observed in the phenytoin group at moderate level and slight improvement was observed in the order and orientation of fibers in the *avocado* oil group and no improvement was observed order and orientation of fibers in control group. bleeding was seen only in the control group and bleeding was not found in *avocado* oil group and phenytoin group.

Also the highest level of edema and inflammation was observed in control group and it was found at smaller and same levels in *avocado* oil and phenytoin groups.

epithelial regeneration did not occur in any of three control groups of control, *avocado* oil and phenytoin. newly-formed vessels were formed slightly only in the *avocado* oil group and it was not formed in the control and phenytoin groups(Figure4) (Table 2).

A B C

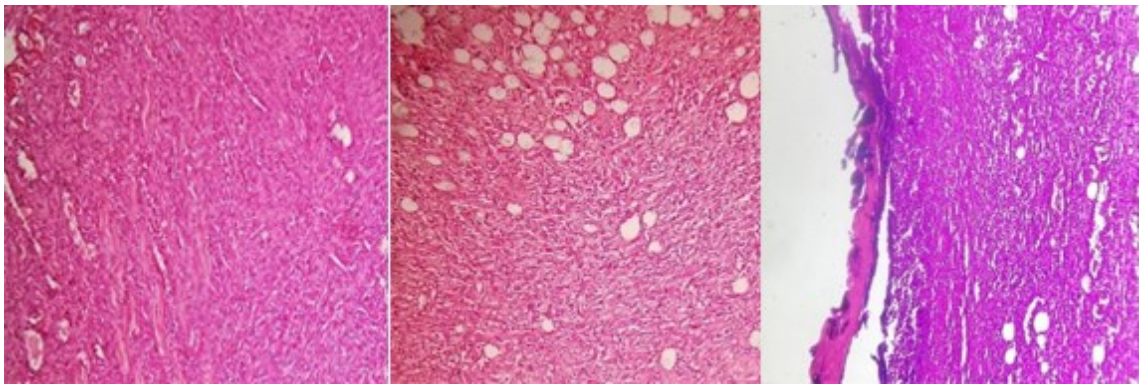

Figure 4. A) Microscopic view of the wound site 7 days after surgery in phenytoin group Formation of relatively appropriate fibers of collagen hyperemia – Edema
B) Microscopic view of the skin wound site 7 days after surgery in avocado group
Existence of edema. Relatively thick fibers of collagen
C) Microscopic view of the wound site 7 days after creating incision in the control group.
Severe inflammation and edema in the tissue – lack of observing proper collagen fibers
(H&E 100x)

Histopathological examination of wounds on day 14

The results showed that collagen fibers were moderate on day 14 in all three groups of control, *avocado* oil and phenytoin.
order and orientation of fibers was at moderate level in *avocado* oil and phenytoin groups and slight improvement was observed in the order and orientation of fibers in the control group. bleeding was observed slightly only in the control group and bleeding was not found in *avocado* oil and phenytoin groups. edema and inflammation were observed at low level only in control group and it was not found in *avocado* oil and phenytoin groups. highest level of epithelial regeneration was observed in *avocado* oil group and it was found at moderate levels in phenytoin group, while it was observed at low levels in control group. newly-formed vessels were not found in any three groups of control, *avocado* oil and phenytoin(Figure 5) (Table 2)

A B C

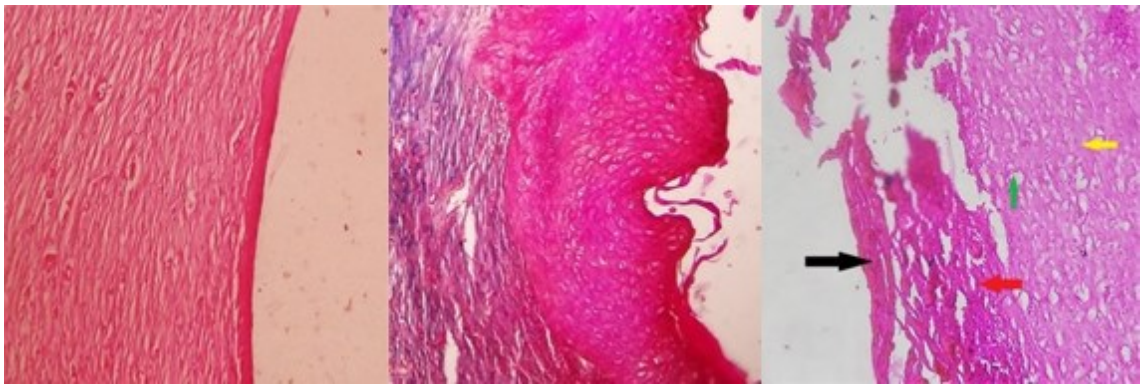

Figure 5. A) Microscopic view of the skin wound site 14 days after surgery in phenytoin group .Formation of epithelium with appropriate thickness, formation of collagen fiber

B) Microscopic view of the skin wound site 14 days after surgery in *avocado* oil group.
Formation of epithelium with appropriate thickness, formation of collagen fiber

C) Microscopic view of skin wound site 14 days after surgery in control group
Existence of clot on the surface of epithelium (black arrow), existence of inflammatory cells (red arrow), existence of edema in tissue (Green arrow) Formation of fine collagen fibers (yellow arrow) (HE.100x)

Table 2. Results of histopathological examinations of wound area 3, 7 and 14 days after wounding

Group	day	Rate of collagen fibers	Order and orientation of fibers	bleeding	Edema and inflammation	epithelium regeneration	Superficial clot	Recently-formed vessels
Control	٣	+	-	+++	++	-	+	+
	٧	+	-	+	+++	-	+	-
	١٤	++	+	+	+	+	-	-
Avocado oil	٣	+	-	+++	++	-	-	+
	٧	++	+	-	+	-	-	+
	١٤	++	++	-	-	+++	-	-
Phenytoin	٣	+	+	+	++	-	-	+
	٧	++	++	-	+	-	-	-
	١٤	++	++	-	-	++	-	-

Discussion

In recent decades, the subject of herbal medicine and the use of chemical plant extracts in wound healing have drawn the attention of researchers around the world. Increasing speed of wound healing has many effects from an economic and health point of view. With increasing the speed of wound healing, the level of wound infection will decrease and accordingly, the whole process of wound healing will be accelerated . Previous studies have indicated that the wound healing process may be accelerated by fatty acids [12]. *Avocado* extract or its oil is used in healing wounds[13], treating psoriasis [14], burns [15], improving wrinkles and harms caused by UVB rays and in taking protective measures of the liver [16]. The non-consumable part of this oil, in addition to improving scleroderma, has the regenerating properties of the epidermis[17] . (Groeber et al 2011).

Avocado oil extracted from fruit paste is rich in unsaturated fatty acids (PUFAs), linoleic acid (9.9-22.6%) and linolenic acids (0.4-0.4%) and unsaturated fatty acids (MUFAs), oleic acid (31.8-69.6%). It also contains beta-sitosterol, beta-carotene, lecithin, minerals and vitamins A, C, D and V[8]. Given its anti-inflammatory, antimicrobial, antioxidant and astringent properties proven in *avocado* oil [18], the present study was conducted to examine the effect of this oil on the healing process of skin wound caused by injury in rats. In general, based on the results of the present study, a significant relationship was observed between the *avocado* oil and phenytoin groups in terms of wound area in rats.

In all three days, the wound area of *avocado* group was slightly smaller than that of the phenytoin group and significantly smaller than that of the control group. It suggests that *avocado* oil was very effective in wound healing and was more effective than phenytoin. The properties of fatty acids (oleic, linoleic and linolenic) have been investigated in several studies, because they seem to be involved in wound healing [12] . The healing process can be assessed by evaluating the rate of wound changes, the level of collagen fibers, tensile strength and histopathology in different wound models , Histological evaluation in the present study revealed that animals treated with *avocado* oil showed a significant increase in epithelial tissue formation. Possible medicinal effects attributed to *avocado* oil in terms of healing process can be associated with compounds of its phytochemicals, such as vitamins A and E, and fatty acids (oleic, linoleic and linolenic acids) . These fatty acids are precursors of active drugs such as prostaglandins, thromboxane, and leukotrienes, which involved in the regulation of cell division and differentiation, angiogenesis and extracellular matrix synthesis [13]. It has been proven that linoleic acid and vitamins have important antioxidant functions in fighting against free radicals, which are responsible for toxicity and delaying tissue healing. Adequate availability of these products provides a favorable environment for wound healing[12].

Topical application of *avocado* oil decreases the number of inflammatory cells in the wound tissue and shows anti-inflammatory activity. Modulation of the inflammatory response can be attributed to the high amount of oleic acid in this oil, since this fatty acid causes less local inflammatory response and is synthesized and mediated by the same linoleic and linolenic acids for the same enzymes (cyclooxygenases and lipoxygenases) and fewer inflammatory mediators are created compared to mediators produced by arachidonic acid [18]. The present study showed a significant reduction in bleeding in animals treated with *avocado* oil and scar tissue maturation (regeneration stage) in the tissue. It is argued that in the physiological healing process, collagen accumulates in the wound area up to 14 days after injury. After this period, the balance between collagen synthesis and degradation is restored with the rapid disappearance of fibroblast cells (apoptosis) [19].Vahedi Larijani et al examined *avocado* fruit extracts in inhibiting the growth of cancer cells compared to normal cells. In this experimental study, after preparing ethanol, chloroform, ethyl acetate and petroleum extracts of *avocado* fruit, the role of each of them on the cell growth of squamous cell carcinoma of the esophagus in comparison with the control group in cell culture medium was investigated using MTT assay. The results of the present study on squamous cell carcinomas of esophagus revealed an inverse linear relationship between the concentration of *avocado* extract and living cells. Also, the results showed that avocado fruit extract plays an inhibitory role in the growth of cancer cells compared to normal cells. This study showed that *avocado* fruit is one of the fruits rich in phytochemicals that plays an effective role in inhibiting the growth of cancer cells. The use of extracts of this fruit is a suitable supplement in treatment of esophageal cancers [4] .

A study conducted by Yamaguchi et al revealed that the fat-containing components of *avocado* fruit inhibited the growth and development of oral cancer cells. Rajkumar et al also reported that the most chemo-protective

effects of *avocado* fruit extract are observed in higher concentrations of this extract, so that it can reduce the genotoxic effects of cyclophosphamide in cancer patients. In other words, this study indicates that the chemical extracts in this fruit can reduce the side effects of chemotherapeutic drugs such as cyclophosphamide in the treatment of various cancers, especially in higher concentrations (200 mg / kg of body weight), due to its chemo-protective components [20] .

Based on the results of the present study, after comparing the wound area in rats, a significant relationship was observed between the group treated with *avocado* oil and the group treated with phenytoin. The wound area in the group treated with *avocado* oil, compared to the group treated with phenytoin, was smaller, indicating that avocado oil had a greater effect on wound healing than phenytoin. Thus, it can be concluded that *avocado* oil contains effective substances in wound healing and can be used in the wound healing process.

Conclusion

The results of present study revealed that when *avocado* oil is used naturally or in drug formulations topically, it can increase collagen synthesis, reduce the number of inflammatory cells, accelerate the coagulation process, accelerate epithelial regeneration during the wound healing process, and accelerate the skin wound healing process and reduce the time takes for wound healing, since *avocado* oil is a rich source of oleic acid and contains essential fatty acids. Hence, it can be considered as a good option for treatment of skin wounds.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of University of Shahrekord (no:01981019)

Conflict of Interest: The authors have no conflicts of interest to declare

Acknowledgements: I acknowledge the efforts of Dr. Theophilus Jarikre and Mr. Israel Busayo who gave their professional support for the histological and statistical analyses of this experiment

References

Balbino, C.A. Pereira, L.M, Curi, R., 2005. Mechanisms involved in wound] 1[revision, Brazilian Journal of Pharmaceutical Sciences 41,27–51.
[2] Falzon, C.C, Balabanova, A, 2017. Phytotherapy: An introduction to herbal medicine, Primary Care: Clinics in Office Practice 44(2):217-27.
[3] Deodhar, A. K,1997. Surgical physiology of wound healing: a review. Journal of Postgraduate Medicin 43, 52–56.
[4] Gupta, N., Jain, U. K, 2010. Prominent wound healing properties of indigenous medicines, Journal of Natural Pharmaceuticals 1: 2–13.
[5] Fulgoni, V. L., Dreher, M., Davenport, A. J, 2013. Avocado consumption is associated with better diet quality and nutrient intake ,and lower metabolic syndrome risk in US adult: results from the national health and nutrition Examination survey, 2001-2008 . Nutrition journal 12: 1-10.
[6] Bruce, A, Schaffer, B, 2012. The Avocado: Botany, Production and Uses, 2nd Edition, Wolstenholme, Antony William Whiley, Boston, USA 45-501.
[7] Camila, A, Eryvelton, F, Rafaella, B, Daniele, C, 2013. Effect of Semisolid Formulation of Persea Americana Mill (Avocado) Oil on Wound Healing in Rats. Evidence-Based Complementary and Alternative Medicine 2013: 1-8.
[8] Nayak, B. S, Raju, S. S, Chalapathi Rao, A. V, 2008. Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats, Journal of Wound Care17: 123–126.
[9] Martin, P,1997. Wound healing-aiming for perfect skin regeneration. Science 276: 75–81.
[10] McDaniel, J. C, Belury, M., Ahijevych, K, Blakely, W, 2008. Omega-3 fatty acids effect on wound healing. Wound Repair and Regeneration 16: 337–345.
[11] Massafera, G, Costa, T. B, Oliveira, J. D, 2010. Fatty acids of mesocarp and seed oils of avocados (Persea americana Mill.) from Ribeirão Preto, SP, Alimentos e Nutriçã 21: 325–331.
[12] Stücker, M, Memmel, U, Hoffmann, M, Hartung, J, Altmeyer, P,2001. Vitamin B12 cream containing avocado oil in the therapy of plaque psoriasis, Dermatology 203:141147.
[13]Vega, R. G, Rivero, R. R, Moreiro, R. G, 2000. Study of avocado action on the process healing in burnt rats, Archivo Médico Camaguey 4: 39–43.
[14] Kawagishi, H, Fukumoto, Y, Hatakeyama, M, 2001. Liver injury suppressing compounds from avocado (Persea americana), Journal of Agricultural and Food Chemistry 49:2215–2221.
[15] Viviane, C, Indiara, B, Mariana, P, Henrique, F, 2018. Persea americana Mill. crude extract exhibits antinociceptive effect on UVB radiation-induced skin injury in mice, Inflammopharmacology 27: 323-338.
[16] Salgado, J. M, Danieli, F, Regitano-D'Arce, M. B, Frias, A, Mansi, D. N, 2008. The avocado oil (Persea americana Mill) as a raw material for the food industry, Ciencia e Tecnologia de Alimentos 28:20–26.
[17] Ghasemi, A, and Ketabi, M,2018. Antimicrobial activity of Avocado leaf extract in subject with poor oral hygiene:an invitro study, Journal Infohan Dent sch 14: 410-418 .
[18] Larijani, L, Ghasemi, M, Abedian Kenari, S, Naghshvar, F, Azadbakht, M, Yazdani Cherati, J, 2012. Investigating the effects of 4 types of avocado fruit extract on squamous cell carcinoma of the mouth and esophagus in comparison with peripheral blood mononuclear cells and control group, Journal of Mazandaran University of Medical Sciences 22 : 48-54 .
[19] Groeber,F, Holeiter, M, Hampel, M ,2011. Skin tissue engineering-In vivo and vitro applications, Advanced Drug Delivery Reviews 128: 352–366.
[20] Hatz, R. A, Niedner,R, Vanscheidt, W. and Westerhof, W, 1994, *Wound Healing and Wound Management*, 4th ed, Springer-verlag, 1-68.