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CERRAHPASA

Histomorphological Examination of Skin Wound Healing Under the Effect of Avocado Oil in Wistar Rats

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Abstract

Given the presence of most fatty acids and proven anti-inflammatory properties in avocado oil, the present study tried to examine its effect on the skin wound healing process in rats. 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 a full-thickness skin wound with an area of 4 cm was created on their backs. Wound healing was examined histologically and the wound area was examined during the 3rd, 7th, and 14th days. 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 ($p < .05$). During the study, 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. 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, thus accelerating the healing process. Hence, it can be an appropriate option for the treatment of skin wounds.

Keywords: Avocado, phenytoin, rat, skin wound healing

Introduction

Maintaining the integrity of the skin in humans and animals is vital to protect against water loss and bleeding, and to prevent the 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." Wound healing in humans and evolved animals occurs with a completely complex and advanced mechanism, passing through various stages such as inflammation, reproduction, repair, and regeneration. Several factors are involved in the speed and quality of the wound healing process, including wound size, local blood supply, age, health and nutritional status of the patient, and the presence of foreign objects or microorganisms (Balbino et al., 2005). One of the therapeutic goals in medicine is wound healing in a short time and with fewer side effects. Several Egyptian, Greek, Indian, and European physicians sought wound healing in the shortest time and with the least side effects in the old days. The use of various types of herbal medications to enhance tissue regeneration and wound healing are gradually becoming popular. As a field of medicine, phytomedicine uses plants as agents either to treat disease or to promote health. Traditional phytotherapies generally preserved the original composition and integrity of the source plant. However, in modern phytomedicine, 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 (Falzon & Balabanova, 2017).

The results reported by various medical communities have been reviewed clinically in the laboratory, but none have been recommended as an effective medicine so far (Balbino et al.,

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2005). Concerning the effect of phenytoin in wound healing, a series of clinical studies has been conducted and the results indicate its positive effect in the healing of all war and non-war wounds (Deodhar & Rana, 1997).

Phenytoin can increase angiogenesis and traction tolerance in wound healing. Studies suggest the effect of topical phenytoin in boosting organ transplant acceptance in rats. In a study conducted by Balbino et al. (2005) 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 and Rana (1997) 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 the wound healing process (Jain & Gupta, 2010). Given the toxic and harmful effects of disinfectants such as betadine, acetic acid, and iodophor for fibroblasts and lymphocytes, the use of medicinal plants shows increasing growth in many developed countries (Fulgoni et al., 2013).

In recent times, the physiological and pharmacological effects of plant extracts and the use of herbal medicines have been increasingly investigated worldwide and especially in Iran. Factors such as fewer side effects, diversity of effective compounds in plants, lower economic costs, and development of industries related to the 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 (Bruce & Schaffer, 2012).

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 (Camila et al., 2013; Fulgoni et al., 2013). Avocado oil is a 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 (Nayak et al., 2008). Avocado oil has been examined due to its ability to treat skin wounds. Studies in rats have shown that avocado oil results in faster healing of wounds. The fatty acids and oleic acids in avocado oil can improve the synthesis of collagen, which is the process of creating new connective tissue. The 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. The epidermis can easily absorb these nutrients and use them to generate new cells. Avocado oil can penetrate and soften the skin, prevent the drying of skin, and keep it moisturized (Nayak et al., 2008).

The results of a study conducted by Camila et al. (2013) entitled "The effect of semi-solid formulation of avocado oil on wound healing in rats," 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. (2008) results showed that avocado fruit extract has a beneficial effect on experimental wound healing in rats (Martin, 1997). Flores et al. (2014) investigated the properties and applications of avocado oil, and their research results revealed that avocado oil supplementation in Wistar rats results in an improvement in mitochondrial function, a decrease in free radical levels, lipid peroxidation, and an improvement in glutathione ratio (McDaniel et al., 2008). Hence, given the beneficial effects of avocado, the study was conducted to evaluate the effect of avocado fruit oil on surgical wound healing.

Method

Animals

To conduct this study, 30 male Wistar rats weighing from 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 20–30°C and light conditions were provided for 12 hours.

Wound Creation

To create full-thickness skin wounds on the backs of rats, they were anesthetized with a mixture of two drugs, 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 the 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 on the back of the rats. The day of surgery was considered to be day zero.

The studied groups were:

- Group 1: Control.
- Group 2: Avocado oil.
- Group 3: 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 were used to treat the wounds in the study groups. The wound area was measured on days 3, 7, and 14 by digital caliper with millimeter precision.

Preparation of Tissue Samples from the Wound Site

During days 3, 7, and 14, the rats were anesthetized using anesthetics. Afterward, 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 day 14, the 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, the tissues were placed in 10% formalin. The tissue samples were then transferred to the 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. During the experiment, an attempt was made to observe the ethical standards of research based on the given commitment (Massafera et al., 2010).

Statistical Analysis

The collected results were analyzed using IBM Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) 2016 software and ANOVA test.

Results

Results of Clinical Evaluation

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

The results revealed that the mean and standard deviation of the wound area in the control group on day three were 348 ± 5.05 mm^2, and the wound area was larger compared to the wound area in the groups treated with avocado oil (249 ± 5.29 mm^2) and phenytoin (252 ± 5.30 mm^2) on day three. In fact, it can be stated that no significant relationship was observed between the control group and the groups treated with avocado oil and phenytoin in terms of wound area on day three. However, on day three, there was a significant relationship between the groups treated with avocado oil and phenytoin in terms of wound area ($p < .05$). The mean and standard deviation of the wound area in the control group on day seven were 268± 5.79 mm^2, and the wound area was still wider compared to the wound area in the two groups treated with avocado oil (137 ± 4.08 mm^2) and phenytoin (142 ± 4.10 mm^2) respectively, on day seven.

In other words, no significant relationship was observed between the control group and the two groups treated with avocado oil and phenytoin in terms of wound area, on day seven. On day seven, there was still a significant relationship between the groups treated with avocado oil and phenytoin in terms of wound area ($p < .05$).

Mean and standard deviation of the wound area in the control group on day 14 were 168 ± 6.31 mm^2. The wound area in this group, compared to the wound areas in the two groups treated with avocado oil (46 ± 7.83 mm^2) and phenytoin (54 54 ± 10.12 mm^2) was higher on day 14. No significant relationship was found between control group and the 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 ($p < .05$). 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. On all three days, the wound area of the group treated with avocado oil was slightly smaller than that of the group treated with phenytoin, and it was significantly smaller than that of the control group, indicating that the avocado oil was very effective in wound healing and was more effective than phenytoin (Figures 1 and 2).

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 three in all the three groups–control, avocado oil, and phenytoin. Histopathological examination showed that the order and

Table 1

Mean and Standard Deviation of Rat Wound Area at Different Times and Under Different Treatments

Treatment	Time		
	Day 3	Day 7	Day 14
Control	348 ± 5.03[a]	268 ± 5.79[a]	168 ± 6.31[a]
Avocado	249 ± 5.29[b]	137 ± 4.08[b]	46 ± 7.83[b]
Phenytoin	252 ± 5.30[b]	142 ± 4.10[b]	54 ± 10.12[b]
Significance level	.001**	.001**	.001**

*The area of wounds is in mm^2.

**The difference between the groups is significant ($p < .05$).

[a,b]Treatments with different Latin letters have a statistically significant difference.

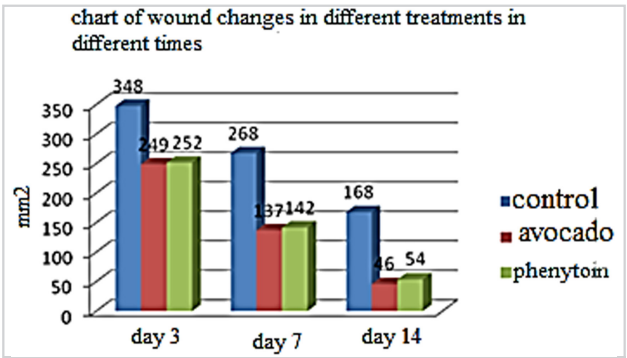

Figure 1

Wound Changes in Different Treatments on Different Days.

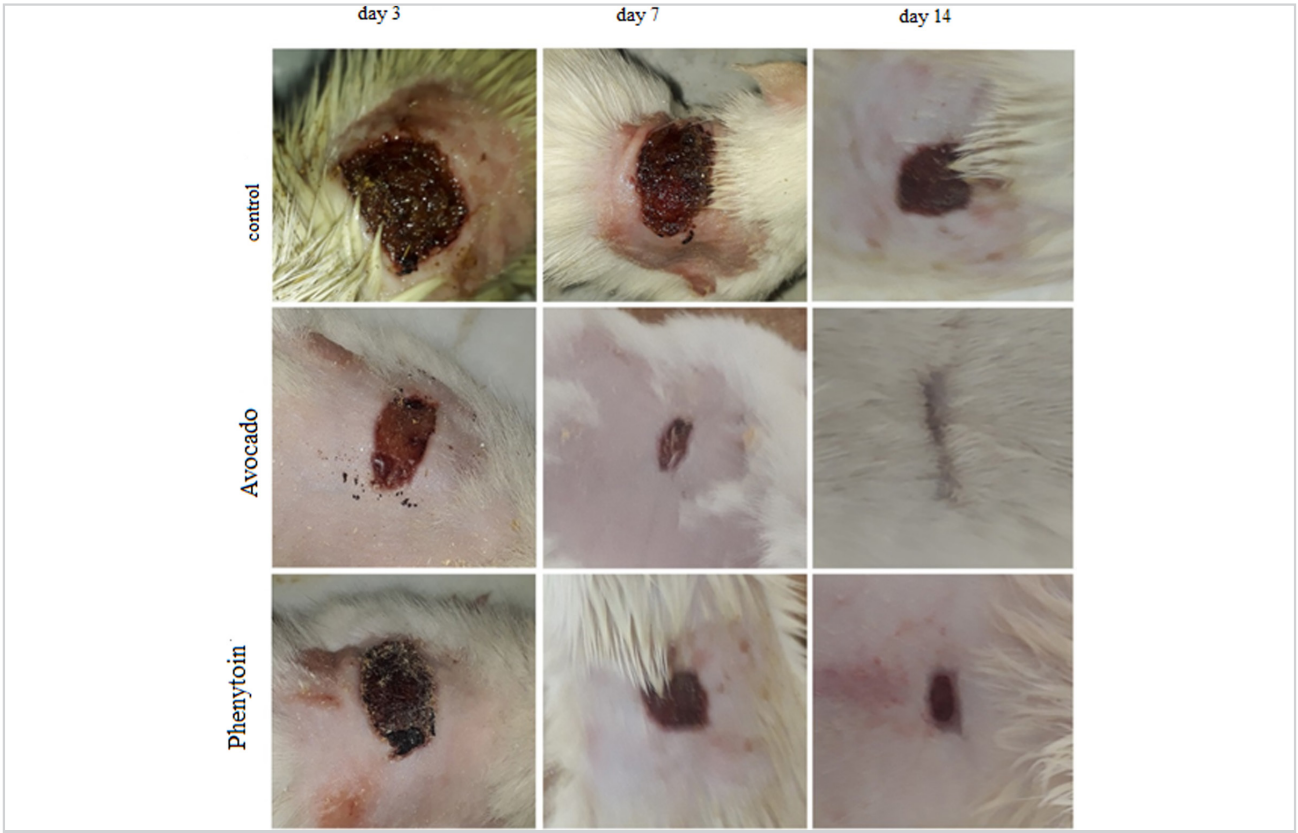

Figure 2
Appearance of Wound in Different Treatments on Days 3, 7, and 14.

orientation of fibers were observed at low levels in the phenytoin group. As a matter of 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 the avocado oil group, and no bleeding was observed in the phenytoin group.

Moreover, the levels of edema and inflammation in all the three groups—control, avocado oil, and phenytoin—were moderate. Epithelial regeneration did not occur in any of the three groups (Table 2).

Evaluation in Terms of Newly Formed Vessels
The results of histopathological examinations on day three revealed that the rate of newly formed vessels was small in all the three groups of control, avocado oil, and phenytoin (Figure 3).

Histopathological Examination of Wounds on Day 7
The histopathological examination indicated that collagen fibers were moderate on day seven in the avocado oil and phenytoin groups, and more than in the control group.

The highest order and orientation of fibers were observed in the phenytoin group at a moderate level; slight improvement was observed in the order and orientation of fibers in the avocado oil group; and no improvement was observed in the order and orientation of fibers in the control group. Bleeding was seen only in the control group, and bleeding was not found in the avocado oil and phenytoin groups.

Additionally, the highest levels of edema and inflammation were observed in the control group and were found at smaller and similar levels in the avocado oil and phenytoin groups.

Epithelial regeneration did not occur in any of the three experimental groups of control, avocado oil, and phenytoin. The formation of new vessels was slight in the avocado oil group, and no newly formed vessels were seen in the control and phenytoin groups (Figure 4, Table 2).

Histopathological Examination of Wounds on Day 14
The results showed that collagen fibers were moderate on day 14 in all the three groups of control, avocado oil, and phenytoin.

The order and orientation of fibers were at a moderate level in the avocado oil and phenytoin groups and a slight improvement was observed in the order and orientation of fibers in the control group. Bleeding was observed slightly only in the

Table 2

Results of Histopathological Examinations of Wound Area at 3, 7, and 14 Days After Wounding

Group	Day	Rate of Collagen Fibers	Order and Orientation of Fibers	Bleeding	Edema and Inflammation	Epithelial Regeneration	Superficial Clot	Recently Formed Vessels
Control	3	+	–	+++	++	–	+	+
	7	+	–	+	+++	–	+	–
	14	++	+	+	+	+	–	–
Avocado oil	3	+	–	+++	++	–	–	+
	7	++	+	–	+	–	–	+
	14	++	++	–	–	+++	–	–
Phenytoin	3	+	+	+	++	–	–	+
	7	++	++	–	+	–	–	–
	14	++	++	–	–	++	–	–

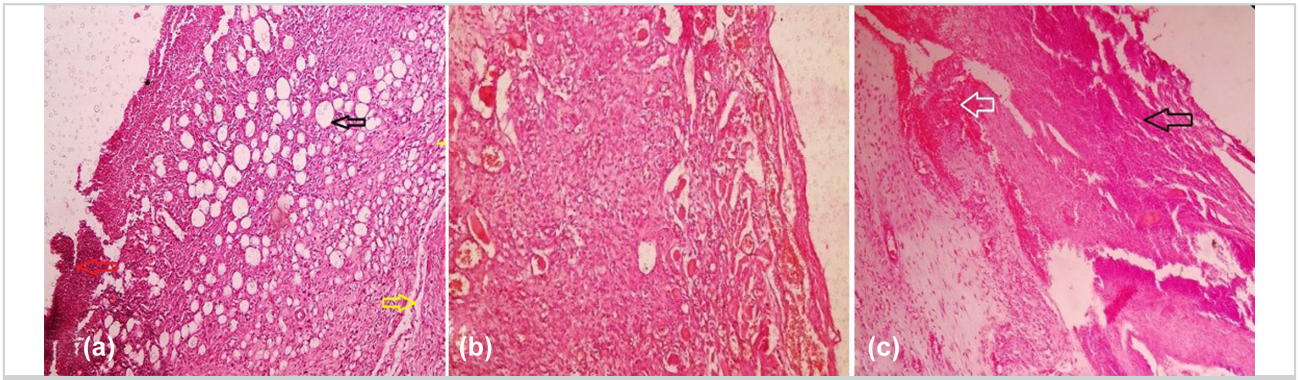

Figure 3

(a) Microscopic View of the Wound Site 3 Days After Creating Incision in the Phenytoin Group. Inflammation and Edema in the Tissue (Red and Black Arrow) - Formation of Fine Collagen Fibers (Yellow Arrow). (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 (White Arrow) Multicellular Granular Tissue with Low Fibers and Edema Inflammatory Cells (Neutrophils) Below the Clot (Black Arrow) (H&E 100 ×).

control group and bleeding was not found in the avocado oil and phenytoin groups. Edema and inflammation were observed at a low level only in the control group, and were not found in the avocado oil and phenytoin groups. The highest level of epithelial regeneration was observed in the 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 of the three groups of control, avocado oil, and phenytoin (Figure 5, Table 2).

Discussion, Conclusion, and Recommendations

In recent decades, the subject of herbal medicine and the use of chemical plant extracts in wound healing have drawn the attention of researchers all around the world. Increasing the speed of wound healing involves numerous effects on the economy and health sectors. With increasing 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 (Stücker et al., 2001). Avocado extract or its oil is used in healing wounds (Vega et al., 2000), treating psoriasis (Kawagishi et al., 2001), burns (Viviane et al., 2018), improving wrinkles and mitigating the harm caused by UVB rays, and in protecting the liver (Salgado et al., 2008). The non-consumable part of this oil, in addition to improving scleroderma, has properties that can regenerate the epidermis (Groeber et al., 2011).

Avocado oil extracted from fruit paste is rich in unsaturated fatty acids (PUFAs), linoleic acid (9.9–22.6%), linolenic acids (0.4–0.4%), unsaturated fatty acids (MUFAs), and oleic acid

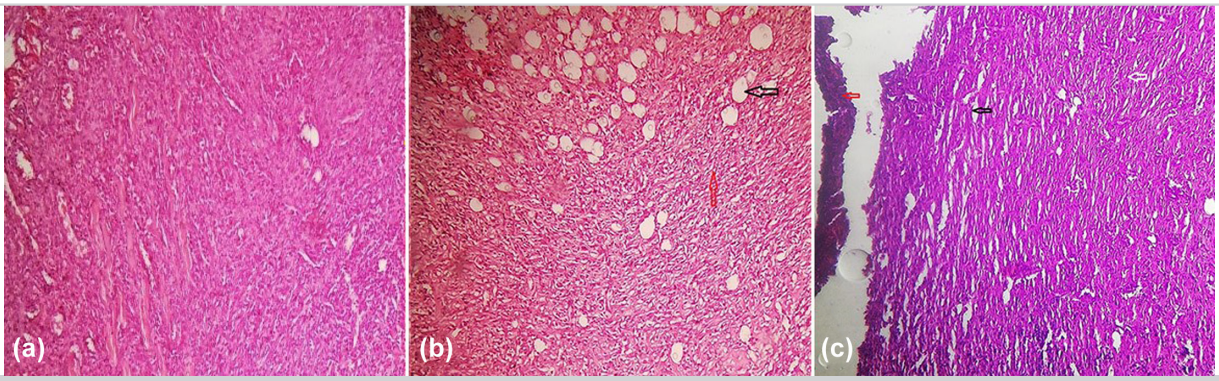

Figure 4
(a) Microscopic View of the Wound Site 7 Days After Surgery in Phenytoin Group Formation of Relatively Appropriate Fibers of Collagen Hyperemia – Edema. (b) Microscopic View of the Skin Wound Site 7 Days After Surgery in Avocado Group. Existence of Edema (Black Arrow). Relatively Thick Fibers of Collagen (Red Arrow). (c) Microscopic View of the Wound Site 7 Days After Creating Incision in the Control Group. Severe Inflammation and Edema in the Tissue (Red Arrow) – Lack of Observing Proper Collagen Fibers (Black Arrow) (H&E 100×).

(31.8–69.6%). It also contains beta-sitosterol, beta-carotene, lecithin, minerals, and vitamins A, C, D, and V (Nayak et al., 2008). Given its proven anti-inflammatory, antimicrobial, antioxidant, and astringent properties (Ghasemi & Ketabi, 2018), the present study was conducted to examine the effect of avocado 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.

On all three days, the wound area of the 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 (Stücker et al., 2001). 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.

A histological evaluation in the present study revealed that the animals treated with avocado oil showed a significant increase in epithelial tissue formation. Possible medicinal effects attributed to avocado oil in terms of the healing process can be associated with compounds of its phytochemicals,

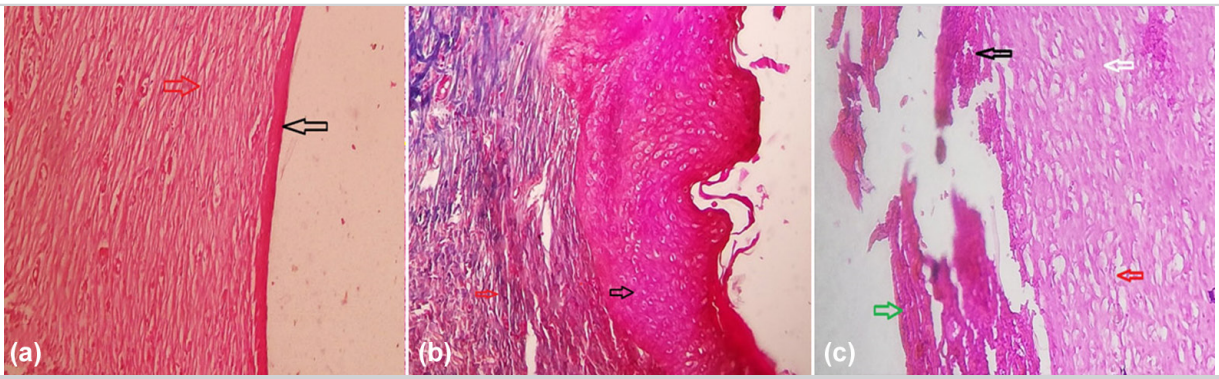

Figure 5
(a) Microscopic View of the Skin Wound Site 14 Days After Surgery in Phenytoin Group. Formation of Epithelium with Appropriate Thickness (Black Arrow), Formation of Collagen Fiber (Red Arrow). (b) Microscopic View of the Skin Wound Site 14 Days After Surgery in Avocado Oil Group. Formation of Epithelium with Appropriate Thickness (Black Arrow), Formation of Collagen Fiber (Red Arrow). (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 (Green Arrow), Existence of Edema in Tissue (Red Arrow) Formation of Fine Collagen Fibers (White Arrow) (H&E.100×).

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 biologically active substances, which are involved in the regulation of cell division and differentiation, angiogenesis, and extracellular matrix synthesis (Vega et al., 2000). It has been proven that linoleic acid and vitamins have important antioxidant functions in fighting against free radicals which are responsible for toxicity and delayed tissue healing. Adequate availability of these substances provides a favorable environment for wound healing (Stücker et al., 2001).

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 the mediators produced by arachidonic acid (Larijani et al., 2012). The present study showed a significant reduction of 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 for 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) (Groeber et al., 2011).

Larijani et al. (2012) 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 the MTT assay (colorimetric assay for assessing cell metabolic activity). 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. Moreover, 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 play an effective role in inhibiting the growth of cancer cells. The use of extracts of this fruit is a suitable supplement in the treatment of esophageal cancers (Jain & Gupta, 2010).

A study conducted revealed that the fat-containing components of avocado fruit inhibited the growth and development of oral cancer cells. Rajkumar et al. (2011) also reported that the most chemo-protective effects of avocado fruit extract are observed in higher concentrations of this extract, which can reduce the genotoxic effects of cyclophosphamide in cancer patients. In other words, this study indicates that the chemical extracts of this fruit can reduce the side effects of chemotherapeutic drugs such as cyclophosphamide in the treatment of various cancers, especially at higher concentrations (200 mg/kg of body weight), due to its chemo-protective components (Hatz et al., 1994).

According to 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 that in the group treated with phenytoin, was smaller, indicating that the avocado oil had a greater effect on wound healing than phenytoin. Thus, it can be concluded that avocado oil contains substances effective in wound healing and can be used in the wound healing process. The results of the present study revealed that using avocado oil naturally or in drug formulations topically 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, reducing the time taken for wound healing. Since avocado oil is a rich source of oleic acid and contains essential fatty acids, it can be considered as a good option for the treatment of skin wounds.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the University of Shahrekord (no. 2020/3/3).

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