Histometric and histopathological study of the healing effects of the alcoholic extract of cloud pomegranate seeds and zinc oxide nanoparticles on skin wound healing in rats.

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Abstract

The skin is the largest organ in the body in terms of surface area and has various functions. One of the most common injuries to the skin is open wounds caused by cuts and injuries. One of the most important topics for researchers has always been to find a way to repair wounds. Today, herbal treatment is very much studied by researchers in the field of wound healing due to the possibility of less side effects. Cloud pomegranate white seed extract is probably effective in healing wounds due to its antibacterial and anti-inflammatory properties. On the other hand, some studies show the antibacterial properties of zinc oxide nanoparticles in local use and reduce contamination and infection in the wound, and suggest re-epithelialization as the most important mechanism of wound healing in this substance. In this research, an attempt was made to investigate the healing effects of zinc oxide nanoparticles in the form of an ointment, the alcoholic extract of white pomegranate seeds in the form of an ointment, and the combined ointment of the two compared to the effects of the control group and Oserin. In this study, twenty-five mice were selected and randomly assigned to five treatment groups. The first group did not receive any medication, the second group received Oserin ointment, the third group received white pomegranate seed extract as an ointment, and the fourth group received zinc oxide nanoparticles. Ointment face and the fifth group received alcoholic extract of white pomegranate seeds along with zinc oxide nanoparticles. Photographs were taken of the wounds created on days 0, 7, 14, and 21 and the extent of wound healing was calculated. On days 0, 1, 3, 7, 14, and 21, mice were killed painlessly and samples were taken from the wounds. Histopathological examinations were performed on the samples collected from the wound. At the end of the study, the amount of mononuclear cells, multinucleated cells, fibroblast and vascularization in the fifth group was less than other groups. The process of wound contraction in the fifth group was more than other groups. Although, in the fifth and fourth groups, the process of wound contraction was more severe than the other groups. Complete healing of the wound was observed on the 21st day only in the fifth group. In this study, the topical application of white pomegranate seed extract along with zinc oxide nanoparticles extract had a positive effect on the stages of wound healing in mice, and therefore it may have a beneficial role on wound.healing.
Keywords: histopathology, wound healing, alcoholic extract of white pomegranate seeds, zinc oxide nanoparticles, Syrian mice.

1. Introduction

Wound healing is a process that starts immediately after the injury, and during the injury, the tissue is destroyed, blood vessels release blood plaques, and blood cells are released into the wound site. The first sign of wound injury is the release of molecules. It is like ATP and the unfolding of collagen in the wall of blood capillaries. The general processes of wound healing have three major stages that are defined during the wound healing process, which include healing processes, formation of epithelial tissue, and wound healing. Healing processes occur in four stages. During the first stage or hemostasis, the damage caused is limited. The clot provides a suitable initial substrate for the transfer of cells to the wound area. In the second stage, inflammation occurs, in this stage, with the increase of blood flow in the wounded area and the entry of different types of white blood cells, the damaged tissues are removed and the wound is protected against microorganisms. In the cell proliferation stage, a fibrinous tissue is formed in the wound site with the cooperation of fibroblasts, which is actually a germ tissue, and simultaneously, epithelial cells begin to multiply from the edges of the wound. In the final stage of maturity, the wound occurs. The final product of healing processes is scar tissue, which can be in three forms: atrophic, hypertrophic, or keloid [1]. Many advances have been made in the management of surgical wounds and various methods including aseptic techniques, prophylactic antibiotics and laparoscopic surgery have been used. Surgical wound infection and wound damage leave complications caused by surgery [2]. Pomegranate with the scientific name Punica granatum L. is one of the members of the dark pomegranate family (Punicaceae) whose origin is believed to be Iran and its neighboring countries[3-4-5]. The number of cultivars of this fruitful plant has been reported to be very numerous and there are more than 1000 cultivars [6-7-8]; While this number is more than 760 based on the latest reports in the country [3-7-8-9]. The edible fruit of this plant is a type of seta called Balosta[3-8-9]. The skin of the fruit of this plant is leathery and thick, which contains a large number of fleshy seeds called aril [10]. The fruit consists of three parts: the seeds, which make up about 3% of the fruit's weight and contain 20% of oil; Fruit juice, which makes up about 30% of the weight of the fruit, and the skin of the fruit, which is attached to the seeds with fleshy parts, is called skin. Pomegranate fruit is consumed in different ways in the world, such as fresh or processed, such as fruit juice, paste, kernel oil, vinegar, pomegranate seeds, etc. [10-3]. Pomegranates, olives, figs and grapes were the first plants cultivated by humans. Pomegranate is one of the oldest edible fruits that is mentioned in the Quran, Bible and Torah [11]. Pomegranate is considered as a manifestation of life, longevity, health, elegance, fertility, knowledge, ethics, immortality and spirituality. 12]. In ancient Egyptian culture, pomegranate fruit was known as a manifestation of success and ambition [10].

[10]. In traditional medicine, the different components of this plant have many therapeutic effects. Since ancient times in our country, the root of this plant has been used as an anthelmintic [3]. In traditional Indian medicine, they knew about pomegranate and used its skin and roots to get rid of worms. Its fruit skin is a strong astringent and is used to treat diarrhea, and its juice is used as a febrifuge and tonic [13]. Some authors [14-15-16]. They believe that the decoction of dried pomegranate skin in water is useful for internal and external use for many injuries that required astringent or antimicrobial properties, especially for pestilence,

diarrhea and blindness. The products obtained from a mixture of seeds, fruit juice and skin not only prevent miscarriage., but it causes pregnancy [17]. In traditional Greek medicine, pomegranate flowers are used to treat diabetes [18]. The new uses of pomegranate-derived products today are for the treatment of AIDS [19] It works; Also for beautifying and increasing beauty [19], hormone replacement therapy [20], diagnosis of allergy symptoms [21], cardiovascular protection [22-23], oral and dental hygiene [24], eye ointments [25] and Weight loss soap [26] is used.

Over the past few decades, there has been considerable research interest in drug transformation using particle delivery systems as carriers of small and large molecules. Particulate systems such as nanoparticles have been used as a physical approach to change and improve the pharmacokinetic and pharmacodynamic properties of different types of drug molecules [27]. Nanotechnology is the science of controlling the unique chemical, physical and biological properties of materials at the nano scale and using them in fundamental and new ways. Nanotechnology can be very important in many fields such as imaging, drug production and tissue engineering. The application of nanotechnology in veterinary medicine and animal reproduction and in other fields has been reported [28]. Nanotechnology in veterinary medicine with emphasis It has been used mostly on biosensors, drug making, chemotherapy devices and also nano material mechanisms for diagnosis, treatment and tracking of diseases. Nanotechnology is the understanding and application of new properties of materials and particles in dimensions of 1 to 100 nanometers, which show new physical effects mainly influenced by the dominance of quantum properties over classical properties. Considering the various characteristics and substances effective on wound healing that are present in white pomegranate seed, the aim of this study is to investigate the healing effect of white pomegranate seed extract along with zinc oxide nanoparticles on the healing of experimental wounds of all thickness in Syrian mice.

2. Materials and work methods

White pomegranate seeds were separated from the fruit and dried in the shade at room temperature (24°C). The dried seeds were ground and turned into powder by an electric grinder. Next, the alcoholic extract (ethyl alcohol) was obtained by the usual method [29]. To carry out this study, 25 Syrian mice were prepared and kept in individual cages. Then the mice were divided into 5 groups including the first group did not receive any medicine, the second group was Oserin ointment, the third group was the sieved extract of white pomegranate seeds in the form of an ointment, the fourth group was zinc oxide nanoparticles in the form of an ointment, and the fifth group was the alcoholic extract of pomegranate white seeds along with zinc oxide nanoparticles. They were divided. In the saline control group, after weighing each mouse (average weight 25 grams), according to the anesthetic dose for Syrian mice, ketamine 50 mg/kg + xylazine 5 mg/kg [30] intraperitoneally and using an insulin syringe. The injection was done. After anesthetization, the area between the two shoulder blades of the animal was weighed and prepared by betadine scrub and a circular piece of skin with a diameter of 2 cm was removed. After removing the skin with all its thickness (epidermis and dermis), the operation site was treated with sterile physiological serum. This treatment process was done for all 12 mice after surgery (12 mice in each group were divided into three subgroups 7, 14 and 21 days after surgery). After the end of each treatment period in each group, the mice were anesthetized using ether and removed from the wound site along with 0.5 cm of the surrounding skin and preserved in 10% formalin and sent to the histopathology laboratory. In the Esrin control and experimental groups, all operations were performed and the duration of treatment was the same as in the saline control group, with the difference that the treatment was performed with Esrin and Esrin ointment base containing white pomegranate seed extract, respectively. Consumption volume of physiological serum In such a way as to clean the wound from possible contamination. Also, the same amount of esrin and esrin containing white pomegranate seeds were used and covered the entire wound area in a thin layer. Sampling was done in the same way as the previous two groups. The samples sent to the pathology laboratory, after staining with hematoxylin-eosin method, were evaluated qualitatively and quantitatively. In histopathology grading, parameters affecting wound healing [31] were used, and the amount of epithelium formation, vascularization, formation of collagen fibers, and the presence or absence of inflammatory cells were investigated. The mentioned criteria were scored as - (absence), +1 (very little), +2 (low), +3 (moderate) and +4 (high). Also, the wound surface was measured on the 7th, 14th and 21st days after the wound was created using Image J software with the unit of 〖mm〗^2. The percentage of wound healing is calculated according to the following formula:

(1)

$$\text{Recovery percentage}=\frac{\text{first day on the surface wound} - \text{The wound surface on the desired day}}{Wound\ surface\ on\ the\ first\ day}\times 100$$

3. Quantitative evaluation

In the microscopic observation of the skin wound healing site on the 7th day of the control group, most of the wound surface was covered by a scab consisting of a blood clot containing fibrin and blood cells, edematous cells and remnants of necrotic tissues. The presence of a large amount of microbial cells on the surface of the scab on the wound was quite clear. The space under the scab was occupied by loose connective tissue. The presence of acute type edematous cells was also evident under the scab as well as the connective tissue itself located in the wound space. The covering tissue of the pavement, which had started to regenerate with the formation of covering lichen, was expanding from the surface under the scab towards the surface of the wound (Figure 1). In the microscopic observation of the skin wound healing site on day 7 of the Oserin treatment group, most of the wound surface was covered by a scab consisting of a blood clot containing coagulated fibrin, edematous cells and necrotic tissue remnants, as in the control group. The wound space was completely occupied by young and newly formed granulation tissue. Acute edematous cells were also seen mainly in the superficial parts of the wound and under the scab, although they were also abundantly visible in the deeper tissues of the wound. The covering tissue of the pavement also started to regenerate with the formation of a covering bud and was expanding from the surface under the scab to the surface of the wound (Figure 2). Microscopic observation of the healing site of the skin wound on the 7th day of the treatment group with zinc oxide nanoparticles showed that the majority of the wound surface was covered with a scab containing a blood clot consisting of fibrin and blood cells, as in the previous groups (control and treatment with Oserin). It is also covered with edematous cells and necrotic tissues. The wound space was completely occupied by the young fleshy bud, rich in vascularity and pericellular. The intensity of the presence of tumor cells was reduced compared to the group treated with Oserin. The covering bud was thicker and more developed compared to the group treated with Oserin, and it was expanding from the surface under the scab to the surface of the wound (Figure 3). In the microscopic observation of the healing site of the skin wound on the 7th day of the treatment group with white pomegranate seed extract, a large part of the wound surface was covered by a scab containing a blood clot consisting of fibrin and blood cells, as well as edematous cells and necrotic tissues. The wound space was completely filled by pericellular and young fleshy buds and was more developed than the previous groups (control, Oserin and zinc oxide nanoparticles). Certain hyperemia was observed in the new blood vessels inside the flesh bud. The presence of tumor cells was also reduced compared to the group treated with zinc oxide nanoparticles. Compared to the treatment group with zinc oxide nanoparticles, the coating texture of the pavement has grown more and it is growing from the bottom surface of the scab. The extension was toward the surface of the wound (Figure 4).

In the microscopic observation of the healing site of the skin wound on the 7th day of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles, many parts of the scab were separated from the wound surface and the wound surface was mainly covered by the remains of necrotic tissues and edematous cells. The wound space was completely filled by vascular fleshy bud and young pericell, and it was more developed than the previous groups. Certain hyperemia was also observed in the new blood vessels inside the flesh bud. The presence of tumor cells was also reduced compared to the group treated with white pomegranate seed extract. Epithelial tissue continued to expand on the wound surface and grew more than the group treated with white pomegranate seed extract (Figure 5).

Microscopic studies of the healing site of the skin wound on the 14th day of the control group showed that a large part of the wound surface was still covered by a scab containing blood clots consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. The wound space was completely occupied by vascularized fleshy bud and young pericell. Many edematous cells were visible on the surface under the scab as well as the tissue of the flesh bud itself. The cobblestone covering tissue was also spreading from the surface under the scab towards the surface of the wound (Figure 6).

In the microscopic evaluation of the healing site of the skin wound on the 14th day of the Oserin treatment group, a significant part of the wound surface was still covered by a scab containing a blood clot consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. The wound space was completely occupied by vascularized fleshy bud and young pericell, and marked hyperemia was observed in the new blood vessels present in it. The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself was reduced compared to the control group. The cobblestone tissue was still expanding towards the surface of the wound (Figure 7). The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself was reduced compared to the control and

Oserin treated groups. The squamous covering tissue was also expanding towards the wound surface and it grew more than the previous two groups and covered more than half of the wound surface (Figure 8). Microscopic studies of the healing site of the skin wound on the 14th day of the treatment group with white pomegranate seed extract showed that parts of the wound surface were covered by scabs containing blood clots consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. The wound space, which is occupied by young granulation tissue, was more orderly than the group treated with zinc oxide nanoparticles. The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself was significantly reduced compared to the group treated with zinc oxide nanoparticles. The squamous covering tissue was also expanding from under the scab towards the surface of the wound and covered more of the surface of the wound than the group treated with zinc oxide nanoparticles (Figure 9).Microscopic observation of the healing site of the skin wound on day 14 of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles showed that the scab was separated from the wound surface and the wound space was covered by granulation tissue, which was more orderly and organized than the group treated with white pomegranate seed extract. It was occupied. The amount of tumor cells was also reduced well compared to the group treated with white seed extract. The cobblestone covering tissue that was expanding towards the wound surface had covered more of the wound surface, but it was not much different compared to the group treated with white pomegranate seed extract (Figure 10).

Microscopic examination of the healing site of the skin wound on the 21st day of the control group showed that the squamous epithelial tissue, spreading from both sides of the wound surface, covered most of it, and the remaining part was partially covered by a scab consisting of blood clots, remnants of necrotic tissues, and a small number of The cells are covered. The wound space was completely occupied by the young granulation tissue with vascularity and pericellularity, and there was a definite blood pool in its vessels. Edema cells were also visible diffusely in the granulation tissue (Figure 11). Microscopic observation of the healing site of the skin wound on day 21 of the oserin treatment group revealed that the squamous epithelium has covered more than half of the wound surface by spreading from both sides of the wound surface. The wound space is completely occupied by young granulation tissue with vascularity and pericellularity, and the collagen strands in it were thicker and more regular compared to the control group. Edema cells were also visible diffusely in the granulation tissue, but their intensity was reduced compared to the control group during this period. The neoplastic vessels of the granulation tissue had relative hyperemia in the deeper parts (Figure 12).

Microscopic evaluation of skin wound repair site on day 21 of zinc oxide nanoparticles treatment group. The cobblestone covering extends from both sides of the wound surface and covers most of it (more than three-fourths) and a small part of it has no covering. The wound space was completely occupied by young granulation tissue with vascularity and pericells, and the collagen fibers in it were thicker and more organized than the group treated with Oserin and had more maturity. As in the group treated with Oserin, the presence of edematous cells was well reduced. The new vessels of the granulation tissue in the deeper parts were still relatively hyperemic as in the group treated with Oserin (Figure 13). Microscopic examination of the healing site of the skin wound on day 21 of the group treated with white pomegranate seed extract showed that the covering tissue of the relatively regular paving of the new foundation completely covered the wound surface. The wound space was completely occupied by relatively mature granulation tissue and fibrous strands. The presence of edematous cells was also greatly reduced and they were rarely visible, and mild to moderate hyperemia was observed in the vessels of the areas around the repair area (Figure 14).Microscopic observation of the healing site of the skin wound on day 21 of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles showed that the covering tissue of the new foundation pavement completely covered the wound surface. Compared to the group treated with white pomegranate seed extract, it is thicker and has more order and organization. The wound space is completely occupied by mature granulation tissue with specific fibrotic fibers and the collagen fibers in it are thicker and more organized compared to the group treated with white pomegranate seed extract. Hyperemia was not visible and edematous cells were not noticeable (Figure 15). The rank comparison of the tissue changes of repair between the studied groups during the test period is given in Tables 1 to 3.

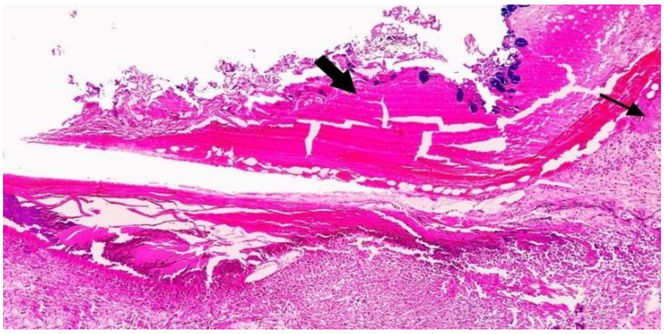

Figure 1. Microscopic view of the healing site of the skin wound on day 7 of the control group. The surface of the wound is covered by a scab (thick arrow) consisting of a blood clot containing fibrin and blood cells, edematous cells and the remains of necrotic tissues. The presence of microbial cells on the surface of the scab on the wound is obvious. The space under the scab is occupied by loose connective tissue. The presence of acute type edematous cells is also evident under the scab as well as the connective tissue itself. The squamous covering tissue that has started to regenerate with the formation of covering mucus (thin arrow) and is visible from the surface under the scab to the surface of the wound (hematoxylin-eosin, 100x magnification).

Figure 2. Microscopic view of the healing site of the skin wound on day 7 of the Oserin treatment group. The surface of the wound is covered by a scab (thick arrow) consisting of a blood clot containing coagulated fibrin, edematous cells and the remains of necrotic tissues. The wound space is completely filled by young granulation tissue (arrowhead). The presence of acute swelling cells is mainly limited to the superficial parts of the wound and under the scab. However, they can be seen in deeper tissues. The squamous covering tissue that has started to regenerate with the formation of covering mucus (thin arrow) and is expanding from the surface below the scab towards the surface of the wound (hematoxylin-eosin, 100x magnification).

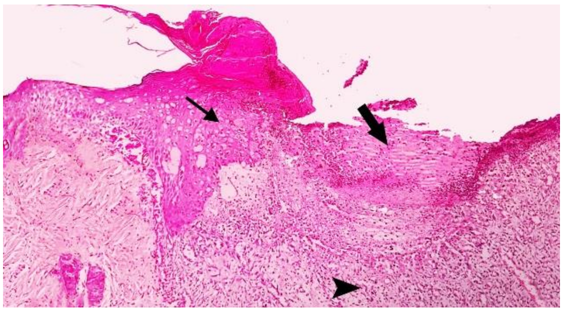

Figure 3. Microscopic view of the healing site of the skin wound on day 7 of the group treated with zinc oxide nanoparticles. The wound surface is covered by a scab (thick arrow) containing a blood clot consisting of fibrin and blood cells, as well as edematous cells and necrotic tissues. The wound space is completely filled by pericellular and young fleshy bud (arrowhead). The presence of tumor cells has decreased compared to the group treated with Oserin. The squamous epithelial tissue that has started to regenerate with the formation of a covering bud (thin arrow) is thicker and more developed compared to the group treated with Oserin, and it is expanding from the surface under the scab towards the surface of the wound (hematoxylin-eosin, 100x magnification)

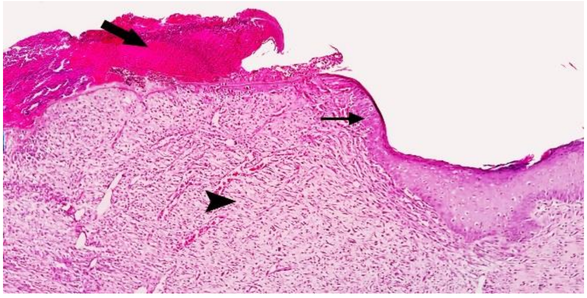

Figure 4. Microscopic view of the healing site of the skin wound on day 7 of the group treated with white pomegranate seed extract. The wound surface is covered by a scab (thick arrow) containing a blood clot consisting of fibrin and blood cells, as well as edematous cells and necrotic tissues. The wound space is completely filled by the pericellular and young fleshy bud (arrowhead) and marked hyperemia is observed in the newly formed vessels inside the fleshy bud. The presence of tumor cells has decreased compared to the group treated with zinc oxide nanoparticles. The squamous covering tissue (thin arrow) has grown more than the group treated with zinc oxide nanoparticles and is expanding from the surface below the scab towards the surface of the wound (hematoxylin-eosin, 100x magnification).

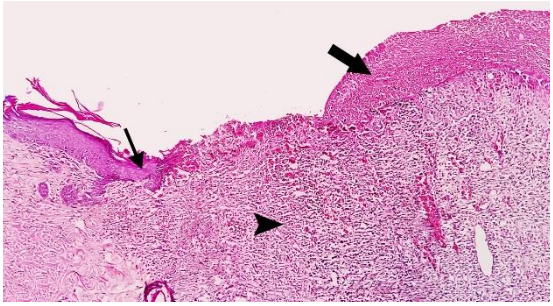

Figure 5. Microscopic view of the healing site of the skin wound on day 7 of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles. The scab is separated

from the surface of the wound and the surface of the wound is mainly covered by the remains of necrotic tissues and edematous cells. The wound space is completely filled by vascular fleshy bud and young pericell, and a specific hyperemia is observed in newly formed vessels inside the fleshy bud. The intensity of the presence of edematous cells has decreased compared to the group treated with white pomegranate seed extract. The squamous covering tissue (arrow) is also expanding on the surface of the wound (hematoxylin-eosin, 100x magnification).

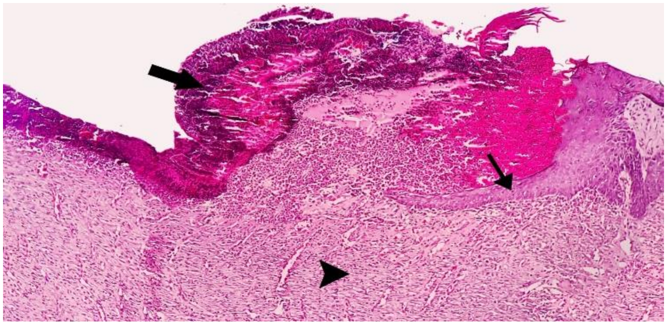

Figure 6. Microscopic view of the healing site of the skin wound on day 14 of the control group. The surface of the wound by a scab (thick arrow) contains a blood clot consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. It is covered. The wound space is completely occupied by vascular fleshy bud and young pericell (arrowhead). Many edematous cells can be seen on the surface under the scab as well as the tissue of the fleshy bud itself. The squamous covering tissue (thin arrow) is expanding from the surface below the scab towards the surface of the wound (hematoxylin-eosin, 100x magnification).

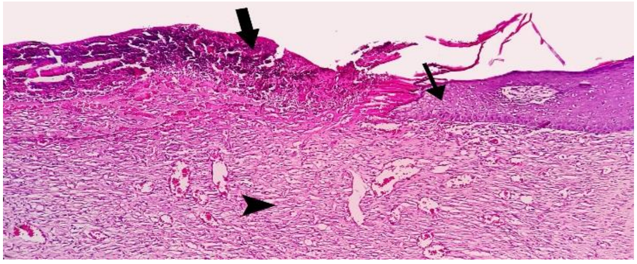

Figure 7. Microscopic view of the healing site of the skin wound on day 14 of the Oserin treatment group. The surface of the wound is covered by a scab (thick arrow) containing a blood clot consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. The wound space is completely occupied by vascular fleshy bud and young pericell (arrowhead). A specific hyperemia is observed in the neovascular vessels of the

flesh bud. The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself has decreased compared to the control group. The squamous covering tissue (thin arrow) is also expanding towards the surface of the wound (hematoxylin-eosin, 100x magnification).

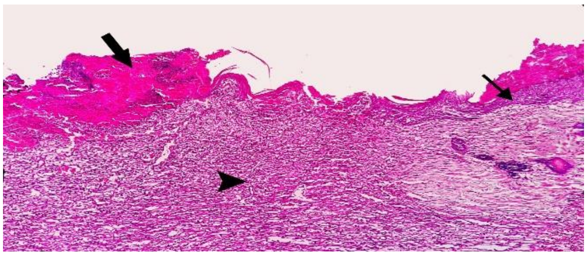

Figure 8. Microscopic view of the healing site of the skin wound on day 14 of the group treated with zinc oxide nanoparticles. Parts of the scab (thick arrow) are separated from the wound surface, but a large part of the wound surface is covered by a scab containing a blood clot consisting of fibrin and blood cells, as well as a large number of edematous cells and necrotic tissues. The wound space is completely occupied by vascular fleshy bud and young pericell (arrowhead). The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself has decreased compared to the control group. The squamous covering tissue (thin arrow) is also expanding towards the surface of the wound (hematoxylin-eosin, 100x magnification).

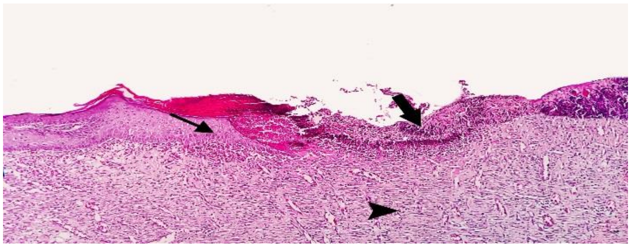

Figure 9. Microscopic view of the healing site of the skin wound on day 14 of the group treated with white pomegranate seed extract. The surface of the wound is covered by a scab (thick arrow) containing a blood clot consisting of fibrin and blood cells, as well as a large number

of edematous cells and necrotic tissues. Wound space occupied by young granulation tissue (arrowhead).Compared to the group treated with zinc oxide nanoparticles, it has more order. The amount of edematous cells on the surface under the scab and the tissue of the fleshy bud itself has been significantly reduced compared to the group treated with zinc oxide nanoparticles. The squamous covering tissue (thin arrow) is also expanding from under the scab towards the surface of the wound (hematoxylin-eosin, 100x magnification).

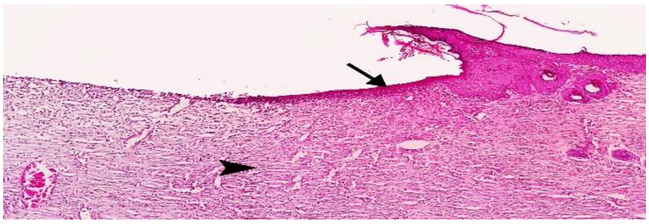

Figure 10. Microscopic view of the healing site of the skin wound on day 14 of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles. The scab is separated from the wound surface and the wound space is occupied by granulation tissue (arrowhead), which is more orderly and organized than the group treated with white pomegranate seed extract. The amount of tumor cells has been reduced well compared to the group treated with white seed extract. The squamous covering tissue (arrow) is also expanding towards the surface of the wound (hematoxylin-eosin, 100x magnification).

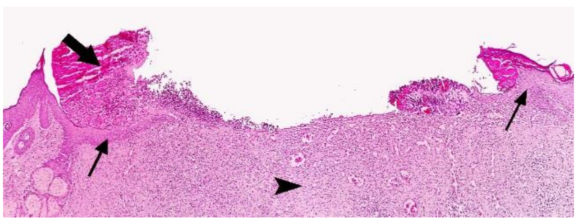

Figure 11. Microscopic view of the healing site of the skin wound on day 21 of the control group. The squamous covering tissue (thin arrows) has covered most of it by spreading from both sides of the wound surface, and the remaining part is partially covered by a scab (thick arrow) composed of blood clots, remnants of necrotic tissues and a small number of cells. The wound space is completely occupied by young granulation tissue with vascularity and pericells

(arrowhead) and a certain amount of blood is observed in its vessels. Edema cells can be seen diffusely in the granulation tissue. (Hematoxylin-eosin, 100x magnification).

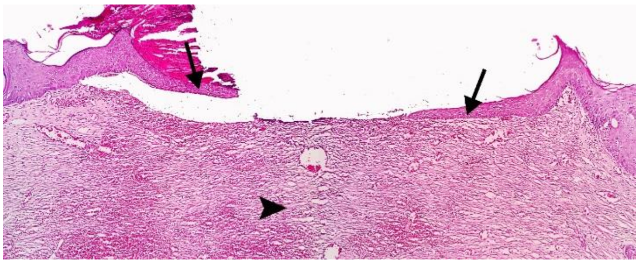

Figure 12. Microscopic view of the healing site of the skin wound on day 21 of the Oserin treatment group. The cobblestone covering tissue (thin arrows) has covered most of the wound surface by spreading from both sides. The wound space is completely occupied by young granulation tissue with vascularity and pericells (arrowhead) and the collagen strands in it are thicker and more regular compared to the control group. Edema cells can be seen diffusely in the granulation tissue. The new blood vessels of the granulation tissue in the deeper parts have relative hyperemia (hematoxylin-eosin, 100x magnification).

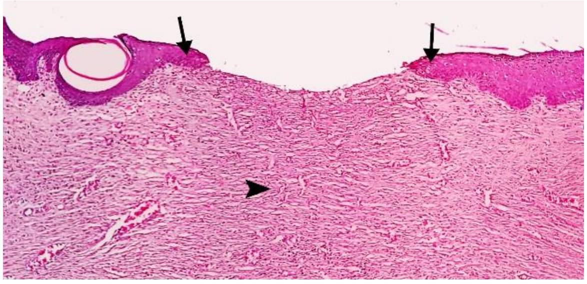

Figure 13. Microscopic view of the healing site of the skin wound on day 21 of the group treated with zinc oxide nanoparticles. The cobblestone covering tissue (thin arrows) by spreading from both sides of the wound surface, covers most of it and a small part of it has no covering tissue. The wound space is completely occupied by young granulation tissue with vascularity and pericells (arrowhead), and the collagen fibers in it are thicker and more organized than in the group treated with Oserin and have more maturity. The presence of edematous cells has also been greatly reduced. The new blood vessels of the granulation tissue in the deeper parts still have relative hyperemia (hematoxylin-eosin, 100x magnification).

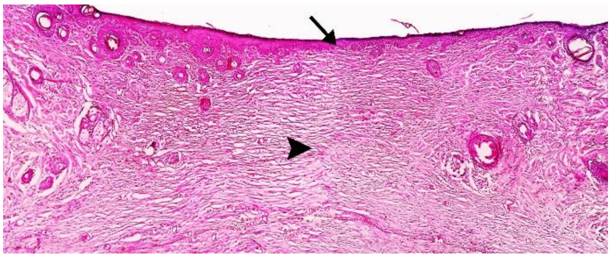

Figure 14. Microscopic view of the healing site of the skin wound on day 21 of the group treated with white pomegranate seed extract. Relatively regular cobblestone covering tissue (thin arrow) has completely covered the surface of the wound. The wound space is completely occupied by relatively mature granulation tissue (arrowhead) and fibrous strands. The presence of edematous cells is greatly reduced and rarely visible, and mild to moderate hyperemia is observed in the vessels around the repair area (hematoxylin-eosin, 100x magnification).

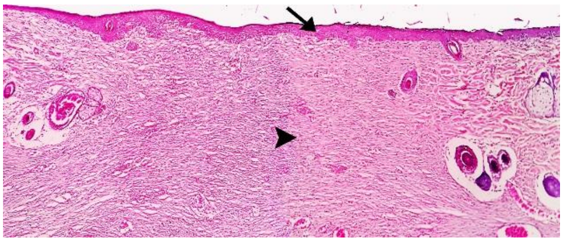

Figure 15. Microscopic view of the healing site of the skin wound on day 21 of the treatment group with white pomegranate seed extract + zinc oxide nanoparticles. The covering tissue of the new foundation pavement (thin arrow) which has completely covered the wound surface is thicker and has more order and organization than the group treated with white pomegranate seed extract. The wound space is completely occupied by mature granulation tissue (arrowhead) with distinct fibrotic fibers, and the collagen fibers in it are thicker and more organized than the group treated with white pomegranate seed extract. Hyperemia was not visible and edematous cells were not seen either (hematoxylin-eosin, 100x magnification).

Table 1- Comparison of changes in restoration parameters between the studied groups on day 7

Epidermal tissue caress	**Fibroplasia process**	**Amas reduction**	**The degree of homeostasis**	**Repair parameters group**
+	+	+	+	the witness
+	+	+	+	Treatment with Oserin
+	+/++	+/++	+/++	Treatment with zinc oxide nanoparticles
++	++	++/+++	++/+++	Treatment with white pomegranate seed extract
+++	+++	+++	+++	Treatment with white pomegranate seed extract + zinc oxide nanoparticles

Epidermal tissue caress	Fibropasia process	Amas reduction	The degree of homeostasis	Repair parameters group
++	++	+/++	+/++	the witness
++	++	++	++	Treatment with Oserin
++/+++	++	++/+++	++/+++	Treatment with zinc oxide nanoparticles

+++	+++	+++	+++	Treatment with white pomegranate seed extract
+++	++++	++++	++++	Treatment with white pomegranate seed extract Zinc oxide nanoparticles

Table 3- Comparison of the changes in restoration parameters between the studied groups on the 21st day

Epidermal tissue caress	Fibroplasia process	Amas reduction	The degree of homeostasis	Repair parameters group
++/+++	++/+++	++	+++	the witness
+++	+++	++/+++	++/+++	Treatment with Oserin
++++	+++	++/+++	+++/++++	Treatment with zinc oxide nanoparticles
+++++	+++/++++	+++/++++	++++	Treatment with white pomegranate seed extract
+++++	++++	++++	++++	Treatment with white pomegranate seed extract Zinc oxide nanoparticles

3. Conclusion

The differences of these cells counted in this study can indicate the acceleration of the healing process due to the effect of the fourth group and the fifth group, because the higher the number of inflammatory cells, the faster the healing process of the wound [32]. Also, the healing process has an inverse relationship with the number of multinucleated cells[32], which is consistent with the findings of this study and indicates the effectiveness of the extract used. After 14 days of treatment with white pomegranate seed extract and zinc oxide nanoparticles extract, The effects of wound healing were observed with a decrease in the wound surface and an increase in contraction of 99 percent on the 14th day and 100 percent on the 21st day compared to the 7th day, which may be the response to the partial effect of tannins and polyphenols such as ellagic acid, Gallic and couric acid are present in the extract of white pomegranate seed [33]. In the macroscopic examination of the treated wounds of the fourth and fifth groups, the production of granule tissue and the contraction of the outer edge of the wound were evident. In the contracted wound, myofibroblast cells play an important role in closing the wound and bringing the edges of the wound closer [34]. The results show that wound treatment with white pomegranate seed extract and zinc oxide nanoparticle extract causes the arrangement of myofibroblast cells in the treated wound areas. These cells are highly active in matrix synthesis and show little organization at the edge of the wound [35-36]. Wound repair is a series of regular events that lead to the formation of the integrity of the injured tissue. Wound repair causes the damaged tissues to be protected from the invasion of pathogenic substances and causes for the restoration of the damaged tissue to be provided. Different phases are involved in wound healing, including inflammatory phases, tissue budding, fibroblastic tissue formation and proliferation, new blood vessel formation, wound consolidation, and epithelial tissue formation [37]. The wound healing process takes place through the use of plants and substances found in nature that contain compounds such as flavonoids, alkaloids, tannins, and other biomolecules. These factors often affect one or more phases of the repair process. Peel, pulp and seed, pomegranate peel has the highest antioxidant activity. There are many polyphenols in pomegranate peel, for example ellagic tannins, ellagic acid and gallic acid. They have been used in the preparation of recipes for food, cosmetics, tinctures and therapeutic drugs. The effectiveness of dried fruit skin in the treatment of respiratory and urinary tract infections and diarrhea has been reported. Cytotoxic activity, hepatoprotective activity and hypoglycemic activity have also been reported. Ethanol extract of pomegranate peel has an ameliorating effect against oxidative stress caused by chlorpyrifos-ethyl in rats. It also has a strong hepatoprotective action and suppresses oxidative kidney damage caused by iron nitrilotriacetate. Reactive oxygen species (ROS) damage cells and tissues, hence are detrimental to wound healing. ROS peroxidize lipids, inactivate enzymes and degrade DNA. Studies have shown that vitamin C and E are effective antioxidants to eliminate free radicals. The antioxidant and anti-inflammatory properties of pomegranate fruit, which can accelerate the wound healing process, can be attributed to some extent to these vitamins. In addition, tannins and polyphenols such as ellagic acid, gallic acid and couric acid are found in pomegranate fruit [20]. In addition, it should be said that this effect was identified as a significant increase in the rate of wound contraction and aggregation, an increase in the formation of epithelial tissue, and an increase in hydroxyproline [37]. In the present study, as it was mentioned, it was seen that the number of blood vessel intersections decreased in the experimental group, which is an ideal stage from the point of view of the process of reconstruction and organization of collagen in the wound site, which is probably due to the presence of flavin in the extract of pollen [37]. Free radicals produced in the damaged area cause damage in the repair process through damage to the cell membrane, nucleotides, proteins and lipids. Antioxidants such as yellow wood pigment, vitamin E, etc. have been mentioned, whose antioxidant properties have been proven in damaged tissues [39-38]. The subject of the use of antioxidants in wound healing and improvement The wound healing process has been proven [40-41]. Many plant extracts have antioxidant power. Flavonoids are the main components of many plant extracts. Flavonoids are strong neutralizers against free radicals [42]. It has been proven that the antioxidant capacity of plant extracts is related to the presence of vitamins C and E, carotenoids and flavonoids [43]. Considering the effect of carotenoids in increasing the power of wound healing, it may be possible to know the wound healing activity of this seed in relation to carotenoids [44]. Wounds treated with flavones show an increase in glutathione, which is an oxidant, and ascorbic acid. It has been observed that the increase in the amount of these two in the site roughly improves the repair process [43]. Ascorbic acid has a confirmed role in collagen metabolism. In addition, ascorbic acid is a stimulus for collagen gene transcription, collagen mRNA levels and collagen production in cultured human fibroblasts [45]. Considering the absorption of mineral materials from the soil by plants such as manganese, iron, copper and zinc, their use in wound healing may be through the provision of necessary elements for the formation of enzymes. In fact, these elements are the catalysts for the synthesis of enzymes that are involved in the wound healing process[46].

In conclusion, the topical application of white pomegranate seed extract along with zinc oxide nanoparticles has a positive effect on the different stages of wound healing, so it may play a beneficial role in wound healing.

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