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The effect of alcoholic extract of Nettle leaves, Urtica dioica, on histomorphology and structural alterations during dermal wound healing in rat

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Abstract

Nettle (*Urtica dioica*) is one of the most important native plants that have been used for the treatment of a variety of diseases, but limited data is available about its beneficial effects on histological changes of skin during wound healing. In the present study, the effects of 5% and 10% alcoholic cream of nettle leaf extract as an experimental drug in wound healing were investigated. The rats (n=30) were randomly divided into five experimental groups. The back of the animals using a puncture machine was wound. Then all of them were treated twice a day for two weeks. The control group received saline at the site of the normal wound. The sham group received the eucerin solvent and the phenytoin group received 1% ointment. Two experimental groups received 5% and 10% of Nettle extracts. Finally, 14 days after the start of the study histological changes in the skin of the wound site, granulation tissue amount, granulation tissue fibroblast maturation, collagen deposition, re-epithelialization, neovascularization, hydroxyproline content, and the percentage of wound healing in different experimental groups were analyzed. On day 14 of the experiment, the phenytoin and NE-treated groups had better effects on wound healing compared with the control group. Hydroxyproline content in dried wounds was significantly increased in the NE-treated groups compared to control groups. These results in 10% NE-treated group were significantly better than the 5% NE-treated group. These findings were confirmed by histological examination as well. According to the obtained results, nettle extract, to some extent similar to common chemical wound healing drug such as phenytoin, had a positive effect on skin changes during wound healing and these effects were dose-dependent.

Key words: Rat, re-epithelialization, Skin, *Urtica dioica*, Wounds healing

Introduction

The skin is the largest and heaviest organ in the human and animal body, which in addition to its important role in protecting and excreting wastes as an important sensory organ, can also play an essential role in maintaining the body's homeostasis. Histologically, the skin is made up of three layers, the epidermis, which is the epithelial layer and component of the epithelial cells, and this layer has an ectodermal origin. Beneath it is a layer called the dermis, which is a connective tissue full of cells and collagen fibers that originates in the mesoderm. The last layer and the lowest

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layer of the skin is the hypodermic layer, which is full of fat and other connective tissues, and the role of this layer is to connect the skin to the surrounding tissues (Mescher 2018).

Rupture and opening of part of the skin can cause lesions and diseases that lead to extensive infections and even death. For this reason, one of the most serious subjects in medical science in the treatment of wounds is repairing them in the shortest time and with the least place of the wound effect in terms of beauty (Blanks et al. 1998; Lazarides and Giannoukas 2007). The nettle (Urtica dioica) plant, from the genus Urticaceae, is a member of the Urtica family of Perennial plants the length of this plant is relatively short. The stem is straight and square and its leaves are covered with biting hairs. In most parts of the world, it grows as a weed in the fields, and in Iran, it is distributed in the northern, western, and central regions (Gucker 2020; Rawat 2020). Almost all parts of this plant including rhizome and dried root, leaves, and seeds, the dried flowering plants can be used for the treatment of several diseases. The leaves of the plant are used to treat urinary tract infections, kidney and bladder stones, and inflammatory diseases. The topical forms of this plant are used to treat dandruff and control sebum (Salih 2014, Piers 2006). The active ingredients in Nettle leaves include flavonoids such as camphor and quercetin, vitamins C, B, and K, calcium, iron, manganese, potassium, especially glucokinin nitrate, chlorophyll, carotene. It also contains amines such as histamine, serotonin, and choline in its young stems and leaf blades (Harborne 1998, Gutowska et al. 2014). Due to the presence of this plant in Iran and the world and the growing tendency to use medicinal plants instead of drugs and chemicals, the current study was performed to investigate the healing effect and compare different doses of Nettle extract on wound model of rat.

Materials and Methods

Extract preparation

First, the fresh leaves of the Nettle plant (The specimen voucher number was 2365.) were collected from the farms of Amol city (52° 19′ 32.17″E, 36° 27′ 36.50″N) in Mazandaran province, north of Iran, at an altitude of 76 m above sea level. The leaves of the upper part of the collected plants were used for research. Leaves were washed thoroughly in water, cut into small pieces using a mixer, and soaked in a 70% aqueous ethanol solution in a large container for 48 hours with occasional shaking. The extracts were filtered through a 0.45 μm filter membrane (Whatman Company, UK) and concentrated in a rotary evaporator (Hosseinzadeh et al. 2021).

Animals

Thirty male Wistar rats (220- 250 g) were obtained from the animal house of Jundishapur University of Medical Sciences of Ahvaz. The rats were kept in polypropylene cages and fed with standard rat chow and drinking water ad libitum. The animals were maintained in a controlled condition at 20 ± 2°C with a 12-hour light/dark cycle. The investigation was performed according to the animal ethics committee guidelines for the use of experimental animals. A schematic view of the research design is shown in Fig. 1.

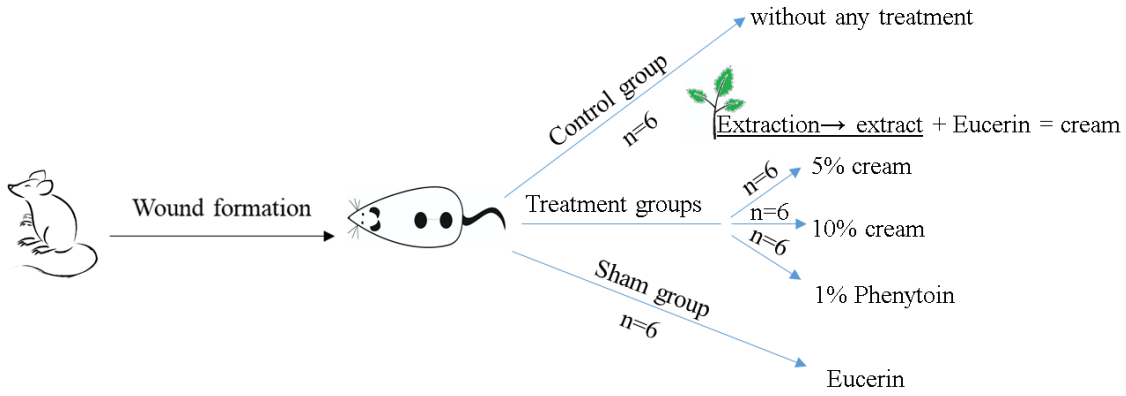

Figure 1: Schematic view of the experimental protocol

Preparation of Nettle Extract Cream

Creams were prepared by dissolving different amounts of alcoholic extract of nettle leaves (5 and 10 % in eucerin (Pasteur Institute, Iran (w/w)) (Zeković et al. 2017). Eventually, the centrifugal method was used to investigate the uniformity of the topical cream formulation produced. 6 g of the produced cream was placed in a centrifuge tube and centrifuged at 4000 rpm for 10 minutes and checked its concentration and drainage (Rayburn et al.,2009, Javadi et al.,2015).

Excision Wound Model

Initially, the rats were anesthetized with 60 mg/kg ketamine and 6 mg/kg xylazine. Then the hairs in the lumbar region on the dorsal surface of each rat were thoroughly shaved using an electric shaver. Then, under completely sterile conditions and in full compliance with the principles of surgery, the circular 6-mm diameter full-thickness excisional wounds were generated using a puncture machine (BIOPSY PUNCH 6.0 mm KAI, Japan) (two wound/rat). (Kamar and Rashed 2019. These wounds were created at a distance of 2 cm from each other dorsal region of the rat and the wounds were disinfected with Povidone-iodine and sterile gauze. The rats were then kept in cages with soft straw beds. All of the rats were injected with 0.2 mg of penicillin (Razi chemical, Iran) and 0.2 mg of gentamicin (Daya Elixir, Iran) as a single dose for preventing possible infection. The day of operation was considered to be zero-day and the beginning of the period.

Experimental groups

The animals were randomly divided into 5 groups (n=6). Group 1 (control group): The wound areas in this group remained without any treatment. Each animal in Groups 2-5 received Phenytoin 1% (Daropakhsh, Iran), eucerin, and two different doses of Nettle extract (NE) cream (5 and 10 % in eucerin, w/w), respectively topically on to the surface of wound areas, twice a day for 14 consecutive days (Priyadarshi et al. 2022). All treatments were performed by one person under the same conditions.

Histological analysis

At the end of the experiment (14th day), animals were anesthetized with 60 mg/kg ketamine and 6 mg/kg xylazine (Mescher, 2018). The hair at the test site is shaved and samples of the skin of the repaired area are collected from all the animals. Samples were placed in a 10% formalin buffer (Merck, Germany) for fixing, and after the usual processing, 5 µm-thick sections were cut. Then the slides were stained with Hematoxylin and Eosin (Merck, Germany) for general morphological. Also, the slides for the detection of collagen fibers, and specific staining of Masson's trichrome

(Merck, Germany) were performed. The slides were examined semi-qualitatively using an optical microscope (Olympus BH-2, Japan) and using a Dino-Eye lens (AM7023, Taiwan) and with the help of Dino capture 2.0 computer software (Fatemi et al. 2021). The slides were examined with a magnification of 400 and the results were expressed according to the method of Abramov et al using the scale from 0 to 3 for the following histological parameters: inflammation (mono and polymorphonuclear leucocytes); granulation tissue amount (mm); granulation tissue fibroblast maturation; collagen deposition; re-epithelialization, and neovascularization. The count was measured in 10 non-overlapping microscopic fields (×100) in each rat and the means were calculated as well (Abramov et al. 2007).

Estimation of hydroxyproline content

The skin tissue formed was carefully removed under anesthesia on the 14th day of the experiment. These tissue samples were dried at 60ºC for 12 h and weighed to determine the dry skin tissue weight. The samples are then hydrolyzed with 5ml 6 N HCl for 4 hours at 130 ° C. The pH of the hydrolyzed samples is raised to 7 and subjected to oxidation of Chloramine T trihydrate (Merck, Germany). The color complex with the Dimethylaminobenzaldehyde (Ehrlich S) reagent (Merck, Germany) forms at 60° C and is read at spectrophotometrically 557 nm. Standard hydroxyproline is also prepared and measured at the same wavelength. Values are reported in µg/ mg tissue dry weight (Alsarayreh et al. 2021). All steps were performed according to the agenda of the manufacturer of the hydroxyproline measurement kit (Kiazist, Iran).

Determination of wound healing percentage

Wound extent (mm^2) was measured on days 1, 7, and 14 with the help of a trans-paper, the wound environment is drawn and with the help of a ruler with an accuracy of 1 mm, the wound area is measured. To measure wound healing on specific days, the skin of the surgical site was photographed by a digital camera under similar conditions. Then Image J software was used to calculate wound size with high accuracy. The percentage of wound healing was determined using the following formula (Nigussie et al. 2021):

Wound healing percentage = (wound area on day 1 -wound area in a day x / wound area on day 1) ×100.

Statistical analysis

All statistical analyses were performed using Graph Pad Prism (V.5.03. San Diego, CA, USA). Data are expressed as mean±SD and the results were statistically evaluated using a one-way ANOVA test. The Tukey test was used to identify significant differences between individual groups. In all cases, $P<0.05$ was considered significant.

Results

Histological study

Finally, on the fourteenth day after the injury, histological studies were performed to detect the repair process (Fig. 2, 3). Histological examination of wound tissue in the Hematoxylin-Eosin (H&E) stained sections in the control group showed irregular epithelialization with fibrous scarring. The epithelium tissue in this group covers the wound surface with less qualification and dried tissue. Also, the surface of the epidermis was full of inflammation cells that showed delayed healing (Fig. 2A). There was a significant difference between the granulation tissue amount and granulation tissue fibroblast maturation in the group receiving 1% phenytoin ointment with the control group, while this group itself had a statistically significant difference with the groups treated with the Nettle extracts in the collagen deposition, re-epithelialization,

neovascularization. In the eucerin-treated group, many fat droplets were seen in the epidermis and dermis, and in Masson's trichrome staining, the density of collagen fibers in the dermis was low in comparison with the control group (Fig. 3C). Following microscopic evaluation of the improved skin in NE-treated rat, it can be concluded that following the increase in fibroblast maturation, wound contraction was quite evident in these groups (Fig. 2D, 3D). In the NE-treated group, the connective tissue with low cells is located in the wound space. Collagen bundles were organized to some extent. There are no signs of inflammatory cells. The epithelium tissue covers the entire surface of the wound. In the NE-treated group, the epithelium tissue was in terms of thickness and organization better than in the control groups. In Masson's trichrome staining, the number of collagen blue filaments was increased and collagen bundles appear to be clearer and thicker in groups treated with the extract, and the vascular structure is seen between them. This condition was more obvious in the 10% NE-treated group (Fig. 2E, 3E). Table 1 showed the histological alterations of the important indicators of wound healing in different groups, the fibroblast cell numbers in granulation tissue were significantly different between the control group and the phenytoin (3.0 ± 0 vs. 2.1 ± 0.16, $P<0.05$) and between the phenytoin group and the NE-treated groups were significantly different ($p<0.05$). It was found no significant differences in fibroblast maturation of granulation tissues were between different experimental groups ($P> 0.05$). In re-epithelization and neovascularization were significantly different between the control group and phenytoin or NE-treated groups ($P<0.05$). The collagen deposition and inflammation were significantly different between the control groups and NE-treated groups ($P<0.05$) and in the 10% NE-treated group more than the 5% NE-treated group.

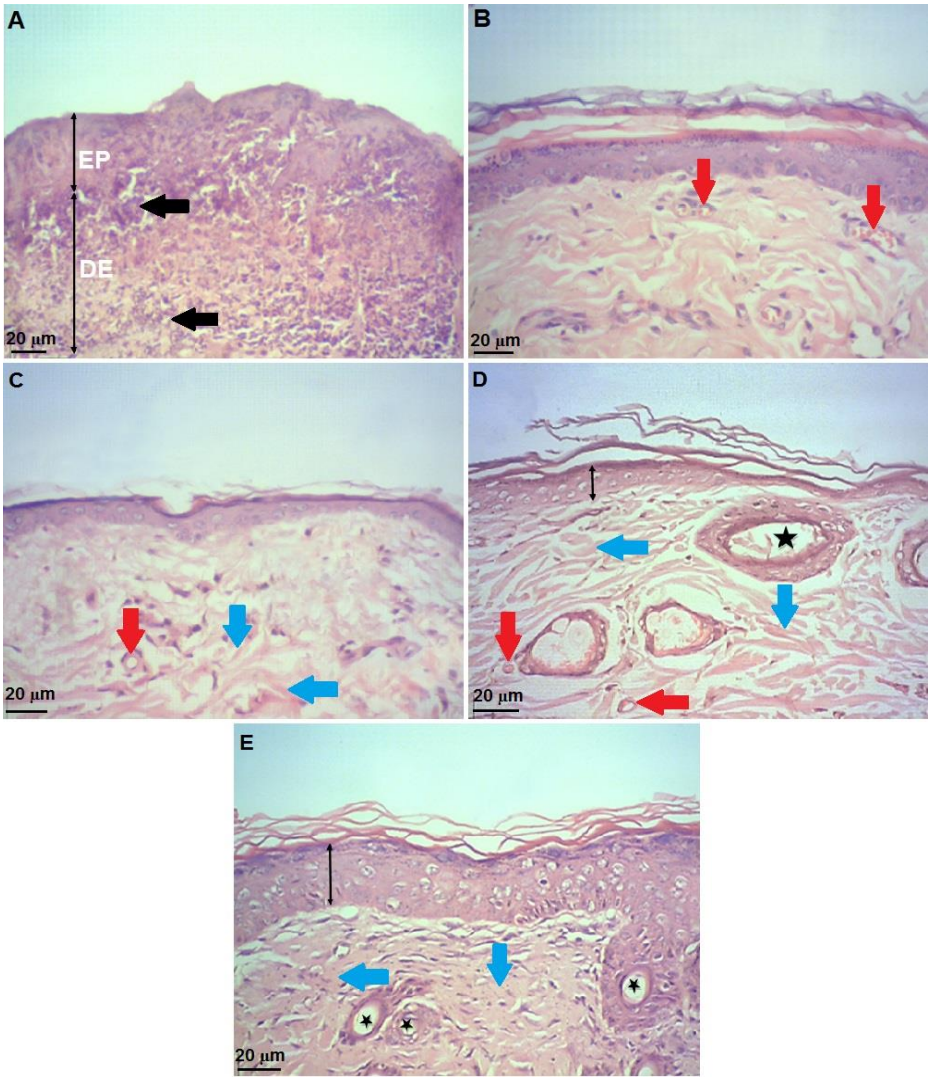

Figure 2: Hematoxylin-eosin staining of healed wounds in different groups after 14 days from wound creation (×200). (A) Untreated wound; (B) Wound treated with 1% Phenytoin; (C) Wound treated with Eucerin; (D) Wound treated with 5% NE-treated and (E) Wound treated with 10% NE-treated. In the untreated group (A), the healed epidermis structure after 14 days is normal whereas the main structural constituent of the epidermis and dermis is relatively mature granulation tissue (black arrows) and the absence of skin adnexal structures is evident. In the wounds treated with the 1% Phenytoin (B), the epidermal structure is closed to normal tissue, but there are still no visible skin structures. In the wounds treated with Eucerin (C), Eucerin was used as a sham group, in which collagen fibers were higher in the dermis than in the control group, and the epidermal tissue was better. Wounds treated with 5% NE-treated (D) and 10% (E) showed a rather normal structure and thickness of the epidermis compared with other groups (two-way arrow). Also, dermal structures including hair follicles and collagen fibers have a significant number and thickness that these structures are more evident in the group treated with the extract of 10%. Two-way arrow Ep: Epidermis layer, Two-way arrow DE: dermis layer, black arrow: granulation tissue, red arrow: blood vessel, blue arrow: Collagen filaments, and star: hair follicles. Scale bar for all images: 20.0 µm.

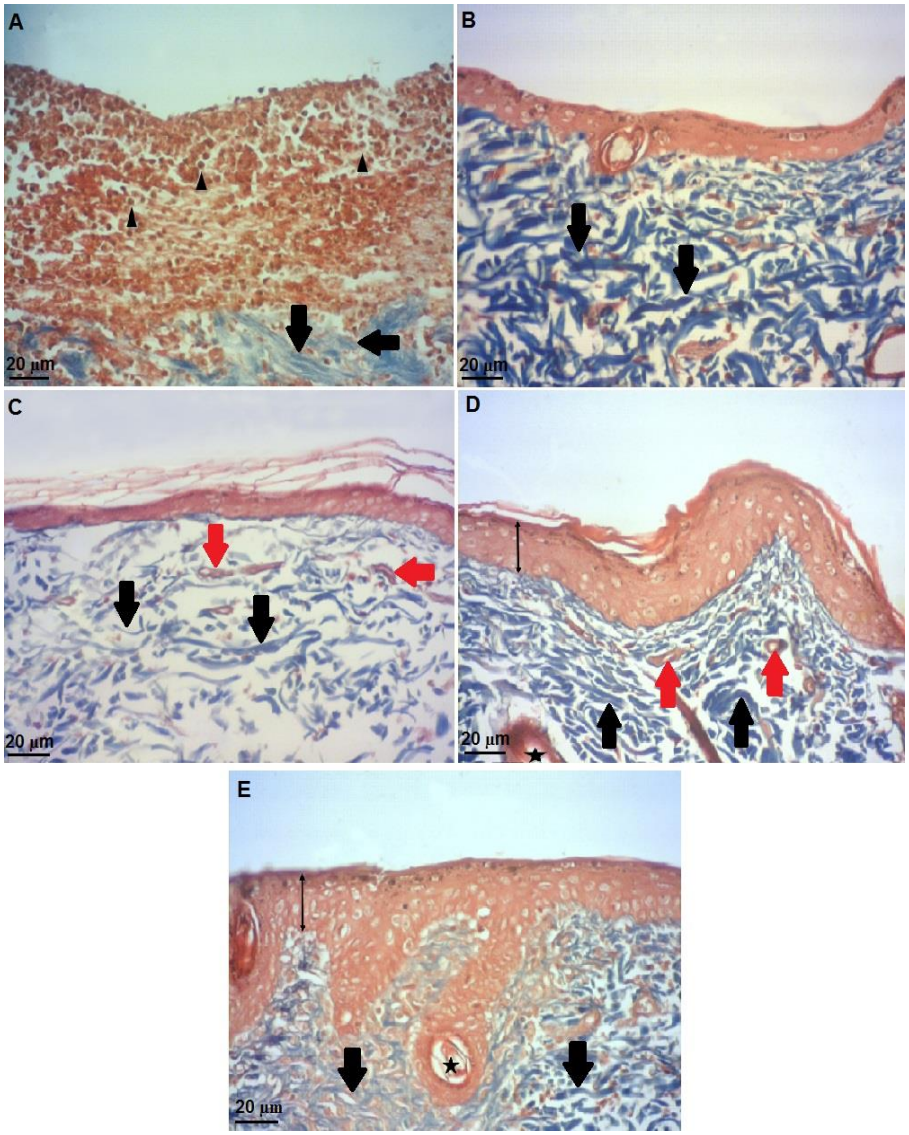

Figure 3: Masson's trichrome staining of the healed wound areas after 14 days from wound creation (×200). (A) untreated wound; (B) wound treated with 1% Phenytoin; (C) wound treated with Eucerin, (D) wound treated with 5% NE-treated, and (E) wound treated with 10% NE-treated. Collagen fibers in this staining are stained with blue color. In the Phenytoin and 10% NE-treated, the density of collagen fibers in the dermis tissue increases and the dermis structure becomes closer to the healthy skin tissue. Two-way arrow Ep: Epidermis layer, arrowhead: granulation tissue, red arrow: blood vessel, black arrow: Collagen filaments, and star: hair follicles. Scale bar for all images: 20.0 µm.

Table 1: The semiquantitative analysis of Histological alteration assessment of parameters in deferent groups on day 14

Parameters/Groups	Control	Phenytoin	Eucerin	5% NE-treated	10% NE-treated
Inflammation	2.3±0.16	1.1±0.22 [a]	2.2±0.21 [b]	2.0±0.21 [b]	1.5±0.22 [a,c]
Granulation tissue amount (mm)	3.0±0	2.1±0.16 [a]	2.8±0.21 [b]	2.6±0.21 [a,b]	2.3±0.21 [a]
Granulation tissue fibroblast maturation	2.1±0.16	2.8±0.21 [a]	2.1±0.14 [b]	2.5±0.26 [a]	2.6±0.31 [a]
Collagen deposition	2.0±0.10	2.8±0.14 [a]	2.2±0.26 [a,b]	2.5±0.17 [a,b]	2.9±0.16 [a,c]
Re-epithelialization	1.3±0.18	2.1±0.25 [a]	1.4±0.12 [b]	1.7±0.19 [a,b]	1.9±0.32 [a]
Neovascularization	2.2±0.16	2.8±0.22 [a]	2.7±0.11 [b]	2.4±0.19 [a,b]	2.7±0.21 [a,c]

[a] Compared with the control group ($p<0.05$), [b] compared with phenytoin group ($p<0.05$),
[c] compared with 5% NE-treated group ($p<0.05$). Data are expressed as mean±SD (n = 6).

Hydroxyproline content

The hydroxyproline concentrations in dried skin tissue are shown in Fig. 4. The results showed that the amount of hydroxyproline in the control group was significantly different from the phenytoin group ($p<0.05$). Also, the phenytoin group with the 5% NE-treated group was significantly different ($p<0.05$). The amount of hydroxyproline concentration in the 10% NE-treated group was more than the 5% NE-treated group (12.7 ± 1.5 vs. 9.8 ± 0.4, $p<0.05$).

Percentage of Wound Healing

By measuring the wound surface and obtaining the wound healing percentage, it was found that on the seventh day, the healing percentage in the phenytoin group and 10% NE-treated has a significant difference from the control group ($p<0.05$). On the fourteenth day, there was a significant difference in wound healing percentage between the NE-treated groups and the phenytoin group in the control group ($p<0.05$). In comparison between the two groups, NE-treated wound healing was performed in the 10% NE-treated group faster than in the 5% NE-treated ($p<0.05$) (Fig. 5)

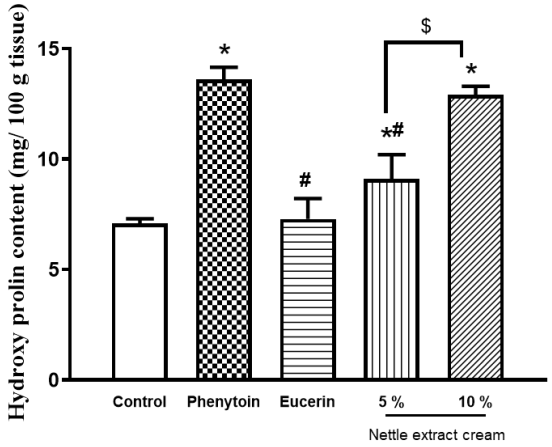

Figure 4: Hydroxyproline content in rat skin tissue in different groups. * Compared with the control group ($p<0.05$), # compared with phenytoin group ($p<0.05$), $ compared with 5% NE-treated group ($p<0.05$). Data are expressed as mean±SD (n=6).

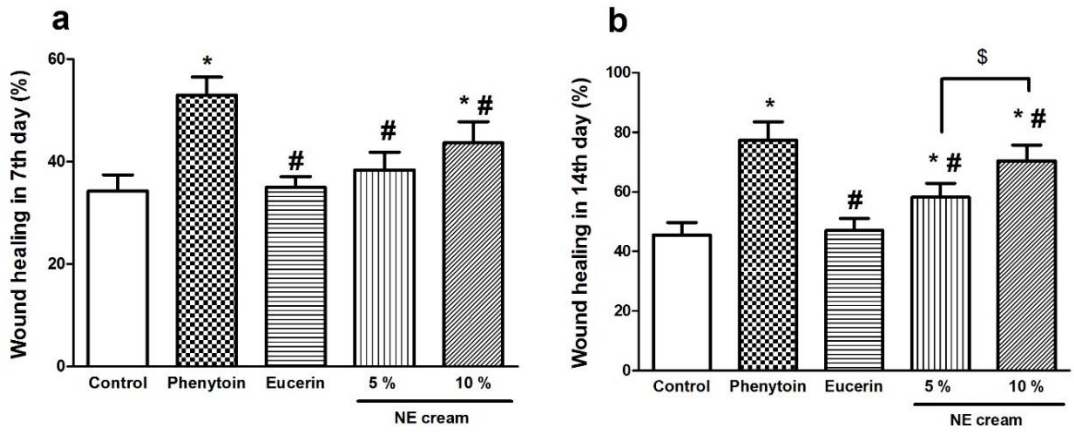

Figure 5: Wound healing on 7th and 14th days after. * Compared with the control group ($p<0.05$), # Compared with phenytoin group ($p<0.05$), $ Compared with 5% NE-treated group ($p<0.05$). Data are expressed as mean±SD (n=6).

Discussion

Wound healing is a complex process that occurs after injury. Wound healing is a necessary physiological process to maintain a stable state and hemostasis of the body after wound formation (Alsarayreh et al. 2021, Huang et al. 2022). This process is disrupted in some diseases like diabetes and causes many pathological problems such as the formation of ulcers, especially in the area of the foot, followed by infection. Thus finding a way to faster treat skin wounds and prevent possible infections can be an interesting area for future investigations by researchers (Alsarayreh et al. 2021). Nettle is known as an irritating herb because the leaves and stems of this plant (in some of its subspecies) have long hairs that, if touched, contain a set of chemicals such as histamine, formicholine, acetylcholine, and serotonin that can irritate the skin (Mozaffarian 1996, Nemati 2021). In the present study, the effect of Nettle, Urtica dioica on histomorphology and structure alterations during dermal wound healing in the rat was investigated. In line with the results of the present study, tissue analysis on the 14th day of the experiment demonstrated in the control group, a presence of a large number of inflammatory cells, inflammation granulation tissue, and neovascularization in the derm. On the other hand, it showed the presence of a low amount of collagen deposition and re-epithelialization that represented the wound healing slow process (Kamar and Rashed 2019, Huang et al. 2022). In our results, the granulation tissue fibroblast maturation in the phenytoin and NE-treated groups was more amount than in the control group, and a comparison between the two treated groups in the 10% NE-treated group was more than the 5% NE-treated. Granular tissue formation is one of the important steps of wound healing repair. Scientists have already stated that, when the fibroblasts proliferate, the amount of collagen produced by them in the tissue increases (Chen et al. 2001, Babaei et al. 2017, Malayeri et al. 2022). In our study of the phenytoin and NE-treated groups, more collagen fibers were seen, which was evident in trichrome staining and this increase was more obvious in the 10% NE-treated group.

Previous works of others have shown that during the healing process fibroblasts of connective tissue differentiate into myofibroblasts and the presence of myofibroblasts is a characteristic of contractile tissue (Moulin et al. 2000, Monika et al. 2021, Tai et al. 2021). Repithelization and neovascularization of wounds, the other one of the signs of the proliferative phase, in our histological findings, completely was obvious and were in the10% NE-treated group more than in the 5% NE-treated group. Ishida et al. stated that re-epithelialization is an important step for wound healing that involves migration and proliferation of surrounding epidermis keratinocytes and the formation of new skin with all of its skin appendicular-like hair follicles and sweat glands (Ishida et al. 2018). The Nettle plant contains histamine. Various studies have shown that histamine, by activating fibroblast growth factor can increase angiogenesis in the healing tissue and ultimately accelerates the healing process of skin lesions (Numata et al. 2006, Raziyeva et al. 2021, Bacci 2022). Some scientists have proven that histamine increases vascular endothelial growth factor production and the amount of connective tissue in the tissue, which in turn accelerates the healing process of the wound. Histamine also causes the small blood vessels to dilate at the site of injury, which in turn increases blood flow and, consequently, increases growth factors such as fibroblast growth factor and epidermal growth factor (Eming et al. 2007, Kajdaniuk et al. 2011, Dvorak 2021, Čoma et al. 2021). Although it has been found that the moving and migration of fibroblasts into the wound environment produce a wide range of cytokines expression and increases the movement of

fibroblasts into the environment (Tottoli et al. 2020). Keratin growth factor (KGF), vascular endothelial growth factor (VEGF), and tissue conversion growth factor (TGF) are among the growth factors that are released from fibroblasts (Peplow and Baxter 2012, Huang et al. 2022, Priyadarshi et al. 2022). The obtained research showed a significantly high content of chlorophyll, carotenoids, soluble peroxidases (POD), and proteins in young Nettle leaves (Gutowska et al. 2014, Rawat et al. 2020, Nemati et al. 2021).

hydroxyproline (4-hydroxyl-proline) is a non-protein amino acid, that is synthesized by post-translational hydroxylation of proline during collagen biosynthesis. The determination of hydroxyproline provides useful information for determining the amount of collagen, which is useful in the prognosis of collagen disorders (Kumar et al. 2016, Tottoli et al. 2020). In our results, the hydroxyproline content in the phenytoin and NE-treated groups was more than the control group, which indicates the presence of large amounts of collagen in the wound environment. Accordingly following this increase in collagen, a faster-wound healing pathway will be formed and fewer scars have remained. Corresponding with our results, a study by Alsarayreh et al. (2022) on the effect of *Globularia arabica* leaf in wound healing demonstrated that going up the amount of hydroxyproline in tissue was Synonyms with a lot of presence of collagen fibers in the dermis in histological findings. Results presented here in line with the numerous studies showed that topical administration of phenytoin ointment increased wound traction, new blood vessels, and collagen synthesis, ultimately creating a better and more rapid recovery than the control group (Babaei et al. 2017, Kamar et al. 2019, Hosseinzadeh et al. 2021, Priyadarshi et al. 2022). On that side, phenytoin has many side effects on various organs of the body, including cardiovascular, urogenital, hematology and oncology, neurological, musculoskeletal, skin, and ocular, and also interferes with the use of some drugs and foods.

Numerous studies have shown the importance of the synthesis of histamine and hydroxyproline in wound healing and epithelialization (Abramov et al. 2007, Kumar et al. 2016, Alsarayreh et al. 2022). Therefore, since histamine and other flavonoid compounds and vitamins are the main components of the Nettle plant, and considering the positive role of these substances in wound healing, it can be stated that the positive effects of recovery in the treatment group with nettle extract compared to other groups are related to the existence of these materials. The results of this study showed that by examining the tissue indices, in the groups consuming the extract, the wound healing rate increased because the time to reach the maximum density of fibroblasts and repair of the epithelial tissue had reduced in these groups. Of course, the time to reach this maximum recovery and density of collagen fibers depends on the dose of the extract, so with increasing the concentration of plant active ingredient, the amount of repair also increases significantly. The current laboratory research is still not enough to fully determine the pharmacological effects, and more clinical and in vivo tests are needed to justify the proper medicinal use of this plant.

Based on the present study, the use of nettle herbal cream to a similar extent as the standard chemical drug phenytoin in wound healing leads to rapid healing and recovery, and its use depends on the dosage and without any side effects. These results were clearly evident in histological studies of the skin and measurement of hydroxyproline content, as with an increasing dose of NE-treated, the 10% NE-treated rats, showed a stronger effect in comparison to the control groups. Therefore, this natural plant can also be used for the rapid healing of skin wounds, but nevertheless, more supplementary studies are needed to clarify the field.

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Conflict of interest

The authors declare that they have no conflict of interest.

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تأثیر عصاره‌ی الکلی برگ گزنه، بر تغییرات هیستومورفولوژی و ساختاری حین ترمیم زخم پوستی در موش صحرایی

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خلاصه

گزنه (Urtica dioica) یکی از مهم‌ترین گیاهان بومی است که برای درمان انواع بیماری‌ها مورد استفاده قرار می‌گیرد، اما اطلاعات محدودی در مورد اثرات مفید آن بر تغییرات بافت‌شناسی پوست در طول بهبود زخم در دسترس است. در مطالعه‌ی حاضر اثرات کرم الکلی ۵ درصد و ۱۰ درصد عصاره‌ی برگ گزنه به عنوان یک داروی آزمایشی در بهبود زخم مورد بررسی قرار گرفت. موش‌های صحرایی به تعداد ۳۰ عدد به طور صادفی به پنج گروه آزمایشی تقسیم شدند. ناحیه پشت حیوانات با استفاده از دستگاه پنچر زخمی شده بود. سپس همه‌ی آن‌ها دو بار در روز به مدت دو هفته تحت درمان قرار گرفتند. گروه کنترل در محل زخم نرمال سالین دریافت کردند. گروه شم حلال یوسرین و گروه فنی توئین پماد ۱ درصد دریافت کردند. دو گروه آزمایشی عصاره گزنه ۵ و ۱۰ درصد را دریافت کردند. در نهایت ۱۴ روز پس از شروع مطالعه تغییرات بافتی در پوست محل زخم، مقدار بافت گرانوله، بلوغ فیبروبلاست بافت گرانوله، رسوب کلاژن، اپیتلیال شدن مجدد، نئوواسکولاریزاسیون، محتوای هیدروکسی پرولین و درصد بهبود زخم در گروه‌های مختلف آزمایش‌های مختلف مورد تجزیه و تحلیل قرار گرفت. در روز ۱۴ آزمایش، گروه‌های تیمار شده با فنی‌توئین و عصاره‌ی گزنه در مقایسه با گروه گروه کنترل تأثیر بهتری بر بهبود زخم داشتند. محتوای هیدروکسی پرولین در زخم‌های خشک شده در گروه‌های تحت درمان با عصاره‌ی گزنه در مقایسه با گروه کنترل به طور قابل توجهی افزایش یافت. این یافته‌ها در گروه تحت درمان با عصاره‌ی ۱۰ درصد به طور قابل توجهی بهتر از گروه تحت درمان با عصاره‌ی ۵ درصد بود. این نتایج با تصاویر بافت‌شناسی نیز به خوبی تأیید شدند. با توجه به نتایج به دست آمده، عصاره‌ی گزنه تا حدودی شبیه به داروهای شیمیایی رایج ترمیم‌کننده زخم مانند فنی‌توئین، تأثیر مثبتی بر تغییرات پوستی حین بهبود زخم داشته و این اثرات وابسته به دوز بوده است.

کلمات کلیدی: موش صحرایی، اپیتلیال مجدد، پوست، گزنه، ترمیم زخم

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