A comparison study on the Effects of Oak Extract, Hyper Perforatum, and silver sulfadiazine 1% on the second grade burn wounds in male

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Abstract: Burn is one of the major causes of mortality in the world, and its prevalence in the developing countries is multiple times the developed countries.In traditional medicinal plants were used to treat injuries and subside pains and accelerate the healing of burn wounds.Oak (Jaft) and Hypericum Perforatum as antioxidants have antimicrobial and antiseptic effects, the extracts of which were used in this design to repair second grade burns in male rats.**Materials and methods:** Some 50 male rats of wistar race with a weight of 200-250 grams were purchased, and were kept in the nest for one week for adaptation, with open (free) access to water, food, and appropriate ventilation, then the rats were divided randomly in five groups. The first group, burn control which were washed daily with physiology serum. The second, third, fourth, and fifth groups were treated after burn with Oak (Jaft) extract, Hypericum Perforatum, a mixture of Oak (Jaft) and Hypericum Perforatum, and silver- sulfadiazine ointment 1% respectively to create second grade burn, the animals were anesthetized with sodium thiopental (40 mg/kg/IP) and were exposed to boiling water in the arranged containers with a hole of 2 square centimeters area for 10 seconds so that the shaved surface of skin was in contact with the boiling water.After 35 days of treatment and after weighing the rats were anesthetized with Sodium thiopental. The specimen of the skin tissue of the injured area were taken and transferred to Formalin 10% solution.After tissue processing, the specimen were moulded, sections of 5 micron were prepared and staining was done with hematoxylin - eosin. Then, the tissue changes were studied using light microscope, and the obtaineddata were analyzed using SPSS software and unilateral variance analysis test.**Results:** The resultsdon't show statistical difference is in the average burn surface, thickness of epiderm and the rate of malondialdehyde in Oak extract, Hypericum Perforatum and the combination of these two, in comparison to other groups ($P<0.05$).**Conclusion:** It is probable that Oak extract and Hypericum Perforatum with their antioxidant properties are able to fortify each other's effects, with more appropriate dosages and repair the injuries of skin and its appends.

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Introduction

In addition to severe physical and mental Consequences, burns are among the most expensive diseases, because of the need to numerous plastic surgeries and rehabilitation process. Skin demolition, dermal injuries and deep wounds are item of attention in burns. Wound healing is a regenerative process which takes place after injuries to skin and soft tissues. After injuries, inflammatory responses arise and the cells begin to increase sub dermal collagen generation, and then the epithelial tissue is gradually regenerated (repaired).For treatment of dermal injuries, antiseptic solutions such as betadine, acetic acid. Washing with physiology serum and antibiotic ointments are used. Recent studies show that many of the solutions used in dermal injuries are poisonous for fibroblasts, lymphocytes, and the cells which need to be repaired. Also in the traditional medicine, various efforts have been made to find a medicine (treatment) for accelerating wound healing and dermal injuries. But because no certain medicine has been introduced to accelerate the healing trend of wounds, research on herbal medicines, and their effects on the regeneration of wounds continually goes on.Researchers have explained that the tannin in the herbal compounds is able to give good result in regeneration of skin and healing of wounds. Oak is among the plants which exist in many mountainous regions of the world, and has various specimens. Oak of Quercus Branti type is the major specimen in the

forest of kohgilooyeh and Boirahmad province. With respect to the studies carried out, this type of oak contains 5% Tannin. Which respect to abundance of oak- trees, increase of burn cases, and also increasing use of traditional treatments? And with respect to the fact that many of these treatment methods have been used for centuries, with good results. Herbal Hypericum Perforatum with long and wide leaves is among other medicinal plants which is used for treatment of wounds and dermal inflammations, and with respect to the current use of silver sulfadiazine ointment as a standard treatment of burns, we decided to study the effects of Oak(Jaft) extract, and Hypericum Perforatum with silver sulfadiazine (1%) ointment on the healing of dermal wounds on second grade burns in the male rats.

Materials and methods

1000 grams of Oak and 2500 gram of Hypericum Perforatum were milled, and were separately mixed with 4 litters of distilled water and alcohol 70% in proper containers, and were macerated for 48 hours. Then the mixtures were paper filtered, and by transfer to over machine with a temperature of 40 centigrade, were insisted, dried, and ultimately collected. Daily 4 mg/kg of dry extract, Oak and Hypericum Perforatum were prepared and used. (8).In this experimental study, 50 male rats of wistar race were used with an average weight of 200-250 grams. The animals were kept with open access to water, food, and 12 hour period of dark- and light, temperature of 22±2 °C and appropriate ventilation. The animals were randomly divided in 5 groups of 10 as follows, first group, control, second group, extract of Oak (Jaft) , third group, extract of Hypericum Perforatum , fourth group, extract of Oak (Jaft) and Hypericum Perforatum , and the fifth group silver sulfadiazine 1% After weighing the animals were anesthetized by sodium thiopental (40 mg/kg/IP) using intraperitoneal method, for creating second grade burns, the process was followed based on a formula proposed by walker as: ($A=K.W^{2.3}$). A is the body surface (area) at cm^2, k, is a fix coefficient, which is equal to 10 for rats, and w is the weight of animal at gram. Then the lumbar hair were shaved entirely, and the animals were placed in a device, designed with dimensions of $2cm^2$ so that the skins of animals were exposed to direct boiling water (97ºC) for 10 seconds. To prevent hypovolemic shock 2.5 ml of physiology serum was injected using, intraperitoneal method. After, the procedure, the animals were kept singly, in very clean and antisepticised shelves. Every day, after cleaning the wounds with a piece of sterilized bandage, once daily the extracts of Hypericum Perforatum - Oak (Jaft), sulfadiazine ointment 1%, and Oak(Jaft), sulfadiazine ointment 1%, and physiology serum were used for treatment of the relevant groups. Then at the end of the day 35, the animals were anesthetized by sodium thiopental, and photography of the burns surfaces was done, and analyzed (studied using image software. The following formula was used to determine the healing percent of wounds:

Percent of wounds= (wound area on the desired day) wound area on the first day) -100

Percent of healing= 100 - percent of wounds.

Then, the skin of the injured area along with a part of healthy skin was removed, and a part of it was transferred to liquid nitrogen tank inside a micropipette (for malondialdehyde test) and another part was transferred to formalin 10% solution. The samples were processed using tissue processing machine, and after moulding, by microtome set, five micron, serial sections were prepared, and then coloration was done with Hematoxiline-eosin.On all tissue sections the changes of epiderm were studied using Olympus Bx 51 microscope and Olysia program. The obtained results and data were assessed using unilateral variance analysis and Tukey test. The results were provided as Mean±SEM, and the difference between data was deemed as a significant difference by considering P<O .05

Results

With respect to table 1, the thickness of epiderm in the groups receiving extracts of Oak (Jaft) Hypericum Perforatum extract, Oak- Hypericum Perforatum extract, and silver sulfadiazine had increased in comparison to the control group, but the increase is not statistically significant (P>0.05). The compounds which exist in the Oak extract showed better regenerative effects on the extant of wound caused by burn, so that the burn area in the Oak extract group had reduced in comparison to the control group, and the reduction is statistically significant (P<0.05). While the extent of burn area in the control group had increased in comparison to Hypericum Perforatum extract, Oak extract, Hypericum Perforatum and silver sulfadiazine groups, but the increase is not statistically significant (P>0.05), showing that regeneration is slower in the epidermal layers of the control group (Table 1). The rate of tissue surface of malondialdehyde in the groups receiving Oak extract, Hypericum Perforatum extract, Oak- Hypericum Perforatum extract and sliver sulfadiazine group had reduced in comparison to the control group, but the reduction is not statistically significant (P>0.05) (Table1). Assessment of histology change: In the assessment

using light microscope, the arrangement of regeneration region in the control group is clearer, compared with the groups under treatment, which arise from more dispersion of the matrix of solidarity tissue and swelling response. Accumulation of collagen filaments, and its more appropriate structural arrangement and also lower accumulation of fibroblast cells in the dermal layer of the groups under treatment with the extracts and silver sulfadiazine is more distinguished in comparison to the control group. The trend of regeneration in formation of epiderm in the groups under treatment showed faster and better quality of regeneration with more regular arrangement and lower inflammatory accumulation.

Discussion

In traditional medicine, it is usual to use medicinal plants for recovery and treatment of dermal injuries, and the purpose of all these treatments, either in traditional medicine, or in the modern scientific procedures, is quick recovery of dermal injuries. Therefore, the objective of the present research is to compare the effects of the extracts of Oak, Hypericum Perforatum, and a compound of the extract of oak Oak-Hypericum Perforatum with silver

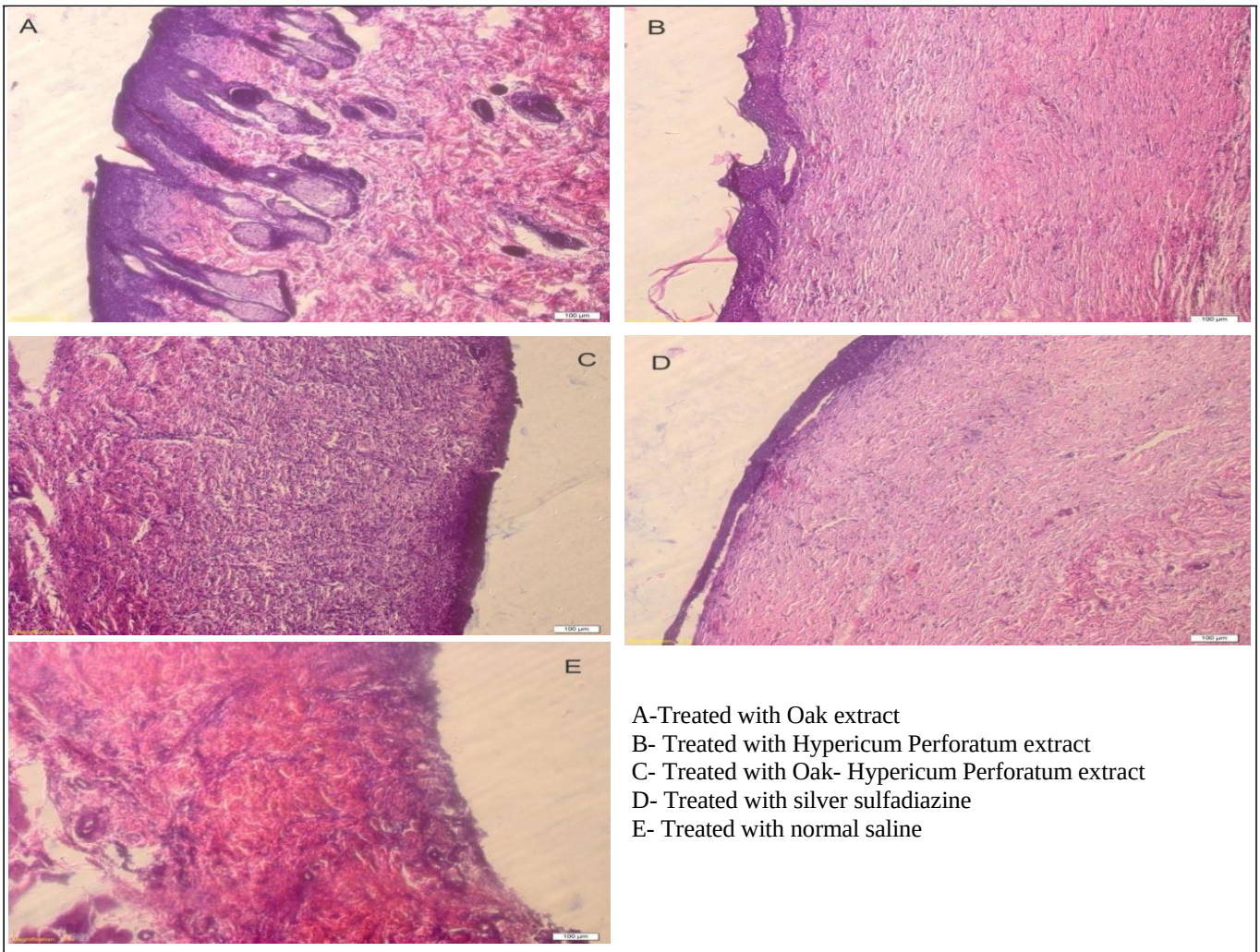

Figure 1- A comparison of the trend of regeneration of skin injured by burn wounds in the studied groups. (Hematoxilin-eosin stain magnifying X40 light microscope)

Table 1- Mean and standard error of mean of variable of skin tissue with second grade burning in male rats (n=10)

	Oak	Hypericum Perforatum	Oak and Hypericum Perforatum	Silver sulfadiazine	Control
Burn area (cm^2)	0	0.36±0.09	0.4±0.29	0.44±0.04	0.72±0.52
Thickness of epiderm (µ)	52.14±8.25	50.15±7.95	41.37±6.90	34.49±9.14	29.14±5.87
Malondialdehyde(µ mol/l)	3.24±1.11	2.46±1.05	4.22±1.21	3.93±0.29	4.69±0.31

sulfadiazine 1% ointment on dermal regeneration in second grade burns. Numerous factors interfere in the trend of skin regeneration, including Hyaluronic acid as a compound that, the skin by absorbing it will be able to accelerate the trend of healing. Hyaluronic acid is one of the main components of Glucose, Aminoglucane sequences which exist in skin. Among other inflammatory factors in dermal injuries which could be mentioned are cytokines No. IL-17 and INF-Y.IL-12, TNF-a created by endothelial of injured blood vessels which are effective in the trend of inflammation and subsequent regeneration. These items affect the injured vessels in dermal injuries an facilitate the out flow of from vessels. Consequently cause inflammation and swelling in the region which is consistent with the findings of the present research. The rate of angiogenesis and expansion of blood vessels is actually one of the most effective factors of wound healing (regeneration), and if a factor be able to multiply or intensify the formation of new blood vessel in the right time, and be able to change the blood circulation of tissues in the region, it could be said that the factor leads the trend of wound healing towards success, and prevents the deepening of wound. Increase of clotting property of endothelial and increase of proliferation velocity of fibroblasts. Activation of tissue fibroblasts and increase of producing extra cellular beds by the activated fibroblasts is able to reduce the extent of burn area and accelerate the regeneration trend of skin epithelium. Moreover, the phenomenons of contraction is because of the existence of fibroblast cells, and due to their contraction property pull the epidermal layer and care the reduction of the dimensions of wound. The rate of dwindling of a wound is a good criterion to assess the healing rate of wound. The wound area dwindles simultaneous with its healing, the cause of which is the existence of the phenomenon of shrinkage of wound and sediment of graft tissue. The findings of this research show that the extent of burn wound area in the group receiving Oak extract are consistant with the findings of the previous research cases. Studies show that tannin is an antioxidant which causes the increase of fibroblast proliferation which could secret large quantifies of T N F. T N F in turn cause to produce No and V E G F in line with accelerating wound healing. No and V E G F are among the main regulating factors of the formation of tissue granulation, and also streng then the angiogenesis process. Fibroblasts are also responsible for creation of collagen fibers. Collagen is a protein which causes scar to resemble the initial tissue (before injuries) after healing (regeneration), and consequently white colour scars remain. More over collagen fibrous cause the increase of wound resistance. Therefore it seems that with respect to the effects of tannin which exist in the Oak extract in most pathes of wound healing and increase of collagen tissue of dermal layer of skin has affected proliferation and activity of fibroblasts and is able to increase the making of collagen filaments, and biosynthesis of nucleic acids and creation of protein, and contribute to the resistance of wound in the trend of regeneration. Some researchers believe that the medicines of silver sulfadiazine with their poisonous complications could negatively affect the growth and number of fibroblasts, reduce formation of collagen, and consequently cause the slow regeneration of the injured area, which is consistant to the present research. Modification of inflammation and use of anti-oxidants accelerates regeneration (healing) of wound. Infection, anti-oxidants, reduction of T GF-b from T-cells and free radicals cause the seriousness of dermal wounds. Oak, with tannin, flavonoid, Alkaloid, and several aromatic components, and tea-grass with phenols of carboxylic acid including caffeine, chlorogenic, and folic have anti-oxidant properties and are able to activate the factors of epidermal. Growth by reducing inflammation, reducing anti-oxidants, and increasing TGF-b.Since the reduction of inflammation accelerates the regeneration (healing) of wounds, it could be inferred that Oak (Jaft) and Hypericum Perforatum accelerate regeneration of wounds caused by burning, because of containing anti-inflammatory substances.

Conclusion and Proposals

With respect to the findings obtained in the present research, the extracts of Oak and tea-grass accelerate the regeneration trend of wounds caused by burn, and shorten the period needed for full recovery of the wounds. But combination of the extracts of the two substances did not show a stronger effect, which entails further research to study the affecting mechanism, and type of their effective substance with more appropriate dosages on regeneration of dermal wounds caused by burn. With doubt, clinical application of the findings of this research depends upon probable side effects, nontoxic dosages, and affecting mechanism of the medicine, that in case of confirming the non-toxicity, by preparing ointment or extract of the two plants, could be used in the treatment of wounds caused by burn.

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