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Research Article

Investigating Healing Effects of Pot Marigold Oil and ProsopisFarcta Extract on Skin Lesions in Rats

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ABSTRACT

It has been a common practice to use herbal plants in Iran and other countries around the world since past years. Today with unfavorable impacts and side effects of chemical drugs, traditional medicine and prescription of herbal plants have attracted specific attention. Treatment and healing of lesions can be considered among major issues which human has faced from the first of creation. Using pot marigold oil and powder of ProsopisFarcta fruit has been long common in Iran for treatment of skin lesions. Therefore, the present study aims to investigate quick healing and restoration effects of ProsopisFracta fruit extract as well as pot marigold oil on laboratory rats. The research was a laboratory trial which was done on 50 rats which were divided randomly into five groups including ten rats after complete anesthesia and creating spinal injuries in completely similar conditions and characteristics. For treatment, five interventions were used as follows: 1. Normal saline solution (0.9% physiologic serum); 2. ProsopisFracta fruit extract (with density of 0.5 to 99.5% solvent); 3. Pure pot marigold oil; 4.Combination of ProsopisFracta fruit extract and pot marigold oil (95% oil and 0.5% extract); and 5.Nitroforazone 1% ointment (positive control). Features including 1. Rats' body temperature, 2.Testing injury site blood, 3.Lesion extent, 4.Histology microscopic experiment during sixteen days of treatment were measured and collected data were analyzed using SPSS statistical software by mean+-SEM as well as survival analysis tests at significance level of 0.05. According to the results, intervention by combination of ProsopisFracta fruit extract and pot marigold oil is significantly better regarding all features studied, while given the feature of lesion extent it shows a significant difference in treatment and healing of the lesion. Results of a study done by Nakhai Moghadam and colleagues confirm the results obtained by the present study.

Keywords: laboratory rats, lesion, healing, ProsopisFracta, pot marigold

INTRODUCTION

It has been a common practice to use herbal plants in Iran and other countries around the world since past years. Today with unfavorable impacts and side effects of chemical drugs, traditional medicine and prescription of herbal plants have attracted specific attention (Soleimani, 1387). Treatment and healing of lesions can be considered among major issues which human has faced from the first of creation (kalantar,1390). Today, fast healing of lesions is considered an important principle in therapeutic science and increasing the quality of lesion treatment has been always interested researchers. In this regard, using herbal plants in order to provide new harmless, useful, healing drugs which are more compatible with human body is considered as an important responsibility for researchers.ProsopisFracta and pot marigoldhave been long used in southern cities of Iran to heal workers' wounds (Ghasemi, 1388). In pot marigold, effective therapeutic products are made in flower organs and include water-soluble flavonoids, carotenoids, mucilage products, and lauric acid.

Therapeutic benefits of pot marigold include anti-inflammatory, healer of lesions, antifungal, anti-dry skin, anti-sunburn, and treatment of varicose ulcers (Barnes etal, 2007)Tehranipur and colleagues in 2008 stated that volatile oils of pot marigold have medicinal effects along with therapeutic functions such as anti-inflammatory and anti-tumor (Tehranipouretal, 2012). Iva and colleagues Reports that pot marigold is an important skin lotion due to its numerous benefits for skin, while it is also beneficial for treatment of inflammatory diseases, skin lesions, and burns, so that it can be used in gradual treatment of lesions (Evaetal,2009). Reported that pot marigold oil can reduce the time of lesion healing through increasing production of hydroxyl praline, more wound tensile strength, and accelerating epithelialization (Prasad etal, 2011). ProsopisFracta fruit ash has been used as anti-infectious. NakhaiMoghadam and colleagues reported that ProsopisFracta fruit extract can accelerate wound healing in addition to different benefits such as reduction of cholesterol and glucose. In a study done by NakhaiMoghadam and colleagues in 1388, it was shown that in the sample treated by ProsopisFracta, epithelial thickness and the rate of epithelialization increased in lesion margins compared to control group(Nakhai Moghadam,1388)Mehranipur and colleagues investigated the effect of ethanol extract of ProsopisFracta fruit and stated that this extract includes tamine, tryptamine, and quercetin(Thranipour etal.,2012)Given the importance of lesion healing and impossibility of spontaneous treatment of pen lesions along with the possibility of local infection and side effects, the present study aims to investigate the effects of influencing products of ProsopisFracta, pot marigold independently and in combination in order to treat local skin lesions.

MATERIALS AND METHODS

The present laboratory research was done on 50 laboratory male Sprague rats at the age range of 3-6 months and weight range of 250-300 mg. the rats were previously examined to ensure absence of every kind of infection. In this research, rats were first anesthetized completely using ether after which their back hair was shaved with sterile blade. Then, at spinal side of each animal two holes with a diameter of 4 mm including derma and epidermal were made by a punch, so that for lesions were resulted at the back of each animal. In the next stage, animals were randomly divided into five groups including ten rats. After preparation of therapeutic materials, five interventions were used with the same quantity as follows: 1. Normal saline solution (0.9% physiologic serum); 2. ProsopisFracta fruit extract (with density of 0.5 to 99.5% solvent); 3. Pure pot marigold oil; 4.Combination of ProsopisFracta fruit extract and pot marigold oil (95% oil and 0.5% extract); and 5.Nitroforazone 1% ointment (positive control); the solvent used in all groups was propylene glycol, alcohol when needed. In all groups, change and treatment were repeated every other day at a specific time and by a special person. It should be mentioned that all steps of creating lesions, cuts were made based on moral principles and by permission of ethics committee of Yazd ShahidSadughi University of Medical Sciences. Features measured during sixteen days of treatment period from the second day and every other day included: 1. Body temperature, 2.Bood test, 3.Lesion extent, and 4.Histology microscopic experiment. Rats' body temperature was measured and recorded every other day in the intervention group by placing children's thermometer in animals' rectal.In order to examine blood samples from lesion site, animal blood was taken by syringe and sent to medical laboratory. In order to examine lesion extant, caliper was used as length indicator of healed part to the overall area of the lesion by a specific person every other day, while the following formula was used:

Mean area=[(D1+D2)/4]× π

Here, D1 is the biggest diameter between two edges of the lesion and D2 represents the smallest diameter between two edges of the

lesion. For better and more exact calculation of lesion area in morphometric evaluations, lesion process imaging software was used in addition to caliper. Imaging fromlesions conditions was done by a digital camera in all groups from the second day and continued to the end of the period every other day.

After image transmission to software and performing calibration of system's measurement software, a cursor's movement showed the site whose skin tissue was not completed while the site was selected and its area was calculated and recorded by the software.

Along the treatment period from the second day, animals were anesthetized every other day and some parts of their skin were separated and sent to medical laboratory for microscopic histologic examination.

RESULTS, DISCUSSION, AND CONCLUSION

Results of lesion extent

Lesion extent of intervention group with normal saline solution showed an increasing trend in the first three days and later showed the surface of a smaller lesion slowly. In intervention group withProsopisFracta fruit extract, the results showed an increase in epithelium thickness compared to control group which represents positive effects of ProsopisFracta fruit extract on healing process. In intervention group with pot marigold oil it was shown that the area of the lesion didn't have a significant increase compared to control group during the first and second days, while later the area of the lesion decreased faster than control group, so that at the eighth day the surface of the lesion was extremely small and it was eventually treated at the twelfth day with no symptoms of lesion at sixteenth day.

In treatment group with 5% of ProsopisFracta fruit extract and 95% of pot marigold oil, during the first days the least increase in initial lesion area was observed compared to control group while later the extent of the lesion reduced faster than other intervention groups and the results showed significant difference.

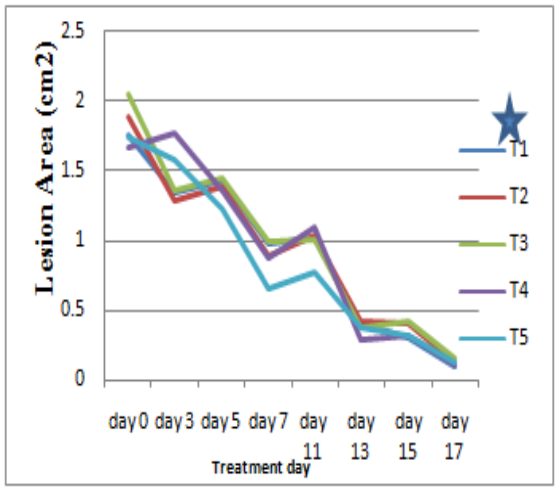

Diagram1. Investigating the effects of different interventions on improvement of lesion extent

Body temperature

Given that one of clinical symptoms in lesion healing is the host's body temperature, clinical investigations and statistical comparisons of means in lesion groups showed that the sample treated with ProsopisFracta fruit extract and pot marigold oil had better healing effects compared to other intervention groups, but it didn't show a significant difference.

Blood test

Examining mean scores of intervention groups showed that none of the groups had a significant difference with control group.

Histologic microscopic investigation

Microscopic investigation of provided sections and counting fibroblasts and vascular sprouts at the center of granulation tissue in control and intervention groups showed that the process of cell transformation in the lesion site was similar to the overall process mentioned in lesion healing including inflammation, proliferation, and contraction. However, there are some differences regarding the time each of these steps are completed in various groups. Comparison of the number of fibroblasts and vascular sprouts in granulation tissue of control and intervention groups showed that intervention withProsopisFractafruit extract and pot marigold oil can increase the speed of lesion healing and treatment. On the other hand, the time of reaching this maximum fibroblastic density reduced and then increased significantly and represented a significant

difference. Higher proliferation of cells and increasing epithelium thickness can be regarded as histologic justification for increased speed of restoration of lesion cut in the sample treated with ProsopisFracta fruit extract and pot marigold oil.

Figure1. Therapeutic view after sixteen days of treatment

DISCUSSION

The results of this study are consistent with Nakhai and colleagues' report in 1388 regarding epithelial diameter increase. The difference in the speed of lesion healing with combination of extract and pot marigold oil compared to control group is in cell proliferation and epithelialization. Unsaturated fatty acids have an important role in transmission and regulation of intracellular signals as well as proliferation of epithelial cells (NakhaiMoghadam etal,1388) and are considered main precursors for effective inflammatory mediators in lesion healing (yaqoob,1998). Cardoso and colleagues showed that using local unsaturated fatty acids can accelerate lesion healing compared to control group Therefore, it can be concluded that the combination of pot marigold oil and extract is better for improvement of lesion healing. Regarding the sample treated with ointment, macroscopic investigation shows positive effects of the ointment. It was clear in microscopic investigations that in intervention sample, epithelium thickness and epithelialization at the edges of the lesion were higher compared to control and extract groups. These findings were consistent with observations done by NakhaiMoghadam and RanjbarHeidari(Kumar and colleagues,2007), showed that flavonoids and triploids increase lesion contraction and epithelialization.

Given that the plant used in the present research has these combinations; results obtained regarding epithelialization consistent with the above mentioned findings are due to presence of flavonoids, triploids, and tannins. (Choudhary and colleagues,2008) investigated lesion healing under the effects of ethanol extract of terminaliabelliri plant and showed tannins as an important combination in this plant, basically responsible for lesion contraction, higher epithelialization rate, and increase in fibroblasts (choudhary etal,2008). Given that ProsopisFracta contains tannins, probably this combination is responsible for contractive and anti-microbial features which lead to lesion healing. However, in complete

extract of a plant, mass effects of different combinations may lead to different results compared to the effects of separate items. Therefore, it seems that the results of the present study are consistent with explained mechanisms of tannins, flavonoids, and triploids in other studies. (Latif-andcolleagues,2010), showed that extract of momordicacharanti plant accelerates lesion healing in diabetic people, which is probably due to photochemical materials such as poly phenols, flavonoids, and triploids. Studies performed by Tsegahun and colleagues on Lotus corniculatus showed that this plant contains high amounts of flavonoids, alkaloids, and tannins like ProsopisFracta(Dicko,2006). Therefore, it is probable that fast lesion healing in rats is due to active combinations in ProsopisFracta fruit. Therefore, according to a report by (Harris Fasser,2002), presence of proteins is necessary for activation of macrophages, production of specific T lymphocytes for each cytotoxic anti-gene, and releasing cytokines such as growth factors. (NakhaiMoghadam and colleagues, 1388), showed that the sample treated with butter had an increase in epithelium thickness compared to control group in pathologic investigation which indicates positive effects of butter on lesion healing process. Moreover, a mixture of animal butter and powder ofProsopisFracta fruit shows a faster healing process than butter. In microscopic investigations there was a significant increase in epithelium thickness and the number of blood vessels in intervention group compared to control group [6]. In a research performed by (GhodratiAzadi, 1390), the results indicated that shallot could accelerate lesion healing process through the rate of epithelialization which is consistent with the present study. In a report by YazdanAsadi and colleagues, extract of herbal tea significantly decreased the time needed for healing surgical wounds and burns.

Like ProsopisFractathis material can be used effectively for treatment of surgery wounds and burns. Comparison of survival and healing of lesion during the study showed significant effects of the combination of ProsopisFracta fruit extract and pot marigold oil on fast healing of lesions compared to normal saline solution alone. It seems that this fast healing is due to unsaturated fatty acids which have an important role in proliferation of epithelial cells and can be main precursors for inflammatory mediators in lesion healing process. Cardoso indicated that local use of unsaturated fatty acids could lead to faster lesion healing compared to control group which shows therapeutic potential for healing of skin lesions (cardoseetal, 2004).

CONCLUSION

Given the results and statistical analyses among practical interventions, it can be concluded that intervention with combination of ProsopisFracta fruit extract and pot marigold oil is more effective than other intervention in treatment of lesions in laboratory rats and the results show a significant difference.

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