

Effects of Myrtus Plant on Histometric and Histopathologic Healing Process in Rats

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Abstract: Wound healing in some chronic diseases and disorders has become one of the challenges of medical science and for this reason using the new compounds to accelerate wound healing is produced will be welcomed. In the meantime, the Myrtus herb has antibacterial and anti-inflammatory, antiseptic and anti-congestive effects. Based on this information, the purpose of this study was to studying the effect of this plant extract on process of wound healing. In this study, after induction of anesthesia in 48 male rats weighing 200 to 250 g and approximately one and a half months of age, using a biopsy punch with a diameter of 23 mm circular wound was created on the dorsal skin region of each of them. Then the rats were divided into four groups, each groups continued healing process with 5% and 10% extract of Myrtus herb mg/cm², 35 dose once-daily, Osrin (drugs) and control (distilled water). Test duration was 28 days, wound diameter measurement using digital image analysis and histopathological study on days 0, 3, 7, 14, 21 and 28 with biopsy two rats from each group were took. this rats were removed from the investigation process after obtaining biopsies. Obtained Results were analyzed with one-way ANOVA and Kruskal Wallis statistical test ($p < 0.05$). The results revealed that treatment with low-dose group of Myrtus herb extract (5%) most shrinkage of wound area and also from the perspective of histopathological change. in the second and third week, tissue had better organization than most other groups ($P < 0.05$), but the group treated with high doses of Myrtus herb extract (10%) had less wound shrinkage and Organizatio

Key words: Plants • Healing • Skin • Rats

INTRODUCTION

Healing the wound has become one of the major problems in today medical science. Since the outbreak of this kind of wounds in modern societies with a higher incidence of diseases such as diabetes mellitus, bed sores, obesity and ETC [1-3] for go up progressively, so trying to introduce new drugs to accelerate wound healing is more interestingly. In this direction, approaches to herbal compounds to produce new and more effective and compatible with living bodies placed on the agenda of researchers [4]. One of the old plants as disinfectant, anti-inflammatory, analgesic, astringent and vascular wound dressings were used is plants with scientific name of Myrtus commanis from Myrta ceae family [5-8].

Several compounds in these plants identified are: Kaufman, diPenten, cineol, Mirtenol, Troll, Zheratpol and compounds in the leaves of this plant, easy tannins, flavonoids and vitamin C and alkaloids and was be free of cardiac alkaloids and glycosides [3, 9-14]. The best

season to collect leaves of this plant is mid-summer, that the plant does most photosynthesis [3,5,11].

In traditional medicine, Myrtus used as a local treatment for herpes simplex type I and II. This herb was used as an antiseptic and in treating inflammation of the nasal mucosa, astringent and disinfectant booster and anti-parasite treatment was used, hemorrhoids, gum infections, pseudoiasis and wound healing. The purpose of this research was recognizing healing effects of Myrtus herb on rats.

MATERIALS AND METHODS

For this study, 48 male Wistar rats with approximate weight 200 to 250 g and approximately one and a half months of age were divided into 4 groups (12 rats for each group). Rats were maintained in the same conditions of temperature, humidity, feeding and lighting. Myrtus Plant was purchased from breeding and propagation of medicinal plants center of Kerman, after drying and

milling, the extraction process via distillation were done in the Pharmacology Department, Medical Sciences University of Tabriz. After weighing the ointment, 5% and 10% were produced Osrin based.

Then, for induction of anesthesia in rats in order to create a separate surgical wounds, first drug was Zylazine 2% to pre-anesthesia with 10 mg/kg dose and then 10% ketamine drug with 40 mg/kg dosage for both drug manufactured by Netherlands Alfasan company were used for total anesthesia (Injected into muscle).

After induction of anesthesia, rats sleeping on the rear wing was preparing for a trauma, Then on the spine (scapula to the posterior edge of the Ilium bone) circular wound in three layers of skin with 23mm diameter in all three layers (dermis, epinephrine and Hypoderm) and the superficial muscles fascia was created with a sterile biopsy punch.

The rats were divided into four groups were numbered via non-toxic colors, for first group high doses of Myrtus plant extract (10%) was administered once 35 mg/cm² daily and group two of rates was used low dose of plant extract to the extent of (5%), third group only received Osrin and the fourth group did not receive any medication and wounds surface were washed with distilled water.

During the treatment period was 28 days and the topical medication on the day was done, for histo-metrical study daily digital image were taken from wound and finally the wound area was calculated using digital image analysis software.

For histopathologic study in the 0, 3, 14, 21, 28 days after testing, tissue samples was taken from the full thickness skin biopsy, in each group we used 2 rats and then this two rats were removed after sampling of current research and all pathologic slides were prepared and stained with Haematoxin and Eosin (H&E).

	n = 12	n = 10	n = 8	n = 6	n = 4	n = 2
N=48	0 day	3 day	7 day	14 day	21 day	28 day
High D.	G1 -	-	-	-	-	-
Low D.	G2 -	-	-	-	-	-
Euc.	G3 -	-	-	-	-	-
Con.	G4 -	-	-	-	-	-
Biopsy	1	1	1	1	1	1

Statistical Analysis: Histo-metrical and histopathological results of studies were analyzed using ANOVA and Kraskal Wallis statistical Tests in SPSS software V.17 at significant level of ($P \leq 0.05$).

RESULTS

Histo-Metric Findings: During the 1-3 days of treatment period, wound diameter in all 4 groups in comparison with pre test was significant that this phenomenon is justified with due to compliance with the inflammatory phase of wound repair. On the third day of the test, group treated with low doses of Myrtus plant extract had the lowest levels and over time, very high levels accelerated to decreasing So that in seventh days at least area was found. In 14th day, except the control group and groups receiving high doses of plant extract Osrin also showed reduced wound diameter and 21 day after healing process, control group also showed close results to other groups. Finally, the wound surface on day 28 in most rats were almost completely closed. Only unpredicted result compromised the results of low-dose group treated with high doses of plant extract (10%).

Results of the one way ANOVA and Kruskal-Wallis was indicative of their significance.

Histo-Pathological Findings: On the third day there wasn't a significant difference between groups in the opinion of histopathological and inflammatory cells, hyperemia Sediment and hemorrhage and fibrin fund scattered in the wound, to be seen in controls and Osrin groups. Bleeding was observed in the deeper parts and there were no pustules and no recovery.

In the group treated with low doses of Myrtus extract that had been covered by pustules, blood and pus and fibrin cells, regeneration was observed lining the sides and fibromyalgia blast infiltration into the tissue with fibrin deposition, but in the group treated with high doses of the Myrtus extract tissue regeneration was not seen in epithelial and Inflammatory cells and were present in most sections of the pre experimentation day and fibromyalgia blast infiltration was still somewhat visible. Seventh-day observations show changes in all groups and filled with blood and the bleeding was identified, in the group treated with Osrin wound was covered by pustules, regeneration in the sides of the pustules had begun to the surface of wounds and purulent inflammatory cells were seen in many tissues in the wound site was composed of young granules.

Wound surface covered by pustules in low doses of Myrtus extracts group and inside the scar tissue was filled by fibrin and connective tissue arnular and in inflammatory cells, fibrin and connective tissue was observed and the tissue was full of regenerated vessels.

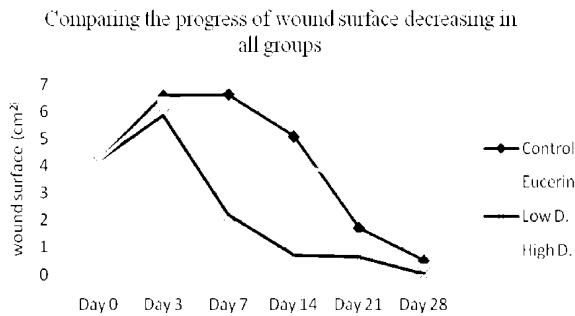


Chart 1: Reduction in wound area between the five groups during the experiment

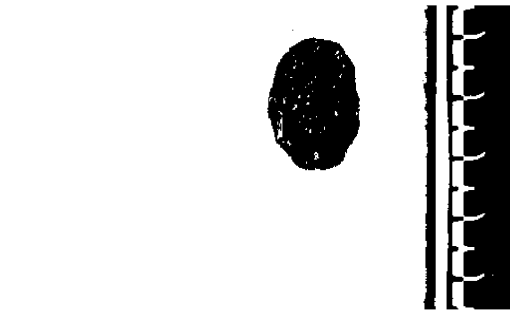


Fig 4: In healing wound, Myrtus treatment group (high dose: 10%), twenty-eight day

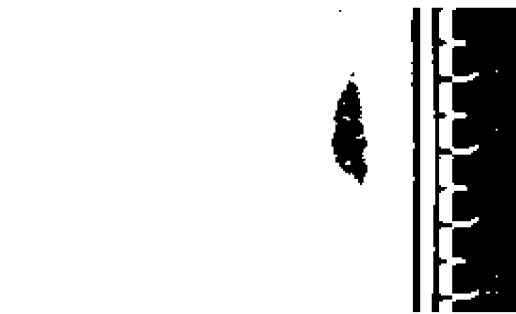


Fig. 1: In healing wound, control group, twenty-eight day

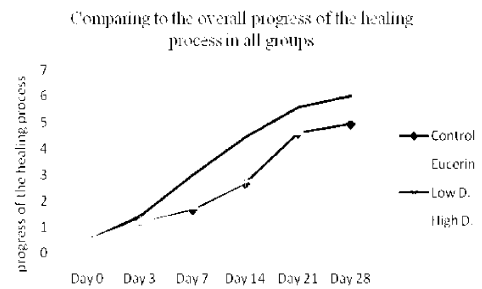


Chart 2: Increase the overall outcome of wound healing among the five groups during the experiment.



Fig. 2: In healing wound, Osrin treatment group, twenty-eight day



Fig 3 In healing wound, Myrtus treatment group (low dose: 5%), twenty-eight day Fig 4. In healing wound, Myrtus treatment group (high dose: 10%), twenty-eight day



Fig. 5: View of the microscope's skin healing position in the control group on day 28 of treatment duration (H&E. X40).



Fig. 6: View of the microscope's skin healing position in the group treated with Osrin 28 treatment duration (H&E. X40).



Fig. 7: View of the microscope's skin healing position in the treated group with 5% plant extract used in the duration of 28 treatments (H&E. X40).

In the group treated with high doses of *Myrtus* extracts, pustules on the wound is still not completely dry and Clearly, the wound covered with blood clots, regeneration was begun and the amount of inflammatory cells was not changed and pus cells number was increased. In fourteen days, marginal ulcers in the control group was covered the lines and immature connective tissue cells and fibers was completely filled the repair membrane in the dermis and there wasn't skin appendage in healing area Fig 8.

In the group treated with Osrin, scar tissue around the wound is occupied by the young cell and the various aspects of regeneration were continued. In the group treated with low doses of plant extract, the repair tissue was more organized and epithelial was thickening, collagen fibers was thicker and there was no clot. Also new annular and vascular tissue was cut and wound surface in high-dose group was covered with a layer of moist.

No differences were found between the twenty and day and fourteenth day and fibroblasts activated for collagen making, collagen fibers were thin and low dense. In the group treated with Osrin, the healing area is filled by a series of small vessels and cells, collagen fibers is still thin and there aren't skin appendage. In the group treated with low doses of *Myrtus* extract, wound surface is largely covered and wound space is filled with granular tissue and slight edema was observed in the lining and collagen fibers were thicker and dense. In the high-dose treatment groups, ulcer size was much different from other groups and histopathological results are not so salient. Situation is almost the same with other groups middle days obtained results (Fig 3).

In the twenty-eighth day, control group haven't degeneration hydropic connective tissue, activated fibroblasts, are in process of collagen making and a number of skin appendages such as hair follicles is infiltrated from adult tissue old into the new repairing tissue. In Osrin group, attachments increased and were



Fig 8: View of the microscope's skin healing position in the treated group with 10% plant extract used in the course of 28 treatments (H&E. X40).

seen more skin collagen fibers. In low-dose group, the wound area is filled by mature granular connective tissue and without regenerated vessel. Also inflammatory cells was observed in individual into the scar tissue and wound surface is covered by connective tissue and collagen fibers were thicker and denser than the days before and skin appendages, was formed in the center of the tissue. In the group treated with high doses, situations were the same as previous days, but there is more severe inflammation in wound place.

The overall results suggest significant differences in the healing process.

DISCUSSION

Anti inflammatory drugs especially non-steroids is highly consuming after surgery due to pain, inflammation decreasing roles and promotion of patients public situation in recovery. Based on abovementioned information and importance of healing process in surgery wounds, for controlling secondary infection and fast recovering of tissue physiologic function, many studies were done on the effects of anti- inflammatory drugs on promotion of healing process.

The study was based on that in several studies, effects of antioxidant [2] and anti-inflammatory [13] *Myrtus* extract herb is known, its therapeutic effect is due to essence those found in the various organs, especially in the leaves. *Myrtus* plant also has anti-infective effects and has been used in treatment of external and internal infections of many diseases like diarrhea and dysentery [10]. There are many study results on its antibiotic effects on *pseudomonas* [1] that best results is for *Myrtus* extract.

In present study, increasing wound diameter in first study days was justifiable via chronological accommodations with swelling of wound healing. In addition to inflammation and swelling, skin and muscle tension in the wound area in these days are involved. In

geometric results there was no abnormality in terms of coordination with the theoretical hypotheses and decreasing wound size was due to reduced inflammation and wound healing progress and in the results presented, finally the best response belongs to the group was treated with the low dose extract.

Based on results of this trial, extract about 5% cream is accelerated process of skin wound healing. However the zinc oxide ointment also been shown to have good effect. Only unpredictable weak results was the 10% Myrtus plant extract ointment that finally shown weak effects on wound healing than control groups. independent study is needed to justify this results but According to the author's personal experiences and studies it is likely that Toxic effects of high doses of Myrtus plant extract caused the poisoning of treated animals.

Fibroblasts are responsible for the synthesis of collagen and connective tissue fibers and Penetration into the connective tissue formed in tissue repair annular, cases the maturation of connective tissue. Since the Myrtus extract stimulate the activity of fibroblasts and macrophages, this two cells in interaction with each others with the stimulation of regenerated vessel to granulation tissues, Makes to healing more quickly [8].

Osrin in total duration showed better results than the control group. Although Osrin is considered a neutral substance but there are certainly legitimate reasons for this significant difference that possibly creating a protective layer over the wound and reduce entry into the wound infectious agents can be effective in this. However for obtaining accurate results, specific experiments are requiring.

CONCLUSION

In conclusion, stated that based on the results of this trial, low dose Myrtus extract ointment is accelerated the skin healing process, However high doses of the extract cream does not have any ability to heal wounds and also may be cause delays in wound healing. In control group, is shown the lowest healing rate during the whole period and to end during of treating phase, the Osrin group had better than control group.

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