Nawar Bahjet Kamil et al /J. Pharm. Sci. & Res. Vol. 10(5), 2018, 1030-1035

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation the effects of local exogenous application of a mixture of Myrrh and Sage oil on incisional wound healing on the skin of the face (Histological, Histochemical and Histomorphpmetrical study in rabbits)

Nawar Bahjet Kamil, B.D.S.
Department of Oral Diagnosis, College of Dentistry, University of Baghdad/Iraq.

Nada M. H. Al-Ghaban, B.D.S, M.Sc., Ph.D.
Department of Oral Diagnosis, College of Dentistry, University of Baghdad/Iraq.

Abstract

Aim of the study: To evaluate the effect of topical application of a mixture of myrrh oil and sage oil on the skin wound healing histologically, histochemically and histomorphometrically.

Material and methods: Twenty male white New Zealand rabbits will be used in this study. A surgical incisional wounds with full skin thickness depth and 2 cm length will be done in both sides of the skin of the cheek for each rabbit. The left side incision(control group) will be irrigated with (10µL) distilled water. The right side incision(Experimental group) will be treated with (10µL) mixture of myrrh oil and sage oil. Then each group will be subdivided into 4 subgroups according to healing intervals into 1,3,7 and 14 days(5 rabbits for each).

Results: Histological findings of the current study showed a high significant difference between both groups in inflammatory cells account with the highest mean value recorded at 1, 3days and 7,14 days for control and experimental groups respectively. Histomorphometrical findings showed that the epithelial thickness nearly completed at 7 days for experimental group and at 14 days for control. Blood vessel account recorded a high significant difference between groups at 1 and 3days only. Histochemical findings showed that collagen fiber remodeling recorded a high significant difference between control and experimental groups at 7 and 14 days.

Conclusion: The present study revealed that the mixture of myrrh oil and sage oil are more effective in accelerating the wound healing than control one in rabbits.

Key word: Myrrh oil, Sage oil, Masson's Trichrome staining.

INTRODUCTION:

The skin is considered as a barrier or interface between the body of the human and the external environment [(1)]. A wound can be defined as a loss of the integrity and function of the tissues of the body. The injury is usually caused by an external force (surgical or traumatic/accidental) and it can involve any type of tissue or organ [(2)].

Wound healing, which is a normal biological process in the body, is achieved by four precise, strict and highly programmed phases. These includes: hemostasis, inflammation, proliferation, and remodeling. For any wound to heal successfully, the all four phases must occur in a proper sequence and accepted time frame [(3)].

Myrrh oil is the hardened sap that oozes from the stem of *Commiphora molmol* (Family Burseracea) tree, collected from the natural cracks or can be from human made cuts in the bark of trees [(4)]. Myrrh had multiple uses in the recorded history and had been valued to be a fragrance and as well as a medical agent by the old Egyptians and by the ancient Chines [(5)]. Caryophyllene C15H24 which is present in myrrh oil also has an antibacterial, anti-tumor and anti-inflammatory action [(6)].

Sage is used in skin care to heal sores, bumps, wounds and other skin injuries [(7)]. A study was performed for the approval that the plant extracts can act as an inhibitor for the formation of the dental plaques [(8)]. The thujonein which is present in sage oil has an antibiotic and antiseptic action and when it is taken as a mouthwash, the sage can deal effectively with infections of the throat, dental abscesses, mouth ulcers and infected gums [(9)].

MATERIAL AND METHOD:

Twenty male New Zealand rabbits, with a body weight of (1.5–2kg) and aging from (4-7) months were used in this study. All rabbits were maintained under controlled ventilation conditions, temperature, housing and feeding, and were given a standard diet (pellet and barseem) with an easy access to the tap water. Animals were kept in standard separate cages and they were kept for 2 weeks in the same suitable environment before the surgical procedure.

All of the animals were examined by (veterinarian staff in the animal house of Biotechnical Research Center at Alnahrain Unniversity) to evaluate their general health in order to exclude unhealthy animals. The animals were fasted prior to the operation for about 6-8 hours. All of the procedures in the experiment were done in accordance with the animal experimentation ethical principles [(10)].

A surgical incisional wounds with full skin thickness depth and 2 cm length will be done in both sides of the skin of the cheek for each rabbit [(11)]. Then the animals will be randomly divided according to healing intervals in to(1,3,7,14) (5 rabbit in each) and each group was divided in to:

1- Control group: The left side incision will be irrigated with distilled water.

2- Experimental group: The right side incisions will be treated with 10µL of a mixture of myrrh oil and sage oil.

The specimens were taken and prepared for histological (H&E stain) and histochemical examination using masson's trichrome stain for assessment of collagen fibers density.

Assessment of wound healing parameters

clinical assessment

- Wound contraction:

At each period interval of day 3,7 and 14 the wounds were measured by ruler [(11,12)].

2 Histological analysis

1. Analysis of Inflammatory cells:

Using power x40 lens, we counted the number of the inflammatory cells in five field and then the mean number of cells was recorded [(13)].

2. Epithelial thickness:

Using power x40 lens, the epithelial thickness measurement was performed by the measurement of the distance from outermost layer of the keratin to the inner most basal layer of the epidermis

at the wound edges as a mean of two readings by using Image J computer software [14].

3. Analysis of blood vessels

This process was done using Image J software. Using light microscope, under power x40[15].

4. Analysis of collagen fiber density

The slides stained with masson's trichrome stain were examined under light microscope, power x40, at period intervals of 3,7 and 14 with the help of image J software, the intensity of collagen fibers was measured by measuring the collagen surface area percent [16].

RESULT:

Estimation of wound contraction

Table (1) showed that the recorded mean values decreased with time, and showed high significant difference between control and experimental group in all healing period($p<0.01$). Figure (1)

Table (1) Descriptive statistics of wound contraction at different healing period

Time/day	Control Mean±SD	Myrrh and Sage Mean±SD	F	P value	LSD
1 Day	11.80	26.80	11.63	0.000**	-15.0
3 Days	17.60	25.20	4.71	0.010**	-7.6
7 Days	26.40	16.60	11.15	0.000**	9.8
14 Days	22.80	10.60	31.07	0.000**	12.2

Inflammatory cell parameter

The study revealed as shown in table (2) that the highest mean values recorded for the experimental group was at day 3 and the lowest mean value was at day 14. For control group the highest mean value recorded was at day 7 and the lowest value was at day 1, also it showed a high significant difference between control and experimental groups at all healing period.

Table (2) Descriptive statistics of inflammatory cells account in each period interval

Time/day	Control Mean±SD	Myrrh and Sage Mean±SD	F	P value	LSD
1 Day	0.95	4.1	19.2	0.000**	-3.62
3 Days	5.81	9.7	7.7	0.002**	-20.52
7 Days	17.69	31.59	42.11	0.000**	-6.73
14 Days	42.11	60.01	61.85	0.000**	-23.92

Epithelial thickness parameter

The study revealed as shown in table (3) that the highest mean epithelial thickness values was recorded for control and experimental groups at day 14 and the lowest mean value for both control and experimental groups was at day 1, also it showed a high significant difference between control and experimental groups at all healing periods($p<0.01$).

Blood vessel account

The study revealed as shown in table(4) that the highest mean blood vessels acount value was recorded for all experimental group at day 3, and for control groups at day 7. The lowest mean values recorded for control and experimental groups was at day 1also it showed a high significant difference between control and experimental groups at day 1and 3 and there was no significant difference at day 7 and 14($p<0.01$).

Collagen fiber density :

The result revealed that the highest mean values was recorded for control and experimental groups at day 14 and the lowest mean values was recorded at day 3 as shown in table(5),also it showed a high significant difference between control and experimental groups at different healing period ($p<0.01$).

Experimental group (M&S): Histological view showed clot keratinocyte cells proliferation at wound edges, clot formation above necrotic debris, infiltration of large number of inflammatory cells and few blood vessels also present.Fig(2)

Table (3) Descriptive statistics of epithelial thickness (µm) in each period interval

Time/day	Control Mean±SD	Myrrh and Sage Mean±SD	F	P value	LSD
1 Day	0.80	2.20	7.07	0.003**	-1.40
3 Days	3.60	8.20	38.12	0.000**	-4.60
7 Days	7.80	6.80	2.97	0.063	1.00
14 Days	6.40	6	1.31	0.31	0.40

Table (4) Descriptive statistics of blood vessels account in each period interval

Time/day	Control Mean±SD	Myrrh and Sage Mean±SD	F	P value	LSD
3 Days	1.86	1.68	15.3	0.000**	0.18
7 Days	1.50	1.32	14.53	0.000**	0.18
14 Days	0.80	0.22	19.95	0.000**	0.58

Table (5) Descriptive statistics of the collagen fiber density (%) in each period interval

Time/day	Control Mean±SD	Myrrh and Sage Mean±SD	F	P value	LSD
3 Days	18.83	37.33	44.48	0.000**	-18.90
7 Days	24.19	56.16	62.94	0.000**	-32.00
14 Days	44.11	68.72	76.23	0.000**	-23.4

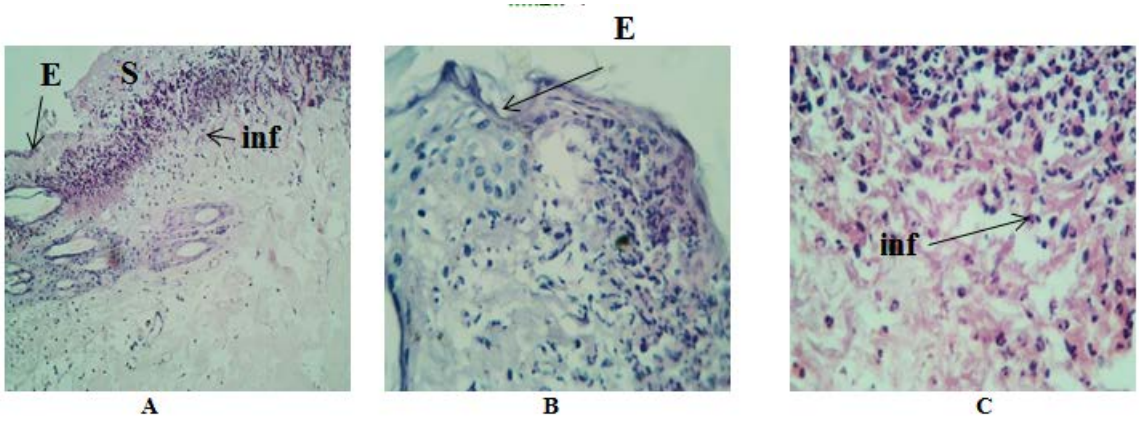
Histological finding (H&E and Masson's Trichrome chemical stain)

- **One day duration: (Control group)**

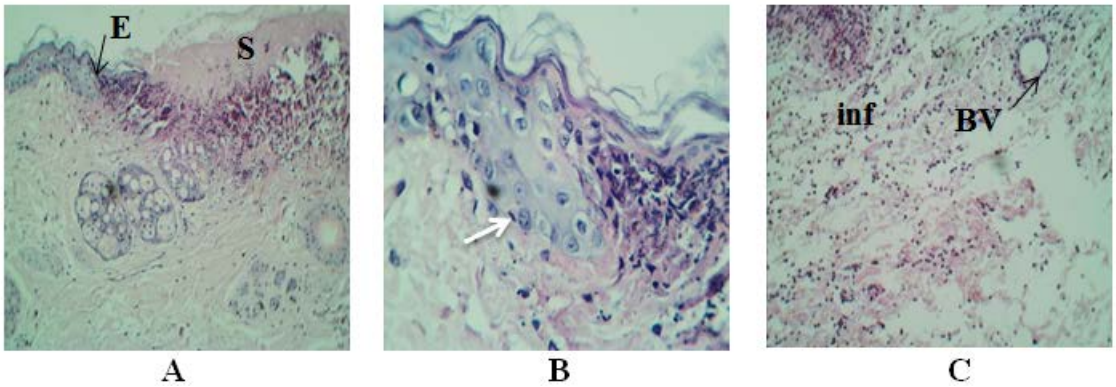
Histological view showed clot formation above necrotic debris and inflammatory cells infiltrated beneath wound area **.**Fig(1)

- **Three days duration:Control group:**

Histological view showed dominant and intense inflammatory reaction, scab formation and new blood vessels. The epidermis was thickened at its cut edges as a result of mitotic activity of keratinocyte cells in basal cells.Fig(3) MTstain: illustrated very little fibrin network filling out the incisional space. This network contained blood cells and created a scaffold for migrating fibroblasts.



Fig(1): View of control group at day 1showed **A:**E:Cutting edge of epithelium, inf: inflammator cells infiltration, S:Scab,H&E,X10.**B:**Magnification of **A** showed E: cutting edge of epithelium.H&E,40.**C:** Magnification View showed Inf: inflammatory cells, H&E,X40.



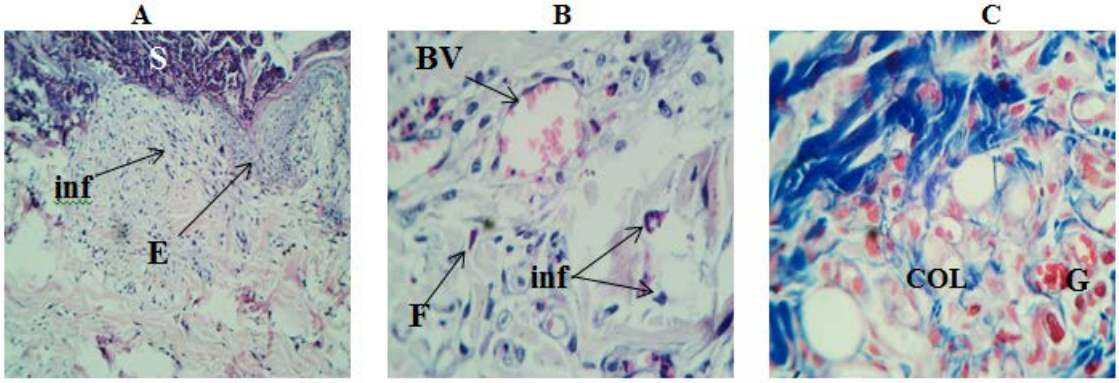
Fig(2): View of M&S group at day 1showed **A:** E : Epithelial cutting edge, S: Scab, H&E,X10.**B:** Magnification view of previous fig. showed migration of basal cell (white arrow) D: dermis,H&E,X40.**C:** BV: Blood vessel and infiltration of Inflammatory cells in dermis as black points,H&E,X10.

Experimental group(M&S):

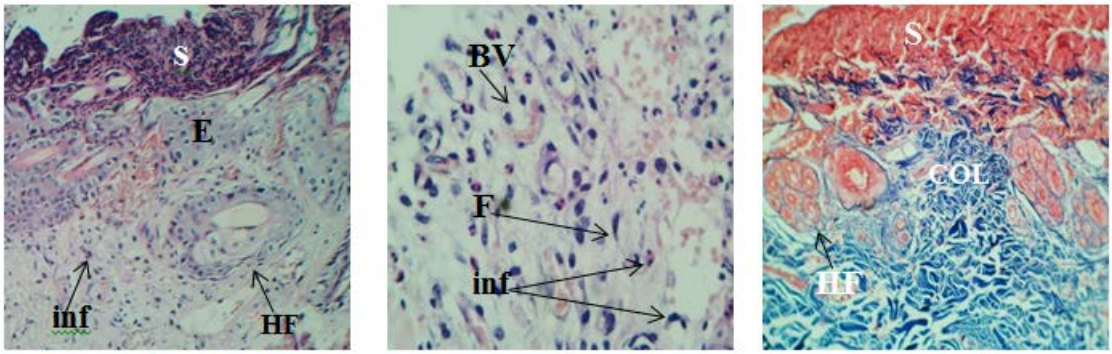
Histological view showed tissue regeneration from the cut edges of the wound and re-epithelialization from hair follicles, on the other hand, in some of the superficial and deep parts of the wound, there was diffuse bleeding and inflammatory cells within the dermis area.MT stain: showed increased dermis cellularity mainly due to fibroblasts proliferation and new matrix deposition.Fig(4)

- **Seven day duration:Control group:**

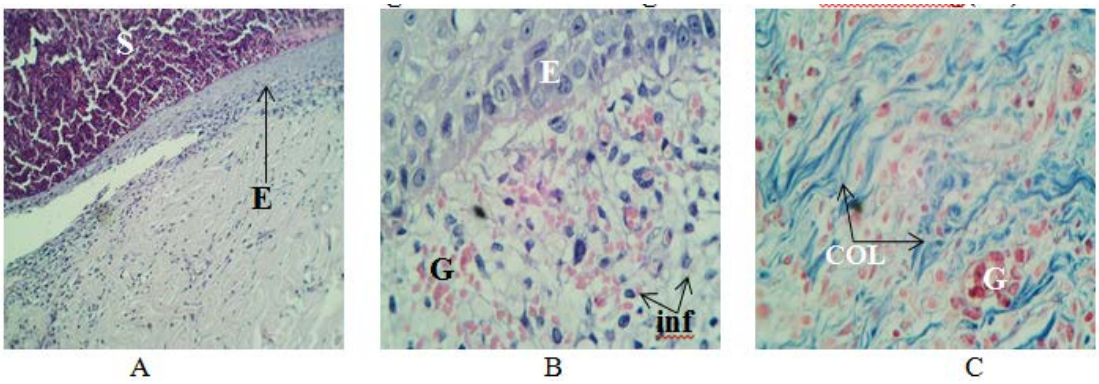
Histological view showed new epithelial formation. The dermis area showed granulation tissue formation, different type of inflammatory cells ,fibroblast cells were infiltration and mitotic division of basal cells was observed.MT stain: illustrated formation of collagen fibers with congested blood vessels.Fig(5)



Fig(3): View of control group at day 3 showed **A:** E:Epithelial migration, Inf::Inflammatory cells, S:Scab formation, H&E,X10.**B:** Showed Inf::Inflammatory cells, BV: Blood vessel , F:Fibroblast cell H&E,X10. **C:** Showed COL: fine collagen fiber ,G: granulation tissue formation, MT,X40.



Fig(4): View of M&S at day 3 showed,**A:** E: new epithelium, S: scab, Inf: inflammatory cells, HF: hair follicle, H&E,X10.**B:** Inf: different types of inflammatory cells F: Fibroblast cell, BV: blood vessels, G: granulation tissue,H&E,X40.**C:** S; Scab, Col: new fine collagen fiber, HF: hair follicles,MT,X40.



Fig(5): View of control group at day 7 showed, **A:** E: new epithelium formation, S: scab, H&E,X10. **B:** Magnification of previous figure E: basal cell layer of epithelium Tissue, Inf: inflammatory cells, G: granulation tissue,H&E,X40.**C:** G: granulation tissue, blue area: coarse of Collagen fibers, MT, X40.

Experimental group:
Histological view showed that the wound is completely lined by hyperplastic epidermis, decreased scab size due to wound contraction, observed keratin layer, few number of inflammatory cells in the dermis area and also can see the union of epithelial hair follicles with epidermal layer. Also can seen numerous blood capillaries surrounded by inflammatory cells and fibroblast cell.MT stain: showed numerous strands of collagen fibers that still had not achieved complete organization. Fig(6)

- **Fourteen day duration: Control group:**

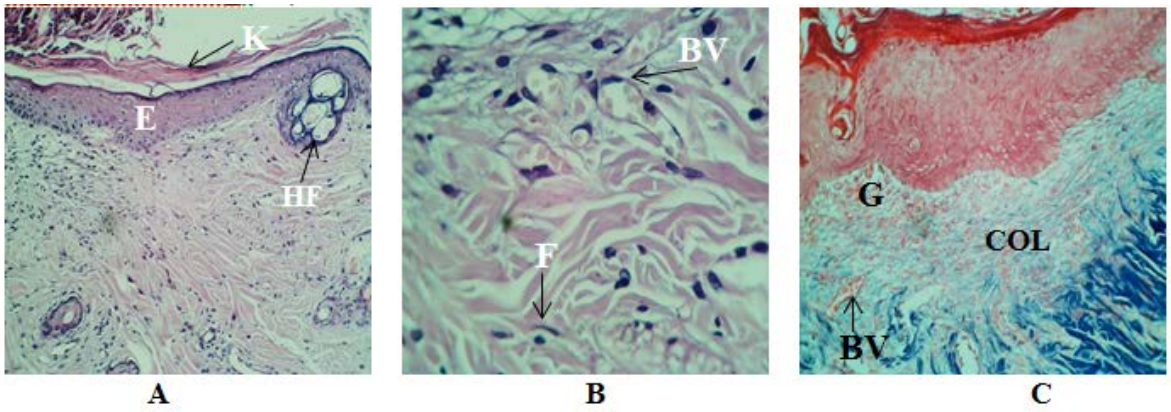
Histological layers showed visible keratin layer with Re-epithelialization of the wounds that was incomplete with the presence of granulation tissue , few number of inflammatory cells, developing hair follicles and congested blood vessels.MT stain: showed disoriented collagen fibers and granulation tissue.Fig(7)

Experimental group:
Histological view showed thick keratin layer and complete epithelial layers that were continuous with the epithelium of the hair follicles also can see epidermis layers and large blood vessels in the dermis area.MT stain: showed thick and oriented collagen fiber bands.Fig(8)

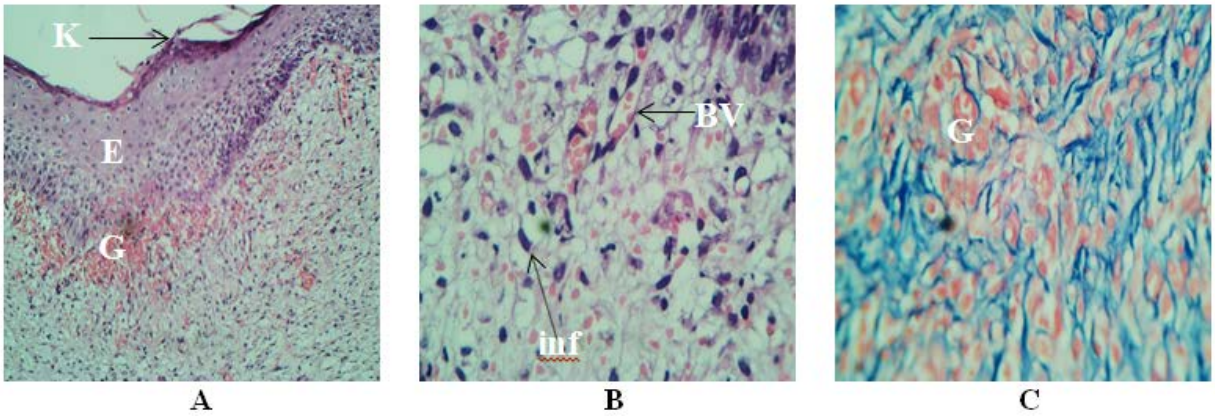
DISCUSSION

Today, herbal medicine is considered to be a branch of the alternative and complementary medicine. Herbal therapies use of for caring of injuries and wounds had been popular since the ancient civilization [17,18].
Present study showed that wound contraction was accelerated in experimental group when compared with control group. This acceleration in wound contraction may be due to increased proliferation and progression of epidermal cells in the experimental group and because of their anti-inflammatory effects of these oils [19, 20].

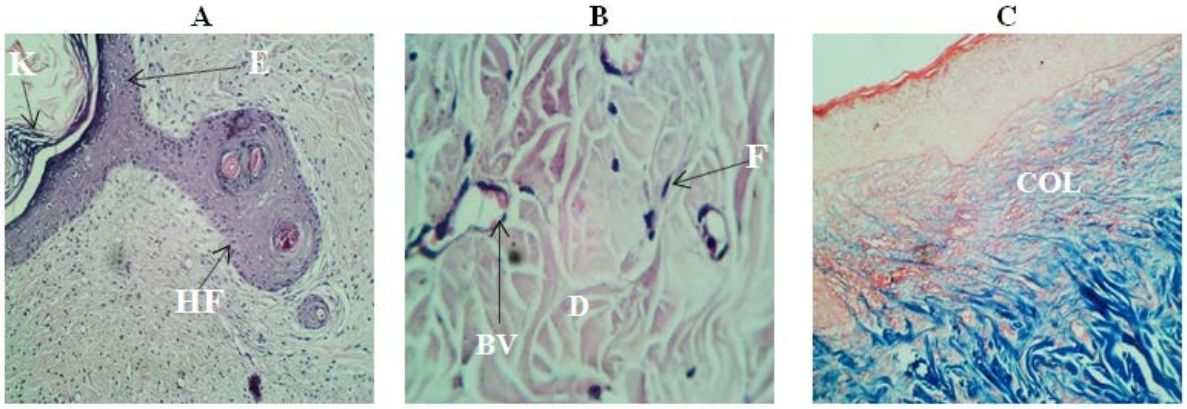
Histological and histomorphometrical evaluation: In this study experimental group displayed variable degrees of inflammatory reaction in the first 24 hours, in contrast to control group which displayed a more prolong and sever inflammatory response extending up to the 7 days attributed to bacterial colonization and lack of immunomodulation and anti-inflammatory activity. This agrees with [21,22].
The density of inflammatory cells in the wound area was found to be predominant and the intensity of the inflammatory reaction was sever in experimental group as compared to control group in the first and third day, while in the seventh and fourteenth day the inflammatory cells became mild because of the completion of the inflammatory stage and the beginning of the remolding stage in experimental groups while they were recorded high mean value in the control groups at the 7th day and decrease in 14th day . That was may be because of the anti- bacterial effect of myrrh [23,24] and sage [25] and this led to the promotion and acceleration of the healing process.



Fig(6): View of M&S at day 7 showed, **A:** K: keratin E: New epithelium, HF: new hair follicle, H&E,X10.**B:** Magnification of previous figure F: fibroblast, BV: blood vessels, H&E,X40.**C:** BV: blood vessels, Col: coarse of collagen fibers, G: granulation tissue, MT, X40.



Fig(7): View of control group at day 14 Showed, **A:** K:keratin layer, E: epidermis, G: granulation tissue, H&E,X10.**B:** Magnification of previous figure ,BV: blood vessel, Inf: inflammatory cells,H&E,X40.**C:** G: granulation tissue and blue area: remodeling of collagen fiber. MT,X40.



Fig(8): View of M&S group at day 14 Showed, **A:** K: keratin layer,HF: developing hair follicle, E:complet formation of epithelium, H&E, X10.**B:** BV: blood vessel , D: Dermis layer, F:fibroblast cell, H&E,X40.**C:** large area occupied by remodeling collagen fiber (Col) and invaded by blood vessel, MT, X10.

Neovascularization was another important event that takes place in the second stage of the wound repair [26]. In our study, in 1st day, neovascularization in the control group was found to be nearly absent, while in the experimental groups, it was found to be present but to a little extent,high significant difference recorded between control group and experimental group in day 1,3 but there was none significant difference in day 7,14. The early Neovascularization in experimental group lead to promot healing because new vessel usually supplies the oxygen and required nutrient and remove the waste products [27].

The present study showed that re-epithelialization was happened faster in experimental group and recorded high significant difference compared as to the control group because of increased proliferation and progression of epidermal cells with an increased in the amount of neovascularization, fibroblast cells and the dermal collagen fiber in experimental group this agrees with [21], the current study also agrees with a previous study done by [22].

The remodeling stage started earlier in experimental groups than in control group which agrees with previous study[19,21].

The masson's trichrome stain was used in this study for the assessment of the collagen fiber density, this agree with previous study[16].Experimental groups were found to have higher collagen density than control group at day 3,7 and 14. This agrees with a previous study [28-30].

CONCLUSION

Mixture of myrrh and sage oil are able to accelerate wound healing because of faster wound contraction, re-epithelization, early neovascularization and higher collagen density than control group Mixture of myrrh and sage oil group showed anti-inflammatory effect which is identified by the decrease in the inflammatory cells account with time.

REFERENCES:

1. Hongbo ,Z. and Maibach, HI. 2004. Dermatotoxicology. CRC Press LCC, USA, 6: 938-955.
2. Rose, L.and Hamm,DPT.2015.text and atlasof wound diagnosis and treatmeant,New york McGrow Hill,chapter (1):1-39.
3. Thiruvoth ,FM., Mohapatra, DP., Kumar, D., Chittoria ,S R K., Nandhagopal, V.2015. Current concepts in the physiology of adult wound healing.plastic and esthetic research. Indea, 2:250-6.
4. Marshall,S. 2004. Myrrh: Magi, medicine and mortality. The pharmaceutical J. 273: 919-921.
5. Etman, M., Amin, M., Nada, AH., Shams-Eldin ,M. and Salama, O .2011. Emulsions and rectal formulations containing myrrh essential oil for better patient compliance. Drug Discov Ther, 5, 150 - 156.
6. Suad, A. Gadir , Ibtisam, M.and Ahmed, J. 2014. Chem. Pharm. Res., 6(7):151-156.
7. Fluck, H. 1988. Medicinal Plants. W. Foulsham & Co. Ltd., Marlow, United Kingdom, 20: 480-482.
8. Smullen, J., Finney, M., Storey, DM.and Foster, HA. 2012. Prevention of artificial dental plaque formation in vitro by plant extracts. J Appl Microbiol, 113:964-973.
9. Narayanan,N.andThangavelu,L. 2015. Salvia officinalis in dentistry. Dental Hypotheses,6 (1):27-29.
10. Rollin, A. and Bernard,E.2006.The regulation of animal research and the emergence of animal ethics :A conceptual history. Theoretical medicine and bioethics,27(4):285-304.
11. AL-Kadhimi,B.,J. 2014. Effect of topical application of estrogen hormone on wounds healing in ovariectomized rabbits, university of Baghdad,M.Sc.thesis.
12. Kumar, A.Chomwal, R., Kumar, P. and Sawal, R. 2009. Anti inflammatory and wound healing activity of curcuma aromatic salisb extract and its formation. Journal of chemical and pharceutical research, 1(1):304-310.
13. Accorinate, M., Holland, R., Reise, A., Bortuluzzi, M. and Murate, S.2008. Union of mineral trioxide cement as pulp capping agent in teeth ,Journal of endo,34:1-6.
14. Gal,P.,Vidinsky,B. and Toporcer,t.2006.Histological assessment of the effect of laser irradiation on skin wound healing in rats.Photomed. Las. Surg.,24(4):408-488.
15. Wosgrau, ACC., Jeremias, TdS., Leonardi ,DF.,Pereima, MJ Di., Giunta, G.and Trentin, AG .2015.Comparative Experimental Study of Wound Healingin Mice: Pelnac versus Integra. Plose one journal.pone ,10(3).
16. Suvik, A. and Effendy A.W.M. 2012. The use of modified Masson's Trichrome staining in collagen evaluation wound healing study. Malaysian Journal of Veterinary Research 3 (1): 39-47.
17.. Hajiaghaalipour,F.,Kanthimathi,MS.,Abdulla,MA. And Junedah Sanusi, J.2013.The effect of Camellia sinesis on wound healing potential in an animal model .Evidence- based complementary and alternative medicine research article,2:1-7.
18. Nezhad, HR., Sahhri, NM. and Rakhshandeh.2013.The importance of tomeric extract on wound repaire in rate. Animals of biological research,4(12):123-128.
19. Rouy,MB.,Azarsina,m.,Soufi,LR.and Alikhani,MY.2015.The antibacterial effect of sage extract mouth wash against Streptococcus mutans in dental plaque: arandomized clinical trial.Iranian J. of microbiological,7(3):173-177.
20. Shalaby ,M A. and Hammouda ,A A-E. 2014. Analgesic, anti-inflammatory and anti-hyperlipidemic activities of *Commiphora molmol* extract (Myrrh).J Intercult Ethnopharmacol , 3(2): 56–62.
21. Al-Mobeeriek,A.2011.Effect of myrrh on intra oral mucosal wounds compared with tetracycline and chlorhexidine based mouth washes. Clinical, cosmetic and Investigational Dentistry,3:53-58.
22. Fawzi, M., Kamel, Z. and Farhan, S.2017. Anti-Inflamatory Effect of Salvia Officinalis Extracts on Oral Health. Iraqi Dental Journal, 39(1):1-5.
23. Almekhlafi,S., Anes, A. M., Thabit, Ameen, M. I., Alwossabi, Awadth,N. Abdulbaqi, A. M. and Zaid Algaadari,Z.2014. Antimicrobial activity of Yemeni myrrh mouthwash. Journal of Chemical and Pharmaceutical Research, 6(5):1006-1013.
24. Elsarag, SMM. 2008. The activity of commphora myrrh against microorganism recoverd rfom wounds.msc thesis, Sudan University of Science and Technology.
25. Stanojevic, D., Comic, L., Stefanovic ,O., Solujic-Sukdolak, S. 2010. In vitro synergistic antibacterial activity of Salvia officinalis and some preservatives. Arch Biol Sci Belgrade,62:175–83.
26. Gurtner, GC. Werner, S. Barrandon, Y. Longaker, MT. Wound repair and regeneration. Nature. 2008; 453 (7193):314–21.
27. Shaykhiev, R., Beisswenger, C.and Kandler ,K. 2005. Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. *Am J Physiol Lung Cell Mol Physiol*. 289(5):L842–848.
28. Kondo, T. 2007. Timing of skin wounds. Legal Medicine 9:109–114.
29. Reinke ,JM. and Sorg, H. 2012. Wound repair and regeneration. Eur Surg Res., 49: 35-43.
30. Enoch, S. and Leaper, D.J. 2007. Basic science of wound healing. Surgery; 26(2):31-37.