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Original Research Article

Formulation of Herbal Gel Preparations from Medicinal Plants and Evaluation of their Wound Healing Activities

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Abstract

Aims: The present study aimed to formulate herbal gel preparations containing alcoholic extracts of *Withania somnifera* (Ashwagandha), *Allium sativum* (garlic), and *Curcuma longa* (turmeric), and to investigate their wound healing activities upon topical application on full-thickness skin wounds induced surgically in Rabbit. ***Methods:*** Experimental study was conducted using thirty adult male rabbits. Animals were randomly allocated into 6 groups each containing 6 animals. Animals in group 1 were without treatment and served as control. Animals in group 2 received the carbomer gel base not containing drugs. Animals in group 3 received herbal gel containing withania extract. Animals in group 4 received herbal gel containing garlic extract. Animals in group 5 received herbal gel containing curcuma extract. Animals in group 6 received gel containing ciprofloxacin. Gel was applied on wound once daily up to 14-18 days starting from the second day of wounding. ***Results:*** The wound healing was slower in the untreated control animals as well as the animals treated with carbomer gel base where wounds completely healed by the 18th day of experimental period. In the other hand, wound healing in animals treated with withania gel reached about 90% by the 12th day and completely healed by the 14th day. Notably, wound healing reached about 90% by the 10th day and 97% by the 12th day and completely healed by the 14th day. Additionally, wounds in animals treated with curcuma gel cured about 90% by the 14th day and completely cured by the 16th day. Moreover, wound healing reached more than 90% by the 12th day and completely healed by the 14th day in animals treated with ciprofloxacin gel. ***Conclusions:*** Topical application of gels containing the extracts of withania, garlic, and curcuma has fastened the rate of healing in wounds induced surgically in rabbits.

Keywords: Wound healing; *Withania somnifera*, *Curcuma longa*, *Allium sativum*; Topical gel.

INTRODUCTION

Wound is a term applied to the disruption of the anatomic continuity of a tissue with or without microbial infection; they mostly occur as a result of detrimental exposure of tissues to physical, chemical, thermal, microbial or immunological stimuli. The wound-healing process involves four highly integrated and overlapping phases: hemostasis (immediate), inflammation (days 1-3), proliferation (days 4-21), and tissue remodeling or resolution (21 days to 1 year) [1]. These phases and their physiological functions must occur in a proper sequence, at a precise time, and continue for a specific duration at an optimal intensity [2]. Wounds that exhibit impaired healing have failed to progress through the normal stages of wound healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process [3]. Non-healing wounds urge seeking medical help and result in enormous health care expenditures. For instance, the total cost in the US is estimated at more than $3 billion per year [2, 4]

Most single synthetic drug formulations in the market are not reliable for their wound healing properties. Besides, synthetic drugs available are either bacteriostatic or bactericidal rather enhancing the normal stages of wound healing. Several medicinal plants have been found to have wound healing activities. Some plant extracts have been formulated for clinical use in wound management and have proved safe and efficacious [5]. Garlic is one of the most ancient vegetables and its medicinal used are dated back more 5,000 years [6]. Garlic possesses antimicrobial, antioxidant, anticancer, and anti-inflammatory activities [7, 8]. Also, curcuma, turmeric or Indian saffron, which consists of dried as well as

fresh rhizomes of the plant known as Curcuma longa. Traditionally, it has been proved as anti-inflammatory, antioxidant, anticancer, and antiseptic [9, 10]**.** Moreover, *Withania somnifera* has been shown to possess anti-inflammatory, antimicrobial, and immunomodulaty effects [11-13].

The present study aimed to formulate herbal gel preparations containing alcoholic extracts of Withania somnifera (Ashwagandha), Allium sativum (garlic), and Curcuma longa (turmeric), and to investigate their wound healing activities upon topical application on full-thickness skin wounds induced surgically in rabbits.

MATERIALS AND METHODS

Preparation of plant extracts

The aerial parts of Ashwagandha "withania" (*Withania somnifera* Dunal; *Solanaceae*), and garlic rhizomes (*Allium sativum*; *Amaryllidaceae*) were collected from agricultural nurseries located in Ibb city, Yemen. Turmeric rhizomes (*Curcuma longa*; *Zingiberaceae*) were obtained from a local supplier. The plant specimen was air-dried in a shade, powdered and were subjected to cold extraction method by maceration with methanol as a solvent. The weighed powdered was macerated in methanol 95% (1:4w/v) with shaking for 72 hours. After maceration, the extract was filtrated and treated in a rotary evaporator at 40°C to separate the alcohol under vacuum. The obtained plant extract was kept in a tight container till further investigations.

Preparation of the herbal gels / ciprofloxacin gel

Topical herbal gels containing either plant extracts 1% were prepared using carbomer as a gelling agent. The composition of formulation is showed in Table (1). Carbomer gel was prepared by dispersing carbomer powder in sufficient quantity of deionized water with the aid of magnetic stirrer (1500 rpm). The drug or the plant extract were added to make 1% concentration, then the pH was adjusted to pH 7 to 7.5 using NaOH 10% with continuous stirring till gel was formed. Ciprofloxacin gel was prepared by a similar method. The obtained gel preparations were tested before application to animals. The tested parameters included color, consistency, washability, pH, spreadability, extrudability, and irritancy test according to previously described methods [14**,** 15, 16].

Table-1: Formulation of topical gel containing the plant extracts 1%:

Excipient	Quantity
Plant extract	1 g
Carbomer	0.8 g
NaOH 10%	3.2 ml
Deionized water	89.2 ml

Experimental wounding

Adult male rabbits weighing 1000-1500 g were used in the study. Animals were kept under controlled environmental conditions; diet and water were allowed *ad libitum*. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). All efforts were exerted to minimize animal suffering.

All animals were anaesthetized by open mask method with anesthetic ether before wound creation. Hairs were removed from dorsal thoracic central region of anaesthetized animals and the area sterilized with 70% alcohol. Full thickness from the demarcated area was excised to produce wound measuring around 1cm^2. The wound was left undressed to the open environment. In this model wound contraction and wound closure time were monitored [17, 18].

Animals were randomly allocated into 6 groups each containing 6 animals. Animals in group 1 were without treatment and served as control. Animals in group 2 received the carbomer gel base not containing drugs. Animals in group 3 received herbal gel containing withania extract. Animals in group 4 received herbal gel containing garlic extract. Animals in group 5 received herbal gel containing curcuma extract. Animals in group 6 received gel containing ciprofloxacin. Gel was applied on wound once daily up to 14-18 days starting from the second day of wounding.

Percent wound contraction and epithelialization time

After wound creation the wound margin were measured every two days until complete healing. Healed area was calculated very two days by subtracting the unhealed area from the initial wound area. Wound healing was represented as percent wound contraction and epithelialization time was observed after complete healing. The area of the wounds on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound area on the first day. The percent wound contraction was calculated using the following formula:

$$\%\ Wound\ Contraction = \frac{Healed\ Area}{Total\ Wound\ Area} \times 100$$

Statistical analysis

All values were expressed as mean ± SEM and the statistical significance of differences among groups in terms of rate of wound healing were evaluated. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.

RESULTS

During first days of treatment, the rate of wound healing was seen slightly faster in animals subjected to topical application of carbomer gel base as compared to the untreated animals. Then, the rate of wound healing was nearly similar in both groups during the last days. However, the wounds were completely healed in both groups by the 18th day of the experimental period (Figure 1).

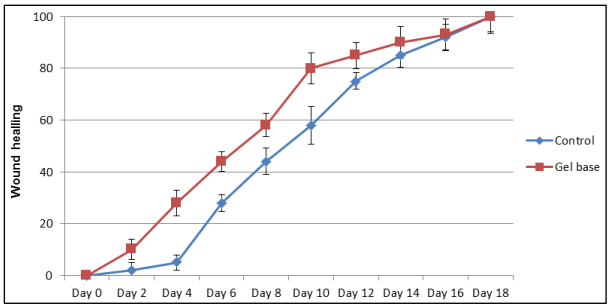

Fig-1: Wound healing in untreated control animals as compared with animals treated with carbomer gel base.

Wound healing in animals treated with withania gel reached about 90% by the 12th day of treatment and completely healed by the 14th day of the experimental period. Wounds in the untreated control group were not completely healed by the 14th day. Thus, it is obvious from the results that withania extract gel has shortened the time of wound healing to 14 days (Figure 2).

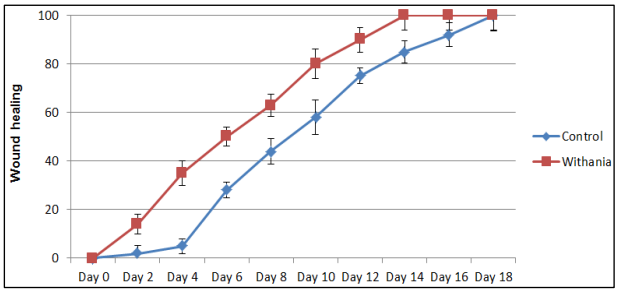

Fig-2: Wound healing in animals treated with withania gel as compared to the untreated control animals

The fastest rate of wound healing was observed among animals treated with garlic extract gel as compared to the control group. Wound healing reached about 90% by the 10th day of treatment and 97% by the 12th day and completely healed by the 14th day of treatment (Figure 3).

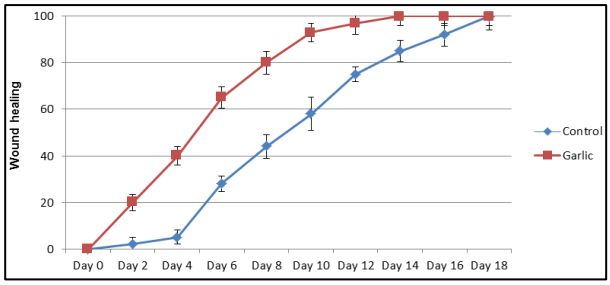

Fig-3: Wound healing in animals treated with garlic gel as compared to the untreated control animals

The wound healing rate in animals treated with curcuma gel was faster than that observed in control group but slower than that of animals treated garlic gel. Wounds in animals treated with curcuma gel cured about 90% by the 14th day and completely cured by the 16th day of treatment (Figure 4).

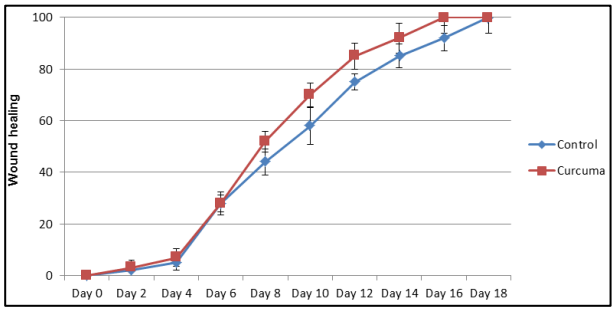

Fig-4: Wound healing in animals treated with curcuma gel as compared to the untreated control animals

The wound healing effect of 1% ciprofloxacin was faster than untreated control animals. Wound healing reached more than 90% by the 12th day of treatment and completely healed by the 14th day of the experimental period (Figure 5). Animals treated with ciprofloxacin gel 1% showed faster wound healing rate than that observed in animals treated with curcuma 1% gel and nearly similar rate as compared with animals treated with either gel containing either withania or garlic extracts.

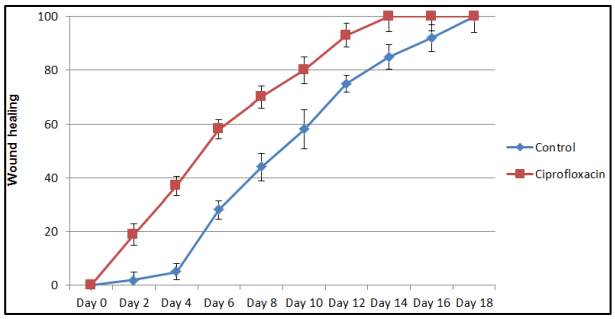

Fig-5: Wound healing in animals treated with ciprofloxacin gel as compared to the untreated control animals

Representative photographs of wound healing among different animal groups were shown in figure (6).

Fig-6A: Wound healing in untreated control animals.

Fig-6B: Wound healing in treated with carbomer gel base.

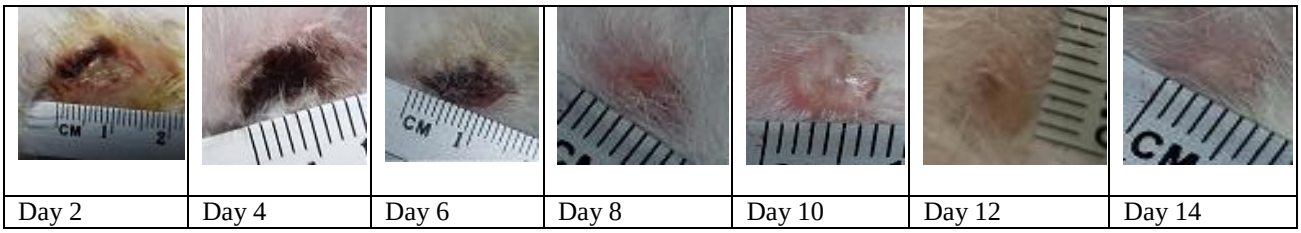

Fig-6C: Wound healing in treated with withania gel base.

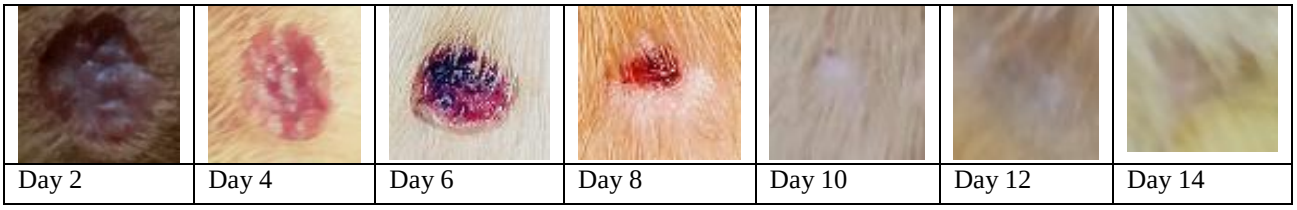

Fig-6D: Wound healing in animals treated with garlic gel.

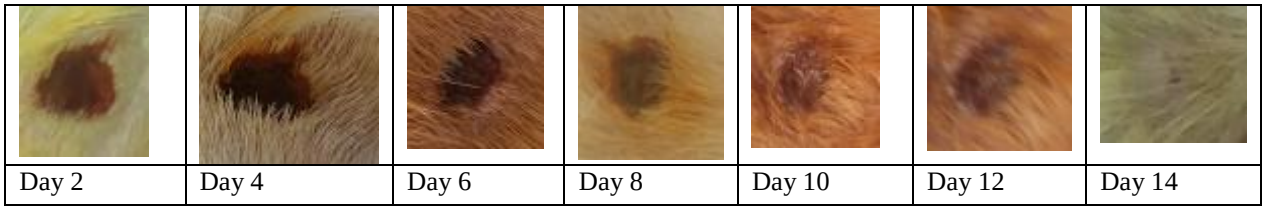

Fig-6E: Wound healing in treated with curcuma gel base.

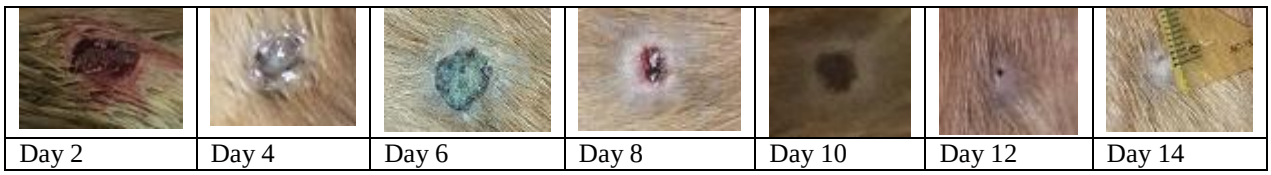

Fig-6F: Wound healing in animals treated with ciprofloxacin gel.

DISCUSSION

In the present study, an excision skin wound model was employed in rabbits and the healing effect of gel preparations containing 1% herbal extracts of withania (*Withania somnifera*), garlic (*Allium sativum*), turmeric (*Curcuma longum*), and ciprofloxacin 1% were investigated.

The wounds in both control untreated animals and animals treated with carbomer gel base were completely healed by the 18th day of the experimental period. These results show that the wound healing rate is the same in both groups. Wound healing involves several interdependent phases including an early inflammatory phase (prominent within the first 2 days), a late inflammatory phase (commencing 2-3 days after injury), a proliferative phase (days 4–21), and a tissue

remodeling phase (21 days to 1 year) [19, 20]. Including surgical incisions, acute wounds usually pass through these phases relatively quickly and wounds tend to heal rapidly especially when wounds are closed immediately with sutures. However, when the tissue loss is more extensive, the wound edges cannot be approximated and the reparative process is prolonged as the defect must be filled with extensive granulation tissue. The process of wound healing in such type of wounds is called closure by secondary intention [21].

The obtained results showed that wound healing in animals treated with withania gel reached about 90% by the 12th day of treatment and completely healed by the 14th day of the experimental period. These results demonstrated that withania extract gel reduced the time required for wound healing, as compared with untreated wounds in the control animals which did not completely healed by the 14th day of treatment. Treatment with withania extract gel was also equipotent to ciprofloxacin gel, which is a potent antimicrobial agent. These effects could be attributed to immunomodulator, antioxidant, and anti-oxidative stress effects [22], as well as the antimicrobial effects exerted by withania [23].

Garlic is one of the most ancient spices and its medicinal uses are dated back more five thousand years [6]. Garlic possesses various pharmacological activities including antimicrobial, antioxidant, anticancer, and anti-inflammatory activities [7, 8]. In the current study, the fastest rate of wound healing was observed among animals treated with garlic extract gel as compared to the control group. Wound healing reached about 90% by the 10th day of treatment and 97% by the 12th day and completely healed by the 14th day of treatment. The activity of garlic in acceleration of wound healing was similar to ciprofloxacin gel. These effects could reflect that garlic accelerated the re-epithelization in wound healing process. The effect of garlic on wound healing process may be contributed of some active constituents of garlic including allicin, flavonoids and triacremonone as anti-inflammatory agents [24]. The anti-inflammatory effect of garlic accelerates the proliferative phase of wound healing that is characterized by the occurrence of re-epithelization and the formation of new blood vessels and fibroblasts.

Notably, the wound healing rate in animals treated with curcuma gel was faster than that observed in control group. Wounds in animals treated with curcuma gel cured about 90% by the 14th day and completely cured by the 16th day of treatment. Having antioxidant and anti-inflammatory properties, curcumin reduces expression of inflammatory cytokines, restores the disturbed antioxidant status, shortens the inflammatory phase, and promotes the collagen synthesis, fibroblasts migration and differentiation [25]. Several in vitro and in vivo studies have reported that curcumin might modulate physiological and molecular events during the inflammatory phase. Curcumin reduces the expression of pro-inflammatory cytokines such as and interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) [26]. In the proliferative phase, curcumin facilitates collagen synthesis **[27]**, fibroblasts differentiation [28].

Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic active against a broad range of bacteria. The results of the present study showed that wound healing effect of 1% ciprofloxacin was faster than untreated control animals. Wound healing reached more than 90% by the 12th day of treatment and completely healed by the 14th day of the experimental period. These findings reflect effectiveness of incorporation ciprofloxacin into topical gels. It has been reported that ciprofloxacin exhibits slow in vitro diffusion rate from solutions which could be attributed to the low aqueous solubility of ciprofloxacin at pH close to 7. Surprisingly, incorporation of ciprofloxacin into gel caused an increase in its release. In an attempt to explain the reasons for these findings, chemical structure of carbomer suggests formation of ionic pairs between the zwitterionic species of ciprofloxacin and the carboxylic groups of carbomer. The dissociation of ionic pairs has contributed in facilitating the drug release from gel matrices [29].

CONCLUSIONS

The study discloses that topical application of gels containing the alcoholic extracts (1%) of *Withania somnifera* (Ashwagandha), *Allium sativum* (garlic), and *Curcuma longa* (turmeric), has fastened the rate of healing in wounds induced surgically in rabbits. Also, similar results have been observed for ciprofloxacin 1% gel.

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Conflicts of interest: There are no conflicts of interest.

REFERENCES

1. Gosain, A., & DiPietro, L. A. (2004). Aging and wound healing. *World journal of surgery*, *28*(3), 321-326.
2. Mathieu, D., Linke, J. C., & Wattel, F. (2006). Non-healing wounds. In *Handbook on hyperbaric medicine* (pp. 401-428). Springer, Dordrecht.
3. Guo, S. A., & DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of dental research*, *89*(3), 219-229.
4. Menke, N. B., Ward, K. R., Witten, T. M., Bonchev, D. G., & Diegelmann, R. F. (2007). Impaired wound healing. *Clinics in dermatology*, *25*(1), 19-25.
5. Ogwang, P. E., Nyafuono, J., Agwaya, M., Omujal, F., Tumusiime, H. R., & Kyakulaga, A. H. (2011). Preclinical efficacy and safety of herbal formulation for management of wounds. *African health sciences*, *11*(3).

6. Gamboa- León, M. R., Aranda- González, I., Mut- Martín, M., García- Miss, M. R., & Dumonteil, E. (2007). In vivo and In vitro Control of Leishmania mexicana due to Garlic- induced NO Production. *Scandinavian journal of immunology*, *66*(5), 508-514.
7. Lang, A., Lahav, M., Sakhnini, E., Barshack, I., Fidder, H. H., Avidan, B., ... & Chowers, Y. (2004). Allicin inhibits spontaneous and TNF-α induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. *Clinical nutrition*, *23*(5), 1199-1208.
8. Bramanti, I., & Purwono, S. (2013). The acceleration of garlic (Allium sativum L) ethanolic extract on gingival wound healing process in Wistar rats. *Journal of the Medical Sciences (Berkala ilmu Kedokteran)*, *45*(02).
9. Agrawal, D. K., & Mishra, P. K. (2010). Curcumin and its analogues: potential anticancer agents. *Medicinal research reviews*, *30*(5), 818-860.
10. Thangapazham, R. L., Sharad, S., & Maheshwari, R. K. (2013). Skin regenerative potentials of curcumin. *Biofactors*, *39*(1), 141-149.
11. Alam, N., Hossain, M., Mottalib, M. A., Sulaiman, S. A., Gan, S. H., & Khalil, M. I. (2012). Methanolic extracts of Withania somnifera leaves, fruits and roots possess antioxidant properties and antibacterial activities. *BMC complementary and alternative medicine*, *12*(1), 175.
12. Mwitari, P. G., Ayeka, P. A., Ondicho, J., Matu, E. N., & Bii, C. C. (2013). Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus. *PloS one*, *8*(6), e65619.
13. Giri, K. R. (2016). Comparative study of anti-inflammatory activity of Withania somnifera (Ashwagandha) with hydrocortisone in experimental animals (Albino rats). *J Med Plants Studies*, *4*, 78-83.
14. Panigrahi, L., Ghosal, S. K., Pattnaik, S., Maharana, L., & Barik, B. B. (2006). Effect of permeation enhancers on the release and permeation kinetics of lincomycin hydrochloride gel formulations through mouse skin. *Indian journal of pharmaceutical sciences*, *68*(2).
15. Aswal, A., Kalra, M., & Rout, A. (2013). Preparation and evaluation of polyherbal cosmetic cream. *Der Pharmacia Lettre*, *5*(1), 83-88.
16. Mendhekar, S.Y., Thorat, P.B., Bodke, N.N., Jadhav, S.L., Gaikwad, D.D.(2017) Formulation and evaluation of gel containing neem, turmeric, aloe vera, green tea and lemon extract with activated charcoal and honey. Ejpmr, 4(12),439-443.
17. Hemmati, A. A., & Mohammadian, F. (2000). An investigation into the effects of mucilage of quince seeds on wound healing in rabbit. *Journal of herbs, spices & medicinal plants*, *7*(4), 41-46.
18. Zaki, A. A., El-Bakry, H. M., & Fahmy, A. A. (2005). Effect of licorice on wound healing in rabbits. *Egypt J Hosp Med*, *20*, 58-65.
19. Mitchell RN. Acute and chronic in flammation in: Kumar V, Cotran RS, Robbins SL. Editors. Robbins bastic pathology.
20. Birdane, L., San, T., Muluk, N. B., Burukoglu, D., & Cingi, C. (2014). Efficacy of Curcumin in the healing of paracentesis in rats. *International journa of pediatric otorhinolaryngology*, *78*(2), 280-284..
21. Young, A., & McNaught, C. E. (2011). The physiology of wound healing. *Surgery (Oxford)*, *29*(10), 475-479.
22. Singh G, Sharma PK, Dudhe R, Singh S. Biological activities of Withania somnifera. Ann Biol Res. 2010;1(3):56-63.
23. Jana, S.N., Charan, S.M. (2018). Health Benefits and Medicinal Potency of Withania somnifera: A Review. Int J Pharm Sci Rev Res, 48(1),22-29.
24. Ban, J. O., Oh, J. H., Kim, T. M., Kim, D. J., Jeong, H. S., Han, S. B., & Hong, J. T. (2009). Anti-inflammatory and arthritic effects of thiacremonone, a novel sulfurcompound isolated from garlic via inhibition of NF-κB. *Arthritis research & therapy*, *11*(5), R145.
25. Barchitta, M., Maugeri, A., Favara, G., Magnano San Lio, R., Evola, G., Agodi, A., & Basile, G. (2019). Nutrition and wound healing: An overview focusing on the beneficial effects of curcumin. *International journal of molecular sciences*, *20*(5), 1119.
26. Wu, N. C., & Wang, J. J. (2014, May). Curcumin Attenuates Liver Warm Ischemia and Reperfusion–Induced Combined Restrictive and Obstructive Lung Disease by Reducing Matrix Metalloprotease 9 Activity. In *Transplantation proceedings*(Vol. 46, No. 4, pp. 1135-1138). Elsevier.
27. Gopinath, D., Ahmed, M. R., Gomathi, K., Chitra, K., Sehgal, P. K., & Jayakumar, R. (2004). Dermal wound healing processes with curcumin incorporated collagen films. *Biomaterials*, *25*(10), 1911-1917.
28. Jagetia, G. C., & Rajanikant, G. K. (2012). Acceleration of wound repair by curcumin in the excision wound of mice exposed to different doses of fractionated γ radiation. *International wound journal*, *9*(1), 76-92.
29. Al-Kassas, R. S., & El-Khatib, M. M. (2009). Ophthalmic controlled release in situ gelling systems for ciprofloxacin based on polymeric carriers. *Drug delivery*, *16*(3), 145-152.