

**OFLOXACIN POLYHERBAL TOPICAL GEL****Pappu Kr. Chaurasiya\*, Praveen Kumar Ashok, Deepika Joshi and Pratima Jayasawal**

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**\*Corresponding Author****Pappu Kr. Chaurasiya**Gyani Inder Siingh Institute  
of Professional Studies,  
Dehradun.**ABSTRACT**

According to Ayurveda, individual herbs are insufficient to achieve a desired therapeutic effect. When it is optimized as multiple herbs composition in a particular ratio it will give a therapeutic effect in a better way with reduced toxicity. In order to develop such an intervention, the present study was intended to develop a polyherbal drug from methanolic extracts of *Plumbago zeylanica* Linn, *Datura stramonium* Linn and *Argemone Mexicana* Linn. The study also aimed to evaluate the impact of polyherbalism on antimicrobial and antioxidant effect, thereafter the ratio of individual plant extracts was optimized accordingly to treat the wound. The polyherbal drug was put

on preclinical trial to assess the anti-inflammatory and wound healing activity as 2% and 5% polyherbal carbopol-940 gels. The antimicrobial activity was assessed by agar well diffusion and broth dilution method while wound healing activity was evaluated by excision and incision wound models. Topical anti-inflammatory activity was assessed by carrageenan induced paw oedema. The findings of the study revealed the synergistic antimicrobial potential of Polyherbal drug against gram-positive and negative strains. Polyherbal carbopol-940 gels (2% and 5%w/w) promoted the wound healing and anti-inflammatory effect. The high rate of wound contraction ( $< 0.0001$ ), early epithelialization period ( $< 0.0001$ ) and increased wound breaking strength ( $< 0.0001$ ) were observed in 2% and 5% polyherbal gel treated group when compared to the normal control and negative control group. The antimicrobial and anti-inflammatory effect of Polyherbal drug provoked and promoted the wound healing process through accelerated remodelling of damaged tissue.

**INTRODUCTION**

In Indian society, Ayurveda is also known as “Goddess of All Healing” and is considered as one of the most effective traditional system of medicine with many curing and healing properties. Several plant extracts and their phytoconstituents are known as a promising

alternative for wound healing agents due to the presence of diverse active components, ease of access and minimal side effects. The Ayurvedic literature “Sarangdhar Samhita” has also highlighted the concept of polyherbalism in which products with combined extracts of plants are considered more effective rather than individual ones. The active phytoconstituents of individual plants have been identified but are generally present in a small quantities, which is insufficient to produce the desired therapeutic action for curing wounds. Medicinal plants with antimicrobial, antioxidant and anti-inflammatory properties have mitigated the wound healing process. Polyherbalism results in cheaper medication by reducing the duration of therapy or individual cost for anti-inflammatory and antimicrobial medications.

Wound is defined as the any damage or mechanism failure in the defensive mechanism of the skin due to external condition as surgery, cuts, and accidental. Due to damage condition the loss of consistent existence and change the anatomy of the epithelial tissue or epithelium, may or may not loss of the latent connective tissue as like muscles, bone, and nerve. Types of the wound are also required for the diagnosis of these. There is cut wound, surgical wound, accidental wound, shape as curved wound, straight cut. Season is also role play in wound. High heat and high cold are negative effect on the wound. Wounds are infected by external environmental condition as heat, cold, and other bacterial attack, Microbes will be grow. In the autumn season minimum chances of infection or microbial grow.



**Figure no. 1.6: image of wound on skin.**

### **1.5.2 Wounds are classified as following**

#### **1. Infected wound**

This type of wound is older, which are long term treatment. It consist dead tissue and cells. These type of wounds are required carefully treatments or maintained.

## 2. Contaminated wound

It is wound as like open and may be surgical that is major cut or surgery with the help of sterile process which are allow to visible the inflammation.

## 3. Clean wound

Such type of wounds is operational or surgical in hospital or trauma. On the surface of such type wound no microbes are stable due to their maintenance and treatment.

### Clean and contaminated wound

This type of the wounds is not infected that's non inflammation. It is surgical wound and under the supervision of doctor, and hospital staff as junior doctor.

### 1.5.3 Wound healing

Wound healing is the fundamental process that response to connective tissue. The Process of wound healing is the recovery of normal cells or tissue from the injured or damage tissue. Wound size and shape are also important for the incision or medication.

A long time ago a physician from Egypt who treats the open wound by using the grease, honey paste and skin removed for the wound healing. 400 BC Hippocrates discusses, in the Greek medication system for the wound healing, surgical method applies and for that a tinny pipe are push in the wound cavity and wound healing drainage. This method is not existence to long time. Every-one know about that cleaning washing and bandage according to the type of wound. Now a day Wound are cleaning and washing by the hot water or sterile water, and other chemical like savlon antiseptic liquid, Dettol. Ancient time a Greek physicians treat the patient for wound healing using wine that is contains 98% of alcohol, grease and fragrant which is bactericidal. First manufacturer of sterilize cotton, gauze, and other product for the treatment of wound and other surgical equipment was Johnson and Johnson company.

In the wound healing process, there are four types mechanism are study

1. Haemostasis [blood cloth]
2. Inflammation
3. Proliferation [tissue growth]
4. Maturation [tissue remodeling]

## WOUND HEALING

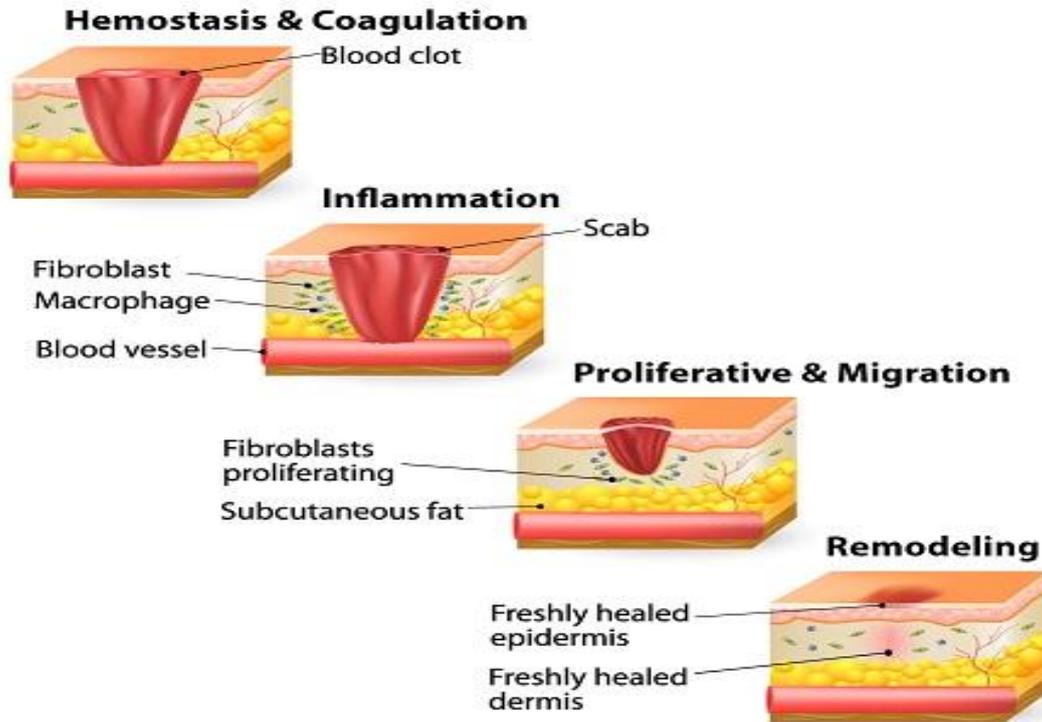


Figure no. 1: Image of wound healing phases.

### Haemostasis

#### Hemostasis

- Series of reactions to stop a bleed
- Phases:
  1. Vascular spasm
  2. Platelet plug
  3. Coagulation (clotting)
  4. Clot Retraction
  5. Fibrinolysis (Clot Eradication)

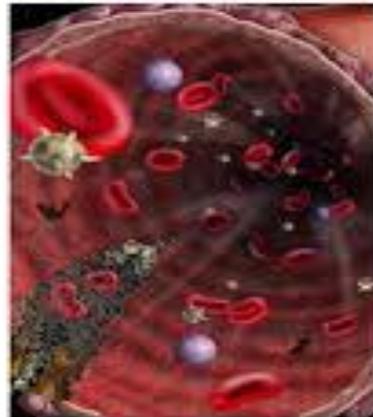
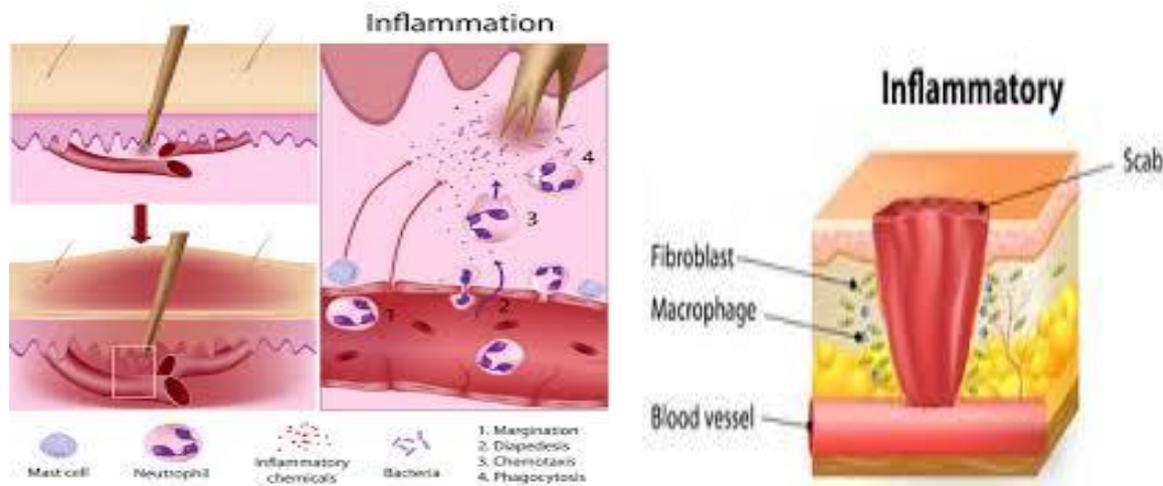


Figure no. 2: Image of haemostasis.

Haemostasis is primary phase of wound healing process. When the skin are damage by any caused there is protective mechanism are appear as haemostasis. It is the defensive

mechanism of body which is exposed as time of any accidental, or damage of the tissues. When this is exposed after any damage on the body there is blood clot and that tissue is red.

### Inflammation

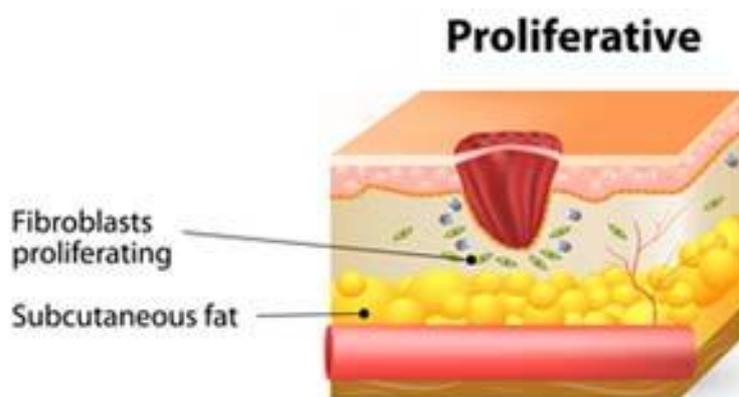


**Figure no. 4: Images of inflammation.**

Starting phase of wound healing process include an acute inflammatory phase that is travel by synthesis of collagen and additional extracellular macromolecule which are change the structure to form a scar as a mark left on the skin. In the healthy skin epidermis which is surface layer and dermis which is deeper layer to provide a protective layer or barrier against the outer environment of the body. If the protective barrier is broken, the biochemical events of body are set a motion to recovery the damage of tissue or cells.

In the inflammation when the breakdown the epithelial cells release cytokines which enhance integrands on the circulatory lymph.

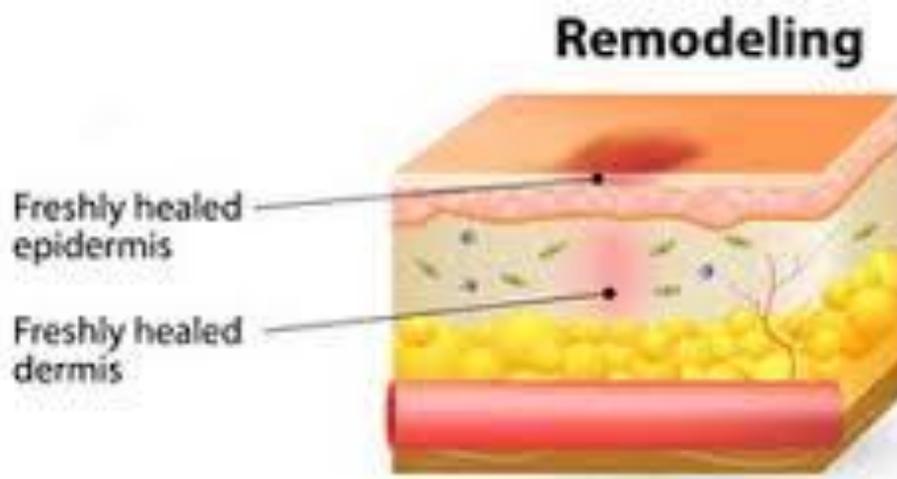
### Proliferative phase



**Figure no. 5: Image of proliferative stage.**

In such types phase the tissue are growing for the recovery of normal tissue. When the tissue Injured and it is break down, then for changing of damage or dead tissue to normal cell it will tissue recover.

### Maturation



**Figure no. 6: Maturation stage.**

This is the last phase of recovery the normal tissue from damage tissue. In this phase the tissue growth in the proliferation phase but there is a mark left. While this phase re-building of the injured tissue and recovery to normal tissue. In this final state of phase maturation of tissue recovery start from the third week to process recommended to long time as 9-12<sup>th</sup> month. In this phase collagen III are change in the form of collagen I which is tensile strength become make a greater size and recovery the 80% of normal tissue.

## 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 /ofloxacin 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.

### **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<sup>2</sup>. The wound was left undressed to the open environment. In this model wound contraction and wound closure time were monitored.

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 contain in ofloxacin. 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**

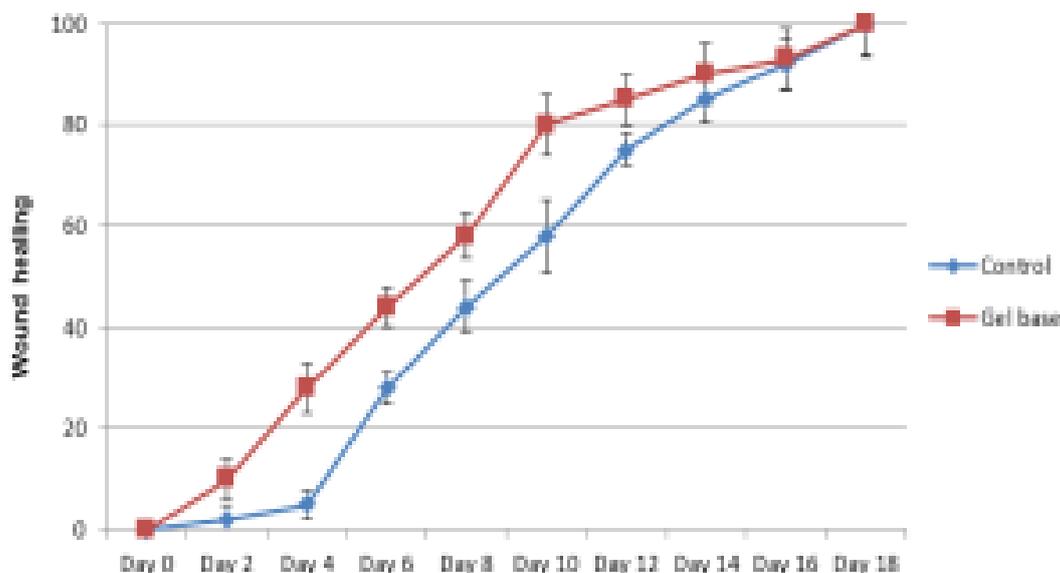
After wound creation the wound margin were measured every two days until complete healing. Healed area was calculated very two days by subtracting the unhealed area from the

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:

### Statistical analysis

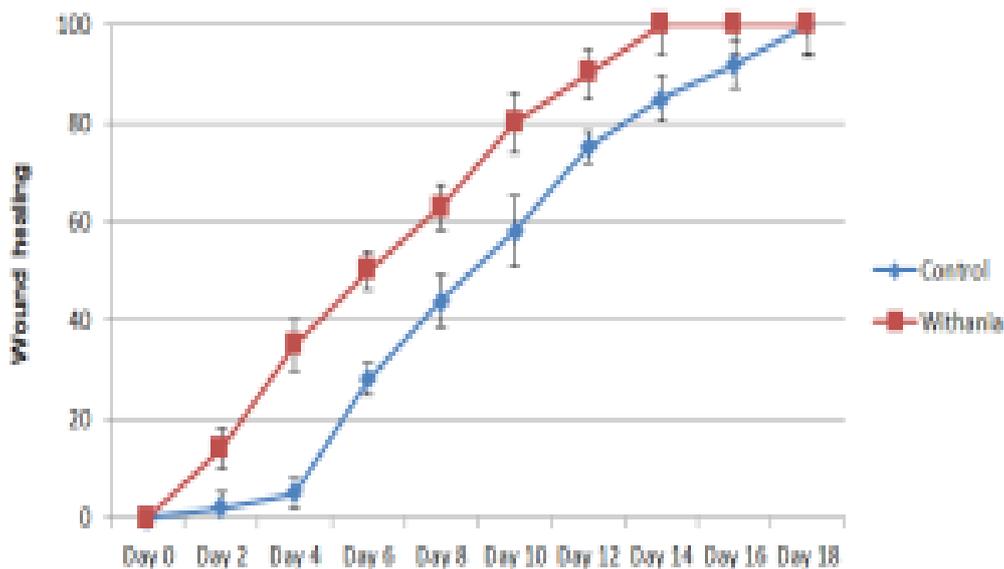
All values were expressed as mean  $\pm$  SEM and the statistical significance of differences among groups in terms of rate of wound healing were evaluated. A value of  $p < 0.05$  was considered significant. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS)

## RESULTS



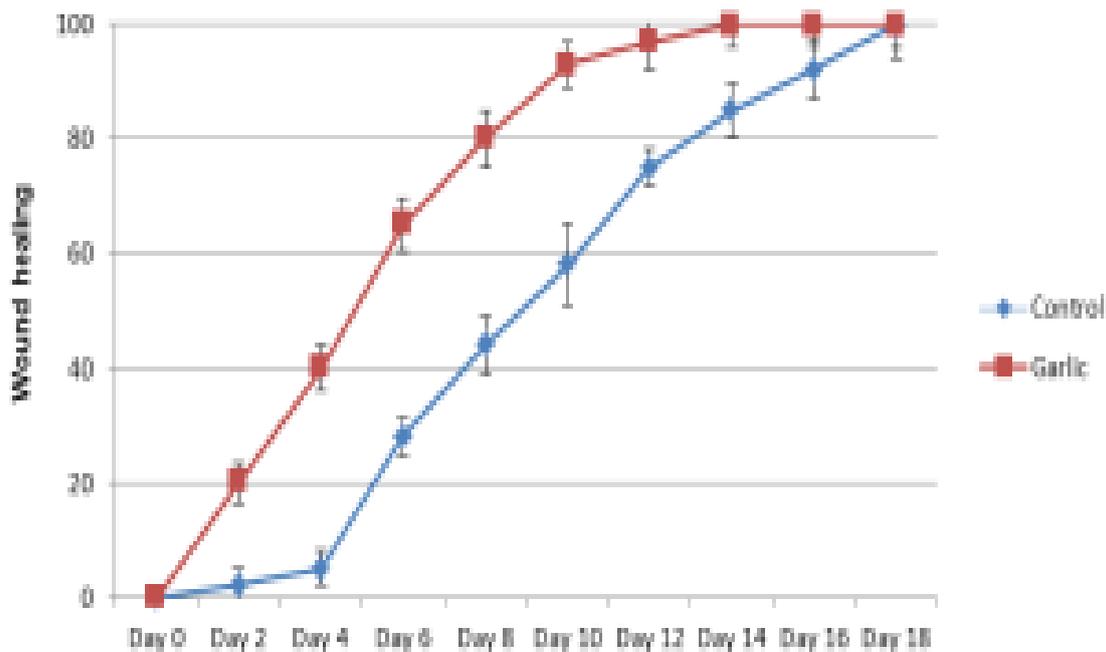
**Fig-7: Wound healing in untreated control animals as compared with animals treated with carbomer gel base.**

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.



**Fig. 8: Wound healing in animals treated with withania gel as compared to the untreated control animals.**

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.



**Fig. 9: 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.

## DISCUSSION

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.

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).<sup>[19,20]</sup> 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.<sup>[21]</sup>

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,<sup>[22]</sup> as well as the antimicrobial effects exerted by withania.<sup>[23]</sup>

Garlic is one of the most ancient spices and its medicinal uses are dated back more five thousand years.<sup>[6]</sup> Garlic possesses various pharmacological activities including antimicrobial, antioxidant, anticancer, and anti-inflammatory activities.<sup>[7,8]</sup> 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 ofloxacin 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 triacremone as anti-inflammatory agents.<sup>[24]</sup> 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.<sup>[25]</sup> 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- $\alpha$ ).<sup>[26]</sup> In the proliferative phase, curcumin facilitates collagen synthesis,<sup>[27]</sup> fibroblasts differentiation.<sup>[28]</sup>

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.<sup>[29]</sup>

## 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 ofloxacin 1% gel.

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**Conflicts of interest:** There are no conflicts of interest.

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