Original Research Article

# Formulation Development and Evaluation of Polyherbal Ointment For Wound Healing Activity: Incision Wound Model

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## Abstract

Wound healing is a challenging clinical problem which needs efficient wound management. Wound is a disruption of the typical anatomical structure and function of skin which can also leads to the organ damage. The wound healing can be divided into 4 major phases according to their mechanism of action *i.e* Hemostasis, Inflammation, Proliferation and Remodeling. Various allopathic formulations are available for wound healing but with possible drawbacks, So,now a days herbal plants are gaining much popularity for treatment of various ailments. This research article focused on the role of herbal plants in wound healing which was accompanied by formulating and evaluating the wound healing assessment by using incision wound model. In this research article polyherbal ointment was prepared using honey, *Ocimum sanctum* extract,*Aloe vera* gel extract and Lemon peel extract. The formulation was evaluated for various parameters and finally subjected to animal study for assessing its wound healing property. Formulation F-C (7%w/w) showed significant($P<0.001$) result when compared with negative control. Marketed formulation was also showed significant result.

**Keywords:** Wound Contraction, Collagen, Flavonoids, Tensile strength, hydroxyproline.

## 1. INTRODUCTION:

Wounds are the inevitable events of life due to a physical or chemical injury or bacterial infection. Wound healing often deviates from the Normal course and lacks healing or excessive healing or it is common for wounds not to heal [1]. A wound is a damage to biological tissues that generally results from surgery, violence, or accidents. It can be open or closed depending upon the cause. Searching for drugs that stimulate wound healing is a developing field of modern biomedical sciences. Different medications Obtained from plant sources are known to increase the wound healing process or reduce the frequency of wound healing time.[2]

A detailed description of wound treatment is mentioned in various classical texts of Ayurveda, in particular Sushruta Samhita, as "Vrana" or Wounds. Different Classical Ayurvedic Texts, medicinal plants, minerals, and animal products described wound healing activity under the term "Vranaropaka"[3]

In the present research different polyherbal formulations containing honey, aloe vera powder, lemon peel extract and ocimum sanctum was taken for preparation of ointment. The main constituents which are responsible for wound healing are flavonoids, glycosides, Vitamin C, saponins, tannins and terpenoids etc. All herbal drugs which are used in the study contain various phytoconstituents showing wound healing property[4].

## 2. MATERIAL AND METHOD

Ocimum sanctum leaves was collected from the herbal garden of the Department of Pharmaceutical Education and Research, Sonipat, Haryana. Lemon Fruits was collected from HAU, Hisar, Haryana. Honey was freshly collected and treated to remove impurities.

Ocimum Sanctum leaves were washed with distilled water and dried in shade. The dried leaves were placed in a thimble of the soxhlet apparatus. The extraction was done using ethanol as solvent. Extraction was continued till a clear solvent was visible in the thimble. The extract obtained in the flask was concentrated in a water bath to form a dark green residue. The extract was subjected to a different test for qualitative phytochemical screening.

Aloe vera gel was used in the dired state. The Lemon was thoroughly washed with distilled water, peeled and the peels dried in shade. Dried lemon peels were ground to form a coarse powder and placed in a thimble of soxhlet apparatus for extraction of phytochemical constituents using ethanol as solvent. The prepared extract was concentrated and subjected to a different test for qualitative screening of phytochemical constituents [5-6].

**Fig.1.1: *O.sanctum* leaves Powder**

**Fig. 1.2: Lemon Peel Powder**

### 2.1 Preliminary phytochemical screening of Phytoconstituents:

The extracts/plant material were investigated chemically for identification of phytoconstituents like phenolic compounds, flavonoids, saponins, alkaloids, glycosides, sterols, carbohydrates, protein & amino acids, acidic compounds, gums & resins, *etc*. [7-10]

### 2.2 Preparation of Polyherbal Ointment [11,12]

For the preparation of ointment, first of all simple ointment was prepared as per the formula given in British Pharmacopoeia. Polyherbal ointment of 3%, 5% and 7% w/w, were prepared by incorporating the sufficient quantity of simple ointment in melted state to other ingredients of formulation.

After preliminary screening polyherbal ointment was prepared using honey, aloe vera gel powder, extract of Ocimum sanctum, and lemon peel. For the preparation of ointment, the trituration method was followed by incorporating a sufficient quantity of simple ointment in a melting state to different concentrations of the extract. Polyherbal ointment of different concentration was prepared and the wound healing activity of prepared polyherbal ointment was compared with the marketed formulation against control group treated with simple ointment base.

**Table 1:**Formula for preparation of simple ointment

| S. No. | Ingredients | Quantity required for 100 gm. |
|---|---|---|
| 1. | Wool Fat | 5gm. |
| 2. | Hard Paraffin | 5gm. |
| 3. | White Soft Paraffin | 85gm |
| 4. | Cetostearyl Alcohol | 5gm |

## 3. Evaluation of Polyherbal Ointment [13-15].

**3.1. Color and Odour:** Color and odour were determined using sensory organs.

**3.2. pH:** pH of the prepared formulation was determined by diluting 1gm. of sample with 100 ml of distilled water reading was taken in a digital pH meter. The procedure was repeated thrice to get the a*verag*e.

**3.3. Diffusibility:** For the diffusion study agar nutrient media was prepared, and poured into a Petri dish. A hole was bored in the Centre of the agar media to place the prepared ointment. The diffusibility was measured after 60 minutes.

**3.4. Sensitivity:** A portion of the ointment was applied on the forearms of 8 volunteers. Observed the occurrence of any toxicity after 20 minutes.

**3.5. Washability:** A portion of ointment was applied on the skin and allowed to flow under the flow of tap water. The time of complete removal of ointment from skin was noted.

**3.6 Stability Study:** The stability study was checked by placing the prepared formulation at different temperature conditions (25±2°C and 40±2 °C) for 3 months in hot air oven and changes if any were noted.

## 4. INCISION WOUND MODEL [16,17]

### 4.1 Wound Healing model Experimental Conditions:

**Table 2:** Experimental conditions

| | |
|---|---|
| **Animal used** | Albino Wister rats |
| **Total no. of animals used for study** | 30 |
| **Number of animals/ Group** | 6/group |
| **Age** | 10-12 weks |
| **Room condition** | 22 ± 2°C |
| **Diet** | Regular Standar Diet |
| **Water** | *ad libitum* |
| **Route of Administration** | Topical |
| **Frequency of Administration** | Once in a day |
| **Acclimatization period** | 12 days |
| **Treatment duration** | 24 days |

### 4.2 Surgical Procedure for incision wound model:

Albino rats (190-210 g) were selected in this study and five groups of six animals each were used. The rats were anesthetized prior to and during creation of the wounds, with diethyl ether. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 5 cm long was made through the skin and cutaneous tissue on the back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed. Animals were kept in separate cages by cleaning their wound using cotton swabs soaked in 70% alcohol. The extract was topically applied to the wound once a day. Sutures of all the animals were removed on 7th post wounding day and the tensile strength was measured on 10th post wounding day using tensiometer The Experimental work on animal was conducted after approval from animal ethical committee vide Animal ethical committee no. PBRI/IAEC/10-09-22/005.

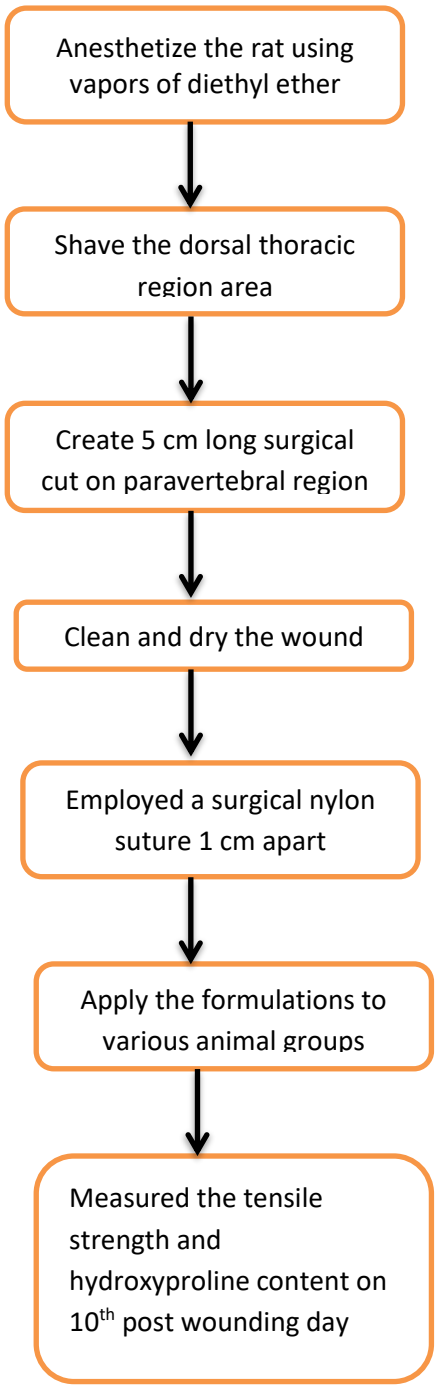



**Fig. 1.3: Schematic Diagram of Incision wound model**

## 5. RESULT AND DISCUSSION:

Preliminary Phytochemical screening showed the presence of various phytoconstituents such as carbohydrates, saponins, flavonoids, glycosides, tannins and phenols. The result is shown as table given below:

**Table 3** Preliminary phytochemical screening of Phytoconstituents

| S. No. | Phytoconstituents | Crude Extract of selected Plants | | |
|---|---|---|---|---|
| | | ***O. sanctum*** | **Lemon Peel** | ***A. vera*** |
| 1 | Carbohydrates | + | + | + |
| 2 | Tannins | + | + | + |
| 3 | Phenols | - | + | + |
| 4 | Flavonoids | + | + | + |
| 5 | Saponins | + | + | + |
| 6 | Glycosides | + | + | + |
| 7 | Terpenoids | + | - | + |
| 8 | Alkaloids | + | - | - |
| 9 | Steroid | - | + | - |
| 10 | Fixed Oils and Fats | - | - | - |
| 11 | Proteins and Free amino acids | - | - | - |

+ means present - means absent

**Table 4:** Evaluation Parameters of Polyherbal Ointment

| Parameters | F-A | F-B | F-C |
|---|---|---|---|
| Color | Yellowish Green | Yellowish Green | Yellowish Green |
| Odour | Characteristic | Characteristic | Characteristic |
| Loss of Drying | 23%w/w | 24%w/w | 26%w/w |
| pH | 6.11±0.5 | 5.93±0.4 | 5.25±0.3 |
| Diffusion Study (After 60 Min.) | 0.9 cm | 0.8 cm | 0.9 cm |
| Storage (25±2°C and 40±2 °C) | Stable | Stable | Stable |

### 5.1 Tensile strength:

In incision wound model tensile strength was measured to observe the extent of wound healing. The standard drug, F-A, F-B and F-C showed significant ($P<0.001$) increase in tensile strength as compared to control group which may be due to the increase in collagen concentration.

**Table 5:** Tensile strength of different groups

| S No. | Group | Tensile strength (g) |
|---|---|---|
| I | **Negative control** | 237.67±5.574 |
| II | **Std MF** | 525.00±3.521** |
| III | **F-A** | 395.17±8.280** |
| IV | **F-B** | 485.67± 11.272** |
| V | **F-C** | 502.00±9.230** |

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *$P<0.050$, **$P<0.001$ and $^{NS}P>0.001$ compared to the negative control.

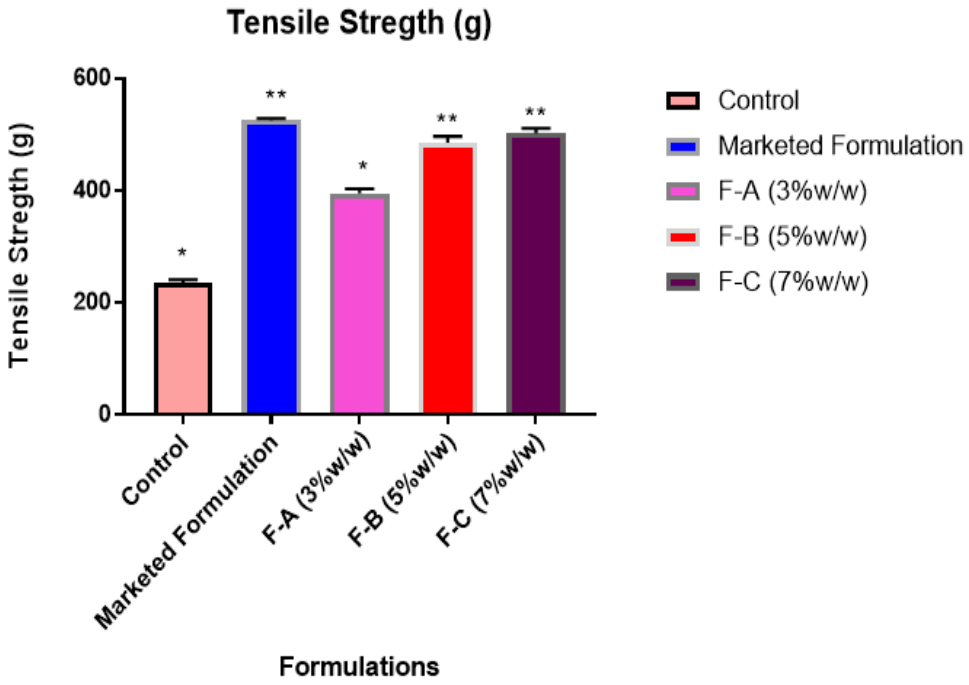
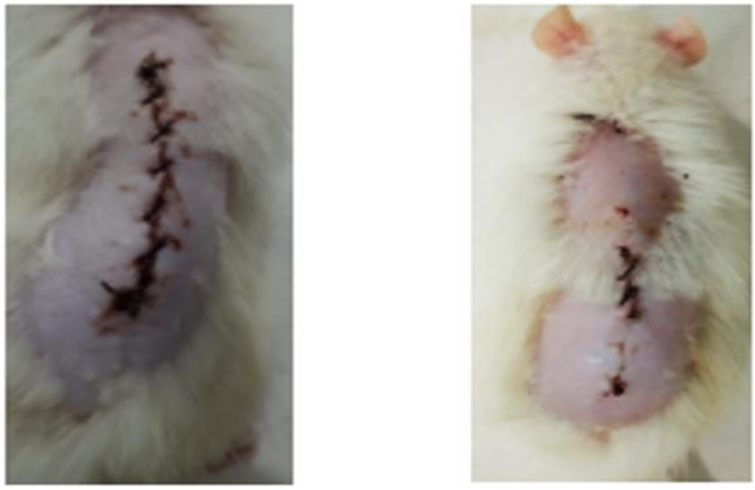
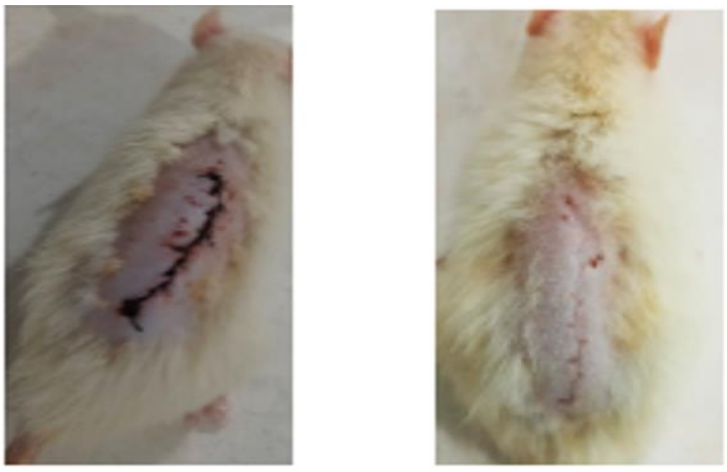



**Fig 1.4** Graph showing comparison of tensile strength b/w different formulation and control group (* Non significant result, ** Significant result)

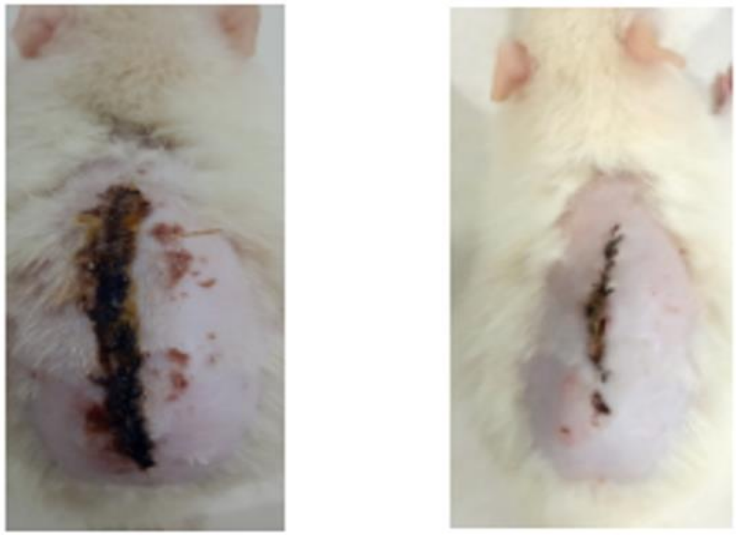
## Incision Wound Model



**Group I** Negative control



**Group II** Standard Marketed Formulation



**Group III** treated with formulation F-A

**Group IV** treated with formulation F-B

**Group V** treated with formulation F-C

## 6. DISCUSSION:

In this research polyherbal ointment containing honey, *Aloe vera* gel powder, *Ocimum sanctum* extract and lemon peel extract was prepared and its wound healing activity was compared with marketed formulation. The wound healing activity was assessed by topically applying the different formulations on rat using incision wound model. As per the result it was concluded that with increasing the concentration of active constituents, the wound closure time decreases. There was a significant increase in wound breaking strength when compared with control. In incision wound model tensile strength was measured to observe the extent of wound healing. The standard drug, F-A, F-B and F-C showed significant ($P<0.001$) increase in tensile strength as compared to control group which may be attributed to the phytoconstituents such as flavonoids,saponins,carbohydrates,phenols, tannins and terpenoids. These phytoconstituents may be responsible to increase collagen synthesis and fibres stabilization.

## 7. CONCLUSION:

Wound healing process is a set of series which comprised of different steps. The aim of our research is to formulate a polyherbal formulation which provides complete closure of wound in minimum period of time without showing any toxic effect. In this rsearch wound healing activity is mainly due to the combination effect of different phytoconstituents. Herbal drugs used in this research contains different bioactive constituents such as flavonoids, Vitamins, Minerals, Saponins, Tannins, Glycosides which shows synergistic wound healing effect and increased collagen and cellular proliferation.

### Declaration of Competing Interest:

No competing interest.

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