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**Original Research Article**

# Enhanced *In-Vivo* Wound Healing Efficacy of a Polyherbal Gel Formulation

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**Abstract:** ***Background:*** Haemostasis, inflammation, proliferation, and remodeling represent the various stages of the wound healing process. Insufficient blood circulation to the wound site and the presence of microbial infections are two additional factors that can impede healing. Improving both the systemic and local health of the patient, along with establishing an optimal environment for healing, are crucial elements of effective wound management. Various products have been created to modify the wound environment, aiming to establish a safe, moist, and infection-free milieu for recovery. In the wound healing cascade, newer materials are being introduced to complement or replace older substrates. ***Methodology*****:** Carbopol 940 (Carbomer Homopolymer USP), Disodium Edetate IP, Sodium Methylparaben IP, Sodium Propylparaben IP, and Sodium Hydroxide IP were used to manufacture the Polyherbal Gel, which was then tested for wound healing activity. For evaluation of wound healing potential of different Polyherbal gel formulations various parameters like wound contraction, tensile strength and Hydroxyproline as a biomarker were assayed. ***Result*****:** It has been observed that animals given the F1 and F3 formulations experienced the quickest wound healing. It was discovered that the epithelization duration of F1 and F3 was essentially typical. The incision wound models demonstrated that, when compared to the standard, the mean tensile strength of the recovered incision wound on the tenth post-injury day showed F1 & F3 formulation to be most significant. ***Conclusion*****:** The investigation's results showed that the synergistic effects of flavonoid and plant phenolic present in the extracts, the results of the current study demonstrated that polyherbal formulations containing ethanolic leaf extract of *M. oleifera, O. sanctum, and A. indica* showed potent wound healing efficacy.

**Keywords:** Wound Healing, *M. oleifera, O. Sanctum & A. Indica* & Polyherbal Gel.



## INTRODUCTION

A wound is characterized as the damage to cellular structures and anatomical continuity within a tissue [1]. This tissue can sustain injury from various sources, including chemical, physical, thermal, microbiological, or immunological factors. Complications such as infected wounds and other problematic conditions are more prone to arise in wounds that are deemed uncomfortable [2]. Wound healing involves a series of coordinated processes aimed at restoring the integrity of damaged tissue [3]. Researchers are exploring alternatives, as herbal-based remedies for wound healing are often not only more affordable but also associated with various issues such as drug resistance and allergic reactions [4]. Despite this, over 80% of individuals globally still rely on traditional medications for their health concerns. These traditional treatments are particularly significant in wound management, as they promote the creation of an optimal healing environment by maintaining moisture. The traditional medicinal framework posits that many medicinal plants can aid in wound healing, although their mechanisms of action and effectiveness have not been thoroughly studied. The initial stage of this healing process is the acute inflammatory phase, which is succeeded by collagen production and the synthesis of other extracellular macromolecules, ultimately leading to scar formation [5-7]. This mechanism fundamentally represents a connective tissue response. When the skin experiences tearing, slicing, burning, or puncturing [8],

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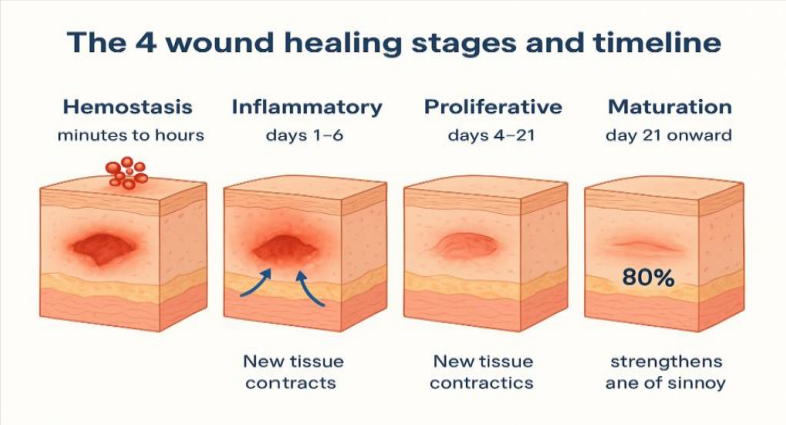
it leads to the formation of a wound. Various physiological systems, including white blood cells [9], fibroblasts, keratinocytes, and others, typically engage in the healing process at the wound site. There is an increase in protein, lipid, and glucose metabolism as resting energy expenditure (RES) rises. Additionally, antibiotics and anti-cancer medications may exert an effect. Other conditions, such as diabetes and others, also influence the wound healing process. In less developed countries, wound infection is one of the most prevalent ailments due to inadequate hygienic conditions [10, 11]. The restoration of disrupted anatomical continuity and compromised functional status of the skin relies on an effective wound healing method. Physical injuries that result in the skin tearing or opening are referred to as wounds [12]. In the treatment of burns and wounds, numerous traditional healers globally, especially in nations such as India and China, possess essential knowledge of various lesser-known, previously unrecognized wild herbs. One of the emerging fields of research in biomedical sciences today is the investigation of drugs aimed at wound treatment. Traditional medical practices that have been employed for many years in Asia and Africa to address wound-related conditions are now undergoing scientific examination. According to various traditional medical practices worldwide, wounds are frequently treated topically with different medicinal plants or their extracts, either alone or in combination with other plant parts. [13].




**Phases of Wound Healing**

Disruptions in skin integrity lead to disorientation, while tissue damage can cause the formation of either acute or chronic wounds. In traditional medicine systems such as Ayurveda, polyherbal formulations are commonly utilized for wound healing. The integration of these traditional approaches with clinical treatments has facilitated the creation of numerous wound-healing products.

**Experimental Works**

**Collection and Authentication of Plant**

The plant was collected in the month of February from the local area of Shivpuri (M.P.). Herbarium file of plant part was prepared and authenticated.

**Animal Care and Handling**

All of the rats were in good health and ranged in weight from 150 to 200 grammes. The temperature was kept at 22°C (plus or minus 3°C) and the animals were kept in an air-conditioned setting. Every fourth day, animal bedding was changed.

**Formulation of Polyherbal formulation (Gel)**

Prepared three gel of 200g each. Firstly, individual weight of drug extract was calculated & an approximate amount was rounded around.

**Table 1: Formulation of Herbal gel**

| Ingredient | F 1 | F 2 | F 3 |
|---|---|---|---|
| Shewaga | 10gm | 5gm | 5gm |
| Tulsi | 5gm | 10gm | 5gm |
| Neem | 5gm | 5gm | 10gm |
| Carbopol 940 (Carbomer Homopolymer USP) | 1.25% w/w | 1.25% w/w | 1.25% w/w |
| Disodium Edetate IP | 0.05% w/w | 0.05% w/w | 0.05% w/w |
| Sodium Methylparaben IP | 0.1% w/w | 0.1% w/w | 0.1% w/w |
| Sodium Propylparaben IP Sodium Hydroxide | q.s. for pH adjustment | q.s. for pH adjustment | q.s. for pH adjustment |
| IP | 0.05% w/w | 0.05% w/w | 0.05% w/w |
| Purified Water IP | q.s. to 100% w/w | q.s. to 100% w/w | q.s. to 100% w/w |

20gm of drug extract & to its quantity 8 times of volume of water was added *i.e.* 160ml.Whole extract was kept overnight with continuous stirring to get smooth gel. Required quantity of methyl paraben was dissolved by heating on water bath. Then volume was reduced to its 1/4$^{th}$ i.e 40ml & finally, full mixed ingredients were mixed properly with the carbopol 940 gel with continuous stirring and NaOH was added drop wise to the formulation for adjusting the required skin pH (pH: 6.8-7.0) and to obtain required consistency.

***In-Vivo* Screening of Wound Healing Activity**
**Excision Wound Model on Albino Rat-**
**Animal:** Albino rat 150gm-200gm
**Drug:** Betadine ointment 25gm (Marketed Formulation)

**Table 2: Selection of Groups for Investigation**

| S. No. | Group |
|---|---|
| Group 1(Control) | - No treatment given |
| Group 2(Standard) | Treated with standard marketed formulation betadine |
| Group3(Test Formulation1) | Treated with test formulation containing<br>Sheawaga- 10gm<br>Tulsi- 5gm, Neem- 5gm |
| Group 4(Test Formulation 2) | Treated with test formulation containing<br>Shewaga- 5gm, Tulsi- 10gm &Neem- 5gm |
| Group 5(Test Formulation 3) | Treated with test formulation containing Shewaga- 5gm, Tulsi- 5gm &Neem- 10 gm |

For the excision wound study, 5 group was made & each group has 4 animals respectively. A circular wound of about 2.5 diameter made on the depilated dorsal thoracic region of the rats under light chloroform anesthesia in aseptic condition. The gel formulations were applied for 12 days the observation of percentage wound closure were made on 4 th,8 th,12 th post wounding days. Along with percentage wound closure epithelization period was also determined. Grouping of animals were done as follows. The percentage wound contraction was determined using the following formula:

$$\% \text{ Closure} = \frac{\text{Wound area on corresponding days - Wound area on day zero}}{\text{Wound area on day zero}} X100$$

On 12$^{th}$ day of post wounding days some parameters were evaluated that were percentage wound contraction & epithelization period.

**Evaluation of Parameters**

The area that has contracted over a predetermined length of time is transformed into a % unit to calculate percentage wound contraction. The epithelization period is the amount of time until a scar forms and new skin grow in.

***Incision Wound Model*:**

With the use of a sharp blade, 6 cm paravertebral incisions were made on both sides of the vertebral column while under a light ether anaesthesia, cutting through the entire thickness of the skin. With the aid of a hetero spherical body needle, the wounds were sutured using Ethicon 4-zero silk thread. On the eighth post-injury day, sutures will be removed, and on the tenth post-injury day, a tensiometer will be used to determine the tensile strength [14].

**Estimation of Biochemical Marker: Hydroxyproline**

An approximately (500 mm2) circular wound was made, as shown in the incision model. For 19 days, the ointment was topically applied to the surgical wound. The scab was taken off after 20 days and dried in an oven at about 110 ° C. kept in a test tube with a glass stopper after being weighed (10 mg). To each tube containing 10 mg of dry scab, 1 mL of 6 NHCl was added. The tube was thereafter heated for hydrolysis for 24 hours (12 hours per day for two days). The hydrolysis was then cooled, and using phenolphthalein as an indicator, excess acid was neutralised with 10N NaOH. Using pure water, the neutral hydrolyzate was diluted to a concentration of 20 mg/ml. Using the final hydrolyzate to hydroxyproline. The hydroxyproline (HPR) level was found. Each tube received 1 ml of hydrolyzate, 2.5 N NaOH, 0.01 M $CuSO_4$, and 6 percent $H_2O_2$. The tube was given a thorough shaking before being immediately submerged in an 80 °C water bath. The tube was withdrawn after 15 minutes and chilled with cold water for 5 minutes. 41.2 mL of 3NH 2SO and 0.6 mL of freshly made para-dimethylaminobenzaldehyde in n-propanol solution were added. The test tube was once more heated to 75 ° C in a water bath for 15 minutes before being cooled under running water for 5 minutes. A spectrophotometer was used to evaluate colour intensity at 540 nm in comparison to a blank. An estimation of the tissue hydroxyproline content using a standard curve made with standard 4-hydroxyproline was 10-100 # g/ml [15].

## RESULT & DISCUSSION

Injuries to the skin that affect surrounding soft tissue led to the formation of wounds. The intricate and prolonged process of tissue remodelling and repair in response to injury is what constitutes wound healing. Throughout the years, numerous plant-derived products have been employed for wound treatment. Herbal

extracts that facilitate wound healing also contribute to blood clotting, infection management, and overall wound recovery. It is essential to identify and evaluate phytoconstituents derived from plants for their antibacterial properties in wound care. A thorough analysis from various perspectives has indicated that herbal gel is a natural product suitable for extended use. The development of a polyherbal wound healing gel represents a significant advancement in wound management, harnessing the diverse therapeutic properties of multiple herbal ingredients. Moringa oleifera is renowned for its valuable pharmacological attributes, including anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, antimicrobial, antioxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic effects. Tulsi (Ocimum sanctum Linn) is a prominent herb in Ayurveda, and contemporary research is now corroborating its health advantages. Tulsi has also been shown to alleviate psychological stress by enhancing memory and cognitive function, as well as metabolic stress by normalizing blood glucose, blood pressure, and lipid levels, alongside its anxiolytic and antidepressant properties. The extensive antimicrobial activity of Tulsi, which encompasses effects against various human and animal pathogens, indicates its potential use as a hand sanitizer, mouthwash, and water purifier, in addition to applications in animal husbandry, wound healing, food preservation, and the maintenance of herbal raw materials, as well as promoting traveler health. Furthermore, it can be utilized for animal husbandry, wound treatment, and the preservation of food and herbal materials. Neem (Azadirachta indica) has demonstrated anti-inflammatory, anti-arthritic, antipyretic, and hypoglycemic properties. For evaluation of wound healing potential of different Polyherbal gel formulations various parameters like wound contraction, tensile strength and Hydroxyproline as a biomarker were assayed. Fig. 1 and Table 3 represent the reduction of wound area of the different groups over the period of 4$^{th}$, 8$^{th}$, 12$^{th}$, 16$^{th}$ days (Fig. 2). It had been seen that fastest healing of wound took place in case of animals treated with F1 and F3 formulations. The period of epithelization of F1 & F3 was found to be more or less similar to standard (Fig. 3 & Table 4). The incision wound models revealed that mean tensile strength of resutered incision wound on 10$^{th}$ post wounding day showed F1 & F3 formulation found to be most significance as compared to be standard (Table 5, Fig. 4 & 5). The synthesized collagen was deposited at the wound site and cross-linked to form fibers. Collagen not only provides the strength and integrity of the tissue matrix, but also plays an important role in late homeostasis and epithelialization of the healing stage. Collagen is the major extracellular protein in the scab of healing wounds, and the synthesis of this protein in the wound area immediately after injury is rapidly increasing. When collagen is broken down, free hydroxyproline and its peptides are released. The measurement of hydroxyproline serves as an indicator of collagen turnover. The result revealed that hydroxyproline content of F1 treated animal showed 41.58±0.45 as compared to standard. Although F2 treated group showed hydroxyproline content 40.86±0.90. The result was tabulated in Table 6 & Fig 6.

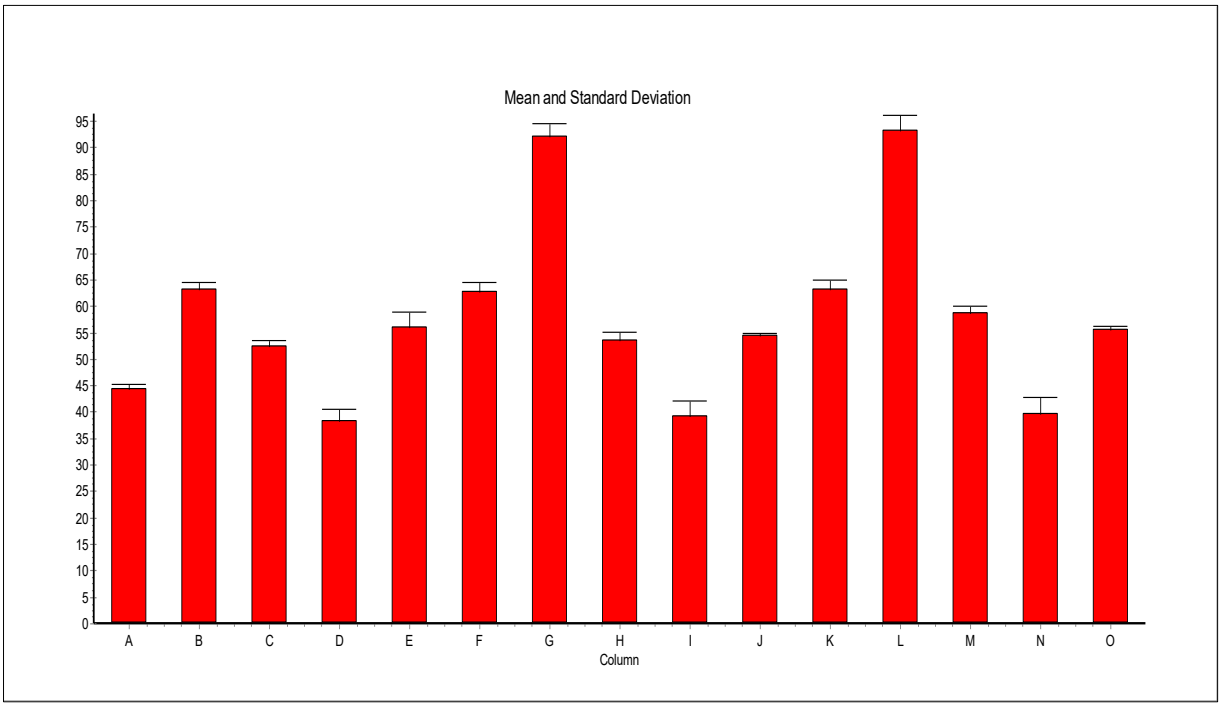



**Fig. 1: Graph showing mean & standard deviation of wound contraction**

**Table 3: Effect of topical application of drug on Excision wound**

| DAYS | CONTROL | STANDARD | TEST F-1 | TEST F-2 | TEST F-3 |
|---|---|---|---|---|---|
| 4th Day | 43.3875±0.9446 | 65.13±1.335<br>a*** | 53.33±1.244<br>a***<br>b*** | 40.225±2.264<br>a**<br>b*** | 54.947±3.003<br>a***<br>b** |
| 8th Day | 61.71±1.914 | 93.98±2.493<br>a*** | 56.44±1.643<br>a***<br>b*** | 39.27±2.961<br>a***<br>b*** | 55.447±0.415<br>a***<br>b*** |
| 12th Day | 62.237±1.837 | 96.31±1.408<br>a*** | 9.802±1.34<br>ns<br>b*** | 68.61±3.277<br>a***<br>b*** | 86.582±0.610<br>a***<br>b*** |

*P<0.05, **P<0.01, ***P<0.001, P>0.05 ns-Non-Significant, n=4

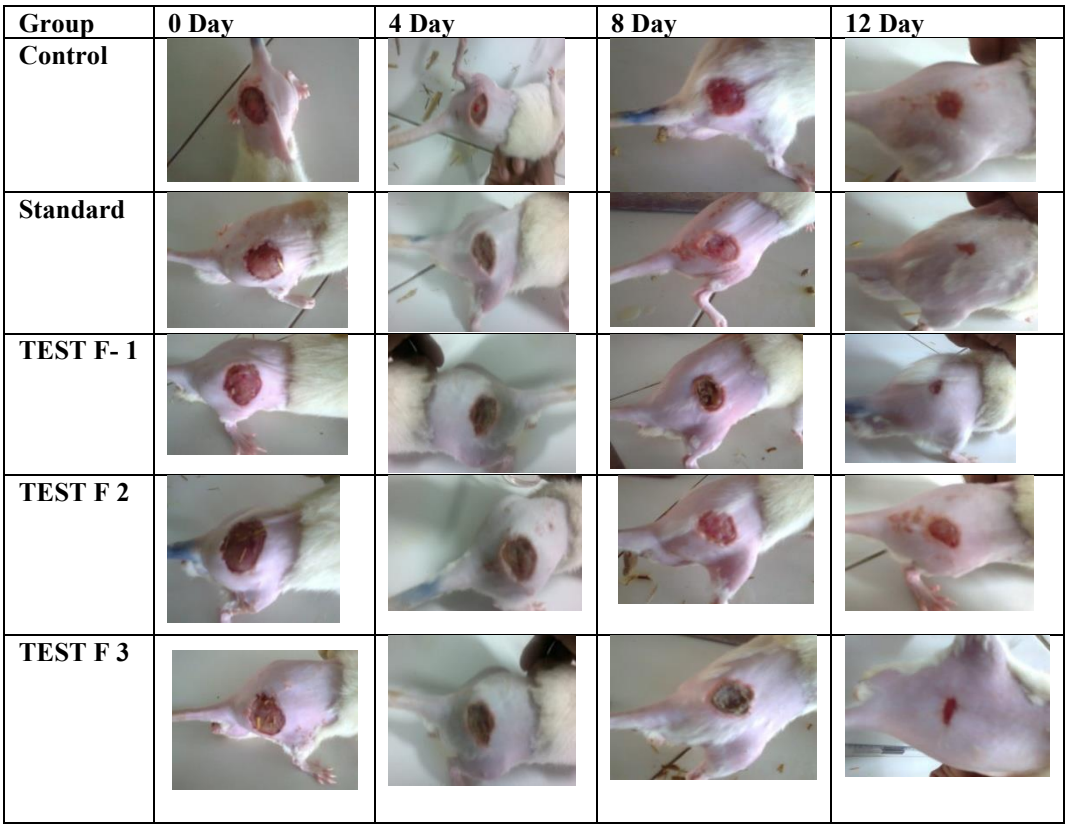



**Fig. 2: Effect of topical application of drug on Excision wound**

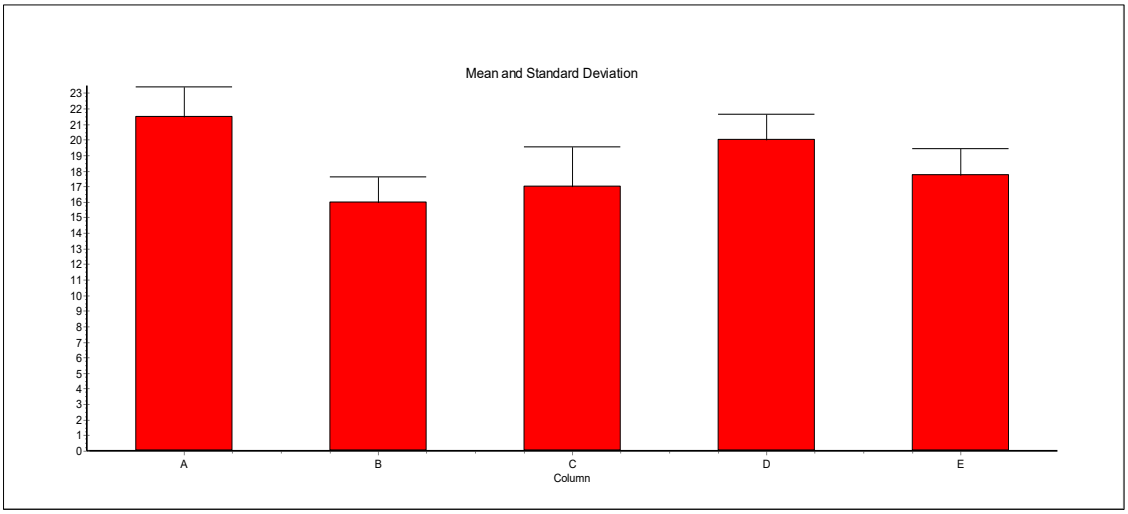



**Fig. 3: Graph showing mean & standard deviation of epithelization period**

**Table 4: Effect of topical application of drug on epithelization period**

| Group | Control | Standard | Test F-1 | Test F-2 | Test F-3 |
|---|---|---|---|---|---|
| **Epithelization Period** | 21.500±1.915 | 16±1.633 a** | 17±2.582 a* | 20±1.63 Ns | 17.75±1.708 Ns |

*P<0.05, **P<0.01, P>0.05 ns-Non-Significant, n=4

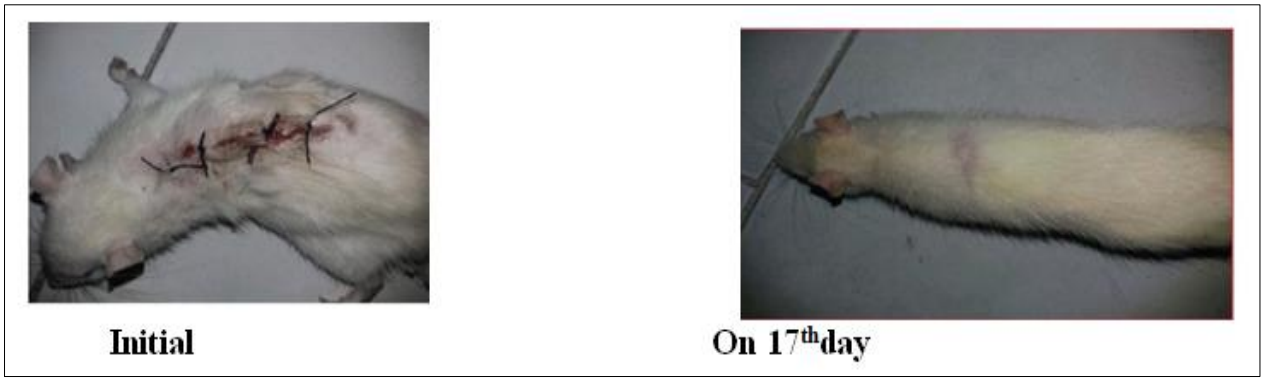



**Fig. 4: Incision wound evaluation**

**Table 5: Mean Tensile strength of resutured incision wound on 10th Post Wounding Day**

| S. No. | Groups | Breaking strength (gm) |
|---|---|---|
| **1.** | **Control** | 244.86 ± 1.47 |
| **2.** | **F1** | 411.42±0.79* |
| **3.** | **F2** | 392.2±0.36 |
| **4.** | **F3** | 409.96±01.29 |
| **5.** | **Standard** | 419.2±3.8 |

Value are expressed as the Mean ± SEM, n = 6 in each group P< 0.001 significance Vs control

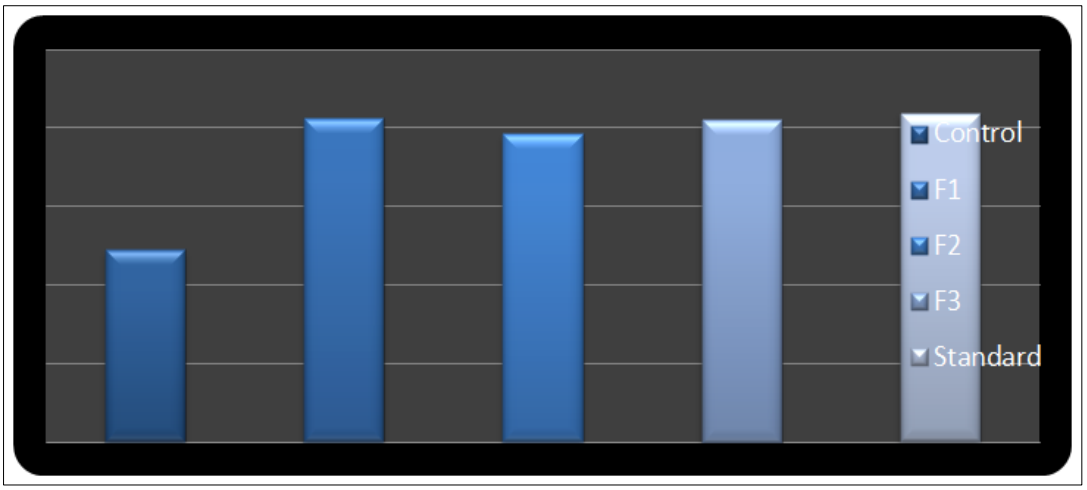



**Fig. 5: Mean Tensile strength of resutured Incision wound on 10th Post Wounding Day**

**Table 6: Effect of applying different Polyherbal formulation on content of hydroxyproline in the eschar of excision wound**

| Group (N= 6) | Hydroxyproline (µg/ 500 mg) |
|---|---|
| **Gr I(Control)** | 29.22 ±0.67 |
| **Gr II(Standard)** | 43.02 ±0.70 |
| **F1** | 41.58 ±0.45*** |
| **F2** | 38.45±0.15** |
| **F3** | 40.86±0.90** |

SO, simple gel base; n = 6 animals in each group. The treated groups are compared by Student t-test with the control group.** P < 0.01, *** P < 0.001

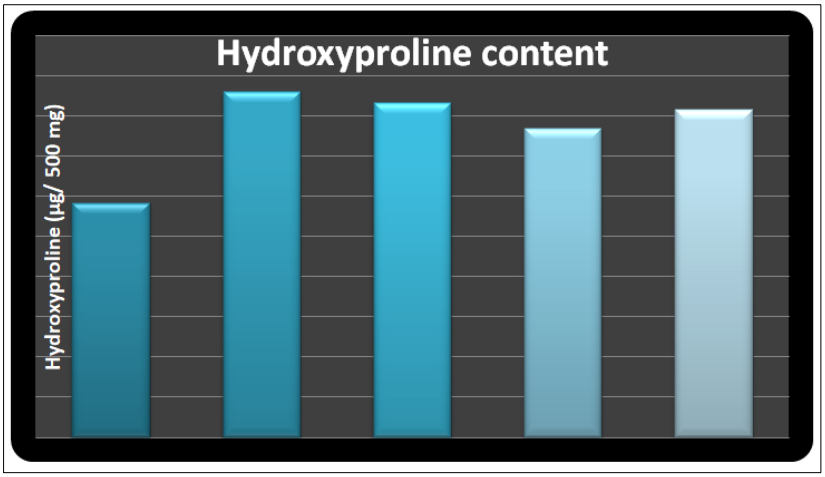



**Fig. 6: Hydroxyproline content**

## CONCLUSION

Carbopol 940 (Carbomer Homopolymer USP), Disodium Edetate IP, Sodium Methylparaben IP, Sodium Propylparaben IP, and Sodium Hydroxide IP were used to manufacture the Polyherbal Gel, which was then tested for wound healing activity. It has been observed that animals given the F1 and F3 formulations experienced the quickest wound healing. It was discovered that the epithelization duration of F1 and F3 was essentially typical. The incision wound models demonstrated that, when compared to the standard, the mean tensile strength of the recovered incision wound on the tenth post-injury day showed F1 & F3 formulation to be most significant. Collagen turnover is shown by the measurement of hydroxyproline. The outcome showed that the F1-treated animal had significantly higher hydroxyproline content than the control. Despite this, the F2 treated group displayed hydroxyproline that was quite similar to that of F1. Numerous studies have focused on the many advantages of polyherbal formulations, but to fully utilise them, a systematic method is needed. But it is clear from the major findings of the recent study that polyherbal formulations are very effective at accelerating wound healing. They have the capacity to start a variety of physiological processes that quicken wound healing. Due to the synergistic effects of flavonoid and plant phenolic present in the extracts, the results of the current study demonstrated that polyherbal formulations containing ethanolic leaf extract of *M. oleifera, O. sanctum, and A. indica* showed potent wound healing efficacy due to synergetic efficacy of phenolic and flavonoid present in selected herbs.

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