

Synergistic Wound Healing and Anti-Inflammatory Effects of a Polyherbal Methanolic Gel: Evidence from In Vivo Experimental Models

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Abstract:

Aim: To explore the in-vivo wound healing activity of a polyherbal methanolic gel prepared from the roots of *Plumbago auriculata*, leaves/flowers of *Calotropis gigantea*, and roots of *Arctium lappa*. **Objective:** The present study investigated the in-vivo wound healing and anti-inflammatory activities of a polyherbal methanolic gel (PHMG) formulated from selected medicinal plants traditionally used for wound management. **Material and Method:** A carbomer 940-based polyherbal methanolic gel (5%) was formulated using methanolic extracts of *Plumbago auriculata*, *Calotropis gigantea*, and *Arctium lappa*. Acute dermal toxicity studies were conducted according to OECD Guideline 402 to evaluate the safety profile of the formulation. Anti-inflammatory activity was assessed using the carrageenan-induced paw edema model in experimental animals. Wound healing efficacy was evaluated using excision and incision wound models by monitoring wound contraction, epithelization period, and wound breaking strength. **Result:** Acute dermal toxicity studies demonstrated that PHMG was safe up to 2000 mg/kg, with no mortality, morbidity, or signs of dermal irritation observed during the study period. In the excision wound model, PHMG-treated animals showed significantly enhanced wound contraction and a reduced epithelization period compared to the control group ($p < 0.0001$). In the incision wound model, a significant increase in wound breaking strength was observed, indicating improved tissue regeneration and collagen maturation. Furthermore, PHMG exhibited marked anti-inflammatory activity in the carrageenan-induced paw edema model, particularly during the late phase of inflammation, suggesting modulation of inflammatory mediators involved in tissue repair. **Discussion:** The findings indicate that the polyherbal methanolic gel possesses significant wound healing and anti-inflammatory properties with an excellent safety profile. The synergistic interaction of bioactive constituents present in the selected medicinal plants may contribute to enhanced tissue repair, accelerated wound closure, and reduction of inflammatory responses. These observations support the traditional use of the constituent plants in wound management and provide scientific evidence for their therapeutic potential. **Conclusion:** The polyherbal methanolic gel demonstrated significant wound healing and anti-inflammatory activities while maintaining a favorable safety profile. The formulation may serve as a promising natural topical therapeutic for effective wound management and warrants further investigation through advanced preclinical and clinical studies.

Keywords: Polyherbal formulation, Methanolic gel, wound healing activity, Anti-inflammatory activity, Excision wound model, Incision wound model, Dermal toxicity.

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Introduction:

Wound healing is a complex and highly coordinated biological process that restores the integrity and function of damaged tissues following injury. It involves a sequence of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling, which collectively contribute to tissue repair and regeneration. Although the human body possesses a remarkable capacity for self-repair, various factors such as infection, prolonged inflammation, poor vascularization, and chronic diseases can significantly delay the healing process. Consequently, the development of effective therapeutic strategies that promote rapid and complete

wound healing remains an important area of biomedical and pharmaceutical research^{1, 2}. In recent years, increasing attention has been directed toward the use of medicinal plants and herbal formulations for wound management. Traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine have long employed plant-based remedies for the treatment of cuts, burns, ulcers, and other skin injuries. Herbal preparations are often preferred because of their wide therapeutic potential, biocompatibility, affordability, and reduced risk of adverse effects compared with synthetic drugs^{3, 4}.

Among the various approaches investigated for wound care, polyherbal formulations have emerged as particularly promising therapeutic systems. The concept of polyherbalism is based on the synergistic interaction of multiple medicinal plants, which may produce superior therapeutic effects compared with individual plant extracts. By combining herbs with complementary pharmacological activities, polyherbal formulations can target different biological pathways involved in tissue repair, including inflammation control, stimulation of fibroblast proliferation, collagen synthesis, angiogenesis, and epithelialization.⁵

The increasing prevalence of chronic wounds, including diabetic ulcers, pressure ulcers, and venous leg ulcers, has created a significant healthcare burden worldwide. These wounds are characterized by prolonged inflammation, delayed tissue regeneration, and an increased risk of secondary complications. Conventional treatment strategies such as wound dressings, debridement, and topical medications are often insufficient to achieve complete healing. Therefore, there is a growing need for alternative therapeutic interventions that not only accelerate wound closure but also improve the quality of tissue regeneration.⁶

The present study focuses on the development and evaluation of a polyherbal methanolic gel formulated from selected medicinal plants traditionally recognized for their wound healing potential. The formulation contains methanolic extracts of *Plumbago auriculata* roots, *Calotropis gigantea* leaves and flowers, and *Arctium lappa* roots. These plants were selected based on their historical use in traditional medicine and their reported ability to support tissue repair and skin regeneration.

Plumbago auriculata (Cape leadwort) belongs to the family Plumbaginaceae and has been traditionally employed in folk medicine for the treatment of skin disorders, wounds, and inflammatory conditions. Species of the genus *Plumbago* are known for their medicinal importance and have been reported to possess wound healing and tissue regenerative properties^{7, 8}

Similarly, *Calotropis gigantea* (family Apocynaceae) has a long history of medicinal use in Asian traditional medicine. Various parts of the plant have been utilized in the treatment of wounds, ulcers, inflammation, and skin diseases. Experimental studies have demonstrated that extracts of *C. gigantea* promote wound contraction and epithelialization, thereby supporting tissue repair processes^{9, 10}

Arctium lappa L. (Burdock), a member of the family Asteraceae, is widely recognized for its beneficial effects on skin health and tissue regeneration. The roots of the plant contain bioactive compounds that

have been reported to facilitate wound contraction, enhance collagen deposition, and improve tissue remodeling. These properties make *A. lappa* a promising candidate for inclusion in wound healing formulations^{11, 12}

The combination of medicinal plants in a single topical formulation may provide complementary or synergistic therapeutic effects that support the wound-healing process, as reported for polyherbal preparations in preclinical studies.^{13, 14} The use of herbal medicines in wound management has also been supported by their diverse biological activities and potential to promote tissue repair.^{15, 16} A gel-based topical dosage form offers important advantages, including ease of application, prolonged contact with the wound surface, and the ability to provide a suitable moist environment that supports wound healing and tissue regeneration.^{13, 14} In view of the increasing scientific interest in plant-based therapeutics and the need for effective topical wound-management approaches, the present investigation was undertaken to evaluate the wound-healing potential of a polyherbal methanolic gel and to generate scientific evidence regarding its efficacy as a topical wound-healing preparation.

Material and method

Plant collection, authentication and extraction

The roots of *Plumbago auriculata*, leaves/flowers of *Calotropis gigantea*, and roots of *Arctium lappa*., were procured and were authenticated by the Botanical Survey of India (BSI), Ministry of Forest and Climate Change, Chatham Lines, Prayagraj (Allahabad), Uttar Pradesh India (Approval No. 2506230006479) Before the extraction process, steps such as pre-washing, drying of plant materials, or freeze-drying, followed by grinding to achieve a uniform sample, are carried out. The extraction was conducted separately for various selected plants. The plant materials underwent a shade-drying process for 2 weeks, were then pulverized into a coarse powder, and subsequently passed through a 20-mesh sieve to ensure uniformity. The coarsely dried powder underwent an initial treatment with petroleum ether (60–80 °C) for 24 hours to eliminate fatty materials, followed by re-extraction with methanol utilizing a Soxhlet apparatus at specified temperatures. Extracts were gathered, subjected to filtration using Whatman filter paper, and subsequently concentrated or dried utilizing a rotary vacuum evaporator^{17, 18} The extracts were preserved in a desiccator for subsequent applications, and the percentage yield was determined.

Phytochemical screening of the plant extract:

Abhilasha Shete et al. (2026) carried out a comprehensive phytochemical, antioxidant, and antimicrobial evaluation of selected plant extracts and a polyherbal formulation. The plant materials were

extracted using methanol or a methanol:water mixture (70:30) and subjected to qualitative phytochemical screening for major secondary metabolites, including alkaloids, glycosides, flavonoids, tannins, and volatile oils. TLC profiling was performed to characterize the phytoconstituents by comparing the observed *R_f* values with reference values.

The rutin content of the extracts was quantitatively estimated by UV–visible spectrophotometry using a rutin calibration curve. Extract samples were analyzed at 260 nm, and the rutin content was expressed as a percentage of the extract weight. The in-vitro antioxidant activity of individual extracts and the polyherbal combination was assessed using the DPPH free-radical scavenging assay. Different concentrations of the extracts were reacted with freshly prepared 0.1 mM DPPH solution, while ascorbic acid was used as the standard antioxidant. After incubation in the dark, absorbance was measured at 517 nm, and the percentage of free-radical scavenging activity was calculated. Measurements were performed in triplicate under controlled conditions to minimize light-induced degradation of DPPH. The in-vitro antimicrobial activity of the polyherbal extracts was evaluated against *Bacillus subtilis* (MTCC 1305) as a Gram-positive bacterium and *Pseudomonas aeruginosa* (MTCC 1688) as a Gram-negative bacterium using the agar well diffusion/cup-plate method. Bacterial cultures were maintained in nutrient broth and incubated at 37°C for 24 h. Agar plates were inoculated with the test organisms, and wells containing different concentrations of the extracts (50, 100, 150, and 200 mg/mL) were prepared using DMSO as the solvent. Ofloxacin (10 µg/mL) was used as the reference drug. Following 24 h incubation, antimicrobial activity was assessed by measuring the zones of inhibition.

In addition, the minimum inhibitory concentration (MIC) was determined by the broth dilution method. Serial concentrations of the extracts were prepared ranging from 50 to 0.19 mg/mL, while appropriate concentrations of ofloxacin were used as the reference. The bacterial strains were inoculated into nutrient broth containing the test samples and incubated at 37°C for 24–48 h. The MIC was defined as the lowest concentration at which visible bacterial growth or turbidity was absent compared with the appropriate controls.

The previously reported phytochemical profile, rutin content, antioxidant activity, and antimicrobial potential of the polyherbal extracts provide scientific evidence supporting their potential role in wound healing. The identified bioactive constituents, together with their free-radical scavenging and antimicrobial activities, may contribute to controlling oxidative stress and microbial infection, which are important

factors in tissue repair and wound regeneration. Collectively, these findings provide a rational basis for further evaluation of the polyherbal formulation as a potential wound-healing agent.¹⁹

Preparation of carbomer 940-based PHMG gel formulation

Abhilasha Shete et al. (2026) prepared 2% and 5% polyherbal methanolic gels using Carbopol-940 as the gelling agent, incorporating the polyherbal extract, propylene glycol, preservatives, sodium metabisulphite, and triethanolamine. The prepared gels were evaluated for their physicochemical properties, stability, and release characteristics and showed suitable properties for topical application. The reported phytochemical, antioxidant, and antimicrobial activities of the formulation provide a scientific basis for its potential in wound-healing applications. Thus, the same polyherbal gel further used in this study for evaluation of wound-healing efficacy.²⁰

In-vivo Pharmacological activity of prepared formulation

Animal ethical approval and housing conditions

In this investigation, Wistar albino rats ranging in weight from 180 to 200 g were utilized. Institutional Animal Ethics Committee (Approval number METFOP/IAEC/12/25) gave its stamp of approval to the research project in accordance with CPCSEA regulations in India. The preliminary research was carried out at the approved animal facility of the Moradabad Educational Trust (MET) Faculty of Pharmacy in Moradabad, Uttar Pradesh, with the following registration number: 1867/PO/ReBi/S/16/CPCSEA. The animals were prepared for the investigation by adjusting to the typical laboratory settings, which included a temperature range of 25 ± 20°C, a relative humidity of 44–56%, a light and dark cycle of 12:12 hours, and the provision of water and the prescribed meal.

Acute dermal toxicity

Following the OECD recommendations no. 402, the acute dermal toxicity test of the extracts was conducted. Both male and female adult Wistar rats were utilized. There are a total of six rats; three from each group (n=3). Using appropriate depilatory preparation (Veet), the test animals had approximately 10% of their body hair removed from the dorsal region around 24 hours before to the test. The first group served as a control, while the second group had a topical application of poly-herbal gel (5% concentration) at a dose of 2000 mg/kg of body weight. For fourteen days, we tracked the animals' hair, eyes, behaviour, and toxic dermal responses²¹

Grouping of animals

The rats were divided into 5 groups (n=6) and were subjected to pre-treatment scheduled

Group-I: Normal control (left untreated).

Group-II: Gel base considered as Negative control

Group-III: Standard (5% w/w povidone iodine ointment USP for wound healing model and diclogel in Anti-inflammatory model)

Group-IV: Polyherbal Methanolic Gel-PHMG (2%) treated

Group-V: Polyherbal Methanolic Gel-PHMG (5%) treated

All the treatments were given once daily (OD).

In-vivo Excision Wound Model

A modified version of the reported approach was used to construct the excision wound. Following a depilatory cream (Veet) shave on the dorsum, the animals were put to sleep with ketamine hydrochloride (100 mg/kg, i.p., body weight). The area of the incision to be made was indicated and an imprint was formed on the shaved dorsal region. Along the marking, a 500 mm² circular excision incision was made using sharp scissors, a surgical blade, and toothed forceps. The rats were placed in an open, sterile environment without clothing. From the day of the procedure until the last day of healing, the patient was instructed to apply the basic gel base, designed extract gel, and standard medicine once daily. Wound contraction and epithelialization duration were assessed in this excision model. On the tenth day following wound creation, the percentage of contraction was recorded. After the experiment was over, the rats were put to sleep and tissue samples were taken from the hot wounds in each group, being sure to leave a 5mm margin of skin around the wound borders. Tissue samples were put in a 10% formalin solution before being utilized for biochemical and histological analyses²²

In-vivo incision wound model

The previously reported approach was used to make incision wounds. The procedure for grouping and treating the animals was identical to that of the excision wound model. Ketamine hydrochloride was administered intraperitoneally to all rats at a dosage of 100 mg/kg. On either side of the rats' spinal column, a sharp scalpel was used to make a 4-centimeter-long paravertebral incision that went through the whole thickness of the shaved skin. Following full haemostasis, the wounds were repaired using interrupted sutures—black silk surgical thread (number 000) and a curved needle (number 11)—placed about 1.5 cm apart. The animals were treated daily for 9 days after the stitches were removed, and they were left nude to heal. In order to quantify wound breaking strength (WBS), all rats were put to sleep and their sutures were removed on the tenth day²³

Wound Healing Evaluation Parameters

Measurement of Wound Contraction

All groups in the excision wound model had their wound areas measured on days 0, 10, and 20 by tracing the wound using digital vernier callipers. The percentage of healing wound area was used to depict wound contraction, which was assessed every ten days until the wound healed completely. We used the following formula²⁴ to determine the percentage of wound contraction, starting with a 100% initial wound size:

$$\% \text{ wound contraction} = \frac{\text{Initial wound area} - \text{specific day wound area}}{\text{Initial day wound area}} \times 100$$

Epithelialization period

The number of days needed for the dead tissue remains of the wound to come out without any raw wound remaining was used to determine it²⁵

Hydroxyproline estimation

We used the conventional approach to estimate the hydroxyproline. For the purpose of hydroxyproline measurement, the surgically removed tissues from each rat were examined. A hot air oven was used to dry the tissues to a constant weight at 60° C. After that, they were hydrolyzed in 6 N HCl at 130° C for 4 hours. After that, they were neutralized to a pH of 7.0 and treated to Chloramine-T oxidation for 20 minutes. Colour was produced at 60° C using Ehrlich reagent after 5 minutes, and the reaction was ended by adding 0.4 M perchloric acid. The materials were subjected to UV spectrophotometer analysis at 557 nm following complete swirling. Using a standard curve of pure L-hydroxyproline, the hydroxyproline content in the tissue samples was determined²⁶

Histology

Standard procedures 105, 106 were used for the histopathological analysis. Before being fixed in paraffin wax, the obtained tissue samples underwent a series of processing in alcohol at concentrations ranging from 25% to 100% to ensure full drying. After being cut into 5µm slices using a microtome, the paraffin-fixed skin tissue was dried in an oven overnight so that it could be stained with Hematoxylin-Eosin and Masson's trichrome.

Measurement of wound breaking strength (WBS)

Under local anaesthetic, the sutures were removed on the tenth day. The incision is precisely in the centre of the previously cut area since just a little portion of the wound was excised. Two on the fixed side and one on the free end of three tiny curved needles (No: 14) were inserted into the skin. On one side, a rod held two needles, while on the other, a weighing pan dangled freely. The incision was left to heal while gradually increasing weights were applied to the pan. As an indirect measure of wound tensile strength, the weight at which the wound merely breaks is taken into consideration.²⁷ A comparison was made between the

tensile strength of wounds treated with usual procedures, polyherbal gels (2% and 5%), and normal and negative control groups.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

The carrageenan-induced edema model was used to assess the anti-inflammatory effects of the medication under investigation. The plantar surface of the right hind paw of the negative control, standard, and test drug treatment groups was coated with gel base, standard diclogel, and polyherbal 2.5% and 5% gel, respectively. The coats were delicately rubbed 50 times with the index finger. A sub-plantar injection of 0.1 mL of a 1% carrageenan solution in normal saline was administered to the treated paw three hours subsequent to dosing. Nonetheless, 0.1 cc of saline was administered to the left hind paw. After inducing inflammation, paw edema was assessed every 60 minutes for up to four hours. The measurement of the difference in footpad thickness between the right and left foot was taken using digital Vernier callipers (Digi calliper). Statistical methods were used to compare and assess the mean values of the treatment groups with those of the control group.²⁸

Measurement of paw thickness

At one, two, three, and four hours following inflammatory induction, digital vernier callipers were used to quantify the paw volume up to the tibiotarsal joint. To measure the inflammation, we used the following calculation to determine the percentage increase in left paw thickness compared to right paw thickness in each rat (Figure.1):

$$\% \text{ increased in paw thickness} = \frac{\text{Thickness of right paw} - \text{Thickness of left paw}}{\text{Thickness of right paw}} \times 100$$

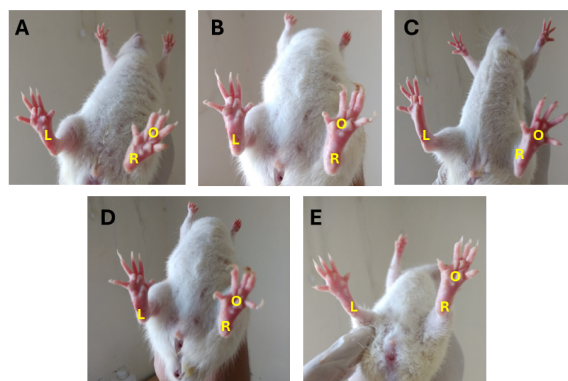


Figure 1 Comparative illustration between left (L) paw and Right (R) paw thickness. (A) Normal Control, (B) Negative Control, (C) Standard, (D) PHMG 2%, (E) PHMG 5%. The right paw of all groups except Normal control was injected with carrageenan that experience paw edema (O), while the left paw remains untreated. However, the right paw of normal control animals was injected with normal saline.

Result:

Optimization of polyherbal proportion for *in-vivo* activity

The methanolic extracts PAME, CGME, ALME and PHME showed presence of both polyphenols and flavonoids both by qualitative and quantitative estimation. The antioxidant effect of sample PHME was found to be effective synergistically as compared to individual plant extracts. The results of *in-vitro* antimicrobial assay were found to show antimicrobial efficacy (average zone of inhibition of each test sample) in order viz. PHME>PAME>CGME>ALME. The MIC values of selected plant extracts and polyherbal were found to be inverse of their zone of inhibition (ALME>CGME>PAME>PHME). The proportion of the individual extract used for the preparation of polyherbal gel (PHMG) was based on their antioxidant and antimicrobial potency.

Acute dermal toxicity of PHMG

In accordance with OECD 402 recommendations, the primary test was substituted with the limit test at 2000 mg/kg to ascertain acute dermal toxicity. When the main data points to the possibility that the test substance is not toxic—that is, having toxicity below regulatory limit doses—then the limit tests are employed for the *in vivo* investigations. Information on the toxicity of the test material was sourced from literatures for comparable tested compounds or mixes or products as the present study focused on natural/non-synthetic chemicals. Based on the existing literature, we can confidently say that the medicine under study is harmful. Animals treated with the 5% polyherbal methanolic gel PHMG showed no side effects and were able to maintain their normal behaviour, eyes, and fur. The maximum safe dose was 2gm/kg. No abnormalities, such as skin irritation or unpleasant responses, were seen in the treated animals, and they remained healthy and active throughout the experiment. This provided the groundwork for the formulation to be deemed dermatologically safe.

Treatment with PHMG resulted in accelerated epithelial recovery along with enhanced wound contraction

On the 10th and 20th day, the wound area of each group was measured in the excision wound model. Animals treated with PHMG exhibited enhanced wound contraction, in contrast to the normal control and negative control group rats, which exhibited reduced wound contraction ($P < 0.0001$). Figure 9 and 10 revealed that the rate of wound contraction was substantially greater in the PHMG 5% treatment group compared to the normal and negative control groups. The animals in the normal control and negative control groups showed slower epithelization recovery compared to the rats treated with PHMG (2% and 5%), as seen in Figure 11 ($P < 0.0001$). Figure 12 showed

that PHMGs epithelize more quickly than normal and negative control groups, which may be because the newly produced epithelial cells are more viable. Based on the results compared to the normal and negative control groups, it was determined that PHMGs had a substantial role in wound contraction and epithelization, leading to successful healing.

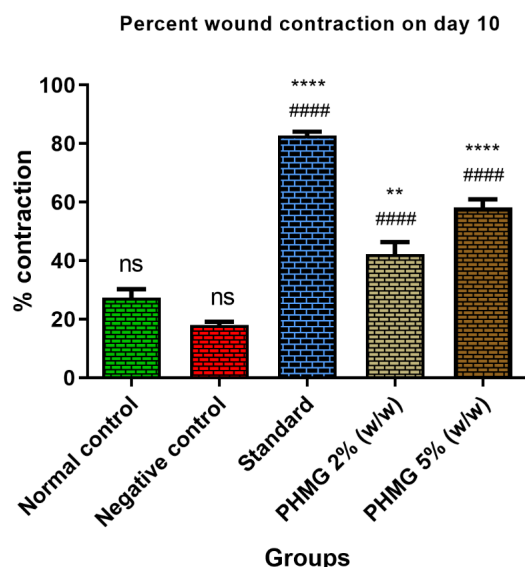


Figure 9 The effect of PHGs on percent wound contraction on day 10. All values are represented as mean $\pm$ SEM, n= 6 animals in each group. All values are represented as mean $\pm$ SEM, n=6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

*Denotes significant difference as compared to Normal group (** $P < 0.01$, **** $P < 0.0001$).

#Denotes significant difference as compared to Negative control group (##### $P < 0.0001$).

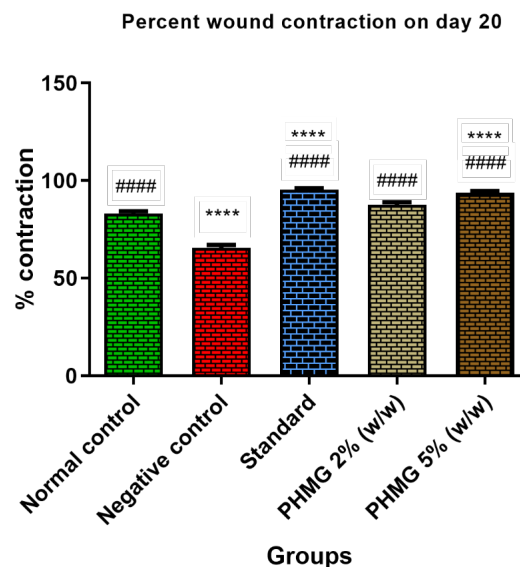


Figure 10 The effect of PHGs on percent wound contraction on day 20. All values are represented as mean $\pm$ SEM, n= 6 animals in each group. All values are represented as mean $\pm$ SEM, n=6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

*Denotes significant difference as compared to Normal group (**** $P < 0.0001$).

#Denotes significant difference as compared to Negative control group (##### $P < 0.0001$).

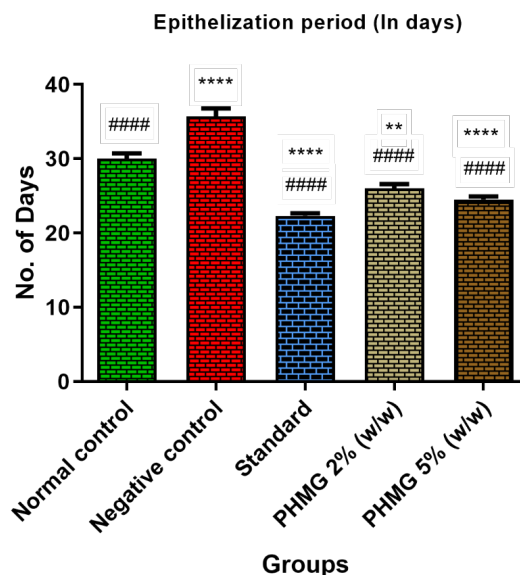


Figure 11 The effect of PHMGs on epithelization period as compared to normal control and negative control. All values are represented as mean $\pm$ SEM, n= 6 animals in each group. All values are represented as mean $\pm$ SEM, n=6 animals in each group. Data were

analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

*Denotes significant difference as compared to Normal group (** $P < 0.01$, **** $P < 0.0001$).

#Denotes significant difference as compared to Negative control group (## $P < 0.0001$).

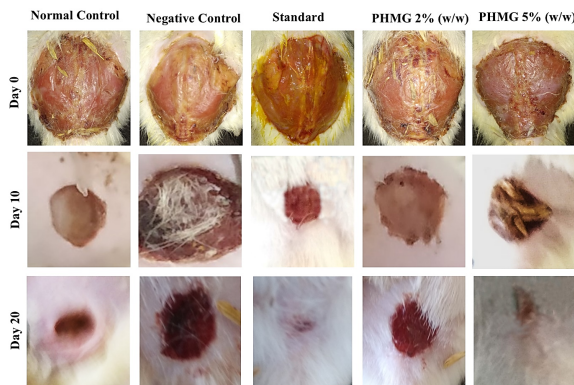


Figure 12 Assessment of Wound healing in terms of wound contraction in Wistar albino rats. The macroscopic appearance of wound closure of Normal control, Negative control, Standard, PHMG 2% (w/w) and PHMG 5% (w/w) at 0, 10th and 20th day.

PHMGs maintain collagen viability with improved hydroxyproline level

An essential process in the process of tissue healing is collagen turnover. It is possible to assess the collagen turnover's viability using a number of biochemical indicators. One possible biomarker to evaluate the collagen viability of test medications for improved healing quality is tissue hydroxyproline level. The current investigation evaluated the extent of skin tissue healing with an estimation of hydroxyproline derived from granulated tissue in an excision wound model across all animals (Figure 13). A higher concentration of hydroxyproline was discovered in the tissues of the groups treated with PHMG (2% and 5%), in contrast to the negative control group (# $P < 0.05$ and #### $P < 0.0001$) which shows that the increased collagen depots in the injured region were caused by the elevated hydroxyproline level in the PHMG groups.

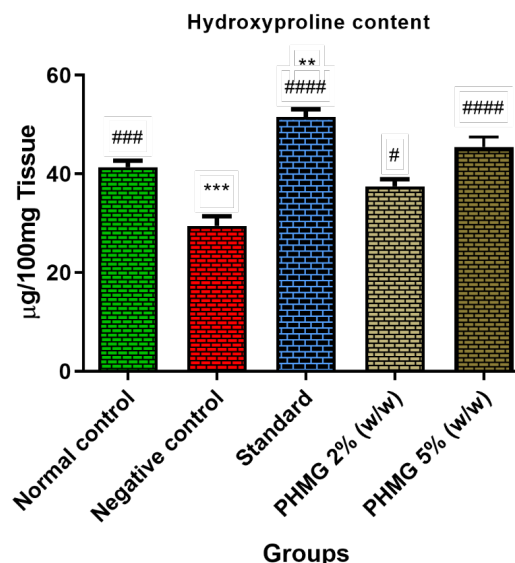


Figure 13 Hydroxyproline content of treated tissue samples.

All values are represented as mean $\pm$ SEM, $n=6$ animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

*Denotes significant difference as compared to Normal group (** $P < 0.01$, **** $P < 0.0001$).

#Denotes significant difference as compared to Negative control group (# $P < 0.05$, ## $P < 0.001$, #### $P < 0.0001$).

Treatment with PHMGs achieved optimum wound healing potential with thick collagenous and increased epithelization

Masson trichrome (MT) staining have been applied to wound tissues from every animal group (Figure 14A to 14E). Compared to normal control (Figure 14A) and negative control (Figure 14B) animal tissue, maximum epithelization and a condensed collagen bed were seen in several treatment groups of standard-treated animal tissue (Figure 14C), however, the PHMGs treated animal tissue revealed thick collagenous and increased epithelization (Figure 14D and 14E). Areas of hemorrhage with dispersed erythrocytes were less prevalent in the dermal area of skin tissues of normal control and negative control animals compared to the skin tissues of standard and PHMG group animals. Figures 15A and 15B show the skin tissue of the negative control group and normal control animals stained with H&E. These animals' skins had more disturbed granulation and blood capillaries compared to the skin tissue of the treated animals (Figures 15C to 15E). Comparing the normal control and negative control skin tissues to those treated with standard (Figure 15C) and PHMG 2% and

5% revealed ordered skin tissue micrographs with concrete collagen production (Figure 15D and 15E).

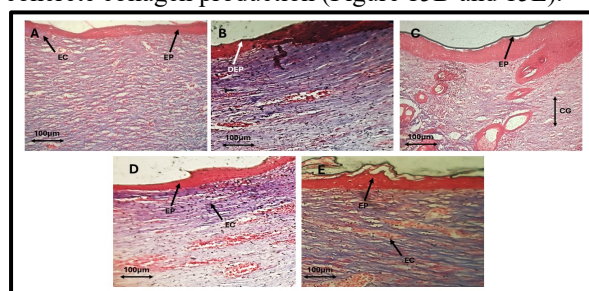


Figure 14 Masson trichrome (MT) histomicrographs of the wound tissues after 20 days of the treatment, representing epithelization area, collagen remodelling and erythrocytes. (a) Normal control, (b) Negative control (Gel base treated), (c) Standard, Povidone iodine 5% (w/w), (d) PHG 2% (w/w), (e) PHG (5% w/w).

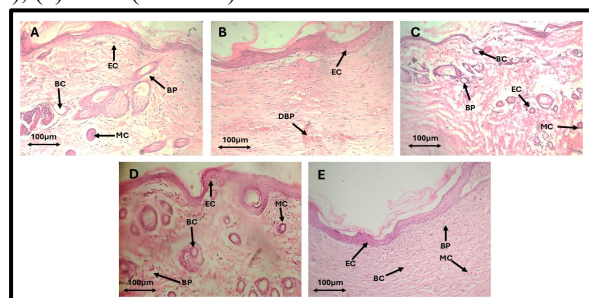


Figure 15 Haematoxylin & Eosin (H&E) stained wound tissues after 20 days of treatment for (a) Normal control, (b) Gel base treated, (c) Standard, Povidone iodine 5% (w/w), (d) PHG 2% (w/w), (e) PHG 5% (w/w). Reformed Blood capillaries-BC, Monocytes-MC, Blood platelets-BP and Erythrocytes-EC are indicated by arrows.

PHMG treatment improved wound breaking strength (WBS)

In order to further assess the PHMGs' therapeutic effectiveness, we used an incision wound model that measured wound breaking strength (WBS) to determine the degree of healing. A higher tensile strength was used to gauge the wound stability. The weight suspension method was used to quantify it on the tenth day. Figure 16 shows that the mice treated with PHG (2% and 5% concentrations) and standard drugs had significantly higher WBS compared to the animals in the normal and negative control groups ($P < 0.0001$). The enhanced hydroxyproline level in the PHGs treated animals was primarily responsible for the greater wound stability, as measured by tensile strength, compared to the negative and normal control group animals. This, in turn, was mostly influenced by the degree of collagen turnover and stabilized fibres. Collagen turnover, as measured by tissue hydroxyproline level, was responsible for the incision wound stability seen in animals treated with PHMGs.

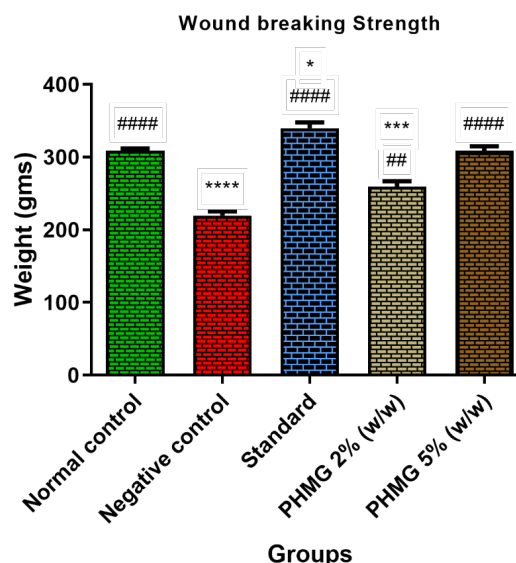


Figure 16 The wound breaking strength (WBS) in grams of different treatment groups in which PHG% showed maximum tensile strength after standard treated group.

All values are represented as mean $\pm$ SEM, $n=6$ animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

*Denotes significant difference as compared to Normal group (** $P < 0.01$, *** $P < 0.001$).

#Denotes significant difference as compared to Negative control group (# $P < 0.05$, ### $P < 0.001$, #### $P < 0.0001$).

Treatment with PHMGs possess significant anti-inflammatory activity

There are typically three stages to the inflammation that carrageenan induces, which can continue up to five hours. At 60, 120, 180, and 240 minutes following inflammation induction, observations were taken from all the groups at 60, 120, 180 and 240mins and paw thickness from tibiotarsal joint was measured by using digital vernier caliper (Figure 17). Figure 18A–D shows the percentage increase in paw thickness for the Standard diclogel and PHMG (2% and 5%) treated groups. Figure 18B reveal that the animals treated with the conventional drugs had no increase in paw thickness and reached their maximal anti-inflammatory effect 120 minutes after receiving the carrageenan injection. After 180 minutes of the carrageenan administration, PHMG (2% and 5% concentrations) demonstrated a notable and strong anti-inflammatory impact in comparison to the normal and negative control animals ($P < 0.0001$, Figure 18C). As shown in Figure 18D, the irritation returned after more than 240 minutes and subsided after 5 hours. Results showed that compared to animals treated with

the conventional medication, those in the PHGs group had a worse reaction in the initial phase (1 h) after post treatment. Inadequate histamine and serotonin release can be the cause. The second phase (1-2 hours) of the anti-inflammatory impact was uniform across all treatment groups, with the standard medication group showing the greatest benefit, which may have been due to the release of a mediator in this phase. On the other hand, during phase three (2-3 hours), which corresponds to the substantial release for phase 3 mediators, the groups treated with PHGs at all dosage levels were determined to be efficacious.

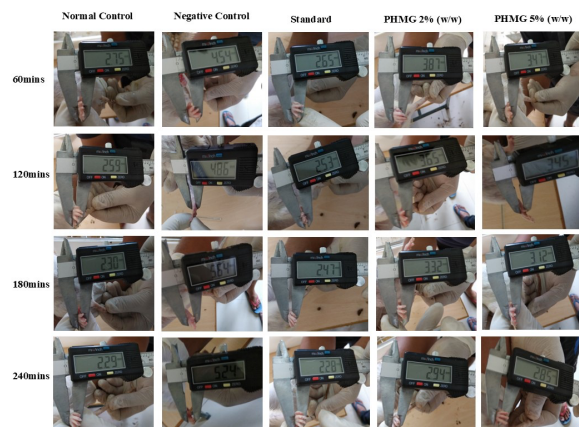


Figure 17 Observations were taken from all the groups at 60, 120, 180 and 240mins and paw thickness from tibiotarsal joint was measured by using digital vernier caliper.

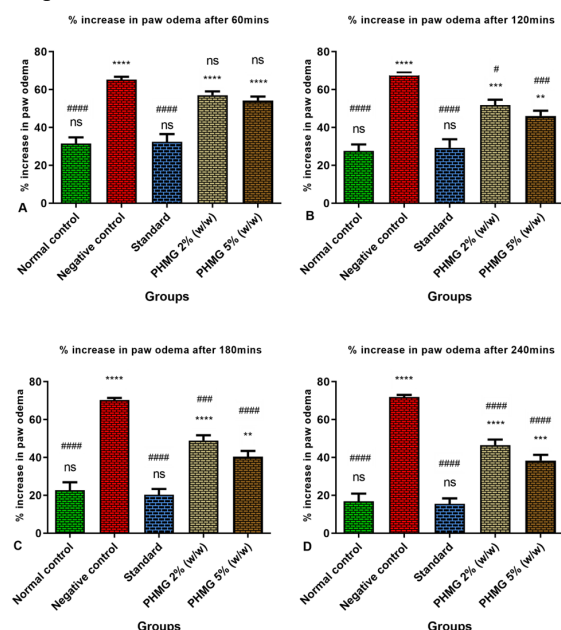


Figure 18 Effects of the polyherbal gels against carageenan-induced paw edema in rat as measurement of swelling thickness at (A) 60, (B)120, (C) 180 and (D) 240mins after induction of inflammation. All values are represented as mean $\pm$ SEM, $n=6$ animals

in each group. Data were analyzed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.

Denotes significant difference as compared to Normal group ($P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

#Denotes significant difference as compared to Negative control group (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$).

Discussion:

The present study was undertaken to evaluate the wound healing and anti-inflammatory potential of the polyherbal methanolic gel (PHMG) formulated from selected medicinal plants traditionally used for the treatment of skin disorders and wounds. The findings demonstrated that PHMG possesses significant therapeutic efficacy in promoting tissue repair and reducing inflammation, thereby supporting its potential as a topical herbal formulation.

Acute dermal toxicity studies conducted according to OECD guideline 402 confirmed the safety profile of PHMG. No mortality, behavioral abnormalities, erythema, edema, or signs of dermal irritation were observed throughout the study period, indicating that the formulation is safe for topical application. The absence of toxic manifestations suggests good dermal compatibility and supports the further development of PHMG as a wound management product, consistent with the safety requirements of topical wound healing formulations reported by Sharma et al. (2021).²⁸

The wound healing efficacy of PHMG was assessed using the excision wound model. The treated groups exhibited significantly enhanced wound contraction and reduced epithelization period compared to the control group. Among the tested formulations, the higher concentration of PHMG demonstrated superior healing activity, indicating a dose-dependent therapeutic response. Accelerated wound contraction reflects improved tissue regeneration and restoration of the damaged skin architecture. Similar observations have been reported for herbal formulations rich in bioactive phytoconstituents capable of stimulating fibroblast proliferation, angiogenesis, and extracellular matrix formation, thereby promoting rapid wound closure.²⁹

Hydroxyproline estimation revealed significantly increased levels in PHMG-treated animals compared to controls. Hydroxyproline is a major constituent of collagen and serves as a reliable biochemical marker for collagen synthesis and turnover. Increased hydroxyproline content indicates enhanced collagen deposition at the wound site, which contributes to improved tensile strength and structural integrity of the regenerated tissue. Collagen plays a crucial role in wound healing by providing a scaffold for cellular migration and tissue remodeling. Therefore, the

elevated hydroxyproline levels observed in the present study suggest that PHMG effectively promotes the proliferative phase of wound repair. Similar findings have been reported by Chattopadhyay and Raines (2014)³⁰ who emphasized the importance of collagen synthesis and maturation during tissue regeneration. Histopathological examination further supported the wound healing findings. Tissue sections from PHMG-treated animals showed well-organized granulation tissue, dense collagen fiber deposition, re-epithelialization, and increased neovascularization. The regenerated tissue exhibited a more organized structure with reduced inflammatory cell infiltration compared to the control group. These observations indicate accelerated progression from the inflammatory phase to the proliferative and remodeling phases of wound healing. Enhanced fibroblast proliferation and collagen maturation are important indicators of effective tissue repair and were clearly evident in the treated groups. Similar histological characteristics have been described in successful wound healing models by Gaspar-Pintilieșcu et al. (2019)³¹

The incision wound model demonstrated a significant increase in wound breaking strength (WBS) in PHMG-treated animals. Wound breaking strength is an important parameter reflecting the quality and maturity of newly formed collagen fibers. Increased WBS suggests improved collagen cross-linking and stabilization of connective tissue, leading to stronger and more resilient healed skin. The superior tensile strength observed in treated animals confirms that PHMG not only accelerates wound closure but also improves the quality of tissue regeneration. Enhanced collagen maturation and cross-linking have previously been correlated with increased wound tensile strength and tissue integrity.^{29, 30}

The anti-inflammatory activity of PHMG was evaluated using the carrageenan-induced paw edema model. The formulation produced significant inhibition of edema formation, particularly during the later stages of inflammation. The anti-inflammatory effect observed during this phase suggests modulation of inflammatory mediators responsible for sustaining edema and tissue injury. Reduction in inflammatory response is highly beneficial during wound healing, as prolonged inflammation can delay tissue repair and contribute to excessive tissue damage. By controlling inflammation, PHMG creates a favorable environment for cellular proliferation and tissue regeneration. Similar mechanisms have been reported for medicinal plant-derived wound healing formulations by Hosseinkhani et al. (2017)³²

The wound healing and anti-inflammatory effects observed in the present study appear to be closely interconnected. Inflammation is an essential

component of wound healing; however, excessive or prolonged inflammatory responses can impair tissue repair. The ability of PHMG to reduce inflammation while simultaneously promoting collagen synthesis and tissue remodeling may account for its superior therapeutic performance. The observed improvements in wound contraction, epithelialization, hydroxyproline content, histological architecture, and wound breaking strength collectively indicate effective enhancement of the healing process. Contemporary evidence suggests that successful wound management requires a coordinated balance between inflammation control, collagen deposition, angiogenesis, and tissue remodeling.²⁹

Overall, the findings of the present investigation demonstrate that PHMG is a safe and effective topical formulation with significant wound healing and anti-inflammatory properties. The formulation accelerated tissue repair, enhanced collagen deposition, improved tensile strength, and promoted histological recovery of damaged skin. These results support the traditional use of the constituent medicinal plants and highlight the potential of PHMG as a promising herbal therapeutic agent for wound management. Further studies involving detailed molecular investigations and clinical evaluation are warranted to establish its mechanism of action and therapeutic applicability in human wound care.

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