GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Assessment of wound healing activity of polyherbal gel, by "Excision wound Model" in Wistar rats

Yograj Mahajan [1,*] and Vishal Gupta [2]

[1] *Associated Professor, Faculty of Pharmacy, Mansarovar Global University, Sehore M.P. 466111.*
[2] *Dean, Faculty of Pharmacy, Mansarovar Global University, Sehore, M.P. 466111.*

GSC Biological and Pharmaceutical Sciences, 2025, 32(01), 025-035

Publication history: Received on 26 April 2025; revised on 11 June 2025; accepted on 13 June 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.32.1.0228

Abstract

Objective: This study was done for comparative evaluation of polyherbal gel formulations for topical application of healing agents at the time of wound to rapidly skin repair shortly duration possible, with minor pain, malaise and scarring to the patient over the wound healing stage.

Methods: Topically utilization of standard marketed ointment (Betadine) and polyherbal gel formulation prepared from the hydroalcoholic extracts (Ethanol/Methanol and water 1:1 ratio) from *Azadirachta indica* leaves, Calendula officinalis flower, Turmeric rhizome and Aloe-vera pulp, were prepared and assess for its effectiveness and safety. General formulation approaches towards dermal delivery feature for a wound healing medication. In the present study excision wound model was applied on wistar rats.

Result: All the selected polyherbal gel formulations exhibited good stability during the storage. The skin irritation result indicates the topical application of formulated gel preparations was safe, there was no sign of any dermal reaction such as edema and erythema. Differences in wound healing were detected between various formulated polyherbal gel preparations ($P<0.0001$). compared to the standard marketed preparation. Which had promoting effect on the wound healing mechanism.

Conclusion: The result and data obtained from the study were encouraging and hydroalcoholic extracts (ethanol and water) formulated polyherbal gel preparation (F-6 and F-9) produced better wound healing than methanol and water extracts gel preparation (F-2) and negative controlled treated animals' groups. The result demonstrates that the formulated polyherbal gel preparations has accelerated the wound healing process and efficient in skin injuries.

Keywords: Excision Wound; Hydroalcoholic; Skin Irritation; *Azadirachta indica*; Calendula; Wistar Rat

1. Introduction

A wound is an injury to the living tissue due to accident, surgery, violence and some chronic disorder; that generally defined by fracturing of the skin membrane and usually injury to underlying tissue or organs. Wound may be developing due to various mechanical, physical, chemical and biological factors such as animal bite, accidental trauma, burn etc. [1]. The basic principle of the wound healing is reducing tissue trauma by providing proper perfusion and oxygen to the damage tissue and providing sufficient nutrient supplementary. Restore the anatomical progression and function of the injured part. Wound can be classified according to depth, etiology of injury, location and appearance. [2]. Most of the medicinal plant commonly contain flavonoids, phenolic compound, lignans, and coumarins that shows variety of biological activity against pathogens, [3, 4]. Despite enormous work has been performed on wound healing activity, still

* Corresponding author: Yograj Mahajan.

the healing of burn, diabetic wound, bed sore etc. has remained a challenge to modern medicine. In present study, we intend to prepare polyherbal gel that may show wound healing activity, anti-inflammatory, antibacterial and analgesic activity in together combination.

1.1. The wound healing processes

Wound healing is a normal biological phenomenon in living organism. Wound healing may be defined as a process in which living organisms replace damaged or destroyed tissue by newly generated tissue, in which body restore their normal structure and function. [5]. Acute wound generally heals in an orderly and systematic manner and developed smoothly through the four definite, but overlapping phase of wound healing: hemostasis, inflammation, proliferation and remodeling. (Figure 1). In distinction, chronic wound will similarly start the wound healing process, but will have prolonged inflammatory, proliferative and remodeling phase, due to that reason in tissue fibrosis and in non-healing ulcers. [6].

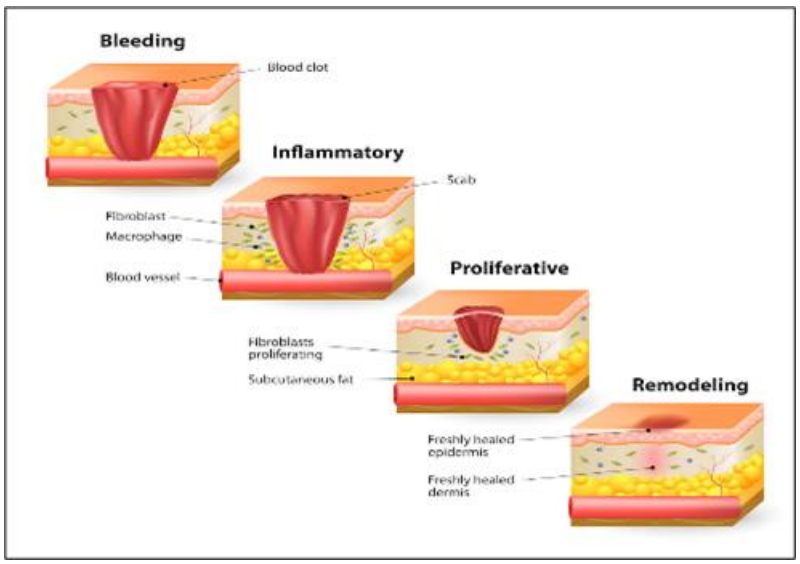

Figure 1 Steps of wound healing

1.1.1. Hemostasis

Hemostasis is the primary response to injury that stops blood loss. To prevent exsanguination, vasoconstriction by the activation of platelets density, adhesion and aggregation at the site of injury, to the formation of fibrin clot. Once in contact with collagen, platelet secret the soluble mediator's sticky glycoprotein. Platelet start aggregation, clotting factors are liberated resulting in the accumulated of a fibrin clot at site of injury. Which not temporary cover the wound but also prevent the wound against pathogens. [7].

1.1.2. Inflammation

the next steps of wound healing begin within the first 24 hours after the injury. After a wounding take place, the inflammatory phase start wound repairing which may up to a few days, during this period which show the following symptoms like edema, erythema, heating sensation and pain etc. Granules are release from the mast cells, filled with enzymes, histamine and active amines. That's responsible for the characteristic four signs of inflammation they are following; the rubor (redness), calor (heat), tumor (swelling), and dolor (pain) at the site of injury. [8].

1.1.3. Proliferative Phase

proliferative phase begins and overlaps inflammatory phase and identified by proliferation of epithelial cells and resettlement over the provisional matrix within the wound, this phase also known as re-epithelialization. The landmarks through the proliferative phase involve replacement of the provisional fibrin matrix within new matrix of collagen fibers, fibronectin and proteoglycans to replace the structure and function of the tissue. Angiogenesis process also happened during this phase, growth of new capillaries to replace earlier damage vessels and restore circulation. In proliferative phase platelets liberate various growth factors such as platelet-derived growth factor and transforming growth factor etc. [9].

1.1.4. Remodeling phase

Remodeling is the finishing phase of the wound healing activity; in this phase the granulation tissue gets mature into scar and the tensile strength of tissue increased. During the maturation of granules tissue decreases the quantity of capillaries by accumulation into huge vessels and also reduced the quantity of glycosaminoglycans and the water connect with the proteoglycans and glycosaminoglycans. In this phase regeneration of many newly generated capillaries founded. [10, 11].

1.2. Gel

Topical gels are homogeneous, semi-solid formulation apply for healing and anticipation of skin condition. Due to the aquaphilic kind of gels, the medicament and their active components was released rapidly. Gels are prepared in two stages: a cross linked and 3D material that hold a great amount of fluid. [12].

The word "gel" is procured from the Latin word "gelu" for "frost" and gelare means freeze or congeal. That's means a fluid position to a solid-like substance that does not move but is having elastic nature and keep hold some liquid feature.

2. Materials and Methods

2.1. Materials

2.1.1. Selection procurement and authentication of plant material's

Leaves of Neem and Aloe-vera, and flower of calendula herb was collected from the Herbal Garden of Mansarovar Global University campus sehore M.P. The turmeric was purchase from the local market of Bhopal. The authentication of plants done from the department of botanical science, Government Jaywanti Haksar post graduate college Betul Madhya Pradesh India.

2.1.2. Chemicals

all the chemicals and reagents utilize in the study were analytical grade.

2.1.3. Method

the extraction of selected plant materials was done by using continuous hot Soxhlet extraction method and cold maceration process. Hydroalcoholic solvent (Ethanol/Methanol and water) are used as extraction solvent. The extracted extract was further subjected to phytochemical investigation for the detection and identification of various secondary metabolites present. Gel was prepared by using dispersion method.

2.1.4. Animals

the wistar rats, weight between 150 to 180 gm of both genders were procured from animal house of Truba Institute of pharmacy Bhopal M.P.

2.2. Methods

2.2.1. In-vivo Animal Activity

Animal

The experimental protocol for the in-vivo wound healing activity was approved by Institutional Animal Ethical Committee (IAEC) TIP/IAEC/02/13.Under the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA). In present study wistar rats' weight between 150 gm.to 200 gm. of both sexes was used for the experiment. The experimental animals were kept in animal house in good environmental condition, provided normal food and water during the experimental study. Maintain 12 hrs. light dark cycle. The animal was acclimatized for 5 days before the experiment was started. [13].

Skin Irritation Test

Skin irritation test is done for the determine risk of allergic reaction on skin due contact between skin and substance present in the preparation. Following factor is responsible for that adverse reaction such as concentration of drug, duration and frequency of dosing etc. This test was performed on Wistar rats. Take wistar rat of any sex weight between

150 to 185 gm. The hairs of rat's skin were clean 3 days before the experiment. The poly-herbal gel formulation (F-2, F6 and F-9) was applied on the skin of experimental animal. Simple gel base was used as a control and applied on back of rats. The experimental animal was daily treated up to 1 week. After a week treated skin was visually examined for edema and erythema present on skin. Primary Dermal Irritation Index (PDII) = PDII observed on 12+24+48+72 hrs. / 4

Primary dermal irritation index scores for erythema (no erythema-0, very slight erythema -1, well-defined erythema-2, moderate to severe erythema -3, severe erythema-4) and edema (no edema-0, very slight edema -1, well-defined edema -2, moderate to severe edema -3, severe edema -4). [14, 15, 16].

2.2.2. In Vivo Wound Healing Study

Excision wound model

Excision wound model is used for the determination of the rate of contraction and epithelization. In this model drugs are applied on dorsal thoracic region of the animal. For the excision wound healing model study, 5 groups (Wistar rats 150- 185 gm) were made and each group contain 6 animals respectively. The dorsal fur of the wistar rats were trimmed and depilated with the help of clean razor blades and the shaved region were treated with 90% ethanol. [19]. Animal was anesthetized by using local anesthetic agent (light Chloroform) in aseptic condition, then remove the hairs of back region of animal; hollow was made on the dorsal thoracic region of animal. Remove the 300 mm circular area with the help of sterilize surgical blade or scissor. The depth of wound approx. 2 mm. the wound area created uniform in all animals. Treatment of animal started from the day first of experiment to 15 days. Group 1 were treated with simple gel base for negative control. Group 2 treated with 5 % Povidone iodine ointment as a reference standard for positive control and group 3, 4, and 5 was treated with poly-herbal gels (F-2, F6 and F-9) preparations. Determine the area of wound, and duration of epithelization, observation of percentage wound closure along with percentage wound closure epithelialization periods. The measurement of wound closure was done by charting the wound area on alternative days with the help of transparent paper until wound completely healed. Wound tracings were drawing on a millimeter scale graph paper for determination of wound area. Calculate the wound contraction by using following formula- [13, 17, 18 20].

$$\textbf{Percentage of Wound Closure} = \frac{\textbf{Initial Wound Size} - \textbf{Specific day Wound Size}}{\textbf{Initial Wound Size}} \times 100$$

The area that contracted over a predetermined duration of time is convert into a % unit to determine percentage wound contraction. The epithelialization period is the duration of time until a scar formation and new skin generate in.

3. Result and Discussion

3.1. In-Vivo Animal Activity

3.1.1. Skin Irritancy

The skin irritancy test was conducted on 20 wistar rats for 7 days and the result of skin sensitivity study indicated that the optimize polyherbal gel formulations were free from dermatological reaction. Compared to this, neither the placebo nor the polyherbal gel formulations caused irritation for up to 3 days, after which there was minor erythema and light redness at the skin surface. All the gel formulations didn't provoke any dermatitis such as erythema and edema for about 72 hrs. when topically applied over the skin. This result of the in-vivo skin irritation study indicates that polyherbal gel formulations F-2, F-6 and F-9 does not cause any major irritation on rats' skin for 7 days. The result of the dermatitis test show in table 1 and 2.

Table 1 Skin irritation study data day 1 to day 7

Sr.no	Treated Groups	Reaction	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Gel Base	Erythema	0	0	0	1	0	1	1
		Edema	0	0	0	0	0	1	1
2	F-2	Erythema	0	0	0	1	1	0	0
		Edema	0	0	0	0	0	0	1
3	F-6	Erythema	0	0	0	0	0	1	0
		Edema	0	0	0	0	1	0	0
4	F-8	Erythema	0	0	0	1	0	0	0
		Edema	0	0	0	0	1	0	0

Table 2 Skin irritation study of prepared herbal gel formulations

Treated Groups	Reaction	Primary Dermal Irritation Index score PDII observed on 12+24+48+72 hrs. /4
Negative (Gel Base)	Erythema	0.55
	Edema	0.45
F-2	Erythema	0.35
	Edema	0.20
F-6	Erythema	0.25
	Edema	0.15
F-9	Erythema	0.15
	Edema	0.05

<0.5: non-irritating, 0.5- 2.0: slightly irritating, 2.1-5.0: moderately irritating and >5.0: severely irritating.

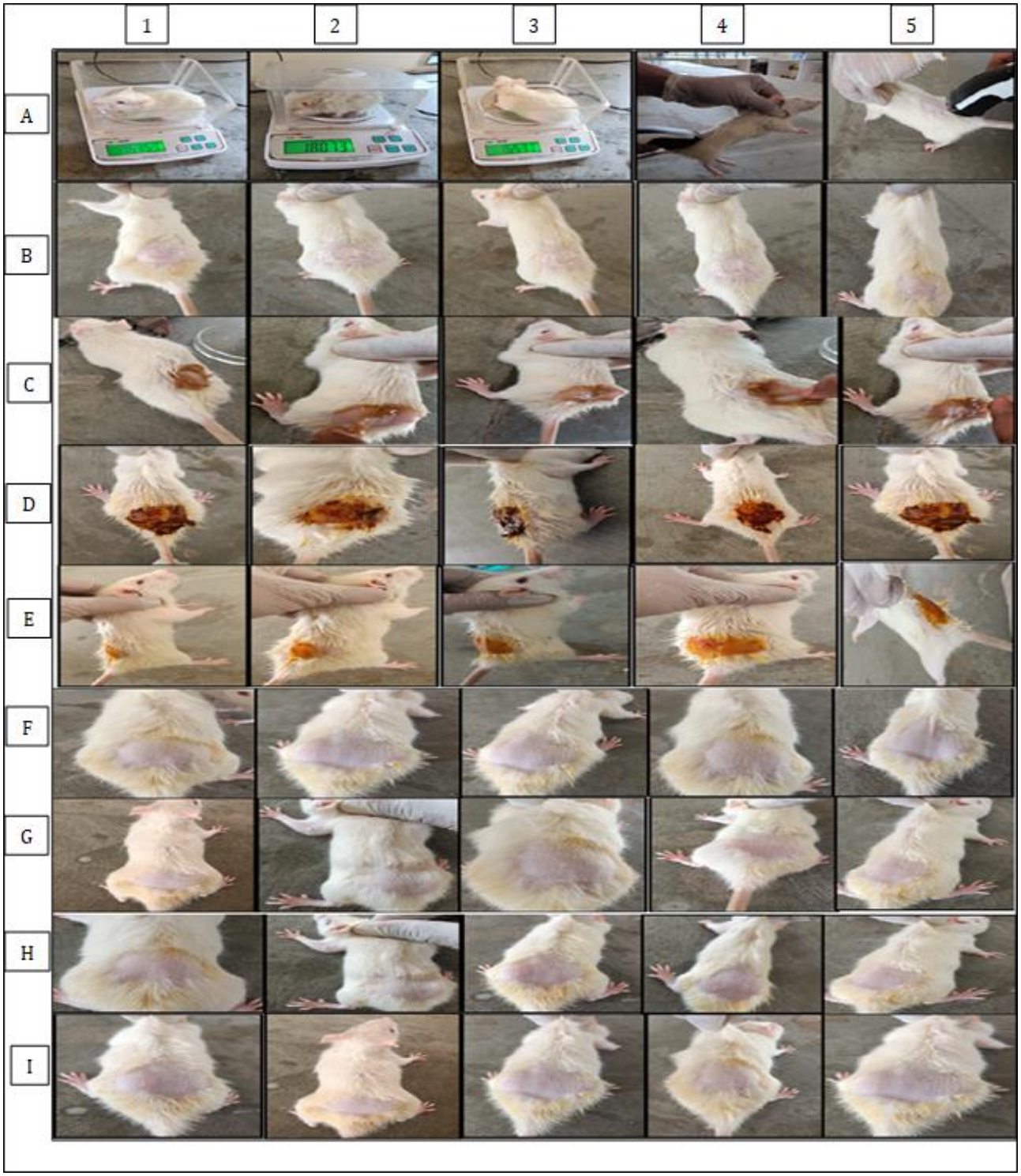

Figure 2 Skin irritation study of selected gel formulations. Image A represent weighting of experimental animals and hair removal stage. Image B represent the animals treated with normal gel base treated group. Image C, D, and E represent the animals treated with polyherbal gel formulation F-2, F-6 and F-9 respectively. Image F, G, H and I represent animals group treated with various selected gel formulations after 7 day of skin irritation study

3.2. Evaluation of In-vivo Wound healing activity of Polyherbal gels

3.2.1. Excision wound model

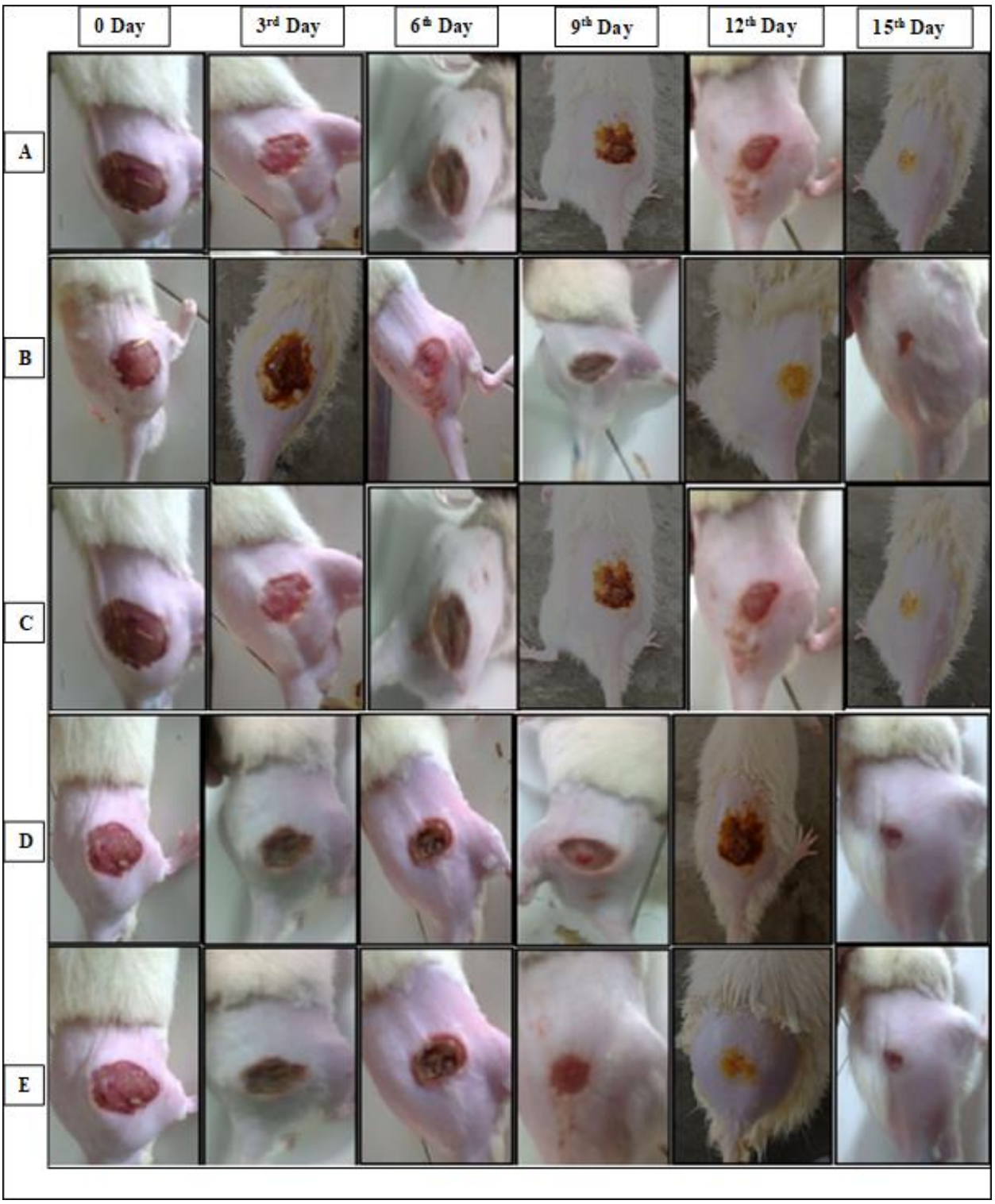

Figure 3 The excision wound contraction area at day 0, 3rd, 6th, 9th, 12th and 15th respectively. Image A represent the animals treated with negative control (Gel base). Image B represent the animals treated with standard (5% Povidone-Iodine) marketed formulation. Image C, D and E represent the animal groups treated with polyherbal gel formulation F-2, F-6 and F-9 respectively

Polyherbal gel having accelerated epithelization healing and wound contraction. Wound contraction and epithelialization period were used to evaluate the wound healing potential of various polyherbal gel formulation. table and graph exhibit the decrease in wound area of the various treatment groups over 3rd, 6th, 9th, 12th, and 15th days. Based on data analysis, the result of the mean changes in wound length on every 3rd, 6th, 9th, 12th and 15th day. It was conducted that each of the polyherbal gel formulation that were effective against changes in wound length in rats. the measurement of wound healing progression was accompanied using the standard drug Cipladine. Animals group treated with F-6 and F-9 polyherbal gel formulation demonstrated significantly higher wound healing (80.3±1.74% and 83.72±2.07%) compared with negative control gel base (59.83±1.56%) and F-3 formulation (77.78±1.98%). Povidone Iodine show maximum wound healing (86.07±1.30%) potential ($P<0.0001$). The wound contraction area of various treatment groups was observed on 3rd, 6th, 9th, 12th, and 15th days for excision wound healing model showed in figure 51, while the result on the 15th day all groups experienced a minimizing in wound size, all the polyherbal gel formulations treatment groups F-2, F-6 and F-9 had a relatively smaller compared to the negative control group. The 7th and 14th are classified into the proliferation phase. The wound healing based on the rate at which the contraction of wound like happen and also enhance the re-epithelialization of the injured tissue via collagen recovery. Data represented in table 3,4 and 5.

Table 3 Effect of topical application of drug on Excision wound (wound contraction area means ± standard deviation)

Days	Negative Control	Positive Control (standard)	Test F-2	Test F-6	Test F-9
Day 1	25.43±0.39	25.12±0.29	24.77±0.53	24.94±0.53	24.95±0.54
Day 3	22.53±0.39	20.62±0.48	21.03±0.44	20.76±0.31	20.58±0.47
Day 6	19.64±0.5	17.08±0.51	18.51±0.45	18.89±0.48	17.26±0.54
Day 9	16.16±0.51	13.90±0.53	15.96±0.48	15.39±0.42	13.67±0.49
Day 12	14.03±0.52	9.42±0.5	11.73±0.53	10.70±0.51	9.18±0.45
Day 15	10.22±0.51	3.50±0.33	5.51±0.53	4.92±0.48	4.06±0.51

3.3. Average mean wound area closure

The wound area was manually detected and photographed at 3-day intervals, and the wound healed area was assessed by reducing it from the original wound area. The initial mean excision wound area on day first were write down as follows: group I: 25.43±0.39 mm^2, Group II: 25.12±0.29 mm^2, Group III: 24.77±0.53 mm^2, Group IV: 24.94±0.53 mm^2, and Group V: 24.95±0.54 mm^2. The result for each group is exhibited in the table 4.

During the starting 3rd days, no significant wound contraction was detected in both the normal control gel base and standard control groups as well as the other experimental groups. This condition is reasonable because the wound healing process that happen on the day 1 to 3 day is an inflammatory mechanism that causes edema. However, on days 6th significant wound contraction was showed in the standard and experimental group F-9. On days 9th the wound contraction rate significantly faster in standard and F-9 treated group. That action possible by reducing the expression of cyclooxygenase (COX-2) compared to 3rd and 6th. On the same 12th days, the wound contraction areas were also measured, the respective measurement were group I: 14.03±0.52, Group II: 9.42±0.5, Group III: 11.73±0.53, Group IV: 10.70±0.51 and for Group V: 9.18±0.45.

By the end on the 15th days, these measurements reduced to group I: 10.22±0.51, Group II: 3.50±0.33, Group III: 5.51±0.53, Group IV: 4.92±0.48 and Group V: 4.06±0.51 respectfully from the initiation of the wound. It is known that curcumin can inhibit the COX-2 enzyme, besides that they also suppress inflammation by decreasing the expression of TNF-α. The analysis of these result indicates that all three polyherbal gel formulations, when topically applied, demonstrated a significant efficacy on wound closure compared to marketed formulation. It was observed that the polyherbal gel formulation F-9 exhibited similar effects to the marketed allopathic formulation with a 5% concentration of povidone iodine.

Table 4 Percentage wound Contraction in Excision wound.

Days	Negative Control	Positive Control (5% Povidone-Iodine)	Test F-2	Test F-6	Test F-9
Day 1	0%	0%	0%	0%	0%
Day 3	11.41±0.94%	17.80±1.14%	15.1±0.79%	16.73±0.96%	17.44±1.82%
Day 6	22.77±1.26%	32±1.48%	25.27±1.94%	28.23±2.65%	30.78±1.78%
Day 9	36.47±1.74%	44.67±1.94%	35.56±0.76%	38.26±1.75%	45.18±2.14%
Day 12	44.86±1.69%	62.48±1.90%	52.63±1.92%	57.09±1.60%	63.17±2.08%
Day 15	59.83±1.56%	86.07±1.30%	77.78±1.98%	80.3±1.74%	83.72±2.07%

All values are represented as mean± standard deviation, n= 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey's Multiple Comparisons Test. A significant difference as compared to negative control and standard treated group. P<0.0001.

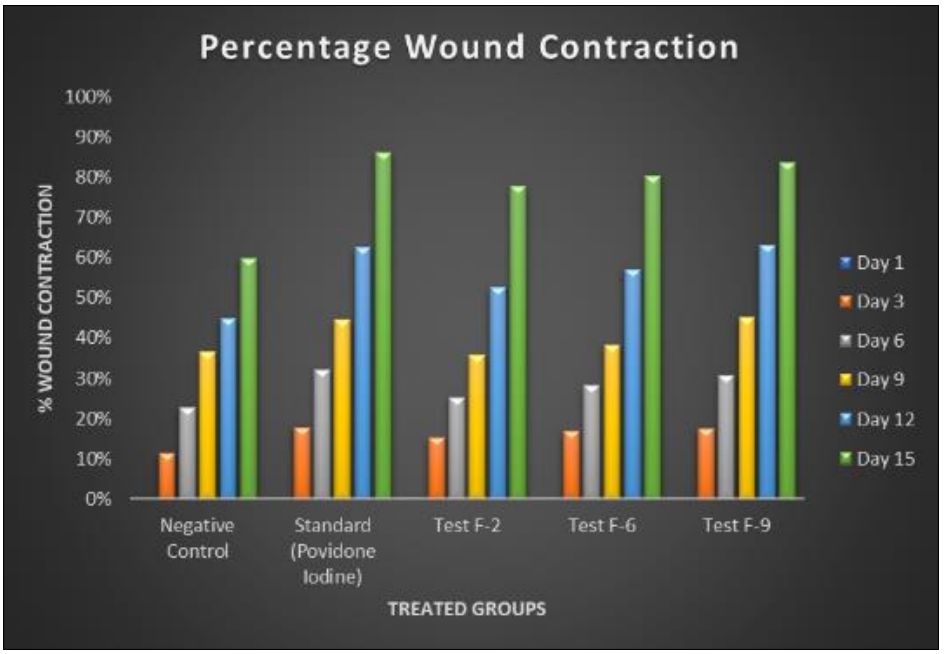

Figure 4 Percentage wound healing for various gel formulation treated groups compared with negative control and standard

3.4. Evaluation of period of Epithelialization

The result of the epithelialization period for different treatment groups are presented in the table. It was observed that the rate of epithelialization for group V (F-9 formulation) demonstrated fastest epithelization (19.33±1.21) compared with negative (25.83±1.94) gel base, F-2 (23.67±1.63) and F-6 gel (22.17±1.47) groups might be due to adequate viability of newly generated epithelial cells. The epithelialization period was 17.5±1.38 days in the standard (Povidone-Iodine) control group.

Table 5 Epithelialization periods among various treatment groups in days

Group no.	Treated Groups	Epithelialization periods (Days)
1	Negative Control (Gel base)	25.83±1.94
2	Standard (P. Iodine)	17.5±1.38
3	F-2	23.67±1.63
4	F-6	22.17±1.47
5	F-9	19.33±1.21

All values are represented as mean± standard deviation, n= 6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey's Multiple Comparision Test. P<0.0001, A significant difference as compared to negative control and standard group.

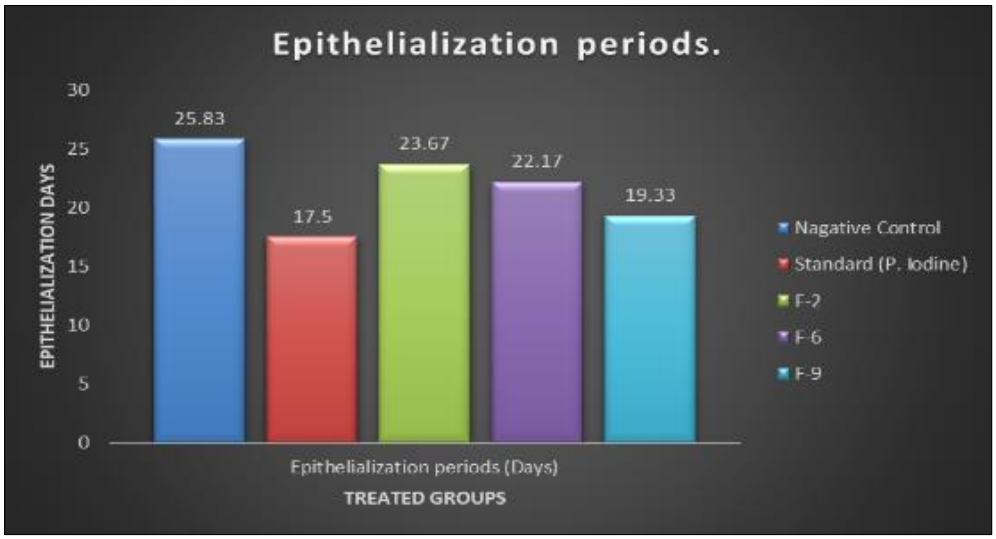

Figure 5 Epithelialization days in different treatment groups

These finding indicate that the polyherbal test gel formulations F-9 showed comparable effectiveness in boosting epithelialization time as the reference drug. The result suggested that the effect of polyherbal gel formulations in wound contraction and accelerated epithelialization consider for significant and efficient wound healing as compared to negative control (gel base) group. However, formulation F-2 exhibited a slightly longer epithelialization period. Data showed in table 5.

4. Conclusion

Wound consist of a homeostasis operation including re-epithelization and remodeling of tissue matrix collagen. Phytogenic biologically actives provide a better and alternate therapy via-rapid wound healing by rapid wound contraction, tiny epithelialization period and dense collagen regeneration. They also foster sufficient tensile strength to the newly healed wound.

An effort was made in this study to develop and assess the wound healing activity of polyherbal content from hydroalcoholic extract (Ethanolic/Methanolic and Water) 10% fraction of *Azadirachta indica*, Calendula officinalis, Curcuma longa and Aloe-vera barbadensis. It was concluded the result of skin irritation study the selected gel preparations, which are topically applied didn't provoke any dermatitis like erythema and edema, there was minor erythema and light redness showed at the skin surface due the present of sodium hydroxide in the gel formulation.

In the excision wound model, polyherbal gel preparation F-9 was found to exhibited significantly enhance in percent wound contraction as compared to negative control group and F-2 formulation treated animal's groups. The period of wound healing depends upon the rate of wound contraction. The shrinking of a wound increases the wound closure through rapid re-epithelialization, via shortening the migrating space traveled by keratinocytes and also decrease the extracellular matrix necessary to recover the injured epithelium. The epithelialization process includes migration and proliferation of the neo-generated cells towards injured wound bed. The shorter epithelialization duration exhibited by F-9 gel formulation might be due the sufficient viability of epithelial cells as compared to negative control and F-2 gel formulation treated groups. The effective treatment and wound care and are also related with efficient antimicrobial and anti-oxidant activity

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained

References

[1] Barua C.C., Talukdar A., Barua A. G., Chakraborty A., Sarma R. K. and Bora R S. Evaluation of the wound healing activity of methanolic extract of *Azadirachta indica* (Neem) and Tinospora Cordifolla (Guduchi) in Rats. Pharmacologyonline, 2010, (1): 70-77.

[2] Kordestani S. S. Atlas of Wound Healing a Tissue Regeneration Approach. Elsevier publication 2019: 1-31.

[3] Baktas N., Senel B., Yenilmz E., Ozatik O. and Arslan R. Evaluation of wound healing effect of chitosan-based gel formulation containing vitexin. Saudi pharmaceutical journal, Elsevier, 2020, (28), 1:87-94.

[4] Stan D., Tanase C., Avram M., Apetrei R, Mincu N-B., Mateescu A. L and Stan D. Wound healing application of creams and "smart," hydrogels. Experimental Dermatology, 2021, 30 (9): 1218-1232.

[5] Shedoeva A., Leavesley D., Upton Z. and Fan C. Wound Healing and the use of Medicinal Plants" Evidence Based Complementary and Alternative Medicine, 2019, 1-30. Doi: 10.1155/2019/2684108.

[6] Schultz G. S., Chin G. A., Moldawer L. and Diegelmann R. F. Principles of Wound Healing Mechanism of vascular Disease- NCBI Bookshelf. National Library of Medicine, 2011, 1-36.

[7] Ehtesabi H., Fayaz M., Hosseini-Doabi F. and Rezaei P. The application of green synthesis nanoparticles in wound healing: a review. Materials Today Sustainability, 2023, (21) 1-39, 100272.

[8] Mohan H. Textbook of Pathology. 7th edition, Jaypee Brothers Medical Publishers (P) Ltd. 2015: 116-163.

[9] Nandhini J., Karthikeyan E. and Rajeshkumar S. Nanomaterials for wound healing: Current status and futuristic frontier. Biomedical Technology, 2024, (6): 26-45.

[10] Yi Li, Luo H., Li Y., Huang P., Xu J., Zhang J., Cai P., He H., Wu J. and Li X. Surface Biofunctional bFGF – loaded electrospun suture accelerates incisional wound healing. Materials & Design, Elsevier, 2023, 225. 111451.

[11] C. Wells C. Newfoundland and Labrador Skin and Wound Care Manual. Labrador-Grenfell Health, 2008, 1-11.

[12] Sharma GS., Anusha L., Shireeh R., Geetha K. and Rama Rao T. A Review on Pharmaceutical Gels. YMER Publisher, 2022, (21): 12, 1338-1351.

[13] Ramya Devi D., Sowmiya Lakshna S., Veena Parvathi S. and Vedha Hari B.N. Investigation of wound healing effect of topical gel of Albizia amara leaves extract. South African Journal of Botany, Elsevier, 2018, 119, 400-409.

[14] Lakshmi P. K., Thangellapalli N., Chennuri A., Prasanthi D. and Veeresh B. Wound healing activity of topical lawsone gel on rat model. International Journal of Pharmaceutical Sciences and Research, 2017, (8): 7, 3162-3169.

[15] Toppo F. A and Pawar R. S. Development, Optimization and Evaluation of Different herbal formulations for wound Healing. International Journal of Pharmacy and pharmaceutical Sciences, 2014, (7): 3, 1-6.

[16] Madiwalar M. B, Shindhe P. S, Hiremath R. R. and Killedar R. S. Wound healing efficacy of novel Ayurveda formulation – Pentabark Kashaya: In wistar rats using excision wound model- an in vivo study. Journal of Ayurveda and Integrative Medicine, 2022, (13): 100602.

[17] Al- Nadaf A. H., Awadallah A. and Thiab S. Superior rat wound –healing activity of green synthesized silver nanoparticles from acetonitrile extract of Juglans regia L: Pellicle and leaves. Heliyon, 2024. (10): 1-12, e24473.

[18] Boakye Y. D., Agyare C., Ayande G. P., Titiloye N., Asiamah E. A. and Danguah K. O. Assessment of Wound-Healing properties of Medicinal Plants: The Case of Phyllanthus muellerianus. Frontiers in Pharmacology, 2018. 1-21.

[19] Verma R., Gupta P.P., Satapathy T. and Roy A. A Review of wound healing activity on different wound models. Journal of applied pharmaceutical research. 2019, (7), 1, 1-7.

[20] Rodríguez-Acosta H. Chronic wound healing by controlled release of chitosan hydrogels loaded with silver nanoparticles and calendula extract. Journal of Tissue Viability, 2022, (31):1, 173-179.