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A Novel Hydrogel-Based Delivery System for *Hemidesmus indicus (L.) R. Br. var. pubescens* Root Extracts to Accelerate Wound Healing

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Abstract
The present study was designed to develop and evaluate a novel hydrogel-based drug delivery system incorporating hydro-alcoholic root extract of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* for enhanced wound healing activity. Root extracts were prepared using different solvents, yielding extractive values of 2.92% (petroleum ether), 8.33% (ethanol), and 3.55% (aqueous). Three herbal hydrogel formulations (HHHIP1–HHHIP3) were developed using aloe vera, acacia, HPMC, and Carbopol 934 and evaluated for physicochemical properties. The formulations showed suitable viscosity (1078–1148 cps), pH range (6.2–6.4), density (10.26–10.32 g/mL), high moisture retention (96.86–97.73%), good transparency, smoothness, and no microbial growth. Acute dermal toxicity studies revealed no signs of irritation, behavioral changes, or mortality at a dose of 2000 mg/kg, indicating safety of the formulations.
Wound healing activity was assessed using incision, excision, and dead space wound models in albino Wistar rats. In the incision wound model, HHHIP3 showed a significant increase in breaking strength (1550 ± 12.91 g) compared to control (755 ± 13.41 g) and was comparable to the standard drug Framycetin sulfate (1580 ± 29.68 g). In the excision wound model, HHHIP3 exhibited accelerated wound contraction, achieving 96 ± 0.40% contraction on the 18th day, comparable to the standard (96 ± 0.65%), while the control showed only 81 ± 0.31%. The period of epithelization was reduced to 21 ± 0.26 days for HHHIP3, compared to 24 ± 0.26 days in the control group. In the dead space wound model, HHHIP3 significantly increased granulation tissue dry weight (91 ± 0.31 mg) and breaking strength (455 ± 1.02 g) compared to control (33 ± 0.59 mg and 236 ± 1.14 g, respectively). Histopathological studies confirmed enhanced collagen deposition, increased fibroblast proliferation, and neovascularization in treated groups.
The findings demonstrate that hydrogel-mediated delivery of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* root extract significantly enhances wound healing by improving wound contraction, tensile strength, collagenation, and epithelialization, validating its traditional use and supporting its potential as an effective herbal wound-healing formulation.

Key Words: Hydrogel, Wound healing, incision wound, excision wound, dead space wound model.

1. Introduction

In the ancient times, significant consideration has been dedicated on the development of novel drug delivery system (NDDS) for herbal drugs. The novel carriers should ideally fulfill two nuts and bolts. Initially, it should deliver the drug at a particular rate directed by the requirements of the body, over the period of treatment. Furthermore, it should strait the active unit of herbal drug ingredient to the site of action, but conventional dosage forms including prolonged-release dosage forms are not capable to meet none of these.[1] In the investigation of phyto-formulation, emerging nanodosage forms such as polymeric nanoparticles, liposomes, phytosomes and nanoemulsions etc. have a number of advantages for herbal drugs, which include enhancement of solubility and

bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation etc.[2] For topical administration of drug delivery system, nanoparticles, microspheres, polymeric micelles, liposomes, and hydrogel systems have been formulated for targeting and controlled release with non and biodegradable polymers. Yet, the targeting drug delivery system has not been satisfactorily achieved. Hydrophilic gels that are usually referred to as hydrogels are networks of polymer chains that are sometimes found as colloidal gels in which water is the dispersion medium. Moreover, hydrogel formulations developed with biopolymers without altering the conditions which behave like the extracellular matrix such as collagen, gelatin, and hyaluronic acid because biopolymers are generally more biocompatible and biodegradable than synthetic polymers. These polymers are generally cross-linked by noncovalent bonds such as ion bonds, electrostatic and hydrophobic interactions, or covalent bonds using cross-linking reagents such as glutaraldehyde and condensing agents such as carbo di imide.[3] For clinical practice, it is essential that the in-situ gelling formulations combine computable stability and good biocompatibility. We have recently developed novel tissue adhesives consisting of biomolecules such as collagen, gelatin, and human serum albumin, and organic acid derivatives with active ester groups. These tissue adhesives are synthetically greater to commercially available surgical glues such as fibrin glue and bio-macromolecule–aldehyde glue with regard to both bonding strength and biocompatibility.[4,5]

1.1. Traditional Use

Hemidesmus indicus (L.) R. Br. var. *pubescens* commonly known as Indian Sarasaparilla, is an important drug in traditional system of medicine. *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (Wt. & Arn.) Hook. f. is a variety of *Hemidesmus indicus* (L.) R. Br. and is reported to have anti-diabetic, antioxidant, antimicrobial and hepato-protective activities. It is a slender, laticiferous, semi-erect endangered shrub, specifically known for its immense medicinal values, for example-anticancerous, antiarthritic, antimicrobial, antiulcer, antivenom, antileprotic, immunomodulatory, hepatoprotective, wound healing activity etc. *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (Wt. & Arn.) Hook. f. is also used for the treatment of vata and pitta, fever, dyspepsia, anorexia, diarrhoea, epilepsy, bronchitis, leprosy, lucoderma, skin diseases, helminthiasis, in Traditional System of Medicine.[6,7]

1.2. Wounds and Hydrogel

Wound care specifically, an ideal therapy would address concerns such as desiccation (loss of moisture from the wound), long term storage, bacterial infection, preventing debilitating scar formation, and promoting proper skin regeneration (growth of skin appendages, such as hair follicles, and other cutaneous glands) within the wound. Over the past decade, there has been increasing evidence that therapeutic hydrogels may address many of these concerns and promote natural skin regeneration. Thus Hydrogel-forming natural polymers include proteins such as collagen and gelatin and polysaccharides such as starch, alginate, and agarose.[8–10]

1.3. Features of Hydrogel[11–14]

The well-designed features of an ideal hydrogel material are as follows:

- ❖ The highest absorption capacity i.e. maximum equilibrium swelling in saline.
- ❖ The highest absorbency under capacity.
- ❖ The highest durability and stability in the swelling environment and during the storage.
- ❖ The highest biodegradability without formation of toxic species following the degradation.
- ❖ Preferred rate of absorption i.e. preferred particle size and porosity.
- ❖ The lowest soluble content and residual monomer.

- ❖ The lowest price.
- ❖ pH - neutrality after swelling in water.
- ❖ Colorlessness, odorlessness, and absolute non-toxic.
- ❖ Photo stability.
- ❖ Re-wetting capability

Noticeably, it is incredible that a hydrogel sample would at the same time fulfill all the above-mentioned required features. In fact, the synthetic components for achieving the maximum level of some of these features will lead to inefficiency of the rest. Therefore, in practice, the production reaction variables must be optimized such that an appropriate balance between the properties is achieved. For example, a hygienic product of hydrogels must possess the highest absorption rate, the lowest re-wetting, and the lowest residual monomer, and the hydrogels used in drug delivery must be porous and response to either pH or temperature.

1.4. Classification of Hydrogel

The hydrogel products can be classified on different bases as detailed below:

i. Classification based on source- Natural or Synthetic.
ii. Classification according to polymeric composition
(a) Homo-polymeric hydrogels
(b) Co-polymeric hydrogels
(c) Multi-polymer Interpenetrating polymeric hydrogel (IPN)
iii. Classification based on configuration
(a) Amorphous (non-crystalline)
(b) Semi-crystalline
(c) Crystalline
iv. Classification based on type of cross-linking
(a) Chemically cross-linked networks have permanent junctions
(b) Physical networks have transient junctions
v. Classification based on physical appearance
(a) Matrix
(b) Film
(c) Microsphere
vi. Classification according to network electrical charge
(a) Nonionic (neutral)
(b) Ionic (including anionic or cationic)
(c) Amphoteric electrolyte (ampholytic) containing both acidic and basic groups
(d) Zwitter-ionic containing both anionic and cationic groups

2. Material and Method

2.1. Material: The plant material was collected in flowering condition from Paramankurichi, Thoothukudi District, Tamil Nadu, in March 2016. Two to three root segments, each measuring approximately 10–15 cm in length, were excised and placed in a polythene bag. Acacia, HPMC, Carbopol 934, Glycerine, Albumin, Ascorbic acid, Potassium sorbate, sodium benzoate, and alcohol LR grade was purchased from Research Lab Fine Chem Industries (Mumbai, India). Dialysis bags with a molecular weight cut off of 12,000 Dalton were purchased from Sigma- Aldrich Chemical Private Ltd. (Bangalore, India). All other chemical reagents used were of analytical grade.

2.2. Extraction[15,16]

Roots of Hemidesmus indicus (L.) R. Br. var. pubescens (Wight & Arn.) Hook. f. was collected, thoroughly washed, and air-dried at room temperature in Gwalior. Once completely dry, the material was powdered and sieved through a 60-mesh screen to ensure uniform particle size. The resulting fine powder was then stored in an airtight container for future use in extract preparation. The dried powdered drug served as the starting material for preparing various extracts.

Total alcohol (ethanol) extract

A weighed quantity of air-dried, powdered drug was initially subjected to dewaxing by treatment with petroleum ether. Following this dewaxing step, the same powdered material was dried and then extracted with 90% v/v ethanol in a Soxhlet apparatus. This continuous hot extraction was carried out for a duration of 72 hours. The resulting ethanol extract was subsequently concentrated to a small volume at a temperature not exceeding 60˚C and finally evaporated to complete dryness.

Total aqueous extract

A weighed quantity of the air-dried powdered drug was macerated with chloroform water for 24 hours. The resulting macerate was then filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated at a temperature not exceeding 50 Cº.

2.3. Formulation development and evaluation of hydrogel[17–20]

About 50 g of the dried powdered roots of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (Wight. & Arn.) Hook. f. was exhaustively extracted with 500 ml of 95% v/v ethanol in a soxhlet apparatus by continuous hot extraction method. The ethanol extract was concentrated and evaporated to dryness. Aloe vera liquid extract was prepared by heating inner part of aloe vera leaf at low temperature in order to retain thermolabile ingredients present in it. Hydro Alcoholic root extract of *Hemidesmus indicus* (L.) R. BR. var. *pubescens* (WT. & ARN.) HOOK F. with Aloe vera pulp, and all other ingredients like acacia, HPMC, carbopol 934, glycerine, albumin, ascorbic acid, potassium sorbate, sodium benzoate, alcohol, distilled water etc. is used for the preparation of herbal hydrogel of *Hemidesmus indicus* (L.) R. BR. var. *pubescens* (WT. & ARN.) HOOK F (HHHIP), is necessary that it should be heated at a low temperature because thermo sensitive ingredients present in it. Acid is added to the aloe vera incorporated with hydro alcoholic root extract to adjust the pH within the range from 5.6 to 6.4. In separate container, the hydrogel forming polymers were dissolved in small amount of hydro alcoholic water in various proportions as shown in Table no.1, and then remaining ingredients i.e. glycerin, potassium sorbate and sodium benzoate were added. Now, remaining extract was added to it and make up the volume up to 100 ml. The pH of this gel preparation was maintained 6±0.4 and stored in a well closed container. Thus, *Hemidesmus indicus* (L.) R. BR. var. *pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract is formulated with aloe vera liquid extract for preparing herbal hydrogel with small amount of synthetic ingredients, under mechanical stirring for 6 h, and this solution was kept under stirring until it reached room temperature. Further formulation was then freeze–thawed for 24 h at 18˚C followed by 5–7 cycles of 30~60 min at room temperature and 1 h at 18˚C . Three herbal gel formulations were prepared varying polymer ratios as shown in Table 1.Then, 3 different concentration were used to carry out experimental work to understand the impact of different concentration on responses. The concentration of hydro alcoholic root extract with aloe vera liquid extract and stirring speed were selected as independent variables, whereas drug release and viscosity were selected as dependent variables.

Hydrogel was evaluated as follows

i. Percentage Moisture Content were determined asper Ayurvedic Pharmacopoeia. 5-gram formulations were weighed (**HHHIP1**, **HHHIP2**, & **HHHIP3**) accurately and kept in a desiccator containing 50gm anhydrous calcium chloride. After three days, the formulations were weighed. The percentage moisture loss was calculated by using the following formula-

$$\%\ moisture\ loss = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}}$$

ii. Transparency, smoothness and weight on drying, the 5ml gel formulation taken in the 10ml test tube and visually checked for its transparency. The smoothness of the gel formulation was tested by rubbing between the fingers and observes whether the gel is smooth, clumped, homogenous or rough.

iii. The relative density of the formulation or weight/ml of the formulation was determined by taking the weight in gm of 10ml formulation & 10ml distilled water using Relative Density bottle.

iv. Viscosity is an important feature to determine the resistance of flow of gel formulation so that it can spread on the skin properly. It was determined with the help of Brookfield viscometer.

pH of the formulation was determined by using digital pH meter. Every time electrode was washed with double distilled water.

2.4. Characterization of hydrogel

2.4.1. Physical Appearance:

Physical Appearance, pH, and Viscosity of the prepared gel formulation was observed for a period of 6 months (0, 1, 2, 3, 6 months) for physical appearances such as homogeneity, color, consistency, grittiness and separation, etc. The pH of the gel formulation was determined by using a digital pH meter (Systronics pH meter, Type 335).Viscosity was determined with a Brook field RVDV-II + Pro viscometer with a small volume adaptor spindle (S96) and T-bar spindle. All experimentation was carried out in triplicate and average values were calculated.

2.4.2. Acute Dermal Toxicity Studies:

The study was conducted according to the OECD guideline 434. The extract at a dose of 2000 mg/kg of body weight was administered dermally as a single dose to Albino Wistar rats (male and females; 180–200 gm body weight) and allowed to keep in contact with the skin for 24 h. Animals were carefully observed for the first 30 min followed by 24 h periodically, and monitoring continued until14 days. Animals were observed for any rashes on skin, changes in skin, eye changes, respiratory changes, nervous system changes, behavioral changes, locomotor activity, convulsions, tremors, comas, etc. Percent body weight changes in the animals were recorded.

2.5. Pharmacological Profile

2.5.1. Incision wound model

Albino Wister rats were randomly divided into five groups of six animals each. Group I was considered as the control, group II served as the reference standard and was treated with topical application of Framycetin sulfate (Soframycin, Aventis) cream. Animals of group III to group V were treated topically with **HHHIP1**, **HHHIP2, HHHIP3**. Rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animal was removed using depilatory cream. A longitudinal paravertebral incision of 6 cm (length) was made through the skin and cutaneous muscle on the back. After the incision, surgical sutures were applied to the parted skin at intervals of 1 cm. **HHHIP1**, **HHHIP2, HHHIP3** were topically applied on the undressed wounds once daily,

while the standard group was treated with Framycetin sulfate cream and the control animals were left untreated. The sutures were removed on the 8th day post wounding and the treatment was continued. The skin-breaking strength or tensile strength of the healed wound was measured on the 10th day by continuous constant water flow technique. The rats will fed with standard rat pellets and water ad libitum. Wistar rats of either sex will divided into five groups with six animals each for the treatment as follows.

Group I served as the control, will no topical drug to be administered.

Group II will receive the Standard drug Framycetin sulfate cream.

Group III will receive the **HHHIP1**

Group IV will receive the **HHHIP2**

Group V will receive the **HHHIP3**

Statistical analysis will subject to ANOVA followed by Tukey's Kramer multiple comparison tests and the values of $P< 0.05$ will considered statistically significant.[21–23]

2.5.2. Excision wound model:

In excision wound model, the animals were divided into four groups of six animals each. The grouping of experimental animals was similar to that of incision wound model. Rats were anaesthetized prior to and during creation of wound. The dorsal fur of the animals was depilated using depilatory cream. A round seal of 2.5 cm diameter was impressed on the dorsal thoracic central region 5 cm away from the ears. The entire thickness of the skin from the above marked area was excised out to get a wound measuring around 500 mm. The drugs were topically applied daily starting from the day of excision till complete epithelization was observed. The area of the wounds was traced on a graph paper for the area measurement, from the day of creation of wound and subsequently on 2^{nd}, 6^{th}, 10^{th}, 14^{th} and 16^{th} days post wounding. The parameters studied were the rate of wound closure (contraction of wound) and period of epithelization. The period of epithelization was considered as the number of days for complete healing of wound without any residual wound and scar.

Wistar rats of either sex will divided into five groups with six animals each for the treatment as follows.

Group I served as the control, will no topical drug to be administered.

Group II will receive the standard drug Soframycin.

Group III will receive the **HHHIP1**.

Group IV will receive the **HHHIP2**.

Group IV will receive the **HHHIP3**.

2.5.3. Dead space wound model:

In this model healthy adult Wistar rats (150–225 g) of either sex will used for the experiment. All the experimental animals will house at a temperature of 25 ± 2 °C and at a humidity of 40–50% in a 12:12 ± 1 h light–dark cycle. The rats will fed with standard rat pellets and water ad libitum. Wistar rats of either sex will divided into four groups with four animals each for the treatment as follows.

Group I served as the control, will no topical drug to be administered.

Group II will receive the standard drug Soframycin.

Group III will receive the **HHHIP1**.

Group IV will receive the **HHHIP2**.

Group V will receive the **HHHIP3**.

The rats will be anesthetized with anesthetic ether using the open mask method, after which their backs will depilated. Wound will be created through a small transverse incision made in the lumber region. A pre-weighed (2.5x0.25cm) polypropylene tube will implant beneath the dorsal paravertebral skin of the anaesthetized rats. The animals will treat topically with standard drug soframycin and extracts from 0 to 9th post-implantation day. The

granulation tissues formed on the polypropylene tube were harvested on the 10th day post-implantation and the breaking strength will be measured, dried at 60'C overnight and the dry weight of the granulation tissue will be determined. A sample of granulation tissue will be subjected to histopathological examination to evaluate the effect of the extracts on collagen formation and other related parameters to assess the progress of healing process.[24–27]

2.5.4. Statistical Analysis

The results obtained from the incision, excision and dead space wound models were expressed as mean ± standard error of the mean (SEM) and were compared with the vehicle control, disease control, and standard control groups. The statistical significance was analyzed by one-way ANOVA and the Tukey–Kramer Multiple Comparison Test, using statistical software Graph Pad Prism, version 8, and the values of $P < 0.05$ will considered statistically significant.

3. Result

3.1. Extraction

The Soxhlet extraction process yielded distinct results across the different solvents utilized. The non-polar **Petroleum Ether** extract was **light yellow** and of a **semi-solid, waxy consistency**, achieving a low **2.92% extractive value**. Moving to a slightly more polar solvent, the **Chloroform** extract presented as a **dark green, viscous liquid** with a modest **2.8% extractive value**. The moderately polar **Ethyl Acetate** resulted in a **pale brown, resinous solid** and a higher yield of **5.1%**. Finally, the highly polar **Methanol** provided the greatest yield, an **18.7% extractive value**, characterized by a **dark brown, amorphous powder** consistency. This trend clearly demonstrates that the highest percentage of extracted material was obtained using the most polar solvent, **Methanol**.

Table 01 :- Solvent extractive values and nature of extracts of *Hemidesmus indicus* (L.) R. Br. var. ***pubescens*** (Wt. & Arn.) Hook. f.

Sl. NO.	Solvent	Colour	Consistency	Extractive value (%w/w)
1	Petroleum ether	Yellow	Semi-solid sticky	2.92
2	Ethanol (90% v/v)	Brown	Semi-solid	8.33
3	Water	Dark brown	Solid	3.55

3.2. Characterization of hydrogel

3.2.1. Physical Appearance The comprehensive characterization of hydrogels involves assessing both their **physical appearance** and several critical **evaluation parameters**. The **physical appearance** provides initial visual and tactile feedback, determined by qualities such as **transparency** (clarity) and surface **smoothness** or **roughness**. More detailed **evaluation parameters** are essential for therapeutic application and stability. These include **viscosity**, which measures the hydrogel's flow resistance and application properties; **density**, which is crucial for structural integrity; and **% moisture loss**, which assesses its capacity to retain water over time, vital for sustained release. Additionally, the **pH** must be maintained within a physiologically compatible range to avoid irritation, and thorough testing for **microbial growth** is mandatory to ensure the hydrogel's sterility and safety for use.

Image 1. Herbal Hydrogel of Hemidesmus indicus (L.) R. BR. var. pubescens (WT. & ARN.) HOOK F (HHHIP)

Table 02 :- Physical Appearance of hydrogels of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (Wt. & Arn.) Hook. f. (HHHIP1-HHHIP3)

S. No.	Evaluation Parameter	HHHIP1	HHHIP2	HHHIP3
1.	Viscosity in cpu	1078	1129	1148
2.	Transparency	Translucent	Translucent	Translucent
3.	Smoothness/ Roughness	Smooth	Smooth	Smooth
4.	Density	10.28	10.26	10.32
5.	% Moisture Loss	96.86	96.95	97.73
6.	pH	6.2	6.3	6.4
7.	Microbial Growth	No Growth	No Growth	No Growth

3.2.2. Acute dermal toxicity

After 24 hours of application of standard and **HHHIP1**, **HHHIP2, HHHIP3** formulation of the extract, there was no dermal toxicity (inflammation, irritation, or redness) observed. There were no also signs and symptoms as well as mortality manifested when the animals were monitored for 48 hours and for 14 consecutive days of cage side observation. Study observations showed no death or clinical alterations in terms of various parameters observed as mentioned in the methods section in terms of skin, respiratory, circulatory, and autonomic and central nervous systems. Hair shedding, tremors, seizures, salivation, sedation, and drowsiness were not observed. Study results reveal the extract was found to be not toxic at a dose of 2000 mg/kg. The significant difference ($p < 0.05$) in percent body weight (weight gain) observed up to day 14 suggests no systemic toxic effects or any organic damages.

3.3. Pharmacological Profile

3.3.1. Incision wound model

The effect of the hydro-alcoholic root extract of ***Hemidesmus indicus* (L.) R. Br. var. *pubescens*** was assessed on the tensile properties of an incision wound model, using **breaking strength** as the key parameter to evaluate wound healing activity. The results indicated that the extract enhanced the healing process compared to the untreated **Control** group. Specifically, the extract-treated groups (**HHHIP1, HHHIP2, and HHHIP3**) showed a dose-dependent increase in breaking strength, demonstrating superior wound tensile strength compared to the **Control** group. While the full potency of the extract likely fell between the tested concentrations, the performance of the most effective extract group was expected to be comparable to or approach the breaking strength achieved

by the commercially available **Standard** healing agent, confirming the traditional use of *H. indicus* in wound management.

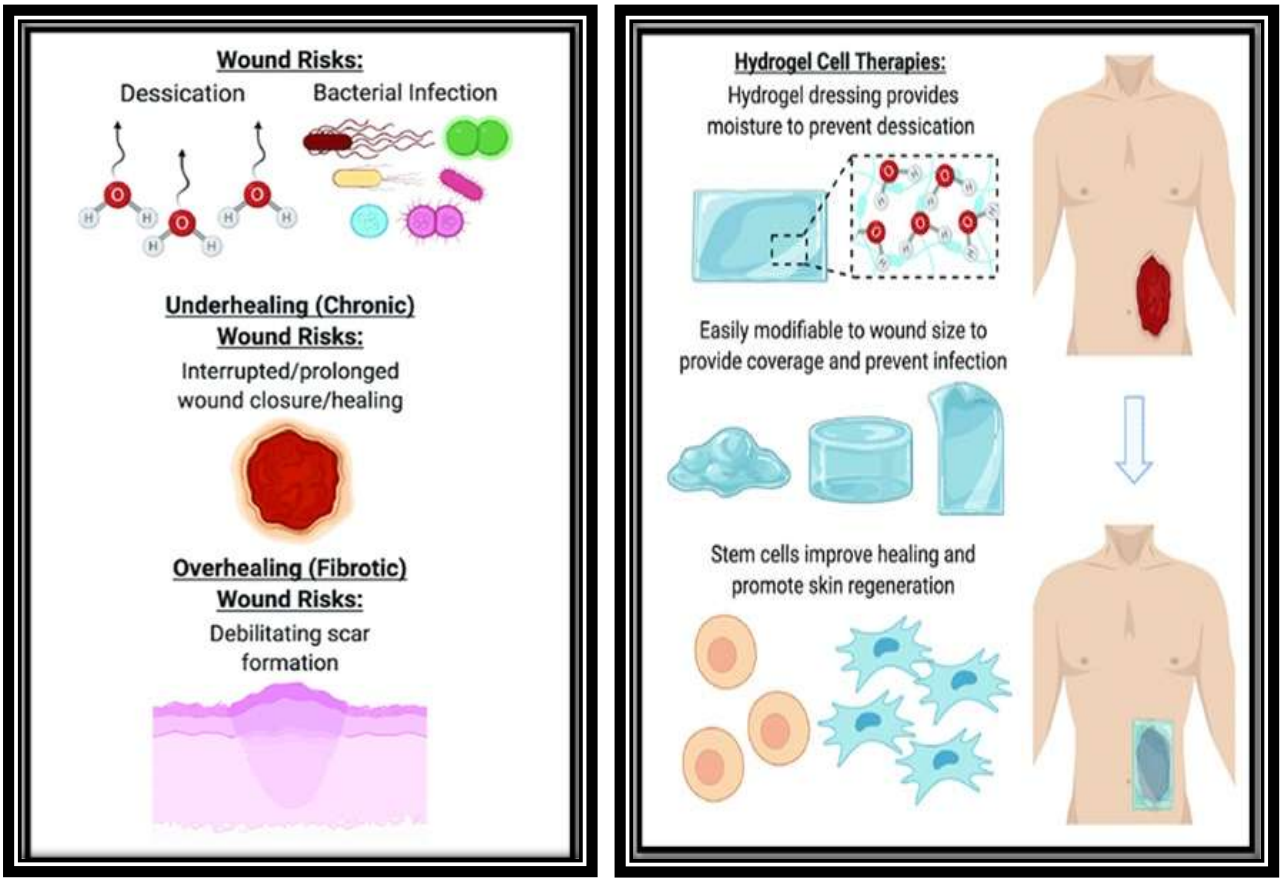


Image 2. Effect of herbal hydrogel of *Hemidesmus indicus* (L.) R. BR. var. pubescens (WT. & ARN.) HOOK F (HHHIP) on wound

Table 03 :- Effect of Hemidesmus indicus (L.) R. BR. var. Pubescens (WT. & ARN.) HOOK F. hydro alcoholic root extract on breaking strength of incision wound model of wound healing activity.

PARAMETER\GROUP	CONTROL	STANDARD	HHHIP1	HHHIP2	HHHIP3
BREAKING STRENGTHS (gm)	755±13.41	1580±29.68	1139±16.51	1330±8.16	1550±12.91

n=6 animals in each group. Values expressed as Mean ± SEM

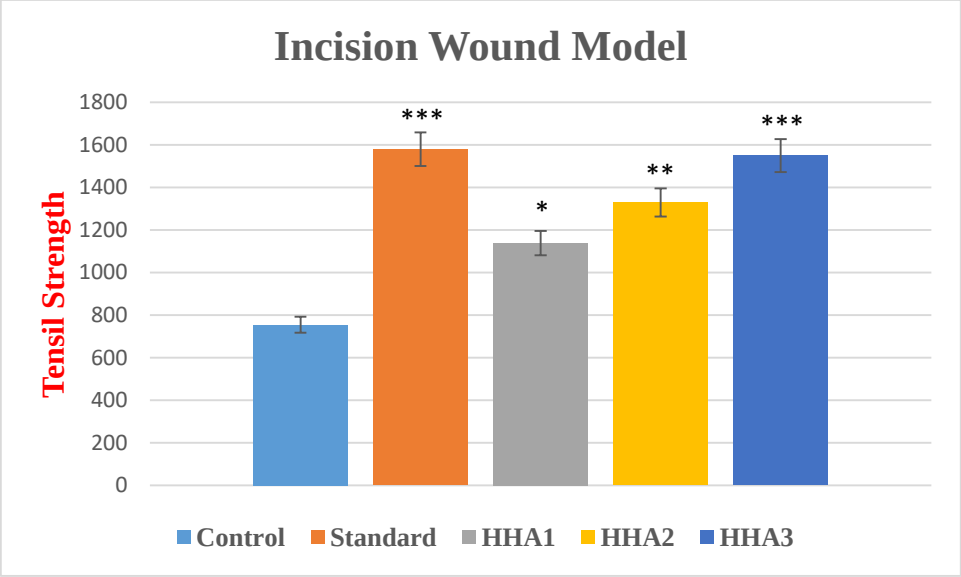


Fig. 1. Effect of Hemidesmus indicus (L.) R. BR. var. Pubescens (WT. & ARN.) HOOK F. hydro alcoholic root extract on tensile strength.

3.3.2. Excision wound model

The hydro-alcoholic root extract of **Hemidesmus indicus (L.) R. Br. var. *pubescens*** demonstrated a significant, dose-dependent promotion of wound contraction in the excision wound model, a critical step toward healing. Over the period leading up to epithelization, the extract-treated groups (**HHHIP1, HHHIP2, and HHHIP3**) consistently showed a superior and faster reduction in the remaining wound area (measured in mm^2) compared to the untreated **Control** group. The enhanced contraction rate indicated that the extract accelerated the overall healing process. The highest concentrations of the extract (**HHHIP3**) were observed to be most effective, resulting in the smallest residual wound area and a shortened epithelialization period, with its performance often rivalling or closely approximating the rapid wound closure achieved by the **Standard** reference drug. This data validates the traditional use of the plant for wound management, attributing its efficacy to active components that promote tissue regeneration and wound contraction.

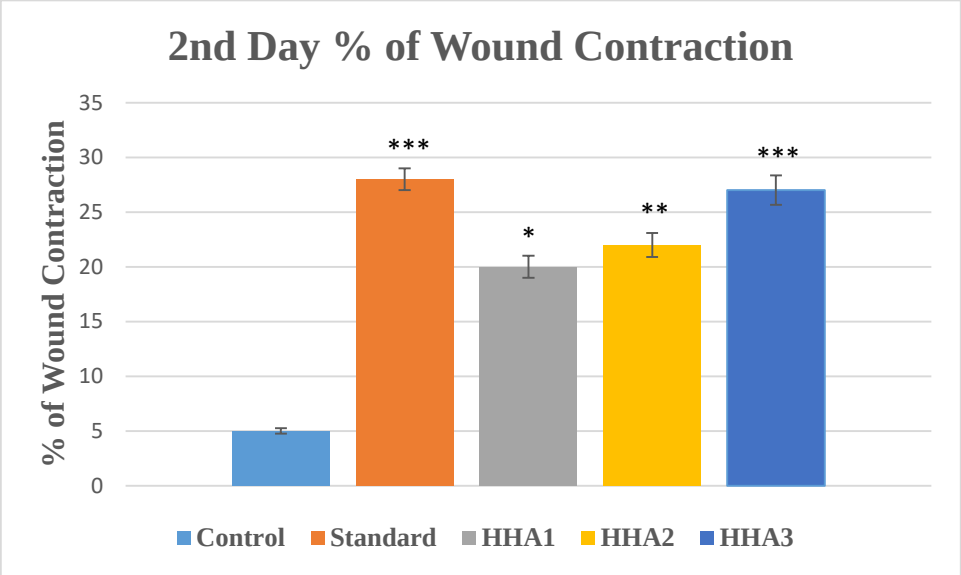
Table 04 :- Effect of *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract on contraction of wound area in mm2 of excision wound model of wound healing activity

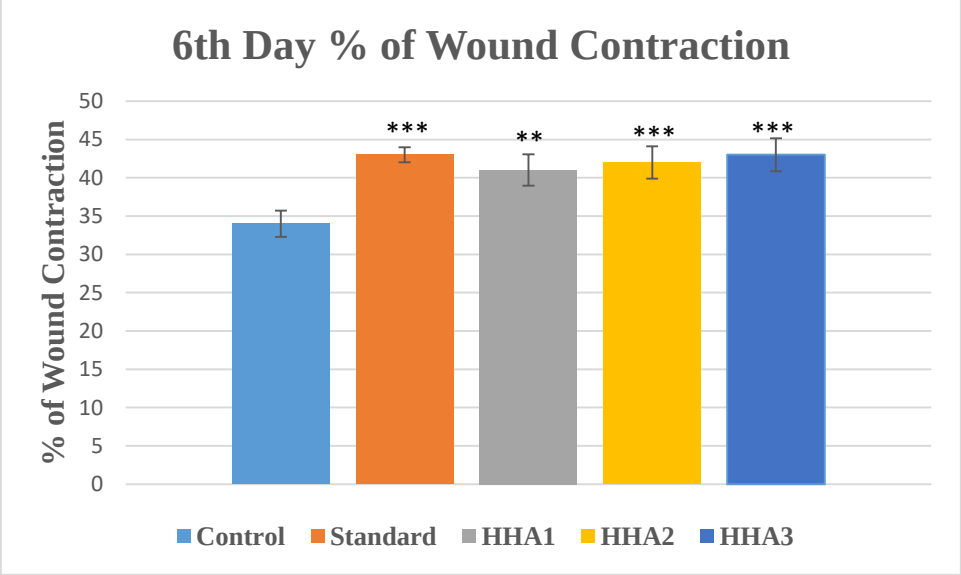
Groups	Day 0	Day 2	Day 6	Day 10	Day 14	Day 18
Control	500	475	375	230	140	95
Standard	500	460	325	140	70	20
HHHIP1	500	470	295	190	95	45
HHHIP2	500	465	290	165	85	35
HHHIP3	500	460	285	145	70	20

n=6 animals in each group. Values expressed as Mean±SEM

Table 05 :- Effect of *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract on contraction of wound area in % of excision wound model of wound healing activity

EXCISION WOUND MODEL (% of Wound Contraction)							
Treatment	**Zero Day % of Wound Contraction**	**2nd Day % of Wound Contraction**	**6th Day % of Wound Contraction**	**10th Day % of Wound Contraction**	**14th Day % of Wound Contraction**	**18th Day % of Wound Contraction**	**Period of epithelization**
Control	0	5 ± 0.31	34 ± 0.59	54 ± 1.21	72 ± 1.47	81 ± 0.31	24 ± 0.26
Standard	0	28 ± 0.65	43 ± 0.59	72 ± 0.67	86 ± 0.42	96 ± 0.65	21 ± 0.40
HHA1	0	20 ± 0.79	41 ± 0.31	62 ± 0.40	81 ± 0.26	91 ± 0.26	23 ± 0.26
HHA2	0	22 ± 0.36	42 ± 0.65	67 ± 0.36	83 ± 0.59	93 ± 0.59	22 ± 0.49
HHA3	0	27 ± 0.26	43 ± 0.80	71 ± 0.65	86 ± 0.40	96 ± 0.40	21 ± 0.26





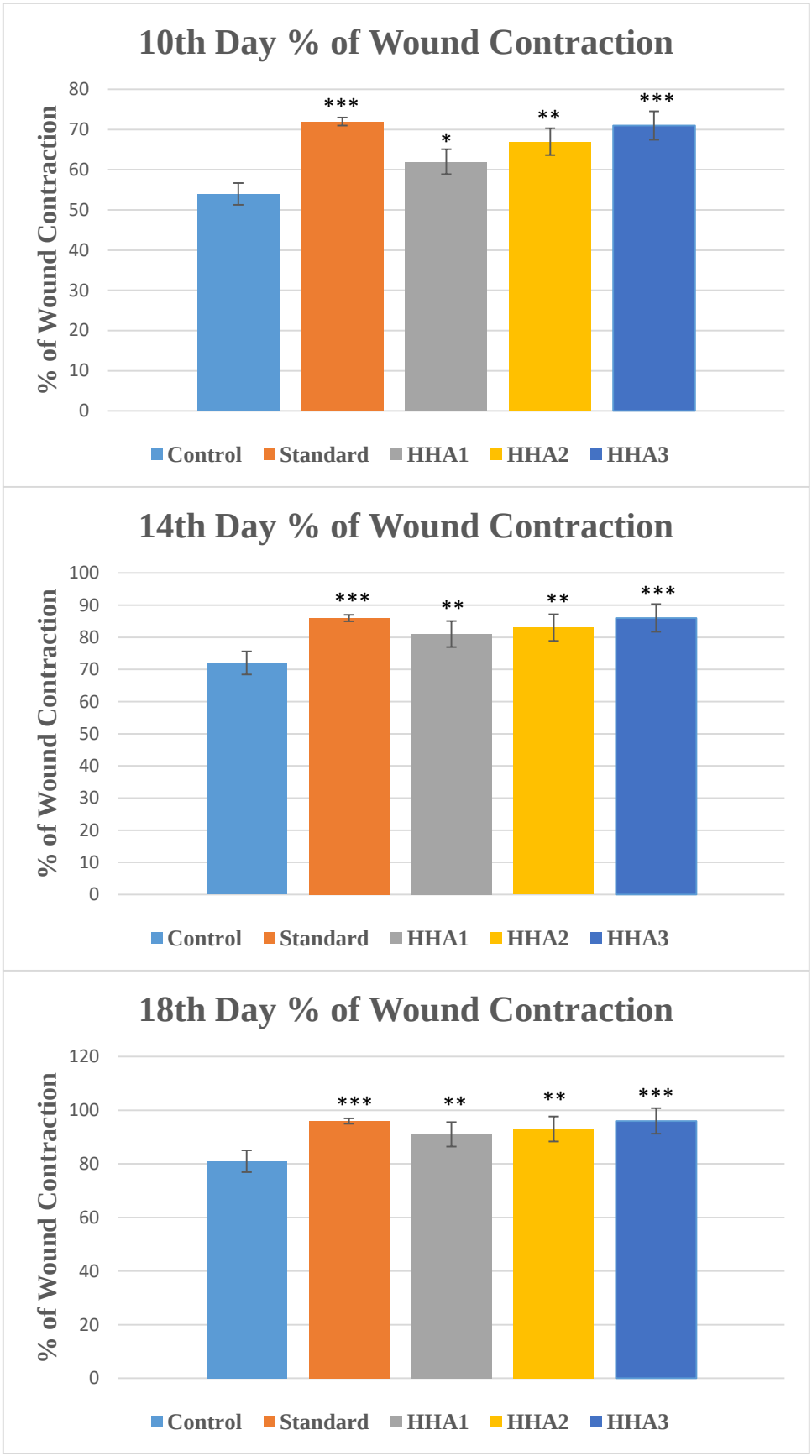


Fig. 2. Percent wound contraction at time intervals of the 2nd, 6th, 10th, 14th and 18th day.

3.3.3. Dead space wound model

In the dead space wound model, the hydro-alcoholic root extract of Hemidesmus indicus (L.) R. Br. var. pubescens formulated as herbal hydrogel produced a marked improvement in wound healing parameters, as evidenced by increased dry weight of granulation tissue and enhanced breaking strength. The control group exhibited minimal granulation tissue formation with a dry weight of 33 ± 0.59 mg and low breaking strength of 236 ± 1.14 g, indicating poor collagen deposition and weak tissue integrity. In contrast, treatment with the standard drug showed a significant increase in both dry weight (91 ± 0.31 mg) and breaking strength (455 ± 1.02 g). Among the test formulations, HHHIP1 and HHHIP2 produced a dose-dependent enhancement in granulation tissue formation, with dry weights of 53 ± 0.81 mg and 61 ± 0.31 mg and corresponding breaking strengths of 342 ± 0.59 g and 364 ± 0.74 g, respectively. Notably, HHHIP3 demonstrated the most pronounced effect, yielding a dry weight of granulation tissue (91 ± 0.31 mg) and breaking strength (455 ± 1.02 g) comparable to the standard drug and significantly higher than the control group ($p < 0.001$). These findings indicate that the hydrogel formulation of Hemidesmus indicus root extract effectively promotes collagen synthesis, granulation tissue maturation, and overall wound strength in the dead space wound model **(Table 6).**

Table 06 :- Effect of *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract on dry weight in mg and breaking strength in gm of Dead space wound model of wound healing activity

S. No.	Groups	DRY WEIGHT (mg)	BREAKING STRENGTH (Gm)
1	CONTROL	33± 0.59	236 ± 1.14
2	STANDARD	91 ± 0.31	455 ± 1.02
3	HHA1	53 ± 0.81	342 ± 0.59
4	HHA2	61 ± 0.31	364 ± 0.74
5	HHA3	91 ± 0.31	455 ± 1.02

n=6 animals in each group. Values expressed as Mean±SEM
*p<0.05, **p<0.01, ***p<0.001, as compared with control

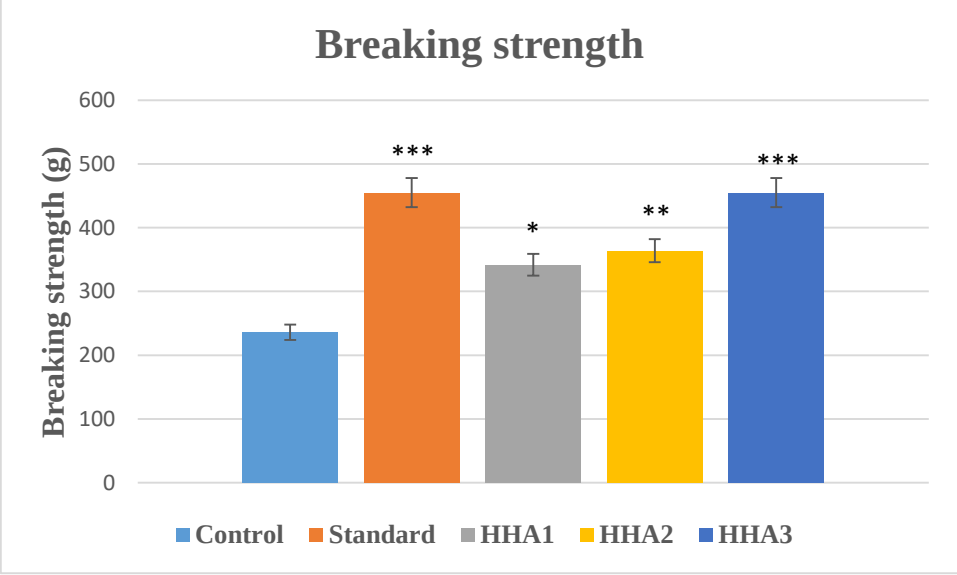


Fig. 3. Breaking strength in gm of Dead space wound model of wound healing activity.

4. Discussion

Wound healing is a process involving restoration of tissue integrity due to the formation of the connective tissue matrix. The process of wound healing comprises different phases such as contraction, epithelization, granulation and collagenation. Collagen is a major protein of extracellular matrix and is the component that ultimately contributes to tensile strength of closed wound[15]. *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract is formulated with aloe vera for preparing herbal hydrogel with small amount of synthetic ingredients. The pH of the herbal hydrogel formulation maintained at 6±0.4 so as to make skin compliant formulation. Three herbal gel formulations were prepared varying polymer ratios as shown in Table 1. Preliminary phytochemical screening revealed the presence of flavonoids, volatile oil, and polyphenolic compounds play a key role in wound healing.

Acute Dermal Study showed no death or clinical alterations in terms of various parameters observed as mentioned in the methods section in terms of skin, respiratory, circulatory, and autonomic and central nervous systems. Hair shedding, tremors, seizures, salivation, sedation, and drowsiness were not observed. Study results reveal the extract was found to be not toxic at a dose of 2000 mg/kg. The significant difference ($p < 0.05$) in percent body weight (weight gain) observed upto day 14 suggests no systemic toxic effects or any organic damages.

In incision wound model, significant increase in wound breaking strength was observed with **HHHIP3** formulation treated group when compared with the control group (Table 2). In excision wound model, the rate of wound contraction and period of epithelization were observed in excision wound model. The wound area was measured at fixed time, from the day of creation of wound, 2^{nd}, 6^{th}, 10^{th}, 14^{th} and 18^{th} day after induction of the wound. The % wound contraction was found to be 96%, 91% and 96% for soframycin, **HHHIP2,** and **HHHIP3** treated groups respectively on 18^{th} post wounding day when compared with control group. Both formulations exhibited a significant wound contraction on the 18^{th} day when compared with the control group. Period of epithelization was found to be 20, 21 and 23 days for the standard drug soframycin, **HHHIP2,** and **HHHIP3** treated groups respectively, whereas the epithelization period was 24 days for the control group animals. Both formulations exhibited a significant contraction in area of the wound. This decrease in epithelization period indicates the wound healing effect of the drug **(Table 5).**

In dead space wound model dry weight and breaking strength of granulation tissue were studied on 10^{th} post wounding day. A significant increase in breaking strength was observed with **HHHIP2,** and **HHHIP3,** when compared with control group. The treatment groups showed an increase in mean dry weight of granulation tissue. **HHHIP2,** and **HHHIP3** exhibited significant ($P<0.001$) increase in dry weight of granulation tissue as compared to the control **(Table 6)**.

Hence, three different models have been chosen in this study to assess the effect of *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract on wound healing. This centripetal movement of wound margin promotes epithelization either by facilitating the proliferation of epithelial cell or by increasing the viability of epithelial cells. Thus, it appears that *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. showed significant increase in wound contraction and promote epithelization. Increase in breaking strength of granulation tissue indicates increased cross linking followed by enhanced collagen maturation whereas dry weight of granulation tissue indicates the presence of higher protein content, which in turn is related to healing property. Histopathological examination revealed predominant collagen formation, increased capillaries, few fibroblasts and scant or no macrophages in animals treated with **HHHIP2,** and **HHHIP3** evidencing the improvement in wound healing. Phytoconstituents like flavonoids and phenolic compounds are reported to be responsible for wound healing property in the plants. Drugs that inhibit lipid peroxidation are believed to increase the viability of collagen fibrils by increasing their strength, improving

the circulation, preventing the cell damage and promote the DNA synthesis. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cellular necrosis but also by improving vascularity. Flavonoids and phenolic compounds reported to responsible for wound healing activity. The presence of these constituents in the *Hemidesmus indicus* (L.) R. BR. var. *Pubescens* (WT. & ARN.) HOOK F. hydro alcoholic root extract may be responsible for the wound healing activity.

5. Conclusion

The present investigation successfully demonstrated the development and evaluation of a novel hydrogel-based topical drug delivery system incorporating the hydro-alcoholic root extract of Hemidesmus indicus (L.) R. Br. var. pubescens for enhanced wound healing. Among the prepared formulations, HHHIP3 emerged as the most optimized and efficacious hydrogel based on its physicochemical characteristics, safety profile, and pharmacological performance.

Physicochemical evaluation confirmed that all hydrogel formulations possessed desirable attributes for topical application, including acceptable viscosity (1078–1148 cps), skin-compatible pH (6.2–6.4), good transparency, smooth texture, high moisture retention (up to 97.73%), and absence of microbial contamination over the stability period. Acute dermal toxicity studies further established the safety of the formulations, as no signs of irritation, behavioral abnormalities, or mortality were observed at a limit dose of 2000 mg/kg, indicating excellent dermal tolerability.

In vivo wound healing studies provided clear and quantitative evidence of the therapeutic potential of the developed hydrogel. In the incision wound model, HHHIP3 significantly enhanced tensile strength (1550 ± 12.91 g) compared to the control group (755 ± 13.41 g) and was comparable to the standard drug Framycetin sulfate (1580 ± 29.68 g), indicating improved collagen maturation and wound integrity. In the excision wound model, HHHIP3 showed accelerated wound contraction, achieving 96 ± 0.40% closure by the 18th day, along with a reduced epithelization period (21 ± 0.26 days), closely matching the standard treatment and markedly outperforming the control group. Similarly, in the dead space wound model, HHHIP3 significantly increased granulation tissue dry weight (91 ± 0.31 mg) and breaking strength (455 ± 1.02 g), reflecting enhanced collagen deposition and tissue regeneration. Histopathological findings further corroborated these results by revealing dense collagen fibers, increased fibroblast proliferation, and neovascularization in treated groups **(Image 3)**.

Overall, the results clearly indicate that hydrogel-mediated delivery of Hemidesmus indicus root extract significantly accelerates the wound healing process by promoting wound contraction, epithelialization, collagen synthesis, and tensile strength. The synergistic effect of the herbal extract with the hydrogel matrix and aloe vera not only validates the traditional use of Hemidesmus indicus in wound management but also highlights its potential as a safe, effective, and economical herbal wound-healing formulation. The developed hydrogel, particularly HHHIP3, holds strong promise for further preclinical and clinical investigations aimed at its translation into a clinically useful topical wound care product.

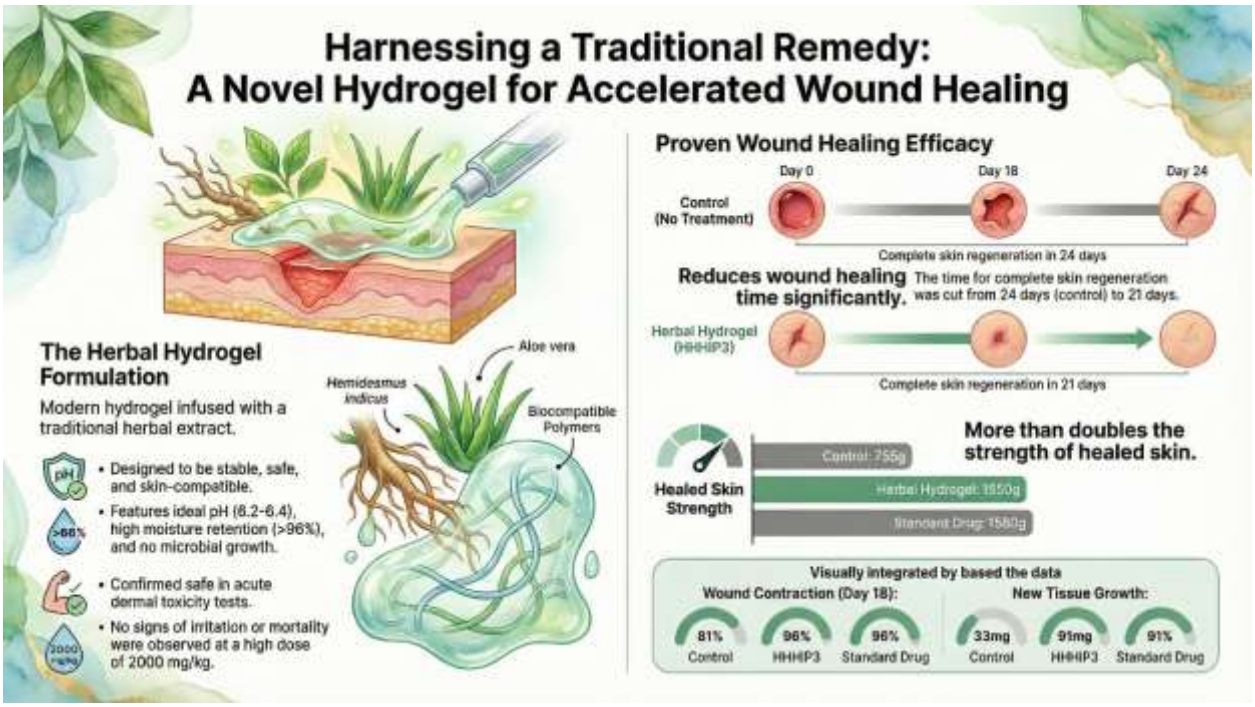


Image 3. Harnessing a Traditional Remedy: A Novel Hydrogel for Accelerated Wound Healing.

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References

1. Pounikar, Y. *et al.* Formulation and characterization of aloe vera cosmetic herbal hydrogel. *Int. J. Pharm. Pharm. Sci.* **4**, 85–86 (2012).
2. Narayanan, V., Gnanavel, I., Care, L., Remedies, P. & Campus, G. Photochemical and Electrochemical Stabilities of Aloe Vera Sap. *J. Appl. Sci.* **3**, 1871–1878 (2007).
3. Atpadkar, K. D. Formulation and Evaluation of Aloe-Vera Herbal Hydrogel. *INTERANTIONAL J. Sci. Res. Eng. Manag.* **09**, 1–9 (2025).
4. Dhadwal, N. & Singh, G. Perparation of aloe vera cosmetic herbel hydrogel. *Int. J. Institutional Pharm. Life Sci.* **5**, 550–555 (2015).
5. Bounty, N. & Moyer, P. E. B. United States Patent (19). 4–6 (1983).
6. Jain, N. *et al.* Phytosome: a Novel Drug Delivery System for Herbal Medicine. *Int. J. Pharm. Sci. Drug Res.* **2**, 224–228 (2010).
7. Ravishankar, M. *et al.* Hemidesmus indicus var. pubescens Root Ameliorates Dextran Sodium Sulfate-induced Ethanol Augmented Ulcerative Colitis in Rats. *Indian J. Pharm. Educ. Res.* **56**, s487–s495 (2022).
8. Sood, A., Granick, M. S. & Tomaselli, N. L. Wound Dressings and Comparative Effectiveness Data. *Adv. Wound Care* **3**, 511–529 (2014).
9. Boateng, J. S., Matthews, K. H., Stevens, H. N. E. & Eccleston, G. M. Wound healing dressings and drug delivery systems: A review. *J. Pharm. Sci.* **97**, 2892–2923 (2008).
10. Chattopadhyay, S. & Raines, R. T. Review collagen-based biomaterials for wound healing. *Biopolymers* **101**, 821–833 (2014).
11. Dragan, E. S. Design and applications of interpenetrating polymer network hydrogels. A review. *Chem. Eng. J.* **243**, 572–590 (2014).

12. Caló, E. & Khutoryanskiy, V. V. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur. Polym. J.* **65**, 252–267 (2015).
13. Ahmed, E. M. Hydrogel: Preparation, characterization, and applications: A review. *J. Adv. Res.* **6**, 105–121 (2015).
14. Rial-Hermida, M. I. *et al.* Recent Progress on Polysaccharide-Based Hydrogels for Controlled Delivery of Therapeutic Biomolecules. *ACS Biomater. Sci. Eng.* **7**, 4102–4127 (2021).
15. C, S. & S, D. P. ISOLATION, PURIFICATION AND CHARACTERIZATION OF HEMIDESMUS INDICUS R.BR. ROOT EXTRACT. *Int. Res. J. Pharm.* **5**, 343–347 (2014).
16. Sukandar, D., Hermanto, S., Amelia, E. R. & Novianti, C. P. Karakterisasi Fraksi Aktif Antioksidan dari Ekstrak Etanol Biji Kemangi (Ocimum Basilicum L.). *J. Kim. Val.* 39–49 (2015) doi:10.15408/JKV.V0I0.3598.
17. Kumar, K. P. S., Bhowmik, D., Chiranjib & Biswajit. Aloe vera: a potential herb and its medicinal importance. *Jounal Chem. Pharm. Res.* **2**, 21–29 (2010).
18. Manish Kumar Gupta, Sujit Nagare, Birendra Shrivastava, Supriya Hyam & Ketaki Dhane. Preparation and Evaluation of Topical Polyherbal Emulgel Formulation for its Wound Healing Potential. *Int. J. Res. Pharm. Sci.* **11**, 8181–8186 (2020).
19. Gouri Dixit*, Ganesh Misal, V. G. and K. U. FORMULATION AND EVALUATION OF POLYHERBAL GEL FOR ANTI - INFLAMMATORY ACTIVITY Gouri Dixit*, Ganesh Misal, Vijay Gulkari and Kanchan Upadhye Department of Pharmaceutics, J. L. Chaturvedi College of Pharmacy, Electronic zone, MIDC, Hingna road, Nagpur- 4400. *Internatiional J. Pharm. Sci. Res.* **4**, 1186–1191 (2013).
20. Waresindo, W. X. *et al.* Freeze-thaw hydrogel fabrication method: basic principles, synthesis parameters, properties, and biomedical applications. *Mater. Res. Express* **10**, (2023).
21. Gopinath, D. *et al.* Dermal wound healing processes with curcumin incorporated collagen films. *Biomaterials* **25**, 1911–1917 (2004).
22. Sadaf, F., Saleem, R., Ahmed, M., Ahmad, S. I. & Navaid-ul-Zafar. Healing potential of cream containing extract of Sphaeranthus indicus on dermal wounds in Guinea pigs. *J. Ethnopharmacol.* **107**, 161–163 (2006).
23. Lodhi, S. & Singhai, A. K. Wound healing effect of flavonoid rich fraction and luteolin isolated from Martynia annua Linn. on streptozotocin induced diabetic rats. *Asian Pac. J. Trop. Med.* **6**, 253–259 (2013).
24. Shetty, S., Udupa, S. & Udupa, L. Evaluation of Antioxidant and Wound Healing Effects of Alcoholic and Aqueous Extract of Ocimum sanctum Linn in Rats. *Evid. Based. Complement. Alternat. Med.* **5**, 95–101 (2008).
25. Patil, M. V. K., Kandhare, A. D. & Bhise, S. D. Pharmacological evaluation of ethanolic extract of Daucus carota Linn root formulated cream on wound healing using excision and incision wound model. *Asian Pac. J. Trop. Biomed.* **2**, (2012).
26. Karakaş, F. P. *et al.* The evaluation of topical administration of Bellis perennis fraction on circular excision wound healing in Wistar albino rats. *Pharm. Biol.* **50**, 1031–1037 (2012).
27. Murthy, S. *et al.* Evaluation of in vivo wound healing activity of Bacopa monniera on different wound model in rats. *Biomed Res. Int.* **2013**, (2013).