OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences

Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Pharmacy**

A Novel Herbal Hydrogel Loaded with Lupeol Formulation for Wound Healing

Rekha Kumari[1]*, Jitender K Malik[1]

[1]Faculty of Pharmacy, P. K. University, Shivpuri (MP)

DOI: https://doi.org/10.36348/sjmps.2026.v12i05.003 | **Received:** 11.03.2026 | **Accepted:** 05.05.2026 | **Published:** 06.05.2026

***Corresponding author:** Rekha Kumari
Faculty of Pharmacy, P. K. University, Shivpuri (MP)

Abstract

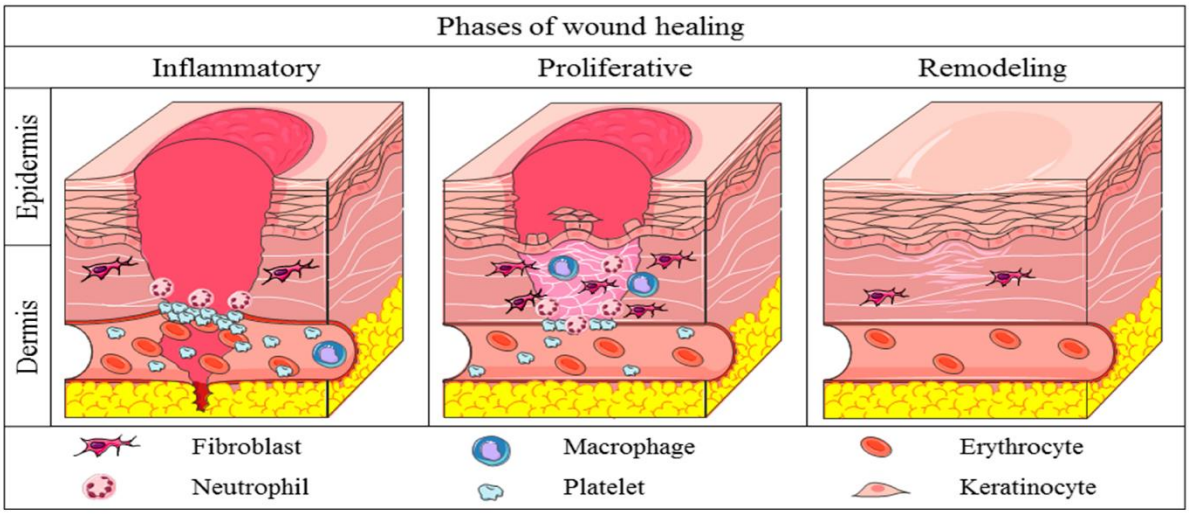
Background: Proper treatment of wounds is crucial, as untreated wounds can potentially become fatal. Ethnomedicinal herbs possess the ability to heal wounds without causing any adverse consequences, unlike chemical medications which are increasingly associated with negative effects. Hydrogels are highly promising and extensively employed in the realm of biomedicine. Hydrogel dressings have made tremendous advancements in their ability to reduce inflammation, effectively addressing many clinical problems encountered in efforts to enhance wound healing. Lupeol, a triterpene phytoconstituent, is present in numerous fruit plants and medicinal plants that have been extensively researched for its potential in treating various ailments, including skin wounds. ***Objective:*** The aim of present investigation is to assessed the wound healing efficacy of lupeol loaded hydrogel. ***Method:*** A hydrogel was synthesized by combining Carbopol 934 with HPMC polymers in a 1:1 ratio. The wound healing potential was assessed using both the excision and incision models, as well as by measuring the hydroxyproline content. ***Result:*** The excision wound study demonstrated that the H1 formulation including lupeol exhibited a substantial outcome that was comparable to the usual treatment. The duration of epithelization was determined to be 18 days. The incision wound model demonstrated that the tensile strength of H1 was much greater than the standard. The current study determined that the hydroxylproline content of formulation H1, which contains lupeol, was measured to be 43.52 +0.42, which is comparable to the standard. ***Conclusion:*** The exploration has demonstrated that the formulation has the ability to enhance the activity of wound healing. The discovery indicated that lupeol promotes the formation of new blood vessels, the expansion of fibroblast cells, and the production of cytokines and growth factors that are essential for the process of wound healing.

Keywords: Hydrogel, Lupeol, excision, incision model & hydroxylproline content.

INTRODUCTION

A wound is characterized by the disruption of cellular and anatomical structures in a tissue [1]. Tissue can sustain damage from exposure to chemicals, physical force, heat, microorganisms, or immune system responses. Uncomfortable wounds are more prone to developing infected wounds and other problematic issues [2]. Several factors such as diabetes, immune system disorders, ischaemia, malnutrition, aging, local infections, and local tissue damage from burns or gunshots might impede the process of wound healing. Infection is a prevalent consequence of burn injuries, contributing to 50-75% of deaths in hospitals. The user's text is "[3]". Wound healing is a systematic series of processes that restore the structural integrity of injured tissue. Scientists are seeking alternatives to herbal-based therapies for wound healing due to their tendency to be both less costly and fraught with issues such as drug resistance and allergic reactions. The user's text is "[4]". Worldwide, over 80% of individuals continue to rely on traditional medications to address their health conditions [5]. They play a crucial role in wound management [6] as they promote the creation of an ideal environment by providing moisture. The traditional system of medicine claims that several medicinal plants can aid in wound healing, despite the lack of sufficient investigation into their mechanisms of action and effectiveness. The initial stage of this process involves an intense inflammatory response, which is then followed by the synthesis of collagen and other large molecules outside of cells. These molecules are eventually modified to form a scar [7]. In essence, this process is a response of the connective tissue. A wound is the outcome of the skin being torn, slashed, burned, or punctured [8]. Various physiological systems, such as white blood cells [9],

fibroblasts, and keratinocytes, typically contribute to the process of wound healing at the location of the wound. Resting energy consumption (RES) is associated with increased metabolism of proteins, lipids, and glucose. Antibiotics and anti-cancer medications can also exert an effect. Various illnesses, including diabetes and others, impact the process of wound healing. Wound infection is prevalent in underdeveloped nations due to unsanitary conditions [10–13]. An effective wound healing approach is necessary to restore the damaged anatomical continuity and altered functional condition of the skin. Wounds are physical injuries that result in the splitting or opening of the skin [14]. Traditional healers in countries such as India and China possess valuable knowledge about other lesser-known wild herbs that can be used to cure burns and wounds. A contemporary field of research in the biomedical sciences is the investigation of pharmaceuticals designed to heal wounds [15–18]. Scientific research is currently being conducted on traditional medical practices that have been used for a long time in Asia and Africa to treat disorders associated to wounds. Traditional medical practices from various cultures commonly use topical application of medicinal plants or their extracts, either alone or in combination with other plant components, to cure wounds [19].



Phases of wound healing

A hydrogel is a three-dimensional structure composed of hydrophilic polymers, characterized by its elasticity and porosity. It typically contains a minimum of 10% water, often exceeding 90%. Hydrogels are categorized based on the technique of formation, the characteristics of the polymers, the ionic charge, or the types of links between the polymer chains. Hydrogels offer numerous advantages in the treatment of wounds for patients, including the prevention of complications, accelerated wound healing, improved patient quality of life, and enhanced therapeutic outcomes. Hydrogels has advantageous characteristics that render them well-suited for the management of profound wounds, including non-adhesiveness, moisture retention, gas permeability, exudate absorption, biocompatibility, and patient comfort. Furthermore, they bear a resemblance to dermal tissue, exhibiting a structure similar to the extracellular matrix. They possess the capability to facilitate cell migration and can cause partial tissue regeneration. An important benefit is that the inherent characteristics of hydrogels can be enhanced by including active substances, such as antibiotics, nanoparticles, stem cells, and growth hormones [20-21].

Lupeol is a pentacyclic triterpenoid with the chemical formula $C_{30}H_{50}O$. It has a melting point of 215–216 °C. The structural analysis of Lupeol reveals that it has a molecular weight of 426.7174 g/mol, 1 H-Bond donor, 1 H-Bond acceptor, 1 rotatable bond count, an accurate mass of 426.386166, and a mono isotopic mass of 426.386166 [22].

Experimental Work

Preparation of Hydrogel systems for Pharmacological evaluation

The hydrogel producing polymers were dissolved in a separate container using different quantities of double distilled water, as indicated in Table no.1. Subsequently, glycerine and sodium benzoate were added to the mixture. Subsequently, an active ingredient was included into the mixture, resulting in a final volume of 100 ml. Next, the sample was subjected to sonication using a Lark probe sonicator at a frequency of 6φ for a duration of 20 seconds at a temperature of 280°C. The aforementioned composition was left undisturbed for 24 hours at ambient temperature. The pH of this gel mixture was consistently maintained at 6±0.4 and stored in a tightly sealed container. No detrimental or dangerous effects, nor death of experimental animals, were detected when administering the maximal dose of 2000 mg/kg b.w. of Cleome viscosa Linn. seeds extracts [23].

Table 1: Composition of Hydrogel formulations

S. No.	Components	Formulation H1
1.	Carbopol 934	500mg
2.	HPMC	500mg
3.	Acaica	500mg
4.	Glycerine	2.ml
5.	Sodium benzoate	100mg
6.	Lupeol	30mg

***In – vivo* Evaluation for Wound Healing Activity [24-25]**

Selection and procurement of animals

We obtained albino rats and chose rats of both genders (weighing 150-200 g). The rats were kept at a temperature of 24-28°C, housed individually, and provided with unlimited access to food and water. The rats were provided with a regular food and housed in a well-ventilated animal facility. The rats were subjected to a 12-hour cycle of alternating darkness and light for the duration of the study. In order to conduct the experiment, the mice were separated into seven groups, with each group including six animals.

Group I - CONTROL
Group II - TEST (H1)
Group III - Standard [Standard Povidone-Iodine (5% w/w) Ointment]

EXCISION WOUND MODEL:

In the excision wound investigation, six animals were chosen for each group. A circular wound with a diameter of approximately 2.5 cm was created on the depilated dorsal thoracic area of rats. The rats were depilated while under light ether anesthesia in a semi-aseptic environment. They were then monitored for the duration of the study. The rats were individually housed. The various groups were segregated and subjected to hydrogel systems on the wound once daily for a duration of 14 days, commencing from the day of injury. The assessment of wound closure percentages will be conducted on the 4th, 8th, 12th, and 16th days following the injury. Additionally, the process of epithelization, as well as the size and shape of the scar, will be documented. The samples were administered daily for a duration of 16 days, commencing on the day of injury. They were then assessed for the specified criterion.

Wound contraction And Epithelization time: -

Wound contraction was measured plannimetically using a transparent paper at four days interval. It was observed that after simple ointment treatment, % wound contraction reached to maximum in 22 days, but in standard group 18 days were found to be required for 100% contraction or complete wound healing. Mean of epithelization time of extract was found to be lesser (18day) compared to control groups (22 days).

The percentage wound contraction was determined using the following formula

$$\% \text{ Closure} = \frac{\text{Wound area on corresponding days - Wound area on day zero X 100}}{\text{Wound area on day zero}}$$

***INCISION WOUND MODEL*:**

Using a sharp blade, two 6 cm incisions were made on both sides of the vertebral column, going through the entire thickness of the skin, while the patient was under light ether anesthesia. The cuts were stitched using Ethicon 4-0 silk thread using a straight round bodied needle. The sutures will be extracted on the 8th day after the wound, and the tensile strength will be measured on the 10th day after the wound using a tensiometer.

Measurement of tensile strength:

Tensile strength refers to the ability of a material to withstand breaking when subjected to tension. The tensile strength of the recently regenerated tissue, including the scar, was measured using a Tensiometer after excising it.

ESTIMATION OF HYDROXYPROLINE AS BIOCHEMICAL MARKER

Collagen is the main protein found outside of cells in the scab of a healing wound. After an injury, there is a quick rise in the production of this protein in the area of the wound. Collagen breakdown results in the release of hydroxyproline and its peptides. Hydroxyproline assessment is used as a measure of collagen turnover.

A circular wound of approximately 500 mm2 was produced, following the excision wound concept. Over a span of 19 days, topical application of ointments was performed on excision wounds. On the 20th day, the eschar was extracted and dehydrated in an oven at around 110 °C. The substance was measured, with a weight of 10 milligrams, and stored in test tubes with glass stoppers. 1 mL of 6 N HCl was added to each tube holding 10 mg of the dried eschar. The tubes were subsequently placed in a boiling water bath for a duration of 24 hours, with 12 hours allocated to each day over a span of two days, in order to facilitate hydrolysis. After cooling, the hydrolysate was treated with an excess amount of acid, which was then neutralized using 10 N NaOH. Phenolphthalein was used as an indicator. The neutral hydrolysate was diluted with distilled water to

achieve a concentration of 20 mg/mL. The ultimate hydrolysate was utilized to determine the hydroxyproline content.

Hydroxyproline (HPR). 1 mL of hydrolysate, 2.5 N NaOH, 0.01M $CuSO_4$, and 6% H_2O_2 were added to each tube. The tubes were aggressively agitated and promptly placed in a water bath set at a temperature of 80°C. After a duration of 15 minutes, the tubes were taken out and allowed to cool for a period of 5 minutes in cold water. 0.6 mL of a recently made 5% solution of paradimethyl aminobenzaldehyde in n-Propanol and 1.2 mL of 3 N H_2SO_4 were included. The test tubes were once again immersed in a hot water bath at a temperature of 75°C for a duration of 15 minutes, followed by a cooling period of 5 minutes under a running stream of water. The spectrophotometer was used to measure the color intensity at 540 nm by comparing it to the blank. The hydroxyproline content in the tissue was determined using a standard curve generated with a 4-Hydroxy-L-proline standard (CDH Laboratories Pvt. Ltd., New Delhi, India), ranging from 10 to 100 g/mL.

RESULT AND DISCUSSION

Modern drug delivery systems (DDS) are developed employing cutting-edge technology to expedite the administration of drugs across the body to a particular target location, hence optimizing the effectiveness of treatment and reducing the buildup of drugs in unintended areas. Consequently, they have a significant impact on the management and treatment of diseases. Recent drug delivery systems (DDS) offer significant benefits compared to traditional methods. These advantages arise from their improved performance, automation, precision, and effectiveness. Hydrogel dressings Hydrogel dressings consist of hydrophilic polymers arranged in three-dimensional networks. They provide a damp environment at the wound site, which enhances the growth of new tissue through the processes of granulation and re-epithelialization. Hydrogel dressings have been effectively utilized for the management of persistent wounds. Hydrogel-based dressings have the advantage of being easily modifiable compared to other techniques for treating chronic wounds. A formulation including lupeol (H1) was produced for the study of its pharmacological potential. The efficacy of the selected plant in hydrogel dosage form was tested (table 1). The hydrogel containing lupeol demonstrated an epithelization time of 18 days, as depicted in Figure 2. The percentage of wound contraction was determined to be 99.16% on the 16th day, which closely matched the standard values (as shown in Table 1 and Figure 1). The incision wound model demonstrated that the tensile strength of H1 was significantly higher than the standard, as indicated in table 3 and figure 3. Collagen, the primary protein in connective tissue, is mainly made up of glycine, proline, and hydroxyproline. Collagen production necessitates the hydroxylation of lysine and proline, as well as the presence of co-factors like ferrous iron and vitamin C. Collagen breakdown releases unbound hydroxyproline and associated peptides. Therefore, the quantification of hydroxyproline can serve as a biochemical indicator for the presence of tissue collagen and as a measure of collagen metabolism. An elevation in hydroxyproline levels signifies an augmentation in collagen production, which is associated with improved wound healing. The current study determined that the hydroxylproline content of formulation H1, which includes lupeol, was 43.52 +0.42, which is comparable to the standard (as shown in table 4 and figure 4).

Table 2: Mean Percentage closure of Excision wound area by different formulations

Groups	% Wound contraction				Period of epithelization(days)
	4^{th}day	8^{th}day	12^{th}day	16^{th}day	
Control	17.65 ±1.28	38.99 ±1.35	56.92 ±1.58	76.52 ±1.75	27
H1	29.92 ±1.80*	58.12±1.25*	87.58 ±1.26**	99.16***	18
Standard	31.15 ±1.18	57.88 ±1.85	87.79 ±1.00	99.82 ±0.68	20

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

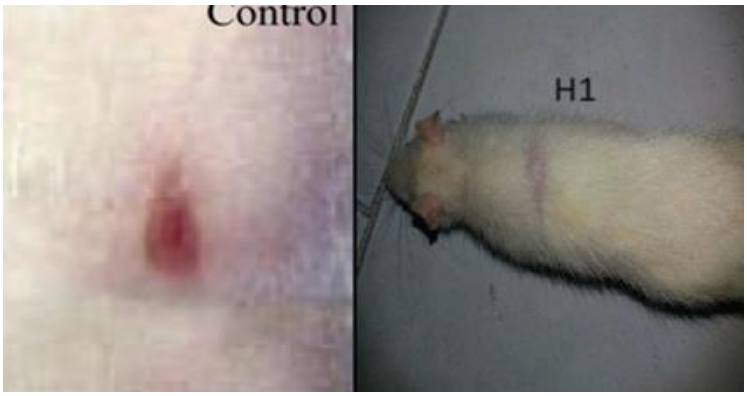


Fig. 1: Excision wound model

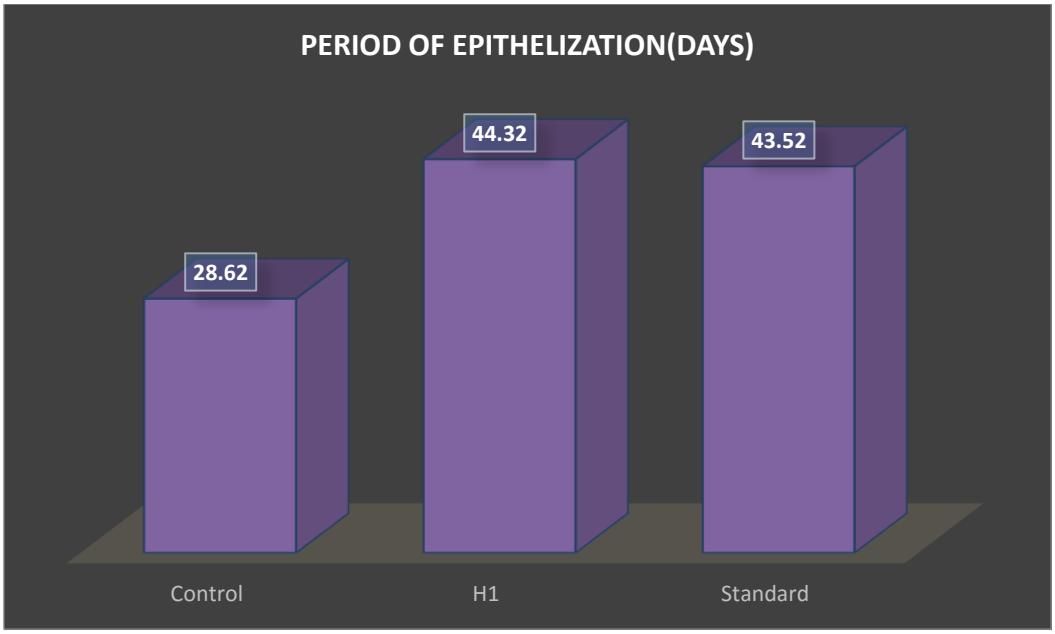


Fig. 2: Period of epithelization of various formulation treated group

Table 3: Mean Tensile strength (Incision model)

S. No.	Groups	Breaking strength (gm)
1.	**Control**	243.56 ± 1.27
2.	**H1**	726.54 ±2.18
3.	**Standard**	727.12 ±4.02

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

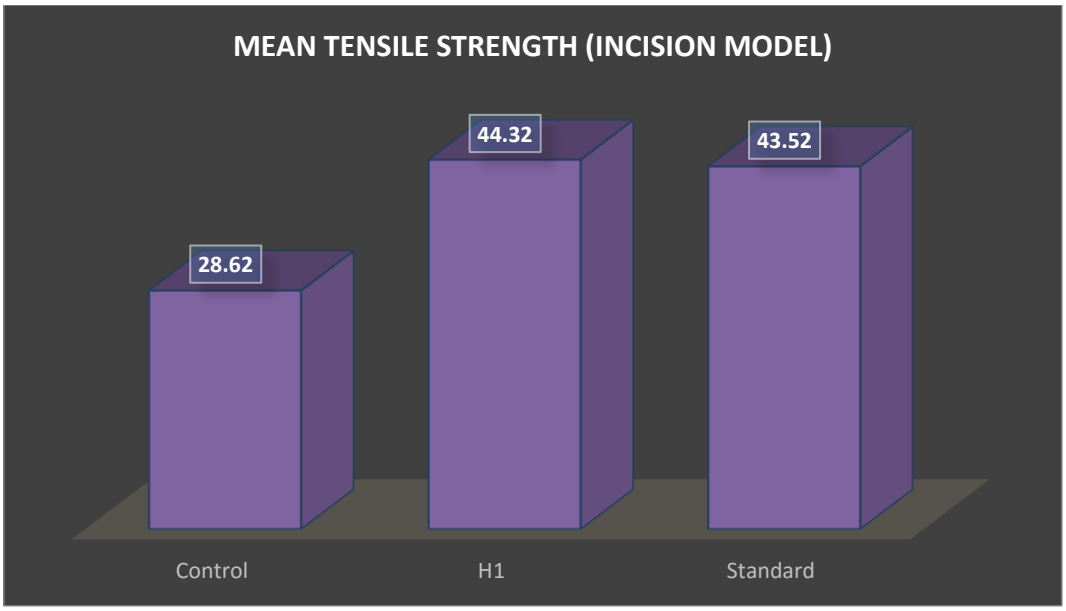


Fig. 3: Tensile strength of various formulation

Table 4: Hydroxyl proline content determination

Group (N= 6)	Hydroxyproline (µg/ 500 mg)
Control	28.62 ±0.57
Standard	44.32±0.82
H1	43.52 ±0.42

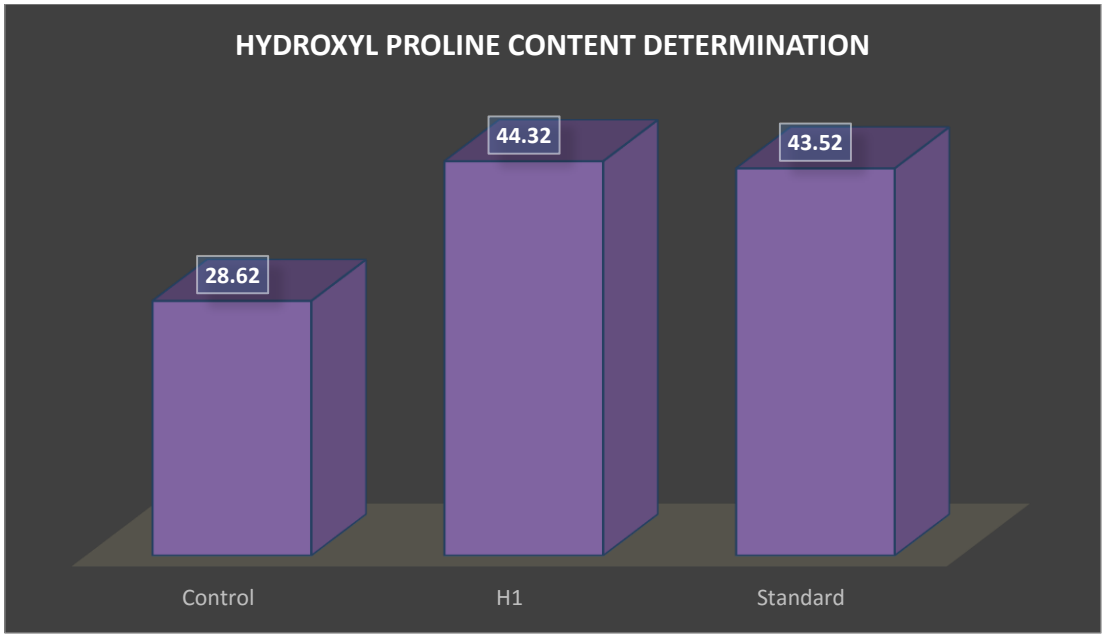


Fig. 4: Hydroxyl proline content of various formulation

CONCLUSION

The current research study focused on developing a novel topical herbal hydrogel that promotes moist wound healing for therapeutic purposes. Based on the findings, it was determined that the hydrogel containing lupeol exhibited significant effects on the healing of skin wounds.

REFERENCES

1. Bennet, R.G. *Fundamentals of cutaneous surgery*. St. Louis C.V Mosby, 1988, p.78
2. Ingold, W.M. *Wound therapy: growth factors as agents to promote healing. Trends in Biotechnol*, 1993, 11: 387-392.
3. Mokadas, E., Rotimi V O, Sanyal SC. *In vitro activity of Piperacilin itazobactam versus other broad –spectrum antibiotics against nosocomial gram-negative pathogen isolated from burn patients.* J. Chemotherapy, 1998, 10: 208-214.
4. Sai, K. P and Babu M. *Traditional medicine and practices in burn care: need for newer scientific perspectives. Burns*, 1998, 24:387-388.
5. Kumara, P.D, Jayawardane G L, and Aluwihare AP. *Complete colonic duplication in an infant Ceylon*. Med. Med. J., 2001, 46: 69-70.
6. Purna, S.K and Babu M, *Collagen based dressing-a review. Burns,* 2000, 26: 54-62.
7. Chitra P., Sajithal GB and Chanrakaran G., Influence of Aloe vera on collagen turnover in healing of dermal wounds in rats. Ind J Exp Biol., 1998, 36, 896.
8. J. W. Rope, J. A. R Smith and June Edhouse, (Eds), in; *the Management of Wounds*
9. *and Burns*, 2nd Edn, Oxford University Press, London, (1999) pp. 1-3
10. Dohanics J and Lintar EA., *Osmotic stimulationeffect of dexamethasone peptides*. Ann J Physiology, 1990, 1(10), 51.
11. Ranonnsky AJ and Bagloy GJ., *Increased muscle glucose uptake and lactate release after endotoxin administration.* Ann J Physiology, 1980,3 (11), 23.
12. Fain JN and Kovacev V., *Effect of growthhormone and dexamethasone or lypolysis and metabolism in isolated fat cells of the Rat*. J Bio Chem., 1965,13(12), 35.
13. Moore FD. *Bodily changes during surgical convalescence*. J Ann Surg., 1953,13(11),28
14. Senthil Kumar M, Sripriya R, Vijaya Raghavan H, Sehgal P. 2006. *Wound Healing Potential of Cassia fistula on Infected Albino Rat Model*. *J Surg Res* 131: 283–289.
15. Meenakshi S, Raghavan G, Nath V, Ajay Kumar SR, Shanta M. 2006. *Antimicrobial, wound healing and antioxidant activity of Plagiochasma appendiculatum* Lehm. et Lind. *J Ethnopharmacol* 107: 67–72.
16. Enoch S, John Leaper D. 2005. *Basic science of wound healing. Surgery* 23: 37 – 42.
17. Sumitra M, Manikandana P, Suguna L. 2005. *Efficacy of Butea monosperma on dermal wound healing in rats*. *Int J Biochem Cell Biol* 37: 566–573
18. Biswas TK, Mukherjee B. 2003. *Plant Medicines of Indian Origin for Wound Healing Activity:* A Review.*Lower Extr Wounds* 2: 25–39.
19. Himesh Soni etal. Evaluation of Wound Healing Activity of Methanolic extract of Annona Squamosa Leaves in Hydrogel delivery system. Am. J. PharmTech Res. 2018; 8(3).
20. Himesh Soni *etal*. Recent Update of Botanical for Wound Healing Activity. IRJP,3(7) (2012)1-7.
21. Calo E, Ballamy L, Khutoryanskiy VV. Hydrogels in wound management. In Hydrogels: Design,

Synthesis and Application. Drug Delivery and Regenerative Medicine; 2018. London: CRC Press.
22. Stan D, Tanase C, Avram M, Apetrei R, Mincu NB, Mateescu AL, Stan D. Wound healing applications of creams and "smart" hydrogels. Exp Dermatol. 2021 Sep;30(9):1218-1232.
23. Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett. 2009 Nov 28;285(2):109-15.
24. Himesh Soni *et al.,* Efficacy of Hydrogel Containing Rutin in Wound Healing. EAS J Pharm Pharmacol; Vol-3, Iss-6 (Nov-Dec, 2021): 161-167.
25. Himesh Soni etal (2022). Potential of Polyherbal Formulation in Burn Wound Model. Sch Int J Tradit Complement Med, 5(1): 19-23.
26. Cissell DD, Link JM, Hu JC, Athanasiou KA. A Modified Hydroxyproline Assay Based on Hydrochloric Acid in Ehrlich's Solution Accurately Measures Tissue Collagen Content. Tissue Eng Part C Methods. 2017 Apr;23(4):243-250.