Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14,issue.33s (2025)
https://www.jneonatalsurg.com

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Evaluation Of Wound Healing Efficacy Of Hydrogel Containing Dalbergia Latifolia (Roxb) Bark Extract.

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Cite this paper as Dhanush KR, Ashoka Babu VL, Damodar Nayak A (2025) Evaluation Of Wound Healing Efficacy Of Hydrogel Containing Dalbergia Latifolia (Roxb) Bark Extract.. .Journal of Neonatal Surgery, 14, (33s), 1-17

ABSTRACT

Skin is vulnerable to a variety of injuries, necessitating effective wound healing solutions. The present study aims to evaluate wound healing properties of Dalbergia latifolia (Roxb) bark. Alcoholic extract was prepared and analysed by GC–MS and further subjected to molecular docking studies. The hydrogel was prepared with 5%, 7.5%, and 10% concentrations of Dalbergia latifolia (Roxb) bark extract and characterized by pH, viscosity, swelling index, drug content, in-vitro drug release and kinetic study. Wound healing potential of alcoholic extract was evaluated by incision and excision wound models following acute dermal irritation studies. Histopathological studies and haematological parameters were determined for excision model, tensile strength was performed for incision wound skin. Phytochemical screening showed the presence of flavonoids, phytosterols, phenolics, and tannins in alcoholic extract. GC-MS analysis revealed multiple phytoconstituents, Molecular docking studies performed with targets TGF-β, EGFR, GSK-3β, IL1, and FGFR1, 5QU0 revealed high binding affinities. ADMET studies showed better pharmacokinetic and drug likeliness property. Acute dermal irritation tests showed no significant irritation, while in vivo studies proved that 7.5 % hydrogel has potential wound healing efficacy. Histopathological examination confirmed enhanced fibroblast proliferation and collagen formation in treated wounds. In conclusion, hydrogels contain- D. latifolia extract demonstrated significant potential for wound healing, supported by both chemical analysis and biological evaluation..

Keywords: Dalbergia latifolia (Roxb), Hydrogel, GC-MS, In-vitro drug release and kinetic, hydroxyproline and hematology

1. INTRODUCTION

Skin, the outermost layer of the body, is easily damaged through mechanical, chemical, physiological, and radiation injuries. Mild injuries heal quickly, while extensive damage may require therapeutic therapy. Hydrogels are three-dimensional, water-absorbent network structures found naturally in polymer networks like collagen or gelatin or synthesized. Hydrogels, sensitive to environmental changes like volume, have various applications due to their adaptability, with traditional stimuli like pH, temperature, and ionic strength triggering reactions. Hydrogel reactions, triggered by analyses and biomarkers like glucose, proteins, and DNA, are utilized in various applications like sensors, drug delivery systems, biosensors, and artificial organs [1].

Dalbergia latifolia (Roxb), a tropical Asian timber tree, is renowned for its decorative and fragrant wood, rich in aromatic oils, and is primarily found in Nepal, India, and Indonesia. Traditionally used for treating diarrhoea, indigestion, and leprosy, it has ethanomedical properties like vermicidal, appetite-increasing, cough suppressant, nausea, gastric upset, blood-borne diseases, antibacterial, and skin disorders [2, 3]. *D. latifolia's* medicinal properties are attributed to flavonoids, isoflavonoids, glycosides, and steroids, including dalbinol, sisafolin coumarin, β-sitosterol, lupeol, latifolin, dalbergin, latinone, and neoflavonoid dalcriodon [4].

Recent studies shows that topical agents with flavonoids and triterpenoids can improve wound healing by preventing infection in burns or wounds [5, 6]. The presence of flavonoids, phenolic compound tannins made us to choose this plant for the present studies.

Material and Methods:

Plant material

Plant material was collected in December 2023 from Karehalli, Hassan district, India. The collected plant material was identified and authenticated by Dr. Noorunnisa Begum, Curator, FLRHT, Bangalore (FRLHT-Acc.No. 6152).

Preparation of alcoholic extract: 250g of dried powdered drug was extracted in soxhlet apparatus with 70% alcohol for 48 hours, filtered, and concentrated using rotary film evaporator. The prepared extract was subjected to preliminary phytochemical analysis.

GC-MS analysis:

The GC-MS analysis of *Dalbergia latifolia* (Roxb) extract was conducted using an Agilent 8890 MSD at SAIF IITM, Madras. The phytoconstituents were identified using open lab CDS 2.5 software and NIST library.

Formulation of hydrogel

Using a magnetic stirrer, 1gm of Carbopol-934 was dissolved in 100 ml of distilled water at 38-55 ^{o}C with 0.5g sodium benzoate. The prepared solution was stored overnight and stirred with 10ml glycerine and 5ml ethanol, 2-3 drops of triethanolamine was added and mixed until a gel formed. 5%, 7.5% and 10% hydrogel were prepared by dissolving 5g, 7.5g and 10 g of extract in distilled water respectively and then mixed with prepared hydrogel. [7]

Characterization of Hydrogel

The prepared hydrogel was tested for various physicochemical properties, including visual examination, pH, viscosity, spreadability, swelling index, percentage content analysis, biodegradation and ATR IR. [7]. The prepared formulation's colour, consistency, homogeneity, and lump presence were examined. A hydrogel was dissolved in distilled water, and the pH was measured using a digital pH meter. The experiment was repeated three times, and the average was calculated. The viscosity of the bioactive hydrogel system containing D.latifolia was measured using a Brookfield DV II+ viscometer at 25 ^{o}C and 12 revolutions per minute. The hydrogel's spreadibility was assessed by sandwiching it between two glass slides, applying weight, and measuring its diameter, which was repeated three times to determine the average. The hydrogel sample was weighed, soaked in a pH 7.4 phosphate buffer solution, and then wiped with tissue paper. The swelling percentage was determined using a formula

$$\text{Equilibrium swelling ratio} = \frac{(Wi - Wf)}{Wi} \times 100$$

Whereas: Wi: Initial weight, Wf- Final weight

Analysis of Percentage content of *Dalbergia latifolia* uniformity in hydrogel

The sample was dissolved in methanol for half an hour, then centrifuged at 3000 rpm for 5 minutes. The supernatant was filtered, and the content was verified through UV spectrophotometric analysis at λ max 234 [8].

Biodegradation:

A hydrogel sample was dried in a hot air oven at 50 ^{0}C until a constant weight was achieved. The sample was then immersed in a pH 7.4 phosphate buffer solution for 48 hours, then dried again. The weight of the hydrogel was calculated before and after degradation [8].

$$\text{Water loss} = \frac{(Wi - Wf)}{Wi} \times 100$$

ATR-IR:

Functional group analysis of hydrogel samples was conducted using attenuated total reflectance (ATR-IR) spectroscopy (ALPHA II, Bruker Lab India), ranging from 4000 to 400 cm^{-1} Peaks obtained from the graphs were used to identify the functional groups present in the hydrogel and extract.

***In-Vitro* drug release studies:** *In-vitro* drug release study was performed using dialysis bag technique. The dialysis membrane (Dialysis membrane-70, molecular weight: 12000 to 14000 Da, HiMedia Laboratories Pvt. ltd, Indian) was soaked in distilled water at room temperature for overnight. The dialysis membrane was attached in the Franz diffusion cell. 20 mg of hydrogel sample were dissolved in 10 ml of methanol, and 2ml (2mg) of the sample was deposited in the upper compartment of the Franz diffusion cell. The down compartment is filled with phosphate buffer at pH 7.4. The entire assembly was kept at 37°C and agitated at 150 rpm. 1ml of sample were collected at 5min, 10min, 20min, 30min, 1hr, 2hr, 3hr, 4hr, 5hr, and 6 hours, we added the same amount of release medium to restore the original volume. Then, their absorbance was measured at λ max 234nm [8].

Drug release kinetics of prepared hydrogels

The obtained result of *in-vitro* drug release was applied to the software to get the drug release kinetic studies for hydrogels using the mathematical models equations given below. The various models used for kinetic studies are (first-order, higuchi,

and Hixson-crowell) [8].

First-order, $logCo - \frac{kt}{2.303}$, Higuchi model, $ft = Q = K_H \times t^{1/2}$, Hixson-rowell model, $W^{1/2}-W^{1/3} = kt$

Acute dermal irritation study:

Experimental protocol was approved by IAEC (XXIX/MSRFPH/COG/PG-12/15.02.2024), a study involved healthy, 12-month-old New Zealand white rabbits, a small area of 6 cm^2 shaved for test topical formulation application. The untreated area was a control. Animals were observed for 4 days for signs of edema, erythema, redness, and inflammation [9].

2.12 *In-vivo* wound healing activity

Albino Wister rats (150-200g) were housed under controlled conditions for a week. They were fed a conventional mouse pellet diet and provided with water, with a light and dark cycle. Prior to the studies an approval was obtained from IAEC, (Institution Animal Ethical Committee) Faculty of pharmacy MSRUAS. (IAEC No. XXIX/MSRFPH/COG/PG-12/15.02.2024)

Wound healing activity was assessed by incision and excision wound models

Grouping: Total numbers of groups 6 (Each group has 6 rats)

- Group 1: Normal Control
- Group 2: Positive Control
- Group 3: Standard group
- Group 4: Hydrogel formulation of 5%
- Group 5: Hydrogel formulation of 7.5%
- Group 6: Hydrogel formulation of 10%

Incision wound model

Rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animal is removed using animal hair trimmer. A longitudinal paravertebral incision, 1.5 cm in length were made through the skin and cutaneous muscle on the back. After the incision, surgical sutures was applied to the parted skin. The wounds will be left undressed. The rats were topically applied with extracts once daily. The control animals were left untreated, the sutures were removed on the 8th post wounding day and treatment was continued. The skin-breaking strength or tensile strength was measured on the 14th day [1].

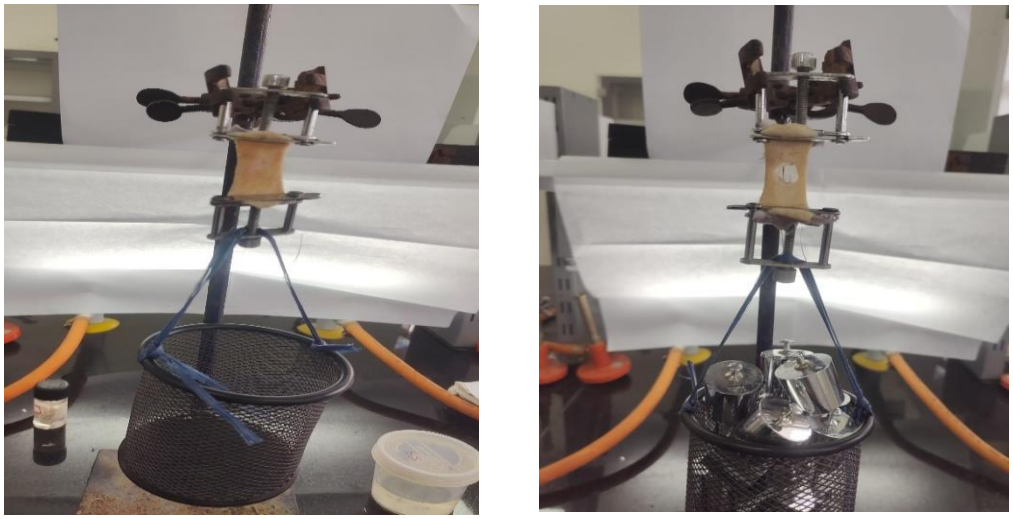
Excision wound model

Rats were anaesthetized prior to and during creation of wound. The hairs were removed the dorsal thoracic region of the rats using animal hair trimmer. To create wound using a Biopsy punch seal of 6mm in diameter were impressed. The drug was topically applied once a day till complete epithelization, starting from the day of the excision. The area of the wounds were measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 2^{nd},4^{th}, 6^{th}, 8^{th},10^{th} and 14^{th} day post wounding. The number of days for falling of scar without any residual raw wound gives the period of epithelisation [1].

Evaluation of parameters

Measurement of Tensile Strength

For determining how much the wound had healed, tensile strength was employed. The instrument used for the measurement of tensile strength had two clamps one was attached to solid support. The excised skin was fixed to one end of the clamp and connected to the empty bag shown in fig 2. On the skin's incised area, the two clamp were firmly placed onto a line facing each other, 1cm away from the healed tissue. Weight was gradually applied to the incision site, causing the outer edges of the wound to separate. Weight application was stopped as soon as wound gaping developed. Calculated and recorded as an approximate measurement of breaking strength in grams. Percentage of tensile strength for standard drug and test drug with respect to negative control treated [5].



(a) (b)

Figure 2. Tensile strength determination (a) Before addition of weight, (b) After addition of weight

Wound reduction size

The wound closure rate was calculated by tracing the wound on different days (2nd, 4th, 6th, 8th, 10th, 12th, and 14th), with wound contraction measured for 14th days in an infected wound model. Changes in wound area were assessed, revealing the rate of contraction and epithelialization period. The surface area was utilized to determine the proportion of wound contraction [7].

$$\% \text{ Wound contraction} = \frac{(\text{Wound area on 1st day} - \text{Wound area on day (n)})}{\text{Wound area on 1st day}} \times 100$$

Where n is number of days (2nd 4th, etc.).

Period of epithelialization

The epithelialization period was defined as the number of days required to reach the end point of complete epithelialization, which was the falling of the scab, leaving no raw wound behind. The average number of days required for wound epithelialization was calculated for each group [10].

Estimation of hydroxyproline

250 mg of soaking tissue was dried for 24 hours at 80°C in a hot air oven. It was weighed and stored in test tubes sealed with aluminium foil. 1ml of 6 N HCl was added to each tube containing 40 mg of dry granulation tissue. To enhance hydrolysis, the tubes were immersed in a hot water bath for a whole day. After cooling the hydrolysate, extra acid was neutralized with 10 N NaOH, and phenolphthalein was utilized as an indicator. The resulting hydrolysate was used to compute hydroxyproline using a standard curve generated using the appropriate substrate [11]. Each tube received 0.3 mL hydrolysate, 2.5 N NaOH, 0.01 M CuSO4, and 6% H2O2. The tubes were shacked thoroughly and placed in an 80°C water bath. The tubes were removed after 15 minutes and cooled in cold water for five minutes. Then, 1.2 mL of 3 N H2SO4 and 0.6 mL of Elhrich reagent were added and immersed in a hot water bath heated to 75°C for fifteen minutes. The test tubes were cooled for five minutes with a steady flow of water. Color intensity was measured at 540 nm and compared to a blank. The standard curve was developed using standard 4-hydroxy L-proline (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The tissue's hydroxyproline concentration was measured from 75 to 900 μg/0.3 mL with a working solution of 3 mg/mL.

2.14 Histopathological studies

All the animals were treated topically with *Dalbergia latifolia* (Roxb) extract. After completion of incision and excision wound healing studies skin tissue was collected for histopathological analysis.

2.15 Statistical analysis

Statistical analysis was performed via one-way and two-way ANOVA using Graphpad prisim version 8.4.2 software was used to analyse the experiments, with the data presented as the mean± the standard error of the mean(SEM). Statistical significance were represented as ns- non-significance, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Results:

Total alcoholic extract

The percentage yield, color, consistency of total alcoholic was tabulated in Table 1

Table 1: Nature of total alcoholic of *D.latifolia* (roxb) bark

Sl. No	Solvent	Color	Consistency	Percentage Yield (% w/w)
1	Alcoholic extract	Dark brown	Semisolid	18.34

Phytochemical analysis

The results of phytochemical screening is presented in Table 2

Table 2. Phytochemical screening of total alcoholic extract of *D.latifolia* (roxb) bark

Sl. No	Test	Alcoholic extract
1	Alkaloids	-
2	Glycosides	+
3	Phytosterols	+
4	Phenolic compounds	+
5	Flavonoids	+
6	Fixed oils and fats	-
7	Saponins	-
8	Protiens and amino acids	-
9	Gums and mucilages	-

GC-MS analysis: The extract was analyzed for its phytochemicals using GC-MS, and revealed 38 compounds. The data was analyzed for retention time, peak area, and score (>500) with reference to the NIST database library to identify important secondary metabolites. Most compounds were eluted within 20-40 minutes. The GC-MS chromatogram and spectrum for identified compounds are shown in fig-3.

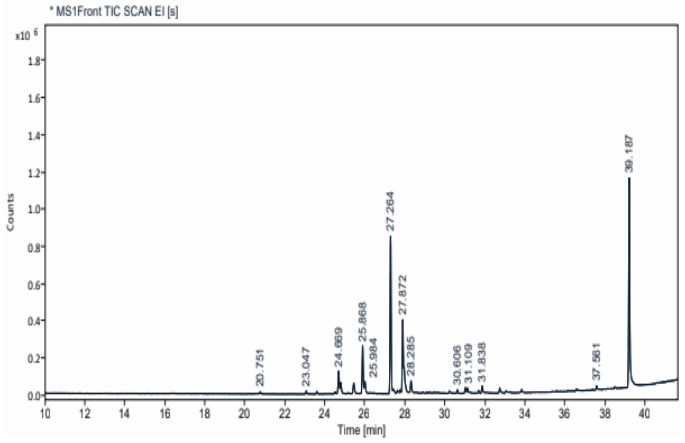


Figure 3. GC-MS chromatogram for extract

Table 3. Phytochemicals identified in ethanol extract by GC-MS

Retention time	Name	Synonyms	Category
20.75	2H-1-Benzopyran, 3,4- dihydro-2-phenyl	Flavanone	Flavonoids
20.75	ibogaine	-	Alkaloid
23.04	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	Panaxynol	Triterpenoid
25.86	Panaxynone	-	Triterpene
37.56	9-Methoxy-6a,11a dihydro-6H benzofuro[3,2-c]chromen-3-ol	Medicarpin	Isoflavonoid
39.187	6-Hydroxy-7-methoxy-4- phenylcoumarin	Dalbergin	Flavonoids
39.187	7-Hydroxy-4-(4-methoxyphenyl)chromen 2-one	-	Flavonoids
39.187	Aurantio-obtusin	-	Anthraquinone

Formulation of Hydrogels

The percentage composition of the optimized formulation system for wound healing application is shown in Table 6.

Table 6. Percentage composition of *Dalberia latifolia* (roxb)

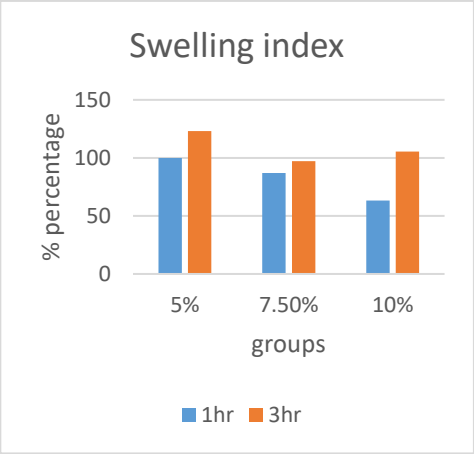
Sl. No	Ingredients	Role	% composition (w/w)
1	Carbopole-934	Gelling agent	1.0
2	Glycerin	Humectant	10
3	Ethanol	Vehicle	5
4	*Dalbergia latifolia* extract	Bioactive compound	(5,7.5,10)
5	Triethanolamine	pH adjuster	qs
6	Sodium benzoate	Preservative	0.5
7	Distilled water	Vechicle	qs to 100

Characterization of Hydrogel

D.latifolia- hydrogel was characterized by various physicochemical properties. Results are tabulated in Table 7, and figure no 6.

Table 7. Characterization of prepared hydrogels

Formulae	General appearances	Homogeneity	pH	Spreadability	% content	Biodegradation
5% hydrogel	Transparent coffee brown colour	Homogenous	7.08±0.03	80.66±1.76	86.48±0.26	43.07±0.64
7.5% hydrogel			7.19±0.11	81.33±1.76	85.88±0.29	47.15±1.36
10% hydrogel			7.48±0.11	81.33±1.33	86.33±0.34	46.77±0.88



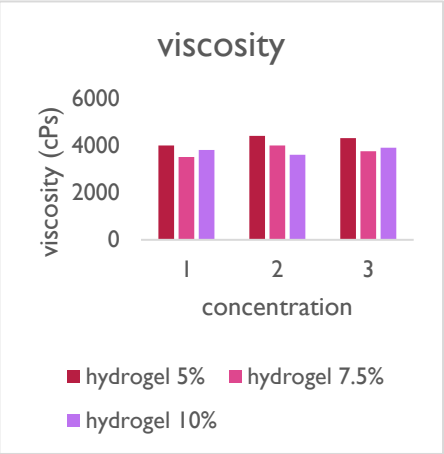


Fig 6.1 : Swelling index profile

Fig 6.2: Viscosity of Hydrogel

ATR-IR

ATR IR was carried out for *D.latifolia* (roxb) extract, carbopol-934, blank, 5% hydrogel, 7.5% hydrogel, 10% hydrogel are illustrated in Fig- 7. Detailed peak position are expressed in table 8.

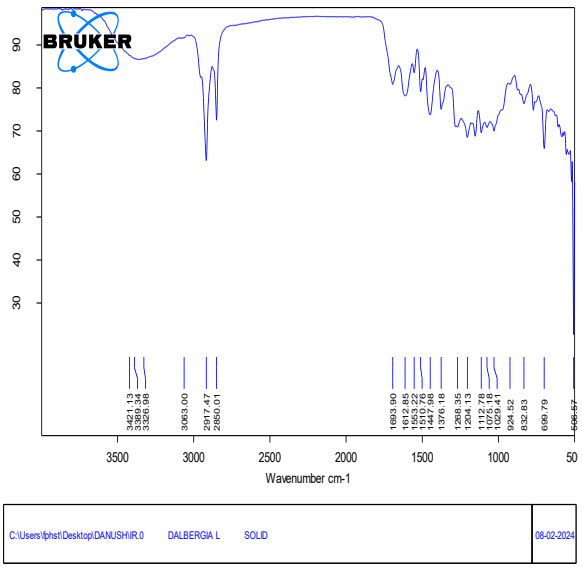


Fig. 7.1 Attenuated total reflectance infrared spectroscopy (ATR IR) spectrum of

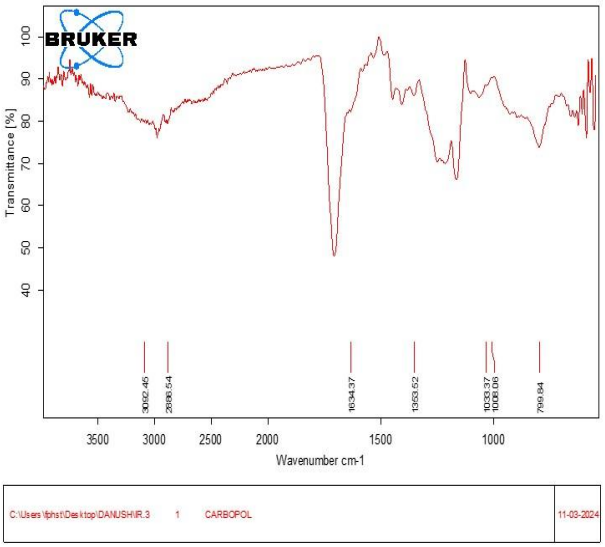


Fig. 7.2. Attenuated total reflectance infrared spectroscopy (ATR IR) spectrum of carbopol-934 polymer.

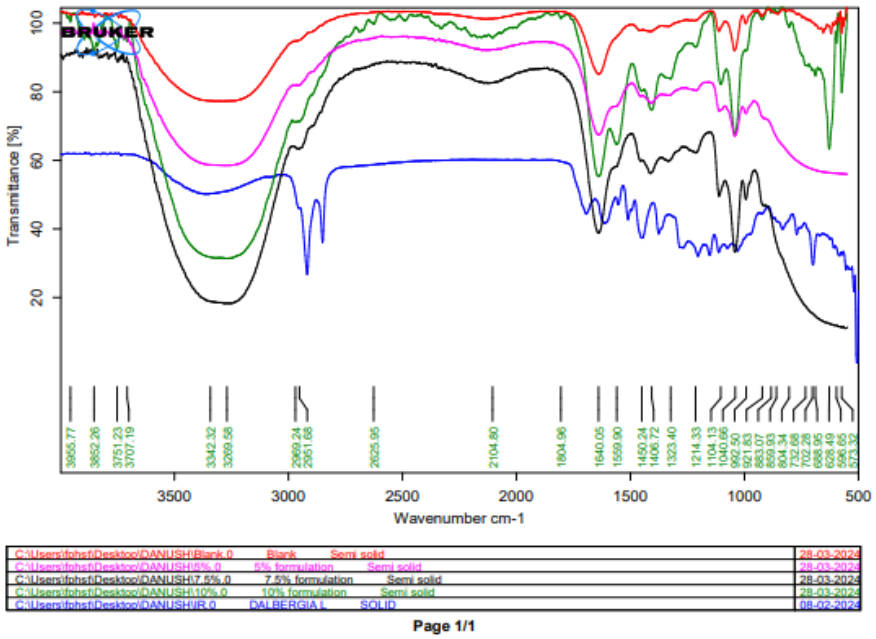


Fig 7: Attenuated total reflectance infrared spectroscopy (ATR IR) spectrum of hydrogels contain D.latifolia (roxb) extract, blank, 5%, 7.5%, and 10% hydrogel formulation

Table 8: ATR IR spectra

Functional groups	Standard wave number (Cm^{-1})	Carbopol-934 (Cm^{-1})	Extract (Cm^{-1})	Blank (Cm^{-1})	5% hydrogel (Cm^{-1})	7.5% hydrogel (Cm^{-1})	10% hydrogel (Cm^{-1})
O-H	3650-3200	-	3326	3284	3276	3255	3269
Aromatic C-H	3150-3050	3092	3063	-	-	-	-
Aliphatic C-H	3000-2850	2886	2917	2951	2955	2955	2951
CH_3	1200-1000	1033	1112	1111	1166	1154	1104
CH_2	1500-1200	1353	1447	1459	1456	1455	1450
C-O-C	1300-1000	-	1268	1219	1249	1261	1214

***In-vitro* drug release**

The drug release from 5%, 7.5% and 10% hydrogels was investigated in phosphate buffer solution using franz diffusion to understand the in-vitro drug release studies. Results are shown in figure no 8

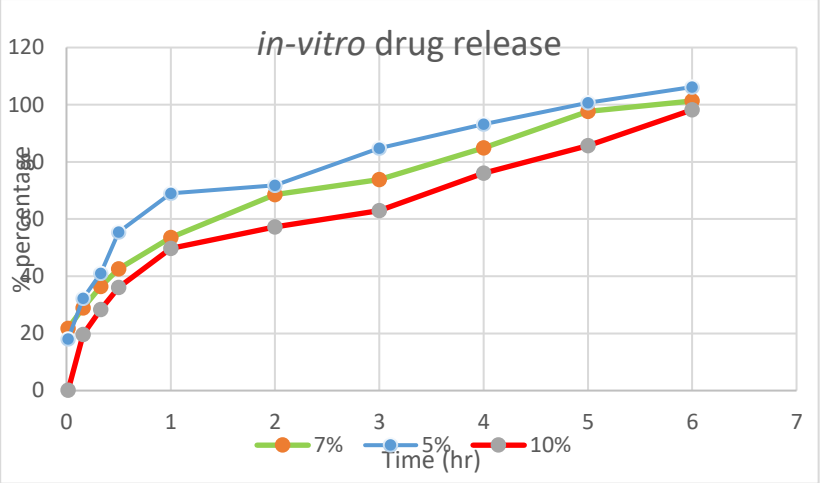


Fig No 8: in-vitro drug release studies for prepared hydrogels

Drug release kinetics of prepared hydrogels

To determine the release mechanism of prepared hydrogels, three models were studied, namely zero-order, higuchi, Hixson-crowel models .The results of the correlation coefficient R^2 of curve of the each model were shown in the Table 9. The first-order and higuchi models of drug release had a higher R^2, showed in figure no 9

Table 9. Drug release kinetic study of the *in-vitro* release data of hydrogels

Kinetics	5% hydrogel (R^2)	7.5% hydrogel (R^2)	10% hydrogel (R^2)
First order	0.8480	0.7942	0.7816
higuchi	0.8036	0.8869	0.8925
Hixson-crowel	0.7295	0.7139	0.7114

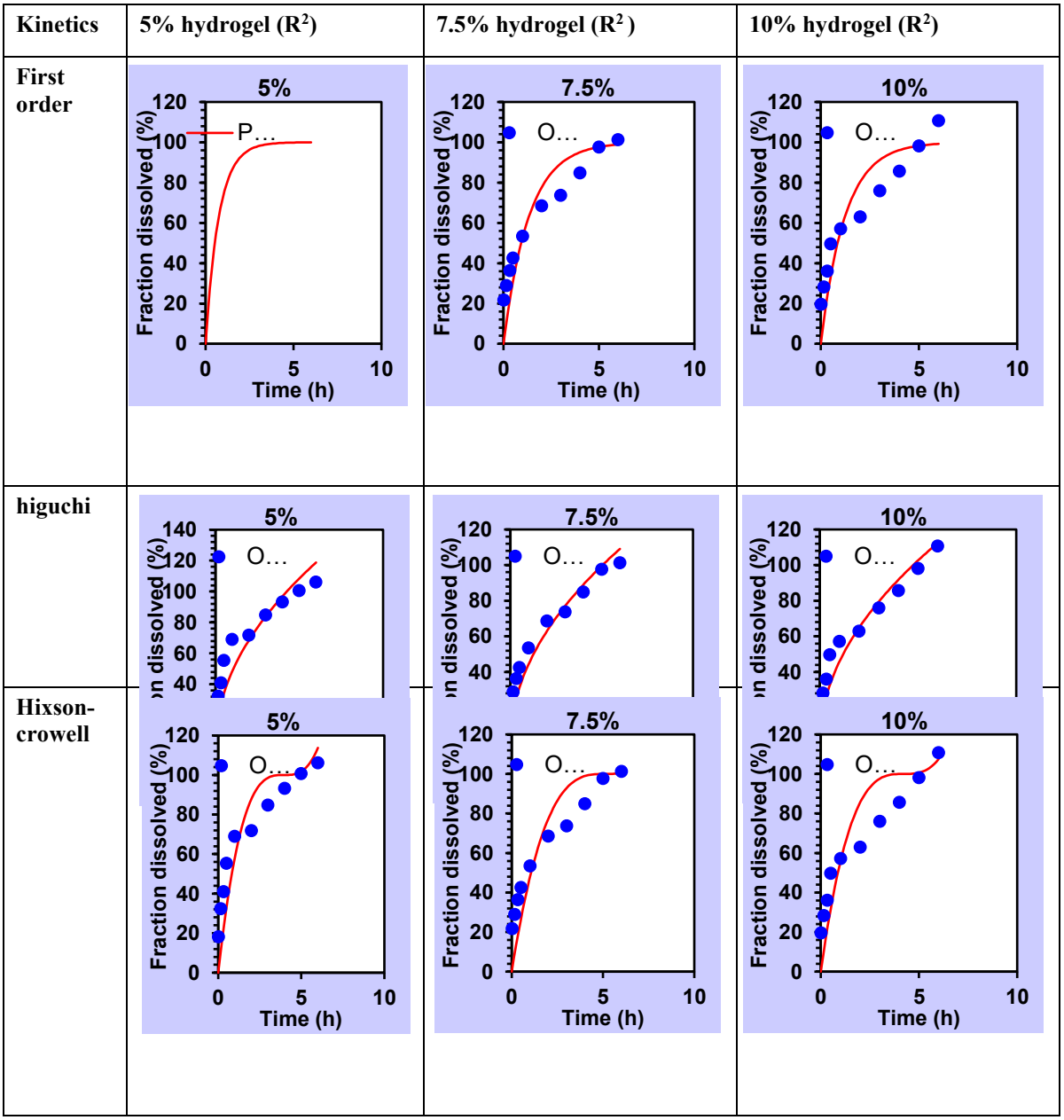


Figure 9. Drug release kinetics models (first-order, higuchi, Hixson-crowell for drug release) for drug release kinetic studies from prepared (5, 7.5, 10%) hydrogels.

Acute dermal irritation study

The acute dermal irritation study was observed on the skin sites of the rabbit one hour after applying hydrogel. Two treated skin area appeared normal at 24 hr observation and the remaining treated skin site appeared normal at the 72-hr observation shown in figure 10.

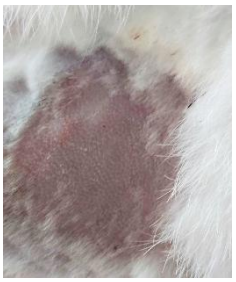
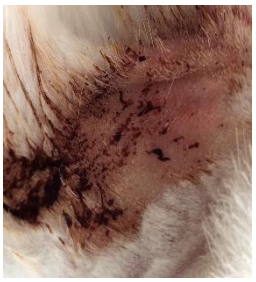
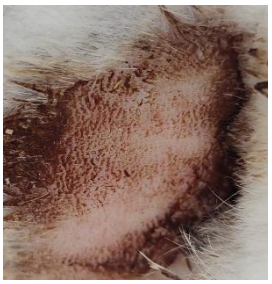
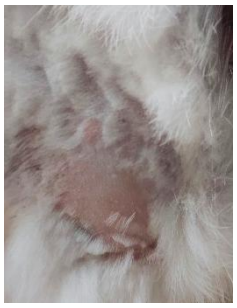
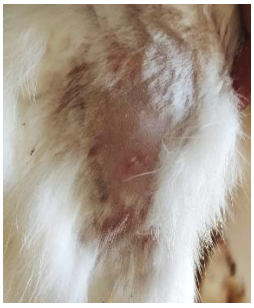
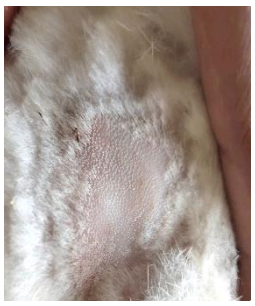
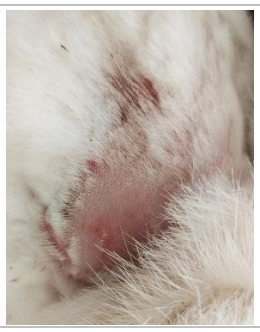
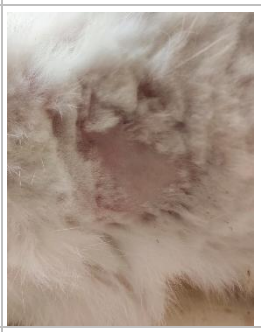
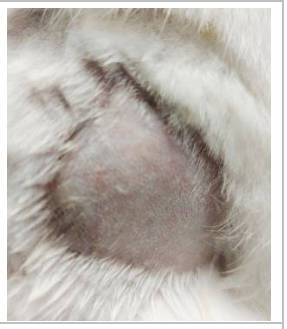
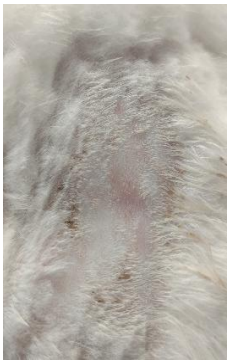
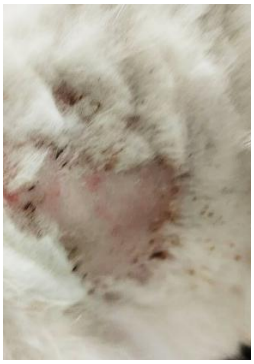
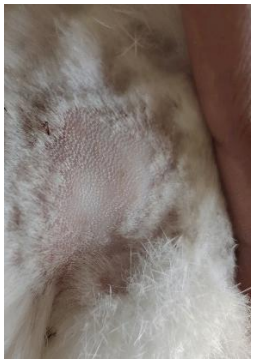
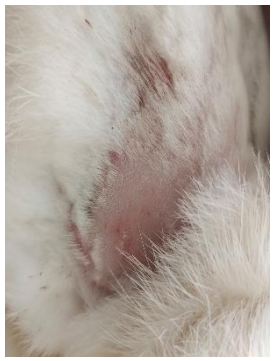
Time duration (hr)	Normal Control	5% Hydrogel	7.5% Hydrogel	10% Hydrogel
1hr				
24 hr				
48 hr				
72 hr				

Figure No 10. Acute dermal irritation study of control and test groups in rabbits

Incision wound model in rats

For the evaluation of wound healing activity, five groups of animals were used.

In the incision model, tensile breaking in grams was used to determine the wound healing efficacy of *D.latifolia* (roxb)contain hydrogels on 14th day, test groups shown fast recovery in 8-10th day.

Measurement of Tensile Strength

The minimum tensile strength of the wound in the control group was 6.68± 0.64g/mm2. The mean tensile strength of a wound treated with 5% w/w hydrogel was 8.28 ± 0.29g/mm2, whereas a wound treated with 7.5% w/w hydrogel had 9.41 ± 1.05 g, and 10% hydrogel had 9.1±0.49. The significant results shown in figure no 11.

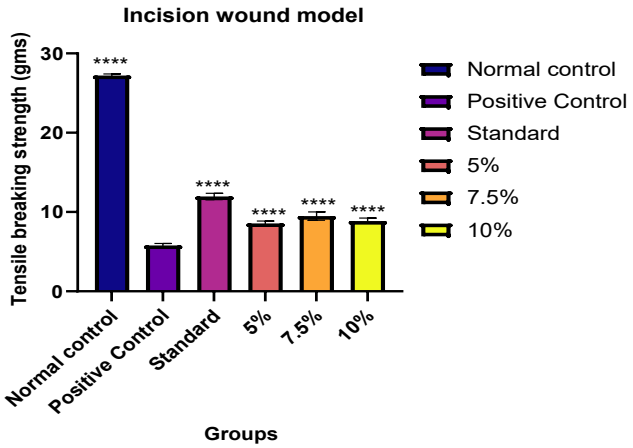


Figure 11. Effect of topical application of *D.latifolia extract* contain hydrogels tensile breking strength of incision wound expressed as Mean±SEM, n=6 one way ANOVA was applied and comparison between positive control Vs all the groups shows significant (**p<0.0001) at 14th Day.**

Excision wound model in rats

For the evaluation of wound healing activity five groups of animals was used. The first group control, the second group was standard, third fourth and fifth were 5%, 7.4% and 10% hydrogel contain *D.latifolia* extract of barks, respectively. The digital photographs of wound area of each treated group taken on 1, 4,6,8,10,12 and 14th are shown in Figure 12.

Days	Positive control	Standard	5% hydrogel	7.5% hydrogel	10% hydrogel
2					
4					
6					
8					
10					

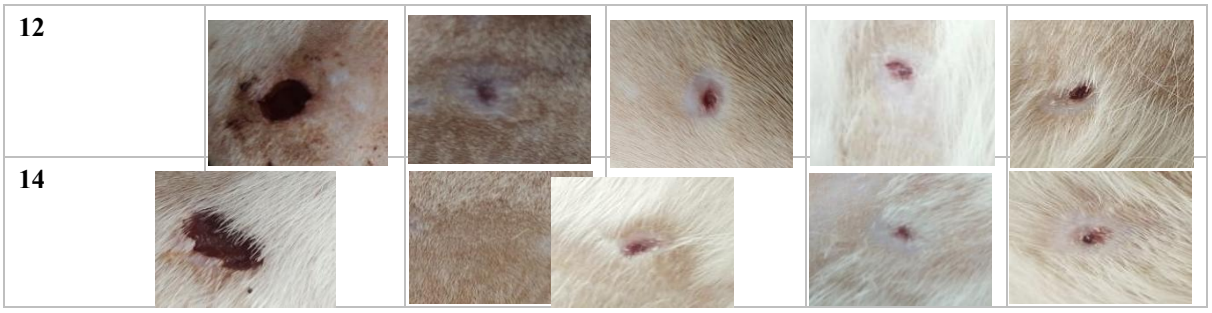


Figure No 12: Representation of excision wound healing model of hydrogels

Wound reduction size

Measurement of wound diameter was a main criteria for indication of progressive excision wound healing was expressed as a decrease of the original wound diameter showed in figure 11. % of wound contraction and Wound contraction area (mm) of excision wound was performed at different time interval, i.e 2, 4, 6, 8, 10, 12 and 14th day of wound created.

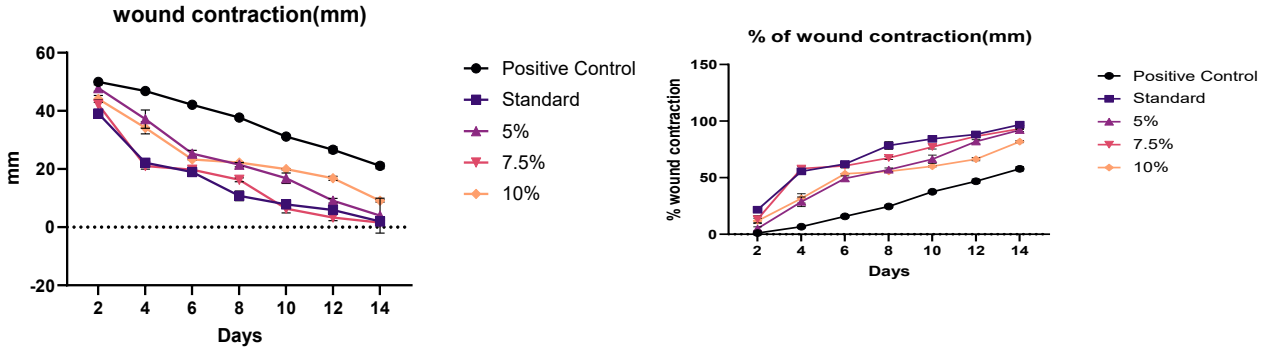


Figure 13. Wound diameter ($mm^{2)}$ and percentage of wound reduction of excision wound expressed as Mean±SEM, n=6 Two way ANOVA was applied and comparison between positive control Vs all the groups shows significant (**p<0.0001) at 14th Day.**

Period of epithelialization

The epithelialization period was reduced in a dose-related manner from 13.15±2.24 for the 5% hydrogel, 13.31±5.37 for 7.5% hydrogel to 11.69±2.91 for 10% hydrogel treated groups. And 13.82±4.13 for standard drug, were compared with the 8.26±1.46 for positive control showed significant (****p<0.0001 vs positive control) in figure 14.

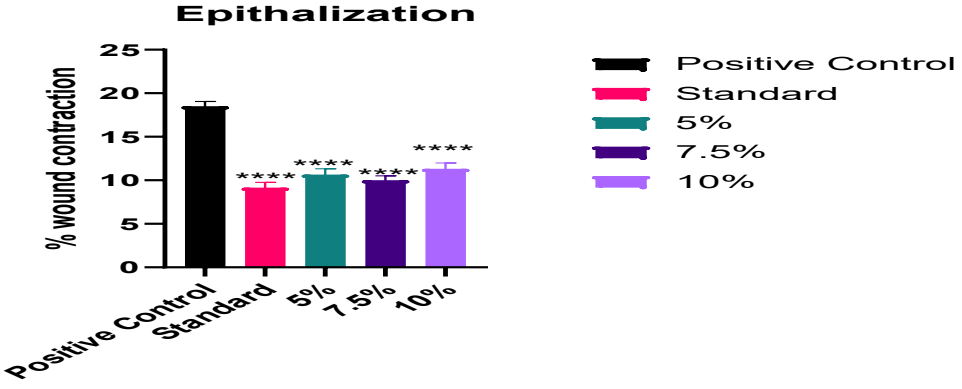


Figure 14. Epithelialization of excision wound, Mean±SEM n=6, One way ANOVA was applied and comparison between positive control Vs all the groups shows significant (**p<0.0001) at 14th Day**

Hydroxyproline content

Hydroxyproline content levels in granuloma tissue treated with hydrogels formulation is represented in figure 15. The value was calculated from the relationship obtained from the standard curve (y= 0.0011x +0.4386; R2= 0.993) in the present study, the topical application of hydrogels (standard, 5, 7.5 and 10%w/w) significantly Increased (****p<0.0001) hydroxyproline concentrations when compared to the positive control group.

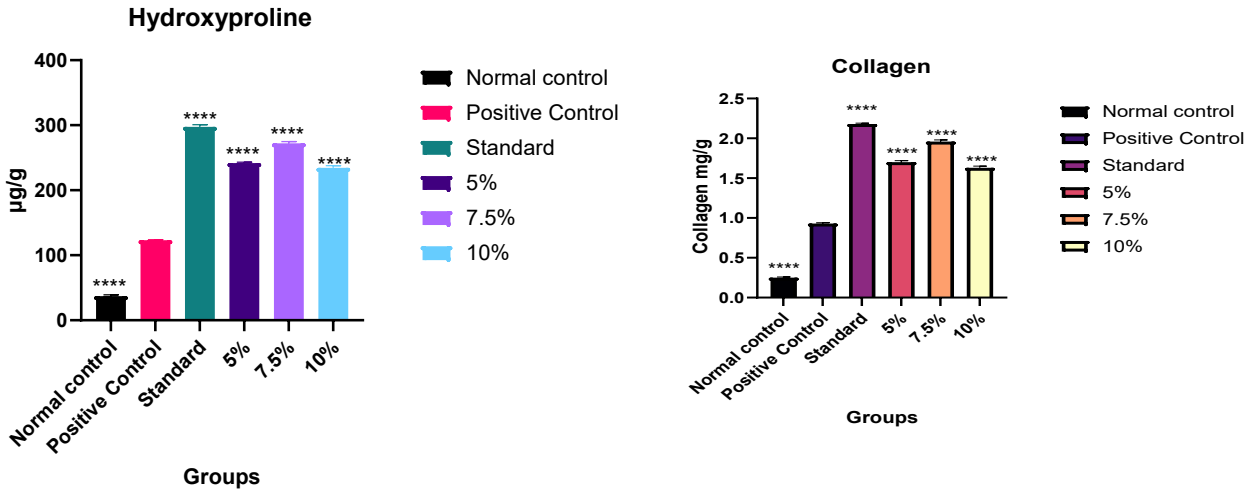


Figure 15. Hydroxyl proline and Collagen content, Mean ± SEM, n=6, One way ANOVA was applied and comparison between positive control Vs all the groups using multiple comparison test analysis shows significance (****p<0.0001).

Histopathological studies

Skin showed healing response were significantly noticed shown in figure 16, when compared to the positive control group showed dermis morphology disrupted. Standard, 5% hydrogel group, 7.5% and 10% hydrogel treated groups showed more proliferations & Collagen & restoration of Fibroblasts, skin tissue score and report tabulated in table 10.

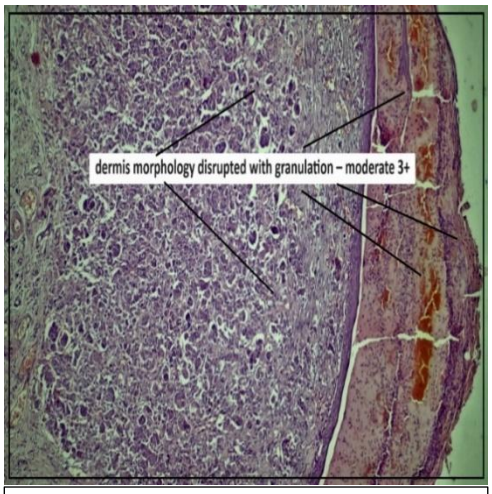


Fig a. Positive control

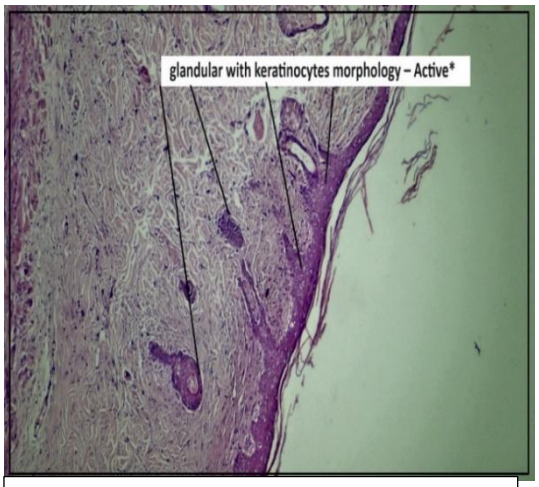


Fig b. Standard

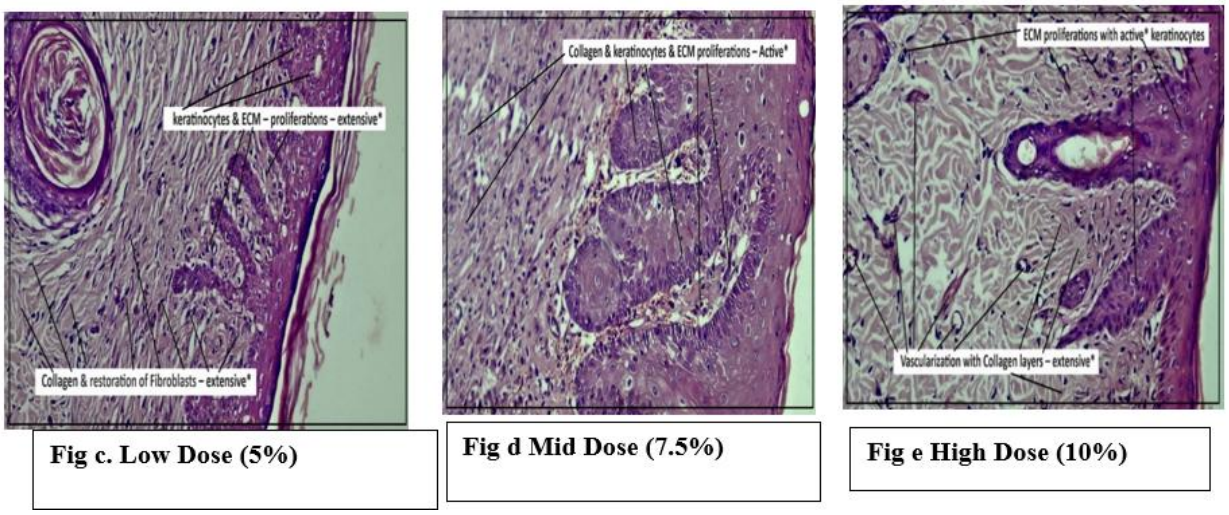


Fig c. Low Dose (5%) | **Fig d Mid Dose (7.5%)** | **Fig e High Dose (10%)**

Figure 16. Histopathology of skin at day 14th stained with H&E

Table 10: Histopathological evaluation of the rat skin tissue score

Group	Histopathological findings Rat Skin	Scorings / gradation
Normal control	keratinocytes with glandular morphology – normal	NAD+
Disease Induced	dermis morphology disrupted with granulation – moderate 3+ hyperkeratinisation with congestion & glandular hyperplasia – moderate 3+	moderate 3+
Standard	keratinocytes & ECM – proliferations & Collagen & restoration of Fibroblasts – Active* moderate+	Active* moderate+
Low Dose (5%)	keratinocytes & ECM – proliferations & Collagen & restoration of Fibroblasts – Active* extensive*	active* extensive*
Mid Dose (7.5%)	keratinocytes & ECM – proliferations & Collagen & restoration of Fibroblasts – Active* moderate+	Active* moderate+
High Dose (10%)	keratinocytes proliferated resulting in epithelialization & ECM restoration, active* keratinocytes & ECM proliferations – healing response* Vascularization with Collagen layers – extensive* in entire dermis area (Drug induced beneficial response*)	active* extensive*

2. DISCUSSION

Wound healing is a complex natural process, which is characterized by tissue regeneration. The purpose of wound management is always to enhance the healing process while minimizing risk factors like infection, which have a substantial effect on it. Studies in this area are conducted using a variety of medicinal plants with wound-healing characteristics [12, 13].Total alcoholic extract was prepared and phytochemical screening of the extract revealed the presence of flavonoids, phytosterols, phenolic and tannins. Recent research shows that topical agents, including flavanoids and triterpenoids, can improve wound healing by preventing infection and preventing infection in burns or wounds [14].

Qualitative screening is very important to determine the phytochemical compounds present in herbal plants. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation [15]. The extract was analysed for its phytochemicals using GC-MS, and identified 38 compounds. Analysis of GC-MS data for retention time, peak area and Score (>500) with reference to NIST database library helped to identify the presence of important secondary metabolites. The compounds identified are, 2H-1-Benzopyran, 3,4- dihydro-2-phenyl; Ibogaine; (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol; Panaxyone; 9-Methoxy-6a,11a dihydro-6H benzofuro[3,2- c]chromen-3-ol; 6-Hydroxy-7-methoxy-4- phenylcoumarin; 7-

Hydroxy-4-(4-methoxyphenyl)chromen 2-one and aurantio-obtusin of compounds).Most of the compounds were eluted between 20 -40 min analysis time.

The % drug content of hydrogel was performed to calculated actual drug loaded in the hydrogels (85-86%). Formulated hydrogels are similar biodegradation behaviours resulting (43-47%) in different weight loss. Hence all the formulated hydrogel shows successful cross-linking networking on biodegradation.

To determine drug release kinetics mechanism of prepared hydrogels, three models were studied, namely zero-order, higuchi, Hixson-crowel models [16,17]. The results first-order (0.848, 0.7942, & 0.7816) and higuchi models (0.8036, 0.8869 & 0.8925) of drug release had a higher R2.

Acute dermal irritation studies examine the actual skin irritation and to determine lethal dose/ concentration of the drug. There is no erythema and oedema was observed on the skin sites of the rabbit one hour after applying hydrogel, in the incision model, tensile breaking in grams was used to determine the wound healing efficacy of *D.latifolia* contain hydrogels on 14th day. The minimum tensile strength of the wound in the normal control group was 27.20± 0.22g/mm2 and 6.68± 0.64g/mm2 in positive control. The mean tensile strength of a wound treated with 5% w/w hydrogel was 8.28 ± 0.29g/mm2, whereas a wound treated with 7.5% w/w hydrogel had 9.41 ± 1.05 g, and 10% hydrogel had 9.1±0.49. Tensile strengths in 7.5% hydrogel reported high recovery compared to other test groups shown fast recovery in 8-10th day with the significance(****$p<0.0001$).

% of wound contraction and wound contraction area (mm) of excision wound was performed at different time interval, i.e 2, 4, 6, 8, 10, 12 and 14th day of wound induction. The comparison between positive control Vs all the groups shows significant (****$p<0.0001$) at 14th Day. 7.5 % hydrogel group showed the faster recovery of wound compared to other test groups.

The period of epithelialization was high in 7.5% hydrogel and standard as compared to positive control. The epithelialization period was reduced in a dose-related manner from 13.15±2.24 for the 5% hydrogel, 13.31±5.37 for 7.5% hydrogel to 11.69±2.91 for 10% hydrogel treated groups. And 13.82±4.13 for standard drug, were compared with the 8.26±1.46 for positive control showed significant (****$p<0.0001$).

Hydroxyproline is measure of concentration of collagen in which increase in the concentration leads to faster rate of healing wound [7]. The topical application of hydrogels (standard, 5, 7.5 and 10%w/w) significantly Increased (****$p<0.0001$) hydroxyproline concentrations when compared to the positive control group. Hence, hydroxyproline content indicating the wound healing potential in prepared hydrogels.

3. CONCLUSION:

From the above results, hydrogel with 7.5% concentration was found to have significant wound healing activity compare to other concentrations and it could be incorporated in hydrogels following further studies...

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