The Pharma Innovation Journal 2024; 13(11): 36-42

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2024; 13(11): 36-42

www.thepharmajournal.com
Received: 17-09-2024
Accepted: 28-10-2024

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Fucidin topical gel formulation and evaluation by using biopolymers chitosan & sodium alginate

Gowtham Mandadapu, Prachetha Kolli, Kurra Venkata Gopaiah and Ramya Teja Medarametla

Abstract
The study focused on Fucidin, a topical antibacterial agent used to treat bacterial infections by disrupting bacterial protein synthesis. The researchers aimed to formulate a topical gel incorporating various gel-forming polymers, including chitosan, to assess their effects on gel characteristics and wound healing.
Using Carbopol 934p as the gelling agent, the gel formulations were prepared through a soaking method. Infrared spectroscopy indicated that the formulations were physical mixtures, confirming the compatibility of Fucidin with the other components. The gels were evaluated for drug content, pH, viscosity, spread ability, and extrudability.
Results showed that the plain gel released Fucidin at a slower rate compared to other formulations, with the best performing formulation (F8) achieving a release rate of 99.56%. All gel formulations demonstrated significant wound contraction, with one formulation containing Fucidin, 1% chitosan, and 1% sodium alginate achieving a remarkable 99.4% reduction in wound area over 12 days.

Keywords: Fucidin, vitamin-c, sodium alginate, aloe vera, chitosan, wound healing

Introduction
Topical drug delivery for skin disorders has become increasingly popular due to its effectiveness and ability to bypass the first-pass metabolism associated with oral medications. This alternative route helps to avoid gastrointestinal irritation and metabolic degradation, with only a fraction of orally administered drugs reaching systemic circulation. By applying medications directly to the skin, patients can benefit from more localized treatment without the disadvantages of oral administration [1].
Gels, as defined by the USP, are semisolid systems that consist of a liquid interspersed with either small inorganic particles or larger organic molecules. Dermatological gels possess advantageous properties such as thixotropism, excellent spreadability, a non-greasy feel, and ease of removal, making them suitable for a variety of skin applications.
Wound healing is a complex process involving multiple cellular and biochemical activities. Natural polysaccharides like chitosan have garnered attention for their beneficial properties, including biodegradability and the ability to maintain homeostasis. Chitosan can be formulated into various forms, such as sponges or films, and is effective in managing wounds due to its biocompatibility and moisture-retentive qualities. Similarly, alginate, derived from seaweed, is recognized for its ability to promote healing and skin regeneration.
Aloe vera, known for its rich array of bioactive compounds, is particularly effective in enhancing wound healing and reducing inflammation. Its growth factor-like substances play a crucial role in these processes. Collagen, a key component in tissue repair, consists primarily of amino acids like proline, glycine, and lysine, and serves as the structural framework for new tissue formation. Vitamin C, essential for collagen synthesis, also acts as a potent antioxidant and supports immune function [2, 3].
In the context of wound care, the use of antibiotics is critical for preventing infections, especially in vulnerable cases such as diabetic foot ulcers and surgical wounds. Fucidin, a topical antibacterial agent derived from the fermentation of *Fusidium coccineum*, is effective against a range of bacterial infections and is available in various formulations, including creams and ointments [4, 5].
This study aims to formulate and assess a topical gel containing Fucidin, aloe vera, and vitamin C, targeting the effective management of diverse wound types. By leveraging the combined properties of these ingredients, the formulation is expected to enhance healing while minimizing the risk of infection [6].

Materials and Methods

Chitosan was obtained as gift sample from National Drug Bank, Hyderabad, Telangana and Fucidin was obtained as gift sample from Azanta Chemi Ltd. (Pharma division) Hyderabad, Telangana. Aloe vera was obtained from the Sanju chemicals Ltd., Viajyawada. Sodium Alginate and Vitamin C was obtained from SD Fine Chem. Ltd. Mumbai and Carbopol 934 from Lobachem Pvt Ltd, Mumbai [7, 8, 9, 10, 11].

Formulation of Fucidin gels

Different gel formulations were prepared containing 1% w/w of Fucidin using carbopol 934p as gel base according to the formula mentioned in the table 1 and 2.

Table 1: Formulation details of Fucidin gels with different wound healing modifiers

Ingredient (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8
Drug	1	1	1	1	1	1	1	1
Carbopol	1	1	1	1	1	1	1	1
Chitosan	-	2	-	-	-	1	1	1
Sodium Alginate	-	-	2	-	-	1	-	-
Vitamin-c	-	-	-	2	-	-	1	-
Aloevera	-	-	-	-	2	-	-	1
Ethanol	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Triethanol Amine	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Glycerine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methyl Paraben	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048
Propyl Paraben	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.080
Water	43.249	42.82	42.82	42.82	42.82	42.82	42.82	42.82

Table 2: Formulation details of Fucidin gels with different wound healing modifiers

Ingredient (%w/w)	F9	F10	F11	F12	F13	F14	F15
Drug	1	1	1	1	1	1	1
Carbopol	1	1	1	1	1	1	1
Chitosan	-	-	-	0.5	0.5	0.5	-
Sodium Alginate	1	1	-	0.5	-	0.5	0.5
Vitamin-c	-	1	1	-	0.5	0.5	0.5
Aloevera	1	-	1	0.5	0.5	-	0.5
Ethanol	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Triethanol Amine	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Glycerine	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methyl Paraben	0.048	0.048	0.048	0.048	0.048	0.048	0.048
Propyl Paraben	0.080	0.080	0.080	0.080	0.080	0.080	0.080
Water	42.82	42.82	42.82	42.82	42.82	42.82	42.82

Procedure [12, 13, 14, 15, 16]

The preparation of the gels involved initially soaking 1% carbopol in 25 ml of water for 24 hours, followed by neutralization with an appropriate amount of triethanolamine. This mixture was thoroughly mixed using a glass rod and allowed to sit for 15 minutes. Meanwhile, the drug was dissolved in an adequate quantity of ethanol.

To incorporate vitamin C, an accurately measured amount (2%) was dissolved in distilled water and subsequently added to the neutralized carbopol solution while stirring continuously. The drug solution was then integrated into the carbopol mixture with ongoing stirring for approximately 30 minutes, resulting in a clear gel. The final volume was adjusted to 50 ml with distilled water while ensuring continuous stirring. Periodic pauses in stirring were implemented to allow any trapped air to escape.

This same methodology was applied to prepare additional formulations incorporating various wound healing agents, including sodium alginate, chitosan, and aloe vera, following the established steps to ensure consistency and efficacy in the final products.

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Analytical Method [17, 18]

Fourier-transformation infrared spectroscopy (FTIR)

The interactions between the drug and polymer, as well as between different polymers, were analyzed using Fourier Transform Infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum 100, Japan). A sample of 2% w/w was prepared with potassium bromide, ground into a fine powder, and then compressed into discs using a hydraulic press. Each disc underwent 16 scans at a rate of 2 mm/sec, with a resolution set at 4 cm^{-1}. The resulting characteristic peaks were recorded for further analysis.

Evaluation of Fucidin Gels

Determination of pH [19, 20]

1gm of the gel formulation was dispersed in 10 ml of distilled water and the pH was determined by digital pen pH meter[10].

Drug content

The drug content was quantified by accurately weighing 10 mg of the gel and transferring it to a 100 ml volumetric flask containing phosphate buffer at pH 7.4. The mixture was then sonicated and filtered. From this solution, a 1 ml aliquot was pipetted out and further diluted to 10 ml. The concentration of Fucidin was measured using a Shimadzu UV-visible spectrophotometer at a wavelength of 204.60 nm, with a blank for comparison. The analysis was performed in triplicate to ensure reliability of the results.

Rheological properties

a) **Viscosity:** The viscosity was determined using Brookfield LVDV-III ultra-programmable rheometer. The spindle No (CP-52) was used for the measurement. An optimum speed (2 rpm) was used to measure the viscosity of the preparation.

b) **Spread ability:** The spreadability of the formulations was assessed using a modified apparatus based on the method proposed by Mutimer *et al*. This setup comprised a wooden block with a pulley at one end and a rectangular ground glass plate fixed on top. Approximately 2 grams of the gel formulation were placed on the lower glass plate. The gel was then sandwiched between this plate and an upper glass plate of the same dimensions, which had a hook attached.

To create a uniform gel film and eliminate air, a 1 kg weight was applied to the upper plate for 5 minutes. Any excess gel was removed from the edges. Subsequently, a pull of 50 grams was applied to the upper plate using a string connected to the hook, and the time (in seconds) taken for the upper plate to travel a distance of 10 cm was recorded. A shorter time interval indicates improved spreadability of the gel formulation.

The spread ability was calculated using the formula:

$$S = m.l/t.$$

Where S = spread ability

m = weight tide to upper side

l = length moved on the glass slide t = time taken in seconds.

Extrudability

In this study, extrudability of the gel formulations was evaluated based on the percentage of gel extruded from a tube under a specific load. Higher amounts of extruded gel indicated better extrudability. The formulation was filled into a clean, lacquered aluminium collapsible one-ounce tube equipped with a nasal tip featuring a 5 mm opening.

The tube was positioned between two glass slides and secured in place. Extrudability was assessed by applying a constant load of 1 kg on the slides and measuring the amount of gel that extruded through the tip. The extruded gel was collected and weighed, and the percentage of gel extruded was calculated. Grades were then assigned based on the extrudability results. (+++ good; ++ fair and + poor) [12, 13].

***In vitro* Diffusion study by using Cellophane Membrane [21]**

The apparatus used for this study consisted of a cylindrical glass tube with an internal diameter of 14 mm and a height of 62 mm, open at both ends. A gel formulation equivalent to 10 mg of Fucidin was evenly spread over a cellophane membrane that had been soaked in distilled water overnight. This membrane was secured to one end of the tube, ensuring that the gel adhered to the inner circumference.

The entire assembly was positioned so that the gel touched the surface of the diffusion medium, which consisted of 100 ml of phosphate buffer at pH 7.4, maintained at a temperature of 37±2 ˚C. The cellophane membrane served as a barrier between the gel and the phosphate buffer. The medium was stirred using a magnetic stirrer set to 50±5 rpm.

At predetermined intervals of 1, 2, 3, 4, 6, and 8 hours, 5 ml samples were withdrawn from the receptor fluid and replaced with fresh phosphate buffer at pH 7.4. The drug release was quantified using a Shimadzu UV-Visible spectrophotometer at a wavelength of 204.60 nm. All experiments were conducted in triplicate to ensure accuracy and reliability of the results.

***In vivo* wound healing activity [22, 23]:**

In this study, male Wistar albino rats weighing between 150 and 250 g were utilized. A total of 16 groups, each consisting of three animals, were formed. The rats were maintained under standard laboratory conditions. The *in vivo* wound healing activity was assessed using an excision wound model for the gel formulations, with marketed soframycin serving as the standard treatment.

Excision wounds were created under light ether anesthesia by removing a circular piece of full-thickness skin (18 mm²) from the dorsa interscapular region. Wound contraction was monitored by measuring the wound area using planimetric methods every other day until complete healing was observed. The percentage of wound contraction was calculated based on the reduction in wound area [15, 16, 17, 18].

Results & Discussion

In this study, topical gels containing Fucidin were formulated with various wound healing modifiers to enhance wound management. The modifiers used included vitamin C, aloe vera, sodium alginate, and chitosan. All gel formulations were prepared using a soaking method. Characterization of the gel formulations through FTIR analysis indicated that the resulting formulations are physical mixtures.no chemical reaction took place between drug and polymer as depicted in figure 1 and figure 2.

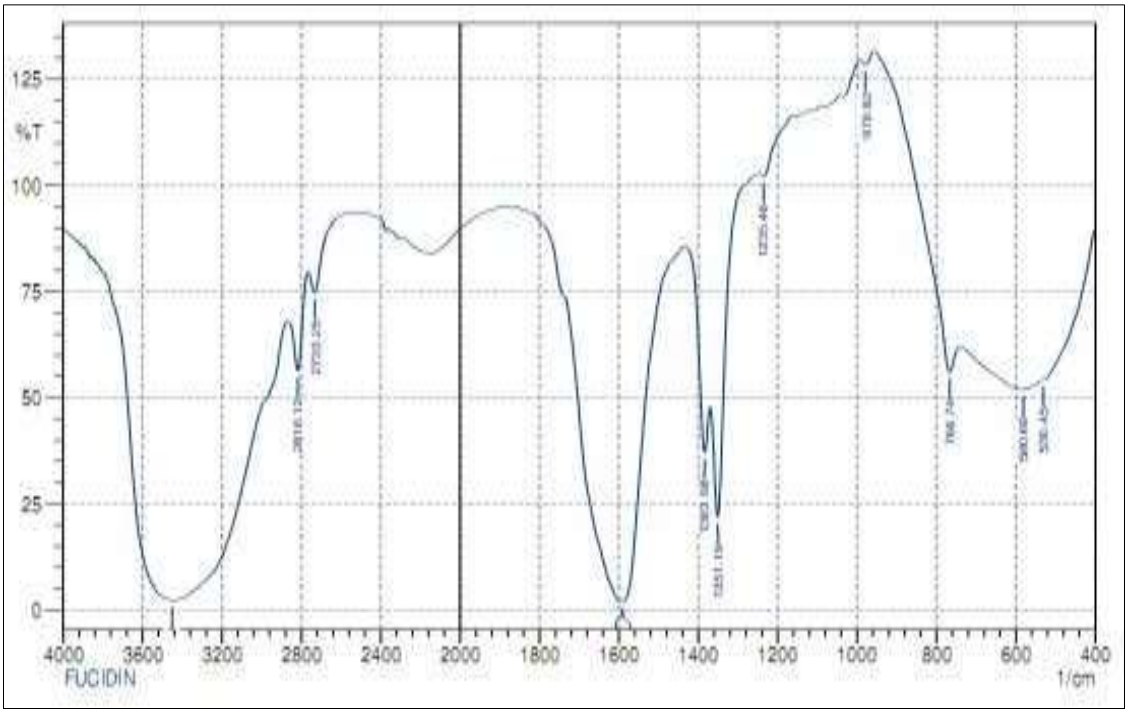

Fig 1: IR spectra of pure drug Fucidin

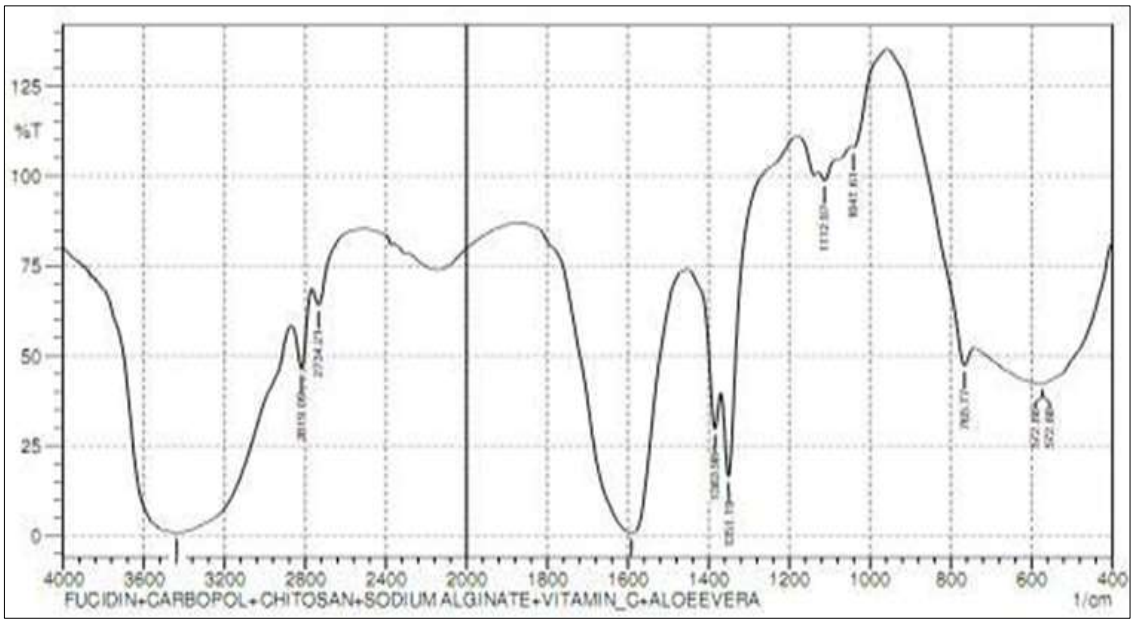

Fig 2: IR spectra of Fucidin + Carbopol 934 + Chitosan + Sodium Alginate + Vitamin-c + Aloe vera

The prepared gel formulations are evaluated for drug content, pH and rheological parameters like viscosity, spread ability and extrudability and results are tabulated in table 3. The drug content was found in the range of 94.75 to 99.83% for all gel formulations suggested uniform distribution of Fucidin in gels. The pH of all gel formulation was more than 6.

Table 3: Evaluation of Fucidin gel formulations

Formulation Code	pH	Drug content%	Viscosity (cps)	Extrudability	Spread ability (gm.-cm/sec)
F1	6.8±0.15	97.89±0.50	6829.32	+++	21.21
F2	6.5±0.10	96.6±0.43	7600.12	+++	12.45
F3	7.1±0.13	94.75±0.45	6158.18	+++	15.55
F4	6.8±0.15	99.48±0.70	3246.31	++	28.13
F5	7.4±0.15	99.13±0.45	6891.64	+++	20.11
F6	6.8±0.20	98.39±0.35	5767.48	+++	16.30
F7	7.1±0.12	95.80±0.43	6012.36	+++	14.28
F8	6.23±0.15	95.06±0.15	6820.12	+++	20.88
F9	7.2±0.10	96.90±0.55	5820.32	+++	18.97
F10	6.9±0.16	99.63±0.15	5087.29	+++	14.51
F11	7.20±0.12	98.40±0.13	4983.21	++	19.23
F12	7.00±0.14	97.66±0.18	5832.13	+++	17.23
F13	6.5±0.15	98.90±0.27	4646.31	+++	20.21
F14	6.00±0.10	99.83±0.39	4712.64	+++	22.12
F15	6.92±0.17	96.10±0.42	4524.57	+++	17.23

All formulations demonstrated good viscosity, ranging from 3246.31 to 7600.12. The spreadability of the gels varied between 12.21 and 28.13 gm.cm/sec. Each gel was easily extruded from the tube, indicating favorable consistency.

In vitro drug release was assessed using a cellophane membrane in phosphate buffer at pH 7.4. The formulation designated as F8, which contained 1% Fucidin along with 1% chitosan and 1% aloe vera, exhibited the highest percentage of Fucidin diffusion compared to formulation F1 (which contained only Fucidin) and other formulations with different wound healing modifiers, as shown in Table 4 and Figure 3. Selected gel formulations were also subjected to stability studies, with results summarized in Table 5.

Wound healing involves the restoration of damaged tissue to its normal state, with wound contraction representing the reduction in wound area. This process is influenced by tissue repair capabilities, which can be impaired by infection. Wound contraction data, expressed as a percentage, is presented in Table 6 and Figure 5, while additional results are illustrated in a bar graph in Figure 4. All gel formulations achieved over 80% reduction in wound contraction, indicating their effectiveness.

Table 4: Comparative *in vitro* diffusion of Fucidin from gel containing pure drug with gels containing Fucidin along with different wound healing modifiers (F1, F2, F6, F8, F14) Cumulative Percent Drug Release

Time (hrs.)	F1	F2	F6	F8	F14
0	0.00	0.00	0.00	0.00	0.00
1	6.13	36.27	36.27	51.28	25.80
2	7.58	44.19	45.64	65.37	36.57
3	8.78	51.08	58.34	76.71	40.47
4	9.99	64.43	76.73	83.58	56.40
6	10.64	78.31	85.53	90.62	65.21
8	11.84	91.15	90.50	99.56	81.23

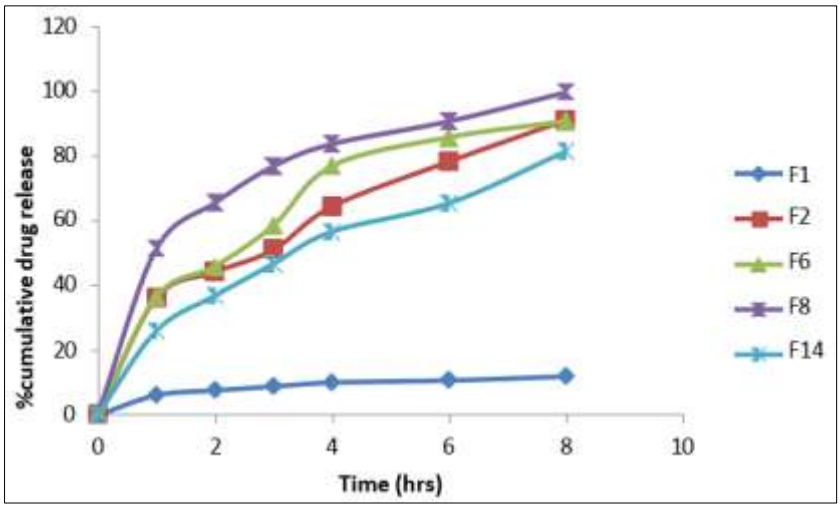

Fig 3: *In vitro* drug release profile of selected formulations

Table 5: Stability studies of selected Fucidin gel formulations (F2, F6, and F14)

Time interval (months)	F2			F6			F14		
	pH	% Drug Content	Viscosity (cps)	pH	% Drug Content	Viscosity (cps)	pH	% Drug Content	Viscosity (cps)
0	6.5	96.60	7600.12	6.3	98.39	5767.48	6.00	99.83	4712.54
1	6.3	96.59	7600.11	6.8	98.39	5767.48	5.99	99.83	4712.54
2	6.2	96.56	7600.08	6.6	98.35	5767.45	5.97	99.80	4712.51
3	6.00	96.48	7600.00	6.3	98.33	5767.42	6.96	99.78	4712.49

Table 6: Wound healing data of Fucidin gel formulation containing different wound healing modifiers (F2, F6, F8, F14) Wound Area in (mm)

Post- Wounding Days	Control	Standard	F2	F6	F8	F14
0	20.03±0.81 (0%)	20.35±0.76 (0%)	19.98±0.7 (0%)	19.25±0.65 (0%)	19.92±0.86 (0%)	19.98±0.65 (0%)
2	18.06±0.77 (9.7%)	18.02±0.52 (9.9%)	16.12±0.06 (33.25)	15.95±0.24 (20.25%)	19.92±0.86 (15.05%)	15.92±0.77 (2.4%)
4	16.09±0.32 (19.6%)	15.69±0.53 (21.55%)	13.25±0.55 (84.7%)	12.92±0.14 (35.25%)	16.99±0.28 (29.4%)	13.13±1.11 (34.35%)
6	15.03±0.88 (24.55%)	13.46±1.20 (32.7%)	6.12±0.99 (84.7%)	6±0.29 (70%)	11.98±0.6 (40.01%)	6.14±0.28 (69.3%)
8	14.07±1.04 (29.65%)	12.5±0.5 (37.5%)	3.06±0.84 (84.7%)	2.98±0.94 (85%)	9.8±0.82 (51%)	2.92±0.29 (85.4%)
10	13.04±0.62 (34.85%)	9.35±0.94 (53.25%)	1.53±0.28 (92.35%)	1.32±0.46 (93.4%)	5.56±0.68 (72.2%)	1.4±0.22 (93%)
12	10.09±0.40 (49.55%)	4.04±0.38 (79.55%)	0.76±0.24 (96.5%)	0.12±0.04 (99.4%)	2.02±0.29 (89.9%)	0.22±0.06 (98.9%)

Values are mean ±SD of three animals in each group. Numbers in parenthesis indicates wound contraction.

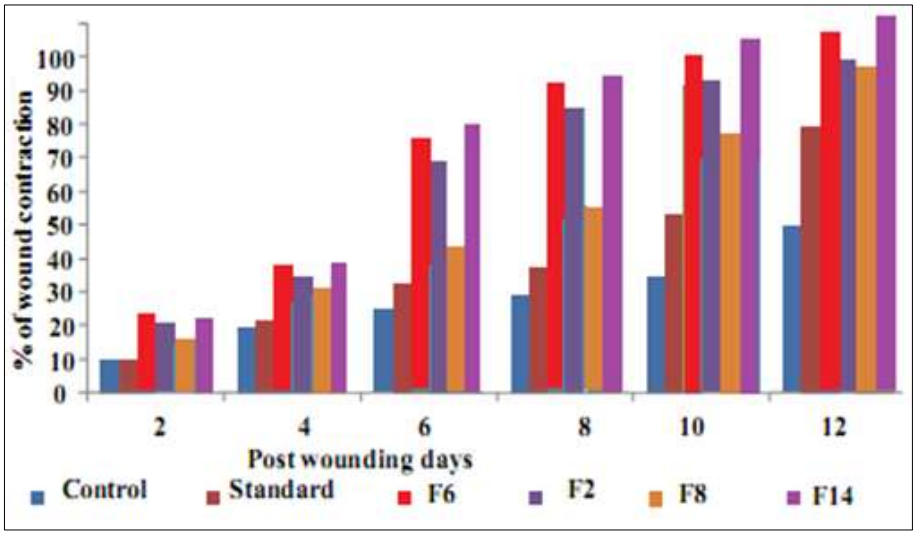

Fig 4: Wound healing activity Fucidin gel (F2, F6, F8, F14)

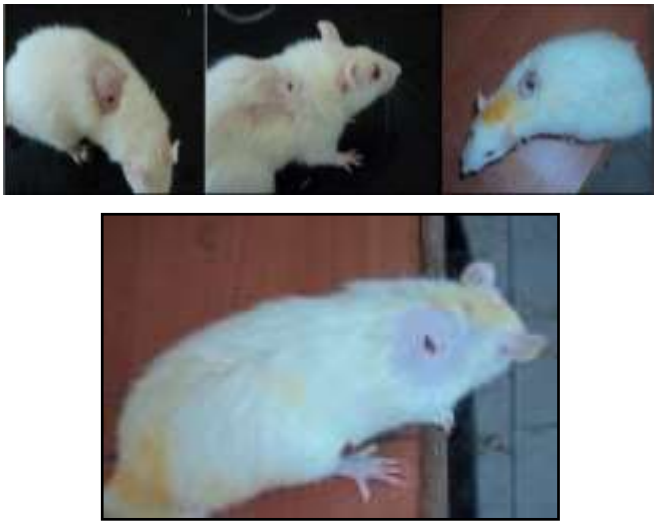

Fig 5: Wound area in mice after 12 days of application of formulations F2, F6, F8 & F14 respectively

Conclusion

The topical gel formulations incorporating 1% Fucidin with various wound healing modifiers, including chitosan, aloe vera, vitamin C, and sodium alginate at different concentrations, were prepared using carbopol 934P as the gel base. All prepared gels fell within acceptable limits for drug content, pH, and rheological properties.

An *in vitro* diffusion study was conducted using a cellophane membrane, revealing that formulation F8, which contained 1% Fucidin along with 1% chitosan and 1% aloe vera, exhibited the highest percent diffusion of Fucidin compared to formulation F1 (Which contained only Fucidin) and other formulations with different modifiers.

In vivo wound healing activity was evaluated for all gel formulations, with each demonstrating more than 80% reduction in wound area. Specifically, the formulation containing Fucidin, 1% chitosan, and 1% sodium alginate achieved a remarkable 99.4% reduction in wound area by day 12. Other formulations also showed significant reductions: F10 exhibited a 95.5% reduction, F11 showed 90.4%, and F15 demonstrated a 94.65% reduction in wound area after 12 days.

Stability studies on selected gel formulations confirmed their ability to maintain activity over time. Overall, the findings suggest that Fucidin gels combined with wound healing modifiers represent a promising approach for effective wound management.

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