AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Fucidin Topical Gel Containing Wound Healing Modifiers

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ABSTRACT

Fucidin, a topical antibacterial agent indicated in the treatment of bacterial infections. It works by interfering with bacterial protein synthesis, specifically by preventing the translocation of the elongation factor G (EF-G) from the ribosome. The aim of the present study was to prepare topical gel by using gel forming polymers along with chitosan and to study the effect of the concentration of polymers, effect of alone Fucidin, combined effect of Aloe Vera with vitamin C on different gel parameters and wound healing activity. The gel formulations were prepared by soaking method using Carbopol 934p as gelling agent/gel base. IR spectroscopy studies suggested that the formulations prepared is a physical mixture and the drug is compatible with other excipients. The prepared gel formulations were evaluated for drug content, pH and rheological parameters like viscosity, spreadability and extrudability. The percentage release of Fucidin from plain gel was slow as compared to other drug loaded gel formulations. The formulation (F8) showed maximum percentage release (99.56%). All the gel formulations showed more than 80% reduction in wound contraction. The gel formulation containing Fucidin along with 1% chitosan and 1% sodium alginate showed 99.4% reduction in wound area after 12 days.

Keywords: Fucidin, Vitamin-C, Sodium Alginate, Aloe Vera, Chitosan, Wound healing.

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Received 21 August 2015, Accepted 27 August2015

Please cite this article as: Saleem MA *et al.*, Formulation and Evaluation of Fucidin Topical Gel Containing Wound Healing Modifiers. American Journal of PharmTech Research 2015.

INTORDUCTION

Delivery of drugs on to the skin is an effective and targeted therapy for local dermatological disorders. This route of drug delivery has gained popularity because it avoids first pass effect, gastrointestinal irritation and metabolic degradation associated with oral administration. Due to the first pass effect only 25-45% of the orally administered dose reaches blood circulation. Topical application of drug can be a strong alternative mode of drug delivery which can overcome the disadvantage of first pass metabolism[1]. The USP defines gels as semisolid systems consisting of either suspension made up of small inorganic particles or large organic molecules interpenetrated by a liquid. The favorable properties of dermatological gels are thixotropism, good spreadability, greaseless, easily removable, emollient, demulcent, non-staining and comparable with number of excipients[2]. Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical process which occur in living organism[3]. Chitosan, natural polysaccharide, has desirable qualities such as homeostasis and biodegradability properties and is being investigated for various biomaterial and biomedical applications. It is commonly accepted that ideal wound covering should mimic many properties of human skin and thus is used in different forms of filament, powder granules, sponge and a composite with cotton or polyester. Due to their biocompatibility, ability to absorb exudates and film forming properties chitosan products are good candidates for burn and wound management[4]. Alginate a linear polymer of β-D- mannuronic acid and α-L-glucoronic acid is known to facilitate wound healing and epidermal regeneration[5]. Aloe Vera is a complex plant containing biologically active substance. It is effective in wound healing and inflammation reduction. This is attributed to a growth factor like substances in aloe that activates wound healing and inflammation reduction process[6]. The important component in wound healing is collagen, which is composed of the amino acids lysine, proline and glycine. Collagen forms the structure of the connective tissue that becomes the frame work around which the new tissues are rebuilt. Vitamin C is an important co-factor, with the enzyme involved in the formation of collagen. Besides stimulating the formation of collagen in healing wound, vitamin C also act as powerful antioxidant and immune system modulator[7]. The purpose of applying antibiotic and other antibacterial agent is mainly to prevent or combat infections specially for diabetic foot ulcers, surgical and accidents wound where the incident of infections can be high due to reduced resistance resulting from extreme trauma[8]. Fucidin is a topical antibacterial agent indicated in the treatment of bacterial infections. Fucidin is isolated from the fermentation broth of *Fusidium coccineum*. Commercially Fucidin is

available in the form of Cream, ointment and may also be given orally as tablets or parenteral as injections[9]. Hence, in the present study an attempt will be made to prepare and evaluate topical gel of Fucidin containing aloe vera along with vitamin C for the effective management of different types of wounds.

MATERIALS AND METHOD

Chitosan was obtained as gift sample from India Sea Foods, Cochin, Kerala and Fucidin was obtained as gift sample from Shivam Enterprises (Pharma division) Gurgaon, Delhi. Aloe vera was obtained from the Rajesh chemicals, Mumbai. Sodium Alginate and Vitamin C was obtained from SD Fine Chem. Ltd. Mumbai and Carbopol 934 from Lobachem Pvt Ltd, Mumbai.

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

The gels were prepared by soaking 1% carbopol in 25ml of water for 24h and then neutralize with sufficient amount of triethanolamine, mixed well with glass rod and kept for 15 minutes. The drug was dissolved in sufficient quantity of ethanol. Accurately weighed quantity of vitamin-C (2%) was dissolved in sufficient quantity of distilled water and then added to the neutralized carbopol with continuous stirring. Finally the drug solution was added to the neutralized carbopol solution with continuous stirring for about half an hour to get a sparkling clear gel. Finally make up the volume up to 50ml with distilled water with continuous stirring. The stirring was stopped periodically to expel the entrapped air during the process of stirring. Same procedure is followed for other remaining formulations containing different wound healing modifiers like sodium alginate, chitosan, aloe vera.

Analytical method

Fourier-transformation infrared spectroscopy (FTIR)

The drug-polymer and polymer-polymer interaction were studied using FTIR spectrometer (Perkin-Elmer (spectrum-100) Japan) by taking 2% w/w of the sample with respect to potassium bromide disc, ground in to a fine powder and then compressed in to a discs in a hydraulic press. Each disc was scanned 16 times at 2mm/sec at a resolution of 4 cm^{-1} using adoptization. The characterization peak was recorded.

EVALUATION OF FUCIDIN GELS

Determination of pH

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

Drug content was determined accurately weighed quantity of 10mg gel and transferred to 100 ml volumetric flask containing 7.4 pH phosphate buffers and allowed to sonicated and filtered, from which 1 ml of aliquot was pipette out and diluted to 10 ml. The content of Fucidin was determined by using Shimadzu UV-visible spectrophotometer at 204.60nm against blank. The test was carried out in triplicate[11].

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) Spreadability

Spreadability of the formulations was determined by an apparatus suggested by Mutimer et al, which was suitably modified in the laboratory and used for the study. It consists of a wooden block which was provided by a pulley at one end. A rectangular ground glass plate was fixed on the block. An excess of gels (about 2 gm) under study was placed on the lower plate. The gel was then sandwiched between lower glass plate and another upper glass plate having the same dimensions, provided with the hook. A 1 Kg weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the gel between the plates. Excess of gel was scrapped off from the edges. The upper plate was then subjected to a pull of 50 gm. With the help of a string attached to the hook and the time (in sec) required by the upper plate to cover a distance of 10 cm was noted. A shorter the time interval indicates better spreadability.

The spreadability was calculated using the formula:

$S = m.l/t.$

Where S = spreadability

m = weight tide to upper side

l = length moved on the glass slide

t = time taken in seconds.

c) Extrudability

In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel extruded from tube on application of certain load. More the quantity extruded better was extrudability. The formulation under study was filled in clean, lacquered aluminum collapsible one- ounce tube with a nasal tip of 5 mm opening. It was then placed in between two glass slides and was clamped. Extrudability was determined by weighing the amount of gels extruded through the tip when a constant load of 1 Kg was placed on the slides and gel extruded was collected and weighed. The percentage of gel extruded was calculated and grades were allotted (+++ good; ++ fair and + poor)[12, 13].

d) *In Vitro* Diffusion study by using Cellophane Membrane:

The apparatus consists of cylindrical glass tube with 14 mm internal diameter and 62 mm height, which was opened at both ends. The gel formulation equivalent to 10 mg of Fucidin was spread uniformly on the surface of cellophane membrane (previously soaked in distilled water for overnight) and was fixed to the one end of the tube such that preparation occupies inner circumference of the tube. The whole assembly was fixed containing gel was touched (1-2 mm

deep) the surface of diffusion medium i.e., 100 ml of 7.4 pH phosphate buffer and maintained temperature 37±2˚C. The cellophane membrane acts as barrier between the gel and 7.4 pH phosphate buffer. The medium were stirred using magnetic stirrer at 50±5 rpm. A quantity of 5 ml sample was withdrawn from receptor fluid at the time interval of 1, 2, 3, 4, 6 and 8 hours and replaced at each time with 7.4 pH phosphate buffer. The release of drug was estimated by using Shimadzu UV–Visible spectrophotometer at 204.60 nm. The study was carried out in triplicate[14].

e) *In vivo* wound healing activity:

Male Wister albino rats (150-250g) were used in the study. A total of 16 groups of each having three animals were used. Animals were housed under standard conditions of laboratory. In vivo wound healing activity was carried out by excision wound model for gel formulations. Marketed soframycin was used as a standard. Excision wounds were inflicted under light ether anaesthesia by excising a circular piece of (18 mm^2) of full thickness skin from the dorsa interscapular region. Wound contraction was monitored by measuring wound area planimetrically, every alternate day till the wound was completely healed. Wound contraction was calculated as a percentage reduction in wound[15, 16, 17, 18].

RESULTS AND DISCUSSION

In the present study Fucidin topical gels were prepared containing different wound healing modifiers for the effective management of wound. Vitamin C, aloe vera, sodium alginate, chitosan were used as wound healing modifiers. All the gel formulation was prepared by soaking method. The gel formulations were characterized by FTIR study suggested that the formulations prepared is a physical mixture; no chemical reaction took place between drug and polymer as depicted in figure 1 and figure 2.

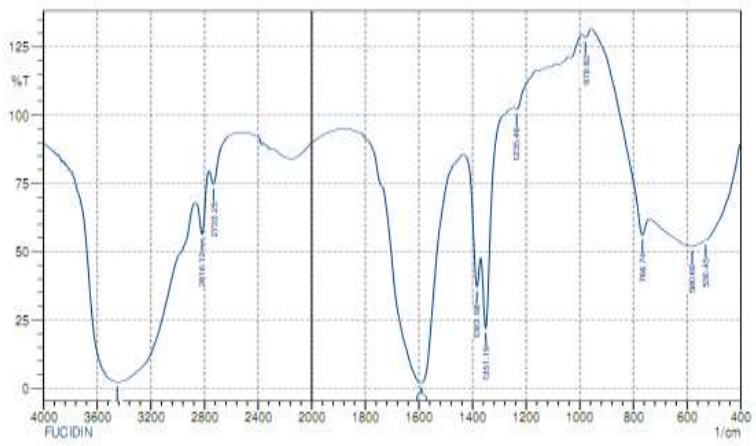

Figure 1: IR spectra of pure drug Fucidin

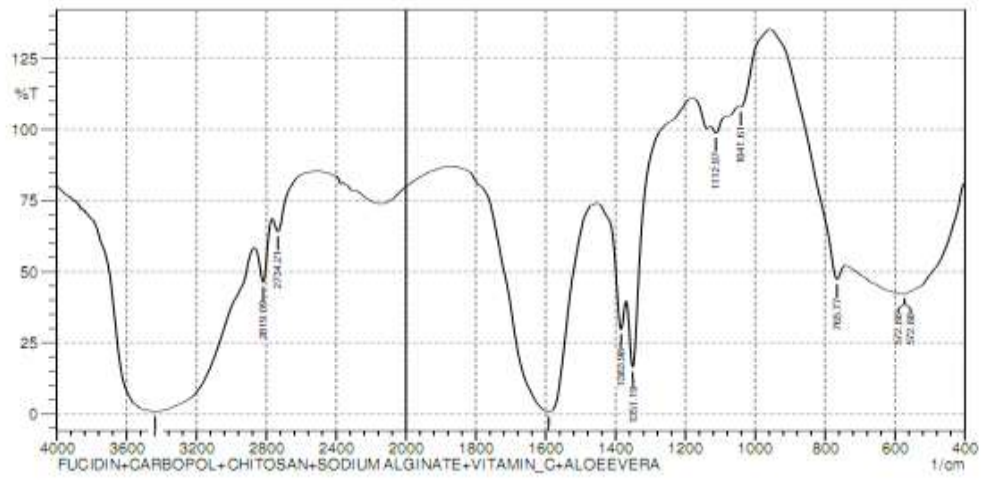

Figure 2: IR spectra of Fucidin + Carbopol 934 + Chitosan + Sodium Alginate + Vitamin-c + Aloe vera

The prepared gel formulations were evaluated for drug content, pH and rheological parameters like viscosity, spreadability 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	Spreadability (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 the formulations showed good viscosity in the range of 3246.31 to 7600.12. The spreadability of all the gels was found between 12.21 to 28.13 gm cm/sec. All the gels were easily extruded out from the tube suggested that the gel have good consistency.

In vitro percent drug release was also determined. The gels were evaluated for in vitro diffusion

study using cellophane membrane in phosphate buffer pH 7.4. The formulation (F8) containing 1% Fucidin along with 1% chitosan and 1% aloe vera showed highest in vitro percent diffusion of Fucidin compared to formulation (F1) containing alone Fucidin and other formulations containing different wound healing modifiers as depicted in table 4 and figure 3. The selected gel formulations were also subjected to stability studies and the results are depicted in table 5. Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state. Wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of tissue, which may be reduced due to infection. The results were expressed in percent contraction in wound data as depicted in table 6 and figure 5. Results were also represented in bar-graph in figure 4. All the gel formulations showed more than 80% reduction in wound contraction.

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)

Time (hrs)	Cumulative Percent Drug Release				
	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

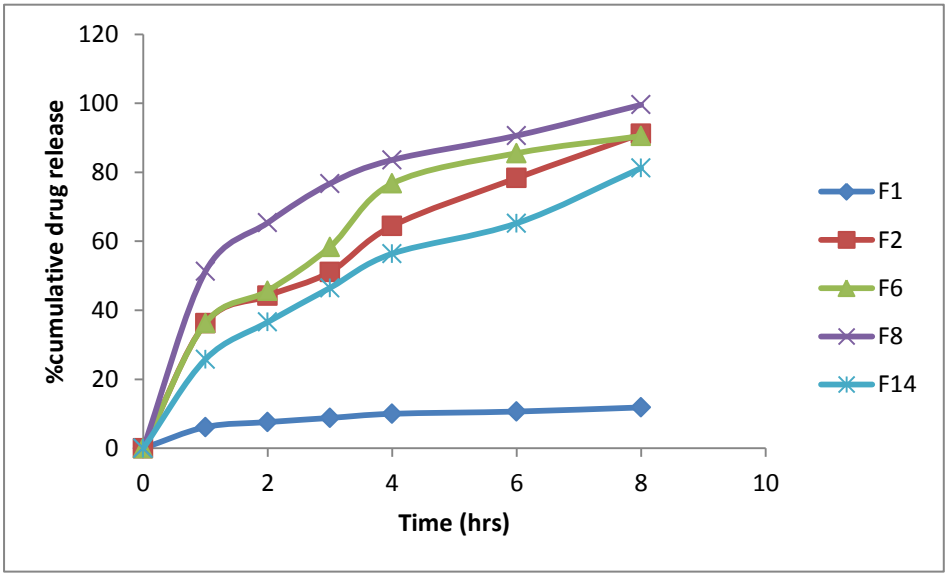

Figure 3: *In vitro* drug release profile of selected formulations

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)

Post-Wounding Days	Wound Area in (mm)					
	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.

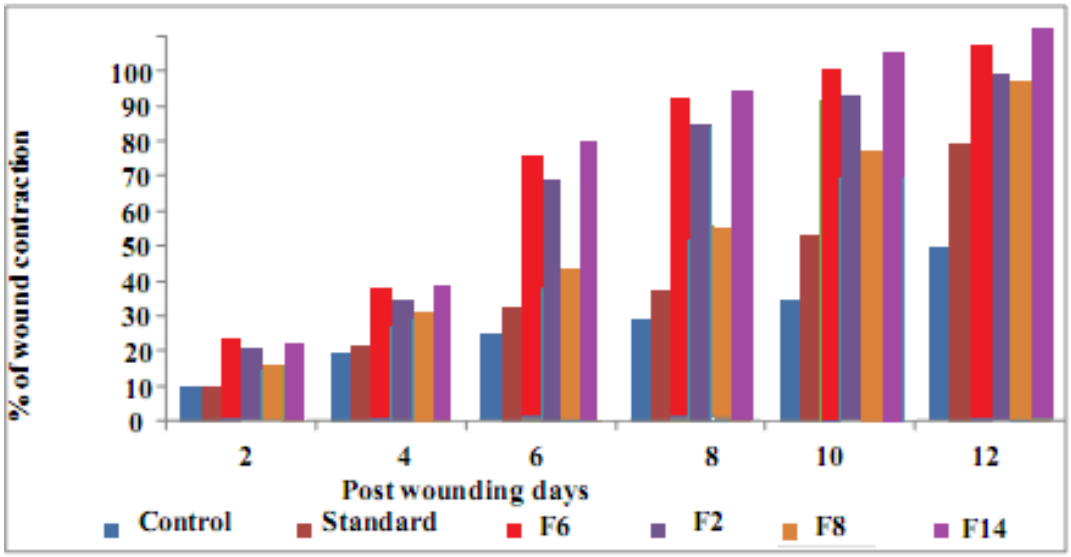

Figure 4: Wound healing activity Fucidin gel (F2, F6, F8, F14)

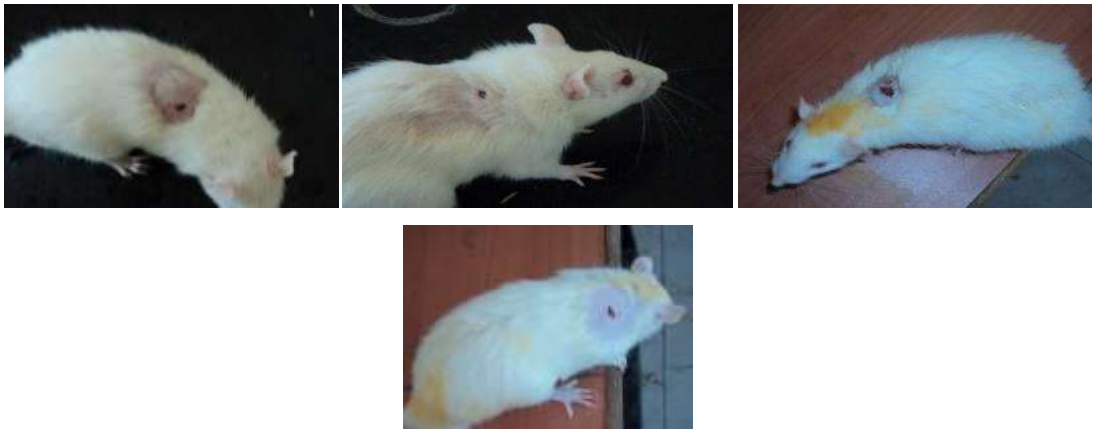

Figure 5: Wound area in mice after 12 days of application of formulations F2, F6, F8 & F14 respectively

CONCLUSION

The topical gel formulation containing 1% Fucidin along with different wound healing modifiers like chitosan, aloe vera, vitamin C and sodium alginate in different concentrations were prepared by using carbopol 934p as gel base/gallant. All the prepared gels were in acceptable range of drug content, pH, and rheological parameters. The in vitro percent diffusion study was carried out using cellophane membrane. The formulation (F8) containing 1% Fucidin along with 1% chitosan and 1% aloe vera showed highest in vitro percent diffusion of Fucidin as compared to formulation (F1) containing alone Fucidin and other formulations containing different wound healing modifiers. The gel formulations were evaluated for the in vivo wound healing activity. All the gel formulation showed more than 80% reduction in wound healing activity. The gel formulation containing Fucidin along with 1% chitosan and 1% sodium alginate showed 99.4% reduction in wound area after 12th day. The gel formulation (F10) showed 95.5% reduction in wound area after 12th day. The gel formulation (F11) showed 90.4% reduction in wound area after 12th day. The gel formulation (F15) showed 94.65% reduction in wound area after 12th day. Stability studies of selected gel formulations were performed to assure that the formulation retains its activity. The formulations were found to be stable. Hence, from the overall study it can be concluded that Fucidin gels along with wound healing modifiers would be promising in the effective management of wounds.

ACKNOWLEDGEMENT

The authors are thankful to Shivam Enterprises (Pharma division) for providing Fucidin and Luqman College of Pharmacy, Gulbarga for providing all the necessary facilities to carry out the research.

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