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Development and evaluation of ofloxacin topical gel containing wound healing modifiers from natural sources

Sravani Kota[1], Mohammed Asadullah Jahangir[1], MD. Mazher Ahmed[1], Imran Kazmi[2], P. Durga Bhavani[3], Abdul Muheem[4] and M. A. Saleem*[1]

[1]Department of Pharmaceutics, Luqman College of Pharmacy, Gulbarga, India
[2]Department of Pharmacognosy, Glocal University, Saharanpur, India
[3]Department of Pharmaceutics, SSJ College of Pharmacy, Hyderabad, India
[4]Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

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ABSTRACT

Ofloxacin is a synthetic fluorquinolone (fluoroquinolones) antibacterial agent that inhibits the super-coiling activity of bacterial DNA gyrase, halting DNA replication. Topical gel formulations of Ofloxacin were developed by using gel forming agent like Carbopol 934P. Chitosan, Neem, and Turmeric were used as wound healing modifiers in different concentrations. Glycerin was used as humectants. The gel formulations were characterized by IR study suggested that the formulations prepared are a physical mixture. The prepared gel formulations were evaluated for drug content, pH and rheological parameters like viscosity, spreadability and extrudability. The prepared gels were also evaluated for in-vitro diffusion study. The percent release of Ofloxacin from plain gel containing alone Ofloxacin was slow as compared to other gel formulations containing different wound healing modifiers. The formulation (F2) containing 1% Ofloxacin along with 2% chitosan showed maximum percent release (96.48%). The gels were also evaluated for in vivo wound healing activity. All the gel formulations showed more than 89% reduction in wound. The gel formulation (F7) containing Ofloxacin along with 1% chitosan and 1% turmeric showed 99.1% reduction in wound area after 12thday. Hence, from the overall study it was concluded that Ofloxacin gels along with wound healing modifiers would be promising in the effective management of wounds.

Keywords: Ofloxacin, Turmeric, Neem, Chitosan, Aloevera, Carbopol 934P.

INTRODUCTION

Semisolids constitute a significant proportion of pharmaceutical dosage forms [1]. They serve as carrier for drugs that are topically delivered by way of the skin, cornea, rectal tissue, nasal mucosa, vagina, buccal tissue, urethral membrane and external ear lining [2]. Because of their peculiar behavior, semisolids can adhere to the application surface for sufficient long period before they are washed off. This property helps to prolong drug delivery at the application site [3]. A semisolid dosage form is advantageous in terms of its easy application, rapid formulation and ability to topically deliver a wide variety of drug molecules. Semisolids are available as a wide range of dosage forms each having unique characteristics [4].

Ointments are semisolid preparation for external application to skin or mucous membranes. Their composition softens but does not melt upon application to the skin. Therapeutically, ointment function as skin protective and emollients, but they are used primarily as vehicle for topical application of drug substances. Creams are semisolid dosage forms that contain one or more drug substances dissolved or dispersed in a suitable base. Usually an oil-in-water emulsion or aqueous micro crystalline dispersion of long chain fatty acids or alcohol that are water

washable and cosmetically acceptable. Gels are semisolid systems that consist of either suspension of small in organic particles or large organic molecules interpenetrated by a liquid. Gel can be either water based (aqueous gels) or organic solvent based (organo gels) [5].

Pastes are semisolid dosage forms that contain one or more drug substances incorporated in a base with large proportions of finely dispersed solids [3]. Semisolid dosage forms usually are intended for localized drug delivery. Localized drug delivery by semisolid dosage form constitutes to be a major area of research. Advances in formulation approach have led to increase drug stability as well as improvement in aesthetic appeal of semisolid dosage form. Turmeric (*Curcuma longa*) is also known as Indian saffron. It belongs to the family of zingiberaceae. It has been traditionally used as an anti-inflammatory, anticancer and antiseptic agent [6].

Chitosan is currently receiving a great deal of attention for medical and pharmaceuticalapplications due to its beneficial intrinsic properties. Chitosan is a deacetylated chitin derivative containing amino sugar. Chitosan possess many properties that are advantageous for wound dressing, biocompatibility, biodegradability, haemostatic activity, anti-infection and wound acceleration properties. Alginate is another polymer which is biodegradable and obtained from natural origin having wound healing property and good bio-adhesion which is necessary for more properties.
Ofloxacin is a synthetic fluoroquinolone antibacterial agent that inhibits the super-coiling activity of bacterial DNA gyrase, halting DNA replication [7]. Commercially it is available in the form of solution 0.3% (ear and eye drops), tablets (200-400mg).

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 [8].

Neem (*Azadirachta indica*) is indigenous to India and is cultivated in many parts of the world because its plethora of medicinal use. The alcoholic extract of neem has been proved to be useful in eczema, ringworm and scabies. The extract of neem leaf and oil from seeds has proven anti-microbial effects. They are helpful in prevention of any secondary infection microorganism [6].

Topical application of antimicrobial agent is useful tool for the therapy of skin and soft tissue infections. It has several potential merits compared with systemic therapy. Firstly it avoid an unnecessary exposure of the gut flora (e.g. by the oral route), which may exert selection for resistance. Secondly it is expected that the high local drug concentration in topical application should overwhelm many nutritional resistance. Thirdly topical applications are less like than systemic therapy to cause side effects [9]. At present there are several kinds of antimicrobial agent used in topical applications such as β-lactams, quinolones, aminoglycosides, macrolides, tetracycline and fusidic acid. Hence, in the present work an attempt will be made to prepare and evaluate topical gel of Ofloxacin containing turmeric, neem oil and aloe vera for the effective management of different types of wounds.

MATERIALS AND METHODS

Materials:
Chitosan was obtained as gift sample from India Sea Foods, Cochin, Kerala and Ofloxacin was obtained as gift sample from Macleods Pharmaceuticals Ltd, Mumbai. Turmeric and Neem oil was obtained from the Jajee Stores, Gulbarga. Aloe vera was obtained from the Rajesh chemicals, Mumbai. Carbopol 934 was obtained from Lobachem Pvt Ltd, Mumbai. All other chemical used were of analytical grade.

Method:
Formulation of Ofloxacin gels
Different gel formulations were prepared containing 1%w/w of Ofloxacin using Carbopol 934P as gel base according to the formula mentioned in the table 1 and 2.

Procedure:
The gels were prepared by soaking 1% carbopol in 25ml of water for 24hrs and then neutralized with sufficient amount of triethanolamine, mixed well with glass rod and kept for 15 minutes. The drug was dissolved in sufficient quantity of methanol. Accurately weighed quantity of turmeric (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 30 mins to get a sparkling clear gel. Finally the volume was made up to 50ml with distilled water with continuous stirring. The stirring was stopped periodically to expel the air entrapped during the process of stirring. Same procedure

was followed for other remaining formulations containing different wound healing modifiers like chitosan, aloe vera and neem.

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

Ingredient (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8
-	1	1	1	1	1	1	1	1
-	1	1	1	1	1	1	1	1
-		2	-	-	-	1	1	1
Neem		-	2	-	-	1	-	-
Turmeric		-	-	2	-	-	1	-
Aloevera	-	-	-	-	2	-	-	1
Methanol	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	42.82	42.82	42.82	42.82	42.82	42.82	42.82	42.82

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

Ingredient (%w/w)	F9	F10	F11	F12	F13	F14	F15	F16
Drug	1	1	1	1	1	1	1	1
Carbopol	1	1	1	1	1	1	1	1
Chitosan	-	-	-	0.5	0.5	0.5	-	0.25
Neem	1	1	-	0.5	-	0.5	0.5	0.25
Turmeric	-	1	1	-	0.5	0.5	0.5	0.25
Aloevera	1	-	1	0.5	0.5	-	0.5	0.25
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	42.82	42.82	42.82	42.82	42.82	42.82	42.82	42.82

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 Ofloxacin gels:

1) Appearance:

Gel formulations were visually inspected for clarity, colour, homogeneity, presence of particles and fibers.

2) Determination of pH:

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

3) Drug content:

Drug content was determined by accurately weighing 10 mg gel and transferring to 100ml volumetric flask containing 7.4 pH phosphate buffer, which was then sonicated and filtered, from which 1 ml of aliquot was pipette out and diluted to 10 ml. The content of Ofloxacin was determined by using Shimadzu UV-visible spectrophotometer at 293.0 nm against blank. The test was carried out in triplicate [11].

3) 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 a dean, 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].

4) *In vitro* Diffusion Study
In vitro release of Ofloxacin from gel was done by using modified apparatus.

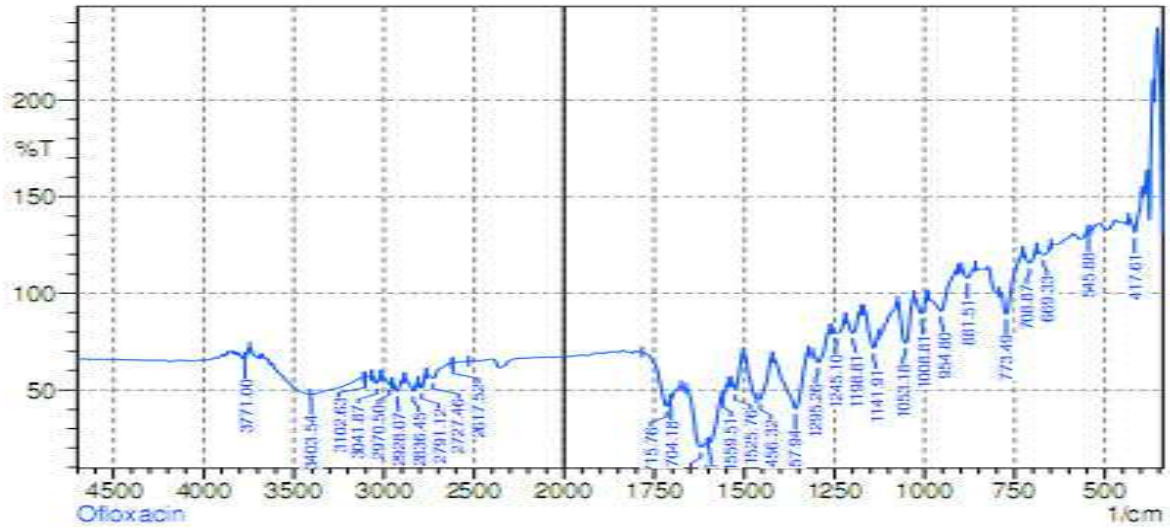
By using Cellophane Membrane:
The apparatus consists of cylindrical glass tube with 14mm internal diameter and 62 mm height opened at both ends. The gels formulation equivalent to 10 mg of Ofloxacin was spread uniformly on the surface of cellophane membrane (previously soaked in distilled water for overnight) 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 acted 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 a time interval of 1 hr and replaced at each time with 7.4 pH phosphate buffer [13]. The study was carried to 8 hrs. The release of drug was estimated by using Shimadzu UV visible spectrophotometer at 293.0 nm. The study was carried out in triplicate.

5) In vivo wound healing activity:
Male Wister albino rats (150-250gm) were used in the study. A total 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 anesthesia by excising a circular piece of (18 mm^2) of full thickness skin from the dorsal interscapular region. Wound contraction was monitored b y measuring wound area planimetrically, every alternate day till the wound was completely healed [14], [15], [16], [17]. Wound contraction was calculated as percent reduction in wound.

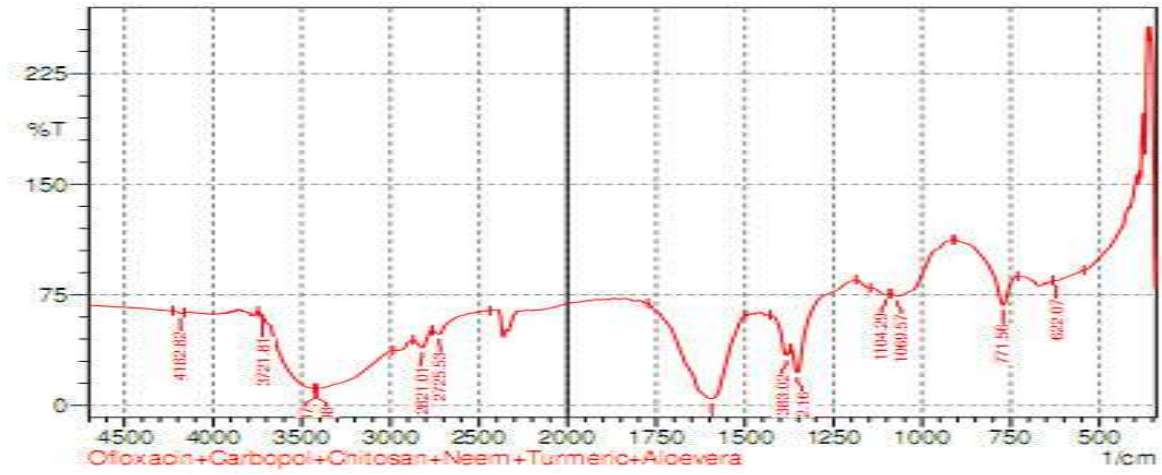
6) Stability studies:
The stability of selected formulation was studied as per ICH guidelines. The gel formulation was packed in container and kept at temperature 30±2ºC and relative humidity 65±5% for 3 months [18]. Stability Studies were carried out for the drug content, viscosity, and pH of the formulations.

RESULTS AND DISCUSSION

The FTIR spectra of drug mixtures which contains pure drug Oflaxacin and polymers were shown in figure 1. The FTIR study of Oflaxacin and polymers revealed that all the peaks with respected wave numbers of pure drug are retained and showed no significant shift or reduction in intensity of peaks of Oflaxacin. Hence, it is clear that the drug has not undergone any type of structural change or any chemical reaction with the polymers and other excipients used.



(A)



(B)

Fig 1: IR spectra of (A) Ofloxacin (B) Drug + Polymers + Wound healing modifiers

All the gels were evaluated for appearance, pH and content uniformity and data is present in table 3. The drug content was found in the range of 95.43 to 99.39 % for all gel formulations suggested uniform distribution of Ofloxacin in gels. The pH of all gel formulation was more than 6. The gels were subjected to rheological studies and various rheological parameters are presented in table 3. Viscosity is an important parameter for characterizing the gel as it affects the spreadability, extrudability and release of drug. All the formulations showed good viscosity between (5329.43 to 7800.12). Spreadability is important property of gel formulation. A good gel takes less time to spread. The spreadability of all the gels was found between 11.21 to 40.23 gm cm/sec. All the gels were easily extruded out from the tube suggested that the gel have good consistency.

Table 3: Evaluation of Ofloxacin gel formulations

Formulation code	pH	Drug content%	Viscosity (cps)	Extrudability	Spreadibility gm-cm/sec
F1	6.8±0.15	99.13±0.44	7800.12	+++	11.21
F2	6.5±0.10	95.43±0.38	7158.18	+++	14.65
F3	7.1±0.13	95.65±0.49	6983.31	+++	19.23
F4	6.8±0.15	95.48±0.70	5912.64	++	26.13
F5	7.4±0.15	98.13±0.88	6143.64	+++	24.88
F6	6.8±0.20	99.39±0.35	6945.36	+++	19.86
F7	7.1±0.12	97.77±0.49	5486.51	+++	35.28
F8	6.23±0.15	98.88±0.19	6143.24	+++	24.88
F9	7.2±0.10	97.99±0.23	6435.76	+++	22.12
F10	6.9±0.16	98.99±0.15	5087.29	+++	40.23
F11	7.20±0.12	98.33±0.13	5426.21	++	38.21
F12	7.00±0.14	98.33±0.33	5832.13	+++	28.00
F13	6.5±0.15	96.66±0.72	5646.31	+++	32.16
F14	6.00±0.10	98.83±0.77	5324.43	+++	24.12
F15	6.92±0.17	97.30±0.42	5333.57	+++	36.98
F16	6.55±0.22	98.99±0.33	5812.99	+++	29.12

(mean±SD, n=3)

The gels were evaluated for in vitro diffusion study using cellophane membrane in phosphate buffer pH 7.4. Hence, from over all in vitro percent diffusion study it can be concluded that the formulation (F2) containing 1% Ofloxacin along with 2% chitosan showed highest (96.48) in vitro percent diffusion of Ofloxacin compared to formulation (F1) containing alone Ofloxacin and other formulations containing different wound healing modifiers. Figure 2 shows the graphical in vitro diffusion data.

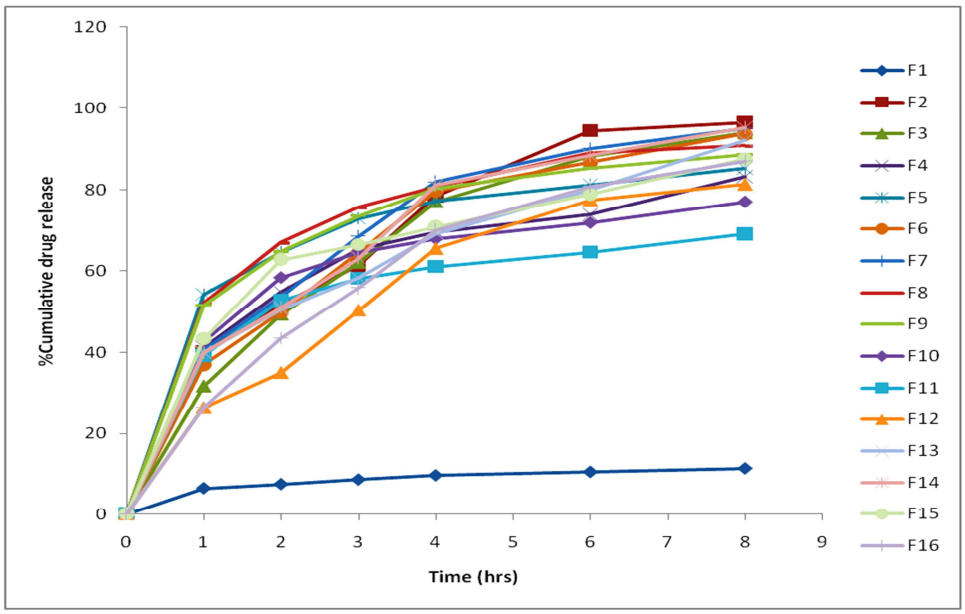


Fig 2: In vitro drug release profile of Formulations (F1-F16)

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 and are represented in bar-graph in figure 3. All the gel formulations showed more than 85% reduction in wound contraction.

Hence, from overall in vivo wound healing study it was concluded that the gel formulation (F7) containing 1% Ofloxacin along with 1% chitosan and 1% Turmeric showed 99.1% reduction in wound area after 12th day as compared to control showed (46.55%), standard (80.15%). The formulation containing (F8) 1% Ofloxacin along with 1% Chitosan and 1% aloe vera showed 98.1% reduction in wound area after 12th day. Addition of turmeric to the formulation (F11) containing 1% Ofloxacin along with 1% aloe vera and 1% turmeric showed improvement in the reduction of wound area to 97.4%. The formulation (F10) containing 1% Ofloxacin along

with 1% turmeric and 1% neem showed 96.9% reduction in wound area after 12th day.

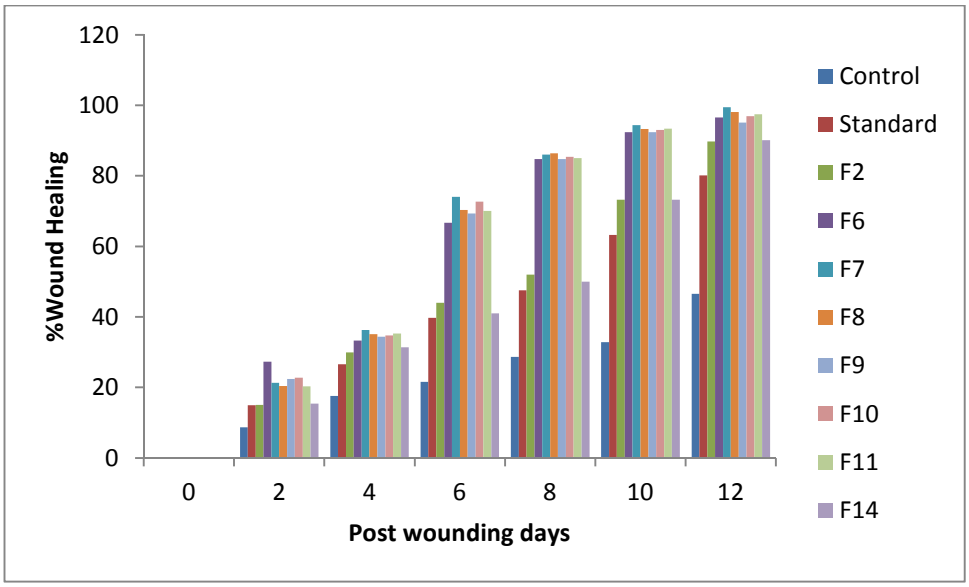


Fig 3: Wound healing activity of Ofloxacin gel with wound healing modifiers

The stability study of selected gel formulation was performed for a period of 3 month at 30±20˚C with RH 65±5%. The gels were checked periodically for the pH, drug content and viscosity and the result was depicted in table 4. The stability study performed for a period of 3 month indicated that the prepared gel formulations are stable.

Table 4: Stability studies of selected Ofloxacin gel formulations (F2, F8, and F12)

Time Interval (months)	F2			F8			F12		
	pH	%Drug content	Viscosity (cps)	pH	%Drug content	Viscosity (cps)	pH	%Drug content	Viscosity (cps)
0	6.5	95.43	7158.18	6.23	98.88	6143.24	7.00	98.33	5832.13
1	6.4	95.40	7157.99	6.20	98.88	6143.22	6.99	98.31	5832.11
2	6.5	95.38	7157.97	6.20	98.85	6143.20	6.97	98.30	5832.11
3	6.4	95.38	7157.99	6.19	98.83	6143.0	6.97	98.30	5832.09

Hence, from the overall study it can be concluded that Ofloxacin gels along with different wound healing modifiers would be promising in the effective management of wounds.

CONCLUSION

In a successful attempt to develop topical gel of Ofloxacin along with different wound healing modifiers it was observed that the topical gel of Ofloxacin along with wound healing modifiers from natural source can provide clinicians with a new choice of topical delivery system for the effective management of wound. However, there is further scope of pharmacokinetic and pharmacodynamic evaluation.

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