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Research Article

STUDY OF FORMULATION, CHARACTERISATION AND WOUND HEALING POTENTIAL OF TRANSDERMAL PATCHES OF CURCUMIN

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ABSTRACT

The aim of this study was to investigate the feasibility of Curcumin patches formulation (CPF) as a transdermal therapeutic system for wound healing potential. A combination of Poly Vinyl Pyrrolidone (PVP) and Ethyl Cellulose (EC) most strongly enhanced the permeation of *Curcumin* patch which permeated through the skin could effectively pass into the systemic circulation and attend therapeutic concentration . All formulation showed good physicochemical properties like thickness, weight variation, drug content, folding endurance, moisture content .The drug release through the transdermal patches of *Curcumin* follows first order kinetics with diffusion controlled mechanism. The results showed wound healing and repair is accelerated by applying CPF-1 formulation of the wound area by an organized epidermis. Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epithelialization. The animals treated with Vicco-turmeric Cream and CPF-1 Formulation showed significant (* $p < 0.01$) wound healing results when compared with control groups. The treated wound after nine days itself exhibit marked dryness of wound margins with tissue regeneration. Group treated with CPF-1 formulation showed better wound closure compared to control group. Histopathological studies of *Curcumin* patches showed well-organized collagen fibers, increased in fibroblast cells and new blood vessels formation as compared to control group.

Keywords: Curcumin, formulation, transdermal patches, wound healing.

INTRODUCTION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Healing is a complex process initiated in response to an injury that restores the function and integrity of damaged tissues. Wound healing involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phase's viz. inflammation (0-3 days), cellular proliferation (3-12 days) and remodeling (3-6 months). The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part.[1-4].

Recently several technical Advancements have been done and resulted in new techniques for drug delivery. Transdermal delivery constitutes one of the most important routes for new drug delivery system (NDDS). Transdermal delivery, that traditionally uses a patch containing drug substances pressed onto the skin, is non-invasive, convenient and painless, and can avoid gastrointestinal toxicity (e.g. peptic ulcer disease) and the hepatic first pass metabolism.[5] These techniques are capable of controlling the rate of drug release. The transdermal drug delivery has advantage to deliver medicines via skin to systemic circulation at a predetermined rate and maintain therapeutic concentration for prolong period of time. [6,7]

Transdermal drug delivery is theoretically ideal for many injected and orally delivered drugs, but many drugs cannot pass through the skin because of skin's low permeability. Transdermal administration of drugs that undergo first pass metabolism can improve the bioavailability and reduce the dosing frequency compared with the oral route.

Curcumin is the principal curcuminoid of the popular Indian spice turmeric which is a member the ginger family (Zingiberaceae). which have low oral bioavailability i.e. 40-85 percent of an oral dose of Curcumin passes through the gastrointestinal tract unchanged, with most of the absorbed flavonoid being metabolized in the intestinal mucosa and liver[14]. Low oral bioavailability motivates us to study topical preparation. A combination of Poly Vinyl Pyrrolidone (PVP) and Ethyl Cellulose (EC) most strongly enhanced the permeation of *Curcumin* transdermal patch which permeated through the skin could effectively pass into the systemic circulation and attend therapeutic concentration

MATERIALS AND METHODS

Animals

The healthy albino rats of either sex (200-250 g) with no prior drug treatment were selected to carry out all the present in vivo studies. The animal was used after an acclimatization period of 10 days to laboratory environment. They were housed in standard metal cages and provided with food and water *ad libitium*. Animal study was performed in division of pharmacology, VNS Institute of Pharmacy, Bhopal (M.P) with due permission from institutional ethical committee. The protocol of the study was approved by the Local Ethical Committee for animal experimentation. For excision wound model, 18 animals of either sex weighed between 200-250 g were divided into three groups in each groups consisting of 6 animals as follows. Group I is (untreated) control group, group II is (vicco-turmeric cream) standard group, group III (CPF-1F Formulation) treated group.

Optimization of Formulation

Preparation of casting solutions

Poly Vinyl Pyrrolidone and Ethyl cellulose (CPF-1, CPF-2 and CPF-3)

The casting solutions were prepared by dissolving different weighed quantities polymer ratio such as PVP (Poly Vinyl Pyrrolidone) 300 mg and EC (Ethyl Cellulose) 100 mg of polymers (formulation code is CPF-1), PVP 200 mg and EC 150 mg of polymers [formulation code is *Curcumin* patch formulation) CPF-2] and PVP 100 mg and EC 200 mg of polymers (formulation code is CPF-3) in chloroform. The Curcumin drug 20 mg was dissolved in chloroform and added to the above polymer solution along with propylene glycol (0.5 ml), as plasticizer, and 0.1 ml of DMSO (disodium methyl sulfoxide) as penetration enhancer which was thoroughly mixed to form a homogeneous mixture. The volume was made up to 10 ml with chloroform. Entrapped air bubbles were removed by applying vacuum.

Preparation of Transdermal Patches

The casting solution (10 ml) was poured into glass moulds and dried at room temperature for 24 h for solvent evaporation. The patches were removed by peeling and cut into squares with dimension of 2x2 cm^2. These patches were kept in desiccators for 2 days for

further drying and wrapped in aluminium foil, packed in self-sealing covers. Transdermal patches were prepared with different polymer ratio, with constant plasticizer concentration and permeation enhancers.[8]

Pre-formulation study of the Transdermal Patches

Pre-formulation study is the one step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of pre-formulation study is to generate information useful to the formulator in developing stable and bio available dosage forms that can be produced.

In vitro drug release studies

The fabricated patches was placed on the rat skin and attached to the diffusion cell such that the cell's drug releasing surface was towards the receptor compartment which was filled with phosphate buffer solution of pH 7.4 at 37±1 ^{0}C. The elution medium was stirred magnetically. The aliquots (5ml) were withdrawn at predetermined time intervals and replaced with same volume of phosphate buffer of pH 7.4. The collected samples were diluted with equal volumes of ethanol and the absorbance was recorded at 416.0 nm.[9]

***In vivo* study for wound healing activity**

Excision wound model

All the animals were divided into 3 groups and the animals were kept in separate cages. Three groups of animal containing 6 in each group were anaesthetized by open mask method with anesthetic ether before wound creation. Group I, II, & III were assigned as control, standard, and CPF-1Formulation. The particular skin area was shaved one day prior to the experiment. An excision wound inflicted by cutting away a 300 mm^2 full thickness of skin from a predetermined shaved area. Rat's wounds were left undressed to the open environment. The patches were topically applied once in a day, till the wound was completely healed. In this model wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.[10,11]

Wound healing evaluation parameters

Wound contraction measurement

The progressive changes in excision wound area were measured in mm^2 by tracing the wound boundaries on transparent paper on each 2 days interval until complete wound healing, wound creation. The wound areas in all groups were recorded on graph paper. Wound contraction was expressed as reduction in percentage of the original wound formula

% wound contraction = healed area / total wound area ×100

Wound area was measured by tracing the wound margin using a transparent paper in each 2 days interval and healed area calculated by subtracting from the original area.[12]

Epithelialization period

Epithelialization period was monitored by noting numbers of days required for the Escher to fall off from the wound surface without leaving a raw wound behind. The epithelialization period was measured from initial day.[13]

STATISTICAL ANALYSIS

The relative wound area results were compared using one- way analysis of variance (ANOVA) followed by Dennett's tests.

RESULTS AND DISCUSSION

Preformulation studies

For organoleptic property different test were performed on *Curcumin*. The results shows *Curcumin* color was orange powder, taste was acrid and odor was odorless. The melting point of the compounds was determined using VEEGO apparatus. Practically obtained melting points were sharp (specification was 179-185ºC and observation was 182±1ºC) which indicates the purity of the synthesized compounds. The solubility of compounds was determined in polar and non-polar solvents. Solubility of 10 mg *Curcumin* was insoluble in > 100 ml of water, 10 mg Curcumin was insoluble in > 100 ml of ether and 100 mg of *Curcumin* was sparingly soluble in 5-10 ml ethanol.

Determination of Absorption Maxima (λ_{max})

The wave length of light in the ultra violet range at which the compound shows the maximum absorbance is called as λ_{max}. The UV spectra of compounds are shown in figure 3.1. The λ_{max} was 416.0 nm of Curcumin compound.

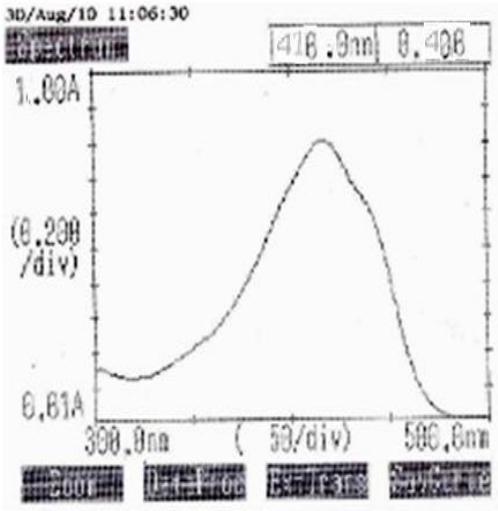


Figure 3.1 Absorption maxima (λ_{max}) of *Curcumin*

Compatibility Study using Fourier Transform Infrared (FTIR) Spectroscopy

The spectra showed no incompatibility between the polymer and *Curcumin* drug.

Table 3.1: IR spectra of *Curcumin* Frequency in cm^{-1}

Frequency in cm^{-1}	Indications
3500.8	OH
2960.0	OCH_3
3004.5	C-H Aromatic
1695.6	C=O
1632.1	C-C
1400.4	C=C

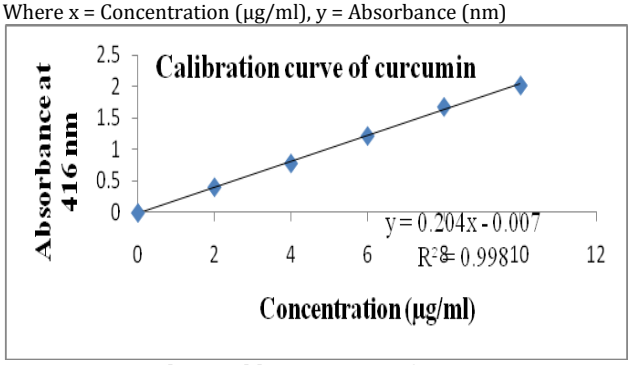
Calibration curve of *Curcumin*

Results obtained are presented in table 3.2 and fig 3.6.

Table 3.2: Absorbance of different dilution at 416.0 nm

S. No.	Concentration (µg/ml)	Absorbance (n=3) I	II	III	Average ± Std. Dev.
1	0	0	0	0	0
2	2	0.404	0.406	0.408	0.406 ± 0.002
3	4	0.787	0.780	0.782	0.783 ± 0.003
4	6	1.213	1.219	1.216	1.216 ± 0.003
5	8	1.675	1.680	1.676	1.677 ± 0.002
6	10	2.014	2.019	2.015	2.016 ± 0.002

Calibration Curve of *Curcumin* at 416.0 nm is taken and curve show in Graph: 3.1
Correlation Co-efficient (r^2) = 0.998
Equation for regressed line y =0.204x-0.007
Slope of regressed line =0.204
Where x = Concentration (µg/ml), y = Absorbance (nm)



Graph 3.1 Calibration curve of *Curcumin*

Physical evaluation.

The values obtained for all the formulations are given in the table 3.3.

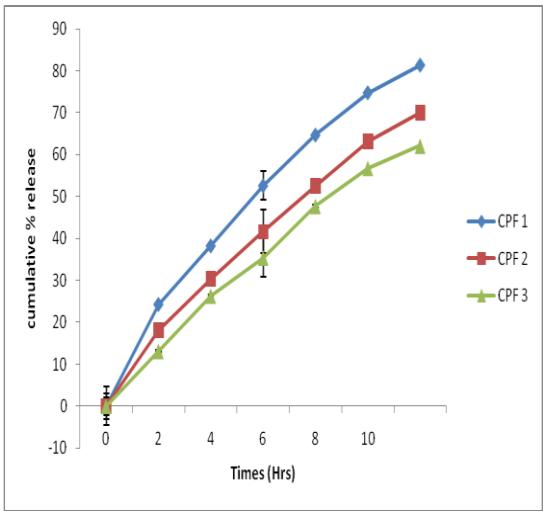
Table 3.3: Physicochemical evaluation of Transdermal patches

Parameters	CPF-1	CPF-2	CPF-3
Weight variation(mg) +SD	211.2 ± 2.1	207.6 ± 4.6	198.2 ± 3.1
Thickness(mm) +SD	0.19 ± 0.1	0.20 ± 0.3	0.18 ± 0.2
% Moisture contents +SD	4.13 ± 0. 6	3.66 ± 0.8	2.80 ± 0.3
Folding endurance +SD	10 ± 3.5	12 ± 5.2	13 ± 4.5
% Drug Content +SD	99.30 ± 0.1	98.20 ± 0.2	97.11 ± 0.3

3.3: In vitro drug release studies

Table 3.4: Comparison between in-vitro releases of different formulation

Time(hrs)	CPF-1	CPF-2	CPF-3
0	0	0	0
2	24.34 ±2.34	18.13 ±2.03	13.13 ±3.67
4	38.29 ±1.56	30.41 ±2.41	26.31 ±1.76
6	52.63 ±2.63	41.61 ±1.53	35.42 ±2.47
8	64.71 ±1.98	52.56 ±3.21	47.72 ±1.33
10	74.81 ±3.11	63.13 ±1.88	56.81 ±2.76
12	81.38 ±1.45	69.93 ±2.06	62.12 ±1.43



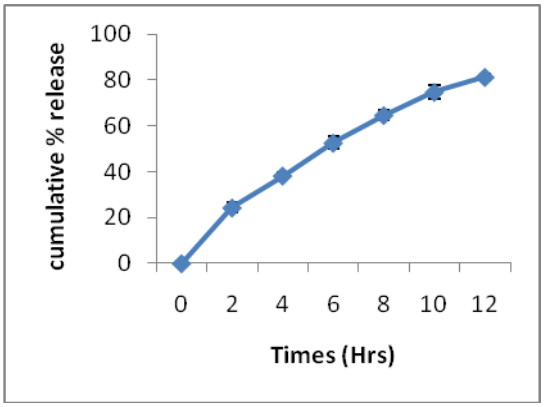
Graph 3.1 Comparison of In vitro release of various formulations

Table 3.5: In-vitro release data of optimize formulation CPF-1

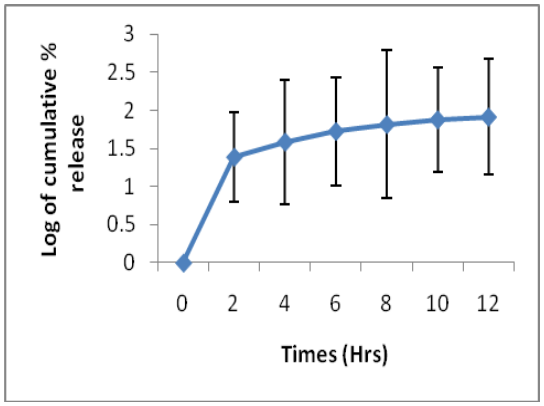
Time	Sq. root of time	Log time	% cumulative release	Log % cumulative release	% cumulative remaining	% log cumulative remaining
0	0	0	0	0	100	2.000
2	1.410	0.301	24.34 ± 2.34	1.386 ± 0.59	76.670 ± 1.89	1.879 ± 0.48
4	2.000	0.602	38.29 ± 1.56	1.583 ± 0.82	61.710 ± 1.32	1.790 ± 0.61
6	2.450	0.780	52.63 ± 2.63	1.721 ± 0.71	47.370 ± 1.73	1.675 ± 0.71
8	2.830	0.903	64.71 ± 1.98	1.812 ± 0.97	35.290 ± 1.48	1.548 ± 0.54
10	3.160	1.000	74.81 ± 3.11	1.874 ± 0.68	25.190 ± 2.01	1.401 ± 0.74
12	3.460	1.079	81.38 ± 1.45	1.910 ± 0.76	18.620 ± 1.11	1.270 ± 0.37

Table 3.6: Kinetic equation parameter (r^2 value) of optimized formulation

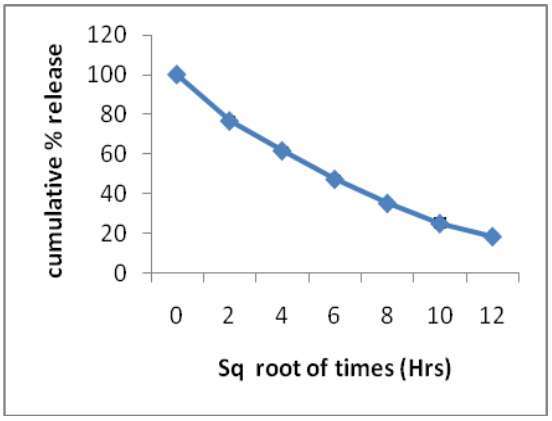
Formulation	Zero order		First order		Higuchi		Peppas	
	R^2	K_0	R^2	K_f	R^2	K_h	R^2	N
CPF-1	0.968	6.634	0.995	0.060	0.981	24.280	0.805	1.532



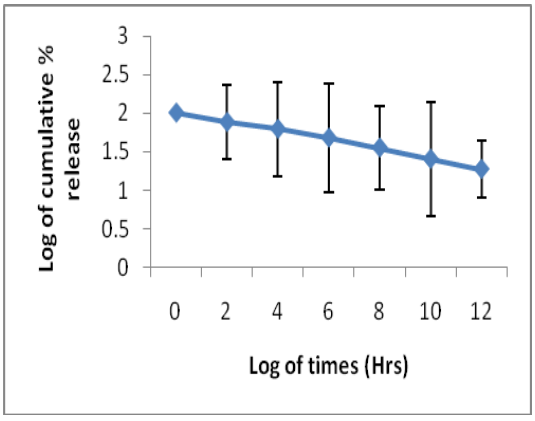
Graph 3.2 Zero order plot for drug release kinetics



Graph 3.3 First order plot for drug release of optimized formulation.



Graph 3.4 Higuchi plot for drug release kinetics



Graph 3.5 Peppas plot for drug release kinetics of optimized formulation of optimized formulation

3.4: In vivo studies for wound healing potential of CPF-1 Formulation

3.4.1 Effect of CPF-1 Formulation and standard cream on % of wound contraction on

Table 3.7: Effect of Vicco-turmeric Cream and CPF-1 Formulation on % wound contraction of Excision wound models in rats.

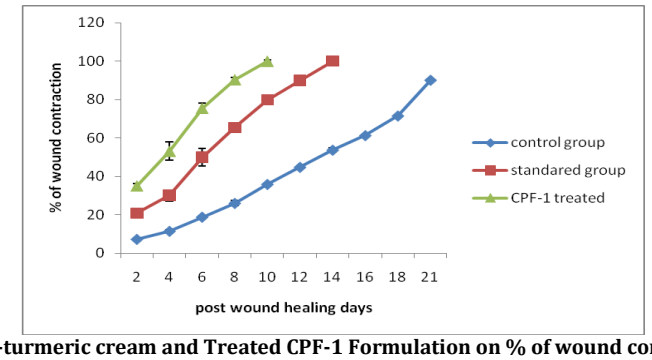
Grps	Post wound days									
	2days	4days	6days	8days	10days	12days	14days	16days	18days	21days
I Grp (Ctrl)	7.37% ±0.38	11.56% ±0.87	18.90% ±0.49	26.09% ±1.23	35.93%±0.42	44.83% ±0.65	53.75% ±1.27	61.32% ±0.38	71.51% ±0.32	90.11%±0.43
II Grp (Std)	20.82% ±1.16	30.08% ±2.92	49.98% ±4.56	65.40% ±1.55	79.80% ±1.26	89.89% ±1.16	99.99%±1.16*			
III Grp (CPF-1)	35.12% ±1.17	53.13%±4.78	75.50% ±2.49	90.4% ±1.16	99.99% ±0.54*					

n=6 albino rats per group; values are represent mean± SEM.*p< 0.01(Comparison of I with II&III)

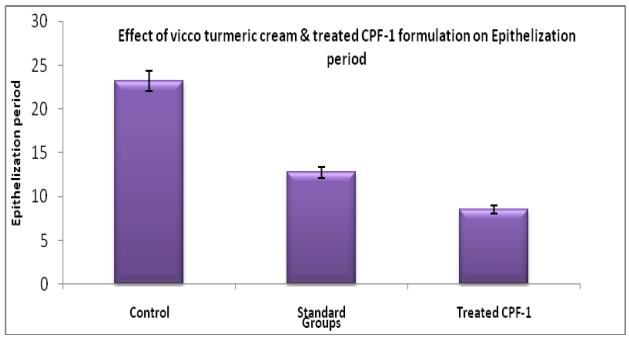
Table 3.8: Effect of topical application of cream & CPF-1 Formulation on wound parameters of excision wound models in rats.

S.NO.	Groups	Epithelialization (Mean time in days)
I	Group (Control)	23.25 ± 0.478
II	Group (Standard)	12.75 ± 0.251*
III	Group (CPF-1)	8.50 ± 0.288*

n=6 albino rats per group; values are represent mean± SEM.* p< 0.01



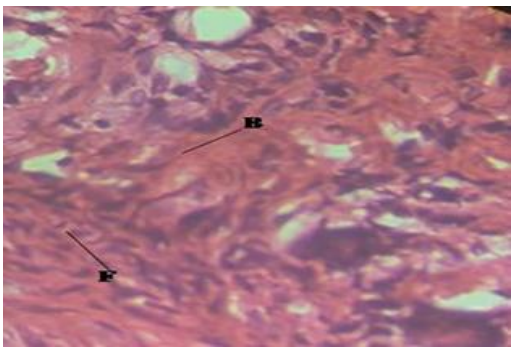
Graph 3.6: Effect of Vicco-turmeric cream and Treated CPF-1 Formulation on % of wound contraction (Excision wound)



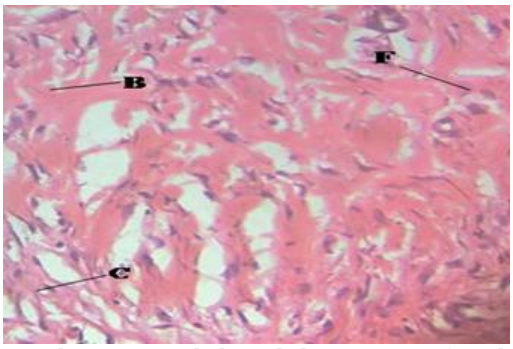
Graph 3.7: Effect of Vicco-turmeric cream and Treated CPF-1 Formulation on Epithelialization period (Excision Wound).

3.5: Histopathological examinations Group I (control) Group II (Standard)

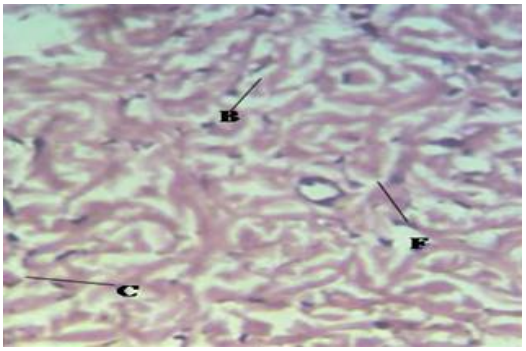
The results of the present study revealed that CPF-1 Formulation of *curcuma longa* have significant (* p< 0.01) wound healing activity in excision wound models. (Table 3.7 & graph 3.6)



Photograph 3.1 Histopathological Characteristic of rat skin of control group.



Photograph 3.2 Histopathological Characteristic of rat skin by treatment with Vicco- turmeric Cream Photo shows poor fibroblast cells (F), Photo shows increased fibroblast cells (F), blood vessels (B), in Excision wound, blood vessels (B), & collagen fibers in Excision wound.
Group III CPF-1 Formulation



Photograph 3.3 Histopathological Characteristic of rat skin by treatment with CPF-1 Formulation. Photo shows increased fibroblast cells (F), blood vessels (B), & collagen fibers in Excision wound.

4: CONCULUSIONS

All formulation showed good physicochemical properties like thickness, weight variation, drug content, folding endurance, moisture content .The drug release through the transdermal patches of *Curcumin* follows First order kinetics with diffusion controlled mechanism.

In control albino rat's excision type of wounds shown incomplete healing as in poor fibroblasts cell. Wound healing is stepwise process, which consists of different phases such as haemostasis, inflammation, proliferative and remodelling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair. Hence in this study, excision wound models were used to evaluate the effect of CPF-1 formulation on various phases.

The results showed wound healing and repair, accelerated by applying CPF-1 formulation of the wound area by an organized epidermis. To further understand its therapeutic effect on wound healing, the antioxidant effects of *Curcumin* on H_2O_2 and hypoxanthine-xanthine oxidase –induced damage to cultured human keratinocytes and fibroblasts were investigated. Exposure of human keratinocytes to *Curcumin* at 10 µg/mL significantly protected against the keratinocytes from H_2O_2 induced oxidative damage.

So concluded that *Curcumin* indeed possessed powerful inhibitory capacity against H_2O_2 induced damage in human keratinocytes and fibroblasts and this protection may contribute to wound healing.

Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epithelialization. The animals treated with Vicco-turmeric Cream and CPF-1 Formulation showed significant (* p< 0.01) results when compared with control groups. The treated wound after nine days itself exhibit marked dryness of wound margins with tissue regeneration.

The results obtained showed that CPF-1 Formulation possesses separate wound healing potential. All the results demonstrated that group treated with CPF-1 Formulation showed better wound closure compared to control group.

Histopathological studies of *Curcumin* patches showed well-organised collagen fibres, increased in fibroblast cells and new blood vessels formation as compared to control group.

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