

Original Article

WOUND HEALING POTENTIAL OF TRANSDERMAL PATCHES CONTAINING BIOACTIVE FRACTION FROM THE BARK OF *FICUS RACEMOSA*

V. RAVICHANDIRAN, S. MANIVANNAN

School of Pharmaceutical Sciences, VISTAS, Vels University, Pallavaram, Chennai 117, Tamilnadu, India
Email: vishnuvardh@gmail.com

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ABSTRACT

Objective: The present study aimed to develop transdermal patches containing alkaloid fraction, flavonoid fraction and tannin fraction from *Ficus racemosa* bark and to assess its wound healing potency.

Methods: In the present investigation, herbal transdermal patches were formulated using natural polymer Protanal LF10/60 by solvent evaporation technique. The physicochemical parameters like flexibility, thickness, smoothness, weight variation, moisture content, hardness and tensile strength were evaluated. Transdermal formulation was exposed to *in-vitro* cytotoxicity studies in Balb/c 3T3 cell line using the direct contact method. Wound healing activity of the developed formulation was carried out by excision and incision wound model.

Results: Thin, flexible, smooth and transparent films were obtained with natural polymer Protanal LF10/60. The formulation was found to be flexible, uniform thickness and weight, smooth, good drug content (92 to 96%) and little moisture absorption. Quantitative evaluation of *in-vitro* cytotoxicity studies using MTT assay for the test items and Blank (Placebo) showed a viability of 86.84%, 85.53%, 86.84% and 92.76% respectively. The obtained values indicated that the transdermal patches were non-cytotoxic. Among the tested formulations, the transdermal patches containing flavonoid fraction (TPFF) and tannin fraction (TPTF) showed significant reduction in wound area and significant increase in wound breaking strength in both excision and incision models when compared with control group.

Conclusion: The developed formulations containing bioactive fractions possess significant wound healing activity in excision and incision wound models. The present investigation offers scientific evidence to the folkloric accounts of the use of bark in treating wounds.

Keywords: Transdermal patches, *Ficus racemosa*, Tannin fraction, Wound healing.

INTRODUCTION

Transdermal drug delivery system (TDDS) is topically administered medicaments in the form of patches or semisolids (gels) that deliver drugs for the systemic effects at a predetermined and controlled rate [1]. Transdermal route has gained more attention in drug delivery due to its flexibility and convenience in comparison to other routes of delivery and it is one of the suitable, convenient, safe and economic ways to deliver drug [2].

Transdermal drug delivery system has many advantages over conventional modes of drug administration, provides a controlled rate of release of medicaments, avoids hepatic metabolism, ease of termination and long duration of action. A drug is applied in a relatively high dosage to the inside of a patch, which is worn on the skin for an extended period of time. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since there is high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, maintaining the constant concentration of drug in the blood flow [3].

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells [4].

The plant *Ficus racemosa* belonging to the family Moraceae is a moderate sized deciduous tree [5]. The bark is highly efficacious in threatened abortion, urological disorders, diabetes, hiccup, leprosy, dysentery, asthma, chronic wound and piles [6]. The plant contains various phytoconstituents like alkaloids, tannins, flavonoids and steroids [7]. Several phytoconstituents like alkaloids, flavonoids and tannins are known to promote wound healing process due to their antioxidant and antimicrobial activities. Hence the present study was designed to test the wound healing activity of the transdermal patches containing alkaloid fraction, flavonoid fraction and tannin fraction of *Ficus racemosa* in experimentally induced excision and incision wound model.

MATERIALS AND METHODS

Plant material

The plant specimen *Ficus racemosa* L (Moraceae) bark for the proposed study was collected from in and around Makali, Bangalore, Karnataka and authenticated by the botanist, Himalaya drug company, Bangalore, Karnataka. Specimen samples were preserved in institution laboratory (N13,10752 E77.469889) for further reference.

Extraction

The shade dried bark of *Ficus racemosa* of about 6 kg was subjected for size reduction to coarse powder.

Isolation of alkaloid rich fraction

The coarsely powdered bark of *Ficus racemosa* (2 kg) was exhaustively extracted with methanol (5×1 L) at room temperature. The methanol extract (40 g) was acidified (pH 2) with 2M hydrochloric acid and the final volume was adjusted to 400 mL. The aqueous acidic solution of methanol extract was then extracted with ethyl acetate (3×200 mL) to remove neutral components. After removal of neutral components, the aqueous layer was then made alkaline (pH 9) with 30% ammonium hydroxide solution and

repeatedly extracted with chloroform (3 × 300 mL). The combined extracts were evaporated under vacuum to yield the alkaloid rich fraction labeled as AF. Total alkaloids [8] were spectrophotometrically estimated in the chloroform fraction which was found to contain total alkaloids.

Isolation of flavonoid fraction

The coarsely powdered bark of *Ficus racemosa* (2 kg) extracted using a soxhlet apparatus with methanol (18 h). The extracted solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). The concentrated extract was again exhaustively defatted by refluxing with n-hexane and benzene (15 h twice). The two fractions were negative for polyphenols. Then the defatted bulk residue was successively extracted by refluxing with ethyl acetate (15 h twice). The solvent was filtered, distilled off and the final traces of solvent were removed under vacuum and the fraction was labeled as FF. Total flavonoids [9] were spectrophotometrically estimated in the ethyl acetate fraction which was found to contain total flavonoids.

Isolation of tannin rich fraction

The coarsely powdered bark of *Ficus racemosa* (2 kg) was defatted with petroleum ether. The defatted bark powder was extracted with acetone: water mixture (70:30) by cold maceration method to obtain tannin rich fraction. The solvent was filtered, distilled off and the final traces of solvent were removed under vacuum and the fraction was labeled as TF. Total tannins [10] were spectrophotometrically estimated in the acetone fraction which was found to contain the bulk of tannins.

Thin layer chromatography (TLC)

The fractions of *Ficus racemosa* were subjected to thin layer chromatographic studies. Each of the fractions was spotted on a percolated silica gel 60F₂₅₄ plate (Merck). The mobile phase employed was Chloroform: Methanol (95:05 v/v) for alkaloid fraction, Chloroform: Methanol: Water (6:3.5:0.5, v/v/v), for flavonoid fraction and Butanol: Acetic acid: water (4:1:1 v/v/v) for tannin fraction. Reagents such as Dragendorff, Vanillin sulphuric acid and Ferric chloride were sprayed for the detection of alkaloids, flavonoids and tannins respectively.

Number of spots was noted and R_f values were calculated using the formula,

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Formulation of transdermal patches

Three transdermal patches were designed with alkaloid fraction, flavonoid fraction and tannin fraction. Matrix type transdermal patches composed of natural polymer protonal LF10/60 were prepared by using solvent evaporation method [11, 12]. Drug matrices were prepared by dissolving required amount of fractions (Alkaloid, Flavonoid, Tannin) in hot water, filtered through filter paper and mixed in natural polymer dispersion. The polymer protonal LF10/60 (optimized concentration of 2.5% solution) was dissolved in water. To this dispersion, starch (0.5% solution in hot water) solution was added and stirred well using Remi stirrer. The uniform dispersion obtained was casted on glass petriplates specially designed for this purpose and dried at RT for 6-8 hrs. The dried films were removed and cut manually for required size and were stored in desiccator until use. The formulated transdermal patches containing alkaloid, flavonoid and tannin fraction were labeled as TPAF, TPF, TPTF respectively. The placebo transdermal patches were prepared in a similar method but without incorporation of the fractions. The working formula was calculated on basis of 200 mg of fraction for every formulation except placebo.

Evaluation of formulated transdermal patches

The formulated patches were evaluated for *in-vitro* and *in-vivo* tests. The formulated transdermal patches were evaluated for thickness, weight uniformity, folding endurance, percentage moisture content,

water vapor permeability, drug content, *in-vitro* permeation of the fractions and *in-vitro* cytotoxicity.

a) Thickness of the patch

The thickness of patch was measured in different points by using digital micrometer and the average thickness was calculated [13].

b) Weight uniformity

The prepared patches were dried at 60°C for 4 hrs before testing. A specified area of patch was cut in different parts and weighed in digital balance [14].

c) Folding endurance

A strip of specific area was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance [15].

d) Percentage moisture content

The prepared films were weighed individually and kept in a desiccators containing fused calcium chloride at RT for 24 hrs. After 24 hrs the films were reweighed and the percentage moisture content was calculated by the given formula [16].

Percentage moisture content = $\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$

e) Water vapor permeability

Glass vials of 5 ml capacity were washed and dried in an oven to a constant weight. About 1 gm of fused calcium chloride was taken in the vials & the polymer films were fixed over the brim with the help of an adhesive tape. Then the vials were weighed and stored in a humidity chamber at 85 % RH condition for a period of 24 hours. The vials were removed and weighed at various time intervals like 3, 6, 12, 18 and 24 hrs to note down the weight gain [17].

f) Drug content

A 1 cm² area of the prepared patches was dissolved in 10 ml of water. Then the solutions were filtered using Whatman filter paper. The collected filtrate was analyzed for the drug content by mass spectrometry [18].

g) *In-vitro* permeation studies

In-vitro skin permeation studies were performed by using a modified Franz diffusion cell with a receptor compartment capacity of 20 ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated patches were cut into size of 1 cm² and placed over the drug release membrane and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37±0.5 °C. The samples of 1 ml were withdrawn at time interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h, analyzed for drug content spectrophotometrically at 376 nm against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal. The cumulative amounts of drug permeated per square centimeter of patches were plotted against time [19].

h) *In-vitro* cytotoxicity studies

The direct contact method using Balb/c 3T3 was used to test the *in vitro* cytotoxicity of the prepared transdermal patches [20]. The samples TPAF, TPF, TPTF and blank (Placebo) were exposed to the *in-vitro* cytotoxicity test. Natural rubber latex and cell culture medium were used as positive and negative control respectively. The samples were stored at room temperature. The cell confluence and morphology were confirmed by microscopy and found to be 80%. The culture media was carefully removed and replaced with fresh media. This confluent culture flask was trypsinised and reseeded on to culture flasks for the cytotoxicity assay at a

concentration of approximately 5.5×10^5 cells per culture flask. All the individual test items were treated separately. Triplicate cultures were set up for negative control, positive control and test items. Each individual, negative, positive control and test item measuring $1 \times 2.5 \text{ cm}^2$ covering 10% of the total surface area were carefully placed in center of each culture flask. The cell cultures were then incubated at 37°C for 24 h in an atmosphere of CO_2 . After 24 h incubation period, the cells were subjected to qualitative and quantitative evaluations viz., cell confluency and morphology and grades of cytotoxicity, respectively.

Qualitative evaluation

After 24 h incubation, the cultures were analyzed for microscopic evidence of cytotoxicity under microscope. Qualitative evaluation was assessed by the above grading system given. If the numerical grade obtained is greater than 2 the test item was considered as cytotoxic.

Quantitative evaluation

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of the blue-violet formazan formed. The test item is considered non-cytotoxic if the percentage of the viable cell count is equal to or greater than 70% of the untreated control. A reduction of more than 30% viability in the test item treated cultures compared to concurrent untreated culture indicates cytotoxicity. Good scientific judgment was used in interpreting the data. Quantitative evaluation using the MTT assay was measured by colorimeter at absorbance at 580 nm.

In-vivo evaluation of formulated transdermal patches

Animals

Wistar rats (150-200 g) and Swiss albino mice (15-25 g) were obtained from the institutional animal house. Animals were kept in polypropylene cages and were left for 5 days for acclimatization to animal room maintained at standard laboratory conditions, air-conditioned with adequate fresh air supply, with 12 h fluorescent light and 12 h dark cycle. Water was provided *ad libitum* throughout the acclimatization and experimental period. All the animals were taken care under ethical consideration as per the guidelines of CPCSEA with due approval from Institutional Animal Ethics Committee (IAEC), approval number IX/290/CPCSEA/PHA-05-09 dated 16.10.2009.

Acute toxicity

Acute toxicity studies were carried out using mice as per Economic Co-operation and Development OECD 425 guidelines [21]. Mice (6/group) were divided into four groups. The first 3 groups were treated at a dose of 2000 mg/kg body weight of alkaloid fraction, flavonoid fraction and tannin fraction isolated from the bark of *Ficus racemosa*. The 4th group received saline (10 ml/kg) as control. The animals were observed for clinical signs and mortality for a period of 15 days and body weight changes were recorded every week.

Evaluation of wound healing activity (Excision method)

All the animals were divided into 4 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 and IV were assigned as control, formulation TPAF, TPFF and TPTF respectively. 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. The measurement of the wound areas of the excision wound model were taken on 1st, 5th, 10th, 15th, 21st days until healing were complete; the percentage of wound closure was calculated [22].

Evaluation of wound healing activity (Incision method)

The method of Enrich and Hunt [23] was adapted for incision wound model. Under light ether anaesthesia, 6 cm long

Paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column. The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into four groups of six animals each. The animals were left undressed and housed separately. The animals of group I left untreated, group II, group III and group IV were treated with formulation TPAF, TPFF, TPTF respectively. The patches were topically applied once in a day from the day of operation till complete healing. The sutures were removed on 8th post wounding day and the skin breaking strength of the wounds were measured on the 10th day according to the continuous constant water flow technique of Lee et al [24].

The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light-weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams.

Three readings were recorded for a given incision wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Statistical analysis

Level of significance of all the parameters was expressed as the arithmetic mean \pm SEM and was analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's "t" test. *P* value less than 0.05 ($P < 0.05$) was the critical criterion for statistical significance.

RESULTS

Isolation of fraction

In the present study, alkaloid fraction, flavonoid fraction and tannin fraction were isolated from *Ficus racemosa* bark and estimated spectrophotometrically. The total alkaloid content, flavonoid content and tannin content was found to be $202.34 \pm 9.26 \text{ mg}$, $480.38 \pm 7.28 \text{ mg}$ and $578.60 \pm 8.48 \text{ mg QE/g dry weight}$ respectively.

Thin Layer Chromatography (TLC)

The isolated alkaloid fraction, flavonoid fraction and tannin fraction were subjected to TLC analysis. Alkaloid fraction showed the presence of 5 well separated spot with Rf value 0.18, 0.21, 0.23, 0.90 and 0.96; Flavonoid fraction showed the presence of 11 well separated spot with Rf value 0.19, 0.20, 0.22, 0.28, 0.36, 0.40, 0.56, 0.64, 0.79, 0.86 and 0.94; Tannin fraction showed the presence of 10 well separated spot with Rf value 0.18, 0.25, 0.34, 0.48, 0.57, 0.63, 0.85, 0.91, 0.94 and 0.97. The results were shown in fig. 1.

Formulation of transdermal patches

Transdermal patches (fig. 2) containing alkaloid fraction (TPAF), flavonoid fraction (TPFF) and tannin fraction (TPTF) were prepared by solvent evaporation method as per the working formula given in table 1. A biocompatible nature polymer protonal LF10/60 was used to prepare the matrices.

Evaluation of the developed transdermal patches

The formulated transdermal patches were evaluated for thickness, weight uniformity, folding endurance, percentage moisture content, water vapor permeability, drug content, *in vitro* permeation of the fractions and *in vitro* cytotoxicity were carried out. From the results it was observed that all batches of transdermal patches were consistent and were within the specified limits.

The thickness of the patches varied from 0.182 to 0.256 for placebo to TPTF, respectively. However the batch consistency was high which was well indicated with calculated standard deviations. The weight uniformity was also consistent among all the batches of the transdermal patches formulated.

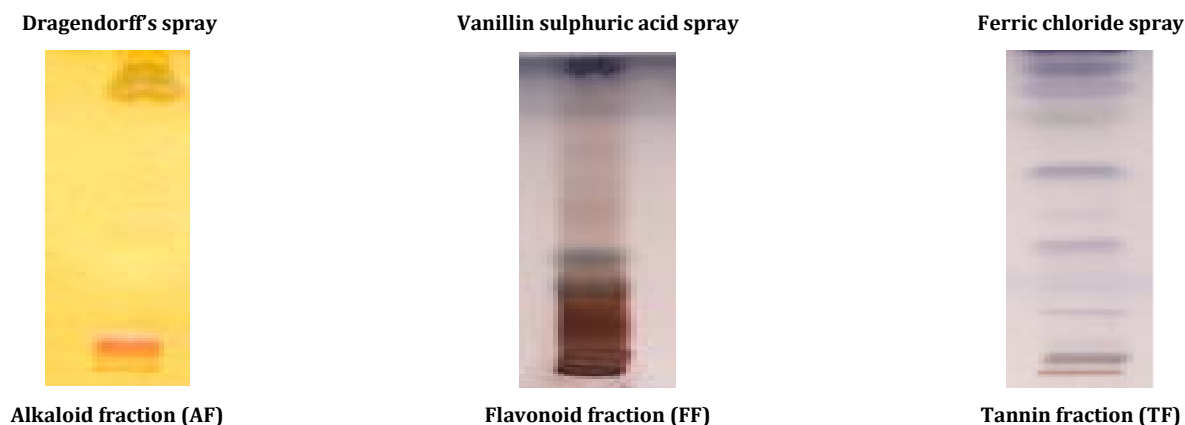
Fig. 1: TLC of the fractions from *Ficus racemosa* bark

Table 1: Formula for preparation of transdermal patches

Ingredients		Placebo (%)	Alkaloid Fraction (%)	Flavonoid Fraction (%)	Tannin Fraction (%)
Active part	Fraction	-	0.2	0.2	0.2
	DM water	15.3	7.5	15	15
Polymer part	Protonal LF10/60	2.5	2.5	2.5	2.5
	DM water	66.7	66.7	66.7	66.7
Additives part	Starch	0.5	0.5	0.5	0.5
	DM water	15	15	15	15

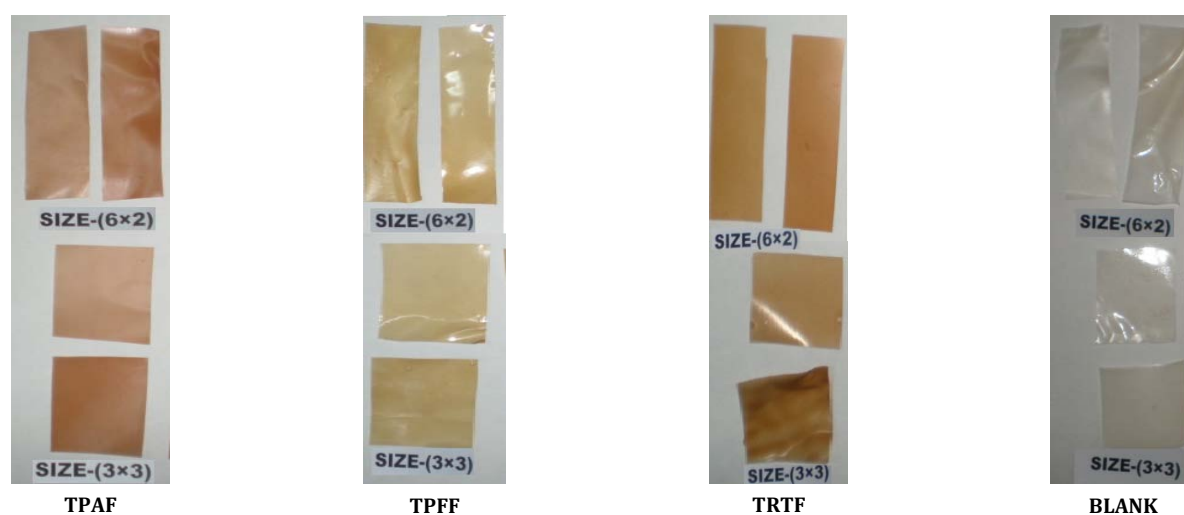


Fig. 2: Formulated transdermal patches

The folding endurance was highest in the TPTF with 84% and was lesser for the placebo with 76%, which indicates that the transdermal patches containing the tannin fraction provides better flexibility to the films and can adhere to the skin without breaking.

The moisture content in all the transdermal patches were below 1.5%, under the specified storage conditions and this ensure better stability of the patches during storage. Higher moisture contents causes not only physical deformation of the patches but also facilitates chemical degradation of the phytoconstituents.

The water vapour permeability of the prepared films was considerably high and this suggests better release of the therapeutic agents from the patches at a constant rate. Further, it will not cause discomfort to the skin as it do not cause occlusion in the skin.

The drug content in the patches was determined by mass spectrophotometry and it was found to high, reliable and consistent throughout. The drug content is an important parameter, as it

determines the capability of any dosage form to serve as a carrier for the therapeutic agent. The results of the above mentioned studies were tabulated in the table 2.

In-vitro release studies

The *in vitro* release studies of the three patches (TPAF, TPFF and TPTF) were carried out by diffusion studies and the samples collected at different time intervals up to 24 hrs. The samples were analyzed by using mass spectrometry. The data of the study reveals that all the three patches released the contents for more than 24 hrs and the release were found to be steady throughout the period of the study. At the end of 24 hrs, 82.6±1.6%, 86.6±1.6% and 90.8±1.2% of the fractions were released from TPAF, TPFF and TPTF respectively. This profile suggests that the release of the fractions from the polymeric matrix takes place by the mechanism of slow diffusion due to the swelling of the polymeric molecules. The results were indicated in the fig. 3.

Table 2: Evaluation of Transdermal patches

Test	Placebo	TPAF	TPFF	TPTF
Thickness (mm)	0.182±0.004	0.234±0.006	0.242±0.004	0.256±0.004
Weight uniformity (mg)	98.1±3.06	104±3.06	102±3.08	106±2.14
Folding endurance (%)	76±3.1	81±3.1	82±2.4	84±2.1
Moisture content (%)	1.28±0.04	1.15±0.04	1.08±0.03	1.10±0.02
Water vapor permeability (%)	2.58±1.22	1.56±1.32	1.46±1.22	1.52±1.46
Drug content (%)	0	94.1±0.1	94.3±0.3	95.2±0.1

Values are in±SD

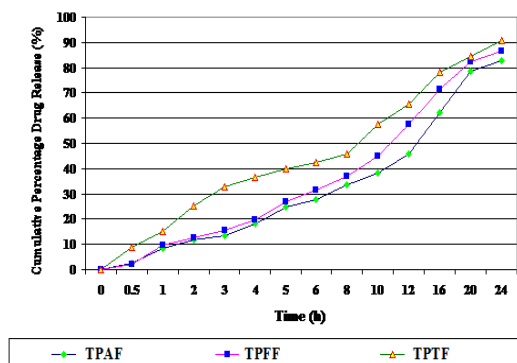


Fig. 3: In-vitro drug release studies of Transdermal patches

In-vitro cytotoxicity

The determination of cytotoxicity of the developed formulation was performed by qualitative evaluation using MTT assay. Cell confluency and morphology were specifically looked into using a grading scheme as described in table 3. The cells treated with the positive control showed a complete destruction of cell layer compared with negative control. Quantitative evaluation using MTT assay for the formulation TPAF, TPFF, TPTF and Blank (Placebo) showed a viability of 86.84%, 85.53%, 86.84% and 92.76% respectively. The cells treated with the negative control did not induce any cytotoxicity and the positive control induced 73.68% cytotoxicity.

Based on the results obtained from the study (test carried out using Balb/c 3T3 cells line), it was concluded that the tested formulations TPAF, TPFF and TPTF (6 x 2 cm) were considered non-cytotoxic. The results were indicated in the table 4 and 5.

Table 3: Grading scheme for cytotoxicity

Grade	Reactivity	Description of reactivity zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending further than 1.0 cm beyond specimen

Table 4: Qualitative evaluation of cytotoxicity of transdermal patches

Sample	Culture	Reactivity	Grade
Negative control	1	None	0
	2	None	0
	3	None	0
TPAF	1	None	0
	2	None	0
	3	None	0
TPFF	1	None	0
	2	None	0
	3	None	0
TPTF	1	None	0
	2	None	0
	3	None	0
Blank	1	None	0
	2	None	0
	3	None	0
Positive control	1	Severe	4
	2	Severe	4
	3	Severe	4

Table 5: Quantitative evaluation of cytotoxicity of transdermal patches (580 nm)

Sample	Replicate 1	Replicate 2	Replicate 3	Mean	Viability (%)
Negative control I	1.53	1.55	1.49	1.52	100
TPAF	1.34	1.32	1.31	1.32	86.84
TPFF	1.32	1.27	1.30	1.30	85.53
TPTF	1.33	1.30	1.34	1.32	86.64
Blank	1.40	1.42	1.41	1.41	92.76
Positive control	0.41	0.39	0.40	0.40	26.32

Acute toxicity study

In the acute toxicity studies, no signs of toxicity or mortality were observed at 2000 mg/kg dose level. So, 200 mg/kg b. w. dose was taken as the therapeutic dose.

Excision and Incision wound model

The transdermal patches TPAF, TPFF and TPTF showed notable wound healing activity in both excision and incision wound model when applied topically. TPTF showed the most significant activity followed by TPFF and TPAF in both Excision and Incision wound models. In the excision wound model, TPFF and TPTF treated animals showed a significant reduction in the wound area and epithelialization period. The percent of

wound closure in TPFF and TPTF treated animals was significantly more (** $P < 0.01$) on 15th and 21st day as compared to that of blank patch. The significant increase in breaking strength of incision wounds were observed in TPFF (** $P < 0.05$) and TPTF (** $P < 0.01$) treated wound of animals compared to that of blank patch. Although required qualities were achieved with herbal formulations TPAF, TPFF and TPTF, transdermal patches containing tannin fraction (TPTF) exhibited better activity in both *in vitro* and *in vivo* studies. The results of the present investigations revealed that the tannin fraction containing transdermal patches (TPTF) possess significant wound healing activity in excision and incision wound models and was considered to be ideal and effective in the management of wound healing. The results were shown in fig. 4, 5 & 6.

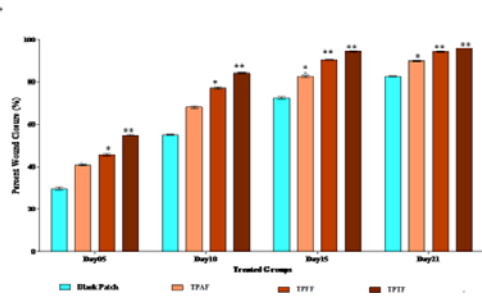


Fig. 4: Percentage wound contraction of transdermal patches on excision wound models in rats
 Values are expressed as Mean±SEM, n = 6; Blank Patch Vs TPAF, TPF, TPTF (*p ≤ 0.01, **p ≤ 0.01)

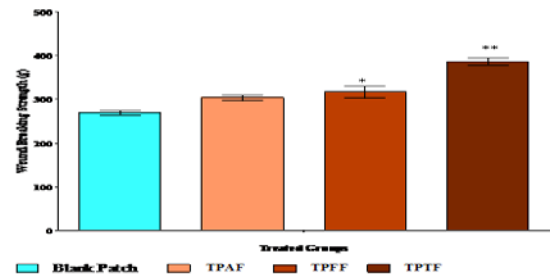


Fig. 5: Wound Breaking Strength of transdermal patches on Incision wound model
 Values are expressed as Mean±SEM, n = 6; Blank Patch Vs TPAF, TPF, TPTF (*p ≤ 0.05; **p ≤ 0.01)

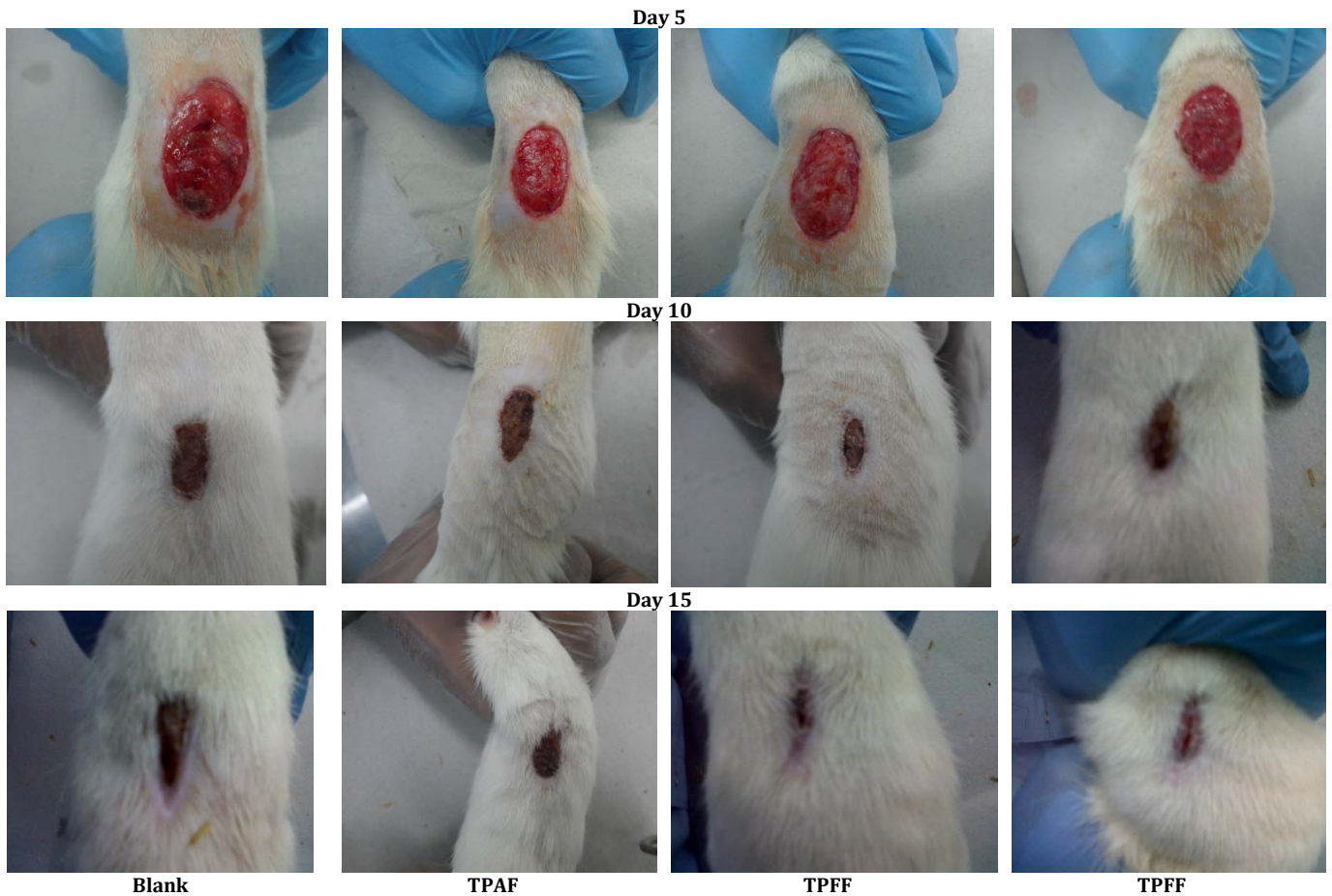


Fig. 6: Photo representation of contraction rate on different days in treatment group

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against wounds so as to exploit them as herbal wound healing agents. Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues [25]. Wound healing involves regeneration of specialized cells by proliferation of surviving cells and connective tissue response characterized by the formation of granulation tissue [26]. It is also characterized by haemostasis, reepithelialization and remodeling of the extracellular matrix. Epithelialization, which is the process of epithelial renewal after

injury, involves the proliferation and migration of epithelial cells towards the centre of the wound while wound contraction is largely due to the action of myofibroblasts [27]. The treated group of wound showed complete healing of wounds with almost normal architecture of the collagen, reticulin. Increase in tensile strength of treated group wound may be due to increase in collagen concentration. Significant increase in skin breaking strength which was a reflection of increased collagen levels by increased cross linking of collagen fibers [28]. Thus, the effect of TPAF, TPF and TPTF on wound contraction and epithelialization suggest it may enhance epithelial cells migration and proliferation, as well as the formation, migration and action of myofibroblasts.

Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis, but also by

improving vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase viability of collagen fibrils by increasing the strength of collagen fibres; increasing circulation; preventing cell damage and by promoting the DNA synthesis [29]. Tannins are known to promote the wound healing process chiefly due to their astringent and antimicrobial properties. Tannins promote the wound healing through several cellular mechanisms; chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts [30, 31].

CONCLUSION

The developed formulation showed good physicochemical properties like thickness, weight variation, drug content, folding endurance, moisture content. In wound healing models, the formulation containing flavonoid fraction (TPFF) and tannin fraction (TPTF) exhibited significant wound healing activity and the promising wound healing activity may be attributed to high flavonoid content and tannin content which seems to be responsible for wound contraction and increase rate of epithelialization.

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CONFLICT OF INTERESTS

Declared None

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