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WOUND HEALING ACTIVITY OF TOPICAL LAWSONE GEL ON RAT MODEL

P. K. Lakshmi *, Narmada Thangellapalli, Amoolya Chennuri, D. Prasanthi and B. Veeresh

Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana, India.

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Correspondence to Author:
Dr. P. K. Lakshmi

Professor, Department of Pharmaceutics. G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana - 500028, India,

E-mail: drlakshmisuresh@gmail.com

ABSTRACT: The objective of present work is to study the wound healing effect of topical lawsone gel. Topical lawsone gel was prepared by using natural and synthetic polymers in different ratios. The gel was prepared with Tara gum and HPMC K15M using dispersion method. Optimized gels were characterized for physicochemical evaluations. Optimised formulations (LT3, LT4) were evaluated for spreadability (10.6±0.02, 13.8±0.28), pH (6±0.58, 5±0.59), viscosity (18000±62.5, 18023±71.3) and drug content (94.51±0.63, 87.08±0.40). The gel was found to be homogeneous and uniform without any formation of lumps. In vitro release of optimized gel (LT3, LT4) showed control release of 81.68% and 87.47% within 7 hours. The formulation was found to follow zero order model dependent kinetics with fickian diffusion possessing a release rate of 198.3 and 202.4 µg /cm^2/$hr^{-1/2}$. Animal studies performed with optimized formulations (LT3, LT4) showed wound contraction of (85.30±1.56%, 99.21±1.32%) when compared to the control gel (76.61±2.15%). The period of epithelisation of LT3, LT4 formulation showed complete epithelisation on 13.4±0.45 and 14.2±0.61 days respectively, when compared to control gel (21.6±0.50) days. The results of LT3&LT4 formulations showed better wound healing activity and can be used for the treatment of excision wounds. In the excision wound model, results showed that the wound contracting capacity of lawsone was significantly greater than ($P<0.05$) the control.

INTRODUCTION: Topical drug administration is the simplest and easiest route of localized drug delivery anywhere in the body by ophthalmic, rectal, vaginal and dermal routes. Mostly, pharmaceutical preparations applied to the skin are expected to serve some local actions which include antiseptic, antifungal, skin emollients and protectants. Topical delivery system is beneficial as it bypasses the first pass metabolism, avoids the risks and inconveniences of intravenous therapy and of the varied conditions of absorption like pH changes, presence of enzymes and gastric emptying time [1].

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The topical drug delivery system is generally used where the other systems of drug administration fail or for treatment of local skin infections such as fungal infection. Topical drug delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorder.

Dermatological products applied to skin are diverse in formulation and range in consistency from liquid to powder but the most popular products are semisolid preparations. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations [2]. Gels are a relatively newer class of dosage forms created by entrapment of large amounts of aqueous or hydro alcoholic liquid in a network of colloidal solid particles. Gel formulations generally provide faster drug release compared to ointments and creams [2].

A wound is defined as a defect or break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition. Based on the nature and repair process of wounds, they can be classified as chronic wounds and acute wounds [3].

Acute wounds are tissue injuries that heal within 8-12 weeks. The primary causes of acute wounds are mechanical injuries (friction contact between skin and hard surfaces), burns and chemical injuries. In the case of burns, the temperature of the source and time of exposure is important to decide the degree of wound. Burn wounds need normally specialist care because of associated trauma. Chronic wounds heal slowly and leave serious scars. There can be different reasons that chronic wound do not heal as fast as acute wounds [3].

Among most common are diabetes, infections and poor primary treatment. A wound is colonized when growth and death of bacterial in the wound is balanced by the host. If the host is not able to keep the bacterial growth in balance, the wound will enter the infection phase. Symptoms for an infected wound are erythema, edema, warmth, pain and exudate. Infections of chronic wounds are often poly bacterial with *Staphylococcus aureus* and anaerobes being the most common in chronic wound [3].

The wound healing process is a series of independent and overlapping stages. In these stages, both cellular and matrix compounds work to re-establish the integrity of damaged tissue and replacement of lost tissue. These overlapping series can be classified in five stages: hemostasis, inflammation, migration, proliferation and maturation [3].

Lawsone (2-hydroxy-1,4-naphthoquinone), also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*) as well as in the flower of water hyacinth (*Eichhornia crassipes*). Dried powdered leaves of henna contain 0.5-1.5% lawsone, traditionally used to produce fast orange, red and brown dyes.

Lawsone is the active constituent of *Lawsonia inermis* which is a derivative of naphthoquinone group. Lawsone possess astringent, aromatic, cooling, anti-fungal, anti-bacterial activities. Studies of lawsone have shown the wound healing activity on animals. So far no study has been reported on topical lawsone in the form of gel. Hence it was hypothesized that topical lawsone gel may have wound healing activity which can be tested on rat model.

MATERIALS AND METHODS:

Preparation of gel: Gels in the industrial scale are normally prepared under room temperature. The topical lawsone gel is prepared using dispersion method.

TABLE 1: FORMULATION OF TOPICAL LAWSONE GELS

Formulation code	Lawsone (mg)	Tara gum (mg)	HPMC K15M(mg)	Propylene glycol (%)	Methanol (%)	Result
LT1	50	100	-	10	10	**
LT2	100	100	-	10	10	**
LT3	50	200	-	10	10	***
LT4	100	200	-	10	10	***
LH1	50	-	300	10	10	*
LH2	100	-	300	10	10	*
LH3	50	-	400	10	10	***
LH4	100	-	400	10	10	***
LTH1	50	100	300	10	10	***
LTH2	100	100	300	10	10	***
LTH3	50	150	250	10	10	***
LTH4	100	150	250	10	10	***

LT = Lawsone with Tara gum, LH = Lawsome with HPMC K15M, LTH = Lawsone in combination with both tara gum and HPMC K15M.

In all the formulations water was added, quantity sufficient to 10g

* = gel not formed, ** = gel formed with less consistency, *** = gel formed with good consistency.

Dispersion method: In this method polymer is dispersed in water for 2 hours so that the polymer gets completely soaked. After that other chemical ingredients are mixed and stirred well until a homogenous mass is obtained. Placebo gels were prepared with different polymers and their combinations. Based on their physical and chemical properties, the type of polymer and its concentration were selected for further optimization of gels containing drug.

Evaluation of topical lawsone gel: [4] Gels were evaluated for their clarity, pH, spreadability, viscosity, drug content, *in vitro* and skin irritation studies [4].

Clarity: It was determined by visual inspection under the black and white background and it was graded as follows: turbid: +, clear: ++, very clear (glossy): +++.

Homogeneity: It was determined by visual inspection for the appearance of gel and presence of any aggregates [9].

Determination of pH: pH of the formulations was determined by dispersing 1 gm of gel in 100 ml of 7.4 phosphate saline buffer. It was checked using digital pH meter at constant temperature. Prior to this, the pH meter was calibrated using standard buffer solution and then electrode was washed with demineralised water. The electrode was then directly dipped in to gel formulation and constant reading was noted [4].

Spreadability: The spreadability of the gel formulations was determined by measuring the spreading diameter of 1g of the gel by 20X20 cm glass plates after 1 min. The mass of the upper plate was standardized at 10g. Spreadability was then calculated using the following formula:

$$S = M \times L/T$$

Where, S = is the spreadability, M = is the weight in the pan (tied to the upper plate), L = is the length moved by the glass slide and T = it represents the time in seconds taken to separate the slide completely [6].

Determination of viscosity: Viscosity of prepared gels was determined by VISCO lab 3000 viscometer. It is the ideal choice for measurement when viscosity testing requires the sample material to be tested at specific temperature. It is a temperature controlled viscometer incorporating an integrated heater that delivers accurate measurements at user-defined temperatures from slightly above ambient to 356F (180 °C).

Utilizing minimal bench space and providing an easy to read digital display in centipoise or centistokes, the VISCO lab 3000 delivers accurate measurements with a small size, less than 2ml of fluid is required [7].

Drug Content: 500 mg of gel was taken and dissolved in 100 ml of pH 7.4 phosphate saline buffer. The placebo gel 500mg was dissolved in the same buffer solution. The volumetric flasks were kept for shaking for15 min. The solution was passed through the Whatmann filter paper no.42 and filtered. Appropriate dilutions were made and drug content was measured spectrophotometrically corresponding placebo gel at 454 nm [8].

***In vitro* diffusion study:** Diffusion study of the topical gel was performed using Franz diffusion cell. The cell was locally fabricated and volume of receptor compartment was 25ml. The dialysis membrane was mounted between the donor and receptor compartments. Gel formulation (1g) equivalent to 10 mg of drug were taken on the dialysis membrane and the compartment clamped together. The receptor compartment was filled with phosphate buffer saline 7.4 pH and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at 450rpm. At predetermined time intervals, 5ml of samples were withdrawn and an equal volume of buffer was replaced. The samples were analyzed after appropriate dilution for drug content spectrophotometrically.

Release rate: Plot Higuchi kinetics- Calculate slope. Slope is release rate. Unit is microgram/cm^2/$hr^{-1/2}$.

Calculation of model dependent kinetics for prepared gel formulations: Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data was fitted into zero order, first order, Higuchi, and

Korsemeyer-peppas release model, to study the drug release from the dosage form.

Animal Studies: [5] The animals study was approved by the IAEC. The institutional ethical approval number is 320/CPCSCEA.

Skin Irritation Studies: Skin irritation is one of the most common adverse effects in humans depend on many factors, including the concentration, duration and frequency of exposure, exposed skin site, rate of penetration and intrinsic toxic potential of the substance. This must be done to determine the risk of irritation due to the contact between these compounds and human skin. The most commonly used test is the rabbit skin irritation test described in the OECD test guidelines [5].

Wound healing activity: Healthy Male albino wistar rats of either sex, weighing between 250-275g were obtained from the animal house. The animals were caged individually after wounding for treatment till completion of wound healing [6].

The animals were anaesthetised prior to the infliction of the experimental wounds. The surgical inventions were carried out under sterile conditions using ether. Animals were closely observed for any infection those which showed signs of infection were separated and excluded from the study. The study was approved by the Institutional animal ethics committee (IAEC) [6].

RESULTS AND DISCUSSIONS: IR study was done to verify if there was any interaction between the pure drug and various excipients used in formulations. The spectrum of lawsone and physical mixture of lawsone and excipients (Tara gum, HPMC K15M) were recorded by the KBr pellet method using IR spectrophotometer in the range of 400-4000cm^{-1} and compared

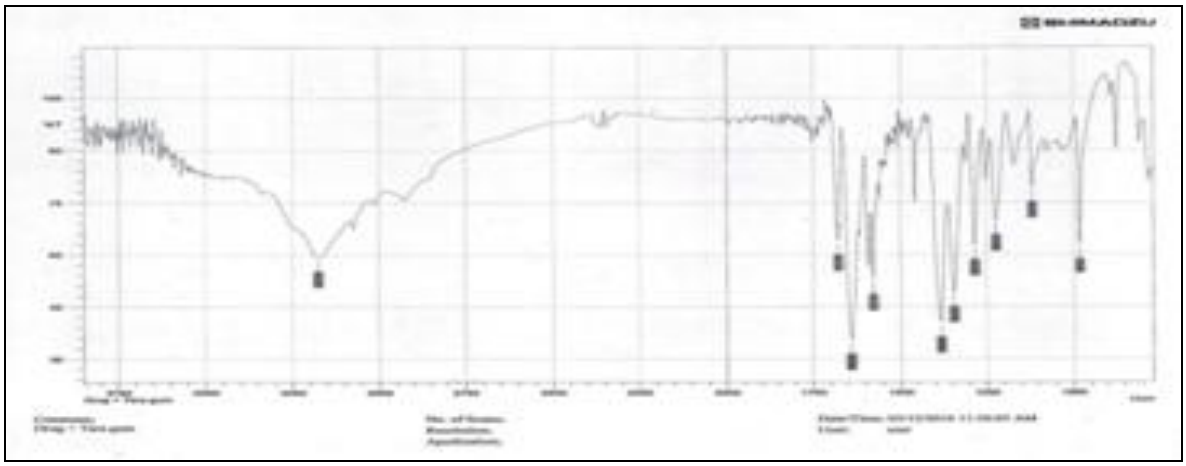

FIG. 1: FTIR GRAPH OF PURE DRUG AND TARA GUM MIXTURE

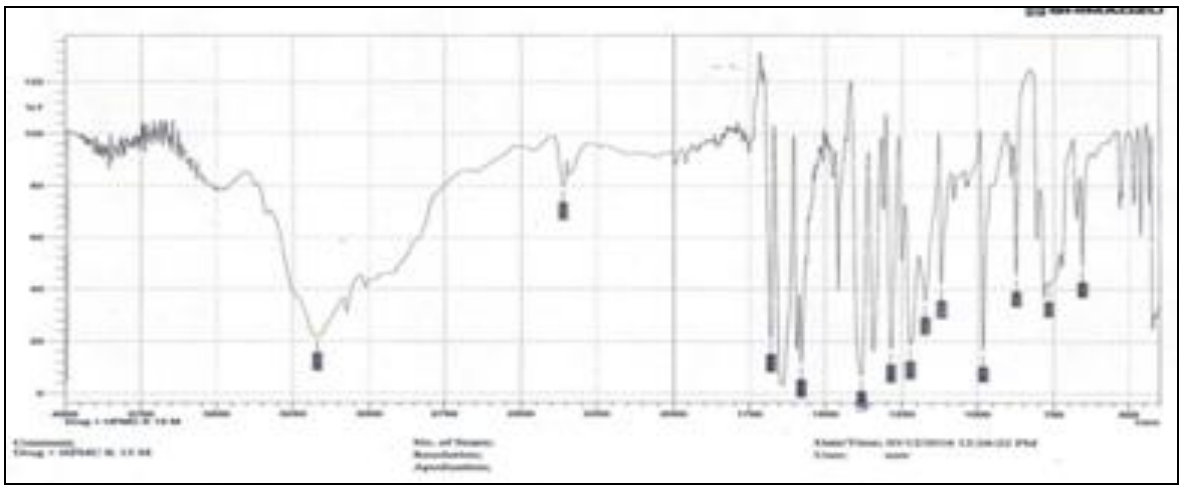

FIG. 2: FTIR GRAPH OF PURE DRUG AND HPMC MIXTURE

Evaluations on the optimized formulations of the gel for spreadability, pH, drug content and homogenesity: [6]

TABLE 2: EVALUATION OF SPREADABILITY, pH, VISCOSITY, DRUG CONTENT, HOMOGENESITY FOR OPTIMIZED FORMULATIONS

Formulation code	Spreadability (g.cm/sec)	pH	Viscosity (cps)	Drug content (%)	Homogeneity
LT1	11.4±0.04	5±0.98	16150±65.3	91.29±0.15	++
LT2	13.5±0.05	6±0.84	16253±68.2	88.48±0.95	++
LT3	10.6±0.02	6±0.58	18000±62.5	94.51±0.63	+++
LT4	13.8±0.28	5±0.59	18023±71.3	87.08±0.40	+++
LH3	10.5±0.04	6±0.67	18856±75.6	90.86±0.63	+++
LH4	12.8±0.21	5±0.26	19104±80.2	91.06±0.74	+++
LTH1	15.2±0.12	6±0.85	21700±67.6	95.01±0.43	++
LTH2	12.4±0.58	5±0.05	21945±78.4	89.51±0.56	+++
LTH3	14.8±0.15	6±0.17	22358±75.3	94.26±0.15	+++
LTH4	13.9±0.31	6±0.28	22987±60.6	90.25±0.25	++

Note – 1) LT = Lawsone with Tara gum, LH = Lawsome with HPMC K15M, LTH = Lawsone in combination with both tara gum and HPMC K15M.
2) + = satisfactory, ++ = good, +++ = excellent

One of the criteria for a topical formulation to meet the ideal qualities is that it should possess good spreadability. The value of spreadability varies from 10.5 to 15.2 g.cm/sec, indicating that the topical lawsone gels were easily spreadable by small amount of shear. All gel preparations indicated a good spreadability.

The pH was found to be in range from 5 to 6 thus indicating suitability for application along with good extrudability and spreadability. The results in the present study showed a good relation between absorption and pH of the lawsone topical gel formulations.

Viscosity studies of various formulations revealed that formulation LT3 and LT4 was better compare to others.

After various formulations of lawsone gel the drug content of the optimized gels was estimated and the results were in the official limits with range of 90 to 95%. The drug content determination also showed that the drug was uniformly distributed ensuring adequacy in the method of preparation of the topical lawsone gel.

All developed gels showed good homogeneity with absence of lumps. The results indicated that the formulation can be applied easily without being runoff.

***In-vitro* drug release profile of different formulations: [10]**

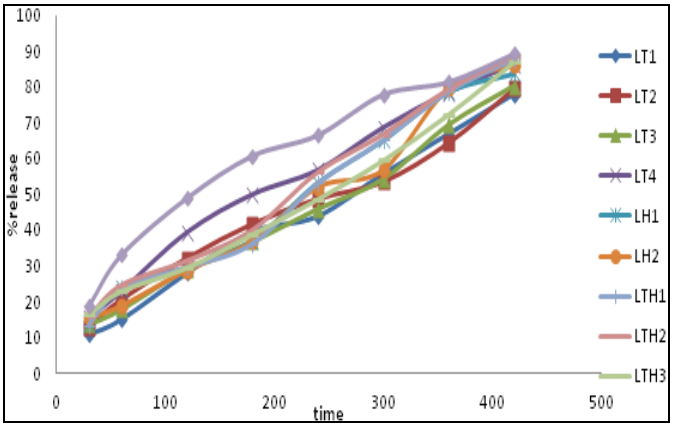

FIG. 3: *IN-VITRO* DRUG RELEASE PROFILE OF DIFFERENT FORMULATIONS

In vitro diffusion studies showed that the formulations with different ratios of drug to polymers has released maximum drug within 5 hours. Among all the formulations LT3 and LT4 are optimized based on different polymer concentrations and the drug release of 81.68% and 87.47% respectively within 7 hours.

Skin irritation studies: Skin irritation study was performed using rabbit. The topical lawsone gel and placebo formulation were applied on the back of rabbit. After every 24 hours, on treatment with the LT3, LT4 formulations showed the score for skin irritation in terms of erythema and edema in all rabbits was found to be zero, and same results were found for placebo gel [11].

TABLE 3: SKIN IRRITATION TEST RESULTS

S. No	Treatment	Day 1	Day 2	Day 3
1	Control	0	0	0
2	Lawsone gel 0.5%(LT3)	0	0	0
3	Lawsone gel 1%(LT4)	0	0	0

Wound contraction studies: A circular piece (200 mm^2 in area) of full thickness skin was excised from the back of the rat. Wound contractions were monitored by measuring wound area, on alternate days till the wound were completely healed.

Application of placebo gel on excision wound:

1st day **4th day** **16th day** **21st day**

FIG. 4: PHOTOGRAPHIC REPRESENTATION OF CONTRACTION RATE SHOWING PERCENT WOUND CONTRACTION AREA ON DIFFERENT POST EXCISION DAYS OF CONTROL GEL

Application of topical lawsone gel on excision wounds:

1st day **4th day** **8th day** **16st day**

FIG. 5: PHOTOGRAPHIC REPRESENTATION OF CONTRACTION RATE SHOWING PERCENT WOUND CONTRACTION AREA ON DIFFERENT POST EXCISION DAYS OF TOPICAL LAWSONE GEL

The aim of the present work is to evaluate the wound healing activity of lawsone gel by *in vivo* and *in vitro* models. Wound healing is an extremely complex phenomenon involving a number of well-orchestrated events including continuous, overlapping and precisely programmed phases [12]. In the excision wound model, significant contraction of wounds was observed in the animals treated with control ($P<0.05$) and lawsone gel ($P<0.0053$, $P<0.006$) was observed from 2nd day onwards [13]. There was 76.6, 85.3, 99.2% wound contraction respectively for control(C), lawsone gel 0.5% (LT3), lawsone gel 1% (LT4) treated animals on 16th day [14].

TABLE 4: EFFECT OF WOUND HEALING ACTIVITY OF LAWSONE GEL EXCISION WOUND MODEL

Groups	% Wound contraction				Period of epithelisation(days)
	Day 3	Day 7	Day 11	Day 16	
Control (C)	28.33±1.10	47.66±1.22	51.42±1.86	76.61±2.15	21.6±0.50
LT3	46.66±1.57	62.33±0.32	76.61±1.48	85.30±1.56	13.4±0.45
LT4	53.15±1.11	80.45±0.23	90.23±0.54	99.21±1.32	14.2±0.61

Note: All values are expressed as Mean ± SEM, n= 3 animals in each group. Tukey-Kramer multiple comparison test: *P<0.05, **P<0.006, ***P<0.0053, as compared with control

TABLE 5: ANOVA RESULTS

Treatment	Significance
C vs LT3	**
C vs LT4	**
LT3 vs LT4	NS

In the excision wound model, significant contraction of wounds was observed in the animals treated with control ($P<0.05$) and lawsone gel ($P<0.0053$, $P<0.006$) was observed from 2nd day onwards. There was 76.6, 85.3, 99.2% wound contraction respectively for control(C), lawsone gel 0.5% (LT3), lawsone gel 1% (LT4) treated animals on 16th day.

The period of epithetlization was reduced significantly ($P<0.01$ and $P<0.001$) for the extracts compared with the control group. The contraction capacity of excision wound model was measured in different time intervals. Initial findings from the results of the three groups [control, lawsone gel 0.5 % (LT3), lawsone gel 1% (LT4)] were reduced on the 16th day. A comparison of these results indicates that the optimized topical lawsone gel is better than untreated control group. In view of the above reports, the wound contraction and healing effects of lawsone might be attributed to its ability to stimulate and increase the synthesis of one or more of the above mentioned cells and factors known to promote wound healing [16].

CONCLUSION: In the present study an attempt was made to formulate topical lawsone gel for wound healing activity on rat model. Topical gel formulations of lawsone were prepared by dispersion technique using natural and synthetic polymers like tara gum and HPMC K15M. Natural polymer (tara gum) based formulation (LT3 and LT4) gave controlled drug release of 81.68% and 87.47% when compared to synthetic polymer (HPMC K15M). Among all the formulations LT3 and LT4 showed release rate of 198.3 $\mu g/cm^2/hr^{-1}$ and 202.4 $\mu g/cm^2/hr^{-1}$ in pH 7.4 phosphate buffer saline for 7 hours. The formulation was found to follow zero order model dependent kinetics with Fickian diffusion. Skin irritation studies have proved that the formulation was non-toxic and non-irritant.

The results of the animal studies have showed that when compared to control (76.61±2.15) on the day 16, LT3 and LT4 (85.30±1.56, 99.21±1.32) showed better wound closure when applied topically. Significant decrease in time of epithelization was observed in groups treated with lawsone. The lawsone topical gel showed complete epithelization on 13.4±0.45 and 14.2±0.61 days, respectively when compared to control (21.6±0.50). This results in better wound healing activity. The statistical analysis of wound contraction by ANOVA showed that significant wound reduction with LT3 and LT4 formulations in comparison with ($P<0.05$) control. But no significance difference was seen between LT3 and LT4 formulations.

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