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PREPARATION AND CHARACTERIZATION OF ALOE VERA BASED *CURCUMIN* LOADED NANOEMULGEL FOR WOUND HEALING ACTIVITY

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Abstract
Hydrogels system being a drug delivery system has great significance particularly for topical application in cutaneous open wound. The specific physicochemical properties such as non-adhesiveness, moisture retention, and exudates absorption and gas permeability make them ideal as a drug delivery vehicle for wound healing treatment. Further, curcumin (a natural bioactive compound) was selected as a therapeutic agent to incorporate into the hydrogel system to design and develop nanoemulgel for wound healing. Although, curcumin possess remarkable anti-inflammatory, antioxidant, and anti-infective activity along with hastening the healing process by acting over the different stages of the wound healing process, but its poor biopharmaceutical (low aqueous solubility and skin penetrability) attributes hamper their therapeutic efficacy for skin applications. The current research study aimed to develop the AL-CUR-NEG formulation system and evaluated to check the improvement in the therapeutic efficacy of curcumin through a nanomedicine-based approach for wound healing activity in albino wistar rats. The curcumin was enclosed inside the nanoemulsion system prepared through a high-energy ultrasonic emulsification technique at using minimum concentration of surfactant required to nanoemulsify the curcumin-loaded oil system having droplet size 385.9nm. The optimized curcumin loaded nanoemulsion was incorporated into aloe vera hydrogel system for topical application. The developed curcumin nanoemulgel exhibited thixotropic rheological behavior and spreadability coefficient a significant ($p<0.05$) increase in skin penetrability characteristics compared to curcumin dispersed in marketed formulation system. Further signify the role of the AL-CUR-NEG formulation in *in-vivo* wound healing efficacy study.

Keywords: Nanoemulsion, Nanoemulgel, Wound healing

Introduction

Physical injuries resulting in a skin break or opening are called wounds **(Singh M. et al. 2006)**.

In order to restore the skin's disturbed anatomical and functional status, wounds must be healed appropriately. One of the most intricate physiological processes is wound restoration, which begins with the body's reaction to an injury and ends with the integrity and functionality of the injured tissues being restored **(Dadekar R. et al. 2012)**. Numerous physiological processes, including clotting, coagulation, inflammation, and the production of new tissues, are involved in wound healing. These processes can occur over a range of timescales, from minutes to several months or years **(Boateng J.S. et al. 2015)**. The formation of an incomplete healing process and the failure of wound healing may be caused by any deviations or delays to the multistage healing process **(Gould L. et al. 2015)**. Prolonged injuries can have a major negative impact on one's quality of life, and they need to be treated with extremely high-quality care **(Stejskalova A. et al. 2017)**. The risk of morbidity and mortality could rise as a result **(Saporito F. et al. 2018)**. This is particularly valid for those with diabetes mellitus and vascular illnesses **(Gould L. et al. 2015)**. Consequently, it's critical to develop a wound healing technique that can reduce tissue damage and quicken the healing process. There are several ways to provide wound healing medicines, such as parenteral and oral; nevertheless, systemic medication administration of these agents may result in undesired systemic adverse effects. Therefore, a topical drug delivery system is a desirable strategy that can enhance the healing of wounds and reduce systemic adverse effects.

Turmeric

Turmeric belongs to the spice family; it's more intriguing for the medicinal/specific and culinary worlds. Curcuma longa is a mismatched herbaceous persistent plant from to the Zingiberaceae family **(Priyadarsini K.I. et al. 2014)**. The sources of curcumin and the medicinal properties of turmeric have been known for millennia. Determining the precise mechanism of action and the bioactive components are therefore periodically investigated **(Gupta S.C. et al. 2013)**. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), is a diferuloylmethane. The primary naturally occurring polyphenol in a Curcuma supplement is derived from Curcuma longa, or turmeric **(Aggarwal B.B. et al. 2003)**. A nation in Asia has long utilized curcuma longa as a medicinal herb because of its antimicrobial **(Phatak A.A. et al. 2012 & Larsson M. et al. 2014)**, antibacterial, anti-inflammatory **(Lestari M.L. et al. 2014)**, anticancer or antimutagenic **(Mahady G.B. et al 2002** & **Reddy R.C. et al. 2005)**, and antioxidant qualities. Three different curcuminoids (curcumin, bisdemethoxycurcumin, and desmethoxycurcumin) are taken at a dose of 12,000 mg per day at a 95% concentration **(Lao C.D. et al. 2006)**.

Turmeric is a divided into following parts.

1. First Variety is curcuma aromatic or kasturi manjal is known for the fine odour. A gastric manila is not used for cooking because its bitter taste.
2. The second variety is often employed in sauce powder, which is extensively utilized in baking. Curry powders are prepared by heating curcumin longa in water and then drying them before using the powder.
3. The third variety has an extended, circular shape that resembles a tiny kuda and is employed in rituals and traditions, where people worship the ultimate spirit and use it as a component of their sacred image at home.

4. The fourth form of turmeric is called black turmeric, and its roots have a little dark color. Turmeric of this kind is usually used to produce arctic medicine.
5. The fifth variety is a vine known as a mara manjal, which is extremely helpful when making a very small amount of medication **(Pillai S. et al. 2018)**. As a result, the turmeric variety is spreading quickly like wildfire in the West.

Curcumin (*Curcuma Longa*)

Turmeric contains the chemical curcumin, also known as diferuloylmethane. Curcumin (CUR), a constituent of *Curcuma longa* (Zingiberaceae Family), is scientifically referred to as diferuloylmethane and has been reported to possess anti-inflammatory **(Simol R.C. et al 1973)**, anti-carcinogenic **(Huang M.T. et al. 1988)**, con-oxidizing **(Sreejayan Rao M.N. et al. 2006)**, and hypocholesterolemia characteristics **(Rao D.S. et al. 1970)**. Thus, curcumin was used to create innovative formulations such as liposomes **(Bangham A.D. et al. 1974)**, solid lipid nanoparticles **(Tiyaboonchai W. et al. 2007)**, transdermal film microspheres **(Vidyalakshmi K. et al. 2004)** and nanoemulsion **(Kumar V. Et al. 2002)**. The digestive system does not effectively use curcumin's capabilities. After consuming curcumin, minimal quantities of the compound are seen in the blood and tissues **(Wang X. et al. 2008)**. Primitive 1900 diagnose curcumin principal over Lampe and Milobadzka. About 2.5% to 6% were found based on its structure and biological investigation **(Siviero A. et al. 2015)**. Pure curcumin understands of turmeric. The chemical and biophysical property of curcuminoids was showed in **Table 1**.

Table 1: Chemical and biophysical properties of curcuminoids **(Garcea G. et al. 2005)**

Characteristic	Cur-I	Cur-II	Cur-III
Chemical Name	Dicinnamoyl Methane	4-OH Cinnamoyl Methane	Bis-4 –OH Cinnamoyl Methane
Common Name	Cur	DemethoxyCur	Bisdemethoxycurcumin
Colour	Bright orange yellow	Bright orange yellow	Bright orange yellow
Amount present (%)	77	17	3
Molecular Mass (g/mol)	368.4	338	308.1
Melting Point (°C)	183-186	172.5-174.5	224.5
Neutral Solvent(water)	Poorly soluble	Poorly soluble	Poorly soluble
Solubility in Organic Solvents	Soluble	Soluble	Soluble
Solubility in Hexane or Ether	Insoluble	Insoluble	Insoluble
Excitation/Emission	420/530nm	420/530nm	420/530nm
Excitation/Emission	536-560nm	Unknown	Unknown

A small molecule produced via reticule copolymerization enhanced as a resolvent, either via biological or physical means, is referred to as a nanogel. Defined cross-linked bifunctional networks of ionic and non-polar polymers, such as polynucleosomes and poly glycol (PEG) **(Dorwal D. et al. 2012)**, gave rise to the term “Nanogel”. The normal size range of nanogel is

20-200nm **(Bencherif S.A. et al. 2009)**. Nanogels are the incised complements of hydrogels that are associated with the ownership of crystallize with the collision in ways such as microhetrogeneous structure, small diameters, and a resurface-volume ratio. Nanogels are characterized by their balance, fluff, and swelling in superior solvents. They are also referred to as "nanoscaler polymer networks", "gel nanoparticles" and "nanoscale hydrogels" etc. Nanogel is a popular, high-profile product that is dedicated to the criticism of being used as a medication delivery system. They combine extraordinary solubilization capability, nearly reduced viscosity, intriguing thermal stability, and the ability to withstand indirect sterilizing methods **(Tan J.P. et al. 2010)**. Drugs and biological substances may be drawn to nanogel. They have a wide range of applications in gene and protein delivery. Because of their hydrofoil structure, nanogels can effectively encapsulate quadraphonic medicines. Nanogel formulations add an advanced greedy drug delivery system for less soluble drugs. The free drug's biological absorption is increased rather than its solubility and strength being increased **(Soni G. et al. 2016)**. Their characteristics include a strong affinity for aqueous solutions, dominance, dormancy in both the internal and fundamental circulation, and usefulness for molecular bulk addition **(Rigogliusosa S. et al. 2012)**. They are also being researched as an advantageous carrier for the delivery and vital uptake of proterons, peptides, and other biological compounds.

Materials and Methods

Drug, chemicals and experimental animals

The drug curcumin was purchased from Loba chemie Pvt. Ltd. (with a purity ≥ 98%). Tween 80 and polyethylene glycol (PEG 400) were purchased from Sigma-Aldrich Pvt. Ltd, India, Gelling agent Aloe Vera was purchased from ND Care Nirogam Pvt. Ltd. Dimethyl Sulfoxide (DMSO) was purchased from ACS laboratory Pvt. Ltd. Loba chemie, ND Care Nirogam and Sigma-Aldrich are recognized for its stringent quality control measures and adherence to international standards, ensuring the reliability and purity of the chemicals procured for research and experimentation purposes. All other chemicals and solvents used in the experimental work were of analytical grade.

In this study male albino wistar rats weighing about 200-250g (10-12 weeks old) were used. Experimental specimens were obtained from the Animal Ethics committee, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut.

Method of preparation of AL-CUR-NEG

The aloe vera based curcumin loaded nanoemulgel formulation was prepared by using the spontaneous emulsification method **(Donsi et al. 2011)**. Optimized nanoemulsion formulation was incorporated into aloe vera gel base to obtain AL-CUR-NEG formulation **(Conxita S. et al. 2012)**. Varying amount of optimized curcumin nanoemulsion concentrations was combined with aloe vera gel base for the synergistic effect of aloe vera gel against wound infection **(Muhtadi et al. 2020)**.

Spectrophotometry

U.V. spectrum of drug sample was measured using U.V-Spectrophotometer (Shimadzu Model No. FTIR8100). Sample (10mg) was weighed and transferred to 100mL volumetric flask and diluted with ethanol suitably. Then stock solution was diluted to 10 times and U.V. spectrum was scanned in the range of 400-800nm **(Hassan S.S.M. et al. 1980)**. U.V. spectrum of the procured sample of curcumin showed a maximum absorption at 425nm which is identical with reported value in certificate of analysis.

Analytical Methodology (Spectrophotometric method)

Accurately weight the quantity (10.0mg) of curcumin on analytical balance (Shimadzu Model No. AY220), and was dissolved in 10mL ethanol taken in amber color volumetric flask. It was marked as the primary stock solution of drug having concentration (1000µg/mL). 1.0mL of this primary stock solution was taken in another volumetric container diluted to 10.0mL with ethanol to obtain concentration of 100µg/mL solution **(Luykx D.M.A.M. et al. 2008)**. This solution was taken in volumetric container. It was denoted as secondary stock solution. Further, ten different aliquots from secondary stock solution was taken in different test tubes (measuring 1, 2, 3, 4 & 5mL) and diluted with ethanol to get the concentration of 1, 2, 3, 4, 5µg/mL). The absorbance of resulting solutions was measured at λ_{max} 425nm by using U.V- Vis spectrophotometer instrumentation (Shimadzu Model No. FTIR8100) keeping ethanol solution used as a blank **(Hassan S.S.M. et al. 1980)**. The entire procedure was repeated thrice. A graph was plotted between absorption v/s concentration value of resulting solution in excel sheet of MS Excel software and statistical parameters were determined as correlation coefficient and regression line.

FT-IR spectroscopy

Fourier transforms infrared absorption spectrum of the curcumin was determined directly through FTIR-Bruker Alpha E ATR model at 900cm^{-1} to 4000cm^{-1} from the faculty of pharmacy, Swami Vivekanand Subharti University, Meerut U.P.

Particle size, polydispersity index and zeta potential

Particle size of aloe vera based curcumin loaded Nanoemulgel formulation was measure by using Zetasizer (Zetasizer 3000 HAS, Malvern Instruments Ltd, Worcestershire, UK). Particle size and polydispersity index (PDI) measurements were done using polystyrene cuvettes containing filtered deionized water, which were then diluted and examined at a fixed 90° angle. Using a laser-based multiple angle particle electrophoresis analyzer, the zeta potential of the optimized formulation was evaluated **(Kawakami S. et al. 1998)**. The optimized formulation was dispersed in distilled water and then put the sample in an electrophoretic cell.

Rheology study

The viscosity of AL-CUR-NEG formulation was measured using a Brookfield viscometer for 3 minutes at 25°C and 10rpm **(Gadkari et al. 2019)**.

Spreadability

This block is fastened with a ground glass slide. Placed on this ground slide is an excess of the nanoemulgel under examination (about 2 gm). Between this glass slide and another with the dimensions of a fixed ground slide, the nanoemulgel was positioned and equipped with a hook. To remove air and create a consistent layer of nanoemulgel between the slides, a 500g weight was placed on top of each of the two slides for 5 minutes. The excess amount of nanoemulgel was removed by scraping off the slide edges. The length of time (in seconds) needed for the top slide to travel a certain distance can be calculated with the use of thread that is fastened to the hook **(Shadab et al., 2020)**. Better spreadability was observed with shorter time interval. Spreadability of AL-CUR-NEG formulation was calculated by using the formula.

$$\text{Spreadability} = \frac{\text{Weight tied to upper slide (M)} \times \text{Length of glass slides (L)}}{\text{Time taken to separate the slides completely from each other (T)}}$$

***Ex-vivo* skin permeation study**

Male albino wistar rats were shave of their dorsal skin hair and sliced into a circle. After that, the removed skin was submerged in phosphate buffer (PB) at pH of 7.4 for 30 minutes. AL-CUR-NEG (5g) was placed on the donor compartment of the Franz diffusion cell, and the receptor solution (PB) pH 7.4 was stirred at 37±0.5°C at 100rpm. Small aliquots (5mL) sample were collected in different time interval and were measured using a U.V-Vis spectrophotometer (Shimadzu Model No FTIR8100) at 425nm. Cumulative AL-CUR-NEG permeated (μg/cm^2) was calculated from the detected AL-CUR-NEG concentration **(Kim B.S. et al. 2008)**.

***In-Vivo* Study**

Experimental animal protocol

The animal protocol to carry out the *in-vivo* wound healing activity examination was approved by the institutional ethical committee (Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut) and followed their guidelines to perform the studies **(1204/PO/Re/S/08/CCSEA/24-11)**. Albino wistar rats (weight about 200-250g) were used for the wound healing study. The animals were kept under standard laboratory conditions (temperature: 25±2°C; relative humidity: 55±5%). The animals were housed in polypropylene cages, with free access to a standard laboratory diet and water. Animals were anesthetized under aseptic conditions, using 50mg/kg Ketamine hydrochloride intraperitoneally **(Chollet J.L. et al. 1999)**. All animals were placed on a plain surface, their back hair was shaved, and a deep wound (about 8mm) was created using a sharp biopsy punch (Acu punch, Acuderma Inc, Louderale, FL, USA).

All animals were divided into four groups with six rats in each group. Group I was the negative control group with no any type of treatment, group II was the treated group with the marketed preparation of 1% w/w Cipladine cream twice a day, group III was the group treated with 0.5% curcumin-gel twice a day, and group IV was the group treated with 0.5% AL-CUR-NEG twice a day. All the treated groups (Group II, III, and IV) received the therapy for 20 consecutive days.

Wound healing activity

Wound healing area activity was performed in terms of wound contraction percentage, wound closure time, and epithelialization period **(Nagar H.K. et al. 2016)**. Percentage of wound contraction was calculated taking the initial size of the wound area as 100% by using the following formula.

$$\% \text{ wound contraction} = \frac{\text{Initial day wound area} - \text{Specific day wound area}}{\text{Initial day wound area}} \times 100$$

Results and discussion

U.V. Spectrophotometer

According to this present study, standard curve showed in **Figure 1** that the maximum absorbance at 0.638 the lowest absorbance at 0.120 and r^2 value (≈0.9964) was near about r^2 value 0.999. It was useful to calculate the concentration of curcumin **(Luykx D.M.A.M. et al 2008)**. So, they are slightly equivalent to the reference standard curve of curcumin.

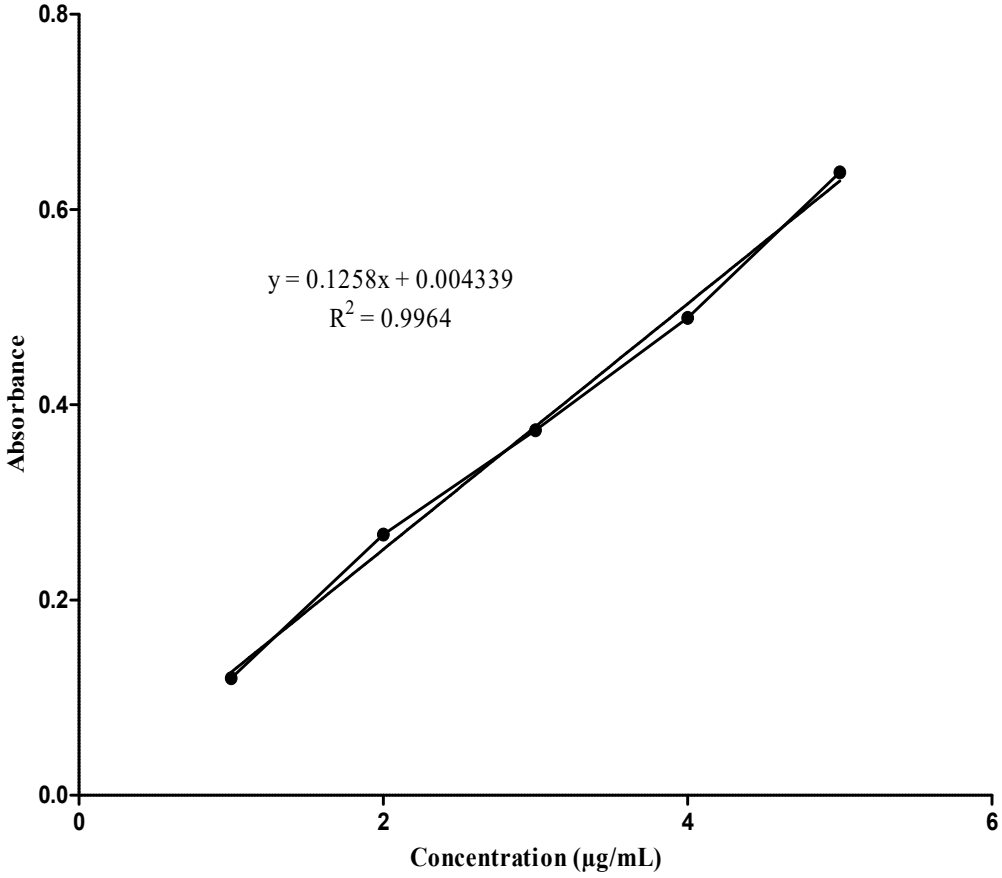

Figure 1: Standard curve of curcumin in ethanol

FT-IR spectroscopy

The **Figure 2 & 3** showed the FTIR spectra of dried powder of curcumin and AL-CUR-NEG from different regions. The main active constituents of curcuminoids present in curcumin. The peak and shoulders was shown the functional groups present in the curcumin and Nanoemulgel formulation. From the analysis, the FTIR spectra showed similar peaks, which can be interpreted as a similar profile of curcumin chemical components **(Vazquez P.P. et al. 2000)**. The differences in obtained peak intensities caused due to the different levels of chemical contents present in the dried powder of curcumin and Nanoemulgel formulation.

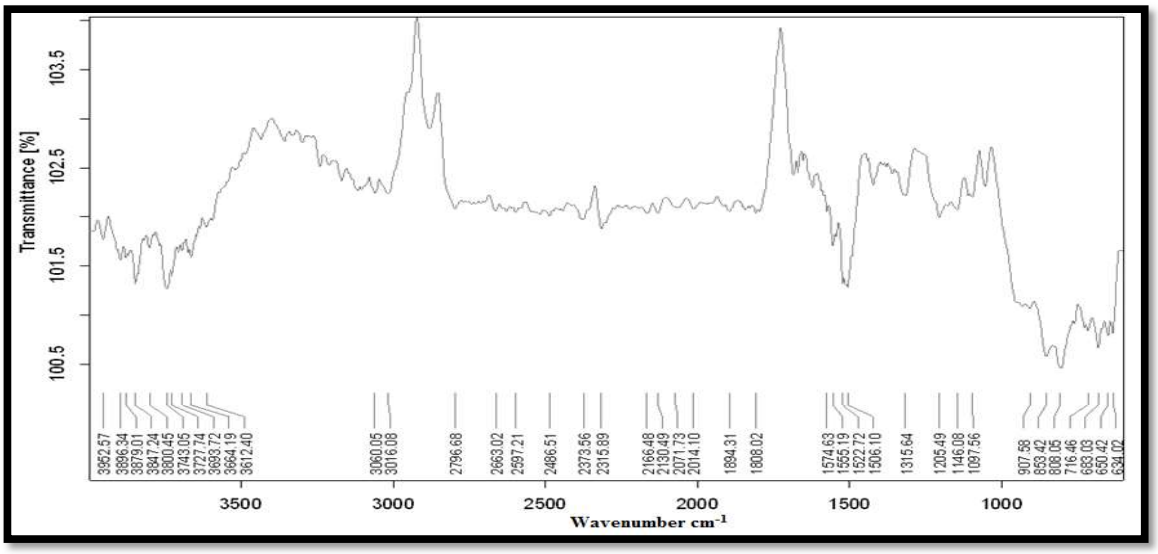

Figure 2: FTIR spectra of Curcumin

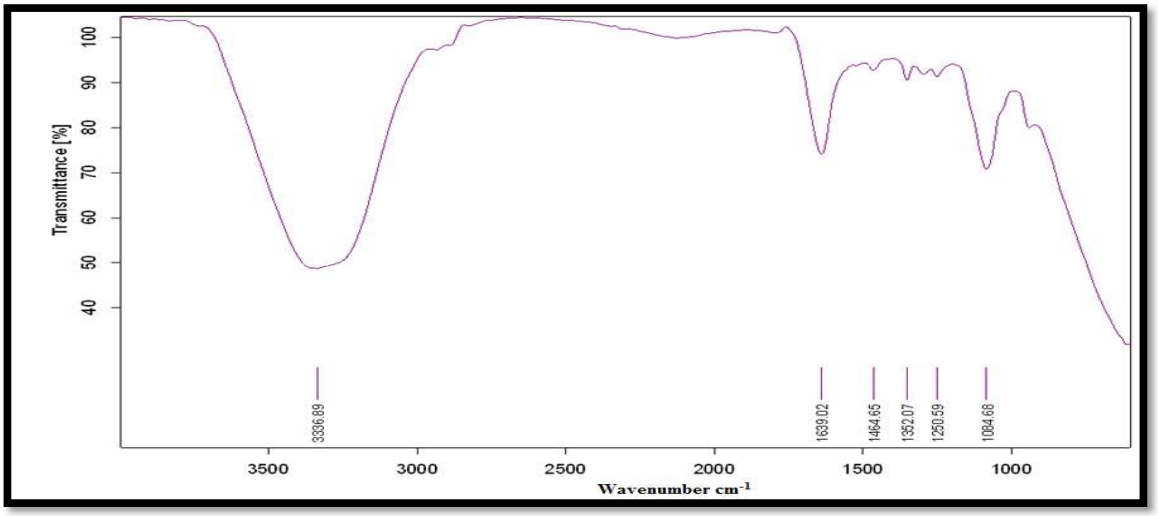

Figure 3: FTIR spectra of Curcumin

The peak obtained at 3,371cm^{-1} to 3,200cm^{-1} may be due to the presence of stretching vibration of the OH bond. The obtained peak at 2800cm^{-1} to 3000cm^{-1} due to C-H aromatic stretching vibration and peaks obtained at 1,624cm^{-1} to 1,800cm^{-1} due to the presence of C=O double bond stretching. CH_2, CH_3 and C-H bending stretching vibration mode due to the obtained peaks 900cm^{-1} to 1,400cm^{-1}.

Particle size, polydispersity index and zeta potential

Droplet size and its polydispersity index of optimized formulation was determined using Malvern zeta sizer. Graphical representation of obtained result was showed in **Figure 4**. Optimized formulation was existed in the droplet size range of 385.9nm with uniform size distribution. Mean droplet size of formulation was good arrangement to the definition of

nanoemulsion (50-200nm) with good polydispersity index indicates uniformity of droplets size **(Kawakami S. et al. 1998)**.

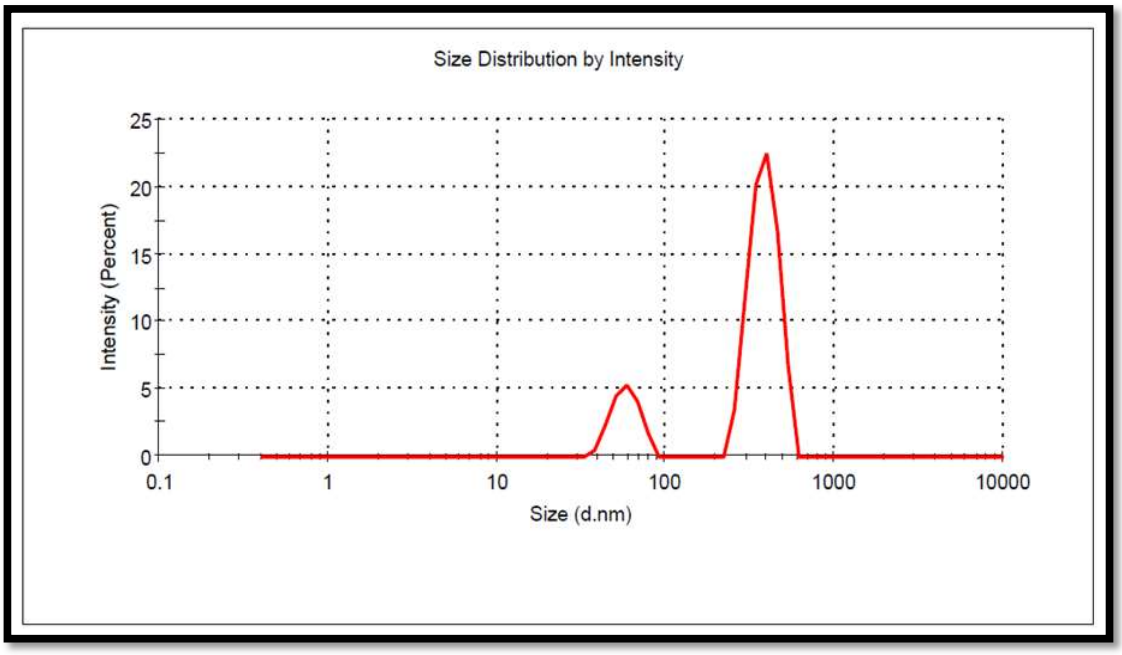

Figure 4: Particle size analysis of optimized formulation

Rheology study and Spreadability of AL-CUR-NEG

AL-CUR-NEG formulation effectiveness was determined in term of several evaluation parameters. Those evaluated parameters were showed in **Table 2**. Spreadability of AL-CUR-NEG formulation was depending on the viscosity. Viscosity of AL-CUR-NEG formulation was determined by using Brookfield viscometer and it was found to be 46800±2.214cps for AL-CUR-NEG formulation and 50876±2.262cps for marketed formulation. Hence spreadability of both formulations was determined using glass slides and it was found to be 30.5±2.17gm.cm/sec. for AL-CUR-NEG formulation and 29.68±1.27gm.cm/sec. for marketed formulation. The prepared AL-CUR-NEG formulation should be easily spreadable in term of marketed formulation.

Table 2: Rheology, spreadability of AL-CUR-NEG & marketed formulation

S. No.	Evaluation Parameters	AL-CUR-NEG formulation	Marketed formulation
1.	Viscosity (cps)	46800±2.214	50876±2.262
2.	Spreadability (gm.cm/sec)	30.5±2.17	29.68±1.27

Ex vivo skin permeation

The cumulative AL-CUR-NEG permeated for 48 hours from the AL-CUR-NEG (106.62±4.20µg/cm^2) was higher than the marketed formulation (93.34±3.54µg/cm^2). The graphical representation was showed in **Figure 5**.

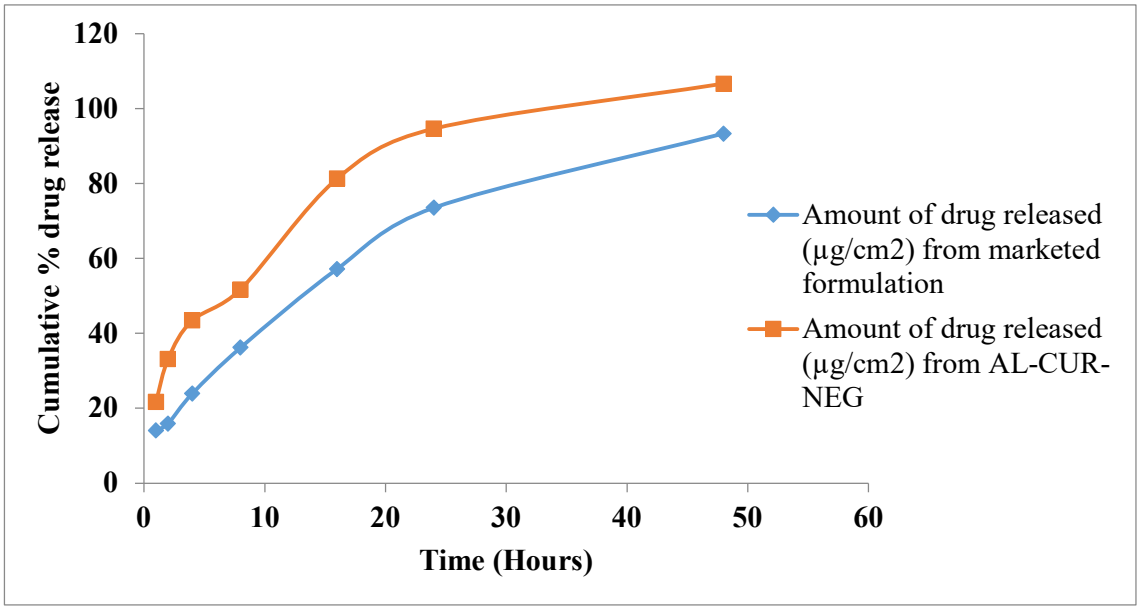

Figure 5: Cumulative % drug releases from AL-CUR-NEG and marketed formulation

In the *ex-vivo* skin permeation study, the presence of tween 80 aided the drug penetration through the skin membrane because tween 80 plays the role of a penetration enhancer, extracting the stratum corneum lipids and subsequently plunging the stratum corneum barrier ability **(Khurana S. et al. 2013)**. Further study is needed to compare the penetration of AL-CUR-NEG formulation with maketed conventional gels formulation so that the difference can be assessed.

***In-vivo* wound healing activity**

Curcumin from the AL-CUR-NEG and traditional curcumin gel was assessed for wound healing effectiveness and contrasted with the widely available Cipladine formulation. The topical administration of the examined formulation in wistar rats was observed for duration of 20 days. Appearance and contraction of the wound was observed at the days 1, 4, 8, 12, 16 and 20. **Figure 6 & 7** showed the wound healing contraction percentage as the wound size at day one was considered 100% **(Nagar, H.K. et al. 2016)**.

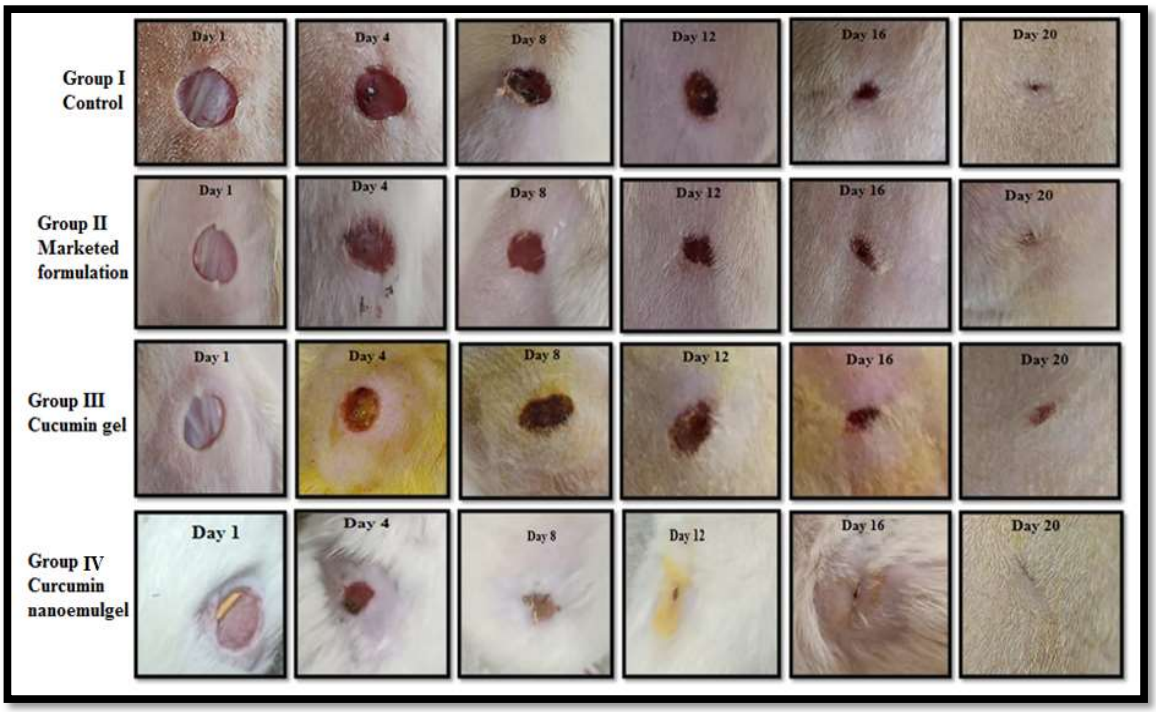

Figure 6: *In-vivo* wound healing activity in albino wistar rats

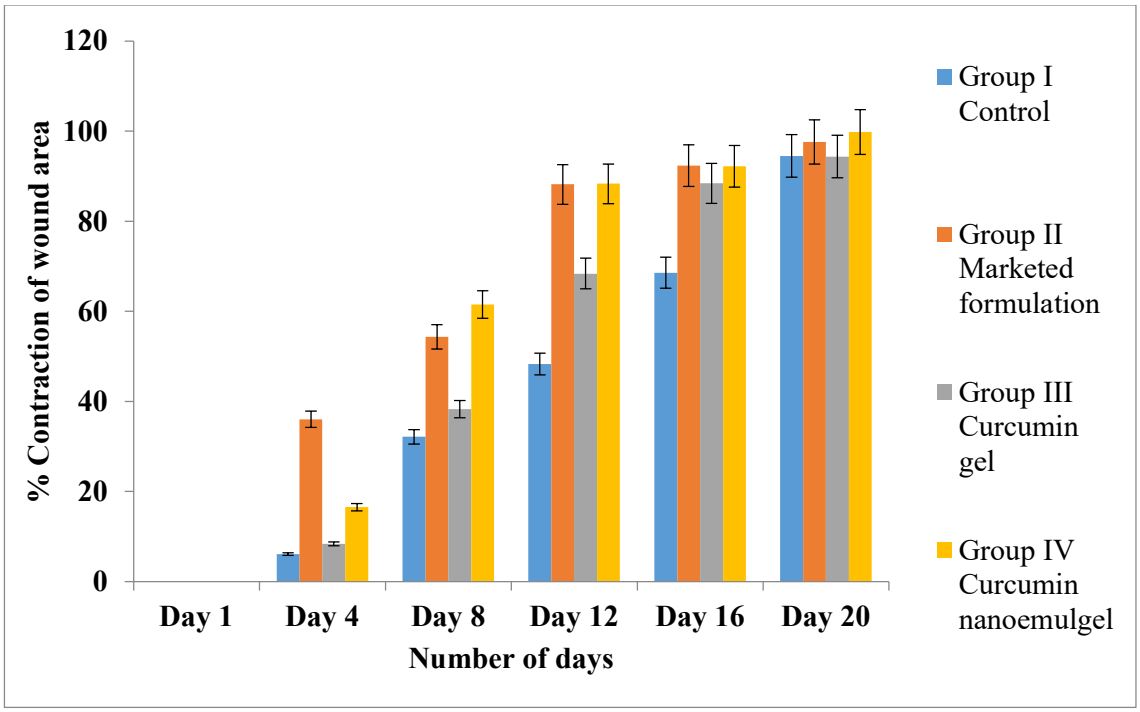

Figure 7: Percentage contraction of wound area for wound healing activity.

On the fourth day of post-wound observations group I animals showed signs of edema and exudates. The three treated groups exhibited decreased inflammation, a soft thrombus without discharge, and a decreasing order of wound healing activity, with Group IV > Group II > Group III. Furthermore, on day eight, brown-reddish tissues were seen forming in the wounds of groups I and III; on day six, however, this structure was noticed forming in groups II and IV

(but not showed in **Figure 6**).

When compared to the untreated control group (Group I), all of the treated groups (Groups II, III, and IV) showed a remarkable wound healing activity **(Nagar, H.K. et al. 2016)**. In particular, the groups treated with formulation AL-CUR-NEG (Group IV) and the groups treated with marketed cream cipladine (Group II) almost completely treated the wound contraction at study end, or day 20. The untreated group needed 16 days to properly epithelize the wound that was observed, while the three treated groups needed 14 days, 11 days, and 10 days, respectively, to properly epithelize their wounds: Group III received curcumin gel treatment, Group II received cipladine cream treatment, and Group IV received AL-CUR-NEG treatment **(Castro Souza J.D. et al. 2017)**.

Figures 6 and **7** show that the animals treated with the AL-CUR-NEG formulation system and cipladine marketed cream (a conventional medicine) had nearly equal wound healing activity. Curcumin is widely recognized for its ability to promote wound healing activity **(Tejada S. et al. 2016)**. However, when compared to the traditional curcumin gel formulation, the AL-CUR-NEG formulation significantly increased curcumin's ability to promote wound healing activity **(Akbik D. et al. 2014)**.

Conclusion

In present study, AL-CUR-NEG formulation was prepared by spontaneous emulsification method. Drug excipient compatibility study was confirmed by FT-IR. Designed AL-CUR-NEG formulation was optimized on the basis of particle size, zeta potential viscosity and spreadability. Optimized AL-CUR-NEG formulation evaluated for ex vivo skin penetrability attributes along with *in-vivo* wound healing efficacy in albino wistar rats. Thus, it can be concluded that nanoemulgel was prepared with enhance solubility, permeability as well as bioavailability of Curcumin was safe, effective and promising formulation for the treatment of wound healing.

List of Abbreviations

NEG: Nanoemulgel; CUR: Curcumin; AL: Aloe Vera; PEG: Poly ethylene glycol; DMSO: Dimethyl Sulfoxide; FTIR: Fourier Transform Infrared Spectroscopy PDI: polydispersity index; ML: Milliliter; nm: Nanometer; mg: Milligram; µm: Micrometer; cm: Centimeter; ppm: Parts per million; RPM: rotations per minute; UV: Ultraviolet; Conc: Concentration; °C: Degree Celsius; Hrs: Hours; Min: Minutes; Lit: Litre; µg: Microgram.

Ethics approval and consent to participate: Yes

Competing interests: The author has declared that no conflicts of interest exist.

Funding: No financial support

Author's Contribution: In the present research article, V, SS given the contribution in the preparation of AL-CUR NEG formulation and perform all the entire experimental studies. MK, GV analyzed the present research study data related to related to wound healing treatments approaches and were the most important contribution in making the manuscript. All authors read and approved the final manuscript.

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