

FABRICATION OF HYPERICIN-LOADED LIPOSOMES IN HYDROGELS FOR ENHANCED WOUND HEALING

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in
Biotechnology

by
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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A" by NAAC
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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of AMRITHA HARINID(37230005) who carried out the project entitled "**FABRICATION OF HYPERICIN-LOADED LIPOSOMES IN HYDROGELS FOR ENHANCED WOUND HEALING**" under my / our supervision from January 2021 to March 2021

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DECLARATION

I Amritha Harini.D hereby declare that the Project Report entitled FABRICATION OF HYPERICIN-LOADED LIPOSOMES IN HYDROGELS FOR ENHANCED WOUND HEALING done by me under the guidance of Dr.Jeyshree Nellore(Internal) and_____(External)at Sathyabama institute of science and technology(Company name and address) is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE:

PLACE:

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Wound healing remains a major health problem worldwide. Recently, wound dressings comprising of biocompatible and biodegradable materials have been synthesised to improve the wound healing process. Hydrogels when injected into the wound bed, quickly transform into semisolid gels that fill the entire wound area, protecting the wound and absorbing wound secretions. Additionally, due to their porous nature hydrogels gradually deliver encapsulated drug into the dermal wound. Liposomes have many advantages as a drug carrier for dermal wound healing, including the ability to codelivery hydrophilic and hydrophobic drugs. However, liposomes are relatively unstable, with low viscosity; therefore, there is a need to develop an insitu matrix for drug-loaded liposomes to enhance their stability and increase contact time of the encapsulated drug in cutaneous wounds. One strategy for overcoming these drawbacks includes dispersal of the liposomes into a hydrogel, which can be used as a platform for targeted delivery. Hence, this study aimed to fabricate Phytochemical-Loaded Liposomes in hydrogels and investigate their therapeutic effects on dorsal full-thickness excisional wounds.

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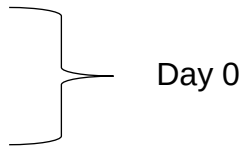
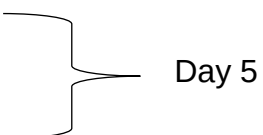
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REFERENCE

LIST OF ABBREVIATIONS

1. KN – Kinetin
2. NAA – Napthalene acetic acid
3. HypR – Hypericum red shoots
4. HypG – Hypericum green shoots
5. HPLC - High-performance Liquid chromatography
6. Hyp – Hypericin
7. NaNO₂ - Sodium nitrite
8. ALCL₃.6H₂O - Aluminium Chloride Hexahydrate
9. NaOH – Sodium hydroxide
10. TFC -Total Flavonoid Content
11. nm – Nanometer
12. M – mole
13. ml – millilitre
14. mg – milligram
15. g – gram

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CHAPTER-1

INTRODUCTION

One-half of deaths in tropical countries square measure due infectious diseases (children below 5 years of age) .The slump worldwide, high value of medicines,inefficient public access to medical aid and facet effects caused by artificial medication square measure a number of the factors to be tackled in health care. there's Associate in Nursing pressing want for the invention and development of recent infection-fighting ways that counteract the indiscriminate and abusive use of artificial antibiotics.

1.1 PHYTOCHEMICALS:

The in depth use of combos has resulted within the development of adverse side-effects and emergence of antibiotic resistance. of these facts underscore the undisputed and pressing got to develop new effective therapeutic approaches for MDRP- a treatment. Recent proof suggests that developing a targeted drug delivery system (using nanostructures to 10deposit medication at intervals specific cells) may bypass the matter of resistance). Liposomes – a synthetic sac composed of supermolecule bilayer (phospholipids Associate in Nursingd cholesterol) which can self associate to encapsulate an binary compound interior (pharmacological drugs).

- The use of liposomes could increase antimicrobial activity of the drug and antibiotics against the bacterium.
- Antidepressants to treat gentle to moderate depression
- widely studied in skin and neoplasm therapies
- viral, fungal, and microorganism infections

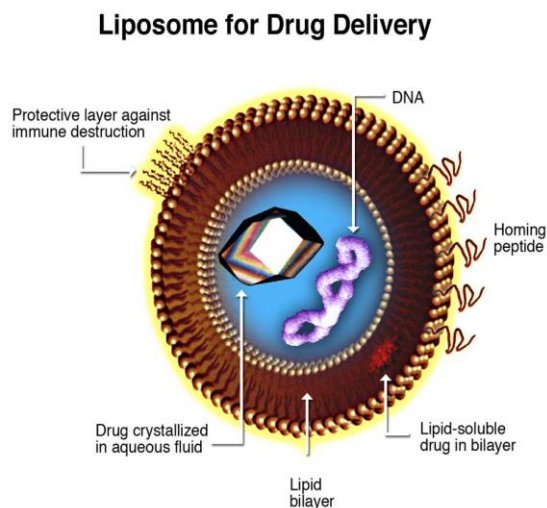


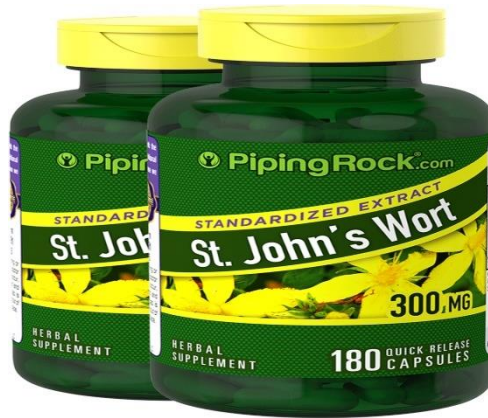
Fig 1.1 Liposomes for drug delivery

1.2 HYPERICIN-*Hypericum hookerianum*

Hypericum hookerianum or Hooker's st. John's Wort may be a perennial ligneous plant within the angiosperm dilleniid dicot family native to Japanese and Southern Asia. Because of its use as a wound healing agent in ancient practices and literature reference, the present study was taken to work out the wound healing potential of this plant.



Fig 1.2 Hypericum hookerium



1.3 SAMPLE COLLECTION AND CULTURE:

The *Hypericum hookerianum* shoots were collected from Nilgiri hills.

1.3.1 SEED GERMINATION:

hookerianum collected from Pambar shola forest (2200 m) adjacent to Pambar Falls in Pambapuram of Kodaikanal city was known by Dr. A.G. Pandurangan of Jawaharlal Nehru Tropical biological science Garden & analysis Institute, Palode, Thiruvananthapuram. Ripe berries were collected from mature shrubs of seeds in terms of 120-140 were distributed on water gelzan, the seeds incubated for germination at $25 \pm 2^\circ\text{C}$ below twelve unit of time photoperiod.

1.3.2 SEEDLING CULTURE:

Cotyledon explants were cleft from the seedlings, and subcultured in agar medium supplemented with KN and NAA.

once four weeks the explants in culture were removed and five hundred mg tissue was extracted with eightieth ethyl alcohol for assaying hypericin.

1.3.3 MICRO PROPOGATION:

Micropropagation is that the method of multiplying plant stock material by growing plantlets in tissue culture to supply an outsized variety of offspring plants so planting them out.

The tissue culture was disbursed on Ms media by adding zero.5mg/mL Napthalene ethanoic acid (NAA) and zero.5mg/mL Kinetin.

1.3.4 GELLAN GUM:

Gellan gum may be an inexperienced biopolymer widely utilized in prescription drugs, cosmetics and food industries as suspending, helpful, and thickening agent. It's ready to type hydrogels with multivalent cations that absorb high volume of water or body fluids and adequately encapsulate medication or cells in opening areas among chemical compound chains.

1.3.5 IN-VIVO STUDIES:

In-vivo refers to the experimental study that is carried out in an animal model.

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LITERATURE SURVEY

H. hookerianum had been studied for his or her wound restoration assets within the form of ointment, the use of kinds of wound fashions in thirty six rats. Ointment of the dried extract of the leaves and stem of the plant had been enforced in exclusive concentrations. This observe confirms victimisation of a locality of hypericin, as a capability wound restoration agent. (The magazine of chance and medicine, 2000)

The observe some the anti-microbial impact, anti-inflammatory, anti-depressive impact, wound restoration impact, phototoxicological impact and additionally bigger observe some the alternative useful and unknown homes of this utile plants. (Sara Kiani et al *Der Pharmacia Lettre*, 2016)

Liposomal encapsulation may be a promising answer to overcome those limitations. Use of liposomes as transport cars for hypericin in antimicrobial photodynamic medical aid (aPDT) is pretty new (Nikola Plenagl, 2018)

The leaves of alcohol extract of *H. hookerianum* confirmed the most effective inhibitor interest additionally to synthetic resin content. This observe indicates that the alcohol extract of leaves of *H. hookerianum* may be a capability provide of flavorer inhibitor (Narayanan ravisankar, 2014)

The gift observe become aimed to assess the medicinal drug interest of hypericin the utilization of RAW 264.7 the consequences exhibited that hypericin appreciably suppressed LPS prompted NO producing with a concomitant lower within the stages of pro-inflammatory cytokines. Conclusion: The observe demonstrates the capability of hypericin as Associate in Nursing powerful anti-inflammatory agent. (Mahendrakumar M, Seeni S, Perinbam. K, 2018)

The antiviral interest of these plant extracts become assessed through cytopathic impact inhibition assay, virus yield assay attended through MTT assay. consistent

with the observations only extracts, leaves of dilleniid dicot genus[dilleniid dicot genus] hookerianum and leaves of Hypericum patulum, out of five extracts indicated life-size microorganism repressing assets at concentrations of 100 microgram / cc while challenged with 100 TCID fifty dose of acute anterior poliomyelitis virus kind one. Others confirmed slight or no interest. (P.K sharma,2007)

Compare the consequences of diphenylhydantoin (PHT) and hypericin (HP) cream on healing of burned wound. The raised VEGF and TGF- β expression when PHT and HP treatment powerfully indicate that PHT and HP treatment induces VEGF and TGF- β production and action within the burn wound space. (Hamide Sayar,2014)

The study have tested that aerial elements of Hypericum possess outstanding wound healing and anti inflammatory activities supporting the assertion of the plant in Turkish folks drugs. On the opposite hand, grain alcohol extract of Hypericum scabrum showed neither outstanding wound healing nor medicinal drug activity demonstrating the importance of correct plant species choice in therapeutic applications. (Ipek Pesin Suntar,2009)

Cotyledon segments derived from H. hookerianum were polite on Murashige and Skoog (MS) media supplemented with Kinetin (KN, 1 mg/l) and hydrocarbon ethanoic acid (NAA, 0.1 mg/l) to induce hypericin-rich red shoots (HypR, 0.87 mg/G DW). extremely stable liposomes (-29.4 mV) were with success developed that encapsulated $63 \pm 0.8\%$ Hyp extracts, severally. (P.S Pradeep,2019)

The medicinal drug activity of the chloroform, ketone and alcohol extracts of Hypericum hookerianum leaves and stems was evaluated. All extracts showed medicinal drug activity against six totally different gram-positive and gram-negative microorganism, the alcohol extracts exhibiting the utmost repressing activity at four hundred $\mu\text{g/ml}$.
(Pulok k Mukherjee,2001)

Wound-healing impact of St. John's Wort (Hypericum perforatum L.) extract was evaluated by examination with dexpanthenol and titrated extract of Centella asiatica

(TECA) on polite chicken embryonic fibroblasts. Wound-healing activity of Hypericum perforatum extract appears to be principally because of the rise within the stimulation of formative cell albuminoid production and also the activation of formative cell cells in polygon, that plays a task in wound repair by closing broken space. The findings incontestable the wound-healing activity of Hypericum perforatum, that has antecedently been supported ethnomedical knowledge. (Nilgun Ozturk,2007)

The study was applied on forty eight male person Wistar unusual person rats. The animals were divided into 2 main teams as management and study teams. 2 main teams were divided into 3 subgroups consistent with the sacrifice days. All rats got streptozotocin sixty mg/kg, when seventy two hours, and people having glucose levels on top of two hundred mg/dL were enclosed within the study. membrane defects were created within the palatal space of the rats. H. perforatum oil was applied locally double each day to the injuries of the rats within the study cluster (Ahmet Altan,2008)

CHAPTER-3

AIM AND SCOPE

3.1 AIM

To fabricate *Hypericum hookerianum* -Loaded Liposomes in hydrogels and investigate their therapeutic effects on dorsal full-thickness excisional wounds.

3.2 OBJECTIVE

Sample collection

- *Hypericum hookerianum* shoots from nilgiri hills
- Micropropagation
- Synthesis of Hypericin

Preparation and characterization of liposomes

- Liposomal hypericin
- Size determination and Zeta potential
- Gellan gum based liposomal hydrogel

***In vitro* studies**

- *In vitro* wound healing activity of liposomal hypericin on keratinocyte cell lines.

***In vivo* studies**

- To determine the toxicity of the synthesized liposomal hypericin in rat model.
- *In vivo* wound healing activity of liposomal hypericin on rat model
- Wound closure rate
- Gene expression studies on growth factors
- Histopathology studies

CHAPTER-4

MATERIALS AND METHODS

4.1 MATERIALS:

4.1.1 PLANT MATERIAL:

1. Seeds from ripe berries of hypericum
2. HypR – Hypericum RED shoots (Hypericin rich shoots)
3. HypG – Hypericum GREEN shoots (Control)

4.1.2 CHEMICAL COMPOUNDS:

- 1.Napthalene acetic acid (NAA)
- 2.Kinetin
- 3.Ethanol
4. Sodium nitrite
- 5.Aluminium Chloride Hexahydrate
- 6.Sodium hydroxide
- 7.Cholestrol
- 8.Chloroform
- 9.Triton X-100
- 10.Distilled water

4.1.3 APPARATUS:

- 1.Mortar and pestle
- 2.Forceps
- 3.Conical flasks
- 4.Petri dishes
- 5.Culture tubes or
- 6.Culture plates
- 7.Filter paper
- 8.Glass pipettes
9. Cotton plug
- 10.Scissors

4.1.4 INSTRUMENTS:

1. Incubator
2. Colorimeter
3. Sonicator
4. Ti probe-sonication
5. UV Spectrophotometer

4.1.5 ANIMAL MODEL

1. Rat model is used in this study

4.2 METHODOLOGY:

4.2.1 MICRO PROPOGATION:

The tissue culture was carried out on Ms media by adding 0.5mg/mL Napthalene acetic acid (NAA) and 0.5mg/mL Kinetin.

Step 1 – preparation of explant plant from stock plant by placing it in the suitable media

Step 2 – multiplication of shoot or embryo from the defined culture media

Step 3 – transferred to next rooting or storage medium.

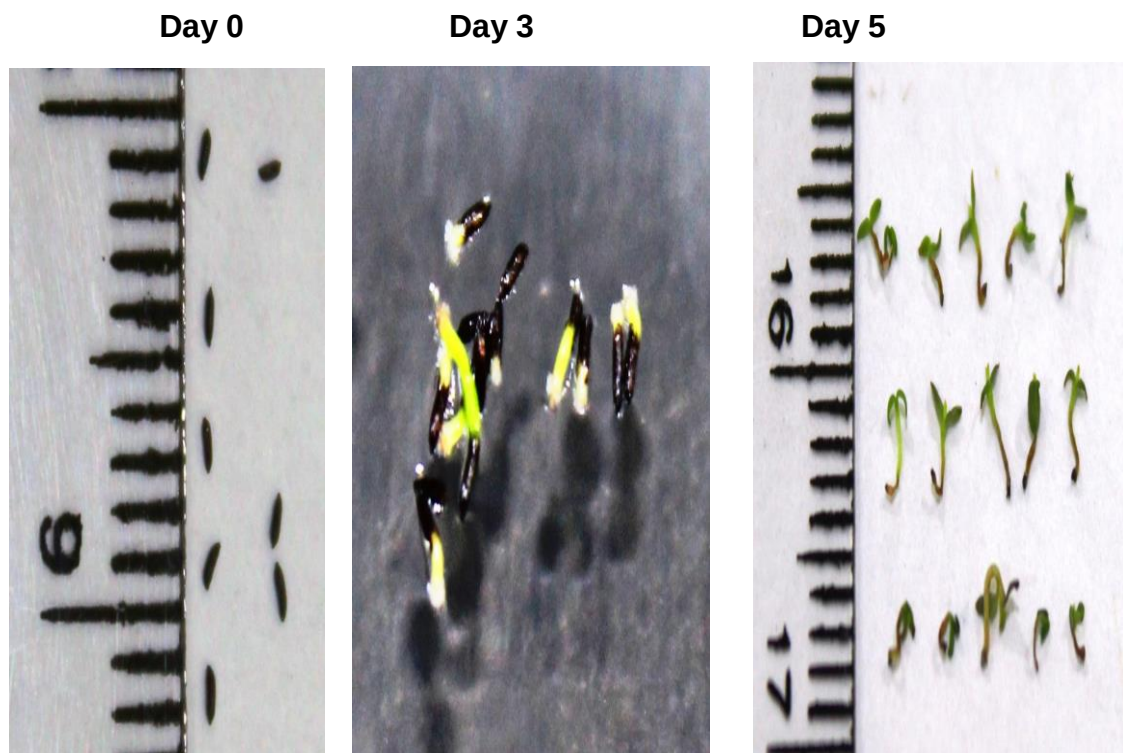


Fig 4.2.1 development of seed in 5 days

4.2.2 HPLC PROFILE:

High-performance Liquid chromatography is a technique used to identify the amount of each components present in the mixture. here, it is used to determine the presence of hypericin. The retention peak at 4.3 mins confirms the presence of hypericin in the sample.



Fig 4.2.2 hypericin rich red shoots.

4.2.3 EXTRACTION AND QUANTIFICATION-HPLC:

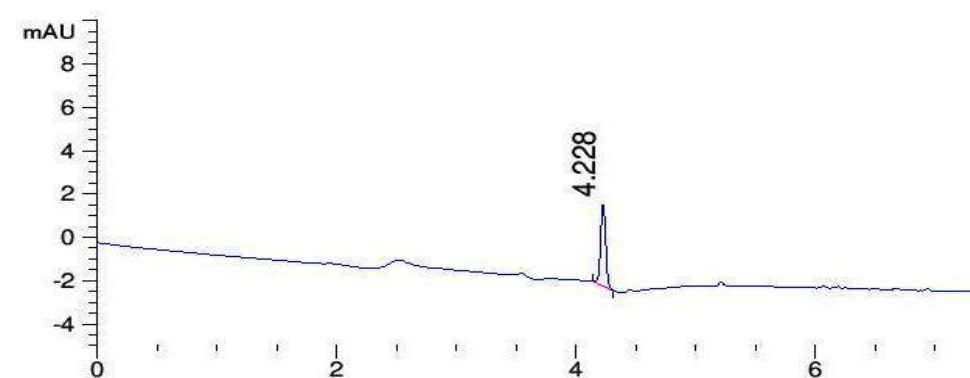


Fig 4.2.3.1 Hypericin – standard

4.2.6 GELLAN GUM BASED LIPOSOMAL HYDROGEL:

Gellan gum based liposome is used in drug delivery. The hydrogel adhesives act as a boundary against microbial infections. This gives the wound an out right protection and healing process. The use of hydrogel minimises the wound area with the increasing rate of wound healing. Success in wound treatment requires careful assessment on the wound and suitable wound dressing.

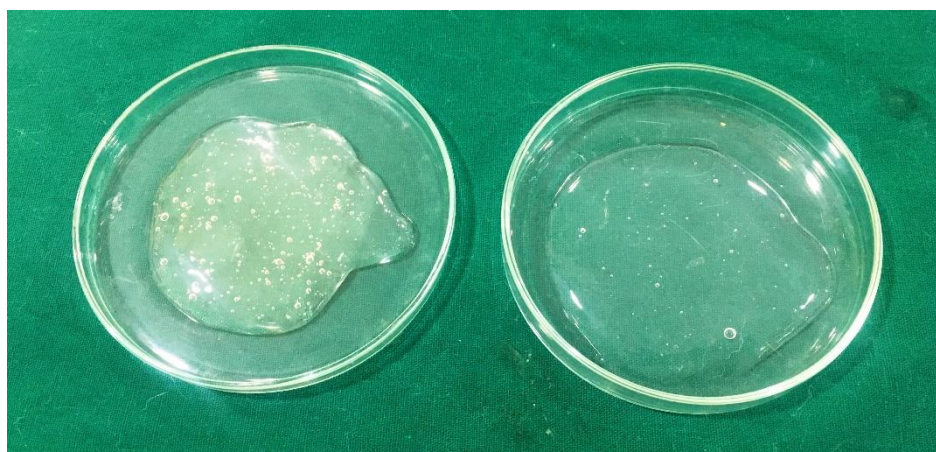


Fig 4.2.6 Gellum gum based liposome

4.2.7 IN-VIVO STUDIES:

To determine the toxicity of the synthesized liposomal hypericin in rat model. *In vivo* wound healing activity of liposomal hypericin on rat model. The rat models were categorized into three groups to determine and have a comparative analysis between the 3 groups and infer the results.

Group I – Control

Group II – HypR gel

Group III – Positive control (Povidone Iodine)



Fig 4.2.7.1 rat model with wound



Fig 4.2.7.2 group I control



Fig 4.2.7.2 group II HypR gel



Fig 4.2.7.3 group III Povidone Iodine

CHAPTER-5

RESULTS AND DISCUSSIONS

5.1 TOTAL FLAVONOID CONTENT(TFC):

The highest flavonoid content was observed in HypR extract at **79.7 mg/g** sample and relatively lesser TFC was detected in HypG extract at **34.1 mg/g** sample.

5.2 PREPARATION AND CHARACTERIZATION OF LIPOSOMAL HYP FORMULATION:

$$\text{Encapsulation Efficiency (\%)} = [H_{\text{Ent}}/H_{\text{Ini}}] \times 100$$

Where H_{Ent} is the hypericum suspension entrapped in liposomes.

H_{Ini} is the initial hypericum concentration utilized in the mixture.

RESULTS: The encapsulation efficiency of Hyp in liposomes was **63±0.8%**

5.3 IN-VIVO WOUND HEALING STUDIES:

5.3.1 WOUND HEALING AT DAY 0:

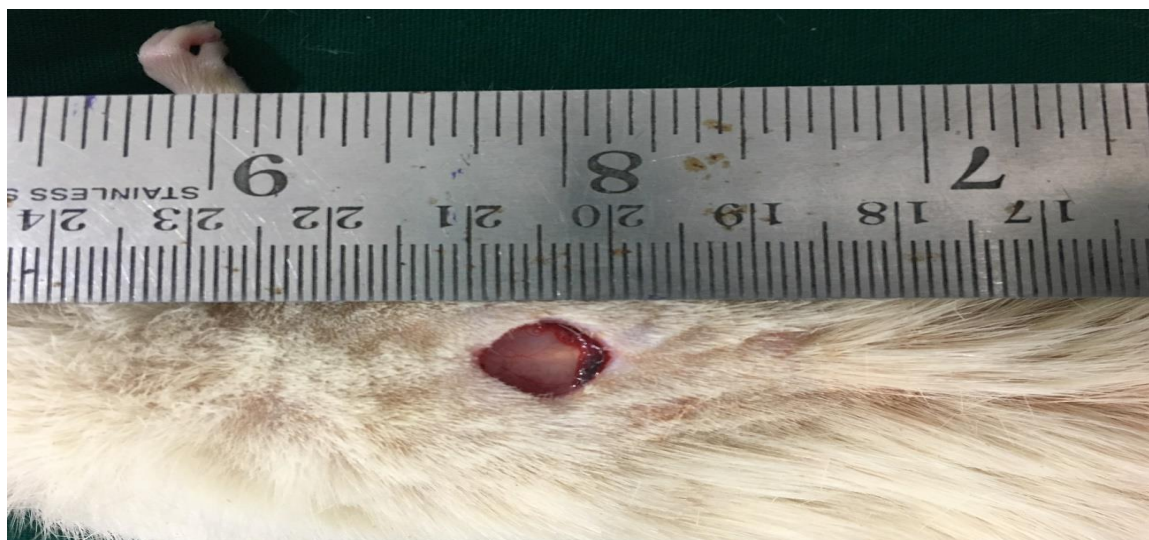


Fig 5.3.1.1 group I

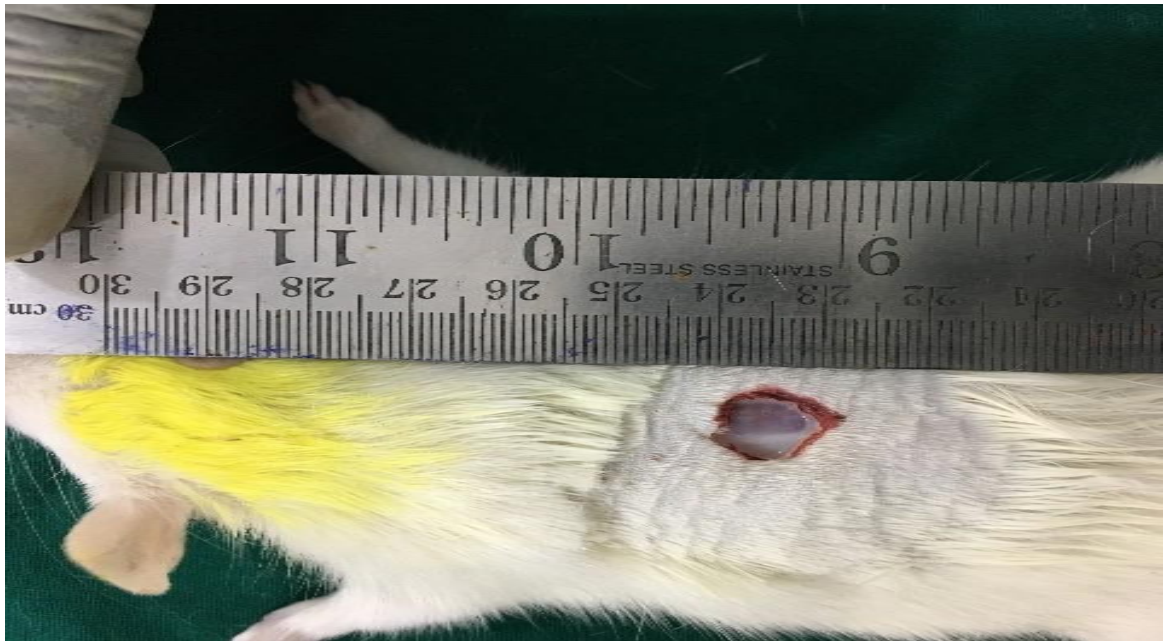


Fig 5.3.1.2 group II

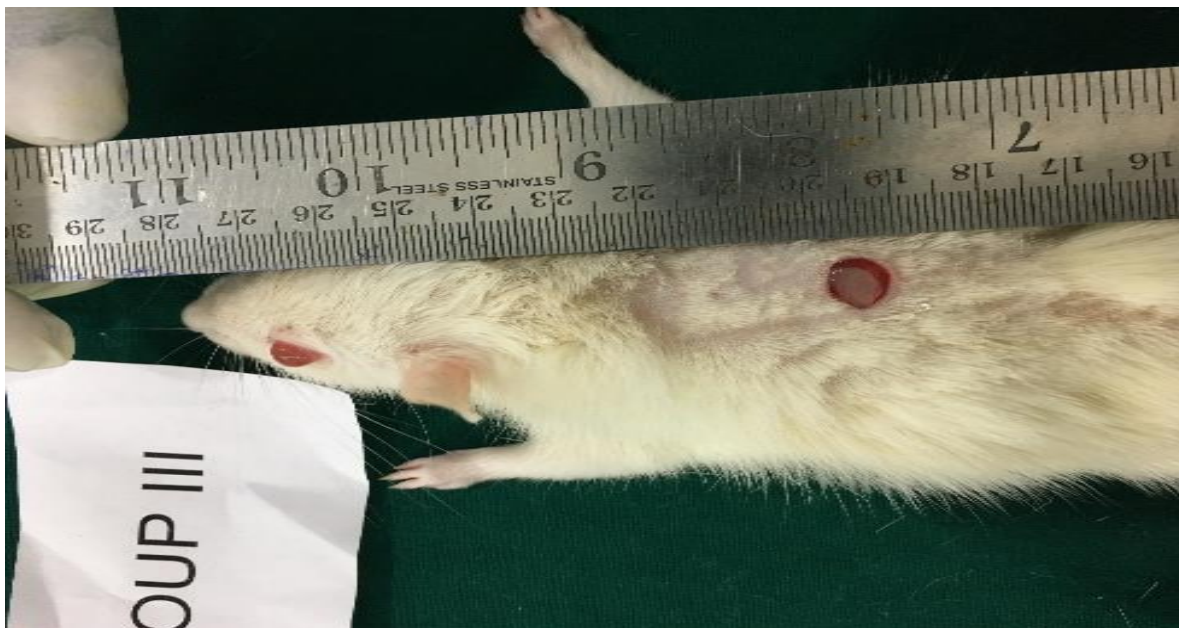


Fig 5.3.1.3 group III

5.3.2 WOUND HEALING AT DAY 5:

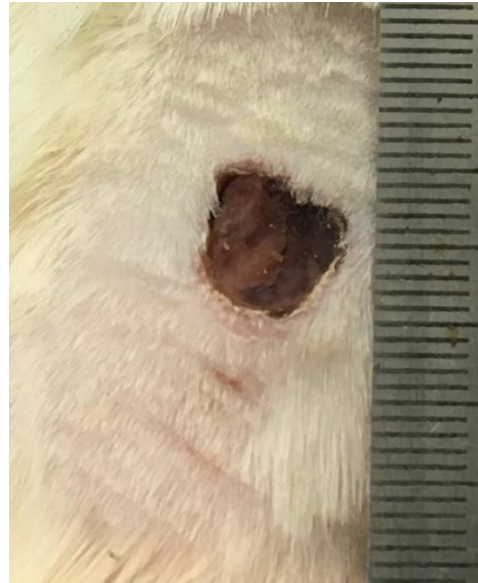
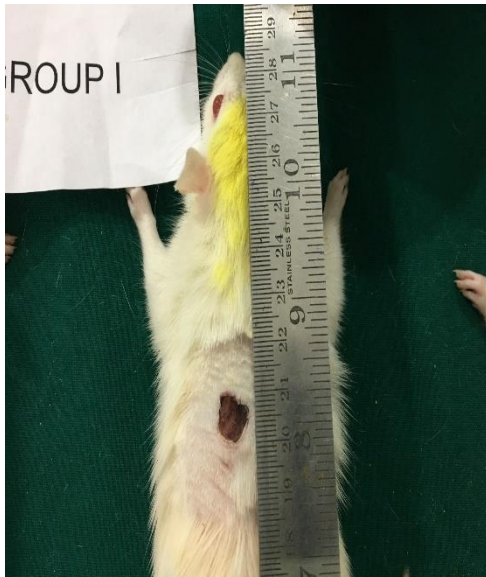


Fig 5.3.2.1 group 1

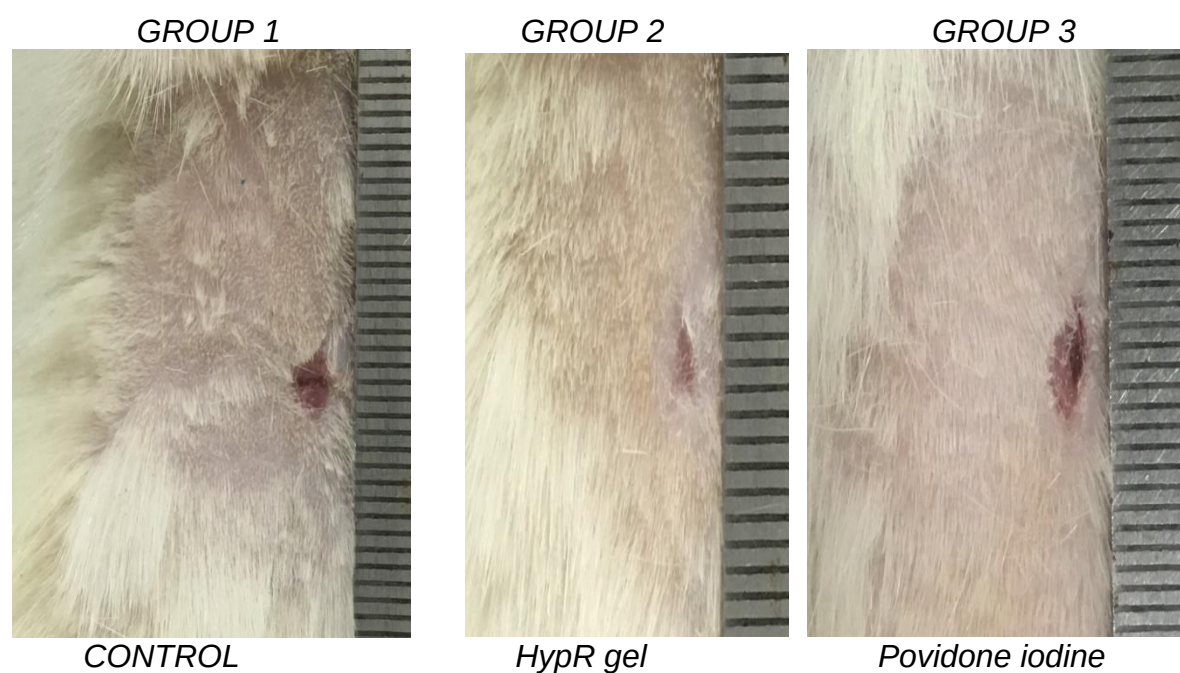


Fig 5.3.2.2 group 2



Fig 5.3.2.3 group 3

5.3.3 WOUND HEALING AT DAY 10 – END OF STUDY:



From this study, we conclude that Hypericum red shoots (Hypericin rich shoots) loaded liposomes in hydrogel for enhanced wound healing.

CHAPTER-6

SUMMARY AND CONCLUSION

6.1 SUMMARY:

Hypericin have higher wound healing potential. The use of liposomes may increase anti-microbial activity of the drug and antibiotics against the bacteria. The in-vivo studies were carried out in rat models. Nine rats were divided into three groups and a second-degree wound was created. The burn in the first,second and third group were treated daily with gellan gum(control) , HypR gel and povidone iodine (positive control) respectively. The rats were kept under observation for 10 days. At the end of the study (on the 10th day) it was found that group-II that is HypR gel had higher healing potential than the other two groups.

6.2 CONCLUSION:

The following results were drawn from the studies:

1. The plant (*Hypericum hookerianum*) roots were rich in hypericin.
2. The in-vivo study further proved that the group II model had quicker healing potential in which HypR gel was used for treating the wound.

CHAPTER-7

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