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Formulation and Evaluation of Topical Semisolid Preparation of Ehretia Laevis Roxb. (Khandu Chakka)

[1]Miss. Nishigandha Kakade*, [2]Dr. Prashant Deshmukh
[1]Research Scholar,[2]Guide,
[1]Department of Pharmaceutics,
1P. R. Pote Patil College of Pharmacy, Amravati, India
Corresponding Author: 1Miss. Nishigandha Kakade

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ABSTRACT: Many herbal plants are mentioned in Ayurveda for wound healing. Folklore medicine for various ailments including wound healing is being practiced in India. The Ehretia laevis Roxb. is rare Indian medicinal plant and member of Boraginaceae family. Local people of vidharbha India using Ehretia laevis Roxb. Commonly known as (Khandu-chakka) plant People uses paste of leaves for wound healing problems like infections, old age stress, diabetes, chemotherapy drug, obesity, alcohol consumption, smoking, malnutrion. Khandu-chakka plant use in covid-19. Ehretia laevis Roxb. called Khandu chakka & Ajan Vruksha and traditionally being used for wound healing. Objectives of the present study is to formulate and evaluate the topical semisolid formulation of Ehretia laevis Roxb. (Khandu Chakka) for wound healing activity and evaluation is done by in vivo study in animal model. This present study has found put the ethanolic leaves extract of Ehretia laevis Roxb. has enhanced the process of wound healing ascertained on the in vivo study of formulated Emulgels formulation. There are not many studies that have experimental with ethanolic extract of E. laevis Roxb. leaves. For the objective assessment for its property of enhanced wound healing. From the present study, the topical semisolid preparation Emulgels of 2.5 % w/w concentration of ethanolic extract of E. laevis had better wound healing contraction than the other formulated (5%w/w and 7.5%w/w) Emulgels formulation.

KEYWORDS: Ehretia laevis Roxb., Emulgels, wound healing, in vivo study

I. INTRODUCTION

1.1. Topical Drug Delivery System

Advances in pharmaceutical technologies have encouraged the formulation scientists to explore alternative routes, besides oral/parenteral, for the delivery of drug efficiently and effectively to the target site. Effective drug administration encompasses the optimal delivery of therapeutics at the site of action within the given time frame. The topical delivery system refers to a method wherein the formulation is applied to the superficial areas such as the skin, eyes, nose and vagina for the treatment of local diseases. The drug application to the topical surfaces evades the hepatic first pass metabolism, gastric pH variations and fluctuations in plasma levels, frequently encountered when a drug is administered through the oral route.[1]

1.2. Semisolid Dosage Form

Pharmaceutical semisolid preparations may be defined as topical products intended for application on the skin or accessible mucous membranes to provide localized and sometimes systemic effects at the site of application. Semisolids are the product for the use on skin and mucous membranes. In general, semisolid dosage forms are complex formulations having complex structural elements. They are often composed of two phases (oil and water), one of which is a continuous (external) phase and the other a dispersed (internal) phase. The active ingredient is often dissolved in one or both phases, thus creating a three-phase system. It may use for the treatment of pathological conditions and may also protect the body from harmful conditions. These semisolid preparations are used to protect over the time and produces the therapeutic effect through the occlusions. [2,3]

1.2.1. Ideal Properties of Semisolid Dosage Forms

1. Physical Properties

•Smooth texture.
•Elegant in appearance.
•Non dehydrating.

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•Non gritty, greasy and non-staining.

2. Physiological Properties
•Non irritating.
•Do not alter membrane / skin functioning.
•Miscible with skin secretion - low sensitization index.

3. Application Properties
•Easily applicable with efficient drug release.
•High aqueous wash ability.

4. Storage Properties
•Storage of semisolids at 25°C.
•It should not allow to freeze and stored in a well-closed container4.

1.2.2. Types of Semisolid Dosage Forms
Semisolid includes ointments, creams, pastes, gels and many more.

1. Ointments
They are soft hydrocarbon based semisolid preparation, composed of fluid hydrocarbon meshedin a matrix of higher melting solid hydrocarbon petrolatum being a tasteless, odorless, unctuous material with a melting range. Since they are greasy nature so they stain cloths. Principle ingredients forming the system hydrocarbon and silicon oil are generally poor solvent for most drugs, seemingly setting a low limit on the drug delivery capabilities of the system.

2. Creams
They are viscous semisolid emulsion system with opaque appearance as contrasted with translucent ointments. Consistency and rheological character depend on whether the cream is w/o or o/w. properly designed O/W creams are elegant drug delivery system, pleasing in both appearance and feel post application. O/W creams are non-greasy and are rinsible. They are good for most topical purpose and are considered particularly suited for application to oozing wounds.

3. Pastes
Pastes are basically ointments into which a high percentage of insoluble solid has been added. Pastes are usually prepared by incorporating solids directly into a congealed system by levigation with a portion of the base to form a paste like mass. The remainders of the base are added with continue levigation until the solids are uniformly dispersed in the vehicle.

4. Gels (Jellies)
Gels are semisolid system in which a liquid phase is constrained within a 3-D polymeric matrix (consisting of natural or synthetic gum) having a high degree of physical or chemical cross-linking. Gels are aqueous colloidal suspensions of the hydrated forms of insoluble medicament. When the coherent matrix is rich in liquid, the product is often called as jelly. Jellies are transparent or translucent non-greasy semisolid gels. Some are as transparent as water itself, an aesthetically pleasing state, other are turbid, as the polymer is present in colloidal aggregates that disperse light. They are used for medication, lubrication and some miscellaneous applications like carrier for spermicidal agents to be used intra vaginally with diaphragms as an adjunctive means of contraception. [5,6,7]

1.3. Wound Healing
A skin wound results from the breakdown of the epidermal layer integrity. Any tissue injury with anatomical integrity disruption with functional loss can be described as a wound. Wound healing mostly means healing of the skin. The wound healing begins immediately after an injury to the epidermal layer and might take years. This dynamic process includes the highly organized cellular, humoral, andmolecular mechanisms. Wound healing has 3 overlapping phases which are inflammation, proliferation, and remodeling. Any disruption leads to abnormal wound healing. Wound healing is occasionally classified as primary healing and secondary healing. Uncomplicated healing of a non-infected, well-approximated wound is defined as primary healing. Surgical wounds are the best example for primary healing. If the wound healing course in this wound is disrupted by infection,dehiscence,wound is disrupted by infection, dehiscence, hypoxia or immune dysfunction, secondary healing stage begins. During secondary healing, granulation tissue formation and epithelization over this new tissue take place. These types of wounds are more susceptible to infections and poor healing.[14]

1.4. Emulgels
Emulgels are dosage forms in which gel and emulsion are incorporated together to form a double release control system for topical delivery. They are made by the dispersion of huge quantity of liquid molecules (either oil in water or water in oil emulsion) in a solid continuous phase (gel base)8. Compared to ointments and creams, gel formulations yield faster release of drugs. The main

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defect of gel formulations is the inability to deliver hydrophobic drugs. Emulgels are formulated to overcome this limitation. Lipophilic drugs are mainly incorporated in Oil in water emulsions, while water in oil emulsions is used to encapsulate hydrophilic drugs. A typical emulsion will get transformed to an emulgel owing to the presence of a gellant in the aqueous phase. Emulgels are elegant preparations that have high penetrability.
They are easily spreadable and easily washable preparation9.

1.4.1. Major Components of Emulgels Preparation

1. Oily Phase

Mineral oils are widely used as the oily phase especially in case of emulsions for external use. Castor oils and fixed oils are widely used in emulsions for oral administration.

2. Aqueous Phase

The aqueous phase of the emulsion may be water or alcohols.

3. Emulsifying Agents

Emulsifiers are the agents used to stabilize the emulsion by preventing the coalescence of the dispersed phase globules. Thus, it increases the shelf life of emulsions. The most widely used emulsifying agents are sodium lauryl sulphate,
Tween 80, sodium dioctyl sulfosuccinate, Span 80, tragacanth etc.

4. Gelling Agent

Gelling agents are the ingredients which are used to convert the product into a gel consistency. They increase the viscosity of emulgels, since they function as viscosity building agents or thickening agent. The main gelling agents used for emulgel preparation are Carbopol polymers (Carbopol 934 and Carbopol 940), Hydroxypropyl methylcellulose (HPMC), Sodium Carboxymethylcellulose (NaCMC) etc.

5. Penetration/ Permeation Enhancers

The outermost layer of skin Stratum Corneum performs as a barricade for the penetration of the drug into the body. Permeation enhancers are agents that help with the penetration of the drug through the stratum corneum. They intermesh with the constituents of the skin and thereby increase the permeability of the skin. They interact with the intracellular protein and thereby reversibly increase the permeability of the skin. They should be pharmacologically inert, non-irritating and non-toxic. Penetration enhancers commonly used for emulgel preparation are Clove oil, Cinnamon, Menthol and Linoleic acid.[10,11]

II. DRUG PROFILE

Ehretia laevis Roxb. (Khandu Chakka)

2.1. Plant Description

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Boraginales
Family: Boraginaceae
Genus: Ehretia
Species: Ehretia laevis (Roxb)
Botanical Name: Ehretia laevis Roxb.
Synonyms: Ehretia laevis Var.platyphylla Merrill.
Common/Local Name: Khanduchakka
Regional and Other Names
English: Ehretia,
Gujarati: Vadhavaradi
Hindi: Bhairi, Chamror, Datranga, Tamoriya
Nepali: Datingal
Konkan: Kalo Gamdo
Marathi: AjaanvrukaDatrang
Tamil: Kuruviccai, Kalvirasu
Telugu: Tellajuvvi, Paldattam
Malayalam: Harandi
Sanskrit: Charmavriksha

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Fig.1: Ehretia Laevis Roxb. Plant

Habit and Habitat: Small deciduous tree, with short stem and grey Bark, occasionally common.
Native: India, China, Bhutan, Pakistan, Laos, Myanmar.
Flowering and Fruiting Time: January to April
Flowers: White, up to 8 mm Fruits: A small drupe, at first red, at length black

Properties and Uses: The inner bark of Ehretia laevis Roxb is used as food. Leaves are applied to ulcers and in Headaches. Fruits are astringent, anthelmintic, demulcent, Expectorant, diuretic, and used in the affection of urinary passages, diseases of Lungs and spleen. Powdered kernel mixed with oil is a remedy for ringworm, Seeds are Anthelmintic this medicinal plant has a light grey or white back with an irregular trunk. The leaves in size and form are variable the length varies between 2 and 6.3 cm and 1.3 to 3.8 cm. These plants flowers are colored white. The calyx of the flowers is 2.5 mm long and the corolla 3-Lobed and 5 corollas lobed is 6-8 mm long. Smaller than Calyx the corolla tube and lobe are longer.[20]

2.2. Chemical compounds useful for wound healing:

E. Laevis contains many useful compounds responsible for wound healing like, naphthoquinone derivative responsible for antimicrobial activity. Ursolic acid responsible for antioxidant property, minerals are responsible for enhancement of immune system and shows antioxidant and antiviral activity. Gallic acid is responsible for antiviral activity. Tannic acid is responsible for antimicrobial activity. Rutin is responsible for antimicrobial activity, enhances immunity and wound healing. Ascorbic acid is responsible for immunity booster. Phytol is antioxidant and responsible for enhancement of immunity. α and β amyrin responsible for antimicrobial activity. Piperazine, betulin, betulinic acid, lupeol, β-sitosterol shows antimicrobial activity. Cysteine is responsible for wound healing. Histidine shows antioxidant activity. Hydroxy proline promotes collagen synthesis. Lysine is responsible for formation of antibodies. 12,15-Octadecadienoic acid, methyl ester shows antimicrobial activity. Benzoic acid acts as an antiseptic. Ethyl Isoallocholate shows antimicrobial activity. Arachidonic acid promotes wound healing.[12]

III. MATERIALS AND METHOD

3.1. Collection of plant

The leaves of the plant Ehretia laevis Roxb. was collected from Amravati (Maharashtra), India in the month of September 2023.The species of Ehretia laevis Roxb. was identified and authenticated by Dr. Ganesh B. Heda, Head of the Department of Botany, Shri Shivaji Science College, Amravati, Maharashtra, India.

3.2. Extraction of E. Laevis Leaves Roxb.

Soxhlet Extraction Method

Extraction of leaves of E. Laevis leaves Freshly collected leaves were washed by using

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distilled water and ethanol. Washed leaves were shade dried under aseptic condition for 6 to 7 days. As lowering the size of particle increases the surface. The dried leaves were powdered up to the size smaller than 0.5mm. Finely powdered sample (100gm) was placed in a "thimble", and thimble was placed inside the chamber of the Soxhlet. Ethanol (500ml) was then boiled in the flask to vaporize and vapors then condensed into the condenser. When it reaches up to the siphon arm, the condensed solvent dropped back into the RBF of Soxhlet apparatus and then same process was done till complete solvent from the RBF get condensed. Then the solvent was evaporated from an extract at room temperature.

Fig.2: Soxhlet Extraction for E. Laevis Roxb

3.3. Characterization of Ehretia laevis Roxb.Leaves Extract:

Characterization of physical and chemical properties of Ehretia laevis Roxb. leaves extract was performed by –

3.3.1. Phytochemical Screening

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analyses were done using the procedures of (Harborne, 1998) and (Khandelwal, 2005). The following tests were performed on extracts to detect various phyto - constituents present in them

3.3.1.1. Detection of Flavonoid (Alkaline Reagent Test)

To one ml solution of the extract 1 N NaOH solution was added to give yellow color. This Color vanishes after addition of few drops of dil. acid indicating the presence of Flavonoid.

3.3.1.2. Detection of Alkaloids

Solvent free extract, 50 mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows

Dragendroff'sTest

To a few ml of filtrate, 1-2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

3.3.1.3. Detection of Carbohydrates

The extract (100 mg) was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

Benedict's Test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic-colored precipitate indicated the presence of sugar.

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3.3.1.4. Detection of Glycosides

For detection of glycosides, 50 mg of extract was hydrolyzed with concentrated hydrochloric Acid for 2 hrs. On water both, filtered and the hydrolysate was subjected to the following tests.

Keller KillianiTest

The test solution with few drops of glacial acetic acid in 2 ml of 5% FeCl3 and concentration H2SO4 from side of the test tube- Lower layer reddish brown and upper layer (bluish green) Indicates the presence of Glycosides.

3.3.1.5. Detection of Saponins by Foam Test

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of Saponins.

3.3.1.6. Detection of Proteins and Amino Acids

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through what man No.1 filter paper and the filtrate were subjected to tests for proteins and amino acids.

Biuret Test

An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this,1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, pink color in the ethanolic layer indicated the presence of proteins.

3.3.1.7. Detection of Phenolic Compounds and Tannins (Ferric Chloride Test)

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% Ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

3.3.2. Thin Layer Chromatography
Preparation of TLC Plates

Weighed 30 gm of silica gel "G", it was suspended homogenously in 60 ml of distilled water for a duration of two minutes. The slurry was applied to the glass plate, which was allowed to air dry until the layer's transparency vanished. After 30 min of drying at 110OC in a hot air oven, the plates were placed in a dry environment and utilized as needed.

Preparation of Sample Solution

Samples are first dissolved in an appropriate solvent before being applied typically in amounts of 1-10micro liter, to the beginnings of a TLC sheet or plate.

Development of the Chromatogram

Following the sample application on the adsorbent, the TLC plate was placed in the solvent within the TLC glass chamber, allowing the mobile phase to the pass through the adsorbent phase to a depth of approximately ¾ of the plate. The separation occurred. And following spaying with the appropriate detecting agents, the colored dots were obtained. The images of the TLC plates and tabulated results of the determination of the Rf values for the various places are included in the table.

3.4. Chemicals

Ethanol (Variety Traders Amravati), Olive oil (DE oleo India Pvt Ltd), Tween 80 Span 80, Methyl Paraben, Propyl Paraben, Triethanolamine, Carbopol 934(Central Drug House (P) Ltd.), Propylene Glycol (Avantor Performance Materials India Limited).

3.5.Preparation of Emulgels

Emulsion has been prepared by adding the oil phase to the aqueous phase. By dissolving span 80 in light liquid paraffin the oil phase was produced and the aqueous phase was by dissolving Tween 80 in purified water. The extract was then dissolved in water by sonication technique. Methyl paraben and propyl paraben were dissolved in propylene glycol. Both systems were heated to 70-75 0c individually. The oil phase was introduced with constant stirring to the aqueous phase and then cooled at room temperature. The prepared emulsions were mixed in 0.5% to 4% concentrations of polymer (Carbopol 934) with constant stirring in homogenizer for theformulation of Emulgels. (Table) and pH of the Emulgels were adjusted using triethanolamine.

The topical semisolid formulation (Emulgels) is prepared by using the different concentrations of extract of plant leaves and the selected excipients.

1.Three batch of different concentration of the extract are formulated.

2.The concentrations of the extracts are as 2.5%,5%and 7.5% for the 15 gm of topical semisolid preparation (Emulgels).

3.Formulation 1(2.5% of ethanolic extract)

4.Formulation 2(5% of ethanolic extract)

5.Formation 3(7.5% of ethanolic extract)

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Table 1: Formula for 15gm Emulgels formulation

Sr. No	Content	Formulation 1	Formulation 2	Formulation 3
1	E. laevis leaves extract	0.375 gm	0.75 gm	1.125gm
2	Olive oil	1.125ml	1.125ml	1.125ml
3	Tween 80	0.225ml	0.225ml	0.225ml
4	Span 80	0.225ml	0.225ml	0.225ml
5	Methyl Paraben	0.0015ml	0.0015ml	0.0015ml
6	Propyl Paraben	0.00225ml	0.00225ml	0.00225ml
7	Propylene Glycol	1.125ml	1.125ml	1.125ml
8	Triethanolamine (TEA)	0.60ml	0.60ml	0.60ml
9	Carbapol 934	0.225gm	0.225gm	0.225gm
10	Water up to 15 gm	11.09ml	10.715ml	9.96ml

3.5. Evaluation of Emulgel

Following parameters were used for the evaluation of Emulgels:

3.5.1. Appearance

The developed Emulgels were tested for appearance by visual observation. This method describes the visual assessment for clear, transparent. 0.2 g of Emulgels was placed on slide and tested for appearance.

3.5.2. Homogeneity

The developed formulations were tested for homogeneity by visual inspection. The emulgelpreparation applied to a piece of glass then the homogeneity of the preparation was observed.

3.5.3. pH Determination

The pH of emulgel formulations was determined by using digital pH meter. One gram of emulgel was dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3.5.4. Spredability

One of the criteria for a topical formulation to meet the ideal qualities is that it should possess good spreadability. It is the term expressed to denote the extent of area to which formulation readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. To determine the spreadability of formulation, 0.5 g of emulgel was placed on a petri plate, over which a second petri plate was placed. A weight of 50 g was allowed to rest on the upper petri plate for 40 sec. The increase in the diameter due to emulgel spreading was noted. It indicates the extent of the area to which emulgel readily spreads on application to the skin or affected part. The therapeutic potency also depends upon spreading value. The time in sec taken by two slides to slip off from gel which is placed in between the slides under the direction of certain load is expressed as spreadability. Lesser the time taken for the separation of two slides, better the spreadability. The following formula is used to calculate the spreadability:

Spreadability (S) = M × L / T

Where,
M = weight tied to upper slide
L = length of glass slides
T = time taken to separate the slides

3.5.5. Viscosity

The viscosity of the developed emulgel was measured using Brookfield viscometer.

Method development: For the measurement of viscosity of emulgel formulation first we develop a method suitable for formulated emulgel.

The Spindle used are L-1, L-4, S-14, S-16, S-64

The Spindle rpm was set at 50 rpm

The Temperature was set to 27 + 2 0 C Steps: First L-1 spindle taken at 0.5rpm and test a sample if it is not showing torque 80-80% first increase the speed 50rpm until it achieved 80% torque. If the torque not achieved with L-1 spindle, then change the spindle to L-4 and so on. Viscosity of emulgel was measured at 27 + 2 0C with L-4 spindle a 50 rpm. 26 g of gel was taken in a test tube and the L4 spindle was dipped in it and then reading was taken.

3.5.6. Grittiness

All the formulations were evaluated microscopically for the presence of particles if any

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no appreciable particulate matter was seen under light microscope. Hence obviously the gel preparation fulfils the requirement of freedom from particular matter and from grittiness as desired for any topical preparation.

3.5.7. Skin Irritation

Test for irritation was performed on human volunteers. About 1.0 gm of gel was applied on an area of 2 square inch to the back of hand and observed for lesions or irritations

3.6. In Vivo Study:

3.6.1. Wound Healing Activity

3.6.1.1. Experimental Animals

Swiss albino mice (180-200g) were procured from animal house of Smt. Kishoritai Bhoyar College of Pharmacy, Kamtee. Mice were kept in proper cages in proper hygienic conditions and fed with standard pellet diet (VRK Nutritional Solution,) and water ad libitum. All Swiss albino mice were maintained under proper conditions, Room temperature was 26±3°C, relative humidity was 45-55% and light were 12:12 hdark cycle. Proper hygienic conditions were maintained to avoid infection in cages while experiment in animal office. Animal studies had an approval of IAEC of Smt. Kishoritai Bhoyar College of Pharmacy, Kamtee. The project proposal no. was 853/IAC/2023-24/52.

3.6.1.2. Experimental Design

The mice were marked and then separated into 4 groups and in each group, there were 3 mice. The Groups were made as per follows and treated accordingly.

Group I (Control): Applied topically by simple emulgel 0.5 g.

Group II (Batch 1): Applied topically by E. laevis extract of 2.5 % w/w emulgel 0.5 g locally.

Group III (Batch 2): Applied topically by E. laevis extract of 5 % w/w emulgel 0.5 g locally.

Group IV (Batch 3): Applied topically by E. laevis extract of 7.5 % w/w emulgel 0.5 g locally.

3.6.1.3. Excision Wound Model:

Excision wound model was used. The mice were anesthetized by diethyl ether. Hairs from dorsal thoracic region were removed by application of a hair removing cream. Hair removing cream was purchased from medical store. (cosmo silky). Approximate 500 mm2 area was marked by an indelible ink and rubber seal. Then it was cleaned by normal saline water and skin was excised and circular wound was created by cutting throughout the marked area through the skin. The wounded mice were kept in different cages. The prepared emulgel of different fractions (2.5%,5.5%, &7.5%) was applied locally once in a day till the wound was completely recovered. The study of wound was done by marking the wound area on a transparent paper on 1st, 3rd, and 7th day, using a millimeter-scale graph paper.

The % of wound contraction was calculated by given formula.

% of wound contraction = (Wound area on day "0"-n (wound area on days) ×100
(Wound area on day "0")

Where, n = no of days (1st ,3rd and 7th days).

3.6.1.4. Statistical Analysis:

Statistical analysis was done by ANOVA test and then followed by Dunnett's t comparison test. The values were expressed as mean±SEM and $p< 0.05$ as considered as significance.

IV.RESULT AND DISCUSSION

4.1. Characterization of Ehretia laevis Roxb. Leaves Extract

4.1.1. Phytochemical Screening

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The results were observed in the phytochemical test showed below fig 3.

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Fig.3: Phytochemical Screening Test Result of E. Laevis Roxb. Leaves Extract

The different phytochemical tests were performed for the characterization of ethanolic extract of E. laevis Roxb. for its chemical composition. The tests show following results in the table 2.

Table 2. Phytochemical Studies of E. laevis Roxb.

Sr. No.	Test Name	Test for	Result
1	Alkaline reagent test	Flavonoids	+
2	Dragendroff's' Test	Alkaloids	+
3	Fehling's test	Carbohydrates	+
4	Benedict's test	Reducing Sugar	-
5	Killer killiani test	Glycosides	+
6	Foam test	Saponins	+
7	Biuret test	Proteins	-
8	FeCl3 test	Phenolic compounds	+

+ Indicates the Presence of Phytochemical
-Indicates the Absence of Phytochemical

4.1.2. Thin Layer Chromatography

The characterization of ethanolic extract of E. laevis Roxb. Leaves was performed by the TLC.The phytoconstituents which are present in the given extract by the phytochemical screening were confirmed by the TLC.The phytochemicals are identified by the TLC method in the extract of E. laevis Roxb. leaves are as shown in the given table 3.

Table 3: TLC Profile for Ethanolic Extract of E. laevisRoxb.leaves

Sr. No.	Groups	Mobile phase	Detection	Rf Values
1	Flavonoids	Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:0.5:0.5:1 v/v/v/v)	AluminumChloride Reagent	0.44
2	Alkaloids	Tolune: Ethyl Acetic: Diethyl amine (7:2:1 v/v/v)	Dragendroff'sReagent	0.76

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3	Saponins	Chloroforms: Acetic acid: Methanol: Water (6.8:3,2:1.4:0.8 v/v/v/v	Vanillin Sulphuric Acid Reagent	0.53
4	Phenolic Compounds	Cyclohexane: ethyl acetate: formic acid (4:6:1 v/v/v)	Gibb's Reagent	0.22

By the TLC method alkaloids, flavonoids, saponins and the phenolic groups were present in the ethanolic extract of E. laevis extract.

4.2. In Vivo Study of Emulgels

4.2.1. Wound Healing Activity

The result of wound healing activity of the formulated ethanolic extract E. laevis Roxb. Emulgels formulation were showed in the given Table 4.

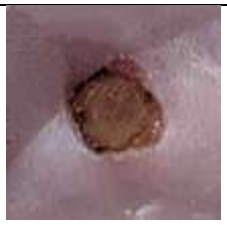
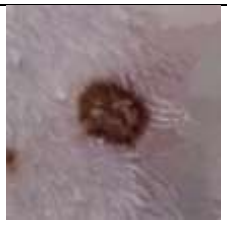
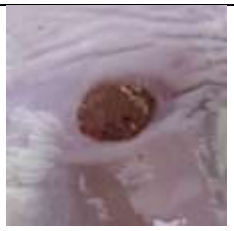
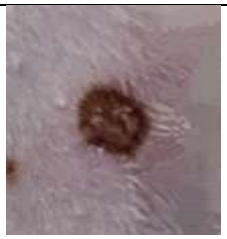
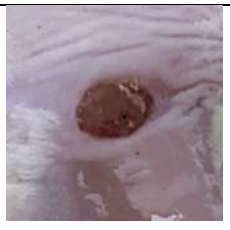
4.2.2. Percentage of Wound Contraction:

Contraction of wound of different groups till the 7th day was calculate & recorded in the Table 5 and. Control groups showed least rate of wound healing (51.38 ± 0.333 %) faster rate of healing was seen in groups treated with 2.5% w/w of ethanolic extract of E. laevis Roxb. emulgels.5% w/w of ethanolic extract of E. laevis Roxbemulgels treated group was 38.11±0.667% and 7.5% w/w of ethanolic extract of E. laevis Roxbemulgels treated group showed 21.78 ±0.822%.

Each value is the mean±SEM, n=6, Batch 1, 2, 3 vs control **P<0.01 on day 1 and day 7. Batch 3 vs control *P<0.05 on day 3 SEM: Standard error of mean.

The Fig 4. showed the % wound contraction of the formulation of different concentration of plant extract of E. laevis Roxb. in which the % contraction of formulation1 had the high-rate wound healing activity according to days of treatment by the Excision wound model method as compared to the other formulation 2 and formulation 3. In this figure 4. The small dotted bar represents the control group, the large black square bar represents the formulation1, the horizontal line bar represents the formulation 2 and the vertical line bar represents the formulation 3. This above fig 4. represents comparison of the % wound contraction in the mice between the different groups of formulations. The * represents the p value between the groups.

Table 4: Result of Wound Healing Activity in Mice

	Control	Batch 1	Batch 2	Batch 3
Day 1				
Day 3				

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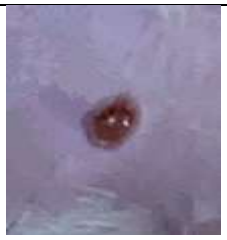
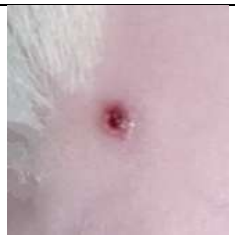
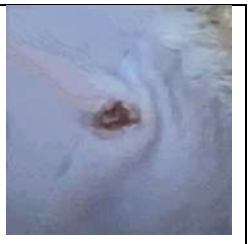
Day 7				

Table 5: Result of Percentage Wound Contraction

Post Wounding Day	Percentage wound Contraction			
	Control	**Batch 1**	**Batch 2**	**Batch 3**
Day 1	9.56±0.033	17.81±0.333	9.52±0.013	9.19±0.333
Day 2	22.78±1.202	30.74±0.667	22.13±0.667	12.18±0.667
Day 3	51.38±0.333	61.61±0.667	38.11±0.667	21.78±0.822

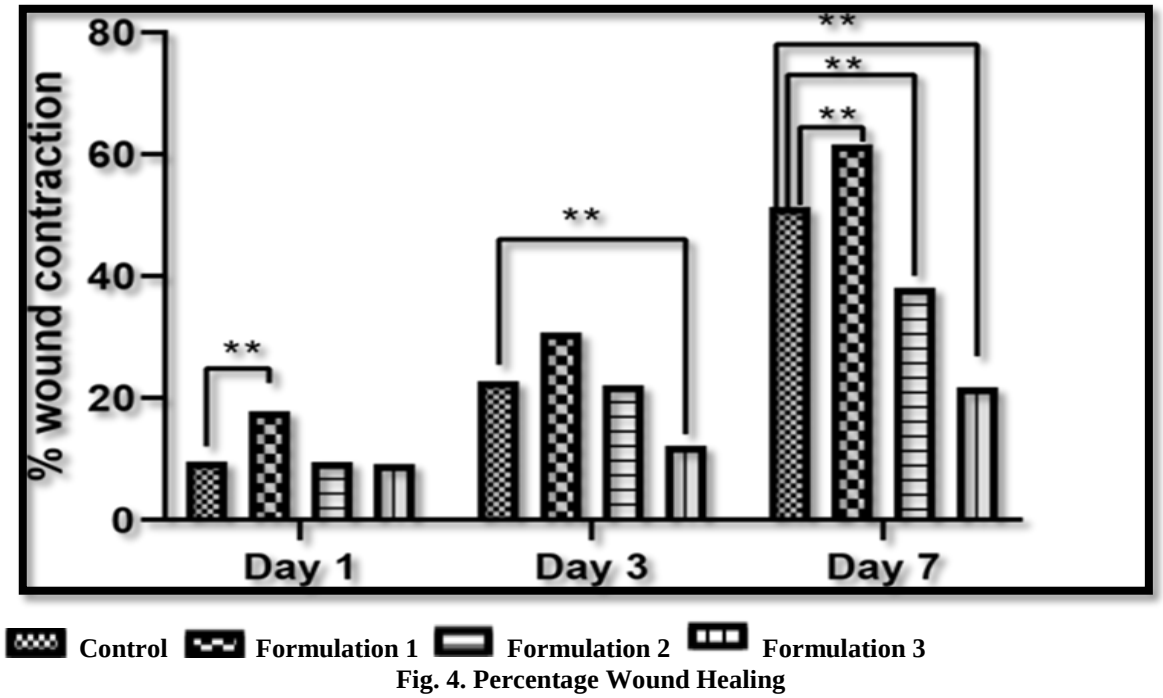


Fig. 4. Percentage Wound Healing

V. CONCLUSION

Topical semisolid formulation of ethanolic extract of E. laevis Roxb. leaves were formulated. In the topical semisolid preparation, we preferred for the Emulgels formulation for the wound healing activity. Three different concentrations of ethanolic extract of E. laevis was used for the Emulgels formulation of three different batch. Emulgels were prepared formulation 1, formulation 2 and formulation 3 (2.5%w/w,5%w/w and 7.5%w/w) using different concentration of extract and were evaluated for appearance, pH, spreadability,

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viscosity, skin irritation, homogeneity and grittiness. These results indicated that the formulations were satisfactory.

The in vivo study of the Emulgels formulation were performed by using the Excision wound healing model with the help of Swiss albino mice. In this excision wound model was performed in 3 groups of 3different concentration of Emulgels. This study revealed that the % contraction of wound healing is higher in the formulation 1 as compared to the formulation 2 and 3. The formulation 1 enhanced the rate of wound contraction. The in vivo study revealed that the developed formulation 1 showed significant wound healing potential in a dose dependent manner compared with untreated group.

This present study has found put the ethanolic leaves extract of Ehretia laevis Roxb. has enhanced the process of wound healing ascertained on the in vivo study of formulated Emulgels formulation. There are not many studies that have experimental with ethanolic extract of E. laevis Roxb. leaves. For the objective assessment for its property of enhanced wound healing.

From the present study, the topical semisolid preparation Emulgels of 2.5 % w/w concentration of ethanolic extract of E. laevis had better wound healing contraction than the other formulated (5%w/w and 7.5%w/w) Emulgels formulation.

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