FORMUALTION AND EVALUATION OF A HERBAL CREAM FOR WOUND HEALING

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In
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CERTIFICATE

This is to certify that the thesis entitled**"Formualtion and Evaluation of a Herbal Cream for Wound Healing"** submitted to Gauhati University for the partial fulfilment of the Master of Pharmacy in Pharmaceutics is a faithful record of bonafide and original research work carried out by **Trailokya Das**, with registration no 048368 of 2007-08& Roll no: MP/11/05 during the academic session 2011-2013 at Pharmaceutics laboratory/Department of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS) under my supervisions and guidance.

I wish him all success in his life.

Signature of Guide
JibanDebnath
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Signature of Guide
Prof (Dr.) Suvakanta Dash
[Principal, GIPS]

DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"Formualtion and Evaluation of a Herbal Cream for Wound Healing"**is a bonafide and genuine research work carried out by me under the joint supervision of ***Jiban Debnath,*** Assistant Professor, and ***Dr.Suvakanta Dash,*** Principal**,** Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

Signature of Student

Trailokya Das

Dedicated to

My Beloved family

Chapter 4

PLANT AND EXCIPIENTS' PROFILE

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

1. PLANT PROFILE:

A. ALOE VERA[53,54,55,56]:

Figure4: Aloe vera Leaves

Scientific Name: Aloe vera, synonyms *Aloe barbadensis*.

Family: Aloaceae or Asphodelaceae.

Common Names: Aloe Capensis, Aloe Latex, Aloe Perfoliata, Aloe Vera Barbenoids, Aloe Vera Gel,Aloes, Barbados Aloe, Burn Plant, Cape Aloe, Chritkumari, Curacao Aloe, Elephant'sGall, Ghee-Kunwar, Ghi-Kuvar, Ghrita-Kumari, GvarPatha, Hsiang-Dan, IndianAloe, Jafarabad Aloe, Kanya, Kumari etc.

Aloe vera (*Aloe barbadensis*) has been used widely to treat external wounds, burns, and skin inflammations. Folk medicine describes the usage of Aloe for an even wider range of conditions, including ulcers, mucoid discharges, dermatitis, fungal infections, anemia, dental pain, and constipation. Scientific research has confirmed Aloe's effectiveness with burns, wounds, frostbite, conjunctivitis, inflammatory bowel disease, digestive disturbances, and several viruses. In addition to being a popular ingredient in first aid products, Aloe also is used in cosmetics and hair care products. Research continues on Aloe's antibacterial, antiseptic, antimicrobial,

antifungal and anti-inflammatory properties, as well as on its effectiveness in treating diabetes, asthma, arthritis, heart disease, and cancer.

Description: Aloe vera, a cactus-like member of the lily family, is a spiky-leafed succulent. While this perennial grows naturally in warm and arid climates, it also is a popular indoor plant due to its wound healing properties. Aloe has short triangular, fleshy leaves with serrated edges that grow from a central base. The fibrous outer part of the leaves protects the softer inner part. This inner layer contains a clear, gel-like sap that may be used topically. The middle of the leaf has a bitter yellow sap containing Anthraquinones that have a laxative effect. Aloe plants may grow six feet tall.

Habitat: The Aloe plant is believed to have originated in Northern Africa and the Middle East. Aloe, mentioned in written records as far back as 1,750 B.C., has been used to treat wounds for thousands of years. Aloe is considered an important medicinal and sacred plant in the Indian Ayurvedic tradition. One of the most frequently prescribed medicines in the U.S. during most of the 18th and 19th centuries, Aloe remains a popular medicinal herb because of its healing properties with skin irritations, burns and wounds. Aloe has long been used in this country to treat colic, indigestion, constipation and urinary ailments in animals. The FDA has approved *Aloevera* as a food flavoring.

Parts use: Leaf.

Constituents: There are over 10 active biological components in Aloe,including:

- Vitamins/Minerals – Vitamins C, A, E, B vitamins, Bcarotene, Calcium, Zinc, Copper, Magnesium, Manganese, and Phosphorous.
- Enzymes – at least five different enzymes have been identified.
- Amino acids – 22 amino acids.

- Gibberellin – a growth factor that assists healing.
- Polysaccharides – including B1-4 and B1-3.

Uses:

- Topically to reduce irritation, inflammation, itching and pain of wounds, burns, sunburn, frostbite, insect bites, and surgical incisions. Follow effective wound cleaning and management protocols *prior* to using Aloe.
- Topically to reduce inflammation and irritation of the external parts of the eye, such as conjunctiva, eyelid edges, cornea, and lachrymal sac. Treats conjunctivitis and keratitis. Consult with veterinarian.
- Stimulates the immune system when given orally.
- Internally to heal minor injuries, irritations, and inflammations of the digestive tract with a very small dose of Aloe vera administered orally. Strive to eliminate cause of gastrointestinal problems, such as wrong diet, overfeeding, and parasites.
- Injections of acemannanis, a compound in Aloe, are approved for feline leukemia.

B. PANAX GINSENG[57,58,59]:

Figure 5: *Panax ginseng* root & leaves

Scientific name:*Panax ginseng* .

Family: Araliaceae.

Common names: American ginseng, Asiatic ginseng, Chinese ginseng, five-fingers, Japanese ginseng, jintsam, Korean ginseng, ninjin, Oriental ginseng, schinsent, seng and sang, tartar root, Western ginseng.

Descriptions: *Panax ginseng* belongs to the Araliaceae family and is found throughout East Asia and Russia. It grows natively in remote forests of Manchuria and North Korea, but has become overharvested in other parts of Asia. It is cultivated in Korea, China, and Japan for export and use as a medicinal herb.*Panax ginseng* is a shade-loving, deciduous perennial with five-fingered leaves, tiny white flowers, red berries, and a yellowish-brown root.The root is utilized medicinally, although active compounds are present in all other parts of the plant. The root of *Panax ginseng* is a thick structure that resembles a human-like form, which is responsible for its name in Chinese, jenshen, or "man-root."Panax is derived from the Latin word panacea, which refers to its historical usage for many conditions. There are two distinct forms of *Panax ginseng*, red and white ginseng.

Habitat: Ginseng favors cool, well-drained soils of rich, moist deciduous woods. It may also be found on rocky talus slopes. Among the specific habitats in Massachusetts are a variety of rocky habitats, including the tops of ledges, rocky talus slopes and jumbles, and rocky rich mesic woods; along a creek at the base of a fern-covered slope; and various rich mesic forest habitats, including ones at the base of a dolomitic limestone ledge and one in a ravine. None of the current sites is in full sun.

Parts use: Roots.

Constituents: Panax ginseng contains triterpene glycosides, or saponins, commonly referred to as ginsenosides. Many activecompounds can be found in all parts of the plant, including amino acids, alkaloids, phenols, proteins, polypeptides, andvitamins B1 and B2.

Use: It has been used to increase physical endurance and lessen fatigue, to improve the ability to cope with stress, and to improve concentration. It is also used foranemia, diabetes, gastritis, neurasthenia, erectile dysfunction, impotence and male fertility, fever, hangover, and asthma. *Panax ginseng* is also used for bleeding disorders, loss of appetite, vomiting, colitis, dysentery, cancer, insomnia, neuralgia, rheumatism, dizziness, headache, convulsions, disorders of pregnancy and childbirth, hot flashes due to menopause, and to slow the aging process. It may also improve your overall being.

C. ARNICA MONTANA[60,61]:

Figure 6: ***Arnica montana***

Scientific name:*Arnica montana.*

Family: Asteraceae.

Common names: Leopard`s Bane, European Arnica, Mountain Tobacco, Wolfsbane, Mountain snuff.

Habitat: Moist, upland meadows of the cooler parts of Europe, a plant of hills in Central Europe. It extends through Russia to Siberia. It is also found sparsely in north western parts of the U.S.

Parts use: Whole plant including the root.

Description: A perennial herb with a creeping, slender, blackish rhizome, 2-5 cm long, 5cm thick. Leaves ovate, sessile, opposite; Stem 25-30 cm high, erect, rough, striated; Flowers yellow, numerous, with tubular corolla with 5 spreading teeth. Blooms in July & August.

Constituents: Sesquiterpene lactones, flavonoids, volatile oil, mucilage and polysaccharides, tannins, misc. substances. Other constituents are Tussilagine, Isotussilagine, Arnicin, HelenalinDihydrohelenalin etc. The roots contain derivatives of thymol.

Use: External :

a)Arnica prevents inflammatory swellings,and relieves the soreness of myalgia and the effects of sprains, bruises, and contusions.

b) It is often serviceable to remove ecchymoses, and it gives grateful relief to sore muscles that have undergone much strain and exertion.

Internal:

a. It has a pronounced action upon the medulla and spinal cord. The keynote for arnica is spinal and vagal enervation. It is brought into service when there is deficient nervous response, sluggish vascular power, and in almost all conditions in which prevails the triad-torpor, debility, and depressed function.

b. In the advanced stages of exhausting diseases, where spinal innervation is poor, control over the sphincters lost, and there is feeble respiration due to central vagal impairment, it is a most important stimulant.

c. It is used when breathing is carried on chiefly only by force of the will, and becomes weak and shallow when the patient drops into sleep; or when the sleeper awakens with a start on account of dyspnoea when automatic respiratory action alone is depended upon. Such a state occurs in the low stage of typhoid and other fevers, and in lobar pneumonia.

d. Arnica will prove useful in the depression occasioned by extreme forms of diarrhoea and dysentery when the discharges escape control.

e. During mild forms of chronic rheumatism, with cold skin and general debility it will stimulate the nervous system, restore normal warmth, re-establish restrained secretion, and thus relieve pain.

f. In painful, bruised or subacute inflammatory disorders arising from injury, with marked lowering of nerve tone, muscular aching and chilly sensations.

D. **CALENDULA OFFICINALIS**[62,63,64,65]**:**

Figure 7: *Calendula officinalis*

Scientific name: *Calendula officinalis.*

Family:Asteraceae.

Common names: Calendula, field marigold, garden marigold, goldbloom, holligold, maravilla, marybud, marygold, pot marigold, Ringelblumen(Ger).

Habitat: Calendula is native to Mediterranean region. Cultivated by the Egyptians, Greeks, Hindus and Arabs, calendula grew in European gardens. . It is probably native to southern Europe, though its long history of cultivation makes its precise origin unknown, and it may possibly be of garden origin. Calendula officinalis is widely cultivated and can be grown easily in sunny locations in most kinds of soils. Although perennial, it is commonly treated as an annual, particularly in colder regions where its winter survival is poor, or in hot summer locations where it also does not survive.Calendula are considered by many gardening experts as among the easiest and most versatile flowers to grow in a garden, especially since they tolerate most soils.

Parts use: Flowers, leaves.

Description: It is a short-lived aromatic herbaceous perennial, growing to 80 cm (31 in) tall, with sparsely branched lax or erect stems. The leaves are oblong-lanceolate, 5–17 cm (2–7 in) long, hairy on both sides, and with margins entire or occasionally waved or weakly toothed. The inflorescences are yellow, comprising a thick capitulum or flowerhead 4–7 cm diameter surrounded by two rows of hairy bracts; in the wild plant they have a single ring of ray florets surrounding the central disc florets. The disc florets are tubular and hermaphrodite, and generally of a more intense orange-yellow colour than the female, tridentate, peripheral ray florets. The

flowers may appear all year long where conditions are suitable. The fruit is a thorny curved achene.The flower heads range from pastel yellow to deep orange, and are 3–7 cm across, with both ray florets and disc florets. Most cultivars have a spicy aroma. It is recommended to deadhead (removal of dying flower heads) the plants regularly to maintain even blossom production.

Constituents:

The petals and pollen of *Calendula officinalis* contain triterpenoid esters and the carotenoids flavoxanthin and auroxanthin (antioxidants, and the source of theyellow-orange coloration). The leaves and stems contain other carotenoids,mostly lutein (80%) and zeaxanthin (5%), and beta-carotene.

Plant extracts are also widely used by cosmetics, presumably due to presence of compounds such as saponins, resins and essential oils. The chemical composition of *Calendula officinalis*flowers comprises variousclasses of active biologic compounds: glicosides of triterpeniquesaponins, (namedcalenduloside) , flavonoids, sterols (colestanol, campestanol, stigmasterol, sitosterol,, neutral lipids (which contain C12-C22 fatty acids chain, especially lauric, miristic,palmitic acids, as well as polyunsaturated fatty acids), carotenoids (β-carotene), sacharides(water soluble polysacharides, pectic substances, hemicelulose).

Use: Marigold flowers (*Calendula officinalis*) often used in food industry for their nutritive qualities as well as for coloring of several culinary products, is well known of centuries in popular medicine because of their biologic properties: anti-inflammatory, diuretic, vessel dilatators activities. It is used for in gastro-intestinal, gynecological, eye diseases, skin injuries, burns, and swellings. Their use for the preparation of cosmeticproducts is well known also.

E. CLERODENDRUM INDICUM[66]:

Figure 8: Clerodendrum Indicum

Scientific name: *Clerodendrum indicum.*

Family: Verbenaceae.

Common names: Tube- flower and Skyrocket (in English), Bharangi (in Hindi), Bharngi in Sanskrit, and Bharangi in Guajarati, Akal bish (Assamese, Bangali).

Habitat: It is generally found in the area that is about 4000 feet in height. It is more commonly found in areas with moderate temperature. It is also found in temperate zone Tube flower is reported to be native to the Malay Archipelago. However it is reported to be native of many other parts of the world too. In India it is found in the eastern region of Himalaya like Kumaun, Assam, Bengal, Bihar and regions of Bihar, Delhi, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Mani Pur, Orissa, Punjab, Sikkim, Tamil Nadu, Tripura, and Uttar Pradesh etc regions. It is also found in Bhutan, Nepal, Srilanka, Indo-China, Cambodia, Laos, Myanmar, Thailand, Malesia, Philippines etc. It is widely cultivated as an ornamental plant and has become naturalized in South America, the West Indies and much of the southern U.S., where it grows in disturbed sites and especially along road shoulders.

Parts use: Leaves, bark, root.

Description: It has a perennial bushy plant that attains a height of 2-8 feet in height. Stem is hollow. Leaves are 3-6 inch in length and 2 to 3 inch in breadth. These are rough having dentate at the edges, spear shaped. Leaves are borne in whorls and have short petioles. The inflorescence is large and bears tubular flowers that bear white or bluish colored flowers and it is about 2 feet in length and one inch broad. These flowers have good odor. The tubes of the flower are about 8 centimeter in length that droops down. Fruit is circular and has a height of ¼ inch in length and 1/6 to ½ inch broad. It is pulpy and when it ripe, it changes to dark purple to black in color. The flowering and fruiting of this plant has been reported to occur during summer and rainy seasons.

Constituents: The bark of the root contains phenolic glycoside and sepogenin as active ingredient. Sepogenin is very helpful as an anti- histamine agent and it is very much effective in preventing the bodies or over active reaction of the body towards any external agent entering the body. The sapogenin mixture contains three major triterpenoidconstitutent'soleonolic acid, queretaroic acid and serratagenic acid. The powdered stem contains D- mannitol, D- glucoside of sitosterol, sitosterol and acetyl alcohol. Alcoholic extract and saponin isolated from root bark caused release of histamine from lung tissue.

Use: It is useful in asthma, cough, fever, worms, burning sensation of the body and wounds.

The roots and leaves of bharngi have great medicinal value. The plant is useful, both, internally as well as externally. The leaves are useful as an external application for cephalalgia and ophthalmia. The pulp of the leaves applied externally, mitigates the glandular swellings and hastens the wound healing. The juice of its leaves is applied on the lesions in erysipelas. The root paste applied on the forehead alleviates

headache. Internally bharngi is used in vast range of diseases. It is an appetizer, lacative and digests ama, hence is beneficial in anorexia, tumours and distaste. The plant works well as a blood purifier.

F. ROSE HIP OIL[67]:

Figure 9: Rose hip oil

Scientific Name:*Rosamoschata, Rosa canina.*

Family: Rosaceae.

Common name: RosaMosqueta, dog rose.

Habitat: It is found in cool, rainy, mountainous region of southern Chile, Europe (Hungary).

Parts Used: Seeds.

Description: The aggregate fruit of dog rose has long been used for medical purposes. The weight of a rose hip ranges from 1.25 to 3.25g from which 71% constitutes the pericarp and approximately 29% the seed. Generally, the fruit and its pericarp is commercially available. The largest rose hip plantation of central Europe is found in Hungary where production ranges from 60-70 ton of rose hips annually. During processing, seeds are crushed and used for animal nutrition or burned.

Constituents: AlphaLinolenic Acid (Omega 3, ALA), Linoleic Acid (Omega 6, LA), Oleic Acid (Omega 9), Vitamin A, Vitamin C.

Use: Scientific studies confirm that Rose Hip Oil is highly effective in the treatment of Surgery, Minor burns, Stretch marks, Acne, Sun exposure.

Rosehip oil offers ease of spreading, great penetration and significant moisture retention. It is very effective in halting and even reversing the effects of sun radiationontheskin.Ingeneral,itisusefulinallkinds of cell regeneration face treatments, pore reduction and acne treatments.

2.1 EXCIPIENTS' PROFILE:

A. Propylene Glycol

i. ***Synonyms***: 1,2-Dihydroxypropane; E1520; 2-hydroxypropanol; methyl ethylene glycol; methyl glycol; propane-1,2-diol; propylene glycolum.

ii. ***Empirical Formula:*** $C_3H_8O_2$

iii. ***Molecular Weight:*** 76.09

iv. ***Functional Category***: Antimicrobial preservative; disinfectant; humectant; plasticizer; solvent; stabilizing agent; water-miscible cosolvent

v. ***Description:*** Propylene glycol is a clear, colorless, viscous, practically odorless liquid, with a sweet, slightly acrid taste resembling that of glycerin.

vi. Typical Properties

Boiling point 188^0C

Density 1.038 g/cm3 at 20^0C

Melting point -59^0C

Osmolarity: A 2.0% v/v aqueous solution is iso-osmotic with serum.

Solubility: Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble at 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils, but will dissolve some essential oils.

vii. ***Stability and Storage Conditions***: At cool temperatures, propylene glycol is stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid.

B. Triethanolamine

i. ***Synonyms***: TEA; Tealan; triethylolamine; trihydroxytriethylamine, trolaminum.

ii. ***EmpiricalFormula***: C6H15NO3

iii. ***MolecularWeight***: 149.19

iv. ***FunctionalCategory***: Alkalizing agent; emulsifying agent.

v. ***Description***: Triethanolamine is a clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacalodor.

vi. ***TypicalProperties***:

Acidity/alkalinity pH = 10.5 (0.1N solution)

Boiling point: 335ºC

Flash point: 208ºC

Freezing point: 21.6ºC

Hygroscopicity: Very hygroscopic.

Melting point 20–21ºC

Moisture content 0.09%

vii. ***Stability and Storage Conditions:***Triethanolamine may turn brown on exposure to air and light.

C. Tween60

i. ***Synonym***: Polysorbate 60, Atlas Armotan PMS 20; Capmul POE-S; Cremophor PS 60; Crillet 3; Drewpone 60K; Durfax 60; Durfax 60K, Liposorb S-20K; Lonzest SMS-20; Montanox 60; Nikkol TS-10; Norfox SorboT-60

ii. ***Empirical Formula***: $C_{64}H_{126}O_{26}$

iii. ***Molecular Weight:*** 1312

iv. ***Functional Category:*** Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.

v. ***Description***: Polysorbates have a characteristic odor and a warm, somewhat bitter taste.

vi. ***Incompatibilities***: Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials.

D. White petrolatum

i. ***Synonyms***: mineral jelly; petroleum jelly; Silkolene; Snow White, vaselinumflavum; yellow petrolatum

ii. ***EmpiricalFormula***: Petrolatum is a purified mixture of semisolid saturated hydrocarbons having the general formula C_nH_{2n+2}, and is obtained from petroleum.

iii. ***Functional Category***: Emollient; ointment base.

iv. ***Applications in Pharmaceutical Formulation***: Petrolatum is mainly used in topical pharmaceutical formulations as an emollient-ointment base; it is poorly absorbed by the skin. Petrolatum is also used in creams and transdermal formulations and as an ingredient in lubricant formulations for medicated confectionery together with mineral oil.

v. ***Description***: Petrolatum is a pale yellow to yellow-coloured, translucent, soft unctuous mass. It is odourless, tasteless, and not more than slightly fluorescent by daylight, even when melted.

vi. Typical Properties:

Solubility: Practically insoluble in acetone, ethanol, hot or cold ethanol (95%), glycerin, and water; soluble in benzene

Viscosity (dynamic): The rheological properties of petrolatum are determined by the ratio of the unbranched chains to the branched chains and cyclic components of the mixture.

vii. ***Stability and Storage Conditions:*** Petrolatum is an inherently stable material owing to the unreactive nature of its hydrocarbon components; most stability problems occur because of the presence of small quantities of impurities. On exposure to light, these impurities may be oxidized to discolour the petrolatum and produce an undesirable odour.

E. Lanolin

i. ***Synonyms***: Adepslanae; ceralanae; E913; lanolina; lanolin anhydrous

ii. ***Functional Category***: Emulsifying agent; ointment base

iii. ***Applications in Pharmaceutical Formulation***: Lanolin is widely used in topical pharmaceutical formulations and cosmetics. Lanolin may be used as a hydrophobic vehicle and in the preparation of water-in-oil creams and ointments.

iv. ***Description***: Lanolin is a pale yellow-coloured, unctuous, waxy substance with a faint, characteristic odour. Melted lanolin is a clear or almost clear, yellow liquid.

v. Typical Properties:

Density 0.932–0.945 g/cm3 at 15^0C

Flash point 238ºC

Refractive index n_D^{40} = 1.478–1.482

vi. ***Stability and Storage Conditions:*** Lanolin may gradually undergo autoxidation during storage. To inhibit this process, the inclusion of butylatedhydroxytoluene is permitted as an antioxidant.

F. Stearic Acid

i. ***Synonyms***: Acidumstearicum; cetylacetic acid; Crodacid; Cristal G; Cristal S Dervacid; E570; Edenor; Emersol; Extra AS; Extra P; Extra S.

ii. ***Empirical Formula***: C18H36O2

iii. ***Molecular Weight:*** 284.47 (for pure material)

iv. ***Functional Category***: Emulsifying agent; solubilizing agent; tablet and capsule lubricant.

v. ***Applications in Pharmaceutical Formulation***: Stearic acid is widely used in oral and topical pharmaceutical formulations. It is mainly used in oral formulations as a tablet and capsule lubricant. It may also be used as a binder or in combination with shellac as a tablet coating. It has also been suggested that stearic acid may be used in enteric tablet coatings and as a sustained-release drug carrier.

vi. ***Description***: Stearic acid is a hard, white or faintly yellow-coloured, somewhat glossy, crystalline solid or a white or yellowish white powder. It has a slight odour (with an odour threshold of 20 ppm) and taste suggesting tallow.

vii. ***Stability and Storage Conditions***: Stearic acid is a stable material; an antioxidant may also be added to it. The bulk material should be stored in a well closed container in a cool, dry place.

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Trailokya Das

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORMS
rpm	Revolution per minute
mg	Miligram
Hrs	Hours
Min	Minutes
Sec	Seconds
w/w	Weight/ Weight
µg/mc	Microgram
^{0}C	Degree centigrade
%	Percentage
I.P.	Indian Pharmacopoeia
U.S.	United states of Pharmacopoeia
B.P.	British Pharmacopoeia
PhEup	European Pharmacopoeia
USP/NF	National Formulary, United states of Pharmacopoeia
SD	Standard Deviation
bw	Body weight
HE	Hetatoxyline eosine
Cps	Centipoise

Chapter 1

INTRODUCTION

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

1.1. WOUND HEALING:

1.1.1. DEFINITION OF WOUND [1,2]:

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue.

Wound healing is a complex but generally orderly process. Sequential waves of specialized cell types first clear the inciting injury and then progressively build the scaffolding to fill in any resulting defect.

Injury to tissue may result in cell death and tissue destruction. Healing on the other hand is the body response to injury in an attempt to restore normal structure and function. The process of healing involves 2 distinct processes.

a. Regeneration is the replacement of lost specialized tissue by proliferation of surrounding undamaged same tissue. For example regeneration of bone and cartilage.
b. Repair is the replacement of lost tissue by granulation tissue, which matures to form scar tissue repair of muscle and skin wounds.

Wound may be produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight.

1.1.2. CLASSIFICATION OF WOUND [1,2]:

Based on underlying cause of wound creation, wounds are classified as open and closed wounds.

A. Open wounds

In this class, bleeding is clearly visible as blood escapes from the body. It is further classified as: Incised wounds, Laceration wounds, Abrasions or superficial wounds, Puncture wounds, gunshot wounds and penetration wounds.

B. Closed wounds

In closed wounds, blood escapes from circulatory system but remains in the body. It involves Contusion or bruises, hematomas or blood tumour, Crush injury etc.

On the basis of physiology of wound healing, wounds are classified as acute and chronic wounds.

A. Acute wounds

Acute wound is an injury to tissue that normally precedes through an orderly and timely reparative process those results in sustained restoration of anatomic and functional integrity. Acute wounds are generally caused by cuts or surgical incisions and complete the wound healing process within the expected time frame.

B. Chronic wounds

Chronic wounds are those wounds that fail to progress through the normal stages of healing and therefore enter into a state of pathologic inflammation. These wounds either require a prolonged time to heal or recur frequently. Most common frequent causes of chronic wounds are Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, malnutrition, immunodeficiency or medications.

1.1.3. PATHOLOGY OF WOUNDS [1,2,3]:

Wounds are physical injuries that results in an opening or breaking of the skin. Proper healing of wounds is very essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Healing is a complex process

initiated in response to an injury that restores integrity and function of damaged tissues of skin. Wound healing involves continuous interactions between cell–cell and cell–matrix that allow the process to proceed in three overlapping phases viz. inflammatory phase(0–3days), cellular proliferation or proliferative phase (3–12 days) and remodelling phase (3–6 months)[1] . Healing requires the collaborative efforts of various tissues and cell lineages. It involves aggregation of platelets, clotting of blood, fibrin formation, and an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. Healing process is not complete until the disrupted surfaces are firmly knit by collagen. The basic principle behind optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition to tissue and moist wound healing environment to restore the anatomical continuity and function of the wound. Cutaneous wound healing is accompanied by sequence of biological events starting with wound closure and progressing to the repair and remodelling of damaged tissue in an order. In spite of tremendous advances in the pharmaceutical industry, the availability of drugs capable of stimulating wound repair processes is still limited. Moreover, due to high cost therapy and presence of unwanted side effects, management of chronic wounds is another major problem. It is agreed that reactive oxygen species (ROS) are deleterious to wound healing process, as they show harmful effects on cells and tissues. Absorbable synthetic biomaterials are considered to be degraded via ROS. Cytoprotective enzymatic group of enzymes called Free-radical-scavenging enzymes (FRSE) plays an essential role in the reduction, de-activation and removal of ROS as well as in regulation of wound healing process. Inflammation, which is a part of acute response, results in a coordinated influx of neutrophils at the site of wound. These cells produce free radicals through their characteristic

"respiratory burst" activity. Free radicals are also generated by wound related non-phagocytic cells by non-phagocytic NAD (P) H oxidase mechanism. Thus, the wound site has rich concentration of both oxygen and nitrogen centered reactive species along with their derivatives. These radicals results in oxidative stress leading to lipid peroxidation, breakage of DNA, and enzyme inactivation, including free-radical scavenger enzymes. Evidence for the role of oxidants in the pathogenesis of many diseases suggests that antioxidants may be of therapeutic use in these conditions. Application of compounds topically with free-radical-scavenging properties in patients has shown to improve wound healing processes significantly and protect tissues from oxidative damage.

1.1.4. MECHANISM OF WOUND HEALING [3,4]:

The response to injury caused either surgically or traumatically, is immediate and the damaged tissue or wound then passes through four phases in order to affect a final repair.

A. First phase

It involves a brief and transient period of vasoconstriction and haemostasis. A 5-10 minute period of intense vasoconstriction is followed by active vasodilatation accompanied by an increase in capillary permeability. Aggregated platelets within a fibrin clot secrete a variety of growth factors and cytokines that set the stage for an orderly series of events leading to tissue repair.

B. Inflammatory phase:

The second phase of wound healing, the inflammatory phase, usually lasts between 24 and 48hrs and in some cases persists for about 2 weeks presents itself as erythema, swelling, and warmth, and is often associated with pain. The response increases vascular permeability, resulting in migration of neutrophils and monocytes into the

surrounding tissue. First line of defence against infection is provided by neutrophils, engulfs cell debris and microorganisms. Migration of neutrophils ceases after the first few days of post-injury if the wound is not contaminated. Acute Inflammatory Phase persists due to wound hypoxia, infection, nutritional deficiencies, medication use, or other factors related to the patient's immune response, it can interfere with the late inflammatory phase. In the late inflammatory phase, monocytes converted to macrophages in the tissues, which digest and kill bacterial pathogens, scavenge tissue debris and destroy remaining neutrophils. Transition from wound inflammation to wound repair is the function of macrophages that secretes a variety of chemo tactic and growth factors that stimulate cell migration, proliferation, and formation of the tissue matrix.

C. Proliferative phase:

The subsequent proliferative phase lasts for about 2 days to 3 weeks after the inflammatory phase, dominated by the formation of granulation tissue and epithelialization. Its duration also depends up on the size of the wound. Chemo tactic and growth factors released from macrophages and platelets stimulate the migration and activation of wound fibroblasts that produce a variety of substances essential to wound repair, including glycosaminoglycans (hyaluronic acid, chondroitin- 4-sulfate, dermatansulfate, and heparin sulfate) and collagen. These form an amorphous, gel-like connective tissue matrix necessary for migration of cells. This phase also includes contraction of wound edges pull together to reduce the defects in the third step epithelial tissues are formed over the wound site.

D. The Remodelling phase

This phase lasts for 3 weeks to 2 years. It involves formation of new collagen. The amount of collagen secreted determines the tensile strength of the wound. Tissue

tensile strength is increased due to intermolecular cross-linking of collagen via vitamin-C dependent hydroxylation. Improper cross-linkage of collagen fibers has been responsible for nonspecific post-operative bleeding in patients with normal coagulation parameters .The scar flattens and scar tissues become 80% as strong as the original.

The various phases of wound healing are sequentially depicted in the following flowchart:

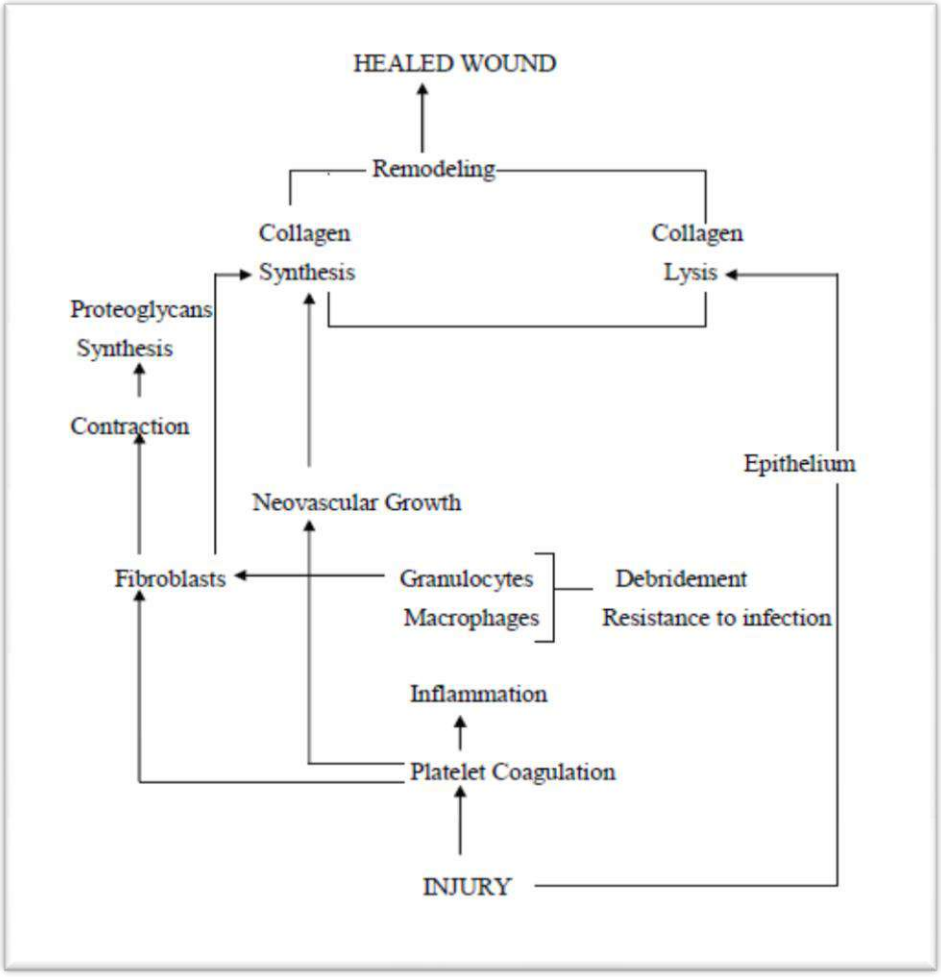

FIG 1: Process flowchart of wound-healing.

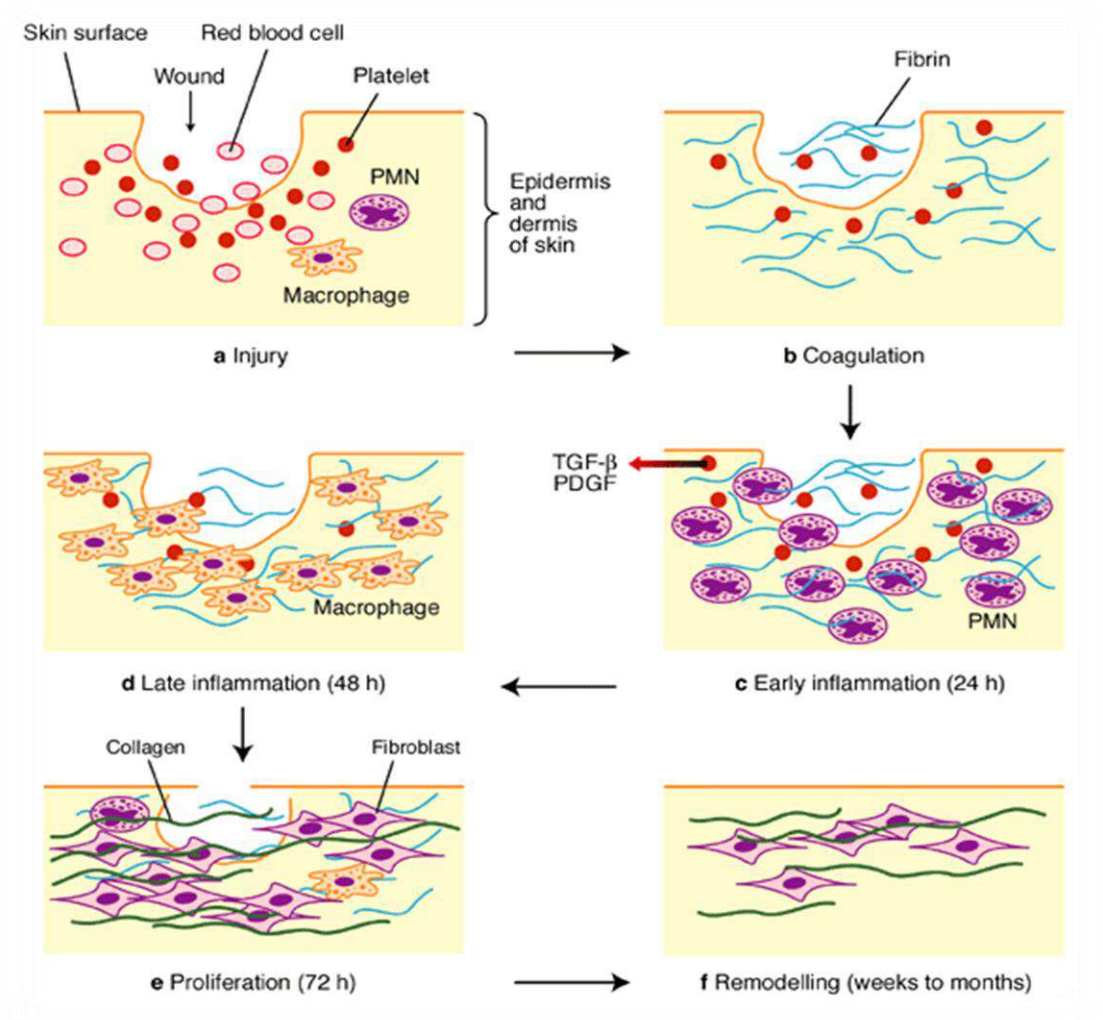

FIG 2: The phases of cutaneous wound healing

1.1.5. CONTRACTION OF WOUNDS[4,5,6,7]:

The wound starts contracting after 2-3 days and the process is completed by the 14th day. During this period, the wound is reduced by approximately 80% of its original size. Contracted wound results in rapid healing since lesser surface area of the injured tissue has to be replaced.

Mechanism of action of wound contraction:

1. Dehydration as a result of removal of fluid by drying of wound was first suggested but without being substantiated.

2. Contraction of collagen was thought to be responsible for contraction but wound contraction proceeds at a stage when the collagen content of granulation tissue is very small.
3. Discovery of myofibroblasts appearing in active granulation in active granulation tissue has resolved the controversery surrounding the mechanism of wound contraction. These cells have features intermediate between those of fibroblasts and smooth muscle cells. Their migration into the wound area and their active contraction decreases the size of the defect. The evidences in support of this concept are both morphological as well as functional characteristics of modified fibroblasts or myofibroblasts as under-

   i) Fibrils present in the cytoplasm of these cells resemble those seen in smooth muscle cells.

   ii) These cells contain actin myosin similar to that found in non –striated muscle cells.

   iii) The nuclei of these cells have infoldings of nuclear membrane like in smooth muscle cells.

   iv) These cytoplasm’s have basement membrane and desmosomes which are not seen in ordinary fibroblasts.

   v) The cytoplasm of these modified cells demonstrates immune-fluorescent labeling with anti-smooth muscle antibodies.

   vi) The drug response of granulation tissue is similar to that of smooth muscle.

1.1.6. FACTORS INFLUENCING HEALING[7,8,9,10,11]:

Two types of factors influence the wound healing: those acting locally, and those acting in general.

A. Local factures. These include the following factors

1. Infection is the most important factor acting locally which delays the process of healing.
2. Poor blood supply to wound d slows healing e.g. injuries to face heal quickly due to rich blood supply while injury to leg with varicose ulcers having poor blood supply heals slowly.
3. Foreign bodies including sutures with healing and cause intense inflammatory reaction and infection.
4. Movement to ionizing radiation delays granulation tissue formation.
5. Exposure to ultraviolet light facilitates healing.
6. Exposure to ionizing radiation delays granulation tissue formation.
7. Type, size and location of injury determine whether healing takes place by resolution or organization.

B. Systemic factors. These are as under.

1. Age. Wound healing is rapid in young and somewhat slow in aged and debilitated people due to poor blood supply to the injury area in the latter.
2. Nutrition Deficiency of constituents like protein, Vitamin C and zinc delays the wound healing.
3. Systemic infection delays wound healing.
4. Administration of gluco-corticoids has anti-inflammatory effect.

5. Uncontrolled diabetics are more prone to develop infections and hence delay in healing.

Hematologic abnormalities like defect of neutrophils functions, and neutropenia and bleeding disorder slow the process of wound healing.

1.2. CREAM:

1.2.1. DEFINITION OF CREAM[12,13]:

Cream is defined as semisolid emulsions which are oil in-water (o/w) or water- in- oil (w/o) type and these semisolid emulsions are intended for external applications.

Creams are often composed of two phases. Oil-in-water (o/w) emulsions are most useful as water-washable bases, whereas water-in-oil (w/o) emulsions are emollient and cleansing agents. The active ingredient is often dissolved in one or both phases, thus creating a three-phase system. Patients often prefer a o/w cream to an ointment because the cream spreads more readily, is less greasy, and the evaporating water soothes the inflamed tissue. O/W creams (vanishing creams) rub into the skin; the continuous phase evaporates and increases the concentration of a water-soluble drug in the adhering film. The concentration gradient for drug across the stratum corneum therefore increases, promoting percutaneous absorption[14]

Creams are basically ointments which are made less greasy by incorporation of water. Presence of water in creams makes them act as emulsions and therefore is sometimes referred as semisolid emulsions. Hydrophilic creams contain large amounts of water in their external phase (e.g., vanishing cream) and hydrophobic creams contain water in the internal phase (e.g., cold cream). An emulsifying agent is used to disperse the aqueous phase in the oily phase or vice versa. As with ointments, creams are formulated to provide protective, emollient actions or deliver drugs to surface or interior layers of skin, rectum, and vagina. Creams are softer than ointments and are

preferred because of their easy removal from containers and good spreadability over the absorption site[15].

1.2.2. TYPES OF CREAM[16,17,18,]:

1. *Hydrophilic creams (Oil-in-Water creams)* contain large amounts of water in their external phase. Example: Vanishing cream.

2. *Hydrophobic creams (Water-in-Oil creams)* contain water in the internal phase. Example: Cold cream.

Types of creams depending on formulation are as follows-

i. *Sterol Creams*: They are water in oil emulsions where emulgent is wool fat or wool alcohol. Classical example is lanolin.

ii. *Soap Creams:*Triethanolamine Creams are neutral soaps, produces o/w emulsion with oleic acid and triethanolamine (good emulsifying agents gents for liquid paraffin).

iii. *Anionic Emulsifying Wax Creams*: These emulsifiers produce oil in water type.

iv. *Cationic Emulsifying Wax Creams:* These emulsifiers produce water in oil type.

v. *Creams Emulsified with Non-ionic Surfactants*: Cream bases prepared with Self emulsifying monostearin, a sorbitan ester, a macrogol ester, a non emulsifying wax containing a macrogol ether etc.

vi. *Divalent Creams*: Classical example is Lime creams which is of water in oil type. Emulgent in these is Oleic acid and Calcium hydroxide.

vii. *Vanishing Creams*: They are oil in water type creams which when rubbed onto the skin and disappear with little or no trace of their former presence.

1.2.3. FORMULATION COMPONENTS OF CREAM:

1.2.3.1.Bases used in the cream[19,20]:

Bases are the Major classes or types of formulation compositions based on composition and physical properties. Bases are classified based on their composition and physical characteristics. The U.S. Pharmacopeia (USP) classifies ointment bases as:

i. Hydrocarbon bases ,
ii. Absorption bases,
iii. Water - removable bases,
iv. Water - soluble bases (water -miscible bases).

i. **Hydrocarbon bases:**

Hydrocarbon bases are Also known as oleaginous bases, the hydrocarbon bases are essentially water-free, incorporating aqueous preparations only in small amounts and with considerable difficulty. The primary features of this type of base include its emollient effect, retention on the skin for prolonged periods, prevention of escape of moisture from the skin to the atmosphere, and difficulty in washing off. They act as occlusive dressings, thus increasing skin hydration by reducing the rate of loss of surface water. Also, they do not dry out or change noticeably on aging. Hydrocarbon-based semisolids comprise fluid hydrocarbons C16 to C30 straight chain and branched, entrapped in a fine crystalline matrix of yet higher-molecular-weight solid hydrocarbons. The high-molecular-weight fraction precipitates out substantially above room temperature, forming interlocking crystallites. The extent and specific nature of this structure determine the stiffness of the ointment. In general, hydrocarbon-based ointments liquefy on heating because the crystallites melt. Moreover, when cooled very slowly, they assume fluidity much greater than when rapidly cooled because

slow cooling leads to fewer and larger crystallites and, therefore, less total structure. Common examples of these bases include:

1. *Petrolatum, USP23*—a mixture of semisolid hydrocarbons obtained from petroleum. It is an unctuous mass, varying in color from yellowish to light amber. It melts at temperatures between 38^0C and 60^0C and may be used alone or in combination with other agents as an ointment base. Petrolatum of varying melting ranges and consistencies are commercially available.

2. *White petrolatum, USP*—a petrolatum that has been decolorized, either partially or wholly. It is used for the same purpose as petrolatum but is more esthetically acceptable to a patient than petrolatum because of its lighter color. White petrolatum is particularly useful to treat diaper rash (impervious to urine and protects the baby's skin) and dry skin (helps the skin retain moisture). Example 1 illustrates an ointment preparation that incorporates 95% white petrolatum resulting in an ointment base with firm consistency.

3. *Yellow ointment, USP*—the purified wax obtained from the honeycomb of the bee. It contains 5% yellow wax and 95% petrolatum in the formulation.

4. *Mineral oil*—a mixture of liquid hydrocarbons obtained from petroleum. These are useful as levigating agents to wet and incorporate solid substances (e.g., salicylic acid, zinc oxide) into the preparation of ointments that consist of oleaginous bases as their vehicle. There are two types of mineral oils listed in the U.S. Pharmacopeia/National Formulary (USP/NF). Mineral oil USP is also called heavy mineral oil with a specific gravity between 0.845 and 0.905 and a viscosity of not less 34.5 cSt (cSt ¼ mm2/s) at 40^0 C. Light mineral oil.

ii. Absorption bases [21, 22]:

These bases contain small amounts of water. They provide relatively less emollient properties than hydrocarbon bases. Similar to hydrocarbon bases, absorption bases are also difficult to remove from the skin due to their hydrophobic nature. Hydrophilic petrolatum USP and lanolin USP are examples of absorption bases.

iii. Water-Removable Bases (Water-Washable Creams):

These are the most commonly used o/w emulsion bases that are capable of being washed from skin or clothing with water. They may contain water-soluble and -insoluble components. From a therapeutic viewpoint, they have the ability to absorb serous discharges in dermatologic conditions. The water-removable bases form a semi-permeable film on the site of application after the evaporation of water. As such, the base consists of three component parts: the oil phase, the emulsifier, and the aqueous phase. The oil phase, also called the internal phase, is typically made up of the petrolatum and/or liquid petrolatum. Other ingredients such as cetyl and stearyl alcohol may be added to make up the oil phase.

iv. Water-soluble bases[23,24]:

Do not contain any oily or oleaginous phase. Solids can be easily incorporated into these bases. They may be completely removed from the skin due to their water solubility. Polyethylene glycol (PEG) ointment National Formulary (NF) is an example of water - soluble base. Selection of an appropriate base for an ointment or cream formulation depends on the type of activity desired (e.g., topical or percutaneous absorption), compatibility with other components, physicochemical and microbial stability of the product, ease of manufacture, pour-ability and spreadability of the formulation, duration of contact, chances of hypersensitivity reactions, and ease

of washing from the site of application. In addition, bases that are used in ophthalmic preparations should be non-irritating and should soften at body temperatures.

1.2.3.2. Emulsifying agent[25,26,27]:

Emulsifying agents reduces surface tension of two phases in an emulsion, preventing coalescence of individual phases. Examples are- Detergent, emulsifying wax (detergent treated wax), cetostearyl alcohol, polysorbate 20.

Emulsifiers used in cream formulations may be classified into three different categories: anionic, cationic, and non-ionic emulsifiers.

Anionic emulsifiers: - The active portion of this class of emulsifiers is the anion. In general, these emulsifiers are more acid-stable and permit adjustment of the emulsion pH level to the desirable range of 4.5 and 6.5. Common examples include sodium lauryl sulfate and soaps such as triethanolamine stearate. Triethanolamine stearate is one of the most popular emulsifiers for creams and lotions in use today. It is usually prepared in situ during manufacture from stearic acid in the hot oil phase and from triethanolamine in the hot aqueous phase. The amount of triethanolamine controls the pH level of the resulting product.

Cationic emulsifiers: - Cationic compounds are highly surface-active but are used less frequently as emulsifiers. The cation portion of the molecule is usually a quaternary ammonium salt including a fatty acid derivative such as di-lauryl-dimethyl-ammonium chloride. These emulsifiers are irritating to the skin and eyes and have a considerable range of incompatibilities, including anionic materials.

Non-ionic emulsifiers: - This class of emulsifiers shows excellent pH and electrolyte compatibility in emulsions, owing to the fact that they do not ionize in solution. Although non-ionic emulsifiers range from lipophilic to hydrophilic members, a typical emulsifier system may include a mixture of both alipophilic and a

hydrophilic member to produce a hydrophilic–lipophilic balance (HLB). Emulsions containing non-ionic emulsifiers are prepared by dissolving or dispersing the lipophilic component in the oil phase and the hydrophilic component in the aqueous phase. The two phases are heated separately and combined. Emulsions containing non-ionic emulsifiers are generally low in irritation potential, stable, and have excellent compatibility characteristics.

1.2.3.3.Humectants:-

Humectants are added to minimize water loss in the finished composition and to add to the overall physical product acceptability. Common examples of humectants used include glycerin, propylene glycol, and a polyethylene glycol.

1.2.3.4.Permeation enhancer:-

Permeation enhancers facilitate diffusion process of active ingredient across the stratum corneum by chemical modification. Example: Ethanol, oleic acid, propylene glycol, polyethylene glycol (400).

1.2.3.5. Preservative:-

Prevents or slows microbial growth; may be one of 4 major compound types: acid, alcohol, quaternary ammonium compounds, or organic mercurial. Example: Acid: benzoic; alcohol: phenylethyl; quaternary ammonium: stearyl dimethyl benzyl ammonium chloride; organic mercurial: thimerosal.

1.2.3.6. Thickening agent:-

Increase viscosity; may be natural, semi-synthetic, or synthetic. Example Natural: cellulose, pectin; semi-synthetic: methylcellulose, (sodium) carboxy methylcellulose; synthetic: Carbopol.

1.2.3.7. Antioxidant:-

Prevents or slows oxidation of other components. Example Tocopherol, butylate dhydroxy toluene, or a reducing agent such as ascorbic acid.

1.2.4. EVALUATION OF THE CREAM[28,29,30,31]:

The cream should be evaluated for the following physical parameters:-

i. Formulation Properties:-

The formulation properties of the cream were studied by visual appearance and characteristics like:-

a. State of the cream

b. Colour of the cream

c. Odour of the cream

d. Appearance of the cream.

ii. pH of the Cream:-

The pH meter calibrated using standard buffer solution with a pH of 7.4 and 9.2. About 0.5g of the cream weigh and dissolve in 50.0 ml of distilled water and measure the pH by using the calibrated digital pH meter.

iii. Drug content uniformity:-

The formulation equivalent to 50 mg of drug is taken and dissolved in small quantity of methanol. Then the formulation is warmed on the water bath so that the drug present in the formulation completely dissolved. Then the solution is filtered through Whattman filter paper in to 50ml vol. flask. The volume is made up to the mark which gives concentration of 1000mcg/ml. From this different concentration of solution was taken in 10ml volumetric flask and volume was made upto 10ml with methanol and absorbance was measured by UV spectrophotometer against blank.

iv. Viscosity:

The viscosity of the formulated o/w cream may be measured by Brook field Viscometer (LVDV-III ultra-programmable Rheometer) using spindle CP-52 at varying speed and shear rates. The measurements made over the range of speed setting from 0.10, 0.20, 0.30, 0.40 and 0.50 rpm with 60sec between two successive speeds as equilibration with shear rate ranging from 0.20 sec-1 to 1.0 sec-1. Viscosity determinations are performed at room temperature. The viscosity data was plotted for Rheogram-

Viscosity in cps v/s shear rate in sec^{-1}.

v. Spreadability:-

Spread ability is a term expressed to denote the extent of area to which the topical application spreads on application to skin on the affected parts. The therapeutic efficiency of the formulation also depends upon its spreading value. Hence, determination of spread ability is very important in evaluating topical application characteristics. For the determination of spread ability, excess of sample (3gm) was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5minute. Thereafter weight (50gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10cm is noted. A shorter interval indicates better spread ability. The spreadability (S) can be calculated using the formula:-

$$S = m.l/t$$

Where,

S – Spreadability

m- Weight tied to upper glass slide.

l- Length moved on glass slide and t- time taken.

vi. Rheological behavioural of the cream:-

The rheological property determined to know the flow behaviour of formulation. The viscosity at different RPMs was measured using Brookfield viscometer. The rheological behaviour of the formulation is studied by taking 100g of the cream in the beaker. The rate of shear is measured and corresponding dial reading is also noted; then, the rate of shear was and the dial reading was recorded. The graph is plotted between percent torque and viscosity to determine type of flow.

vii. Tube extrudability:-

In the present study, the method adopted for evaluating cream formulation for extrudability is based upon the quantity in percentage cream extruded from tube on application of finger pressure. More quantity extruded better was extrudability. The formulation under study is filled in a clean, lacquered aluminium collapsible (5 grams) tube with a nasal tip of 5mm opening and the pressure on the tube to be applied by the help of finger. Tube extrude ability was then determined by measuring the amount of cream extruded through the tip when a pressure was applied on tube.

viii. Primary skin irritation test:-

Laboratory experimental animals:-The animals selected are rabbits,rat and guinea pigs. These animals are keep in different cages and supplied with fresh food and water during the test period, 24 hours prior to test, the hair from the neck and thigh region are shaved to expose sufficient large test area. The test site is cleaned with surgical spirit then o/w cream is applied to test area. The test site is observed for erythema and oedema for 24 hrs; 48 hrs; and 72 hrs after application. This test is conduct to evaluate the irritancy of the prepared cream on the intact skin of animals. None of the prepared

cream showed any erythema or oedema, indicating that the prepared formulation is non-irritant on the skin of animals.

1.3. SKIN[32,33,34,35,36]:

For Understanding the concept of wound healing process, it is important to review the structural and biochemical features of human skin and those characteristics which contribute to the healing process of the skin.

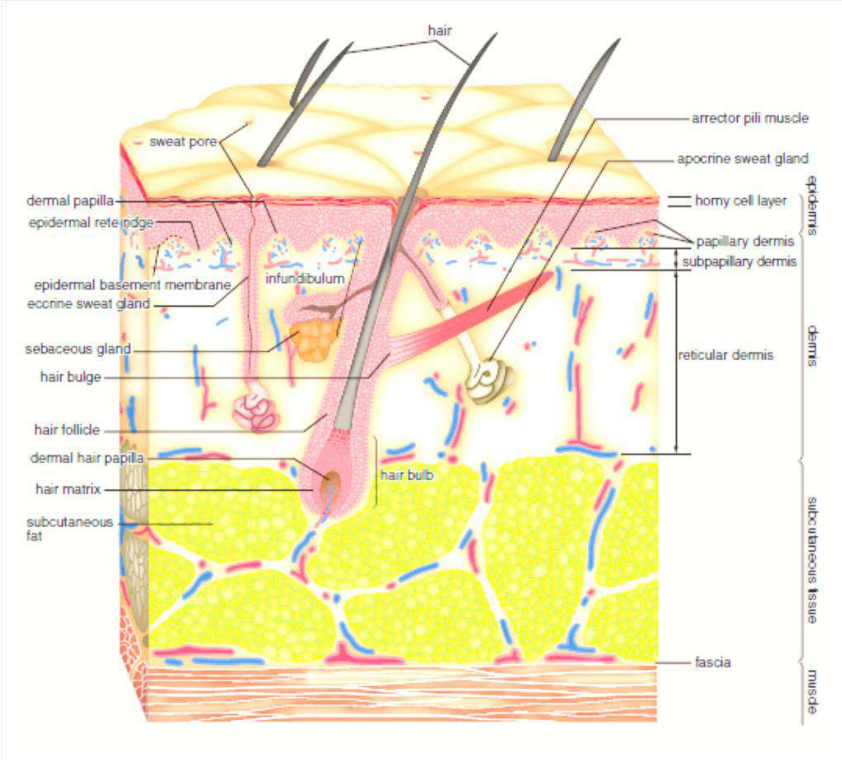

FIG 3: Physiology of skin

1.3.1. PHYSIOLOGY OF SKIN[32,33]:

The skin is one of the most extensive organs of the human body covering an area of about $2m^2$ in an average human adult. This multilayered organ receives approximately one-third of all blood circulating through the body. It has varied functions and properties. With a thickness of only a millimetre, the skin separates the underlying blood circulation network from the outside environment, serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in

maintaining body temperature, protects against harmful ultraviolet rays of the sun and plays a role in the regulation of blood pressure.

Anatomically, the skin has many histological layers but in general, it is described in terms of three major tissue layers: the epidermis, the dermis and the hypodermis. The epidermis results from an active epithelial basal cell population and is approximately 150μm thick. It is the outermost layer of the skin and the process of differentiation results in migration of cells from the basal layer towards the skin surface. The end result of this process is the formation of a thin, stratified and extremely resilient layer at the skin surface. Below this layer are the other layers of the epidermis-the stratum lucidum, stratum granulosum, stratumspinosum and stratum germinativum. Together, these other layers constitute the viable epidermis. The stratum corneum or the horny layer is the rate limiting barrier that restricts the inward and outward movement of chemical substances. The interior of these cells is crisscrossed with densely packed bundles of keratin fibres. Due to this, the dry composition of the horny layer is 75-85% protein, most of which is the intracellular keratin and a part being associated with a network of cell membranes. The bulk of the remainder of the substance of the stratum corneum is a complicated mixture of lipids which lies between the cells. It appears to be organised into bilayers. The stratum corneum thus has two distinct chemical regions, the mass of intracellular protein and the intercellular lipoidal medium. The epidermis rests on the much thicker (2000 μm) dermis.

A rich bed of capillaries is encountered 20 μm or so into the dermal field. Also contained within the dermis are lymphatics, nerves and the epidermal appendages such as hair follicles, sebaceous glands and sweat glands. Each hair follicle is associated with one or more sebaceous glands which are outgrowths of epithelial

cells. The duct of sebaceous gland is filled with a soft, slowly extruded lipoidal medium-sebum. About 1/1000 of the total skin surface is occupied by hair follicles. The sweat glands are divided into the eccrine and apocrine types and are widely distributed over the surfaces of the body. Eccrine glands are particularly concentrated in palms and soles (400glands/cm2). The apocrine glands are found in the axillae (armpits), in anogenital regions and around nipples. These are coiled tubular glands, about ten times larger than eccrine glands and extend entirely through the dermis and well into the subcutaneous layer. The sweat glands serve to control body heat by secretion of a dilute salt solution.

1.3.2. BIOCHEMISTRY OF SKIN[34,35]:

Like other tissues of body, the skin has two metabolic requirements, small molecular weight building blocks and chemical energy. Dissimilarities peculiar to the skin are discussed here, as it might affect transdermal drug delivery.

A. Dermis

Protein synthesis (from amino acid precursors) is key factor in dermal metabolism. Fibroblasts produce and deposit extra-cellularly, huge quantities of collagen and elastin. This becomes important in repair/turnover of dermal proteins altered by environmental sunlight. Extensive protein synthesis also occurs in hair follicles where hair, consisting of approximately 95% protein originates.

The sebaceous glands produce large quantities of liquid. The energy derived from the intracellular aerobic carbohydrate metabolism is used for cellular synthetic process.

B. Epidermis

The surface of energy for the lower portions of the epidermis is also glucose and the end product of metabolism, lactic acid accumulates in skin, which results in a tissue pH from the usual 7 to less than 6.

The cells rely primarily on fatty acids for cellular functions. These fatty acids are derived from the degradation of phospholipids from membranes. The energy derived is used in synthesis of the protein and lipids for construction of stratum corneum.

During differentiation from basal cells to stratum corneum, by degradation of the existing cellular components, the entire cellular make-up changes. Specialised cellular organelles called lysosomes contain a host of lytic enzymes which they release for intracellular lysis.

The epidermis is reservoir of such lytic enzymes. Many of these enzymes are inactivated in upper granular layer; however, many also survive into the stratum corneum. The stratum corneum also has proteolytic enzymes involved in this desquamation.

1.3.3. MAIN FUNCTIONS OF THE SKIN[36]:

A. Protection:

The stratum corneum is a tightly interlocking layer of dead cells that guard against infections entering via the skin. It is also mostly waterproof preventing the loss or entry of fluids. The acidic ph balance inhibits the growth of bacteria. It protects against mechanical damage such as abrasions and friction. It tries to protect against the damaging effects of sunlight (ultraviolet light), through the regulation of melanin which is produced by the Melanocyte cells. When stimulated by sunlight the cells produce more melanin which appears as a sun tan, this is the skins way of trying to protect its self.

B. Heat regulation:

Body temperature is controlled by sensing the environmental temperature via the nerve endings and then adjusting the blood flow and sweat production accordingly to try and maintain the body's optimal working temperature which is around 37 degrees.

Sensation:

With most sensory nerve endings terminating in the dermis, the skin is able to sense many things including, environmental temperature, pressure, vibrations, touch and pain and then reacts accordingly.

C. Absorption:

Although the skin is almost waterproof some lipid soluble materials will penetrate. Included in this list are fat soluble vitamins, oxygen, carbon dioxide, also some drugs and unfortunately toxic materials.

D. Secretion:

Sebum is secreted from sebaceous glands; the sebum balances the skins acid mantle. Sebum makes the skin oily when there is an over production. It moisturises the skin keeping it soft and supple therefore prevents cracks and openings in the skin.

E. Elimination:

Small amounts of urea, uric acid, ammonia and lactic acid are excreted out of the body in our sweat which is produced by the sweat glands.

F. Vitamin D formation:

The skin with the aid of UVB (ultraviolet) a part of sunlight forms vitamin D. The UV light converts a fatty substance called Ergosterol into vitamin D which then circulates around the body and is used in the formation and maintenance of bone along with calcium and phosphorous.

Chapter 2

LITERATURE REVIEW

1. **Kolkane D. D. et al**[37] studied the wound healing activity of the root of *Mimosa pudica*. They studied the wound healing activity of the root by incorporating the methanolic and the total aqueous extract in simple ointment base B.P. in the concentration of 0.5% (w/w), 1% (w/w) and 2% (w/w). They studied the wound healing activity in three typesof model in rats, viz. excision, incision and estimation of biochemical parameter. In case of the excision wound model, wound contraction and period of epithelization was studied. While in incision wound model the tensile strengthand hydroxyproline content in the scab were determined. They saw that ointment containing 2% (w/w) of the methanolic and 2% (w/w) of the totalaqueous extract exhibited significant ($P< 0.001$) wound healing activity.
2. **Akkol E. K. et al**[38]evaluate the wound healing activity of the roots of *Arnebia densiflora*. They prepared extracts from the root with different solvents like hexane, chloroform, ethyl acetate and methanol. They found significant wound healing activity with the ointment formulation prepared byusing hexane extract at 1% concentration on the mentioned models.
3. **Patel N. A. etal**[39] prepared a carbopol 934 gel with containing different concentrations of extract of *Terminalia ajunar*, *Centella asiatica* and *Curcuma longa* to study their wound healing activity on experimentally induced open wounds in albino rats. They prepared formulations containing 1% and 2% herbal extracts and applied topically three times a day to open wounds for 24 days post-operatively and compared it with base control. They observed that treated wounds showed a faster rate of wound contraction compared with controls. The wound contraction studies revealed that the wound contractions increase with an increase in the herbal extract concentration.

4. **Shafiuddin et al**[40] evaluated oils of *Comphora officinarum*, *Shorearobusta beeswax*, *Acacia catechu*, *Sesamum indicum*, and *Azadirachta indicafor* wound healing activity in excision and incision wound models in albino rats. The activity was compared with that of the control and Framycetin sulfate cream 1% w/w as standard drug. They saw that the formulation showed a significantly higher contraction rate and shortened epithelization period in both the models. In excision model, the healing was 99% ($p < 0.001$) on 16^{th}day compared to 85% and 75% of healing with Framycetin sulfate cream and control, respectively. In incision wound model, there was significant increase in tensile strength ($p <0.001$). Thus, they concluded that the formulation has got potential wound healing activity for both the types of wounds; justifying its use in the traditional practice.
5. **Vijay Viswanathan et al**[41] studied the effects of a polyherbal formulation cream on diabetic foot ulcers. A total of 40 (M:F=29:14) consecutive type 2 diabetes patients with foot ulcers were enrolled in this study. They were randomly assigned to two groups of 20 each; Group 1 was treated with polyherbal formulation and group 2 with silver sulphadiazine cream. All the patients were followed up for a period of 5 months. The baseline ulcer size was noted and photograph of the wound was taken at the baseline and at each follow up visit. Number of days taken for healing of the wound was recorded. The mean age of patients, duration of diabetes and HbA1c% were similar in both the study groups. The mean length and width of the ulcers was also similar in both the groups at baseline visit. There was a significant decrease in the size of the wound (length and width) in both the study groups ($P<0.001$).

6. **Sahu Alakh et al**[42] formulated and evaluated a Curcuminoid Based Herbal Face Cream. Curcuminoids from *Curcuma domestica* Val. (turmeric) has been incorporated in the formulation. Turmeric rhizome powder has been extracted with methanol and curcumin content in the methanolic extract has been quantified spectrophotometrically. Evaluation of formulated cream with parameters - type of emulsion, ashing at 600 oC, pH, homogeneity and sensory parameters has been conducted. Accelerated stability testing of 16 prepared formulations has been conducted at elevated temperature of $40^{o}C \pm 1^{o}C$ for 20 days. 4 out of 16 products have shown stability with no signs of bleeding and no change in the color of the product.
7. **Himal Paudel Chhetri et al** [43] formulated and evaluated The ethanolic extracts of the selected plants were taken in different ratio randomly and the antimicrobial tests of the combinations were carried out. The most effective combination was then determined by comparing the results of the zone of inhibition given by the 10 different extract ratios on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species. Then the minimum inhibitory concentration of the effective combination was found out. The ointment base was prepared and formulation of ointment was done by incorporating the active ingredients in most effective ratio in the base by trituration. After the completion of the formulation, quality of the ointment was assessed in terms of irritancy, spreadability, diffusion and stability.
8. **KottaKranthi Kumar et al** [44] carried out developments of formulation and evaluation of Diacerein cream which are designed to enhance the onset of action. The cream is formulated by two-phase system. The oil phase is melted at 90% and then transferred into the heated aqueous phase. The mixture is stirred by stirrer at 200rpm. As the temperature decreases the cream get formed. The cream is formed

by using the fusion technique. The formulation was found to be a best one which gives accurate result. The % of drug content of diacerein was found to be 98.54. The pH was found to be 4.5.Colour was found to be Yellowish semisolid cream. The viscosity was found to be 32727cps, the spreadibility was found to be 9.12, the extrudability was found to be 94.20%.The result shown per stability study after three months, it gives the accurate and satisfactory result.

9. **Prabhudutta Panda et al**[45] formulated and evaluated cream prepared from the chloroform extract of *Pandanusfascicularis* Lamk. Creams are prepared at different concentrations i.e. 5%, 7.5% and 10% (w/w) by fusion method using different excipients. These topical formulations were tested for pH, viscosity, spreadability, drug contents uniformity, in vitro diffusion. The stability study was carried out at 4, 25 and 37^0C. All the formulations were evaluated for its acute skin irritancy, wound healing activity in Swiss Albino rats. These formulations did not produce any skin irritation for about a week when applied over the skin. Comparative studies showed that the viscosity of the formulations increases, spreadability decreases and vice versa. From the stability studies, creams showed no changes in properties after keeping at different temperatures for 90 days.
10. **Ashish Aswal et al**[46] prepared and evaluated polyherbal cosmetic cream comprising extracts of natural products such as *Aloe vera,* Cucumissativus and Daucuscarota. Different types of formulations oil in water (O/W) herbal creams namely F1 to F7 were formulated by incorporating different concentrations of stearic acid and cetyl alcohol. All formulations were evaluated for various parameters like pH, viscosity, spreadability and stability. Formulations F6 and F7 showed ease of removal, spreadibilty, no evidence inflammation and irritation, no

evidence of phase separation and no redness, edema. These formulations are safe to use for skin.

11. **V. V. Paithankar** [47] Formulated and evaluated herbal cosmetic preparation using using the various concentration of safedmusli extract, along with the other ingredients like stearic acid, cetyl alcohol, mineral oil, triethanol amine, glycerin, safedmusli, perfume, preservative, and distilled water, to choose out the best concentration ratio for the creams which will give the better anti ageing result. The ratio of 7: 2: 20: 2:10: 3.5: 1 0.5 was selected after evaluation. This single formulation (cream) is used as anti ageing.
12. **A. A. Baravkar et al**[48] evaluated a cream formulation of the methanolic extract of the leaves of plant *Acacia nilotica* (Fabaceae). The methanolic extract of leaves was obtained by Soxhlet extraction and its medicated as well as non-medicated cream formulation is developed using bees wax, liquid paraffin, borax and water. Commercially available Vicco turmeric cream was used as a standard for this study. All three formulations were evaluated for various pharmaceutical parameters such as Rheological properties, stability, pH and external characters as well as biologically for its wound healing activity on excision wound made on albino rats. Results were found by comparing the wound contraction (in mm2 and in %) as well as period of epithalisation of medicated cream with that of control group, non-medicated cream group and vicco turmeric cream (as standard) and was found faster for medicated cream than control group and non medicated one.
13. **Monica Butnariuet et al**[49] evaluated a number of Biologically Active Compounds from *Calendula officinalis* Flowers using spectrophotometry. The antioxidant capacities of extracts were assessed by using spectrophotometry to measure both absorbance of the colorimetric free radical scavenger 2,2-diphenyl-

1-picrylhydrazyl (DPPH) as well as the total antioxidant potential, using the ferric reducing power (FRAP) assay. pectrophotometric assays in the ultraviolet-visible (UV-VIS) region enabled identification and characterization of the full range of phenolic and flavonoids acids, and high-performance liquid chromatography (HPLC) was used to identify and quantify phenolic compounds (depending on the method of extraction).

14. **Camelia Paula Stefanache et al**[50] studied in vitro regeneration of arnica montana l. from natural populations. For in vitro regeneration, the biological material used to initiate tissue cultures, comes from *Arnica montana* seedlings, resulting from germination of seeds collected from natural populations.
15. **Manjir Sarma Katakiet al**[51] studied the antimicrobial, antioxidant and antihelmentic activity of ethanolic extract of *Ananas comosus* leaves. Antibacterial activity of the extract was evaluated against four bacterial strains at 50 and 100 mg/ml concentrations. Marked inhibitory effect was observed against Staphylococcus aureus, Escherichia coli and Salmonella typhi except Bacillus subtilis. In addition, reducing power assay, determination of total antioxidant activity and DPPH radical scavenging activity were performed to evaluate the in vitro antioxidant activity of the extract. The results indicated higher antioxidant activity of the extract than α-tocopherol but lower than that of BHA. Anthelmintic activity of the extract was evaluated using adult Indian earth worm and the results indicated a dose dependent increase in anthelmintic activity of the extract at 25, 50 and 100 mg/ml concentrations.
16. **Koca U. et al**[52] evaluated the anti-inflammatory and wound healing activities of the aerial part of *Centaurea iberica*. In order to evaluate the anti-inflammatory and wound healing activities of the plant, they prepared the extracts with variety

of solvents like hexane, chloroform, ethyl acetate and aqueous methanol (85%) fromthe aerial parts. They found that the ointment formulation prepared with 1% methanol extract shows wound healing activity. The results of histopathological evaluation also supported the outcome of both incisionand excision wound models. Moreover, they also saw that the methanol extract exerted remarkable wound healing activityand also demonstrated a significant and dose-dependent anti-inflammatory activity.

Chapter 3

AIM, OBJECTIVES & PLAN OF WORK

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

AIM OF THE STUDY:

To formulate and evaluate the herbal cream for wound healing.

OBJECTIVES OF PRESENT STUDY

Although various types of cream is considered for wound healing but these are still appears to be limited in rate of tissue regeneration. Hence after a depth review regarding pathogenesis as well as different traditional and alternative therapy for wound healing, we have taken up the project to develop and formulate an herbal cream which will be effective and has better rate of tissue regeneration. The herbal cream that is planned to be formulated for wound healing will be oil/water emulsion type which will be less oily, less greasy and less sticky in nature so that patient compliance is more and it will be beneficial for all kind of people in our society. The objectives are as follows:

- To formulate an herbal cream for wound healing.
- To evaluate the prepared cream for pharmaceutical properties.
- To study the wound healing activity of prepared cream using rat as animal model.
- To compare the effectiveness of prepared cream against established marketed wound healing cream.

PLAN OF WORK:

I. Review of literature,

II. Procurement of crude drugs in the form of plant parts (leaves, flowers,powder etc.)

III. Extraction of active drug from the crude drugs.

IV. Freeze drying (lyophilization) of liquid extract to obtain dry products.

V. Formulation of an prototype **o/w** cream and evaluation

(a) Selection of various prototype ingredients for cream preparation

(b) Development of prototype formula of cream

(c) Evaluation of prototype creams for type of emulsion, homogeneity, appearance, after feel, type of smear, ease of removal, globule size analysis, spread ability, accelerated stability testing ect.

VI. Formulation of **oil-in-water herbal cream** using the freeze dried, purified drug with varying concentrations of excipients.

VII. Evaluation and characterization of prepared cream

a. *In vitro* evaluations

i. Types of emulsion

ii. Homogeneity

iii. Appearance

iv. After feel

v. Type of smear

vi. Removal

vii. Macroscopic examination

viii. Globule size analysis

ix. Determination of pH,

x. Viscosity

xi. Spread ability

xii. Stability study.

b. *In vivo* evaluations

i. Skin irritation test

ii. Evaluation of wound healing property on rats.

Chapter 5

MATERIAL & METHOD

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

5.1. MATERIALS

5.1.1. Collection of plant materials:

The fresh leaves of *Clerodendrum indicum* were collected from North Eastern Development Finance Corporation Ltd. (NEDFI), Khetri (Assam). The dried crude drug of *Calendula officinalis* were collected from the API Supplier of Kolkata. The dried stem, leaves of *Arnica Montana* were collected from the API Supplier, Kolkata. The extracted powder of Aloe vera was supplied by the API supplier, Kolkata. The dried root of *Panax ginseng* were collected from B. S. Trading, Kolkata. Rose hip oil was collected from Katyani Exports, Delhi.

5.1.2. Chemicals and Reagents:

The chemicals used during the experiments were of analytical grade. Lanolin was purchased from Burgoyne Urbidges& Co., Mumbai, India. White petrolatum was purchased from Yarrowchem Products, Mumbai- 400037, India. Stearic acid was purchased from Himedia Laboratories Pvt. Ltd, Mumbai- 400086, India. Tween 60 was purchased from Himedia Laboratories Pvt. ltd, Mumbai 4000086, India. Mineral oil was purchased from Merck Specialities Pvt. Ltd, Worli, Mumbai. Triethanolamine was purchased from Merck Specialities Pvt. Ltd, Worli, Mumbai. Propylene Glycol was purchased from Merck Specialities Pvt. Ltd, Worli, Mumbai.

5.1.3. Instruments:

Freeze Dryer (INSTIND; PIRANI; IIC Industrial Corp; Kolkata),Homogenizer (Remi Motor, Vasai, India), Thermostat Water Bath (JSGW), Refrigerator (Godrej), Centrifuge (REMI), Viscometer (Brookfield DV-E viscometer),Digital Balance (Denver Instrument), Hot Air Oven (International Commercial

TradersKolkata).Paraffin embedding system, Microtome and Electronic microscope(Dept. Pathology, College of Veterinary Science, Khanapara, Assam.)etc.

5.2. METHODS:

5.2.1. EXTRACTION OF PLANT MATERIALS:

5.2.1.1. Extraction of *Calendula officinalis*: By Simple Maceration[63]:

The extraction of *Calendula officinalis*was done using simple maceration technique. The following procedure was followed for extraction:

a) Fresh leaves of *Calendula officinalis* were collected, washed with clean water and kept for shade drying.

b) After the leaves were dried completely, they were crushed to form coarse or moderately coarse powder.

c) The plant material (powder) is then kept in a closed vessel and the whole of the selected solvent (menstrum) was poured into it.

d) This mixture is allowed to stand for three days with occasional shaking.

e) After 3 days, the liquid is strained off and the solid residue (marc) is pressed.

f) Strained liquid is then mixed with pressed liquid.

g) The liquid mixture is then filtered to get a clear liquid extract.

h) The clear liquid is then subjected to freeze drying in order to get a solid mass to be used in the formulation.

5.2.1.2.Extraction Of *Arnica Montana*[60]:

Extraction of arnica Montana was done using simple maceration technique. The following procedure was followed for extraction:-

a) The dried plant materials were crushed to form coarse or moderately coarse powder.

b) The plant material (powder) is then kept in a closed vessel and the whole of the selected solvent (menstrum) was poured into it.

c) This mixture is allowed to stand for three days with occasional shaking.

d) After 3 days, the liquid is strained off and the solid residue (marc) is pressed.

e) Strained liquid is then mixed with pressed liquid.

f) The liquid mixture is then filtered to get a clear liquid extract.

g) The clear liquid is then subjected to freeze drying in order to get a solid mass to be used in the formulation.

5.2.1.3.Extraction Of *Clerodendrum indicum*[66]:

Extraction of *Clerodendrum indicum* was done using simple maceration technique. The following procedure was followed for extraction:-

a) Fresh leaves of *Clerodendrum indicum* were collected, washed with clean water and kept for shade drying.

b) After the leaves were dried completely, they were crushed to form coarse or moderately coarse powder.

c) The plant material (powder) is then kept in a closed vessel and the whole of the selected solvent (menstrum) was poured into it.

d) This mixture is allowed to stand for three days with occasional shaking.

e) After 3 days, the liquid is strained off and the solid residue (marc) is pressed.

f) Strained liquid is then mixed with pressed liquid.

g) The liquid mixture is then filtered to get a clear liquid extract.

h) The clear liquid is then subjected to freeze drying in order to get a solid mass to be used in the formulation.

5.2.1.4. Extraction of *Panax ginseng* root[58,59]:

Extraction of *Panax ginseng* was done using simple maceration technique. The following procedure was followed for extraction:-

a) The dried roots were crashed in a grinder mixer.
b) The plant material (powder) is then kept in a closed vessel and the whole of the selected solvent (menstrum) was poured into it.
c) This mixture is allowed to stand for three days with occasional shaking.
d) After 3 days, the liquid is strained off and the solid residue (marc) is pressed.
e) Strained liquid is then mixed with pressed liquid.
f) The liquid mixture is then filtered to get a clear liquid extract.
g) The clear liquid is then subjected to freeze drying in order to get a solid mass to be used in the formulation.

5.3. FORMULATION OF A PROTOTYPE CREAM:

The main components of oil in water type emulsion base include: water (55%), petrolatum (>15%), mineral oil (>15%), and cetostearyl alcohol (>10%); all other ingredients are less than 5%.

5.3.1. SELECTION OF VARIOUS PROTOTYPE INGREDIENTS FOR CREAM PREPARATION[12,14]:

i. *Emulsifying agent:* It is used to reduce the interfacial tension between oil and water and thus make them miscible with each other e.g. tween and span etc.

ii. *Oil base:* White petrolatum, Lanolin, Stearic acid etc.

iii. *Oil*: Mineral oil, arachis oil, paraffin oil.

iv. *Water*: pure water, lime water.

v. *Humectants*: It is used to prevent fast drying of water in cream preparation e.g. triethanolamine, propylene glycol.

vi. *Preservative*: It is added in hair cream preparation to avoid the microbial growth because the microbial growth is very high in aqueous phase e.g methyl paraben, propyl paraben etc.

5.3.2. DEVELOPMENT OF PROTOTYPE FORMULA OF CREAMS[15,16]:

After selection of the ingredients, a prototype formula had to be generated. The maximum limits of each ingredient, as per literature which were considered during the development of prototype formula are given below:

Table 1: Maximum permitted limit of ingredients to be used in formulation

Ingredients	Maximum levels (% w/w)
Herbal extract	50
Emollients (oils, waxes, etc.)	30
Humectants (e.g. glycerin, propylene glycols)	15
Emulsifying agents (e.g. sorbitansesquioleate, sorbitan stearate)	5
Cosmetic colorants (e.g. often iron oxides)	2
Preservatives, antimicrobials	0.5
Viscosity controlling agents (e.g. cellulose gum)	1
Perfume	1
Aqua	to 100

Based on these, it was decided to develop 3 prototype formulae, which will be different from each other only in terms of surfactant concentration. Tween 60 was selected as the surfactant whose concentration was to be varied based on the following HLB data.

Table 2: Surfactants and their HLB values

Surfactant	***HLB***
Tween 40	15.6
Tween 60	14.9
Tween 80	15.0
Span 60	4.7
Span 80	4.3
Span 85	1.8

As surfactants having HLB valueabove 10 give **O/W type emulsion,**therefore Tween 60 is selected for the study. Tween 60 was used in three different concentrations, namely 1%, 5% and 10%.

Table 3: Formula for preparation of prototype cream

SL.NO	**PART-A (OILY PHASE) (30%)**		**PART-B (AQUOUS PHASE) (70%)**	
	Ingredients	**(%w/w)**	**Ingredients**	**(%w/w)**
i.	White petrolatum	1	Propylene glycol	5
ii.	Lanolin	1	Triethanolamine	2
iii.	Stearic acid	13	*Tween 60*	*1/5/10*
iv.	Mineral oil	15	Methyl paraben	0.2
v.	-	-	Water	Up to 100

During pre-formulation study first an emulsion is prepared using 70% aqueous phase and 30% oil phase. Each tube is agitated for 10 seconds using a vortex and then examined. All of the samples that lead to a rapid separation of phases are rejected due

to their instability. The emulsions that gave rise to sedimentation were also eliminated (inverse emulsions).

FIG 10: Preparation of prototype cream

5.3.2. EVALUATION OF PROTOTYPE CREAMS[12,14,15,16]:

The above emulsions were evaluated for the following parameters:

i. Type of emulsion:

a. **Dye method:** A portion of the product was taken in a watch glass. To that water soluble dye (methylene blue) was added, mixed properly and observed under microscope the dispersed oil globules in the coloured aqueous phase.

b. **Dilution method:** Fixed amount of cream was diluted with water and mineral oil separately. Then they are observed for separation of both the phases in oil and water.

ii. Homogeneity:

The formulations were tested for the homogeneity by visual appearance and by touch.

iii. Appearance:

The appearance of the cream was judged by its color, pearlscence and roughness and graded.

iv. After feel:

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

v. Type of smear:

After application of cream, the type of film or smear formed on the skin were checked.

vi. Removal:

The ease of removal of the cream applied was examined by washing the applied part with tap water.

vii. Macroscopic examination:

The assessment of the physical stability of an emulsion is made by an examination of the degree of creaming or coalescence occurring over a period of time. This involves the calculation of the ratio of the volume of the creamed or separated part of the emulsion and the total volume. A comparison of these values can be made for different products.

viii. Globule size analysis:

An increase in mean globule size with time (coupled with a decrease in globule numbers), indicates that coalescence is the cause of this behavior. This can be used to

compare the rates of coalescence for a variety of emulsion formulations. For this purpose, microscopic examination is widely used.

ix. pH:

The p^H of various formulations was determined by using Digital p^H meter (Digital p^H meter, systronics).

x. Viscosity:

The measurement of viscosity of prepared cream was carried out with Brookfield Viscometer using Spindle 64.

xi. Spreadability:

Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to skin or affected part. The spreadability is expressed in terms of times in seconds taken by two slides to slip off from cream and placed in between the slides under the direction of certain load.

Lesser the time taken for separation of two slides, resultant the better spreadability. Spreadability was calculated by using the formula. (S= M.L/T). Where S= spreadability, M= Weight tied to upper slide, L= Length of glass slides and T= Time taken to separate the slides completely from each other.

xii. Accelerated stability testing:

The stability studies of formulated cream were carried out at 40^0C /75% RH for 1, 2 & 3 months. The effects of temperature, humidity and time on the physical characteristics of the creams were evaluated for assessing the stability of the prepared formulations. The stability studies were carried out when the room temperature was

20^0C to 25^0C. The pH, viscosity, spreadability and % phase separation in 10 and 40 minutes centrifugation were evaluated for stability after keeping for 1 month.

After analyzing the results of the prepared formulations, the best stable formulation was selected for final formula development of cream.

5.4. FORMULATION OF HERBAL CREAM[14,16]:

O/W emulsion based cream formulation was prepared. Ingredient of oil phase (A) was melted in a beaker by using water bath on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase (up to 70^0 C). The preservative methyl paraben and concentrated aqueous extract of the plants was added into aqueous phase and heated to same temperature. Then oil phase was added to water phase little by little on constant stirring and perfume was added to it when the temperature was 35^0C - 40^0 C.

Topical herbal creams of mentioned herbal extracts were prepared as per the following way. The formulation compositions of the various herbal creams are presented in Table 4.

Table 4: Composition of herbal cream formulations

Ingredients	Formulation %w/w(in grams)					
	F1	F2	F3	F4	F5	F6
Aq. Extract of *Panax ginseng*	2.5	3.0	3.5	4.0	4.5	5.0
Aq. Extract of *Calendula officinalis*	2.5	3.0	3.5	4.0	4.5	5.0
Aq. Extract of *Arnica montana*	0.5	0.75	1.0	1.25	1.5	2.0
Aq. Extract of *Clerodendrum indicum*	0.25	0.5	0.75	1.0	1.0	1.0
Aloe vera	1.0	1.25	1.5	2.0	2.5	3.0
Rose hip oil	1.5	2.0	2.5	3.0	3.5	4.0
White petrolatum	0.8	0.8	0.8	0.8	0.8	0.8
Liquid paraffin	15	13.5	12.5	10.5	9.5	8.3
Lanolin	0.8	0.8	0.8	0.8	0.8	0.8
Stearic acid	11.9	12.9	13.4	14.9	15.4	16.7
Propylene glycol	3.5	3.5	3.5	3.5	3.5	3.5
Triethanolamine	1.0	1.0	1.0	1.0	1.0	1.0
Tween 60	5.0	5.0	5.0	5.0	5.0	5.0
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1
Water	q.s	q.s	q.s	q.s	q.s	q.s

FIG 11: Formulation of Polyherbal cream

5.4.1. SELECTION OF OPTIMIZED FORMUALTION BATCH:

Based on the results of viscosity, spreadability, stability and appearance of prepared formulations (F1 to F6) the best formulation was selected. The stability studies of the prepared cream formulations were carried out at refrigerator temperature (2-8 ºC), room temperature 25 ºC and accelerated temperature of 40º C for one month. After one month the formulations were evaluated for appearance, consistency, phase separation, spreadability, viscosity and the most stable formulation was selected. From the results, stable optimized batch was selected for wound-healing activity.

5.4.2. EVALUATION (*IN-VIVO)* OF HERBAL CREAM:

5.4.2.1. SKIN IRRITATION TEST[37]:

The cream was evaluated for primary skin irritation test on experimental animals (shaved back of the rat) to evaluate the safety of cream. The OECD guidelines number 402 was followed for the study.

5.4.2.2. EVALUATION OF WOUND HEALING ACTIVITY OF PREPARED HERBAL CREAM[37,69,70,71]:

The healthy Wistar albino rats of either sex,weighing 150–200 g, were housed under standard environmental conditions of temperature and humidity (25±0.50^{0}C) and 12 hr (light/dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water *adlibitum*. The animal studies were performed in the institutewith due permission from Institutional Animal Ethical Committee (registration no- GIPS/IAEC/MPH/2013/3).

The rats were divided into three groups namely control (base cream treated group), standard (povidone iodine treated group/betadine) and test (formulated hearbal cream) group. On the day of experiments rat were anesthetized by administering ketamine (50 mg/kg, b.w. i.p.). A full thickness of the excision wound of circular area (1.5 cm)

was made on the shaved back of the rats, 10 min later the administration of ketamine injection. The wounding day was considered as day 0. The wounds were treated with topical application of the cream till the 18th days. The wounds were monitored and the area of wound was measured on 3, 6, 9, 12, 15 & 18 of post-wounding days and the mean % wound closure/contraction is reported on 9, 18 of post-wounding days.

The percentage of wound closure was calculated using the following formula:

$$\%\ \text{of wound closure} = \frac{\text{Wound Area on Day } '0' - \text{Wound Area on Day } 'n'}{\text{Wound Area on Day } '0'} X100$$

Where*n* =number of days 9th and 18thday.

5.4.2.3.HISTOPATHOLOGICAL STUDIES OF WOUNDED SKIN [69,70]:

At the day 18th the experiment was terminated and the wound area was removed from the surviving animals for histopathological examination. Sample tissues were fixed in 10% formalin and were embedded in paraffin wax. Serial sections (5µm thickness) of paraffin embedded tissues were cut. The tissues stained by haematoxylin and eosin, and after that they were examined by electronic microscope.

This part of the experiment was carried out with due help fromDepartment of Pathology, College of Veterinary science, Assam Agricultural University (AAU), Khanapara, Guwahati-22. Assam.

Chapter 6

RESULT & DISCUSSION

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

6. RESULTS OF PROTOTYPE FORMULATION STUDY:

6.1. PREPARATION OF VARIOUS PROTOTYPE O/W TYPE CREAMS:

Three creams were prepared successfully using the concentration as shown in table5. It is observed that creams containing 1% and 10% w/w Tween 60 give rise to rapid separation of phases and they are rejected. Cream containing 5% emulsifier (Tween 60) is white cream and stable, which is selected for further evaluation process.

Table 5:The ingredients selected for the prototype cream formulations.

SL.NO	PART-A (OILY PHASE) (30%)		PART-B (AQUOUS PHASE) (70%)	
	Ingredients	**(%w/w)**	**Ingredients**	**(%w/w)**
i.	White petrolatum	1	Propylene glycol	5
ii.	Lanolin	1	Triethanolamine	2
iii.	Stearic acid	13	*Tween 60*	*1/5/10*
iv.	Mineral oil	15	Methyl paraben	2
v.	-	-	Water	Up to 100

6.2. RESULTS OF PHYSICAL EVALUATION OF PROTOTYPE CREAM:

i. ***Type of cream:***

The prepared cream was oil in water (O/W) type as water soluble dye methylene blue was seen in continuous phase. The type of cream was also confirmed by the result of dilution tests. In the dilution test, when it is diluted with water, there is no phase separation as it gets mixed with the continuousaqueous phase of the cream.

ii. ***Homogenicity***:

The prepared cream was found to be homogeneous by visual experiment and touch.

iii. ***Appearance***:

The cream was found to be milky in appearance.

iv. ***After feel:***

The cream formulation was found to be pearlescent, emollient, non-greasy and easily removed after the application.

v. ***Globule size analysis:***

The globule sizes of cream was varied from 100-500 microns, which indicates formation of fine cream.

vi. ***Viscosity***:

The viscosity of cream was in the range of 21400 – 1074 cps which indicates that the cream is easily spreadable by small amounts of shear. The viscosity of the prepared cream was presented in the table below:

Table 6: Viscosity data of formulation batches

RPM	Viscosity in centipoises(cps)
0.5	21400
1.0	16300
5.0	8110
10	5410
50	1730
100	1074

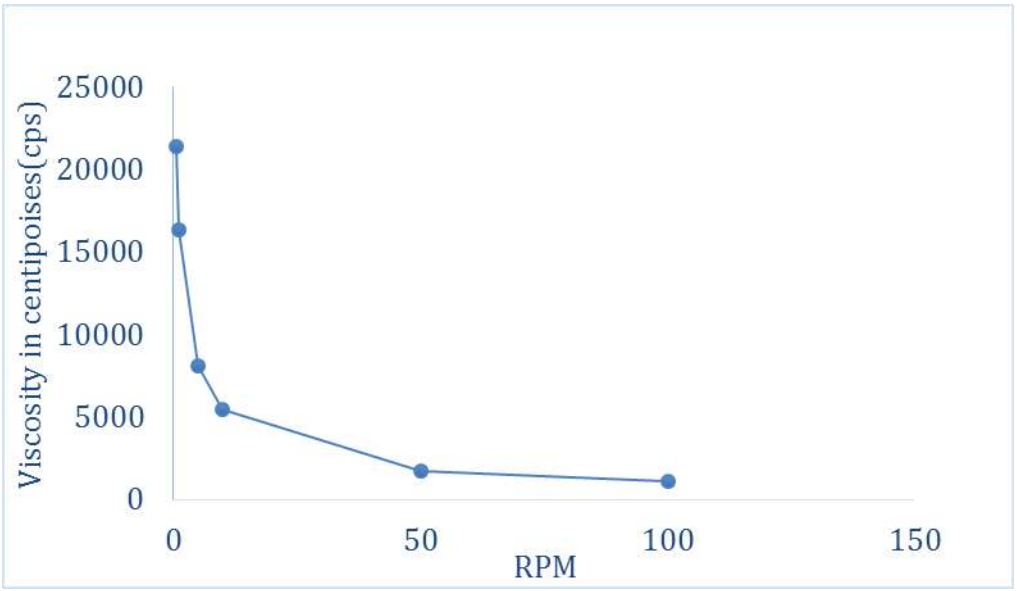

FIG 12: Viscosity of prototype cream

vii. ***Spreadability***:

The spreadability of the cream was found to be 13-16 seconds which revealed ease of spreading over skin at small amount of shear and its washability.

viii. ***Stability***:

From the stability study, the cream was found to be stable up to 24 hr and 7 days with respect to p^H, viscosity, spreadability, emolliency and phase separation. There was no sign of phase separation after 10 and 40 minutes centrifugation at 2000 rpm.

6.3. FORMULATION OF POLYHERBAL CREAM:

Topical herbal creams of mentioned herbal extracts were prepared as per the compositions of base presented in Table5.

6.4.EVALUATION RESULTS OF HERBAL CREAM:

6.4.1. Preliminary studies data of herbal cream:

The results of the present research work showed the creams were light brown in appearance and gave cool and smooth feel on application which was maintained after tested the stability study. The p^H of all the formulations was found to be in the range of 6.70-6.80, which is good for skin (p^H=6.8). The creams also showed good spreadability when measured using slides and found to be less variation with the initially prepared creams. After application of the cream the type of smear formed on the skin was found to be non-greasy and easily removed on washing with tap water. All the formulations showed uniform distribution of the extracts in the cream and they were homogeneous. Physico-chemical properties of the prepared formulations showed good spreadability with proper viscosity which is needed for cream based formulations.

Table 7:Preliminary studies data of herbal cream

Formulations	Physical Parameters			p^H	Spreadability (g.cm/sec)
	Colour	Appearance	Feel on application		
Control	White	Opaque	Cool and smooth	6.70	15.14
Cream (F3)	Light brown	Opaque	Cool and smooth	6.79	16.12
Cream (F6)	Light brown	Opaque	Cool and smooth	6.80	16.17

6.4.2. Viscosity results

The viscosity of the creams wasfound to be in the range of 109800-5300 cps, which indicates that the prepared creams were easily spreadable with small amount of shear. The viscosity results of prepared cream formulations are shown in table 8. Figure

13shows the effects of shearing forces on viscosity of various formulations. At low shear the viscosity of the creams was greater than higher shearing rates. As the shear was progressively increased the viscosity of the creams were also get reduced which indicates that a minimum force or shear is required to initiate the flow exhibiting good pseudo plastic flow behavior.

Table 8: Viscosity data of formulation batches

RPM	Viscosity in cps		
	Control	Cream (F3)	Cream (F6)
5	119800	109800	109790
10	59160	53160	53154
25	27390	21207	17650
50	17470	15470	15464
75	11700	10700	10692
100	10500	8500	8490
150	7200	5300	5287

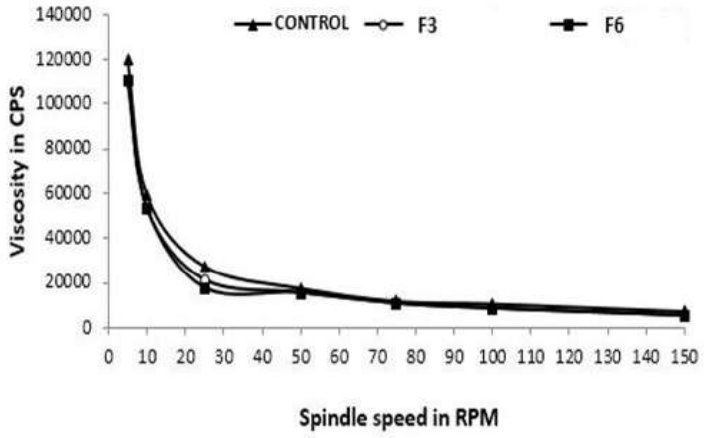

Figure 13. Effects of viscosity of cream formulations at different shearing rates

6.4.3.Stability study results:

From the stability study, the creams were found to be stable with respect to p^H, viscosity, spreadability, emolliency and phase separation. There was no sign of phase separation after 10 and 40 minutes centrifugation at 2000 rpm.

6.4.4. Selection of Formulation: From the above study we have selected the best formulation on the basis of physical stability and the desired percentage of active ingredients. The formulation that was taken for further pharmacological evaluation was **formulation 6 (F6)**.

6.4.5. Results of skin irritation test:

This test was conducted to evaluate the irritation caused by the prepared cream on the intact skin of animals. The results showed that the formulation (F6) was devoid of any primary skin irritation or sensation or erythema, or edema even after 48 hrs of application on the rat skin. None of the animal shows any skin reaction.

6.5.Results of wound healing activity:

The results of wound healing activity of the prepared Polyherbal cream (F 6) on the animal model are presented in the following figure:

Figure 14: wound at day 0

Figure 15: wound healing after day 9

Figure 16: wound healing after day 15

6.6.Excision wound study:

The results of wound healing activity by excision wound model are presented in Table 9 and figure 17, 18. The values of wound area are presented in cm at 0, 3, 6, 9, 12, 15

and 18 days. The results indicate that standard cream (povidone iodine treated group/betadine) and test herbal cream both significantly reduces the wound area as compared to the control group (simple base treated group).

The other table. Table 10 and figure 19, 20 represents percentage wound healing (wound contraction) at 9 and 18 days for control, standard and the test groups. It is observed that wound contracting ability of animals treated with herbal cream and standard cream significantly higher ($P < 0.01$) on days 9 and 18 as compared to the control group.

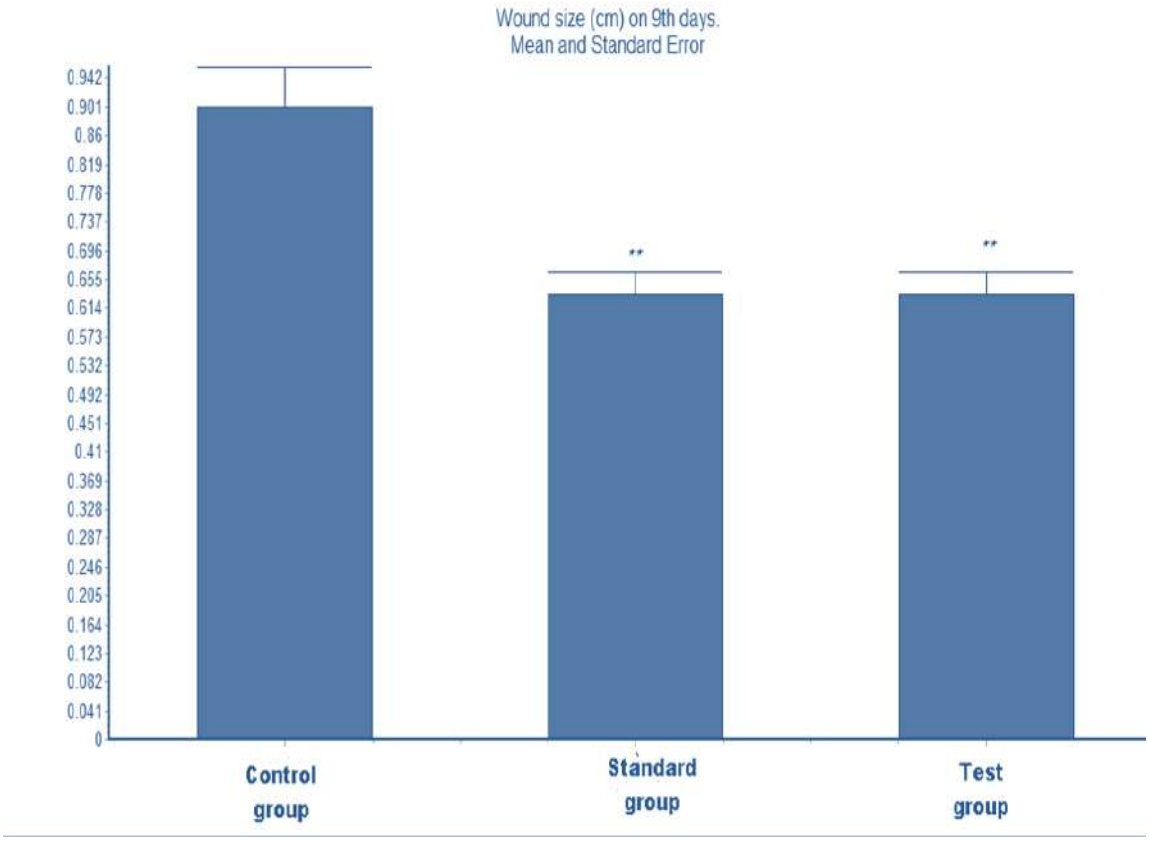

Figure 17: Wound size on 9th days (cm) with mean and standard error.

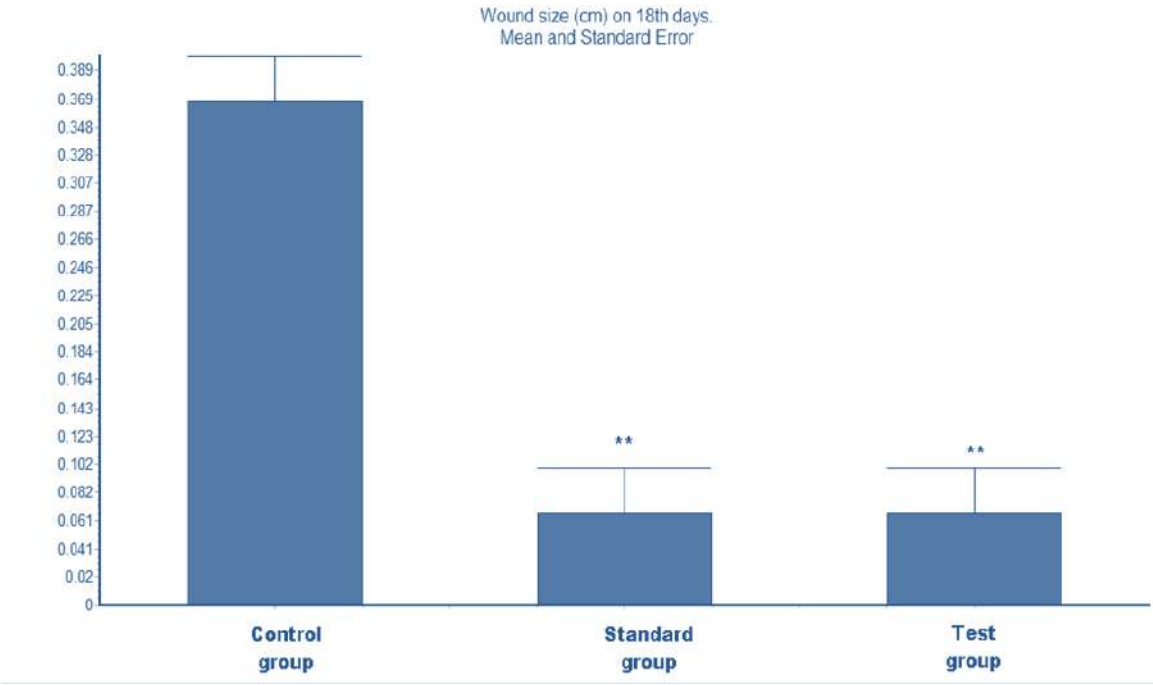

Figure 18: Wound size on 18th days (cm) with mean and standard error.

Table 9: Wound size/area in cm (mean ± SEM) at different days interval

Groups	Wound size/area in cm (mean ± SEM) at different days interval						
	0 days	**3 days**	**6 days**	**9 day**	**12 days**	**15 days**	**18 days**
Control	1.5	1.46 ± 0.33	1.20 ± 0.05	0.90 ± 0.05	0.70 ± 0.05	0.50 ± 0.05	0.36 ± 0.03
Standard	1.5	1.33 ± 0.05	0.93 ± 0.03	0.63 ± 0.03	0.43 ± 0.03	0.20 ± 0.05	0.06 ± 0.03**
Test	1.5	1.33 ± 0.05	0.96 ± 0.03	0.63 ± 0.03	0.46 ± 0.06	0.20 ± 0.05	0.06 ± 0.03**

The treated groups are compared with the control group.
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

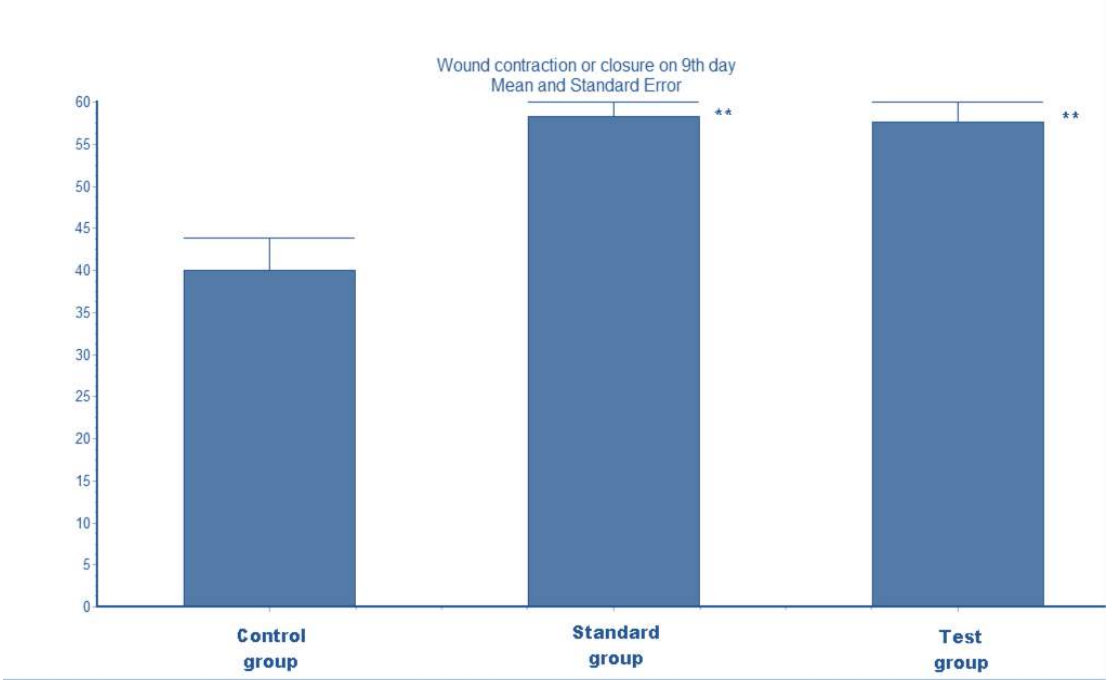

Figure: 19: Wound size on 9th days (cm) with mean and standard error.

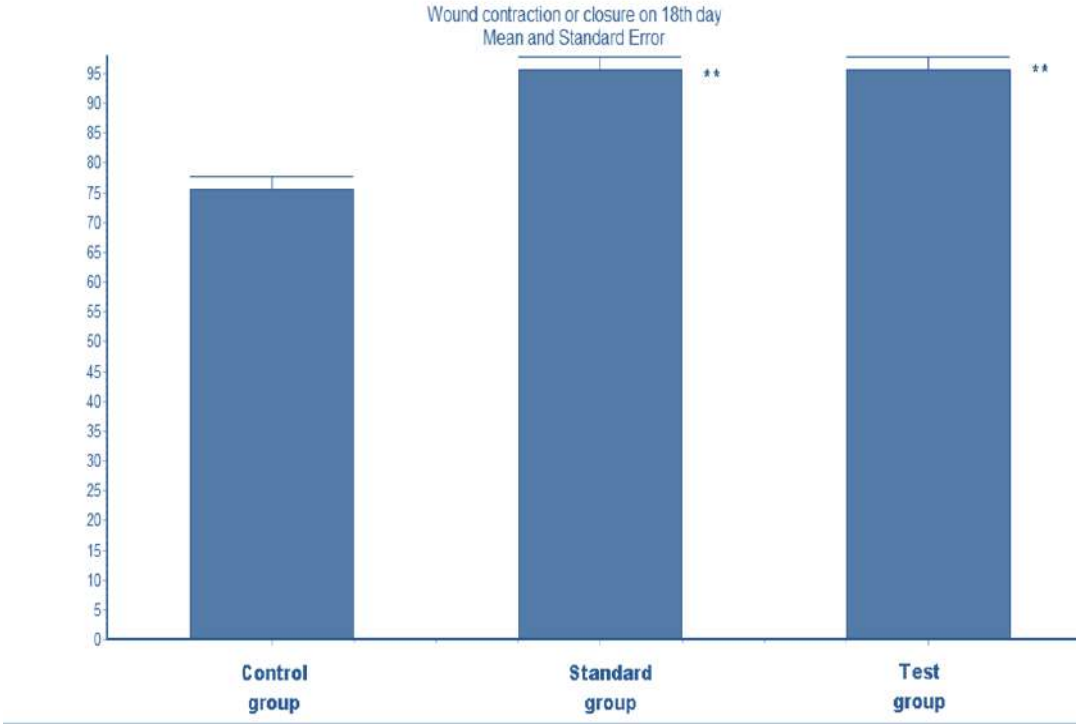

Figure 20: Wound size on 18th days (cm) with mean and standard error.

Table 10:Effect of herbal cream on wound contraction of excision wound (mean percentage ± SEM) at different days interval

Groups	Effect of herbal cream on wound contraction of excision wound (mean percentage ± SEM) at different days interval		
	0 days	**9 day**	**18 days**
Control	00	39.96 ± 3.83	75.33 ± 3.85
Standard	00	58.33 ± 1.66	95.53 ± 3.85 **
Test	00	57.66 ± 2.33	95.53 ± 3.85 **

The treated groups are compared with the control group.
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

6.7.Results of histopathological study:

Histopathological study report:-

On the histopathological examination of the wound sections, the main activity observed was the proliferation of fibroblasts, granulation tissue, collagen fibre and tissue remodeling.

When compared to control groups the above mention parameters were more prominent in case of both standard and test group. This histopathological observation provided additional evidence for the experimental wound healing studies based on the contraction value of wound areas. The details of histopathological data are given below.

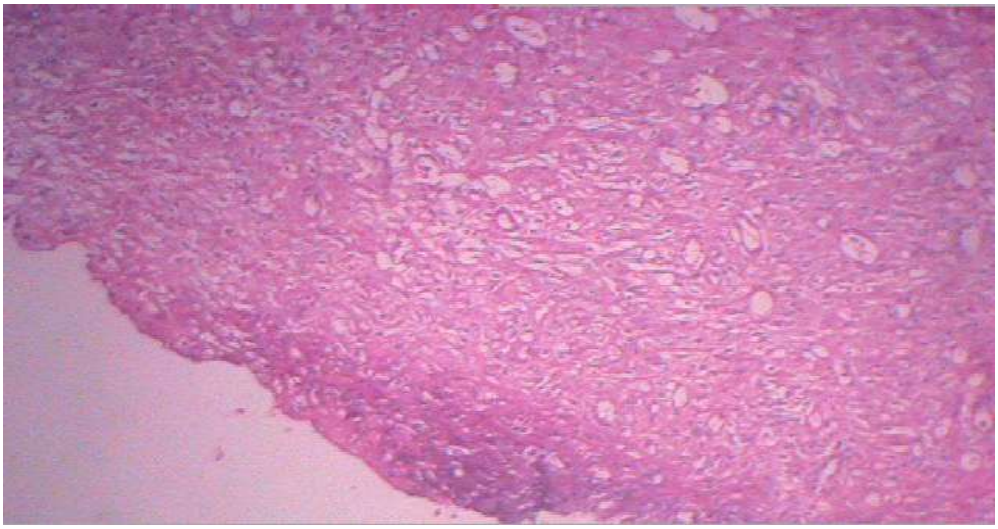

Figure 21: Histological image of control at HEX 10

The granulation tissue showing large number of newly formed blood vessels with RBC inside the lumen. The granulation tissue constituting of fibroblast shows many tinny empty spaces indicating incomplete link of the injured part. Though fibroblast with collagen are also seen in the lesion.

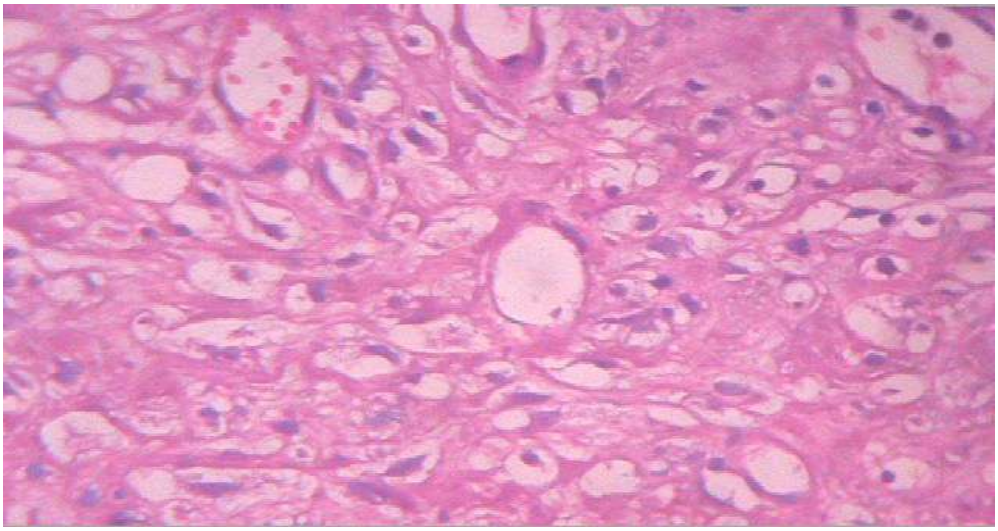

Figure 22: Histological image of control at HEX20

In the higher magnification of figure 21 showing the dilated and newly formed capillaries with number of fibroblast and immature collagen with plenty of empty spaces in between.

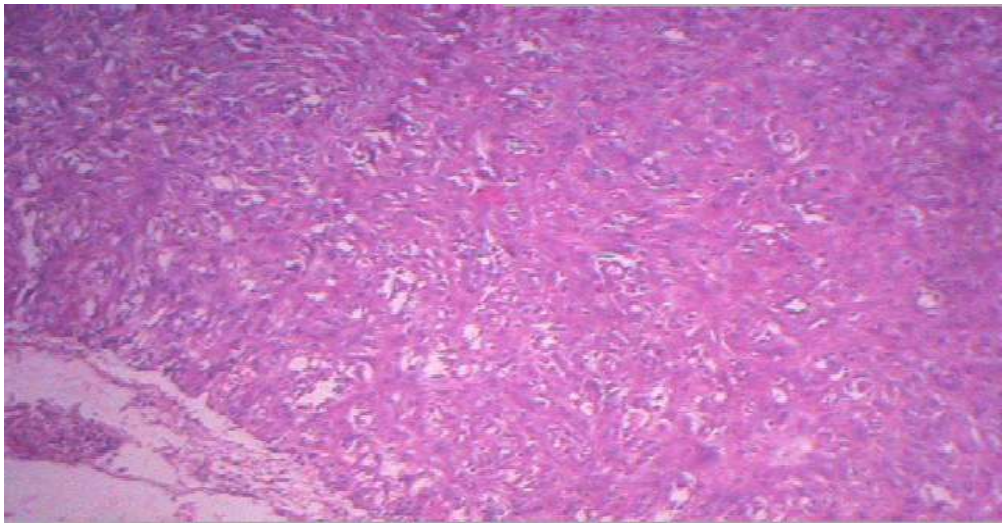

Figure 23: Histological image of standard at HEX10

Granulation tissue at top of the wound with newly formed blood vessels and parallel arranged fibroblast.

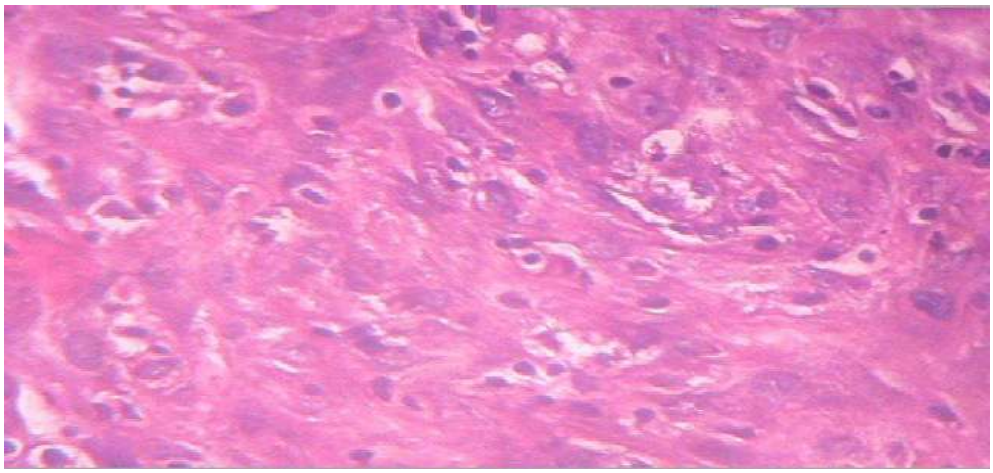

Figure 24: Histological image of standard at HEX20

The arrangement of fibroblast with collagen in the deeper position of the granulation tissue. The collagen has almost filled the gap.

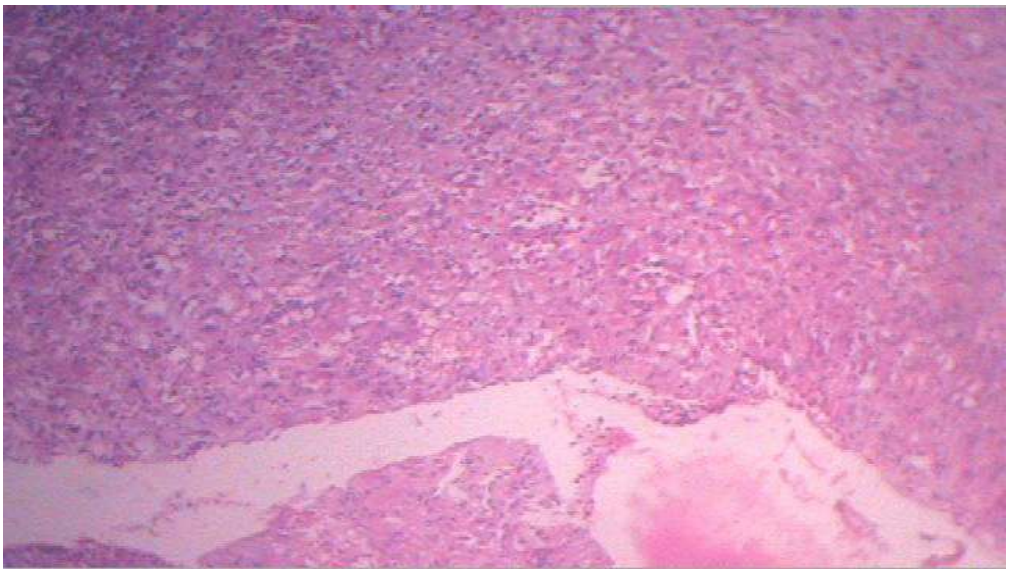

Figure 25: Histological image of test at HEX10

The granulation tissue with scar formation. The granulation tissue is almost filling the injured part having abandoned fibroblast with organized collagen and only a very few blood vessels and some phagocytic cells involve in remodeling of the granulation tissue.

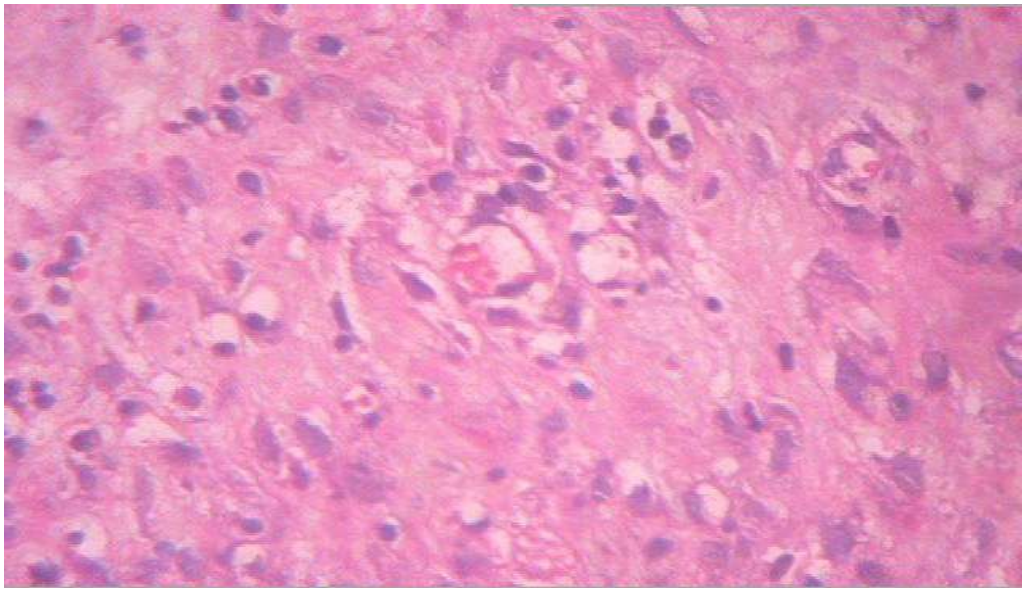

Figure 26: Histological image of test at HEX20

The higher magnification of figure 25 showing organization of the granulation tissue. Showing a few blood vessels matured collagen, fibroblast and some inflammatory cells.

Chapter 7

CONCLUSION

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CONCLUSIONS

Our present study it concludes that the herbal extract cream based formulations will be useful for the treatment of affected wound when experimented on lab animals. The prepared cream was pleasant, coolant, effective, easily washable thereby increases the patient compliance. However, further structured study, especially in human phase, would be beneficial to assess its usefulness more exactly. Not only that, this study can be helpful for upcoming researchers to select this herb for the formulation and evaluation of other cosmetic applications which can be claimed for their efficacy with scientific data, which shall further give strength to our herbal and cosmetic industries eminence in global market.

REFERENCE

REFERENCES:-

1) Reddy AKG.,Saranya SC., Kumar ACK. "Wound healing potential of Indian medicinal plants",International Journal of Pharmacy Review & Research,2012; 2(2):75-87.
2) Kumar B., Kumar VM., Govindarajan R., Pushpangadan P. "Ethnopharmacological approaches to wound healing—Exploring medicinal plants of India", Journal of Ethnopharmacology, 2007;114:103–113.
3) Rajsekhar S. "Unseen aspects of wound healing: an overview", International Journal of Pharma and Bio Sciences,2011;2(4):275-287.
4) Mekonnen A.,Sidamo T., Asres K., Engidawork E. "In vivo wound healing activity and phytochemical screening of the crude extract and various fractions of *Kalanchoe petitiana* A. Rich (Crassulaceae) leaves inmice", Journal of Ethnopharmacology, 2013; 145: 638–646.
5) Pandey DK., Dwivedi PK., Sharfuddin C., Kumar P. "Wound healing potential of a herbomineral skin ointment: a comparative study" International Journal of Life Sciences Biotechnology and Pharma Research, 2012; 3: 41-49.
6) MacKay D., Miller AL., "Nutritional support for wound healing", Alternative Medicine Review, 2003; 8: 359-377.
7) Soni H., Singhai AK. "A recent update of botanicals for wound healing activity", International Research Journal of Pharmacy, 2012; 3(7): 1-7.
8) Geol A., Kumar S., Singh DK., Bhatia AK. "Wound healing potential of *Ocimum sanctum* Linn. with induction of tumor necrosis factor- α", Indian Journal of Experimental Biology, 2010; 48: 402-406.

9) Sanwal R., Chaudhary AK. "Wound healing and antimicrobial potential of *Carissa spinarum*Linn. in albino mice", Journal of Ethnopharmacology, 2011; 135: 792-796.

10) Sreenivasan S., Rajoo N., Rathinam X., Lachimanan YL., Rajoo A. "Wound healing potential of *Elaeisguineensis* Jacq leaves in an infected albino rat model", Molecules, 2.010; 15: 3186-3199.

11) Nayak BS., Pereira LP., Maharaj D. "Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats", Indian Journal of Experimental Biology, 2007; 45: 739-743.

12) Jagtap NS.,Khadabadi SS., Farooqui IK., Nalamwar VP., Sawarkar HA. "Development and evaluation of herbal wound healing formulations", International Journal of Pharmaceutical Technology and Research, 2009;1(4):1104-1108.

13) Gidwani B., Alaspure RN., Duragkar NJ., Singh V., Rao SP., Shukla SS. "Evaluation of a Noval herbal formulation in the treatment of eczema with psoraleacorylifolia", Iranial Journal of Dermatology , 2010; 122-127.

14) Shah C., Nayak B., Gaudani R., Patel J., Modi H. "Formulation and evaluation of antimicrobial polyherbal cream", An International Journal of Pharmaceutical Sciences,2012; 2715-2722.

15) Singh M., Sharma S., Khokra LS.,Kumar SR. "Preparation and evaluation of herbal cosmetic cream", Pharmacologyonline,2011; 5(2):1258-1264.

16) Rao PK., Khaliq K., Sagare P., Patil SK., Kharat SS., Alpana K. "Formulation and evaluation of vanishing cream for scalp psoriasis", International Journal of Pharma Science Technology,2010;4(1): 32-41.

17) Kaale E., Schepdael AV., Roets E., Jos H. "Determination of capsaicinoids in topical cream by liquid- liquid extraction and liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 2002; 3(30): 1331-1337.

18) Das K., Dang R., Machale MU., Ugandar R.,Lalitha B. "Evaluation for safety assessment of formulated vanishing cream containing aqueous Stevia extract for topical application, Indian Journal of Novel Drug delivery,2012;4(1):43-51.

19) Khalid A S .,Saringat H J., Khan GM. "Haruan (*Channastriatus*) incorporated palm-oil creams: Formulation and stability studies",Pakistan Journal of Pharmaceutical Sciences,2005;18(1):1-5.

20) Mahalingam R C., Xiaoling L., Bhaskara RJ. "Semisolid Dosages: Ointments, Creams and Gels", Pharmaceutical Manufacturing Handbook, 2006; 2(3): 267-274.

21) Upadhyay N K., Kumar R., Mandotra S K., Meena RN.,Siddiqui MS.,Sawhney RC., Gupta A. "Safety and healing efficacy of Sea buckthorn (*Hippophaerhamnoides* L.) seed oil on burn wounds in rats", Food and Chemical Toxicology, 2009; 3(47):1146–1153.

22) Lipipun V., Sasivimolphan P. Yoshida Y.,Daikoku T.,Boonchoo S.,Garnpimol R.,Likhitwitayawuid K.,Pramyothin P.,Hattori M.,Shiraki K. "Topical cream-based oxyresveratrol in the treatment of cutaneous HSV-1infection in mice,Antiviral Research, 2011; 7(91):154-160.

23) Guru B.,Prabhu S. "Semisolid preparations",Encyclopedia of PharmaceuticalTechnology, 2008; 3(1):3257-3273.

24) Gupta A., Steven PS., Paul BM."An HPLC method for quantitation of perillyl alcohol in a topical pharmaceutical cream formulation", Journal of Pharmaceutical and Biomedical Analysis, 2005; 8(37): 447–452.

25) Singh AP., Ashwinkumar VM "Clinical evaluation of a herbal formulation 'Scavon vet cream' on the wound healing in domestic animals", Indian Veterinary Medical Journal, 2002; 2(26):73-74.

26) Flower A., Lewith G., Paul L. "Combining rigour with relevance: A novel methodology for testing Chinese herbal medicine", Journal of Ethnopharmacology, 2011; 4(134): 373–378.

27) Saleea R.,Rizwana R., Ahmed M., Sadaf F.,Ahmad SI., Zafar N., Khan SS., Siddiqui BS., Ansari F., Khan SA.,Faizi S. "Effect of cream containing *Meliaazedarach* flowers on skin diseases in children", Phytomedicine, 2008; 3(15): 231–236.

28) Nagulwar DB., Bhoyar PK., Baheti JR., Biyani DM., Mundhada DR., Prasad PK."Development and validation of herbal antiseptic topical formulation", World Journal of Pharmacy and Pharmaceutical Sciences,2009;1(2): 674-692.

29) Kataki MS. "A_Tibacterial activity, *In Vitro*activity of ethanolic extract of *Ananascomosus leaves*",Pharmacologyonline, 2010; 2(1): 308-319.

30) Nair SS., Mathew M., Sreena K. "Formulation and evaluation of herbal cream containing *Curcuma longa*", International Journal of Pharmaceutical and Chemical Sciences, 2012; 1(4):1362-1368.

31) Rajvanshi A., Sharma S., Khokra SL., SahuRK.,JangdeR. "Formulation and evaluation of *Cyperusrotudus*and *Cucumissativus* based Herbal Face Cream", Pharmacology online, 2011; 2:1238-1244.

32) Richards GM., Oresajo CO., Halder RM. "Structure and function of ethnic skin and hair", Dermatol Clin, 2003; 21: 595-600.

33) Montaga W. "The architecture of black and white skin", Journal of American Academic Dermatol, 1991; 24: 29-37.

34) Fleming ID., Barnawell JR., Burlison PE., Runkin JS. "Skin cancer in black patients", Cancer, 1975; 35: 600-605.

35) Olson RL., Gaylor J., Everett MA. "Skin color, melanin and erythema", Architecture Dermatol, 1973; 108: 541-544.

36) Chakravorty RC., Dutta-Chowdhury R. "Malignant neoplasms of the skin in eastern India", Indian Journal of Cancer, 1968; 5: 133-144.

37) Kolkane DD., More RY., Kale MB., Nehete MN., Mehendale PC., GadgoliCH. "Evaluation of wound healing activity of root of *Mimosa pudica*",Journal of Ethnopharmacology,2009;124: 311–315.

38) Akkol K. E., Koca U., Pesin I., Yilmazer D. "Exploring the wound healing activity of *Arnebia densiflora* (Nordm.) Ledeb by in vivo models", Journal of Ethnopharmacology, 2009; 124: 137-141.

39) Patel NA., Patel M., Patel RP. "Formulation and evaluation of Polyherbal gel for wound healing" International Research Journal of Pharmaceuticals, 2011; 1: 15-20.

40) Shafiuddin Md., Khan A., Ali S. "Wound healing activity of traditional herbalformulation", International Journal Chemical and Science, 2009; 7(2): 639-643.

41) Viswanathan V., Kesavan R., Kavitha KV.,Kumpatla S. "A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers", Indian Journal of Medical Research, 2011; 3(134):168-173.

42) Sahu AN., Jha S., Dubey SD. "Formulation & evaluation of curcuminoid based herbal face cream, Indo-Global Journal of Pharmaceutical Sciences, 2011;1(1): 77-84.

43) Chhetri HP., Yogol NS., Sherchan J., Anupa KC.,Mansoor S., Thapa P. "Formulation and evaluation of antimicrobial herbal ointment", Kathmandu University Journal Of Science, Engineering And Technology,2010;6(1):102-107.

44) Kotta KK., Sasikanth K., Sabareesh M., Dorababu N. "Formulation and evaluation of diacerein cream", Asian Journal of Pharmaceutical and Clinical Research, 2011;4(2):93-98.

45) Panda PD., Nayak SS., Panda DP."Formulation and evaluation of cream prepared form *Pandanus fascicularis* Lamk and their wound healing activity",Drug Invention Today, 2010;2(9):417-420.

46) Aswal A., Kalra M., Rout A. "Preparation and evaluation of polyherbal cosmetic cream", Asian Pacific Journal of Tropical Biomedicine, 2012; 1-4.

47) Paithankar VV."Formulation and evaluation of herbal cosmetic preparation using *Safedmusli*", International Journal of Pharmaceutical Technology and Research, 2010; 2(4): 2261-2264.

48) Baravkar1 AA., Kale RN., Patil RN., Sawant SD. "Pharmaceutical and Biological Evaluation of formulated cream of methanolic extract of*Acacia nilotica* leaves", Research Journal of Pharmacy and Technology, 2008; 1(4): 56-63.

49) Butnariu M., Coradini ZC. "Evaluation of biologically active compounds from *Calendula officinalis* flowers using Spectrophotometry", Butnariu and Coradini Chemistry Central Journal, 2012;5(7):1-7.

50) Stefanache CP., Danila D., Necula R., Gille E.“Studies regarding *in vitro* regeneration of *Arnica montana*, from natural populations”, National Institute of R&D for biological sciences, 2010; 33.

51) Kataki MS. “Antibacterial activity, *In Vitro* antioxidant activity and antihelmintic activity of ethanolic extract of *Ananas comosus* tender leaves”,Pharmacologyonline, 2010; 2: 308-319.

52) Koca U., Suntar P.I. Kelesb H., Yesilada E., Akkola EK. “In vivo anti-inflammatory and wound healing activities of *Centaurea iberica*”, Journal of Ethnopharmacology, 2009; 126: 551–556.

53) Ghosh AK., Banerjee M., Mandal TK., Mishra A., Bhowmik MK. “A study on analgesic efficacy and adverse effects of *Aloe vera* in Wistar rats”, Pharmacologyonline,2011;1:1098-1108.

54) Nandal U., Bhardwaj RL. “*Aloe vera* for human nutrition, health and cosmetic use -A review”, International Research Journal of Plant Science, 2012; 3(3): 38-46.

55) Yadav KCH., Kumar JR., Basha S., Deshmukh GR., Gujjula R., Santhamma B. “Wound healing activity of topical application of *Aloe vera* gel in experimental animal models”, International Journal of Pharma and Bio Sciences,2012;3(2):63-72.

56) Davis RH., Mark GL., Joseph M., Megan E. “Wound Healing, oral and topical activity of *Aloe vera*”, Journal of The American Podiatric Medical Association,1989;79(7):559-562.

57) Edzard E.“*Panax ginseng*: An overview of the clinical evidence”, Journal on Ginseng Researsh, 2010; 34(4):259-263.

58) Attele AS., Wu JS.,Chun SY. "Ginseng pharmacology multiple constituents and multiple actions", Biochemical Pharmacology, 1999; 58(3):1685-1693.

59) Nocerino E., Amato M., Angelo A. "The aphrodisiac and adaptogenic properties of ginseng", Fitoterapia, 2000; 71: 1-5.

60) Stefanache CP., Danila D., Necula R., Gille E. "Studies regarding *in vitro* regeneration *of arnica montana* from natural populations - bistrita valley (eastern carpathians)", National Institute of R&D for Biological Sciences, 2010; 33-42.

61) Asdal A., Labokas J., Olsson K., Radušienė J., Bladh JR. "Ecotypic exploration and characterization trials to promoteconservation of *Arnica montana* L. in Northern Europe", European Crop Wild Relative Diversity Assessment and Conservation Forum, 2010; 60-65.

62) Akhtar N., Zaman S., Khan BA., Haji M., Khan M., Mahmood A.,Rasool F., Mahmood T.,Rasul A. "Evaluation of various functional skin parameters using a topical cream of *Calendula officinalis* extract", African Journal of Pharmacy and Pharmacology,2011; 5(2): 199-206.

63) Bernatoniene J.,Masteikova K., Davalgiene M., Peciura J., Gauryliene R., Bernatoniene R.,Majiene J., Lazauskas R., Civinskiene G., Velziene S., Muselik J.,Chalupova Z. "Topical application of *Calendula officinalis*:Formulation and evaluation of hydrophilic cream with antioxidant activity", Journal of Medicinal Plants Research,2011;5(6):868-877.

64) Gazim ZC., Rezende CM.,Fraga SR., Benedito B.,Filho P., Nakamura CP., Cortez DA. "Analysis of the essential oils from *Calendula officinalis* growing in Brazil using three different extraction procedures", Brazilian Journal of Pharmaceutical Sciences, 2008; 44(3): 391-395.

65) Albulescu M., Alexa N.,Cojan J. "*Calendula officinalis* flowers, Source of extracts with antioxidant activity", Series Chemistry,2004;13(2): 169-176.

66) Raiman SZ, Biswas P, Monir MM, biswas SK, chowdhury A., Rahman AKMS. "Phytochemical investigation and *in-vitro*antinociceptive activity of *Clerodendrum indicum* leaves", Pakistan Journal of Biological Sciences, 2012; 15(3): 152-155.

67) Malec IS., Civeira ME., Vigo MS. "Seeds of *Rosa rubiginosa* L.(*Rosa mosqueta)* Chemical composition of seed oil and seed meal residue", Anales de la Asociacion Quimica Argentina, 1993; 81: 445-450.

68) Babu AV., Govindarao M., Chandra R., Reddy D., Harish B., Vishwanath J., Reddy G. "Antidiabetic activity of hydroalcoholic extract of *Ananas comosus* leaves in Streptozotocin induced Diabetic rats", International Journal Of Pharmacy,2012; 2(1): 142-147.

69) Ilango K., Chitra V. "Wound healing and anti-oxident activities of the fruit pulp of *Limonia acidissima* Linn (Rutaceae) in rats", Trop Journal of Pharmaceutical Research, 2010; 9(3): 223-230.

70) Karodi R., Jadhab N., Rub R., Bafna A. "Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L.(Indian madder) in mice, International Journal of Applied Research in Natural Products, 2009; 2(2): 12-18.

71) Nayak B.S., Pereira L.P., Maharaj D. "Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats", Indian Journal of Experimental Biology, 2007; 45: 739-743.

Appendix

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)

(AUnit of ShrimantaShankarAcademy)

Approved by AICTE, New Delhi, Affiliated to GauhatiUniversity

N.H. 37, Hathkhowapara, Azara, Guwahati-781017

Telephone: (361) 2843405,09957184005(Principal) E-mail: gips_guwahati@rediffmail.com

CERTIFICATE

This is to certify that the project entitled "Formulation and Evaluation of a Herbal Cream for Wound Healing" has been approved by the IAEC, GIPS with the animal and number as per condition given below:

1. Proposal number:- Mph-3
2. Approval No:- *GIPS/IAEC/MPH/20013/3*
3. Date of approval:- 3.5.2013
4. Expiry date: - 3.6. 2013
5. Species of animal used:- Rat
6. No of Animal permitted: - 9 (nine only).

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE AZARA, GUWAHATI-17

29/5/13

Chairman, IAEC, GIPS
(Dr. Suvakanta Dash)

Dr. Suvakanta Dash M. Pharm, Ph. D.
Principal
Girijananda Chowdhury Institute
of Pharmaceutical Science
Azara, Guwahati - 17

Histopathology of samples of skin wound healing brought by Sri Trailokya Das , a M. Pharm student of Girijananda Choudhury Institute of Pharmceutical Science.

Standard:-

The injured part shows mark proliferation of granulation tissue where the fibroblast at the top of the wound are arranged parallel to the newly formed blood vessels. In the deeper layer the fibroblast are arranged in perpendicular position to the blood vessels. These are abundant secretion of collagen by the fibroblast which are still having vesiculated and oval shaped nuclei. A few areas so presence of scattered infiltration by mono- nuclear cells.

Control:-

The granulation tissue shows a large number of newly formed and dilated blood vessels containing RBC. in the lumen. Fibroblast with varying amount of collagen are found running parallel at the top and perpendicular at the deeper layers respectively are seen. The granulation tissue is not fully organized.

Test:-

The granulation tissue made up of a few newly formed blood vessels with a large number of fibroblast with abundant collagen filling most of the injured part. The collagen fibres are arranged parallel to the blood vessels at the top and perpendicular to the blood vessels at the deeper layers. The granulation tissue is in the process of remodelling in the upper layers where a number of mono- nuclear cells are seen. The scar tissue is slightly detached from the top of the lesion

T. N. Upadhyaya

30/5/13

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