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PREPARATION AND EVALUATION OF HERBAL GEL FORMULATION

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Abstract:-

Herbal medicines is still the mainstay of about 75-80% of the world's population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. Herbal medicines consist of plant or its part to treat injuries, disease or illnesses and are used to prevent and treat diseases and ailments or to promote health and healing. It is a drug or preparation made from a plant or plants and used for any to such purpose. Herbal medicines are the oldest form of health care known to mankind. Gel formulations prepared with Carbopol 934, HPMC K 100 M and Xanthan gum showed good homogeneity, no skin irritation, good stability and anti-inflammatory activity. However, the Xanthan gum based gel proved to the formula of choice, since it showed the highest percentage of extrudability, good spreadability and rheological properties. Formulation F_5 with 1 % leaves extract and F_{11} with 1% root extract of *Clerodendrum serratum* showed the best formulation with significant anti-inflammatory activity. Formulation $_5$ and F_{11} shows approximately equal anti-inflammatory activity. Hence, there is no need to used roots for the preparation of medicines for anti-inflammatory action.

INTRODUCTION:-

Inflammation:

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to response that results from other type of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion.[1] Inflammation dilutes, destroys, or walls off harmful agents that have entered the body. It activates a sequence of biological events to heal the damage. The most common causes of inflammation are infections, burns and trauma, and many types of immune reactions.

Classification of inflammation:

Inflammation may broadly classify into three categories;

1. Acute inflammation;
2. Chronic inflammation;
3. Miscellaneous;

Topical Drug Delivery System:

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.[3]

•External topical that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area.

•Internal topical that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

Advantages of Topical Drug Delivery System: [5]

•Avoidance of first pass metabolism.

•Convenient and easy to apply.

•Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes,

•Achievement of efficacy with lower total daily dosage of drug by continuous drug input.

•Avoids fluctuation in drug levels, inter- and intrapatient variations.

•Ability to easily terminate the medications, when needed.

•A relatively large area of application in comparison with buccal or nasal cavity

•Ability to deliver drug more selectively to a specific site.

•Providing utilization of drugs with short biological half-life,

•Improving physiological and pharmacological response.

•Improve patient compliance.

•Provide suitability for self-medication.

Disadvantages of Topical Drug Delivery System:[5]

•Skin irritation of contact dermatitis may occur due to the drug and/or excipients.

•Poor permeability of some drugs through the skin.

•Possibility of allergenic reactions.

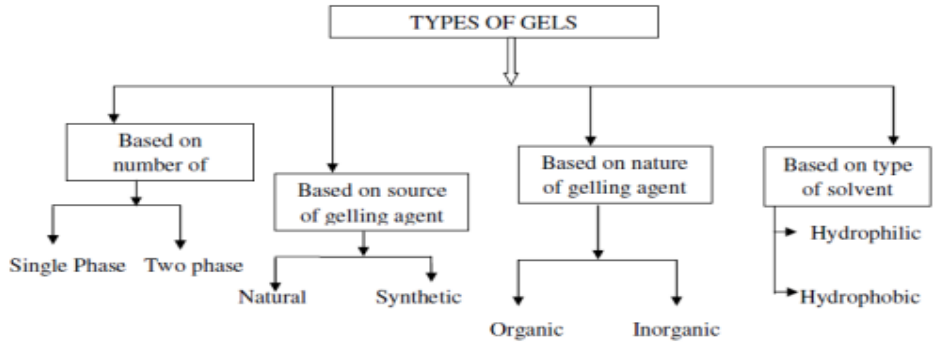
•Can be used only for drugs which require very small plasma concentration for action

•Enzyme in epidermis may denature the drugs

•Drugs of larger particle size not easy to absorb through the skin

Classification of Topical Drug Delivery System:

Classification of Topical Drug Delivery System based on physical state-



Introduction to Herbal Medicines: [6-8]

Ever since the birth of mankind of there has been a relationship between life, disease and plants. There is no record that people in prehistoric times used synthetic medicines for their aliments but they tried to make use of the things they could easily procure. The most common thing they could find was there in environment i.e. the plants and animals.

World Health Organization (WHO) has defined herbal medicines are finished, labeled medicinal products that contain active ingredients, aerial or underground parts of the plants or other plant material or combination. Herbal formulations have reached widespread acceptability as therapeutic agents like anti-microbial, anti-diabetic, anti-ageing, anti-arthritic, anti-depressant, anti-anxiety, anti-inflammatory, anti-HIV, treatment of cirrhosis, asthma, migraine, Alzheimer's disease and memory enhancing activities.

Skin

The skin is a most extensive and readily accessible organ of the human body. The skin of the average human being cover an area of about 2 square meter and weighs 4.5-5 kg, about 16 % of body weight. It also receives 1/3 rd of the total blood supply. Most topical preparation are meant to be applied to the skin and hence basic knowledge of skin and its physiological function and biochemistry is very important for designing topical formulations. The pH of the skin varies from 4 to 5.6. Sweat and fatty acids secreted from sebum influence the pH of the skin surface. It is suggested that acidity of the skin helps in limiting or preventing the growth of pathogens and other organisms.[9,10]

Anatomy-Physiology of skin:-[9,11]

The skin is multi-layered organ and anatomically has many histological layers. Skin is an anatomic barrier between the body and its environment and contributes to about 16-18% of normal body weight. The overlaying outer layer is called epidermis; the layer below epidermis is called dermis. Beneath the dermis are subcutaneous fatty tissues.

Gel:

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass

enclosing and interpenetrated by a liquid. Gels and jellies are composed of small amount of solids dispersed in relatively large amount of liquid, yet they posses more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some form of cutaneous structure, which provides solid-like properties.

DRUG AND POLYMER PROFILE

Plant Profile:

Clerodendrum serratum [1-4]

Bharangi is botanically termed as *Clerodendrum serratum*. *Clerodendrum serratum* Linn. Is a genus of flowering plants in the **Verbenaceae. family. Bharangi** grows throughout India.

Ayurvedic Properties and Actions:

Rasa : Katu, Tikta, Kasaya
Guna : Laghu, Ruksa
Virya : Usna
Vipaka : Katu
Karma : Dipana, Kaphahara, Pacana, Vatahara, Swasahara

•Habit: [5]

Clerodendrum serratum is a perennial shrub 0.9-2.5 m high.

Stem- Scarcely woody not much branched, bluntly quadrangular and young parts are usually glabrous.

Leaves- are sessile or nearly so and opposite or sometimes ternate, passing upwards into bracts. 12.52-15 by 5.7-6.3 cm, sometimes reaching up to 28 cm long, narrowly obovate-oblong or sub-elliptic, acute base, acuminate tip, coarsely and sharply serrate margins and glabrous. Petioles are very stout and 6 cm long.

Flowers:-

Numerous, in lax pubescent dichotomous cymes with a pair of acute bracts at each branching and a flower in the fork, each in the axial of a large leafing bract and collectively forming a long lax terminal usually pyramidal erect penicle 15-25 cm long; pedicels often twisted so as to make the large lower corolla.

Bracts-

1.3-3.8 cm long, from obovate to lanceolate, pubescent, and often coloured.

Fruit-

Fruit is drupe 6 cm long, somewhat succulent, broadly obovoid, dark purple When ripened.

EXPERIMENTAL:

Methods:

Preformulation study:

Preformulation studies are needed to ensure the development of a stable as well as effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and its interaction with various formulation components. Goals of Preformulation study:

- To determine the necessary physicochemical parameter of a new drug substance.
- To establish its incompatibility with excipients of formulation.

PHARMACOGNOSTIC INVESTIGATION:

1. Collection and Authentication:

Collection of *Clerodendrum serratum* (Linn) moon.

The fresh leaves and roots of *Clerodendurm serratum* (Linn) moon (Verbenaceae) Were collected at the flowering stage in August from side of Koyana Ricer, K o y a n a Tal-Patan (Dist- Satara), Maharashtra State, India.

B. Organoleptic Characterization:

Colour, odour, shape, test and size of the rhizomes and bark were observed

C. Physicochemical Characters:

After botanical evaluation, the shade-dried plant material were subjected to size reduction to get coarse powder and then passed through sieve no. 43 to get uniform powder. Then, the uniform powder was subjected to standardization with different parameters as per literature.

1. Extractive values:[1,2]

a) Alcohol soluble extractive value:

Macerated 5 gm of the air dried drug coarsely powdered drug (leaves and roots), with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and followed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvents, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105^0c to constant weight and weighed.

b) Water Soluble extractive value:

Macerated 5 gm of the air dried drug, coarsely powdered (leaves and roots), with 100 ml of Chloroform-water the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and followed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvents, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105^0C, to constant weight and weighed. Calculated the % of water-soluble extractive with reference to the air dried drug.

Determination of Ash value:

a) Determination of total ash:

Incinerated about 2-3 gm accurately weighed, of the ground drug in a tared silica dish at

a temperature not exceeding 450^0C until free from carbon, cool and weight. If a carbon free ash cannot be obtained in this way, exhaust the charved mass with hot water, collected the residue on an ashless filter paper, incinerated the residue and filter paper, added ignited at a temperature not exceeding 450^0C. Calculated the % of ash with reference to the air dried drug.

b) Determination of Acid-Insoluble ash:

To the crucible containing total ash, add 25 ml of *dilute hydrochloric acid*. Collected the insoluble matter on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Transfered the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes and weighed without delay. Calculated the content of acid insoluble ash with reference to the air-dried drug.

3. Determination of Foreign Matter:

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. Take a representative portion from a large container, or removed the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray. Examined in daylight with unaided eye. Transfer suspected particles, if any, to a petri dish, and examined with 10x lens in daylight.

II. EXTRACTION:

Preparation of Ethanolic Extract of Clerodendrum serratum Linn moon.

In the present study, the leaves and roots were carefully selected washed to remove impurities and shade dried. The dried material was reduced to fine powder in the mechanical grinder. The fine powder was passed through sieve no.43 and stored in an airtight container for further use. About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus. The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed. After each extraction, the extract was evaporated to dryness in rotary vacuum evaporator. Moreover, some part of the extract was preserved for preliminary Phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of gel batches.

PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

The alcoholic extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, saponins and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. The following procedures were adopted to test for the presence of various chemical constituents in extract.

1. TEST FOR SAPONINS

Foam test

A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of Saponin.

2. TEST FOR ALKALOIDS

1. **Dragendroff's test:** Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.
2. **Mayer's test:** 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.
3. **Wagner's test:** 2-3 ml of filtrate with few drops of Wagner's reagent gives reddish brown colour.
4. **Murexide test for purine alkaloid:** To 3-4 ml. test solution add 3-4 drops of conc.HNO_3. Evaporate to dryness. Cool and add 2 drops of NH_4OH. Purple colour is observed.

3. TEST FOR CARBOHYDRATES.

Fehling's test: Mix 1ml. Fehling's A and 1ml. Fehling's B solutions boil for one minute. Add equal volume of test extract solution. Heat in boiling water bath for 5-10 min. Appearance of orange red precipitate indicates presence of carbohydrates.

ii. **Benedict's test:** Mix equal volume of Benedict's reagent and test extract in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

4. TEST FOR FLAVANOIDS :

i. **Ferric chloride test:** To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

ii. **Shinoda Test:** To dry extract, add 5 ml. of 95% ethanol, few drops conc. HCL and 0.5 g magnesium turnings. Pink colour observed.

iii. **Zinc**-hydrochloric acid-reduction test: Test solution with zinc dust and few drops of HCL shows magneta red colour

iv. **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

v. **Lead acetate solution test:** Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

5. TEST FOR PROTEINS :

i. Biuret test:

a) Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath. Appearance of red or violet colour indicates presence of proteins.

b) To 3 ml. extract add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violate or pink colour appears.

ii. Million's test: Test solution treated with million's reagent and heated on a water bath,

iii. Xanthoprotein test: Test solution treated with conc. nitric acid and on boiling gives yellow precipitate.

iv. Ninhydrine test: Test solution treated with Ninhydrine reagent gives blue colour.

6. TEST FOR GLYCOSIDES

i. Baljets test: Treat the extract with sodium picrate solution. Appearance of yellow to orange colour indicates presence of glycoside with lactone ring.

ii. Keller-killiani test: The test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and conc. sulphuric acid is added from the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.

iii. Bromine water test: Test solution dissolved in Bromine water gives yellow precipitate.

iv. Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

7. TEST FOR AMINO ACIDS

i. **Ninhydrine test:** Heat 3 ml extract and 3 drops of 5% Ninhydrine solution in boiling water bath 10 min. Purple or bluish color appears.

ii. **Test for Tyrosine:** Heat 3 ml extract and 3 drops of Million's reagent. Solution shows dark red color.

iii. **Test for cysteine:** To 5 ml of extract add few drops of 40% NaOH and 10% lead acetate solution. Boil. Black ppt. of lead sulfate is formed.

8. TEST FOR STEROIDS

i. Salkowski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml of conc. H_2SO_4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

ii. **Liebermann's reaction:** Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H_2SO_4. Blue color appears.

IV. In-vitro anti inflammatory study of extract

• Inhibition of albumin denaturation

The anti inflammatory activity of *Clerodendrum Serratum* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al* and Saket *et al* followed with minor modifications. The reaction mixture was consists of test extract and 1% aqueous solution of albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCL. The sample extract were incubated at 37^0C for 20 min and then heated to 51^0C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition= (abs Control-Abs Sample) × 100/ Abs control

V. Drug-Excipients compatibility study:-

Study of interaction of the drug with excipients by physical compatibility study:-

Each excipients used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each excipient was thoroughly blended with drug

extract to increase drug-excipients molecular contacts and also to accelerate the reaction if possible. Each drug extract excipients blend was taken separately into vials and kept for one month study at 40^0c and at 75% RH for 2 weeks and observe the changes. After 30 days storage of drug extract with excipients in various ratio at room temperature, samples were observed for physical changes but there were no physical changes observed in the mixture of Clerodendrum Serratum extract and polymer combination.

7.2 Experimental design:

During formulation three gelling agents used at two different concentrations, resulting in six different batches of gels for leaves extract and six batches for root extract, total twelve batches prepared. In this case Carbopol 934, HPMC K 100 M and Xanthan gum, these three types of gelling agents were taken. Three gelling agents were used as follows:

a. Carbopol 934 (at concentration 1% and 1.5%)

b. HPMC K 100 M (at concentration 1% and 1.5%)

c. Xanthan gum (at concentration 1% and 1.5%) Gel composition was finalized after doing many trial and errors. And the composition finalized is described here. Same experimental design was applied for both types of extract which was results in total twelve batches of gel formulations. All the batches were prepared according to the experimental design.

7.3 Preparation of Gel:[9]

a) Preparation of gel with Carbopol 934: Accurately weighed Carbopol 934 was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take 5 ml of propylene glycol and required quantity of Extract. Take 5 ml propylene glycol in another beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. After all Carbopol dispersed, 1 gm extract and preservatives solutions were added with constant stirring. Finally volume made upto 100 ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

b) Preparation of gel with HPMC K 100 M: Accurately weighed 1 gm of extract was transferred to a beaker and dissolved in 10 ml of propylene glycol into which preservatives were added. HPMC K 100 M was made to disperse in distilled water then heated up to 80-90^0C with continuous stirring and it was allowed to cool. he 1 %w/v extract loaded propylene glycol solution were added to HPMC K 100 M preparation and stirred vigorously to mix in cold condition and water was added to make up the volume up to 100 ml and stirred in mechanical stirred well to get a uniform gel.

c) Preparation of gel with Xanthan gum: Accurately weighed Xanthan gum was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell the Xanthan gum for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take 5 ml of propylene glycol and required quantity of Extract. Take 5 ml propylene glycol in another beaker and add weighed quantity of

propyl paraben and methyl paraben to it and stirred properly. After all Xanthan gum dispersed, Extract and preservatives solutions were added with constant stirring.

Physicochemical evaluations:[10-13]

Physical appearance:

The prepared gel formulations containing Clerodendrum Serratum were inspected visually for their color, homogeneity, consistency and phase separation.

Measurement of pH:

The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Spreadability:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis on slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weighted was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.Spreadability was calculated using the following formula**:**

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

Where, S= Spreadability,

M= weight in the pan (tied to upper slide),

L= Length moved by the slide,

T= Time (in sec.)

Apparatus

Rheological Study:

The viscosity of the developed gel formulations was determined by using Brookfield viscometer (Brookfield viscometer RVT) with spindle No. 7.

Extrudability:

The gel formulations were filled in standerd capped collapsible aluminum tubes and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).

Optimization:

The batches were optimized by checking, and by studying physical evaluation to their

pH, viscosity, Spreadability and Extrudability of all formulation batches. By studying the evaluation parameters off all batches, batch F_5 from leaves extract gel formulation and F_{11} from root extract gel formulation were be optimized.

In-vitro skin irritation study:[14]

HET-CAM (Hen's Egg Test on the Chorioallantoic Membrane)

History: Basis was Chicken-embryo models used by embryo toxicologists and Virologist. - Test method first proposed by Luenken (1985), and Lesepke and Kemper (1986). The HET-CAM test is routinely used to evaluate the potential eye irritation of raw materials but can in some cases be used to evaluate skin irritation. Irritation causes alterations in the vascular system of the HET-CAM that result in membrane discoloration, haemorrhaging and increased perfusion.

Materials:

- White Hen's Eggs
- Fresh (not older than 7 days), fertile, clean eggs between 50 and 60 gms.
- Incubator

Preparation of test system:

Fertile 50-60 g eggs were selected and candled. The eggs which were defective were discarded.-

Incubation of the egg's for 9 days:

Eggs should be rotated at least 5 times daily for the first 8 days of incubation. Incubation for additional 24 hours without rotation.Prior to use removal of the eggshell along the air cell by means of a saw. Moistening of the white inner membrane with 0.9 % (w/v) Nacl solution; keeping egg's warm until use. Carefully removal of the inner membrane immediately prior to use (not more than 20 min after removal of the egg shell).

Treatment with the test substance: Application of test substance directly to the CAM

- Exposure of the CAM to the test substance for at least 300 sec.

End point measured by visual inspection:

- **Haemorrhage:** Bleeding out of the blood vessels of the CAM with red blood dots around the vessels.
- **Lyesis:** Optical disappearance of small blood vessels in the CAM cave. This is not a real Lyesis according to principles of general pathology.
- **Coagulation:** Thrombosis (Intravascular dark spots), extravascular blood coagulation (dark spots), denaturation of albumin.

In-vitro anti-inflammatory activity of prepared herbal gel:[15]

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of gel solution so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000µg/mL. A similar volume of double distilled water served as the control. Next, the mixture were incubated at 37 ± 2^0C in a BOD incubator for 15 minutes and then heated at 70^0C for five minutes. After cooling, their absorbance was measured at 660nm by using the vehicle as a blank. Diclofenac sodium in the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500µg/mL) was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following

formula:% inhibition= 100× [V_t/V_c-1]

Where, V_t = absorbance of the test sample

V_c = absorbance of control

Stability study:[16]

The optimized gel formulations were prepared; packed in aluminum collapsible tubes and subjected to stability studies at 40^0 C/75% RH for a period of 3 month as per ICH Guidelines. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, spreadability and extrudability.

RESULT AND DISCUTION

The present work aimed to increase stability of gel and to increase anti inflammatory activity of gel formulation with Carbopol 934, HPMC K 100 M and Xanthan gum as well as to compare natural gelling agent to synthetic gelling agent. The prepared formulations were characterized for physical appearance, pH, spreadability, viscosity, in-vitro anti inflammatory study and in-vitro skin irritation study.

Preformulation study:

Organoleptic Characterization for Leaves and Root powder:

Table No.5: Organoleptic characteristics of extract

Leaves powder	characteristics	Roof powder	Characteristic
Colour	Light reen	colour	External surface light brown
Odour	odourless	odour	Characteristic
Taste	pungent	taste	Bitter, pungent and astringent

Extraction of Clerodendrum serratum:

Table No.6: Extractive values of *Clerodendrum serratum*

sample	Extraction method	Solvent use	Wt. of sample	Extraction values(%w/w)
Clerodendrum Serratum Leaves powder	Solvent extraction	Ethanol	200	24.13
Cleordendrum serratum root powder	Soxhlet extraction	Ethanol	200	22.5

8.1.3 Determination of solubility for Leaves and Root extract:-

Table No.7: Solubility of Extract

Medium	Extract
Distilled water	soluble
Ethonal	Soluble
Chloroform	Soluble
Propylene glycol	Soluble

8.1.4 Physico-chemical Characters:- For leaves:

Table No.8: Physico-chemical Characters of Root Extract

Test	Observed value in %	Standard value
Total ASH	9	Not more than 11%
Acid insoluable ash	0.6	Not more than 1%
Alcohol soluable extraction value	505	Not more than 6%
Water soluable extract	14	Not more than 12%
Foreign matter	1.8	Not more than 2%

For roots:

Table No.9: Physico-chemical Characters of Leaves Extract

test	Observed valus
Total ASH	9.5%
Acid insoluable ash	0.4%
Alcohol soluable extraction value	6%
Water soluable extract	14.45%
Foreign matter	1.3%

No standard values are available for the leaves of Clerodendrum Serratum in Ayurvedic Pharmacopoeias. Preliminary Phytoch

Table No.10: Preliminary Phytochemical investigation

Sr.no	Chemical test	observation	Inference
1	Test for Saponin 1. Forum Test . Bromine water test	Foam persist The test solution gives yellow ppt	+ +
2	Test or alkaloid . Dragondroff's test 2. Mayer's reagent	 Orange red ppt The test solution gives cream colourppt	+ + +

	3. Hager reagent 4. Wagner's reagent 5. Murexide test	The test solution reddish brown ppt Brown ppt Purple coloured observed	+ +
3.	Test for carbonate 1. Fehing test 2. Benedict's test	Orange red ppt Sol green, yellow	+ +
4	Test for flavanoid	Appears green colour	+
5	Test for protein	Red	+
6	Test for glycoloid	Yellow	+
7	Test for amino acid	Purple	+
8	Test for steroid	Greenish yellow flurenscence	+

8.2 In-vitro Anti inflammatory study:-

Due to Restrictions of CPCSEA for the use of animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use whenother suitable methods are available. Hence, in the present study the protein/ albumin denaturation bioassay was selected for in vitro assessment of the anti inflammatory property of the herbal extract of *Clerodendrum serratum.* Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Albumin denaturation is a well documented cause of inflammation. As a part of the investigation on the mechanism of the anti inflammatory activity, ability of extract to inhibit protein denaturation was studied. the present finding exhibited a concentration-dependent inhibition of protein (albumin) denaturationby Clerodendrum serratum extract through-out the concentration range of 100 to 500µg/mL. Indomethacin, in the concentration of 100µg/mL, was used as a reference drug which also exhibited concentration-dependent inhibition of protein denaturation. There is anti-inflammatory study for leaves extract and root extract separately.

Table No.11: Effect of albumin denaturation

sample	abs	% inhibition	Root extract conc(ug/ml)	abs	% inhibition
Control	0.3905	-	Control	0.2410	-
100	0.2731	30.06	100	0.1831	24.02
200	0.2243	42.56	200	0.1443	39.61
300	0.1610	58.77	300	0.1367	42.44
400	0.1287	67.04	400	0.1029	56.57
500	0.1129	71.08	500	0.0705	70.71
indomethacin	0.1014	74.03	indomethacin	0.0615	73.53

8.3 Drug-Excipients compatibility study:-

After 30 days storage of drug extract with excipients in various ratio at room temperature, samples were observed for physical change but there is no physical change observed in the mixture of extract and polymer combination.

Table no.12: Physical Observations of Compatibility Study

batch	caking		discoloration		Liquification	
	initial	1 mon	initial	1mon	initial	1 mon
extract	No	No change	No	No change	No	No change
Carbopol 934	No	No change	No	No change	No	No change
Extract + car 934	No	No change	No	No change	No	No change
HPMC K100M	No	No change	No	No change	No	No change
Extract + HPMC K100M	No	No change	No	No change	No	No change
Xanthol gum	No	No change	No	No change	No	No change
Extract+xanthol gum	No	No change	No	No change	No	No change

8.4 Experimental design:

During formulation three gelling agents used at two different concentrations, resulting in six different batches of gels for leaves extract and six batches for root extract, total twelve batches prepared. In this case Carbopol 934, HPMC K 100 M and Xanthan gum, these three types of gelling agents were taken. Three gelling agents were used as follows:a. Carbopol 934 (at concentration 1% and 1.5%) b. HPMC K 100 M (at concentration 1% and 1.5%) c. Xanthan gum (at concentration 1% and 1.5%) All the batches were prepared according to the experimental design. For leaves extract and root extract there is same experimental design used which results in total 12 gel batches into which 6 batches for leaves extract and 6 batches for root extract.

Table No.13: Quantitative composition of leaves extract gel formulation

Composition	F1	F2	F3	F4	F5	F6
Leaves extract	1	1	1	1	1	1
Carbopol 934	1	1.5	-	-	-	-
HPMC K 100M	-	-	1	1.5	-	-
Xanthan gum	-	-	-	-	1	1.5

Propylene glycol	**10**	**10**	**10**	**10**	**10**	**10**
Methyl paraben	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**
Propyl paraben	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
Purified water	**100**	**100**	**100**	**100**	**100**	**100**
Menthol oil	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Triethonal	**q.s.**	**q.s.**	**q.s.**	**q.s.**	**q.s.**	**q.s.**

Table No.14: Quantitative composition of root extracts gel formulation

Composition	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Root extract	**1**	**1**	**1**	**1**	**1**	**1**
Carbopol 934	**1**	**1.5**				
HPMC K 100M			**1**	**1.5**		
Xanthan gum					**1**	**1.5**
Propylene glycol	**10**	**10**	**10**	**10**	**10**	**10**
Methyl paraben	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**
Propyl paraben	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
Purified water	**100**	**100**	**100**	**100**	**100**	**100**
Menthol oil	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Triethonal amine	**q.s.**	**q.s.**	**q.s.**	**q.s.**	**q.s.**	**q.s.**

8.5 Physicochemical evaluation:

8.5.1. Physical Appearance:-

All formulation batches were found to be homogeneous light green gel preparations

8.5.2. Measurment of pH:-

The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Table No.15: pH of leaves extracts formulation

formulation	**pH**
F1	**6.5**
F2	**6.3**
F3	**6.8**
F4	**6.8**
F5	**6.8**
F6	**6.4**

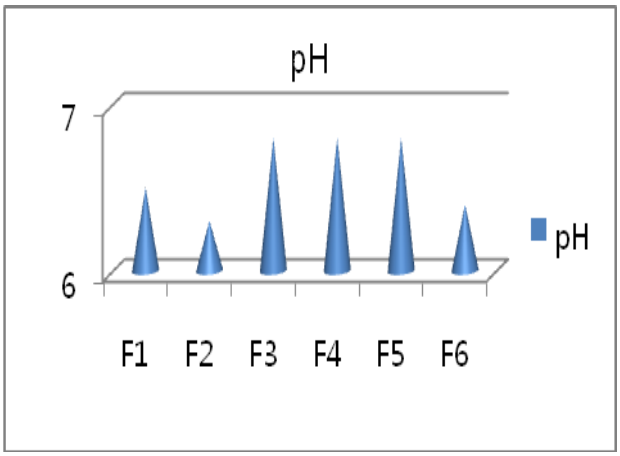
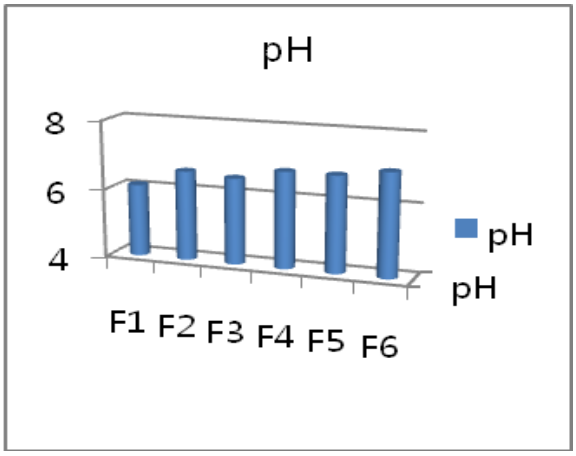


Table No.16: pH of root extracts formulation

formulation	pH
F1	6.1
F2	6.6
F3	6.5
F4	6.8
F5	6.8
F6	7



8.5.3. Spreadability:-

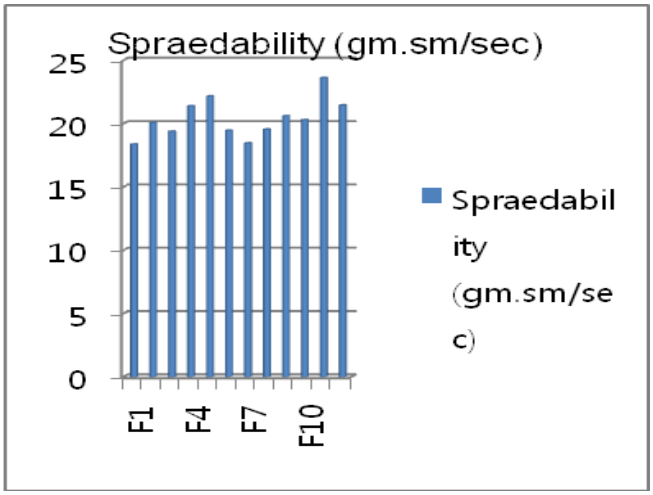
The spreadability of Leaves extract gel formulation and of root extract gel formulation is depicted in table no.15 from the combined graph of all formulation it was concluded that all the developed formulation showed acceptable spreadability (fig.No.3). Xanthan based formulation showed better spreadability than the carbopol 934 and HPMC K 100 M formulations. All formulation shows good spreadability after compare with marketed gel formulation.

Table No.17: Spreadability of gel formulations (mean±S.D)

batch	Spraedability (gm.sm/sec)	batch	Spraedability (gm.sm/sec)
F1	18.36	F7	18.45
F2	20.06	F8	19.54
F3	19.37	F9	20.59
F4	21.38	F10	20.29
F5	22.16	F11	23.61
F6	19.45	F12	21.44

Spreadability of marketed gel:

MARKETED gEL	SPREADABILITY
Diclofenac sodium gel	24.82



Rheological Study:-

The measurement of viscosity of the prepared gel was done with Brookfield viscometer of (Brookfield Engineering Laboratories). In all these formulations the highest viscosity was found in formulation batch F_5 and F_{11}

viscosity of gel formation, mean SD, n=3

Batches	Viscosity(Cps)	Batches	Viscosity(Cps)
F1	4237±0.11	F7	4215±0.54
F2	4124±0.43	F8	4183±0.75
F3	3714±0.21	F9	3815±0.98
F4	3688±0.69	F10	3664±.015

F5	**3671±0.58**	**F11**	**3549±0.65**
F6	**3984±0.25**	**F12**	**3684±0.27**

5. Extrudability:-

The Extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly and hence suitable consistency is required in order to extrude the gel from the tube. Extrudability of Xanthan gum gel formulations was found to be good. Whereas, Extrudability of Carbopol 934 and HPMC K 100 M gels were satisfactory.

Table No.18: Extrudability of Leaves extract gel formulations (mean S.D., n=3)

batches	wt. of formulation	Wt. of gm extrudded	Extradibility amount	appearance
F1	**16.77**	**15.06**	**89.80**	**++**
F2	**17.52**	**14.51**	**82.81**	**++**
F3	**17.06**	**14.60**	**82.58**	**++**
F4	**16.78**	**14.44**	**86.05**	**++**
F5	**17.50**	**16.05**	**91.71**	**++**
F6	**17.56**	**15.80**	**89.97**	**++**

Table No.19: Extrudability of root extract gel formulations (mean S.D., n=3)

batches	wt. of formulation	Wt. of gm extrudded	Extradibility amount	appearance
F1	**16.65**	**14.83**	**86.06**	**++**
F2	**17.02**	**15.18**	**89.18**	**++**
F3	**17.51**	**14.30**	**81.66**	**++**
F4	**16.85**	**14.53**	**86.23**	**++**
F5	**17.43**	**15.86**	**90.99**	**++**
F6	**17.39**	**15.42**	**88.67**	**++**

Note: + fair, ++ good, +++ excellent

8.6. Optimization of Batch:

After analysis of all batches of formulations for their evaluation parameters like pH, Viscosity, Spreadability, and Extrudability, the formulation batch F_5 from leaves extract gel and F_{11} from root extract gel showed good results. The batch F_5 optimized with the good viscosity,

Spreadability and Extrudability and F_{11} shows good spreadability hence, these two batches were used for further evaluation like in-vitro skin irritation study and in-vitro anti-inflammatory study.

The parameters from batches as follows:

Optimization Batch	pH	Viscosity (Cps)	Spreadability (gm.cm/sec)	Extrudability (%)
F5	**6.8**	**3671±0.58**	**22.16±0.3**	**91.71**
F11	**6.8**	**3549±0.65**	**23.61±0.4**	**90.99**

Skin irritation study:-

Absence of skin irritation in gel formulation is acceptable by patient. Skin irritation test performed by using in-vitro skin irritation test method. There are certain problems associated with the use of animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study in-vitro skin irritation method were used to skin irritation study of optimized gel. All gel formulations were found to be free from irritation. Observation indicates acceptability of these gels for topical use.

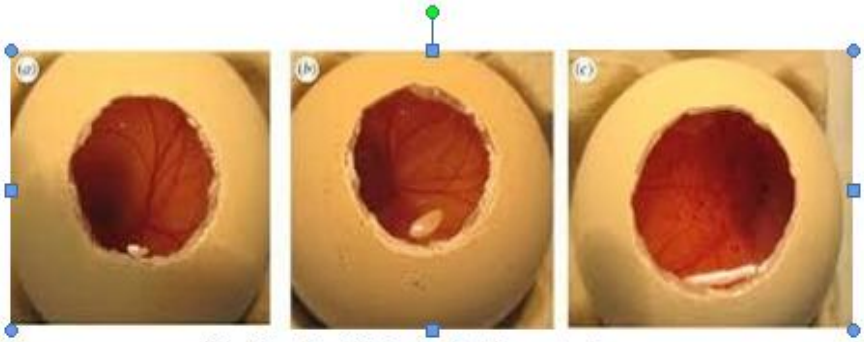


Fig No. 9: Skin irritation study

End point measurement for skin irritation test:

Table No. 20: End points for Skin irritation

End point	Observation
Hemorrhage	-
Lyesis	-
Coagulation	-

Note: - Indicates absence of end point

In-vitro Anti-inflammatory study of gel:

This study was conducted by applying in-vitro anti inflammatory study by using % inhibition of albumin. The anti inflammatory action of formulation F_5 and F_{11} was calculated and it was compared with marketed preparation (Diclofenac sodium gel). The % inhibition of marketed formulation and both gel formulations are given in table no.22 and 23. The statistical and graphical analysis of results shows that there was no significant difference in the inhibition of inflammation in between the gel F_5, F_{11} and marketed gel. So the prepared herbal gel formulations are as effective as marketed formulations

% inhibition of Batch F5

Antiinflamatory activity

concentration	absorvation	% Inhibition
Control	0.1987	-
1000	0.3417	71.96
500	0.3181	60.09
250	0.2753	38.55
125	0.2636	32.66
62.5	0.2401	20.83
31.25	0.2129	70.14
Diclofenac sodium gel	0.3530	77.65

% inhibition for batch F11

concentration	absorvation	% inhibition
Control	0.1987	-
1000	0.3324	72.40
500	0.3172	69.70
250	0.3016	56.43
125	0.2961	53.57
62.5	0.2718	40.97
31.25	0.2605	35.11
Diclofenac sodium gel	0.3530	77.65

Stability studies:

Accelerated stability studies indicated that the physical appearance, rheological properties, extrudability, spreadability in the prepared gel remained unchanged upon storage for 1 month. The pH observed of prepared gel through 1 month storage was in between 6-7. Rheological properties and spreadability was obtained uniformly. Gel formulation was maintaining drug level after 1 month of accelerated stability.

Evaluation	Batch F5		Batch F11	
	initial	after	Initial	After
pH	6.8	6.7	6.8	6.8
Viscosity(CPs)	3673643543551	5	9	2
Spreadability(gm.cm/sec)	22.16	22.14	23.61	23.60
Extrudability (%)	91.71	91.60	90.99	90.82

SUMMARY:

Utility of gel based drug delivery systems are being employed in recent past for therapeutic effectiveness of topical applied drugs. *Clerodendrum serratum* is tradition anti-inflammatory agent. It has analgesic, anti-oxidative properties. Topical route for *Clerodendrum serratum* was selected up to avoid GIT irritation and to maximize the drug concentration at the site of action. The transdermal delivery of the drug is limited by the barrier properties of the skin that needs inclusion of penetration enhancers in the formulation.In the present study an attempt were made to formulate and evaluate topical gels of *Clerodendrum serratum*. In our preliminary study the standardization of *Clerodendrum serratum* was carried out for purity and identity. The authentication and taxonomical identification done by Dattajirao Kadam Arts, Science and Commerce College, Ichalkaranji, Dist. Kolhapur. The Preformulation studies include identification, phytochemical evaluation, physicochemical evaluation, extraction, solubility were carried out. Drug extract-excipient study was done by physical method and the result showed that the drug extract is compatible with all three polymers, Carbopol 934, HPMC K 100 M and Xanthan gum. All the developed gels were evaluated for their physicochemical properties like appearance, pH values, rheological properties, spreadability, extrudability properties, skin irritation test, stability studies and anti-inflammatory activity studies. The pH range of carbopol 934 gels, HPMC K 100 M gels and Xanthan gum gels were found to be suitable for topical application. The viscosity measurement was done for selected gels using Brookfield Viscometer at room temperature. Extrudability of Xanthan gum shows better result HPMC K 100 M gels were comparable with marketed gel (Diclofenac Sodium Gel). The order of spreadability of Xanthan gum is greater than other polymers. Anti-inflammatory activity of formulated gel was carried out with marketed gel using in-vitro anti-inflammatory method. The inhibition of albumin denaturation method will be used. The percentages of inhibition of both extract gels are comparatively equal. The stability study of formulated gels where carried out for three months as per ICH norms at a temperature 40^0C ± 2^0C, 75% ± 5% RH and were analyzed for the changes in appearance, pH, spreadability, viscosity. Result showed no significant variation with respect to evaluation parameters. Thus, *Clerodendrum serratum* gel formulation was stable in topical gel formulation and indicating better results in treatment of inflammation.

CONCLUSION:

It can be concluded from the present investigation that proper selection of polymers and drug is a prerequisite for designing and developing a transdermal drug delivery. The physical compatibility studies suggest that polymers selected i.e. Carbopol 934, HPMC K 100 M and Xanthan gum were found to be compatible with drug *Clerodendrum serratum*. The varying concentration of the three polymers was found to affect the gel parameters like viscosity and spreadability. Gel formulations prepared with Carbopol 934, HPMC K 100 M and Xanthan gum showed good homogeneity, no skin irritation, good stability and anti-inflammatory activity.

However, the Xanthan gum based gel proved to the formula of choice, since it showed

the highest percentage of extrudability, good spreadability and rheological properties. Formulation F_5 with 1 % leaves extract and F_{11} with 1% root extract of *Clerodendrum serratum* showed the best formulation with significant anti-inflammatory activity. Formulation F_5 and F_{11} shows approximately equal anti-inflammatory activity. Hence, there is no need to used roots for the preparation of medicines for anti-inflammatory action.

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