Formulation and Characterization of Novel Polyherbal Anti-Inflammatory Gel

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Abstract **- Aim: The aim of the present study was to explore about the formulation and characterization of novel polyherbal gel containing gaultheria ,clove & cinnamon oil Methods: All ingredient mixed together, gaultheria, clove and cinnamon oil . Other ingredients except triethanolamine were added in water and stirred well using a mechanical stirrer. To this the extracts were added and stirred. Then triethanolamine and perfume was added, and the volume was made up using alcohol. Physico-Chemical Evaluation: By varying the concentration of herbal drug extracts different formulations were prepared and evaluated for physical appearance, pH and anti-microbial study. Results: Based on results S-4 formulation was selected as an optimized formulation which showed a promising result. Conclusion: The efforts taken in this study add optimistic approach for herbal drugs. Hence the herbal formulations can be a potential alternative to available marketed preparation.**

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 defence, 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.[2]

Classification of inflammation:[2]

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 interpatient 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:

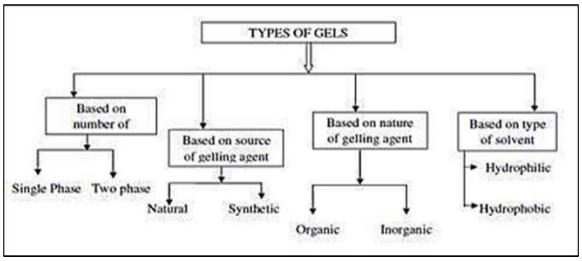

Fig 1: Classification of Topical Drug Delivery System based on physical state

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, labelled 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 covers 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 possess 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.

MATERIALS AND METHOD

Materials:
All oils were taken from shri krishna medical store, Paithan gate, Aurangabad. Oils were from Baidhyanath and Rex company. All other ingredients used were of analytical grade.

Method:
Identification Methods for Gaultheria Oil:
Thin layer chromatography
Stationary phase: Silica Gel
Mobile phase: n Hexane– Ethyl acetate (95+5)
Standard used: methyl salicylate
Test sample: Gaultheria oil
All components were selected as mentioned above. The chromatograms were developed in a given mobile phase. Identification of spots was done by keeping TLC plates in an iodine chamber and RF value was calculated.

PHYTOCHEMICAL TESTS:
1.Test for Carbohydrate:
Molisch Test Treat test sample with few drops of alpha naphthol. 0.2 ml of conc. Sulphuric acid was added slowly along the side of test tube. Purple to violet color ring appears at the junction.[13]
2.Test for Terpenoids: Salkowaski Test sample was mixed with 2 ml of chloroform. Concentrated sulphuric acid (3ml) was added by the wall of the test tube to form layer. Reddish brown coloration at interface shows presence of terpenoids.[13]
3.Determination of Specific Gravity: Specific gravity of Gaultheria oil was determined by using density bottle. First the density of oil was calculated and by taking ratio of density of oil to the density of water specific gravity was calculated.
Preparation of Standard Stock Solution and calibration curve:
The standard stock solution was prepared by dissolving Gaultheria oil in methanol to make final concentration of 100 µg/ml. Different aliquots were taken from stock solution and diluted with methanol separately to prepare series of concentrations. Absorbance was measured at 237 nm against methanol as blank. The calibration curve was prepared by plotting absorbance versus concentration.

Method of preparation for Gel For topical gel preparation:
Dispersion method was used because polymers selected for gel preparations can be easily dispersed in water by stirring at room temperature. All the ingredients were accurately weighed as shown in Table no. 3. Then polymer was dispersed in 50 ml of distilled water with constant stirring. Methyl Paraben and Propyl Paraben were dissolved separately in 5 ml of distilled water by heating on water bath. The solution was cooled and then added Propylene Glycol 400. After this 100 mg of Gaultheria oil was added and then this solution was mixed in polymer solution and volume was made up to 100 ml by distilled water. Finally, sufficient quantity of Triethanolamine (TEA) was added in the mixture by continuous stirring for adjustment of required gel strength. Then weight and pH of gel formulation was determined.
Table 1: Formula for different gel preparations

Ingredients	Formulation 1	Formulation 2	Formulation 3
Gaultheria oil microemulsion	3 %	3 %	3 %
Carbapol 940	2 %	-	-
Carbapol 934	-	2%	-
Sod. CMC	-	-	5%
Methyl paraben	0.2 %	0.2%	0.2%
Propyl paraben	0.1%	0.1%	0.1%
Triethanolamine	q.s.	q.s.	q.s.
Propylene glycol	5 %	5 %	5 %
Dist. water	q.s. up to 100 ml	q.s. up to 100 ml	q.s. up to 100 ml

Evaluation [19, 20]

- Homogeneity

The developed gel was tested for homogeneity by visual inspection after the gel has been set in the container. It was tested for appearance and presence of aggregates.

- Grittiness

The formulation was evaluated microscopically for the presence of particles if any.

- Extrudability study

Aluminum collapsible tube was filled with 10 g of gel using universal tube filing machine and was hold between fingers. The tube was compressed by applying finger pressure and Extrudability of the formulation was determined by measuring the amount of gel extruded in percentage on application of finger pressure.

- pH

The pH of formulation was determined by using digital pH meter. 1 g of gel was dissolved in 100 ml of distilled water and stored for 2 h. The pH measurement of formulation was done in triplicate.

- Viscosity study

The measurement of viscosity of the prepared gel was done with Brookfield viscometer. The gels were rotated at 25 rpm using spindle no. 64 and the corresponding dial reading was noted.

- Spread ability study

The Spread ability of the gel was determined by measuring the spreading diameter of gel (1 g) between two horizontal plates of 20 cm × 20 cm after one min of time. The standard weight of 125 g was applied on the upper plate to determine spread ability.

- Drug content

1 g of gel was taken and dissolved in 100 ml of phosphate buffer pH 5.5 in a volumetric flask. The flask was kept for 2 h and shaken well in a shaker to mix it properly. The solution was filtered. 1 ml of the filtered solution was taken and diluted to 10 ml with phosphate buffer in a 10 ml. This solution was measured spectrophotometrically at 237 nm against phosphate buffer as blank. The drug concentration in gel was determined by comparing the absorbance of gel solution to slope of standard curve of Gaultheria Oil in methanol.

- Drug diffusion study

The *in-vitro* drug diffusion from prepared gel formulations was studied using Franz diffusion cell. A dialysis membrane was sandwiched between donor and receptor compartment of Franz diffusion cell. The temperature was kept constant at 37ºC. One gram of gel was spread on dialysis membrane, phosphate buffer pH 7.4 added in receptor compartment. Diffusion medium was continuously stirred using magnetic stirrer to avoid diffusion layer effect. Samples were withdrawn at 30 min. intervals. The withdrawn samples were analyzed by UV spectrophotometer at 237 nm by taking phosphate buffer as blank. [15]

IN VITRO ANTI- INFLAMMATORY ACTIVITY

In vitro anti-inflammatory activity of all prepared formulation was studied by inhibition of protein denaturation method.

Inhibition of Protein denaturation method

Protein denaturation related with the inflammatory diseases like Rheumatoid arthritis. Protein denaturation destroys the biological activity of protein molecules. Therefore, the ability of substance to prevent protein denaturation may be helps to prevent inflammatory disorders.

Method

In this method either egg albumin or serum bovine albumin used as a protein. The reaction solution (5ml) consists of 0.2ml of protein, 2.8 ml of phosphate buffered saline solution (pH 6.4) and 2 ml of test sample. Similar volume double distilled water served as a control. Mixture heated at 70ºc for 5 min. After cooling Absorbance was measured at 660 nm by using UV- spectrophotometer taking vehicle as blank. Diclofenac (1mg/ml) served as a reference and treated similarly for determination of absorbance. The percent protein inhibition calculated by following formula [16]

Protein inhibition = (Abs. Control – Abs. Sample) / Abs. Control ×100

RESULTS

IDENTIFICATION OF GAULTHERIA OIL

THIN LAYER CHROMATOGRAPHY

The TLC of Gaultheria oil was performed by taking Methyl Salicylate as standard and RF values of std. and test were compared. RF value of Gaultheria oil was found to be nearly equal to RF value of Methyl salicylate hence it confirms that Gaultheria oil shows presence of Methyl salicylate.

(A) (B)

Fig.2. Thin layer chromatography

A. TLC of Methyl salicylate

B. TLC of Gaultheria oil

Table 2: RF value comparison of Methyl salicylate and Gaultheria oil

Sample	Rf Value
Methyl salicylate (std.)	0.8
Gaultheria oil	0.83

PHYTOCHEMICAL TESTS

The phytochemical screening of Gaultheria oil was done for the presence of Carbohydrate and Terpenoids. From results both the phytochemical tests was found to be positive, it confirms that Gaultheria oil shows the presence of terpenoids and methyl salicylate which is present in a form of Gaultherin as carbohydrate.

Table 3: Phytochemical screening of Gaultheria oil

Test	Inferance	
Molish test for carbohydrate	Positive	
Salkowaski test for terpenoids	Positive	

VISCOSITY OF GAULTHERIA OIL

Viscosity of Gaultheria oil was determined by using Ostwald Viscometer and it was found to be 3.92 cp

Table 4: Viscosity of Gaultheria oil

Sample	Time of flow (sec.)			Mean time (sec.)	Density(gm/ml)	Viscosity (cp)
	1	2	3			
Distilled water	23.40	23.89	24.41	23.9 sec.	0.997 gm/ml	0.890 cp
Gaultheria oil	59.13	59.20	60.30	59.54 sec.	1.77 gm/ml	3.92

EVALUATION OF GAULTHERIA GEL

The observations of various evaluation parameters for *Gaultheria* topical gel are given in Table 2. The gel was found smooth, particle, having good homogeneity and spread ability. The drug content in gel was determined with the help of standard curve of *Gaultheria fragrantissima oil*. All the formulations shows drug content in standard acceptable range that is plus-minus 15%.

Table 5: Evaluation parameters for topical gel.

Parameter	Observation		
	Formulation 1	Formulation 2	Formulation 3
Gel appearance	Light pink	Light pink	Light pink
Homogeneity	Good	Good	Good
Extrudability	Good	Good	Good
pH	6.79	6.81	6.92
spread ability	3.2 cm	3.2cm	3.4 cm
Grittiness	Absent	Absent	Absent
Viscosity	1986 cps	1724 cps	1998 cps
Drug content	90.72%	86.67%	85.92%

Drug diffusion study

All the prepared gel formulations were evaluated for in-vitro drug diffusion, among all three formulations the formulation 1 contains Carbapol-940 as a gelling agent shows highest drug release as 99.57% at 120 min. Formulation 2 and formulation 3 contains gelling agent Carbapol-934 and Sod. CMC shows highest drug release as 93.86% and 76.95% at120 min. respectively.

Table 6: Percent drug release of formulation 1

Time (Min.)	Absorbance	Conc. (mg/ml) *10	Conc. In 22ml (mg/ml)	Cumulative drug conc.(mg/ml)	%drug release
30	0.058	10.6	234.60	234.60	17.25 %
60	0.091	15.32	337.14	571.14	41.99 %
90	0.104	17.16	377.54	948.68	69.75 %
120	0.113	18.43	405.50	1354.18	99.57 %

Table 7: Percent drug release of formulation 2

Time (Min.)	Absorbance	Conc (mg/ml) *10	Conc. In 22ml (mg/ml)	Cumulative drug conc.(mg/ml)	%drug release
30	0.043	8.54	187.99	187.99	14.46 %
60	0.079	13.62	299.85	487.84	37.52 %
90	0.098	16.41	361.07	848.91	65.30%
120	0.102	16.87	371.32	1220.30	93.86%

IN VITRO ANTI-INFLAMMATORY ACTIVITY OF TOPICAL GEL FORMULATIONS

All the formulated topical Gaultheria gels (F1-F3) were studied for in-vitro anti- inflammatory activity, taking Diclofenac sod. as a standard. All formulations were studied for its activity with increasing drug concentration that is 200, 400, 600, 800, 1000 microgram per ml. same is done for the standard. All

formulations show activity in increasing manner as the concentration of drug increases. Among the formulations, formulation F1 containing Carbapol-940 as gelling agent shows highest activity at 1000 microgram per ml concentration. Hence we can confirm that formulation F1 may be the optimum formulation for desired therapeutic effect.

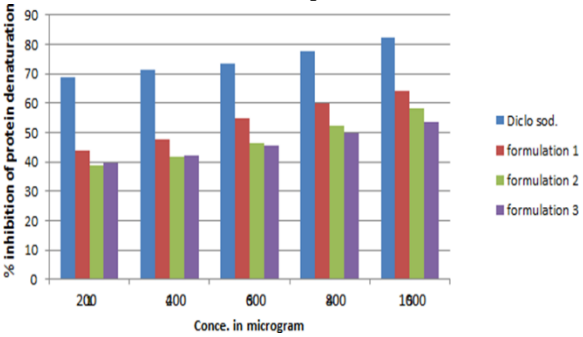

Fig.3 . In-vitro anti-inflammatory activity for prepared Gaultheria topical gels

STABILITY STUDY:

The stability study was performed as per ICH guidelines. The formulated gel was filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz. 25ºC ± 2ºC / 60% ± 5% RH, 30º C ± 2ºC / 65% ± 5% RH, 40ºC ± 2ºC / 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and spreadibility.

Table 12: Stability testing at 25oC ± 2oC/60% ± 5% RH (3rd months) of Polyherbal gel

Formuation	Colour	Appearance	Spreadability	pH
F1	Pinkish	Homogenous	17	6.9
F2	Pinkish	Homogenous	18	7.1
F3	Pinkish	Homogenous	20	7.0

Table 13: Stability testing at 30oC ± 2oC/65% ± 5% RH (3rd months) of Polyherbal gel

Formuation	Colour	Appearance	Spreadability	pH
F1	Pinkish	Homogenous	18.5	7.1
F2	Pinkish	Homogenous	16	7.1
F3	Pinkish	Homogenous	20.2	7.2

Table 14: Stability testing at 40oC ± 2oC/75% ± 5% RH (3rd months) of Polyherbal gel

Formuation	Colour	Appearance	Spreadability	pH
F1	Pinkish	Homogenous	17.2	6.8
F2	Pinkish	Homogenous	20.6	7.2
F3	Pinkish	Homogenous	19	7.0

CONCLUSION

All the prepared topical gel of Gaultheria, Cinnamon And clove oil that is topical Gel has shown good promising results for all the evaluated parameters with good drug content and drug diffusion for topical gels. In-vitro anti-inflammatory activity of all formulations by inhibition of protein denaturation assay shows more than 50% of inhibition of protein denaturation as compared to standard Diclofenac sodium. Hence, we can conclude that, Gaultheria oil, Cinnamon Oil and clove oil can be topically used as an anti-inflammatory agent as alternative to NSAIDs.

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