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FORMULATION AND EVALUATION OF POLYHERBAL EMULGEL FOR RHEUMATOID ARTHRITIS

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ABSTRACT

The present study was conducted to develop an emulgel formulation containing potential herbal anti-inflammatory agent viz., turmeric, ginger, black paper, menthol. Inflammation and rheumatism remain serious problem in the present era. Although there are number of allopathic formulation available in market for the treatment of inflammation, but these suffer from side effects like heartburn, stomach pain, nausea, vomiting, diarrhea, constipation, liver damage, fluid retention, nephrotoxiciy etc. It is considered that the herbal medication as safer as compared to that of allopathic medicine in the market. The herbal components ginger, turmeric, black paper menthol has been selected for the development of anti-inflammatory formulation, as from literature review it revealed that these are effective in the treatment of inflammation. Ultrez 20 (corbomer) was used as gelling agent ultrez 20 has several advantages over traditional corbopol, ultrez 20 wet within a 5 min whereas traditional corbopol take 12-15 hrs for wetting. The emulgel were subjected for evaluation on the basis of appearance, pH, spreadability, extrudability, rheological behavior, in vitro release performance, anti-inflammatory study and were compared with marketed preparation containing diclofenac sodium. The anti-inflammatory study suggests that formulation emulgel is superior to that of all formulation including marketed gel and emulgel.

KEYWORDS: curcumin, gingerol, polyherbal, carrageenan induced rat paw edema.

INTRODUCTION

A B Garrod in 1858 named the disease rheumatoid arthritis replacing the old terms arthritis deformans and rheumatic gout. He is thus credited to make a distinction between rheumatoid arthritis and osteoarthritis and gout. Rheumatoid arthritis RA is an autoimmune disease that can cause joint pain and damage throughout your body. The joint damage that RA causes usually happens on both sides of your body. So if a joint is affected in one of your arms or legs, the same joint in the other arm or leg will probably be affected, too. This is one way that doctors distinguish RA from other forms of arthritis, such as osteoarthritis (OA). RA is a long-term or chronic disease marked by symptoms of inflammation and pain in the joints.

RA symptoms, which can occur throughout the body, includes

1) Joint pain
2) Joint swelling
3) Joint stiffness
4) Loss of joint function

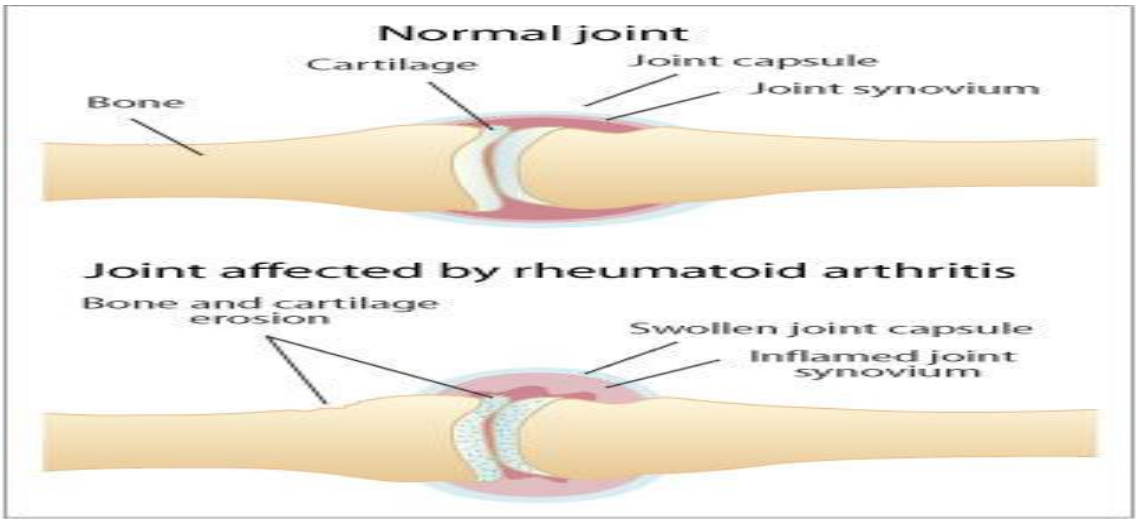

Fig. 1 Normal joint and joint affected by rheumatoid arthritis.

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. 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 preparations are applied to the skin for surface, local or systemic effects. In some cases, the base may be used alone for its therapeutic properties, such as emollient, soothing or protective action.

Skin is the largest organ of the body. It is not uniformly thick. At some places, it is thick and in some places, it is thin. The average thickness of the skin is about 1 to 2mm.

Formulations containing two or more than two herbs are called polyherbal formulation. Drug formulation in Ayurveda is based on two principles: Use as a single drug and use of more than one drug. The latter is known as polyherbal formulation.

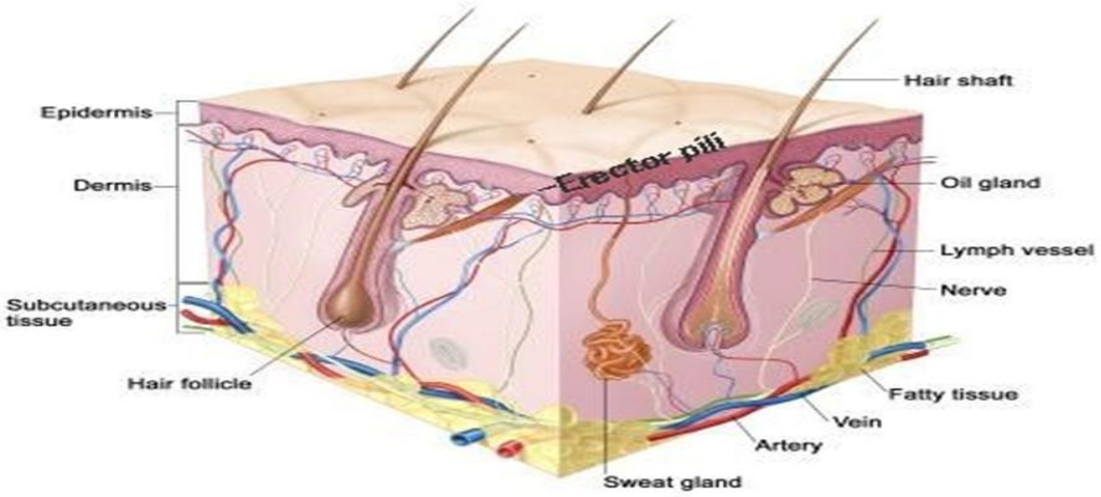

Fig. 2 Structure of skin.

The concept of polyherbalism in this ancient medicinal system. In the traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. Even though the active phytochemical constituents of individual plants have been well established, they usually present in minute amount and always, they are insufficient to achieve the desirable therapeutic effects. For this, scientific studies have revealed that these plants of varying potency when combined may theoretically produce a greater result, as compared to individual use of the plant and also the sum of their individual effect. This phenomenon of positive herb-herb interaction is known as synergism. Certain pharmacological actions of active constituents of herbals are significant only when potentiated by that of other plants, but not evident when used alone.

Emulsion as the name suggest, they are the combination of gel and emulsion. Both oil-in-water and water-in-oil type of emulsion used as a vehicle to deliver various drugs to the skin. They also have a high ability to penetrate the skin. The presence of the gelling agent in water phase converts a classical emulsion into an emulgel. Emulgel for dermatological use has

several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent and pleasing appearance.

MATERIALS AND METHODS

The following drug, plants, excipients and chemicals were used for the formulation and evaluation studies of Emulgel.

Table 1: List of drug and supplier.

Sr.no.	Drug	Supplier
1.	Turmeric	Local market, Nagpur
2.	Ginger	Local market, Nagpur
3.	Black paper	Local market, Nagpur
4.	Menthol	UNIJULES Life Sciences Ltd., Nagpur
	Excipients	**Supplier**
7.	Ultrez 20	UNIJULES Life Sciences Ltd., Nagpur
7.	Cetosteryl alcohol	S D Fine Chem Limited
8.	Cetomacrogal	UNIJULES Life Sciences Ltd., Nagpur
9.	Glyceryl-monostearate(GMS) (self-emulsifying)	UNIJULES Life Sciences Ltd., Nagpur
10.	Disodium edetate	UNIJULES Life Sciences Ltd., Nagpur
11.	Triethyl amine	Loba Chemie, Mumbai.
12.	Benzyl alcohol	Ranbaxy Fine Chemicals Limited

Table 2: List of equipments used.

Sr.no	Instruments/Equipments	Manufacturer
1.	Electronic balance	Shimadzu ATY224
2.	pH meter	Elico Pvt. Ltd. India
3.	Viscolead Pro (Viscometer)	Fungilab, Spain
3.	Hotplate Magnetic Stirrer	Labman Scientific Instruments
4.	UV spectrophotometer	V-730 Jasco2000 series, Japan
5.	Soxhlet extractor	-
7.	Diffusion cell	Orchid Scientific, Nashik
7.	Dialysis Membrane (12000-16000 D)	HiMedia, Mumbai
8.	High Speed Homogenizer	IKA Ultra turrex T18, Germany

Table 3: Authentication of plants.

Plant Name	Authentication No.
Turmeric	10159
Ginger	10160
Black paper	10161
Menthol	10158

Table 4: Details of Voltaren Gel purchased from medical shop.

Name of product	Dt. of Mfg.	Dt. of Exp.	Quantity
Voltaren Gel GenericName: diclofenac sodium gel Brand Name: Voltaren Gel	**Jan 2019**	**Jan 2021**	**15g**

1. 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 0^{c} and at 75% RH for 2 weeks and observe the changes. After 30 days storage of drug extract with excipients in various ratios at room temperature, samples were observed for physical changes but there were no physical changes observed.

2. UV spectroscopic determination of λmax of Turmeric

Preparation of standard solutions

Standard stock solution contain 100 µg/mL was prepared, 10 mg of Turmeric was accurately weighed and transferred into 100 mL volumetric Flask and made up to the mark with Methanol, standard solutions were prepared in the concentration range of 1-5 µg/mL by further dilution with methanol.

Maximum absorbance (λmax) of Turmeric

Wavelength of maximum absorption (λmax) was determined by scanning 10 µg/mL solution of turmeric using UV-visible double bean spectrophotometer from 400-600 nm using methanol as blank.

Determination of wavelength of maximum absorbance (λmax) of Ginger

Preparation of standard solutions

Standard stock solution contain 100 µg/mL was prepared, 10 mg of vGinger extract was accurately weighed and transferred into 100 mL volumetric Flask and made up to the mark with Methanol, standard solutions were prepared in the concentration range of 1-5 µg/mL by further dilution with methanol.

Maximum absorbance (λmax) of Ginger

Wavelength of maximum absorption (λmax) was determined by scanning 10 µg/mL solution of ginger using UV-visible double bean spectrophotometer from 400-600 nm using methanol as blank.

Determination of wavelength of maximum absorbance (λmax) of Black paper

Preparation of standard solutions Standard stock solution contain 100 µg/mL was prepared, 10 mg of Black paper extract was accurately weighed and transferred into 100 mL volumetric Flask and made up to the mark with Methanol, standard solutions were prepared in the concentration range of 1-5 µg/mL by further dilution with methanol.

Maximum absorbance (λmax) of Black paper

Wavelength of maximum absorption (λmax) was determined by scanning 10 µg/mL solution of black paper using UV-visible double bean spectrophotometer from 400-600 nm using methanol as blank.

Determination of wavelength of maximum absorbance (λmax) of menthol

Preparation of standard solutions

Standard stock solution contain 100 µg/mL was prepared, 10 mg of Menthol was accurately weighed and transferred into 100 mL volumetric Flask and made up to the mark with Methanol, standard solutions were prepared in the concentration range of 1-5 µg/mL by further dilution with methanol.

Maximum absorbance (λmax) of menthol

Wavelength of maximum absorption (λmax) was determined by scanning 10 µg/mL solution of menthol using UV-visible double bean spectrophotometer from 400-600 nm using methanol as blank.

3. Standard calibration curve

3.1 Standard calibration curve (Turmeric extract)

Calibration curve in methanol

10 mg of turmeric extract was dissolved in 100 mL of methanol to obtain working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug concentration were transferred to 10 mL volumetric flask and the volume was

adjusted to mark with methanol. Absorbances of the above solution were taken at λmax 418 nm against the blank solution prepared in the same manner without adding the drug.

Calibration Curve in pH 7.4 Phosphate Buffer

10 mg of turmeric extract was dissolved in 100 mL of pH 7.4 buffer to obtained working standard of 100µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug were transferred to 10 mL volumetric flask and the volume was adjusted to mark with pH 7.4 buffer. Absorbances of the above solution were taken at λmax 418 nm against the blank solution prepared in the same manner without adding the drug.

3.2 Standard calibration curve (ginger extract)

Calibration curve in methanol

10 mg of ginger extract was dissolved in 100 mL of methanol to obtain working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug concentration were transferred to 10 mL volumetric flask and the volume was adjusted to mark with methanol. Absorbances of the above solution were taken at λmax 291 nm against the blank solution prepared in the same manner without adding the drug.

Calibration Curve in pH 7.4 Phosphate Buffer

10 mg of ginger extract was dissolved in 100 mL of pH 7.4 buffer to obtained working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug were transferred to 10 mL volumetric flask and the volume was adjusted to mark with pH 7.4 buffer. Absorbances of the above solution were taken at λmax 291 nm against the blank solution prepared in the same manner without adding the drug.

3.3 Standard calibration curve (Black paper)

Calibration curve in methanol

10 mg of Black paper was dissolved in 100 mL of methanol to obtain working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug concentration were transferred to 10 mL volumetric flask and the volume was adjusted to mark with methanol. Absorbances of the above solution were taken at λmax 340 nm against the blank solution prepared in the same manner without adding the drug.

Calibration Curve in pH 7.4 Phosphate Buffer

10 mg of Black paper was dissolved in 100 mL of pH 7.4 buffer to obtained working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug were transferred to 10 mL volumetric flask and the volume was adjusted to mark with pH 7.4 buffer. Absorbances of the above solution were taken at λmax 340 nm against the blank solution prepared in the same manner without adding the drug.

3.4 Standard calibration curve (menthol)

Calibration curve in methanol

10 mg of menthol was dissolved in 100 mL of methanol to obtain working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug concentration were transferred to 10 mL volumetric flask and the volume was adjusted to mark with methanol. Absorbances of the above solution were taken at λmax 248 nm against the blank solution prepared in the same manner without adding the drug.

Calibration Curve in pH 7.4 Phosphate Buffer

10 mg of menthol was dissolved in 100 mL of pH 7.4 buffer to obtained working standard of 100 µg/mL. Aliquots of 0.5 mL to 2.5 mL from the stock solution representing 5 to 25 µg/mL of drug were transferred to 10 mL volumetric flask and the volume was adjusted to mark with pH 7.4 buffer. Absorbances of the above solution were taken at λmax 248 nm against the blank solution prepared in the same manner without adding the drug.

4 Formulation of Polyherbal Emulgel

4.1 Extraction Procedure for Raw material

- The Raw material of Turmeric, Ginger, Black paper and Menthol were collected, clean and dried.
- The herbs were crushed, powdered, and stored in air packed container.
- Extraction of all the crude raw material in 70% ethanol with soxhlet extraction process individually.

Table 4.1: parts used for extraction.

Sr.no	Herbs	Parts used	chemical constituens	uses
1	Peppermin(*Mentha piperita*)	leaves	Menthol	Topical analgesic Penetration enhancer
2	Ginger(*Zingiber officinale*)	rhizomes	Zingerol	Anti-infllamatory
3	Blackpepper (*piper nigrum*)	fruits	piperine	Anti-inflamatory
4	Turmeric(*Curcuma longa*)	rhizomes	Curcumin	Anti-inflamatory

- **Process of Extraction**
- Plants material (Crushed or cut small or moderately coarse powered)
- Placed in extraction thimble
- Mix the liquid sample with sand and sodium sulfate
- Closed the extraction thimble with a fat free cotton wad, insert the thimble into the soxhlet extractor
- Fill the solvent into the solvent vessel. extract at a temperature of 70-80^{o}c for 20-30 extraction cycles (4-7 hours).
- Collect the extract Evaporate and Concentrate.

5 Preparation of Polyherbal Emulgel

Table 5.1: Ingredients and its quantity.

Each 5gm emulgel contains:		
Sr.no.	**Ingredients**	**Quantity**
1	Turmeric	1g
2	Ginger	1g
3	Black paper	0.5g
4	Menthol	100mg
5	Excipients	qs.

Step 1: Formulation of emulsion either O/W or W/O.

Step 2: Formulation of gel base.

Step 3: Incorporation of emulsion into gel base with continuous stirring.

Emulgel was prepared by the method reported by Mohammad et al. (2004) with minor modification. The gel in formulations was prepared by dispersing ultrez 20 in purified water with constant stirring at a moderate speed then the pH is adjusted to 7 to 7.5 using triethanolamine. The oil phase of the emulsion was prepared by heating cetostearyl alcohol, cetomacrogol, GMS, while the aqueous phase was prepared by dissolving disodium edetate in purified water. Whereas drug extract was dissolved in purified water and both solutions were mixed with the aqueous phase.

Both the oily and aqueous phases were separately heated to 70°–80°C; then the oil phase was added to the aqueous phase with continuous stirring until cooled to room temperature and add benzylalcohol, menthol eutectic mixture in during of mixing of gel at 40 ^{0}c and emulsion to obtain the Emulgel [Figure 7.1].

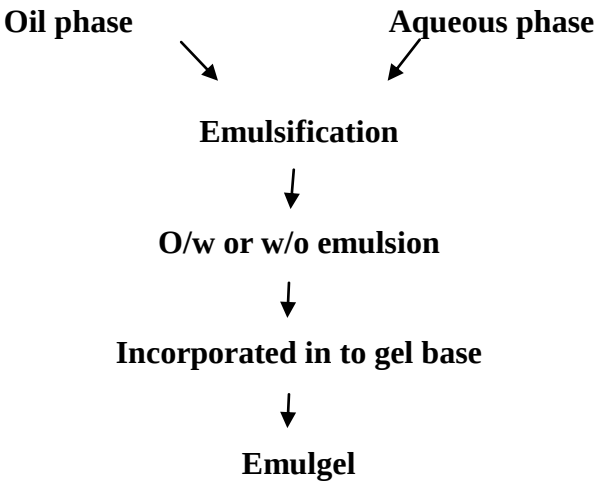

Fig: 5.1: Emulgel preparation method.

6. Experimental Design and Analysis

A computer-generated experimental design was built for statistical analysis by ANOVA generating model equations, using software Design-Expert®, Version 11, Stat-Ease, Inc., Minneapolis to evaluate the effects of the concentration of preparation factors on the formulation of Emulgel. Three-dimensional (3D) surface response plots and contour plots were constructed to establish the relationship between independent variables and dependent variables. According to preliminary experiments, the main independent variables that affected the properties of Emulgel in the desired Formulation were the concentration of gelling base i.e. ultrez 20 (% w/v) (X1), and concentration of Cetostearyl alcohol (% w/v) (X2). A total of four formulation batches were prepared by considering these two independent factors at three different level values. The two dependent variables consisted of the % cumulative drug release (R1) and spreadability (R2). The two-level values of each independent factor are shown in Table no. 6.1.

Table 6.1: Two-level values of each independent and dependent factor.

Independent factors	**Factor code**	**Level**	
		medium	High
Ultrez 20	X1	0.3	0.5
Cetostearyl alcohol	X2	5	7.2
Dependent factors	R1: % Cumulative Drug Release		Target up to 100
	R2: Spreadability (cm)		Optimum spreadability

Table 6.2: Formulation composition of an Emulgel.

Sr.no	Ingredients (Qty in %)	Batch Codes			
		F1	**F2**	**F3**	**F4**
1	Turmeric	Ext. of 1g	Ext. of 1g	**Ext. of 1g**	Ext. of 1g
2	Zinger	Ext. of 1g	Ext. of 1g	**Ext. of 1g**	Ext. of 1g
3	Black paper	Ext. of 0.5g	Ext. of 0.5g	**Ext.of 0.5g**	Ext. of 0.5g
4	Menthol	100mg	100mg	**100mg**	100mg
5	Ultrez 20	0.5	0.3	**0.5**	0.3
6	Cetostearyl alcohol	5	7.2	**7.2**	5
7	Glyceryl Monostearte	2	2.5	**2**	2.5
8	Benzyl Alcohol	0.2	0.1	**0.1**	0.2
9	Triethanolmine	0.5	1	**0.5**	1
10	Disodium edetate	0.1	0.05	**0.05**	0.1
11	cetomacrogol	1.8	2	**1.8**	2

7. Evaluation of qualitative and quantitative parameters

Characterization of Emulgel

Emulgel was evaluated for parameters like:

7.1 Physical examination

The prepared Emulgel formulations were visually inspected for colour, appearance and homogeneity.

7.1.1 Determination of pH

pH of the formulation was determined using digital pH meter. pH meter electrode was washed by distilled water and then dipped into the formulation to measure pH, and this process was repeated 3 times.

7.1.2 Spreadability

The spreadability of Emulgel formulations were determined by placing 0.5 g of respective Emulgel within a circle of diameter 1 cm, pre-marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for about 15 seconds. The diameter due to spreading of the Emulgel was noted.

7.1.3 Viscosity

The viscosities of formulations were determined by Brookfield viscometer using spindle no 7. The hydrogel sample was taken in a beaker and the dial reading was noted at 100 rpm.

7.1.4 Extrudability

The method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudabilitys of each formulation was in triplicate and the average values were presented. The extrudability was than calculated by using the following formula:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm^2)

7.1.5 Swelling Index

Emulgel of known weight (1 gram) was wrapped with Aluminium Foil (pricked with a pin to make holes) and placed in phosphate buffer pH 7.4 for 7 hours. After 7 hours, the gels were scrapped and the wet weight of the swollen gel was determined by first blotting the gels with filter paper to remove absorbed water on surface and then it was immediately weighed on an electronic balance. The weight of the swollen gels was determined using an electronic balance.[12] The swelling index of was calculated using the following formula,

$Sw = [(Wt – Wo) / Wo] \times 100$

Where, Sw = percentage of swelling of Emulgel

Wt = the weight (g) of the gels at time t

Wo = initial weight (g) of the Emulgel.

7.2 Thin layer chromatography (Wagner, 1997)

The Emulgel was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them. The details of the procedure are as follows:

- **Preparation of the plates**

The adsorbent/stationary phase used for thin layer chromatography was silica gel G. About 25 g of silica gel G was taken in a glass mortar and about sufficient water was added to it. The mixture was stirred with glass rod until it became homogeneous and allowed to swell for 15 minutes. Then additional water was added to it with stirring. This suspension was then uniformly spreader immediately on plates.

- **Drying and storage of plates**

The freshly coated plates were then air dried and stacked in a drying rack and were heated in an oven for 30 minutes at 110° C. Activated plates were kept in a desiccator, till required for further use.

Sample and Standard preparation Sample and Standard stock solution where prepared in Methanol.

Application of the sample

The test samples were applied in the form of a band, with the help of fine capillaries.

- **Developing solvent system**

i) **for turmeric:** Cloroform:Methanol (9.25:0.75)

ii) **for ginger:** n-hexane: Diethyl ether(4:7)

iii) **for black paper:** Toluene : ethyl acetate (7:3)

- **Development of TLC plates**

Chromatographic rectangular glass chamber was used in the experiments. To avoid insufficient chamber saturation. Different mobile phase where tried but the satisfactory resolution was obtained in the solvent systems given above. After development of plates, they were air-dried and numbers of bands were noted & Rf values were calculated. The Rf value (Retention Factor) was calculated as follows,

$$RF = \frac{\text{Distance travel by the sample}}{\text{Distance travel by the solvent}}$$

7.3 Drug Content

The quantity of polyherbal emulgel sample equal to 1 gm was dissolved in sufficient quantity of methanol and volume was made up to 100 ml. The absorbance was measured by UV spectrophotometer at λmax-418 nm, 219 and 340 nm the drug content was calculated using standard graph.

7.4 In-vitro diffusion studies

In-vitro diffusion study of topical polyherbal emulgel formulations was carried using on Franz diffusion cell having 2 cm outer diameter and 23 mL capacity. Dialysis membrane-50, LA-393 having cut of molecular weight 12000-14000k Da (Himedia) was used as membrance. Pieces of dialysis membrance were soaked in pH 7.4 phosphate buffer, the dialysis membrane was mounted on cell. The temperature was maintained at 37 ^{o}C. In topical polyherbal emulgel formulation was placed in donor compartment. The 1mL sample was withdrawn from the acceptor compartment, replacing the sampled volume of phosphate buffer pH 7.4 after each sampling up to required time period. The samples withdrawn was filtered and used for analysis. Blank sample was run simultaneously throughout the

experiment to check for interference. The amount of drug diffused was calculated using UV-spectrophotometer at individual absorbance at 418 nm, 219 nm and 340 nm.

7.5 Skin irritation study

The study was carried out using 7 albino mice of either sex weighing between 20 to 30g. Animals were divided into two groups of 3 animals in each (one as control and another as test). Hairs were depleted from the back of mice with the help of depilatories and animal were used after 24 h after hair depletion. Emulgel was applied once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as

0 – No reaction

1 – Slight scaly and erythematous plaques

2 – Moderate scaly and erythematous plaques

3 – Severe scaly and erythematous plaques

7.6 In vivo anti-inflammatory study

The study was conducted in accordance with the approval of the Animal Ethical Committee, university department of pharmaceutical sciences Edema was induced on the left hind paw of the Rat by sub planter injection of 1% w/v carrageenan. Emulgel formulation F5 and standard (diclofenac gel) were applied 30 min after carrageenan injection. The paw volume was measured at intervals of 30, 70, 90, 120 and 180 min by vernier caliper.

Group 1 (control group): carrageenan (1% w/v) was injected in the plantar surface of Rat.
Group 2 (standard group): topical marketed Diclofenac emulgel +carrageenan (1% w/v).
Group 3 (test group): polyherbal emulgel formulation F5+ carrageenan (1% w/v).

Each group contained four Rat. The % inhibition of paw edema in drug treated group was compared with carrageenan control group and calculated using the formula:

%inhibition =V_c- V_t/V_c x100

Where, V_c is the inflammatory increase in paw volume control group and
V_t the inflammatory increase in paw volume in test group

7.7 Stability study

Formulation showing optimum viscosity, pH, and in-vitro drug release was selected for stability studies. Stability studies carried out on polyherbal emulgel formulation. Sufficient

quantities of formulation in aluminium tube were stored in stability chamber, at 40± 2 ^{o}c and 75 + 5%RH. The physical stability of topical polyherbal emulgel was observed periodically for the occurrence of turbidity and gelation. Polyherbal emulgel formulation was evaluated at interval of 1month, 2 month for the viscosity, pH, percent drug content, in-vitro drug release.

RESULTS AND DISCUSSION

1. Drug-Excipients compatibility study

After 30 days storage of drug extracts 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 extracts and polymer combination.

Table 1.1: Physical Observations of Compatibility Study.

Batch	Caking		Discolor-ationn		Liquification	
Extracts	Initial	1 mon	Initial	1 mon	initial	initial
Ultrez 20	No	No change	No	No change	No	No change
Extrcts+Ultrez20	No	No change	No	No change	No	No change
Extracts+glyceryl monostearate	No	No change	No	No change	No	No change
Extracts+cetosteryl alcohol	No	No change	No	No change	No	No change
Extracts+cetomacrogol	No	No change	No	No change	No	No change
Extracts+Benzyl alcohol	No	No change	No	No change	No	No change
Extracts+Triethyl amine	No	No change	No	No change	No	No change
Extrcts+Menthol	No	No change	No	No change	No	No change
Extracts+Disodium edentate	No	No change	No	No change	No	No change
2. UV spectroscopic determination of λmax						

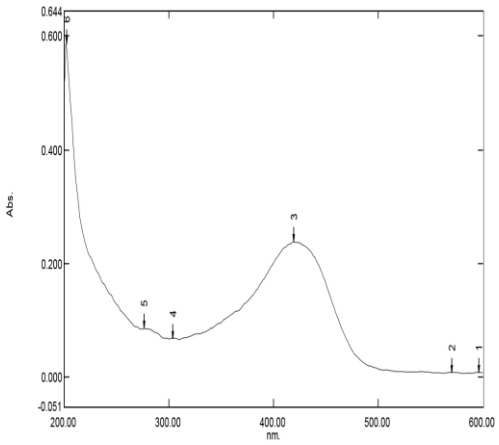

Fig: 2.1 Absorption spectra of Turmeric.

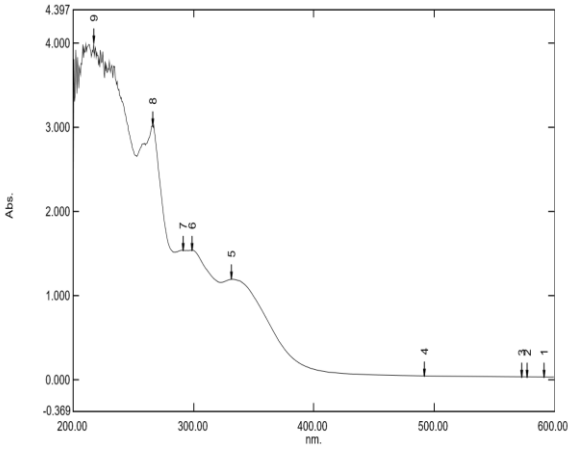

Fig: 2.2 Absorption spectra of Ginger.

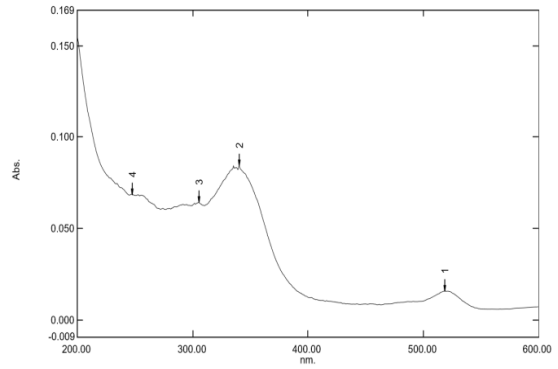

Fig: 2.3 Absorption spectra of Black pepper.

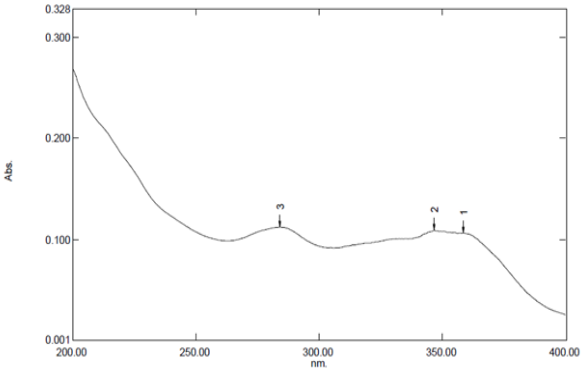

Fig: 2.4 Absorption spectra of menthol.

3. Standard calibration curves

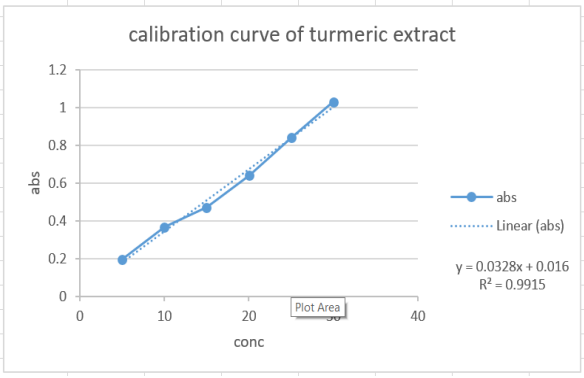

Fig: 3.1 turmeric in methanol.

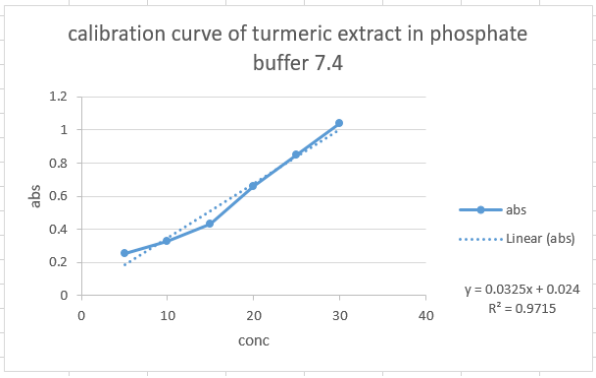

Fig: 3.2 turmeric in phosphate buffer.

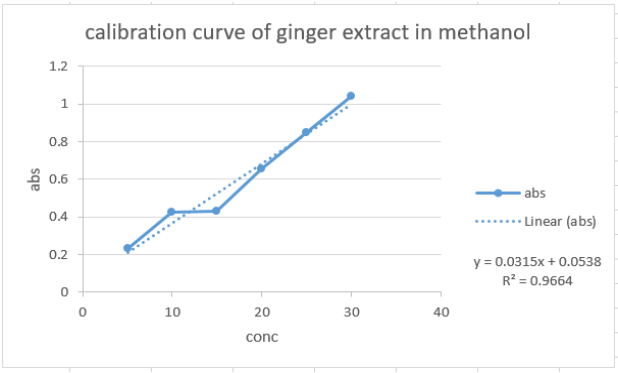

Fig: 3.3 ginger in methanol.

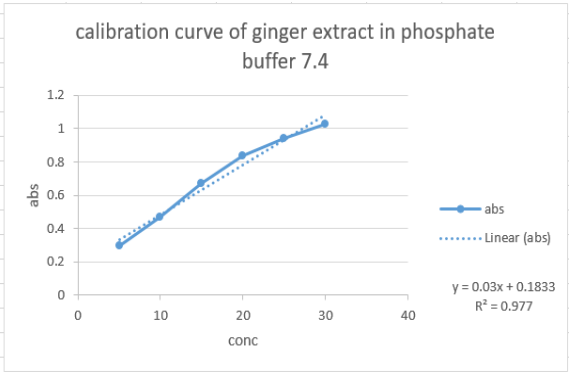

Fig: 3.4 ginger in phosphate buffer.

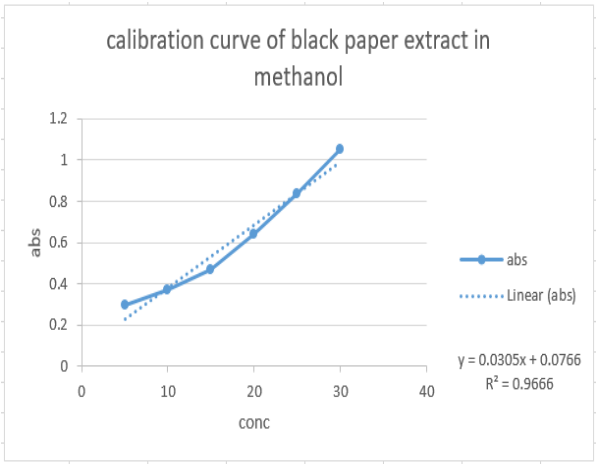

Fig: 3.5 black paper in methanol.

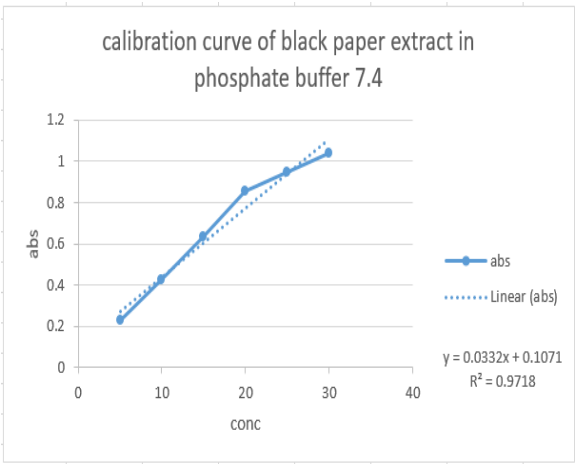

Fig: 3.6 black paper in phosphate buffer.

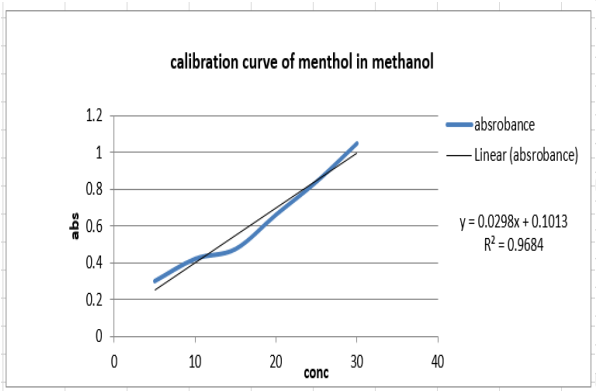

Fig: 3.7 Menthol in methanol.

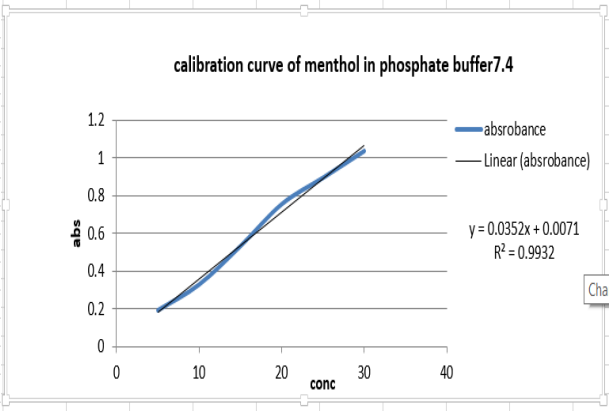

Fig: 3.8 menthol in phosphate buffer.

4. Optimization of polyherbal emulgel by factorial design

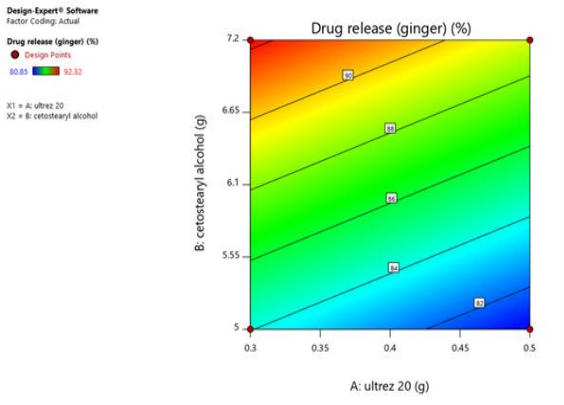

Fig: 4.1 Contour plots for % Cumulative Drug release of ginger.

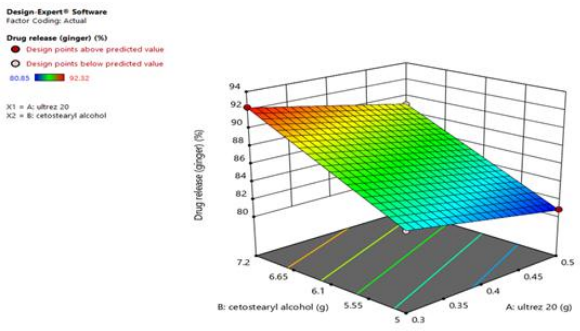

Fig: 4.2 3D Response surface plot for % Cumulative drug release of ginger.

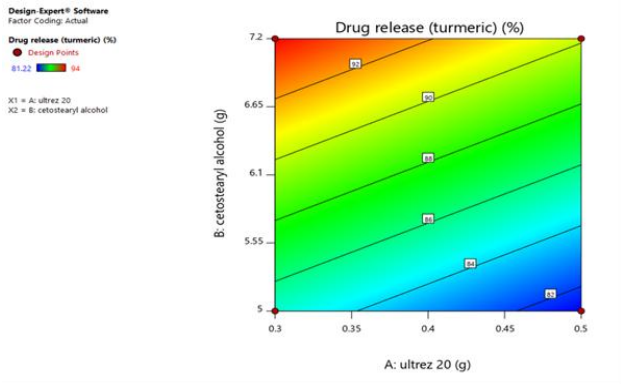

Fig: 4.3 Contour plot for % Cumulative drug drug release of turmeric.

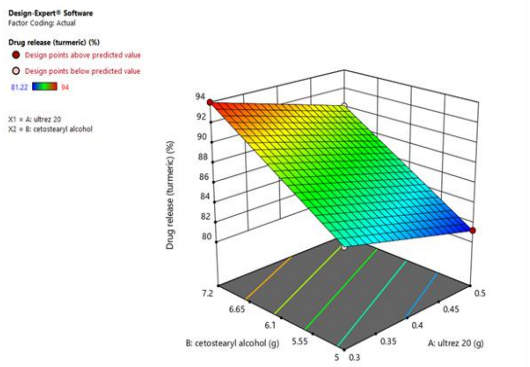

Fig: 4.4 3D Response surface plot for release of turmeric.

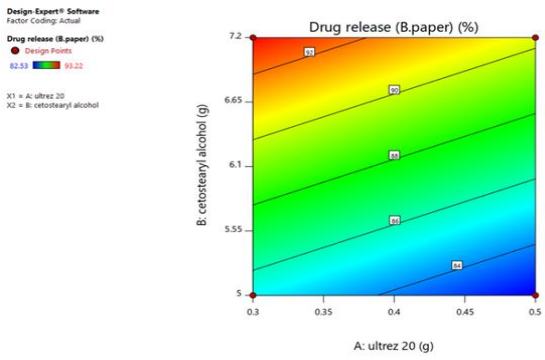

Fig: 4.5 Contour plot for % Cumulative drug drug release of black paper.

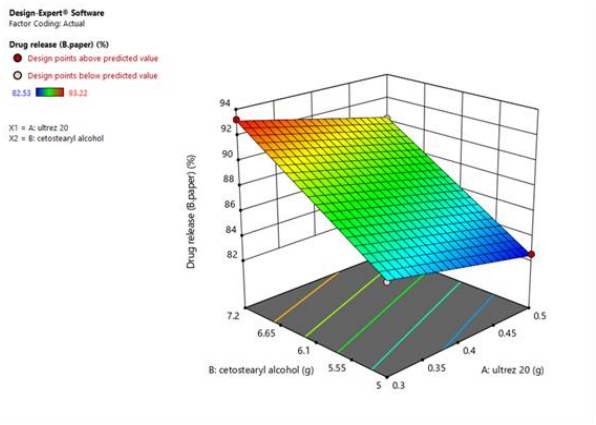

Fig: 4.6 3D Response surface plot for release of black paper.

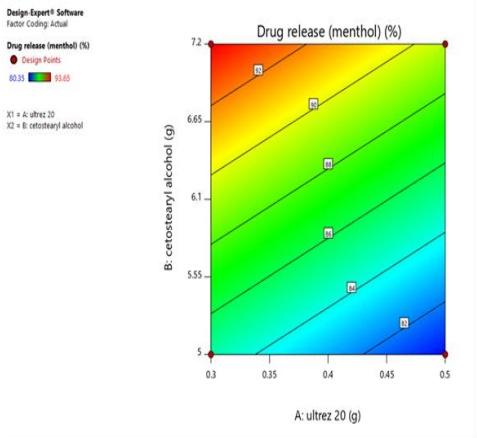

Fig: 4.7 Contour plot for % Cumulative drug drug release of Menthol.

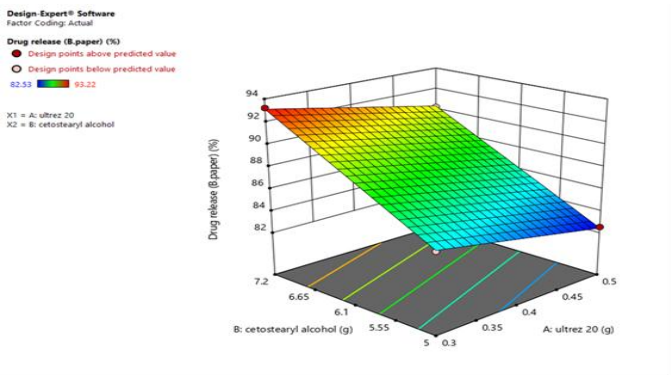

Fig: 4.8 3D Response surface plot for release of Menthol.

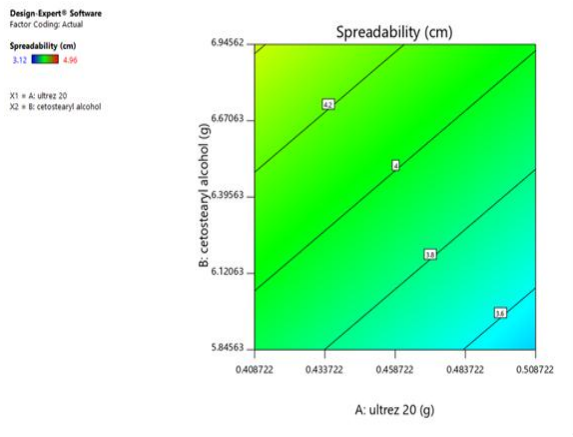

Fig: 4.9 Contour plot for spreadability.

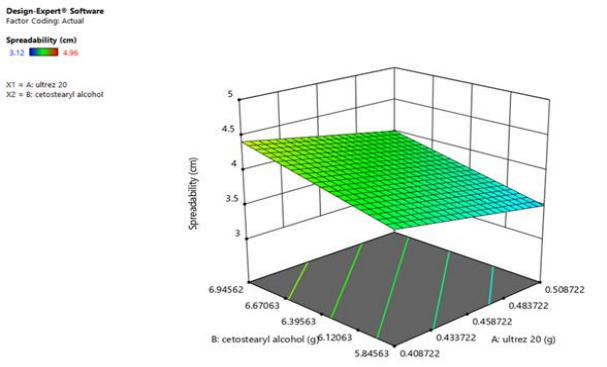

Fig: 4.10 3D Response surface plot for spreadability.

5. The prepared Emulgels were yellow in color free from grittiness and had good consistency.

Table 5.1: shows the appearance characteristics of the Emulgels.

Formulation Code	Color	Grittiness	Consistency
F1	Yellow	-	++
F2	Yellow	-	+++
F3	**Yellow**	-	**+++**
F4	Yellow	-	++

Table 5.2: Spreadability, pH and viscosity of emulgel formulations.

Formulation Code	Spreadability(cm)	Viscosity (cps)	pH
F1	3.63	32,451	6.46
F2	4.33	31,331	6.33
F3	**4.33**	**31,312**	**6.68**
F4	4.33	31,312	6.62

6. Extrudability Test

The extrudability was determined as per the method mentioned using a weight of 20 gms most of the gels exhibited excellent extrudability characteristics. (+ fair, ++ Average, +++ Excellent).

Table 6.1: Table indicating Extrudability Swelling Index results.

Formulation Code	Extrudability	Swelling Index
F1	++	38.3%
F2	+	33.7%
F3	**+++**	**48.23%**
F4	++	44.82%

7. Thin Layer Chromatography (TLC)

R_f Values for methanolic extract of Turmeric

Solution	Solvent-Front Height (cm)	No. of spots	Spot height(cm)	Rf Value
Turmeric extract	5.9	1	3.1	0.52
Polyherbal Emulgel	5.6	1	2.9	0.51

Fig: 7.1 Curcuma longa TLC Rf Values for methanolic extract of ginger.

Solution	Solvent-Front Height (cm)	No. of spots	Spot height(cm)	Rf Value
ginger extract	6.5	1	2.3	0.35
Polyherbal Emulgel	8.7	1	3.2	0.36

Fig: 7.2 *Zingiber officinale* TLC.

R_f Values for methanolic extract of black paper

Solution	Solvent-Front Height (cm)	No. of spots	Spot height(cm)	Rf Value
blackpaper extract	4.3	1	3.5	0.81
Polyherbal Emulgel	4.2	1	3.4	0.80

Fig: 7.3 *Pipper nigrum* TLC.

8. Drug content

Table 8.1: Percent drug content of polyherbal emulgel formulations.

Formulation	%Drug content of Turmeric	%Drug content of ginger	%Drugcontent of black pepper	%Drug content of menthol
polyherbal emulgel	94.72%	93.62%	90.43%	92.23%

9. In-Vitro diffusion study

In vitro studies of formulation were performed using the Franz diffusion cell with dialysis membrance. Phosphate buffer pH 7.4 was used as diffusion media. Dialysis membrane-50, LA-393 having cut off molecular weight 12000-14000 kDa (Himedia) was used for diffusion membrane.

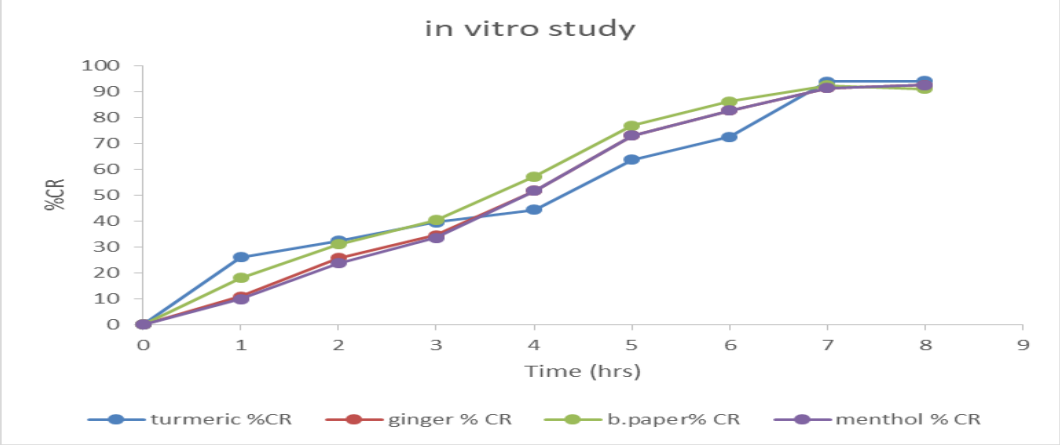

Fig: 9.1 % Cumulative drug release of polyherbal emulgel (F3 formulation).

10. Release kinetic study

Drug the drug release data of the In-vitro drug release studies were analyzed with various kinetic models like zero order, first order, Higuchi model, Korsmeyeer-Peppas model. Correlation coefficient values were calculated for the linear curves by regression analysis for above plots.

Table 10.1: Drug release kinetics study.

Release-kinetic model	R^2 value			
	turmeric	ginger	Black pepper	menthol
First order	0.9917	0.9587	0.93	0.9528
Zero order	0.9951	0.9862	0.9686	0.9883
Higuchi	0.9948	0.9665	0.9378	0.9698
Korsrmeyer peppas	0.9945	0.9951	0.9786	0.9852

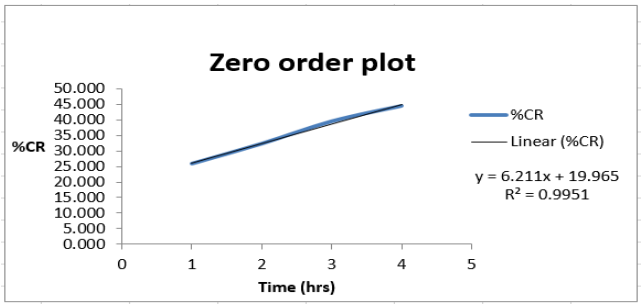

Fig: 10.1 Zero order kinetics (Turmeric)

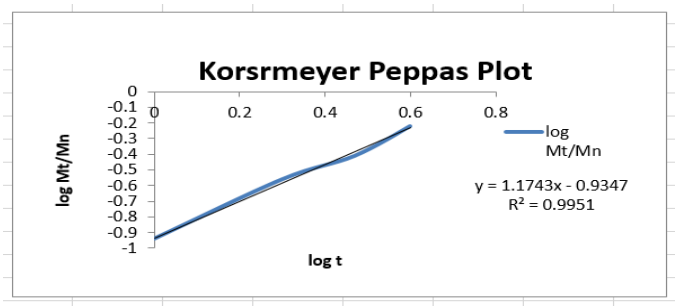

Fig: 10.2 Korsmeyeer-Peppas plot (ginger)

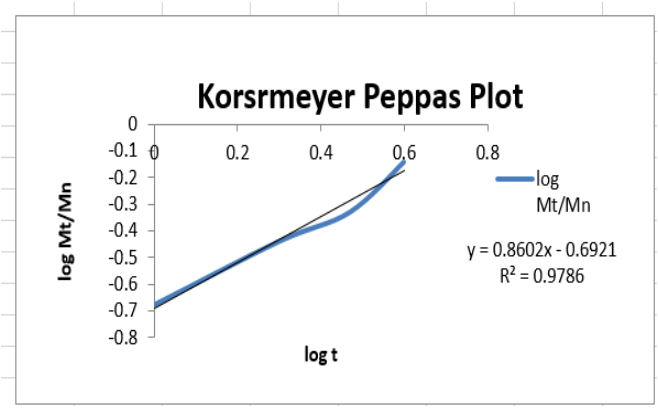

Fig: 10.3 Korsmeyeer-Peppas plot (black pepper).

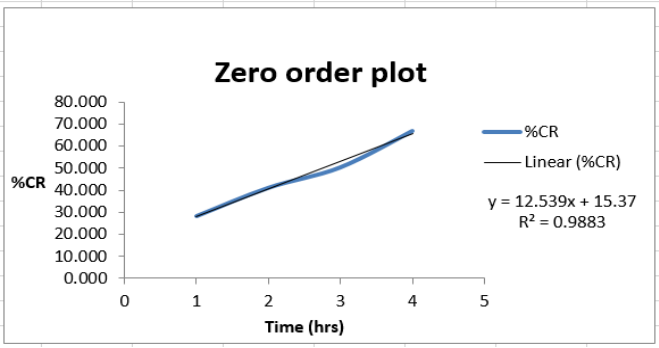

Fig: 10.4 Zero order kinetics (menthol).

11. study Skin irritation

Skin irritation studies carried out on mice revealed that the formulation F3 shows no reactions (t No detectable level of skin irritancy in mice indicates the compatibility of formulation F3 with skin. Thus preliminary test for irritation resulted in selecting the gel for further tests because it proved to be non-irritant.

Table 11.1: Skin irritation study of selected polyherbal emulgel (batch F3)

Treatment	Treatment Scores on respective days						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0	0	0	0	0	0	0
F3	0	0	0	0	0	0	0

12. In vivo anti-inflammatory study

The anti-inflammatory activity of the polyherbal emulgel formulation F3 (test) was compared with marketed Diclofenac emulgel i.e., standard group. The % inhibitions of standard and test group were 65.92% and 51.08%, which indicated that formulated emulgel was more effective than marketed emulgel.

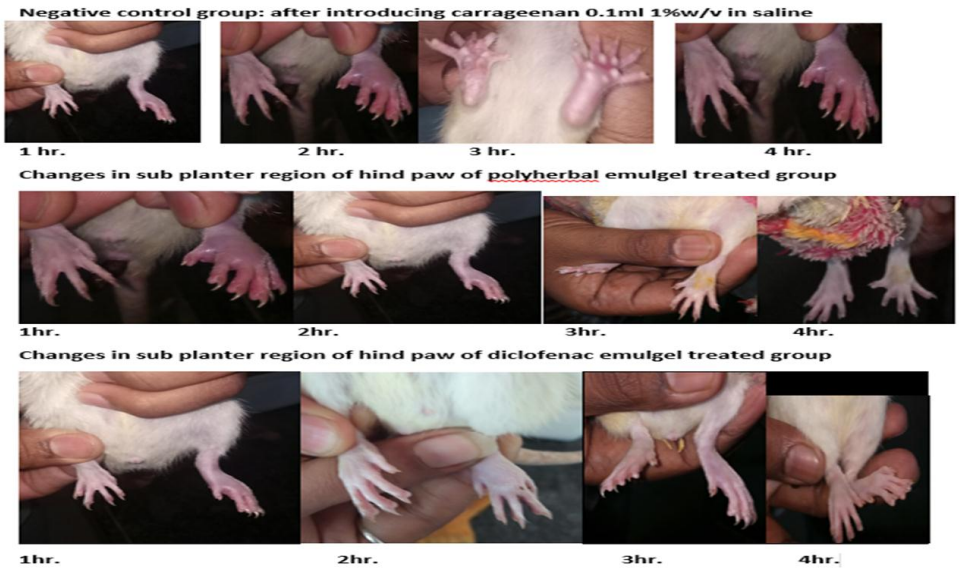

Fig: 12.1 Changes in sub planter region of hind paw after applying polyherbal emulgel and diclofenac emulgel.

Table 12.1: Percent inhibition of paw edema.

Group	Paw thickness (mm)					Percent inhibition
	0min	1hr	2hr	3hr	4hr	
Control	2.43	4.26	4.57	4.7	4.82	0
Standard	2.58	4.24	3.8	3.33	3.52	65.92
Test	2.2	4.02	3.62	3.32	3.48	51.08

13. Stability study

The polyherbal emulgel formulation was evaluated for stability studies stored at 40 ± 2 ^{o}C temperature and 75 ±5% RH for 2 month. The formulation shows good stability with no remarkable changes in viscosity, gel strength, and drug content, and in-vitro drug released profile was shown in Table 8.25.

Table 13.1: Stability study of optimized F3 batch.

Sr. no.	Parameter	Storage period 2 month at 40 ± 2^{o}C temp. and 75 ±5%RH		
		Initial	After 1 month	After 2 month
1	Viscosity (Cps)	31,312	31.303	31,300
3	Drug content (%)	92.72%	92.12%	92.00%
4	In –vitro drug released (%)			
	Turmeric	90.12	90.00	90.00
	Ginger	88.96	88.22	88.00
	Black pepper	90.23	89.85	89.80
	menthol	89.55	89.00	89.00
5	pH	6.68	6.62	6.66

CONCLUSION

The aim of the study was to formulate and evaluate polyherbal Emulgel as a topical drug delivery system for the treatment of rheumatoid arthritis by using anti-inflammatory, drugs like turmeric, ginger, black paper, menthol as these are lipophilic and hydrophilic drugs having problem to incorporate directly into, gelling base or cream base.

So Emulgels are a unique approach for the hydrophobic drugs as compared to conventional gels. They overcome the drawback hydrophobicity because the Emulgel possess both phases hence it is suitable for both hydrophilic and lipophilic drugs.

In case of skin diseases, skin become inflamed, itchy, red, hard, dry and patchy, the topical emulgel containing cetostearyl alcohol and glyceryl monostearate which acts as a self-emulsifier as well as moisturizer, menthol used as active plays both roles active as well as penetration inhancer. Topical drug delivery system has great advantage that it allows to target the site and reduces the dose of drug owing to it topical treatment is choice of treatment for rheumatoid deseases. According to plan of work the preformulation studies were done to characterize the drug. All the results of the preformulation studies were found in range given in pharmacopoeial standards. The calibration curves of all the drugs in various solvents were found to obey the Beer-Lambert's law. Prior to formulation of an Emulgel, Drug polymer

compatibility studies were performed by visualisation, no alteration occurred in drug structure and physicochemical properties.

The optimized batch was evaluated for appearance, viscosity, Spreadability and drug content, all physical evaluation parameters results were found in acceptable range. Invitro diffusion study of gel containing pure drug was also performed. The gel showed increase in drug release and permeability. Animal model was performed, rat paw edema model was used to evaluate the effectiveness of formulation and it compared with marketed formulation, Emulgel reduces inflammation as marketed formulation. According to the results obtained, it was observed that, increase in concentration of ultrez 20 resulted in decreased spreadability and % drug release whereas Increase in concentration of cetostearyl alcohol and glyceryl monostearate (emulsifier) was resulted in increased spreadability with increase in % cumulative drug release.

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