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FORMULATION AND EVALUATION OF ANTI-ARTHRITIC POLY HERBAL EMULGEL

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Abstract

Herbal molecules are known for their potent action and fewer side effects but they have draw backs like low bioavailability, High molecular weight, Low lipophilicity etc. To overcome such drawbacks novel drug delivery systems are employed. One of such an approach is Emulgel. Aim of this work was to formulate an Emulgel of Guggulsterones, Liquorice and Serrapeptidase and investigate their anti-inflammatory and anti-arthritic activity. Formulations were prepared using polymers like Tragacanth, Xanthum gum and Guar gum which are of natural origin. The influence of the gelling agent and its concentration on the drug release from the prepared emulgel was investigated. The prepared Emulgels were evaluated for different parameters and it was concluded that formulations F1, F2, F3 were found to be more promising as it showed desirable physicochemical characteristics. F3 formulation showed better anti inflammatory and anti arthritic activity with 70% inhibition when compared with standard which showed only 62 % of inhibition.

Key Words: Guggulsterones, Liquorice, Serrapeptidase, Tragacanth, Xanthum gum, Guar gum.

Introduction

Arthritis is a progressive, disabling, chronic multisystem disease of unknown cause characterized by pain, swelling and stiffness of synovial joints. An inflammatory reaction, increased cellularity of synovial tissue and joint damage are the pathological hallmarks of arthritis[1]. Though conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs like Ibuprofen, Naproxen, Diclofenac sodium and mesoprostol, Celecoxib, Duloxetine have all been associated with adverse effects. Because of this reason, patients

suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief and are amongst the highest users of complementary and alternative medicine [1, 2].

(Reference 1: http://www.arthritis.com/osteoarthritis_symptoms).

(Reference 2: http://www.medicinenet.com/rheumatoid_arthritis/article.html).

Herbal system suggests a number of poly herbal drugs as being effective in the treatment of this condition. Gum Guggul and Liquorice are such efficacious drugs which are low at cost and show a potent anti inflammatory activity. [3, 4]. The repression of NF-κB activation through inhibition of IKK activity is the mechanism of the anti-inflammatory effect of Guggulsterone. Liquorice acts by inhibiting PG synthesis. Serratiapeptidase acts by proteolytic action on clots.

Serrapeptidase is a multifunctional enzyme which is potent in low amounts showing profound ability in reducing the pain by blocking the release of pain inducing enzymes.[5]

When gels and emulsions are used in combined form the dosage forms are referred as Emulgel. The presence of a gelling agent in the water phase converts a classical emulsion into an emulgel. [6, 7, 8].Emulgels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent & pleasing appearance.

Materials & Methods

Guggulsterones (5%) and Liquorice (20 %) extract were obtained as gift sample from Ayush herbals, Himachal Pradesh. Serrapeptidase was obtained as gift sample from Unique Biotech Limited, Shameerpet. Xanthum gum, Tragacanth and Guar gum were obtained from Loba Chemie as gift sample. Dialysis membrane was obtained from Hi media Ltd. Solvents used were of lab analytical grade. Adult albino wistar male rats weighing between 175-200 grams are used for the study. The animals were divided into six groups of three animals each. Studies are carried out in Albino Labs, Hyderabad.

Ethical committee approval No: ***CPCSEA/IAEC/EXP/25/50/2013/EXP-015***.

Characterization:

Organoleptic properties: The Drugs procured are tested for their organoleptic properties like color, odour and appearance. Results are given in Table 1.

Solubility profile:

Solubility of Guggulsterones, Liquorice and serrapeptidase was checked in water, ethanol, methanol, DMSO (Di methyl sulfoxide), chloroform, acetone, phosphate buffer 6.8 pH[9, 10].

Identification of Drugs Procured:

TLC Procedure:

Guggulsterones: A TLC plate is prepared using silica gel G as a coating substance. A mobile phase mixture of 3 volumes of light petroleum (boiling point 60-80 ^{0}) and 1 volume of ethyl acetate is prepared. Apply separately 5 µl of each solution on the plate containing 0.25 % w/v of gugulipid RS allow it to dry in air until the odour of the solvent is no longer detectable and spray with a 50% w/v solution of sulphuric acid. The principal spots in the chromatogram obtained with reference solution should correspond to those in the chromatogram obtained with standard solution. [9]

Liquorice:

TLC Identity test: Test solution: Shake 1 gm of drug with 20 ml of chloroform for 15 min and filter it. Reflux the marc for 1 hour with 30 ml of 0.5 M H_2SO_4 .Cool and shake the unfiltered mixture with chloroform (2*20ml) and concentrate the combined $CHCl_3$ extract. Dissolve the residue in 1 ml $CHCl_3$ extract. Dissolve the residue in 1 ml $CHCl_{3:}$ MeOH (1:1) mixture.

Reference solution: Reflux 5 mg of glycyrrhizin with 20 ml 0.5 M H_2SO_4. Cool and extract with chloroform (2*10 ml) .Evaporate with combined $CHCl_3$ extract and dissolve the residue in 1 ml $CHCl_{3:}$ MeOH (1:1) mixture.

Solvent system: Toulene: ethyl acetate: glacial acetic acid (12.5: 7.5: 0.5)

Procedure: Apply 5 µl each of test and reference solutions in 2 different tracks on a pre coated silica gel G F_{254} plate (5*15 cm) of uniform thickness (0.2 mm) .Develop the plate in the solvent system to a distance of 12 cm. Scan densitometrically at 254 nm both reference and test solution tracks and record the fingerprint profiles. Under UV at 254 nm Spots are visualized when the plate is sprayed with Anisaldehyde-sulfuric acid reagent and heat at 110 ^{o} c for 5-10 min.

Evaluation: 1) Under UV 254 nm (before spraying): two spots (0.41, 0.45) exhibiting quenching are visible in the sample solution track, one of which (Rf 0.41) corresponds to glycyrrhetic acid of reference track.

2) In day light (after spraying): Glycyrrhetic acid is visible as a dark violet spot in both reference and test solution tracks. Other spots visible in the test solution indicates two dark yellow spots (Rf 0.45, 0.49), two violet spots (Rf 0.27, 0.70) and a dark blue spot running along with the solvent front.[9]

FTIR studies:

Drug-polymer interactions are carried out by infrared spectral analysis. The drug excipients compatibility study was determined by FTIR (shimadzu) using KBr pellets of 0.1 mm. The IR spectrum of the pure drug was compared with IR spectrum of combination of drug and all the excipients to check the interaction. (Figure: 1- 4)

Analytical Methodology:

The UV absorption spectrum was obtained for a solution of Guggulsterone, Liquorice and Serra peptidase by mixing the drugs in a mixture of DMSO (Di methyl sulfoxide) and 6.8pH phosphate buffer (2:8) over a wavelength range of 200-400nm[11] using UV spectroscopy (Lab India UV 3000+). Wavelength maxima values are obtained and a calibration graph is plotted.[10]

Wavelength maxima of Guggulsterone: 245nm

Wavelength maxima of Liquorice: 255 nm

Wavelength maxima of Serrapeptidase: 229 nm.

Iso bestic point: 236 nm.

Formulation of Emulgels:

The required quantities of Guggulsterones are taken in a beaker and added with sufficient amounts of propylene glycol/ Capmul. Add span 20 and sonicate to prepare the oil phase. A required quantity of Serrapeptidase is added to some amount of water and Liquorice is added to ethanol. Sonicate the drug solutions and mix them to make aqueous phase. Add preservatives to the oil phase. Heat the two phases separately to 60-70 $^{\circ}$ c and add the oil phase to aqueous phase to make an emulsion (o/w).Prepare a gel using appropriate amount of gelling agent and water. To ensure proper mixing and remove air bubbles the gel is kept on a magnetic stirrer. Repeat the same process to prepare Xanthum Emulgel and Guar gum Emulgel. Add the prepared emulsion to the gel and stir gently to prepare an Emulgel [12, 13, 14]. Prepared Emulgels are evaluated for parameters like color, homogeneity, consistency, spreadability, pH determination, in vitro

diffusion studies, Rheological studies and Drug content. Formulation table is given in Table 2. Results are given in Table 3.

Evaluation[14, 15]:

Homogeneity*:*

All developed Emulgels were tested for homogeneity by visual inspection after they have been set in the container. They are tested for their appearance and presence of any aggregates, particles and fibers. Results are given in Table 3.

Color Change:

All Emulgels are checked for any color change by visual inspection. Results are given in Table 1.

pH Determination:

The pH of 1% aqueous solutions of the prepared Emulgels was measured by using a calibrated digital pH meter. Results are given in Table 3.

Spreadability: The spreadability of the prepared formulations was determined by measuring the spreading diameter of 1 gm of the emulgel between 20x20cm glass plates after 1 minute. The mass of the upper plate is standardized at125gm.The spreadability is calculated using the formula

$$S=M.L/T$$

Where, S= spreadability, M=weight tied to the upper slide, L=length of glass slide T=time taken to separate the slide completely from each others. Results are in Table 3.

Viscosity*:* The viscosity of the formulation was determined using cone and plate viscometer with spindle number 63 at different rpm (Brookfield programmable rheometer DV-III). (Results shown in Table 3).

Drug Content: 1gm of the Emulgel was taken and dissolved in 100 ml of Phosphate buffer 6.8 pH and kept aside for 1-2 hrs. The solution was passed through the Whatmann filter paper no. 42 and filtered. Appropriate dilutions were done if required and the drug content was determined spectrophotimetrically against pH6.8 at 236nm. (Results shown in Table 3)

Extrudability study:

The formulations are filled in the collapsible tubes. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5cm ribbon of gel in 10 seconds.

In vitro diffusion studies:

Drug release studies are carried out using Franz diffusion cell (with dimensions 20mm: 15ml volume - 3.14 square cm area) with Dialysis membrane. The membrane was first hydrated in distilled water for 24hrs. The membrane was then clamped between donor and receptor compartments of the cells. The receptor solution is pH 6.8 phosphate buffer (15ml) and is magnetically stirred throughout the experiment. The donor compartment contained appropriate amount of the formulation. (Equivalent to 1 gm of Emulgel).

Aliquot (5ml) of sample is withdrawn from the receptor compartment at specified time intervals and is replaced with fresh receptor solution. The samples are analyzed at 236nm using UV-visible double beam spectrophotometer. The drug concentration is calculated using standard calibration curve. The release studies are conducted and a graph of cumulative % release versus time was plotted. Results are given in Table 3 (Figure 5).

*In vivo Anti Inflammatory Studies***:**

In vivo performance of optimized formulation was assessed by carrageenan induced rat paw edema test. The study was performed in Albino Labs, Bachupally, Hyderabad. Male wistar rats (150-200 gm) are used for the study. Animals were fasted overnight and are divided into 6 groups each of 3 animals. Except in control group other groups were injected with 0.5ml of 1% carrageenan solution in the sub-planatar region of the left hind paw before half an hour of administration/application of formulation. Emulgel was applied on the paw area in sub planatar region. Take initial hour reading for paw volume and paw thickness using plethysmograph followed by 1^{st}, 2^{nd}, 3^{rd}, 4^{th} hour readings and measure the % inhibition was measured using formula

$$\% \text{ inhibition} = \frac{To - Tt}{To} \times 100$$

Where T_t is the thickness of paw of rats with treated at corresponding time and T_o is the paw thickness of rats of control group at the same time [16, 17]. Results are given in Table 4. (Figure 6)

Group I: Control (normal animals)

Group II: Standard (marketed formulation)

Group III: Disease induced

Group IV: Formulation-I

Group V: Formulation-II

Group VI: Formulation-III

Skin Irritation Studies:

Mice are chosen to perform this test .The placebo Emulgel and the formulation were applied onto the backside of mice and checked for 8 hours to observe any signs of irritation are present or not. These results are compared with control [17].

Stability studies:

The stability study was performed as per ICH guidelines. The formulated gels were stored at different temperatures, viz. 4 ºC ± 2ºC, 25ºC ± 2ºC, 40ºC ± 2ºC for a period of three months and studied for appearance, pH, spreadability, extrudability, drug content and in vitro diffusion studies at one month intervals. Results are given in Table 5.

Results:

Characterization of powdered drugs: The drugs are characterized by performing specific tests to determine their organoleptic properties, Limit tests, Physico parameters and IR studies.

Table 1: Characterization of Drugs:

Identification physical parameter	**Limits for Guggulsterones (5%)/ Inference**	**Limits for Liquorice (20%)/ Inference**	**Reference**	**Limits for Serratiapeptiase/ Inference**	**Reference**
Color	Pale yellow/creamish	Light brown	Herbal pharmacopeia	White pale to brown	USP
Loss on drying	Not more than 5 %	Not more than 5%	Herbal pharmacopeia	Not more than 7 %	USP
Total Ash	Not more than 5 %	Not more than 5%	Herbal pharmacopeia	Not more than 1.5 %	USP
Acid-insoluble ash	Not more than 1 %	Not more than 2%	Herbal pharmacopeia	-	-
Alcohol-soluble extractive	Not less than 27 %	Not less than 65 %	Herbal pharmacopeia	-	-

The drugs are characterized according to Herbal pharmacopeia and it was found to be complied within the limitations

FTIR STUDIES[18]:

FTIR Spectra of Guggulsterones: An FTIR spectrum of Guggulsterones is compared with the standard graph of the drug and is observed to be having no extra new groups in it.

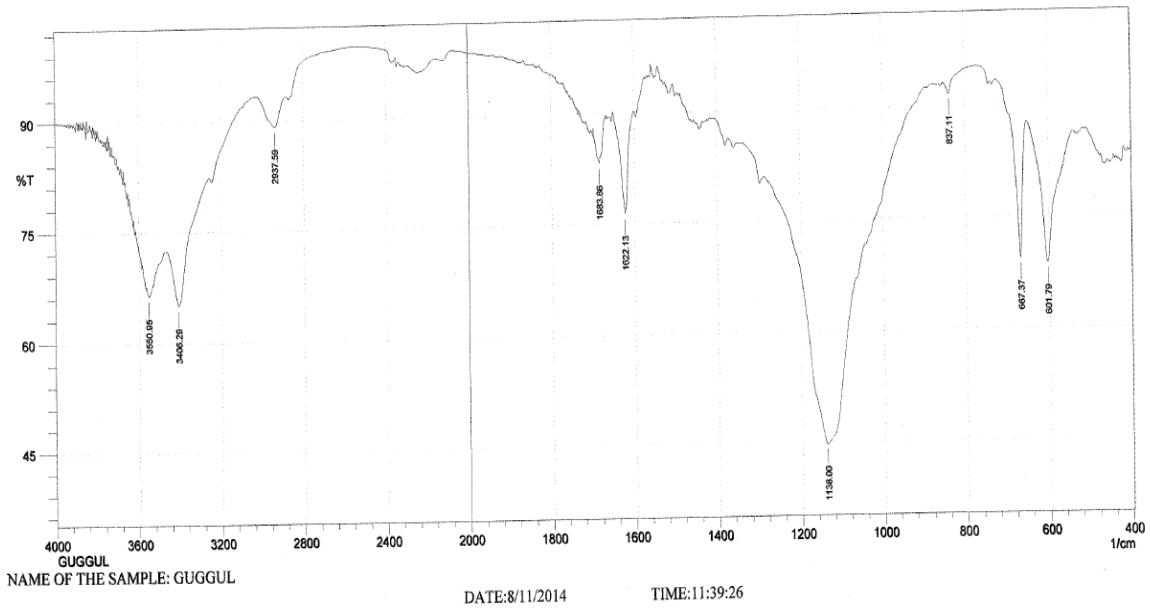


Figure 1: FTIR Spectra of Guggulsterones (as per IP).

FTIR spectra of Liquorice drug: An FTIR spectrum of Liquorice is compared with the standard graph of the drug and is observed to be having no extra new groups in it.

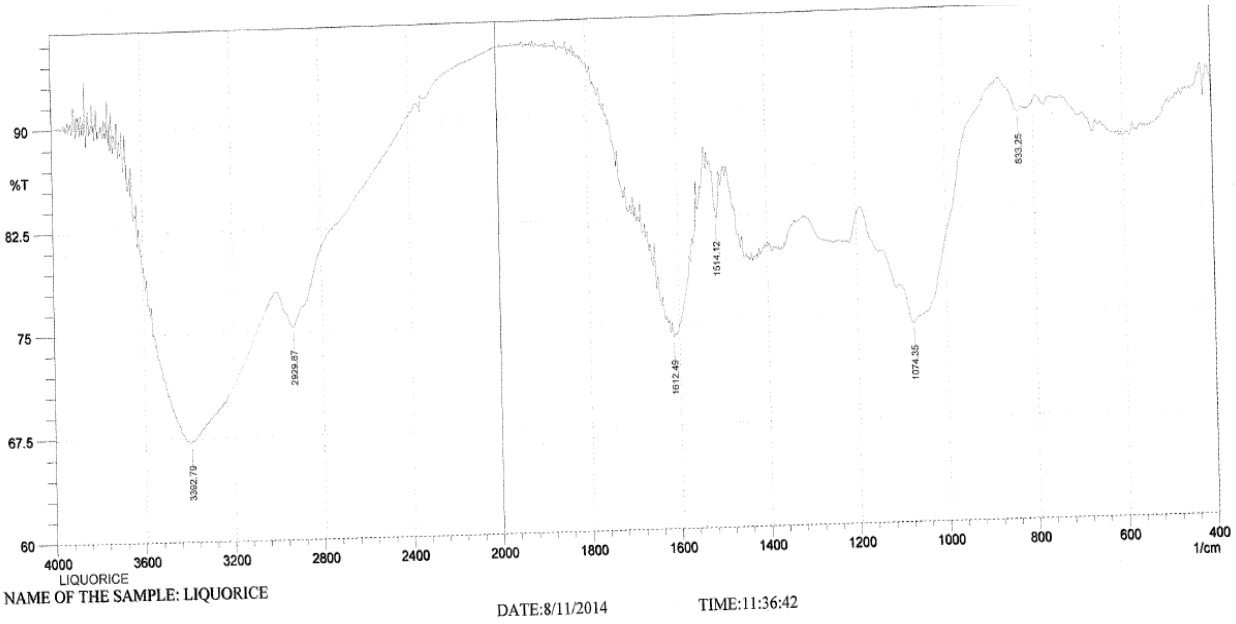


Figure 2: FTIR spectra of Liquorice (as per IP).

FTIR Spectra of Serrapeptidase: An FTIR spectrum of Serrapeptidase is compared with the standard graph of the drug and is observed to be having no extra new groups in it.

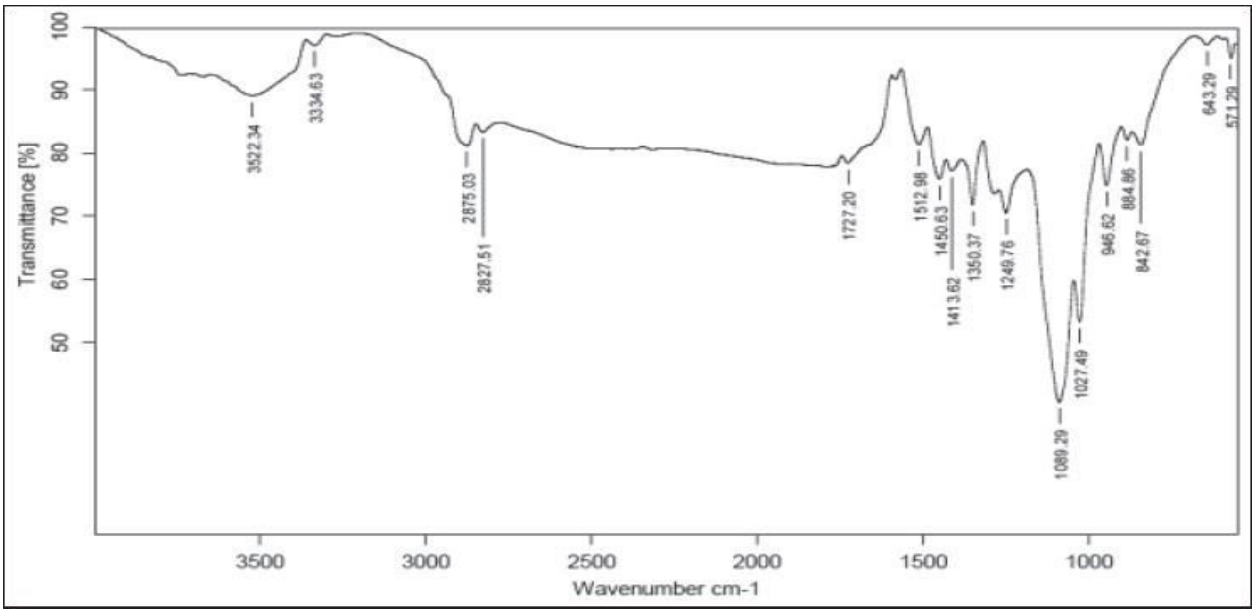


Figure 3: FTIR Spectra of Serrapeptidase.

IR Spectra of Drugs and polymers: According to the data obtained from the spectra it is understood that there is no drug polymer interaction and no new groups are found and is compared with the standard.

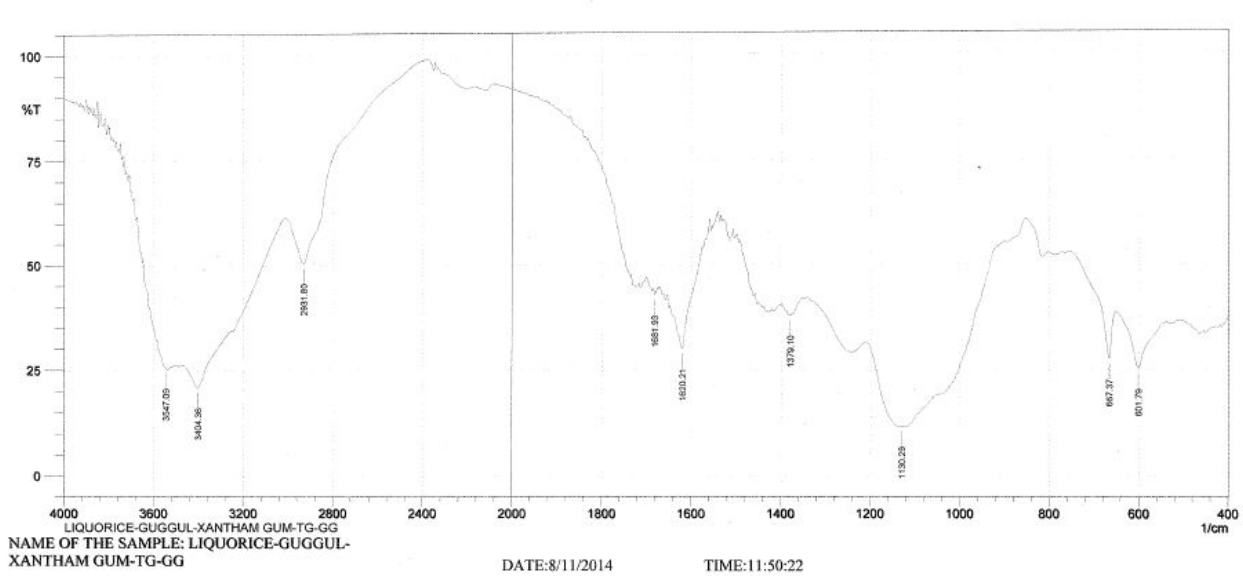


Figure 4: FTIR Spectra of all drugs & polymers.

Solubility profile: Guggulsterones are insoluble in water but soluble in ethyl acetate, chloroform and methanol. Liquorice is soluble in ethanol. Serratiapeptidase is soluble in water

Formulation of Polyherbal Emulgels:

Various formulations using different natural polymers and oils were prepared, evaluated and optimized for further In vivo studies.

Table 2: Formulation Table.

INGREDIENTS	**F1**	**F2**	**F3**
Guggulsterones (mg)	200	200	200
Liquorice (mg)	200	200	200
Serratiapeptidase(mg)	20	20	20
Tragacanth (%)	3	3	3
Xanthum gum (%)	0.4	-	-
Guar gum (%)	-	1	-
Propylene glycol(ml)	2	2	-
Capmul (ml)	-	-	2
Clove Oil(ml)	1	1	1
Ethanol(ml)	2	2	2
Span-20(ml)	0.05	0.05	0.05
Methyl Paraben (%)	0.09	0.09	0.09
Propyl Paraben (%)	0.011	0.011	0.011
Water (up to 5gm)	Qty up to 5gms	Qty up to 5gms	Qty up to 5gms

Table 3: Evaluation for optimized formulations:

Formulation code	Homogeneity	pH	Spreadability (gm.cm/sec)	Viscosity (cps)	Drug content	% Drug Release
F1	+++	6.68	48.25	26274	97	83.59
F2	+++	6.78	46.89	23245	96	85.6
F3	+++	6.80	51.23	1740	97	91.2

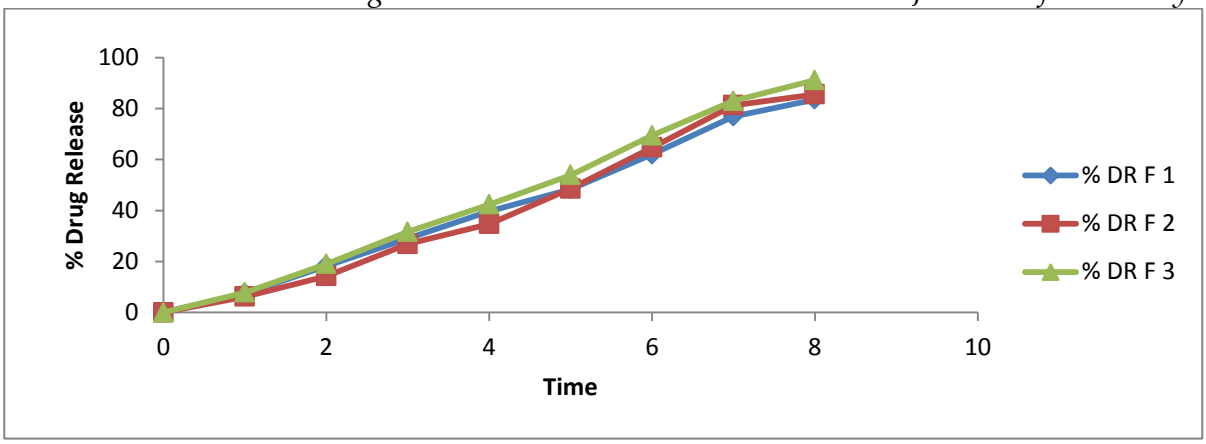
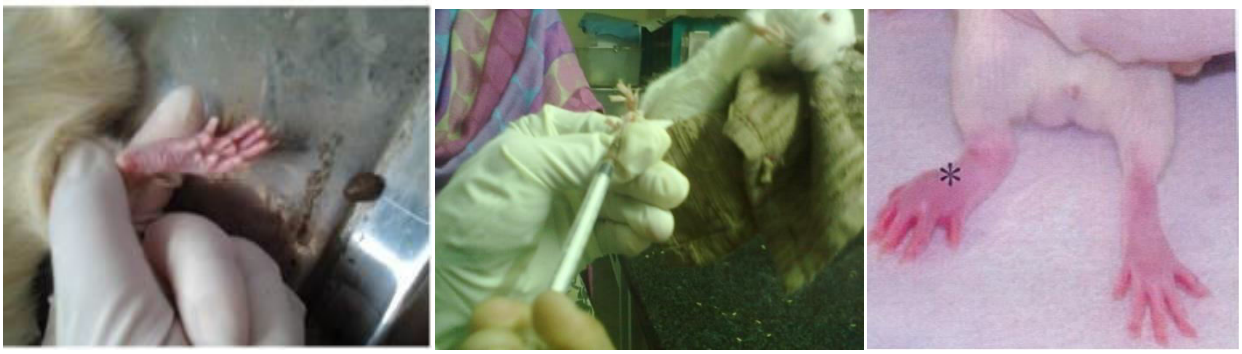


Figure 5: Dissolution profiles of optimized formulations.

Table-4: In vivo anti inflammatory studies.

Sno	Formulation	% inhibition at 30 min	% inhibition at 1 hr	% inhibition at 2 hr	% inhibition at 3 hr	% inhibition at 4 hr
1	F1	19.54	26.12	35.01	43.5	52.7
2	F2	24	30.45	37.45	47.8	62.59
3	F3	27	33.59	42.05	48.05	70.2
4	STD	30.45	35.02	41.99	50.23	62.5

Figure-6: Pictures Showing Anti inflammatory study performed in wistar rats.



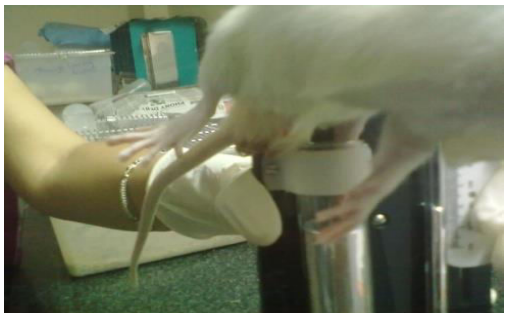


Table 5: Stability Studies of optimized formulations.

Formulation code	Parameter	1st month	2nd month	3rd month
F1	Visual appearance pH Drug content	No change 6.67 96±0.18	No change 6.67 96±0.42	No change 6.67 96±1.11
F2	Visual appearance pH Drug content	No change 6.78 96±2.12	No change 6.78 96±0.43	No change 6.78 95±0.12
F3	Visual appearance pH Drug content	No change 6.80 97±2.84	No change 6.80 97±0.81	No change 6.80 97±0.59

Discussions: Rationale of the present study was to prepare an Emulgel to treat Arthritis. Poly Herbal Emulgel was successfully formulated using different natural polymers. The prepared gel formulations were evaluated and optimized based upon their pH, spreadability, extrudability, viscosity, drug content, invitro drug release. The optimized Emulgel formulations showed transparent appearance. The values of spreadability indicate that the gel is easily spreadable by small amount of shear. They showed good homogeneity with absence of aggregates. pH of optimized formulations was in the range of 6-7 which was considered acceptable to avoid the risk of irritation upon application to the skin. The drug content determination showed that the drug was uniformly distributed throughout the Emulgel. F1, F2 and F3 formulations showed highest % drug content. Formulation F1 showed 83.59% drug release where as formulation F2 and F3 showed 85.90% and 91.2% drug release at the last of 8 hours. Viscosity of the formulations shows that the gel is easily spreadable. The optimized formulations were subjected to stability studies. It was observed that there was no phase separation in any of the formulations.

In vivo anti-inflammatory studies were performed and the results showed that F3 was more active and % inhibition was reduced to 70.4% and is compared to marketed formulation which reduced to 62.5%.

Conclusion:

The Polyherbal Emulgel formulations containing Guggulsterones, Liquorice and Serrapeptidase are successfully formulated and evaluated. Improvement in bio availability and controlled release was achieved by using clove oil as a penetration enhancer. From the above study it is concluded that Polyherbal Emulgel formulations of Guggulsterones, Liquorice and Serrapeptidase can be used as a potential candidate for treatment of arthritis when compared with NSAID's which are potentially dangerous and cause many side effects like Stomach ulcers , Stomach bleeding etc.

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