Research Article

Formulation and Evaluation of Poly Herbal Emulgel for Rheumatoid Arthiritis

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ABSTRACT

The polyherbal emulgel formulations were formulated to treat arthritis from hydroalcoholic extracts of four medicinal plants: *Rubia cordifolia, Vitex negundo, Piper nigrum,* and *Myristica fragrans* (RVMP). These herbs have been reported to have excellent antioxidant, anti-inflammatory and anti-arthritic properties. Carbopol 934, Carbopol 940, HPMC K100M and Sodium CMC were used as gelling agents along with herbal extracts. Formulations were evaluated for physical appearance, spreadability, viscosity, pH, extrudability and *in vitro* drug release. The results showed that the G6 formulation containing Carbopol 940 by weight exhibited better physicochemical properties and better drug release (91.85% at 8 hours) as compared to the other formulations. A polyherbal blend of RVMP hydroalcoholic extracts exhibited a 78.71% inhibition of protein denaturation at 100 µg/ml, indicating a good anti-arthritic candidate.

Keywords: Arthiritis, Emulgel, *Myristica fragrans, Piper nigrum*, Polyherbal formulation, Protein denaturation, *Rubia cordifolia, Vitex negundo.*

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INTRODUCTION

Free radicals can be detrimental when they are produced in excess. They can cause inflammation, cancer, ischemia, lung damage, rheumatoid arthritis, ageing, cardiovascular disease, and other degenerative diseases. [1,2] Rheumatoid arthritis (RA) is an autoimmune disease with no known cause; however, various pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, play a critical role in the development of symmetric joint inflammation, synovial proliferation, cartilage loss, and systemic manifestations such as haematological, pulmonary, neurological, and cardiovascular abnormalities via free radical generation. Patients with Rheumatoid Arthritis suffer from joint pain, which is broadly linked to physical impairment, reduced mobility, fatigue, sleep disruption, and increased medical expenses, resulting in a decline in quality of life and a significant impact on society.[3] The most commonly prescribed drugs for Rheumatoid Arthritis are steroidal, nonsteroidal anti-inflammatory, disease-modifying antirheumatic, and immunosuppressant drugs, all of which have been linked to gastrointestinal problems, immunodeficiency, and humoral disturbances.[4] As an alternative to oxidative damage, natural antioxidants, such as polyphenols found in medicinal and dietary plants are becoming increasingly popular. Our earlier study on a polyherbal mixture consisting of roots *of Rubia Cordifolia*, the fruit of *Piper nigrum*, leaves of *Vitex Negundo*, and seeds of *Myristica fragrans* showed antioxidant potential by eliminating free radicals generated. [5]

Topical preparations have a localized effect at the application site by penetration of the drug into the underlying layer of skin or mucous membrane via ophthalmic, rectal, vaginal, or skin routes. The main advantage of the topical delivery system is to bypass first-pass metabolism. [6] Topical delivery indirectly delivers the drug to the site of action via diffusion, and the absorption occurs directly on the skin's surface. The result is an increased bioavailability and consistent drug delivery for a more extended duration.[7,8,9] Percutaneous absorption of drugs can be improved by increasing the release rate of drugs from dosage forms.[10] The rate at which topical preparations release their active ingredients depends on the carrier and the medication's combined physical and chemical properties. [11] Emulsion gels are becoming increasingly popular as a semi-solid dosage form in the pharmaceutical industry. Due to the wide use of emulsion systems in dermatological formulae, they are also commonly used as pharmaceutical dosage forms. A gelling agent is mixed with emulsions, either water-in-oil or oil-in-water, to form Emulgel. [12]

In the direct (oil-in-water) system, lipophilic drugs are encapsulated, while hydrophilic drugs are encapsulated in the reverse (water-in-oil) system. [13] In addition to being elegant, emulsions can be easily washed off. Furthermore, they have a high penetration capability. Dermatological emulgels are characterized by several favourable characteristics such as being thixotropic, greaseless, easily

spreadable and easily removed, emollient, non-staining, water-soluble, longer shelf life, biodegradable, transparent and pleasing to the eye. [14]

The focus of this research was to formulate a topical emulgel and exploit its antioxidant properties for the treatment of RA using a polyherbal mixture consisting of roots of *Rubia Cordifolia*, the fruit of *Piper nigrum,* leaves of *Vitex Negundo*, and seeds of *Myristica fragrans*.

MATERIALS AND METHODS

Carbopol 934, Carbopol 940, HPMC K100M, Sod. CMC, Tween 20, Span 20, Methyl Paraben, Propylene Glycol, Liquid Paraffin, and Triethanolamine (TEA) were procured from SD Fine Chemicals.

Plant materials and extract preparation

Rubia cordifolia and *Vitex negundo* were collected from our college's herbal garden, and *Piper nigrum* and *Myristica fragrans* were purchased from a local market in Vadodara, Gujarat. Botanists identified and authenticated the various parts of plants. The roots, fruits, leaves, and seeds were shade dried for 3-4 weeks before being finely powdered in a mixture and sieved twice to obtain a fine powder. All plant materials hydroalcoholic extracts were dried until they reached a constant weight. 30 mg of *Rubia Cordifolia*, *Vitex Negundo*, *Myristica fragrans*, and 10mg of *Piper nigrum* extract were combined and labelled as Polyherbal mixture (RVMP). The powdered extracts were used for emulgel preparation.

Preparation of Emulgel

Gels are transparent to opaque semi-solids containing a high solvent ratio to a gelling agent. The emulgel was formulated in three different steps.[15]

Step 1: Preparation of o/w emulsion.

Step 2: Preparation of gel phase.

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

Composition of Formulation

The gel was prepared (Table 1) using various ratios of Carbopol 934, Carbopol 940, HPMC K100 M and Sodium CMC in an aqueous solvent with continuous stirring on a magnetic stirrer. The oil phase of the emulsion was prepared by dissolving Span 20 in Liquid Paraffin, while the aqueous phase of the emulsion was prepared by dissolving Tween 20 in purified water. Methyl Paraben was dissolved in propylene glycol, whereas extracts were dissolved in water, and both solutions were mixed with the aqueous phase. The oily and aqueous phases were heated separately at 70-80 °C. Then the oil phase was added drop wise to the aqueous phase with continuous stirring with the help of a magnetic stirrer, and the resultant emulsion was cooled at room temperature. The obtained emulsion was mixed with the gel in a 1:1 ratio with moderate stirring to obtain the emulgel. The pH of all formulations was adjusted to 6-6.5 using triethanolamine (TEA).

Table 1: Composition of Polyherbal Emulgel formulation containing the RVMP mixture

Ingredients %W/W	G1	G2	G3	G4	G5	G6	G7	G8
Carbopol 934 (gm)	0.25	0.75	1	-	-	-	-	-
Carbopol 940 (gm)	-	-	-	0.25	0.5	1	-	-
HPMC K100M (gm)	-	-	-	-	-	-	0.75	-
Sod.CMC (gm)	-	-	-	-	-	-	-	1
Tween 20 (mL)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Span 20 (mL)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Methyl Paraben (gm)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propylene Glycol (mL)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Liquid Paraffin (mL)	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Triethanolamine (TEA)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Polyherbal Mixture RVMP(mg)	100	100	100	100	100	100	100	100
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of Emulgel Formulation [15,16,17]

Appearance

The prepared emulgel was inspected visually for clarity, colour, phase separation, state and presence of any particle.

Homogeneity

After the emulgel was set in the container, all developed formulations were visually inspected for homogeneity.

Extrudability

The formulation's extrudability was assessed using a squeezable aluminium tube filled with 10 g of emugel. The tube was fixed in place by two clamps. The tube was squeezed, and the formulation's extrudability was calculated as the weight in grams required to extrude a 0.5 cm long gel ribbon in 10 seconds. The extrudability is then calculated by using the following formula:

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

Spreadability

Spreadability refers to how far the emulgel spreads after being applied to the skin or affected area. The spreading value of an emulgel formulation also influences its bioavailability efficiency. The spreadability of semi-solid preparations was determined using the parallel plate method, widely used for determining and quantifying spreadability. After fixing the two slides to stand without any movement, only the upper slide could slip off freely due to the force of the weight tied to it. G1, G2, G3, G4, G5, G6, G7, and G8 formulations were pressed between two 20 x 20 cm glass slides. The experiment was repeated three times, and the average time for spreadability was determined using the following formula.

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

Where, S = Spreadability

M =Weight tied to the upper slide (20gm)

L = Length of the glass (7.5 cm)

T = Time taken in seconds

Viscosity

The viscosity of the polyherbal emulgel was measured at 10 rpm using a Brookfield viscometer, model DV-||+pro. 20 grams of the gel were taken in a beaker, and the spindle No. 64 was immersed for about 5 minutes before taking the reading.

Determination of pH

A digital pH metre was used to determine the pH of the formulation. One gram of formulation was dissolved in one hundred millilitres de-mineralized water and stored for two hours. The pH of the formulation was measured in triplicate. Before use, the instrument was calibrated with standard buffer solutions at pH 4, 7, and 9.

The in vitro drug release studies [18,19]

The *in vitro* drug release studies were carried out in modified Franz diffusion (FD) cells using a dialysis membrane. The membrane was soaked in phosphate buffer pH 7.4. The membrane was clamped carefully to one end of the hollow glass tube of the dialysis cell for 8-12 hr. Then emulgel was spread uniformly on the dialysis membrane. PBS pH 7.4, used as dissolution media, was added to the receptor compartment. The donor compartment was kept in contact with the receptor compartment. This whole assembly was kept on a magnetic stirrer, and the solution on the receptor side was stirred continuously using a magnetic bead, and the temperature of the cell was maintained at 37± 1°C. The sample (5 ml) was withdrawn at suitable intervals and replaced with equal amounts of fresh dissolution media. Samples were analyzed spectrophotometrically at 285 nm, and the cumulative % drug release was calculated. The difference between drug release and control readings was used as the actual reading in each case.

In Vitro Anti-Arthritic Study

Inhibition of protein denaturation using bovine serum albumin [20]

The reaction mixture (0.5 ml) contained 0.45 ml BSA (5 percent aqueous solution) and 0.05 ml of various concentrations polyherbal mixture of RVMP (10, 20, 40, 60, 80, 100 µg/ml) and Diclofenac (reference drug). The samples were incubated at 37 °C for 20 minutes before being raised to 57 °C for 3 minutes. 2.5 ml of phosphate buffer saline was added to the above solutions after they had cooled. A UV-Visible spectrophotometer set to 255 nm was used to measure absorbance. In control, 0.05 mL of distilled water was used in place of the test extract, and Diclofenac sodium served as the standard. The percentage inhibition of protein denaturation was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Abs Control Solution} - \text{Abs Test solution}}{\text{Abs Control solution}} \times 100$$

RESULTS

Organoleptic Properties

The evaluation was carried out, and no change in the organoleptic characteristics of the formulations was observed, as shown in Table 2.

Table 2: Characterization of Polyherbal Emulgel Formulations for Color, State, Homogeneity, Phase separation and Extrudability

Batch code	Color	State	Homogeneity	Consistency	Phase separation	Extrudability
G1	Pale Yellow	Semi-solid	Excellent	Excellent	None	Good
G2	Light Yellow	Semi-solid	Excellent	Excellent	None	Good
G3	Light Yellow	Semi-solid	Excellent	Excellent	None	Excellent
G4	Light Yellow	Semi-solid	Excellent	Excellent	None	Good
G5	Light Yellow	Semi-solid	Excellent	Excellent	None	Good
G6	Light Yellow	Semi-solid	Excellent	Excellent	None	Excellent
G7	Pale Yellow	Semi-solid	Excellent	Excellent	None	Excellent
G8	Light Yellow	Semi-solid	Excellent	Excellent	None	Good

Homogeneity

After setting the gels in the container, all emulgel formulations were visually inspected for homogeneity and showed uniform extract distribution, as shown in Table 2.

Extrudability

Extrudability was assessed for all emulgel formulations, and it was discovered that G3, G6, and G7 had excellent extrudability, as shown in Table 2.

Spreadability Studies

According to spreadability studies, all formulations have excellent spread, as shown in Table 3.

Viscosity

All emulgel formulations had viscosities between 2000 and 11000 cps, suggesting that modest application of shear would quickly spread them, as shown in Table 3.

pH of Emugel

All the emulgel formulations pH was reported to be approximately 6.5 to 7.1, indicating skin compatibility, as shown in Table 3.

In vitro drug release

In vitro cumulative % drug release profile, showed the control release of optimized formulations for 8 hours. G6 showed the highest percentage of drug release of 91.85% shown in Figure 1.

In vitro Anti-arthritic study

In vitro inhibition of protein denaturation using bovine serum albumin

The anti-arthritic activity of the polyherbal mixture was performed by this method, and it was observed that concentrations of 10, 20, 40, 60, 80, and 100 µg/ml showed 3.99, 12.45, 26.43, 40.89, 58.76, and 78.71 per cent inhibition of protein denaturation (bovine serum). In contrast, standard Diclofenac at concentrations of 10, 20, 40, 60, 80, 100 µg/ml showed 15.11, 24.22, 39.45, 56.22, 70.56, and 87.36% inhibition of protein denaturation shown in Figure 2.

Table 3: Characterization of Polyherbal Emulgel Formulations for pH, Spreadability, Viscosity

Formulation	pH*	Spreadability (cm/sec) *	Viscosity (cps)*
G1	6.7 ± 0.01	18.13 ±0.21	4579±0.34
G2	6.07 ±0.01	20.28 ±0.19	6812±0.21
G3	6.86 ±0.11	25.21 ±0.14	8558±0.18
G4	7.1±0.23	23.48 ±0.31	4602±0.27
G5	6.6±0.12	20.62 ±0.24	7069±0.18
G6	6.6±0.25	29.21 ±0.21	10947±0.24
G7	6.5±0.3	22.74 ±0.18	2442±0.32
G8	6.53±0.31	19.15 ±0.11	5599±0.13

*All values represent mean ± standard deviations (SD), n=3

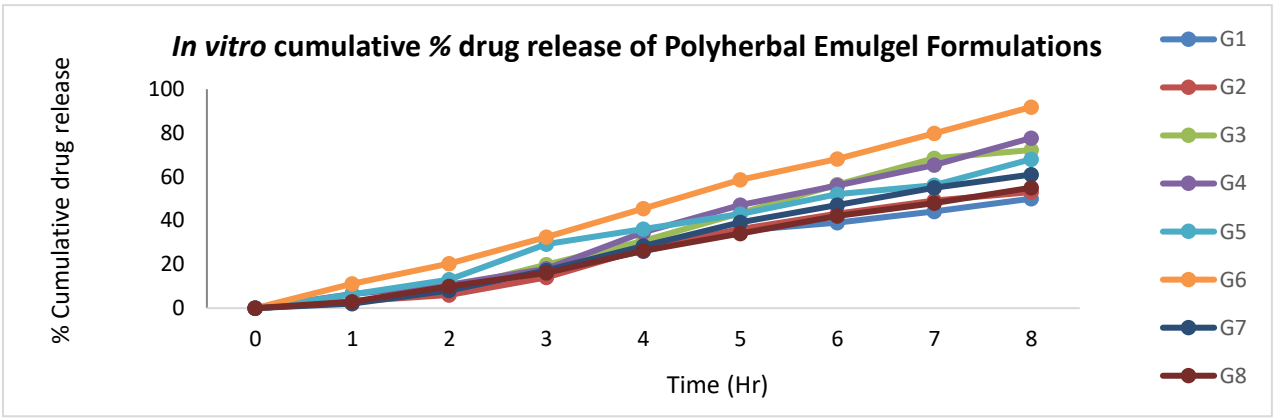

Figure 1: *In-vitro* cumulative % drug release profile of polyherbal Emulgel formulations

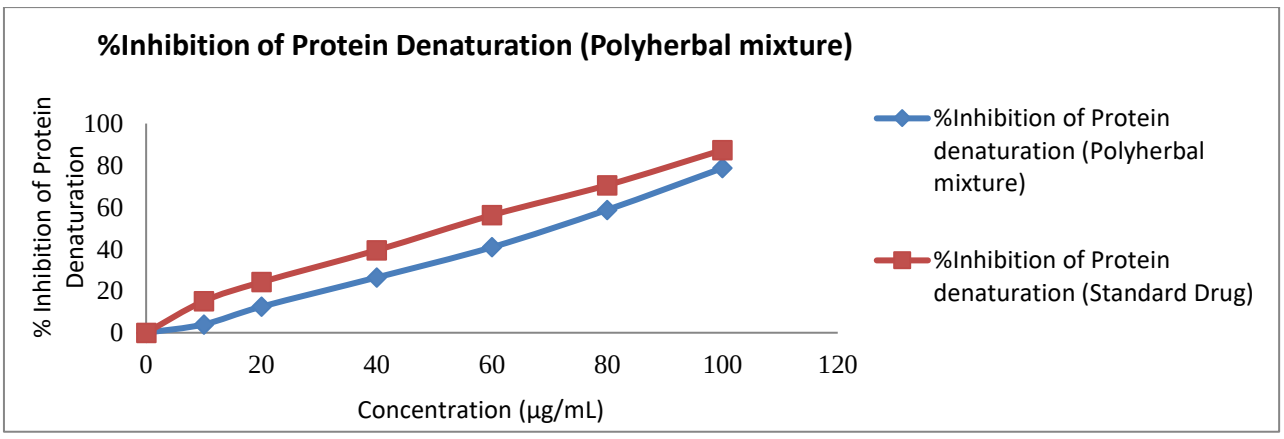

Figure 2: *In vitro* anti-arthritic activity of optimized emulgel formulation in comparison with standard drug.

DISCUSSION

Emulgel of a polyherbal RVMP mixture for treating Rheumatoid Arthritis were successfully prepared using various concentrations of Carbopol 934, Carbopol 940, HPMC, and Na CMC. The formulations were all pale to light yellow, with a smooth, homogeneous texture and a glossy appearance. [13] All prepared formulations had pH values ranging from 6.5 to 7.1, indicating that they could be used on adult skin with a lower risk of skin irritation.[14] Spreadability is the degree to which the gel can spread at the application site, and it is an essential parameter in determining the gel's efficacy because the spreadability of the emulgel formulation is dependent upon the viscosity of the formulation and the physical properties of the gelling agent.[16] The emulgel containing 1 percent carbopol 934 and 940 had the best spreadability. [6] The rheological behaviour of the emulgel indicated that they followed the shear-thinning effect with thixotropic properties. The viscosity of the formulation increased with an increase in the gelling agent.[21] *In vitro* drug release of the optimized emulgel formulation (G6) showed control release for 8 hours with maximum drug release of 91.85%. *In vitro* antiarthritic activity of the polyherbal RVMP mixture showed dose dependent inhibition of the protein denaturation as a result of the antioxidant potential of the polyherbal mixture to reduce inflammatory response due to free radical generation.

CONCLUSION

Results of the studies revealed that the prepared polyherbal formulations which comprised of hydroalcoholic extract of roots of *Rubia Cordifolia*, the fruit of *Piper nigrum*, leaves of *Vitex Negundo*, and seeds of *Myristica fragrans* the physical analysis and rheological studies of the prepared polyherbal gel proved to be potent and efficacious. The effective anti-arthritic activity exhibited by the polyherbal formulations may be attributed to the synergistic action of the plant constituents present in the formulation.

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