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RESEARCH ARTICLE

Formulation and Evaluation of an Anti-inflammatory Topical Polyherbal Gel

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Abstract

Inflammation is currently treated with NSAIDs. Unfortunately, these drugs increase the risk of blood clots, heart attacks, and strokes. Therefore, the development of potent anti-inflammatory drugs from natural products is currently being investigated. Natural products made from medicinal plants play an important role in curing many diseases associated with inflammation. Conventional anti-inflammatory drug available in the market has various side effects. Because of these side effects, there is a need to look for newer drugs with fewer or no side effects. The objective of the present study was to develop polyherbal gel containing hydroalcoholic extract of *Berberis aristata* root, *Rubia cordifolia* root and *Boswellia serrata* gum by using Carbopol 934 and Propylene glycol. The 3^2 factorial design was constructed using concentration of polymer (carbopol 934) and penetration enhancer (Propylene glycol) as independent variables while Viscosity (m.Pas), % *in vitro* release of Berberine, Rubiadin and AKBA as dependent variables, total 9 possible experimental runs formulate and evaluate. The optimized gel was selected by design of expert employing the overlay plot with desirability approach. Optimized gel showed 39568 m.Pas viscosity, drug content of Berberine 0.48 mg, Rubiadin 0.42 mg and AKBA 0.51 mg. *In vivo* and histopathology study revealed that prepared gel showed good anti-inflammatory activity.

Keywords: Anti-Inflammatory, Design of Expert, Polyherbal Gel

1. Introduction

Inflammatory diseases include a wide range of disorders and conditions, characterized by inflammation. Fatty liver disease, endometriosis, diabetes mellitus, Inflammatory Bowel Disease (IBD), asthma, rheumatoid arthritis, obesity, Alzheimer's disease, Parkinson's disease, and cancer are the major conditions on the list. Indian System Medicine (ISM) has practiced herbal drugs against inflammation for thousands of years. A few among many of the herbal drugs used for inflammation according to ISM are *Berberis aristata*, *Boswellia serrata*, and *Rubia cordifolia*.

Berberis aristata of the family Berberidaceae is commonly known as Daru Haldi or Daruharidra, Indian barberry, Chitra, Tree turmeric, etc. It is used traditionally for wound healing, rheumatism, skin diseases, menorrhagia, and jaundice[1,2]. The main pharmacological activity like anti-inflammatory, analgesic, antipyretic, and hepatoprotective effects have been attributed to berberine, a benzylisoquinoline alkaloid[3]. Several reports have shown the anti-inflammatory effects of *B. aristata*[4,5].

Boswellia serrata of the family Burseraceae is commonly known as Indian Frankincense. Extracts of the gum resin and other components have long been widely used in folk medicine. Specially, they have used for asthma, inflammatory bowel disease, pain and osteoarthritis, and pain[6-10].

Boswellic acids are pentacyclic triterpene acids found in *B. serrata*. Early studies suggested that the anti-inflammatory effects of *B. serrata* are mediated by six main boswellic acids[11].

Rubia cordifolia of the family Rubiaceae is commonly known as Common Madder. This flowering plant has used

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traditionally for treating various inflammation associated disorders. Many previous studies have proved the anti-inflammatory potentila of *R. cordifolia*[12-14].

Till date, no studies have been reported in combination of *Berberis aristata*root, *Rubia cordifolia* root and *Boswellia serrata* gum. Considering the abundance of supporting literatures, the present work was designed to formulate and evaluation an anti-inflammatory gel made of *Berberis aristata* root, *Rubia cordifolia* root, and *Boswellia serrata* gum for a synergistic effect in the treatment of acute inflammation.

2. Materials and Methods

2.1 Collection and Authentication of Crude Drugs

The raw materials for preparation of polyherbal formulation; roots of *Berberis aristata* DC. (Darihaldi) and *Rubia cordifolia* L. (Majith) were collected from Local Market in Vadodara and exudates of *Boswellia serrara* Roxb. (Salai guggul) was procured from FL enterprise, dhoop loban company near haridham sokhada, Vadodara. The plants parts were positively identified and authenticated by Dr. Sucheta Gohrai Giri, Botanist, Parul Institute of Applied Sciences, Parul University, Vadodara (PIAS/Stud/Plant/Authcerti/70/2017-2018).

2.2 Preparation of Extracts of Crude Drugs

The hydroalcoholic extracts (70% ethanol and 30% water) of *Berberis aristata* root and *Rubia cordifolia* root were prepared by using Maceration with stirrer and hydroalcoholic extract of *Boswellia serrata* gum was prepared by using orbital shaker. All the extracts were concentrated by distilling the solvent and the extracts were lyophilized and stored for further use.

2.3 Preparation of Polyherbal Gel

2.3.1 Method of Preparation of Gel[15,16]

Different concentration of carbopol 934 was diffused in 70 ml distilled water and set aside overnight to get a smooth gel. Preservative methyl paraben and propyl paraben were incorporated into gel base. Penetrating enhancer propylene glycol and hydroalcoholic extracts of *Berberis aristata* root, *Rubia cordifolia* root, and Boswellia *serrata* gum were dissolved in 30 ml water. Drug solution poured into gel base with continuous stirring. Triethanolamine was added drop wise to the formulation for to obtained normal skin pH to 7.

2.3.2 Preliminary Trials Batches for Optimization of Polymer Concentration

Preliminary trial bacthes were prepared without extracts with varying concentration of different carbopol 940 and 934 and evaluated by its pH and viscosity of gels.

2.3.3 Physiochemical Compatibility of Extracts with Selected Excipients[17,18]

Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) were used to check physicochemical compatibility between extracts of *Berberis aristata* root, *Rubia cordifolia* root, *Boswellia serrata* gum, and polymers in the gel.

2.3.4 Optimization of Polyherbal Gel by 3^2 Factorial Design[19]

The optimization of polyherbal gel was carried out by using Design Expert software (V.13.0.0. Stat-Ease Inc) employing overlay plot with desirability approach.

2.4 Evaluation of Polyherbal Gel

2.4.1 pH of Gel[20]

With the aid of a digital pH meter, the pH of the formulation was determined. 100 ml of distilled water was utilized to dissolve 1 gm of gel. The formulation's pH was measured three times, and the standard deviation was computed. In order to prevent any irritation, the pH of the gel should preferably be close to that of the skin.

2.4.2 Viscosity[21]

Viscosity final optimized polyherbal gel was measured using digital viscometer LMDV-60 and gels were rotated at 12 rpm using spindle no. L4.

2.4.3 In Vitro Drug Release[22]

Franz diffusion cells with a diffusional area of 3.4 cm^2 and a receptor compartment volume of 15 ml were used for the *in vitro* drug release study. In the receptor compartment, phosphate buffer solution (pH 7.2) was utilized and kept at 100 rpm at the room temperature. A sigma dialysis membrane (60 mm x 60 mm) was used as the diffusion membrane. Spread 1 gm of gel over the dialysis membrane in the donar compartment. To keep the volume in the receptor compartment consistent, the 2

ml aliquots were analysed by HPLC and replaced with the same volume of fresh phosphate buffer pH 7.2.

2.4.4 Drug Content[23]

1 gm gel dissolved in 20 ml methanol solution and sonicate for 10 mins and filter the solution with 0.45 µm filter paper. From filtrate solution take 1 ml filtrate and dilute upto 10 ml with methanol and again filter and from filtrate solution was injected for HPLC.

2.4.5 Spreadability[24]

When gel applied or rubbed on the skin surface, it should have a sufficient spreading coefficient. Spreadability was evaluated by placing 1 gm of gel on a glass slide. Another glass slide of the same length was placed above that and mass of 50 gm was put on the glass slide so that the gel gets sandwiched between the two glass slides and spread certain distance. The time taken for separating two slides from each other was noted.

It was determined by following formula:

$$S = \frac{M \times L}{T}$$

where,

S = Spreadability
M = Weight put on the upper slide
L = Length of glass slide
T = Time taken for separation of two slides

2.4.6 Stability Study[25]

Accelerated stability study was performed as per ICH Q1A(R2) guideline by storing the gel at 40° ± 2 °C and 75 ± 5 % Relative Humidity (RH) in stability chamber (Bio-Tech) for 6 months.

2.5 Pharmacological Activity of Optimized Polyherbal Gel

2.5.1 Acute Dermal Toxicity[26,27]

The Acute Dermal Toxicity test of polyherbal formulation was performed according to the OECD guidelines no. 402.

Female Wistar rats (nine weeks old) were used for the study. Three groups of three animals each were formed from the nine total animals. 10% of the body's hair was removed by hair remover from the test animals' dorsal area about 24 hrs before test. Group I animals were considered as control, Group II animals received optimized polyherbal gel formulation (0.5 gm of 2% gel) and Group III animals received again for confirmatory optimized polyherbal formulations topically at 0.5 gm of 2% gel. All animals were monitored for 14 days for changes in fur, eyes, behaviour, and toxic dermal reactions.

2.5.2 In Vivo Anti-Inflammatory Study of Optimized Polyherbal Gel

2.5.2.1 TPA Induced Anti-Inflammatory Study[28]

The ear edema was induced by 1 mg TPA dissolved in acetone (2.5 µg/ear) in mice. The inner and outer surfaces of mouse ears were treated with TPA (2.5 µg/ear) dissolved in 20 µl of acetone using a micropipette. Total 24 animals (mice) were distributed between groups.

Group 1: No treatment
Group 2: Disease control (TPA)
Group 3: Standard (1% Diclofenac gel)
Group 4: Gel (1%)
Group 5: Gel (2%)

Standard (Diclofenac gel) and Polyherbal gel (1%, 2%) was applied after the TPA induction. Ear edema was evaluated at 0 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 180 min and 240 min. Ear edema was measured in terms of thickness before and after induction of inflammation by micrometer gauge. After 4 hrs treatment, mice was subjected for anaesthesia and scarified by cervical dislocation and each ear biopsy was done.

2.5.2.2 Histopathological Analysis of Mouse Ear Tissue[29]

Biopsies from the control and treated ears of mice in each treatment group were taken and preserved in 4% formaldehyde for the evaluation of skin inflammation. The tissues were then dried, covered with paraffin, and serially cut with a microtome at a thickness of 5.0 µm (Leica Microsystems, USA). Hematoxylin-eosin (H and E) staining was used for depicting the histopathological alterations in a representative section from each group of animals. Bright field microscopy (E.S.AW1500) was used to evaluate some sections, and images were taken at a 10x magnification.

3. Result and Discussion

3.1 Preliminary Trials Batches for Optimization of Polymer Concentration

Initial trial batches developed with various concentrations of carbopol 934 and 940 were assessed for pH and gel viscosity. From different evaluation parameters, Carbopol

934 have been selected for gel preparation than Carbopol 940.

3.2 Physiochemical Compatibility of Extracts with Selected Excipients

The physical and chemical interactions between the plant extracts and the excipients have been investigated using FTIR and DSC techniques (Figures 1 and 2).

3.2.1 By Fourier Transform Infrared Spectroscopy (FTIR)

From FT-IR spectra of extracts and polymers mixture, prominent peaks are, i.e., -OH, -NH stretching (3213.51 cm^{-1}), C-H stretching (2858.60 cm^{-1}) and C=O stretching (1600.97 cm^{-1}) were noticed.

3.2.2 By Differential Scanning Calorimetric Analysis (DSC)

The DSC thermograph of extracts and polymer are shown in Figure 2. The endotherm A (*Berberis aristata* extract) appeared melting point around 141.30°C, endotherm B (*Rubia cordifolia* extract) appeared melting point around 131.20, 148.50°C, endotherm C (*Boswellia serrata* extract) appeared melting point around 148.16°C, endotherm D (Carbopol 934) appeared melting point around 130.26, 231.72°C and endotherm E (Mixture of Polymer and extracts) appeared melting point around 133.42°C.

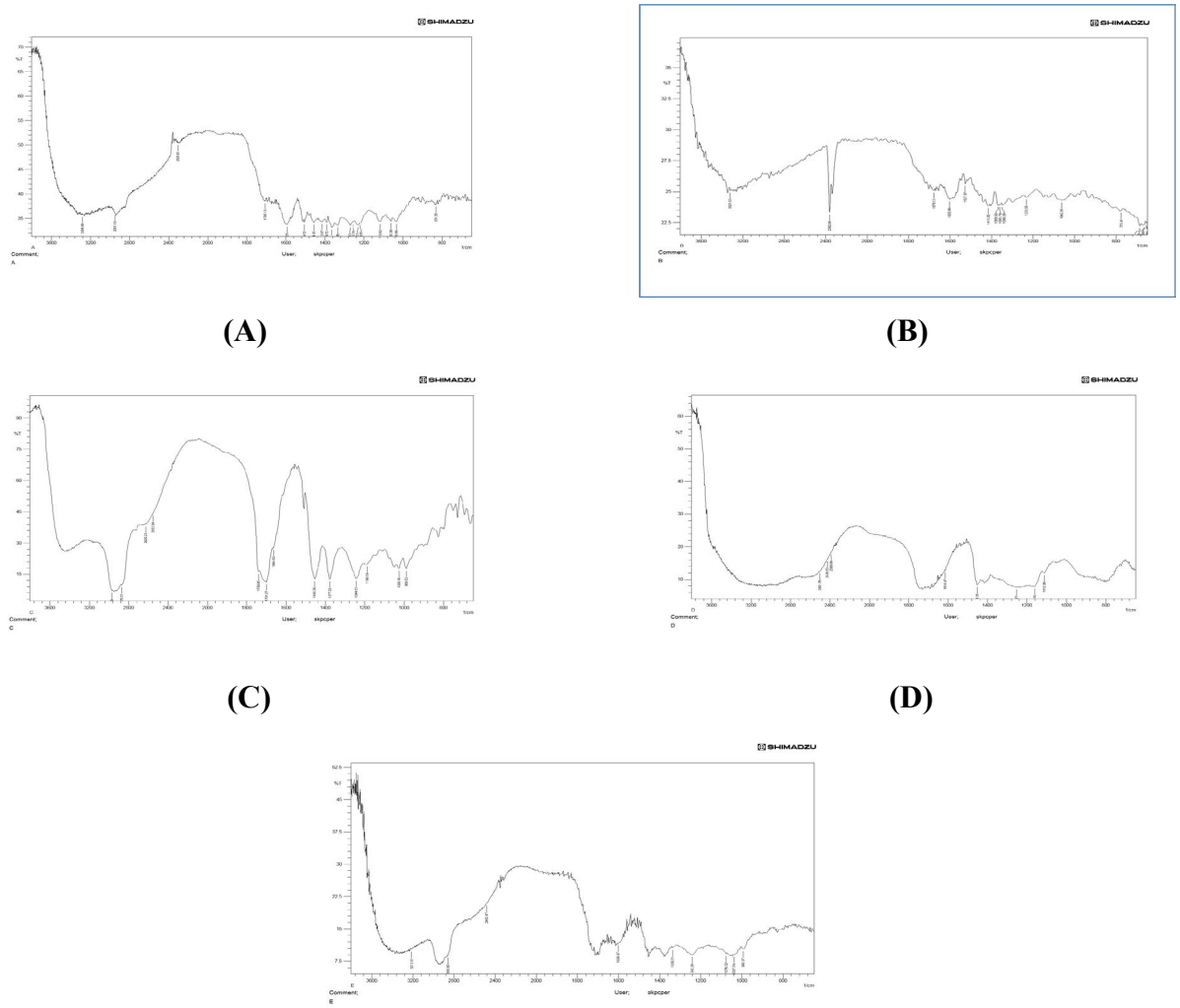

Figure 1. FT IR and DSC study graphs. **(A).** FT-IR Spectra of Berberis aristata extract. **(B).** FT-IR Spectra of Rubia Cordifolia extract. **(C).** FT-IR Spectra of *Boswellia Serrata* extract. **(D).** FT-IR Spectra of Carbopol 934. **(E).** FT-IR Spectra of combination of extracts with polymer.

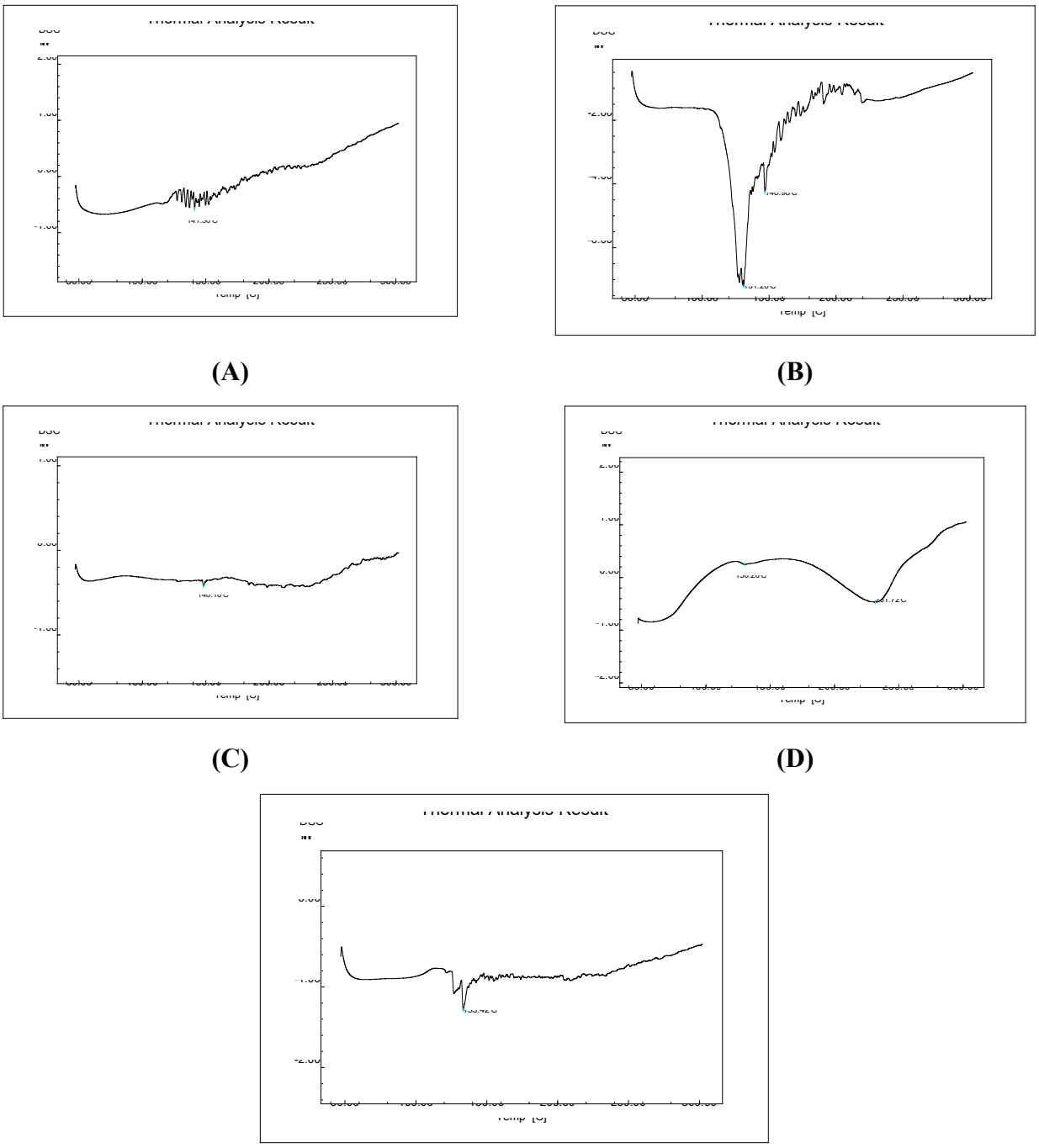

Figure 2. DSC thermograph of extracts and polymer. **(A).** DSC of *Berberis aristata* extract. **(B).** DSC of *Rubia cordifolia* extract. **(C).** DSC of *Boswellia serrata* extract. **(D).** DSC of Carbopol 934. **(E).** DSC of extracts and Carbopol 934.

3.3 Optimization of Polyherbal Gel by 32 Factorial Design

From design of expert, nine formulations were obtained from selected responses and are reported in Table 1.

The optimization of the formulation was carried out by I-optimal design using design expert version 13.0 with study type response surface (randomization). The obtained total run was nine without including block effects. The % Carbopol (A) and % PG (B) were taken as

Table 1. 3^2 factorial design batches and responses noted for polyherbal gel formulation

Batch No.	Concentration of Carbopol 934 (%) X_1	Concentration of Propylene glycol (mg) X_2	R1 Viscosity(mPa.s) ±SD	R2: %CDR of Beberine at 12 hr	R3: %CDR of Rubiadin at 12 hr	R4: %CDR of AKBA at 12 hr
F1	0.75	4.6	11490.33 ± 1.53	84.9	80.34	83.85
F2	1	4.6	22646.33 ± 2.08	85.05	82.06	87.15
F3	1.25	4.6	37747 ± 3.61	82.92	81.26	85.07
F4	0.75	4.8	16478 ± 2.65	86.29	84.23	88.69
F5	1	4.8	42791.33 ± 1.53	89.31	86.06	90.09
F6	1.25	4.8	52270.33 ± 4.73	88.27	83.35	86.45
F7	0.75	5	17861 ± 1.00	80.7	80.25	79.15
F8	1	5	62247.67 ± 2.52	80.39	79.24	81.06
F9	1.25	5	82210.67	77.67	77.68	79.3

variables factor and its response measures on viscosity (R1), % CDR at 12 hr for Berberine (R2), % CDR at 12 hr for Rubiadin (R3), and % CDR at 12 hr for AKBA (R4).

Final equation in terms of actual factor: The equation in terms of actual factors can be used to make a prediction about the response for given levels of each factors.

The equation of response R1 has showed that we conclude that the concentration of Carbopol and PGi ncreases theviscosity of formulation increases.

R1: Viscosity = +38415.85+21066.45A+15072.61B+9 523.25AB

The equation of response R3 has showed that %CDR of Berberine at 12hr tends to increase with increase in Propylene glycol (factor B) from low to middle level, while %CDR of Berberine at 12 hr decrease with increase in Propylene glycol (factor B) from middle to highlevel. While increase carbopol concentration (factor A) decrease release of Berberine.

R2: %CDR of Berberine at 12 hr = +87.96-0.5050* A-2.35* B-6.02* B^2

The equation of response R3 has showed that %CDR of Berberine at 12hr tends to increase with increase in Propylene glycol (factor B) from low to middle level while %CDR of Berberine at 12 hr decrease with increase in Propylene glycol (factor B) from middle to highlevel. While increase carbopol concentration (factor A) decrease release of Rubiadin.

R3: %CDR of rubiadin at 12 hr = +85.39-0.4217* A-1.08* B-0.8725* AB-1.27* A^2-4.41* B^2

The equation of response R3 has showed that %CDR of AKBA at 12hr tends to increase with increase in Propylene glycol (factor B) from low to middle level, while %CDR of AKBA at 12 hr decrease with increase in Propylene glycol (factor B) from middle to highlevel. While increase carbopol concentration (factor A) decrease release of AKBA.

R4: %CDR of AKBA at 12 hr = +89.98 – 0.1450* A-2.76* B-2.35* A^2-5.81* B^2

Analysis of Variance: Analysis of Variance shows that viscosity, % CDR of Berberine, Rubiadin and AKBA shown statistically significance difference ($P < 0.05$).

Response surface analysis: The objective of optimization is to discover the levels of optimization of various variables which affect the method so that production of formulation with required qualities can be achieved easily and reproducibly. Optimization region identification was possible using selected response surface with constraint (Viscosity and %CDR of Berberine, Rubiadin and AKBA). Figure 3 shows three dimensional relationship between factors and responses.

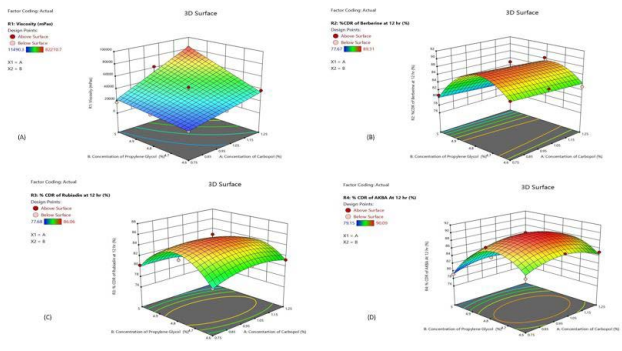

Figure 3. Three dimensional surface plot of. **(A).** R1: Viscosity. **(B).** R2: CDR of Berberine. **(C).** R3: %CDR of Rubiadin. **(D).** %CDR of AKBA.

3.4 Experimental Validation of Design Space for Formulation Variables

DoE trials for formulation variables validation was carried out by formulation at the check point batch suggested by software in Overlay plot. Figure 4 shows the overlay plot representing design space. The optimized parameters as check point batch were suggested by DoE software to obtain the desirable responses.

Table 2 shows that the close resemblance between observed and predicted response value assessed the robustness of predictions. These value indicate the validity of used model.

3.5 Evaluation of Optimized Polyherbal Gel

The final optimized gel (Figure 5) were evaluated for required parameters (Table 3).

3.6 Pharmacological Activity of Optimized Polyherbal gel

3.6.1 Acute Dermal Toxicity

There was no significant changes observed after application of the topical polyherbal gel.

No mortality and morbidity were seen.

3.6.2 In Vivo Anti-Inflammatory Study of Optimized Polyherbal Gel

3.6.2.1 TPA Induced Anti-Inflammatory Study

TPA, standard, and gel application was done at 9:00 am to 9:30 am and ear edema was evaluated at 0 min, 15 min, 30 min, 45 mins, 60 mins, 90 mins, 120 mins, 180 min and 240 mins. After 4 hrs treatment, mice was subjected for chloroform, anesthesia and sacrified by cervical dislocation and each ear biopsy was done. Ear edema was measured in terms of thickness before and after induction

Table 2. Evaluation of check point batch

X1(%)	X2(ml)	Responses	Predicted	Actual	%Error
1	4.8	Viscosity	40839.2	39568.11±1.25	1.78
		% CDR of Berberine	88.72	86.85±1.60	2.10
		% CDR of Rubiadin	85.27	86.14±1.81	1.02
		% CDR of AKBA	89.77	88.15±1.21	1.80

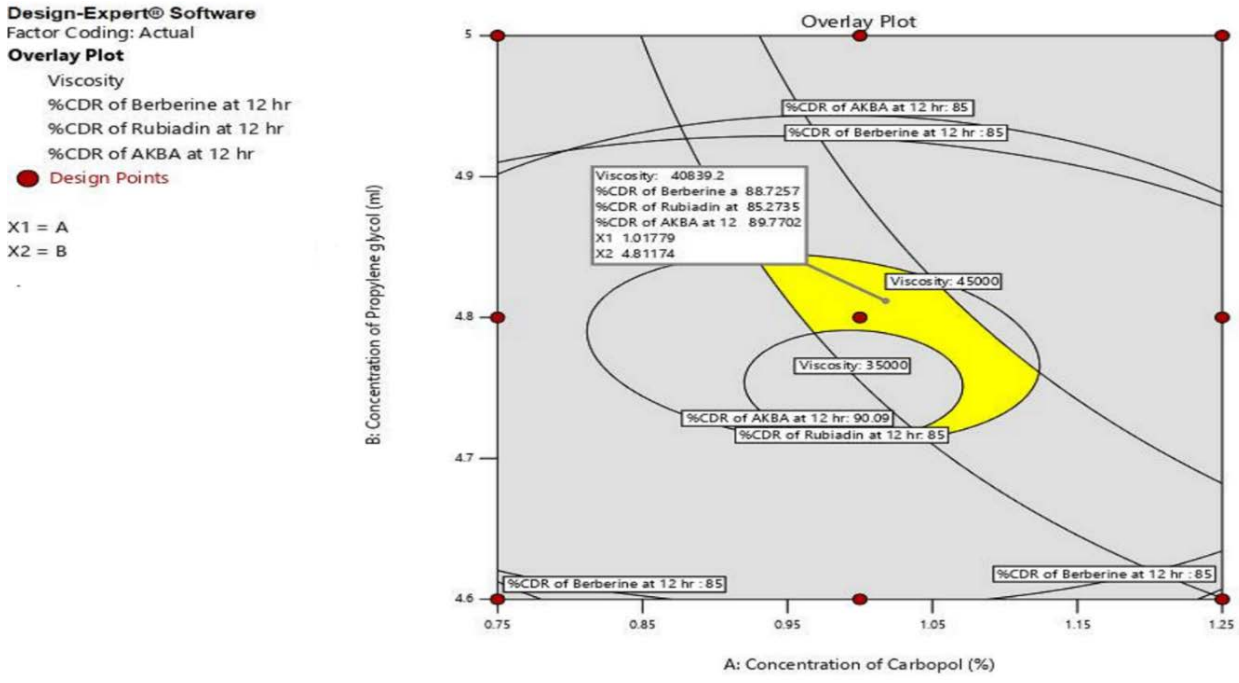

Figure 4. Overlay plot for optimization of formulation.

Figure 5. Final optimized gel

Table 3. Evaluation of gel

Evaluation parameters	Results of optimized gel
Viscosity	39568.11 ± 1.25 m.Pas
Drug content of Berberine	0.48 ± 0.03 mg
Drug content of Rubiadin	0.42 ± 0.01mg
Drug content of AKBA	0.51 ± 0.02mg
Spreadability	35.54 ± 0.41

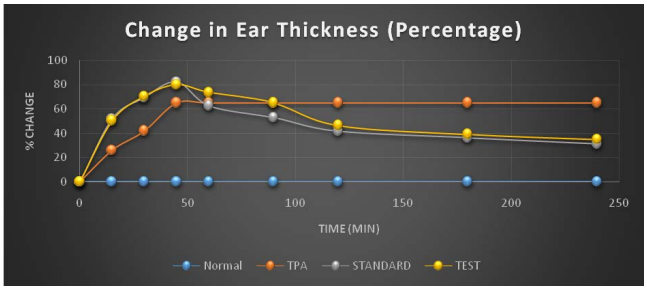

Figure 6. Change in ear thickness (Percentage).

of inflammation by micrometer guage (Tables 4 and 5) (Figures 6 and 7).

3.6.2.2 *Histopathological Analysis of Mouse Ear Tissue*

(A) Normal: Size of ear seems normal. The level of epithelial cells are properly internally connectively and properly dense.

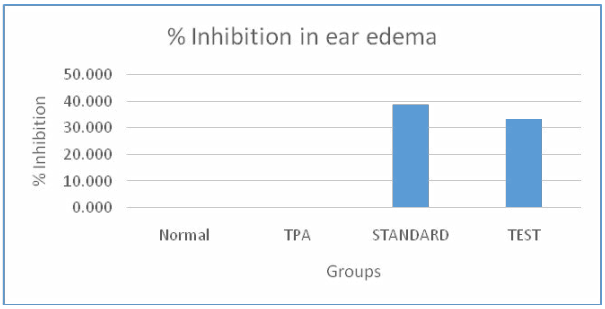

Figure 7. % Inhibition comparison between animal groups.

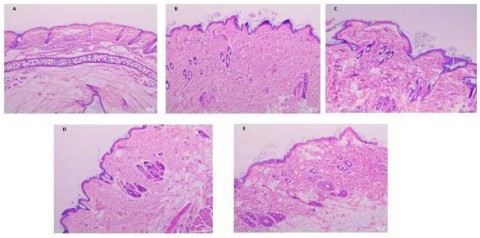

Figure 8. Histopathological analysis of mouse ear tissue. **(A).** Normal. **(B).** Disease control. **(C).** Standard-diclofenac. **(D).** 1% Gel. **(E).** 2% Gel.

Table 4. Ear thickness of animals

Ear thickness (MEAN ± SEM) (mm)									
Time	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**90 min**	**120 min**	**180 min**	**240 min**
Normal	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012	0.613± 0.012
TPA	0.63 ± 0.008	0.793± 0.012	0.893± 0.03	1.04 ± 0.057	1.04 ± 0.057	1.04 ± 0.057	1.04 ± 0.057	1.04 ± 0.057	1.04± 0.057
Standard	0.616± 0.012	0.936± 0.03	1.043± 0.04	1.123± 0.033	1.003± 0.009	0.943± 0.041	0.873± 0.046	0.84 ± 0.029	0.81± 0.037
Test	0.63 ± 0.008	0.946± 0.028	1.073± 0.024	1.133± 0.04	1.093± 0.033	1.04 ± 0.042	0.923± 0.047	0.876± 0.024	0.85 ± 0.04

Table 5. % Inhibition in ear edema

Groups	% Inhibition
Control	0
Disease	0
Standard	38.68
Test	33.33

(B) TPA induced-control: It is showing irregular and less number of epithelial cells, increasing size of ear, and showing some cavities filled with fluids.

(C) Standard: The size of ear is reduced compared to control. There is increasing of number epidermal cells and mild deposition of fluid and loosing of junction between epithelial cells**.**

(D) 1% Gel: The size of ear is reduced compared to control but still more than standard. Number of epidermal cells are more than control but less than standard**.**

(E) 2% Gel: The size of ear is less than control and near to standard. Number of epidermal cells are more than control and 1% gel and less than standard (Figure 8).

Conclusion: Reduction in the oedema and the infiltration of mononuclear and polymorphonuclear cells seen in the 2% gel treated group was found comparable to the Diclofenac Sodium Treated group. While 1% gel treated group showed reduction in oedema but infiltration of mononuclear and polymorphonuclear cells and connective tissue disruption were found to be less than desirable indicating 1% gel treated group while showing reduction in ear thickness is not as effective as the 2% gel treated group.

4. Conclusion

Berberis aristata root, *Rubia cordifolia* root, and *Boswellia serrata* exudate were selected for formulating a gel to treat inflammatory conditions. The phytochemical studied revealed the presence of alkaloids, glycosides, saponins, steroids and triterpenoids, tannins, and carbohydrates. The markers compounds Berberine, Rubiadin, and AKBA were quantified in the extracts by HPTLC and a simultaneous HPLC method was also develop and validated. The gel formulation was prepared with carbopol 934, and it did not show any dermal toxicity. The TPA induced anti-inflammatory study indicates that the 2% gel treated group is comparable to Diclofenac Sodium in treating inflammation conditions.

5. Acknowledgement

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