RESEARCH PAPER

Development, Characterization of Herbal Emulgel Containing Piper Betle Leaf Extract with In Vivo Studies

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ABSTRACT
Background
The present study aimed to formulate, characterize, and evaluate the in vivo performance of a herbal emulgel containing aqueous extract of Piper betle leaf for topical application.
Materials and Methods
Emulgel formulations were prepared using Carbopol 940 as gelling agent along with suitable emulsifying agents and excipients. The prepared formulations were evaluated for physicochemical parameters including appearance, homogeneity, pH, viscosity, spreadability, extrudability, globule size, drug content, and in vitro drug release. In vivo studies including skin irritation, acute dermal toxicity, and hair growth activity were performed using albino mice models.
Results
The optimized formulation exhibited satisfactory physicochemical properties, good stability, and enhanced drug release characteristics. The formulation was found to be non-irritant and safe for dermal application. Significant hair growth promoting activity was observed in comparison with the control group.
Conclusion
The results suggested that the developed Piper betle leaf emulgel possessed promising potential as a topical herbal formulation for dermatological and hair growth applications.
Keywords: Piper betle, Emulgel, Herbal formulation, Hair growth, Topical delivery, In vivo studies.
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1. Introduction

Topical drug delivery systems are widely used for localized treatment because they provide direct therapeutic action at the site of application while minimizing systemic side effects. Conventional semisolid preparations such as ointments and creams often exhibit disadvantages including stickiness, poor spreadability, and reduced patient compliance. Emulgels have emerged as a novel topical drug delivery system combining the advantages of emulsions and gels, thereby improving stability, spreadability, drug release, and patient acceptability[(1)]. *Piper betle* containing triterpenoids present a promising botanical option for offering a natural and holistic approach to managing hair loss. Its diverse pharmacological properties, including anti-inflammatory, antioxidant, immunomodulatory, and anti-androgenic effects, make it a valuable ally in promoting scalp health and hair regrowth. [(2)]

Despite their therapeutic effectiveness, conventional topical formulations have several limitations, including the potential for skin irritation caused by the drug or excipients, which may result in contact dermatitis or allergic reactions. Additionally, poor permeability, larger particle sizes, and stickiness can reduce drug absorption through the skin and cause discomfort for the patient. These challenges can be addressed by formulating the drug as an emulgel, which allows for faster drug release and improved dermal delivery. Emulgels offer multiple advantages for topical application, including thixotropic behavior, ease of spreading, simple removal, enhanced patient compliance, and an extended shelf life, making them a more acceptable and efficient dosage form compared to conventional semisolids.

2. Materials and Methods

2.1 Selection of plant

Piper betle leaves were were collected from Dept. of Botany, Maharana Pratap Government PG College, Chittorgarh, Rajasthan.

2.2 Chemicals
All chemicals and solvents were of analytical grade and were obtained from Oxford Lab Fine Chem LLP and Central Drug House (P.) Ltd.

2.3 Plant Extraction
The collected leaves were washed thoroughly with distilled water and shade dried for several days. The dried leaves were powdered using a mechanical grinder. The powdered material was subjected to aqueous extraction by maceration for 72 hours with intermittent shaking. The extract was filtered and concentrated using a rotary evaporator. The concentrated extract was stored in airtight containers until further use. [3]

2.4 Formulation of Emulgel
The emulgel was prepared using the fusion method according to the measured quantities (Table 1). The oil phase consisting of liquid paraffin and Span 80 was heated to 70–75°C [4]. Simultaneously, the aqueous phase containing Tween 80, propylene glycol, preservatives, and *Piper betle* extract was heated to the same temperature. [5]
The aqueous phase was gradually added to the oil phase with continuous stirring to form an emulsion. The gel base was prepared separately by dispersing Carbopol 940 in distilled water followed by neutralization using triethanolamine. The prepared emulsion was mixed with the gel base in a 1:1 ratio with continuous stirring to obtain the emulgel. [6]

Table 1: Formulation of Emulgel using aqueous extract of *Piper betle* leaf [6]

Ingredients	P1	P2	P3	P4	P5	P6	P7	P8	P9
Extract (gm)	5	5	5	5	5	5	5	5	5
Carbopol 934 (gm)	0.8	1	1.2	0.8	1	1.2	0.8	1	1.2
Liquid Paraffin (ml)	6.5	7	7.5	7.5	6.5	7.0	7.0	7.5	6.5
Tween 80 (gm)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Span 80 (gm)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Propylene Glycol (ml)	5	5	5	5	5	5	5	5	5
Triethanolamine (gm)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water upto 25 ml	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

3 Characterization of Emulgel
3.1 Physical Appearance
The prepared formulations were visually evaluated for color, consistency, homogeneity, grittiness, and phase separation. [7]

3.2 Measurement of pH
The pH of the emulgel formulations was measured using a digital pH meter. The gel was dissolved in distilled water in the ratio of 1:10 and allowed to stand for 2 hours. The measurements were performed in triplicate and the average value was calculated. [8]

3.3 Spreadability study
A weighed amount of the emulgel was placed on one glass plate, and the second plate was dropped from a height of 5 cm. The diameter of the spread emulgel circle was then measured. The spreadability was calculated using the formula:

$$\text{Spreadability} = M \times L / T$$

where M is the weight applied, L is the length of spread, and T is the time taken for spreading. This test was performed in triplicate for each formulation. [8]

3.4 Extrudability study
Extrudability was determined using collapsible aluminum tubes filled with emulgel. The tubes were pressed manually and the amount of formulation extruded upon application of pressure was noted. Extrudability was evaluated based on the ease with which the emulgel was extruded from the tube. [9]

3.5 Rheological study
The viscosity of the formulations was measured using a Brookfield viscometer fitted with suitable spindle. Approximately 50 g of emulgel was placed in a beaker and allowed to equilibrate at room temperature before analysis. The spindle was immersed into the formulation and viscosity was measured at different rotational speeds. The readings were recorded in centipoise (cP). [9]

3.6 Swelling Index
The swelling index of the emulgel formulations was determined to evaluate the water absorption capacity of the gel matrix. About 1 g of emulgel was

accurately weighed and placed on a porous aluminum foil or pre-weighed filter paper. The sample was then immersed in 50 mL of distilled water contained in a Petri dish at room temperature. At predetermined time intervals, the formulation was removed carefully and excess surface water was eliminated using filter paper without pressing the gel. The swollen emulgel was weighed again and the increase in weight was recorded. The formula used for calculating swelling index:

$$SW\ \% = [Wt - Wo / Wo] \times 100$$

Where, SW % = Equilibrium percent swelling,
Wt = Weight of the swollen emulgel after time t,
Wo = Initial weight of emulgel at time zero. [8-9]

3.7 Drug Content Determination

Drug content was determined using UV-visible spectrophotometry. About 1 g of emulgel was accurately weighed and dissolved in a suitable solvent with continuous stirring. The solution was filtered and appropriately diluted. Absorbance was measured at 272 nm using a UV-visible spectrophotometer. Drug content was calculated using the calibration curve of the extract.
Drug content was calculated using the formula:
Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor. [10]

3.8 Globule Size determination

0.1 gm of the prepared emulgel was taken and diluted with a 3 ml of distilled water to obtain a uniform dispersion. The diluted sample was mixed gently to avoid the formation of air bubbles. A drop of the dispersion was then placed on a clean glass slide and covered with a coverslip. The prepared slide was observed under an optical microscope at 40× magnification. The diameters of approximately 50–100 dispersed oil globules were measured using the calibrated ocular micrometer. The values obtained were recorded in micrometers (μm), and the average globule size was calculated by dividing the sum of individual globule diameters by the total number of globules measured. [11]

Average Globule Size= Sum of diameters / Number of globules measured

3.9 *In vitro* drug release

The *in vitro* drug release study was performed using Franz diffusion cells. The dialysis membrane was mounted between donor and receptor compartments. Phosphate buffer pH 7.4 was used as receptor medium and maintained at 37 ± 0.5°C with continuous stirring. Emulgel weighing 0.5 gm was placed in the donor compartment. 1 ml of samples were withdrawn from the receptor compartment at predetermined time intervals (1, 2, 3, 4, 6, and 8 hours) and replaced with fresh buffer to maintain sink condition. The samples were analyzed spectrophotometrically at 272 nm. The cumulative percentage drug release was calculated and plotted against time. [9,11]

Cumulative % Drug Release= Total drug content in emulgel (mg)/Cumulative amount of drug released (mg) ×100

4. Pharmacological Studies

4.1 Animals

Swiss albino mice were obtained in the animal house of Pinnacle Biomedical Research Institute (PBRI) and were acclimatized to the experimental room having temperature 23 ± 2 °C, controlled humidity conditions, and 12- hr light - dark cycle. Animals were caged in poly acrylic cages with maximum of six animals per cage. The mice were fed with standard food pellets and water ad libitium.[12]

4.2 Acute toxicity study

Acute toxicity studies were conducted to determine the safe dose by following OECD guideline 423 and 402 [20] for limit testing of herbal extracts. The overnight fasted female rats weighing between 20-30 g, were fed with dispersed emulgel formulations of leaves and fruits of *Aegle marmelos* and leaves of *Piper betle* at the dose of 5, 50, 300, and 2000 mg/kg body weight. Three animals were used in the initial step. Based on the absence of mortality, the same dose was administered to an additional three animals. The prepared emulgel formulation was dispersed in distilled water containing 0.5% w/v carboxymethyl cellulose (CMC) to obtain a uniform suspension. After administration the animals were observed for their skin, fur, eyes, salivation, lacrimation, behavioural patterns, neurological and psychological symptoms continuously for 1 hr, frequently for the next 4 h and then and then continuously for 14 days. [13-14]

4.2 Methodology

4.2.1 Dose selection

Based on acute toxicity study, the test doses were selected for animal study. As there was no lethality observed up to 2000 mg/kg in the animals[15]. Hence $1/10^{th}$ dose i.e., 200 mg/kg body weight were used. The dose $1/20^{th}$ dose i.e., 100mg/kg body weight of the emulgel were used.

4.2.2 Skin Irritation Test

The dermal irritation study (OECD 404) [16] was carried out using healthy adult albino mice of either sex, weighing between 25–30 g. The animals were acclimatized under standard laboratory conditions with free access to food and water. Prior to application, the dorsal surface of each mice was carefully shaved approximately 24 hours before the experiment to expose an area of about 2 cm^2. The animals were divided into groups corresponding to the test formulations, standard irritant (0.8% v/v aqueous formalin) [22] and a control group. 0.1 gm of

each formulation was applied uniformly over the shaved area. The application site was left uncovered to simulate normal usage conditions. The treated areas were observed for signs of erythema (redness) and edema (swelling) at 24, 48, and 72 hours after application. [17-19]

4.2.3 Hair Growth Activity

The hair growth activity of the prepared emulgel formulations was evaluated and compared with a Standard (2% Minoxidil) [21] and a control group. The study was carried out on healthy albino rats. The dorsal area of each rat was shaved carefully using a Veet cream to remove hair without injuring the skin. The test formulations were then applied topically once daily for a period of 30 days. The animals were divided into five groups of six animals in each group. The animals were kept under standard laboratory conditions with free access to food and water. Hair growth was assessed by measuring the length of newly grown hair at 10, 20, and 30 days from the day of application. Hair samples were plucked randomly from the treated dorsal area using fine forceps and measured using a micrometer scale. [23]

5. Result and Discussion

The objective of the present study was to carry out formulation and evaluation of leaves and *Piper betle* containing natural Triterpinoids.[18] The studies performed provided valuable information for the identification and authentication of plant material. The data represented the mean ± standard error (S.E.M.) of the indicated number of experiments. These findings may serve as standards for future investigations. The *Piper betle* leaf [2] was easily distinguished by its glossy, smooth surface, characteristic aroma, and mildly pungent taste. The incorporation of the aqueous extracts into the emulgel base[5] resulted in homogenous systems with smooth consistency, good appearance, and satisfactory texture suitable for dermal application. [19]

The *Piper betle* leaf emulgel displayed a fresh green coloration with a more pronounced characteristic aroma and a glossy, homogenous texture indicating efficient emulsification[7]. The pH evaluation[8], spreadability[8], extrudability study[9] and rheological studies[9] of emulgels formulations indicated that the incorporation of each plant extract did not adversely affect the mechanical properties of the formulated emulgels along with the predictable flow characteristics during application. The swelling index[8-9] evaluation of emulgel formulations provided insight into the water uptake capacity and hydration potential of the gels, which is important for skin moisture retention and controlled release of active constituents[11]. Emulgel formulated from *Piper betle* leaf extracts provided controlled swelling suitable for sustained topical application (Table 2)

Table 2: pH values, Spreadability, Extrudability, Rheological studies and Swelling Index of Emulgels

Sr. No.	Formulation Code	pH value	Spreadability (gm.cm/sec)	Extrudability (gm/cm^2)	Rheology (cp)	Swelling Index (%)	
						2 hr	4 hr
1	P1	6.05±0.035	22.43±0.028	17.45±0.212	18249 ± 2.12	18.70 ± 0.078	29.68 ± 0.042
2	P2	5.82±0.014	20.55±0.021	16.60±0.141	18256 ± 1.41	18.80 ± 0.021	31.78 ± 0.127
3	P3	5.37±0.028	17.78±0.049	18.25±0.212	17687 ± 3.54	19.82 ± 0.092	39.38 ± 0.064
4	P4	5.86±0.035	19.17±0.057	19.60±0.141	17429 ± 5.66	17.64 ± 0.057	26.09 ± 0.106
5	P5	5.72±0.021	20.19±0.014	17.75±0.212	18746 ± 4.95	15.92 ± 0.071	25.25 ± 0.156
6	P6	5.25±0.049	19.55±0.057	19.35±0.071	17991 ± 2.12	18.63 ± 0.092	31.73 ± 0.049
7	P7	5.74±0.021	21.08±0.042	18.35±0.212	16989 ± 2.12	17.40 ± 0.134	28.11 ± 0.035
8	P8	5.55±0.014	20.18±0.078	20.30±0.141	18767 ± 2.12	16.56 ± 0.064	22.72 ± 0.106
9	P9	5.95±0.049	18.51±0.049	17.25±0.071	18702 ± 5.66	19.36 ±	32.66 ±

						0.021	0.170

Table 3: Percent drug content, drug release and mean globule size

Formulation Code	Drug Content (%)	Mean Globule Size (µm) ± SD	% Drug Release (Mean ± SD)
P1	95.50 ± 0.12	6.54 ± 0.57	48.17 ± 1.02
P2	95.95 ± 0.08	5.48 ± 0.49	54.92 ± 1.14
P3	97.55 ± 0.09	4.86 ± 0.45	60.45 ± 1.09
P4	98.95 ± 0.06	2.96 ± 0.38	78.63 ± 0.96
P5	97.35 ± 0.10	4.52 ± 0.44	65.34 ± 1.25
P6	98.05 ± 0.07	5.12 ± 0.52	59.27 ± 1.18
P7	97.75 ± 0.11	5.94 ± 0.63	52.40 ± 1.31
P8	97.55 ± 0.09	5.36 ± 0.50	56.82 ± 0.87
P9	98.25 ± 0.08	6.01 ± 0.58	50.16 ± 1.07

The percent drug content values ranged from 95.50 ± 0.12% to 98.95 ± 0.06%, suggesting that each formulation successfully incorporated the active constituent in near-uniform amounts. The minor variations among batches reflect uniform incorporation of the extracts into the emulgel base and high reproducibility in formulation. According to table, formulation P1 exhibited the lowest drug content of 95.50 ± 0.12%, while the highest value was recorded for formulation P4 (98.95 ± 0.06%).Among all batches, P4 exhibited the smallest mean globule size (2.96 ± 0.38 µm) and highest percentage drug release (78.63 ± 0.96%). The sustained release pattern observed in all optimized formulations demonstrated superior emulsification efficiency and finer droplet distribution compared to the other formulations. The reduction in droplet size was likely associated with optimized surfactant concentration and improved interfacial stabilization, which prevented coalescence and aggregation. The studies indicated superior drug diffusion and enhanced release characteristics. The formulation parameters appeared to significantly influence the diffusion kinetics. [(1)]

The acute toxicity evaluation, skin irritation and hair growth promotion activity was performed on P4 formulation based on the data obtained (Table 2 and 3) of the emulgel formulations, and it revealed no mortality or moribund behavior at all tested doses, ranging from 5 mg/kg to 2000 mg/kg body weight. Observations across all dose levels showed that the animals remained healthy with no signs of toxicity, behavioral changes, or adverse effects throughout the study period. The dermal irritation study was conducted to evaluate the safety of the formulated emulgel following topical application. The control group did not exhibit any signs of erythema or edema throughout the observation period, confirming that the experimental conditions did not induce spontaneous skin reactions. In contrast, the standard irritant (0.8% v/v aqueous formalin) group showed visible redness and swelling, validating the sensitivity and reliability of the experimental model. P4 formulation exhibited slight to moderate redness at 24 and 48 hours; however, the reaction diminished by 72 hours, and no severe irritation was noted.

Table 4: Average Hair growth of the Emulgel formulation at 10, 20 and 30 days (mm)

S. No	Formulations	Hair Length at 10 days (mm)	Hair Length at 20 days (mm)	Hair Length at 30 days (mm)
1	Standard (2% Minoxidil)	3.63 ± 0.210	9.24 ± 0.196	13.37 ± 0.577
2	P4	3.53 ± 0.512	9.4 ± 0.155	12.58 ± 0.226
3	Control	3.2 ± 0.155	8.75 ± 0.487	11.8 ± 0.071

The evaluation of hair growth activity revealed the plant-based emulgel formulation promoted hair growth over 30 days when compared to the untreated control (Table 4). The control (untreated) group displayed the least increase in hair length, with values of 3.2 ± 0.155 mm (10 days), 8.75 ± 0.487 mm (20 days), and 11.8 ± 0.071 mm (30 days) at corresponding intervals. Overall, the data revealed that the formulation P4 with values 3.53 ± 0.512 (10 days), 9.4 ± 0.155 (20 days) and 12.58 ± 0.226 (30 days), promoted hair growth over 30 days, results most comparable to the standard (2% Minoxidil) treatment. The results clearly showed that the Emulgel formulation was successful in reducing the time taken for hair growth initiation. As it achieved an average hair length of 12 mm towards 30th day of the study, concluded to be promising when compared to the standard Minoxidil treated group which showed 13 mm of length for the same study. This concludes that P4 exhibited the potent hair growth-promoting activity; comparable to the standard 2% Minoxidil, suggesting that *Piper betle* leaf contains bioactive compounds capable of stimulating hair follicle activity and accelerating hair growth.

6. Summary and Conclusion

On the basis of the results obtained, it was concluded that the developed plant-based emulgel formulations which was undertaken to develop, and evaluate plant-based emulgel formulations prepared from *Piper betle* leaf extracts for enhanced topical delivery and hair growth activity. The prepared emulgels were evaluated for pH, spreadability, extrudability, rheology, globule size distribution, drug content uniformity, *In vitro* drug release using the Franz diffusion method, acute toxicity, skin irritation potential, and pharmacological activity in terms of hair growth promotion. The results demonstrated among all batches, P4 emerged as optimized formulations due to their smaller globule size, superior drug release profile, excellent stability, absence of skin irritation, and significant pharmacological activity.

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