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Formulation and Evaluation of Polyherbal Gels for Antinociceptive Activity

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Abstract

In the present study three medicinal plants *Vitex negundo* Linn (Verbenaceae), *Bryophyllum pinnatum* Linn (Crassulaceae) and *Centella asiatica* (Umbelliferae) reported to have significant analgesic potential were selected to be formulated as polyherbal gels with and without permeation enhancers and were investigated for their antinociceptive potential. The dried finely ground herbs were extracted in distilled water and combined in different ratios and incorporated in 5% gel base of carbopol 934 (GF1-GF15). Antinociceptive activity was evaluated using two animal models, viz. acetic acid induced writhing response and formalin induced hind paw licking and was compared against control and standard diclofenac diethylamine gel (1.16%). All the formulations significantly ($P<0.001$) reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid. The topical application of polyherbal gel formulations GF1-GF15 significantly ($P<0.001$) inhibited the licking time induced by the injection of formalin. The study established these polyherbal gels as suitable alternatives to synthetic analgesic agents.

Keywords: Antinociceptive, gel, polyherbal, writhing

1. Introduction

Herbal medicine has become an integral part of standard healthcare based on combination of traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety[1]. Natural products and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic efficacy[2]. *Vitex negundo* Linn (Verbenaceae), a large aromatic shrub with bluish purple flowers has been used for various medicinal purposes in Ayurvedic and Unani systems of medicine. Analgesic and anti-inflammatory effects of *Vitex negundo* have been reviewed thoroughly[3]. Presence of various flavonoid compounds like 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone and 3,5- dihydroxy-3',4',6,7-tetramethoxy flavonol are reported in the leaves of *Vitex negundo*[4]. *Bryophyllum pinnatum* Linn (Crassulaceae) is a shrub with simple or trifoliate petiolate leaves used mainly in folk medicine to alleviate pains of various intensities and etiologies. The analgesic potency of the aqueous extract of the leaves of *Bryophyllum pinnatum* demonstrated strong analgesic potency comparable in a time and dose-dependent manner to a nonsteroidal anti-inflammatory drug.

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Presence of various flavonoids, polyphenols, triterpenoids are reported in the leaves of *Bryophyllum pinnatum*[5]. *Centella asiatica* (Umbelliferae) is a perennial, herbaceous creeper used in traditional medicine in the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly. Various bioactive terpene acids such as asiatic acid and madecassic acid are reported from the water-methanol extraction of *Centella asiatica*. These phytocompounds may be present in the crude extract of *Centella asiatica* that may account for the antinociceptive and antiinflammatory activities[6]. The objective of the present investigation is to formulate topical gels containing these herbs with and without permeation enhancers and screen their antinociceptive potential.

2. Materials and methods

2.1. Plant Material

The leaves of *Bryophyllum pinnatum* were collected locally from Indore in the month of July 2008. The dried leaves of *Vitex negundo* and dried leaves of *Centella asiatica* were purchased from the local market and the above herbs were identified by comparing with standard herbarium specimens available in AICRP on Medicinal & Aromatic plants, J.N. Krishi Vishwa Vidyalaya, College of Agriculture, Indore, Madhya Pradesh. Voucher specimen (SCOPE/Phcog/08/11(a-c)) of the plant was deposited in the Herbarium of the Department of Pharmacognosy, Smriti College of Pharmaceutical Education Indore, Madhya Pradesh.

2. 2. Preparation of aqueous extract

The powdered leaves of *Bryophyllum pinnatum*, *Vitex negundo* and *Centella asiatica* were soxheleted individually with distilled water and the percentage yields obtained were 15.6%, 14% and 21% respectively.
To find the most effective combination for a polyherbal gel formulation, the obtained extracts were mixed in different proportions as defined in Table 1.

2. 3. Preparation of polyherbal gel formulations

Polyherbal gel formulations were prepared by incorporating the extract combined in different ratios in 5% gel base of carbopol 934. Menthol and oleic acids in concentration of 2.5% and 5% were incorporated in the gels as permeation enhancers. Glycerin 5% w/w was incorporated as humectant, methyl paraben 0.02 % w/w as preservative, triethanolamine q.s. for pH in the range 6-7 and distilled water q.s. up to 100 ml. The formulation code of different extract combinations with and without permeation enhancers are given in Table 2.

2. 4. Animals

Swiss albino mice of either sex weighing 20-25 gm, from Veterinary College, Mhow (Madhya Pradesh) were used for these studies. The animals were maintained at 28±2^{o}C at a relative humidity of 50-55% and at 12 h light and dark cycles. Animals were housed in groups of six per cage and were fed with standard pellet diet *ad libitum* and allowed free access to drinking water. The animals were acclimatized to laboratory conditions prior to experimentation. All experimental protocols were approved by Institutional Animal Care and Ethics Committee headed by CPCSEA (Committee for Purpose of Control and Supervision of Experiments on Animals).

2. 5. Antinociceptive Activity

Two models, viz. acetic acid induced writhing response and formalin induced hind paw licking using albino mice were employed to study the antinociceptive effect of the polyherbal gel formulations. The animals were divided into seventeen groups of six animals each. Group I served as control and received 1% v/v acetic acid intraperitoneally, group II served as standard group and was topically treated with diclofenac diethylamine gel (1.16%), groups III- VII were treated with polyherbal gels (GF1-GF5) with extract combination (1:2:3), groups VIII-XII (GF6-GF10) and groups XIII- XVII (GF11- GF15) were treated with polyherbal gels with extract combination (2:3:1) and (3:1:2) respectively. In all the treatment groups the polyherbal gel with different combination of extracts, with and without permeation enhancers were topically applied on the abdomen region 15 minutes prior to administration of acetic acid 1%v/v. In formalin test standard and polyherbal gel formulations were topically applied on the dorsal surface of the hind paw of mice 30 min prior to administration of dilute formalin.

2. 6. Acetic acid induced writhing response[7]

Acetic acid (1% v/v) was administered intraperitoneally to all the groups at the dose of 1 ml/kg body weight 15 min after the application of polyherbal gels. A writhe is indicated by abdominal constriction and full extension of hind limb. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted for 30 minutes after administering acetic acid. Percent protection against writhing movement was taken as index of antinociception. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pre-treated (n=6) with the polyherbal gel formulations using the formula

(Control mean – Treated mean) x 100 / Control mean

2.7. Formalin induced hind paw licking in mice[8]

Nociception was induced by injecting 20µl dilute formalin (1% in saline solution) under the skin of the dorsal surface of the hind paw of the mice. Each mice was challenged with formalin 30 minutes after being pretreated with the standard and test polyherbal formulations GF1-GF15 and then placed into a transparent plastic cage and observed. The licking response was monitored until 30 minutes starting immediately after the injection of formalin. The amount of time spent licking the injected paw was considered as indicative of pain. Antinociception was defined as a statistically significant reduction in the time spent in licking the injected paw in comparison with the control group during 30 min.

3. Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Tukey Kramer multiple comparison tests. All the results obtained in the study were compared with the control group. P values <0.001 were considered to be statistically significant.

4. Results and discussion

Preliminary screening of aqueous extracts of leaves of *Bryophyllum pinnatum*, *Vitex negundo* and *Centella asiatica* showed significant analgesic potential in accordance with the findings of other workers. The topical application of polyherbal gel formulations GF1-GF15 significantly inhibited the writhing reaction induced by acetic acid. All the formulations significantly (P<0.001) reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid as compared

to control (Table 3). GF5 and GF10 exhibited the highest writhing inhibition percentage of 95.43% and 93.38% respectively with oleic acid 5% as the permeation enhancer. The highest writhing inhibition percentage was found in the formulations where *Centella asiatica* and *Vitex negundo* were taken in higher concentrations.

The topical application of polyherbal gel formulations GF1-GF15 significantly inhibited the licking time. All the formulations significantly ($P<0.001$) reduced the paw licking induced by the injection of formalin as compared to control. The percentage protection in formalin test is shown in Table 4. GF3 exhibited the highest percentage protection of 77.60% with menthol 5% as the permeation enhancer.

Flavonoids are known to inhibit the enzyme prostaglandin synthetase. Since prostaglandins are involved in the pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins caused by flavonoids in *Vitex negundo*[4] and *Bryophyllum pinnatum*[5] might be responsible for the analgesic effect. Similarly the terpene acids found in *Centella asiatica* might also be responsible for the antinociceptive effects[6].

The primary role of the skin is to act as a barrier for drug absorption. There is ample proof that the stratum corneum is the primary barrier to drug absorption through skin[9]. Menthol is a natural skin permeation enhancer with relatively low skin irritancy. Mechanism of skin permeation enhancement by menthol can be attributed to the increase in skin flux by altering the barrier properties of the stratum corneum[10]. Oleic acid a mono-unsaturated fatty acid, because of its kinked or twisted shape and structure, is supposed to insert itself into the lipid matrices of the skin, widening the non-aqueous channels, and thereby increasing permeation[11]. Menthol and oleic acid both enhanced the skin permeation of the polyherbal gels.

5. Conclusion

The polyherbal gels with highest concentration of *Vitex negundo* and *Centella asiatica* demonstrated significant antinociceptive potential. Menthol and Oleic acid in a concentration of 5% also enhanced the antinociceptive effect when compared with gels with no permeation enhancers.

Table 1: Combination of extracts for polyherbal gel formulations

S.No.	Code	Ratio		
		Bryophyllum pinnatum	***Vitex negundo***	***Centella asiatica***
1.	F1	1	2	3
2.	F2	2	3	1
3.	F3	3	1	2

Table 2: Formulation of different extract combinations with and without permeation enhancers

Code	**Extract combination (*Bryphyllum pinnatum:Vitex negundo: Centella asiatica*)**	**Permeation enhancers**	
		Menthol	**Oleic acid**
GF1	1:2:3	-	-
GF2	1:2:3	2.5	-
GF3	1:2:3	5.0	-
GF4	1:2:3	-	2.5
GF5	1:2:3	-	5.0
GF6	2:3:1	-	-
GF7	2:3:1	2.5	-
GF8	2:3:1	5.0	-
GF9	2:3:1	-	2.5
GF10	2:3:1	-	5.0
GF11	3:1:2	-	-
GF12	3:1:2	2.5	-
GF13	3:1:2	5.0	-
GF14	3:1:2	-	2.5
GF15	3:1:2	-	5.0

Table 3: Effect of polyherbal gels on acetic acid induced writhing in mice.

Groups	**Number of contractions**	**Percentage inhibition**
Control	73.0 ± 0.96	0.00
Standard	28.5 ± 1.40*	60.95
GF1	34.66 ± 1.33*	52.52
GF2	12.5 ± 0.95*	82.87
GF3	6.16 ± 0.98*	91.56
GF4	17.83 ± 0.54*	75.57
GF5	3.33 ± 0.49*	95.43
GF6	41.0 ± 0.36*	43.83
GF7	18.66 ± 0.49*	74.43
GF8	13.0 ± 0.73*	82.19
GF9	22.66 ± 0.55*	68.95
GF10	4.83 ± 0.6*	93.38
GF11	39.16 ± 0.79*	46.35
GF12	15.16 ± 0.47*	79.23
GF13	12.0 ± 0.57*	83.56
GF14	24.16 ± 0.60*	66.90
GF15	8.5 ± 0.76*	88.35

n=6, the values are expressed in Mean ± SEM; * = $p < 0.001$ when compared with control group (One way ANOVA followed by Tukey's multiple comparison tests).

Table.4 Antinociceptive effect of polyherbal gels on formalin test in mice.

Groups	Mean licking time	Percentage protection
Control	61.0 ± 0.36	0.00
Standard	20.83 ± 0.4*	65.85
GF1	38.33 ± 0.21*	37.16
GF2	20.16 ± 0.30*	66.95
GF3	13.66 ± 0.33*	77.60
GF4	17.0 ± 0.25*	72.13
GF5	14.66 ± 0.33*	75.96
GF6	42.16 ± 0.47*	30.88
GF7	21.83 ± 0.30*	64.21
GF8	14.33 ± 0.21*	76.50
GF9	19.33 ± 0.42*	68.31
GF10	15.33 ± 0.42*	74.86
GF11	45.83 ± 0.30*	24.86
GF12	25.66 ± 0.33*	57.93
GF13	17.5 ± 0.56*	71.31
GF14	24.16 ± 0.47*	60.39
GF15	23.16 ± 0.47*	62.03

n=6, the values are expressed in Mean ± SEM; * = $p < 0.001$ when compared with control group (One way ANOVA followed by Tukey's multiple comparison tests).

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