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Original Article

FORMULATION AND EVALUATION OF AN HERBAL ANTI-INFLAMMATORY GEL CONTAINING *TRIGONELLA FOENUM GREACUM* SEED EXTRACT

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ABSTRACT

Objective: The present study was aimed to develop topical gel containing *Trigonella foenum greacum* (fenugreek) seed extract using carbopol-934, Hydroxypropyl methylcellulose K4M (HPMC K4M) as gelling agents and to investigate the anti-inflammatory activity of suitable gel formulation.

Methods: Gels were prepared using carbopol-934, HPMC K4M individually as well as in combination as gelling agents. Prepared formulations were evaluated for various physicochemical properties. Based on *in vitro* permeation study, the best gel formulation was chosen and it was subjected to *in vivo* anti-inflammatory activity studies using carrageenan-induced rat paw edema model and kept for stability studies for a period of three months.

Results: Gels prepared with a combination of carbopol-934 & HPMC K4M as gelling agent showed highest drug release of 88.02±0.06 % after 8 h of *in vitro* release study when compare to other formulations. Among the prepared formulations of fenugreek, gels prepared with a combination of carbopol-934 & HPMC K4M showed significant reduction of paw edema (57.78%) when compared to the control topical base group after 3 h from carrageenan injection.

Conclusion: Fenugreek has high potential as an anti-inflammatory agent when formulated as an herbal gel for topical use and can be effective in acute inflammatory disorders.

Keywords: Fenugreek, Gels, Ethanolic extract, Anti-inflammatory activity.

INTRODUCTION

Topical drug delivery systems are gaining increased in popularity, and several drugs have been successfully delivered by this route for both local and systemic action. Gels have better potential as a vehicle to administer drug topically in comparison to the ointment because they are non-sticky, requires low energy during formulation [1]. Drug delivery through the skin has been a promising concept for a long time because the skin is easy to access, has a large surface area with vast exposure to the circulatory and lymphatic networks and the route is non-invasive. Gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates leaving the drug entrapped in a thin film of the gel–forming matrix physically covering the skin [2].

The presence of a network formed by the interlocking of particles of the gelling agent gives rise to the rigidity of a gel. The nature of the particles and the type of form that is responsible for the linkages determine the structure of the network and the property of the gel [3].

The available anti-inflammatory drugs (steroidal and non-steroidal) present a wide range of side effects. Therefore, many studies are being directed to find anti-inflammatory agents from natural sources. Fenugreek (*Trigonella foenum-graecum*; Fabaceae) is a plant whose seeds and leaves are used in traditional medicine. Fenugreek act as powerful antioxidant mainly due to the presence of flavonoids and polyphenols [4,5]. Fenugreek has been reported to possess anti-inflammatory activity mainly due to the presence of flavonoids because flavonoids act as antioxidant and potential inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase [6].

Even though fenugreek has been used for the treatment of inflammation, no report exists on the development of gel formulations from an extract of fenugreek. Hence, the present study is aimed at formulating and investigating the effective anti-inflammatory gel formulation from the ethanolic extract of Fenugreek.

MATERIALS AND METHODS

Preparation of ethanolic extract of fenugreek

The seeds of *Trigonella foenum greacum* were obtained from local market which was identified, authenticated by Prof (Dr) Nagalakhmamma St. Aloysius College, Mangalore. Seeds were, pulverized, sieved through 40 mesh to obtain a coarse powder. Hundred grams of powdered seeds were extracted with ethanol as a solvent by hot extraction method using soxhlet apparatus. The resulting extract was cooled and filtered. The filtrate was evaporated in vacuum to give a residue.

Preparation of gel formulations

Various gel formulations were prepared from fenugreek seed extract using carbopol-934 alone, HPMC K4M alone and a mixture of carbopol-934, HPMC K4M as gelling agents. Gels were prepared by cold mechanical method described by Schmolka *et al.* [7]. Required quantity of polymer (carbapol-934, HPMC K4M) was weighed individually, and sufficient amount of distilled water were mixed in a separate beaker, after which it was continuously stirred by mechanical stirrer till the polymer is soaked in the water and kept for 24 h at room temperature.

With continuous stirring, now the appropriate quantity of methyl paraben and propyl paraben was added which acts as a preservative. Small quantities of triethanolamine were added with continuous stirring to achieve neutral pH. Finally extract was added to gel with continuous stirring till drug get dispersed completely. The prepared gel was filled and sealed in the aluminium collapsible tube. A similar procedure was followed for base control gel without the extract.

Evaluation of gel formulations

Prepared formulations were evaluated for various physicochemical parameters such as colour, homogeneity, pH, spreadability, viscosity and drug content (total phenolic content).

Measurement of pH

5 gm of gel formulation was dispersed separately in 45 ml of water, and the pH of the suspension was determined using digital pH meter (digital pH meter, 335, systronics, Noroda, Ahmedabad). Measurements of pH of all formulations were carried out in triplicate and the averages of three readings were noted [8].

Homogeneity

Formulations were tested for homogeneity by visual inspection after the formulations have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of viscosity

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand [9]. 50 g of gel was filled in a 100 ml beaker. T-bar spindle (T95) was used for the measurement of viscosity of all the gels. The helipath T-bar spindle was moved up and down and viscosity was measured at 2.5, 4, 5 and 10 rpm.

Spreadability

Spreadability was determined by wooden block and glass slide apparatus [10]. The apparatus consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of slip and drag characteristics of formulations. An excess of the formulation (about 2 g) was placed on this ground slide and then formulation was sandwiched between this slide and another glass slide (movable) having the dimension of fixed ground slide and provided with the hook.

A weight of 1 kg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. The top plate was then subjected to pull of 80 g with the help of string attached to the hook and the time (in seconds) required by the top slide to (movable) to separate completely from the fixed slides was noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula:

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

Where, S = Spreadability

M = Weight in the pan (tied to the upper slide),

L = length of glass slide

T = Time (in sec.) taken to separate the slide completely each other

Table 1: Composition of gel formulations

Ingredients (%w/w)	Gel formulations		
	G1	G2	G3
Fenugreek extract	5	5	5
Carbopol-934	1.0	-	1.0
HPMC K4M	-	1.0	1.0
Triethanolamine	q. s	q. s	q. s
Methyl paraben (0.5%)	1.5 ml	1.5 ml	1.5 ml
Propyl paraben (0.2%)	0.5 ml	0.5 ml	0.5 ml
Purified water	100 ml	100 ml	100 ml

Drug content determination

Drug content was determined by dissolving accurately weighed 1 g of gel in phosphate buffer of pH 6.8. After suitable dilution, total phenolic content were determined spectrophotometrically following Folin-Ciocalteau method described previously with minor modification [11]. Absorbance was recorded by using UV-visible spectrophotometer at 765 nm and the concentration is determined for estimating drug content.

***In vitro* release study of polyphenolic content from topical formulations**

Diffusion studies of the all the formulations were carried out in Franz diffusion cell through a sigma membrane [12]. In diffusion cell, sample (0.1 g) was applied on dialysis membrane in donor compartment. The entire surface of membrane kept in contact with the receptor compartment containing phosphate buffer (pH 6.8) as the dissolution medium. Magnetic stirrer was used for stirring the receptor compartment. The temperature maintained was 37±1 °C. The study was carried out for 8 h with samples removed at 0.5, 1, 2, 4, 6, 8 h. The sample was withdrawn at a predetermined period of time and the same volume was replaced with fresh phosphate buffer (pH 6.8). Samples were analyzed for total phenolic content according to the Folin-Ciocalteu method and absorbance values were measured at 765 nm using UV/Visible spectrophotometer.

***In vivo* study**

Experimental animals

Pharmacological studies were conducted on animals after obtaining approval from the institutional animal ethics committee (reference number KSHEMA/AEC/39/2011). Albino Wister rats of both sexes weighing 150-200 g was used. They were obtained from the central animal house KSHEMA Deralakatte, Mangalore, India. The animals were maintained under controlled conditions of temperature (22±2 °C), humidity (50±5%) and 12 h light-dark cycles. They were fed commercial stock diet and water *ad libitum*.

Acute skin irritation study of topical formulations

This test was performed on albino rats weighing between 150-200g. The animals were given standard animal feed and had free access to water *ad libitium*. Animals are divided into three groups, each batch containing five animals. Dorsal hairs at the back of the rats were removed one day prior to the commencement of the study and kept individually in cages to avoid contact with the other rats. Two groups of each were used for control (plain gel base) and standard irritant. Other group was used as a test. The 50 mg of the formulation were applied over one square centimeter area of whole and abraded skin of different animals. Aqeous solution of 0.8% formalin was used as standard irritant. The animals were observed for seven days for any signs of edema and erythema.

Investigations of anti-inflammatory activity by carrageenan-induced rat paw edema method

In the present study, rats of either sex weighing 150-200 g were used. Animals were allowed to free access to feed and water before the experiment. Animals are divided into control, Standard and test group containing six animals in each group.

Approximately 50 µl of a 1% suspension of carrageenan in saline was prepared 1h before each experiment and was injected into the plantar surface of the right hind paw of rat. To the test group 0.2g of gel containing ethanolic extract of fenugreek was applied to the plantar surface of the right hind paw by gently rubbing with the index finger. Rats of the control groups received only the gel base; Voltaren gel applied, in the same way, was used as a standard. One hour after the application of the gel base, topical preparation of fenugreek and standard; 50 µl of a 1% suspension of carrageenan in saline was administered into plantar surface of the right hind paw of rat. Paw volume was measured immediately after carrageenan

injection and at 1h, 2h, 3h and 4h after the administration of the noxious agent by using a plethysmometer [13]. The paw volume was recorded at different time points.

The percentage inhibition in paw volume was calculated by using the formula:

$$\% \text{ Inhibition} = \frac{\text{Paw volume (Control)- Paw volume(Test)x 100}}{\text{Paw volume (Control)}}$$

Statistical analysis

The results of various studies were expressed as mean±SEM. Data analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test using "Graph pad Instat" version 3.00 for Windows 95, Graph Pad Software. Probability values of 0.05 ($p<0.05$) or less were considered statistically significant.

RESULTS AND DISCUSSION

Formulation and evaluation of herbal gels from fenugreek extract

Gel formulations were prepared using polymers such as carbopol-934 and HPMC K4M as gelling agent. Triethanolamine was used in formulations to neutralize the pH and methyl paraben; propyl paraben were used as preservatives.

Gel formulations showed green colour, aromatic odour, good homogeneity and spreadability. The pH of gel formulations was in the range of 6.9-7.1 which lies in the normal pH range of the skin. The viscosity of gel formulation containing carbopol-934 alone as gelling agent was found to be high with less spreadability than gels formulated with HPMC K4M. Drug content of all the formulation was found to be more than 94%.

Table 2: Physicochemical evaluations of topical formulations of fenugreek

Formulation code	Colour	Homogenity	pH	Spreadability g. cm/sec	Drug content (%)
G1	Green	Good	6.9±0.04	20.30±1.11	94.6±0.3
G2	Green	Good	7.1±0.01	50.05±1.06	95.9±0.1
G3	Green	Good	7.0±0.01	31.25±1.51	97.2±0.2

All values are expressed as mean±SD, n=3.

Table 3: Measurement of viscosity of topical formulations of fenugreek

Formulation code	Viscosity (cps) at rpm			
	2.5	4	5	10
G1	20108±20	15300±11	10,014±16	8600±18
G2	2345±10	2100±12	1980±12	1425±15
G3	15163±11	10986±18	9030±10	7800±13

Table 4: *In vitro* release study of polyphenolic content from topical formulations

Time (mins)	Formulation code					
	G1		G2		G3	
	% CDR	Av. Flux (µg/cm²/min	% CDR	Av. Flux (µg/cm²/min	% CDR	Av. Flux (µg/cm²/min
30	1.10±0.01	0.60	2.01±0.03	1.09	4.00±0.05	2.17
60	10.50±0.05	5.11	18.05±0.07	8.72	29.01±0.07	13.59
120	21.13±0.09	2.89	30.06±0.06	3.26	49.65±0.02	5.61
240	48.69±0.02	3.75	56.09±0.03	3.54	79.31±0.06	4.03
360	59.12±0.06	1.42	68.13±0.05	1.64	84.85±0.01	0.75
480	70.05±0.06	1.49	75.13±0.03	0.95	88.02±0.06	0.43

All values are expressed as mean±SD, n=3.

In vitro drug release studies were carried out for all gel formulations for 8 h and amount of polyphenolic content released were estimated which is shown in table 4. Gel formulation G3 showed higher percentage release of phenolic content (88.02%) after 8 h than G1 (70.05%) and G2 (75.13%). Gels prepared with combination of carbopol-934 & HPMCK4M as gelling agent showed better release of polyphenolic content when to compare to other topical formulations. Amount of drug permeated through unit area of the membrane was determined on the basis of *in vitro* drug release data. For all formulations, average flux was maximum in the first one hour of drug release and thereafter flux decreased gradually with time which is shown in table 4. It is also observed that among the gel formulations, average flux values were greater for G3 containing mixture of carbopol-934 and HPMC K4M as gelling agents.

Pharmacological investigation of topical formulations

Acute skin irritation study of topical formulations

Gel preparations of fenugreek extract were subjected to acute dermal toxicity to check for the skin reactions. There were no any signs of oedema and erythema was observed till seven days after the treatment which indicated that absence of skin toxicity after topical application of formulations.

Investigation of anti-inflammatory activity of prepared gel formulation

Anti-inflammatory activity of selected gel formulation was investigated and results obtained are shown in table 5.

Table 5: Measurement of carrageenan-induced paw edema volume in rats treated with topical formulations

Treatment	Paw volume(ml)[a] (Percentage inhibition of edema)			
	1h	2h	3h	4h
Control (gel base)	1.21±0.02	1.2±0.17	1.22±0.02	1.09±0.03
Diclofenac gel	0.70±0.07** (42.07)	0.52±0.02** (56.16)	0.36±0.03** (70.16)	0.26±0.08** (75.00)
G3	0.91±0.04** (24.75)	0.75±0.02** (37.5)	0.51±0.04** (57.78)	0.60±0.02** (45.35)

[a]Values are expressed as mean±SEM (Number of animals, n=6); one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability values of 0.05 ($p<0.05$) or less were considered statistically significant; p**<0.01 p***<0.001 Vs control.

Treatment with formulation G3 showed 57.78% reduction of paw edema when compared to the control topical base group after 3 h from carrageenan injection. Gel formulation of fenugreek significantly reduced the paw oedema throughout the entire period of observation in comparison to control ($p<0.01$). Fenugreek gel formulation containing combination 1% carbopol 934 and 1% HPMC K4M was found to be best for topical application as anti-inflammatory agents. Poly phenolic compounds mainly flavonoids present in fenugreek extract may be responsible for producing anti-inflammatory activity which acts as antioxidant and potential inhibitors of cyclo-oxygenase, lipoxygenase and nitric oxide synthase [14]. Research has shown that topical application of fenugreek is effective for various skin problems such as abscesses, boil, burns and eczema. Fenugreek seeds contain galactomannans which acts as humectants thereby improves the mechanical property of the skin such as skin elasticity, hydration and fatigue [15]. Fenugreek, when applied to the skin, found to decrease skin pain, reduce swelling. Therefore, fenugreek extract containing several anti-inflammatory constituents has been made into gel formulation that is suitable for topical application which can provide multiple benefits for skin disorders along with treating inflammation.

Table 6: Stability study of gel formulation (G3) at 40±2 °C and 75±5% RH

Time in days	G3 Formulation		
	Colour	Viscosity (cps)	Percentage drug remaining
0	Green	15163±11	97.23±0.2
15	Green	15287±09	97.05±0.4
30	Green	15890±12	96.86±0.1
45	Green	16200±09	96.05±0.3
60	Green	16478±10	95.93±0.2
90	Green	16780±09	95.01±0.2

Stability studies shows that there is no significant change in viscosity and drug content which indicates that prepared gel formulation of fenugreek were found to possess good stability on storage up to 3 mo.

CONCLUSION

Topical gels containing fenugreek extract can be successfully prepared using carbopol-934 and HPMC K4M as gelling agents. The topical gel prepared from mixture carbopol-934 and HPMC K4M will be better gelling agent for making an ideal topical preparation. *Trigonella foenum greacum* seed extract in the form of gel possess significant topical anti-inflammatory properties, supporting their traditional use for the treatment.

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CONFLICT OF INTERESTS

Declared none

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