



FORMULATION AND CHARACTERIZATION OF ANTI-INFLAMMATORY GEL FROM FENUGREEK SEEDS EXTRACT

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Background- The *Trigonella foenum-graecum* plant is also known as methi and used in Ayurvedic medicines for the treatment of bronchitis, rheumatoid arthritis, abscesses and digestive abnormalities. The review article has been made for the standardization and phytochemical evaluation of Fenugreek seeds and this one is comprises of powder microscopy, fluorescence analysis. In modern food technology Fenugreek is used as food stabilizer, adhesive and emulsifying factor because of its gum, protein and fiber content. It is a rich source of calcium, iron and other vitamins. In this study after a general discussion of physio-chemical constituents, the biological and pharmacological reactions of Fenugreek. And to investigate the anti-inflammatory activity of suitable gel formulation.

Method - The polyherbal gel formulation was designed by using Carbopol 934, Sodium CMC, *Trigonella foenum-graecum*, Fenugreek extract, Ethanol, propylene glycol 400, methyl paraben, propyl paraben, and required amount of distilled water. The skin pH (6.8-7) was maintained by drop wise addition of triethanolamine. The prepared polyherbal gels were evaluated for physical appearance, pH, spreadability, skin irritation to observe side effect. It was inferred from the results that polyherbal gel formulations were good in appearance and homogeneity. The overall result of this research the prepared poly herbal gel formulation shows significant anti-inflammatory activity.

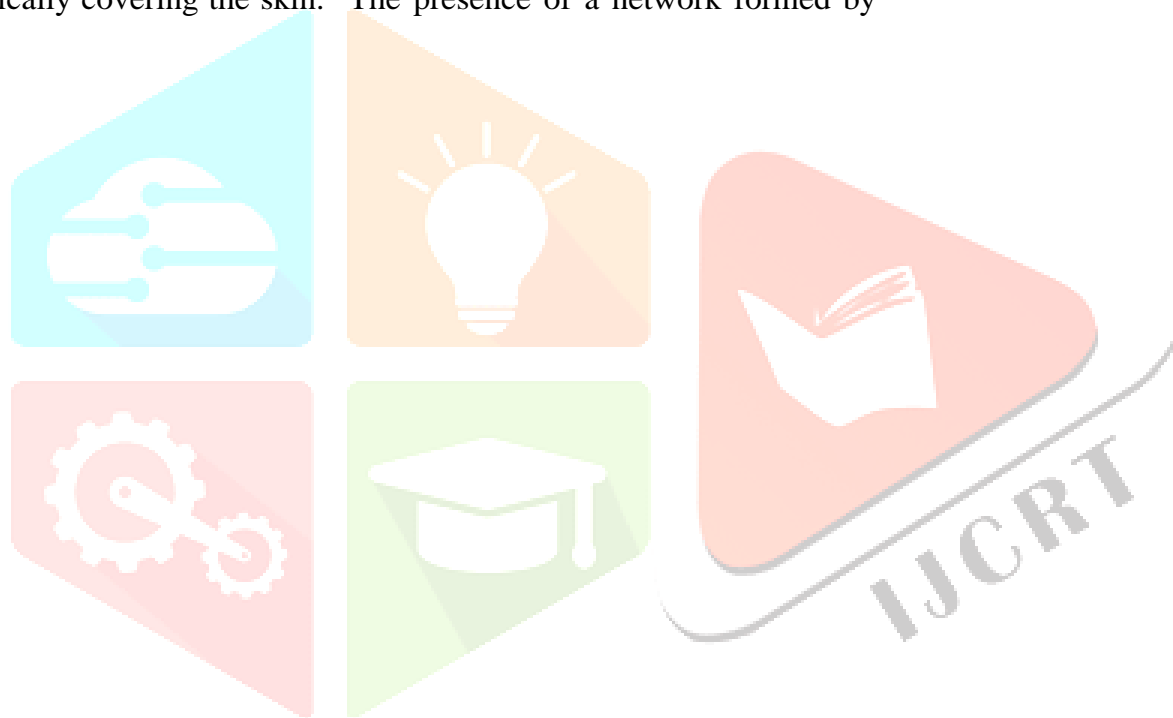
Results - Gels were prepared using carbopol-934, HPMC K4M individually as well as in combination as gelling agents. Prepared formulations were evaluated for various physicochemical properties. The topical gel prepared from mixture carbopol-934 and HPMC K4M will be better gelling agent for making an ideal topical preparation. F1 batch shows the good results than other batches so F1 batch is suitable for anti-inflammatory use. The prepared formulation was evaluated various parameters. All of the evaluation tests were passed by the formulation.

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. The main purpose of this study was to formulate a stable and functionally effective anti-inflammatory gel with addition herbs with synthetic chemicals. The research concluded that natural remedies are more acceptable and are safer with minimum side effects than synthetic preparation.

KEYWORDS - Fenugreek seeds, Gels, Ethanolic extract, Carbopol934, Anti-inflammatory activity.

I. INTRODUCTION

Topical drug delivery system are becoming more and more popular and thus multiple drug have been effectively administered for each local and systemic action a maximum of non-steroidal anti-inflammatory drugs (NSAIDs) in recent years to raise awareness of these drug to deliver drugs topical gel protect against gastrointestinal irritation overcome " first pass " effect and maximize their action since the gel is not sticky and require less strength throughout the composition it has a higher dosage than topical formulation when evaluating an ointment. 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. 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. 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 acts as a powerful antioxidant mainly due to the presence of flavonoids and polyphenols. Fenugreek has been reported to possess anti-inflammatory activity mainly due to the presence of flavonoids because flavonoids act as antioxidants and potential inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase.

GEL INTRODUCTION

Gel is defined as semi-rigid systems in which the strength of the dispersion medium is limited by the three-dimensional interaction of particles or the solubility of macromolecules in the dispersed phase.

The word "gel" is derived from "gelatin", and both "gel" and "jelly" can be traced back to. In Latin *gelu* means "drop" and *gel* means freeze or "freeze". This origin shows the basic principle of liquids as solids that do not flow but are elastic and retain some liquid properties. Use of the term "gel" to describe began in the late 1800s, when chemists tried to separate the semisolids based on their phenomenological properties rather than their molecular composition. The analytical methods required to identify drug samples are not currently available.

Gels are harder than jellies because they have more cross links, higher physical density or simply less liquid. Gel-forming polymers form materials of different hardness, starting from sols to slimes, jellies, gels and hydro gels.

Some gel systems are as clear as water, while others are cloudy because the material is not completely molecularly dispersed (soluble or insoluble) or does not form light-scattering aggregates. With some exceptions, the concentration of gelling agent is usually less than 10% and is usually in the range of 0.5% to 2%. 0% more.^[2]

ANATOMY OF HUMAN SKIN

SKIN

The human skin is the body's outer covering and the largest organ of the integumentary system. The skin has up to seven layers of ectodermal tissue and protects the muscles, bones, ligaments, and internal organs beneath. Human skin is similar to that of most other mammals, and it is very similar to that of pigs. Despite the fact that nearly all human skin is covered in hair follicles, it can appear hairless. There are two types of skin: hairy skin and glabrous skin (hairless). The adjective cutaneous means "of the skin" (from Latin *cutis*, skin).

Skin plays an important immunity role in protecting the body against pathogens and excessive water loss because it interfaces with the environment. Its other functions include insulation, temperature regulation, sensation, vitamin D synthesis, and vitamin B folate protection. Skin that has been severely damaged will attempt to heal by forming scar tissue.

It is frequently discoloured and depigmented. Skin pigmentation varies between populations in humans, and skin types range from dry to oily. Such skin variety provides a rich and diverse habitat for bacteria, which number around 1000 species from 19 phyla and are present on human skin.^[7]

DRUG PROFILE



Fig No. Fenugreek Seeds

Biological source:

The dried ripe, nature seeds of fenugreek is its biological source. Fenugreek scientific name is *Trigonella foenum-graecum*. It belongs to family *fabaceae*.^[14]

Taxonomical Classification:

- **Kingdom** – Plantae
- **Division** – Magnoliophyta
- **Class** – Magnoliopsida
- **Order** – Fabales
- **Family** – Fabaceae
- **Sub-family** – Trifolieae
- **Genus** - *Trigonella*
- **Species** – *Trigonella foenum-graecum*.

Morphological features:

The fenugreek seeds sown in well prepared soil sprout in three days. Seedling grows, erect, semi-erect or branched and attains a height of 30 to 60 cm.

It contains pinnate compound, trifoliolate leaves, auxiliary white to yellow flower, and 3 to 15 cm long with pointed beaked pods and every pod contains 10 to 20 oblong greenish brown seeds with unique hooplike grooves.

- **Appearance** - Solid-rhomboidal seeds, 3 to 5 mm long, 2 mm thick, hard, pebble-like.
- **Colour** - Yellowish brown-light brown.
- **Odour** - Characteristics spicy.
- **Taste** - Slightly Bitter and mucilaginous.

Parts used: Seeds.

Chemical Constituent:

It consists of vitamins and saponins. The most prevalent alkaloids are trigonelline and coumarins include cinnamond scopoletin.^[15]

Uses: 1. It is used as anti-inflammatory activity.

2. It promotes hair growth and shows anti-fungal activity.

REVIEW OF WORK DONE ON FORMULATION AND CHARACTERIZATION OF ANTI-INFLAMMATORY GEL

In 2015 The present study was aimed to develop topical gel containing *Trigonella foenum-graecum* (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. **Divya Jyothi. et al.**^[28]

In 2023 The present study has been undertaken with the aim to formulate and evaluate the new polyherbal gel formulation containing 0.3 % hydroalcoholic extract from *Trigonella foenum-graecum* and *Glycyrrhiza glabra* for its topical anti-inflammatory activity against carrageenan induced rat paw edema method. The studies were conducted on Wistar rats of either sex (150-180g). The polyherbal gel formulation was designed by using Carbopol 934, Sodium CMC, *Trigonella foenum-graecum*, Ethanol, propylene glycol 400, methyl paraben, propyl paraben, and required amount of distilled water. The skin pH (6.8- 7) was maintained by drop wise addition of tri- ethanolamine. The prepared polyherbal gels were evaluated for physical appearance, pH, spreadability, skin irritation to observe side effect. It was inferred from the results that polyherbal gel formulations were good in appearance and homogeneity. **A.W.Baitul. et al.**^[2]

RESEARCH METHODOLOGY

EXTRACTION METHOD

100 gm of seeds of fenugreek was reduced to fine powder in mechanical grinder. The fine powder passed through sieve no.43. The finely grounded powder was placed in filter paper bag & kept in a Soxhlet apparatus and add 100ml of ethanol in it. The extraction solvent in flask was heated & the vapors were condensed. When the level of liquid in chamber raises to the top of siphon tube the liquid contents of chamber siphon was collected into flask.

This process was continued and carried out until the siphon tube was emptied. The collected extract was then evaporated by rotavapor to remove the solvent completely and crude extract were obtained in the round bottom flask.^[28]

METHOD OF PREPARATION OF GEL FORMULATION

1. 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.
2. Required quantity of polymer (carbopol-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.

3. With continuous stirring, now the appropriate quantity of methyl paraben and propyl paraben was added which acts as a preservative.
4. 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.
5. The prepared gel was filled and sealed in the well container.
6. A similar procedure was followed for base control gel without the extract.^[28]

Anti-inflammatory Test ^[33]

➤ Procedure

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of Synthetic compound (1000µg/ml). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C ±2) in a incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1000 µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

FORMULATION OF GEL

The gel was formulated using the ingredients as specified The plant material used in the formulation is rich in various phytochemicals. A good gel must have ideal consistency to facilitate application on the skin. Carbapol934 and HPMC K4M was added as gelling agent, Methyl paraben and propyl paraben is added as preservative, triethanolamine added to the formulation for emulsifier and rose oil is added to the perfume.

EVALUATION TESTS FOR THE GEL

2 Evaluation of the anti-inflammatory gel is done using various physiological and chemical tests. These tests provide with information regarding various parameters of the formulation. .

5.3 PHYTOCHEMICAL SCREENING

The naturally occurring chemical compounds are phytochemicals. They give organoleptic properties and colour to the plant. Some phytochemicals are known to reveal medicinal and physiological activities which are flavonoids, alkaloids, etc. Tests were carried out to detect the presence of secondary metabolites such as alkaloids, flavonoids, terpenoid, anthraquinone and compounds.

Table No. 01 Phytochemical investigation of fenugreek seeds

SR NO.	Phytochemicals	<i>Trigonella foneum–graceum</i> (Methi)
1.	Alkaloids	+
2.	Flavonoids	+
3.	Terpenoids	+
4.	Anthraquinones	-
5.	Saponins	+
6.	Tannins	+
7.	Glycosides	+

(+) indicates presence whereas (-) indicates absence of the phytochemical

PHYSICAL APPEARANCE

1. Colour and odour

The prepared formulation is examined visually. The formulation must be visually appealing for greater customer satisfaction. The prepared formulation must be free from any agglomerates and must be uniform in nature. Proper care was taken while preparing the formulation to ensure that there was no formation of lumps. The formulation was also inspected for other physical attributes such as colour and odour which also play an important role while usage of any cosmetic product. It was observed that the formulation exhibits white milky colour and has an aromatic odour due to addition of herb.



F1



F2

Appearance of the anti-inflammatory gel

2. Spreadability

Good spreadability can be guarantee the distribution of gel when apply to the skin; good spreadability ranges from gm.cm/sec. The spread test result of the anti- inflammatory gel preparations reveal a value between 4.5 to 5 gm.cm/sec. which indicates that F1 batch as good spreadability.

3. pH

The topical skin is extremely sensitive to the pH variation of the products applied in its surface. The pH of the anti-inflammatory gel is important for high potential. An herbal gel for topical use and can be effective in acute inflammatory disorders. Balanced pH is one of the ways to minimize damage to the topical skin. Most anti-inflammatory gel is neutral or slightly acidic. The pH of formulated gel was determined using pH meter.

PHYSIOCHEMICAL ANALYSIS

1. Determination of Viscosity

Viscosity of the developed gel formulation was determined by using the Brookfield viscometer. Measuring the viscosity of formulation is an essential part of quality control of the product. Product viscosity plays an important role in defining and controlling many attributes such as shelf life, stability.

Table No.02 Viscosity by Brookfield Viscometer

BATCH NO.	Rpm	Viscosity (cP)
F1	2.0 rpm	9450 cP

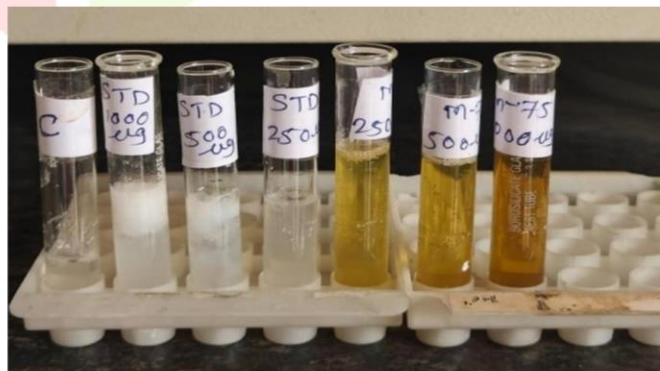
2. Stability testing

Stability testing was carried out to check the quality of the product at room temperature which was kept for the period of one month. Formulated gel containing open container when expose to ambient room temperature then syneresis was observed it means concentration of gel by separating out of liquid syneresis it means form of instability in aqueous gels.

Table No.05 Stability testing

Open container	Closed container
Not stable	stable

Anti-inflammatory testing



Denaturation of tissue protein is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of protein in vivo. Agents that can prevent protein denaturation therefore could be worthwhile for anti-arthritic and anti-inflammatory drug development F1 sample showed Good activity as compared with standard drug.

The anti-inflammatory properties of fenugreek seeds extract were evaluated by the anti-protein denaturalizing method of egg albumin. The anti-inflammatory activity of the gel by protein coagulation method in egg albumin showed greater inhibition 250µg, 500 µg, 1000 µg of fenugreek extract when compared with diclofenac sodium 0.15 /Ibuprofen as shown in table No.15.

Table No.04 Anti-inflammatory activity result

Sr. No.	Sample (1000µg/ml)	Conc.	O. D.(Once Time)	Mean	Percent inhibition
1.	Control	-	0.42 0.43 0.46	0.43	-
2.	Standard Diclofenac sodium 0.15 /Ibuprofen	250µg	0.18 0.15 0.16	0.16	62.79
		500µg	0.11 0.12 0.10	0.11	74.41
		1000µg	0.09 0.08 0.07	0.08	81.39
3.	Sample	250µg	0.36 0.38 0.34	0.36	16.27
		500µg	0.28 0.25 0.24	0.25	41.86
		1000µg	0.25 0.22 0.20	0.22	48.33

Evaluation results**Table No.06 Evaluation results**

Evaluation Characteristics	Formulation	
	F1	F2
Colour	Milky	Milky
Odour	Characteristics as a rose	Characteristics as a rose
Nature	Semi-solid	Semi-solid
Smoothness	Good	Good
Consistency	Good	Good
Greasiness	Good	Good
Washability	Washable	Washable
Spreadability	3.44 g.cm/sec	3.25 g.cm/sec
pH	6.82	6.46
Homogeneity	good	good
Viscosity(cP)	9450 cP	-

CONCLUSION

The phytochemical component of the seeds *Trigonella foenum-graecum* plant of was analyzed. 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.

The *Trigonella foenum-graecum* seeds extract in the form of gel possess significant topical use anti-inflammatory. Formulation F2 was better than that of F1. From among all the developed formulation, F1 shows better spreadability, consistency and physical appearance. pH of the F1 batch is Sufficient to treat the pain, also shows anti-inflammatory activity. **F1 batch shows the good results** than other batches so F1 batch is suitable for anti-inflammatory use. The prepared formulation was evaluated various parameters. All of the evaluation tests were passed by the formulation.

The main purpose of this study was to formulate a stable and functionally effective anti-inflammatory gel with addition herbs with synthetic chemicals. The research concluded that natural remedies are more acceptable and are safer with minimum side effects than synthetic preparation. The data presented in this study, it was demonstrated that the developed gel processes significant, therapeutically efficacious, suitable vehicle for drug delivery in low cost but definitely with high potential.

The formulated anti-inflammatory gel was show the good scope in future about research in natural remedies.

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