



## Isolation And Evaluation Of *Trigonella Foenum Graecum* Mucilage As Gelling Agent In Diclofenac Potassium Gel

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*The objective of present work is to find out the gelling potential of mucilage from Trigonella foenum graecum (Fenugreek seeds). Mucilage was isolated from Trigonella foenum graecum by soaking, extraction and boiling with distilled water as solvent and precipitating by addition of acetone as non-solvent and drying at 50-60°C. Mucilage extracted from Fenugreek seeds were subjected to its safety and preformulation studies for its suitability as a gelling agent. Physico-chemical characteristics such as solubility, ash values, swelling index, loss on drying and pH were studied. In the present study five batches of Diclofenac gel were prepared with different concentration of mucilage (viz; 4.0%, 5.0%, 6.5%, 8.0%, 10%w/w), Diclofenac sodium 1%w/w, Glycerin 10%w/v, Methyl paraben 0.02%. The gels prepared with 8.0% of mucilage were found to be ideal and comparable with commercial preparation. The gels were evaluated for drug content, viscosity determination, in-vitro permeation, skin irritation etc. The prepared gels did not produce any dermatological reactions and were well tolerated by the albino mice. The gels were found to be stable with respect to viscosity, drug content and physical appearance at all temperature conditions for 3 weeks. Studies indicate that the extracted mucilage may be a good substitute as a pharmaceutical excipient specifically as a gelling agent.*

**Keywords:** Fenugreek, Mucilage.

### INTRODUCTION:

Herbs serve as a gelling agent as an alternative to synthetic products because of eco-friendly, economical and renewable resource. Majority of investigations on herbs in drug delivery system are centered on polysaccharides due to above mentioned benefits. Mucilages are most commonly used adjuvant in pharmaceutical preparations. They consist of sugar and uronic acid units. They swell in water and form a gel such a phenomenon is often called as rheology synergism. They possess variety of pharmaceutical properties such as emulsifying, suspending, binding and gelling agents<sup>1,2</sup>.

Fenugreek (FG) or Methi (*Trigonella foenum graecum* Linn.) is an erect annual plant (height 30-60 cm.) belonging to family Leguminosae (Fabaceae). Various parts of fenugreek, mainly its leaves and seeds have been widely used in the Indian food. It has several cosmetic and medicinal uses like gastroprotective, antiurolithiatic hypoglycemic, diuretic, anti dandruff agent, anti-inflammatory agent and as antioxidant. Mucilage of various seeds has been used as granulating and binding agent due to its nontoxicity, low cost, free availability, emollient and non irritating nature<sup>3</sup>.

Fenugreek seed endosperm galactomannan was not in industrial production till 1993. Currently some industries are producing and marketing a sizable amount of fenugreek gum and other fenugreek products are finding increasing application. Guar and LBG have been in industrial use for quite some time. Similar to guar gum fenugreek also produces from widely grown annual agricultural crop<sup>4</sup>.

It is an old cultivated spice bean crop indigenous to India, Middle East, Southern Europe North Africa and North America. It has thin 10- 15 cm. long sword shape pods with 10- 20 seeds. The oblong yellow brown seeds (2-3 mm long) are hard with wrinkled surface<sup>5,6</sup>. Dicotyledonous Fenugreek seeds consist of brown

husk mainly composed of soluble galactomannan polysaccharides. Dry seeds contain 3-6% moisture, 25-30% protein, 20-25% insoluble fibres and 3-4% ash. The mucilages possess various pharmaceutical

properties such as binding, gelling, emulsifying and suspending agents. The present work uses the most economical and simple method for the separation of mucilage from Fenugreek seeds<sup>7, 8</sup>.

Diclofenac possess potent anti-inflammatory antipyretic and analgesic activity belongs to Non- Steroidal Anti- inflammatory Drug<sup>9</sup>. Diclofenac is widely prescribed for the treatment of osteoarthritis, rheumatoid arthritis, acute lumbago and dental pin condition. It is well tolerated with minor adverse effect including gastric upset, dizziness, vertigo and tremor<sup>10</sup>.

### **OBJECTIVES:**

The present study was performed with following objectives:

- a. Isolation of mucilage from seeds of *Trigonella-foenum graecum* Linn.
- b. Preparation of gels.
- c. Evaluation of prepared gels.
- d. Comparison with marketed gel preparation.

### **MATERIALS AND METHODS:**

#### **Materials:**

The Fenugreek seed mucilages prepared in our laboratory. Diclofenac potassium was obtained as gift sample from Aristo Pharmaceuticals Ltd, India. All other materials, solvents and reagents were of analytical grade.

#### **Animals:**

Healthy adult wistar albino rats weighing about 200-250g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12h light and 12h dark cycle, 25±2°C, 40-60% humidity) The study was approved by institutional animal ethical committee (Reg. No.751/03/abc/CPCCSEA)

#### **Methods:**

#### **Isolation of Mucilage:**

The mucilage of fenugreek seeds was isolated with following established methods. 20gm of fenugreek seeds dissolved in 200ml double distilled water and boiled with stirring up to slurry formation, kept it to cool for 3 to 4 hr. to separate supernatant liquid. The upper clear solution was decanted and centrifuged at 500rpm for 20 minutes. The supernatant was separated and concentrated at 60°C on water bath. The solution was cooled to the room temperature and was poured into thrice the volume of acetone with continuous stirring. The precipitated material was washed with distilled water and dried at 50-60° under vacuum.

#### **Preformulation studies:**

The study was performed on solubility, loss of drying, swelling index, total ash, acid insoluble ash etc. according to Indian Pharmacopoeial Procedures. The pH of mucilage was determined using digital pH meter. Physicochemical parameters are evaluated as per shown in Table 1.

#### **Color change:**

The study was performed to assure whether the mucilages are prone to oxidation or not by following established methods. Sample 1 containing 2 ml of 2% w/v mucilage with 1 ml of 0.1% ascorbic acid and Sample 2 containing 2 ml of 2% w/v mucilage with 1 ml of 0.1% sodium bisulphite as antioxidant. Sample 3 containing 2 ml of 2% w/v mucilage without any antioxidant. Keep all above samples exposed to sunlight as well as dark for the period of 12 h. After 12 hr they were observed for oxidation or color changes.

#### **Toxicity studies:**

The toxicity studies were performed by the method of Knudsen and Curtis. The male albino rats of Wister strain weighing of 160- 200 gm were divided into two different groups comprising four animals each. Group I (control group) received normal saline 20 ml/ kg i.p. Group II received 500, 1000, 1500 and 2000 mg/Kg

of mucilage suspension in normal saline orally. The animals were observed for mortality if any for 48 h. Since no mortality, no toxic manifestation were observed. In chronic toxicity studies, 6 animals are used and

divided equally in Group I (control) and Group II (test). To the Group II 250 mg/kg of mucilage suspension was administered daily for period of 30 days. Body weights and haematological parameters were recorded for both the groups at an interval of 10 days.

#### **Preparation of gels:**

Gels were prepared by using different concentration of drug mucilage, methyl paraben as a preservative and glycerine as a plasticizer as shown in table no- 2.

Evaluation of prepared gels:

The gel was evaluated for various parameters including;

**Consistency:** The measurement of consistency of the prepared gel was done by dropping a cone attached to a holding a rod from fixed distance of 10 cm in such a way that it should fall on the centre of beaker filled with the gel. The permeation by cone was measured from the surface of gel to tip of the cone inside the gel. The distance travelled by cone was noted down after 10 sec.

#### **Homogeneity:**

The gels were tested for appearance and presence of any aggregate by visual inspection after the gels were poured and settled in the container.

#### **pH:**

The pH of gels was recorded by using digital pH meter.

#### **Spreadability:**

The Spreadability was determined by using wooden block and glass slide apparatus. Apply about 15 gm of gel to the fixed slide and note down the time take to separate the movable slide from the fixed sl

Spreadability was then calculated by using formula

$$S = M.L/T$$

Where,

S = Spreadability

M = Weight applied to upper slide (30 g)

L = Length of glass slide (6 cm)

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

#### **Viscosity:**

Viscosity was measured by using Brookfield viscometer with shear rate of 5 rpm for 5 min.

#### **Drug content:**

100 mg of prepared and marketed gel dissolved separately in 100 ml of phosphate buffer of pH 7.4 with continuous stirring until the complete solubility of drug. Then filter the solution and determined the drug content spectrophotometrically at 274 nm. using phosphate buffer (pH 7.4) as blank.

#### **Skin irritation study:**

Wister albino rats 150-200 g were used to study skin irritation. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard condition. Hair are shaved on two sides, one side is subjected to control and other to the test. Gel was applied twice a day for 7 day and observed for irritation. The animals are found with no irritation and sensetivity

#### **In vitro diffusion study:**

This study was performed with the help of diffusion cell

apparatus. The prepared gel formulations (F3, F4, and F5) and marketed gel preparations are studied using egg permeation membrane. Glass cylinders with donor and receptor compartment open on both ends. 10 cm

height and 3.7 cm outer diameter was used as permeation cell apparatus. An egg permeation membrane (washed with distilled water and soaked in phosphate buffer of pH 7.4) was fixed to acceptor cell. One gram of prepared gel was taken in donor cell and the cell was immersed in a beaker containing 100 ml of phosphate buffer of pH 7.4 (receptor cell). The cell was immersed into a depth of 1 cm below the surface of buffer, which was stirred by magnetic stirrer and the temperature was maintained at  $37^{\circ} \pm 1^{\circ} \text{C}$  throughout the experiment. Sample were withdrawn from receptor compartment periodically (0.5, 1, 1.5, 2 hr). After each withdrawal, the volume of liquid in the receptor compartment was replaced by phosphate buffer of pH

7.4. The concentration of drug was determined double beam spectrophotometrically (UV Elico Ltd.) at 274 nm.

#### Anti-inflammatory activity:

The anti-inflammatory activity of gel formulations was evaluated by the carrageenan-induced rat hind paw edema method<sup>11</sup>. The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 751/03/abc/CPCSEA) was obtained. Healthy Wistar rats of either sex weighing between 150-200 g were obtained from the disease free small animal house of CCSHAU, Hisar. The animals were housed in institutional animal house under standard conditions with free access to food and water. Anti-inflammatory activity of the diclofenac loaded *Trigonella foenum graecum* gel was compared to the marketed gel of diclofenac (VoveranA Emulgel). Fifteen albino Wistar rats were divided into three groups of five animals each as follows: Group 1 (Control group): animals were treated with plain gel. Group 2 (Standard group): animals were treated with diclofenac gel B.P. Group 3 (PNG2): animals were treated with *Trigonella foenum graecum* gel formulation. Inflammation was induced by sub-plantar carrageenan injection and after 1 hour, formulations were applied topically on the inflamed paw of rats by gently rubbing with index finger and the volume of the paw was measured<sup>11, 12</sup>. The thickness of paw was measured at 1 h time intervals till 5 h after carrageenan injection. A digital vernier caliper (Aerospace, China) was used for measuring paw thickness of rats. The percentage inhibition of inflammation was calculated by the following formula:

$$\text{Percentage inhibition} = [(C-T)/C] \times 100$$

where C = control paw edema, T = test paw edema.

#### RESULTS AND DISCUSSION:

The supernatant of *Trigonella foenum graecum* on precipitation with acetone produces about 53% of mucilages. No change in color was found when the solution of mucilages was subjected to sunlight for specific time period. The Mucilage was characterized for various properties and specifications were set as Pharmacopoeial guidelines and are summarized in table 1.

Table 1: Physicochemical Characterisation of Mucilages

Sr. No.	Parameters	Specification
1.	Solubility	It is sparingly soluble in warm water but insoluble in organic solvents like chloroform, methanol, ethanol etc.
2.	Swelling index(ml)	21
3.	Loss on Drying	7.4%
4.	pH	6.9
5.	Total ash	3.1%
6.	Test for carbohydrates(Molisch's test)	+
7.	Test for proteins(Biuret test)	+
8.	Description	Powder yellow- brown color
9.	Test for mucilage (Ruthenium red test)	+
10.	Tapped density	0.71
11.	Bulk density	0.49

Five batches of gel were prepared corresponding to 4.0, 5.0, 6.5, 8.0 and 10%w/w of *Trigonella foenum graecum* mucilages, 1%w/w Diclofenac sodium, 0.02 % w/w methyl paraben as a preservative and 10% w/w glycerine as a plasticizer with the help of formula given in table 2.

Table 2: Formulation of Diclofenac gel

Ingredients	F1	F2	F3	F4	F5
F.G. Mucilage (%)	4.0	5.0	6.5	8.0	10
Diclofenac (%)	1	1	1	1	1
Glycerin (%)	10	10	10	10	10
Methyl Paraben (%)	0.2	0.2	0.2	0.2	0.2
Purified water q.s. to (g)	50	50	50	50	50

The toxicity study was performed to assure the safety of isolated mucilages. The rat animals were observed with no toxic symptoms, mortality and behavioural changes within 48 hours. The haematological properties were studied after the 10 day of continuous administration of mucilages and found to be comparable with control group as summarized in table 3.

Table 3: Hematological values

Sr. No.	Parameters	Control	Sample
1.	RBC	8.4±0.37	8.1±0.38
2.	Haemoglobin	15.98±0.69	16.49±0.83
3.	WBC	2.19±0.85	3.13±0.40
4.	Platelet	923±139	922±143

The prepared gels were evaluated for toxicity studies, haematological properties, pH, Spreadability, Consistency, Homogeneity, Skin irritation test Drug content, Physical Appearance and *in-vitro* diffusion study. The results are shown in table 4.

Table 4: Evaluation of prepared gel and marketed gel

Batch no.	pH	Spreadability (g.cm/sec)	Consistency (60 sec)	Homogeneity	Skin irritation test	Drug content (%)	Physical appearance
F3	6.7±0.03	6.0	6.0	Homogeneous	Nil	99.40	opaque
F4	6.5±0.07	6.2	7.0	homogeneous	Nil	99.60	Yellow-opaque
F5	6.4±0.05	7.0	6.0	Homogeneous	Nil	99.80	opaque
Marketed gel	6.5±0.03	6.6	8.0	Homogeneous	Nil	99.96	Yellow-opaque

The percent hind paw inhibition of ideal batch was studied and the results are shown in table

Table 5: Paw thickness and percentage inhibition in different groups at various time intervals.

Group	Treatment	Paw volume (mm)* (Percentage inhibition)				
		1 h	2 h	3 h	4 h	5 h
I	Control	-	-	-	-	-
II	Standard	0.103±0.013 (44.62%)	0.118±0.011 (68.86%)	0.098±0.09 (78.74%)	0.089±0.017 (81.26%)	0.079±0.016 (82.78%)

III	FNG	0.108±0.020 (41.93%)	0.149±0.016 (60.68%)	0.114±0.013 (75.27%)	0.101±0.09 (78.73%)	0.093±0.015 (79.73%)
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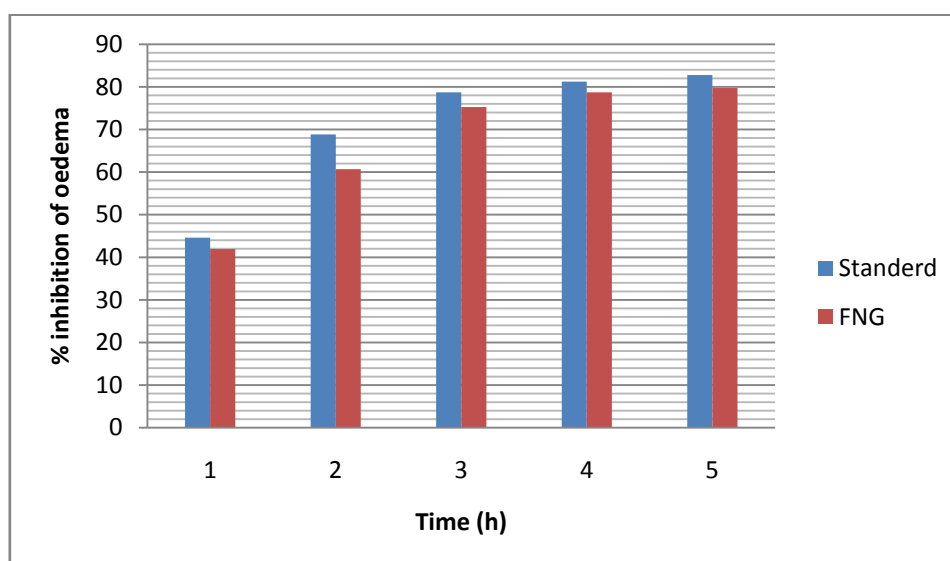


Fig. 1: Percent inhibition of hind paw oedema.

The pH values of those batches were determined. There was no significant difference in pH between pure mucilage solution and different batches of gels formulated. Among the prepared gels, the batch F4 containing 8.0% w/w mucilage had opaque colour without any characteristic odour and pH of 6.4. Therefore this was considered as ideal batch.

The gels exhibited pseudoplastic flow. The viscosity was found to be ideal for topical application. Spreadability of prepared gel was 6.2 g.cm/sec while marketed preparation having 6.6g.cm/sec indicating Spreadability of mucilage was good as compared to the marketed gel. Consistency of prepared gel (7mm) was better as compared to the marketed products (8mm). The prepared gel was clear and opaque as compared to the marketed preparation. The prepared gel showed good homogeneity without presence of lumps. No irritation was observed when prepared gel applied on healthy rat animal skin. The gels prepared with FG mucilages were easily washable because of its water solubi

### CONCLUSION:

From the present study, it can be concluded that FG seed mucilage (6-8%) may be good, biocompatible and biodegradable substitute for synthetic gelling agent for topical as well as systemic gel preparations. The anti-inflammatory activity of this gel formulation in rat hind paw edema model reveals that diclofenac was delivered to the inflammation site at a control rate over a period of 2-4 hr. Hence the mucilage 7-8% can be used for sustained drug release as in-situ gelling dosage form.

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