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Screening of aqueous extract of fenugreek seeds for its anti-inflammatory potential in albino rats

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ABSTRACT

Background: Inflammation is a common defense mechanism that involves a complicated network of cell-cell, cell-mediator, and tissue interaction. Fenugreek (*Trigonella foenum-graecum* L.) seeds are used for the treatment of many inflammatory conditions. **Aims and Objectives:** The aim of the study was to analyze the anti-inflammatory properties of aqueous extracts of fenugreek seeds in albino rats and compare it to control and standard anti-inflammatory treatments in animal models of acute and chronic inflammation. **Materials and Methods:** The methods employed to study the anti-inflammatory activity are carrageenan-induced rat paw edema and turpentine induced arthritis model in rats for acute inflammatory model and cotton wool pellet granuloma in rats for chronic inflammatory model following acquisition of the Institutional Ethics Committee approval. The control group was given 1% gum acacia, standard group was given 10 mg/kg Indomethacin dissolved in distilled water and test group was given 200 mg/kg of aqueous extract of fenugreek seeds suspended in 1% gum acacia orally. **Results:** The test group displayed almost equal increase in paw volume edema as that of the standard group under experimental conditions. In Carrageenan-induced rat paw edema model and turpentine induced arthritis model, aqueous extract of fenugreek seeds and indomethacin had comparable anti-inflammatory effect. The anti-granuloma effect of aqueous extract of fenugreek seeds was of moderate degree compared to the standard under the present experimental conditions. **Conclusion:** When compared to standard indomethacin, the aqueous extract of fenugreek seeds demonstrated significant anti-inflammatory activity in acute models of inflammation and moderate activity in chronic models of inflammation.

Key words: Anti-inflammatory; Fenugreek seeds; Indomethacin

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INTRODUCTION

Fenugreek is a member of the Fabaceae family and was given the name *Trigonella*, which is Latin for "small triangle," because of its triangular and yellowish-white blooms.[1] Fenugreek (*Trigonella foenum-graecum* L.) dates back to 4000 BC and is one of the ancient medicinal plants from the family, Fabaceae that originated in the Central Asia.[2] Its description and advantages were mentioned in the Ebers Papyrus, one of the first preserved medical documents, in Egypt in 1500 BC.[3] Commercial cultivation of fenugreek is being done in Argentina, France, Spain, Turkey, Morocco, North Africa, Pakistan, Afghanistan, Iran, Nepal, and India.[1,2]

Inflammation is a common defense mechanism that involves a complicated network of cell-cell, cell-mediator, and tissue interaction. Celsus initially described the characteristics of inflammation; Galen and Hunter later contributed to this list that includes Rubor (Redness), Tumor (Swelling), Calor (Heat), Dolor (Pain), and Functio laesa (loss of function).[4] Most inflammatory diseases are characterized by exudation of fluid and abnormal accumulation of various inflammatory cells which are regulated by inflammatory mediators such as growth

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factors, histamine, leukotrienes, prostaglandins, reactive oxygen species, bradykinin, cytokines, and lysosomal contents of neutrophils.[5] A compound that decreases one or more aspects of the inflammatory process is known as an anti-inflammatory agent. Given the complexity of the processes involved in inflammation, the medications used will differ in their structure, mode of action, and level of efficacy.

Non-steroidal anti-inflammatory drugs (NSAIDs) act through the suppression of cyclooxygenase (COX) enzyme and correspondingly the synthesis of prostaglandins. Glucocorticoids inhibit the Phosholipase-A2 enzyme by which they inhibit both COX and lipo-oxygenase pathway and correspondingly the synthesis of prostaglandins and leukotrienes, and hence serve as powerful anti-inflammatory agents. Disease-modifying antirheumatic drugs are used specifically against a particular inflammatory disease and not widely used in the therapy of inflammation such as gold salts, leflunomide, chloroquine, anakinra, and IL-1 receptor antagonist for rheumatoid arthritis.[6,7]

Anti-inflammatory drugs that are now on the market have drawbacks and undesirable side effects. According to estimates, between 34 and 46% of NSAID users will experience some gastrointestinal harm as a result of the gastric mucosa's protective COX enzyme being inhibited.[8] Despite being stomach-friendly, recently created selective COX-2 inhibitors have a prothrombotic tendency that could result in MI and mortality. Continuous administration of glucocorticoids causes a variety of adverse effects. New class of drugs in pipeline for the management of inflammation and pain is COX-3 antagonists, CGRP antagonists, N-acetylcholine receptor antagonists, Vallinoid 7, and neurokinin receptor antagonists. Fenugreek seeds' alcoholic extract is shown to have anti-inflammatory properties.[9]

Aims and objectives

The aim of the study is to analyze the anti-inflammatory properties of aqueous extracts of fenugreek seeds in albino rats and compare it to control and standard anti-inflammatory treatments in animal models of acute and chronic inflammation.

MATERIALS AND METHODS

Preparation of fenugreek seed extract

Fenugreek seeds identified as *T. foenum-graecum* were collected from Mysore market and dried under shade. After optimum drying, it was coarsely powdered and stored until future usage, in an airtight container. The aqueous extract of fenugreek seeds was obtained using cold maceration method by extracting the coarsely ground seeds with water, and water was then used to extract the marc. After four iterations of the procedure, the filtrates were mixed, distilled, and evaporated. Indomethacin, belonging to a known and established anti-inflammatory group of NSAIDS, was taken as a standard drug and compared with the results obtained using aqueous extract of fenugreek seeds.

Methods to study anti-inflammatory activity

Adult healthy albino rats of Wistar strain of similar characteristics of either sex, non-pregnant weighing 150–250 g were selected from the central animal facility of the institution after acquiring approval from the Institutional Animal Ethical Committee, J.S.S Medical College, Mysore.

The methods employed to study the anti-inflammatory activity are carrageenan induced rat paw edema and turpentine induced arthritis model in rats for acute inflammatory model and cotton wool pellet granuloma in rats for chronic inflammatory model.

Albino rats of similar characteristics were grouped into three sets of six each. The first group was used as control, the second group as standard and third as test group. The control group was given 1% gum acacia, the standard group received a dose of 10 mg/kg Indomethacin dissolved in distilled water and the test group received 200 mg/kg of aqueous extract of fenugreek seeds suspended in 1% gum acacia orally.

Acute inflammatory models

Carrageenan-induced rat paw edema

Oral administration of all the drugs was done 1 h before carrageenin injection. The animals were not allowed either food or water throughout the period of experimentation. At the same level each time, a convenient anatomical landmark was noted. An hour after gum acacia was administered to the test groups, a subplantar injection of 0.05 ml 1% carrageenin was made into the right hind paw of rats in each group using a tuberculin syringe (Figure 1).[10] The volume

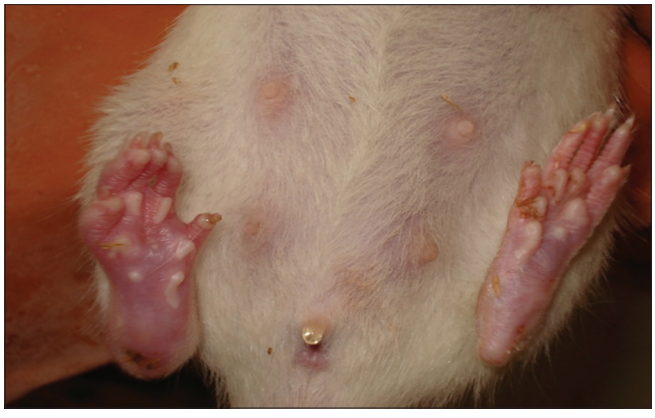

Figure 1: Carrageenan-induced rat paw edema model

of the right hind paw was measured immediately using the Mercury plethysmograph (0 h- volume) and at the end of 4 h indicating the actual edema. On the angle of the rat's ankle joint, a fixed point was marked with ink. The limb was then carefully placed inside the mercury container. The vessel's mercury level mark and the limb's mark were made parallel to one another. The extra mercury quickly passed and settled in the linked capillary where the mercury level was measured. The mercury level mark on the glass vessel, the actual level of mercury in the vessel, and the ink mark on the animal limb accurately fell on the same line when the reading was taken. The mercury capillary level indicates the amount of mercury that has been displaced by the animal's paw indicating the paw volume.[11]

The average paw edema in animals treated with drugs was compared with that of the untreated control group on a group-by-group basis.

$$\text{Percentage of edema inhibition} = 100(1 - Vt/Vc)$$

Where, Vc=Average volume of paw edema in the control group.
Vt=Average volume of paw edema in the drug treated group.

Turpentine induced arthritis model in rats

Oral administration of all the drugs was done 1 h before injecting 0.01 ml of turpentine oil into to the right knee joint's synovial cavity to induce joint inflammation. Screw gauge was used to measure the knee joint lateral diameter immediately (0-h reading) and at 6 h after injecting the turpentine oil into the knee joint cavity (Figure 2).[12] The pitch, least count, and zero error of the instrument were noted. The screw head was rotated till the gap was sufficient to hold the knee joint. The knee joint was gently placed in the gap between the screw tip and the plug; the screw was slowly turned till the knee joint was held in position, firmly but gently. The pitch scale reading PSR and the head scale reading HSR were noted.

The total reading was calculated using the formula,

$$TR = PSR + (HSR \pm Z)\ LC.$$

Where, TR=Total reading, PSR=Pitch scale reading, HSR=Head scale reading, Z=Zero error, LC=Least count.

The average increase in the knee joint diameter of control group and drug treated groups was compared.

Percent anti-arthritic activity=100 (1-Dt/Dc).

Where Dc=Average increase in lateral diameter of knee joint in the control group and Dt=Average increase in lateral diameter of knee joint in the drug treated group

Chronic inflammatory model

Cotton wool pellet granuloma in rats

On the 1st day, all medications were administered orally 1 h before the cotton pellet implantation, and then once every day for 6 days straight after that. Under aseptic conditions, small linear incisions of 1 cm were made in each axilla and groin of the rats that were under mild ether anesthesia. Four pellets of sterile cotton wool that weighed 10 mg each were injected subcutaneously in each of these locations to cause a foreign body granuloma. Black silk thread was used to suture the wounds while using aseptic measures (Figure 3). Throughout the duration of the trial, food and water were provided to the animals in clean cages. The animals were later sacrificed on the 8th day and the cotton pellets with granulation tissue were taken out, thoroughly cleaned, and dried in a hot air oven

Figure 2: Turpentine induced arthritis model

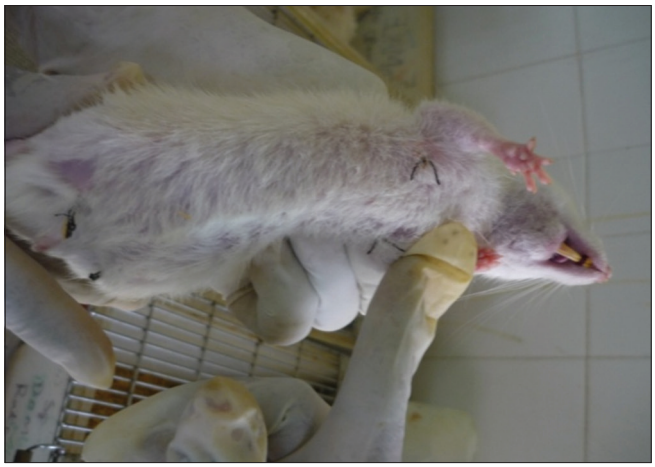

Figure 3: Cotton wool pellet granuloma model

to a specific weight.[13] The variation in cotton pellet dry weight between measurements taken before and after implantation was used to compute the volume of actual granulation tissue generated.

Percentage of anti-granuloma activity=100(1-Wt/Wc).

Where, Wt=Average dry weight of granuloma in the drug treated group
Wc=Average dry weight of granuloma in the control group.

All the statistical methods were done using SPSS for Windows (version 21.0).

RESULTS

Carrageenan-induced rat paw edema method

In our study, mean rat paw volume of control group was found to be 0.66 mm at 0 h and 8.39 mm at 4 h and showed a maximal increase in mean paw volume edema of 7.73 mm indicating maximum inflammation. Standard group treated with indomethacin showed a mean paw volume of 1.3 mm at 0 h and 5.13 mm at 4 h, with an inflammatory edema of 3.83 cm, indicating good anti-inflammatory activity. The test group treated with fenugreek seeds showed a mean paw volume of 2.28 mm at 0 h and 7.14 mm at 4 h with an inflammatory edema of 4.86 mm. This indicated that the test group displayed almost equal increase in paw volume edema as that of the standard group under experimental conditions. A significant change in rat paw volume from 0 h to 4 h was observed (P<0.001) (Table 1).

Mean percentage inhibition of paw edema in Carrageenan-induced rat paw edema model in different groups for standard and test drug was 51% and 37%, respectively, when the percentage inhibition of the control was considered as 0% (Table 2).

Turpentine induced arthritis model

The mean lateral knee diameter measured in mm using screw gauge in turpentine-induced arthritis model of control group was 3.33 mm at 0 h and 8.05 mm at 4 h with a mean increase in knee-joint diameter of 4.71 mm, displaying maximum increase in knee diameter; standard group treated with indomethacin showed a knee diameter of 3.5 mm at 0 h and 5.4 mm at 4 h and displayed a minimum increase in knee diameter with a mean increase in knee joint diameter of 1.9 mm. The test group treated with aqueous extract of fenugreek seeds showed a knee diameter of 3.38 mm at 0 h and 6.24 mm at 4 h with a mean increase in knee joint diameter of 2.86 mm. This shows that the test medication had anti-inflammatory effects similar to those of indomethacin (Table 3).

Mean percentage inhibition of knee arthritis in turpentine-induced arthritis model in different groups for standard and test drug were 59.7% and 39.3%, respectively, when the percentage inhibition of the control was considered as 0% (Table 4).

Cotton wool pellet induced granuloma model

Cotton pellet-induced granuloma shows that control group showed a maximum dry granuloma weight of 90.33 mg indicating maximal granuloma formation and inflammation. Standard group showed a dry granuloma weight of 46 mg indicating minimal granuloma formation and maximum anti-granuloma activity. Test groups treated with fenugreek seed aqueous extract showed the granuloma weight of 65 mg (Table 5).

DISCUSSION

Inflammation is a crucial component of the body's defense system. Exudation of plasma, vasodilatation,

Table 1: Mean rat paw volume (mm) at 0 h and 4 h in different drug groups

Time	N	Group	Mean	SD
0 h	6	Ctrl	0.66	0.16
	6	Std	1.3	0.28
	6	Fenugreek	2.28	0.029
4 h	6	Ctrl	8.41	0.45
	6	Std	5.13	1.03
	6	Fenugreek	7.14	0.35

F=44.48, P=0.0001

Table 2: Mean difference in rat paw volume (mm) in different drug groups

Groups	N	Mean	SD	%
Ctrl	6	7.73*	0.58	0
Std	6	3.83*	1.08	51
Test	6	4.86*	0.37	37

*Indicates P<0.05 (Significant), t test=12.847, P=0.0001

Table 3: Mean lateral knee diameter (mm) at 0 h and 4 h in different groups

Time	N	Group	Mean	SD
0 h	6	Ctrl	3.33	0.51
	6	Std	3.5	0.54
	6	Fenugreek	3.38	0.08
4 h	6	ctrl	8.05	0.23
	6	std	5.4	0.35
	6	Fenugreek	6.24	0.20

F=65.802; P=0.001

Table 4: Mean difference in lateral knee diameter (mm) measured at 0 h and 4 h in different drug groups

Groups	N	Mean	SD	%
Control	6	4.71	0.60*	0
Std	6	1.9	0.39*	59.7
Fenugreek	6	2.86	0.10*	39.3

*Indicates P<0.05 (Significant), t test=5.312, P=0.001

Table 5: Average dry granulation tissue weight (mg) across different drug groups

Groups	N	Mean	SD
Ctrl	6	90.33*	2.94
Std	6	46.33*	3.44
Fenugreek	6	65*	0.89

*Indicates P<0.05 (Significant), F=411.5; P=0.001; T=12.847

release of several inflammatory mediators, growth factors, cytokines, and leukocyte emigration are all indications of acute inflammation. Fibroblast proliferation, mononuclear cell infiltration, enhanced connective tissue development, and blood vessel growth are all characteristics of chronic inflammation. Inflammatory response is modeled after tissue infection. Different anti-inflammatory medications block inflammation at various stages.

Fenugreek seeds are used as an anti-inflammatory agent externally for local inflammations such as ulcers, eczema, and internally for upper respiratory tract infections.[14] Accordingly in light of the aforementioned information, the anti-inflammatory potential of fenugreek seeds' aqueous extract was studied and it was compared to the standard reference drug indomethacin.

When compared to the control, indomethacin inhibited carrageenan-induced rat paw edema by 51%, while the test drug only inhibited it by 37%. The test group's percentage of paw edema inhibition, calculated using the standard as 100%, was 78.80%. Thus, in a model of rat paw edema caused by carrageenin, aqueous extract of fenugreek seeds and indomethacin had comparable anti-inflammatory effect.

The standard group and test group showed reduced risk of knee arthritis by 59.7% and 39.3%, respectively, in turpentine induced arthritis model. Therefore, as compared to the conventional drug, fenugreek seeds showed good anti-arthritic effect. The percentage of knee arthritis inhibition in the test group was 70.76% when the percentage inhibition of the standard group was taken to be 100%. Thus, in a model of arthritis caused by turpentine, aqueous fenugreek seed extract had good anti-inflammatory effect comparable to that of the widely used drug indomethacin.

In comparison to the control group, indomethacin inhibited dry granulation tissue weight by 49%, while the test group only had a 28% reduction. Fenugreek seeds have a somewhat strong anti-granuloma activity compared to the standard drug. The test group's percentage inhibition of dry granuloma weight, calculated with the standard's percentage inhibition as 100%, was 70.76%. Thus, in a cotton wool pellet induced granuloma model, aqueous extract of fenugreek seeds demonstrated mild anti-inflammatory activity when compared to that of indomethacin.

In arthritis model induced by turpentine under the current experimental conditions, change in the rat knee joint diameter from 0 h to 6 h was significant (P<0.001).

The anti-granuloma effect of aqueous extract of fenugreek seeds was of moderate degree compared to the standard under the present experimental conditions. Overall P value was found to be <0.05, showing a significant difference between the study groups with regards to the variable (mean dry granuloma weight) taken into consideration.

The anti-inflammatory action of indomethacin was strong in the turpentine-induced arthritis model, but slightly weaker in the paw edema and arthritis models caused by carrageenan. The anti-inflammatory effects of fenugreek seeds were good and nearly identical in models of carrageenan and turpentine-induced paw edema and granuloma, respectively.

In the current investigation, aqueous extract of fenugreek seeds demonstrated remarkable benefits in both experimental chronic and acute models of inflammation when compared to indomethacin.

The plausible mechanism of the acute anti-inflammatory effect of fenugreek seeds could be because of the inhibition of synthesis of prostaglandins, decrease in myeloperoxidases, cyclooxygenases, and other inflammatory mediators. The chronic anti-inflammatory impact may result from downregulation of JNK, CK2, NF-Kappa b and inhibition of inducible nitric oxide (NO) synthase (iNos) protein, NO, collagen synthesis, mucopolysaccharides, and fibroblast proliferation during the granuloma formation.[15]

Our research is in accordance with the study by Sindhu et al.,[16] wherein fenugreek mucilages showed highest percentage of edema inhibition at a dosage of 75 mg/kg on the 21st day of adjuvant arthritis with decreased activities of inflammatory enzymes myeloperoxidases, cyclooxygenases. Paw tissue histopathology revealed reduced edema development and cellular fenugreek mucilage infiltration.

Our results were in par with that of a study by Debranjan and Tara[9] which emphasized the anti-inflammatory activity of fenugreek seeds concluding that fenugreek seeds' alcoholic extract demonstrated anti-inflammatory effects on rat paw edema caused by carrageenan at a dosage of 100 mg/kg of body weight.

A study by Jung et al.,[15] observed that pre-treatment with diosgenin led to a concentration-dependent suppression of iNos protein, NO, and mRNA expression. It decreases LPS and INF gamma-induced NF- Kappa b and AP-1 activity and inflammatory mediators are inhibited at the transcriptional level. Another study by Kawabata et al.,[17] found that steroidal saponins glycopyranoside, minutoside B, and pseudoprotodioscin-3 suppress the production of inflammatory cytokines.

Limitations of the study

There are no limitations to this study.

CONCLUSION

When compared to standard indomethacin, the aqueous extract of fenugreek seeds demonstrated significant anti-inflammatory activity in acute models of inflammation and moderate activity in chronic models of inflammation. Hence, aqueous extract of fenugreek seeds can be used alone or in combination with other conventional anti-inflammatory drugs including NSAIDs, steroids, LT receptor antagonists, Mast cell stabilizers, cytotoxic drugs, and cytokine modulator during the period of administration to treat inflammation. Fenugreek's capacity to stop the generation of pro-inflammatory mediators such as prostaglandins, histamine and serotonin, cyclooxygenase, myeloperoxidases, antioxidant effects inhibition of inducible iNos protein, and NO accounts for its anti-inflammatory properties.

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Authors Contribution:
BM- Concept and design of the study, review of literature, acquisition of data, original draft preparation, preparation of manuscript, statistical analysis and interpretation of results, and revision of manuscript.

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