

STUDY OF FENUGREEK EXTRACT ON THE OEDEMA

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ABSTRACT

Fenugreek *Trigonella foenum-graecum* Linn is a spice and medicinal plant that is native to areas around the eastern Mediterranean and central Asia. In centuries past it was grown in India and South Africa. Its leaves, pods, and seed have several medicinal benefits and it is commonly used in unani medicine for its anti-inflammatory and immunodulatory activity. The various pharmacological activities of fenugreek include anti-inflammatory activity. The various pharmacological activities of fenugreek include antimicrobial, analgesic, antioxidant, antiulcer and anti-diabetics properties while it is used in treating arthritis, diabetes, cough and metritis. To assess anti-inflammatory activity of ethanolic extract of dried leaves of *Trigonella foenum-graecum* Linn. in male wistar rats. All of the studies used adult male wistar (250-500 mg/kg) body weight in normal rats. There are alkaloids flavonoids vitamin K and C, Calcium iron and nicotinic acid in fenugreek leaves. The aims of the present studies was to explore the anti-inflammatory role of fenugreek. The extraction of dried fenugreek leaves with 70% ethanol by maceration method. In acute studies the methanolic extract exhibited anti-inflammatory activity by significantly reducing paw oedema volume in a dose dependent manner when compared with the control standard drug. *Trigonella foenum-graecum* Linn. Ethanolic extract markedly and dose dependently reduced frequent number of writhing induced by acetic acid and significantly increased paw licking latency in hot plate method. Statistical analysis was performed using one way ANOVA and subsequently Tukey's test. Ethanolic extract of *Trigonella foenum-graecum* Linn. Possess anti-inflammatory activity in dose dependent manner.

KEYWORDS: Fenugreek, anti-inflammatory, extract, ethanolic, pharmacological activities.

INTRODUCTION

The general definition of inflammation is the local response of living mammalian tissue to injury by an agent. The body's defense mechanism eradicate or contain the damaging agent. Inflammation

typically arises when infectious microorganism – fungi, bacteria and viruses – invade the body, nest in certain tissues, and/or spread through the bloodstream.

Inflammation can also occur in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration. Both the innate and adaptive immune responses are involved in the formation of inflammation. The innate immune system is the primary defense mechanism against invading microorganism and cancer cells. The adaptive immune system includes the activity of more essential cells, such as B and T cells, which are responsible for eliminating invading pathogens and cancer cell by producing specific receptors and antibodies (K. Lai). 2011). Inflammation is a protective mechanism and provides a safe response of vascular tissue to stimuli, such as pathogens, damaged cells, allergens, irritants. It is characterized by redness, swelling, pain and warmth in the affected area (Ghosh 1984). Mechanism converge in inflammation, such as the release of histamine, bradykinin and prostaglandin. The enzyme clooxygenase 2 may be responsible for elevated PG levels in many inflammatory conditions. The selective COX-2 inhibitor is a form nonsteroidal anti-inflammatory drugs NSAIDs) that directly target COX-2 (Bhrahmbatt 2010).

Causes of inflammation

There are numerous causes of inflammation some of which include

Infective agents- like bacteria, viruses, and their toxins, fungi, parasites.

Immunological agents – like cell mediated and antigen anti-body reaction.

Physical medium – like heat, cold, radiation, mechanical trauma.

Chemical medium – like organic and inorganic toxic.

Inter material - such as foreign bodies

External injuries like scapes or damages through foreign object (for example a thron in your finger)

Diseases that cause inflammation typically have names ending “-itis” .examples includes cystitis (bladder inflammation), bronchitis (bronchi inflammation), otitis media (middle ear inflammation), and dermatitis (skin inflammation). (Garcia- Pastor P, Randazzo A.1999)

Inflammation result from immune system reaction to physical injuries and does not always includes cystitis (bladder inflammation), bronchitis (bronchi inflammation), otitis media (middle ear inflammation) and dermatitis (skin inflammation).

Inflammation results from immune system reaction to physical injuries and does not always indicate an inflammation.

Three key processes occur during acute inflammation.

Arteries enlarge to increase blood flow to the damaged area.

Capillaries become more permeable, allowing liquid and proteins to more move between blood and cells.

Neutrophils, a type of white blood cell contain enzymes to digest microorganisms, are released by the body.

Types of inflammation

Mainly are of two types

Acute inflammation

Acute inflammation is characterized by a short duration and involve a range of physiological reactions, including the collection of fluid and plasma at the affected site, intravascular activation of platelets and the presence of polymorph nuclear neutrophils as the primary inflammatory cells. Conditions that can lead to acute inflammation include acute bronchitis, infected ingrown toenails, laryngeal irritation from colds, skin scratches or cuts, high-intensity exercise, acute appendicitis, dermatitis, tonsillitis, meningitis, sinusitis, and physical trauma. (Ghosh MN, 1984).

Chronic inflammation

Chronic inflammation is characterized by a prolong duration and the persistence of the causative agent from acute inflammation. It may last from week to years, involving macrophages, lymphocytes and plasma cells, leading to fibrosis or scarring. Condition that predispose individuals to chronic inflammations include asthma, chronic peptic ulcers, tuberculosis, rheumatoid arthritis, periodontitis, ulcerative colitis, crohn's disease, sinusitis and active hepatitis.(Pandian SR et al ., 2002).

Figure 1.1 Events obseened during the process of inflammation

Symptoms

Inflammation can be acute or chronic, each presenting different symptoms, acute inflammation is characterized by the acronym perish soreness. Redness, immobile, swollen, and hot, indicating pain, loss function, fluid buildup, and increased warmth in the affected area. These signs primarily pertain to skin inflammation. In contrast, chronic inflammation may cause fatigue, mouth sores, chest and abdominal pain, fever, rash and joint pain, reflecting a broader range of systemic effects.

Figure 1.2 The response to inflammatory stimuli

Treatments

The various strategies used for treating inflammation are –

Anti-inflammatory medication: NSAIDs are effective in reducing pain from inflammation by counteracting an enzymes involved in the inflammatory process. Common include naproxen, ibuprofen, and aspirin. However, long term use can lead to serious side effects, such as stomach ulcers, kidney damage and an increased risk of stroke or heart attack. (Gyurkovaska, V.et al., 2011)

NSAIDs should not be used long term unless prescribed by a doctor, as they may worsen asthma symptoms, lead to kindly damage and increase the risk of stroke or heart failure. Acetaminophen is effective for pain relief without affecting inflammation, making it suitable for those who wish to manage pain while allowing the natural healing process of inflammation to occur(Mehrafarin A A, Rezazadeh SH et al ., 2011).

Corticosteroid: corticosteroid such as cortisol are a class of steroid hormones which prevents by number of mechanisms involved in inflammation. Corticosteroid have two sets glucocorticoids, mineralocorticoids (Garcia- Pastor Pet al., 1999).

Glucocorticoids: these are prescribed for a range of condition, including

Temporal arteritis

Dermatitis

Inflammatory bowel disease(ibs)

Systemic lupus

Hepatitis

Asthma

Allergies reaction

Cancer: Chronic inflammation cells, especially macrophages and t-lymphocytes could also be the morphological expression of associates response to malignant cell therapy might suppress traditional inflammatory responses, diseases tighter.(Pedraza –Alva,G et al ., 2005) (Na, Y.R.; Je, S.; Seok, S.H., 2018).

Immune factor: Several response disease tighter with rheumatoid arthritis, thyroiditis characterized by chronic inflammation. (Straub, R.H.; Schradin, C.,2016)

Plant Introduction

Fenugreek is an annual herbaceous and aromatic plant from the leguminosae family. Its edible leaves are commonly used as a vegetable throughout India. The seeds possess several health benefits, including anti-diabetics, anti-fertility, anti- cancer, anti-microbial, anti-parasitic properties, as well as functions as a lactation stimulant and hypo cholesterolaemic agent (Murlidhar,M. and Goswami, T. K 2012). In Ayurveda, both fenugreek seeds and leaves are utilized to create medicinal extracts or powders (Basch et al., 2003). According to a WHO study, over 80% of the global population relies on herbal medicines for healthcare (Shanmugasundaram, 2005). Excessive production of reactive oxygen species, such as hydrogen peroxide and superoxide anion radical, can lead to oxidative stress (Hafeez B et al., 2003).

History Of Plants – plant cultivation originated in the near east, with evidence of domestication linked to the trigonella genus. Charred seeds dated to 4000 BC have been found at Tell Halal, Iraq and Bronze Age sites like Lachish and Tutankhamen’s tomb. Romans utilized fenugreek in wine flavouring and its significant as a food staple is noted by Josephus in “The Wars of The Jews”(Basch.E,2003)

FG 1.3 TRIGONELLA FOENUM GRAECUM WITH LEAVES AND SEEDS

Habitat – annual herbs are native to the eastern Mediterranean and Central Asia and have been cultivated for seasoning and as pot herbs in India and North Africa for centuries (Wealth of India,

1976; CSIR,1986). They are also used as bait and for soil improvement in various regions, including the Southern U.S., California and Egypt (Wealth of India, 1976; Bentley and Trimen,1990). In ancient Greece and Italy, these herbs were primarily used for medicinal purposes (Wealth of India,1976). In India, they are grown in several states including Punjab, Assam, and Tamil Nadu (Wealth of India ,1976; Kirtikar,1987;Sala,1997)

Discription

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : Trigonella botanical

Name : Trigonella foenum graecum

Morphology – It is an annual herb with 1-2 feet height .It is an erect, slightly branched, cylindrical, hollow smooth stem, root (Sala, 1997).1/4th inch long triangular-acuminate leaflets shortly stalked blunt at the apex. (Bentley and Trimen, 1990).

Chemical constituents – Fenugreek leave contain seven saponins known as graecunin's. These compounds are glycosides of diosgenin leave contain moisture 86.1%, protein 4.4%, fats 0.9%, minerals 1.5%, fibre 1.1%, carbohydrates6%. The mineral and vitamin content are calcium, iron, phosphorous, carotene, Vicenin-I, (Wagner et al.,) hiamine, riboflavin, niacin and vitamin C. .(RAO, 2003). (Shang et al., 2000) found fresh leaves contain ascorbic acid about 220.97 mg per 100g of leaves and β -carotene is present about 19mg/100g. (Yoshikawa M, Murakami T, Komatsu H,et 1997)

Fig 1.4 Structure of Trigoneoside Ib

Fig 1.5 Structure of Vicenin-I

FIGURE 1.6- Structure of some major constituent of Trigonella foenum-Graecum

Quercetin

Luteolin

Kaempferol

Naringenin

Research

Fenugreek contains various phytochemicals, including alkaloids, flavonoids, saponins, amino acids, tannins, and steroidal glycosides, which serve as natural bioactive compounds that protect plants from stress and pathogens. (Anand S C, Nagranju B et al.,2012) these compounds can be utilized individually or in combination for drug development. (rashmi et al., 2011) research indicates that fenugreek exhibits numerous health benefits, such as antimicrobial, wound healing, antifungal, anti-diarrheal, hypoglycaemic, hepatoprotective, and antioxidant activities. (McCarthy TL, Kerry JP et al .,

2001).The current study aims to explore the medicinal benefits of fenugreek leaves for inflammation(Ahmadiani A, Javan M, Semnanian S,et al,2001). Using carrageenan as an inflammatory agent, the anti-inflammatory effects of trigonella foenum graecum (200 mg/kg) were measured plethysmographically and compared to diclofenac sodium as a reference standard. Results showed a significant difference compared to control at the 2nd, 3rd, and 4th hours ($p<0.05$), concluding that fenugreek leaves possess significant anti-inflammatory properties.

Extraction procedure the fenugreek leaves were first dried in the shade and ground into a powder. A 100 gm portion of this powder was defatted with petroleum ether for 24 hours, then dried. The defatted powder was subsequently extracted with ethanol for 48 hours. The solution was filtered and evaporated at 40°C until a viscous liquid remained, which was stored at 4°C for future use.(Arya V ., 2012)

Preliminary phytochemical screening the preliminary phytochemical screening of total ethanolic extract of dried leaves trigonella foenum-graecum were subjected to chemical test for identification of various plant constituents .the ethanolic extact of dried leaves trigonella foenum graecum showed positive results for alkaloids ,proteins ,terpenoid ,flavonoid, tannin, and carbohydrate as in tables.(Harbone JB .,1998)

Test for alkaloids - mayer's test, dragenddorff's test, wagner's test,

Test for carbohydrates - molisch's test

Test for steroids - liebermann's burchard test , salkowski test:

Test for saponins

Test for proteins- millon's test, biuret test, ninhydrin test

Test for terpenoid

Test for flavonoids

Test for tannins

Table 1 -preliminary phytochemical screenining of ethanolic extract of trigonella foenum – graecum

(+) = present. And (-) = absent

In vivo activity

MATERIAL USED

The ethanolic extract of fenugreek leaves was used in this work. Polypropylene ether, pethidine, were ibuprofen purchased from lupin limited, mumbai, was obtained from usv limited, mumbai, acetic acid was purchased from centre drug house pvt. Ltd, new delhi, carrageenan purchased from cdh pvt. Ltd . New delhi. All chemicals used in the studies.

Animals

Wistar rats (250 g-500g) of either sex were used the animal were housed in polypropylene cage prior of pharmacological studies with free access to standard diet and water ad libitum for 2 a week on a

12/12 h light/dark cycle. All animal were fasted overnight before test, tap water was supplied ad libitum. The control temperature was 25-30 °C. All the studies were approved by institutional animal ethics committees.

Selection of dose

Fenugreek leaves (500g) was extracted with 500ml of petroleum ether at the room temperature for 24 hours. Then the leaves was filter and dried then dipped in ethanol (90%) in the soxhlet apparatus at 40°C. And finally 4g of extract was receive. The extract was freeze-dried and store in a vacuum desiccator.

The Hot Plate test

The test followed a procedure based on likely et al. (1950), measuring animals' reactions to thermal heat. Animals displaying a fore paw licking or jumping response within 68 seconds were selected for the study. Thirty minutes post-administration of test and reference compounds, the animals were exposed to a hot plate at 55±1°C for a maximum of 60 seconds to prevent paw damage. Reaction time, defined as the latency until the animal licked its paw, jumped, or lifted a hind paw, was recorded at 30-minute intervals up to 120 minutes.

The Tail Flick test

The test involved administering fenugreek extract (250-500 mg/kg i.p.) and piroxicam i.p. to rats, followed by placing their tails on a hot wire analgesiometer. The time taken for the rats to flick their tails was measured, with a cut-off period of 15 seconds to prevent tissue damage. The results compared the tail flick times of saline-treated control rats with those treated with fenugreek extract and the standard.

Acetic acid –induced writhing response

The study investigates the effects of acetic acid-induced writhing responses in mice, characterized by abdominal muscle contractions and hind limb stretching. Mice were administered the ethanolic extract of fenugreek (250-500 mg/kg) and indomethacin (10 mg/kg) via intraperitoneal injection 15 minutes before the acetic acid injection. The number of abdominal constrictions was recorded for 20 minutes post-injection, comparing saline-treated controls to those receiving the fenugreek extract.

Carrageenan induced paw oedema

The study investigated the anti-inflammatory effects of fenugreek ethanolic extract using a carrageenan-induced rat hind paw oedema model. Male wistar rats (100-200g) were fasted overnight and divided into four groups of six. Group I served as a control, group ii received a standard treatment with piroxicam (10 mg/kg), and group's iii and iv were given oral fenugreek extract at doses of 250

mg/kg and 500 mg/kg, respectively, one hour before carrageenan injection. The oedema was induced with 0.1ml of 1% carrageenan in the left hind paw, and the paw perimeter was measured at 1, 2, and 3 hours post-injection to assess the treatment effects.

Table 2.1: Effect of *Trigonella foenum-gracecum* ethanolic extract on hot plate activity in wistar rats.

Figure 2.1: Effect of fenugreek extract on hot plate activity in wister rats. Results are given as mean $\pm$ SEM of six animals in each group. Control group compared with rest of the treated groups. Significance at *P < 0.05; **P < 0.01; ***P < 0.001

Table 2.2 Effect of *Trigonella foenum-gracecum* ethanolic extract on Tail flick activity in rats

FIGURE 2.2 Effect if fenugreek extract on tail fick in wister rats. Result are given as mean $\pm$ SEM of six animal in each group . Control group compared with rest of the trated groups. Significance at *p < 0.05; **p < 0.01; ***p < 0.00 .

Table 2.3: Effect of *Trigonella foenum-gracecum* ethanolic extract on acetic acid induced writhing in wistar rats.

Figure 2.3: Effect of fenugreek extract on acetic acid induced writhing in wister rats. Results are given as mean $\pm$ SEM of six animals in each group. Control group compared with rest of the treated groups. Significance at *P < 0.05; **P<0.01; ***P < 0.001.

Table 2.4: Effect of *Trigonella foenum-gracecum* ethanolic extract on carrageen induced Paw oedema in rats

Figure 2.4 Effect of fenugreek extract on carrageenan induced Paw Oedema in wister rat . Result are given mean $\pm$ SEM of six animal in each group. Control group compared with reat of the treated groups. Significance at *P<0.05;**P<0.01; ***P< 0.001.

Statistical analysis the results are expressed as mean values and standard error mean (sem) or standard deviation(sd).all the activities were analysed using one –way analysis of variance (anova) (graph pad in stat 3) software and all the result obtained in the study were compared with the control group

RESULTS

1) Hot plate test the result of the hotplate test [figure4.1] professed that the reaction time for the rats was appreciably enhanced from the dose of 250 mg/kg and above. This was somewhat dose retainer. Pethidine at a dose of 4 mg/kg significantly increased the pain latency.

2) Tail flick test the ethanolic extract of fenugreek demonstrated a dose-dependent inhibition of tail flick response in mice compared to the control group. A mild response was noted at 250 mg/kg, with a significantly greater effect at 500 mg/kg (p > 0.05). The standard diclofenac at 10 mg/kg also showed maximal response against the tail flicking time in mice.

3) Acetic acid-induced writhing response ethanolic extract of fenugreek elicited a dose dependent of inhibition of constrictions compared with control group. Ethanolic extract of fenugreek produced 23.18% inhibition at 250mg\kg dose and the maximum inhibition 40.57% i.p.

4) Carageenan induced paw oedema piroxicam is used as standard reference drug as it is reported to inhibit inflammation by its effect upon exudation associated with carrageenan mediated inflammation. Ethanolic extract of fenugreek showed a maximum oedema inhibition at 3h at the dose of 250mg/kg and the effect tested for 3h for fenugreek.

Discussions

The study evaluated the anti-inflammatory effects of ethanolic extract of fenugreek using various methods, including acetic acid-induced writhing, tail flick, and hot plate tests. In the hot plate test, the extract demonstrated a dose-dependent increase in latency time, indicating a reduction in thermal stimulation. (stai et al., 1995) The tail flick test confirmed similar results, with increased latency times for both fenugreek extract and the standard drug, idomethacin. Acetic acid administration produced pain, which was significantly reduced by the extract in a dose-dependent manner, with fenugreek showing a notable impact compared to idomethacin. (Srinivasan K et al., 2003). The results also indicated that fenugreek reduced carrageenan-induced paw edema, likely by inhibiting the synthesis of inflammatory mediators like histamine and prostaglandins. Phytochemical analysis revealed the presence of various compounds, including alkaloids and flavonoids, in the extract.

SUMMARY & CONCLUSION

Inflammation, originated from the latin "inflammare," refers to a complex host response in vertebrates characterized by heat, redness, and swelling. Historically, it was described by roman doctor cornelius celsus, who identified key signs of inflammation. Fenugreek, or *trigonella foenum-graecum*, is a traditional remedy noted for its medicinal properties, particularly in addressing metabolic syndrome. The who's focus on diabetes further emphasizes public health concerns related to life style disorders. The study examined the anti-inflammatory and analgesic effects of fenugreek's ethanolic extract. Preliminary phytochemical screening revealed several constituents, including alkaloids and flavonoids. Behavioral tests demonstrated that fenugreek extracts at doses of 250 mg/kg and 500 mg/kg significantly increased latency times in pain response tests and reduced acetic acid-induced writhing, indicating notable analgesic and anti-inflammatory effects, albeit milder than the reference drug piroxicam. Overall, fenugreek showed potential as a mild anti-nociceptive and anti-inflammatory agent.

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