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Evaluation of Ethnopharmacological Activity of Trigonellafoenum Graceum

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ABSTRACT

Trigonella foenum-graecum (Fenugreek) (Leguminosae) is employed as a herbal medicine. Its seeds are known for their carminative, tonic and antidiabetic effects. A curative dose of *Trigonella foenum-graecum* also produces antiulcer action[1]. In this study we have investigated the Ethnopharmacological activities of the aqueous extract of the seeds *Trigonella foenum-graecum* in normal mice using different route of administration . The methanolic extract administered through the same route produced various effect only at the dose of 1 g/kg body weight. The aqueous extract is under further investigation to determine the chemical structure of the active component. The presence of hypoglycaemic, antiflamatory, antianalgesic activity in aqueous and methanolic extract indicates that the active compounds are polar in nature. The effects of fenugreek seed extract on the alterations in serum thyroid hormone concentrations were studied in adult male mice and rats. Simultaneously, hepatic lipid peroxidation and the activities of the antioxidant enzymes, viz superoxide dismutase and catalase were examined. Present study reports analgesic activity of petroleum ether, chloroform, ethyl acetate and methanolic extracts of leaves and seeds of Trigonella foenum-graecum Linn.

Key word: Evaluation, Ethno pharmacological Activity, methi seed.

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INTRODUCTION

Fenugreek is an annual Mediterranean and Asiatic herb with aromatic seeds. It grows to two feet in height with brownish seeds contained in sickle shaped pods. It is used worldwide as a culinary spice as well as a medicinal herb to soothe the stomach and help maintain blood sugar levels. The seeds are rich in protein.Fenugreek is one of the primary supplements used to support type II diabetics or noninsulin-dependent diabetes mellitus (NIDDM). Most NIDDM patients typically have enough insulin but it is not used effectively. Research as to the cause seems to indicate high levels of body fat, too many calorie consumed from refined foods, lack of polyunsaturated fats and chromium deficiencies. Fenugreek Seed helps by not only reducing blood sugar levels with its high concentrations of phytochemicals, but it has also helped reduce low density cholesterol's and triacylglycerols. Present study reports analgesic activity of petroleum ether, chloroform, ethyl acetate and methanolic extracts of leaves and seeds of Trigonella foenum-graecum Linn. (Fabaceae) using various method for Evaluation of Ethno pharmacological Activity of Trigonellafoenum Graceum induced writhing test for evaluation of peripheral analgesic activity in mice. All the extracts of Trigonella foenum-graecum showed significant central and peripheral analgesic activity at the dose of 50 mg/kg intra peritoneally.[1]

MATERIAL AND METHOD

Plant Material

Seeds of *Trigonella foenum-graecum* were collected from collected from the local Market (400gm.)and its identification was confirmed by NISCAIR and a specimen has been deposited of the seeds vide voucher number.

Chemicals and reagents Used:

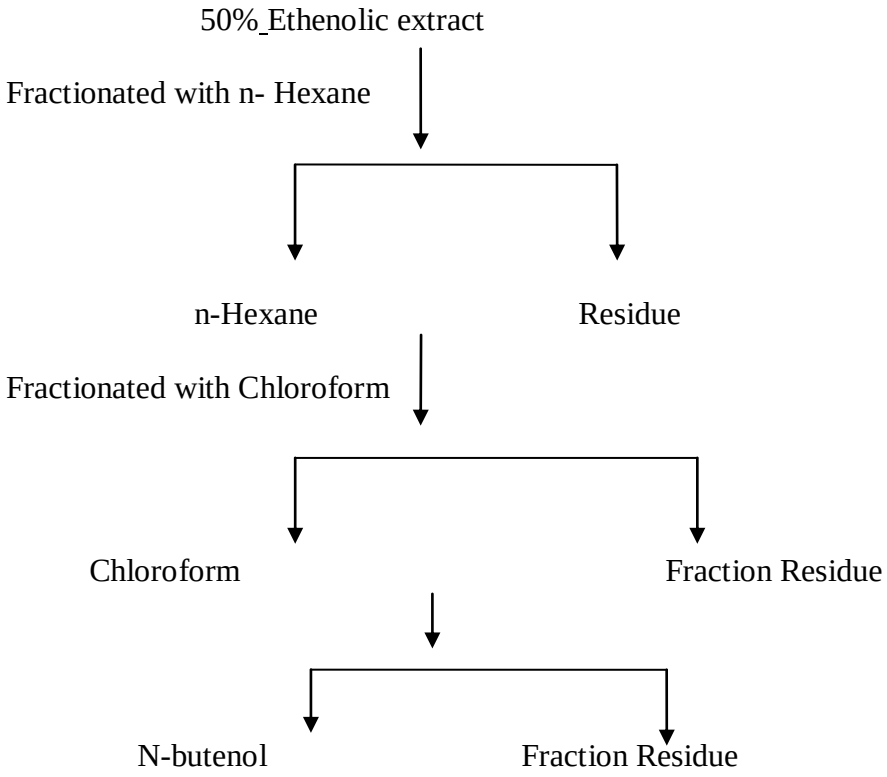
n-Butanaol ,*n*-hexane ,chloroform and silica gel was purchased from Research Lab Fine chemical, menthol was purchased from Burgoyne lab, methyl salicylate was purchased from Research Lab Fine chemicals. All other chemicals and solvents were of analytical grade.

Animals used:

Animals were procured from National Toxicological Center, Pune. Male Swiss Albino mice, weighing between 25-30 g were used for all the experimental protocols. The animals were housed at least one week in the laboratory animal room prior to testing. Food and water were given ad libitum. All procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Extraction & Physical Evaluation of Extracts:-

Preparation of fenugreek seed extract Fenugreek seeds (400g) were finely powdered, mixed with 80% methanol and kept at room temperature for 5 days. After 5 days it was filtered and the solvent was evaporated. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/l). Extraction of polyphenols was carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated. The residue was lyophilized and stored at −70°C. This yielded about 6–8 g per 100 g of seed powder. An aqueous extract (FPEt) was prepared and used for the studies. The polyphenolic content of the extract was assayed using the method of the extract was fractionated successively with *n*-hexane chloroform and *n*-butanol with the help of separating funnel for 4 times each, to obtain n-hexane fraction chloroform faction, n- butanol faction and aqueous portion. [1-2]



Flow diagram for fractionation

Isolation procedure of compound(s) from Trigonella foenum-graecum *:*

On the basis of % yield and review on phytoconstituents, n-Butanaol fraction 50gm was subjected to silica gel column chromatography (silica gel 0.5 kg) and eluted with 2.5L each of 100% chloroform, 25% ethyl acetate in chloroform, 50% ethyl acetate in chloroform, 75% ethyl acetate in chloroform, 100% ethyl acetate, 2% methanol in ethyl acetate, 5% methanol in ethyl

acetate, 10% methanol in ethyl acetate, 20% methanol in ethyl acetate, 30% methanol in ethyl acetate, 40% methanol in ethyl acetate, 50% methanol in ethyl acetate, 100% methanol. The fractions were checked on TLC plate and similar fractions were pooled (Fig. 1). Preparative TLC was performed in silica gel 60 F254 preparative TLC plates (20 × 20 cm with 4 × 20 cm concentration zone, 0. mm layer thickness an fluorescence at 254 nm) (Merck) pre-heated at 105 ± 5 ºC for 30 min for the separation o compounds from*fraction B* (436 mg). A relatively large amount offraction (50 mg/ml) was applied on the plates with a TLC sample applicator. The plates (×5) were developed with the solvent ethyl acetate: Methanol (8:2).[2]

Four day suppression test:

Peters' 4-day suppressive test against *P. berghei* infection was used (Peters et al, 1975). After 3-hrs of infection with parasites, the mice were randomly assigned into treatment groups of five. One group was given 5 ml/kg body wt 30% DMSO in 1% CMC, one group was given chloroquine (10 mg/kg body wt) and the remaining groups were given between 25 - 2400 mg/kg body wt of 80% ethanolic extracts of the plants, with intermediate doses interpolated using a log scale. The extracts were administered orally once daily for four consecutive days. [2]

Qualitative Analysis:-

Determination of parasitaemia:

Percentage suppression of parasitaemia was calculated using the following formula:

% Suppression = Parasitaemia in negative control - Parasitaemia in study group/ Parasitaemia in negative control

Quantitative Analysis:-

Calculations for ID50:-

The mean results of percentage suppression of parastaemia against the logarithms of doses were plotted using the computer program , which also gives the regression equations. The regression equations were used to calculate ID50 values. [2]

PHYSICAL EVALUATION OF PLANT*:*

Physicochemical Parameters:-

The description of the plant is given below

Color: Yellowish color dry powder

Texture: Smooth and powdery

Odor: Strong and pungent

Taste: Bitter

Determination of pH value:-

The drug, *trigonella foenum graceum seed* powder was made as a 10%solution in water & ehtenol, and the pH of the liquid was determined with the help of pH meter and electrode system. The pH value of this drug was 5.87. [2]

Determination of Ash value:

Ash value used to determination of quality and purity of crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium calcium. [2]

Total Ash:

About 5 g of the ground air-dried material was placed in a previously ignited and weighed crucible (usually of platinum or silica). The material was spread in an even layer and ignited by gradually increasing the heat to 500-600ºC, until it is white, indicating the absence of carbon. The heating, cooling and weighing were repeated until the weight of the crucible becomes constant. The content of total ash was calculated in milligrams per gram in comparison to air-dried material. Thus, the total ash was found to be: 9.1%w/w. [2]

Acid insoluble Ash:

The total ash was collected and boiled with 25 ml of dilute HCl for 5 minutes. This solution was filtered with the Whatman (No.40) filter paper. Along with the insoluble ash, the filter paper was burnt in a Gooch crucible. Heating, cooling and weighing of the crucible was done until the weight of the crucible comes constant. The percentage of acid insoluble ash was calculated with reference to the air-dried drug (5 g) and it was: 0.3%w/w. [2]

Water soluble Ash:

Determination of water soluble: 3.7%w/w.

Sulphated Ash:

Determination of Sulphated Ash: 0.6%w/w.

Moisture content of the Crude drug:

The crude drug was introduced into the Karl-Fischer celland Moisture content was determined by Merck Iyer Moisture balance, which was found to be 8.58%. [2]

Extractive value of drug:

Useful for the evaluation of crude drug.

a). Alcohal soluble extractive value:

Exactly 5 g of sample was mixed with 100 ml of 90% alcohol and shaken frequently for 6 hours and kept for 18 hours without disturbing. The above procedure was followed and the percentage of alcohol soluble extractive with reference to the moisture free drug was calculated. The alcohol soluble extractive value so obtained was: 40%W/w. [2]

b).Water soluble extractive value:

Exactly 5 g of sample was mixed with 100 ml of chloroform water, shaken frequently for 6 hours and kept for 18 hours without disturbing and filtered rapidly taking precautions against loss of solvent. Twenty-five milliliters of filtrate was taken using a pipette, and evaporated in a tared shallow bottom dish and dried on a water bath up to constant weight. The percentage of water soluble extractive was calculated with reference to the moisture free drug. The water soluble extractive value was observed to be : 30%w/w

General Limit Test

Limit tests for heavy metals: The test for heavy metals is designed to determine the content of metallic impurities that are colored by sulfide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of the heavy metal per million parts of the substance (by weight), as determined by visual comparison of the color produced by the substance with that of a control prepared from a heavy metal standard solution.[2,3]

Limit test for arsenic:-

The glass tube was lightly packed with cotton wool, previously moistened with lead acetate solution and dried, so that the upper surface of the cotton wool was not less than 25 mm below the top of the tube. The upper end of the tube was then inserted into the narrow end of one of the pair of rubber bungs to a depth of about 10 mm.

A piece of mercuric chloride paper was placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band in such a manner that the borings of the two bungs (or the upper bung and the glass tube) met to form a true tube, 6.5 mm in diameter, interrupted by a diaphragm of mercuric chloride paper. The test solution was prepared as per the norms and placed in a wide-mouthed bottle. Then, 1 g of potassium iodide AsT and 10 g of zinc AsT were added and the prepared glass tube was placed quickly in position. The action was allowed to proceed for 40 minutes. The yellow stain which was produced on the mercuric chloride paper was compared by day light with the standard stains produced by operating in a similar manner with known quantities of dilute arsenic solution AsT.

The comparison of the stains was made immediately at the completion of the test. By matching the depth of color with that of the standard stains, the proportion of arsenic in the substance was determined. A stain equivalent to the 1-ml standard stain, produced by operating on 10 g of substance, indicates that the proportion of arsenic is 1 part per million. [2-3]

Limit test for lead:-

The prepared sample was added to 6 ml of ammonium citrate solution Sp (special solutions prepared as per method specified in API for Limit test for Lead), and 2 ml hydroxylamine hydrochloride solution Sp. Two drops of phenol red solution were added and the solution was made just alkaline (red in color) by the addition of strong ammonia solution.

The solution was cooled and added to 2 ml of potassium cyanide solution Sp. Immediately, the solution was extracted with several quantities each of 5 ml of dithizone extraction solution, draining off each extract into another separating funnel, until the dithizone extraction solution retained its green color.

The combined dithizone solutions were shaken for 30 seconds with 30 ml of a 1% w/v solution of nitric acid and the chloroform layer was discarded. The solution was added to exactly 5 ml of standard dithizone solution and 4 ml of ammonia cyanide solution Sp. and shaken for 30 seconds; By this a chloroform layer is developed. [2-3]

Thin layer chromatography:

Evaporate 20 ml of the homoeopathic mother tincture on a water bath to remove alcohol. Extract the residue with 3 X 20 ml petroleum ether and concentrate the extract to 2 ml. Carry out the thin layer chromatography (TLC) of petroleum ether concentrate using petroleum ether:diethyl ether (9:1 v/v) as solvent system. In UV light, one spot appeared at Rf 0.13 (blue). After spraying with antimony trichloride reagent, the following spots appeared at Rf 0.09 (violet),0.15 (violet). [2-3]

Sample preparation:-

Exactly 3 g of sample was refluxed with 3 X 50 methanol for 1 hour, filtered and concentrated to form a residue and made to 10 ml. With methanol, 5 ˝l was spotted using Stationary phase (application) The prepared sample was applied over the pre-coated silica gel 60 F_{254} plate of 0.2 mm thickness .

Development (mobile phase):-

The sample was developed with the help of mobile phase, i.e., ethyl acetate:methanol:water (100:13.5:10).

Visualization (scanning):-

For visualization, the plate was dried at 100^0C and scanned at 254 nm UV.

Observations:-

The sample plate scanned under UV wavelength 254 nm showed two peaks (spots) and the observed Rf values were 0.32 and 0.36. [2-3]

Analgesic tests on the fractions

The five fractions obtained (that is, dichloromethane fraction (DCF), n-hexane fraction (nHF),

ethyl acetate fraction (EAF), n-butanol fraction (nBF) and aqueous fraction (AQF)) were screened for analgesic activities using the hot plate test and the formalin induced paw licking test as in previous study.

In the Hot plate test animals were orally administered 50 mg/Kg of the fractions or saline (control) or 5 mg/Kg Indomethacin (Reference drug). The animals were each placed on a hot plate (maintained at 50 ± 1o) after 30 min of the administration of the fractions, drug and Saline and the time (Reaction time) it takes each of the animals to jump off the hot plate was noted. Animals were again placed on the hot plate at 60 and 90 min post fractions, Saline and drug administration. The mean of the responses for the animals (5 per group) administered each fraction was compared with the control group. In the Formalin test the method of Hunskaar and Hole, (1997) was used. The paws of the animals were injected with 100 µL of 3% colouration of flavonoids. In the Ferric chloride test, 2 ml of water and three drops of freshly prepared FeCl3 were added to 50 mg of the isolated compound (in a test tube). The mixture was warmed and observed for the bluish green coloration of flavonoids. [2,3,4]

Phytochemical Analysis:

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugars, saponins, tannins, carbonyls, flavonoids, phlobatannis and steriods ,

Test for Alkaloids

Dragendorffs reagent test;

Weigh about 0.2 gm of plant extract in separate test tube and warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorffs reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids. [2,3,4]

Test for Cardiac glycoside

Keller-Killani Test

Weigh about 0.5 gm of plant extract in a separate test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo sulphate (VI) acid. And observe for brown ring formation at the interface (Finar, 1983).

Test for Terpenoids

Weigh about 0.5 g plant extract in separate test tubes with 2 ml of chloroform. And add concentrated Sulphuric acid carefully to form a layer. And observe for presence of reddish brown color interface to show positive results for the presence of terpenoids. [2,3,4]

Test for reducing sugars

Take a test tube and add 2 ml of crude plant extract and add 5 ml of Distill water and filter. The

filtrate was boiled with 3-4 drops of fehlings solution A and B for 2 minutes. Observe for orange red precipitate which indicates the presence of reducing sugars.

Test for Saponins

Weigh about 0.2 gm of plant extract in the test tube and add 5 ml of distilled water and then heat to boil. Observe for the occurrence of frothing (appearance of creamy mass of small bubbles) which then indicates the presence of Saponin. [2,3,4]

Test for Tannin

To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. And observe for dark green solutions that indicate the presence of a tannin.

Test for Carbonyl

Take 2 ml of plant extract in separate test tubes and add few drops 2,4, di nitro phenyl hydrazine solution and shake. And observe for the presence of yellow crystals immediately for the presence of an aldehyde. [2,3,4]

Test for Flavonoids

Weigh about 0.2 gm plant extract in separate test tubes and dissolved diluted Sodium hydroxide and add diluted Hydrochloride. And observe foryellow solutions that turn colorless. This indicates the presence of flavonoids. [2,3,4]

Test for Phlobatanin

Weigh about 0.5 gm of plant extract in a test tube and dissolve with distilled water and filter. The filtrate was boiled with 2% Hydrochloric acid solution. Observe for a red precipitate that shows the presence of Phlobatanin

Test for Steroids

To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid .Observe for the color change from violet to blue or green in samples indicating the presence of steriods

Pharmacological screening:

Experiments were performed on albino mice of either sex weighing around 2025g, divided into five groups each containing six animals. Test extract was prepared as a fine homogenized suspension in tween-80 (2% w/v). Aspirin (10 mg/kg) was used as standard drug.

All the animals were approved by the ethics committee of the institute.

Antibacterial activity

Bacterial strains and Growth conditions

The following cultures were used: *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 3471), *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200). The cultures are obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. Cultures of these bacteria were grown in nutrient broth at 37˚C and maintained nutrient agar slants <12˚C. [7,8]

Reference antibiotic

Reference antibiotic penicillin was obtained from the authorized medical shop, "Chennai."Preparation of Antibiotic and plant extract for the experiment: The antibiotic and dried plant extract were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of 0.5, 1.0, 1.5, 2.0, 2.5mg/ml. [7,8]

Preparation of Inoculum:

Inoculum of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were prepared in nutrient broth medium and kept incubation at 35˚C for 8 hours. [7,8]

Preparation of Medium

The required amount of Mueller-Hinton plates (Hi media) is prepared as per manufacturer instruction.

Procedure for performing the Disc Diffusion test (Bayer et al., 1986)

A sterile cotton swab was dipped into the turbid culture suspension. The dried surface of Muller-Hinton agar plate were inoculated by streaking two more times rotating the plate approximately 60˚each time. The lid may be left aside for 3-5 minutes and allow to dry for the excess surface moisture content.

The previously prepared discs were poured with different concentrations of the above prepared antibiotic and plant extract solutions, the discs were placed on the medium and the plates were incubated at 5˚C for 1 hour to permit good diffusion, and then transferred to an incubator at 37˚C for 24 hours. The negative control was included without adding the cultures to know the sterile conditions. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc.

S. No	Test parameters									
	Alkaloids	Cardiac glycosides	Terpenoids	Reducing sugars	Saponins	Tannis	Carbonyl	Flavonoids	Phlo-batanin	Steriods
1	+	+	+	-	+	+	-	+	-	+

Key: + = Positive, - = Negative

The crude ethanolic extracts of *Aegle marmelos* and the control drug penicillin were subjected to antimicrobial activity. The results are tabulated and discussed below in Table 2-2 In the case of

Escherichia coli, the control drug penicillin showed less activity (about 22.0mm) when compared with the plant extract of *Aegle marmelos* (this 25.7mm). At a higher dilution of about 2.5 mg/ml, the plant extract is effective against gram negative Escherichia coli.The same dilutions were tested on *Pseudomonas aeruginosa*. The plant extract showed a 19.9mm zone of inhibition, but the control drug Penicillin exhibited 18.9mm, and hence this plant extract is effective against gram negative *Pseudomonas aeruginosa* In gram positive *Staphylococcus aureus*, *Aegle marmelos* and the control drug penicillin, the organisms exhibit a similar zone of inhibition (about 29.0mm), hence they are considered as resistant. The same dilutions were subjected to *Bacillus subtilis*, the zone of inhibition of which is about 28.1mm, but as the control drug penicillin exhibits 26.2mm, the plant extract is considered as susceptible. In this study, the results of the investigation show that the plant extracts from *Aegle marmelos* have good antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* due to the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids, and steriods. However, *Staphylococcus aureus* is considered resistant at different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml) against the control drug Penicillin. [8,9]

Table-1 : Minimum inhibitory concentration of *Trignell foenum Greacum* and the control drug Penicillin with the cultures of *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus* and *Bacillus subtilis.*

S. No	Name of organism	Concentration (mg/ml)	Zone of Inhibition(mm)	
			Aegle marmelos	Penicillin
1	*Escherichia coli*	0.5	15.3	12.5
		1.1	17.2	14.0
		1.5	20.1	16.0
		2.0	21.7	18.0
		2.4	24.7	22.0
2	*Pseudomonas aeruginosa*	0.6	12.3	08.5
		1.0	12.7	10.3
		1.2	15.6	12.0
		2.0	14.7	16.7
		2.5	16.9	15.9
3	*Staphylococcus aureus*	0.5	17.4	14.2
		1.0	21.1	19.4
		1.5	23.4	22.5
		2.0	26.1	26.1
		2.5	29.0	29.0
		1.0	21.0	15.7
		1.5	22.6	20.8

Acute toxicity studies

The acute toxicity study of the extracts of A. marmelos was performed as described by Turner

(1965). The dead animals obtained from primary screening studies, [LD.sub.50] value determination experiments, and the acute studies were subjected to postmortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted and any apparent and significant features or differences from the norm were recorded. [7,8]

Determination of [LD.sub.50] values by graphical method:

Wistar albino rats of both sex and of approximately the same weights were divided into four groups, each containing four animals for the purpose of determining the [LD.sub.50] value of a single extract. Each group was caged separately. Four different doses of 50, 70, 90 and 100 mg/kg body wt were employed for each test drug. Each animal in every group was administered with an extract of a pre-determined dose intraperitoneally. About 24 h later, the number of dead animals in a group was recorded. The data were tabulated. The toxicological effect was assessed on the basis of mortality, which was expressed as an [LD.sub.50] value. [7,8]

ANALGESIC ACTIVITY :

Hot plate test :

All the extracts of Trigonella foenum-graecum showed significant analgesic activity at 50 mg/kg, i.p. dose (Table 1). Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, methanolic extract of leaf of Trigonella foenumgraecum showed highest increase in reaction time. In the present study, all the extracts showed significant ($p<0.05$ and $p<0.0001$) analgesic activity but among all the extracts, methanolic extract of leaves of Trigonella foenum-graecum showed highest increase in reaction time. Thermic painful stimuli are known to be selective to centrally active drugs .Prostaglandins and bradykinins were suggested to play an important role in analgesia .Flavonoids and tannins are reported to inhibit prostaglandin synthesis.A number of flavonoids and tannins have beenreported to produce analgesic activity . As phytochemical tests showed presence of flavonoids and tannins in methanolic extract of leaf of Trigonella foenumgraecum, they might suppress the formation of prostaglandin and bradykinins or antagonize their action and exert its activity. [10,11,12]

Writhing test

All the extracts of Trigonella foenum-graecum at dose of 50 mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.6% acetic acid (Table 2). Methanolic extract of leaf of Trigonella foenum-graecum showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol. Peripheral analgesic activity was assessed by acetic acid-induced writhing test,

which showed significant ($p<0.05$ and $p<0.0001$) suppression of writhing by all the extracts, but methanolic extract of leaf of Trigonella foenum-graecum showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts and standard drug paracetamol (Table 2). It was observed that onset of writhing was delayed and duration of writhing was shortened. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [18]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. Overall we can say that leaves of Trigonella foenumgraecum posses better antinociceptive activity. Peripheral analgesic activity was evaluated using acetic acid-induced writhing test Mice were divided into ten groups of six animals each. The animals received petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of leaves or petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of seeds of Trigonella foenum-graecum or standard drug paracetamol (50 mg/kg, i.p.) or vehicle, 30 min before intraperitoneal injection of 0.1 ml of 0.6% solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for the period of 30 minutes and then number of writhes recorded for each animal. [10,11,12,14,15]

Statistical analysis

Results are expressed as mean±SEM. The data from experiments were analysed separately using oneway ANOVA option in the SPSS: 11.5 software. The differences between means were compared using Tukey's honest significant test.

RESULTS AND DISCUSSION:

Effect of the methanolic extract of fenugreek on carrageenan-induced paw Edema Figure 1 shows the mean differences of volume of right and left foot paw before and 4 h after *i.p.* administration of normal saline and carrageenan, respectively. As shown in this figure the index of carrageenan-induced edema is completely different from the ones for normal saline. Methanolic extract of fenugreek seeds at the doses of 100 and 200 mg/kg has shown the most anti-inflammatory activity 1, 2 and 4 h after carrageenan administration. As shown in Figure 2, these extracts notably inhibited paw edema in rat when given *i.p.* at 3 h and 4 h after carrageenan injection. *Effect of the different topical preparations of fenugreek creams on carrageenan-induce paw edema* The results of anti-inflammatory activity after topical administration of fenugreek are

reported in Table 1. Statistical analysis showed that the edema inhibitions of preparations of fenugreek creams are significantly different from the control group. Significant differences between the 1% hydrocortisone ointment (positive control) and the formulations of 3 and 5% fenugreek cream are not observed (p>0.05).

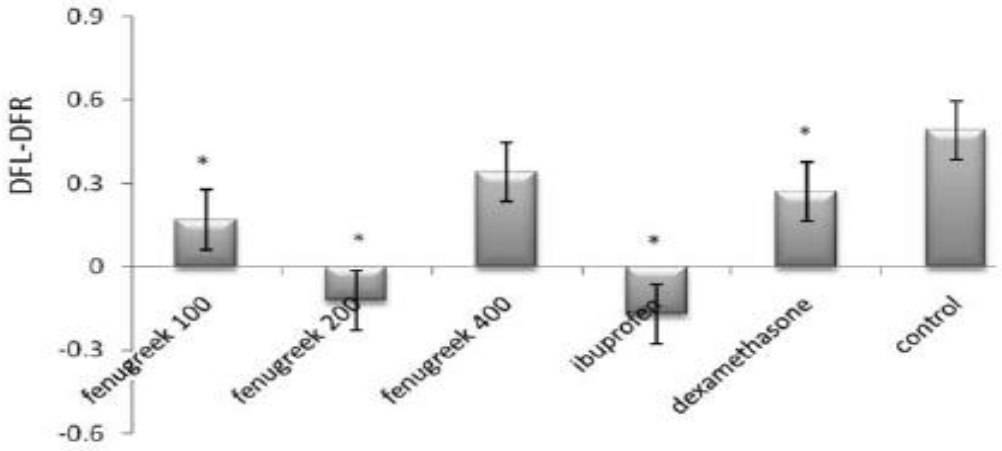


Figure 2: Effects of the methanolic extract of fenugreek on cargeenan induced paw edema in rat.

The DFL-DFR show the carrageenan-induced edema in the left foot paw. Different doses of plant extract (100,200 and 400 mg/kg). *T. foenum graecum* seeds at the dose of 100 and 200 mg/kg have shown the most anti-Inflammatory activity at 1, 2 and 4 h after carrageenan administration. Results are expressed as mean± SEM. Differences between the control and treated groups were tested for significance using a one-way analysis of variance(ANOVA).*Significant differences with Ibuprofen group (p<0.05).

CONCLUSION :

Carrageenan-induced paw edema in mice has been accepted as a useful phlogistic tool for investigating anti-inflammatory agents. It is suggested that there are biphasic effects in carrageenan-induced edema. The earlyhyperemia results from the release of histamine and serotonin, the delayed phase of carrageenan-induced edema results mainly from the potentiating effects of bradykinin on mediator release, and also from prostaglandins which produce edema after the mobilizationof leukocytes. The edema was reached its highest thickness 4 h after the application ofthe stimulus. The present work showed that *T. foenum- graecum* at the doses of 100 and 200 mg/kgsignificantly reduced the paw oedema throughout the entire period of observation in comparison to control (p<0.05). This activity showed no significant difference in comparison to ibuprofen, thus suggesting an anti-inflammatory activity for this plant (Figure 2).

Presence of saponins and flavonoids as the major compounds in *T. foenum-graecum* can approximately explain anti-inflammatory activity of the plant. From these overall results, we can conclude that the topical preparations containing 3% and 5% of fenugreek seeds extract possess an anti- inflammatory effect. The seeds of *T foenum-graecum* contain flavonoids [act as potential inhibitors of cyclooxygenase, lipoxygenase, and nitric oxide synthase as well as being antioxidants . Antipyretic and anti-inflammatory activity of the leaves of *T. foenum-graecum* has been reported .The results here support the traditional uses of the seeds of *T. foenum-graecum* for treatment of inflammations. However, it is needed to do more toxicological research for clinical studies. It has been reported that the fenugreek seeds exert antiulcer activity through the flavonoids since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents.The latter experiments are running.

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REFERENCES

1. Adetuyi et al., 2001; Trease and Evans, 1989; Sofowora, 1982).
2. Kassem A, Al-Aghbari A, AL-Habori M, Al-Mamary M. Evaluation of the potential anti-fertility effect of fenugreek seeds in male and female rabbits, Contraception 2006;73:301-6.
3. Kirtikar KR and Basu BD. Indian medicinal Plants, Vol. I, International Book Distributors, Deharadun, 2nded, 1987; p.700-1.
4. Sur P, Das M, Gomes A, Vedasiromoni JR. Trigonella foenum graecum (fenugreek) seed extract as an antineoplastic agent.Phytother Res. 2001; 15:257-9.
5. Arayne MS, Sultana N, Mirza AZ, Zuberi MH, Siddiqui FA. In vitro hypoglycemic activity of methanolic extract of some indigenous plants. Pak J Pharm Sci 2007; 20:268-73.
6. Boban PT, Nambisan B, Sudhakaran PR. Hypolipidaemic effect of chemically different mucilages in rats: a comparative study. Br J Nutr 2006; 96:1021-9.
7. Dixit P, Ghaskadbi S, Mohan H, Devasagayam TP. Antioxidant properties of germinated fenugreek seeds. Phytother Res2005;19:977-83
8. Jung K, Richter J, Kabrodt K, Lucke IM. The antioxidative power AP--A new quantitative time dependent (2D) parameter for the determination of the antioxidant

capacity and reactivity of different plants. Spectrochim Acta A Mol Biomol Spectrosc2006; 63:846-50.

9. Rastogi RP and Mehotra BN. Compendium of Indian Medicinal Plants Vol. IV, CDRI, Lucknow, 1985-1989:740-741.
10. Harborne JB. Phytochemical methods. In: A guide to Modern Techniques of Analysis, Chapman and Hall Publishers, London, 1973: 4-7.
11. Woolfe G and MacDonald AD. The evaluation of the analgesic action of pthedine hydrochloride (Dermol). J Pharmacol Exp Ther 1944; 80: 300-30.
12. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959; 18:412-8.
13. Chau T. Pharmacology methods in the control of inflammation. In: Modern Methods in Pharmacology, Vol. V, Alan. R. Liss., Inc., New York, 1989: 195-212.
14. Dray A and Perkin M. Bradykinin and inflammatory pain. Trends Neurosci, 1993; 16: 99-104.
15. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol Exp Ther 1969; 166: 96-103**.**