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The Screening of Analgesic and Anti-inflammatory activities of Trigonella foenum- graecum seeds

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Abstract: *Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. So many herbal drugs are there which uses now a days for the treatment of different diseases. Fenugreek seed contains carbohydrates, mucilaginous fiber, proteins; alkaloids are mainly trigonelline, gentianine, flavonoids, apigenin, luteolin, orientin, quercetin, vitexin, isovitexin and free amino acids, and also Vitamins A, B & C, saponins, steroids. This study was based on the evaluation of the Analgesic and Anti-inflammatory effects of the Trigonella foenum- graecum seeds. The analgesic activity was performed on Wistar rats at the dose 1000mg/kg bodyweight using Tail flick method and Dioclofenac sodium 10mg/kg body wt. was used as standard drug. The Methanolic extract showed significant result p<0.001, when compared it with the control. The Anti- inflammatory activity was performed at the doses 250mg/kg bodyweight and 800mg/kg bodyweight using carrargeenan induced rat paw edema modal, Indomithacin 20mg/kg bodyweight was used as standard. In this activity Methanolic extract at the 250mg/kg bodyweight and 800mg/kg bodyweight showed significant result p>0.001, p>0.001 as compared to control respectively. The results indicate the potential of this plant extract to treat acute inflammation and pain.*

Keyword*: Trigonella foenum-graecum, Analgesic potential, Tail flick method, anti-inflammatory, Carrageenan induced paw edema.*

I. Introduction

Plants were frequently used in therapeutics from times immemorial. The documentation of therapeutic utility of plants can be seen from the Vedic period. Now a days therapeutic utility of many plants has been identified. (Ayurvedic Pharmacopoeia, 1996)

Trigonella foenum- graecum belonged to family Fabaeace. It is one of the spices of our kitchen also known as methi and fenugreek. It is an Annual herb 50-70 cm high, oblong compressed, smooth, dark yellow to light brown. The plant is generally cultivated all over the world and naturally grows in sandy, salty and clay soils. Fenugreek seed contains carbohydrates, mainly mucilaginous fiber (galactomannans), proteins, fixed oils (lipids), alkaloids, flavonoids, free amino acids, Vitamins A, B & C, saponins and steroids (Annida B *et al*., 2004). Fenugreek seeds are medicinally used as Anti-diabetic**,** Antispasmodics**,** Hypolipidemic, Immunological, Antibacterial, Anthelmintic, Anti-inflammatory, Analgesic and Antioxidant (Zia *et al*., 2001, Sharma *et al*., 1990, Ahmadiani *et al*., 2001, Pandian *et al.,* 2002, Risley *et al.,* 1962).

Pain is a sensation triggered by the nervous system in response to tissue damage. Pain is a warning signal, which produced discomfort, excessive pain is unbearable and leads to sinking sensation, sweating, nausea, rise (or) fall in BP, tachypnoea. Inflammation, redness, hot occurs at the affected area Analgesic is defined as a state of reduction awareness to pain and analgesics are the substance that are used to decreased the pain sensation by increasing threshold to painful stimuli (Rangri *et al*., 2007).

Inflammation is a defense mechanism and protective response of vascular tissues to harmful stimuli like pathogens, damaged cells, allergens, irritants. This is typically characterized by redness, swelling, pain and heat at the affected area (Ghosh 1984). The mechanisms involved in the inflammation such as release of histamine, bradykinin, and prostaglandins. An enzyme Cyclooxygenase-2 (COX-2) may be responsible for the high levels of PGs in much inflammatory condition. COX- 2 selective inhibitor is a form of non-steroidal anti-inflammatory drug (NSAID) that directly targets COX-2. The inflammation reaction is readily produced in rats in form of paw edema with the help of irritant substance like as carrageenan, formaline etc.The study was done on the evaluation of the anti-inflammation potential of fenugreek seeds using carrageenan induced rat paw edema model (Brahmbatt 2010).

II. Material And Method

Plant material

The plant material was collected from local market of New Delhi. The plant was identified as ***Trigonella foenum- graecum*** family- Fabaeace by Dr. H.B Singh Taxonomist, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi. A Voucher herbarium specimen (specimen no.RIT/MP/G/2010/279).

Preparation of extract

Dried coarse powder was taken and successive extraction was done by methanol by soxhlet apparatus unit. Than methanol extract was concentrated by distillation and vacuum. Dried extract was stored at 2-8^{0}C in dark place.

Phytochemical screening

Phytochemical screening was carried out with the Methanolic extract to determine the presence of different chemical constituents using chemical tests related to them. The study showed that Methanolic extract was give positive result for the group of constituents like alkaloids, steroids, flavonoids, saponins, coumarins, protein, mucilage, tannins, amino acids, and some glycosides (Kokate *et al.*,2005).

TLC profile

Stationary phase: - Glass plates (10×5cm) precoated by silica gel G

Mobile phase: - Many solvent systems were taken for this study but the one give the more spots that was chloroform: methanol (4: 0.5).

Detecting agent: - Iodine chamber was used for the detection of the spots 6 spots were observed (Stahal 1965).

Animals

Wistar rats (150-200 g) were used for analgesic and anti-inflammatory studies. They were housed at the temperature 24 ± 2^{OC} with 12 h light/dark cycles in polypropylene cages in groups of six animals each. The animals were fasted overnight before the experiment (Kulkarni 2007).

III. Analgesic Activity

Tail flick method

Analgesic activity was performed on rats using tail flick method. In this method the pain induced in the tail of rats by placing the last 1-2 cm tail on analgesiometer. The tail withdrawal time from the source of heat was calculated. The cut off period taken was not more than 23 seconds to prevent the damage of tail. (Sur *et al.*, 2007)

Procedure

Rats were divided into 3 groups and 6 rats in each.

- Control group - Saline solution.
- Standard group - Diclofenac sodium (10mg/kg body wt.)
- Test group - Methanolic extract (1000mg/kg body wt.)

Firstly rats were weighed. Than animals were fasted overnight before experiment. On the day of experiment standard drug, extract drug were prepare using 2% CMC. Than before the 1 h prior of pain induction drug was administered. Rats are divided in to 3 groups like as control, standard and test group. Then respectively after time period 0, 30, 60,120, minutes calculate the time of tail flick from the analgesiometer. The cut off time was fixed 23seconds.

IV. Anti-Inflammatory Activity

Carrageenan induced rat paw edema method

This is the method which was used for the inflammation determination. Mercury was filled in Plethysmometer equipment rat paw dipped into it and displaced amount of mercury was noted (Winter *et at.*, 1962, Risley *et al.*, 1962).

Procedure

Rats were divided into 4 groups and 6 rats in each

Group 1:- Vehicle control (distilled water).

Group2:- Standard drug (Indomithacin 20 mg /kg body wt).

Group 3:- Test drug (Methanolic extract of 250 mg/kg body wt).

Group 4:- Test drug (Methanolic extract 800 mg/kg body wt).

The initial paw volume (both right and left) of each rat was noted by mercury displacement method. First the standard and test drugs were given orally. Then after 1 hr. carrageenan was induced in left paw of rats and marked. The right paw was used as reference (non-inflammed paw) for comparison. The left paw volume of rats in each group was noted at the time interval 30, 60, 120 minutes respectively. The acute difference in edema volume was calculated in each control, test and standard group and compared with the control group for determination of the percentage of inhibition of the paw edema at each time interval was calculated. All values were subjected to the statistical analysis.

$$\% \text{ inhibition} = \frac{\text{Mean paw volume of test- mean paw volume of control} \times \mathbf{100}}{\text{Mean paw volume of control}}$$

V. Result

No adverse effect or mortality was detected in Wister rats up to 1000mg/kg, orally of Methanolic extract of Fenugreek seed *during* 24 h observation period. In the Radiant heat Tail Flick model, as summarized in Table 1, the test drug in high doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center. The results of anti-inflammatory activity are summarized in Table 2. The Methanolic extract of Fenugreek seed in doses of 250 and 800 mg/kg significantly suppressed carrageenan-induced paw edema in rats (Table 2).

In TLC profile high resolution was observed in chloroform: methanol (4: 0.05) solvent system and showed maximum spot at Rf values 0.06, 0.14, 0.49 and 0.87.

VI. Discussion

Phytochemical screening of the separated fraction (MTH) from methanolic extract obtained from *Trigonella foenum-graecum* seeds was found to be water-soluble and alkaloids, steroids, flavonoids, saponins coumarins, protein, mucilage, tannins, and amino acids. Methanolic extract (1000mg/kg b.w.) of fenugreek seeds used for the evaluation of the analgesic activity. Methanolic extract showed the significant effect ($p<0.001$) when compared with control. Diclofenac sodium also showed the significant effect ($p<0.001$) when compared with control.

In the Anti- inflammatory activity both doses of Methanolic extract 250mg/kg b.w and 800mg/kg b.w showed significant ($p>0.001$), ($p>0.001$) and dose dependent effect as compared to control respectively. The extract at the dose 800mg/kg b.w and 250mg/kg b.w showed 44.90%, 26.67 % inhibition of inflammation whereas the standard showed 87.52 % inhibition of inflammation. The results are present in the Table 1, 2 and Fig.1.

Based on these results, we can say that the fenugreek seeds extract shows promising anti inflammatory and analgesic activity. It is also suggested that the mechanism of action of Methanolic extract of fenugreek seeds might be associated with the inhibition of prostaglandin synthesis, as observed for non- steroidal drugs. These effects are present due to the presence of saponins, flavonoids, tannins in methanolic extract. Further investigations are required to understand its influence on various pain and inflammatory mediators.

Table 1. Evaluation of Analgesic activity of fenugreek seeds: Mean Analgesic effect at different time of intervals.

S .no	Groups	Drugs	After 30 mins	After 60 mins	After 120 mins
1	Control	Saline solution	0.767±0.448	8.767±0.448	8.767±0.448
2	Standard	Diclofenac sodium (10mg/kg body wt.)	15.333±0.333***	14.167±0.401***	11.917±0.320***
3	Test	Methanolic extract (1000 mg/kg body wt.)	13.283±0.321***	11.867±0.4485***	10.433±0.319**

Values indicate mean ± S.E.M. (ANOVA test followed by Dunnett's *t*-test). Significance variation against control at, *P<0.05, **P<0.01, ***P<0.001

Table 2. Evaluation of Anti-inflammatory activity of fenugreek seed: % Inhibition of paw edema at different time interval.

Groups	Dose	After 30 mins	% Inhibition	After 60 mins	% Inhibition	After 120 mins	% Inhibition
Control	Saline solution	0.576±0.007	---	0.5667±0.007	---	0 .475±0.012	--
Standard	Indomithacin (20mg/kg body wt.)	0.420 ±0.023 ***	27.17	0.343 ±0.023***	39.42	0.253 ±0.021***	87.52
Test Dose 1st	Methanolic extract (250mg/kg body wt)	0.498 ±0.022*	13.59	0.428 ±0.029***	24.42	0.348 ±0.031**	26.67
Test Dose 2nd	Methanolic extract (800mg/kg body wt)	0.423 ±0.012***	26.59	0.348 ±0.011 ***	38.53	0.2617 ±0.017***	44.90

Values indicate mean ± S.E.M. (ANOVA test followed by Dunnett's *t*-test). Significance variation against control at, *P<0.05, **P<0.01, ***P<0.001

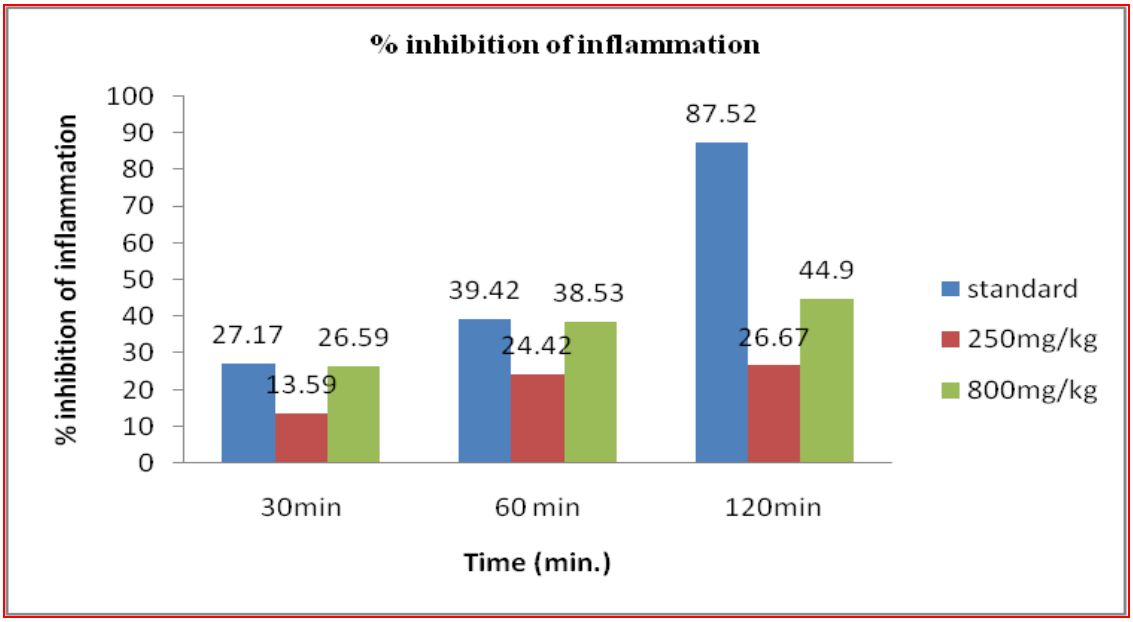

Fig.1: % inhibition of inflammation of different groups at different time.

VII. Conclusion

Fenugreek seeds extract shows promising anti inflammatory and analgesic activity. These data support the possible pharmacological activity to be performed in future in support of relief from NSAIDs adverse effect.

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