



A Comparative Study of Analgesic, Antipyretic and Anti-inflammatory Effect of Ethanolic Extract of *Trigonella foenum-graecum* with Indomethacin and Diclofenac Sodium

Nahid Abbas^{1*}, Mamuna Naz² and Manar Najeeb AISulaim³

¹Department of Medicinal Chemistry, College of Pharmacy, Qassim University, Qassim 51452, KSA.

²Department of Pharmacology, College of Pharmacy, Qassim University, Qassim 51452, KSA.

³College of Pharmacy, Qassim University, Qassim 51452, KSA.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Many drugs are available for the treatment of pain and inflammation but their utility is associated with various adverse effects. To eliminate such adverse effects natural products having medicinal effects are given more priority. This study was done to compare anti-inflammatory, analgesic and antipyretic effect of alcoholic extract of seeds of *Trigonella foenum-graecum* (fenugreek) on albino mice with indomethacin and diclofenac sodium. Initially, seeds were collected, air dried, reduced to no: 36 powder and was extracted with 70% ethanol.

This study was done to compare analgesic activity of alcoholic extract of seeds of *T. foenum-graecum* on albino mice with indomethacin and diclofenac sodium using hot plate method and acetic acid induced writhing method. The animals were pretreated with indomethacin (10 mg/kg b.w.) i.p, diclofenac sodium (10 mg/kg b.w.) i.p and *T. foenum-graecum* (200 mg/kg b.w.) i.p for 4

*Corresponding author: E-mail: nahid.is.abbas@gmail.com;

days and latency time on hot plate without licking or jumping was checked at 30 minutes after the administration of last dose. *T. foenum-graecum* (200 mg/kg b.w.) i.p differed significantly from the control ($P<0.01$). The latency time of fenugreek had increased by 16.39%. In the acetic acid-induced writhing test the maximum analgesic effect was observed in fenugreek.

A further study was done to compare antipyretic activity of alcoholic extract of seeds of *T. foenum-graecum* on albino mice with indomethacin and diclofenac sodium. The temperature was measured rectally using a digital thermometer. The animals were pretreated with indomethacin (10 mg/kg b.w.) i.p diclofenac sodium (10 mg/kg b.w.) i.p and *T. foenum-graecum* (200 mg/kg b.w.) i.p for 4 days. Pyrexia was induced by subcutaneous administration of 20% brewer's yeast suspension (10 ml/kg). Twenty four hours after injection of the yeast, the body temperature was recorded. Maximum temperature reduction was observed in animals treated with fenugreek.

A further study was done to compare anti-inflammatory activity of alcoholic extract of seeds of *T. foenum-graecum* on albino mice with indomethacin and diclofenac sodium. The acute anti-inflammatory activity of *T. foenum-graecum* (200 mg/kg b.w.) i.p. was measured plethysmographically using carrageenan as an inflammatory agent, and comparing it with indomethacin (10 mg/kg b.w.) i.p and diclofenac sodium (10 mg/kg b.w.) i.p as a reference standard. *Trigonella foenum-graecum* (200 mg/kg b.w.) differed significantly from the control ($P<0.05$) at the 2nd, 4th, 6th hour. It was concluded that Diclofenac has a better anti-inflammatory effect than indomethacin and fenugreek.

It is concluded that *T. foenum-graecum* has significant analgesic and antipyretic effect, and can be used as a substituent for diclofenac sodium and indomethacin for minor pains.

Keywords: Pyrexia; analgesia; writhing; normal paw volume.

ABBREVIATIONS

i.p. : *intra peritoneal*

NPV : *Normal Paw Volume*

IASP : *International Association for Study of Pain*

1. INTRODUCTION

The International Association for Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Pain is a physical as well as an emotional experience varying from person to person. In past various treatments strategies have been applied for the management of pain. These strategies involve the use of various medications and herbs. Many drugs are available for the treatment of pain and inflammation but their utility is associated with various adverse effects. To eliminate such adverse effects natural products having medicinal effects are been given more priority. *Trigonella foenum-graecum*, commonly known as fenugreek, is a common herb used to reduce pain. It is an erect annual herb 20-130 cm long, belonging to the Leguminosae family. This herb is mostly cultivated in the warm areas of India and Pakistan. Seeds of fenugreek contain rich amounts of the *Polysaccharide galactomannan*. These seeds are also a good source of saponins such as *yamogenin*, *diosgenin*, *tigogenin*,

gitogenin and *neotigogens*. It also contains volatile oils, mucilage and alkaloids such as *choline* and *trigonelline* [1].

Seeds and leaves of this plant are used as an ingredient of food and in various medical formulations. The green part of the plant is used as vegetable, dried herb is used as flavoring agent and seeds for the treatment of different ailments, such as lymphadenitis, diabetes, fever, hypercholesterolemia etc. Petroleum ether, ethyl acetate, chloroform and methanolic extracts of the seeds and leaves of *T. foenum-graecum* possess various pharmacological activities including: aphrodisiac effect [2], anti-inflammatory [3], anti-diabetic effect, anti-bacterial effect, and anti-nociceptive [4] effects [5,6].

They are several classes of drugs currently available to relieve pain of all kind and reduce inflammation. But unfortunately, the uses of these drugs are not satisfactory because of their numerous adverse side effects. These adverse effects related to the use of NSAID's like indomethacin, diclofenac etc, encourage us to opt for other alternatives from natural botanical sources that may have minimal side effects to control pain associated with inflammation [7].

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) commonly used as a prescription

medication to reduce fever, pain, stiffness, and swelling. It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms. It is available in the market with 12 different trade names, such as Indocin, Tivorbex, Indocin SR, Indocin IV etc. Indomethacin has also been used clinically to delay premature labor, reduce amniotic fluid in polyhydramnios and to close patent ductus arteriosus [8].

Indomethacin is a potent drug with many serious side effects and should not be considered an analgesic for minor aches and pains or fever. The medication is better described as an anti-inflammatory, rather than an analgesic [9,10].

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. It is supplied as or contained in medications under a variety of trade names. The name "diclofenac" derives from its chemical name: 2-(2,6-dichloranilino) phenyl acetic acid. Diclofenac was originally developed by Ciba-Geigy (now Novartis) in 1973 It was first introduced in the UK in 1979. [11,12]

The aim of this study is to evaluate the anti-inflammatory, analgesic and antipyretic of ethanolic extract of *T. foenum-graecum* in comparison with indomethacin and diclofenac sodium.

2. METHODOLOGY

2.1 Plant Material

Seeds of *T. foenum-graecum* (500 g) were air dried, grinded, reduced to no: 36, This powder was extracted by maceration method using 70% ethanol at room temperature for 10 days with occasional shaking. The alcoholic extract was filtered and concentrated in water bath at 45°C. The prepared extract was preserved for further use in experimental procedure.

2.2 Chemicals

Indomethacin, Diclofenac sodium, Ethanol, acetic acid, carrageenan, Brewer's yeast, normal saline.

2.3 Animals

Albino mice (20-25 g) of either sex were used in this experiment. Animals were provided to the

pharmacology section of Pharmacy Department, Qassim University, Kingdom of Saudi Arabia after IRB approval. Animals were placed in standard laboratory conditions (12/12 light and dark cycle at 25°C). Animals were fed with standard food and water.

Animals were divided into four different groups, with each group containing 5 animals.

Group 1: Negative control group (Receiving normal saline)

Group 2: Receive Diclofenac sodium

Group 3: Receive Indomethacin

Group 4: Receive ethanolic extract of *T. foenum-graecum*.

2.4 Analgesic Studies

To evaluate analgesic activity of these drugs, following tests were performed:

2.4.1 Hot plate method

In this method, mice of either sex weighing 20-25 g were divided into four different groups. These animals were pre administered with normal saline (10 ml/kg) i.p, *T. foenum-graecum* (200 mg/kg) i.p. diclofenac sodium (10 mg/kg) i.p. and indomethacin (10 mg/kg) i.p for 4 days. After four days, 30 minutes after the administration of last dose, the animals were subjected hot plate test for analgesia. Temperature of hot plate was set at 55°C. The latency time on hot plate without licking or jumping was calculated for each group. In order to prevent tissue damage a cut-off time of 30 sec. was set for the animals.

The following formula was used to calculate the percent analgesia

$$\% \text{ Analgesia} = (\text{Test latency} - \text{Control latency}) / (\text{Cut off time} - \text{Control latency}) \times 100$$

% Analgesia is calculated for each drug to evaluate the analgesic activity of each drug.

2.4.2 Acetic acid induced writhing method

In this method, mice of either sex weighing 20-25 g were divided into four different groups. These animals were pre administered with normal saline (10 ml/kg)i.p, *T. foenum-graecum* (200 mg/kg)i.p diclofenac sodium (10 mg/kg)i.p. and indomethacin (10 mg/kg)i.p for 4 days. After four days, 30 minutes after the administration of last dose, the animals were treated with 1% acetic acid (i.p.). After 5 minutes of acetic acid injection,

the number of abdominal constrictions was counted for 10 minutes.

% inhibition of pain was evaluated by using the following formula

$$A-B/A \times 100$$

A= no. of writhing in control group
B= no. of writhing in tested group

2.5 Antipyretic Activity

In this method, mice of either sex weighing 20-25 g were divided into four different groups. Before the administration of dose, normal body temperature for each animal was measured by rectal route using digital thermometer. Then these animals were administered the normal saline (10 ml/kg) i.p, *T. foenum-graecum* (200 mg/kg)i.p, diclofenac sodium (10 mg/kg) i.p. and indomethacin (10 mg/kg)i.p. for two days. After two days, pyrexia was induced by subcutaneous administration of 20% Brewer's yeast suspension (10 ml/kg). Twenty four hours after the injection of yeast, body temperature was again evaluated rectally using a digital thermometer.

% reduction of pyrexia was calculated by using the following formula:

$$\text{Percent reduction} = A-B/A \times 100$$

A= Temperature before treatment
B= Temperature after treatment

2.6 Anti-inflammatory Studies

In this method, mice of either sex weighing 20-25 g were divided into four different groups. These animals were pre-administered with normal saline (10 ml/kg) i.p, *T. foenum-graecum* (200 mg/kg) i.p, diclofenac sodium (10 mg/kg) i.p. and indomethacin (10 mg/kg)i.p for 4 days. Normal paw volume (NPV) was calculated for each animal using plethysmograph. 30 minutes after the administration of the last dose, animals were injected carrageenan (1% 0.05 ml) subcutaneously in the sub planter region of right hind paw. Inflammation was measured after 2, 4 and 6 h. of carrageenan injection.

% inhibition of edema was evaluated by using the following formula

$$A-B/A \times 100$$

A= edema volume of control group
B= edema volume of tested group

3. RESULTS

3.1 Data is Reported as Mean Result of Five Animals

3.1.1 Analgesic activity

3.1.1.1 Hot plate method

Table 1 depicts the latency period of animals on hot plate. Results of hot plate method revealed that, the latency time for group 2 (treated with diclofenac) had increased by 14.06%, while the latency time for group 3 (treated with indomethacin) had increased by 14.35% and the latency time was increased by 16.39% in animals of group 4, treated with *T. foenum-graecum*. Graph 1 displays the latency period of animals measured by the hot plate method.

3.1.1.2 Acetic acid induced writhing test

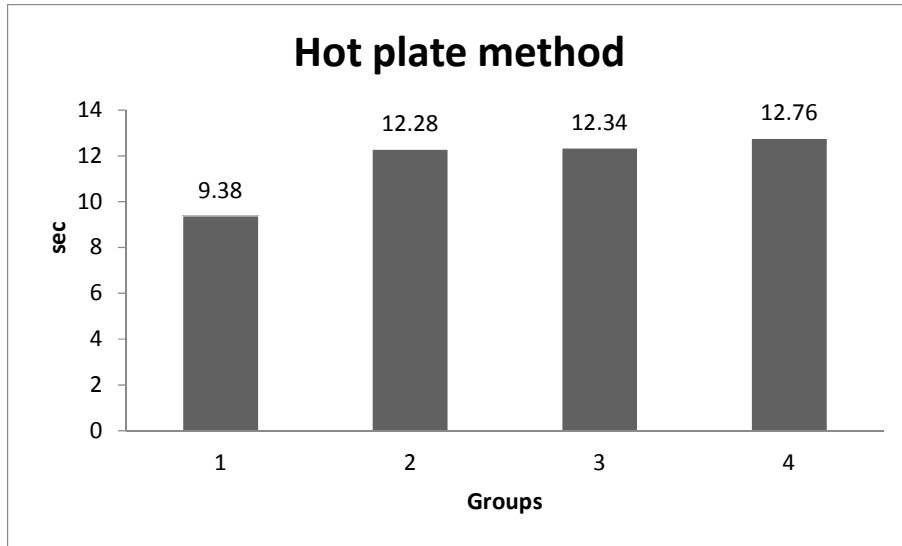
Table 2 depicts the writhing's observed in animals after acetic acid induction. The result of this test revealed that the animals of positive control group had more pain after the injection of acetic acid. Maximum analgesic effect (81.25%) was observed in group 4 treated with *T. foenum-graecum*. A minimum analgesic effect was observed in group 2 treated with diclofenac sodium (77.13%), animals of group 3 treated with Indomethacin (10 mg/kg) had a marked reduction in analgesic effect by 77.43%. Graph 2 displays the writhing's produced by the animals of various groups after the induction of glacial acetic acid.

3.1.2 Antipyretic activity

Table 3 depicts the temperature of animals before and after treatment. The result of this test revealed that the animals of positive control group raised the temperature by 2.33% after the induction of pyrexia. Animals of group 2, treated with diclofenac sodium (10 mg/kg) had a rise in their body temperature by 1.36%, while animals in group 3 treated with Indomethacin (10 mg/kg) had a rise in their body temperature by 1.19%. Maximum temperature reduction was observed in group 4 treated with fenugreek (*T. foenum-graecum*), as their body temperature had risen by 1.09%. Graph 3 displays the temperature readings measured in °C before and after inducing pyrexia.

Table 1. The latency period of animals on hot plate

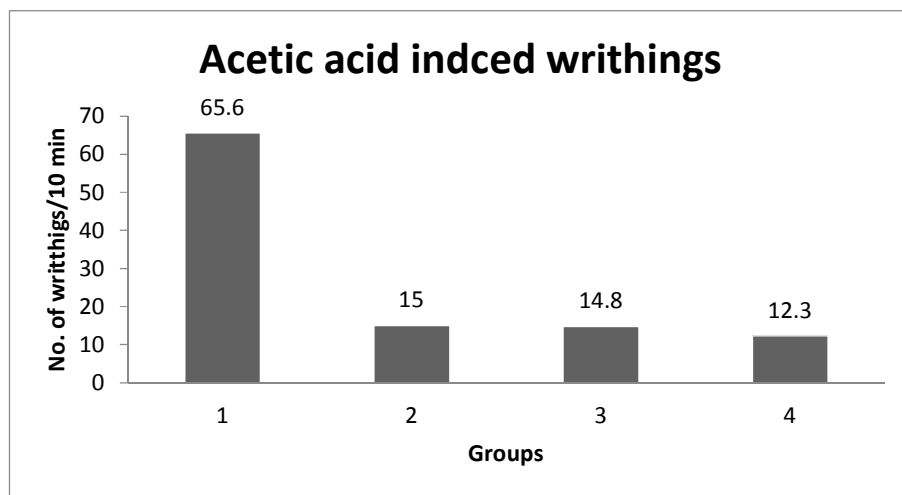
n=5	Group 1	Group 2	Group 3	Group 4
Time (sec) mean±SD	9.38± 0.5	12.28±0.5	12.34±0.5	12.76±0.6
P	----	0.004	0.001	0.002



Graph 1. The latency time(sec) of animals measured by hot plate method

Table 2. Acetic acid induced writhing method

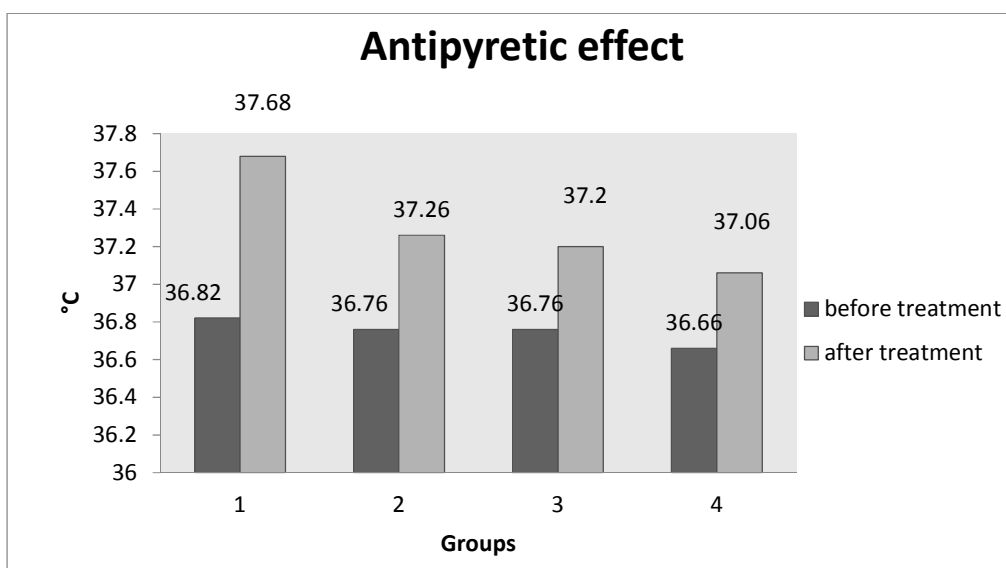
n=5	Group 1	Group 2	Group 3	Group 4
No. of writhes/10min	65.6±4.2 (mean±SD)	15±3.7	14.8±2.5	12.3±4.6
% Inhibition	---	77.13%	77.43%	81.25%



Graph 2. Number of writhings/10 minutes by animals in acetic acid induced writhing method

Table 3. Temperature of animals before and after treatment

n=5	Group 1 Temperature °C	Group 2 Temperature °C	Group 3 Temperature °C	Group 4 Temperature °C
Before	36.82±0.2	36.76±0.2 (P=0.4)	36.76±0.2 (P=0.6)	36.66±0.4 (P=0.4)
After	37.68±0.3	37.26±0.1 (P=0.04)	37.2±0.6 (P=0.02)	37.06±0.1 (P=0.01)



Graph 3. Temperature measured in °C before and after inducing pyrexia

3.1.3 Anti-inflammatory activity

Table 4 depicts the paw volume of the mice at regular intervals of 2 hours. The result of anti-inflammatory activity is shown in the following table, showing the value of average paw volume of treated and control group animals at 0, 2, 4 and 6 hours after the injection of carrageenan. Carrageenan increased the paw volume gradually. Maximum anti-inflammatory activity was observed in group 2, treated with diclofenac sodium (10 mg/kg) as the reduction in paw volume was 23.80%. Group 3 treated with Indomethacin (10 mg/kg) had reduce the paw volume by 14.28% and finally 9.5% group 4 treated with fenugreek (*T. foenum-graecum*) had reduce the paw volume by 9.5%. Hence a minimum anti-inflammatory activity was observed in group4. Graph 4 displays the measurements of paw volume in cm at 0, 2, 4 and 6 hours during anti-inflammatory study.

4. DISCUSSION

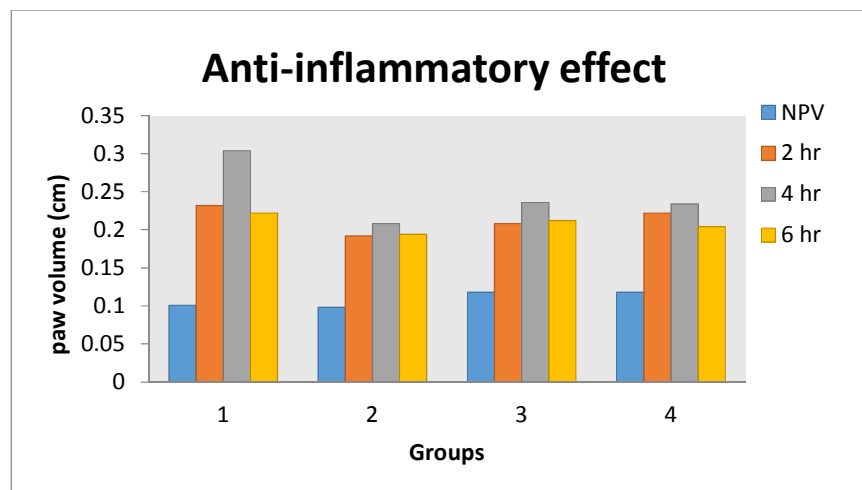
This study involves the investigation of anti-inflammatory, antipyretic and analgesic effect of

Trigonella foenum-graecum (Fenugreek) (200 mg/kg) in comparison with standard drugs. Indomethacin (10 mg/kg) and Diclofenac sodium (10 mg/kg) were used as standard drugs for anti-inflammatory, antipyretic and analgesic effect. As per Tables 1 and 2 pretreatment with *Trigonella foenum-graecum* significant maximum pain reduction in both hot plate and acetic acid induced writhing method, whereas minimum analgesic effect was observed in group2 treated with Diclofenac sodium (10 mg/kg). Pain is processed by the release of various chemical mediators i.e. bradykinins, prostaglandins etc. In the acetic acid induced writhing method, constrictions in the stomach occur by the release of PGE2 and PGF2α in the peritoneal cavity, hence *T. foenum-graecum* may have ability to suppress these mediators to treat the pain [13,14]. Antipyretic activity of *T. foenum-graecum* was evaluated by inducing pyrexia using Brewer's yeast, body temperature of the animals was measured by rectal route using digital thermometer. As per Table 3, *T. foenum-graecum* significantly showed maximum antipyretic effect and diclofenac sodium had minimum antipyretic effect. Anti-inflammatory

Table 4. The anti-inflammatory activity of different treatments in mice

n=5	Group 1 Paw volume (cm) mean±SD	Group 2 Paw volume (cm) mean±SD	Group 3 Paw volume (cm) mean±SD	Group 4 Paw volume (cm) mean±SD
NPV	0.10±0.005	0.09±0.002	0.11±0.03	0.11±0.03
2hr	0.23±0.02	0.19±0.01	0.20±0.02	0.22±0.03
4hr	0.30±0.06	0.20±0.01	0.23±0.04	0.23±0.02
6hr	0.22±0.02	0.19±0.02	0.21±0.03	0.20±0.01

NPV = Normal paw volume

**Graph 4. The measurement of paw volume in cm at 0, 2, 4 and 6 hours during anti-inflammatory study**

activity of *T. foenum-graecum* was studied by inducing edema using carrageenan. Inflammation is supposed to occur in two phases, first phase involve the release of histamine, bradykinin and serotonin, whereas second phase involve the release of prostaglandins [3]. As per Table 4 it was found that *T. foenum-graecum* have a moderate anti-inflammatory activity as compared to diclofenac sodium and indomethacin. Maximum anti-inflammatory activity was observed in animals treated with diclofenac sodium.

5. CONCLUSION

Hence it is concluded that *T. foenum-graecum* has significant analgesic and antipyretic effect and can be used as a substituent for diclofenac sodium and indomethacin for minor pains. *T. foenum-graecum* has a moderate anti-inflammatory effect as compared to diclofenac and indomethacin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This is to certify that Dr. Nahid Abbas, carried research experiments using albino mice after the necessary approval of the Ethical Research Committee in the Collage of Pharmacy, Qassim University, during the year 2015-2016.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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