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Study of anti-inflammatory analgesic and antipyretic antimicrobial effect of aqueous extract of *Trigonella foenum-graecum* sprouts and effect of glacial acetic acid on blood cells of mice

[1] Nahid Abbas, [2] Abrar Rsheed Al Rubaish, [2] Sarah Ali

[1] Qassim University, Medicinal Chemistry Department, College of Pharmacy, Qassim, Saudi Arabia.
[2] Qassim University, Clinical Pathology Department, College of Pharmacy, Qassim, Saudi Arabia.

Abstract
Pain and inflammation can be eradicated by using various drugs. Herbal drugs can be opted to control pain, as they have few side effects. This study was done to compare anti-inflammatory, analgesic and antipyretic of aqueous extract of *T. foenum-graecum sprouts* (fenugreek) on albino mice with diclofenac sodium as a standard of reference. A microbial screening was conducted. Initially, seeds were collected, soaked in water, sprouts were formed then the active constituents was extracted with water.
The analgesic activity of aqueous extract of *T. foenum-graecum sprouts* (fenugreek) on albino mice was found using hot plate method and acetic acid induced writhing method. Complete blood count (CBC) was also done.
The animals were pretreated with diclofenac sodium (10mg/kg b.w.) i.p and *T. foenum-graecum sprouts* (200mg/kg b.w.) i.p for 4 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 sprouts* (200mg/kg b.w.)i.p differed significantly from the control (P<0.01) The latency time of fenugreek sprouts had decreased by 12.06% In the acetic acid writhing test maximum analgesic activity was observed in the mice treated with diclofenac sodium (10mg/kg b.w.) i.p. CBC was also done for all the animals.
A further study was done to compare antipyretic activity of aqueous extract of *T. foenum-graecum sprouts* (fenugreek) on albino mice with diclofenac sodium. Temperature was measured by rectal route using digital thermometer. The animals were pretreated with diclofenac sodium (10mg/kg b.w.) i.p and *T. foenum-graecum sprouts* (200mg/kg b.w.) i.p for 4 days. Pyrexia was induced by subcutaneous administration of 20 % brewer's yeast suspension (10ml/kg). Twenty four hours after injection of yeast, body temperature was recorded. Maximum temperature reduction was observed in animals treated with fenugreek sprout extract.
The acute anti-inflammatory activity of *T. foenum-graecum sprouts* (200mg/kg b.w.) i.p. was measured plethysmo graphically using carrageenan as inflammatory agent and comparing with diclofenac sodium (10mg/kg b.w.) i.p as reference standard. *T. foenum-graecum sprouts* (200mg/kg b.w.) has significant difference with control (P<0.05) at the 2nd, 4th, 6th hour.
Finally an antimicrobial screening was done. It was found that the sprouts had no antifungal activity and was having antibacterial effect on gram negative bacteria.
It was concluded that *T. foenum-graecum sprouts* has mild analgesic effect, significant antipyretic effect, anti-inflammatory effect and antibacterial effect.

Keywords: Pyrexia, analgesia, writhing, normal paw volume

1. Introduction
Mankind has used medicinal plants in order to cure diseases and relieve physical suffering from the earliest times. According to World Health Organization, about 80% population of the developing countries relies on traditional medicines, mostly plant originated drugs [1].
Medicinal plants are the source of primary health care throughout the world for thousands of years. However in the middle of 20th century, the use of medicinal plants was reduced one fourth because researchers favor the use of synthetic chemicals for curing diseases. But, now the trend is changing and people favor the medicinal plants as they contain natural products which are effective, chemically balanced and have fewer side effects as compared to synthetic chemicals [2].
Pyrexia or fever is the increase in body temperature above normal physiological range, which may results due to physiological stress such as during ovulation, increased thyroid secretion, excessive exercise, any lesions to central nervous system, due to leukemia and mostly in microbial infections. Natural defense system of the human body is activated whenever body finds any infectious agent in order to create an unfavorable environment for the survival of infectious agent. The infectious agent or damaged tissues initiate the increase production of pro-inflammatory mediator's cytokines such as interleukin 1β, β, α and TNF-α which enhance the formation of prostaglandin E2 (PGE2) near the peptic hypothalamus area and the prostaglandin in turn act on the hypothalamus to elevate the body temperature (Figure 1). As the temperature of the body is controlled by nervous feedback mechanism so whenever body temperature will be high, blood vessels will be dilated and sweating will be increased to reduce the high temperature. But when the body temperature will be low then vasoconstriction occurs to protect the internal body temperature. Increase temperature as in case of fever leads to faster disease progression due to increased tissue catabolism, dehydration and persisting complaints as in case of HIV infection and other chronic infections [3, 4].

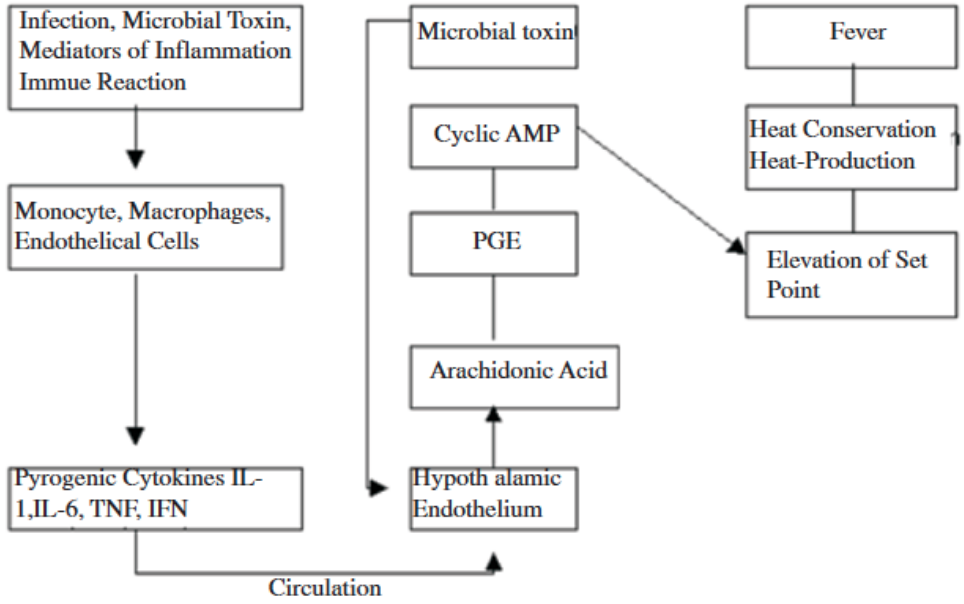

Fig 1: Pathogenesis of fever

Trigonella foenum-graecum (TFG; Fenugreek), from the family Fabaceae, is extensively cultivated in most regions of the world. Fenugreek is known to have several pharmacological effects such as hypoglycemia [5, 6, 7, 8], hypocholestrolemia [9, 10], antioxidation [11], laxation [12], fungicide [13] and appetite stimulation [14, 15].

There are several reports concerning the anti-inflammatory, antipyretic and antinociceptive effects of the plant TFG under the name of ‘‘Shanbalileh’’, in Iranian traditional medicine [16, 17]. This plant is known to contain alkaloids [18], flavonoids [19], salicylate [20], and nicotinic acid [21, 22]. On the other hand, the available anti-inflammatory and antipyretic drugs (steroidal and nonsteroidal) present a wide range of side effects for which the major reason is nonselective inhibition of cycloxygenase I (cox I) and cyclooxygenase II (cox II) [23]. So many studies are being directed to find a selective cox II inhibitor or compounds acting with other mechanisms and little side effects. We have previously shown that the plant extract possess antinociceptive effects [24]. Therefore, the present study was designed to investigate, if the TFG sprouts extract also has anti-inflammatory and antipyretic effects as well.

2. Methodology

Plant Material

Seeds of *T. foenum-graecum* (500g) were taken. Allow seeds to soak for 6-12 hours. Empty the seeds into your sprouter. Rinse thoroughly with cool water. Drain thoroughly. Set your sprouter anywhere out of direct sunlight at room temperature or at 150 Watts of incandescent light. Sprouts are formed.

Sprouts of seeds of *Trigonella foenum-graecum* (500g) were grinded and powder was extracted by maceration method using water at room temperature for 10 days with occasional shaking. The Aqueous extract was filtered and concentrated in water bath at 45 °C. The prepared extract was preserved for further use in experimental procedure.

Chemicals

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

Animals

Albino mice (20-25g) of either sex were used in this experiment. Animals were provided to the pharmacology section of Pharmacy Department, Qassim University, and 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 three different groups, with each group containing 6 animals.

Group 1: Negative control group (Receiving normal saline)

Group 2: Receive Diclofenac sodium,

Group 3: Receive aqueous extract of *T. foenum-graecum Sprouts.*

Analgesic studies

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

Hot plate method

In this method, mice of either sex weighing 20-25 g were divided into three different groups. These animals were pre administered the normal saline (10ml/kg) i.p, *T. foenum-graecum sprouts* (200mg/kg) i.p. diclofenac sodium (10mg/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.

Following formula is used to calculate the percent analgesia

% Analgesia = (Test latency - Control latency) / (Cut off time - Control latency) × 100

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

Acetic acid induced writhing method

In this method, mice of either sex weighing 20-25 g were divided into three different groups. These animals were pre administered the normal saline (10ml/kg) i.p, *T. foenum-*

graecum Sprouts (200mg/kg) i.p, and diclofenac sodium (10mg/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 were counted for 10 minutes.

% inhibition of pain was evaluated using following formula

A-B/A× 100

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

Antipyretic activity

In this method, mice of either sex weighing 20-25 g were divided into three 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 (10ml/kg) i.p, *Trigonella foenum-graecum* Sprouts (200mg/kg) i.p, diclofenac sodium (10mg/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 using digital thermometer by rectal route.

% reduction of pyrexia was calculated using following formula:

Percent reduction = A-B/A× 100

A= Temperature before treatment
B= Temperature after treatment

Anti-inflammatory studies

In this method, mice of either sex weighing 20-25 g were divided into three different groups. These animals were pre administered the normal saline (10ml/kg) i.p, *T. foenum-graecum Sprouts* (200mg/kg) i.p, diclofenac sodium (10mg/kg) i.p. for 4 days. Normal paw volume (NPV) was calculated for each animal using screw gauge. 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 using following formula

A-B/A× 100

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

Antibacterial activity of *T. foenum-graecum Sprouts* in *vitro*

To evaluate the antibacterial activity of *T. foenum-graecum Sprouts*, paper disc diffusion method was adopted. Agar plates were prepared by using 20 ml of blood agar (sheep blood) and poured into sterile petri-dishes. Agar plates were inoculated with bacterial stock suspension by using sterile cotton swab, and then placed in incubator for 15 minutes at 37. After 15 minutes, sterilized filter paper discs of 6 mm diameter and disc of positive control drug (Amoxicillin 10 mcg) were placed on the surface of the test bacterial plate. Dilution of *T. foenum-graecum Sprouts* (500mg/ml, 250mg/ml, 125mg/ml, and 75mg/ml) placed on the surface of filter paper disc (75mcl) by the help of micropipette and the plates were incubated for 48 hours at 37 °C, then inhibition zone were measured.

Antifungal activity of *T. foenum-graecum in vitro*

To evaluate the antifungal activity of *T. foenum-graecum Sprouts*, paper disc diffusion method was adopted. Agar plates were prepared by using 20 ml of molten agar and poured into sterile petri-dishes. Agar plates were inoculated with fungal stock suspension by using sterile cotton swab, and then placed in incubator for 15 minutes. After 15 minutes, sterilized filter paper discs of 6 mm diameter and disc of positive control drug (Clotrimazole10 mcg) were placed on the surface of the test fungal plate. Dilution of *T. foenum-graecum Sprouts* (500mg/ml, 250mg/ml, 125mg/ml, and 75mg/ml) placed on the surface of filter paper disc (75mcl) by the help of micropipette. Then these plates were incubated for 72 hours at 37 °C, then inhibition zone were measured.

3. Results

Data is reported as mean of six animals

Analgesic activity

1. Hot plate method.

Table 1 depicts the latency period of animals on the hot plate. Results of hot plate method revealed that, the latency time for group 2 (treated with diclofenac) had increased, 14.1% analgesia was observed. The latency time had decreased in animals of group 3, treated with *T. foenum-graecum* sprouts, % analgesia was -12.06%. Graph1 displays the latency period of animals measured by the hot plate.

Table 1: Showing the latency period of animals on hot plate

N=6	Group 1 Mean± S.D	Group 2 Mean± S.D	Group 3 Mean± S.D
Time (sec)	9.38± 0.45	12.28± 0.49	6.9± 0.85
P	**----**	0.025	0.068

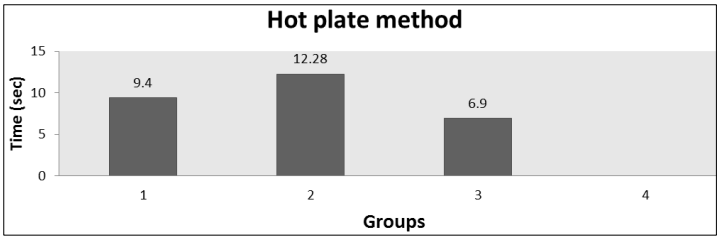

Fig 1: The latency time (sec) of animals measured by hot plate method.

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 less pain after the injection of acetic acid. Maximum analgesic effect (77.13%) was observed in group 2 treated with diclofenac sodium, animals of group 3 treated with *T. foenum-graecum* sprouts was 54.6%.Graph2 displays the writhing's produced by the animals of various groups after the induction of glacial acetic acid. Table 3 depicts the CBC of all the animals after the injection of glacial acetic acid. It was observed that group 3 shows more leukocytosis and thrombocytosis after the administration of glacial acetic acid

Table 2: Showing the result of acetic acid induced writhing method

N=6	Group 1 Mean± S.D	Group 2 Mean± S.D	Group 3 Mean± S.D
No. of writhes/ 10min	65.6± 4.22	14.8± 3.71	29.5± 1.67
% Inhibition	---	77.13%	54.6%

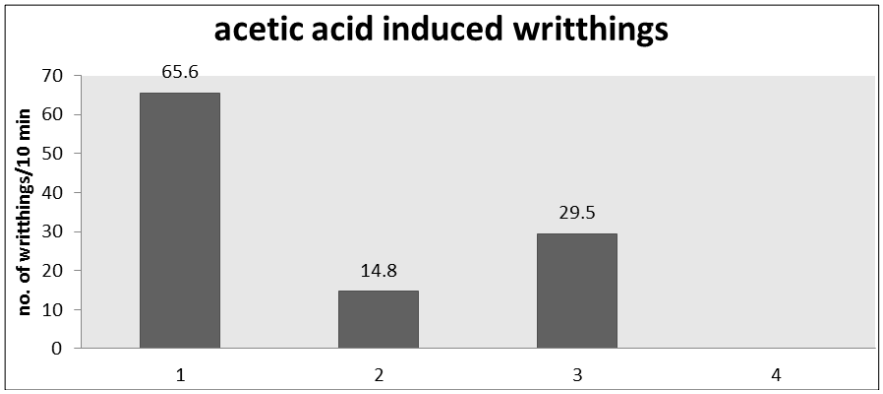

Fig 2: Number of writhings/10 minutes by animals in acetic acid induced writhing method.

Table 3: Showing the result of CBC after acetic acid induction.

CBC	Group 1 Mean± S.D	Group 2 Mean± S.D	Group 3 Mean± S.D
--×10^3/ µl- WBC	6.5±0.05	19.5± 0.54	23.3± 0.54
--×10^6/ µl-RBC	8.9±0.5	6.24± 0.01	6.13± 0.005
Hgb g/dl	14.01±0.04	12.5±0.04	10.1± 0.054
HCT%	48.8± 0.05	39.1± 0.83	33.8± 0.51
×10^3/ µl-PLT	160±0.1	193.1± 4.38	245± 0.44
RDW- SDRL (fL)	36.3± 0.19	43.5± 0.5	48.6± 0.05

Antipyretic activity

Table 4 depicts the temperature of animals before and after treatment. The result of this test revealed that the animals of positive control group reduced the temperature by 1.4% after the induction of pyrexia. Maximum temperature reduction was observed in group 3 treated with fenugreek sprouts (T. foenum-graecum), as their body temperature had fallen by by 2.11%. Graph3 displays the temperature readings measured in °C before and after inducing pyrexia.

Table 4: Showing the temperature of animals before and after treatment

N=6	Group 1 Temperature °C	Group 2 Temperature °C	Group 3 Temperature °C
Before	36.82± 0.16 --	36.76± 0.13 P= 0.721	36.8± 0.27 P= 0.962
After	37.68± 0.26	37.26± 0.11 P= 0.170	36.02± 0.39 0.038 =P

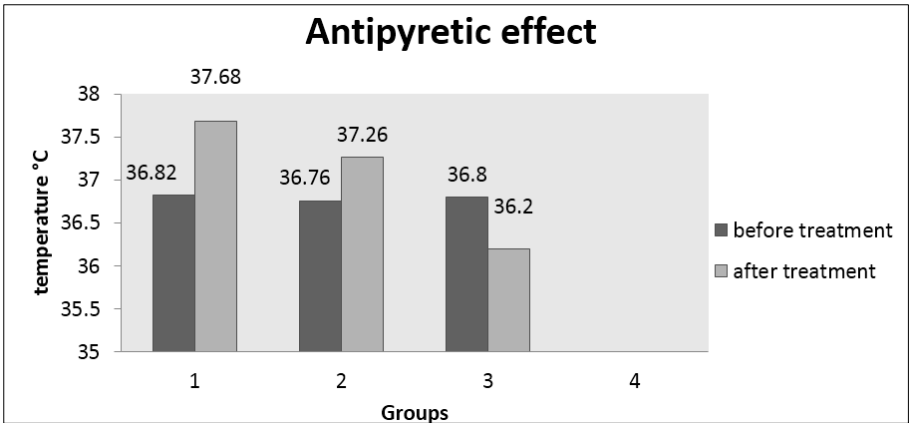

Fig 3: Temperature measured in °C before and after inducing pyrexia.

Anti-inflammatory activity

Table 5 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 5, 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 (10mg/kg) as the reduction in paw volume was 34.4%. Group 3 treated with fenugreek sprouts (T. foenum-graecum)) had reduce the paw volume by 24.1%.Hence a minimum anti-inflammatory activity was observed in group3. Graph 4 displays the measurements of paw volume in cm at 0, 2, 4, and 6 hours during anti-inflammatory study.

Table 5: Showing the anti-inflammatory activity of different treatments in mice

N=6	Group 1 Paw volume (cm) Mean± S.D	Group 2 Paw volume (cm) Mean± S.D	Group 3 Paw volume (cm) Mean± S.D
NPV	0.10±0.005	0.09±0.002	0.12±0.03
2hr	0.23±0.02	0.19± 0.01	0.21±0.02
4hr	0.30±0.06	0.21±0.01	0.25± 0.03
6hr	0.29±0.02	0.19±0.02	0.22±0.02

NPV = normal paw volume

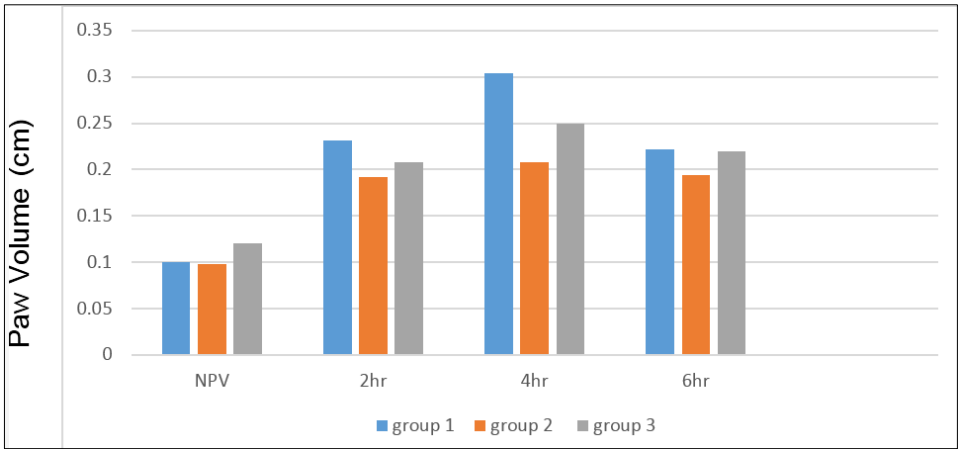

Fig 4: The measurement of paw volume in cm at 0, 2, 4, and 6 hours during anti-inflammatory study.

Antibacterial and antifungal activity

Table 6 depicts the antimicrobial screening of *T. foenum-graecum sprouts*. Antibacterial and antifungal activity of *T. foenum-graecum* sprouts was evaluated by using paper disc diffusion method. Following table shows the measurement of zone of inhibition (mm) of different dilutions of the drug and standard drug (amoxicillin 10mcg). Maximum antibacterial activity was observed for H. pylori (9mm) at 500mg/ml. minimum inhibition zone of *was* found in E. coli. (4.3mm) at 500mg/ml. No anti-bacterial effect was observed at the dose of 75mg/ml. This study also demonstrates that *T. foenum-graecum* has dose dependent anti-bacterial effect. Results show that *T. foenum-graecum* sprouts has no antifungal activity. Graph 5 displays the antimicrobial activity of *T. foenum-graecum* sprouts at various concentrations.

Table 6: Showing the antimicrobial effect of *Trigonella foenum-graecum* against various microbes

	Klebsiella Pneumonia I.Z (mm)	Proteus mirabilus I.Z (mm)	S. Aureus I.Z (mm)	E. coli I.Z (mm)	H. pylori	Candida albicans I.Z (mm)
500mg/ml	0	0	0	4.3	9	0
250mg/ml	0	0	0	4.1	7.8	0
125mg/ml	0	0	0	0	0	0
75mg/ml	0	0	0	0	0	0
+ve control	8	9	8	12	8	0

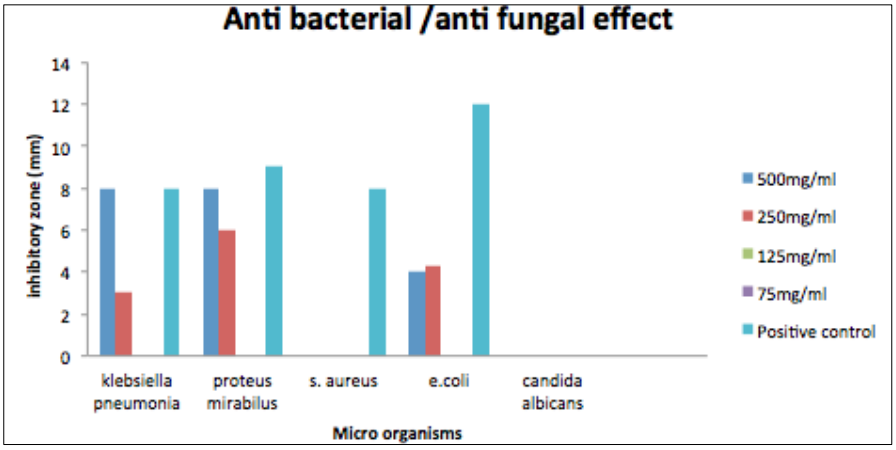

Fig 5: The antimicrobial activity of *T.foenum graecum* sprouts at various concentrations.

4. Discussion

This study involves the investigation of anti-inflammatory, antipyretic, analgesic and antimicrobial effects of *Trigonella foenum-graecum sprouts* (Fenugreek) (200mg/kg) in comparison with standard drug. Diclofenac sodium (10mg/kg). Whereas amoxicillin (10 mcg) and clotrimazole (10 mcg) were used as standard drugs for the evaluation of antibacterial and antifungal activity respectively.

As per table 1 the hot plat method used for testing analgesic activity. It was observed that diclofenac sodium (10 mg/ kg) i.p has 14% analgesic activity and the analgesic effect was very low for the group 2 treated with *Trigonella foenum-graecum sprouts.*

As per table 2 the acetic acid induced writhing test. The percentage inhibition of pain observed by group 2 treated with diclofenac was found to be 77.13% and group 3 treated with aqueous *Trigonella foenum-graecum sprouts* was 54.6%.

As per table 3 group 3 shows more leukocytosis and thrombocytosis after the administration of glacial acetic acid.

As per table 4 the antipyretic activity was observed. The % reduction of pyrexia was 1.19% in diclofenac sodium (10 mg/ kg).

The % reduction of pyrexia was 2.11% in group 3 treated with fenugreek sprouts extract.

Table 5 depicts the anti-inflammatory activity 34.4% reduction of edema was seen in group 2 treated with diclofenac sodium and 24.1% reduction of edema in fenugreek sprouts.

Table 6 depicts the antimicrobial activity. It was observed that aqueous fenugreek sprout extract is have antibacterial effect, only on gram negative bacteria, mainly H. pylori and E. coli. Antifungal activity was absent.

5. Conclusion

Aqueous extract of *T. foenum-graecum sprout* has mild analgesic activity on albino mice when compared with the activity of animals treated with diclofenac sodium.

Antipyretic effect was very high in animals treated with *T. foenum-graecum sprout* extract than the animals treated with diclofenac sodium.

Anti-inflammatory effect was more in animals treated with diclofenac than the animals treated with *T. foenum-graecum sprout* extract.

Antibacterial effect was observed, only on gram negative bacteria H. pylori and E. coli.

Thus the sprouts can be used for fever and any H.pylori stomach infection

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