Evaluation of Analgesic Activity of Aqueous Extract of Fenugreek Seeds in Albino Mice

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Research Article

Abstract: **Objective:** To evaluate the analgesic activity of aqueous extract of fenugreek seeds on chemical and mechanical painful stimulus in albino mice. **Methods:** Albino mice (20-25g) were divided into three groups of 6 animal each as control -1st group, standard-2nd group and test-3rd group.1stgroup was Control and it received Gum acacia (2 g in 100 ml of distilled water), 2nd group was Standard and it received (indomethacin 10 mg/kg),3rd group was test group and it received aqueous extract of fenugreek seeds 200 mg/kg orally in the below mentioned methods. Acetic acid Induced Writhing Test (Chemical Stimulation). The writhing movements were observed and counted for 10 min after intraperitoneal administration of 0.2ml of 1%acetic acid. Tail clip method (mechanical stimulus). An artery clip is applied to the root of the tail to induce pain. The animal quickly responded by biting the clip or the tail. The time between stimulation onset and response is measured and the method repeated after 15 mins and 30 mins. **Results:** In acetic acid induced writhing test mean value of number of writhes is 46.8 in control group, 11.8 in standard group, 19 in test group and the percentage of inhibition is 0%, 75% and 60% respectively. In tail clip method at 0 min the mean value of reaction time in control group was 1.16secs, in standard group was 7.63 secs and in test group it was 4.74 secs. At 15 mins the mean value of reaction time in control group was 1.19 secs, in standard group was 8.67secs and in test groups it was 6.7secs. At 30 mins the mean value of reaction time in control group was 1.24 secs in standard group 9.62secs and in test group it was 7.10secs .Statistical analysis done by ANOVA in both the methods and p value obtained was <0.001 and was statistically significant. **Conclusion:** Based on the results aqueous extract of fenugreek seeds exhibited analgesic activity.

Keywords: Fengureek, analgesic, indomethacin, acetic acid, writhes, tailclip.

Introduction

The most common presentation of patient to the doctor is pain[1] .Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Drugs used to treat such conditions are used extensively worldwide. Drugs in clinical use as analgesics belong to two main group Narcotic or morphine group and analgesic-antipyretic (Non steroid Anti-inflammatory drugs)group.[2]Non Steroidal Anti-Inflammatory Drugs (NSAIDS) are among the largest groups of pharmaceutical agents used all over the world. Anti inflammatory drugs are commonly associated with untoward effects. They are also one of the most common cause of Adverse Drug Reactions reported to drug regulatory agencies and are highlighted in many clinical and epidemiological studies[3]. Indigenous medicinal drugs are in use since ages on such conditions. Eg. aloevera, boswellia, argemone, pricklypoppy, calendula, balsumfir, chagamushroom etc. Study on such herbs not only help in assessing their efficacy but can also throw light on development of newer well tolerated drugs. The added advantages of using these herbs is that it can be given along with other drugs like NSAIDS, opiods making them safer and well tolerated requiring low doses in painful conditions. The ethanolic extract of Fenugreek seeds were known to have analgesic activity[4]. The serotoninergic purinoreceptors of the spinal cord of mice were involved in inducing analgesia in mice[5]. Hence an earnest attempt is being made in this study to explore the analgesic activity of aqueous extracts of Fenugreek seeds.

Objectives of the Study

To evaluate the Analgesic activity of aqueous extract of fenugreek seeds in experimental analgesic animal models.

Materials and Methods

Source of Data

Adult albino mice of either sex weighing between 20 to 25 grams were randomly selected from central animal facility, J S S Medical College, Mysore after prior approval from institutional animal ethical committee.

Exclusion Criteria

Pregnant and Diseased animals are not included in this study.

Chemicals Used

Fenugreek seeds (200mg/kg of body weight), Indomethacin (10mg/kg)of body weight, 1% Acetic acid.

Instruments Used

Artery clip, Tuberculin syringe.

Models of Experiment

The animals were randomly divided into 3 groups of 6 each, one group will serve as control and will receive 2% gum acacia suspension orally (without drug).

Other two groups will receive drug Indomethacin10mg/kg of body weight and aqueous extract of Fenugreek seeds(200 mg/kg of body weight) ,respectively.

Writhing Test[6]

Test drug is administered orally 0.2 ml of 1% Acetic acid is injected intraperitoneally in each animal. The mice were placed individually in glass chambers and number of writhes was recorded for 10 minutes in each animal. Percentage inhibition is average writhes in control group minus writhes in test group divided by writhes in the control group times 100.

Hafner's Tail Clip Method[6]

Test drug is administered orally.An artery clip is placed at the root of the tail of mice to apply noxious stimuli. A quick response of the animal is seen as biting the clip where it was attached to the tail. After 15, 30 or 60 mins the same procedure is repeated and reaction time is measured.

Results and Observations

Statistical methods applied

The effect of the drug under study was presented by calculating mean and SD of the outcome parameters. One way ANOVA, post hoc test and repeated measures ANOVA was applied to see the differences between any two groups at a time.Test of significance were carried out at 5%level. SPSS for windows (version 21) was applied in the statistical analysis.

Acetic acid induced writhing test:

Group	Mean	Standard deviation	Standard Error	ANOVA	Percentage of inhibition of writhes of standard and test group with respect to control	Percentage of inhibition of writhes of test group with respect to standard
Control	46.8	2.8	1.1	F=257.574	-	-
Standard	11.8	1.9	0.7	P=0.000	75%	-
Test	19	3.08	1.2		60%	62.1%
TOTAL	29.7	14.9	3.51			

Interpretation

ANOVA analysis shows a statistical association between the groups

Hence the analgesic activity of the test drug is comparable with standard.

HAFNER'S TAIL CLIP METHOD

Table showing mean reaction time of control, standard and test group at 0,15 and 30 minutes and ANOVA analysis of the results.

Groups	Mean	Std. Deviation	ANOVA	N
Ctrl	1.1667	.02733		6
Std	7.6383	.03656		6
test	4.7400	.01789	F=85320.8 P=0.0001	6
ctrl	1.1933	.01751		6
Std	8.6750	.17248		6
test	6.7000	.17889		6
ctrl	1.2400	.02828		6
Std	9.6200	.06812		6
test	7.100	.07874		6

Interpretation

ANOVA analysis shows a statistical association between the groups.

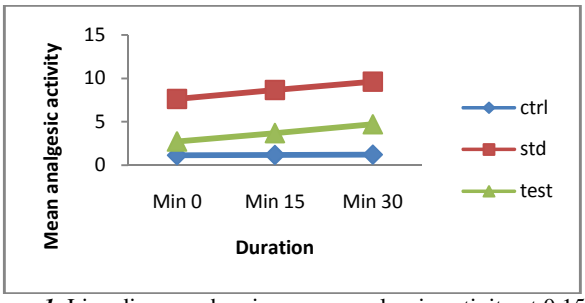

Figure 1: Line diagram showing mean analgesic activity at 0,15,30 mins-Tail clip method

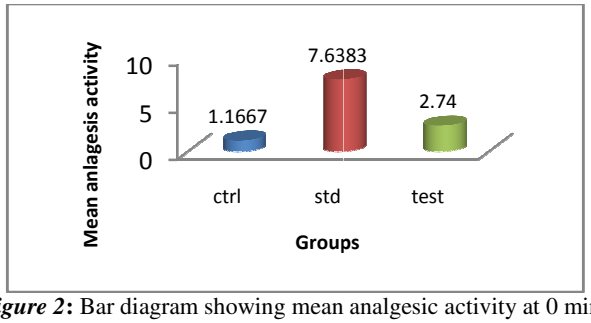

Figure 2: Bar diagram showing mean analgesic activity at 0 mins

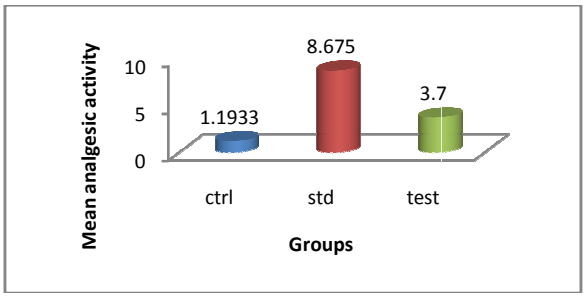

Figure 3: Bar diagram showing mean analgesic activity at 15 mins.

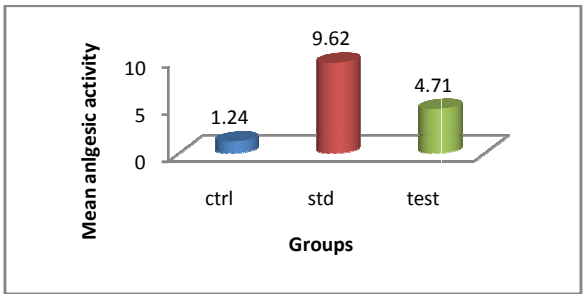

Figure 4: Bar diagram showing mean analgesic activity at 30 mins.

Discussion

Aqueous extract of fenugreek seeds have been investigated in this study for its analgesic activity and compared with the standard drug indomethacin. In the present study the analgesic models studied includes tail clip method and acetic acid induced writhing model.Indomethacin was used as standard drug. In the acetic acid induced writhing mode the percentage of inhibition of writhes by Indomethacin compared with control was 75% and test group when compared with control was 60% .The percentage of inhibition of writhes by the test group considering the percentage of inhibition of standard as 100% was 62%.Thus aqueous extract of fenugreek seeds showed good analgesic activity compared with the standard drug. In Hafners tail clip method,at 0 min the mean reaction time of control was 1.16sec ,in standard it was 7.63 sec,and in test group it was 6.7 secs. At 15 minutes the mean reaction time of control group was 1.19 sec,in standard group it was 8.67 sec and in test group it was 6.7 sec. At 30 mins the mean reaction time of control group was 1.24 secs,in standard group it was 9.62 secs and in test group it was 7.1 secs. Thus there is a increase in threshold of pain in the test group at 0 ,15 and 30 mins comparable with standard. Thus aqueous extract of fenugreek seeds showed good analgesic activity comparable with standard. Our study is in accordance to the previous study 'Probable role of spinal purinoreceptors in the analgesic effect of Trigonella foenum leaf extract' wherein fenugreek leaf extract exert analgesic effect in both formalin and tail flick test and serotoninergic system of spinal cord of mice was involved in inducing analgesic effect[5]. In the present study aqueous extract of fenugreek seeds showed promising results in tail clip method and in acetic acid induced writhing method.Further studies are required to support these findings in human beings.

CONCLUSION:

The substances in nature, has got its own medicinal value. Different parts of plants are having different therapeutic action. Procurement of right part is very much essential to get best result. There are a few studies on fenugreek plant which have showed analgesic properties**.** In the present study, aqueous extract of fenugreek seeds has showed good analgesic- activity in acetic acid induced writhing model and in Hafner's tail clip method in comparison to the standard Indomethacin**.** The analgesic property of fenugreek seeds is due to its action on serotoninergic system. The present study envisaged that the use of fenugreek seeds either as monotherapy or along with the conventional medications may have an added benefit of analgesic activity in various painful conditions. These studies are valuable for identifying lead compounds for analgesic drugs, keeping in mind the side effects of NSAIDs and opiods. Further studies need to done in various other analgesic models along with the human studies to strengthen the results and prove the safety and efficacy of long term administration of aqueous extract of fenugreek seeds as potential analgesic agent in routine clinical practice.

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