ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Analgesic and Anti-Inflammatory Effect of *Foeniculum vulgare.*

Arul Amutha Elizabeth*, Glory Josephine, NS Muthiah, and M Muniappan.

Department of Pharmacology, Sree Balaji Medical College, Chrompet, Chennai, Tamil Nadu, India.

ABSTRACT

To study the analgesic and anti-inflammatory action of *Foeniculum vulgare.* Wistar rats and Swiss Albino mice were used for studying analgesic and anti-inflammatory activity of ethanolic extract of *Foeniculum vulgare,* at doses of 50,100 and 200mgm/kg. Analgesia was studied in albino rats using formalin test and in albino mice using writhing test. Anti-inflammatory activity of the ethanolic extract of *Foeniculum vulgare was* investigated by carrageenan- induced hind paw edema.The ethanolic extract of *Foeniculum vulgare* [50,100 and 200 mgm/kg, i.p] produced significant ($p<0.001$) dose-dependent inhibition of pain response elicited by acetic acid and formalin tests. In respect of anti-inflammatory activity, *Foeniculum vulgare* caused significant ($P< 0.001$) dose dependent inhibition of edema development in the carrgeenan induced inflammation. The effects of the ethanolic extract of *Foeniculum vulgare* were generally comparable to those of the standard drugs used. The findings in this study suggest that the ethanolic extract of *Foeniculum vulgare* possess analgesic and anti-inflammatory activities possibly mediated through central and peripheral mechanisms. These results justify the use of the extract for the treatment of painful and inflammatory conditions.

Keywords: analgesic, anti-inflammatory, *Foeniculum vulgare*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The drugs which are used recently for pain and inflammation are either opiods or non-opioids. Even though these drugs give immediate relief, they produce side effects. Many medicinal plants have been used for a long time for these effects with no adverse effects. It is therefore essential to put efforts to develop new herbal drugs from plants. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs.

Foeniculum vulgare Mill. is a biennial medicinal and aromatic plant belonging to the family Apiaceae (Umbelliferaceae). It is a hardy, perennial–umbelliferous herb with yellow flowers and feathery leaves. The leaves grow up to 40cmlong. The flowers are produced in terminal compound umbels. The fruit is a dry seed 4–10mm long. It is generally considered indigenous to the shores of Mediterranean Sea but has become widely naturalized in many parts of the world especially on dry soils near the seacoast and on the river banks. Some authors distinguish two sub-species of fennel, piperitum and vulgare: sub-species piperitum has bitter seeds, while sub-species vulgare has sweet seeds which are used as flavouring agents in baked goods, meat and fish dishes, ice creams, alcoholic beverages, etc due to their characteristic anise odour. The bulb, foliage and seeds of the fennel plant are widely used in many of the culinary traditions of the world. Dried fennel seed is an aromatic, anise-flavoured spice, brown or green in colour when fresh, slowly turning a dull grey as the seed ages. For cooking green seeds are the best. The bulb is a crisp, hardy root vegetable and may be sauteed, stewed, braised, grilled or eaten raw. Fennel features predominantly in Mediterranean cuisine, where bulbs and fronds are used, both raw and cooked, in side dishes, salads, pastas, vegetable dishes. Many cultures in the Indian subcontinent and the Middle East use fennel seeds in their cooking. Fennel is one of the most important spices in Kashmiri Pandit and Gujarati cooking. [1]

Figure 1

Use in local and traditional medicine

On account of its carminative properties, fennel is chiefly used medicinally with purgatives to allay their side effects and for this purpose forms one of the ingredients of the well known compound liquorice powder. Fennel water has properties similar to those of anise and dill water, mixed with sodium bicarbonate and syrup; these waters constitute the domestic ‘gripe water’, used to correct flatulence of infants. Fennel tea, also employed as a

carminative, is made by pouring boiling water on a teaspoonful of bruised fennel seeds. In the Indian Subcontinent, fennel seeds are eaten raw, sometimes with some sweetener to improve eyesight. Extracts of fennel seeds have been shown in animal studies to have a potential use in the treatment of glaucoma, as a diuretic and a potential drug for the treatment of hypertension.[2]. The fruit and root infusions are used as relaxant, estrogenic, analgesic, and anti-inflammation agent.[3]

Fennel is used in herbal remedies for respiratory tract disorders and indigestion and is also used to increase milk flow in nursing mothers.[4,5] Fennel seeds have been alleviate the symptoms of dysmenorrhoea .[6]

Phytochemistry

F. vulgare has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibre and 42.3% carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin

Essential oil

F. vulgare is well known for its essential oil. The characteristic anise odour of F.vulgare which is due to its essential oil makes it an excellent flavouring agent in baked goods, meat and fish dishes, ice-cream and alcoholic beverages. The major components of *F. vulgare* seed essential oil have been reported to be trans-anethole, fenchone, estragol. The essential oil composition of F.vulgare exhibits considerable chemo diversity depending upon the method of extraction and geographical origin. The accumulation of these volatile compounds inside the plant is variable, appearing practically in any of its parts viz. roots, stem, shoots, flowers and fruits.[7] In one study it was reported that the essential oil content and composition varies during the different maturation stages of *F. vulgare*. The essential oil content was reported to decline with fruit maturity. The content of trans-anethole, the main component, varied between 81.63% and 87.85%. [8]

The other classes of phytochemicals present in *F. vulgare* are phenols and phenolic glycosides.

In spite of the reported uses of *Foeniculum vulgare*, there is no major investigative reports available pertaining to its analgesic and anti-inflammatory action. So this study is taken to evaluate the analgesic and anti-inflammatory action of *Foeniculum vulgare.*

MATERIALS AND METHODS

Collection of plant material

The fennel seeds were purchased from local market and identified by The Director, National Institute of herbal science, Tambaram, Chennai.

Preparation of the plant extract

The fennel seeds were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder (200gms) was taken and soaked in1000ml of ethanol for 72 hours after which the filtrate is obtained and concentrated to dryness at room temperature. The extract is stored at 4ºC for future use.

Phytochemical screening

Freshly prepared fennel extract was subjected to standard phytochemical screening tests for various constituents by standard methods. It showed the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenols, tannins, phytosterols, fixed oils, protein, amino acids, flavanoids, saponins, gums and mucilage.

Animals and treatment regimens

Animals

Albino rats of *Wister strain* (150-200gms), *Swiss albino mice* (15-20gms) were used for the study. Animals were purchased from King Institute of Preventive Medicine, Guindy, Chennai and maintained in the central Animal House, Sree Balaji Medical College and Hospital, Chennai, India. The animals were housed in polypropylene cages with sterilized rice husk as bedding material. The animals had free access to food and water. The care and maintence of the animals were as per the approved guidelines of the Committee for the purpose of control and supervision of Experiments on Animals in India. The animals were fasted overnight before the experiment. The protocol was approved by Institutional Animal ethical committee (IAEC.NO.01/04/2011)

Investigational Drugs and Chemicals

Carrageenan was obtained from sigma, Bangalore; the other drugs like Pentazocin, Diclofenac Sodium, and Ketorolac were obtained from the hospital Pharmacy. All the chemicals and solvents were of analytical grade

Toxicity studies

Acute toxicity study was carried out according to the Organization of Economic Corporation development (OECD) guidelines no 425, *Foeniculum vulgare* was administered i.p in doses of 100,200,400,800, 1000mgm/kg to the group of mice (n=3) and the percentage mortality was recorded for a period of 24 hours. During the first 1 hour after the drug administration, the mice were observed for any gross behavioral change and the parameters observed were hyperactivity, grooming, convulsions, sedation and loss of righting reflex. Respiration, salivation, urination and defecation were also noted. Based on the above toxicity study, direct limit test was done.[9]

Initially a particular dose, on the basis of the above study, was administered to single female rat and the rats were observed for 48 hours with close surveillance up to initial 4

hours. After 48 hours the same dose was administered to 2 more female rats and the observation was done same as for the previous rat. The rats were observed for 14 days and no adverse observation was found morphologically. The weight of the animal was recorded on 7^{th} and 14^{th} day.

Analgesic activity

Acetic acid induced writhing in mice

The animals are divided into five groups of six animals each. Group 1 served as the control, Group 2 was used as the reference standard (Diclofenac Sodium 10mgm/kg).[10] Group 3, Group4, Group5 received the ethanolic extract of *Foeniculum vulgare* in doses of 50mgm/kg, 100mgm/kg, and 200mgm/kg respectively. The experiment was carried out according to the method of Koster et al.1959, Taber et al 1969. [11, 12] Drugs were administered by the intra peritoneal route 30 minutes prior to the injection of acetic acid. Writhing was induced in animals by injecting acetic acid0.5 ml of 0.6% i.p. Each mouse was then put into a big glass cylinder and the total number of writhing episode for a period of 20 minutes after the injection of acetic acid was counted. The percentage of inhibition of the writhing count of the treated group was calculated from the mean writhing count of the control group.

Formalin induced hind paw-licking

The animals are divided into six groups of six animals each. Group 1 served as the control, Group 2 and group 3 was used as the reference standard (Diclofenac Sodium and Pentazocin respectively). Group 4, Group5, Group6 received the ethanolic extract of *Foeniculum vulgare* in doses of 50mgm/kg, 100mgm/kg, and 200mgm/kg respectively.

The formalin induced hind-paw licking was performed as per the method described by Hunskar and Hole. [13] 30 minutes after administration of the treatment drugs by the i.p route, 5% formalin was injected sub-plantar in the right hind paw. The duration of paw licking as an index of nociception was counted in periods of 0-5 minutes (early phase) and 15-30 minutes (late phase).[8].

Anti-inflammatory activity

Carrageenan –induced hind paw edema in rat

The animals are divided into five groups of six animals each. Group 1 served as the control, Group 2 was used as the reference standard (Diclofenac Sodium). Group 3, Group4, Group5 received the ethanolic extract of *Foeniculum vulgare* in doses of 50mgm/kg, 100mgm/kg, and 200mgm/kg respectively. This test was conducted as per the method described by Winter et al. [14]. The mice were fasted overnight. The paw thickness (0 hour) was measured, in millimeters using plethysmometer. The test substances and the standard drugs were administered i.p 30 minutes prior to the administration of carrageenan. Carrageenan 1%was injected subcutaneously into the right hind paw of each mouse. The thickness of injected paw was measured three hours later. From the mean edema volume,

ISSN: 0975-8585

the percentage inhibition of the edema was calculated between the treated and the control group.

Percent (%) inhibition= Vc-Vt× 100/Vc

Where Vc and Vt represent the average paw volume in the control and treated group respectively.

Statistics

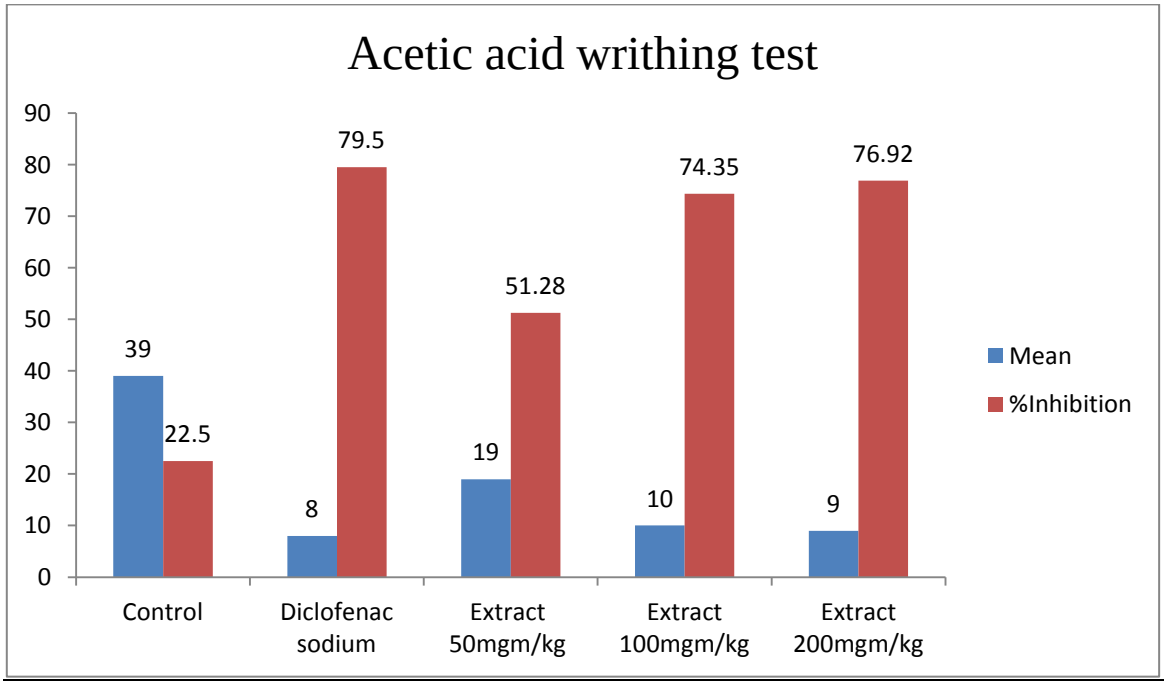
The results were analyzed by One way Analysis of Variance followed by the student Newman Keul's test .The probability of 0.05 or less was considered statistically significant. For the statistical analysis Sigma Stat (SPSS Inc, USA) was used.

RESULTS

Acetic Acid-induced writhing

The results are shown in Table:1, Figure: 2.The writhing counts decreased significantly in the group treated with the ethanolic extract of *Foeniculum vulgare* 200mgm/kg to about 76.92% inhibition, which was comparable to the standard drug Diclofenac Sodium having 79.5% inhibition. The groups treated with *Foeniculum vulgare* 50mgm/kg and 100mgm/kg showed significant reduction in writhing due to acetic acid, the percent of inhibition being 51.28 and 74.35 respectively.

Figure 2: Effect of ethanolic extract of *Foeniculum vulgare* on acetic acid induced writhing test on mice



ISSN: 0975-8585

Table 1: Effect of ethanolic extract of *foeniculum vulgare* on acetic acid induced writhing test on mice

Treatment	Dosage	Mean±SD	%Inhibition
Control	-	39± 2.3	
Diclofenac sodium	10mgm/kg i.p	8± 3.2**	79.5 **
Extract	50mgm/kg i.p	19± 0.9*	51.28*
Extract	100mgm/kg i.p	10±1.4**	74.35*
Extract	200mgm/kg i.p	9±2.1**	76.92**

Values expressed in mean ± SEM, Significant * P <0.01, ** P< 0.001 (n=6).

Formalin induced paw licking

The paw licking phases were divided into two parts, the early phase (0-5 minutes) and the late phase (15-30minutes).In the early phase there was reduction in the number of episodes of licking, and it is stastically significant when compared with the control. The second phase also there is significant reduction in the episodes of licking for the standard drugs Diclofenac sodium and Pentazocin, and also for the ethanolic extract of *Foeniculum vulgare* in doses of 50mgm/kg, 100mgm/kg, and 200mgm/kg. The analgesic effect of *Foeniculum vulgare* 100mgm/kg was comparable with the standard drugs Diclofenac Sodium and Pentazocin. The extract 200mgm/kg is found to be more effective when compared with the standard drugs.

Figure 3: Effect of ethanolic extract of *Foeniculum Vulgare* on formalin induced paw licking in rats

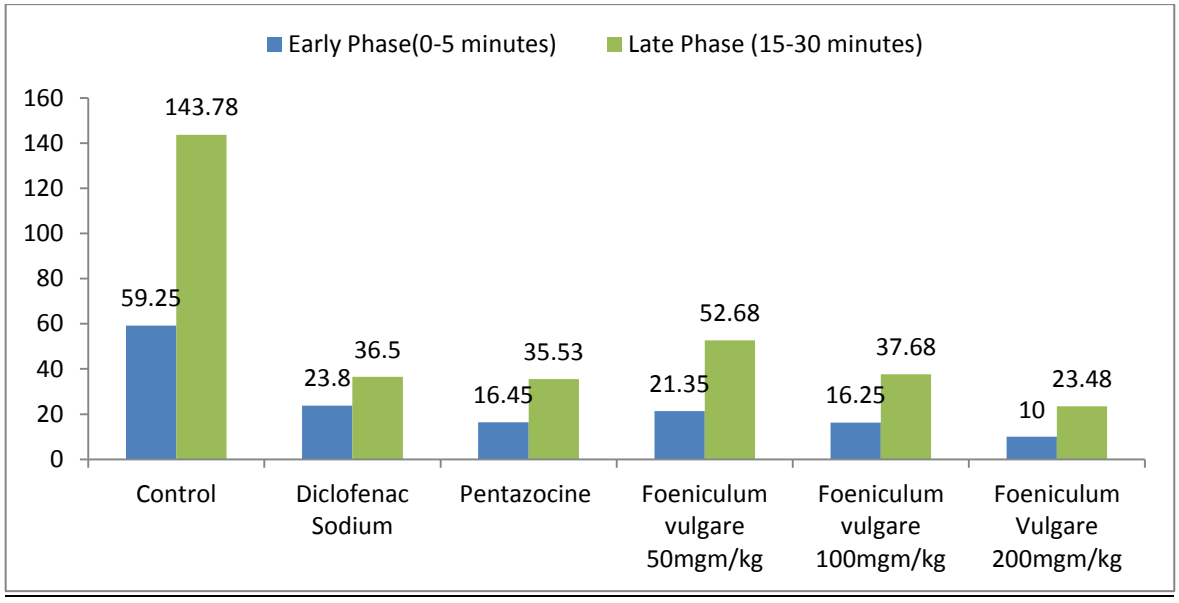


Table: 2. Effect of ethanolic extract of *foeniculum vulgare* on Formalin induced paw licking in rats

Treatment	Dosage	Early Phase(0-5 minutes)	Late Phase (15-30 minutes)
Control	-	59.25±1.07	143.78±1.14
Diclofenac Sodium	10mgm/kg	23.8±1.23**	36.50±2.46**
Pentazocine	5mgm/kg	16.45±1.2**	35.53±0.25**
foeniculum vulgare	50mgm/kg	21.35±1.26*	52.68±1.96*
foeniculum vulgare	100mgm/kg	16.25±2.34**	37.68±1.25*
foeniculum vulgare	200mgm/kg	10±1.22**	23.48±1.25**

Values expressed in mean ± SEM, Significant * P <0.01, ** P< 0.001 (n=6)

ISSN: 0975-8585

Carrageenan-induced paw edema

The results are shown in table: 3, figure: 4&5.The paw edema showed significant reduction in the group treated with standard drug Diclofenac sodium and also with the other treatment groups. The percentage of inhibition for Diclofenac sodium, *Foeniculum vulgare* extract 50mgm/kg, 100mgm/kg and 200mgm/kg are stastically significant. The 100mgm/kg of the extract was comparable with the standard drug and the 200mgm/kg of the extract was found to be more effective than the standard drug.

Figure 4: Effect of ethanolic extract of Foeniculum Vulgare on Carrageenan induced paw edema in rats

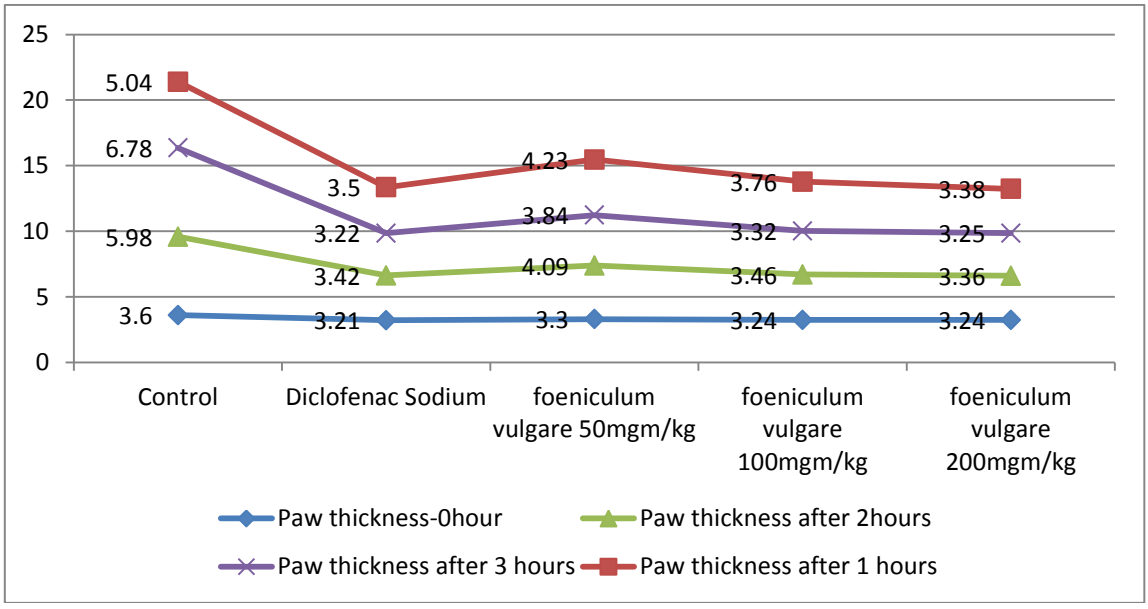
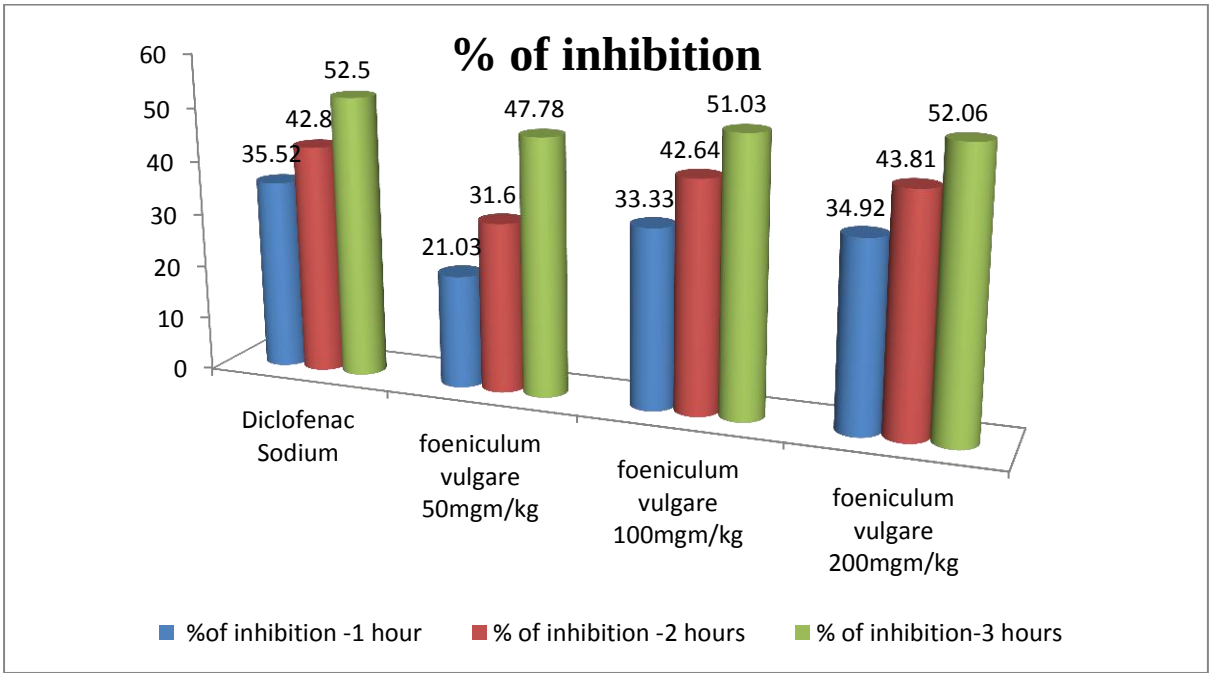


Figure 5



ISSN: 0975-8585

Table 3: Effect of ethanolic extract of *foeniculum vulgare* on Carrageenan induced paw edema in rats

Treatment	Dosage	Initial Paw thickness-0hour	Paw thickness after 1 hours	Paw thickness after 2hours	Paw thickness after 3 hours
Control	-	3.60±0.11	5.04±0.14	5.98± 0.23	6.78±1.23
Diclofenac Sodium	10mgm/kg i.p	3.21±0.01	3.30±0.16 (34.52%)	3.42±0.67 (42.80%)	3.22±2.1 (52.50%)
foeniculum vulgare	50mgm/kg i.p	3.30±0.16	4.23±1.26 (21.03%)	4.09± 1.24 (31.60%)	3.84±1.09 (47.78%)
foeniculum vulgare	100mgm/kg i.p	3.24± 2.05	3.76±0.6 (33.33%)	3.46±0.08 (42.64%)	3.32±1.07 (51.03%)
foeniculum vulgare	200mgm/kg i.p	3.24± 1.16	3.38±0.15 (34.92%)	3.36±2.01 (43.81%)	3.25±0.78 (52.06%)

*=Significant at P<0.05.Significantly different compared with the negative control

DISCUSSION

Natural products of plant origin are used in folk medicine all over the word. They exhibit a wide range of pharmacological activities and may provide relief of symptoms often comparable to that obtained from allopathic medicines. In current decades, traditional herbal medicines have been becoming more and more popular in the world, both for maintaining health and treating chronic complicated or refractory diseases as a complementary or alternative to conventional western medicine. Efficacies of those traditional treatments have been obtained in many cases including chronic inflammatory and arthritic illnesses.

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulse at chemoreceptor's site of pain. While centrally, acting analgesics not only raise the threshold of pain but also alter the physiological response to pain and suppress the patient's unease and anxiety.

Acetic acid induced writhing response in mice is not only the simple and reliable but also affords rapid evaluation of peripherally acting analgesics. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings [15]. Acetic acid is also known to increase PGE1 and PGE2 peripherally. NSAIDs can inhibit COX in peripheral tissue and therefore interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic activity of *Foeniculum vulgare* could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Diclofenac Sodium and NSAIDs. Thus the reduction in the number of writhing indicates that *Foeniculum vulgare* might exert anti-nociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandin.

The formalin- induced paw licking model comprises of early phases and late phase. The early phase (0-5minutes) seemed to be caused by C-fibre activation due to the peripheral stimulus[16,17] The late phase (20-30 minutes) appear to depend on the combination of inflammatory reaction, activation of NMDA receptors and non NMDA

receptors in the peripheral spinal cord[18,19]. In our study the early phase a was more abolished by Pentazocine and the extract *Foeniculum vulgare* 100mgm/kg and 200mgm/kg.In the late phase the extract *Foeniculum vulgare* decreased the reaction time in a dose dependent manner, comparable with the standard drugs diclofenac sodium. The extract in the dose of 200mgm/kg was found to be more effective than the standard drugs in causing analgesic effect. This late phase effect may be due to inactivation of NMDA and non- NMDA receptors. Thus *Foeniculum vulgare* is found to have effect on both central and peripheral pain.

Carrageenan-induced rat paw edema has been a popular inflammatory model to investigate anti-inflammatory effects. The edema induced in the rat paw by the injection of carrageenan is brought about by the autacoids, histamine, and 5-hydroxy tryptamine in the early period after which the kinins act to increase the vascular permeability upto two and a half hours[20,21]. The maximum inflammation is seen approximately three hours post the carageenan injection after it begin to decline, this is caused by the action of prostaglandins. The extract *Foeniculum vulgare* shows a significant inhibition of inflammation, which is comparable to standard drug Diclofenac Sodium.

Toxicity studies have shown that the extract of *Foeniculum vulgare* was safe. The Phytochemical screening of the extract showed the presence of various phyto-constituents like alkaloids, glycosides, carbohydrates, phenols, tannins, phytosterols, fixed oils, protein ,amino acids, flavanoids,saponins, gums and mucilage.

Analgesic and anti-inflammatory effects of flavonoids and tannins have been reported. The flavonoids exhibit potent anti-inflammatory by inhibiting prostaglandin synthesis. These might be responsible for the analgesic and anti-inflammatory activities of the plant extract seen in the study.

The above results therefore support the use of *Foeniculum vulgare* by traditional healers for various forms of pains and inflammatory conditions such as arthritis and rheumatism.

SUMMARY

The *Foeniculum vulgare* which was used as a traditional medicine for the treatment of fever, sprain, rheumatism, dental pain etc is found to be having analgesic and anti-inflammatory activity similar to the standard drugs. This new novel herbal drug product will be useful without any adverse effects.

REFERENCES

[1] Grieve M. 1931. A Modern Herbal: the Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-lore of Herbs, Grasses, Fungi, Shrubs & Trees with their Modern ScientificUses. Brace & Company, Harcourt.
[2] Agarwal et al. Indian J Physiol Pharmacol 2008; 52(1):77–83.
[3] Ozbek H, Uğraş S, et al. Fitoterapia 2003;74: 317-319.
[4] Choi EM and Hwang JK. Fitoterapia 2004;75: 557-565.
[5] Amjad H, Jafary HA. Am J Gastroenterol 2000;95:24915.
[6] Rather M, Dar AB, et al. Arab J Chem 2012.

[7] Diaz- Maroto MC, Diaz- Maroto Hidalgo IJ, Sanchez-Palomo E, Perez-Coello MS. J Agric Food Chem 2005;53(13):5385-5389.
[8] Telci I, Demirtas I, Sachin A. Ind Crops Prod 2009;30:126–130.
[9] OECD (Organisation for Economic Cooperation and Development) 1996.Guideline 423: Acute oral toxicity-Acute toxic class method. Paris: OECD.
[10] Antigona S Hasani et al. ME J Anesth 2011;21(3):355-60.
[11] Koster R, Anderson M, de Bear EJ. Fed Proc 1959;18:412.
[12] Taber RI, Green house DD, Rendell JK, Irwin, S. J Pharm Exp Ther 1969;169:29-38
[13] Hunskar S, Hole K. Pain 1987;30:103-14
[14] Winter CA, Risley, EA, Nuss GW. Proc Soc Exp Bio Med 1962;111:545-547.
[15] Franzotti EM, et al. J Ethnopharmacol 2002; 72:273-8.
[16] Heapy CG, Jamieson A and Russel NJW. Br J Pharmacol 1987; 90:164.
[17] Dickenson AH and Sullivian AF. Pain 1987; 30:349-60.
[18] Coderre TJ, Melzack R. J Neurosci 1992;12:3665 – 70.
[19] Petrenko AB, Yamakura T, Baba H, Shimoji K. Anesth Analg 2003; 97:1108 – 16.
[20] Chang HY, Sheu MJ and Yang CH. E-Cam 2011; 478246:1-8.
[21] Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL and McKenzie KK. Fed Proc 1987; 46(1): 118–26.