Antinociceptive and Anti-Inflammatory Properties of *Daucus carota* Seeds Extract

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The ethanolic extract of *Daucus carota* seeds (DCE) was investigated for anti-inflammatory and analgesic activity at the doses [per oral (p.o.)] of 100, 200 and 400 mg/kg body weight. For evaluation of inflammation carrageenan-, histamine- and serotonin-induced paw edema served as acute models and formaldehyde-induced arthritis served as a chronic model in rats. The acetic acid-induced writhing response and formalin-induced paw licking time in the early and late phases of mice were used to assess analgesic activity. The higher doses of DCE (200 and 400 mg/kg, p.o.) were inhibiting carrageenan, histamine and serotonin-induced paw edema as well as formaldehyde-induced arthritis successfully. In addition, DCE (200 and 400 mg/kg, p.o.) significantly attenuated the writhing responses induced by an intraperitoneal injection of acetic acid and late phase of pain response induced by an subplantar injection of formalin in mice.

Key words ——— *Daucus carota*, antinociceptive, anti-inflammatory, paw edema, writhing

INTRODUCTION

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases.[1] However, studies have been continuing on inflammatory disease and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use.[2] Therefore, development of newer and more anti-inflammatory drugs with lesser side effects is necessary.

Daucus carota Linn. commonly known as "Carrot" belongs to the family Apiaceae (Umbelliferae) and is cultivated almost all over the world as a useful vegetable. Carrot is widely consumed as an aphrodisiac and nervine tonic and its scraped root is used as a local stimulant for indolent ulcers.[3] The plant has undergone extensive phytochemical studies and a large number of active ingredients have been iso-

lated. These include volatile oils, steroids, triterpenes, carbohydrates, glycerides, tannins, flavonoids, amino acid, carotene and hydrocarotene.[4–6] Pharmacological studies showed that *Daucus carota* seeds (DCE) exhibit antifertility properties.[7,8] The crude extract of root of the plant has been found to be hypoglycemic[9] and hepatoprotective.[10] Cholinergic activity was also found in water-soluble fraction of the alcoholic extract of seeds.[11] Different parts of this plant are used in Indian systems of medicine for the treatment of a broad spectrum of aliments including kidney dysfunction, asthma, dropsy, inflammation, leprosy and worm troubles.[12,13] Whether these claims are valid is a subject of great interest and should be probed scientifically. With this aim we carried out an investigation of this plant's potential as an anti-inflammatory and analgesic in rodents.

MATERIALS AND METHODS

Plant Materials ——— The seeds of *Daucus carota* were obtained from local market of Hisar, Haryana (India) in the month of January 2005, which were taxonomically identified and authenticated by The Head, Department of Plant Breeding, Chaudhary Charan Singh Agricultural University, Hisar,

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Haryana (India). A voucher specimen (GJU/PHARM/09/2005) has been preserved at Pharmacology Division of Department of Pharmaceutical Sciences, G. J. University, Hisar, India for ready reference.

Preparation of the Extract —— Collected seeds were powdered in hand grinder and defatted with petroleum ether (b.p. 60–80°C). The defatted seeds (2 kg) were extracted with 95% ethanol using a soxhlet extractor, at room temperature. After exhaustive extraction, the ethanolic extract was filtered and concentrated by distillation process. A brownish-green colored residue was obtained (yield 8.4% w/w), which was kept in a desiccator. This ethanolic extract of DCE was used for further experiments.

Animals —— All the experiments were carried out using male, Swiss Albino mice (25–30 g) and Wistar rats (150–200 g) procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana, India). The animals had free access to food and water, and they were housed in a natural (12 hr each) light-dark cycle. Food given to animals consisted of wheat flour kneaded with water and mixed with a small amount of refined vegetable oil. The animals were acclimatized for at least 5 days to the laboratory conditions before doing experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of committee for the purpose of control and supervision on experiments on animals (CPCSEA), Ministry of Forests and Environment, Government of India (registration number 0436).

Drugs and Chemicals —— The drugs and fine chemicals were purchased from Sigma-Aldrich, U.S.A. All other chemicals and solvents were obtained from local firms (India) and were of highest pure and analytical grade.

Vehicle —— Plant extract (DCE), indomethacin and aspirin were suspended in 0.5% w/v carboxymethylcellulose sodium (CMC) and administered orally to animals. Carrageenan, histamine, serotonin, formaldehyde, acetic acid and formalin diluted separately in normal saline and injected.

Acute Toxicity Studies —— Acute toxicity studies were performed according to organization for economic co-operation and development (OECD/OCDE) guidelines.[14] Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 hr with free access to water only. DCE was administered orally at a dose of 5 mg/kg initially and mortality if any

was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then only higher (50, 300, and 2000 mg/kg) doses of DCE were employed for further toxicity studies.

Acute Anti-Inflammatory Studies —— Carrageenan, histamine and serotonin induced paw edema models were used for evaluating potential of DCE on inflammation. For each model, rats were divided in five groups ($n = 6$). DCE (100, 200, and 400 mg/kg) and indomethacin (10 mg/kg) were administered orally one hour before the subplantar injection of edematogenic agent. The control groups of animals were received vehicle (1 ml/kg) orally. A digital vernier caliper (Model 2061, Mututoyo Digimatic Caliper, Japan) used for measuring paw thickness (mm) of rats.[15,16] Edema (ΔT) was calculated as follows

$$\Delta T = T_t - T_0$$

Where T_t is the right hind paw thickness (mm) at time 't', T_0 is hind paw thickness (mm) before subplantar injection.

Carrageenan Induced Edema in Rats —— In this method, acute inflammation was produced by the subplantar administration of 0.1 ml of 1% w/v carrageenan in the right paw of the rat. The thickness (mm) of the paw was measured immediately and at 1, 2, 3, and 4 hr intervals after the injection of the carrageenan.[15,17–20]

Histamine and Serotonin Induced Edema in Rats —— Edema in rats was induced by injecting 0.1 ml of 0.1% w/v histamine or 0.2% w/v serotonin in subplantar region of the right hind paw. The thickness (mm) of the paw was measured immediately and at 1, 2, 3, and 4 hr intervals after the administration of the histamine or serotonin.[17,21]

Formaldehyde Induced Arthritis in Rats —— Experimental arthritis was induced in male rats according to the method described by Selye.[22] The animals were divided into three groups of six rats each. A subplantar injection of 0.1 ml of 2% v/v formaldehyde was administered to the right hind paw on the first and third day of the experiment. Plant extract (400 mg/kg) or indomethacin (10 mg/kg) or vehicle (0.5% w/v CMC) was administered orally once daily for 10 days. The paw thickness (mm) of each group was measured for 10 days using a digital vernier caliper (Model 2061, Mututoyo Digimatic

Caliper, Japan). On day 3 of the treatment, the paw thickness (mm) was measured before the injection of formaldehyde.[23] Edema (ΔT) was calculated as follows:

$$\Delta T = T_d - T_0$$

Where T_d is the right hind paw thickness (mm) on day 'd', T_0 is hind paw thickness (mm) before subplantar injection.

Acetic Acid-Induced Writhing Response in Mice —— Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster *et al.*[24] Male albino mice were divided in to five groups of six animals each. The animals were pretreated with DCE (100, 200, and 400 mg/kg, p.o.) or aspirin (200 mg/kg, p.o.) used as a standard drug, one hour prior to intraperitoneal injection of 1% v/v acetic acid (0.1 ml/10 g). Five minutes after the intraperitoneal injection of acetic acid, the number of writhing during the following 10 min was counted. Control mice received drugless (0.5% w/v CMC; 10 ml/kg) vehicle.[20,25]

Formalin Test —— The antinociceptive activity of the drugs was determined by using the formalin test described by Dubuisson and Dennis.[26] Male albino mice (overnight fasted, with free access to water) were divided into five groups ($n = 6$). DCE (100, 200, and 400 mg/kg) and aspirin (200 mg/kg, standard drug) were administered orally one hour before formalin injection. Control group received (10 ml/kg) only drugless vehicle (0.5% w/v CMC). One hour before testing, the animal was placed in a standard case ($30 \times 12, \times 13$ cm^3) that served as an observation chamber. 20 μl of 1.0 % v/v formalin injected into dorsal surface of the right hind-paw. The mice were observed for 40 min after the injection of formalin, and the amount of time spent licking the injected hind-paw was recorded. The first 5 min post formalin injection is known as the early phase and the period between 15 and 40 min as the late phase.[27]

Statistical Analysis —— All the results were expressed as mean ± standard error (S.E.). Data was analyzed using one-way analysis of variance test (ANOVA) followed by Dunnett's *t*-test. *p*-values < 0.05 were considered as statistically significant.

RESULTS

Acute Toxicity Studies

All the doses (5, 50, 300, and 2000 mg/kg, p.o.)

of DCE employed for acute oral toxicity studies were found to be non-toxic. *Daucus carota* extract did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. Three submaximal doses (100, 200, and 400 mg/kg, p.o.), which were found to be safe in mice were employed for further psychopharmacological and biochemical investigations.

Carrageenan Induced Edema in Rats

Subplantar injection of carrageenan in rats showed to a time-dependent increase in paw thickness (Fig. 1); this increase was observed at 1h and was maximal at 3 hr after administration of carrageenan injection in the vehicle treated groups. However, carrageenan-induced inflammation was significantly ($p < 0.001$) reduced in all phases of the experiment by treatment with DCE 200 and 400 mg/kg as well as indomethacin. The lower dose of extract (100 mg/kg, p.o.) did not show any considerable change in paw edema as compared with vehicle treated group (Fig. 1).

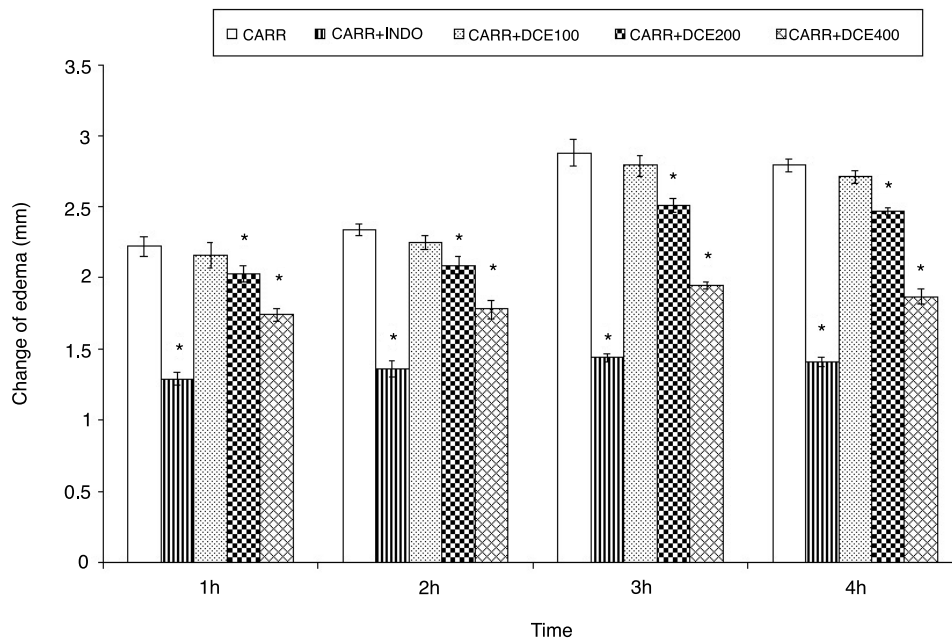**Histamine and Serotonin Induced Edema in Rats**

Figures 2 and 3 show the effects of histamine and serotonin induced paw edema in rats. The maximal paw thickness was observed at 1 hr of after subplantar injection in vehicle treated groups of both the models. The animals treated with higher doses of DCE (200 and 400 mg/kg, p.o.) as well as indomethacin (10 mg/kg, p.o.) produced statistically significant ($p < 0.001$) inhibition of the edema induced by histamine or serotonin in all the phases, when compared to the vehicle treated groups.
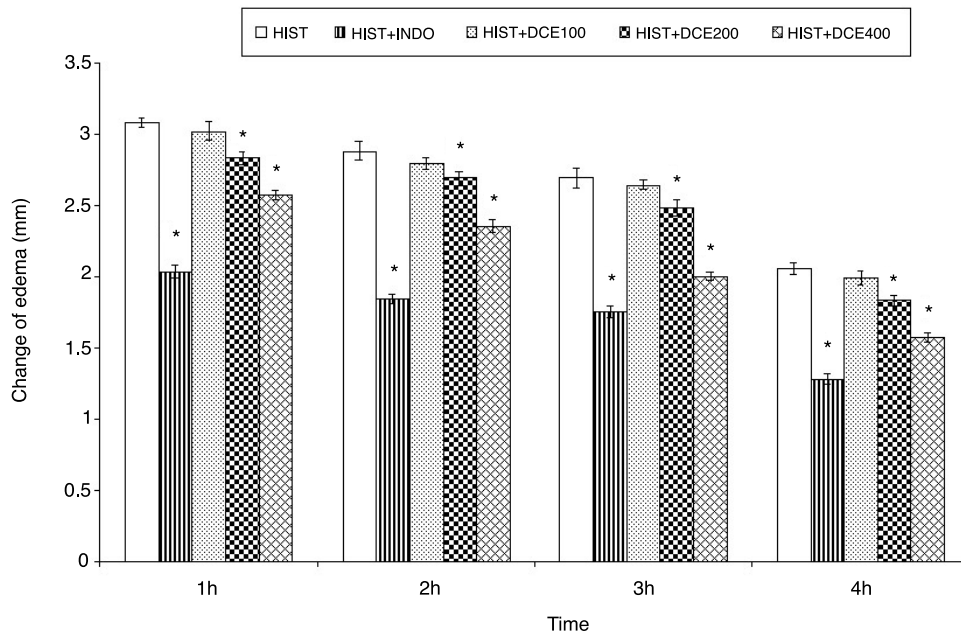
Formaldehyde Induced Arthritis in Rats

Continuous oral treatment (10 days) with plant extract (400 mg/kg) and indomethacin (10 mg/kg) remarkably reduced the paw edema induced by formaldehyde in rats. The significant difference ($p < 0.001$) in paw thickness were observed from first day and throughout the period of the experiment as compared with vehicle treated group (Fig. 4).

Acetic Acid-Induced Writhing Response in Mice

Figure 5 shows the pain behavior of writhing response which was presented as cumulative abdominal stretching response. The treatment of animals with DCE (200 and 400 mg/kg, p.o.) produced a significant ($p < 0.001$) and dose depend inhibition in abdominal writhes produced by acetic acid. The inhibition by DCE (400 mg/kg, p.o.) was nearly simi-

Fig. 1. The Effects of DCE and Indomethacin (INDO) on Rat's Hind Paw Edema Induced by Carrageenan (CARR)
Data represented as mean ± S.E. ($n = 6$). *$p < 0.001$ as compared with the CARR group.
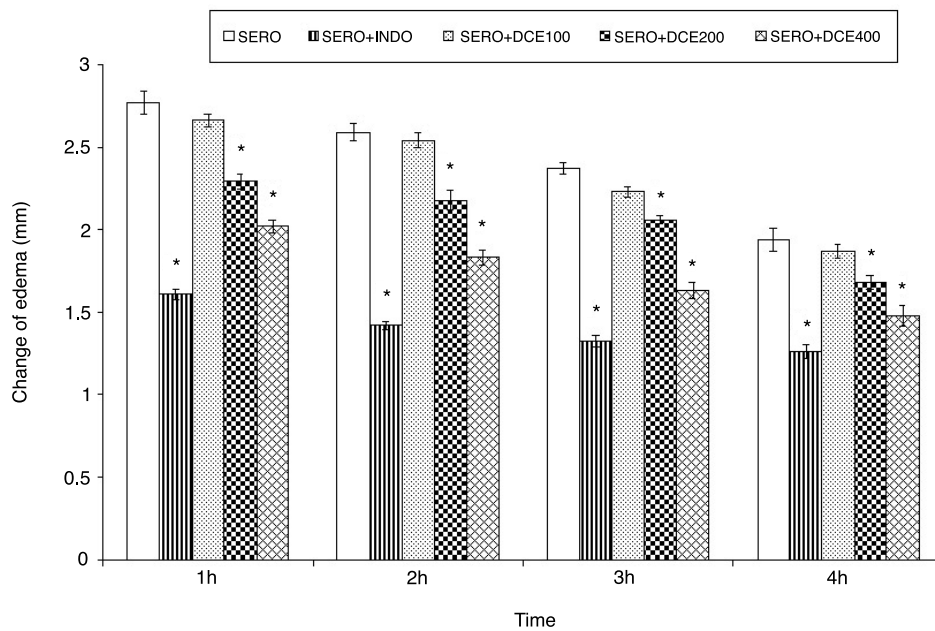

Fig. 2. The Effects of DCE and INDO on Rat's Hind Paw Edema Induced by Histamine (HIST)
Data represented as mean ± S.E. ($n = 6$). *$p < 0.001$ as compared with the HIST group.

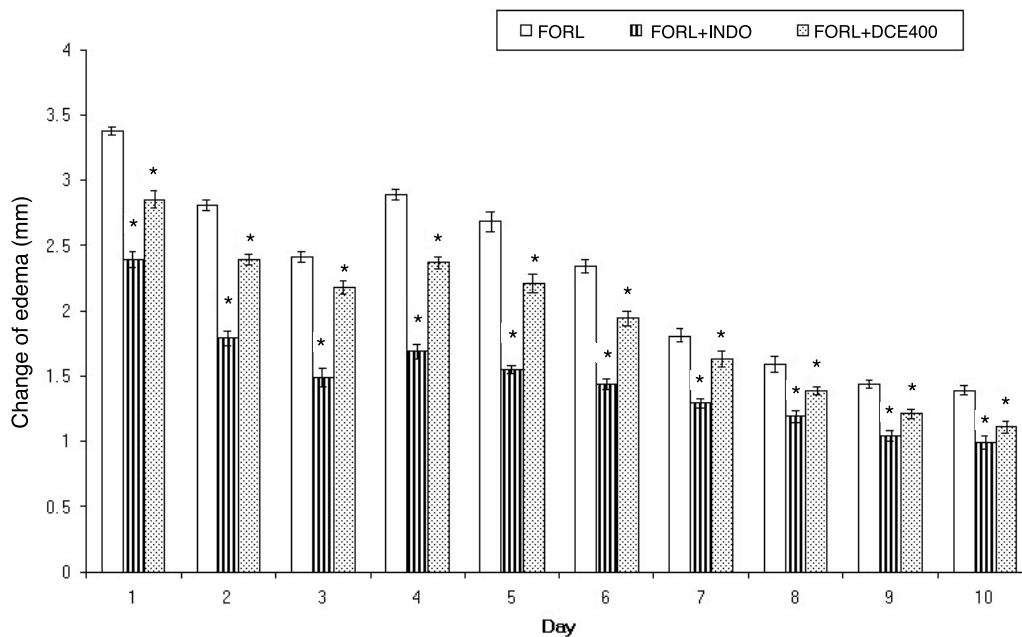lar to that produced by aspirin (200 mg/kg, p.o.), used as a standard drug.

Formalin-Induced Paw Licking in Mice

In Fig. 6, it is shown that pretreatment with aspirin or DCE (100, 200, and 400 mg/kg, p.o.) could not produce any significant changes of paw licking time in the early phase of pain response. However, in the late phase, a dose-dependent and significant ($p < 0.001$) reduction in licking time was observed in mice treated with DCE (200 and 400 mg/kg, p.o.) as well as with aspirin (200 mg/kg, p.o.).

Fig. 3. The Effects of DCE and INDO on Rat's Hind Paw Edema Induced by Serotonin (SERO)
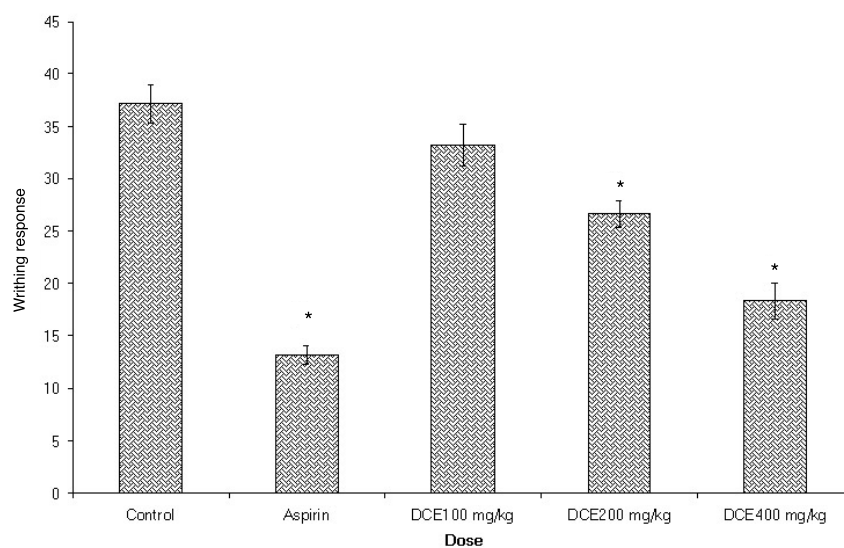Data represented as mean ± S.E. ($n = 6$). *$p < 0.001$ as compared with the SERO group.

Fig. 4. The Effects of DCE and INDO on Rat's Hind Paw Edema Induced by Formaldehyde (FORL)
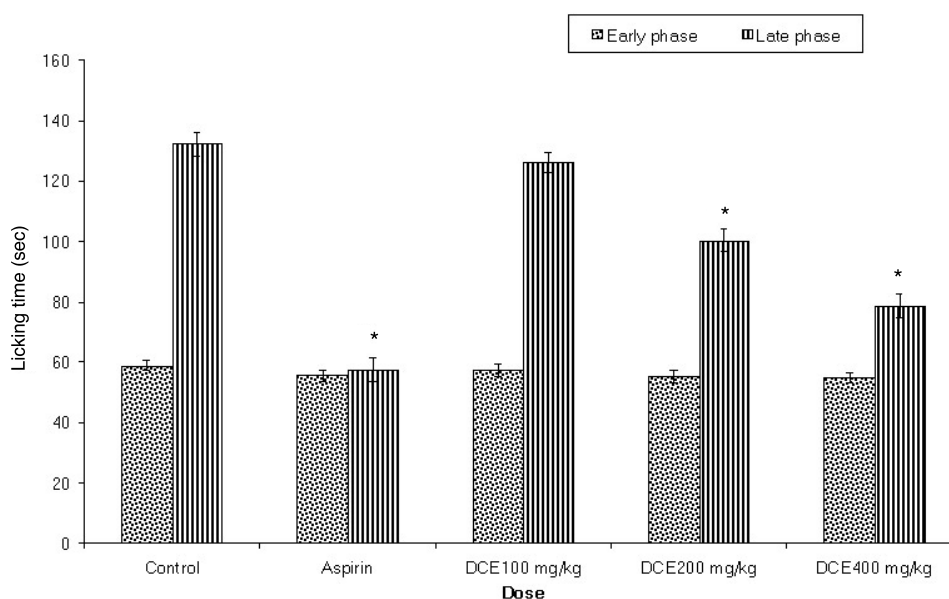Data represented as mean ± S.E. ($n = 6$). *$p < 0.001$ as compared with the FORL group.

DISCUSSION

In the present study, the anti-inflammatory activities of the ethanolic extract of DCE has been established in both acute and chronic inflammation models, which were employed. Carrageenan-in-

duced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products.[28] Development of edema in the paw of the rat after injection of carrageenan is a biphasic event.[29] The initial phase observed during the first

Fig. 5. The Effects of DCE on 1% v/v Acetic Acid-Induced Writhing Response in Mice
Data represented as mean ± S.E. ($n = 6$). $*p < 0.001$ as compared with the control group.

Fig. 6. The Effects of DCE on the Early Phase and Late Phase in 20 μl of 1% v/v Formalin Test in Mice
Data represented as mean ± S.E. ($n = 6$). $*p < 0.001$ as compared with the control group.

hour is attributed to the release of histamine and serotonin.[30] The second phase of edema is due to the release of prostaglandins, protease and lysosome.[29,30] The result of the present study indicates that DCE (200 and 400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation.

Histamine and serotonin are important inflammation mediators and they are potent vasodilator substances as well as increase the vascular permeability.[31–33] This study showed that DCE (200 and 400 mg/kg, p.o.) also effectively suppressed the edema produced by the histamine and serotonin. It is well known that inhibition of formaldehyde induced pedal edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis.[34] Thus formaldehyde-induced arthritis is a model used for the evolution of an agent with anti-inflammatory activity.[35] This experiment is associated with probable anti-inflammatory activity. Injection of formaldehyde subcutaneously into hind

paw of rats produces localized inflammation. DCE (400 mg/Kg, p.o.) and indomethacin (10 mg/kg, p.o.) administered continuously for 10 days successfully inhibited edema induced by formaldehyde.

The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins.[36,37] The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesic. This response is thought to involve local peritoneal receptors.[38,39] The treatment of animals with DCE (200 and 400 mg/kg, p.o.) showed a significant inhibition of the writings which was similar to that of the effect produced by Aspirin (100 mg/kg, p.o.). This result indicates that the analgesic effect of DCE might be mediated by its peripheral effects.

Extract (200 and 400 mg/kg, p.o.) also produced significant inhibition in the late phase of formalin induced pain. The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. The formalin test produced a distinct biphasic response and different analgesics may act differentially in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic.[40] Centrally acting drugs such as opioids inhibit both phases equally.[41] But peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs.[20,42,43] The effect of DCE on the late phase of formalin test suggests that its activity may be resulting from its peripheral action, when compared with aspirin activity in this respect.

In conclusion, the data obtained in this study demonstrated that DCE extract might have analgesic and anti-inflammatory activities. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

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