



Effect of *Cuminum cyminum* L. Seed Extracts on Pain and Inflammation

Sangeeta P. Bhat*, Waseem Rizvi, Anil Kumar

Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh-202002, India.

Abstract

Our objective was to evaluate the analgesic and anti-inflammatory activity of aqueous and ethanolic extracts of *Cuminum cyminum* L. (*C. cyminum*) seeds. Aqueous and ethanolic extracts of *C. cyminum* seeds were prepared using Soxhlet apparatus. Acute toxicity study was performed as per OECD-425 Guidelines and doses of 200 mg/kg and 500 mg/kg of each extract were selected. Swiss Albino mice were used for Acetic-acid induced writhing method and Wistar Albino rats for Eddy's hot plate, Carrageenan-induced paw oedema and Cotton-pellet granuloma methods. The animals were divided into 6 groups (n=6). Group 1 (Distilled water), Group 2 (Standard drug), Group 3 (Aqueous, low dose), Group 4 (Aqueous, high dose), Group 5 (Ethanolic, low dose) and Group 6 (Ethanolic, high dose). Statistical significance ($p < 0.05$) was analyzed using ANOVA with post-hoc Dunnett's test. Both the aqueous and ethanolic extracts showed highly significant analgesic activity in Acetic-acid induced writhing, while the ethanolic extracts, were effective only in Eddy's hot plate method. Both the aqueous and ethanolic extracts showed significant anti-inflammatory activity in Carrageenan-induced paw oedema and Cotton-pellet granuloma models when compared to the control group. In conclusion, the aqueous extracts of *C. cyminum* seeds show predominantly anti-inflammatory activity while the ethanolic extracts show predominantly analgesic activity. However, further evaluation is required for analysis of phytochemical constituents involved in its analgesic and anti-inflammatory activities.

Keywords: Analgesic, anti-inflammatory, aqueous, cumin, ethanolic, seeds

1. Introduction

Cumin (*Cuminum cyminum* L.) is an aromatic plant included in the Apiaceae family and is used to flavor foods, added to fragrances, and for medicinal preparations [1]. In Unani system of medicine, the fruit of *C. cyminum* is used as an astringent, carminative and emmenagogue, for the treatment of corneal opacities, ulcers, boils, styes and to relieve cough and inflammation [2]. In folk medicine, cumin seeds are used for the treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice [3]. In Ayurveda, cumin seeds have long been considered a stimulant, carminative, stomachic, astringent and useful in diarrhea, dyspepsia as well as

in relieving sleeplessness, common cold, and fever [4]. In Iranian traditional medicine, cumin is used for the treatment of toothache, diarrhea and epilepsy [5]. As this plant has been used for relieving painful, inflammatory conditions since ancient times, we decided to investigate its analgesic and anti-inflammatory activity.

2. Materials and Methods

2.1 Plant Materials

The seeds of *C. cyminum* were obtained from the local market, washed and shade-dried. The sample was authenticated by a botanist and the sample voucher specimen number 47592 was obtained.

*Author for correspondence

Email: sweetsan18@yahoo.com

2.2 Preparation of the Extracts

The seeds were finely powdered in a grinder. Aqueous and ethanolic extracts were prepared with the help of a soxhlet apparatus using 100 g of the seeds per extract. The aqueous extract was a light thick, brownish semi-solid material with a yield of 16.48% and the ethanolic extract was a light brown, oily viscous liquid with a yield of 2.66%. The extracts were sealed in an air-tight manner and preserved at 4°C till further usage.

2.3 Experimental Animals

Wistar Albino rats of either sex (100–200 g) and Swiss Albino mice of either sex (18–30 g) were availed from the Central Animal House. They were housed under standard conditions (Temperature = 27 ± 2°C, Humidity = 30–70%) with a 12 hr light-dark cycle. Standard laboratory pellet diet and water ad libitum was provided. The diet was withheld for 12 hours prior to the administration of standard and test drugs. They were acclimatised to laboratory conditions for seven days prior to the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (8335/CAH, dated 16 April 2013) and performed in accordance with NIH guidelines. The animals were grouped as mentioned in Table 1.

2.4 Acetic-acid Induced Writhing

The analgesic activity was evaluated by the method described by Taber et al [6]. Mice of either sex (18-20 gm) were divided as in Table 1. The standard drug

Table 1: Grouping of animals for the experiments

Group (n = 6)	Drug and route (single dose)
Normal control	Distilled water 1 ml/kg p.o.
Standard control	Pentazocine/Aspirin/Diclofenac*
Low dose of aq. ext. of C.cyminum	200 mg/kg p.o.
High dose of aq. ext. of C.cyminum	500 mg/kg p.o.
Low dose of eth. ext. of C.cyminum	200 mg/kg p.o.
High dose of eth. ext. of C.cyminum	500 mg/kg p.o.

*Pentazocine 30mg/kg i.p. for Eddy's Hot Plate method; Aspirin 100 mg/kg p.o. for Carrageenan-induced paw oedema and Cotton-pellet granuloma methods, Diclofenac 5 mg/kg p.o. for Acetic-acid induced writhing method; p.o. – per orum.

used was Diclofenac 5 mg/kg (Tab. Voveran, Novartis, India). The test and control drugs were administered 60 minutes before the injection of 1% acetic acid (G.S. Labs, New Delhi, India) 10 ml/kg i.p. The mice were placed individually into a glass cage for five minutes for acclimatisation. They were, then, observed for a period of ten minutes and the number of writhes was recorded for each animal. A writhe was indicated by characteristic abdominal contraction with extension of at least one hind limb. The % inhibition of writhes was calculated using the formula -

% inhibition =

$$\frac{\text{Av no.of writhes in control group} - \text{Av.no.of writhes in test group}}{\text{Av no.of writhes in control group}} \times 100$$

The animals were rehabilitated by administration of the standard control drug for a week.

2.5 Eddy's Hot Plate

The analgesic activity was evaluated as described by Eddy and Leimbach [7]. Rats of either sex (100-200 gm) were divided as in Table 1. The standard drug used was Pentazocine (Inj. Fortwin, Ranbaxy Lab. Ltd., India) 30 mg/kg i.p. They were placed individually on an electrically heated aluminium plate with a temperature ranging between 55° to 56°C in the analgesiometer (Orchid Scientifics, India) and screened for an initial reaction time of six seconds. Rats responding later than six seconds were discarded. The control and the test drugs were administered orally and the response (licking of the paws) was observed at 0, 15, 30, 60, 90 and 120 minutes. The cut-off time for the reaction was 15 seconds so as to avoid injury to the paw. The plate was wiped clean with saline each time after urination/defecation by rats.

2.6 Carrageenan-induced Paw Oedema

The anti-inflammatory activity was evaluated as described by Winter et al [8]. Rats of either sex (100–200 gm) were divided as in Table 1. The control and the test drugs were administered orally. The standard drug used was Aspirin (Tab. Disprin, Reckitt Benckiser Ltd., India) 100 mg/kg p.o. One hour later, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of Carrageenan (Sigma Aldrich, USA) in normal saline in the left hind paw of the rats. The

paw volumes were measured at zero, one, two and three hours after the Carrageenan injection with the help of Digital Plethysmometer (Orchid Scientifics, India). The percentage inhibition of oedema at each time interval was calculated by using the following formula:-

$$\% \text{ inhibition} = \frac{(Vt - Vo)_{\text{control}} - (vt - vo)_{\text{test}}}{(vt - vo)_{\text{control}}} \times 100$$

Where,

Vo = Paw volume at zero hr

Vt = Paw volume at that particular time interval

2.7 Cotton-pellet Induced Granuloma

The anti-inflammatory activity was evaluated as described by Fukuhara et al [9]. Rats of either sex (100-200 gm) were divided as in Table 1. The control and the test drugs were administered orally. The standard drug used was Aspirin (Tab. Disprin, Reckitt Benckiser Ltd., India) 100 mg/kg p.o. One hour later, the animals were anaesthetized using Inj. Pentobarbitone (Sigma Aldrich, USA) 50 mg/kg i.p. The dorsal skin was shaved and cleaned with alcohol to maintain aseptic conditions. An incision was made in the lumbar region and a subcutaneous tunnel was formed using a blunted forceps. A sterilized cotton pellet (Ten mg, placed in hot air oven at 120°C for two hours) was implanted in each rat. The incisions were sutured by silk 2.0 sutures and Betadine solution applied over wounds to prevent infection.

The animals were treated with fixed doses of drugs once a day for seven days including the day of implantation of pellets. On the eighth day, the animals were anaesthetized and under aseptic conditions, the cotton pellets were removed and made free from extraneous tissues and dried at 60°C for 24 h. Mean weight of granuloma tissue formed around each pellet was evaluated. Percent inhibition was calculated by using the following formula-

$$\% \text{ inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where,

Wc = Weight of the cotton pellets in control animals

Wt = Weight of the cotton pellets in drug treated animals

2.8 Acute Toxicity Study

This study was performed on healthy, adult female rats (150–200 g) as per OECD Guidelines 425. The animals were observed for acute (24 hrs) and subacute (14 days) toxicity.

2.9 Statistical Analysis

The data is expressed as Mean \pm S.E.M. and analyzed using One-way ANOVA with post hoc Dunnett's test. P value <0.05 was considered significant.

3. Results

3.1 Acute Toxicity Study

The LD50 was found to be more than 2000 mg/kg for both aqueous and ethanolic extracts of *C.cyminum*. There was no change in animal behavior/weight either.

3.2 Effect of Extracts of *C.cyminum* Seeds on Acetic-acid Induced Writhing in Mice

The mean number of writhes seen in control group was 31.66 ± 0.66 while Diclofenac sodium provided around 81% ($p < 0.001$) protection in the standard drug group as shown in Fig. 1. The low dose aqueous extract provided around 16% protection with the mean number of writhes being reduced to 26.5 ($p < 0.001$) while the high dose

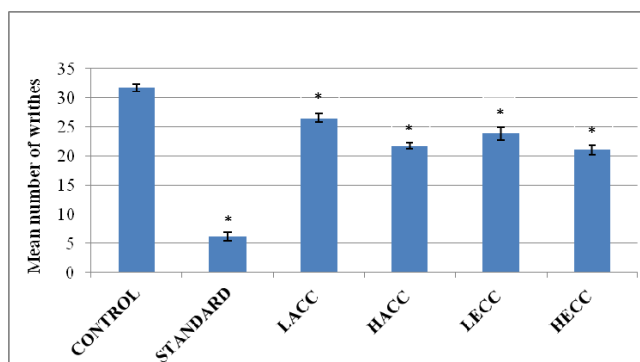


Fig. 1. Analgesic effect of aqueous and ethanolic extracts of *Cuminum cyminum* L. seeds on acetic-acid induced writhing in mice.

* $p < 0.001$; Control – Distilled water 1ml/kg p.o.; Standard – Diclofenac 5mg/kg p.o.; LACC – aqueous extract of *C.cyminum* at 200mg/kg p.o.; HACC – aqueous extract of *C.cyminum* 500mg/kg p.o.; LECC – ethanolic extract of *C.cyminum* at 200mg/kg p.o.; HECC – ethanolic extract of *C.cyminum* at 500mg/kg p.o.; p.o. – per orum.

aqueous extract provided around 32% protection with the mean number of writhes being 21.66 ($p < 0.001$). Both the low and high doses of ethanolic extracts produced highly significant ($p < 0.001$) reduction in the mean number of writhes, 23.83 (24.73%) with the low dose group and 21 (33.67%) with the high dose group.

3.3 Effect of Extracts of *C.cuminum* Seeds on Eddy's Hot Plate Test in Rats

As shown in Table 2, the rats in the control group responded at all intervals (0, 15, 30, 60, 90, 120 min) by six seconds. The Standard group showed highly significant results ($p < 0.001$) at 15, 30, 60, 90 and 120 min with the mean response time being 6.15s, 8.32s, 13.41s, 14.17s

and 13.79s respectively. No significant response was obtained with the aqueous extract groups. The low dose ethanolic group showed a significant ($p < 0.01$) increase in mean response time to 4.85s at 90 min while the high dose ethanolic group increased the same to 4.71s ($p < 0.05$) at 60 min and 4.87s ($p < 0.01$) at 90 min.

3.4 Effect of Extracts of *C.cuminum* Seeds on Carrageenan-induced Oedema in Rats

As shown in Table 3, the rats in the control group showed gradually increasing oedema development from 0.15 ml at 1 hour, 0.23ml at 2 hours and 0.31ml at 3 hours. The Aspirin group showed significant inhibition of oedema development by 40% at one hour, 65% at two hours and

Table 2: Analgesic effect of aqueous and ethanolic extracts of *Cuminum cyminum* L. seeds on Eddy's hot plate in rats

Group (n = 6)	Mean Reaction time (s)					
	0min	15min	30min	60min	90min	120min
Control	4.04 ± 0.24	4.10 ± 0.09	4.03 ± 0.01	4.00 ± 0.26	4.04 ± 0.08	4.10 ± 0.03
Pentazocine 30mg/kg	3.93 ± 0.10	6.15 ± 0.08***	8.32 ± 0.15***	13.41 ± 0.64***	14.17 ± 0.33***	13.79 ± 0.33***
AECC 200mg/kg	3.71 ± 0.15	4.05 ± 0.18	4.24 ± 0.15	4.13 ± 0.11	3.99 ± 0.04	3.65 ± 0.25
AECC 500mg/kg	3.75 ± 0.19	3.70 ± 0.10	4.42 ± 0.15	4.59 ± 0.13	3.82 ± 0.13	3.84 ± 0.10
EECC 200mg/kg	3.86 ± 0.11	3.75 ± 0.08	3.85 ± 0.11	4.58 ± 0.19	4.85 ± 0.10**	4.64 ± 0.21
EECC 500mg/kg	3.88 ± 0.08	3.92 ± 0.08	4.07 ± 0.09	4.71 ± 0.16*	4.87 ± 0.25**	4.63 ± 0.12

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Control – Distilled water 1ml/kg p.o.; AECC – aqueous extract of *C.cuminum*; EECC – Ethanolic Extract of *C.cuminum*; p.o. – per orum.

Table 3: Anti-inflammatory effect of aqueous and ethanolic extracts of *Cuminum cyminum* L. seeds on Carrageenan-induced paw oedema in rats

GROUPS (n = 6)	Mean Change in paw volume (ml) with % inhibition of oedema		
	1 HOUR	2 HOUR	3 HOUR
CONTROL	0.15 ± 0.01	0.23 ± 0.00	0.31 ± 0.01
ASPIRIN 100 mg/kg	0.09 ± 0.02 (40.00%)	0.08 ± 0.01*** (65.21%)	0.08 ± 0.01*** (74.19%)
AECC 200mg/kg	0.08 ± 0.00* (46.66%)	0.12 ± 0.02*** (47.82%)	0.09 ± 0.01*** (70.96%)
AECC 500mg/kg	0.08 ± 0.01* (46.66%)	0.11 ± 0.00*** (52.17%)	0.07 ± 0.01*** (77.41%)
EECC 200mg/kg	0.13 ± 0.01 (13.33%)	0.17 ± 0.02** (26.08%)	0.16 ± 0.02*** (48.38%)
EECC 500mg/kg	0.13 ± 0.02 (13.33%)	0.13 ± 0.01** (43.47%)	0.15 ± 0.01*** (51.61%)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Control – Distilled water 1ml/kg p.o.; AECC – aqueous extract of *C.cuminum*; EECC – ethanolic extract of *C.cuminum*; p.o. – per orum.

74% at three hours. Both the low and high aqueous doses produced significant inhibition in the development of oedema from the very first hour. The low dose group exhibited significant inhibition of oedema by 46.66% ($p < 0.05$), 47.82% ($p < 0.001$) and 70.96% ($p < 0.001$) when compared to the control group at the 1st, 2nd and 3rd hours respectively while, the high dose group inhibited oedema by 46.66% ($p < 0.05$), 52.17% ($p < 0.001$) and 77.41% ($p < 0.001$) at the 1st, 2nd and 3rd hours respectively.

The low dose ethanolic group demonstrated a 26.08% ($p < 0.01$) and 48.38% ($p < 0.001$) inhibition at the 2nd and 3rd hours respectively while the high dose ethanolic group inhibited the development of oedema by 43.47% ($p < 0.01$) and 51.61% ($p < 0.001$) at the 2nd and 3rd hours respectively when compared to the control group.

3.5 Effect of Extracts of *C. cyminum* Seeds on Cotton-pellet Induced Granuloma in Rats

The mean weight of the granuloma in the control rats was found to be 31.70 ± 1.05 mg while in the rats given the Standard drug, the mean weight of the granuloma was 17.44 ± 1.20 mg indicating a significant reduction ($p < 0.001$) in the inflammation by 44.98% as shown in Fig. 2. The low dose aqueous group exhibited a 16.52%

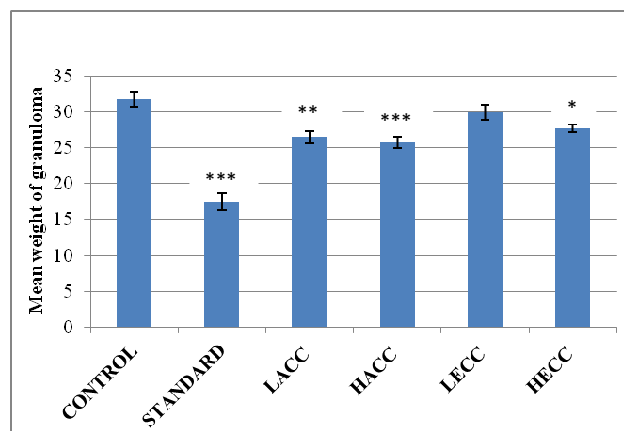


Fig. 2. Anti-inflammatory effect of aqueous and ethanolic extracts of *Cuminum cyminum* L. seeds on Cotton-pellet induced granuloma in rats.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Control – Distilled water 1ml/kg p.o.; Standard – Aspirin 100mg/kg p.o.; LACC – aqueous extract of *C. cyminum* at 200mg/kg p.o.; HACC – aqueous extract of *C. cyminum* 500mg/kg p.o.; LECC – ethanolic extract of *C. cyminum* at 200mg/kg p.o.; HECC – ethanolic extract of *C. cyminum* at 500mg/kg p.o.; p.o. – per orum.

($p < 0.01$) inhibition in granuloma development while the high dose group showed an 18.80% ($p < 0.001$) inhibition when compared to the control group. Only the high dose ethanolic extract inhibited the development of granuloma significantly ($p < 0.05$) by 12.68% when compared to control.

4. Discussion

The current research article deals with the analgesic and anti-inflammatory activities of the aqueous and ethanolic extracts of *C. cyminum* seeds in established animal models.

This is probably the first paper demonstrating the analgesic effects of the aqueous and ethanolic extracts of *C. cyminum*. Regarding the analgesic activity, all the extracts demonstrated significant results in the Acetic acid induced writhing test. Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P from the nerve endings leading to abdominal constrictions [10, 11]. Srivastava et al [12] demonstrated the inhibition of Thromboxane A2 synthesis by ether extract of *C. cyminum* with a simultaneous increase in the formation of Lipoxygenase derived products. Lorenzetti et al [13] established that the essential oil of *C. cyminum* possessed a compound called Myrcene, which was responsible for the analgesic effect in Prostaglandin-induced hyperalgesia in the rat paw. Findings similar to those in our study were reported by Sayyah et al [14] who found significant analgesic activity of the essential oil of fruit of *C. cyminum* in the doses of 0.0125 and 0.20ml/kg, in the late phase of Formalin induced pain model. Thus, there is a strong possibility that the analgesic effect produced by *C. cyminum* extracts may have an Aspirin-like effect, involving the inhibition of COX enzyme.

Significant results were only obtained with the ethanolic extracts of *C. cyminum* in Eddy's hot plate model, thus, indicating that the analgesic action of the extracts might be due to the potentiation of the opioid pathway. Our findings are in contrast to the report by Sayyah et al [14] where in no significant result of the essential oil of *C. cyminum* was obtained by the tail flick model. Another major component of Cumin is Linalool [5]. Paena et al [15] reported significant increase in response times in Eddy's hot plate method at 100 mg/kg

which was completely reversed by Naloxone. The analgesic activity was also attributed to the significant inhibition of iNOS enzyme by Linalool [16]. Thus, the analgesic effect produced by *C.cyminum* extracts may also be due to their Opioid-like effect and decrease in Nitric oxide production and release.

With respect to the findings of *C.cyminum* extracts on inflammatory models, it can be mentioned that the aqueous extracts have a more significant effect on acute inflammation as shown in the Carrageenan-induced oedema model and subacute inflammation as shown in the Cotton-pellet granuloma model as compared to their ethanolic counterparts.

Carrageenan induced paw oedema is a biphasic event [17–19]. The significant anti-inflammatory activity of the aqueous and ethanolic extracts of *C.cyminum* might suggest that they have an inhibitory action on the release of Prostaglandins. Additionally, the aqueous extracts might be acting in the early phase of inflammation by inhibiting the release of histamine, serotonin and kinins. The presence of monoterpene compounds, linalool, γ -terpinene α -pinene and β -pinene [5] contribute largely to the anti-inflammatory activity of the plant [20]. Our findings are in accordance with those of Shivakumar et al [2] who reported significant anti-inflammatory activity of the essential oil of fruit of *C.cyminum* in Carrageenan-induced paw oedema in rats at the 2nd, 4th and 6th hours.

This paper is also the first to report the effect of *C.cyminum* extracts on subacute inflammation by Cotton-pellet induced granuloma model. The anti-inflammatory activity was in the following order of significance – aqueous high-dose > aqueous low dose > ethanolic high dose > ethanolic low dose. Thus, the extracts inhibit the proliferative phase of inflammation as well.

There are also several reports on the anti-oxidant properties of this plant [4, 21, 22]. Apart from the specific phytochemical constituents, this property may also contribute to the anti-inflammatory activity by combating the free radicals generated due to injury.

Thus, we would conclude this study by reporting that the extracts of *C.cyminum* possess significant analgesic and anti-inflammatory activities, the aqueous extracts being more strongly anti-inflammatory and the ethanolic extracts being more strongly analgesic. However, further

studies are required to support the above findings and establish a correlation with the phytochemical constituents of this plant.

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