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Research Article

CORIANDRUM SATIVUM ON PAIN AND INFLAMMATION

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ABSTRACT

Objective- To evaluate the analgesic and anti-inflammatory activities of aqueous and ethanolic extracts of Coriandrumsativum (C.sativum) seeds. **Material and methods -** The aqueous and ethanolic extracts of C.sativum seeds were prepared using Soxhlet apparatus. After an acute toxicity study performed in a previous study as per OECD-425 Guidelines, doses of 250mg/kg and 500mg/kg of each extract were selected. Swiss albino mice were used for Acetic-acid induced writhing method and Wistar albino rats for Carrageenan-induced paw oedema and Cotton-pellet granuloma methods. Statistical significance ($p<0.05$) was analyzed using ANOVA with post-hoc Dunnett's test. **Results -** Both the aqueous and ethanolic extracts of C.sativum seeds showed significant analgesic activity in Acetic-acid induced writhing method and significant anti-inflammatory activity in Carrageenan-induced paw oedema while only the high-dose aqueous group exhibited significant results in Cotton-pellet granuloma model when compared to the respective control group. **Conclusion -** The aqueous and ethanolic extracts of C.sativum seeds demonstrated significant analgesic and anti-inflammatory activities. However, further evaluation is required for analysis of phytochemical constituents involved in these activities.

Keywords: Coriandrum, seeds, analgesic, anti-inflammatory, aqueous, ethanolic.

INTRODUCTION

Coriander (*Coriandrum sativum* Linn.),belonging tofamily Umbelliferae, is a highly reputed medicinal herb. It originated from the Mediterranean and is now cultivated all over India, Italy, Netherlands, Central and Eastern Europe, China and Bangladesh[1].

It is used in the preparation of many household medicines to cure cold, seasonal fever, nausea, vomiting, stomach disorders and also as a drug for indigestion, helminthic infestations, rheumatism and pain in the joints. Also, in some parts of India, it has traditionally been used for its anti-inflammatory properties[2]. In Ayurvedic system of medicine, it has been used to treat local swelling and pain, headache, burning sensation, lymphadenopathy, stomatitis, conjunctivitis, vertigo, syncope, memory loss, digestive disorders, bleeding disorders, cough, dyspnoea and as a diuretic[3-5].

We have already demonstrated that the aqueous and ethanolic extracts of C.sativum at 100, 250 and 500mg/kg p.o. have a significant and dose-dependent effect on reaction time in rats by Eddy's hot plate method,[6] but the predominant use of *Coriandrum sativum L.* (C.sativum) in painful, inflammatory conditions motivated us to further investigate the analgesic and anti-inflammatory potential of its seeds.

MATERIALS AND METHODS

Plant material - The seeds of C.sativum were obtained from the local market, washed and shade-dried. The sample was authenticated by a botanist and the sample voucher specimen number 47796was obtained.

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 100g of the seeds per extract. The aqueous extract was a lightthick, brownish semi-solid material with a yield of 27.10% and the ethanolic extract was an oily, greenish-brown semi-liquid material with a yield of 17.42%. The extracts were sealed in an air-tight manner and preserved at 4ºC till further usage.

Experimental animals - Wistar albino rats of either sex (100-200g) and Swiss albino mice of either sex (18-30g) were obtained from the Institutional Central Animal House, housed under standard conditions (Temperature = 27 ± 2^0C, 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 7 days prior to the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (8335/CAH, dated 16 April 2013) and performed as per CPCSEA guidelines.

Experimental design - The animals were divided into 6 groups (n=6). Group 1 (Distilled water 1ml/kg p.o.), Group 2 (Standard drug - 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), Group 3 (Aqueous, 250mg/kg p.o.), Group 4 (Aqueous, 500mg/kg p.o.), Group 5 (Ethanolic, 250mg/kg p.o.) and Group 6 (Ethanolic, 500mg/kg p.o.).

Acetic-acid induced writhing

The method followed was as described by Taber et al, 1969[7]. The standard drug used was Diclofenac 5mg/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 5 min for acclimatisation and observed for a period of 10 min. The number of writhes was recorded for each animal. A writhe was indicated by characteristic abdominal contraction with stretching of atleast one hind limb. The % inhibition of writhes was calculated using the formula

% inhibition =

$$\left(\frac{Avno.of\,writhesincontrolgroup - Av.no.of\,writhesintestgroup}{Avno.of\,writhesincontrolgroup}\right) X\ 100$$

Carrageenan-induced paw oedema

The anti-inflammatory activity was evaluated as described by Winter et al, 1962[8]. The control and the test drugs were administered orally. The standard drug used was Aspirin (Tab. Disprin, Reckitt Benckiser Ltd., India) 100mg/kg p.o. One hour later, 0.1ml of freshly prepared 1% suspension of Carrageenan(Sigma Aldrich, USA) in normal saline was injected in the sub-plantar region of the left hind paw of the rats. The paw volumes were measured at 0, 1, 2 and 3 hours later by a Digital Plethysmometer (Orchid Scientifics, India). The percentage inhibition of oedema at each time interval was calculated by using the following formula

% inhibition = $\left(\frac{(Vt-Vo)control-(Vt-Vo)test}{(Vt-Vo)control}\right) X\ 100$

Where,

Vo = Paw volume at 0 hr

Vt = Paw volume at that particular time interval

Cotton-pellet induced granuloma

The method used was as described by Fukuhara and Tsurufuji, 1969[9]. The standard drug used was Aspirin (Tab. Disprin, Reckitt Benckiser Ltd., India) 100mg/kg p.o. One hour after oral administration of the drugs, the animals were anaesthetized using Inj. Pentobarbitone (Sigma Aldrich, USA)50mg/kg i.p. The dorsal skin was shaved and cleaned with alcohol to maintain aseptic conditions. An incision was made in the lumbar region, a subcutaneous tunnel was formed using a blunted forceps and a sterilized cotton pellet (10 mg, placed in hot air oven at 120^{0}C for 2 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 7 days including the day of implantation of pellets. On the eighth day, the cotton pellets were removed under anaesthesia and aseptic conditions, made free from extraneous tissues and dried at 60˚C for 24 h. Meanweight of granuloma tissue formed around each pellet was evaluated. Percent inhibition was calculated by using the following formula

% inhibition = $\left(\frac{Wc-Wt}{Wc}\right) X\ 100$

Where,

Wc = Weight of the cotton pellets in control animals

Wt = Weight of the cotton pellets in drug treated animals

Statistical analysis

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

RESULTS

Acute Toxicity Study - The LD50 was found to be more than 2000mg/kg for both aqueous and ethanolic extracts of C.sativum. There

was no change in animal behavior/weight either[6].

Effect of extracts of C. sativumseeds 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 Figure 1. Both the low and high doses of aqueous extracts produced highly significant (p<0.001) reduction in the mean number of writhes, 10.5 (66.84%) with the low dose group and 7.17 (77.35%) with the high dose group while, only the high-dose ethanolic group, inhibited writhing significantly by 22.62% with a mean of 24.5 writhes (p<0.001).

Effect of extracts of C. sativumseeds on Eddy's Hot Plate test in rats

The results as published previously in one of our publications[6] have been shown in Table 1. The rats in the control group responded at all intervals by 6 seconds. The Standard group showed highly significant results (p<0.001) at 15, 30, 60, 90 and 120 min. The low and high dose aqueous extracts showed significant results at 15, 30 and 60mins with increase in mean response times being 5.11s (p<0.01), 5.92s (p<0.001) and 5.18s (p<0.05) with the low dose group and 5.25s (p<0.01), 6.10s (p<0.001) and 5.24s (p<0.05) with the high dose group. The peak effect was seen at 30mins.

The ethanolic extracts showed peak analgesic activity at 60 min, the low dose significantly increasing the mean response time (p<0.001) to 6.52s and 6.09s at 60 and 90 min respectively and the high dose increasing the mean response time to 5.27s (p<0.05), 6.75s (p<0.001), 6.55s (p<0.001) and 4.79s (p<0.01) at 30, 60, 90 and 120 min respectively. The ethanolic extract at 500mg/kg produced maximum increase in mean response time among all extracts.

Effect of extracts of C. sativumseeds on Carrageenan-induced oedema in rats

As shown in Table 2, the rats in the control group showed gradual increase in paw volume upto 3 hours. The Aspirin group showed significant inhibition of oedema development by 40% at 1 hour, 65% at 2 hours and 74% at 3 hours. The aqueous extract, only in the high dose, significantly inhibited the development of oedema at the 3rd hour with the mean change in paw volume being 0.14ml (54.83%). The mean change in paw volume with the low dose ethanolic group was 0.15ml indicating a 51.61% inhibition (p<0.001) at the 3rd hour and with the high dose ethanolic group was 0.13ml (43.47%) and 0.04ml (87.09%) at the 2nd (p<0.001) and 3rd (p<0.001) hours respectively.

Effect of extracts of C. sativumseeds on Cotton-pellet induced granuloma in rats

The mean weight of the granuloma in the control rats was found to be 31.70 ± 1.05mg while the Standard drug limited the mean weight of the granuloma to 17.44 ± 1.20mg indicating a significant 44.98% (P<0.001) reduction in the inflammation as shown in Figure 2. Only the high dose aqueous extract treated group demonstrated significant inhibition of the proliferative phase of inflammation by limiting the mean weight of the granuloma to 25mg (p<0.01).

DISCUSSION

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

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]. Both the aqueous and ethanolic extracts of C.sativum produced significant inhibition of writhing, although, the aqueous extracts were more effective. There is only one report on the activity of C.sativum on acetic acid-induced writhing so far, where significant activity was noted with the essential oil but not with the hydroalcoholic and flavonoid extracts of its seeds[12]. A major monoterpene compound called Linalool is reported to be present in C.sativum[12,13]. Linalool, by itself, exhibited significant inhibition of writhing at doses of 25, 50, 75 and 100 mg/kg[14]. The mechanism of Linalool, when further studied, was reported to be due to inhibition of iNOS enzyme and reduction in NO production and release[15]. Thus, the analgesic action might be attributed to its inhibition of NO production and Aspirin-like effect by inhibition of COX enzyme.

Both the aqueous &ethanolic extracts of C.sativum showed significant results in Eddy's hot plate test. Our findings corroborate with other studies where analgesic activity of aqueous extracts of seeds were studied using thermal pain models[16,17]. Opiate receptors are probably involved in the mechanism of analgesia of C.sativum as administration of the opioid receptor antagonist, Naloxone, reversed the anti-nociceptive effect and this was partially attributed to the presence of Linalool[17]. Therefore, certain constituents of C.sativum extracts like Linalool may be exhibiting an opioid-like effect.

Carrageenan induced paw oedema is a biphasic event[18-20]. The ethanolic extracts demonstrated better anti-inflammatory activity as compared to their aqueous counterparts. Additionally, the high dose ethanolic extract might be acting in the early phase of inflammation by inhibiting the release of histamine, serotonin and kinins and later may have an inhibitory action on the release of Prostaglandins. Junior et al, 2011[21] reported significant anti-inflammatory activity of hydroalcoholic extracts of leaves of C.sativum at doses of 200, 400 and 600 mg/kg p.o. in Carrageenan-induced pleurisy and croton-oil induced ear edema methods, while Hashemi et al, 2003[12] reported no significant anti-inflammatory effect of C.sativum essential oil on Carrageenan-induced paw edema. Moreover, ethanolic extracts from both stem and leaf of C. sativum demonstratedsignificant reduction in Lipopolysaccharide (LPS) induced nitric oxide and prostaglandin E2 production as well as inducible nitric oxide synthase, cyclooxygenase-2, and pro-interleukin-1β expression, indicating a strong anti-inflammatory property by suppressing NF-κB activation and MAPK signal transduction pathway in LPS-induced macrophages[22]. Linalool, at doses of 25, 50 and 75 mg/kg body wt.s.c. significantly inhibitedCarrageenin induced paw oedema in rats at the 1st, 3rd and 5th hours[23].The presence of γ-terpinenein C.sativum[2] was found to significantly inhibit ovine cyclooxygenase 1 and 2 enzymes[24].

This paper is also the first to report the effect of C.sativum extracts on subacute inflammation by Cotton-pellet induced granuloma model. Since, the anti-inflammatory activity was significant with the aqueous high-dose group, it might be that the amount of Linalool is more in the aqueous extract of C.sativum as compared to the ethanolic and this may be responsible for inhibiting the proliferative phase of inflammation as well.

There are also several reports on the anti-oxidant properties of this plant[25-28]. 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.

CONCLUSION

We would, therefore, conclude this study by reporting that the extracts of C.sativum possess significant analgesic and anti-inflammatory activities. However, further studies are required to support the above findings and establish a correlation with the phytochemical constituents of this plant, especially because of its rich history of use in painful, inflammatory conditions.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

Table 1: It shows the analgesic effect of aqueous and ethanolic extracts of *Coriandrum sativum* seeds on Eddy's hot plate method in rats

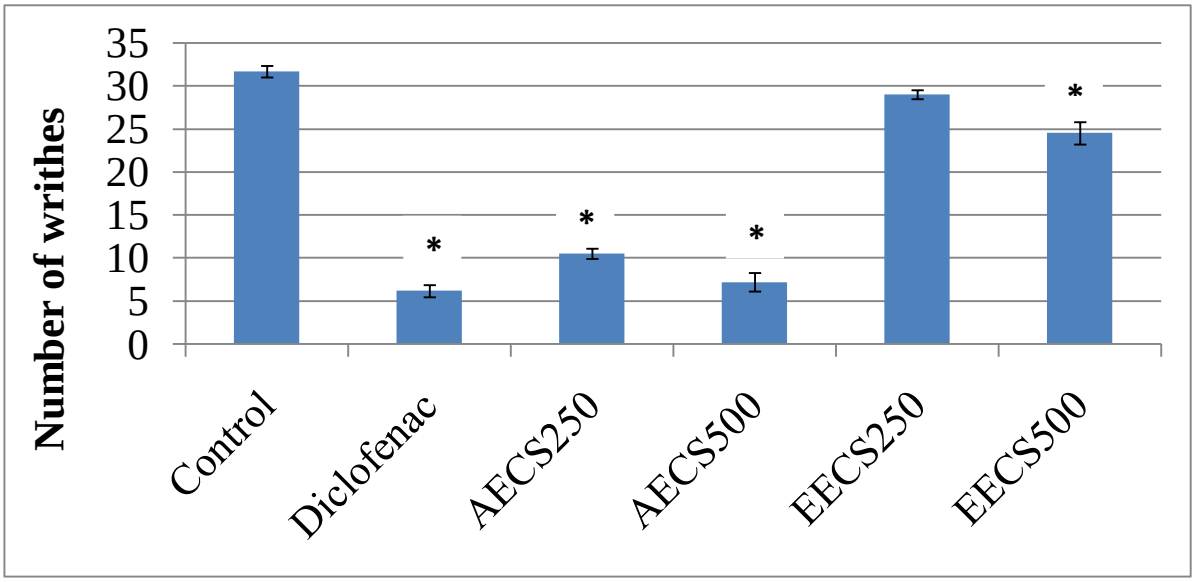
Group (n=6)	Mean Reaction time (s)					
	0min	15min	30min	60min	90min	120min
Control	4.04±0.24	4.10±0.09	4.03±0.01	4.00±0.26	4.04±0.08	4.10±0.03
Pentazocine 30mg/kg	3.73±0.33	8.32±0.75***	12.92±1.53***	14.17±0.82***	14.55±0.44***	13.41±0.74***
AECS 250mg/kg	4.09±0.31	5.11±0.13**	5.92±0.21***	5.18±0.16*	4.38±0.14	4.27±0.12
AECS 500mg/kg	3.93±0.10	5.25±0.21**	6.10±0.18***	5.24±0.24**	4.61±0.10	4.33±0.10
EECS 250mg/kg	3.93±0.10	4.21±0.14	4.99±0.35	6.52±0.31***	6.09±0.39***	4.38±0.13
EECS 500mg/kg	3.74±0.11	4.30±0.13	5.27±0.20*	6.75±0.16***	6.55±0.18***	4.79±0.12**

*P<0.05, **P<0.001, ***P<0.001 when compared to control. Control – Distilled water 1ml/kg p.o.; AECS – aqueous extract of C.sativum; EECS – ethanolic extract of C.sativum.

Table 2: It shows the anti-inflammatory effect of aqueous and ethanolic extracts of *Coriandrum sativum* seeds on Carrageenan-induced paw oedema in rats

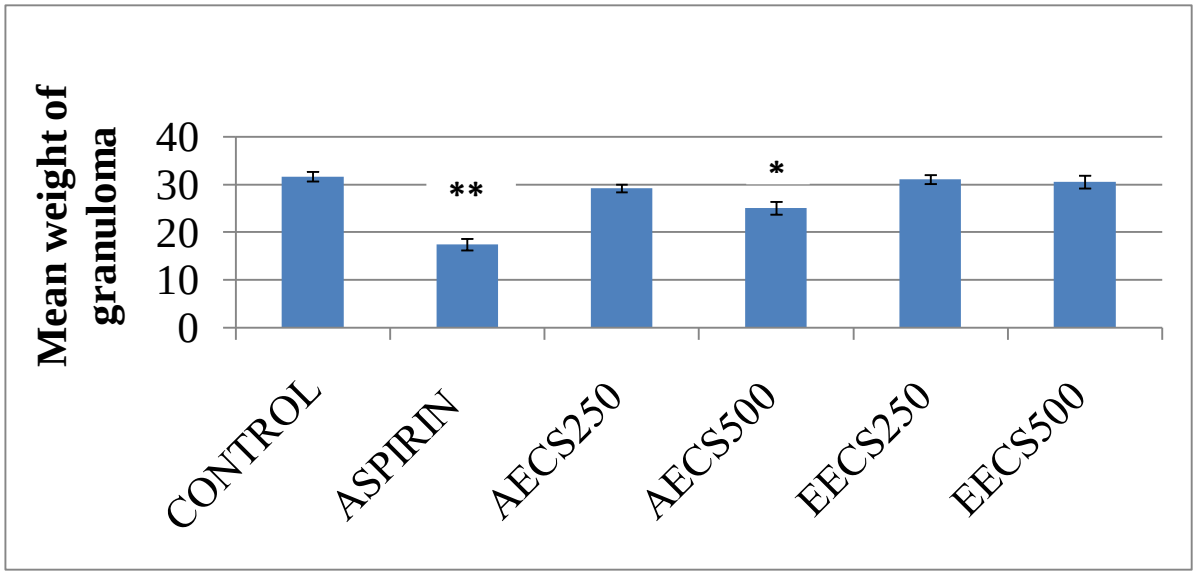
GROUPS (n=6)	Change in paw volume (ml) with inhibition of oedema (%)		
	1 HOUR	2 HOUR	3 HOUR
CONTROL	0.15±0.01	0.23±0.00	0.31±0.01
ASPIRIN 100 mg/kg	0.09±0.02 (40.00%)	0.08±0.01*** (65.21%)	0.08±0.01*** (74.19%)
AECS 250mg/kg	0.15±0.01 (0%)	0.22±0.01 (4.34%)	0.27±0.04 (9.67%)
AECS 500mg/kg	0.13±0.01 (13.33%)	0.21±0.01 (8.69%)	0.14±0.02*** (54.83%)
EECS 250mg/kg	0.15±0.01 (0%)	0.21±0.02 (8.69%)	0.15±0.02*** (51.61%)
EECS 500mg/kg	0.14±0.02 (6.66%)	0.13±0.02** (43.47%)	0.04±0.01*** (87.09%)

*P<0.05, **P<0.001, ***P<0.001 when compared to control. Control – Distilled water 1ml/kg p.o.; AECS – aqueous extract of C.sativum; EECS – ethanolic extract of C.sativum.



*P<0.001; Control – Distilled water 1ml/kg p.o. ; Standard – Aspirin 100mg/kg p.o. ; AECS250 – aqueous extract of C.sativum at 250mg/kg p.o. ; AECS500 – aqueous extract of C.sativum at 500mg/kg p.o. ; EECS250 –ethanolic extract of C.sativum at 250mg/kg p.o. ; EECS500 –ethanolic extract of C.sativum at 500mg/kg p.o.

Fig. 1: It shows the analgesic effect of aqueous and ethanolic extracts of *Coriandrum sativum* seeds on acetic-acid induced writhing in mice



*P<0.01, **P<0.001; Control – Distilled water 1ml/kg p.o. ; Standard – Aspirin 100mg/kg p.o. ; AECS250 – aqueous extract of C.sativum at 250mg/kg p.o. ; AECS500 – aqueous extract of C.sativum at 500mg/kg p.o. ; EECS250 – ethanolic extract of C.sativum at 250mg/kg p.o. ; EECS500 – ethanolic extract of C.sativum at 500mg/kg p.o.

Fig. 2: It shows the anti-inflammatory effect of aqueous and ethanolic extracts of *Coriandrum sativum* seeds on Cotton-pellet induced granuloma in rats

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