

ORIGINAL ARTICLE

Antinociceptive and Anti-inflammatory Activities of *Eruca Sativa* L. Leaves Extract

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ABSTRACT

In recent years, Rocket "*Eruca sativa* L." a member of the Brassicaceae family, has gained greater importance as a salad vegetable and spice, especially among Middle Eastern populations and Europeans. Rocket, is used by local herbal practitioners as a diuretic, stimulant, and in the treatment of stomach disorders and scurvy. In this study, we attempted to identify the possible analgesic and anti-inflammatory activity of methanol extract prepared from the leaves of *Eruca sativa* L. Using tail flick, hot plate and abdominal writhing tests, the analgesic activity of the methanol extract of *Eruca sativa* L. (MEES) at two doses (125 mg/kg and 250 mg/kg) were assessed in mice as animal models. The anti-inflammatory effect was determined by cotton pellet granuloma test. Pentazocin (30 mg/kg; i.p.); Diclofenac sodium (9mg/kg;p.o.); Aspirin (100 mg/kg; p.o) were used as reference analgesic agents. Diclofenac sodium (5mg/kg; p.o.) was used as reference of anti-inflammatory drug. MEES reduced the acetic acid induced writhings by 32 and 46 % at 125 and 250mg/kg ($p>0.05$) doses respectively as compared to Diclofenac sodium (9 mg/kg; p.o.) which induce 57% of reduction in writhing. Hot plate and Tail flick tests shows more positive response at 250 mg/kg dose of test extract. Administration of MEES at 125 mg/kg and 250 mg/kg doses significantly reduced the formation of granuloma tissue induced by cotton pellet at a rate of 42.99 % and 74.96 % respectively whereas Diclofenac sodium (51.35 %). The reduction of granuloma was significant at 250 mg/kg dose ($p>0.05$). The results obtained in this study indicated that MEES (125 mg/kg and 250 mg/kg) possess potent peripheral analgesic activity and it also executed a significant anti-inflammatory activity in chronic models.

Keywords: Anti-inflammatory activity, Analgesic activity, *Eruca sativa*, Brassicaceae

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INTRODUCTION

The inflammatory process involves a cascade of events elicited by numerous stimuli that includes physical or thermal injury, infectious agents, ischemia and antigen antibody interaction. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation and pain. But the greatest disadvantage of the presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drugs and indigenous medicinal plants could be a logical source for such drugs.

Eruca sativa L. (Brassicaceae) commonly consumed as leafy vegetable and locally referred to as Rocket (English), and Jarjeer (Arabic), has gained attention to many researchers because of its diversified medicinal and therapeutic properties^[1,2]. In Arabian countries, *Eruca sativa* L. used as a salad vegetable, also has greater importance in Unani, Ayurvedic and Arab traditional medicine^[3]. It possess medicinal and therapeutic properties that includes astringent, anti-ulcer, hepatoprotective, emollient, depurative, tonic, laxative, anti-secretory, cytoprotective and diuretic activities^[4,5]. It is an effective antioxidant

because of its high content of glucosinolate as well as Vitamin C, carotenoids, and polyphenols [6]. *Eruca sativa* L seeds exert a beneficial antidiabetic effect and the seed oil has greater importance in cosmetics, detergents and polymer production industries due to huge amount of the functionalized glucosinolates along with erucic acid C22:1 (cis -13-docosenoic acid) [7-9]. Earlier studies reported that its leaves possessed sexual desire enhancing power and also alleviate abdominal discomfort and improve digestion [10]. Keeping this in view, the present study has been undertaken to evaluate the analgesic and anti-inflammatory properties of *Eruca sativa* L. on experimental animal models.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of *Eruca sativa* were purchased from a local vegetable market in Sakaka, and the identity of these leaves was confirmed by an expert taxonomist of the Department of Pharmacology College of Medicine, Aljuf University, Saudi Arabia.

Preparation of Plant Extract:

The collected leaves of *E. sativa* were shade dried and reduced to coarse powder using a mechanical grinder. The powdered material of the leaves was exhaustively extracted with methanol under the maceration process. The macerated mixture was filtered by using Whatman No1 filter paper and evaporated at room temperature to yield a solid extract. This extract was kept in refrigerator until the analysis.

Drugs and chemicals

Animals

Albino mice (20-30 gm) were obtained from Animal house, College of Medicine, Al juf University. Animals were housed in groups of six (n=6) at an ambient temperature of 25±1°C with free access to food and water ad libitum. Animals were deprived of food but not water four hours before the experiment. For screening of analgesic and anti-inflammatory activity, mice were divided into four different groups. The first group served as a control group. The second group was used as reference standard. Two groups received ME of *E. sativa* at two different doses (125,250 mg/kg p.o.). The study protocol was approved by Institutional Animal Ethics Committee.

Antinociceptive activity:

Acute toxicity

The method described by Lorke [11] with slight modification was used to determine the safety of the ME of *E. sativa*. Briefly, normal healthy male mice were divided into groups of five mice in each cage. ME of *E. sativa* (125 and 250 mg/kg) or vehicle were intraperitoneally administered. Access to food and water, toxic symptoms and the general behavior of mice were observed continuously for 1 h after the treatment, intermittently for 4 h, and thereafter over a period of 24 h. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality.

Acetic acid-induced writhing

In this method, after an overnight fast of test albino mice, ME of *E. sativa* (125,250 mg/kg p.o.) was administered 30 minutes prior to intraperitoneal administration of 0.6% acetic acid (10 ml/kg i.p.) but Aspirin (100mg/kg; p.o) was administered 15 minutes prior to acetic acid injection. Analgesic activity of ME of *E. sativa* (125,250 mg/kg p.o.) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.) [12,13]. Analgesic activity was recorded by counting the number of writhes after the injection of acetic acid for a period of 20 minutes. A writhe is considered by abdominal constriction and full extension of hind limb. Index of analgesia was determined by percentage protection against abdominal constriction.

It was calculated as:

$$\text{Percent inhibition} = (N - N_t / N) \times 100$$

Where N is the average number of writhing of control per group, and N t is the average number of writhing of test per group.

Hot Plate Test

The hot-plate test was performed according to Franzotti et.al.,[14] method with some modifications. Test animals were placed into the beaker on the Eddays Hot plate surface at a temperature of 55 ± 0.5 °C for a maximum time of 10 seconds as a cut off time to avoid harm to the mice. The reaction time was considered the latency to flick the hind paw or lick or jump from the hot plate at before and at 0, 15, 30 and 45 min followed by oral administration of ME of *E. sativa* (125,250 mg/kg p.o.). Diclofenac sodium (9mg/kg;p.o.) was used as a reference drug. All experiments were performed in a laboratory with an ambient temperature of 22 ± 1°C. Percent analgesia was calculated using the following formula:

$$\% \text{ Analgesia} = (\text{Test latency} - \text{control latency}) / (\text{Cut} - \text{off time} - \text{control latency}) \times 100$$

Tail flick method

In this method the treatments were given to the test animals are same as per hot plate method except the reference drug in this method was Pentazocin (30mg/kg; i.p.). The latency period (reaction time) was determined using tail-withdrawal response when one-third of the tail was placed on the analgesiometer. The reaction time was evaluated at 15, 30, and 45, minutes after the oral administration of ME of *E. sativa* (125,250 mg/kg). and Pentazocin (30mg/kg; i.p.)^[15].

Anti inflammatory activity

Cotton pellet Granuloma test

The cotton pellets-induced granuloma in mice was studied according to the method D'Arcy et al.,^[16]. The animals were divided into four groups of six animals in each group. The mice were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle (0.9% NaCl, 5 ml/kg-1 b.w. The extract MEPA at ME of *E. sativa* 125 and 250 mg/kg b.w was administered orally to groups II and III animals for seven consecutive days from the day of cotton pellet implantation. Group IV animals received sodium diclofenac 5 mg/kg b.w for the same period. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation The antiproliferative effect of ME of *E. sativa* was compared with control.

RESULT

Acute toxicity test

No deaths of animals were observed during the observation period. Test results showed no stereotypical symptoms associated with toxicity, such as ataxy, convulsion,, diarrhea or increased diuresis. Doses of 125 and 250 mg/kg were recorded for i.p and p.o route administration, and the LD50 was estimated to 500 mg/kg.

Acetic acid-induced writhing

The effect of ME of *E. sativa* (125 and 250 mg/kg) on the writhing response in mice is shown in Table 1. The Inhibiton of writhing responses in mice were dose dependent manner and a significant ($P < 0.01$) inhibitory response (46.54%) at the does of 250 mg/kg was observed. However, the test extract dose (250 mg/kg) response was lower as compare to standard drug (57.34 %)in the mice.

Hot Plate Test

ME of *E. sativa* showed maximum analgesic activity 250mg/kg dose. The reaction time in normal control group was found to be 7.33 ± 0.55 Sec. The reaction time (paw licking / jumping response) in mice pretreated with a lower dose of ME of *E. sativa* (125 mg/kg), higher dose of ME of *E. sativa* (250 mg/kg/) and Diclofenac sodium (9 mg/kg;p.o.) was found to be 10.50 ± 0.42 , 12.66 ± 1.08 , 15.50 ± 0.76 sec respectively, when compared to control group mice. The duration of analgesic effect was more in 250 mg/kg compared to 125 mg/kg and reference drug Diclofenac sodium (9 mg/kg;p.o.) dose significantly increased the reaction time shown in figure 1.

Figure 1: Effect of ME of *E. sativa* on Tail Flick Response in Mice

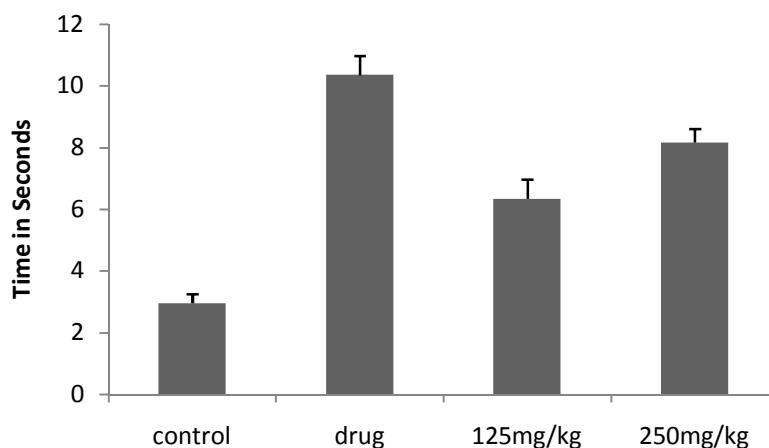
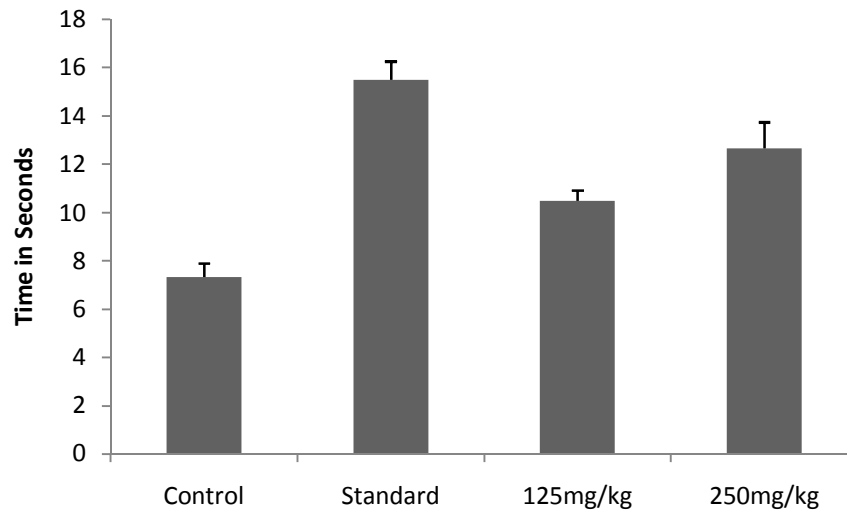


Figure 2: Effect of ME of *E. sativa* on Hot Plate induced Pain in Mice**Table 1: Effect of ME of *E. sativa* on Acetic Acid Induced Writhing Reflex in Mice**

Treatment	Number of Writhings (Mean±SE)	Inhibition (%)
Control	60.16±1.24	-
Standard	25.66±0.88	57.34
125mg/kg	40.66±1.05	32.14
250mg/kg	32.16±1.01	46.54*

*P < 0.05 considered as significant

Table 2: Effect of ME of *E. sativa* on Cotton pellet Granuloma formation in Mice

Treatment	Dry mass (mg)	Inhibition(%)
Control	63.51±6.32	0
125mg/kg	36.21±5.04	42.99
250mg/kg	15.90±1.24	74.96*
Standard	31.08±4.91	51.35

*P < 0.05 considered as significant

Tail flick method

Figure 2 shows the ME of *E. sativa* extract (125 and 250 mg/kg doses) effect of the tail flick test response in mice. All doses of the extract significantly ($P < 0.05$) increased the tail flick test response time compared to the control in dose depended manner. The effect of Pentazocin (30mg/kg; i.p.) was significantly higher (10.35 ± 0.61 sec) ($P < 0.05$) than that produced by the highest dose 250mg/kg) of the ME of *E. sativa* extract (8.16 ± 0.43 sec) (figure 2).

Cotton pellet Granuloma test:

The effects of ME of *E. sativa* extract (125 and 250 mg/kg doses) sodium diclofenac (5 mg/kg b.w) on the proliferative phase of inflammation are shown in table 2. A significant reduction in the weight of cotton pellets was observed with ME of *E. sativa* (250 mg/kg) compared to the vehicle treated mice. Interestingly at the dose of 250mg/kg of ME of *E. sativa* the degree of reduction was higher(74.96%) than the effect caused by sodium diclofenac (5 mg/kg b.w is 51.35%).

DISCUSSION

Currently various phytomedicines are using in traditional healthcare system for the management of pain and inflammation. The results of the present study reveals the Analgesic and Anti-inflammatory Activities Of *Eruca Sativa* L. Extract in animal model. From this study out put, it is evident that the extract has dose-dependent effect on experimental animal model. Inflammation start with the release of several mediators that control adhesion of molecules and process of cell migration, activation and degranulation [17].

Acetic acid-induced writhing test is one of the accurate method to evaluate the peripheral analgesic activity [18]. Acetic acid induce abdominal constriction by liberating endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins etc, that causes activation of peritoneal receptors leads to abdominal constriction [19]. Our results showed that the test extract had dose-dependent effect but has lower effectiveness than standard drug.

Hot plate test and tail flick methods were two well know procedures of thermal nociception. In our study we applied these two methods to double check on possible involvement of spinal, supra-spinal pathways and u-opiate receptor agonists in regulation (CNS modulation) of pain response [20]. Our findings indicates ME of *E. sativa* has significant analgesic activity at 250mg/kg dose as compare to the standard drug, Diclofenac sodium (9 mg/kg;p.o.) in dose-dependent manner. This indicates that ME of *E. sativa* at 250mg/kg dose acts as a good analgesic drug on CNS.

The most routine method to assess transudative and proliferative components of chronic inflammation are cotton pellet-induced granuloma test [21]. In this test the weight of the wet cotton pellets correlates with transudate material and the weight of dry pellet correlates with the amount of granulomatous tissue. In the our study, administration of ME of *E. sativa* has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the higher dose of ME of *E. sativa* exhibited inhibition of inflammation is very significant rather than inhibitory effect of diclofenac sodium (9 mg/kg;p.o.). Diclofenac sodium act as a inhibitor of prostaglandins synthesis at the late phases of inflammation may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide [22]. This effect may Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharids are indicators of the antiproliferative effect by NSAIDs. The test results demonstrate that ME of *E. sativa* at 250 mg/kg dose has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism.

CONCLUSION

The ME of *E. sativa* showed analgesic and antinflammatory activities in the animal models of pain. This supports the anecdotal use of ME of *E. sativa* in the management of pain and inflammation. Furthermore research on this plant will explore more information in management of chronic inflammatory diseases.

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