

Pharmacological and Safety Evaluation Studies on *Lepidium sativum* L, Seeds

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Summary

An ethanolic extract of Cress (*Lepidium sativum* L.) seeds has been studied for anti-inflammatory, antipyretic and analgesic activities and to evaluate the safety of their acute and chronic use in rodents. The extract significantly inhibited carrageenan-induced paw edema and reduced the yeast-induced hyperpyrexia. It also prolonged the reaction time of mice on the hot plate. However, the extract exacerbated indomethacin-induced gastric mucosal damage. The coagulation studies showed a significant increase in fibrinogen level and an insignificant decrease in prothrombin time, confirming its coagulating property. The toxicity tests showed that the administration of extract in single doses of 0.5 to 3.0g/kg did not produce any adverse effects or mortality in mice, whereas the animals treated with extract (100 mg/kg/day) for a period of 3 months in drinking water showed no symptoms of toxicity except a statistically insignificant higher mortality rate. These findings suggest that the seeds of Cress (*L. sativum*) possess significant anti-inflammatory, antipyretic, analgesic and coagulant activities, and are free from serious side or toxic effects.

Key words: *Lepidium sativum* L., Anti-inflammatory, Antipyretic, Analgesic, Haematological effects, Safety evaluation.

Introduction

Cress, *Lepidium sativum* Linn. (Cruciferae), locally known as *Rashad* is an annual tall glabrous herb, with erect stem, widely distributed in many countries of the world (Narkarni, 1954; Morton, 1981). In India, the plant is regarded as a cure for asthma, dysentery, bleeding piles, as a diuretic, and to enhance sexual desire (Dymock, Warden and Hooper, 1890; Chopra, Nayar and Chopra, 1956). In China and other Far Eastern countries the seeds are used for the treatment of abdominal colic, sexual debility, asthma, pleurisy and dropsy (Perry, 1980). *L. sativum* is considered as one of the better medicinal plants in various African countries, where the seeds are chewed to cure throat diseases, asthma, headache and are useful for diuresis and

in menstrual disorders (Kloos, 1976). Hartwell (1982) has reported the seeds of *L. sativum* to be a remedy for uterine tumors, nasal polyps and breast cancer.

A recent survey of different regions of Saudi Arabia showed that the seeds of *L. sativum* are commonly used as febrifuge, anti-rheumatic, diuretic, in menstrual and abdominal discomfort. They are also used for the treatment of rapid bone fracture healing (Ageel, Tariq, Mossa et al., 1987; Ahsan, Tariq, Ageel et al., 1989). The present investigation has been undertaken to study the claimed properties of ethanolic extract, namely anti-inflammatory, antipyretic, analgesic and anti-ulcer activities, haematological effects and acute and chronic toxicity evaluation besides preliminary qualitative phytochemical screening of the seeds of *L. sativum*.

Materials and Methods

Plant material and extraction

The seeds used in this study were purchased from the local market and authenticated by an expert taxonomist, a specimen was deposited in the Medicinal, Aromatic and Poisonous Plants Research Center of this College for future reference.

500 g coarse powder of seeds were macerated in 3 l of 96 % ethanol for 72 hrs. using the percolation method. The solvent was then removed at 40 °C under reduced pressure in a rotavapor. The yield was 6.86 % w/w in terms of dried starting material. The extract was suspended in distilled water with the help of Tween 80 before administration.

Phytochemical screening

A phytochemical analysis of the seeds of *L. sativum* was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, sterols and/or triterpenes, volatiles, cyanogenic glycosides and glucosinolates (Farnsworth, 1966).

Carrageenan-induced paw edema in rats

Pedal inflammation in albino rats (8 to 10 weeks old) of either sex weighing 180-200 g was produced according to the method described by Winter et al. (1962). An injection was made of 0.05 ml of 1 % carrageenan sodium salt (BDH) into the right hind foot of each rat under the plantar aponeurosis. The test groups of rats were treated orally with 500 mg/kg of the ethanolic extract 1 h before the carrageenan injection. At the same time, the control group was given 5 ml/kg of normal saline and the reference group was given 100 mg/kg of an aqueous solution of oxyphenbutazone. The measurements of foot volume were done by the displacement technique using a plethysmometer (Apelex, France) immediately after and +2 and +3 h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:

$$\text{Percent inhibition} = 100 (1 - f^{\wedge})$$

where 'b' is the mean paw volume of control rats after carrageenan injection and 'y' before the injection; whereas 'x' is the mean paw volume of treated rats before injection and 'a' is the mean paw volume after carrageenan injection.

Cotton pellet granuloma in rats

The method of Goldstein et al. (1967) was used with a few modifications. A sterilized cotton pellet weighing 30 mg was introduced s. c. in the groin region of rats. They were treated orally with 500 mg/kg body weight of the extract once daily for four consecutive days. Animals in the

control group received normal saline. Oxyphenbutazone 100 mg/kg (used as standard drug) was given in another test group. On the fifth day, the animals were killed with ether, the pellets were removed, freed from extraneous tissue, dried overnight at 55 ± 0.5 °C and weighed.

Antipyretic activity in mice

Hyperthermia was induced in mice by s. c. injection of 20 ml/kg of a 20 % aqueous suspension of brewer's yeast in the back below the nape of the neck (Loux et al., 1972). The animals were then fasted for the duration of the experiment (approximately 24 hrs), water was made available *ad lib*. Control temperatures were taken 24 hrs after the yeast injection to determine the pyretic response to yeast. Temperatures taken 1 hr prior to drug administration in febrile animals served as a pre-drug control. Plant extract (500 mg/kg) was given 24 hrs after the yeast injection and the temperatures were recorded at 60, 90, and 120 min after its administration.

Analgesic activity

The hot plate method described by Turner (1965) was used. The animals were dropped gently on a hot plate maintained at 55 ± 5.5 °C. The reaction time was taken as the interval extending from the instant the animal reached the hot plate until the moment the animal licked its forefeet or jumped off. The reaction time was measured 10 min before the oral administration of the drug (500 mg/kg) and +30, +90 and +150 min after treatment.

Indomethacin-induced gastric ulcers

Indomethacin was suspended in 1 % carboxymethylcellulose (CMC) in water (6 mg/ml) and administered p. o. at a dose of 30 mg/kg (0.5 mg/100g) to rats fasted for 36 hrs (Bhargawa et al., 1973). The extract was administered 30 min before indomethacin. The rats were killed 6h after the indomethacin administration. Gastric lesions induced by indomethacin were multiple in each stomach. They were evaluated singly according to their dimensions and severity, and scored using a scale of 0 (no visible ulcers) to 10 (deep lesions with a diameter greater than 8 mm). The scores for each single lesion were then summed so that the total score per stomach could exceed the value of 10 (Valcavi et al., 1982). The results refer to the average lesion score ± S.E.M.

Haematological studies

The rats were administered the extract at a dose of 500 mg/kg and killed 2 hrs later. The blood was collected by cardiac puncture and subjected to haematological investigation of effects on prothrombin time, fibrinogen and haemoglobin levels (Caen et al., 1975). Bio Merieux (Lyon,

France) kits were used for the determination of prothrombin time and fibrinogen levels and the final readings were taken on a Saitron-333 Coagulometer.

Safety evaluation studies.

Acute toxicity

Acute toxicity test was performed on 3 groups of mice consisting of 6 animals per group. The ethanolic extract was administered orally in the doses of 0.5, 1 and 3 g/kg body weight. The behavioral changes, symptoms of toxicity and mortality were observed for 24 hrs.

Chronic toxicity

A total of 40 mice (20 male and 20 female) were randomly allotted to different treated and control groups. The extract in each case was administered in drinking water at a dose of 100mg/kg/body weight per day for a period of 90 days (WHO, 1967). The animals were observed for symptoms of toxicity and mortality. At the end of the treatment the animals were sacrificed and the condition of the viscera observed for any gross morphological change and the weight of the vital organs were also recorded.

Results

The preliminary qualitative phytochemical screening of the seeds of *L. sativum* revealed the presence of alkaloids, cyanogenic glycosides (traces), flavonoids, tannins, glucosinolates, sterols and/or triterpenes. The extract was found

to significantly inhibit carrageenan-induced pedal oedema in rats. However, only a weak inhibition of cotton pellet-induced granuloma was observed in extract-treated animals (Table 1).

The mean predrug rectal temperature in yeast-induced febrile mice was found to be 37.13 ± 0.05 °C. The administration of extract reduced the temperature to 36.86 ± 0.04 , 36.68 ± 0.05 and 36.53 ± 0.07 °C at 30, 90 and 150 min following the treatment respectively (Table 2). Administration of *L. sativum* extract (500 mg/kg) significantly prolonged the hot plate reaction time suggesting its analgesic activity (Table 3).

On the other hand the seed extract potentiated the indomethacin-induced gastric mucosal lesions (Table 4). The coagulation studies showed that the extract produced a significant increase in fibrinogen level and insignificant decrease in prothrombin time. However, no significant change was observed in haemoglobin level by extract treatment (Table 5).

The acute toxicity in mice showed no symptoms of toxicity or mortality over a period of 24 hours up to a dose of 3 g/kg orally. The prolonged treatment (90 days) of animals produced significant gain in body weight. However, an insignificant (25 %) mortality of the animals was recorded as compared to control group (15 %) (Table 6).

Discussion

The antiinflammatory, hypothermic and analgesic properties of *L. sativum* are evidenced from the results obtained. The findings of the present study support the use of

Table 1. Effect of the ethanolic extract of *L. sativum* on carrageenan-induced paw edema and cotton pellet granuloma in albino rats.

Group (n = 6)	Dose (mg/kg) orally	Carrageenan-induced edema of right paw		Cotton pellet-induced granuloma	
		Mean increase in paw volume (ml±S.E.)	Percent inhibition	Mean increase in weight of pellet (mg±S.E.)	Percent inhibition
Control		0.202 ±0.012		45.22 ±2.02	
<i>L. sativum</i> extract	500	0.117*0.010	42.08 *	41.96±3.89	7.21
Oxyphenbutazone	100	0.079 ±0.018	61.89*	19.28 ±1.04	57.36"

*p<0.05, Analysis of variance (Newman-Keul's test).

Table 2. Effect of the ethanolic extract of *L. sativum* on yeast-induced hyperthermia in mice.

Group (n = 6)	Dose (mg/kg) Orally	Pre Drug	Rectal temperature °C		
			30 min	Post Drug 90 min	150 min
Control	Saline	36.43 ±0.24	36.41 ±0.22	36.33 ±0.15	36.15*0.06
<i>L. sativum</i>	500	37.13 ±0.05	36.85 ±0.04	36.68 ±0.5	36.53 ±0.07
Aspirin	150	36.70*0.17	35.90*0.07	34.93 ±0.35*	34.46 ±0.30*

*P<0.05, Analysis of variance (Newman-Keul's test).

the seeds in traditional medicine for the treatment of rheumatism, fever and various types of pain. The pharmacological properties of the plant resemble those of the non-steroidal anti-inflammatory drugs (NSAID) which are known to share antipyretic, analgesic and anti-inflammatory activities. One of the major mechanisms involved in the anti-inflammatory activity of NSAID is due to inhibition of prostaglandin biosynthesis (Vane, 1971). Although the prostaglandin (PG) levels were not estimated in this study, the extract was shown to potentiate indomethacin-induced gastric mucosal lesions, as the NSAIDs are known to damage gastric mucosa by inhibiting the PG synthesis (Rafatullah, Tariq, Mossa et al., 1993). On the basis of this finding it may be suggested that the extract produced anti-inflamma-

tory and ulcer promoting activity by inhibiting PG synthesis like other NSAIDs. However, further studies are needed to confirm or rule out this hypothesis. On the other hand diuretic activity may contribute to anti-inflammatory activity. Ageel et al. (1987) have reported a significant diuretic activity of the Cress extract in rats. The diuretics are known to decrease various types of oedema and inflammatory condition by reducing the body fluids (Wiener and Mudge, 1985). Our coagulation studies showed an increased fibrinogen level and a decrease in prothrombin time suggesting coagulant activity of the extract. These findings justify the use of Cress for the treatment of bleeding disorders including piles and menstrual dysfunction (Kritikar and Basu, 1984). The chemical constituents responsible for the phar-

Table 3. Effect of the ethanolic extract of *L. sativum* on hot plate reaction time in mice.

Group (n = 6)	Dose (mg/kg)	Reaction time (seconds)			
		Pre Drug	Post Drug		
	Orally		30min	90min	150 min
Control	Saline	3.91*0.37	4.08 ±0.42	3.83 ±0.16	4.41 ±0.30
<i>L. sativum</i>	500	3.90 ±0.14	5.55 ±0.26*	6.30 ±0.19*	5.58 ±0.24*
Aspirin	150	3.91 ±0.27	5.66±0.55*	7.41 ±0.37*	8.51 ±0.42*

*P<0.05, Analysis of variance (Newman-Keul's test).

Table 4. Effect of the ethanolic extract of *L. sativum* on gastric mucosal damage induced by indomethacin (30mg/kg P. O.).

Treatment (n = 6)	Dose (mg/kg) orally	Ulcer index (Mean ± S.E.)
Control	-	32.16 ±5.25
<i>L. sativum</i> extract	500	39.00 ±2.15

Table 5. Effect of the ethanolic extract of *L. sativum* on haemoglobin and fibrinogen levels and prothrombin time in rats.

Group	Dose (mg/kg)	Haemoglobin (mg/100ml)	Fibrinogen g/l	Prothrombin time (sec)
Control		13.50 ±0.43	31.14±0.66	2.28 ±0.05
<i>L. sativum</i> extract	500	14.01 ±0.26	37.11 ±2.04*	2.09 ±0.05

Six animals were used in each group.

*P<0.05, Analysis of variance (Newman-Keul's test).

Table 6. Effect of chronic treatment (90 days) of ethanolic extract of *L. sativum* on the body weight and mortality in mice.

Treatment and dose (100mg/kg/day)	Pre-treatment body weight		Post-treatment body weight		Percent mortality
	Mean ± S.E.		Mean ± S.E.		
	Male (20)	Female (20)	Male (20)	Female (20)	
Control	27.30*0.65	25.10 ±0.50	32.20 ±2.0*	29.00 ±1.00*	15
<i>L. sativum</i> extract	28.70 ±0.70	23.60 ±0.60	35.30 ±1.0*	28.00 ±1.00*	25

Number in parentheses denote the number of animals.

*P<0.05, Analysis of variance (Newman-Kaul's test).

macological activity of the extract are not known. The seeds were found to contain alkaloids, cyanogenic glycosides (traces), flavonoids, tannins, glucosinolates, sterols and triterpenes. The flavonoid group of compounds have been observed to possess anti-inflammatory activity by various authors (Gabor, 1972; Parmar and Ghosh, 1980; Jaber, Rafatullah and Galal et al., 1993). The safety evaluation studies showed that the extract does not produce any adverse effects or mortality in the animals. These findings suggest a wide margin of safety of the drug for the treatment of various diseases. Chronic treatment of mice in the dose of 100mg/kg/day for 90 days also did not produce any deleterious effects. Hence it may be concluded that the cress, besides having a variety of pharmacological effects, does not cause any serious toxic effects in animals.

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