Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article

Studies on bronchodilatory effect of *Lepidium sativum* against allergen induced bronchospasm in guinea pigs

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ABSTRACT

The ethanolic extract of seeds of *Lepidium sativum* and its various fractions were tested for their bronchodilatory effect against histamine and acetylcholine induced acute bronchospasm in guinea pigs. The ethanolic extract and its all the fractions viz. ethyl acetate, n-butanol and methanol exhibited significant protection against bronchospasm induced by histamine and acetylcholine. However, significant ($p<0.001$) protection was exhibited by n-butanol fraction which was comparable with that of Ketotifen (1 mg/kg) and Atropine sulphate (2 mg/kg) included as reference standard in the study. Thus the results of our study suggest that, the plant *L.sativum* has bronchodilatory activity.

KEYWORDS: *Lepidium sativum*, Bronchodilatory activity, Histamine, Acetylcholine.

INTRODUCTION

Lepidium sativum Linn (Cruciferae) commonly known as Asaliyo, is an erect, glabrous annual herb cultivated as a salad plant throughout India, Europe and United States (1). It is an important medicinal plant since the Vedic era. The seeds of the plant are used by many Ayurvedic practitioners for the treatment of bronchial asthma as it possesses 'Ushna' virya (feeling of warmth) property. In Ayurveda, it is described as hot, bitter, galactogogue and aphrodisiac and claimed to destroy Vata (air) and Kapha (phlegm) (2). In Unani system of medicine, seeds and leaves of *L. sativum* are reported to possess diuretic, aperient and aphrodisiac properties and recommended in inflammation, bronchitis, rheumatism, and muscular pain. It is also reported to be useful in treatment of asthma, cough and bleeding piles (3-4). Although the number of diseases for which *L. sativum* finds use as a medicine is fairly large, yet its curative efficacy has not been scientifically proven in all the diseases. The plant is also reported to possess haemagglutinating, hypoglycemic, antihypertensive, diuretic and fracture healing property (5-8). Recently, we have studied the clinical efficacy and safety of seeds of *L.sativum* in patients with bronchial asthma (9). In the light of above facts the present investigation was conducted to assess the protective effect of the ethanolic extract and its various fractions against histamine and acetylcholine induced bronchospasm in guinea pigs.

MATERIALS AND METHODS

Collection and authentication of Plant Material – Seeds of *L.sativum* were purchased from a commercial supplier, identified and authenticated at Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, India, where a Voucher specimen has been deposited. Dried seeds were powdered and used for the preparation of extracts.

Preparation of extracts

Dried seeds of *L.sativum* were coarsely powdered in electric grinder and the powdered drug was extracted in a Soxhlet apparatus successively with petroleum ether (40-60^{0}C), chloroform and ethanol. The petroleum ether extract after distillation yielded 24 % of light golden yellow oil with a characteristic mustard oil-like odor. The chloroform extract resulted 5 % of a viscous oily residue and the ethanolic extract gave 32% of dark brown residue with a pungent odor. The dried ethanolic extract was taken in minimum quantity of water and was successively extracted with ethyl acetate, n-butanol and methanol to yield 4.92 % w/w, 7.85 % w/w and 8.20 % w/w residue, respectively. The ethanolic extract and its fractions were subjected to the preliminary phytochemical screening for identification of active constituents (10). The ethanolic extract and all the fractions were preserved in refrigerator until further use.

Test Animals

For the experiment, Hartley strain guinea pigs of either sex (500-550 g) were selected and housed in standard conditions of temperature (22±2^{0}C), relative humidity (60±5 %) and light (12 h light/ dark cycle).

The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Histamine and acetylcholine induced bronchospasm in guinea pigs (11-12)

Hartley strain guinea pigs of either sex weighing 500-550 g were selected for the present study. The animals were kept in a closed chamber and exposed to an aerosol of 0.5% histamine hydrochloride using nebuliser and time for preconvulsion dyspnoea (PCD) was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions (13). As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. Time taken for the onset of PCD was taken as basal value. All the animals were randomly divided into six groups each containing six animals. Group I served as control group and received distilled water (vehicle), group II served as standard, received Ketotifen (1mg/kg) against histamine induced bronchospasm. Group III to VI served as test and received ethanolic extract (500 mg/kg), ethyl acetate (100 mg/kg), n-butanol (100 mg/kg) and methanol fraction (100 mg/kg) respectively. All the animals of groups II, III, IV, V and VI were given the respective drug treatment by oral route as a single dose per day. The treatment was continued for seven consecutive days. On day 7, two hours after the last dose, animals were exposed to histamine aerosol and time taken for the onset of PCD was noted. Similar procedure was repeated by exposure of aerosol of 0.5% acetylcholine in another six groups of animals using Atropine sulphate (2mg/kg) as a standard (14). The protection offered by the treatment was calculated by the following formula (15),

% increase in PCD time = $[1-T_1/T_2] \times 100$

where, T_1= time for PCD onset on day 0 ; T_2= time for PCD onset on day 7.

Statistical analysis

Results were reported as mean±SEM; statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test. Though the data was considered statistically significant at $p<0.05$, when data was found to be very ($p<0.01$) or highly ($p<0.001$) significant, this is indicated in the results.

RESULTS AND DISCUSSION

The results of preliminary phytochemical investigation of seed extract of *L.sativum* and its various fractions are shown in Table 1. The ethanolic extract revealed the presence of an array of active constituents including alkaloids, tannins, flavonoids, steroids and sugars. We have developed simple TLC and paper chromatographic techniques to confirm the presence of these phytoconstituents (16).

In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (17). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics are used as quick relief medications in such acute asthmatic attacks (18).

In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Ketotifen (1mg/kg) and atropine sulphate (2 mg/kg) were used as reference standard against histamine and acetylcholine induced bronchospasm respectively (19). The crude ethanolic extract (500 mg/kg), n-butanol (100 mg/kg) and methanol (100 mg/kg) fractions have shown significant bronchoprotection ($p<0.001$) against both the types of spasmogens as compared to control (Table 2). However, highly significant increase in preconvulsion time was observed due to treatment with n-butanol fraction when guinea pigs were exposed to either histamine or acetylcholine aerosol. The bronchodilatory effect of n-butanol fraction was found comparable to the protection offered by both the reference standard drug ketotifen and atropine sulphate.

Ayurveda has recommended a number of plants for the treatment of asthma and other allergic disorders and has been successful in controlling the disease as well (20). Large numbers of medicinal plant preparations have been reported to possess bronchodilatory effects; these include *Adhatoda vasica* (21), *Benincasa hispida* (14), *Albizzia lebbeck* (22), *Cissampelos sympodialis* (23) and *Sarcostemma brevistigma* (24). Phytoconstituents like alkaloids and flavonoids are attributed to possess bronchodilatory activity (21, 24). Qualitative phytochemical investigation of ethanolic extract and its various fractions especially butanol fraction has shown presence of these constituents. In conclusion, the results of present preliminary investigation suggested that, the plant *L.sativum* has significant bronchodilatory activity. However, further studies are suggested to establish the activity and also to isolate and characterize the active principle/s responsible for the action.

Table 1: Preliminary Phytochemical screening of ethanolic extract of L.sativum and its various fractions

Tests	Ethanolic extract	Ethyl acetate fraction	n-Butanol fraction	Methanol fraction
Alkaloids	+	-	+	+
Flavonoids	+	-	+	-
Tannins	+	-	+	+
Terpenoids	-	-	-	-
Steroids	+	+	+	-
Glycosides	+	+	-	-

(+) = Present, (-) = Absent

Table 2: Effect of L.sativum on histamine and acetylcholine induced bronchospasm

Groups	Drug received	Dose	Percentage protection	
			Histamine	Acetylcholine
Control	Distilled water	-	9.6 ± 0.88	8.0 ± 1.29
Standard	Ketotifen	1 mg/kg	59.36 ± 0.62***	-
	Atropine sulphate	2 mg/kg	-	64.65 ± 1.07***
Test	Ethanol extract	500 mg/kg	48.30 ± 5.41***	29.41 ± 1.6***
Test	Ethyl acetate Fraction	100 mg/kg	27.58 ± 2.2 **	23.94 ± 1.74**
Test	n-Butanol Fraction	100 mg/kg	53.28 ± 3.46***	61.24 ± 3.99***
Test	Methanol Fraction	100 mg/kg	46.27 ± 1.57***	42.90 ± 5.27***

*Values are mean ± SEM, (n=6), ***p<0.001; **p<0.01 as compared to control group.*

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