

Original research article

'Neutrophil Adhesion Test' as tool for Immunomodulatory Activity Of Different Extracts of *Lepidium Sativum* Linn. SeedsChetna Baregama¹, Dr. Anju Goyal²¹Ph D scholar, Bhupal Nobles' Institute of Pharmaceutical Sciences, B. N. University, Udaipur, Rajasthan, India.²Professor & HOD, Bhupal Nobles' Institute of Pharmaceutical Sciences, B. N. University, Udaipur, Rajasthan, India.

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Abstract

Currently world wide there is an increase in diseases especially infectious diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since ancient times. *Lepidium sativum* Linn. (Brassicaceae) is annual herb regionally referred to as halon in India, however, usually referred to as garden cress. Immunomodulatory activity of n-hexane, chloroform, ethylacetate and methanolic extracts of *Lepidium sativum* Linn. seeds were studied on albino wistar rats using neutrophil adhesion test. Incubation of blood with nylon fibres produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibres. Among all extracts methanolic extract showed significant increase in the neutrophil adhesion when compared to disease control and standard. Methanolic extract at 250 and 500 mg/kg produced significant increases in adhesion of neutrophils. Among the different doses of methanolic extract, high one (500mg/kg) was more effective in cellular immunity model than low one (250mg/kg). The results of the present study substantiate the belief that *Lepidium sativum* Linn. is an immune system booster.

Keywords: Neutrophil adhesion test, *Lepidium Sativum* Linn., Methanolic extract, Immunomodulatory activity, *Oscimum sanctum*, Methotrexate

1. INTRODUCTION

Currently, world wide, there is an increase in diseases especially infectious diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation. Malnutrition and communicable diseases have remained a challenge especially in developing nations as they greatly cooperate the body's immune system responses in the affected individuals [1]. A global reliance on alternative system of medicine for chronic and acute ailments resulted in an intense area of research and discovery of a number of herbs with potential to curb diseases. Among them, ample number of herbs has been exploited for modulation of immune system from Ayurvedic formulation either alone or in combinations. Environmental pollutants and dietary habits cause disturbances in immune activities and diet containing micronutrients and antioxidants are known to prevent these alterations [2]. The use of herbs as immunomodulators in the indigenous system of medicines, indeed, can change the body's defence mechanism. The following active constituents of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in different experimental models [3].

Lepidium sativum Linn. (Brassicaceae) is annual herb regionally referred to as halon in India, however, usually referred to as garden cress. *L. sativum* is a fast growing edible plant. Seeds, roots, and leaves of garden cress have economic importance; however, the crop is especially cultivated for seeds. It is a therapeutic vital herb in India [4]. The potential therapeutic properties of *Lepidium sativum* Linn. are wide-ranging and include treatment and prevention of airways disorders, gastrointestinal treatment, menstruation cycle regulation, iron deficiency treatment, inflammation cardiovascular disease, diabetes [5]. The medicinal value of a plant is due to the existence of some special substances like alkaloids, glycosides, resins, volatile oils, tannins and gums, flavonoids etc. The active principles usually remain concentrated in the storage organs of the plants [6]. Therefore, the chemical profile indicates *Lepidium sativum* Linn as good sources of immunomodulatory agents. It is widely used as indigenous traditional medicine for variety of disorders including immunodeficiencies [7]. However, till date no scientific evaluations are conducted for confirming its role as immunostimulant. Thus, this study was designed to study the immunomodulatory activity of different extracts of *Lepidium sativum* Linn seeds in experimental models of cellular immunity in animals.

2. MATERIALS AND METHODS

2.1 Experimental animals

Disease-free Wistar albino rats of either sex aged between 6 and 8 weeks were randomized into eleven experimental groups. Albino Wistar rats weighing between 180–220 g were used. All animals were housed at well ventilated animal house. The rats were procured from Central Animal House, B R Nahata College of Pharmacy, Mandsaur (M.P.). The animals had free access to standard food pellets. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep animals clean and dry. Animals were maintained under standard conditions in an animal house approved by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC/BRNCOP/2020/002). The animals were subjected for quarantine (10 days) prior to experimentation.

2.2 Procurement of plant material and extraction

Garden cress seed (*Lepidium sativum*) was purchased from local market of Mandsaur, Madhya Pradesh, India and authenticated by Department of pharmacognosy at B R Nahata College of Pharmacy, Mandsaur. A voucher specimen no. (BRNCP/LS/012/2019/ *Lepidium sativum* seeds) was deposited in the herbarium of the institute. Successive solvent extraction method was used for extraction for *Lepidium sativum* seeds with different solvents like n-hexane, chloroform, ethyl acetate and methanol [8]. The ethanolic extract of *oscimum sanctum* leaves was used as standard immunomodulatory agent [9].

2.3 Preliminary phytochemical testing of extract:

Preliminary phytochemical testing was carried out check and identify the active constituents of all extracts of *Lepidium sativum* Linn. Seeds such as alkaloid, amino acid, carbohydrate, glycoside, inulin, mucilage, tannin, starch, saponin, steroid, triterpenoid and flavonoid [10,11]. Screening of n-hexane extract indicated the presence of amino acid, alkaloids, proteins, carbohydrates, terpenoid and saponins, chloroform extract indicated presence of alkaloids, proteins, carbohydrate, glycoside, triterpenoids and steroids. Ethyl acetate extract indicated presence of alkaloids, amino acid, flavonoids, proteins, carbohydrates, steroids and terpenoids,

methanol extract indicated presence of alkaloid, amino acid, tannin, protein, terpenoid, carbohydrate, flavonoids and saponins [8].

2.4 Acute toxicity studies

For the acute toxicity study, 0.5 –5.0 g/kg body weight of the GC seed powder was administered through diet to rats and obvious symptoms of toxicity and mortality were monitored for 72 h . Acute doses of GC seed powder did not induce any symptoms of toxicity or mortality of rats. In subchronic toxicity study, 1.0 – 10.0% of the GC powder was administered to rats through diet for 14 weeks. Dietary feeding of GC seed powder did not produce any mortality, no significant changes in food intake, gain in body weight, relative weight of organs, hematological parameters, macroscopic and microscopic changes in vital organs, were observed between experimental and control groups. Clinical enzymes viz., LDH, SGPT were within normal levels, however, the serum ALP and SGOT were significantly increased in male rats receiving 5.0 and 10 % of GC seeds. The results showed that acute and subchronic feeding of GC seed for 14 weeks did not produce any toxic effects in male and female rats and thus can be considered non-toxic and safe [12]. According to office of pollution and toxics (OPPT) guidelines (<http://www.epa.gov/oppts/home/guideline.htm>) [13], 1/10th of the maximum safe dose (5g/kg) corresponding to 500 mg/kg was selected as high dose and 250 mg/kg selected as low dose.

2.4.1 Experimental protocol

The drug solutions were prepared in tween 80 (1% solution) for oral administration. Immunomodulatory activity was checked at cellular level. Cellular immunity was evaluated by neutrophil adhesion test. The experimental models had eleven groups consisting of six animals each.

Group I, was served as normal control and received vehicle tween 80 (1 ml/100 g, *p.o*),

Group II, was served as disease control and received Methotrexate (2mg/kg for 7days) [14].

Group III received n hexane extract 250 mg/kg, group IV received n hexane extract 500 mg/kg.

Group V received chloroform extract 250 mg/kg, group VI received chloroform extract 500 mg/kg

Group VII received ethylacetate extract 250 mg/kg, group VIII received ethylacetate extract 500 mg/kg

Group IX received methanolic extract 250 mg/kg, group X received methanolic extract 500 mg/kg

Group XI, received *Ocimum sanctum* (OSE) at a dose of (100 mg/kg, *p.o*) [15].

2.4.2 Neutrophil adhesion test

The rats were pre-treated orally with vehicle or extracts for 14 days. At the end of treatment day 14, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg nylon fibres/ml for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC, respectively to give neutrophil index of blood samples. The percent neutrophil adhesion was calculated as follows:

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIu} - \text{NI}t \times 100}{\text{NIu}}$$

where NIu is the neutrophil index of untreated blood samples and NI_t is the neutrophil index of treated blood samples [16,17].

2.4.3 Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by t test. The values were expressed as mean \pm SEM and $P < 0.05$ was considered significant.

3. RESULT

Incubation of blood with nylon fibres (NF) produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibres. Effect of different extracts of *Lepidium sativum* Linn. seeds on the neutrophil adhesion are shown in table 1.

Table 1 Effect of various extracts of *Lepidium sativum* Linn. Seeds on Neutrophil adhesion

Group	Treatment	TLC (103/mm ³) (A)		Neutrophil% (B)		Neutrophil index (A x B)		Neutrophil adhesion (%)
		UB	NFTB	UB	NFTB	UB	NFTB	
Group I	Normal control	6.8 \pm 0.11	6.6 \pm 0.09	23.5 \pm 0.45	22.6 \pm 0.4	159.8 \pm 3.1	149.16 \pm 2.6	6.7 \pm 0.3
Group II	Disease control	3.0 \pm 0.17	2.9 \pm 0.17	11.1 \pm 0.19	10.8 \pm 0.2	33.3 \pm 0.6	31.32 \pm 0.6	5.9 \pm 0.1***
Group III	n-hexane extract 250 mg/kg	6.3 \pm 0.18	5.7 \pm 0.17	30.3 \pm 0.46	24.3 \pm 0.5	190.89 \pm 2.9	138.5 \pm 2.8	27.4 \pm 0.1***
Group IV	n-hexane extract 500mg/kg	7.1 \pm 0.24	6.5 \pm 0.18	33.2 \pm 0.9	26.0 \pm 0.4	235.72 \pm 6.4	169 \pm 2.6	28.30 \pm 1.6***
Group V	chloroform extract 250mg/kg	4.1 \pm 0.12	3.9 \pm 0.09	19.7 \pm 0.21	19.3 \pm 0.2	80.77 \pm 0.9	75.27 \pm 0.78	6.8 \pm 0.15***
Group VI	chloroform extract 500mg/kg	4.1 \pm 0.12	3.9 \pm 0.12	19.6 \pm 0.69	18.9 \pm 0.5	80.36 \pm 2.8	73.71 \pm 1.95	8.3 \pm 1.1***
Group VII	ethylacetate extract 250mg/kg	4.9 \pm 0.13	4.6 \pm 0.19	23.0 \pm 0.49	21.4 \pm 0.6	112.7 \pm 2.4	98.44 \pm 2.76	12.7 \pm 0.4***
Group VIII	ethylacetate extract 500mg/kg	5.2 \pm 0.08	4.7 \pm 0.12	24.9 \pm 0.15	23.6 \pm 0.2	129.48 \pm 0.8	110.92 \pm 0.94	14.3 \pm 0.1***
Group IX	methanol extract 250mg/kg	7.8 \pm 0.27	6.8 \pm 0.29	39.02 \pm 0.85	32.2 \pm 0.5	304.36 \pm 6.6	218.96 \pm 3.4	28.1 \pm 1.1***
Group X	methanol extract 500mg/kg	8.9 \pm 0.14	7.6 \pm 0.14	43.58 \pm 0.43	30.63 \pm 0.3	387.86 \pm 3.8	232.79 \pm 2.3	39.98 \pm 0.4***
Group XI	standard	7.9 \pm 0.06	7.1 \pm 0.10	40.37 \pm 0.22	31.10 \pm 0.4	318.92 \pm 1.7	220.81 \pm 2.8	30.76 \pm 0.3***

All values are expressed as mean \pm SEM of six observations.

UB- Untreated blood; NFTB – nylon fiber treated blood.

*** $P < 0.001$ when compared to control.

4. DISSCUSSION

Neutrophils are part of the cell-mediated immune responses responsible for the innate immunity that contribute to the clearance of foreign bodies by recognition and migration toward the foreign body, phagocytosis and destroying the foreign agent [18]. Cell adherence property of neutrophils is one of the earliest responses of both immunological and physical injury. In the neutrophil adhesion test the cell adherence property of neutrophils was assessed in blood samples from different groups, by treating with nylon fibers to which the neutrophils adhere [17]. In the present study, neutrophil adhesion of n-hexane, chloroform, ethylacetate and methanolic extracts of *Lepidium sativum* Linn. seeds compared with effect of disease control. This comparison showed that all extracts have more rise in neutrophil adhesion as compared to disease control. Neutrophil adhesion is maximum for methanolic extract and minimum for chloroform extract. At the same time standard compared with disease control. Standard showed great rise in neutrophil adhesion as compared to disease control. Comparison of standard with methanolic extract of high dose (500 mg/kg), methanolic extract showed showed large neutrophil adhesion than standard. The results showed that methanolic extract of high dose (500 mg/kg) showed a substantial rise in the neutrophil adhesion to nylon fibers which was an indication of the boosting the neutrophil migration toward foreign bodies.

5. CONCLUSION

The results of the present study conclude that *Lepidium sativum* Linn. is an immune system booster. Methanolic extract of high dose (500 mg/kg) showed more increase in neutrophil adhesion as compared to standard. The exact constituent responsible for the immunostimulant property of *Lepidium sativum* Linn. seeds is not known. Further work is being undertaken in our lab to evaluate the effect of constituent present in *Lepidium sativum* Linn. seeds on the immune system.

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