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IMMUNOMODULATION EFFECT OF VITEX NEGUNDO LINN. (NIRGUDI)

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Abstract

The current study explores the immunomodulatory activity of *Vitex negundo (VN)* Linn (verbenaceae), known as Nirgudi which is a medicinally important plant having wide variety of phytoconstituents having significant pharmacological activities. Oral administration of extracts of Nirgudi stem and leaves (100 mg/kg) showed a significant increase ($P < 0.05$) in body weight of wistar albino rats- experimental animals. A significant increase in the lymphocyte and total leukocyte count as well as in the percent neutrophil adhesion to nylon fibers was observed. In VN treated rats, the phagocytic index was enhanced. The results led to a conclusion that VN possess potential immunomodulatory activity and has therapeutic potential against auto-immune disorders.

Keywords: *Vitex negundo* (VN), cyclophosphamide, immunomodulatory, Differential leukocyte count- DLC, Total leukocyte count- TLC.

Introduction

Vitex negundo Linn. an important medicinal plant commonly known as Nirgundi belongs to family Verbenaceae. Sinduvaara is the white flowered variety, whereas the blue flowered variety is known as Nirgundi or Sephaali. The Leaves of *Vitex negundo* (VN) contain flavonoids such as vitexin and irridoid glycosides namely negundoside and which is a flavonol glycoside besides casticin and the glycosides, a-D-glucoside of tetrahydroxy monomethoxy flavones and luteolin-7-glucoside. Also two pentacyclic triterpenoids, ursolic acid and betulinic acid along with an aliphatic alcohol, β-sitosterol and p-hydroxybenzoic acid have been isolated from leaves. Stigmasterol and hentriacontane are present in dried powder of roots. The seeds contain triterpenes, p-hydroxybenzoic acid and glucose [1-3]. The water extract of the leaves showed anti-inflammatory, antihistaminic, analgesic activity [4-6] and antioxidant activity [7]. Significant antibacterial activity is exhibited by different extract of leaves and bark of VN [8]. Hepatoprotective activity against carbon tetrachloride using human liver cells was exhibited by the active constituent negundoside isolated from leaves of VN [9-11]. Anti-implantation activity of VN leaves has also been reported in the literature [12]. Significant laxative activity in a dose dependent manner was also shown by VN [13]. *Vitex negundo* also led to attenuation of catractogenesis and calpain activation in selenite induced cataract [14]. The petroleum ether extract of VN served as a potent larvicidal agent [15]. It has been also found that VN extract significantly showed reduction of oxidative stress [16]. In Complete Freund's adjuvant induced paw edema in rats, methanol extract of VN leaves showed significant anti-arthritic activity [17] and as well as anticonvulsant activity [18]. The VN root extract significantly showed activity against snake venom [19] and immunomodulatory potential [20].

Materials and methods

Collection and authentication of Plant

The leaves and stem of VN was collected from Shiroor, Maharashtra and the voucher specimen (08-123) was preserved at herbarium of (Agharkar Research Institue) ARI. The plant material was properly processed by cleaning, shade drying and powdering it to 40 mesh and finally storing in a tightly closed container at 25°C.

Extraction of Plant

VN leaves and stem were washed and dried at 55 °C in an air dryer for 48 h and powdered separately with a Wiley mill (model-4276 M, Thomas, Scientific, USA) to pass through a 20 mesh sieve and stored in an air tight sealed plastic bag. Powdered dried leaf and stem, 500 mg were taken in a 5 ml volumetric flask, 5ml of methanol was added and vortexed for two minutes followed by sonication (33 MHz, Roop telesonic, India) at room temperature for 7-8 min. The process was repeated thrice to ensure complete extraction followed by sonication and finally individual methanol extracts were combined and evaporated to dryness in vacuo. Dried extract was obtained as 1.03 g.

Animals

4 groups of six animals each of Wistar albino rats were used for this study. Group 1 served as a control, to which Polyvinylpyrrolidone (PVP) in water was given. Group II was given cyclophosphamide (standard drug) and group III received VS methanol extract and group IV received VL methanol extract.

Preparation of suspension of drug extract

Suspension of leaves and stem extracts of VN was prepared by suspending weighed quantity of drug in 2% PVP in water.

Dose and route of administration

VN stem and leaf extracts were administered orally at a dose of 100 mg/kg body weight and

cyclophosphamide was given at a dose of 3 mg/kg.

Evaluation of immunomodulatory activity

Determination of hematological parameters

Hematological parameters has been correlated to the ability of drug to stimulate the formation of blood cells (specific immune response). In the current study, the extracts were administered to the animals for 14 days. The blood samples were taken from the animals on 0^{th}, 7^{th} and 14^{th} day of drug administration and hematological parameters such as DLC, differential leukocyte count, (eosinophil, basophil, neutrophil, lymphocyte and monocyte count) and TLC i.e. Total leukocyte count were evaluated. Interpretation of results was done by using the method of Ziauddin *et al.* (1996) [21].

Carbon clearance test

Carbon clearance test is an indicative of phagocytic activity of immune system (non-specific immunity). All the animals received the extracts for a week. On the 8^{th} day of study, 0.2 ml of camel ink was administered i/v through tail vein in all groups, 30 min after the VN extracts were administed orally. Prior to challenge the blood samples were collected and at time interval of 0, 5, 10, 15, 20, 25, and 30 min after challenge from retro-orbital plexus. About 3ml of 25% glacial acetic acid was used to lyse Blood samples (25 µl) and the optical density of lysed samples were recorded spectrophotometrically at 630nm until transparency equivalent to a standard sample that was collected before challenge was observed. Plot was made between optical density and time on a logarithmic scale and the line was regressed [22]. The following formula

Phagocytic index P.I. = K sample/ K standard

Where, K= slope of regressed line.

Neutrophil adhesion test

Neutrophil adhesion test correlates the ability of neutrophils to stimulate non-specific immune response, indicated by peripheral movement of neutrophils. After the determination of neutrophil count and various hematological parameters, blood samples were incubated with 80 mg/ml of nylon fiber for 15 min at 37°C and further the incubated blood samples were analyzed for TLC and DLC determination [23]. Neutrophil index of blood samples was determined as the product of TLC and DLC. Following formula was used to calculate percent neutrophil adhesion:

% Neutrophil adhesion = Neutrophil count of control- Neutrophil count of test / Neutrophil count of control

Results and Discussions

Determination of Haematological parameters

The analysis of hematological parameters TLC and DLC suggests that stem and leaf extracts of VN have a potentiating effect on hematological parameters such as RBC and WBC Count Table (4, 4.1). Increase of TLC was shown by both the extracts of VN such a leaf extract (2.9) but the effect was more prominent on 20 day of drug treatment as compared to control (3.6). The results showed increase in leukocyte count at 20 day of leaves and stem extracts treatment 2.9 and 2.7 thousand/mm^3 respectively as compared to control (3.60). The enhancement in various hematological parameters can be correlated with the immune stimulating nature of the crude drug.

The stem and leaves extracts showed increase in neutrophil count at 20^{th} day of treatment 2.175, 2.05 thousand /mm^3 respectively as compared to control (2.7). The stem and leaves showed increase in eosinophil, basophils and monocyte (mixed) count at 20^{th} day treatment 0.174, 0.162 thousand/mm^3 respectively as compared to control (0.216) Table (1-2) The effect on body weight of experimental animals is depicted in Table 3.

Carbon clearance test

Enhancement of phagocytic index signifies the faster removal of foreign particle from the body, thereby suggesting faster elicitation of the immune response. Both the extracts of VN enhanced phagocytic activity, which is an indicative of immunostimulation. The VN stem extract (0.261) most strongly enhanced rate of carbon clearance (non- specific immunity) that was prominent at 20 min of drug administration as compared to control (0.421) and VN leaf extract (0.301). The results were shown in table (4).

Neutrophil adhesion test

Neutrophil adhesion test determines the adhesion of neutrophil to nylon fiber. The methanol extract of both stem and leaf of VN increased the process of margination of cells in blood vessels. Neutrophil adhesion has been correlated to the ability of neutrophils to stimulate non-specific immune response which is indicated by the peripheral movement of neutrophils i.e. greater adhesion. Leaf extract of VN showed greater neutrophil adhesion (28.1) as compared to stem (32.6) and control (37.9) Table (5).

Different extracts of VN showed stimulatory effect on hematological parameters such as body weight, neutrophil adhesion and carbon clearance. Stimulation of various hematological parameters indicates the effectiveness of drug in the management of various blood disorders anemia and other hematological disorders. The above results showed effectiveness of drug in treatment of immunosuppressive disorders such as severe combined immunodeficiency (SCID), Ataxia telangiectasia, Acquired immunodeficiency syndrome (AIDS), Wiskott Aldrich syndrome (WAS), Bare lymphocyte syndrome, Digeorge syndrome and Gammaglobulinemias.

Conclusion

The current research throws light on immunomodulatory potential of VN leaves and stem extract and the findings prove that VN has therapeutic potential in stimulation of various hematological parameters and could serve as an effective immunomodulatory candidate.

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Table: 1 Effect of VN on hematological parameters

Groups	0				20			
	RBC	WBC	Hb	Platelets	RBC	WBC	Hb	Platelets
Control	3.13	3.4	6.2	549	3.18	3.6	6.3	552
VL	2.91	2.6	5.4	519	3.02	2.9	5.8	541
VS	2.78	2.5	5.26	502	2.81	2.7	5.6	529

Table: 2 Effect of VN extracts on DLC

Groups						
	Neutrophil	Lymphocyte	Mixed	Neutrophil	Lymphocyte	Mixed
Control	2.55	0.646	0.204	2.700	0.684	0.216
VL	1.95	0.494	0.156	2.175	0.551	0.174
VS	1.75	0.475	0.150	2.025	0.513	0.162

Table 3: Effect of VN on body weight in rats

Groups	Body weight	
	0 day	20 day
Control	150	180.3
CP	129.6	124.0
VL	207.6	154.3
VS	184.1	136.2

All values are expressed as mean ± S.E.M, n=6. P*<0.01; P<0.05 considered significant as compared to control**

Table 4: Carbon clearance test of VN

Group	Absorbance		
	0	10	20
Control	0.541 ± 0.02	0.452 ± 0.02	0.421 ± 0.007
VL	0.411 ± 0.01	0.331 ± 0.02	0.309 ± 0.02
VS	0.311 ± 0.02	0.303 ± 0.02	0.261 ± 0.01

Table 5: Neutrophil adhesion data of VN

Group	TLC		% Neutrophil adhesion		Neutrophil index		% NA
	UTB	TB	UTB	TB	UTB	TB	
Control	4.31 ± 0.03	3.4 ± 0.03	3.2 ± 0.05	2.5 ± 0.02	13.79 ± 0.02	8.5 ± 0.02	37.9 ± 0.003
VL	2.6 ± 0.002	1.9 ± 0.007**	1.98 ± 0.02**	19.5 ± 0.02**	51.48 ± 0.28**	37.0 ± 0.19	28.12 ± 0.007**
VS	2.5 ± 0.002**	1.7 ± 0.02**	1.95 ± 0.03**	19.32 ± 0.1**	48.75 ± 0.04**	32.84 ± 0.25**	32.63 ± 0.25**

All values are expressed as mean ± S.E.M, n=6, P* < 0.05, considered significant as compared to control. CP: Cyclophosphamide; UTB: Untreated blood, TB: Treated Blood