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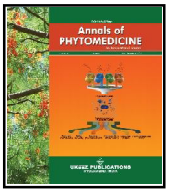

Original Article : Open Access

Immunomodulatory activity of ethanolic extract of *Vitis vinifera* L. in laboratory animal

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Abstract

Globally, *Vitis vinifera* L. is used to make wine. It is also used to cure skin and eye diseases. Ripe grapes were used to cure cancer, cholera, smallpox, kidney and liver diseases. In the present study, phytochemical analysis and immunomodulatory activity of ethanolic extract of the fruit of *V. vinifera* were investigated. Laboratory-based experimental methods were used to evaluate immunomodulatory activity by dividing animals into six different groups, *i.e.*, control, levamisole (Std-1), cyclophosphamide (Std-2), EVV150, EVV300 and EVV450 mg/kg, comprising six animals in each. The immunomodulatory activities were determined by using the carbon clearance test, delayed type hypersensitivity (DTH) test, neutrophil adhesion test, haemagglutination antibody (HA) titer response and T cell population assay.

The response produced by oral administration of ethanolic extracts of the fruit of *V. vinifera* showed a significant dose-dependent increase in phagocytic activity, DTH response, neutrophil adhesion response, augmentation of humoral immune response to sheep red blood cells and an increase in T cell population response. Finally, the results concluded that ethanolic extract of the fruit of *V. vinifera* caused a significant immunostimulatory effect on both the cell-mediated and humoral immune systems in the Wistar albino rats.

1. Introduction

Nowadays, worldwide, there is an increase in the incidence of infectious diseases, which makes individuals more opportunistic to infection, if their immune systems are weaker. Malnutrition, inadequate dietary balance, especially protein and other major causes such as stress, lack of sleep and immunodeficiency disease can make an individual more prone to acquiring the infection. Where malnutrition greatly affected an individual's immune system, which is approximately 14.9%, the immune system is involved in the aetiology and pathophysiology of various diseases. Maintaining a balanced nutritional diet is very important for maintaining the overall immune status of the body to defend against infectious disease or illness (Unicef, 2020; Calder *et al*, 2003; Cooper *et al*., 2005; Chandra, 1997; Calder and Jackson, 2000; Nupur Mehrotra, 2021).

The immune system has been modulated by a number of plant-derived substances such as flavonoids, phenols, tannin, glycosides and vitamins. Phytochemicals are natural substances that play a crucial role in regulating positive immune responses. Immuno-modulation is a therapeutic approach used to auto-regulate the body's defence system to fight against infection. Immunity is also important in maintaining an organism's physiological stability and many serious illnesses, such as cancer, diabetes and rheumatoid arthritis, are caused by an imbalance in the immune system (Kayser *et al.*, 2003; Brindha, 2016; Umaretiya, 2020; Das *et al*., 2020; Girija and Ramadasan, 2019).

V. vinifera common grape vine is a flowering plant containing many phenolic compounds, flavonoids, carotenoids, vitamin C, vitamin B and is rich in unsaturated fatty acids, which help to lower total cholesterol and LDL cholesterol in the blood. Grapes can be eaten fresh or dried as nutritive food which possesses antioxidant, antibacterial, antiviral and antidiabetic activity.

Traditionally, *V. vinifera* is used to increase disease resistance and also exhibit effects on the central nervous system, cardiovascular system, respiratory system and hormone replacement therapy. In ancient times, it was used to cure bronchial disease (Marjan and Hossein, 2016). The scientific literature as an immunomodulator is not available. Hence, the present study was undertaken to evaluate the phytochemical and immunopharmacological activity of *V. vinifera* on experimental animals.

2. Materials and Methods

2.1 Collection of fruit

The fruit of *V. vinifera* were collected from the local market of Miraj, Maharashtra. After collection, it was authenticated by the Botanical Service of India in Pune, Maharashtra (BSI/WRC/IDEN. CER./2020/95, dated October 1, 2020).

2.2 Sample preparation

The fruit of *V. vinifera* was thoroughly clean. They were dried under shade, cut into small pieces and stored in an airtight container for further use.

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2.3 Extraction of a sample

The sample was extracted from the simple maceration and sonication extraction processes by using an ethanolic solvent. The concentrated extract was collected and stored in airtight container for further use (Bhokare *et al.*, 2018; Kumar *et al.*, 2013).

2.4 Preliminary phytochemical investigation

Phytochemical investigations were performed to identify the presence of various chemical constituents in the extracts as per Khandelwal (2012).

2.5 Animals used

The Wistar albino rats (weighing approximately 150 to 200 g) were taken from the animal house of Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra. The experimental protocol was approved by the institutional animal ethics committee (IAEC/ABCP/16/2020-21) and the care of laboratory animals was taken as per the CPCSEA guidelines.

2.6 Acute toxicity studies

An ethanolic extract of the fruit of *V. vinifera* was tested for acute toxicity studies as per OECD guidelines 425. Doses were selected at 150, 300, and 450 mg/kg body weight of animals and were observed for any sign of toxicity or mortality (Ahmed *et al.*, 2011; Shewale *et al.*, 2012).

2.7 Antigenic material

2.7.1 Preparation of sheep RBCs

Sheep blood was collected in sterile Alsever's solution in a 1:1 proportion, Alsever's solution (freshly prepared) blood was kept in the refrigerator and processed for the preparation of SRBCs batches by centrifugating at 2000 rpm for 10 min, washing with physiological saline 4-5 times and then suspending into buffered saline for further use.

2.7.2 Carbon ink suspension preparation

Camlin ink was diluted eight times with saline before being used for carbon clearance tests at a dose of 10 ml/g body weight of rat (Dashputre and Naikwade, 2010).

2.8 Grouping and treatment schedule

Table 1: Grouping and treatment schedule

S.No	Groups	Test substance	Dose (P.O)
1.	Group I	Control	10 ml/kg
2.	Group II	Levamisole (Std-1)	50 mg/kg
3.	Group III	Cyclophosphamide (Std-2)	50 mg/kg
4.	Group IV	Ethanolic extract of fruit of *V. vinifera*	150 mg/kg
5.	Group V	Ethanolic extract of fruit of *V. vinifera*	300 mg/kg
6.	Group VI	Ethanolic extract of fruit of *V. vinifera*	450 mg/kg

2.9 *In vivo* immunopharmacological activity

Carbon clearance test

Delayed type hypersensitivity test

Neutrophil adhesion test

In vivo antibody (HA) titer response to SRBCs

T-cell population assay

2.9.1 Carbon clearance test

Phagocytic activity of the reticuloendothelial system was assayed by a carbon clearance test; the phagocytic index is used to calculate the rate of carbon elimination by the reticuloendothelial system.

2.9.1.1 Procedure

- In this test, animals were divided into six groups, each comprising six animals. Table 1 shows the grouping schedule.
- After seven days of treatment, carbon ink suspension was injected *via* tail vein into each rat for 48 h.
- Blood samples (25 ml) were then withdrawn from the retro-orbital plexus under mild ether anaesthesia at 5 and 15 min after injection of colloidal carbon ink lysed in 0.1% sodium carbonate solution (3 ml).
- The optical density was measured spectrophotometrically at 660 nm.
- The phagocytic activity was calculated using the following formula (Tripathi *et al.*, 2012):

$$K = \text{Log OD1} - \text{Log OD2} / t2 - t1$$

where, OD1 and OD2 are the optical densities at time t1 and t2, respectively.

2.9.2 Delayed type hypersensitivity test

The delayed hypersensitivity reaction is a cell-mediated immunity reaction that manifests itself after 16-24 h.

2.9.2.1 Procedure

- In this test, animals were divided into six different groups, each comprising six animals. As per the grouping schedule mentioned in Table 1.
- The immunised rats were given 0.1 ml of 20% SRBCs in normal saline intraperitonially on the 14th day of the study.
- On day 21st, animals from all groups get a challenge with 0.03 ml of 1% SRBCs in the sub-plantar region of their right hind paw. Foot pad reaction was assessed after 4 h, 8 h and 24 h, *i.e.*, on the 22nd, an increase in footpad oedema was measured with the help of a vernier caliper (Tripathi *et al.*, 2012).

2.9.3 Neutrophil adhesion test

Increase the recruitment of neutrophil adhesion to nylon fibers, which correlates to the process of margination of cells in blood vessels.

2.9.3.1 Procedure

- In this test, animals were divided into six groups, each comprising six animals. As per the grouping schedule mentioned in Table 1.
- On the 16th day of the treatment, blood samples from all the groups were collected by puncturing the retro-orbital plexus under mild anesthesia.

- Blood was collected in vials pre-treated by disodium EDTA and analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC).
- After initial count, blood samples were collected with nylon fibre (80 mg/ml, previously sterilised by 95% alcohol) for 15 min at 37^{0}C the incubated blood samples were analyzed for TLC and DLC. The product of TLC and neutrophil adhesion was calculated as follows (Sharma, and Rangari, 2016):

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIU} - \text{NIT}}{\text{NIU}} \times 100$$

where,

NIU: Neutrophil index before incubation with nylon fibers.

NIT: Neutrophil index after incubation with nylon fibers.

2.9.4 *In vivo* antibody (HA) titer response to SRBCs

The highest dilution of a sample at which clumping is seen is considered haemagglutination.

2.9.4.1 Procedure

- The animals were immunised by injecting 0.1 ml of SRBCs suspension containing 0.5 × 10^9 cells intra-peritoneally on day 0.
- Blood samples were collected in microcentrifuge tubes from individual animals by retro-orbital puncture on day 11.
- The blood samples were centrifuged and serum was obtained.
- Antibody levels were determined by the haemagglutination technique. Equal volumes of individual serum samples from each group were pooled. Two-fold serial dilutions of pooled serum samples made in 25 ml volume of normal saline in microtitration plates were added to 25 ml of a 1% suspension of SRBCs in saline.
- After mixing, the plates were incubated at 37°C for 1 h and examined for haemagglutination under a microscope.
- The reciprocal of the highest dilution of the test serum agglutination was taken as the antibody titre (Shukla and Mehta, 2015).

2.9.5 T cell population assay

Humoral immune response and cell mediated response can be assayed by T cell population assay.

2.9.5.1 Procedure

- Antigen challenge: On 0th day, all groups were sensitised with 0.1 ml of SRBCs containing 1×10^8 cells, i.p. On the 11th day, blood was collected from the retro-orbital plexus and anticoagulated with Alsever's solution in separate test tubes.
- Test tubes containing blood were kept in a sloping position (45^o) at 37^{o}C for 1 h. RBCs were allowed to settle at the bottom and supernatant was collected from each test tube by using a micropipette which contains lymphocytes.
- 50 ml of lymphocyte suspension and 50 ml SRBCs were mixed in the test tube and incubated. The resultant suspension was centrifuged at 200 rpm for 5 min and kept in a refrigerator at 4^{0}C for 2 h.

The supernatant fluid was removed, and one drop of cell suspension was placed on a glass slide. Total lymphocytes were counted and a lymphocyte bound with three or more erythrocytes was considered a rosette and the number of rosettes was counted (Shukla and Mehta, 2015; Sneha *et al*., 2014).

2.10 Statistical analysis

Mean value ± SEM was used to express the test outcome. The one way analysis of variance (ANOVA) technique was used to estimate the variation in a collection of data. The individual comparison of group mean value were done by Dunnet's Test. Statistics were considered significant when the $p<0.05$.

3. Results

3.1 Photochemical investigation

The phytochemical screening of ethanolic extract of *V. vinifera* were done by qualitative chemical test and results obtained were plotted in Table 2.

Table 2: Phytochemical investigation of *V. vinifera*

S. No	Test	Result
1	Test for carbohydrate	
	Molish's test (General test)	++
	Benedict's test	-
2	Test for protein and amino acid	
	Biuret test (General test)	-
	Millon's test	-
	Ninhydrin test	-
3	Test for glycoside	
	1. For cardiac glycoside	
	Baljets test	-
	Legal test	-
	Keller-Killiani test	-
	2. For anthraquinone glycoside	
	Borntargers test	-
	Modified borntargers	-
	3. For saponin glycoside	
	Foam test	-
4	Test for flavonoid	
	Sulphuric acid test	+++
	Shinoda test	+++
	Lead acetate solution test	-
5	Test for alkaloid	
	Dragendroff's test	+++
	Mayer's test	+++
	Hager's test	++
	Wagnar's test	+++
6	Test for tannin and phenolic compound	
	5% Fecl3	+++
	Lead acetate solution	+++
	Bromine water	+++
	Nitric acid	-
7	Test for steroids	
	Salkowaski test	+++
	Libermann burchard test	++
8	Test for vitamin C	+++

Note: (-): Absent, (+): Present, (++): Moderate present, (+++): Strongly present.

3.2 Carbon clearance test

Table 3: Result of carbon clearance test

S. No.	Group	Treatments	Dose and route of administration	Phagocytic index (Mean ± SEM)
1	I	Control	10 ml/kg (P.O.)	0.0122 ± 0.0014
2	II	Levamisole (Std-1)	50 mg/kg (P.O.)	0.0729 ± 0.0038****
3	III	Cyclophosphamide(Std-2)	50 mg/kg (P.O.)	0.0067 ± 0.00005^{ns}
4	IV	EVV	150 mg/kg (P.O.)	0.0487 ± 0.003117****
5	V	EVV	300 mg/kg (P.O.)	0.0557 ± 0.00261****
6	VI	EVV	450 mg/kg (P.O.)	0.0640 ± 0.00256****

EVV= Ethanolic Extract of fruit of *V. vinifera*

Value are expressed as (Mean ± SEM). n=6 **** p<0.0001 statistically significant when compared with control group by ANOVA, followed by Dennett test.

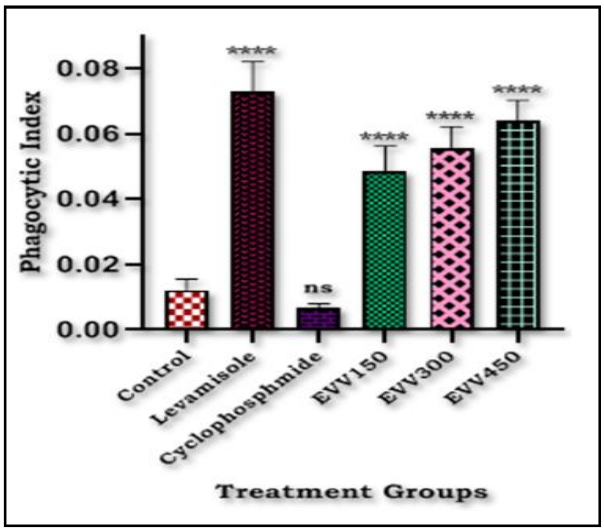

Figure 1: Graphical representation of carbon clearance test.

Phagocytic activity of reticuloendothelial system was studied by carbon clearance test. Test revels that EVV 450 mg/kg dose showed significant increase in phagocytic index 0.0640 ± 0.00256**** when compared with control, which indicate removal of carbon practical from bloodstream.

3.3 Delayed type hypersensitivity test

Table 4: Result of delayed type hypersensitivity test

Group	Treatments	Dose and route of administration	% Increment in foot pad thickness		
			4 h	8 h	24 h
I	Control	10 ml/kg (P.O.)	7.20 ± 0.5510	14.67 ± 0.4261	20.39 ± 0.4597
II	Levamisole(Std-1)	50 mg/kg (P.O.)	26.875 ± 0.57698****	34.94 ± 0.4822****	44.50 ± 0.4191****
III	Cyclophosphamide(Std-2)	50 mg/kg (P.O.)	5.83 ± 0.4890^{ns}	9.382 ± 0.5684****	11.01 ± 0.7774****
IV	EVV	150 mg/kg (P.O.)	13.13 ± 0.1114****	23.69 ± 0.1701****	30.18 ± 0.1782****
V	EVV	300 mg/kg (P.O.)	16.09 ± 0.1499****	27.24 ± 0.1337****	34.74 ± 0.2124****
VI	EVV	450 mg/kg (P.O.)	22.35 ± 0.0976****	33.43 ± 0.1019****	39.08 ± 0.1742****

Values are expressed as (Mean ± SEM). n = 6 **** p<0.0001 statistically significant when compared with control group by ANOVA, followed by Dunnett test.

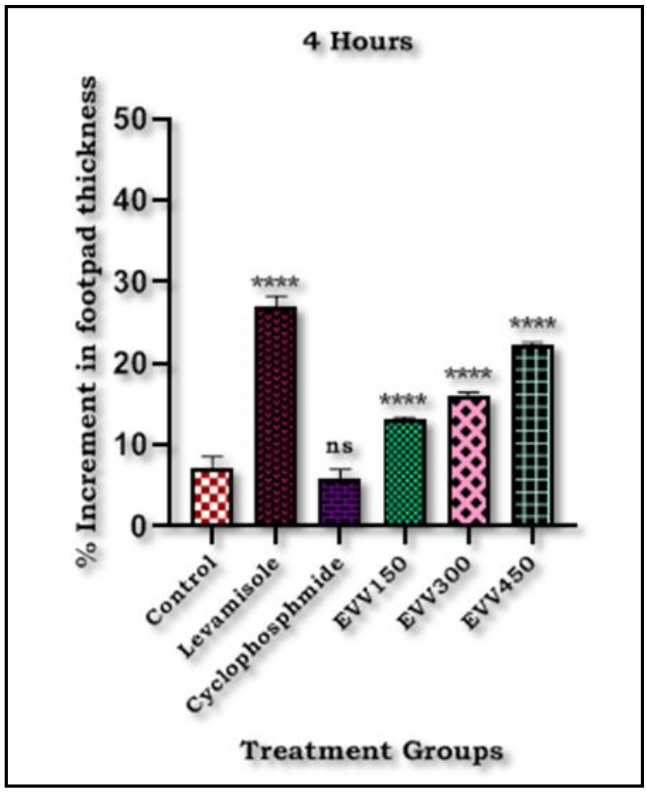

Figure 2: Graphical representation of delayed type hypersensitivity test (4 h).

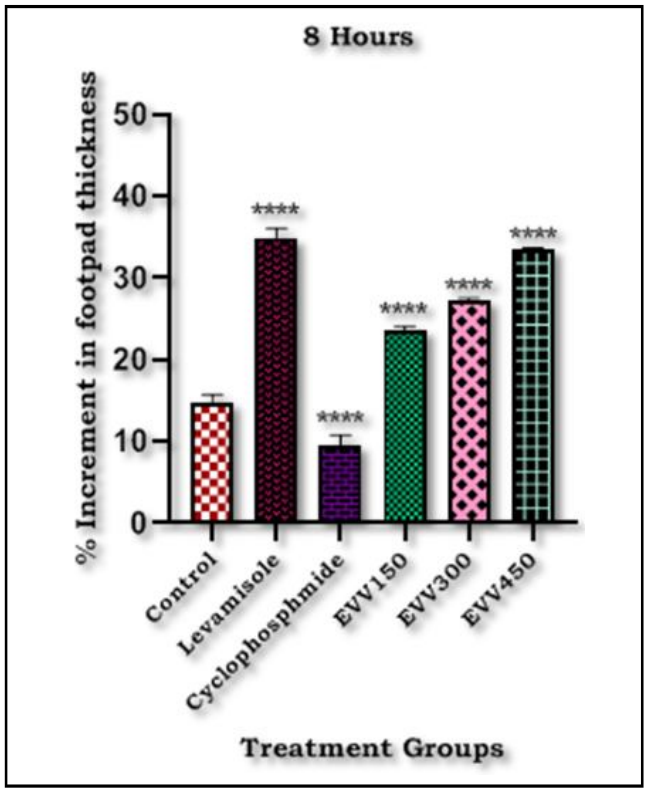

Figure 3: Graphical representation of delayed type hypersensitivity test (8 h).

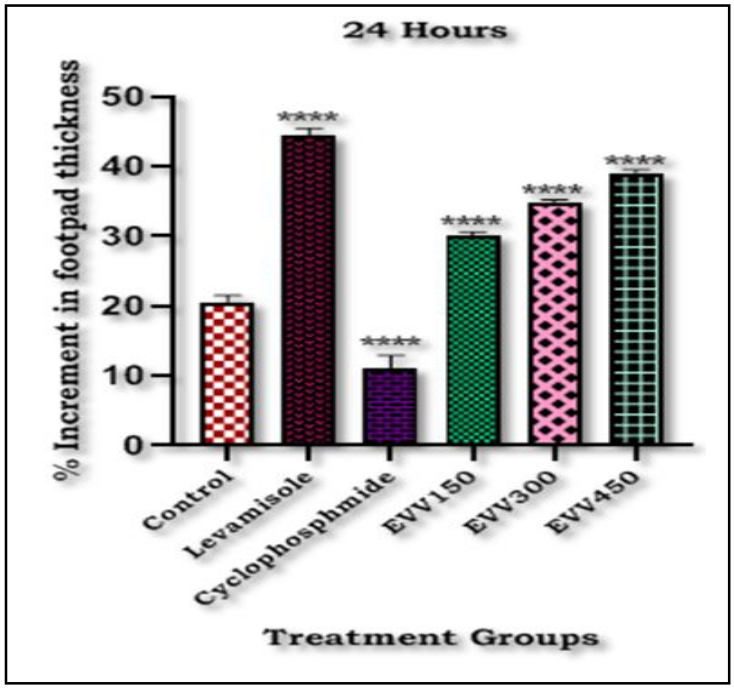

Figure 4: Graphical representation of delayed type hypersensitivity test (24 h).

DTH method indicate cell mediated immune response. The paw oedema values varied significantly across all groups. The ethanolic extract of *V. vinifera* treated groups showed significant increase in paw oedema (p>0.0001), when compared with group I. The EVV 450 mg/kg showed increase in paw volume value (39.08 ± 0.1742****) after 24 h of treatment when compare with control.

3.4 Neutrophil adhesion test

Table 5: Result of neutrophil adhesion test

Group	Treatments	Dose and route of administration	Neutrophil index before treatment (NIU)	Neutrophil index after treatment (NIT)	%Neutrophil adhesion (Mean ± SEM)
I	Control	10 ml/kg (P.O.)	240.31 ± 3.42	216.96 ± 3.27	10.09 ± 0.45
II	Levamisole (Std-1)	50 mg/kg (P.O.)	280.4 ± 4.6	128.16 ± 3.02	54.31 ± 0.45****
III	Cyclophosphamide(Std-2)	50 mg/kg (P.O.)	188.8 ± 7.04	175.98 ± 7.66	6.88 ± 0.79**
IV	EVV	150 mg/kg(P.O.)	269.56 ± 11.20	189.81 ± 0.42	29.54 ± 0.80****
V	EVV	300 mg/kg(P.O)	262.45 ± 10.38	168.81 ± 7.57	35.73 ± 0.68****
VI	EVV	450 mg/kg(P.O)	263.33 ± 8.38	143.35 ± 5.59	45.61 ± 0.73****

Values are expressed as (Mean ± SEM). n=6 **** p<0.0001 statistically significant when compared with control group by ANOVA, followed by Dunnett test.

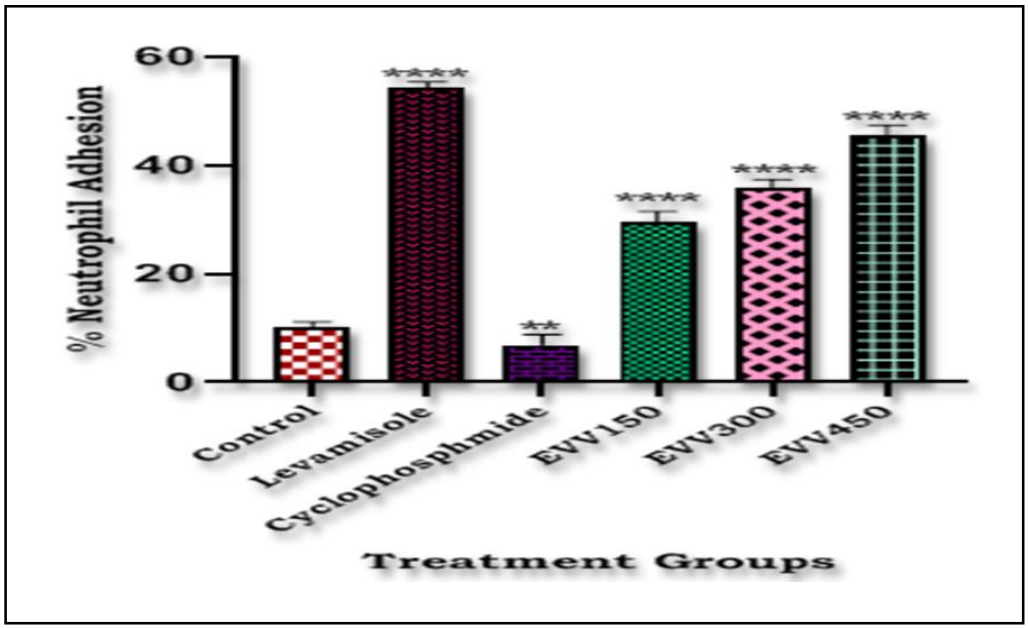

Figure 5: Graphical representation of neutrophil adhesion test.

Decrease in neutrophil count after incubation of blood with nylon fiber and increase in % neutrophils adhesion with nylon fiber which indicated cell mediated immune response which was significantly increase 45.61 ± 0.73**** of EVV 450 mg/kg treated group when compare with control.

3.5 *In vivo* antibody (HA) titer response to SRBCs

Table 6: Result of *in vivo* antibody (HA) titer response to SRBCs

Group	Treatments	Dose and route of administration	HA titer (Mean ± SEM)
I	Control	10 ml/kg (P.O.)	9.33 ± 1.3385
II	Levamisole (Std-1)	50 mg/kg (P.O.)	469.33 ± 42.8326****
III	Cyclophosphamide(Std-2)	50 mg/kg (P.O.)	5.33 ± 0.8465^{ns}
IV	EVV	150 mg/kg (P.O.)	64.00 ± 0.000^{ns}
V	EVV	300 mg/kg (P.O.)	106.66 ± 13.5448*
VI	EVV	450 mg/kg (P.O.)	298.66 ± 42.8326****

Values are expressed as (Mean ± SEM). n=6 **** p<0.0001 statistically significant when compared with control group by ANOVA, followed by Dunnett test.

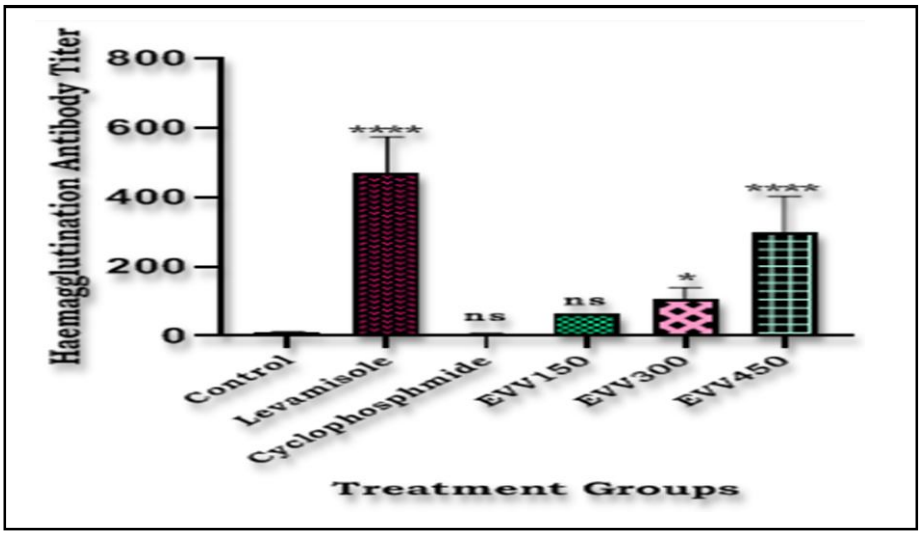

Figure 6: Graphical representation of *in vivo* antibody (HA) titer response to SRBCs.

Humoral antibody response to SRBCs challenge was found to be significantly (p<0.0001) increase by EVV 450 treated groups when compare with control.

3.6 T cell population assay

Table 7: Result of T cell population assay

Group	Treatments	Dose and route of administration	Total lymphocyte count ($10^3/mm^3$) (Mean ± SEM)	Number of rosette (Mean ± SEM)
I	Control	10 ml/kg (P.O.)	4.81 ± 0.1254	6.16 ± 0.3346
II	Levamisole (Std-1)	50 mg/kg (P.O.)	6.4 ± 0.1904****	23.5 ± 0.9255****
III	Cyclophosphamide(Std-2)	50 mg/kg (P.O.)	3.73 ± 0.2035***	5.16 ± 0.4791^{ns}
IV	EVV	150 mg/kg (P.O)	5.53 ± 0.1387*	10.83 ± 0.47911***
V	EVV	300 mg/kg (P.O)	5.71 ± 0.1746**	14.16 ± 0.6565****
VI	EVV	450 mg/kg (P.O)	6.08 ± 0.1452****	18 ± 0.08979****

Values are expressed as (Mean ± SEM). n=6 **** p<0.0001 statistically significant when compared with control group by ANOVA, followed by Dunnett test.

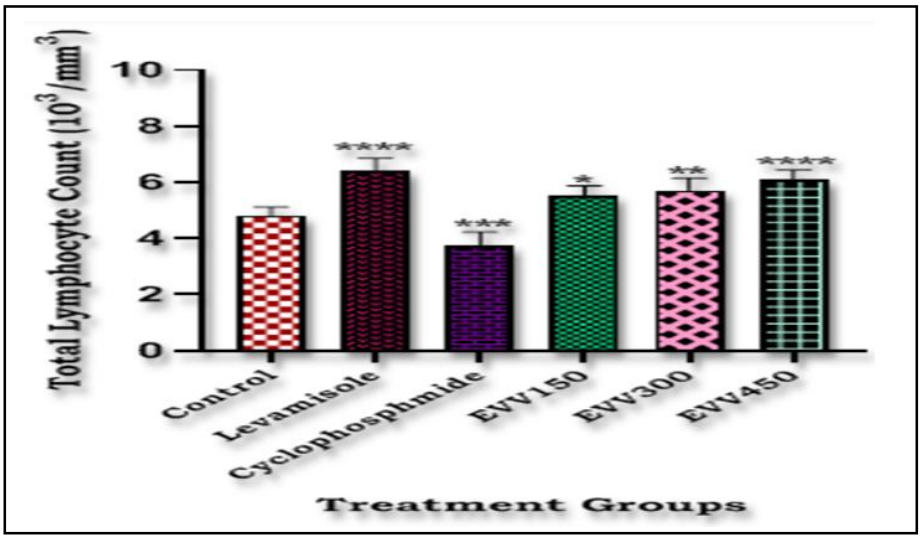

Figure 7: Graphical representation of T-cell population assay (lymphocyte count).

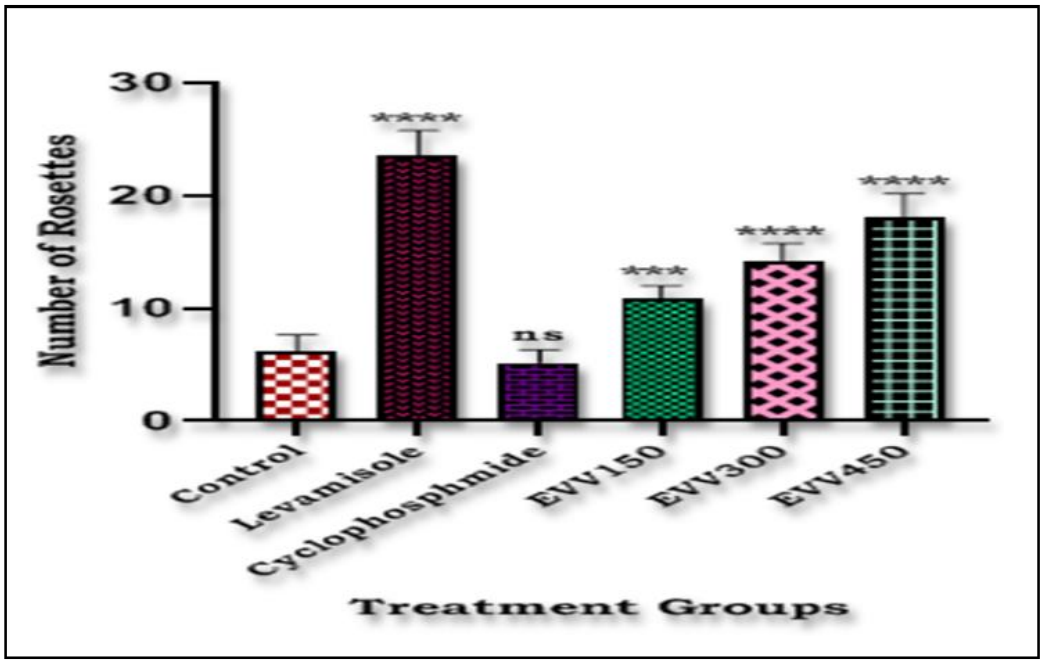

Figure 8: Graphical representation of T-cell population assay (rosettes count).

Ethanolic extract of fruit of *V. vinifera* showed significant effect on increase total lymphocyte count and rosette count after interaction with SRBCs when compare with control group.

4. Discussion

The various fruits, vegetables and nutraceuticals used in the Ayurveda system of medicine in India exhibit a wide range of pharmacological qualities (Prabhakar, 2022). Finding phytochemical plant constituents with immunomodulatory action that could one day be used as alternative medications is gaining popularity in the modern era. The scientific findings from various studies on the *V. vinifera* plant have led to its widespread cultivation for therapeutic purposes **(**Ajith *et al*., 2017; Ghase *et al.,* 2022). The primary goal of this study was to focus on the phytochemical and immunomodulatory activities of an ethanolic extract of *V. vinifera* fruits, with special emphasis on its potential immunomodulatory activity. Acute oral toxicity studies of ethanolic extract of fruits of *V. vinifera*. were performed by OECD guideline 425. We had selected doses three different doses 150, 300 and 450 mg/kg which was safe and does not showed any sign of toxicity and mortality (Pasala *et al*., 2022; Kumar, 2022). The study was conducted using five different methods, each of which offers details on the impact on various immune system components.

Phytochemical investigation

Phytochemical study of ethanolic extract of *V. vinifera* showed presence of carbohydrate, vitamin C, flavonoid, tannin, phenolic compound, steroids and alkaloids which may stimulated overall humoral and cell mediated immune response.

Carbon clearance test

An increase in the removal of carbon particles from the blood stream indicated an increase in phagocytic activity (Patel and Asdaq, 2010). The reticuloendothelial system (RES) consists of mononucleated phagocytes, which are mainly responsible for phagocytosis. Exogenously administered colloidal carbon ink preparations (source of antigen) are recognized as foreign invaders and served by RES through this process of phagocytosis. Table 3 shows the effect of EVV on phagocytic activity as measured by the carbon clearance test. In the carbon clearance test, EVV treated groups exhibited significantly higher phagocytic indexes (p<0.0001) when compared with the control group. The EVV-treated group showed a phagocytic index 0.0487 ± 0.00031**** of dose 150 mg/kg when compared to the control, 0.05570 ± 0026**** of dose 300 mg/kg and 0.06400 ± 002566**** of dose 450 mg/kg, indicated stimulation of the reticuloendothelial system. Similarly, the cyclophosphamide treated group showed a phagocytic index 0.0067 ± 0.00005^{ns} which showed no significant effect on the reticuloendothelial system when compared with the control group. Ethanolic extract of fruit of *V. vinifera* showed increased phagocytic index at a dose of 450 mg/kg, which is close to results obtained by standard levamisole, which is the indicator of increased phagocytic activity and granulopoetic system competency in removing foreign particles (colloidal carbon particles) which is a sign of increased immune response to foreign particles or antigens.

Delayed type hypersensitivity test

The delayed hypersensitivity test (DTH) induces an immune response, specifically memory T cells (SRBCs) that are responsible for margination and inflammation at the site of antigen challenge. DTH is a type IV hypersensitivity reaction that was used to assess skin hypersensitivity after a foreign antigen injection. Further, DTH response also helps to improve lytic enzyme concentration as well as phagocytic activity for more efficient destruction of microbes (Sudha *et al*., 2010). Table 4: shows the effects of DTH-induced footpad edema on EVV on cell-mediated immune response. EVV-treated groups significantly showed an increase in footpad edema (p<0.0001) potentiating delayed type

hypersensitivity response when compared with control groups at an interval of 4 h, 8 h, and 24 h, which indicates the release of cytokines, activation of cellular immune response, increases permeability to vessels and leads to vasodilation and an increase in accumulation of phagocytes, leading to inflammation. Footpad edema increased by a % in the EVV-treated groups 13.13 ± 0.1114****, 16.09 ± 0.1499**** and 22.35 ± 0.0976**** of dose 150, 300, 450 mg/kg at 4 h, respectively. After 8 h, EVV treated group showed % increment in foot pad thickness 23.69 ± 0.1701****, 27.24 ± 0.1337****and 33.43 ± 0.1019****of dose 150, 300, 450 mg/kg, respectively. After 24 h, DTH response was prominently which showed significant repose on foot pad thickness. EVV treated group showed % increment in foot pad thickness 30.18 ± 0.1782****, 34.74 ± 0.2124**** and 39.08 ± 0.1742**** at dose of 150, 300, 450 mg/kg, respectively, when compare with control. Increases DTH response of ethanolic extract of fruit of *V. vinifera* at 450 mg/kg, which is comparable to the results obtained by standard levamisol after 24 h which is an indicator of an increase in cell-mediated immune response to foreign particles or antigens.

Neutrophil adhesion test

Cell-mediated immune responses involve chemotaxis, phagocytosis, exocytosis, intracellular and extracellular killing, and destruction of infected cells by cytotoxic T cells. Neutrophils are the fighting cells of the human body in significantly contributed to the clearance of foreign bodies by recognition and migration towards the foreign body. In the present study, the results showed dose-dependent action over the migration of neutrophil granulocytes into the blood vessels and the accumulation of neutrophils at the site of inflammation, both indicated by neutrophils adhering to the nylon fibers (Pasala *et al*., 2022). Effect of EVV on neutrophils activation by the neutrophils adhesion test is shown in Table 5. Cytokines are secreted by activated immune cell for margination and extravasation of the phagocytes mainly (polymorphonuclear) neutrophils. The percentage neutrophils adhesion was significantly (p<0.0001) increase by EVV 29.54 ± 0.80****, 35.73 ± 0.68**** and 45.61 ± 0.73**** of dose 150,300 and 450 mg/kg, respectively, when compare with control group.

In vivo antibody (HA) titer response to SRBCs

The Haemagglutination antibody response (HA titer) response is used to study humoral immune responses, which involve the interaction of B-cells with the antigen and their subsequent proliferation, differentiation into plasma cells that secrete antibodies **(**Kumar and Vasudeva, 2022) The augmentation of the humoral immune response to SRBCs indicated the enhanced responsiveness of T and B lymphocyte, increase in antibody titre. Humoral antibody response to SRBCs challenge was found to be significantly (p<0.0001) increase by EVV treated groups when compare with control. Table 6 EVV 450 mg/kg showed HA titre response 298.66 ± 42.832**** and EVV 300 mg/kg showed 106.66 ± 13.5448* where EVV 150 mg/kg showed not significant affect over haemagglutination response 64.00 ± 0.0000^{ns} when compare with control.

T cell population Assay

According to Kumar and Vasudeva, T-cells are required for the establishment and maintenance of immune responses, homeostasis and memory. Activation of the T-cell response occurs when any foreign invader enters the body (Kumar and Vasudeva, 2022). EVV treated groups dose showed a significant response to increase in lymphocytes when compared with control. Table 7 EVV 450 mg/kg showed increasing lymphocyte, *i.e.* 6.08 ± 0.1452**** other doses150 mg/kg and 300 mg/kg showed response, *i.e.*, 5.74 ± 0.1746** and 5.53 ± 0.1387*, respectively. Lymphocytes (T-lymphocytes) are involved in both humoral and cell-mediated immune responses. These cells do not secrete the antibody but attack the tissue cells that have been transplanted from one host to another. Therefore, only T cells come into close contact with foreign or infected cells in order to destroy them and to provide cell-mediated immunity. Attachment of lymphocytes to foreign or infected cells is represented as a rosette. Formation of rosette when interaction with antigen showed significantly (p<0.0001) by EVV treated all groups 450 mg/kg, showed response 18.00 ± 0.8979****, 300 mg/kg showed 14.16 ± 0.6565**** and EVV 150 mg/kg showed 10.83 ± 0.4791*** response over rosette formation when compare with control.

Immunomodulatory effects were observed by EVV treated groups at different doses 150, 300, 450 mg/kg, positive response was observed over reticuloendothelial system, DTH response, increase in % of neutrophil adhesion, Increase in antibody response and increase in T-cell count with formation of rosette when interaction with antigen, when compare with control as well as levamisole (Std-1), it indicates ethanolic extract of fruit of *V. vinifera* showed significant immunostimulant activity.

5. Conclusion

Based on study findings, it is reported that the ethanolic extract of fruit of *V. vinifera* increased both the cell-mediated and humoral immune responses in rats. This could be attributed to the presence of different micronutrients, macronutrients and phytochemicals present in the plant. Eating a healthy and balanced diet is crucial to promoting a strong immune system. The immunomodulatory potential of *V. vinifera* could be attributed to the presence of carbohydrates, flavonoids, vitamins, polyphenols, alkaloids and terpenoids which may modulate and potentiate humoral as well as cellular immunity. A detailed investigation needs to be carried out to ascertain its exact mechanism of immunomodulatory action.

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Conflict of interest

The author declares no conflicts of interest relevant to this article.

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