IMMUNOSTIMULANT ACTIVITY OF *COCCULUS HIRSUTUS* ON IMMUNOSUPPRESSED RAT

Bhavna Rastogi [1], Umesh Tiwari[1], Aditi Dubey [1], Bhavana Bawara [1], Nagendra Singh Chauhan [2] and D.K. Saraf *

1. Department of Zoology, Dr H. S. Gour Vishwavidyalya Sagar(M.P) India
2. Department of Pharmaceutical Science, Dr H. S. Gour Vishwavidyalya Sagar(M.P) India

Summary

The aim of the present study is to evaluate the effect of ethanolic and aqueous extract for immunomodulating activity. The ethanolic and aqueous extract of plant of *Cocculus hirsutus* holds potential as a protective agent against cytotoxic drugs. The extracts when studied on humoral and cell mediated immunity in normal, as well as cyclophosphamide-induced immunosuppressed rats. It produced an increase in carbon clearance, humoral antibody (HA) titre, delayed type hypersensitivity (DTH) and WBC count in a dose dependent manner. The present investigation established pharmacological evidence to support the folklore claim that it is an immunomodulating.

Keywords: *Cocculus hirsutus*, Immunostimulant, Cyclophoshamide, Carbon clearance test, Delayed type hypersensitivity, Antibody titre

*Corresponding author
Prof. D.K. Saraf
Department of Zoology,
Dr H. S. Gour Vishwavidyalya Sagar (M.P)
470003 India
Tel: +91-9329787848
E-mail : drdksaraf@gmail.com

Introduction

Plants in Ayurveda are extensively used for the management of neurodegenerative diseases, as rejuvenators, immunomodulators, aphrodisiac and nutritional supplements are known as rasayana herbs [1]. *Rasayana* is a treatment in which the body constituents are prepared to adapt to a selective tissue endowment program. This concept in modern scientific understanding would mean the enhancement of immune responsiveness of an organism against pathogens by nonspecifically activating the immune system with immunomodulatory agents of plant origin [2].

Cocculus hirsutus (Linn.) Diels (family: Menispermaceae) and commonly knows as "Jamti ki bel" by Bundelkhand region of India. It is a climbing shrub. Its various parts are known for their medicinal properties in indigenous system of medicine [3]. *Cocculus hirsutus* is a widely growing plant found in the plains of India in dry localities. The plant is a climber with green flowers bloom in February –March and fruits in May- June. In some places it is found along with water stream, hedges. Tribals of Jhabua, Khargone and Dhar use the fruit of Cocculus hirsutus to cure Jaundice[4]. It contain triterpenoid hirsudiol[5], Alkaloids cohirsinine and Jamtinine[6, 7]. The Plant is in local area people used as tonic but their is paucity of data available on immunomodulatory activity The Plant is in local area people used as tonic but their is paucity of data available on immunomodulatory activity *Cocculus hirsutus* in normal animals. Therefore present work aims at studying effect of polar and nonpolar extract of *Cocculus hirsutus* on immune system. in normal animals. Therefore present work aims at studying effect of polar and nonpolar extract of *Cocculus hirsutus* on immune system.

Materials and Methods

Animals

Albino rats of either sex weighing 120-150 g were housed in a standard environmental condition, fed on standard diet, water ad libtum at 24± 2 ^{0} C and day-night cycle 06:00 h to 18:00 h. All the animal experimentations were out carried after prior permission from the institutional ethical committee of the Dr. H.S. Gour University, Sagar (M.P.) India.

Collection and Identification of Plant Material

The plant *Cocculus hirsutus* was collected from forest near Sagar, M.P. India, in the month of August –September and identified by the Department of Botany, Dr Hari Singh Gour University, Sagar, (M.P.), India. The herbarium collected plants were Deposited in Department of Zoology, Dr Hari Singh Gour University, Sagar (M.P.).Aerial parts of the plant was shade dried, and then dried plant material was reduced to coarse powder and stored in airtight containers.

Extraction of Plant Material

Aqueous extract preparation

Powdered plant material was kept in beaker for 24 hr with water. Filter the content, marc was discarded. Reduce the volume of filtrate in vacuum oven, then dry aqueous extract in Lyophilizer (Heto Drywinner) (Percentage yields 20.3%). The dried aqueous extract was fractionating into ethanol soluble and insoluble portion by pouring ethanol in it. Separate both fractions by filtration and dried it.

Ethanol extract preparation

Powdered plant material was kept in beaker for 24 hr with Ethanol. Filter the content, marc was discarded. Reduce the volume of filtrate in roatatory evaporator (Percentage yields 17.2%). The dried ethanolic extract was fractionating into water soluble and insoluble portion by pouring water in it. Separate both fractions by filtration and dried it.

Treatment

Carbon –Clearance Test for the Determination of Phagocytic Index-

Albino rats were divided in to ten groups having six animals in each. Group I, the control, was given 2ml of 5% normal saline for seven days. Group II.III, IV were administered aqueous /ethanolic extract of 50 mg,100mg,150mg/kgb.w.intraperitonially.Group V, VI, VII were given ethanol soluble /water soluble fraction (50mg, 100mg, 150mg/kg b.w.) of aqueous extract/ethanolic extract ethanol insoluble /water insoluble fraction of aqueous /ethanolic extract was given intraperitonially to group VIII,IX,X of doses 50mg,100mg,150mg/ kgb.w.respectively. After 7 days each mouse given an intravenous injection of 1ml/30g b.w. of Indian ink. Blood samples from retro-orbital venous plexus were taken at intervals of 3,6,9,12,15 min., and transferred in to the centrifuge tubes, the blood in these centrifuge tubes were allowed to coagulate at room temperature. It was then centrifuge at 2000 rpm for 10 minutes and clear supernatant was collected. From each centrifuge tube 20 ml of serum was withdrawn using micropipette and transferred to different volumetric flask (25.0 ml) and volume was made up using distilled water absorbance was measured at 650nm. Recorded absorbance was plotted against the time. This absorbance explains us the rate of elimination of carbon from the blood [8]. The phagocytic index was determined by following formula [9].

$$\text{Phagocytic Index} = \frac{K\ (\text{Immunized})}{K\ (\text{Control})}$$

Where, K = Slope of regression

Delayed Type of Hypersensitivity (DTH)

For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to ten groups, having six animals in each. Group I, the control, was given 2ml of 5% normal saline and to group II.III, IV was administered of 50 mg, 100mg, 150mg/kgb.w. of aqueous /ethanolic extract. Group V, VI, VII received ethanol soluble /water soluble fraction (50mg, 100mg, and 150mg/kg b.w.) of aqueous extract/ethanolic extract. Group VIII, IX, X were injected with ethanol insoluble /water insoluble fraction of aqueous /ethanolic extract intraperitonially for ten days.

On 10^{th} day 0.1ml of SRBC sol. (1% w/v) was injected subcutaneously in to the right foot pad. After 24,48,72,96 hrs, thickness of footpad was measured by plethisometer.Difference in the footpad thickness in control and treated group has been taken as the measure of the DTH reaction [10].

SRBC Agglutination Test

To study humoral antibody response against antigens SRBC agglutination test was performed. Sixty animals were divided in to ten groups having six animals in each group. Group I, was kept as a control and received 2ml of 5% normal saline.intraperitonially for seven days group II.III,IV were given 50 mg,100mg,150mg/kgb.w. aqueous extract/ethanolic extract. Group V, VI, VII were received ethanol soluble /water soluble fraction of aqueous extract/ethanolic extract respectively Ethanol insoluble /water insoluble fraction was administered to groups VIII, IX, X intraperitonially for ten days.

All the animals were injected with 0.25 ml of 5×10. SRBC /ml on 6^{th} 8th and 10^{th} days for achieving maximum titre of antibody. On 11^{th} days blood was collected through retro-orbital venous plexus and centrifuge at 2000 rpm for 15 minutes in order to separate serum.100 ml of serum diluted serially with normal saline in separate test tubes, dilution was made up to 20,40,80, 160 and 320 times. To this 50ml of dextrin coated sheep red blood corpuscles added and incubated at 37.°c for 18 hrs .All the tubes were subjected to examine agglutination visually and compared with control[11,12].

Drug induced Myelosuppression Test

To determine the effect of drug induced myelosuppression**,**Cyclophosphamide was used to produce myelosuppression in albino rats. Albino rats were divided in 11 group of six each. Group I was kept as control and given 2ml of 5% normal saline. Group II was treated with Cyclophosphamide 3mg/kg b.w. for seven days. Group III,IV and V was administered with aqueous extract of 50mg,100mg,150mg/kgb.w. along with the similar dose of Cyclophosphamide as given to group II and group V,VI and VII were treated with ethanol soluble fraction of aqueous extract in increasing doses of 50mg,100mg,150mg/kg b.w. with Cyclophosphamide.Group IX,X and XI were injected with ethanol insoluble fraction of aqueous extract of 50mg,100mg,150mg/kgb.w. doses intraperitoneally with Cyclophosphamide. For the ethanolic extract of various plants the same pattern of groups were followed.

On seventh day blood was taken from retro-orbital plexus and subjected to hematological studies, blood sample of each animal was collected on 15th day, a day after the dose to animal, and again animals were weighed and subjected to hematological studies including hemoglobin count, RBC count, WBC count, Platelet count[13,1]

Results

Carbon –Clearance

Carbon –Clearance depends on time and it is calculated by measuring Phagocytic index and compared with the control. The mean phagocytic Index of control (Group I) was found to be 0.0089 ± 0.021. The aqueous extract of Cocculus hirustus was given to group II, III and IV in a dose of 50 mg,100mg,150mg/kgb.w.intraperitonially for seven days. Phagocytic Index was increased with the increasing dose. It was found to be 0.0092± 0.00027, 0.0113± 0.027 and 0.0115 ± 0.025 (P < 0.025) .Ethanol soluble fractions of aqueous extract has given the phagocytic index as 0.0090± 0.0075,0.0097± 0.021 (P< 0.05) 0.0117± 0.021 (P< 0.025) and ethanol insoluble had shown the phagocytic index as0.0119±0.018(P<0.025)0.013±0.013(P<0.001)with50mg,100mg,150mg/kgb.w.intraper itonially for seven days.

In other experiment Group I was ethanolic extract of Cocculus hirustus was given to the Group II,III and IV have phagocytic Index as 0.0090± 0.027 and 0. 092± 0. 027and 0.0116±0.025 (P< 0.025) in the dose of 50 mg,100mg,150mg/kgb.wt.respectively. water soluble fraction of ethanolic extract had shown phagocytic index as 0.0087 ± 0.052,at the dose of 50 mg,100mg,150mg/kgb.wt.respectively.Water insoluble fraction of ethanolic extract gives phagocytic index as 0.0093±0.027,0.0098 ± 0.027(P<0.05) and 0.0101± 0.025 in 50 mg,100mg,150mg/kgb.wt.respectively when administered intraperitonially for seven days.

DTH

Group I subjected to normal saline subdermally in paw had swallen foot till the solution absorbed in blood. In 24 hrs paw volume increased and then gradually decreases, Group II,III and IV was injected with crude aqueous extract, it increases the paw volume in a dose dependent manner in 24hrs and 48hrs, paw volume in these group were 1.88 ± 0.21,1.95 ± 0.14 and 1.96 ± 0.07ml in 24hrs at the dose of 50 mg,100mg,150mg/kgb.wt.

In later hrs it decrease and follow the pattern as in control group. After 96 hrs paw volume become 0.22 ± 0.15, 0.17± 0.03, 0.13±0.05ml (P<0.001) in dose 50 mg,100mg,150mg/kgb.wt.respectively. Ethanol insoluble fraction also followed the same .pattern. In 24 hrs the volumes of paw become 1.86 ± 0.23, 1.98 ±0.01, 1.95 ±0.17ml in 50 mg, 100mg, 150mg/kgb.wt. In 72 hrs values decreases to 0.59± 0.11, 0.45± 0.25, 0.37 ±0.13 and after 96 hrs these values reduces to 0.20± 0.02 (P< 0.025) 0.15 ± 0.03, (P<0.001). Ethanol soluble fraction did not show any significant activity.

Ethanolic extract also enhance delayed type of hypersensitivity but it was less as compare to aqueous extract. In all the groups paw volume was more as compare to control group in 24 48 hrs and soon its value decreases. Crude ethanolic extract (50mg) showed the following observation in 24,48,72 and 96 hrs 1.88 ± 0.21,1.15 ± 0.22,0.68± 0.07,0.32± 0.24ml similarly) in the dose of 100mg,150mg/kgb.wt paw volume was 1.79±

0.09,1.21± 0.19,0.19 ± 0.22,0.25± 0.05ml and 1.89± 0.07,1.30± 0.14, 0.42 ± 0.18, 0.25± 0.05ml (P< 0.05) respectively. Water soluble fraction did not give significant increase and decrease when compare with control group.
Water insoluble fraction of 150mg/kgb.wt increases the maximum edema and in this way increase the paw volume(1.96± 0.12,1.24± 0.14ml in 24and 48 hrs)Edema and paw volume decreased significantly 0.31± 0.03and 0.41 ± 0.05ml (P<0.001) after 72 and 96 hrs.

SRBC Agglutination Test

Agglutination titre to sheep red blood erythrocyte as calculated and compared with control group (Group I). Group II, III and IV were given crude aqueous extract of *Cocculus hirustus* orally for ten days. The three group were given of 50 mg,100mg,150mg/kgb.w.respectively and on 10^{th} day agglutination titre was esteemed in various serum dilution (X: 20,X: 40, X: 80, X: 160 and X: 320).In the lower dose of crude extract, agglutination titre was observed up to X: 80 which is equivalent to the control group but as the doses increase agglutination titre increases to reach up to the serum dilution of X: 160(P< 0.025) Group V, VI and VII were given to ethanol insoluble fraction at the dose of 50mg,100mg,150mg/kg b.wt and no significant changes in the agglutination titre could be observed. Ethanol insoluble fraction was given 50mg, 100mg, 150mg/kg b.wt which showed a significant elevation in agglutination titre.

Control group was administered 2ml of 5% normal saline and agglutination titre was compared with treated groups. Group II, III and IV were administered ethanolic extract of doses 50mg,100mg,150mg/kg b.wt.respectively and significant increased was observed in IV group(150mg/kg b.wt .). low doses of crude extract did not show remarkable change.

Water soluble fraction also did not show any enhancement in agglutination titre. Water insoluble fraction showed a significant increase in the agglutination titre at the doses (50mg, 100mg, 150mg/kg b.wt.) up to the serum dilution of X: 160.

Drug induced Myelosuppression Test

Cyclophosphamide was given in the dose of 3mg/kg b.wt. orally for seven days to produce myelosuppression in rats. Group I was kept as control and was given 2ml of 5% normal saline. Hematological studies showed that the mean haemoglobin was 12.87± 0.56 gms/dl mean RBC count was 4.32 ± 0.165 million/mm³(P<0.025) WBC count was 12.96 ± 0.687 thousand/mm³ Neutrophills percent was 52.13 ± 1.78, Lymphocyte was 37.66± 1.22%, Monocyte were 3.24 ± 0.24 %, Eosinophil was 2.56 ± 0.36% and platelet count was 3.22 ± 0.256 lacs/ mm³. Group II was treated with Cyclophosphamide given (3mg/kg b.wt.) which caused a significant decreased is in haemoglobin concentration, . WBC count RBC count, Lymphocyte count, Monocyte count, and Platelet count. crude aqueous extract along with cyclophosphamide was given to Group III, IV and V with the dose blood parameters cyclophosphamide,through a good recovery was recorded in the GroupIV and V,with regard to the values of various blood parameters in Group III, IV and V were as follows Haemoglobin was 10.58 0.27,11.18 0.43 and 12.20.12 gms/dl,RBC count was 3.42 0.052,3.86 0.065(P< 0.025) 4.02 0.412 million/mm³(P<0.025) WBC was 10.21 0.103,11.75 0.423 (P< 0.025) 12.10 0.145 thousand/mm³ Neutrophills was 59.50 0.885%,54.83 1.515%,52.93 1.054%,(P< 0.025)

Lymphocyte percent was 30.83 m0.703 ,32.33 0.512 35.66 0.523 Monocyte was 2.86 0.15%, 2.75 0.35%,.3.00 0.11%(P< 0.025) Eosinophil count was 3.10 0.52%,2.62 0.12% 2.65 0.47% platelet count was 2.62 0.012,2.75 0.011 2.98 0.033 lacs/ mm³ respectively. Ethanol soluble fraction showed dose dependent effect on blood parameters. Blood parameters at a dose of 50mg, 100mg, 150mg/kg b.wt. were as follows haemoglobin was 8.22± 0.32, 8.75± 0.42 and 9.42± 0.21 gms/dl,RBC count were 2.85± 0.225, 3.05 ±0.054, 3.20± 0.021 million/mm³(P<0.025) WBC count was 11.35 ±0.322,11.42 ±0.412,10.42 ±0.321 thousand/mm³ Neutrophills were 65.83± 0.55%,64.74± 0.42%, 65.00± 0.65%, Lymphocyte were 30.12±0.11%, 30.42±0.11%,31.23±0.78%, Monocyte were 2.88± 0.55%,2.96±0.22%,2.99±0.35% Eosinophil count were 2.85±0.55%,2.96±0.22%,2.99 ±0.20% and platelet count was 2.72± 0.452,2.85± 0.256, 2.78± 0.452 lacs/ mm³.

Ethanol insoluble fraction showed best results in all three dose (50mg,100mg,150mg/kg b.wt.) mean haemoglobin was found to e 11.07± 0.42 gms/dl(P< 0.025)11.45±0.23 gms/dl (P< 0.025)12.78±0.31 gms/dl,(P< 0.001) RBC count was 3.88±0.033(P< 0.025),3.97±0.041(P< 0.025),4.00±0.321 (P< 0.001) million/mm³(P<0.025) WBC count was 11.21±0.201(P< 0.05),11.86±0.234(P<0.025) 12.95±0.356 thousand/mm³(P< 0.001) Neutrophills was 5.96±0.774%(P<0.025)50.14± 0.556%(P<0.025),52.02 ±0.215%(P<0.025), Lymphocyte % was 32.63± 0.563 (P<0.025), 34.65± 0.578 (P<0.025), 36.87± 0.425(P< 0.001). Monocyte % was2.95 ±0.11(P<0.025) 3.10± 0.35 (P< 0.001),3.35 0.22(P< 0.001).Eosinophil count was 2.78± 0.52 (P< 0.05),2.52± 0.23 (P< 0.001), 2.50± 0.25 (P< 0.001), platelet count was 2.85± 0.072(P<0.025), 3.15 ±0.035 (P< 0.001), and 3.12 0.052 lacs/ mm³(P< 0.001).

In the case of ethanolic extract Group I was administered 2ml of 5% normal saline and various hematological blood parameter were observed mean haemoglobin was 12.87 0.56 gms/dl, RBC count was 4.33± 0.165 million/mm³WBC count was 52.13 ±1.78%, Lymphocyte was 37.66± 1.22% Monocyte was 3.24± 0.24% Eosinophil count was 2.56% platelet count was 3.22± 0.256 lacs/ mm³.

Group II was given cyclophosphamide (3 mg/kg body wt.) and all the blood parameters showed variation as haemoglobin become 7.98±0.11, RBC was 2.99±0.087million/mm^3, WBC count was 10.45±0.244 100/mmq (p<0.05) Neutrophills % was 68.83±0.26, 29.66±0.88 (p<0.05) Monocyte % was 3.00±0.10 Eosinophill count was 2.50±0.34 and platelet count 2.57±0.384. Group III, IV and V were administrated crude ethanolic extract of Cocculus hirsutus of 50, 100 and 150mg/kg body wt. Intraperitoneally and then blood was collected from retro-orbital plexus and haematological studies were found to be the 9.77±0.33 (p<0.05) 11.77±0.31 (p<0.001) RBC count was 3.21±0.012, 3.68±0.014 (p<0.05), 3.85±0.021million/mm^3 (p<0.025),3.85±0.021 millon/mm^3(p<0.025)WBC count was 10.75±0.125, 10.99±0.214, 11.75±0.265 thousand/mm^3 (p<0.025). Neutrophill was 59.95±0.215, 60.32±0.563, 56.93±0.425 (p<0.05), Lymphocyte% was 30.12±0.253 (p<0.025) Monocyte % was 3.01 ±0.12, 2.77± 0.25, 2.55± 0.85 (p<0.025). Eosinophill count was 2.85± 0.32, 2.77± 0.25, 2.55± 0.85(p<0.025) platelet count was 2.77± 0.111, 2.85± 0.023(p<0.05), 3.07± 0.45 lacs/ mm³ (p<0.025).

Water soluble fraction was administered in Group VI,V II and VIII (50,100 and150mg/kg b.wt.) in these groups the blood parameters were as follows haemoglobin concentration was 8.54± 0.65, 8.69 ±0.24,8.95± 0.53 gms/dl RBC count was 2.74± 0.042,

2.98± 0.012, 2.85± 0.032 millon/$mm^{3,}$ WBC count was 10.53± 0.132, 11.00 ±0.122, 10.65± 0.512 thousand/$mm^{3.}$ Neutrophill was 64.00 0.85, 60.12 0.47, 61.32 0.75 Lymphocyte was 29.75 0.75%, 31.01 0.11%, 29.86 0.35%, Monocyte % was 2.77 0.42%, 0.07 0.53%, 3.12 0.65%, Eosinophill count was 2.96± 0.64%, 2.86± 0.46%, and platelet count was 2.68± 0.086, 2.78 ±0.054, 2.62± 0.045 lacs/ mm^3.

Water insoluble fraction was injected to Group IX,X and XI and observe a significant increase in the various blood parameters like haemoglobin concentration became 10.11± 0.32, 11.01± 0.21 ($p<0.05$) and 11.87± 0.42 gms/dl ($p<0.001$) RBC count was 3.54± 0.053, 3.68 ±0.014 ($p<0.05$) 3.79± 0.011 millon/mm^3 ($p<0.025$), WBC count was 11.12± 0.321 ,11.99 ±0.142 ($p<0.025$) , 12.12 ±0.321 thousand/mm^3($p<0.001$), Neutrophill % was 58.78 0.352, 60.32 0.563 , 54.83 0.326 ($p<0.025$), Lymphocyte % was 31.45± 0.415, 29.98± 0.451, 34.00± 0.346 ($p<0.001$), Monocyte % was 3.12± 0.75, 2.75± 0.35, 2.75± 0.12. Eosinophill count was 2.91± 0.44, 2.83± 0.46, 2.54± 0.53 ($p<0.025$), platelet count was 2.77± 0.111, 2.99± 0.053 ($p<0.025$) and 3.10± 0.021 lacs/ mm^3 ($p<0.025$).

Table 1: Effect of Aqueous Extract of *Cocculus hirustus* on Phagocytic Activity In Carbon Clearance Test

S.NO.	GROUPS	ABSORBANCE					PHAGOCYTIC INDEX(K) ±SD
		3min	6min	9min	12min	15min	
1.	Control 2ml Normal saline	0.2524± 0.042	0.2242± 0.052	0.1935± 0.032	0.1687± 0.021	0.119± 0.026	0.0089± 0.021
2.	Crude Aqueous extract (50mg./kg.body wt.)	0.2465± 0.015	0.2154± 0.021	0.1834± 0.028	0.1582± 0.028	0.110± 0.024	0.0092 ±0.027
3.	Crude Aqueous extract (100mg./kg.body wt.)	0.2295± 0.012	0.2082± 0.037	0.1735± 0.015	0.1456± 0.019	0.1035± 0.021	0.0113± 0.027**
4.	Crude Aqueous extract (150mg./kg.body wt.)	0.2270± 0.022	0.2052± 0.037	0.1681± 0.025	0.1392± 0.040	0.0984±0.016	0.0115± 0.025**
5.	Ethanol Soluble fraction (50mg./kg.body wt.)	0.2478± 0.022	0.2187± 0.018	0.1863± 0.017	0.1601± 0.056	0.1131± 0,024	0.0090 ±0.075
6.	Ethanol Soluble fraction (100mg./kg.body wt.)	0.2321± 0.032	0.2101 ± 0.014	0.1725± 0.052	0.1485 ± 0.015	0.1078± 0.032	0.0097± 0.021*
7.	Ethanol Soluble fraction (150mg./kg.body wt.)	0.2256± 0.021	0.2012± 0.025	0.1675± 0.036	0.1387± 0.042	0.0978± 0.030	0.00117± 0.018**
8.	Ethanol Insoluble fraction (50mg./kg.body wt.)	0.2212± 0.014	0.1831± 0.024	0.1654± 0.023	0.1360± 0.017	0.0968± 0.015	0.0119± 0.018**
9.	Ethanol Insoluble fraction (100mg./kg.body wt.)	0.2221± 0.017	0.1825± 0.065	0.1615± 0.055	0.1301± 0.042	0.0945± 0.012	0.0131± 0.013***
10.	Ethanol Insoluble fraction (150mg./kg.body wt.)	0.2107± 0.078	0.1796± 0.044	0.1612 ± 0.021	0.1180± 0.032	0.0834± 0.016	0.0152± 0.019***

n = 6 , value represents mean ±S.D.
* P < 0.05, * * P < 0.025,* * * P < 0.001

Table 2: Effect of Ethanolic Extract of *Cocculus hirustus* on Phagocytic Activity In Carbon Clearance Test

S.NO.	GROUPS	ABSORBANCE					PHAGOCYTIC INDEX(K) ±SD
		3min	6min	9min	12min	15min	
1.	Control 2ml Normal saline	0.2524± 0.042	0.2242± 0.052	0.1935± 0.032	0.1647± 0.021	0.1195± 0.026	0.0089± 0.021
2.	Crude Ethanolic extract (50mg./kg.body wt.)	0.2538± 0.054	0.2154± 0.042	0.1934± 0.035	0.1622± 0.052	0.1121± 0.027	0.0090± 0.027
3.	Crude Ethanolic extract (100mg./kg.body wt.)	0.2395± 0.035	0.2152± 0.041	0.1835± 0.026	0.1596± 0.019	0.1096± 0.021	0.0092± 0.027
4.	Crude Ethanolic extract (150mg./kg.body wt.)	0.2252± 0.038	0.2006± 0.012	0.1722± 0.038	0.1442± 0.022	0.0991± 0.016	0.0116±** 0.025
5.	Aqueous Soluble fraction (50mg./kg.body wt.)	0.2475± 0.058	0.2252± 0.032	0.1695± 0.055	0.1721± 0.022	0.1222± 0.026	0.0087± 0.052
6.	Aqueous Soluble fraction (100mg./kg.body wt.)	0.2454± 0.025	0.2374± 0.056	0.1908± 0.062	0.1687± 0.035	0.1175± 0.055	0.0089± 0.045
7.	Aqueous Soluble fraction (150mg./kg.body wt.)	0.2375± 0.023	0.2098± 0.052	0.1842± 0.062	0.1635± 0.042	0.1265± 0.021	0.0087± 0.052
8.	Aqueous Insoluble fraction (50mg./kg.body wt.)	0.2438± 0.012	0.2178± 0.032	0.1855± 0.035	0.1554± 0.026	0.1087± 0.027	0.0093± 0.027
9.	Aqueous Insoluble fraction (100mg./kg.body wt.)	0.2312± 0.042	0.2012± 0.056	0.1785± 0.032	0.1476± 0.045	0.1023± 0.045	0.0098±* 0.027
10.	Aqueous Insoluble fraction (150mg./kg.body wt.)	0.2241± 0.041	0.2021± 0.012	0.1756± 0.012	0.1465± 0.053	0.1001± 0.045	0.0101± 0.025

n = 6 albino rats Per group, tabular value represents mean ±S.D.

* P < 0.05

* * P < 0.025

* * * P < 0.001

Table 3:Effect of Aqueous Extract of *Cocculus hirustus* on Delayed Type of Hypersensitivity

S.NO.	GROUPS	PAW VOLUME (ML.) ±S.D.			
		24Hrs,	48Hrs,	72Hrs,	96Hrs,
1.	Control 2ml Normal saline	1.80± 0.09	1.22± 0.11	0.86± 0.03	0.35± 0.05
2.	Crude Aqueous extract (50mg./kg.body wt.)	1.88± 0.21	1.17± 0.10	0.68± 0.07	0.22±* 0.15
3.	Crude Aqueous extract (100mg./kg.body wt.)	1.95± 0.14	1.23± 0.20	0.54± 0.12	0.17± 0.03**
4.	Crude Aqueous extract (150mg./kg.body wt.)	1.96± 0.07	1.33± 0.11	0.33± 0.15	0.13± 0.05***
5.	Ethanol Soluble fraction (50mg./kg.body wt.)	1.66± 0.12	1.15± 0.15	0.78± 0.13	0.33± 0.02
6.	Ethanol Soluble fraction (100mg./kg.body wt.)	1.78± 0.11	1.081± 0.22	0.72± 0.12	0.31± 0.13
7.	Ethanol Soluble fraction (150mg./kg.body wt.)	1.59± 0.17	1.181± 0.04	0.75± 0.15	0.31± 0.12
8.	Ethanol Insoluble fraction (50mg./kg.body wt.)	1.86± 0.23	1.29± 0.11	0.59± 0.11	0.20±** 0.02
9.	Ethanol Insoluble fraction (100mg./kg.body wt.)	1.98± 0.01	1.37± 0.22	0.45± 0.25	0.15± 0.03***
10.	Ethanol Insoluble fraction (150mg./kg.body wt.)	1.95± 0.17		0.37± 0.13	

n = 6 albino rats Per group tabular value represents mean ±S.D.
* P < 0.05
* * P < 0.025
* * * P < 0.001

Table 4: Effect of Ethanolic Extract of *Cocculus hirsutus* on Delayed Type of Hypersensitivity

S.NO.	GROUPS	PAW VOLUME (ML.) ±S.D.			
		24Hrs,	48Hrs,	72Hrs,	96Hrs,
1.	Control 2ml Normal saline	1.86± 0.09	1.12± 0.11	0.86± 0.03	0.35± 0.05
2.	Crude Ethanolic extract (50mg./kg.body wt.)	1.88± 0.21	1.15± 0.22	0.68± 0.07	0.32± 0.24
3.	Crude Ethanolic extract (100mg./kg.body wt.)	1.79± 0.09	1.21± 0.19	0.59± 0.22	0.25± 0.05
4.	Crude Ethanolic extract (150mg./kg.body wt.)	1.89± 0.07	1.30± 0.04	0.42± 0.18	0.19± 0.05
5.	Aqueous Soluble fraction (50mg./kg.body wt.)	1.78± 0.15	1.21± 0.03	0.82± 0.09	0.38± 0.13
6.	Aqueous Soluble fraction (100mg./kg.body wt.)	1.90± 0.21	1.12± 0.32	0.96± 0.13	0.37± 0.03
7.	Aqueous Soluble fraction (150mg./kg.body wt.)	1.69± 0.22	1.02± 0.32	0.85± 0.03	0.36± 0.16
8.	Aqueous Insoluble fraction (50mg./kg.body wt.)	1.75± 0.13	1.26± 0.13	0.12± 0.11	0.29± 0.25
9.	Aqueous Insoluble fraction (100mg./kg.body wt.)	1.77± 0.17	1.36± 0.14	0.62± 0.03	0.23± 0.21
10.	Aqueous Insoluble fraction (150mg./kg.body wt.)	1.96± 0.12	1.24± 0.14	0.14± 0.05	0.14± 0.05

n = 6 albino rats Per group, tabular value represents mean ±S.D.

* P < 0.05

* * P < 0.025 * * * P < 0.001

Table 5: Effect of Aqueous Extract of *Cocculus hirustus* on Agglutination Titre to SRBC

S.NO.	GROUPS	SERUM DILUTION IN NORMAL SALINE ± 50µL ANTIGEN				
		× : 20	× : 40	x: 80	x:160	x: 320
1.	Control 2ml Normal saline	+	+	+	_	_
2.	Crude Aqueous extract (50mg./kg.body wt.)	+	+	+	_	_
3.	Crude Aqueous extract (100mg./kg.body wt.)	+	+	+	+**	_
4.	Crude Aqueous extract (150mg./kg.body wt.)	+	+	+	+**	_
5.	Ethanol Soluble fraction (50mg./kg.body wt.)	+	+	+	_	_
6.	Ethanol Soluble fraction (100mg./kg.body wt.)	+	+	+	_	_
7.	Ethanol Soluble fraction (150mg./kg.body wt.)	+	+	+	_	_
8.	Ethanol Insoluble fraction (50mg./kg.body wt.)	+	+	+	+**	_
9.	Ethanol Insoluble fraction (100mg./kg.body wt.)	+	+	+	+	+***
10.	Ethanol Insoluble fraction (150mg./kg.body wt.)	+	+	+	+	+***

n = 6 albino rats Per group, tabular value represents mean ±S.D.
* P < 0.05
* * P < 0.025
* * * P < 0.001

Table 6: Effect of Ethanolic Extract of *Cocculus hirustus* on Agglutination Titre to SRBC

S.NO.	GROUPS	SERUM DILUTION IN NORMAL SALINE ± 50µL ANTIGEN				
		x: 20	x: 40	x: 80	x: 160	x: 320
1.	Control 2ml Normal saline	+	+	+	_	_
2.	Crude Ethanolic extract (50mg./kg.body wt.)	+	+	+	_	_
3.	Crude Ethanolic extract (100mg./kg.body wt.)	+	+	+	_	_
4.	Crude Ethanolic extract (150mg./kg.body wt.)	+	+	+	+**	_
5.	Aqueous Soluble fraction (50mg./kg.body wt.)	+	+	+	_	_
6.	Aqueous Soluble fraction (100mg./kg.body wt.)	+	+	+	_	_
7.	Aqueous Soluble fraction (150mg./kg.body wt.)	+	+	+	_	_
8.	Aqueous Insoluble fraction (50mg./kg.body wt.)	+	+	+	+**	_
9.	Aqueous Insoluble fraction (100mg./kg.body wt.)	+	+	+	+**	_
10.	Aqueous Insoluble fraction (150mg./kg.body wt.)	+	+	+	+**	_

n = 6 albino rats Per group, tabular value represents mean ±S.D.
* P < 0.05, * * P < 0.025,* * * P < 0.001

Table 7: Effect of Aqueous Extract of *Cocculus hirsutus* on Drug induced Myelosuppression Using Cyclophosphamide for 7 days

GROUP	HBG MS/DL	RBC MILLION/ MM³	WBC THOUSAND/ MM³	NEUTROPHIL %	LYMPHOCYTE %	MONOCYTE %	EOSINNOPHILCOUNT %	PLATELETLACS/MM³
I.	12.87± 0.56	4.35± 0.165	12.96± 0.687	52.13± 1.78	37.66± 1.22	3.24± 0.24	2.56± 0.36	3.22± 0.256
II	7.98± 0.11	2.99± 0.087*	10.45± 0.244*	68.83± 0.26	29.66 ± 0. 88*	3.00± 0.10	2.50± 0.34	2.57± .384*
III	10.58± 0.27	3.42± 0.057	10.21 ±0.103	59.50± 0.885	30.83± 0.703	2.86± 0.15	3.10± 0.52	2.62± 0.012
IV	11.18± 0.43**	3.86± 0.065**	11.75± 0.423*	54.83± 1.515	32.33 ±0.512*	2.75± 0.35	2.62±0.12**	2.75± .011*
V	12.22±0.12***	4.01± .412***	12.10±0.145***	52.93± 1.054**	35.66±0.53***	3.00 ±0.11***	2.65±0.47**	2.98±0.033**
VI	8.22± 0.32	2.85± 0.025	11.35± 0.322	65.83± 0.55	30.12± 0.45	2.88± 0.22	2.85± 0.55	2.72± 0.452
VII	8.75± 0.42	3.05± 0.054	11.42 ±0.412	64.74± 0.42	30.42± 0.11	3.00± 0.32	2.96± 0.22	3.85± 0.2 56
VIII	9.42± 0.21	3.20± 0.021	10.42± 0.321	65.00± 0.65	31.23± 0.78	2.99± 0.35	2.99± 0.20	2.78± 0.452
IX	11.07± 0.42**	3.88± 0.053**	11.21± 0.201*	50.96± 0.774**	32.63± 0.563**	2.95± 0.11**	2.78± 0.52*	2.85± 0.012**
X	11.45± 0.23**	3.97± 0.041**	11.86±0.234**	50.14± 0.556**	34.65± 0.578**	3.10± 0.35***	2.52±0.23***	3.15±0.035***
XI	12.78± 0.31***	4.00±0.321***	12.95±0.356***	52.02± 0.215**	36.87±0.425***	3.35± 0.22***	2.50±0.25***	3.12±0.052***

Group I: Control 5%Normal saline
Group II: Cyclophosphamide (3mg/kg b.wt.)
Group III: Crude aqueous extract (50mg/kg b.wt.) + Cyclophosphamide
Group IV: Crude aqueous extract (100 mg/kg b.wt.) + Cyclophosphamide
Group V: Crude aqueous extract (150mg/kg b.wt.) +Cyclophosphamide
Group VI: Ethanol soluble fraction (50 mg/kg b.wt.) + Cyclophosphamide
Group VII: Ethanol soluble fraction (100 mg/kg b.wt.) + Cyclophosphamide
Group VIII: Ethanol soluble fraction (150 mg/kg b.wt.) + Cyclophosphamide
Group XI: Ethanol insoluble fraction (50 mg/kg b.wt.) + Cyclophosphamide
Group XI: Ethanol insoluble fraction (100 mg/kg b.wt.) + Cyclophosphamide
Group XI: Ethanol insoluble fraction (150 mg/kg b.wt.) + Cyclophosphamide
n = 6 value represents mean ±S.D. * $P < 0.05$, * * $P < 0.025$, * * * $P < 0.001$

Table 8: Effect of Ethanolic Extract of *Cocculus hirsutus* on Drug induced Myelosuppression Using Cyclophosphamide for 7 days

GROUP	HBG MS/DL	RBC MILLION/ MM³	WBC THOUSAND/ MM³	NEUTROPHIL %	LYMPHOCYTE %	MONOCYTE %	EOSINNOPHILCOUNT %	PLATELETLACS/MM³
I.	12.87±0.56	4.35± 0.165	12.96± 0.687	52.13± 1.78	37.66± 1.22	3.24± 0.24	2.56± 0.36	3.22± 0.256
II	7.98± 0.11	2.99± 0.087*	10.45± 0.244*	68.83± 0.26	29.66 ± 0. 88*	3.00± 0.10	2.50±0.34	2.57± 0.384*
III	9.77± 0.33	3.21± 0.012	10.75 ±0.125	59.95± 0.215	30.13± 0.253	3.01± 0.12	2.85± 0.32	2.77± 0.111
IV	11.01± 0.21*	3.68± 0.014*	10.99± 0.214*	60.32± 0.563	29.98 ±0.451	2.75± 0.35	2.77 ±0.25	2.85± 0.023*
V	11.77± 0.31***	3.85± 0.021**	11.75± 0.265**	56.93± 0.425*	32.25 ±0.253**	2.89 ±0.42**	2.55± 0.85**	3.07± 0.045**
VI	8.54± 0.65	2.74± 0.042	10.53± 0.132	64.00± 0.85	29.75± 0.75	2.77± 0.42	2.96± 0.64	2.68± 0.086
VII	8.69± 0.24	2.98± 0.012	11.00 ±0.122	60.12± 0.47	31.01± 0.11	3.07± 0.53	2.86± 0.46	2.78± 0.054
VIII	8.95± 0.53	2.85± 0.032	10.65±0.512	61.32± 0.75	29.86± 0.35	3.12± 0.65	2.75± 0.35	2.62± 0.045
IX	10.11± 0.32	3.54± 0.053	11.12± 0.321	58.78± 0.352	31.45± 0.415	3.12± 0.75	2.91± 0.44	2.22± 0.111
X	11.01± 0.21*	3.68± 0.014*	11.99±0.142**	60.32± 0.563	29.98± 0.451	2.75± 0.35	2.85± 0.46	2.99± 0.053**
XI	11.87± 0.42***	3.79± 0.011**	12.12± .321***	54.83± 0.326 **	34.00± 0.346***	2.75± 0.12	2.54± 0.53**	3.10± 0.021 **

Group I: Control 5%Normal saline
Group II: Cyclophosphamide (3mg/kg b.wt.)
Group III: Crude ethanolic extract (50mg/kg b.wt.) + Cyclophosphamide
Group IV: Crude ethanolic extract (100 mg/kg b.wt.) + Cyclophosphamide
Group V: Crude ethanolic extract (150mg/kg b.wt.) +Cyclophosphamide
Group VI: Aqueous soluble fraction (50 mg/kg b.wt.) + Cyclophosphamide
Group VII: Aqueous soluble fraction (100 mg/kg b.wt.) + Cyclophosphamide
Group VIII: Aqueous soluble fraction (150 mg/kg b.wt.) + Cyclophosphamide
Group XI: Aqueous insoluble fraction (50 mg/kg b.wt.) + Cyclophosphamide
Group XI: Aqueous insoluble fraction (100 mg/kg b.wt.) + Cyclophosphamide
Group XI: Aqueous insoluble fraction (150 mg/kg b.wt.) + Cyclophosphamide
n = 6 value represents mean ±S.D. * P < 0.05, * * P < 0.025, * * * P < 0.001

Discussion

The present study was undertaken to verify the traditional use of plant as tonic by studies Immunostimulation activity in immunosuppressed animals using cyclophosphamide and on cellular and humoral immunity.

Cocculus hirsutus increases the rate of phagocytic index with respect to control. It was observed that aqueous extract; ethhanolic extract and their fractions enhance the phagocytic index in terms of clearance of carbon particles from the blood is suggestive of activation of WBC. Increases were dependent on the dose, as the dose increases. Results of these studies clearly indicate that *Cocculus hirsutus* activates the process of phagocytosis. The extract influences the role of neutrophills, digestive enzyme in phagocytic vesicle, and the synthetic processes in the cytoplasm. In treated animal, hyper granulation of WBC is the evidence of it. The secrettory material appeared in the cytoplasm is to meet the necccessity of the cell to phagocytose and digest the antigen, stimulation of was influenced by the macrophages, these secet a number of cytokines such as IL-1, IL-2, etcalso reported aggregation and activation of when expressed to the extracts of different plants[14.15,16].

Cell –mediated immunity is a part of the process of graft rejection, tumour immunity and immunity to many intracellular infections or to microorganisms, which cause chronic diseases. DTH requires the specific recognition of a given antigen by activated T-lymphocytes, which subsequently proliferate and release cytokines .DTH response. Both the extracts and their fraction influenced T –cell activity significantly which in turn increase vascular permeability ,induce vasodilatation ,macrophage accumulation and activation ,and which finally result in the increase in the paw volume which promotes phagocytic activity and also increase the paw volume which promotes phagocytic activity and also increase the concentration of lytic enzymes for more effective killing , this ultimately results in reducing the paw volume after 72 and 96 hrs. The proteoglycons of *Cocculus hirsutus* present in ethanol insoluble fraction when suspended in a solution and injected to the animal strongly behave as chemo attractant especially to monocytes and lymphocytes . This behavior it self is suggestive of activation of immune system at cellular level with lymphocytes, Cytokines, prostaglandulin E etc.are also liberating from the neighboring cells and the cumulative effect is termed as DTH [17].

Ethanol soluble fraction of aqueous extract and water-soluble fraction of ethanolic extract did not make any significant increase. Since it may contain lesser amounts of immunostimulating agents or having some other compounds, which may be partially antagonizing the stimulator. Increases in DTH reaction in mice in response to SRBC revealed the stimulatory effect of aqueous and ethanolic extract on T-lymphocytes and accessory cell types [18, 19, 20]. The results reported here also inconformity it can be opined that the contents present in *Cocculus hirsutus* are much more effective and efficient enough to attract CD4 population of T- lymphocytes, monocytes and other lymphocytes.

The humoral immunity involves interaction of B-cell with the antigen and their subsequent proliferation and differentiation into antibody secreting plasma cells. Antibody functions as the effectors of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross – linking to form clusters that are more readily ingested by phagocytic cell .Aqueous and ethanolic extracts of *Cocculus hirsutus* increase the agglutination titre to SRBC (antigen). In crude aqueous extract agglutination titre increases, as increase in the dose .Ethanol soluble fraction did not show any significant increase in the agglutination titre as compared to control group. On the other hand, Ethanol insoluble fraction showed maximum increase in the agglutination titre at the dose of 100mg and 150 mg/kg b.wt. With these doses the agglutination was observed up to serum dilution of X: 320. Ethanolic extract and Water soluble fraction showed significant increase the agglutination titre in the dose of 150 mg/kg b.wt. .The titre observed up to serum dilution of X: 160.This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis. [21]. Increased level of antibodies gives higher agglutination titre against sheep red blood cells [22].

Cocculus hirsutus antagonices the myelosuppressive effect induced by cyclophsphamide, which produces significant myelosuppression in experimental animal. By the administration of cyclophosphamide Heamoglobin , RBC count , WBC count , Lymphocyte , monocytes ,Eosinophil count and Platelet count decrease significantly [23]. But with the treatment of aqueous and ethanolic extract all the above parameters increase .This indicates the protection produced by the drug against cyclophosphamide .In the case of ethanolic extract and its fraction significant increase was observed in a dose proteoglycons , saponins, flavonoids etc are some of the compounds which are reported to be responsible for the genesis of antibodies still few of them are also to negate the toxic effect of chemicals on haemopoietic tissue , myeloid and lymphoid tissue even some them have both qualities observed with *Cocculus hirsutus* .The compound (s) though the path way ultimately activates B-lymphocytes devoid to form plasma cell, which inturn release particular type of antibodies .Appearance of some protein bands during electrophoresis separation , increase in serum protein concentration and high titer for SRBC strongly indicate about humoral immunity stimulation .Cyclophosphamide is known myelosuppressant agent causes to decrease immunological parameters but some compounds of the extract reduce the toxic effect or the the components of the extract prevent the entry of cyclophosphamide or bind with this compound to make it insoluble or unite to form a molecule to bind receptor site to wash away the effect of the compound in a short period , *Cocculus hirsutus* thus effective in both ways , to stimulate the immune system and to protect it from immunosuppressant.

Finding of these studies suggest that the both the extracts are capable to strengthen the immune system .Both the extract and their fractions modulate immune responses significantly as they increase the phagocytes index , modulate the phagocytic functions of macrophages and phagocytes , which means they have a profound effect over the innate immunity .They also modulate the function of cytotoxic T-cell , that produces delayed type hypersensitivity immune response , which gives a better protection against viruses and tumors. They also increase the antibody titer, which means modulation of humoral immunity.

Acknowledgements

One of the author N. S.Chauhan thanks AICTE , New Delhi for providing National Doctoral Fellowship

References

1. Thakur M, Bhargava S and Dixit VK. Immunomodulatory Activity of *Chlorophytum borivilianum* Sant.F. *Ecam* 2006 ; 4: 419-423.

2. Mungantiwar AA. and Phadke AS . Immunomodulation: Therapeutic Strategy through Ayurveda. In: Scientific Basis for Ayurvedic Therapies .edited by Lakshmi chandra Mishra, CRC Press LLC, 2004; 63-81.

3. Nandkarni KM. Indian Materia Medica, Vol. I, Popular Prakashan, New Delhi, 1976. ; 362.

4. Samvatsar S. and Diwanji NB. Plant sources for the treatment of jaundice in the tribals of western Madhya Pradesh of India. J Ethnopharmacol 2000 ; 73: 313-316.

5. Ahmad VU, Mohmmad FV and Rasheed T. Hirsudiool, A Triterpenoid from *Cocculus hirsutus*. Phytochem 1987l; 26:793.

6. Ahmad VU, Rasheed T. and Iqbal S. Cohirsinine, an alkaloid from *Cocculus hirsutus*. Phytochem 1991; 30:1350.

7. Ahmad VU and Iqbal S. Jamtinine, an alkaloid from *Cocculus hirsutus*. Phytochem. 1993;33:35.

8. Pallabi DE, Dasgupta SC, Gomes A. Immunopotentiating and immunoprophylactic activities of immune 21, a polyherbal product . Ind. J. Pharmacol 1998; 30:163.

9. Das M, Dasgupta SC and Gomes A. Immunomodulatory and antineoplastic activity of common Indian Toad (Bufo melanostictus, Schneider) skin extract. Ind. J. Pharmacology 1998; 30:311.

10. Dikshit V, Damre AS, Kulkarni KR, et al. Preliminary screening of immunocin for immunomodulatory activity. Ind J Pharma Sci 2000; 62 (4):257-260.

11. Nelson DA, Mildenhall P. Studies on cytophillic antibodies. The production by mice of macrophage cytophillie antibodies to sheep erythrocytes; relationship the production of other antibodies and development of delayed like hypersensitivity. Aust J Exp Biol Medi Sci 1967;45 :113.

12. Talwar GP, Gupta SK. Hand book of Practical Immunology. Vikas Publishing House Pvt. Ltd. New Delhi 1983;139-141.

13. Zieuddin M, Phansalkar N, Patki P, et al. Studies on immunomodulatory effects of Aswagandha. J Ethnopharmacol 1996: 50-69.

14. Sonoda Y, Kasahara T, Mukaida N, et al. Stimulation of interleukin -8 production by acidic polysaccharides from the root of *Panax ginseng*. Immunopharmacolo 1998; 38: 287-294.

15. Mugantiwar AA, Nair AM, Saraf MN. Adaptogenic activity of aqueous extracts of roots *Boerhavia diffusa* Linn. Indian drugs 1997; 34 (4):184.

16. Gonda R, Masashi T, Shimizu N, Kanari M. Characterization of an acidic polysaccharide from the seeds of *Malva verticillata* stimulating the phagocytic activity of cells of the RES. Planta Med 1990 ;56 (1) :73-76.

17. Steven HM, Vizi, SE. Immunomodulation. Curr. Opi Pharmacol 2002; 2:425-427.

18. Luster, M.L., Dean, J.H., Boorman, G.A.; Cell-mediated immunity and its application in toxicology. Environ .Healt. Pers 1982;(43):31 -36.

19. Elgert KD. Immunology: Understanding the Immune System, Willey, New York ,1996:306.

20. Kuby, J.; Immunology, Third Edn, W.H. Freeman and Company, New York, 1997 :436.

21. Benacerraf, B.; A hypothesis to relate the specificity of T lymphocyte and the activity of I region-specific Ir genes in macrophages and B-lymphocytes J. Immunol 1978; 120: 1809-1812.

22. Eisen H. Immunology. II Edn, Harper and Row New York,. 1980. 135.

23. Gill HH, Liew FY. Regulation of Delayed hypersensitivity III. Effect of cyclophosphamide on the suppressor cells for delayed hypersensitivity to sheep erythrocytes in mice. Eur J Immunol 1978; 8:172 -176.