CHAPTER-IV

RESULTS AND DISCUSSION
4 RESULTS AND DISCUSSION

4.1 *Cleome gynandra*

4.1.1 Qualitative chemical tests

The aqueous extract of *Cleome gynandra* showed the presence of carbohydrates, glycosides, saponins and proteins and the ethanolic extract showed presence of alkaloids, carbohydrates, glycosides, fixed oils, fat, saponins and flavonoids (Table 3.2).

4.1.2 Toxicity Studies

In toxicity test with *Cleome gynandra*, no mortality was recorded in both the extracts (Table 3.33 and 3.34).

4.1.3: TLC Studies

Thin layer chromatographic studies were performed for aqueous, ethanolic extracts and their fractions. Aqueous extract of *Cleome gynandra* have shown best separation in Acetone: Water (90:10) to give four spots ($R_f$ value - 0.856, 0.721, 0.423 and 0.261) Ethanol soluble fraction have shown three spots ($R_f$ value - 0.811, 0.621, 0.251) in Chloroform: Methanol: Water (65: 35:10) and Ethanol insoluble fraction have also shown three spots ($R_f$ value - 0.541, 0.432, 0.110) in Acetone: Water (90:10) and spots were visualized by $\text{H}_2\text{SO}_4$ (Table3.7 –3.9).

Ethanolic extract of the drug best separates in Benzene: Acetone (80: 20) and give five spots ($R_f$ value - 0.810, 0.65, 0.517, 0.362, 0.224)
Water-soluble fraction gives four spots (Rf value - 0.62, 0.45, 0.33, 0.15) in n-Butanol: Acetic Acid: Water (60:20:20), H2SO4 is used to visualization. Water insoluble fraction separates in Ethanol: Ethylacetate (20: 80) and give five spots and spots were visualized by Dragendorff reagent (Rf value- 0.91, 0.85, 0.75, 0.65 and 0.60) (Table-3.10 -3.12).

4.1.4 Carbon Clearance

Carbon Clearance depends on time and it was calculated as phagocytic index of time interval between the treated groups of animals compared with the control group. The mean phagocytic index of control (Group I) was found to be 0.0087± 0.012. The aqueous extract of Cleome gynandra treated groups (II, III, IV) had shown phagocytic index significantly elevated as 0.0106±0.027, 0.0118±0.017 (P<0.05), 0.0129±0.019 (P<0.025) when animals treated with 50, 100 and 150mg/kg b.wt intraperitoneally for seven days.

Ethanol soluble fraction of aqueous extract has given relatively low phagocytic index as 0.0071±0.01, 0.0083±0.021 and 0.0091±0.018 respectively with 50, 100 and 150mg/kg b.wt. Ethanol insoluble fraction had shown enhanced phagocytic index as 0.0112±0.018, 0.0124±0.013 (P<0.025) and 0.0144±0.019 (P<0.001) with 50, 100, and 150mg/kg b.wt. intraperitoneally for seven days. (Table 3.39 and Fig. 4.1)

The mean phagocytic activity in terms of phagocytic index of Control (Group I) was found to be 0.0087±0.012. The ethanolic extract of Cleome gynandra treated group II, III and IV have shown phagocytic
index as 0.0088±0.031, 0.081±0.027 (P<0.05) and 0.074±0.018 with 50, 100 and 150mg/kg b.wt. respectively.

Water-soluble fraction of ethanolic extract had given the phagocytic index as 0.0091±0.015, 0.0089±0.012, and 0.0087±0.033 respectively with 50, 100 and 150mg/kg b.wt. Water insoluble fraction of ethanolic extract had shown the phagocytic index as 0.0082±0.031, 0.0079±0.027 (P<0.05) 0.0068±0.025 (P< 0.025) with 50, 100 and 150mg/kg b.wt intraperitoneally for seven days. (Table 3.40 and Fig. 4.2)

4.1.5 Delayed Type Hypersensitivity

Delayed type Hypersensitivity response to SRBC was calculated as a measure of paw volume (in mm) for each animal and compared with control group I which was injected 2ml of 5% Normal saline intraperitoneally for seven days. Increase of Paw volume was calculated after 24, 48, 72 and 96 hrs. The increased value for group II, III and IV after 24 hrs were found to be 1.78±0.14 ml, 1.88±0.15 ml, 1.86±0.10 ml and after 48 hrs there were 1.04±010 ml, 1.11±0.12 ml (P< 0.05), 1.07±0.11 ml (P<0.025) after 72 hrs it was 0.59±0.24 ml, 0.49±0.16 ml, 0.37±0.12 ml and finally after 96 hrs Paw volume was reduced significantly 0.27±0.11 ml, 0.19±0.14 ml (P < 0.025) 0.16±0.08 ml (P<0.001). Animals treated with ethanol soluble fraction were not shown any significant activity after 24, 48, 72 and 96 hrs Animals treated with ethanol insoluble fraction have given a significant increase in Paw
volume 1.82±0.16 ml (P<0.025) 1.95± 0.18 ml (P<0.025) and 1.94±0.10 ml after 24 hrs 1.10±0.20 ml, 1.17±0.20 ml (P<0.025) 1.15±0.24 ml (P<0.025) after 48 hrs. After 72 hrs it was recorded to 0.49±0.11 ml (P < 0.05), 0.39±0.16 ml (P < 0.001) and 0.29±0.18 ml with 50, 100 and 150mg/kg b. wt. After 96 hrs paw volume was reduced significantly as compared to control and it was found to be 0.19±0.17 ml (P< 0.025), 0.15±0.24 ml (P<0.001), 0.11±0.11 ml (P<0.001) (Table 3.45 and Fig. 4.7).

DTH response to SRBC for ethanolic extract of Cleome gynandra of Group I (control group) was compared with the treated groups. Control group was given 2ml of Normal saline for 24, 48, 72 and 96 hrs and the test values are found to be 1.66±0.11 ml, 0.96±0.19 ml, 0.69±0.30 ml, 0.29±0.18 ml respectively. Animals of group II, III, IV were shown paw volume 1.35±0.15 ml, 1.20±0.15 ml and 1.15±0.10 ml (P<0.025) after 24 hrs. The paw volumes were 0.92±0.18 ml, 0.86±0.12 ml (P<0.025) and 0.81±0.11(P<0.025) after 48 hrs. After 72 hrs paw volume was 0.78±0.24 ml, 0.82±0.11 ml (P < 0.05), 0.87±0.09 ml and after 96 hrs it was found to be 0.40± 0.11 ml (P < 0.001).

Animals treated with water-soluble fraction of Cleome gynandra didnot show a significant difference with control group. Animals treated with water insoluble fraction had given a following observation after the administration of the extract by 50, 100 and 150mg/kg b.wt. After 24 hrs it was found to be 1.63±027 ml, 1.76±0.07ml (P< 0.05), 1.80±0.05ml
(P<0.025), after 48 hrs it was 1.05±0.05ml (P< 0.025) and 0.85±0.09 ml (P< 0.025) and lastly after 96 hrs Paw volumes were found to be 0.46±0.09ml (P< 0.025), 0.48±0.11 ml (P< 0.001) and 0.47±0.08 ml (P<0.001). *(Table-3.46 and Fig. 4.8)*

### 4.1.6 SRBC Agglutination Test

Agglutination titre to sheep red blood erythrocyte was calculated and compared with Group I (control). Group II, III, IV were treated with crude aqueous extract orally for ten days (50, 100, 150 mg/kg b.wt) and on 10th day agglutination titre were observed in various serum dilution (X: 20, X: 40, X: 80, X: 160, X: 320). Significant increase was observed at the dose of 100 and 150 mg/kg b.wt. while no significant increase observed in 50mg/kg b.wt. Group V, VI and VII were given ethanol soluble fraction at the dose of 50, 100 and 150mg/kg b.wt and no significant change were observed where as ethanol insoluble fraction was given to Group VIII, IX, X (50, 100 and 150mg/kg b.wt. respectively) and caused a significant increase in the agglutination titre. *(Table 3.51 and Fig. 4.13)*

With ethanolic extract Group I was given 5% normal saline and agglutination titre was compared with treated one. Group II, III, and IV that received the ethanolic extract at the dose of 50, 100 and 150mg/kg b.wt respectively, significant increase was observed in the animals of group III which received 150 mg ethanolic extract /kg b.wt. Water-soluble fraction of 50, 100 and 150mg/kg b.wt was given to Group V, VI,
and VII no change was observed. While significant increased in agglutination titre was observed in all the three groups, which were given water insoluble fraction of 50, 100 and 150mg/kg b.wt respectively (Table 3.52 and Fig. 4.14).

4.1.7 Drug Induced Myelosuppression Test

In this study, Myelosuppression was produced in animals with the administration of 3mg/kg b.wt. cyclophosphamide orally for seven days. Group I was kept as a control and given 5% normal saline. The mean haemoglobin was 13.09±0.15 gms/dl mean RBC were 4.66±0.550 million /mm³, Neutrophils were 54.33±1.05%, Monocytes was 2.52±0.56, Eosinophil was 2.30±0.42% and Platelet count was 3.10±0.322 lacs/mm³ were observed in control rats.

Group II (treated with Cyclophosphamide 3mg/kg b.wt.) showed a significant decrease in haemoglobin 8.56±0.27 gms/dl. Mean RBC count was 3.52±0.098 million/mm³, WBCs were 10.93 thousand/ mm³ and Platelets count was 2.57±0.384 lacs/mm³, (P < 0.05) Crude Aqueous extract was given to Group III, IV and V at the dose of 50, 100, 150mg/kg b.wt. which protect them against the effect of cyclophosphamide. There was a good recovery in Group IV and V in which haemoglobin was increased to 11.01±0.28 (P < 0.025) and 12.48±0.24 (P < 0.025) gms/dl respectively. Mean RBC was 3.92±0.057, 4.07±0.026, 4.32±0.118 million/mm³ (P < 0.025) in Group III, IV and V. Mean WBC were 10.21±0.103, 11.96±0.161 (P < 0.025), 12.02±0.115
thousand/ mm³ (P < 0.025). Neutrophils were 59.50±0.885%, 52.83±1.51%, 52.33±1.05%, Monocytes were 1.80±0.30%, 2.81±0.65%, (P<0.05) 3.12±0.33%, (P<0.025). Eosinophil count were 2.10±0.30%, 2.82±0.70%, 1.86±0.47 (P<0.025) and Platelet count were 2.57±0.384 (P<0.05), 2.62±0.012, 2.86±0.035 (P<0.025) 2.98±0.013 lacs/mm³ (P<0.025) respectively in Group III, IV, and V.

Ethanol soluble fraction (50, 100, 150mg/kg b.wt) had shown the protection against cyclophosphamide as in these groups the mean haemoglobin 9.56±0.12, 9.73±0.27, 9.83±0 47 gms/dl, respectively. Mean RBC in the entire three groups were 11.02±0.455, 11.12±0.635, and 11.23 ±0.485 million/mm³, Neutrophils were 61.38±0.29%, 58.83±0.68%, 59.43±0.37%, Monocyte count was 2.20±0.10%, 2.84±0.22%, 2.75±0.29%, Eosinophil count was 3.09±0.45%, 3.50±0.14%, 3.25±0.35%, and Platelet count was 2.82±0.021, 2.87±0.188, 2.57±0.384 lacs/mm³

Ethanol insoluble fraction also showed protection against cyclophosphamide and this was observed in Group IX, X, XI and mean haemoglobin was found to be 11.32±0.11 (P<0.05), 11.01±0.28 (P< 0.025), 12.48±0.24 gms/dl (P<0.025), respectively. Mean RBC count was 3.97±0.018(P<0.05), 4.07 ±0.026 (P<0.025), 4.32 ±0.118 million/mm³ (P<0.025) WBC were 12.41±0.098 (P<0.025) 11.42 ± 0.161 (P<0.029), 11.82±0.0115 thousand/ mm³ (P<0.025) Neutrophils were 58.35±0.135%, 53.49± 1.25%4,56.33±1.184% (P<0.05) Monocytes were
2.80±0.37 %, 2.81±0.65% (P<0.05) 2.79±0.11% (P<0.025) Eosinophil count were 3.86±0.46% 2.82±0.70% 1.76±0.14% (P<0.025) and platelet were 2.38±0.042; 2.86±0.035 (P<0.025) and 3.21 ± 0.013 (P<0.001) lacs/mm³ [Table3.57 and Fig. 4.19-4.23].

In the case of ethanolic extract Group I was control and received as usual 2ml of 5% of Normal saline and various hematological observation were taken. In them the mean haemoglobin was 13.09±0.15 gms/dl, mean RBC count was 4.66±0.145 million/mm³ and mean WBC count was 13.04±0.550 thousand/mm³. Neutrophils count was 54.33% Monocyte count was 2.52±0.56% Eosinophil count was 2.30±0.42% and platelet count was 3.10±0.322 lacs/mm³ In Group II cyclophosphamide (3mg/kg bw) were administered and there was a significant decrease in all hematological parameters studied. Mean haemoglobin was 8.56±0.27 gms/dl, mean RBC count was 3.52±0.098 (P<0.05) WBC count was 10.93±0.355 thousand/mm³ (P<0.05), mean Neutrophils count was 61.83±0.79%, Monocytes count was 3.00±0.16% Eosinophil count count was 2.50±0.34% and platelet were found to be 2.57±0.384 (P<0.05) lacs/mm³.

Group III, IV and V were administered crude ethanolic extract of (50, 100 and 150mg/kg b.wt) intraperitoneally, in them the mean haemoglobin was found to be 9.12±0.14, 8.98±0.09 (P<0.05), 10.24±0.76 gms/dl respectively. Mean RBC count was 3.63±0.029, 3.28±0.026,
3.86±0.237 million/mm³, mean WBC count was 9.89±0.293, 11.96±0.161, 10.02±0.263 thousand/mm³ (P<0.001), Neutrophils count was 62.50±0.321%, 60.45±0.561%, 62.43±0.754%, Monocyte count was 3.40±0.17, %2.93±0.65%, 3.79±0.34%, Eosinophil count count was 3.10±0.23%, 3.52±0.70% (P<0.025) 4.86±0.47% (P<0.001) and platelet count was 2.53±0.012, 2.48 ± 0.042, 2.39 ± 0.013 lacs/mm³ (P<0.025).

Water-soluble fraction was administered in Group VI, VII and VIII, the various blood parameters were; hemoglobin 10.62±0.12, 10.37±0.12, 9.83±0.47 gms/dl, mean RBC count 3.61±0.039, 3.87±0.024, 3.78±0.042 million/mm³, mean WBC count 11.45±0.212, 11.97±0.256, 11.46±0.114 thousand/mm³. Neutrophils count 57.38±0.12%, 59.62±0.13%, 58.68±0.52%, Eosinophil count 2.64±0.37%, 3.10±0.38%, 3.65±0.12% and platelet count was 2.85±0.045, 2.96±0.254 and 2.69±0.314 lacs/mm³.

Water insoluble fraction was injected in the group IX, X and XI and significant increase was observed in various blood parameters, mean haemoglobin was found to be 11.12±0.29, 10.02±0.09 and 9.45±0.52 gms/dl (P<0.025), RBC count was 3.63±0.029, 3.89±0.012, 3.32±0.125 million/mm³ (P<0.025), WBCs were 11.75±0.42, 10.27±0.211 (P<0.05), 9.56±0.256 thousand/mm³, (P<0.001), Neutrophils count were 60.30±0.245%, 60.45±0.561%, 64.45±0.586%, Monocytes count were 3.40±0.17%, 3.93±0.65, %3.33±0.11%, Eosinophil count were
2.86±0.23%, 3.72±0.23%, (P<0.025), 4.01±0.12% (P<0.025) and platelet count were 2.98±0.032, 2.21±0.042 (P<0.025) and 2.14±0.042 (P<0.001) lacs/mm³ (Table 3.58 and Fig. 4.24-4.28).

4.1.8 Discussion

Aqueous extract of Cleome gynandra showed significant immunostimulant activity in carbon clearance test by increasing phagocytic index in a dose dependent manner. Crude aqueous extract, increased the phagocytic index significantly as 0.0106±0.00027, 0.0118±0.00017 (P<0.025) and 0.0129±0.00019 (P<0.025) Ethanol insoluble fraction also enhanced the phagocytic index at maximum dose of 150mg/kg b.wt which is 0.0144±0.00019 (P<0.001). Increase in phagocytic index indicates that phagocytosis is increasing. Stimulation of phagocytosis is influenced by the activation of macrophages, the activated macrophages secrete a number of cytokines, which in turn stimulate other immune cells (Nose et al., 1998). In the same experiment ethanol soluble fraction did not show any significant increase or decrease in the phagocytic effect. This suggests that the active substance, which stimulates the immune system, either is absent or present in such a low concentration that to invocative to phagocytes is generated significantly.

The results depict that aqueous extract of Cleome gynandra has immunomodulatory activity. Both, crude and ethanol insoluble fraction have the phytocomponents for chemostimulation of phagocytosis. Significant clearance of carbon particles from the blood of treated animal in dose
dependent manner is observed. The component(s) of the extract activate the receptors to remove antigen (here the carbon particles) through pinocytosis as the antigen is very small. In case of mouse CR1, CR2, CR3, CR3b and CR3bi are the main receptors. The phenols, flavonoids, terpenes and saponins as reported by Verotta, 2001 are responsible to incite them, which in turn eliminate carbon particles or phagosome, the antigen. The speed and the amount of carbon particles phagocytosed made to assume that the area of plasma membrane of neutrophill and monocytes increases but the microscopical examination of the blood of control and treated animals, show no change in size of monocytes either of cell. Neutrophills or monocytes, which are main phagocytic lecocytes, take up particles through minimum 40 receptors expressed on their surface. These receptors are for IgG complment, mannose and galactose terminated oligosaccharides. It is supposed that many of the receptors become active due to the exposure of the extracts.

The pre exiting and newly formed IgG may be playing their role in the identification of the antigen, activation of MoRC, IgM receptors, and the attachment of the receptors to facilitate phagocytosis. Many Flavones increase phagocytosis through complement C3 and C1. Flavonoides are present in the extract of the plants. Besides them some other compounds are also there which work in association of flavonoides to activate CR3b and CR3bi receptor of phagocytes and ligation of complements with the receptors (Kandaswami and Middleton 1994, Estrada et al., 2000).
Ethanolic extract showed a decline in the phagocytic index. It was 0.0088±0.00031, 0.081±0.00027, 0.0074±0.00018 (P<0.025) at doses 50, 100 and 150mg/kg b.wt. respectively. Water insoluble fraction too followed the same trend and significantly decreased phagocytic index, at the doses of 100, 150mg phagocytic index was 0.079±0.00027 and 0.068± 0.00025 (P<0.025). It is possible that ethanolic extract contains some substances, which suppresses the immune system. But in the case of water-soluble fraction it shows a slight increase in the phagocytic index, this suggested that ethanolic extract might contain some compounds, which are stimulator but are more soluble in water. Moreover, ethanolic extract and its water insoluble fraction decrease phagocytic index significantly. Since the extracts contain varieties of compounds few of them may be inhibiting phagocytosis. Possibly inactivating plasma receptors on binding or IgG with antigen or preventing the binding of antigen-antibody complex with receptors or coating the antigen to prevent its recognition by the phagocytes or the inhibiting the activity of C1 and C3 complement (Millonig et al., 1974).

Delayed Type Hypersensitivity Test was done to study the effect of aqueous and ethanolic extract on cell-mediated immune response to sheep red blood cell (SRBC). Crude aqueous extract first increases the paw edema in 24, 48 hrs and then after 72 and 96 hrs paw volume significantly decreases when compares with control. In the same experiment ethanol insoluble fraction follow the same trend. Ethanol
soluble fraction showed a gradual decrease in the paw volume when compared with control.

Ethanolic extract and its water soluble and insoluble fractions cause to decrease the delayed type hypersensitivity. Paw volume reduced in 24 hrs with respect to control, which continued in later hrs too.

The crude extract exhibits increase in paw volume, in response to sheep RBC the initial response of first an hour is very much suggestive of infiltration of CD4 lines of T-lymphocytes and as usual diapedesis of mononuclear macrophages and liberation of edema causing substances for example serotonin, prostaglandulin E, cytokines etc. The infiltration of lymphocytes is possibly because of the compounds, which perhaps here distort endothelium to accumulated different type of lymphocytes, observed the cell-mediated immune response. Extract of Cleome gynandra, have some compounds that may be different in nature but having potent activity to involve cell-mediated immune response. This indicates that aqueous extract and ethanol insoluble fraction contain some compounds, which activate the T-cell and release vasoactive amines and multiple hormonal substances like lymphokines. These substances then probably function as mediators of the ensuing hypersensitivity response particularly by attracting and activating macrophages. (Roitt 1988, Kulkarni and Desai 2001, Ray et al., 1996) Aqueous extract of the plant contains saponins and according to Liu et al., 1995, Shibata 1977 and Verotta 2001 saponins are immunostimulating agents. In later hrs reduction in paw volume may be
because of a quick action of various enzymes, hormones etc on the invader, simultaneously phagocytosis increases because of activated macrophages and hence reduction in paw volume was observed. Reduction in paw volume after 24 hr. and onwards point to the fact that saponins and similar compounds increase the metabolic activity of the neighbouring cells to release of Serine proteases and immunohormones (Cytokines) these metabolites and activated macrophages eliminate the causative agents hence the edema gradually reduces.

On the other hand ethanolic extract inhibited the inflammatory response. The ethanolic extract and its fraction contain some compound, which show an anti-inflammatory effect. (Nores et al., 1997)

A significant increase in humoral immune response was observed. Agglutination titre to SRBC was increased significantly by aqueous extract. Ethanol soluble fraction also showed agglutination titre up to the same level. Aqueous extract of the plant contains proteins, oligosaccharides and their conjugated compounds besides β-sterols, saponins, flavonoids, flavones etc. the antigenicity to elicit antibodies of first two compounds is well known, but ethanol soluble fraction is devoid of some compounds, other compounds are equally potent for the synthesis of immunoglobulins, since both the extracts develop almost similar effects on the amount of (new) immunoglobulins, the elicitation of the response can be considered as compounded effect. Ethanol soluble fraction did not express any change. Red blood cell at neutral pH possesses negative ions that form cloud, which repel one another.
Immunoglobulins like IgM can overcome the electric barrier and get cross-link with red blood cells, this leads to subsequent agglutination. From the above results it is possible that there is an enhancement in the level of IgM and IgG because antibody titre against SRBC were raised. In many plants similar activities and increased titre of IgM etc. were observed (Rezaeipoor et al., 2000, Frier et al., 2003). The responsible chemicals were alkaloids, flavonoids, polysacchrides and polypeptides as suggested by Gao et al., 1996, Liu et al., 1995 and Pinilla and Luu, 1999.

Ethanolic extract and its water insoluble fraction showed a decrease in the agglutination titre. Crude ethanolic extract and its water-soluble fraction at the dose of 150mg/kg b.wt. showed agglutination titre only up to X: 20 (P<0.025) and with water soluble fraction the agglutination titre remained almost unchanged this indicates that ethanolic extract and its fraction suppress humoral immune response and interfere with antibody formation so less antibody is formed insignificantly, affects agglutination titre against SRBC titre. The study of the results depict that the ethanolic extract and its water soluble fraction surprisingly show almost no change in agglutination titre, perhaps the amount of the compounds that can invoke antibody synthesis is not enough to incite T4 and B lymphocytes or such compounds are not in the extract. This cannot be ignored that immunosuppression may be caused by the other contents of the extract.

Cyclophosphamid suppresses humoral, cellular, non-specific and specific cellular immune response. When animal was treated with
cyclophosphamide then haemoglobin [Hb], RBC counts, WBC count, Lymphocyte% and Platelet count all are reduced significantly (Doherty 1981, Gill and Liew, 1978, Habibullah et al.,1979). The suppressive effect of cyclophosphamide was protected by the administration of aqueous extract and their ethanol soluble and ethanol insoluble fraction. Flavonoids in biological systems tend to adhere with the molecules of cyclophosphamide this causes to increase the size of the molecules and prevent its entry to the stem cells. As already stated that such compounds are detected in the plant extract of the besides this some more compounds are there as it can be assumed which are not only negating the effect of cyclophosphamide, but also accelerating the total WBC and heamoglobin count. Ethanol soluble fraction did not make any significant elevation in the hematological parameters taken for study on the other hand crude aqueous extract of 100, 150mg/kg b.wt. showed significant increase in these hematological parameters. Ethanol insoluble fraction also enhances the Haemoglobin RBC count, WBC count; Lymphocytes and Platelet count in a dose dependent manner. This suggests that the constituent of the plant preventing the access of cyclophosphamide to the stem cells so that synthesis of haemoglobin, WBC and RBC is not inhibited. Another point is that the compounds are neutralizing this immunosuppressant before it could act upon haemopoetic and myeloid tissue and its effective amount is present in 100/150 mg of extract. The crude aqueous extract also enhances the
number and activities of various immune cells and protects the animal from the adverse effect of cyclophosphamide.

On the other hand ethanolic extract of *Cleome gynandra* and its water soluble and insoluble fractions showed a mixed effect, sometimes the values of blood parameters increase or decrease. The ethanolic extract showed a dual nature, stimulatory as well as suppressive effect. In some cases extract protects the animal from the effect of cyclophosphamide but at certain doses of the extract only it shows a slight reduction in the given values. In addition to carbohydrates, glycosides and saponins, proteins also contribute to a large extent in Immunostimulation. More activity of aqueous extract as compare to ethanolic extract can be justified on the basis of denaturation of proteins in ethanolic extract. Overall results with the *Cleome gynandra* showed its immunostimulant as well as immunosuppressant nature (Steven 2000).
4.2 **Cocculus hirsutus**

4.2.1 **Qualitative chemical tests**

Aqueous extract of *Cocculus hirsutus* in qualitative analysis shows the presence of carbohydrates, glycosides, saponins and proteins and in the ethanolic extract alkaloids, carbohydrates, glycosides, fixed oils and fats were found to be present. *(Table 3.3)*

4.3.2 **Toxicity**

Both the extracts of *Cocculus hirsutus* did not show any kind of toxicity and mortality up to the doses of 500mg/kg b.wt. in test animals *(Table 3.35 - 3.36).*

4.2.3 **TLC Studies**

Aqueous extract of *Cocculus hirsutus* have shown best separation in Acetone: Water (90: 10) and give five spots (Rf values are 0.912, 0.856, 0.721, 0.626 and 0.321) Ethanol soluble fraction give best separation in Chloroform: Methanol: Water (65: 35: 10) and four spots were visible having Rf values 0.600, 0.432, 0.312 and 0.222. Ethanol insoluble fraction gave four spots of Rf values 0.900, 0.812, 0.550 and 0.300 in Chloroform: Ethanol (100: 2). H₂SO₄ was used for visualization *(Table 3.15 - 3.17).*

Ethanolic extract of *Cocculus hirsutus* showed best separation in Benzene: Acetone (80: 20) and give six spots (Rf values 0.92, 0.91, 0.89, 0.87, 0.11and 0.08) spots visualized in daylight. On the other hand water-soluble fraction separates in Ethanol: Ethylacetate (20: 80) and
gave four spots (Rf values 0.86, 0.65, 0.43 and 0.15) Spots were visualized by Dragendorff reagent. Water insoluble fraction gave five spots (Rf values 0.95, 0.70, 0.44, 0.22 and 0.11) in Cyclohexane: Chloroform: Diethyl amine (50: 40: 10) spots visualized in daylight (Table 3.18 - 3.20).

4.2.4: 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 hirsutus was given to group II, III and IV in a dose of 50mg, 100mg and 150mg/kg body wt. intraperitoneally 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 fraction of aqueous extract has given the phagocytic index as 0.0090±0.00075, 0.0097±0.021 (P<0.05) 0.0117±0.021 (P<0.025) and Ethanol insoluble had shown the phagocytic index as 0.0119±0.018 (P<0.025) 0.0131±0.013 (P<0.001) 0.0152±0.019 (P<0.001) with 50mg, 100mg, 150mg/kg body wt. intraperitoneally for seven days (Table 3.41 and Fig. 4.3).

In other experiment Group I was control and ethanolic extract of Cocculus hirsutus was given to the Group II, III and IV have phagocytic index as 0.0090±0.027, 0.092±0.027 and 0.0116±0.025 (P<0.025) in the dose of 50mg, 100mg, and 150mg/kg b.wt. respectively. Water-soluble
fraction of ethanolic extract had shown phagocytic index as 0.0087±0.052, 0.0089±0.045, and 0.087±0.052, at the doses of 50mg, 100mg, and 150mg/kg b.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 50mg, 100mg and 150mg/kg b.wt. respectively when administered intraperitoneally for seven days (Table 3.42 and Fig. 4.4).

4.2.5 Delayed Type Hypersensitivity Test

Group I subjected to normal saline subdermally in paw had swollen 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 groups were 1.88±0.21, 1.95±0.14 and 1.96±0.07ml in 24 hrs at the dose of 50, 100 and 150mg/kg b.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 doses 50, 100 and 150mg/kg b.wt. respectively.

Ethanol insoluble fraction also followed the same pattern. In 24hrs the volume of Paw become 1.86±0.23, 1.98±0.01, 1.95±0.17ml in 50, 100 and 150mg/kg b.wt. In 72hrs values decreases to 0.59±0.11, 0.45±0.25, 0.37±0.13ml and after 96hrs these values reduces to 0.20±0.02 (p<0.025)
0.15±0.03 (p<0.001), 0.12±0.08ml (p<0.001). Ethanol soluble fraction did not show any significant activity (Table 3.47 and Fig. 4.9).

Ethanolic extract also enhance delayed type 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, 48hrs 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 and 150mg /kg b.wt paw volume was 1.79±0.09, 1.21±0.19, 0.59±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 compares with control group.

Water insoluble fraction of 150mg/kg b.wt increases the maximum edema and in this way increase the paw volume (1.96±0.12, 1.24±0.14ml in 24 and 48 hrs) Edema and paw volume decreased significantly 0.31±0.03 and 0.41±0.05ml (p<0.001) after 72 and 96 hrs (Table 3.48 and Fig. 4.10).

4.2.6 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 Coecculus hirsutus orally for ten days. The three groups were given of 50, 100 and 150mg/kg b.wt, respectively and on 10th day agglutination titre was estimated in various serum dilution.
(X: 20, X: 40, X: 80, X: 160 and X: 320). In the lower dose of the 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 ethanol soluble fraction at the dose of 50, 100 and 150mg/kg b. wt. and no significant change in the agglutination titre could be observed. Ethanol insoluble fraction was given to group VIII, IX and X of doses 50mg, 100mg, 150mg/kg b.wt. which showed a significant elevation in agglutination titre (Table 3.53 and Fig. 4.15).

Control group was administered 2ml of 5% normal saline and agglutination titre was compared with treated groups. Group II, III and IV were administered with ethanolic extract of doses 50, 100 and 150mg/kg b.wt. respectively and significant increase was observed in IV group (150/kg b.wt.) low doses of crude extract did not show remarkable change. Water-soluble fraction also did not show any enhancement in the agglutination titre. Water insoluble fraction showed a significant increase in the agglutination titre at the doses (50, 100 and 150mg/kg b.wt) up to the serum dilution of X: 160 (Table 3.54 and Fig. 4.16).

4.2.7 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, WBC count was 12.96±0.687 thousand/mm³, Neutrophils percent was 52.13±1.78, Lymphocyte was 37.66±1.22%, Monocyte was 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 (3mg/kg b.wt) which caused a significant decrease is in haemoglobin concentration, RBC count, WBC 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 of 50, 100 and 150mg/kg b.wt. Animals of these groups exhibited protection against the effect of cyclophosphamide, though a good recovery was recorded in the Group IV and V, with regard to the values of various blood parameters in group III, IV and V were as follows Haemoglobin was10.58±0.27, 11.18±0.43 and 12.22±0.12 gms/dl, RBC was 3.42±0.057, 3.86± 0.065 (P<0.025) 4.01± 0.412 million mm³ (P<0.001) WBC was 10.21±0.103, 11.75±0.423 (P<0.05), 12.10±0.145 thousand/mm³ (P<0.001) Neutrophils was 59.50±0.885%, 54.83±1.515%, 52.93±1.054% (P<0.05), Lymphocyte percent was 30.83±0.703, 32.33±0.512 (P<0.05) 35.66±0532 (P<0.001) Monocytes 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% (P<0.025), 2.65±0.47% (P<0.025) Platelet count was 2.62±0.012, 2.75±0.011 (P<0.025) 2.98±0.033 Lacs/mm³ (P<0.001) respectively.

Ethanol soluble fraction showed dose dependent effects on blood parameters. Blood parameters at a dose of 50, 100 and 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. 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±0.22%, 3.00±0.32%, 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 the three dose (50, 100 and 150mg/kg b.wt) mean haemoglobin was found to be 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, 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 % was 2.95±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). [Table 3.59 and Fig. 4.29-4.33]

In the case of ethanolic extract Group I was administered 2ml of 5% Normal saline and various hematological parameter were observed
mean haemoglobin was 12.87±0.56 gms/dl, RBC count was 4.33±0.165 million/mm³, WBC count was 12.96±0.687 thousand/mm³ Neutrophils 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 (3mg/kg body wt.) and all the blood parameters showed variation as haemoglobin become 7.98±0.11, RBC was 2.99±0.087 million/mm³, WBC count was 10.45±0.244 thousand/mm³ (P<0.05) Neutrophils % was 68.83±0.26, 29.66±0.88 (P<0.05) Monocyte % was 3.00±0.10 Eosinophil count was 2.50±0.34 and platelet count was 2.57±0.384. Group III, IV and V were administered crude ethanolic extract of Cocculus hirsutus of 50, 100 and 150mg/kg b.wt. intraperitoneally and then blood was collected from retro-orbital plexus and hematological studies were found to be 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±0.021 million/mm³ (P<0.025) WBC count was 10.75±0.125, 10.99±0.214, 11.75±0.265 thousand/mm³ (P<0.025) Neutrophils 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, 2.75±0.35, 2.89±0.42 (P<0.025) Eosinophil 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, VII and VIII (50, 100 and 150mg/kg b.wt) in these groups the blood parameter were as follows haemoglobin concentration was 8.54±0.65, 8.69±0.24, 158
8.95±0.53 gms/dl, RBC count was 2.74±0.042, 2.98±0.012, 2.85±0.032 million/mm³, WBC count was 10.53±0.132, 11.00±0.122, 10.65±0.512 thousand/mm³. Neutrophils 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% Eosinophil count was 2.96±0.64%, 2.86±0.46% and 2.75±0.35% and Platelet count was 2.68±0.086, 2.78±0.054, 2.62±0.045 lacs/mm³.

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 was 3.54±0.053, 3.68±0.014 (P<0.05) 3.79±0.011 million/mm³ (P<0.025), WBC was 11.12±0.321 11.99±0.142 (P<0.025), 12.12±0.321 thousand/mm³ (P<0.001) Neutrophils % was 58.78±0.352, 60.32±0.563 54.83±0.326 (P<0.025) Lymphocytes % 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. Eosinophil 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/mm³ (P<0.025) (Table 3.60 and Fig. 4.34-4.38).

4.2.8 Discussion

*Cocculus hirsutus* is a member of the family Menispermaceae and commonly known known as “Jamti ki bel”. *Cocculus hirsutus* is a widely growing plant found in the plains of India in dry localities. The plant is a
climber with green flowers. Flowers bloom in February-March and fruits in May-June. In some places it is found along with water stream, hedges. Tribal of Jhabua, Khargone and Dhar uses the fruit of *Cocculus hirsutus* to cure jaundice.

*Cocculus hirsutus* increases the rate of phagocytic index with respect to control. It was observed that aqueous extract; ethanolic extract and their fractions enhance the phagocytic index. Increase in phagocytic index in terms of clearance of carbon particles from the blood is suggestive of activation of WBC. Increase was dependent on the dose, as the dose increases phagocytic index also increases. Results of these studies clearly indicate that *Cocculus hirsutus* activates the process of phagocytosis. The extract influences the role of neutrophils, digestive enzymes in phagocytic vesicle, and the synthetic processes in the cytoplasm. In treated animal, hyper granulation of WBC is the evidence of it. The secretory material appeared in the cytoplasm is to meet the necessity of the cell to phagocytose and digest the antigen. Stimulation of phagocytosis was influenced by the activation of macrophages, these activated macrophages secrete a number of cytokines such as IL-1, IL-2, etc. (Sonoda *et al.*, 1998) and that inturn mobilize the immune cell. Aqueous as well as ethanolic extract significantly influence and activate macrophages. Gao, *et al.*, 1989, Mugantiwar, *et al.*, 1997, Gonda, *et al.*, 1990, Atal, *et al.*, 1986 also reported aggregation and activation of neutrophils when expressed to the extracts of different plants. *Cocculus hirsutus* has saponins, flavones and other compounds as it is mentioned.
earlier the compounds in these plants have different Rf values hence, their chemical composition would be slightly different and so also their effects on the body. The comparative effects of the extract on phagocytosis vary. The mode of action of the compounds on FCRIII, CRb and CRBi may vary in intensity and the same may be in case of antibody and the complement proteins. It is obvious that some of the constituents have definite effect on myeloid tissue directly or through interleukines, besides some compounds exhibiting antigenicity. In the same experiment water-soluble fraction of ethanolic extract did not show any significant immunomodulatory activity.

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. Aqueous and ethanolic extracts of Cocculus hirsutus increase the 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 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 other lymphocytes. This behavior itself is suggestive of activation of immune system at cellular level with lymphocytes. Cytokines, prostaglandulin E etc. are also liberating from the neighbouring cells and the cumulative effect is termed as DTH (Steven, 2002).

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. Increase 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 (Luster et al, 1982, Elgert 1996, Descotes 1999; Kuby1997). 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-cells 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 cells. 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 doses of 100mg and 150mg/kg b. wt. with these doses the agglutination was observed up to serum dilution of X: 320.

Ethanolic extract and Water insoluble fraction showed significant increase of agglutination titre in the dose of 150mg/kg b.wt. The titre was observed up to the serum dilution of X: 160. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis. (Benacerraf, 1998) Increased level of antibodies gives higher agglutination titre against sheep red blood cells (Eisen, 1980).

*Cocculus hirsutus* antagonizes the myelosuppressive effect induced by cyclophosphamide, 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 (Doherty 1981, Gill and Liw 1978). 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 dependent manner. Natural product like polypeptides, oligosaccharides, 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) through the path way ultimately activates B-lymphocytes devoid to form plasma cell, which intern release particular type of antibodies. Appearance of some protein bands during electrophoratic 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 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 immunosuppressants.

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 phagocytic 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 titre, which means modulation of humoral immunity.
4.3 *Lantana camara*

4.3.1 Qualitative Chemical Tests

In qualitative analysis of aqueous extract: carbohydrates, glycosides, saponins, proteins and flavonoids were present. On other hand the ethanolic extract showed the presence of alkaloids, carbohydrates, glycosides, fixed oils, fats and flavonoids.

4.3.2 Toxicity

In the case of Lantana, some taxa are toxic to ruminants. These taxa contain lantadenes A and B, which found to be toxic to sheep. (Sastri et al., 1962) Toxicity is not cumulative and only occurs when sufficient amount of toxic plants consumed at one feed but in albino rats no mortality was recorded up to 500mg/kg b wt. *(Table 3.37- 3.38)*

4.3.3 TLC Studies

Aqueous extract of *Lantana camara* gave best separation in Acetone: Water (90:10) and gave four spots (Rf value 0.67, 0.60, 0.32 and 0.22) Ethanol soluble fraction gave three spots in the solvent Chloroform: Methanol (60:40), Rf value 0.55, 0.213, 0.11 and Ethanol insoluble fraction give three spots of Rf value 0.70, 0.65 and 0.45 for solvent system Chloroform: Methanol (94: 6). H2SO4 used for spot detection. *(Table 3.23-3.25)*

Ethanolic extract of *Lantana camara* gives four spots in Cyclohexane: Chloroform: Di ethylamine [50: 40: 10] (Rf value 0.77, 0.63, 0.52 and 0.30) and were detected in daylight. Water-soluble fraction
gives three spots (R<sub>t</sub> value 0.70, 0.67 and 0.43), in Acetic acid: Water: n-
Butanol (20:20:60), Water insoluble fraction gives three spots in
Benzene: Acetone (80: 20) (R<sub>t</sub> value: 0.72, 0.51 and 0.45) both the above
fractions were visualized by H<sub>2</sub>SO<sub>4</sub>. (Table 3.26-3.28)

4.3.4 Carbon Clearance

Phagocytosis is measured by the carbon clearance method.
Phagocytic index is calculated and is compared with the control group.
Group I was kept as control and the phagocytic index was 0.0085±0.015.
The crude aqueous extract of <i>Lantana camara</i> injected to the Group II, III
and IV with the doses of 50mg, 100mg, 150mg/kg b. wt. and a
significant increase was observed in the phagocytic index in III & IV
group which was 0.0110±0.026 (P<0.05), 0.0121±0.016 (P<0.025)
respectively.

In ethanol soluble fraction of aqueous extract the phagocytic
indices were 0.0084±0.065, 0.0081±0.014 and 0.0083±0.022 in a dose of
50, 100 and 150mg. Ethanol insoluble fraction showed the phagocytic
index as 0.0115±0.027 (P<0.025), 0.0119±0.026 (P<0.025) and
0.0127±0.013 (P<0.001) with 50, 100 and 150mg/kg b.wt. respectively.
(Table 3.43 and Fig. 4.5)

The crude ethanolic extract enhances the phagocytic index. With
the increase in doses phagocytosis increases significantly, the phagocytic
indices with 50mg, 100mg, 150mg/kg b.wt. were 0.0109±0.027 (P<0.05),
0.0126±0.014 (P<0.025) 0.0127±0.016 (P<0.025) respectively. Water-
soluble fraction did not show any significant increase. In these groups phagocytic index follows an irregular trend and it was near to the values obtained in the control group. Water-insoluble fraction again showed a significant activity. In Group VIII, IX, X phagocytic indices were 0.0112±0.02, 0.0128±0.014 (P<0.025) and 0.0131±0.016 (P<0.001) respectively. (Table 3.44 and Fig. 4.6)

4.3.5 Delayed Type Hypersensitivity Test

DTH is performed by giving SRBC and then the paw volume was measure in ml after various interval of time. Group I was kept as control and received 2ml of 5% normal saline intraperitoneally for seven days. Increase in paw volume was measured after 24, 48, 72 and 96 hrs. In control group Paw volume was reduced gradually it was 1.78±0.11, 1.10±0.09, 0.78±0.13 and 0.37±0.12ml in 24, 98, 72 and 96 hrs respectively.

Crude aqueous extract showed slight increase in the paw volume in 24 hrs and 48 hrs as compare to control and then decreased significantly in a dose dependent manner. Ethanol soluble fraction did not make any significant difference but slight decrease was observed in all the cases. Ethanol insoluble fraction again showed the same pattern as in the case of crude aqueous extract in relation to paw volume. In ethanol insoluble fraction doses of 50mg in 24, 48, 72, 96 hrs paw volumes were recorded 1.78±0.08 (P<0.05) 1.11±0.20, 0.64±0.21 (P<0.025), 0.025±0.14ml (P<0.025) and in 100mg/kg b.wt. were
1.88±0.11 (P<0.05), 1.31±0.17ml (P<0.001) respectively and in 150mg/kg b.wt. were 1.94±0.24, 1.34±0.22, 0.60±0.14 and 0.23±0.31ml (P<0.025) respectively. (Table 3.49 and Fig. 4.11)

Ethanolic extract of *Lantana camara* increases the DTH response and hence enhance the activity of immune system. Crude aqueous extract of dose 150mg/kg b.wt showed an increase in paw volume in 24 and 48 hrs (1.98, 1.37) as compare to control and then significant decrease is observed (0.54±0.11, 0.18±0.21ml) in 72 and 96 hrs. Water-soluble fraction showed decrease in the paw volume in 24, 48, 72 and 96hrs gradually. On the contrary water insoluble fraction first causes to increase the paw volume and then decreases in later hrs, this indicates its immunostimulant nature at the maximum dose of 150mg/kg b.wt. paw volume followed the given pattern 1.99±0.35 (P<0.001), 1.34±0.22 (P<0.025), 0.61±0.11 (P<0.025), 0.27±0.24ml (P<0.025) in 24,48,96 and 72 hrs. (Table 3.50 and Fig. 4.12)

### 4.3.6 SRBC Agglutination Test

Group I was a control, in which agglutination titre was observed up to the serum dilution of X: 80. And this result was compared to the treated groups. Crude aqueous extract of *Lantana camara* showed more agglutination in higher doses and exhibited positive results in serum dilution of X: 320. Ethanol soluble fraction of dose 50mg and 100mg did not show any remarkable change but fraction at 150mg/kg b.wt slight increase in the agglutination titre was noticed. Ethanol insoluble fraction
showed a gradual increase in the agglutination titre with the increase in dose. Maximum agglutination was observed at the dose of 150mg/kg b.wt. *(Table 3.55 and Fig. 4.17)*

Ethanolic extract of *Lantana camara* increased the agglutination titre at the maximum dose of 150mg/kg b.wt. On the other hand water-soluble fraction did not increase the agglutination titre by increasing the dose. Water insoluble fraction showed activity only in the dose of 150mg/kg b wt. and agglutination was observed up to the serum dilution of X: 160. In the dose of 50 and 100mg, no significant increase in the agglutination titre was observed when compares with the normal values. *(Table 3.56 and Fig. 4.18)*

**4.3.7 Drug Induced Myelosuppression**

In control group heamoglobin was 12.98±0.11 gms/dl, RBC count was 4.24±0.214 million/mm³, WBC count was 13.40±0.253 thousand/mm³, Neutrophils were 54.23±0.53%, Lymphocyte was 39.96±0.32%, Monocytes were 3.36±0.12%, Eosinophil count was 2.45±0.22% and platelet count was 3.12±0.352 lacs/mm³.

Group II in which only cyclophosphamide is injected intraperitoneally, since cyclophosphamide reduces various blood parameters, hematological parameters were as follows, heamoglobin was 8.21±0.11 gms/dl, RBC count was 2.74±0.024 million/mm³, WBC count was 10.12±0.114 thousand/mm³, Neutrophils was 67.42±0.26%, Lymphocyte was 28.54±0.42%, Monocytes % was 3.12 ± 0.11, Eosinophil
was 2.84 ± 0.22, Platelet count was 2.24 ± 0.211 lacs/mm³. Group III, IV and V were injected with crude aqueous extract of Lantana along with cyclophosphamide at different doses of 50, 100 and 150mg/kg bw respectively. Hemoglobin increased in a dose dependent manner and maximum at 150mg/kg bw 11.74±0.11 gms/dl (p<0.025) Mean RBC count was also increased and at 150mg/kg b.wt. it was 3.86±0.214 million/mm³. Mean WBC count also elevated by the action of crude aqueous extract and showed maximum value of 12.12±0.325 thousand/mm³ (p<0.025) at 150mg/kg b.wt. Neutrophils were gradually decreased and became minimum at 150mg/Kg b.wt Lymphocyte increased as the doses were increased, in the V group its value became 35.69±0.214% (p<0.001) Monocyte and Eosinophil count followed a irregular pattern, by the administration of crude aqueous extract of Lantana camara, they increases up to certain dose then decrease were observed. Platelet count increased by the increasing doses in all the three groups its value were 2.36±0.013, 2.65±0.015 (p<0.025) and 2.78±0.021 lacs/mm³ (p<0.025).

Ethanol soluble fraction gives protection against cyclophosphamide. Hemoglobin increases in a dose dependent manner. Its maximum value is 10.03±0.53 gms/dl at the dose of 150mg/kg b.wt. WBC counts RBC count and Lymphocyte showed a gradual increase but significant values were not obtained Neutrophills and Eosinophil count showed an irregular pattern. Monocytes increase gradually with the
increasing doses of the ethanol soluble fraction. Platelet count first increases and then slight decreases were observed.

Ethanol insoluble fraction showed maximum activity and in this heamoglobin increases and reaches up to 12.55±0.12 gms/dl (p<0.025) which is near to normal values. RBC count also showed a gradual increases, at maximum dose its value become 4.11±0.213 million/mm³ (p<0.001) WBC count were 10.31±0.021, 11.54±0.145 (p<0.021) and 12.65±0.178 thousand/mm³ (p<0.001) Neutrophills decreases 57.65±0.148, 53.49 ± 1.254 and 56.23±1.184% (p<0.05) at 50, 100 and 150mg/Kg b.wt. Lymphocyte become 33.43±0.117 (p<0.025), 34.43±0.321 (p<0.025) and 37.86±0.324% (p<0.001) Monocyte and Platelet count showed fluctuation at different doses, at first with the increases in the dose its value decreases and then in next dose it value increases. On the other hand Eosinophil count increases with the increases in doses. *(Table 3.61 and Fig. 4.39-4.43)*

Group I was control and Group II receive and these groups were common for aqueous and ethanolic extract. Group III, IV and V were administered crude ethanolic extract of *Lantana camara* intraperitoneally at the different doses of 50, 100 and 150mg/Kg b.wt. Heamoglobin increases and were 8.89±0.31, 11.21±0.21(p<0.025) and 11.89±0.33 gms/dl (p<0.001), RBC count was 3.22±0.042, 3.96±0.041 (p<0.025) and 4.16±0.422 million/mm³ (p<0.001) WBC count was 10.42±0.321, 11.87±0.254 (p<0.025) and 12.32±0.231 thousand/mm³ (p<0.001)
Neutrophils was 62.42±0.145, 59.74±0.312 and 57.51±0.421%, Lymphocyte was 30.76±0.425, 34.24±0.341 (p<0.025) and 37.74±0.142 (p<0.001), Monocyte was 3.57±0.13, 3.20 ±0.12 and 3.50±0.11%, Eosinophil was 3.89±0.11 (p<0.025) 3.76±0.32, (p<0.025) and 2.75±0.32% and Platelet count was 2.78±0.024 lacs/mm³ (p<0.001) water soluble fraction showed a slight increases but values were not so significant.

Water insoluble fraction showed gradual increases in heamoglobin and so RBC, Heamoglobin was 10.21±0.14, 11.34±0.57 (p<0.025) and 11.66±0.42 gms/dl (p<0.025) RBC count was 3.65±0.024, 3.88±0.022 (p<0.025) and 3.79±0.262 million/mm³ (p<0.025) WBC count was 11.01±0.421 11.97±0.117 (p<0.025) 12.01±0.511 thousand/mm³ (p<0.025) Neutrophils showed irregular fashion value in different doses were 61.11±0.351, 60.11±0.412 and 60.22±0.257%, Lymphocyte was 32.24±0.358, 35.45±0.213% (p<0.001) and 36.15±0.242% (p<0.001) Monocyte was 3.36±0.13, 2.89±0.17 and 2.78±0.24%, Eosinophil count was 3.25±0.11, 3.11±0.23 and 2.74±0.22%, Platelet count was 2.55±0.024, 3.08±0.011 (p<0.001) and 3.04±0.025 lacs/mm³ (p<0.001) respectively for 50,100 and 150 mg/kg b.wt. . *(Table 3.62 and Fig. 4.44-4.48)*
4.3.8 Discussion

*Lantana camara* has been used in many parts of the world to treat a wide range of disorders. Lantana leaves and twigs are often used in India as green milk. The fruits of *Lantana camara* have been reported to cause fatality in human if consume in sufficient amount but leaves and twigs were used by villagers to treat many disorders. Lantadene A and B are obtained from this plant are also used as antiinflammatory and pain subsidisers.

Phagocytosis and killing of invading microorganism by macrophages constitute body's primary line of defense against infection. (Van Furt 1982) The role of phagocytosis is not only the removal of microorganism and foreign bodies, but also the elimination of dead or injured cells. When the carbon particles are injected intravenously, the rate of clearance of carbon from blood by macrophage was governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. However, *Lantana camara* clears the carbon particles in much faster rate when is compared with control and hence, increases the phagocytic index. Aqueous extract and its ethanol insoluble fraction enhance the phagocytic index in a dose dependent manner. Ethanol soluble fraction was ineffective as there was not any significant increase or decrease in the phagocytic index. Ethanol extract also enhanced the value of phagocytic index. Therefore aqueous and ethanolic extract showed stimulatory effects on macrophages.
Besides them, some oligosaccharides, flavonides have been detected in ethanol soluble and water insoluble fraction. Some of these compounds are responsible for faster clearence of carbon particles from blood. The rate of clearence is highest in case of *Lantana camara* extract among the three plants. The content must be having chemotactic effect or activity. The effect is dose dependent, but the activity does not increase after a particular dose of crude drug. The compounds present in *Lantana camara* (mentioned in results) are water and ethanol soluble.

*Lantana camara* also increases cell-mediated immune response. Delayed Type Hypersensitivity Test (DTH) against SRBC was carried out to study this response. Paw volume is measured after 24, 48, 72 and 96 hrs Crude aqueous extract and its ethanol insoluble fraction show a definite increase in the paw volume up to the 48 hrs, as compared with control and then gradual decrease was observed. Similar trend was generated by ethanolic extract and its fraction. In some cases increase or decrease was not dose dependent, since immune response was not always directly related with the concentration of the dose. This may be because of different constituents present in fraction in different concentration, which interrupts the response at one or other level.

It is possible that the drug activates the mast cells and it leads to release of kinins etc. The developments of delayed type hypersensitivity requires the activation to CD4⁺ and CD8⁺ T-cell, which influence the mechanism of T-Cell through bio-molecules resulting in the increase of
T-cell immune response significantly (Paul 1994, Manfred, et al., 1994; Gorbachev et al., 2001).

*Lantana camara* causes to increase humoral immune response. This was measured by observing agglutination titre to SRBC in various serum dilutions. In the case of control, agglutination titre was observed up to the serum dilution of X: 80. Crude aqueous extract show maximum agglutination with the dose of 100 and 150mg/kg b.wt. In the case of ethanol soluble fraction, increase in agglutination titre was observed with the dose of 150mg/kg b.wt similarly ethanol insoluble fraction showed a gradual increase in the agglutination with increase in the dose. Ethanolic extract showed a maximum increase in crude extract but no significant increase was observed in water-soluble and water insoluble fraction. Maximum dose of 150mg/kg b.wt of water insoluble fraction showed agglutination in the serum dilution of X: 160. It is the possibility that there is an enhancement in IgM and IgG levels because antibody titre was increased significantly. *Lantana camara* is capable to influence B-cell, which in turn synthesize or secret antibodies to increased the antibody molecules link with SRBC that leads to subsequent agglutination. (Roitt, 1984)

Both aqueous and ethanolic extract of *Lantana camara* showed protective effect against cyclophosphamide. Cyclophosphamide is cytotoxic drug that suppresses all sorts of immune system (Specific, non specific) Cyclophosphamide induced myelosuppression and affect the number and concentration of various blood parameter negatively like
Heamoglobin concentration, RBC count, WBC count, Lymphocyte %, platelet count etc. This cannot be ignored that some polypeptides or oligosaccharides or their compounds have strong antigenicity because of the presence of the type of amino acids and their spatial arrangement in a sequence or because of the position of saccharides to configure oligosaccharides. But by the action of *Lantana camara* adverse effects are less and fast recovery is observed but it was not proportional to doses. Results suggest that the drug possesses stimulating properties as it increases the RBC count and hemoglobin percentage. Various haematological and immune parameters like lymphocytes, platelet count, monocytes and Eosinophil count etc. were also increased manifolds.

The plants selected for study exhibit immunostimulatory properties. The compounds detected in plant extract belonging to class of saponin, flavonoids, oligosaccharides, polysaccharides, alkoloids and some phenolic compounds. Each plant has one or more different type of alkoloid, saponin, flavonoids etc. Yet their cumulative effects are immuno stimulatory. The type of test carried is to ascertain their immune modulatory response point to affect some processes of the chain. It may be possible that compounds might be affecting the immune system in many more ways.

DTH is very important in many immune activities such as immunity to many intracellular pathogen, neoplastic tissue and graft rejection. T-lymphocytes proliferate after getting activated on exposure to antigen and increase vasodilation by liberating cytokines besides having
many other properties the extract also cause attachment of cells to the endothelium. If monocytes, lymphocytes are accumulating in one part of the body it should not be forgotten that the neutrophills also adhere to the endothelial cells. The cell adhesion molecule regulates immune system by controlling diapedesis and extravasation of immune cells. The component(s) of the extract as I presume directly or indirectly activating the receptor and participating at early stage of signaling event. A direct application of this property of the extract can be made in its application on a wound to attract the neutrophill to kill the pathogen.

Sheep erythrocytes specific haemagglutination test in this study develop specific agglutinin, which is directly indicative of humoral immune response enhancement. The compound of the extract interact with T and B-lymphocytes. B-lymphocytes after proliferation differentiate to specific plasma cells to liberate new potent antibody against the antigen. (Ananthanarayan, 1996) The extract has the contents, which show different activities in modulating the immunity. Increased haematocrit value, qualitative and quantitative increase in serum protein, activation of lymphocytes, antibody genesis are few features recorded with both aqueous and ethanolic extract.

In present study Lantana camara established as a potent immunostimulator. Both extract exert definite effect over various parameters of immune system. They strengthen innate as well as acquired immunity of experimental animals and protect them from the
adverse effect of cyclophosphamide. The above study reflects induction of immune response by aqueous as well as ethanolic extract and suggests that, the active constituent responsible for stimulation of an antibody response can be extracted with both polar and non-polar solvents.
Fig. 3.2: Standard curve of Indian Ink

\[ y = 0.0205x + 0.0297 \]
\[ R^2 = 0.9994 \]
Fig. 4.1: Effect of Aqueous extract of *Cleome gynandra* on phagocytic activity.

![Aqueous extract graph](image)

Fig. 4.2: Effect of Ethanolic extract of *Cleome gynandra* on phagocytic activity.

![Ethanolic extract graph](image)
Fig. 4.3: Effect of Aqueous extract of Cocculus hirsutus on phagocytic activity.

Fig. 4.4: Effect of Ethanolic extract of Cocculus hirsutus on phagocytic activity.
Fig. 4.5: Effect of Aqueous extract of *Lantana camara* on phagocytic activity.

Fig. 4.6: Effect of Ethanolic extract of *Lantana camara* on phagocytic activity.
Fig. 4.7: Effect of Aqueous extract of *Cleome gynandra* on DTH.

![Graph showing effect of aqueous extract on DTH](image1)

- control
- Crude Aqueous extract 150 mg
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 50 mg
- Ethanol soluble fraction 150 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 50 mg
- Ethanol insoluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 50 mg

Fig. 4.8: Effect of Ethanolic extract of *Cleome gynandra* on DTH.

![Graph showing effect of ethanolic extract on DTH](image2)

- control
- Crude Ethanolic extract 150 mg
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 50 mg
- Water soluble fraction 150 mg
- Water soluble fraction 100 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 150 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 50 mg
Fig. 4.9: Effect of Aqueous extract of *Cocculus hirsutus* on DTH.

![Graph showing the effect of aqueous extract on paw volume over time.](image)

- **Control**
- **Crude Aqueous extract 50 mg**
- **Crude Aqueous extract 100 mg**
- **Ethanol soluble fraction 50 mg**
- **Ethanol soluble fraction 100 mg**
- **Ethanol insoluble fraction 150 mg**

Fig. 4.10: Effect of Ethanoic extract of *Cocculus hirsutus* on DTH.

![Graph showing the effect of ethanoic extract on paw volume over time.](image)

- **Control**
- **Crude Ethanoic extract 50 mg**
- **Crude Ethanoic extract 100 mg**
- **Water soluble fraction 50 mg**
- **Water soluble fraction 100 mg**
- **Water insoluble fraction 150 mg**
Fig. 4.11: Effect of Aqueous extract of *Lantana camara* on DTH.

![Bar chart showing effect of aqueous extract on paw volume over time.]

Fig. 4.12: Effect of Ethanolic extract of *Lantana camara* on DTH.

![Bar chart showing effect of ethanolic extract on paw volume over time.]

Fig. 4.13: Effect of Aqueous extract of *Cleome gynandra* on Agglutination titer to SRBC

![Bar graph showing antibody titer for different extracts.](image)

Extracts:
- control
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 50 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 50 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg

Fig. 4.14: Effect of Ethanolic extract of *Cleome gynandra* on Agglutination titer to SRBC.

![Bar graph showing antibody titer for different extracts.](image)

Extracts:
- control
- Crude Ethanolic extract 50 mg
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Water soluble fraction 50 mg
- Water soluble fraction 100 mg
- Water soluble fraction 150 mg
- Water insoluble fraction 50 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 150 mg
Fig. 4.15: Effect of Aqueous extract of *Cocculus hirsutus* on Agglutination titer to SRBC

![Graph showing antibody titer with different extracts.]

- Control
- Crude Aqueous extract 150 mg
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol soluble fraction 50 mg
- Ethanol soluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
- Ethanol insoluble fraction 50 mg
- Ethanol insoluble fraction 100 mg

Fig. 4.16: Effect of Ethanolic extract of *Cocculus hirsutus* on Agglutination titer to SRBC.

![Graph showing antibody titer with different extracts.]

- Control
- Crude Ethanolic extract 150 mg
- Crude Ethanolic extract 50 mg
- Crude Ethanolic extract 100 mg
- Water soluble fraction 150 mg
- Water soluble fraction 50 mg
- Water soluble fraction 100 mg
- Water insoluble fraction 150 mg
- Water insoluble fraction 50 mg
- Water insoluble fraction 100 mg
Fig. 4.17: Effect of Aqueous extract of *Lantana camara* on Agglutination titer to SRBC.

![Graph showing effect of aqueous extract on agglutination titer](image)

**Extracts**
- control
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 50 mg
- Crude Aqueous extract 100 mg
- Ethanol insoluble fraction 150 mg
- Ethanol soluble fraction 50 mg
- Ethanol insoluble fraction 100 mg

Fig. 4.18: Effect of Ethanolic extract of *Lantana camara* on Agglutination titer.

![Graph showing effect of ethanolic extract on agglutination titer](image)

**Extracts**
- control
- Crude Ethanolic extract 150 mg
- Water soluble fraction 50 mg
- Crude Ethanolic extract 100 mg
- Water soluble fraction 150 mg
- Water insoluble fraction 50 mg
- Water soluble fraction 100 mg
- Water insoluble fraction 150 mg
Fig. 4.19: Effect of Aqueous extract of *Cleome gynandra* on Total WBC count.

![Graph showing the effect of various extracts on WBC count.]

Extracts
- Control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 50 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 50 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg

Fig. 4.20: Effect of Aqueous extract of *Cleome gynandra* on Lymphocyte percentage.

![Graph showing the effect of various extracts on lymphocyte percentage.]

Extracts
- Control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 50 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 50 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
Fig. 4.21: Effect of Aqueous extract of *Cleome gynandra* on Hæmoglobin concentration.

![Graph showing Hb (Gms/dl) vs. Extracts](image)

**Extracts**
- control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg

Fig. 4.22: Effect of Aqueous extract of *Cleome gynandra* on RBC Count.

![Graph showing RBC (million/mm³) vs. Extracts](image)

**Extracts**
- control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 50 mg
- Crude Aqueous extract 100 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
Fig. 4.23: Effect of Aqueous extract of *Cleome gynandra* on Platelet Count.

![Graph showing the effect of different extracts on platelet count.](image-url)
**Fig. 4.24:** Effect of Ethanolic extract of *Cleome gynandra* on Total WBC count.

![Bar chart showing the effect of different extracts on WBC count.]

- Control
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Crude Ethanolic extract 50 mg
- Water soluble fraction 100 mg
- Water soluble fraction 150 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 150 mg
- Water insoluble fraction 50 mg

**Fig. 4.25:** Effect of Ethanolic extract of *Cleome gynandra* on Lymphocyte percentage.

![Bar chart showing the effect of different extracts on Lymphocyte percentage.]

- Control
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Crude Ethanolic extract 50 mg
- Water soluble fraction 100 mg
- Water soluble fraction 150 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 150 mg
- Water insoluble fraction 50 mg
Fig. 4.26: Effect of Ethanolic extract of *Cleome gynandra* on Hemoglobin concentration.

![Hemoglobin concentration graph](image)

**Extracts**
- control
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Water soluble fraction 100 mg
- Water insoluble fraction 100 mg
- Cyclophosphamide 3mg/kg b. wt.
- Crude Ethanolic extract 50 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 50 mg

Fig. 4.27: Effect of Ethanolic extract of *Cleome gynandra* on RBC count.

![RBC count graph](image)

**Extracts**
- control
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Water soluble fraction 100 mg
- Water insoluble fraction 100 mg
- Cyclophosphamide 3mg/kg b. wt.
- Crude Ethanolic extract 50 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 50 mg
Fig. 4.28: Effect of Ethanol extract of *Cleome gynandra* on Platelet Count.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Platelet (Lacs/mm3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide 3mg/kg b.wt.</td>
<td></td>
</tr>
<tr>
<td>Crude Ethanol extract 100 mg</td>
<td></td>
</tr>
<tr>
<td>Crude Ethanol extract 150 mg</td>
<td></td>
</tr>
<tr>
<td>Water soluble fraction 100 mg</td>
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<td>Water soluble fraction 150 mg</td>
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<tr>
<td>Water insoluble fraction 100 mg</td>
<td></td>
</tr>
<tr>
<td>Water insoluble fraction 150 mg</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.29: Effect of Aqueous extract of *Cocculus hirsutus* on Total WBC count.

![Bar graph showing the effect of different extracts on WBC count.]

Fig. 4.30: Effect of Aqueous extract of *Cocculus hirsutus* on Lymphocyte percentage.

![Bar graph showing the effect of different extracts on lymphocyte percentage.]

**Extracts**
- control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
- Crude Aqueous extract 50 mg
- Ethanol soluble fraction 50 mg
- Ethanol insoluble fraction 50 mg
Fig. 4.31: Effect of Aqueous extract of *Cocculus hirsutus* on Heamoglobin percentage.

![Graph showing the effect of various extracts on Hb](image1)

Fig. 4.32: Effect of Aqueous extract of *Cocculus hirsutus* on RBC count.

![Graph showing the effect of various extracts on RBC count](image2)
Fig. 4.33: Effect of Aqueous extract of *Cocculus hirsutus* on Platelet count.

![Bar chart showing the effect of different extracts on platelet count.](image)
Fig. 4.34: Effect of Ethanolic extract of *Cocculus hirsutus* on Total WBC count.

![Graph showing the effect of different extracts on WBC count.]

Fig. 4.35: Effect of Ethanolic extract of *Cocculus hirsutus* on Lymphocyte percentage.

![Graph showing the effect of different extracts on Lymphocyte percentage.]

*Extracts:
- control
- Cyclophosphamide 3mg/kg b. wt.
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Water soluble fraction 100 mg
- Water soluble fraction 150 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 150 mg
- Crude Ethanolic extract 50 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 50 mg*
Fig. 4.36: Effect of Ethanolic extract of *Cocculus hirsutus* on Hemoglobin concentration.

Fig. 4.37: Effect of Ethanolic extract of *Cocculus hirsutus* on RBC count.
Fig. 4.38: Effect of Ethanolic extract of *Cocculus hirsutus* on Platelet count.

![Bar chart showing the effect of different extracts on platelet count.](chart.png)
Fig. 4.39: Effect of Aqueous extract of *Lantana camara* on Total WBC count.

![Bar chart showing the effect of different extracts on WBC count.](chart1)

Fig. 4.40: Effect of Aqueous extract of *Lantana camara* on Lymphocyte percentage.

![Bar chart showing the effect of different extracts on lymphocyte percentage.](chart2)
Fig. 4.41: Effect of Aqueous extract of *Lantana camara* on Hemoglobin concentration.

![Graph showing the effect of various extracts on Hemoglobin concentration.]

**Extracts**
- control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg

Fig. 4.42: Effect of Aqueous extract of *Lantana camara* on RBC count.

![Graph showing the effect of various extracts on RBC count.]

**Extracts**
- control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
Fig. 4.43: Effect of Aqueous extract of *Lantana camara* on Platelet count.

![Bar chart showing the effect of different extracts on platelet count](image)

**Extracts**
- Control
- Cyclophosphamide (3mg/kg)
- Crude Aqueous extract 100 mg
- Crude Aqueous extract 150 mg
- Ethanol soluble fraction 100 mg
- Ethanol soluble fraction 150 mg
- Ethanol insoluble fraction 100 mg
- Ethanol insoluble fraction 150 mg
- Crude Aqueous extract 50 mg
- Ethanol soluble fraction 50 mg
- Ethanol insoluble fraction 50 mg
Fig. 4.44: Effect of Ethanol extract of *Lantana camara* on WBC count.

![WBC count chart]

Fig. 4.45: Effect of Ethanol extract of *Lantana camara* on Lymphocyte percentage.

![Lymphocyte percentage chart]
Fig. 4.46: Effect of Ethanolic extract of *Lantana camara* on Haemoglobin percentage.

![Graph showing effect of Ethanolic extract of Lantana camara on Haemoglobin percentage.]

Fig. 4.47: Effect of Ethanolic extract of *Lantana camara* on RBC count.

![Graph showing effect of Ethanolic extract of Lantana camara on RBC count.]

- Control
- Crude Ethanolic extract 100 mg
- Crude Ethanolic extract 150 mg
- Water soluble fraction 100 mg
- Water soluble fraction 150 mg
- Water insoluble fraction 100 mg
- Water insoluble fraction 150 mg
- Cyclophosphamide 3mg/kg b. wt.
- Crude Ethanolic extract 50 mg
- Water soluble fraction 50 mg
- Water insoluble fraction 50 mg
Fig. 4.48: Effect of Ethanol extract of *Lantana camara* on Platelet count.
Fig. 4.49: Effect of various Aqueous extracts and their ethanol insoluble fraction on Phagocytosis.

Fig. 4.50: Effect of various Ethanolic extracts and their water insoluble fraction on Phagocytosis.
Fig. 4.51: Comparison of effects of various Aqueous Extracts and their Ethanol Insoluble Fractions on WBC count.

Extract
- Cleome Crude Aqueous extract 100 mg
- Cocculus Crude Aqueous extract 100 mg
- Lantana Crude Aqueous extract 100 mg
- Cleome Crude Aqueous extract 150 mg
- Cocculus Crude Aqueous extract 150 mg
- Lantana Crude Aqueous extract 150 mg
- Cleome Water insoluble fraction 100 mg
- Cocculus Water insoluble fraction 100 mg
- Lantana Water insoluble fraction 100 mg
- Cleome Water insoluble fraction 150 mg
- Cocculus Water insoluble fraction 150 mg
- Lantana Water insoluble fraction 150 mg

Fig. 4.52: Comparison of effects of various Aqueous Extracts and their Ethanol Insoluble Fractions on Hb percentage.

Extracts
- Cleome Crude Aqueous extract 100 mg
- Cocculus Crude Aqueous extract 100 mg
- Lantana Crude Aqueous extract 100 mg
- Cleome Crude Aqueous extract 150 mg
- Cocculus Crude Aqueous extract 150 mg
- Lantana Crude Aqueous extract 150 mg
- Cleome Water insoluble fraction 100 mg
- Cocculus Water insoluble fraction 100 mg
- Lantana Water insoluble fraction 100 mg
- Cleome Water insoluble fraction 150 mg
- Cocculus Water insoluble fraction 150 mg
- Lantana Water insoluble fraction 150 mg
Fig. 4.53: Comparison of effects of various ethanolic extracts and their water insoluble fractions on WBC count.

Fig. 4.54: Comparison of effects of various ethanolic extracts and their water insoluble fractions on Hemoglobin concentration.