Immunomodulating Activity of *Cleome Gynandra*

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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 *Cleome gynandra* Linn (Capparidacea) 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. Ethanolic extract of the plant showed significant immunosuppressant activity and administration of extract remarkably decreased the number of WBC and spleenic lymphocytes. It also decreased phagocytic index and both cellular and humoral antibody response. Whereas aqueous extract possess immunostimulant properties. It is concluded that *Cleome gynandra* showed both immunostimulant as well as immunosuppressant nature.

Keywords: *Cleome gynandra*, Immunostimulant, Cyclophoshamide, Carbon clearance test, Delayed type hypersensitivity, Antibody titre

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Introduction

Immunomodulation is a procedure, which can alter the immune system of an organism by interfering with its function, which primarily implies stimulation of non-specific system [1]. Immunomodulators generally act by stimulating both specific and non-specific immunity. Botanical immunomodulating agents might be able to provide an alternative source of immunotherapeutics. Botanicals produce a diverse range of natural products with antimicrobial and immunomodulating potential, including isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, glucans and tannins. Some of the plants with known immunomodulatory activities are *Curculigo orchioides, Viscum album, Panax ginseng, Tinospora cordifolia, Asparagus racemosus* etc [2].

Cleome gynandra L. Syn *Gynandropsis pentaphylla* DC (Capparidacea) is a common weed, which grows in most tropical countries. In India the common names include hurhur and karaila . The leaves and seeds of the plant have long been in use as indigenous medicine for treatment of headaches and stomach aches. Sap from leaves has been used as an analgesic particularly for head ache, epileptic fits and ear ache. A decoction or infusion of boiled leaves and/or roots has been administered to facilitate childbirth. Bruised leaves, which are rubefacent and vesicant, are also used to treat neuralgia, rheumatism and other localized pains [3]. The leaves of Cat's whiskers are used for inflammation[4,5]. Plant contain Cleogynol, 5, 7- dihydroxychromone, 5-hydroxy-3, 7, 4-trimethoxyflavone, luteolin Hexacosanol, sitosterol and kaempferol [6-8]

In the present study an attempt was made to investigate the immunomodulatory effect of *Cleome gynandra* aerial part aqueous and ethanolic extract on the rat.

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 *Cleome gynandra* 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 Dry winner) (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 . 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 3 mg/kg b.w. for seven days. Group III, IV and V was administered with aqueous extract of 50 mg, 100 mg, 150 mg/kg b.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 15^{th} 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]

Results

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 *C. gynandra* treated group (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/kgb.wt.intraperitonially for seven days.

Ethanol soluble fraction of aqueous extract of has given relatively low to the Group II, III and IV have phagocytic Index as 0.0071±0.01, 0.0083±0.021and 0.0091±0.018 respectively with 50,100 and 150mg/kgb.wt. Ethanol soluble fraction had shown enhanced phagocytic index as 0.0012 ± 0.018, 0.0124±0.013, ($P<0.025$) and 0.0144±0.019 ($P<0.001$) with 50 mg, 100mg, 150mg/kgb.wt. 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 was calculated and compared with control group (Group I). Group II, III and IV were treated with crude aqueous extract orally for ten days (50mg, 100mg, 150mg/kgb.wt). and on 10^{th} day agglutination titre were observed in various serum dilution (X: 20, X: 40, X: 80, X: 160 and X: 320).Significant increase was observed at the dose of 100mg and 150mg/kgb.wt.while no significant increase observed in 50mg/kgb.wt.Group V, VI and VII were given to ethanol soluble fraction at the dose of 50mg, 100mg, 150mg/kg b.wt and no significant changes observed where as ethanol insoluble fraction was given to Group VIII,IX,X (50mg, 100mg, 150mg/kg b.wt. respectively) and caused a significant increase in the 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 of 50mg, 100mg, 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 three groups, which were given water insoluble fraction increase of 50mg, 100mg, and 150mg/kg b.wt. respectively.

Drug induced Myelosuppression Test

In this study, Myelosuppression was produced in animals with 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. The mean haemoglobin was 13.09± 0.15 gms/dl mean RBC count was 4.66 ± 0.550 million/mm³ Neutrophills were 54.33±1.05%, Monocyte 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. GroupII (treated with Cyclophosphamide 3mg/kg b.wt.) showed a significant decrease in haemoglobin8.56 0.27 gms/dl. Mean RBC count was3.52 0.098 million/mm³, WBC count was 10.93 thousand/mm³, Platelet count was 2.57 0.384 lacs/ mm³ ($P<0.05$). 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 also 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 b.wt.) in these the various blood parameters were haemoglobin 10.62± 0.12, 10.37 ±0.12,9.83± 0.47gms/dl RBC count was 3.61± 0.024,3.78±0.042,millon/$mm^{3,}$ WBC count was 11.45± 0.212,11.97 ±0.256, 10.65± 0.512 thousand/$mm^{3.}$ Neutrophill was 57.38±0.12%, 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 2.64± 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 to Group IX,X and XI and significant increase was observed in various blood parameters mean haemoglobin was found to be 11.12± 0.29, 10.01± 0.09 and 9.45± 0.52 gms/dl (p<0.025) RBC count was 3.63± 0.029, 3.89 ±0.012 (p<0.05) 3.32± 0.125 millon/mm^3 (p<0.025), WBC were11.75± 0.42,10.27±0.211 (p<0.05) , 9.56 ±0.256 thousand/mm^3(p<0.001), Neutrophill % was 60.30 0.563 , 54.83 0.326 (p<0.001), Lymphocyte % was 31.45± 0.415, 29.98± 0.451, 34.00± 0.346 (p<0.001), Monocyte count were 3.40± 0.17%, 2.75± 0.35, 2.75± 0.12. Eosinophill count were 2.86± 0.23%, 3.72± 0.23%, (p<0.025) 4.01± 0.12% (p<0.025), 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 1: Effect of Aqueous Extract of *Cleome gynandra* 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	0.2457± 0.021	0.2190± 0.014	0.1895± 0.018	0.1527± 0.011	0.1221± 0.015	0.0087± 0.012
2.	Crude Aqueous extract (50mg./kg.body wt.)	0.2402± 0.035	0.2012± 0.044	0.1822± 0.023	0.1485± 0.018	0.1121± 0.025	0.0106 ±0.027
3.	Crude Aqueous extract (100mg./kg.body wt.)	0.2342± 0.039	0.2152± 0.012	0.1798± 0.035	0.1422± 0.019	0.1091± 0.036	0.0118*± 0.017
4.	Crude Aqueous extract (150mg./kg.body wt.)	0.2211± 0.060	0.2012± 0.048	0.1601± 0.015	0.1192± 0.052	0.0954± 0.016	0.0129± 0.019**
5.	Ethanol Soluble fraction (50mg./kg.body wt.)	0.2310± 0.018	0.2122± 0.022	0.1842± 0.032	0.1545 0.028	0.1272± 0.044	0.0071 ±0.010
6.	Ethanol Soluble fraction (100mg./kg.body wt.)	0.2473± 0.098	0.2121 ± 0.034	0.1810± 0.017	0.1528 ± 0.055	0.1293± 0.015	0.0083± 0.021
7.	Ethanol Soluble fraction (150mg./kg.body wt.)	0.2374± 0.021	0.2083± 0.036	0.1960± 0.010	0.1687± 0.023	0.1310± 0.030	0.0091± 0.018
8.	Ethanol Insoluble fraction (50mg./kg.body wt.)	0.2340± 0.012	0.1921± 0.024	0.1822± 0.023	0.1460± 0.015	0.1095± 0.015	0.0112± 0.018
9.	Ethanol Insoluble fraction (100mg./kg.body wt.)	0.2283± 0.017	0.1925± 0.012	0.1755± 0.025	0.1401± 0.029	0.0998± 0.022	0.0124± 0.013**
10.	Ethanol Insoluble fraction (150mg./kg.body wt.)	0.2173± 0.035	0.1905± 0.024	0.1612 ± 0.013	0.1080± 0.032	0.0867± 0.016	0.0144± 0.019***

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

* $P < 0.05$, * * $P < 0.025$, * * * $P < 0.001$

Table 2: Effect of Ethanolic Extract of *Cleome gynandra* on Phagocytic Activity In Carbon Clearance Test

S.No.	Groups	Mean Absorbance ± S D					*Phagocytic Index(K) ±SD*
		3min	6min	9min	12min	15min	
1.	Control 2ml Normal saline	0.2457± 0.021	0.2190± 0.014	0.1895± 0.018	0.1527± 0.011	0.1221± 0.015	*0.0087± 0.012*
2.	Crude Ethanolic extract (50mg./kg.body wt.)	0.2412± 0.015	0.2174± 0.025	0.1822± 0.028	0.1560± 0.018	0.1245± 0.055	*0.0088± 0.031*
3.	Crude Ethanolic extract (100mg./kg.body wt.)	0.2407± 0.013	0.2275± 0.026	0.1838± 0.024	0.1690± 0.010	0.1409± 0.045	*0.0081± 0.027**
4.	Crude Ethanolic extract (150mg./kg.body wt.)	0.2510± 0.010	0.2322± 0.048	0.1999± 0.011	0.1687± 0.023	0.1451± 0.019	*0.0074±** 0.018*
5.	Aqueous Soluble fraction (50mg./kg.body wt.)	0.2388± 0.010	0.2095± 0.045	0.1802± 0.054	0.1625± 0.021	0.1401± 0.044	*0.0091± 0.015*
6.	Aqueous Soluble fraction (100mg./kg.body wt.)	0.2421± 0.098	0.2232± 0.043	0.1758± 0.017	0.1528± 0.012	0.1332± 0.018	*0.0089± 0.012*
7.	Aqueous Soluble fraction (150mg./kg.body wt.)	0.2313± 0.028	0.2112± 0.058	0.1885± 0.029	0.1614± 0.016	0.1253± 0.030	*0.0087± 0.033*
8.	Aqueous Insoluble fraction (50mg./kg.body wt.)	0.2387± 0.023	0.2184± 0.026	0.1798± 0.022	0.1601± 0.037	0.1435± 0.015	*0.0082± 0.031*
9.	Aqueous Insoluble fraction (100mg./kg.body wt.)	0.2445± 0.013	0.2201± 0.026	0.1879± 0.031	0.1765± 0.017	0.1495± 0.014	*0.0079± 0.027**
10.	*Aqueous Insoluble fraction (150mg./kg.body wt.)*	*0.2555± 0.016*	*0.2422± 0.010*	*0.2077± 0.031*	*0.1855± 0.032*	*0.1621± 0.019*	*0.0068± 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 *Cleome gynandra* on Delayed Type of Hypersensitivity

S.No.	Groups	*Paw Volume (ml.) ±S.D.*			
		24Hrs,	48Hrs,	72Hrs,	*96Hrs,*
1.	Control 2ml Normal saline	1.66± 0.11	0.96± 0.19	0.69± 0.30	*0.29± 0.18*
2.	Crude Aqueous extract (50mg./kg.body wt.)	1.78± 0.14	1.04± 0.10	0.59± 0.24	*0.27*±* *0.11*
3.	Crude Aqueous extract (100mg./kg.body wt.)	1.88± 0.15	1.11± 0.12*	0.49± 0.16	*0.19± 0.14***
4.	Crude Aqueous extract (150mg./kg.body wt.)	1.86± 0.10	1.07± 0.11	0.37± 0.12	*0.16± 0.08****
5.	Ethanol Soluble fraction (50mg./kg.body wt.)	1.12± 0.14	0.98± 0.18	0.78± 0.31	*0.32± 0.10*
6.	Ethanol Soluble fraction (100mg./kg.body wt.)	1.02± 0.22	0.90± 0.28	0.67± 0.21	*0.30± 0.18*
7.	Ethanol Soluble fraction (150mg./kg.body wt.)	1.09± 0.25	0.83± 0.31	0.68± 0.31	*0.29± 0.10*
8.	Ethanol Insoluble fraction (50mg./kg.body wt.)	1.82± 0.16	1.10± 0.20	0.49± 0.11	*0.19*±** *0.17*
9.	Ethanol Insoluble fraction (100mg./kg.body wt.)	1.95± 0.18	1.17± 0.21	0.39± 0.16	*0.15± 0.24****
10.	*Ethanol Insoluble fraction (150mg./kg.body wt.)*	*1.94± 0.10*	*1.15± 0.24***	*0.29± 0.18*	*0.11± 0.11****

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 *Cleome gynandra* on Delayed Type of Hypersensitivity

S.No.	Groups	*Paw Volume (ml)* ±*S.D.*			
		24Hrs,	48Hrs,	72Hrs,	*96Hrs,*
1.	Control 2ml Normal saline	1.17± 0.11	0.90± 0.19	0.69± 0.30	*0.29*± *0.18*
2.	Crude Ethanolic extract (50mg./kg.body wt.)	1.35± 0.15	0.92± 0.18	0.78± 0.24	*0.40*± *0.11**
3.	Crude Ethanolic extract (100mg./kg.body wt.)	1.20± 0.15*	0.86± 0.12*	0.82± 0.11*	*0.39*± *0.05***
4.	Crude Ethanolic extract (150mg./kg.body wt.)	1.15± 0.10**	0.81± 0.11**	0.87± 0.09	*0.42*± *0.08****
5.	Aqueous Soluble fraction (50mg./kg.body wt.)	1.52± 0.14	0.98± 0.21	0.71± 0.24	*0.24*± *0.13*
6.	Aqueous Soluble fraction (100mg./kg.body wt.)	1.58± 0.11	0.90± 0.31	0.63± 0.09	*0.30*± *0.07*
7.	Aqueous Soluble fraction (150mg./kg.body wt.)	1.43± 0.25	0.82± 0.15	0.68± 0.04	*0.35*± *0.08*
8.	Aqueous Insoluble fraction (50mg./kg.body wt.)	1.42± 0.13	0.90± 0.17	0.75± 0.16*	*0.46*± *0.09***
9.	Aqueous Insoluble fraction (100mg./kg.body wt.)	1.19± 0.18*	0.88± 0.14*	0.81± 0.21**	*0.48*± *0.11****
10.	*Aqueous Insoluble fraction (150mg./kg.body wt.)*	*1.13*± *0.11***	*0.79*± *0.13***	*0.85*± *0.09***	*0.47*± *0.08****

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 *Cleome gynandra* 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 5% 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 *Cleome gynandra* 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 5% 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 *Cleome gynandra* on Drug induced Myelosuppression Using Cyclophosphamide for 7 days

Group	HbG ms/dl	RBC Million/ mm³	WBC thousand/mm³	Neutrophill %	Lymphocyte %	Monocyte %	Eosinophilcount %	*Platelet Lacs/mm³*
I.	13.09± 0.15	4.66 ± 0.145	13.04± 0.550	54.33± 1.05	41.66± 0.95	2.52 ± 0.56	2.30± 0.42	*3.10± 0.322*
II	8.56± 0.27	3.52± 0.098*	10.93± 0.355*	61.83± 0.79	32.66 ± 0. 88*	3.00± 0.10	2.50± 0.34	*2.57± .384**
III	10.58± 0.27	3.92± 0.057	10.21 ±0.103	59.50± 0.885	33.83± 0.703	1.80± 0.30	2.10± 0.30	*2.62± 0.012*
IV	11.01± 0.28**	4.07± 0.026**	11.96± 0.161*	52.83± 1.515	34.33 ±0.333*	2.81± 0.65*	2.82±0.70	*2.86± 0.035**
V	12.48±0.24**	4.32± .118**	12.02±0.115**	52.33± 1.054**	35.66±0.494***	3.12 ±0.33**	1.86±0.47*	*2.98±0.013***
VI	9.56± 0.12	3.89± 0.078	11.02± 0.455	61.38± 0.29	34.21± 0.36	2.20± 0.10	3.09± 0.45	*2.82± 0.021*
VII	9.73± 0.27	3.91± 0.035	11.12 ±0.635	58.83± 0.68	33.96± 0.88	2.84± 0.22	3.50± 0.14	*2.87± 0.188*
VIII	9.83± 0.47	3.78± 0.042	11.23± 0.485	59.43± 0.37	35.13± 0.57	2.75± 0.29	3.25± 0.35	*2.57± 0.384*
IX	11.32± 0.11*	3.97± 0.018*	12.41± 0.098**	58.35± 0.135	32.93± 0.601	2.80± 0.37	3.86± 0.46	*2.38± 0.042*
X	11.01± 0.28**	4.07± 0.026**	11.42±0.161**	53.49± 1.254	36.43± 0.212**	2.81± 0.65*	2.82±0.70	*2.86±0.035***
XI	*12.48± 0.24***	*4.32±0.118***	*11.82±0.115***	*56.33± 1.184**	*40.26±0.251****	*2.79± 0.11***	*1.76±0.14***	*3.21±0.013****

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 *Cleome gynandra* on Drug induced Myelosuppression Using Cyclophosphamide for 7 days

Group	Hb Gms/dl	RBC Million/ mm³	WBC thousand/mm³	Neutrophill %	Lymphocyte %	Monocyte %	Eosinnophilcount %	*Platelet Lacs/mm³*
I.	13.09±0.15	4.66± 0.145	13.04± 0.550	54.33± 1.05	41.66± 0.95	2.52± 0.56	2.30± 0.42	*3.10±0.322*
II	8.56± 0.27	3.52± 0.098*	10.93± 0.355*	61.83± 0.79	32.66 ± 0. 88*	3.00± 0.10	2.50±0.34	*2.57± 0.384**
III	9.12± 0.14	3.63± 0.029	9.89 ±0.293	62.50± 0.321	31.56± 0.214	3.40± 0.17	3.10± 0.23	*2.53± 0.012*
IV	8.98± 0.09*	3.28± 0.026	11.96± 0.161	60.45± 0.561	33.34± 0.241*	2.93± 0.65	3.52 ±0.70**	*2.48± 0.042*
V	10.24± 0.76	3.86± 0.237	10.02±0.263***	62.43± 0.754	31.21 ±0.257***	3.79 ±0.34	4.86± 0.47***	*2.39± 0.013***
VI	10.62± 0.12	3.61± 0.039	11.45± 0.212	57.38± 0.12	37.42± 0.85	2.91± 0.46	2.64± 0.37	*2.85± 0.045*
VII	10.37± 0.12	3.87± 0.024	11.97±0.256	59.62± 0.13	36.36± 0.42	2.97± 0.51	3.10± 0.38	*2.96± 0.254*
VIII	9.83± 0.47	3.78± 0.042	11.46±0.114	58.68± 0.52	36.17± 0.28	2.69± 0.42	3.65± 0.12	*2.69± 0.314*
IX	11.12± 0.09	3.63± 0.029	11.75± 0.425	60.30± 0.245	35.18± 0.425	3.40± 0.17	2.86± 0.23	*2.98± 0.032*
X	10.02± 0.09	3.89± 0.012	10.27±0.211*	60.45± 0.561	32.21± 0.156**	3.93± 0.65	3.72± 0.23**	*2.21± 0.042***
XI	*9.45± 0.52***	*3.32± 0.125*	*9.56± 0.256****	*64.43± 0.586*	*30.89± 0.354****	*3.33± 0.11*	*4.01± 0.12***	*2.14± 0.042****

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

Aqueous extract of *C. 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. Ethanol insoluble fraction also enhanced the phagocytic index at maximum dose of 150 mg/kg b wt. Increase in phagocytic index indicates that phagocytosis is influenced by the activation of macrophages; the activated macrophages secrete a number of cytokines, which in in turn stimulate other immune cells [14]. In the same experiment ethanol soluble fraction did not show any significant increase or decrease in the immune system, either is absent or present in such a low concentration that to innovation to phagocytes is generated significantly. The result depict that aqueous extract of *C. gynandra* has immunomodulatory activity. Both, crude and ethanol insoluble fraction have the phytocontents for chemo stimulation of phagocytosis. Significant clearance of carbon particles from the blood of treated animal in dose dependent manner is observed.

Ethanolic extract showed a decline in the phagocytic index. It was 0.0o88 0.0031, 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, and 150mg phagocytic index was 0.0779 0.00027 and 0.068 0.00025 .It is possible that ethanolic extracts 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 extracts might contain some compounds, which are stimulator but are more soluble in water. Moreover, ethanolic extracts 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 or on binding of antigen-antibody complex with antigen-antibody complex with receptor or coating the antigen to prevent its recognition by the phagocytes or the inhibiting the activity of C1 and C3 complement [15].

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. 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 in soluble 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 medi8ators of the ensuing hypersensitivity response particularly by attracting and activating macrophages [16-17]. Aqueous extract of the plant contains saponin and according to Shibata 1977 and Verotta et al., 2001 saponins are immunostimulating agents [18-19]. In later hrs reduction in paw volume may be because of a quick action of various enzymes, hormones etc on the invader, simultaneously phagocytes increases because of activated macrophages and hence reduction in paw volume was observed. Reduction in paw volume after 24hrs and onwards point to the fact that 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 24hrs.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 an anti-inflammatory effect [20].

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-sterol's, saponins, flavanoides, 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 immunoglobulin, since both the extracts develop almost similar effect on the amount of (new) immunoglobulin, 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 cloud, which repel one another. Immunoglobulin 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 titer against SRBC was raised. In many plants similar activities and increased titre of IgM etc. were observed [21].

Ethanolic extract and its water insoluble fraction showed a decreased 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 hat can invoke antibody synthesis is not enough to incite T4 and B lymphocyte or such compounds are not in the extract. This cannot be ignored that immunosuppression may be caused by the other contents of the extract.

Cyclophosphamide suppresses humoral, cellular, non specific and specific cellular immune response. When animal was treated with cyclophosphamide then haemoglobulin [Hb], RBC

counts, WBC count, Lymphocyte% and Platelet count all are reduce significantly [9].The suppressive effect of cyclophosphamide was protected by the administration of aqueous extract and their ethanol soluble and ethanol insoluble fraction. Flavonoids in biological system tend to adhere with the molecules of cyclophosphamide this causes to increase 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 100mg/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 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 haemopoitic 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 *C. gynandra* and its water soluble and insoluble fraction 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 *C. gynandra* showed its immunostimulant as well as immunosuppressant nature.

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