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Research Article

PHYTOCHEMICAL SCREENING AND IMMUNOMODULATOR ACTIVITY OF *GREWIA ASIATICA* LINN. LEAVES

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ABSTRACT

The leaves of *Grewia asiatica* were extracted by Petroleum ether and then Ethanol till all the constituents were separated out. Phytochemical screening of Ethanolic extract of *Grewia asiatica* reveals the presence of Flavonoids, Alkaloids, Glycosides, Saponins, Tannins, Carbohydrates, and Phenolic compounds. To find out the Immunomodulator properties of the extract, different parameters of Immunomodulator were performed on Swiss albino rats, like Carbon Clearance study and Phagocytic index, Delayed type of hypersensitivity reaction, Albumin : Globulin ratio, Sheep red blood cell agglutination test, Estimation of Immunoglobulin, and Total leukocytes count.

The results obtained with various experiments suggested that the Ethanolic extract of *Grewia asiatica* leaves exhibited immunomodulatory properties with satisfactory immunostimulation. Preliminary Phytochemical screening of the extract found to contain Flavonoids. It is reported that flavonoids and their derivatives also contribute to macrophage activation. Hence, the presence of flavonoids may contribute to immune stimulating activity.

Keywords: *Grewia asiatica*, immunomodulator activity, Solvent extraction, carbon black as Indian ink

1. INTRODUCTION

The world wide "green" revolution is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Medicinal plants have been used to cure human illness since time immemorial. Certain of these drugs are believed to promote positive health and maintain organic resistance against infections by reestablishing body equilibrium and conditioning the body tissues [1]. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over. Apart from specific stimulative or suppressive activity, certain agents have been shown to possess activity to normalize or modulate the Pathophysiological processes in the immune response [2]. *Grewia asiatica* linn., native species of Uttar Pradesh, has been selected for this study. The genus *Grewia* comprises about 32 species [3]. Various parts of the plant used for various diseases. The bark, flowers, fruits and seeds used as astringent, cooling, anthelmintic, tonic and febrifuge.

Previous chemical studies on this species reported that bark, fruits and flowers contain, Alkaloids, flavonoids, volatile oils, saponins and tannins [4-6]. Leaves of *Grewia asiatica* contain Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates, and Triterpenes.

2. MATERIAL AND METHOD

2.1. Plant Material

The leaves of *Grewia asiatica* were collected from a local form house during the month of December and were authenticated by Dr Gaurav Nigam, Asstt. Professor, Department of Botany, Bundelkhand University Jhansi (U.P.). A voucher no. BU/Bot./Sep/Phar/05-2014/02 was submitted at Botany department, Bundelkhand University, Jhansi, U.P.

2.2. Animals

Swiss albino rats (100-150gm) of Either sex housed in standard conditions of Temp., Humidity and light (12hr light/dark cycle).they were fed with Standard Pellet diet and water *ad- libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Institute of Pharmacy, Bundelkhand

University, Jhansi U.P. India. Approval no. BU/Pharm/IAEC/13/26, as per the guidelines of committee for the purpose of Control and Supervision of experiments on animals, ministry of social justice and Empowerment, Government of India.

Four sets (A, B, C and D) of the animals were taken. Each set contained five groups and each group had six animals.

2.3. Preparation of Extract

Leaves of *Grewia asiatica* Linn. were air dried & made coarse powder with grinder. The Coarsely powdered leaves were packed in Soxhlet apparatus & continuously extracted with Petroleum ether at temp. 100°- 120°C till all fat constituents were separated out then extracted with ethanol at temp. 60°- 80°C till all the constituents were separated out.

2.4. Extractive value

The extractive value of *Grewia asiatica* was found to be 7.23%.

2.5. Phytochemical Screening of Ethanolic extract of *Grewia asiatica* linn. Leaves

The preliminary phytochemical screening was carried out on the Ethanolic extract to reveal the presence of phytochemicals present in the extract.

2.6. Pharmacological Screening

One (1) g of carragenan was dissolved in 100ml. of water for injection. 1% w/v suspension of carbon black as Indian ink was prepared.

2.7. Immunomodulator activity

2.7.1. Carbon Clearance method

Set A animals were selected for this study and divided in to five groups.1ml of Indian ink was administered intravenously to all five groups. Group 1st animals served as control and were administered 0.5 ml of 5% dextrose normal saline. Animals of group 2nd, 3rd, 4th and 5th received Ethanolic extract of *Grewia asiatica* leaves 100, 200, 300, and 400 mg/kg body weight for seven days. Blood samples were collected at 3, 6, 9, and 12 minutes intervals and absorbance was measured at 650 nm. and plotted against the time the slope of line denotes the rate of carbon clearance which is a measurement of Phagocytic activity of RES and termed as Phagocytic index [7].

2.7.2. Delayed type of hypersensitivity reaction

After immunization of set B animals, 0.1 ML of Carrageenan solution (1% W/V) was injected subcutaneously in to the right footpad to all five groups on 10th day. After 24 and 48 hours, thickness of footpad was measured by Plethysomometer and results were recorded into the table. Difference in the foot pad thickness in control and treated groups had been taken as the measure of the DTH reaction [8].

2.7.3. Albumin : Globulin ratio

Albumin to globulin ratio was measured on set D animals on 8th day. Blood samples were collected from retro-orbital plexus and the concentration of albumin and total protein were determined by ERBA CHEM PRO-C5. Difference in the concentration of total protein and albumin was recorded as a globulin concentration (Globulin = Total protein – Albumin). Based upon the albumin globulin ratio were calculated and presented in table 4 and fig.3.

2.7.4. Sheep red blood cell agglutination test

During immunization period with extract, Set C animals were further immunized on second day by injecting 20 µl of 3 X 10^9 SRBC per ml. subcutaneously into the right hind foot pad except group 1st which served as a control. The same volume of SRBC were administered intradermally into left hind foot pad on 8th day. Blood samples were collected from individual albino rats via retro-orbital plexus on 8th day (before secondary challenge) for primary antibody titre and on 15th day for secondary antibody titre. Antibody levels were determined by haemagglutination technique reported by Nelson and Molden hall, 1967. The blood samples were centrifuged to collect the serum. Equal volumes of individual serum samples were pooled. 20 µl of 0.1% suspension of SRBC in BSA saline was added to serial two fold dilution of pooled serum samples made in 20 µl volume of normal saline containing 0.1% BSA saline in V- bottomed micro titration plates. After mixing, the SRBC were allowed to settle at room temperature for 60 to 90 minutes until control wells showed on unequivocally negative pattern (a small button). The value of the highest serum dilution causing visible haemagglutination was taken as the antibody titre. Each pooled serum samples was titrated in five parallel doubling dilutions and the mean titre of these determinations was calculated [9]. Results were as presented in table 5.

2.8. Estimation of Immunoglobulin

Estimation of immunoglobulin was carried on Set D animals after 7 day immunization with extract; 1ml. 1%

w/v BSA was administered intravenously to all above groups.

After 24 hours blood samples were withdrawn and allowed to coagulate at room temperature. These were then centrifuged at 2000 rpm for 10 minutes. Then level of IgM and IgG was estimated in each sample [10]. Results were presented in table 6 and fig. 5.

2.9. Total Leukocyte Count

Total leukocyte count was measured on group animals of set D. Leukocytes were counted using Haemocytometer on 8th day. 0.5ml of blood was withdrawn from the retro-orbital plexus immediately into the W.B.C. pipette, then diluted up to mark with W.B.C. dilution fluid (Qualigens fine chemicals) and it was shaken by rotation using hand palms. A drop of sample was put down on a Neuber's chamber and numbers of leukocytes were determined by observing under the microscope. Same procedure was applied for all the groups [11]. Total leukocytes were represented in table 7.

3. RESULT AND DISCUSSION

Phytochemical screening of Ethanolic extract of *Grewia asiatica* leaves reveals the presence of flavonoids, Triterpenes, Glycosides, Steroids, Alkaloids, Saponins, Carbohydrates, Tannins and Phenolic compounds.

Table 1: Extractive Value of *Grewia asiatica* Linn.

Extract	Powder weight	Extractive value %
Ethanolic Extract	150 gm	7.23%

Phagocytic index was determined by measuring the concentration of Indian ink at different time intervals. The rate of Carbon Clearance is a measure of Phagocytic activity.The results Suggested that in the case of control animals, the concentration of Indian ink obtained after 12 minutes of experimental time was decreased nearly 52% of its initial value as compared with the value recorded initially at 3 minutes. This decreased could be described of the natural course of Phagocytosis to the particles by liver macrophages. On the other hand the Ethanolic extract of *Grewia asiatica* showed significant immunostimulant activity as reflected by lower recovery of carbon particles as compared against that obtained by the control. The same trend was followed even after increasing the dose and the result were found to be directly influence by the increase in dose.

Cell mediated immunoresponse was determined by measuring the footpad thickness of albino rats at different time intervals. This was performed to assess delayed type of hypersensitivity reaction. The results reveal that in case of control the percent inhibition of thickness of foot pad was not increased significantly. But in case of Ethanolic extract at the dose of 100, 200, 300, and 400 mg/kg body weight intraperitoneally, the thickness was decreased significantly and directly proportional to the dose. Percent inhibition was found to be high at 400mg/kg dose.

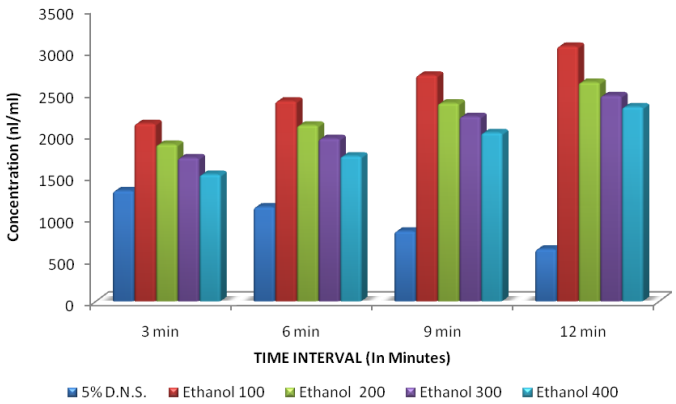

Fig.1: Ethanolic extract of *Grewia asiatica* leaf on macrophage activity by Carbon Clearance method.

Table 2. Effect of *Grewia asiatica* on macrophage activity by Carbon Clearance method

Extract	Dose Mg/Kg	Concentration of Indian ink at different time interval (nc/ml)				Mean phagocytic index/mean slope
		3 min	6 min	9 min	12 min	
Control	5% D.N.S.	1324.38±27	1130.38±64	840.37±34	625.26±37	1.00
Ethanolic extract	100	2130.18±19	2400.34±13	2710.26±18	3055.42±41	3.16
	200	1880.36±49	2110.18±33	2375.18±42	2625.11±23	2.75
	300	1720.18±34	1950.35±43	2214.23±48	2464.38±17	2.57
	400	1520.14±18	1740.16±24	2020.17±36	2330.18±34	2.36

All values are mean ± SD, N = 6 < 0.005 (Significant)

Albumin and Globulin are the Serum components where the later also take part in immune system. The ratio was determined in all the groups, the concentration of albumin was found normal but globulin concentration was significantly increased and this production was directly proportional to the dose of Ethanolic extract ($P < 0.05$) as calculated in all groups by T-test.

The Humoral antibody response was determined after immunization of the animals with SRBC. The data of present study indicate that the ethanolic extract of leaves of Grewia *asiatica* stimulate humoral immunoresponse as evidenced by increased antibody titre in albino rats challenged with SRBC. The ethanolic extract was found effective in all doses.

Immunoglobulin was estimated by Immuno – turbidometric assay. The results suggest that IgG level was increased significantly by the extract at all doses. On the other hand, the IgM level was increased significantly by the extract. It can be concluded that IgG and IgM levels were influenced by Ethanolic extract of *Grewia asiatica* linn. leaves.

Table 3 : Effect of *Grewia asiatic*a leaf on cell mediated immune response in Albino rats.

Extract	Group Mg/Kg	Paw Volume		Difference mean (B-A)	Percent inhibition	Paw Volume ML±SD 48 hours	Difference Mean±SD (C-A)	% inhibition SEM
		Paw volume 0 hours	24 hours					
Control	5% D.N.S.	1.17±0.31	2.17±0.32	1.00±0.17	.....	3.19±0.17	1.02±0.32	.....
Ethanolic extract	100	1.26±0.8	1.95±0.16	0.69±0.12	31±0.13	2.39±0.32	0.44±0.18	50±0.70
	200	1.26±0.12	1.66±0.23	0.40±0.32	60±0.32	1.88±0.34	0.22±0.32	78±0.62
	300	1.28±0.18	1.50±0.11	0.22±0.01	78±0.42	1.65±0.03	0.15±0.18	85±0.42
	400	1.27±0.22	1.39±0.22	0.12±0.04	88±0.12	1.47±0.08	0.08±0.12	92±0.72

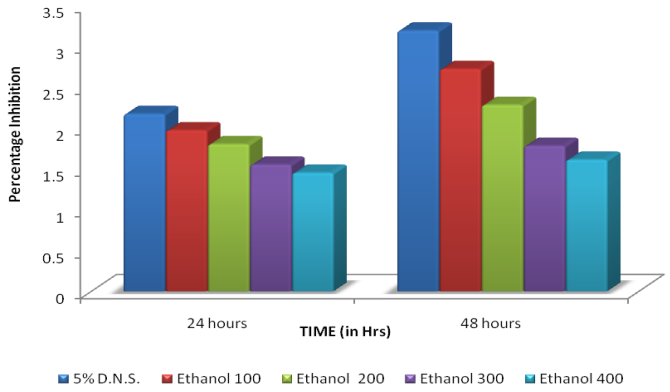

Fig. 2: Ethanolic extract of *Grewia asiatica* leaf on cell mediated immune response in albino rats

Total numbers of leukocytes were determined by using Haemocytometer. The result suggests that the total number of leukocytes in control were normal. On the contrary, in the case of animals treated with Ethanolic extract of *Grewia asiatica,* the numbers of leukocytes were increased. Maximum production was seen at the dose of 400 mg/kg b.w. ($P < 0.05$).

The results obtained with various experiments suggested that the Ethanolic extract of Leaves of *Grewia asiatica* exhibited satisfactory Immunostimulation.

Table 4: Effect of Ethanolic extract of *Grewia asiatica* leaf on protein level

Extract	Dose mg/kg b.w.	Protein level (g/dl)	
		Albumin	Globulin
Control	5% w/v DNS	4.412	2.256
Ethanolic Extract	100	5.298	2.518
	200	4.673	2.936
	300	4.725	3.134
	400	4.523	3.148

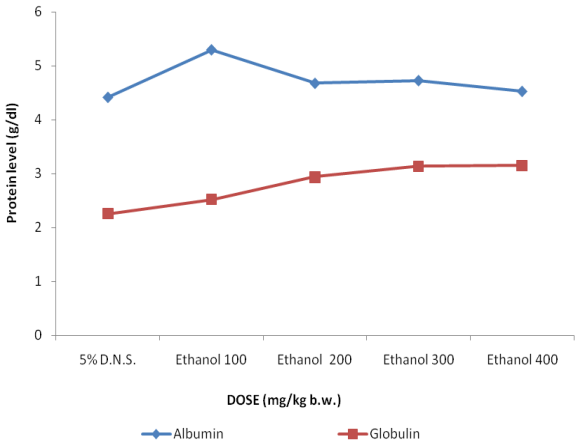

Fig.3: Ethanolic extract of *Grewia asiatica* on Protein level.

Table 5: Effect of *Grewia asiatica* leaf on antibody titre

Extract	Dose mg/kg-b.w. (i.p.)	Antibody Titre			
		Antibody titre range	Primary 7 days	Antibody titre range	Secondary 14 days
Control	5% w/v DNS	192-384	305±73	192-384	296±68
Ethanolic Extract	100	192-384	515±38	192-384	535±34
	200	96-192	583±25	96-192	613±38
	300	96-192	630±12	96-192	675±11
	400	96-192	673±16	96-192	728±36

Table 6: Effect of *Grewia astiatica* leaf on humoral immune responses in albino rats, immunized with BSA

Extract	Dose mg/kg b.w.	Immunoglobulin levels	
		IgG	IgM
Control	5% w/v DNS	1740.52±18.0	750.12±4.32
Ethanolic Extract	100	2490.06±3.8	375.08±7.22
	200	2570.03±12.16	405.11±6.33
	300	2620.36±1.32	440.18±11.22
	400	2660.08±3.21	460.16±16.33

All values are mean ± SD, N = 6 < 0.05 (Significant)

Table 7: Effect of *Grewia asiatica* leaf on leukocyte count

Extract	Dose Mg/Kg b.w. (I.P.)	Total leukocytes
Control	5% w/v D.N.S.	10819.48±215.17
Ethanolic Extract	100	12725.34±315.10
	200	13803.74±340.19
	300	15704.18±290.18
	400	16870.13±190.12

All values are mean ± SD, N = 6 < 0.05 (Significant)

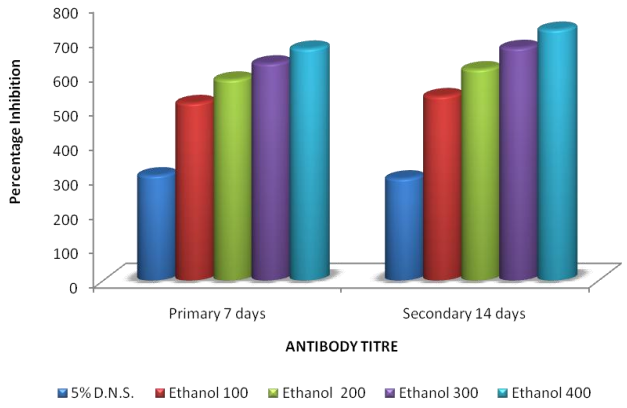

Fig. 4: Ethanolic extract of *Grewia asiatica* leaf on antibody titre

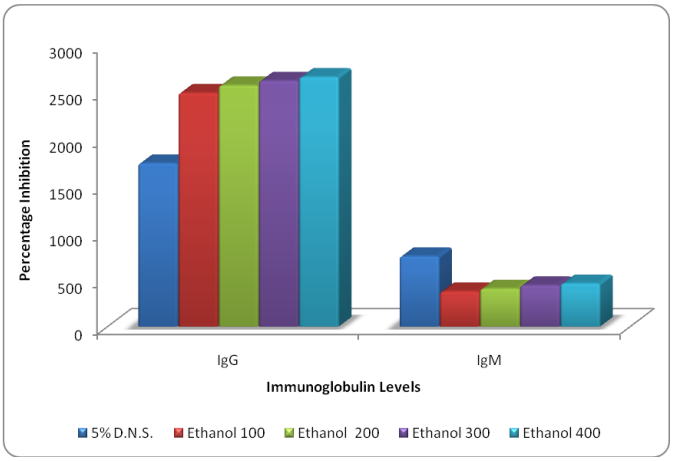

Fig .5: Ethanolic extract of *Grewia asiatica* leaf on humoral immune responses in albino rats, immunized with BSA.

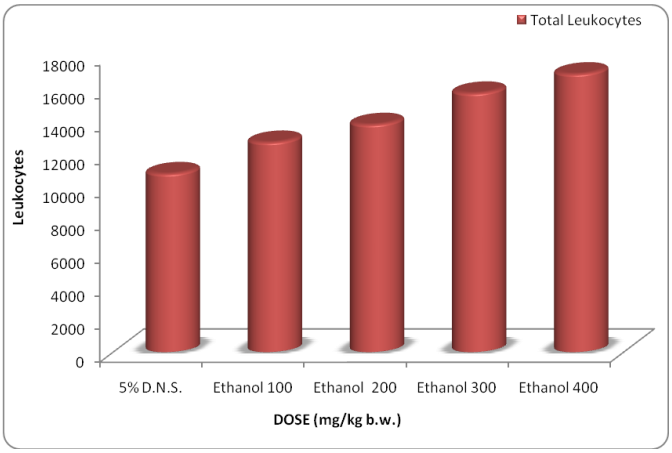

Fig. 6: Ethanolic extract of *Grewia asiatica* leaf on Leukocyte count

Further studies may reveal the mechanism of action responsible for the immunological activities of *Grewia asiatica*.

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