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STUDIES ON THE IMMUNOMODULATORY EFFECTS OF *WEDELIA CHINENSIS*

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ABSTRACT

The aim of the present study was to investigate the immunomodulatory action of ethanolic extract of Wedelia chinensis whole plant (EEWC) in experimental model of immunity. Cellular immunity and humoral immunity was carried out by delayed type hypersensitivity reaction, carbon clearance assay, hematological cell profile and indirect haemagglutination assay. Ethanolic extract of Wedelia chinensis was administered at a dose of 200 and 400 mg/kg orally. Levamisole (50 mg/kg, p.o) was used as standard. Oral administration of Ethanolic extracts of Wedelia chinensis (at 200 and 400 mg/kg) significantly inhibited sheep red blood cells induced delayed type hypersensitivity reactions and significantly increased the phagocyctic index. It also produced a significant dose related decrease in sheep erythrocyte specific haemagglutination antibody titre. The Total Leukocyte Count, lymphocyte and neutrophil count increased significantly. Among the different doses of test drug 400mg/kg was more effective in cellular immunity models than 200mg/kg. However, all the doses exhibited protection in humoral immunity procedures. From the above findings, it is concluded that EEWC possesses potential for augmenting immune activity by cellular and humoral mediated mechanisms.

KEYWORDS: *Immunomodulation, Wedelia chinensis, DTH, Sheep red blood cell etc.*

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INTRODUCTION

Modulatory response of immune system to alleviate the disease condition was the major interest in Ayurveda for development 'Rasayana' drugs. Many plants have been extensively used as 'Rasayana' drugs in 'Ayurveda' for the management of neurodegenerative diseases, as well as rejuvenators, immunomodulators, aphrodisiac and nutritional supplements (1, 2). *Wedelia chinensis* (Osbeck) Merill, Asteraceae is a reputed herbal medicine in Ayurvedic, Siddha and Unani system of medicine (3, 4). The literature reveals that various parts of *Wedelia chinensis* have been used as a folklore medicine for various ailments like its hepatoprotective efficiency, cholagouge, jaundice, diarrhoea, cough,

cephalahgia, diphtheria and pertusis etc (5). The plant is astringent, bitter, acrid, thermogenic, anti-inflammatory, vulnery, ophthalmic, cardiotonic, anthelmintic , diuretic, aphrodisiac, sudorific, febrifuge and trichogenous and is useful in vitiated conditions of Kapha and Vata, inflammation, elephhanmtiasis, otalgia, cephalagia, wounds, ulcers, nyctalopia, dysopia, hepatosplenomagaly, colic, dyspepsia, helminthiasis, strangury, anemia, Seminal weakness, fever, baldness and graying of hair (3). The following active constituents of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in different experimental models (6). The plant of *Wedelia chinensis* is reported to contain many functional and bioactive compounds such as isoflavonoids, bisdesmosidic oleanolic acid saponins and wedelolactones, Norwedelolactone, Norwedelic acid (5, 6-dihydroxy-2 (2', 4', 6'-trihydroxyphenyl)-benzofuran-3-carboxylic acid), fatty acids, melissic and lignoceric acid, stigmasterol and stigmasteryl glucoside, Kauren diterpenes (7,8). Pharmacological investigations have demonstrated that *Wedelia chinensis* possess hepatoprotective wound healing, anticancer, immunostimulant, CNS depressant, antioxidant, adaptogenic, antistress, sedative, anti-osteoporotic (post menopausal), chemopreventive, analgesic, anti-inflammatory, androgen suppressing activity, anthelmintic and febrifuge anticonvulsant, anti ulcerogenic and mucosal protective agent, antibacterial & antimicrobial, insecticidal (9). However scanty scientific evaluations are conducted for confirming its role as immunomodulator. Thus, this study was designed to study the immunomodulatory activity of *Wedelia chinensis* whole plant extract in different experimental models of cellular and humoral immunity in animals.

MATERIALS AND METHODS

Experimental animals

Laboratory bred Wistar albino rats (180–200 g) and albino mice (20–25 g) of either sex were housed at 25° ± 5° C in a well-ventilated animal house under 12/12 h light/dark cycle. The animals had free access to standard food pellets (Hindustan Lever Ltd., Kolkata, India) containing and water ad libitum. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the purpose of control and supervision on experiments on animals (CPCSEA). The animals were subjected for quarantine (10 days) prior to experimentation.

Procurement of plant material and extraction

The whole plant of *Wedelia chinensis* was collected during the month of June 2010 from Tirupathi, Andhra Pradesh, India. The plant material was taxonomically Identified and authenticated by Dr K Madhav Chetty, Department of Botany, Sri Venkateswara University, Tirupathi, India. A voucher specimen (BIT/02/09) has been deposited in the Herbarium of the School of Pharmacy, Bharat Institute of Technology, India, for future reference. The powdered plant material was defatted with petroleum ether and extracted using 95 % ethanol and the solvent was completely removed by vacuum distillation to yield a reddish brown residue (yield 13.5%, w/w). This ethanolic extract of whole plant of *Wedelia chinensis* (EEWC) was examined chemically and it was found to contain flavonoids, terpenoids and sterols which were confirmed by thin-layer chromatography (TLC).The extract was stored in a refrigerator and a weighed amount of EEWC was suspended in 2 % SCMC solution and used for the present study.

Antigen preparation

Fresh sheep blood was collected from the local slaughterhouse. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5×10^9 cells/ml for immunization and challenge (9). The animals were immunized by injecting 1ml of 20% SRBC, i.p. The day of immunization was considered as day zero.

Acute toxicity studies

EEWC was tested for acute toxicity studies as per procedure given in OECD guidelines. Rats (n=6)

were starved for overnight and fed orally with the increasing doses of extract (10, 40, 100, 400, 1000 and 2000 mg/kg .b.w). Animals were observed for next 14 days for behavioral changes and mortality (10).

Experimental protocol

The drug solutions were prepared in distilled water for oral administration. Immunomodulatory activity was checked both at cellular and humoral levels. All the experimental models had four common groups consisting of five animals each. Group I served as control and received (vehicle 0.3 ml/mouse, p.o), group II and group III received the EEWC at a dose of (200 mg/kg and 400 mg/kg p.o), respectively whereas group IV was administered standard drug levamisole at a dose of 50 mg/kg, p.o. (11).

Delayed type hypersensitivity reaction

The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 1X 10^8 cells, intraperitoneally, on day 0. On Day 8, after immunization the thickness of the right hind footpad was measured using a Vernier caliper. The rats were then challenged by injection of 1 X 10^8 SRBCs in the left hind footpad. The footpad thickness was measured again after 24 h of challenge. The difference between the pre- and post challenge footpad thickness, expressed in mm was taken as a measure of the DTH response. The following formula was used to measure the DTH response (12).

$$\frac{\text{(Left foot pad challenged with antigen-Right foot pad control) X 100}}{\text{Left foot pad challenged with antigen}}$$

Carbon clearance test

Swiss albino mice were administered EEWC, vehicle and levamisole orally for 5 days in their respective groups. Forty eight hours after the last dose of the drug, animals of all the groups were injected via the tail vein with carbon ink suspension (10 µl/gm body wt.). Blood samples were drawn (in EDTAsolution 5 µl) from the retro-orbital vein at 0 and 15 min, a 25-µl sample was mixed with 0.1% sodium carbonate solution (2 ml) and its absorbance at 660 nm was determined. The phagocytic index K was calculated using the following equation: K= (Loge OD1-Loge OD2)/15, where OD1 and OD2 are the optical densities at 0 and 15 min, respectively (12).

Humoral antibody titer (Haemagglutination test)

Rats of various groups were pretreated with the drugs for 7 days and all rats of entire groups were immunized with 20% SRBC (0.1 ml) intraperitonially. The day of immunization was referred to as day 0. The drug treatment was continued for 7 more days and blood samples were collected from individual animals by retro-orbital puncture on day 8 and were centrifuged at 2500 rpm for 10 min to separate the serum. Two-fold dilution of 50 µl sera (heat inactivated at 56 °C for 30 min) was performed in RPMI-1640 medium. Serial dilution (taking 50 µl of the aliquot) was performed in 50 µl RPMI-1640 medium into 96 well micro-titre plates. The fresh, SRBC (1.0%; 25 µl) suspension was dispensed into each well and mixed thoroughly. The plates were then incubated at room temperature for 2 h and examined for button formation. The reciprocal of the dilution, just before the button formation, was observed and titer values were calculated (12).

Hematological cell profile

For Total Leukocyte Count and Differential Leukocyte Count, various groups were pretreated with the drugs for 7 days. The drug treatment was continued for 7 days and blood samples were collected from individual animals through retro-orbital plexus and diluted with Turk's fluid in WBC pipette, in which red cells were lysed without affecting the leukocyte and smear of each blood samples was made on a clean glass slide and stained with Leishman's stain respectively for Total Leukocyte Count and Differential Leukocyte Count. Leukocyte count was done using a Neubauer's chamber. For Differential Leukocyte Count Among the hundred leukocytes counted, differentiation of leukocytes was observed based on the cell size and the presence of granules with respect to their size, shape and color of the nucleus present in the different cells. The observation was made under an oil immersion (11).

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by student t test. The values were expressed as mean± SEM and P <0.05 was considered significant.

Results

Delayed type hypersensitivity reaction

SRBC-induced delayed-type hypersensitivity was used to assess the effect of the extract on cell-mediated immunity. Administration of EEWC (200 mg/kg and 400mg/kg) and levamisole produced a significant, dose-related decrease of DTH reactivity in terms of the paw thickness when compared to control group, response at +24 h was taken as a parameter for evaluating the reaction (Table 1).

Table- 1Effect of EEWC and Levamisole on immunomodulation

Treatment	Phagocytic Index	HA Titre	DTHresponse (mm)
Control	0.0521±0.0061	10.5±0.32	22.51±0.05
EEWC (200mg/kg)	0.077±0.0059*	16.45±0.45*	26.13±0.01**
EEWC (400mg/kg)	0.082±0.0029*	20.22±0.74***	24.21±0.08***
Levamisole (50mg/kg)	0.084±0.0029*	25.13±0.74***	20.56±0.080***

All values are expressed as mean ± SEM of five observations.

EEWC,whole plant extract of *Wedelia chinensis*. Group I control; Group II-EEWC 200 mg/kg ; Group III-EEWC 400 mg/kg and Group IV- Levamisole 50 mg/kg. *** as compared to control

Carbon clearance test

Both doses of Wedelia chinensis extract and levamisole showed significant increase in the phagocytic index when compared to control indicating that there was increase in the clearance of colloidal carbon from the blood after administration of these drugs. However, the clearance was best with EEWC at 400mg/kg and levamisole (Table 1).

Humoral antibody titer/ Haemagglutination test

The haemmagglutinating antibody (HA) titre value was significantly increased in animals that received vaccination at 200mg/kg and 400mg/kg of EEWC or Levamisole compared to animals that received vaccination alone (Table 1).

Hematological cell profile

A significant dose related increase in total white blood cell count and a significant increase in Neutrophils (N), lymphocytes (L) and eosinophil (E) (Table 2) was observed in rats treated with EEWC (200 mg/kg and 400mg/kg) and levamisole as compared to control group.

Table- 2 Effect of EEWC and Levamisole on TLC and DLC

Treatment	Lymphocyte(%)	Eosinophill(%)	Neutrophills(%)	TLC(%)
Control	67.00±2.12	4.16±0.30	40.83±0.70**	12.10 ±2.13
EEWC (200mg/kg)	70.00±1.7*	4.5±0.22*	49.17±1.60*	14.28 ±1.83*
EEWC (400mg/kg)	75.00±1.9*	5.16±0.30*	50.17±1.138**	15.18 ±2.32*
Levamisole (50mg/kg)	77.75 ± 2.3*	5.8±0.20*	52.11±1.02**	15.8±2.32*

All values are expressed as mean ± SEM of five observations.

EEWC,whole plant extract of *Wedelia chinensis*. Group I control; Group II-EEWC 200 mg/kg; Group III-EEWC 400 mg/kg and Group IV- Levamisole 50 mg/kg. *** as compared to control.

DISCUSSION

The present study revealed that ethanolic extract of *Wedelia chinensis* (EEWC) posseses immunomodulatory activity in experimental models of cellular and humoral immunity. The extract was found to be most effective at a dose of 400 mg/ kg, p.o, whereas, EEWC at a dose of 200 mg/kg, p.o was moderately effective in modulating immune system. The study was carried out using four different methods, each of which provides information about effect on different components of the immune system. The variety of plant products can modulate immune reaction either by stimulation or suppression and may assist as a supportive therapy along with conventional drugs in immune compromised patients (9).

DTH reaction is characterized by an immune-inflammatory reaction, in which macrophages and these cells play major role. DTH reaction requires a specific antigenic substance which will release cytokines by activation with T-lymphocytes (13). In this study, SRBC was used as the antigenic substance which elicits the hypersensitivity reaction in rats. Therefore, it is anticipated that increase in DTH reaction in mice in response to T-cell-dependent antigen evoked the stimulatory effect of ethanolic extract of *Wedelia chinensis*.

The role of phagocytosis is primarily the removal of microorganisms and foreign bodies, but also the elimination of dead or injured cells. Phagocytic defects are associated with varied pathological conditions in humans (12). In view of the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to B-cells, EEWC was evaluated for its effect on macrophage phagocytic activity and showed a promising clearance of the particulate matter from blood. Immune activation is an effective and protective approach for treating infectious diseases. Among the leukocytes, only antigen specific lymphocytes possess the diversity, specificity, memory and self-reorganization indicating an adaptive immune response (14). It was observed that EEWC caused significant increase in TLC and lymphocyte population indicating its immunological effects.

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 effector 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 (15). The effect of EEWC on humoral response was tested on sheep erythrocytespecific haemagglutination antibody titre in rats and was found to significantly enhance the production of circulating antibody titre. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis.

In conclusion, this result provides primary evidence that whole plant ethanol extract of *Wedelia chinensis* altered the total and differential WBCs count, potentiated the effect on DTH response. Thus the extract showed stimulation of defense system by modulating the immunological parameters and holds the promising therapeutic benefits of the plant parts on immunomodulation. These preliminary results lend to support to the use of this plant in folk medicine alleviating various diseases. Further, investigations are required to clarify the exact active constituents responsible for immunomodulatory effect and their mechanism of action.

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