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Research Article

IMMUNOSTIMULATING ACTIVITY OF METHANOLIC EXTRACT OF *SWIETENIA MAHAGONI* SEEDS

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ABSTRACT

To investigate the immunomodulatory effect of *Swietenia mahagoni* seeds in rats; methanolic extract of *Swietenia mahagoni* seed was administered orally at doses of 50, 100, 200 and 300 mg/kg body weight to healthy rats. The assessment of immunomodulatory activity was carried out by testing the neutrophil adhesion test, phagocytic index by carbon clearance test, Hemagglutinating antibody (HA) titre and delayed type hypersensitivity (DTH) responses. Oral administration of the extract significantly increased in neutrophil adhesion to nylon fibre and delayed type of hypersensitivity responses when compared to control group. On the other hand, it showed significant increased in circulating antibody titre and phagocytic index in carbon clearance assay in a concentration dependent manner. Thus *Swietenia mahagoni* seed significantly potentiated the cellular immunity by facilitating the foot pad thickness responses to the sheep RBCs in sensitized rats as well as humoral immunity by significantly increasing circulating antibody titre. The responses were statistically significant when they were compared with the control (*p<0.05,**p<0.01). The study stated that *Swietenia mahagoni* shown a significant stimulation of the cell mediated immunity and humoral immunity.

Keywords: Immunomodulatory, *Swietenia mahagoni,* Methanolic, Carbon clearance, Cell mediated and Humoral immunity.

INTRODUCTION

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific as well as non-specific immunity (Wagner and Proksh, 1985). Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens. The innate immune system is the first line of defense against an antigenic insult and includes physical (skin), biochemical (complement, lysozyme and interferon) and cellular components (neutrophils, monocytes, macrophages). When the innate immune response is inadequate to cope with infection, the adaptive response culminates in the production of antibodies, which are the effectors of humoral immunity and the activation of T lymphocytes, which are the effectors of cell-mediated immunity. Humoral immunity or B-cell immunity develops circulating antibodies, which are globulin molecules that are capable of attacking the invading agent. Cell-mediated immunity is achieved by the formation of large number of activated lymphocytes that are specifically design to destroy foreign agent (Rang *et al.,* 2003). Several plant products have been reported for immunomodulatory activity and many formulations of these plant products are available to enhance the immune system (Ismail and Mohammed, 2009). Plants are the essential and integral part in complementary and alternative medicine because plants has ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases (Perianayagam *et al.,* 2004).

Swietenia mahagoni (Linn.) Jacq. (Meliaceae) is a large, deciduous, and economically important timber tree native to the West Indies and commonly known as "mahogani". This timber tree is mainly cultivated in tropical zones, such as, India, Malaysia, and Southern China (Mulholland *et al.,* 2000). Plant extract showed platelet aggregation inhibitory activity (Ekimoto *et al.,* 1991), anti-human immunodeficiency virus (HIV) activities (Otake *et al.,* 1995). Ethanolic extract of *S. mahagoni* seeds showed *in vitro* antioxidant and α-amylase inhibitory activity in a concentrations dependent manner (Hajra *et al.,* 2011). However; the claim that *Swietenia mahagoni* seed extracts safe usage in folk medicine is unsubstantiated by scientific studies. Hence, the current study has been undertaken to evaluate the immunomodulatory activity of methanolic extract of *Swietenia mahagoni* seeds using neutrophil adhesion test, phagocytic responses by carbon clearance test, non specific humoral antibody titre and delayed type hypersensitivity response using various *in vivo* experimental model.

MATERIALS AND METHODS

Chemicals

Cyclophosphamide 50 mg/kg was used as a standard immunosuppressant. Carbon ink suspension: Pelican AG ink, Germany, was diluted eight times with saline and used for the carbon clearance test in a dose of 10 ml/kg body weight of rats.

Antigen

Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in large volumes of Alsever's solution and adjusted to a concentration of 0.5×10^9 cells/ml for immunization and challenge.

Plant Material

The seeds of *S. mahagoni* were collected in January 2009 from Hooghly District, West Bengal, India. The authentication of the specimen was done at Department of Botany, Dr. H.S. Gour Central University, Sagar, MP, India. A voucher specimen no. Bot/Her/1001- has been submitted at the Departmental herbarium of Department of Botany, Dr. H.S. Gour Central University, Sagar (MP, India).

Preparation of the extract

The fruits were cut into pieces to obtain seeds, and then the seeds were shade dried at room temperature to prevent the loss of active phytoconstituents. The dried seeds were subjected to size reduction to a coarse powdered using a mechanical grinder. The powdered plant material (35g) was soaked in 500 ml of 75% methanol in a conical flask. This was covered, shaken every 30 min. for 6 hrs, allowed to stand for about 72 hrs. The solution was subsequently shaken and filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator (yield was 5.120% w/w). The extract was then stored below ambient temperature for further studies. The crude extract was dissolved in 5% dimethyl sulfoxide (DMSO) to prepare desired concentrations for the assessment of immunomodulatory activity.

Experimental Animal

All experimental procedures were carried out in strict accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (Reg. No. -379/01/ab/CPCSEA). After performing the experiments all the waste materials disposed in a safe and sanitary manner.

Wister albino rats having body weighing between (age 6-8 week, 140-160gm) of either sex were used. They were maintained under standard environmental conditions (temperature: 23 ± 2°C, relative humidity: 55 ± 10% and 12 h light and dark place) and were fed with standard pellet diet supplied by Hindustan lever Ltd. Kolkata, India, and water *ad libitum*. Fresh animals were used for each experiment.

Acute Toxicity study

Dried methanolic seed extract were administered orally to different groups of rats in dosages ranging from 100 to 1000 mg/kg body weight for the LD_{50} study using the modified method (Ghosh, 1971).

Experimental method

Neutrophil Adhesion Test

Neutrophil adhesion test was done by (Mallurwar *et al.,* 2006). The rats were divided into five groups of six animals in each group. The control group I received 1 ml of 5% DMSO, while animals of treatment groups were administered *Swietenia mahagoni* seed extract at a concentrations of 50, 100, 200 and 300 mg/kg/day, p.o., daily for 14 days. On the 14 day of treatment, blood samples from all the groups were collected by puncturing the retro-orbital plexus under mild ether anesthesia. Blood was collected in pre-treated with disodium EDTA vials. Blood samples were analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC), by fixing blood smears and staining with Field stain I and Leishman's stain. After the initial counts, blood samples were incubated with nylon fibre (80mg/ml of blood sample) for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent of neutrophil adhesion was calculated as follows

$$\text{Neutrophil adhition (\%)} = \frac{\text{NIu} - \text{NIt}}{\text{NIu}} \times 100$$

Where NIu is the neutrophil index of untreated blood samples and NIt is the neutrophil index of treated blood samples.

Carbon clearance assay

Carbon clearance assay was done according to the method suggested by Cheng *et al.,* (2005). Animals were divided into six groups of six rats in each group. Group I animals served as control and received normal saline (10ml/kg b.w.p.o.), Group II animals were treated with Cyclophosphamide (50 mg/kg b.w. i.p for 3 days starting from day 4), Groups III-VI animals were treated with test substances at the concentrations of (50, 100, 200 and 300 mg/kg b.w. p.o.) respectively. All the animals were treated as above from day 0 to day 7. On 7th day of treatment animals of the entire groups received an intravenous injection (10 ml/kg body weight) of Indian ink dispersion (per warmed at 37ºC). Blood samples were collected from retro orbital bleeding by using glass capillaries at an interval of 2 min and 10 min after the injection of ink dispersion. Blood samples were added to 4ml of 0.1% sodium carbonate solution to lyses the erythrocytes. Absorbance of these samples was measured at 675 nm using spectrophotometer, after 10 min of blood collection of each animal. Rate of carbon clearance (K) and phagocytic index (α) were calculated by using following formula:

$$\text{Rate of carbon clearence (K)} = \frac{\text{Log OD2} - \text{Log OD10}}{\text{T2} - \text{T1}}$$

$$\text{Phagocytic index } (\alpha) = \frac{\frac{K1}{3} \text{ x Body weight of animal}}{\text{Liver wt} + \text{spleen wt}}$$

Where OD2 is the log absorbance of blood at 2min; OD10 is log absorbance of blood at 10 min; T2 is the last time point of blood collection; T1 is the first time point of blood collection.

Rate of carbon clearance and phagocytic index of treated group animals were compared with the control group animals.

Hemagglutinating antibody (HA) titre

Hemagglutinating antibody titre was done according to Puri *et al.,* (1993). The animals were immunized by injecting 0.1ml of SRBCs suspension containing 0.5×10^9cells intraperitoneally on 0 day. Blood samples were collected in micro-centrifuge tubes from individual animal by retro-orbital puncture on 7th day. The blood samples were centrifuged at 2500 rpm for 10 minutes and serum was obtained (Kumar and Mishra, 2007). Antibody levels were determined by the hemagglutination technique. Equal volumes of individual serum samples of each group were pooled. Two fold serial dilutions of pooled serum samples made in 25µl volume of normal saline in micro-titration plates was added to 25µl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37ºC for 1h and examined for hemagglutination under microscope. The reciprocal of the highest dilution of the test serum agglutination was taken as the antibody titre.

SRBC-Induced Delayed-type hypersensitivity (DTH) response

SRBC-Induced Delayed-type hypersensitivity (DTH) response was carried out by Puri *et al.,* (1993). The *Swietenia mahagoni* seed extract were administered orally on day 0 and continued till day 7 of challenge. On 7th day the thickness of right hind foot pad was measured using vernier caliper. The animals were then challenged by injecting $0.5x10^9$ SRBCs in right hind foot pad. A foot pad thickness was measured again 24, 48 and 72 h after the challenge. The difference between pre and post challenge foot pad thickness expressed in mm was taken as a measure of (DTH) and the mean value obtained for treatment groups were compared with that of control group. The data obtained was subjected to statistical analysis.

Statistical analysis

Data were expressed as the mean standard deviation (S.D.) of the means and statistical analysis was carried out employing one-way ANOVA. Differences between the data were considered significant at (*p<0.05; *p< 0.01).

RESULTS

Toxicity study

Acute toxicity studies with extracts revealed that LD_{50} is above 1000 mg/kg body weight. There was no lethality in any of the groups after 7 days of treatment. This finding supported the finding of Sahgal *et al.,* (2000), who reported that LD_{50} of methanolic extract was more than 5000 mg/kg body weight is an indication that the extract is safe and has no adverse effect.

Neutrophil adhesion test

Effect of methanolic extract on neutrophil activation by the neutrophil adhesion test is shown in (Table 1). Methanolic extract of *S. mahagoni* seeds with different doses (50, 100, 200 and 300mg/kg b.w.p.o) showed percentage of neutrophil adhesion as 20.49±1.10*, 22.80±2.17**, 26.22±2.53** and 32.03±1.21**, whereas, in case of control group it was 17.79±0.47 (*p<0.05, **p<0.01).

Table 1: Effect of methanolic extract of *S. mahagoni* seeds on neutrophil adhesion test

Animal group (mg/kg)	TLC (10^3mm^{-3}) [X]		Neutrophil [Y]		Neutrophil index [XY]		% of neutrophil adhesion
	UB	FTB	UB	FTB	UB	FTB	
Control	8.13±0.18	7.41±0.21	31.27±2.11	28.14±4.34	253.29±2.13	208.24±3.23	17.79±0.47
50	8.42±0.45	7.55±0.34	35.23±1.57	31.24±2.77	296.64±0.71	235.86±2.13	20.49±1.10
100	9.37±0.67	8.41±0.41	39.67±0.89	34.12±1.07	371.71±0.55	286.95±1.09	22.80±2.17
200	10.72±0.19	9.47±0.52	47.69±2.45	39.83±2.01	511.24±1.23	377.19±1.41	26.22±2.53*
300	12.42±0.23	10.43±0.51	55.76±3.09	45.13±2.34	692.54±0.43	470.71±0.47	332.03±1.2**

Values are mean± S.D., (n=6). One way ANOVA followed by Dunnett's test *p<0.05,**p<0.01 significant; ns= not significant when compared with control group.

Carbon clearance assay

In carbon clearance test, extract treated with all groups showed concentration dependent phagocytic activity when compared to control group. The phagocytic index of (200 and 300mg/kg b.w) showed significant (*p<0.05; *p< 0.01) increased (Table 2) at both the extract. The results showed that methanolic extract showed most potent phagocytic activity (2.89±0.19**, 3.88±0.11**, 4.31±0.09** and 5.07±0.22**) when compared to control group 2.27±0.42 (**p<0.01). Extract possess macrophage stimulatory activity as evidenced by increased phagocytic index in carbon clearance test thus indicates stimulation of the reticuloendothelial system.

Table 2: Effect of methanolic extract of *S. mahagoni* seeds on phagocytic index

Animal Group	Treatment dose (mg/kg) b.wt.	Phagocytic index
1.	Control (10ml/kg vehicle)	2.27±0.42
2.	50	2.89±0.19**
3.	100	3.88±0.11**
4.	200	4.31±0.09**
5.	300	5.07±0.22**

* Values are mean± S.D., n=6, **p< 0.01 significant.

Hemagglutination antibody titre

Oral administration of methanolic extract of *S. mahagoni* seeds produced significant (***p<0.001) humoral antibody titer as compared to control. Methanolic extract with different doses (50, 100, 200 and 300 mg/kg b.w) significantly elevated hemagglutination antibody titre (25.02±0.12**, 29.67±0.37**, 42.22±0.44** and 60.32±0.29**) respectively while control group showed 20.25±0.34 (Table 3).

Delayed type hypersensitivity reaction

Delayed type of hypersensitivity response to SRBC was calculated as a measure of paw oedema thickness (mm) of each animal after the treatment with methanolic extract at the concentrations of 50, 100, 200 and 300 mg/kg b.w and compared with control. An increased in paw oedema thickness was calculated after +24, +48 and +72. In our study, foot volume was enhanced after methanolic extract treatment and suggesting cell mediated immune enhancement by SRBC (Table 3).

Table 3: Effect of methanolic extract (mg/kg bw) of *S. mahagoni* seeds on HA titre and DTH response using SRBCs as an antigen

Group	DTH response (mm)			
(mg/kg bw)	24	48	72	HA titre
Control	0.16±0.010	0.13±0.07	0.07±0.009	20.25±0.34
50	0.20±0.02*	0.15±0.01^{ns}	0.09±0.03^{ns}	25.02±0.12**
100	0.29±0.02**	0.21±0.03*	0.11±0.02^{ns}	29.67±0.37**
200	0.41±0.03**	0.29±0.06**	0.14±0.04*	42.22±0.44**
300	0.50±0.02**	0.44±0.03**	0.26±0.07**	60.32±0.29**

DTH: Delayed type hypersensitivity; HA: Hemagglutination antibody titre

Values are mean± S.D., n=6. One way ANOVA followed by Dunnett's test *p< 0.05, **p< 0.01 significant; ns= not significant when compared with control group.

DISCUSSIONS

Neutrophils are important components in the surveillance and protection systems for a broad spectrum of host defenses. They play the main role as an effectors or killer cell for many types of antigenic challenges especially for infections. The primary functions of the neutrophils in host resistance are the migration towards the challenge, which is called 'chemotaxis' and the intracellular killing of microorganisms by the formation of oxygen radicals (Badway and Karnovski, 1980). Methanolic extract of *Swietenia mahagoni* seeds showed a significant increase in the neutrophil adhesion to nylon fibres. This may be due to the up regulation of the β2 integrins that are present on the surface of the neutrophils through which; they adhere firmly to the nylon fibres. Hence, it can be inferred that *Swietenia mahagoni* causes the stimulation of neutrophils towards the site of inflammation.

The carbon clearance assay was used to evaluate the effect on reticuloendothelial cell mediated phagocytosis (Jayathirtha and Mishra, 2004). When ink containing colloidal carbon is injected intravenously, the macrophages engulf the carbon particles of the ink. Rate of clearance of (carbon particles) ink from blood is known as phagocytic index. When colloidal ink containing carbon particles are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation (Gokhale *et al.,* 2003). Methanolic extract of *Swietenia mahagoni* seeds stimulate the reticuloendothelial system by significant increase in the phagocytic index.

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation to antibody secreting plasma cells (Ose and Muenster, 1968). The results showed that *Swietenia mahagoni* seeds extract significantly increased circulating antibody titre. This indicates the enhanced responsiveness of macrophages, T and B lymphocyte subsets involved in antibody synthesis. The result showed that high values of hemagglutinating antibody titre obtained in the case of methanolic extract of *Swietenia mahagoni* seeds indicated that immunostimulation was achieved through humoral immunity.

The DTH response directly correlated with T-lymphocytes especially T-DTH-lymphocytes, therefore increased the effect on cell mediated immunity. When antigens are challenged T-cells, sensitized T-lymphocytes to convert lymphoblasts and secrete lymphokines, attracting more scavenger cells such as macrophages and basophils and induction becomes apparent within 24-72 h in rats (Waksman, 1979; Poulter *et al.,* 1982). The increased response indicates that seeds extract has a stimulating effect on B-lymphocytes and macrophages killing activity through NO release by stimulating T cell for the hypersensitivity reaction.

Present study indicates that methanolic seed extract of *Swietenia mahagoni* is a potent immunostimulant, stimulating both specific and nonspecific immune mechanisms. Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Herbal agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Wagner, 1984).

CONCLUSION

In the present study, methanolic extract of *S. mahagoni* seeds showed immunostimulant activity. Thus, it can be concluded that *Swietenia mahagoni* seeds has therapeutic potential and could be served as an effective immunomodulatory candidate without any side effects and support the traditional claim of *Swietenia mahagoni* for medicinal purposes.

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