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Full Length Research Paper

Immunomodulatory activity of *Eclipta prostrata* in SRBC immunized mice

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In recent years, there has been an increased clinical use of indigenous drugs due to side effects and expenses associated with allopathic drugs. Indian herb *Eclipta prostrata* is a traditional medicinal plant, known for its anti fungal, anti bacterial and anti hapatitic properties. The present investigation aims at evaluating the immunomodulating properties of ethanol and aqueous extracts of leaves of *E. prostrata*. Swiss albino female mice (40 to 60 g) were used as animal model. The ethanol and aqueous extracts of *E. prostrata* were pretreated orally for 45 days at a dose of 150 to 300 mg/Kg/b.wt. The effect of herbal extracts on body weight was monitored and a significant increase was noticed in ethanol extract than aqueous. After the post treatment of herbal extract for 45 days, animals in all test groups including control were immunized with Sheep Red Blood Cell by *i.p.* route. Twenty-four hours after immunization, various immunological assays, such as neutrophil adhesion, delayed type hypersensitivity and haemagglutination reaction were performed to evaluate humeral and cell mediated immune response. No significant response was noticed in aqueous extract at both 150 and 300 mg/Kg. The ethanol extract showed a dose dependent increase in the entire test performed against SRBC at the concentration of 150 to 300 mg/Kg b.wt. Moreover, the ethanol extract showed very good response in the enhanced antibody production and diminished DTH activity. Hence, the ethanol extract has a strong potential immunostimulant activity, particularly humoral response, against SRBC; although it could be explored further as an immune-based ayurvedic therapy.

Key words: *Eclipta prostrata*, immunostimulant, hypersensitivity reaction, hemagglutination.

INTRODUCTION

The mice host's immune system plays an important role for the prophylactic effect and control of various diseases. A number of agents have been explored with promising immunostimulatory activity, but relatively few studies have been conducted on the clinical applications, indicating the bioavailability to eliminate toxic manifestation of the potent compounds to make them clinically acceptable. In recent years, there has been an increase in the clinical use of indigenous drugs. Herbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of pathophysiological states. Due to the fact that there are no side effects and the low expenses associated with ayurvedic drugs, emphasis is laid on the integration of indigenous health care systems with modern medicine (Ramyadurga et al., 2009). The major concept in ayurvedic medicine is to "increase the body's natural resistance" to the disease/stress. Among the various plants identified, *Eclipta prostrata* has been reported as one of the promising immunostimulant agent. *E. prostrata,* of the family Asteraceae, is widely distributed throughout India, China, Thailand and Brazil. In *ayurvedic* medicine, the leaf extract is considered a powerful liver tonic, rejuvenative, and especially good for the hair (Karthikumar et al., 2007). However, the available evidence is not yet adequate to allow their use in clinical practice.

Thus, the present work aims at evaluating the immunestimulant properties of ethanol and aqueous extracts of leaves of *E. prostrata* by performing various *in-vitro* and *in-vivo* studies in SRBC immunized/challenged mice.

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Table 1. The animal grouping and effect on body weight in animals treated with EtEp and AqEp for 45 days.

Group	Extract treated	Body weight (gram)		
		Initial	After the herbal extract treatment for 45 days	Difference in b.wt before /after treatment
A	EtEp at 150 mg/Kg.b.wt	61.5	65.0	3.5
B	EtEp at 300 mg/Kg.b.wt	54.8	59.5	4.6
C	AqEp at 150 mg/Kg.b.wt	62.3	62.5	0.2
D	AqEp at 300 mg/Kg.b.wt	69.3	68.5	-0.8
E	Sterile Water	43.9	45.6	1.7

EtEp: Ethanolic extract of leaves of *Eclipta prostrata*; AqEp: Aqueous extract of leaves of *Eclipta prostrate*.

MATERIALS AND METHODS

Plant extract

The whole plant of *E. prostrata* was collected from a nearby village and was authenticated by Botanical Survey of India, Coimbatore. A voucher specimen was preserved in the laboratory used for this study. The whole plants were refluxed in running tap water for an hour to remove visible soil and dust particle; then they were shade dried. Leaves were detached and dried leaves were used for sox let extraction with ethanol and water for about 6 to 8 h. The ethanol (EtEp) and aqueous (AqEp) extracts were collected and concentrated under reduced pressure. The residual solvents were removed under vacuum with the addition of an anhydrous salt (calcium chloride) and were stored at 4°C for further use.

Animals

Mus musculus mice, weighing between 40 and 60 g of both sexes were used in this study. Animals were acclimatized to animal house condition, under standard conditions of temperature (23±1°C), 12 h light/dark cycle, and fed with fresh vegetables (carrot and cabbage). All experiments in animals were conducted as per the animal ethical committee norms.

Antigen

Sheep Red Blood Cell (SRBC) were collected in alsever's solution, washed three times in large volumes of Pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5 x 10^9 and 0.25 x 10^9 cells/ml for immunization and challenge, respectively (Chakraborthy, 2009).

Treatment

The animals were divided into five groups consisting of six animals each. Initially, all the animals of groups A, B, C and D were administered different herbal extracts orally for 45 days at different doses, respectively (Table 1). The animals of group E served as healthy control and no herbal extracts were given. After the herbal treatment for 45 days, the body weight of all experimental mice was noted and the animals were subjected to various immunological tests.

Neutrophil adhesion test

On the 45th day of herbal extract treatment, blood samples were collected (before immunization) from all experimental animals including normal control by puncturing the retro-orbital plexus into heparinised vials and analyzed for Total Leukocyte Counts (TLC) and Differential Leukocyte Counts (DLC) by fixing blood smears and staining them with Field stain I and II-Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fiber for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The result of TLC and % Neutrophil gives Neutrophil Index (NI) of blood sample. Percent Neutrophil adhesion was calculated according to Fulzele et al. (2002):

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

Where, NIu = Neutrophil index of nylon untreated blood sample, and NIt = Neutrophil index of nylon treated blood sample.

Delayed type hypersensitivity (DTH) response

After herbal extract treatment on day 46, animals of both the groups and healthy control were immunized by *i.p.* administration (2 ml) of 0.5x10^9 SRBC/ml/mouse. The day of immunization was considered as Day 0. On day 1 (24 h after immunization), animals in all the groups were challenged by subcutaneous administration of 0.2 ml of SRBC (0.025x10^9 SRBC/ml/mouse) into the right hind foot pad, while a 0.2 ml of PBS was administered in the left hind foot pad to serve as control. DTH response was measured at 48 h after the immunization (Day 2) and expressed as percent increase in paw volume. The inflammation percentage was calculated as per Manosroi et al. (2005):

$$\text{Inflammation (\%)} = \frac{\text{(FPS after treatment – FPS before treatment)}}{\text{FPS before treatment}} \times 100$$

* FPS: Foot pad swelling.

Haemagglutinating antibody (HA) titre

Followed by herbal extract treatment for 45 days, animals in all groups including control were immunized by *i.p.* administration of 2 ml of 0.5x10^9 SRBC/ml/mouse. The day of immunization was considered as Day 0. On Day 1, blood samples were collected from all test animals by puncturing the retro-orbital plexus in ependroff tube. The serum was separated at a spin of 2500 rpm for 10 min and used to estimate the haemagglutinating antibody (HA) titre against the antigen SRBC at 0.025x10^9 cells.

Table 2. Effect of oral administration of *Eclipta prostrata* for 45 days on TLC, DLC and nuetrophil adhesion percentage in mice.

Group	Blood Cell count			Neutrophil index (%)		
	TLC	DLC		Untreated	Nylon fiber treated	Neutrophil adhesion (%)
		Neutrophil	Lymphocyte			
	4500	62	38	62	54	12.90
B	4100	70	44	70	57	18.57
C	4200	68	32	68	59	13.23
D	5150	60	40	60	51	15.0
E	4000	56	30	56	54	3.57

Table 3. Effect of EtEp and AqEp extracts of *Eclipta prostrata* on hypersensitivity response and antibody production in mice challenged with SRBC.

Group	HA titer	Left hind (PBS)			Right Hind (SRBC)		
		Before	After	Percentage	Before	After	Percentage
A	128	28	30	7.14	29	35	20.69
B	512	29	30	3.45	31	45	45.16
C	64	37	40	8.11	36	45	25.00
D	128	27	30	11.11	27	40	48.15
Control	16	36	40	11.11	38	43	13.16

RESULTS AND DISCUSSION

Body weight

Body weights of experimental mice were monitored before and after the herbal extract treatment for 45 days. A remarkable change in the body weight was noticed in the animal group treated with EtEp at both concentrations. A dose dependent response in body weight enhancement was noticed in EtEp compared to normal control. An increase of 4.57 g was found in animals treated with EtEp at 300 mg/Kg.b.wt, while an increase of 1.67 g was found in normal control group animals (Table 1). However, AqEp did not show any significant result at both concentrations.

TLC/DLC and neutrophil adhesion test

The blood samples collected after herbal extract treatment from all experimental animals were subjected in to TLC and DLC. A remarkable hike in TLC was noted in all the mice treated with herbal extract than normal control animals (Table 2). Further, the oral administration of herbal extracts for 45 days significantly enhances the adhesion properties of neutrophil to nylon fiber which correlates to the process of activation and migration of cells in blood vessels. Animals treated with EtEp extract at 300 mg/Kg.b.wt showed strong neutrophil adhesion properties when compared to normal control, indicating possible immunostimulant effect.

Hyper sensitivity (DTH) response

Beside herbal extract treatment for 45 days, all experimental animals including control were immunized by *i.p.* administration of 2 ml of $0.5x10^9$ SRBC/ml/mouse, followed by a challenge dose at $0.025x10^9$ SRBC/ml into the right hind foot pad 24 h after immunization. The foot pad swelling was monitored and the results are tabulated in Table 3. No significant results were noticed in both EtEp and AqEp treated animals.

The interaction of sensitized T-cells, with antigen presenting cell, results to the release of cellular mediators, such as histamine, initiation of arachidonic acid metabolism (Grisworld et al., 1982) and eventually to interferon-γ that will lead to DTH reaction. Several inflammatory processes have been suggested as regard these possible mechanisms. For example, activation of complements, releasing of mediators by activated mast cells, kinin, reactive oxygen or nitrogen species from archidonic acid metabolites and pro-inflammatory cytokines (Vinegar et al., 1987). In the present study, the less inhibitory action of both ethanol and aqueous extracts of *E. prostrata* may be due to absence of their influences on these biological mediators or the synthesis process. The diminished DTH activity may also be due to the activation of antibody production, which could assist the phagocytes to eliminate the antigens.

Haemagglutination assay

The successive oral treatment of both EtEp and AqEp for

45 days showed significant response in antibody production against SRBC compared to the control group. The maximum HA titer (1/512) was noted in the animals treated with EtEp at 300 mg/Kg b.wt (Table 3). Similar results were observed in animals treated with AqEp at 300 mg/Kg and EtEp at 150 mg/Kg. The enhancement of antibody responsiveness to SRBC in mice, in this study, indicated the enhanced responsiveness of macrophages and B. lymphocyte subsets involved in the antibody synthesis (Benacerraf, 1978). Therefore, the augmentation of the antibody production response to SRBC confirmed the effectiveness of humoral immune response of EtEp and AqEp.

Conclusion

Human health is always related to their strong immune power. The immune cells and their mediators are directly involved in the processing of antigens and elimination by phagocytosis. Beside the synthetic drugs, herbal preparations are becoming increasingly popular as they are natural and safer to use.

In this study, the oral administration of ethanol and aqueous extract of the leaves of *E. prostrata* for 45 days was found to stimulate the non specific arm of immunity. Moreover, EtEp increases the phagocytic function and efficiency of the neutrophils. The ethanol extract was found to enhance the antibody-mediated immune response than cell mediated immune response. This was evident by high titer value in HA assay and diminished DTH reaction. Further, AqEp did not have any or enough potent immunomodulatory compounds to protect the effect of injected antigen SRBC. Hence, the ethanol extract of leaves of *E. prostrata* has a strong potential to be explored further as an immune-based herbal therapy.

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