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Research Article

Evaluation of Immunomodulatory Activity of Ethanol Extract of *Canscora perfoliata* Lam (Gentianaceae) Whole Plant

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ABSTRACT

Canscora perfoliata Lam an ethnomedicinal plant was studied for its immunomodulatory activity. Immunomodulatory activity of different doses of ethanol extract of *Canscora perfoliata* was evaluated in Swiss albino mice. Mice were treated with two doses (150 and 300mg/kg body weight) for 5 days. Body weight, relative organ weight, delayed type hypersensitivity (DTH) response and Haemagglutinin titre (HT) were studied in various groups of animals. The results obtained show a significant increase ($p<0.05$) in body weight and relative organ weight of spleen, liver and kidney at dose of 300mg/kg. The *Canscora perfoliata* extract eliciated a significant increase ($p<0.05$) in the DTH response at dose of 300mg/kg. In the HT test, the plant extract showed a stimulatory effect at all doses. The doses of 300mg/kg significantly ($p<0.05$) increases the WBC count, compared with the control group. Overall, *Canscora perfoliata* showed a stimulatory effect on both humoral and cellular immune functions in animal models.

Keywords: *Canscora perfoliata*, Delayed type hypersensitivity, Haemagglutinin Titre, Immunomodulation.

INTRODUCTION

The term "immunomodulation" means the alteration of immune response which may increase or decrease the immune responsiveness. Enhancement in the immune responsiveness is called immunostimulation and reduction in the immune responsiveness is called immunosuppression. An immunomodulators may be defined as a substance, biological or synthetic which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response[1,2]. There has been a growing interest in identifying and characterizing natural compounds with immunomodulatory activities. A number of plants used in traditional medicine have been shown to possess immunostimulating activities acting at different levels of the immune system[3-7]. *Canscora perfoliata* Lam is one of the medicinally important plant belongs to Gentianaceae. The juice prepared from the plant is given to treat any poisonous bites by palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu[8]. The biological activities such as hepatoprotective, antidiabetic and antiinflammatory activities were reported[9-11]. Literature reviews indicated that the immunomodulatory activity of whole plant of *Canscora perfoliata* has not been scientifically evaluated so far. In view of this, the present study was aimed at evaluating the immunomodulatory activity of whole plant of *Canscora perfoliata* in mice.

MATERIALS AND METHODS

Plant material

The whole plant of *Canscora perfoliata* Lam was collected from natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin for further reference.

Animals

Study was conducted in Swiss albino female mice (20 - 25 g). The animals were bred and maintained under standard laboratory conditions (temperature 25 ± 2°C and light period of 12 h). The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Treatment protocol

The plant extract was administered i.p. for 5 days at doses of 150 and 300 mg/Kg body weight. The dose volume was 0.2 ml. The control animal group received the same volume of normal saline and left untreated. The animals were divided into four groups (Groups I - IV). Each group comprised of a minimum of five animals. The control group (Group I) was given normal saline and the treatment groups were given the whole plant extract of *C. perfoliata* at the doses of 150 mg/kg and 300 mg/kg body weight (Groups II and III) for five days, respectively. Group IV mice were given dexamethasone, at 10mg/kg body weight. The animals were humanized 24 h after the last dose. Body weight gain (percentage) and relative weight of kidney, liver and spleen (organ weight/100 g of

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Table 1: Effect of whole plant extract of *Canscora perfoliata* on the body weight and relative organs weight

Parameter	Body weight and relative organs weight (mean±SE) in gram				
Treatment	Dose mg/kg	Body weight	Spleen	Liver	Kidney
Group I	Normal Saline	20.45±1.24	0.48±0.12	4.23±0.19	1.14±0.05
Group II	150	22.34±1.65	0.61±0.23	4.90±0.53	1.42±0.01
Group III	300	29.55±1.35*	0.76±0.13*	6.90±0.14*	1.68±0.04*

Each value is SEM of 5 individual observations * P<0.05 compared Normal Control vs Treated groups

Table 2: Effect of whole plant extract of *Canscora perfoliata* on DTH response compared with dexamethasone and on HT titre by using SRBC as an antigen in mice

Treatment Groups	Parameter		
	Dose mg/kg	Food Pad Edema (mm)	HT titre
Group I	Normal Saline	0.29±0.021	2.91±0.022
Group II	150	0.34±0.032	4.83±0.011*
Group III	300	0.44±0.024*	6.74±0.054*
Group IV	10 mg	0.11±0.01	ND

Each value is SEM of 5 individual observations * P<0.05 compared Normal Control vs Treated groups; ND – Not Done

Table 3: Effect of whole plant extract of *Canscora perfoliata* on the Haematological and Serum Liver marker enzymes.

Parameter		Hematological (Blood)			Biochemical (Serum)			
Treatment	Dose mg/kg	Hb (g/dl)	RBC ($X10^6$/ mm^2)	WBC ($X10^6$/ mm^2)	Total Bilirubin (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group I	Normal Saline	13.36±0.84	4.86±0.11	5.35±0.81	0.51±0.04	46.55±3.56	39.14±2.84	123.56±5.74
Group II	150	11.20±0.64	4.05±0.16	7.14±0.91	0.69±0.02	48.14±4.85	38.56±1.45	132.76±6.91
Group III	300	12.56±0.14	4.16±0.12	8.76±0.84	0.74±0.05	53.24±5.16	46.14±1.92	129.54±5.45

Each value is SEM of 5 individual observations * P<0.05 compared Normal Control vs Treated groups.

body weight) were determined for each animal.

Assessment of humoral immune functions

Animals within the experimental groups were challenged with 0.2 mL of 10% sheep red blood cells (SRBC), i.p., on the 10th day of the initiation of experiment. The haemagglutinin titre was also studied in these animals.

Haemagglutinin titre assay

Haemagglutinin titre (HT) assay was performed as per the procedure given by Bin- Hafeez *et al*.[12]. On the fifth day after immunization, blood was collected from the heart of each mouse for serum preparation. Serial two folddilution of serum was made in PBS (pH 7.2) in 96 - well microtitre plates and mixed with 50 µ L of 1% SRBC suspension in PBS. After mixing, the plates were kept at room temperature for 2 h. The value of antibody titre was assigned to the highest serum dilution showing visible haemagglutination.

Delayed type hypersensitivity response

The delayed type hypersensitivity (DTH) response was determined using the method of Raisuddin *et al*.[13]. On the day of termination of the treatment with plant extract, animals were immunized with $1x10^8$ SRBC, subcutaneously. On the fifth day of immunization, all the animals were again challenged with $1x10^9$ cells in the left hind footpad. The right footpad was injected with the same volume of normal saline, which served as the trauma control for non specific swelling. Increase in footpad thickness was measured 24 h after the challenge by using a dial clipper.

Assessment of haematological and liver marker enzymes

Red blood cell (RBC) count, haemoglobin (Hb) content and White blood cell (WBC) count were measured from freely following tail vein blood. Total billirubin was determined as described by Balistrei and Shaw[14]. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalo transaminase (SGOT) and alkaline phosphatase were determined by the method of King and Armstrong[15].

Statistical analysis

All values were expressed as mean ± standard error of mean (S.E.M) and comparison between the groups were made by Analysis of Variance (ANOVA). The Data were analysed using the statistical analysis system SPSS (SPSS Software for windows release 10.0; SPSS Inc., Chicago IL, USA).

RESULTS

After treatment with two different doses (150 and 300 mg/kg body weight) of whole plant ethanol extract of *C.perfoliata* for 5 days, the Swiss albino female mice were evaluated for immunomodulatory activity. Body weight, relative organ weight, delayed type

hypersensitivity (DTH) and haemagglutinin titre (HT) were studied in all the treated animal groups.

Effect of plant extract on Body weight and Relative organ weight

In the present study treatment with the whole plant ethanol extract of *C. perfoliata* was effective in increasing the body weight and also the weight of spleen, liver and kidney (Table 1).

Effect of plant extract on humoral immunity parameters

In the haemagglutinin titre (HT) (Table 2), doses 150 mg and 300 mg/kg showed titre value of 4.83 and 6.74 respectively, while the titre value of control was 2.91, thus showing a significant increase in the titre values with doses of 150 and 300 mg/kg in the treated groups ($p< 0.05$).

Effect of plant extract on cell mediated immunity parameters

The plant extract at dose of 300mg/kg elicited a significant ($p< 0.05$) increase in DTH response (Table 2), compared to the control animals. In this study, dexamethasone (Group IV) decreased DTH response, compared to the control group.

Effects of plant extract on blood parameters and liver enzymes

There was no significant elevation in the levels of SGOT, SGPT and ALP as a result of treatment with C.perfoliata (Table 3). Total bilirubin content was slightly increased. No significant difference in blood parameters was recorded in various test groups. The doses of 150 and 300mg/kg increased the WBC count, compared with the control group.

DISCUSSION

Immunomodulatory therapy represents an important field in the treatment of infectious diseases and is more actual everyday[16]. An immunomodulator is a biological or non biological substance that directly influences a specific immune function or modifies one or more components of the immunomodulatory network to achieve an indirect effect on a specific immune function[17]. In the present study, the immunomodulatory activity of the ethanol extract of *C.perfoliata* has been explored. The present study showed an overall stimulatory effect of whole plant ethanol extract of *C.perfoliata* on the immune function in mice. Stimulatory effects were observed on both humoral and cellular immunity. The humoral immunity involves interaction of B cells with 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. In the HT test, the whole plant extract of *C.perfoliata* showed an increased response with all the tested doses, but this increase was only significant at 300 mg/kg dose. This activity could be due to the presence of flavonoids or coumarins, which can augment the humoral response by stimulating the macrophages and B-lymphocytes involved in antibody synthesis [18]. The treatment with whole plant ethanol extract of *C.perfoliata* improved the haemagglutinin antibody titre reflecting an overall elevation of humoral immune response. The delayed type hypersensitivity reaction was measured as an indicator of T-cell mediated immunity[19] DTH is characterized by large influx of non specific inflammatory cells, mainly macrophages and it is a part of the process of graft rejection, tumor immunity and most importantly immunity to many intracellular infectious microorganisms, especially those causing chronic diseases[19,20]. DTH requires the specific recognition of a given antigen buy activated T-lymphocytes which subsequently proliferate and release cytokines. The significant difference in DTH response observed in the present experimental animals indicates that the *C.perfoliata* extract has a stimulatory effect on lymphocytes and accessory cells required for the expression of the reaction and thus increases cell mediated immunity. Treatment with *C.perfoliata* extract enhanced DTH reaction, which is reflected from the increased foot pad thickness compared to control group suggesting hightened infiltration of macrophages to the inflammatory site. The ethanol extract of *C.perfoliata* enhanced the production of WBC and SGPT, SGOT and ALP. Results of the present study also revealed no significant difference in the other blood parameters. Findings of the present study establish that *C.perfoliata* also have appreciable immunostimulatory activity.

CONCLUSION

Findings of the present study showed an overall stimulatory effect of *C.perfoliata* whole plant extract on both humoral and cellular immunity in mice.

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