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Research Article

Immunumodulatory Potential of Chloroform Extract of *Crateva magna* (Lour.) DC

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ABSTRACT
In the present study, chloroform extract of *Crateva magna* bark was considered and was subjected to Immunomodulatory assays to verify the ethnomedicinal claims against the Plant. The models used for the study included *In vivo* Carbon clearance study, Haem-agglutination titer, Delayed type hypersensitivity study and Neutrophil adhesion test model. The Chloroform extract of the plant has shown potent immunomodulatory activity in all the models, supporting the ethnomedicinal claims against the plant.

KEYWORDS: *Crateva magna*; immunomodulatory; carbon clearance; delayed type hypersensitivity; chloroform extract

INTRODUCTION

Crateva magna plant is densely foliaceous, deciduous tree, upto 10 m tall, fairly smooth with horizontal wrinkles, the wood is yellowish white, close grained, branches are grayish brown when dry, lenticellate [1]. The flower pedicles are upto 6.5 cm with ascending sepals, petals are white with stamens which are 5-5.5 cm ovary is oblong elliosoid, upto 0.4cm long and the stigma are sessile. The berries are sub-spherical, 3.5 cm across, the seeds are dorsally crested and tuberculate [2].

The bark and leaves are astringent, acrid, bitter, diuretic, lithotriptic stimulant, expectorant, demulcent depurative, astiperiodic tonic and detergent. They Leaves and bark are also useful in vitiated conditions of Vata and kapha (Ayur.), dyspepsia, colic, flatulence, helminthiasis, strangury, renal and vesical calculi, cough, bronchitis, asthma, pruritus, skin diseases, intermittent fevers, visceramegaly, scrofula, inflammations and hepatopathy. They leaf paste is applied on piles externally and leaf juice is used as a drink to get relief from bleeding piles [3,4].

From therapeutic point of view, immunomodulation usually refers to any process where an immune response is altered to a desired level [5]. In other words; immunomodulation is change in the body's immune system, due to agents that activate or suppress its function [6]. Initial immune response to any antigenic stimulation, usually induces specific antibody synthesis and also

production of active effector cells. Nature, magnitude and the specific form is determined by a series of modulatory processes which plans and arranges this response. Responses of the organism to slight alterations of immune regulatory pathways may be termed as "Biological response modification." Immunomodulation often can also be achieved by simple low molecular weight chemical substances like Avridine, Imuthiol etc. These are called "Low molecular weight immunomodulators" [7].

Immunomodulators are mainly classified into two groups, immunostimulants and immunosuppressants. Immunostimulants are those which enhance body's resistance against infections through increasing the immune response. These could increase the oxidative activity of neutrophils, increases engulfment activity of phagocytic cells as necessary defense mechanisms. A lot of diseases and disorders could be treated using immunostimulants such as autoimmune diseases, viral infections, and cancer [8]. Immunosuppressants reduce the activation or efficacy of the immune system. They are used to control severe conditions of allergic reaction, autoimmune response and transplant related diseases. Some drug shows a diffusive effect on the immune system while others have specific targets [9].

Ayurvedic system of medicine emphasizes on promotion of health as a concept for prevention of diseases and strengthening both mental and physical health. Ayurveda believes that the immune system is involved in etiology and pathophysiological mechanisms of various inflammatory diseases of respiratory tract, joints, skin, gut, as well as in infectious diseases [10].

Number of medicinal plants has been exploited for modulation or alteration of immune system in the form of Ayurvedic formulations either alone or in combinations. Herbs used for Immunomodulation can provide potential non toxic alternatives to conventional chemotherapies, specially when the host defense mechanism needs to be activated. Variety of phyto constituents like lectins, polysaccharides, alkaloids, peptides, flavonoids, steroids and tannins have been reported to modulate immune system in various *in vivo* models [11].

MATERIALS AND METHODS

Plant material

Chloroform extract of *Crateva magna* bark was used as test drug for the studies.

Preparation of test samples

As test samples, oral suspensions of the plant materials was prepared by suspending the extract separately in 1% solution of sodium carboxy-methylcellulose to obtain separate doses as required [12].

Experimental animals used

Female Swiss albino mice weighing in the range of 17–25 g each were used for the studies. The test animals were housed under standard conditions; temperature (23 ±10ºC) and relative humidity (55±10%); 12hr each of light and dark cycle and fed with standard pellet diet and water *ad libitum*.13 The study was approved by Institutional Animal Ethical Committee of GIPS, Guwahati, vide approval number, GIPS/IAEC/12.

Drugs and Chemicals used

Levimasole tablets (Levasol Tabs of Merindian Medicare) and Cyclophosphamide tablet (Cycloxan 50 -Biochem Pharmaceuticals) were locally purchased from medicine store at Maligaon. Trypan blue was purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Nylon fiber was purchased from local market of Guwahati, Assam, India.

Antigen for Test

Fresh blood sample from healthy sheep was collected from the local slaughterhouse. Sheep red blood cells (SRBCs) were washed with normal saline three times and adjusted to concentration of 1× 108 cells in 0.1 ml for immunization and challenge [14].

Immunomodulatory activity of Chloroform extracts of *Crateva magna* bark

In vivo carbon clearance test

Experimental animals (mice) were divided into six groups, containing six animals each. Grp I was administered with 0.3 ml/mouse of 1% Sodium carboxy-methyl cellulose in water, for seven days and was marked Control, Grp II was administered with 50 mg/kg/day p.o of Levimasole and designated as Positive standard, Grp III was labeled Negative standard was

administered with 2.5 mg/kg/day p.o of Cyclophosphamide and Grp IV, V and VI were administered with 250, 500 and1000 mg/kg/day p.o. dosage of the Chloroform extract for seven days

After seven days, all the mice from all the groups were injected carbon ink suspension (10 µl/gm body weights) via the tail vein. Then, blood samples were drawn (in 5µl EDTA solution) from the retro-orbital vein at intervals of 0 and 15 min, 25 µl of this sample was mixed with 2 ml of 0.1% sodium carbonate solution and the absorbance were measured at 660 nm. Carbon clearance was calculated using the equation:

Carbon Clearance = (Loge OD1 – Loge OD2)/ 15

Where, OD1 and OD2 are the optical densities at 0 and 15 min, respectively [15].

Haemagglutination titer test (HA)

The experimental animals were divided into six groups, each containing six animals. Group I labeled Control was given 0.3 ml of 1% Sodium carboxy methyl cellulose in water for seven days, Group II was given 50 mg/kg/day p.o, Levimasole (standard drug) and marked as Positive Standard, Group III was labeled negative standard administered with 2.5 mg/kg/day p.o of Cyclophosphamide, whereas, Group IV, V and VI were administered with 250, 500 and 1000 mg/kg/day p.o. dosage of the extract for seven days. All the animal groups were immunized by injecting 0.1 ml of SRBC suspension containing 1×108 cells intra-peritoneally on day 0. Blood samples were collected in centrifuge tubes from animal of all the groups by retro-orbital vein puncture at the end of day 7 or on the 8th day. All the blood samples were centrifuged and serums were separated. Antibody levels were determined by the haemagglutination titer technique. Where, 50 µl of individual serum samples of each group were polled. To the serial two fold dilutions of polled serum samples made in 50 µl volumes of RPMI-1640 in microtitration plates, 50 µl of 1% suspension of SRBC in RPMI-1640 was added and mixed well. All the plates were then incubated at 37ºC for 1 h and examined for haemagglutination (button formation) under microscope. The reciprocal of highest dilution, just before the button formation, was observed as the titre values of the test samples [15].

Delayed type hypersensitivity test (DTH)

The DTH experiment of was carried out on the same animals after the Humoral Antibody titer test. On the 8th day, the right hind footpad thickness was measured using vernier calliper. All the mice were then challenged by injecting 1× 108 SRBCs in right hind footpad. Footpad thickness was measured after 24 h and 48 hr. of the challenge. Difference between the pre-chellange and post challenge footpad thickness recorded in mm was considered as measure of DTH response [16].

Neutrophil adhesion test

The experimental animals were orally pre-treated with vehicle and extract at different dose for 14 days like other models. At the end of 14th day, blood samples from the animals were collected from the retro-orbital plexus into heparinized vials and were analyzed for differential leukocyte count (DLC). After the initial counts, all the blood samples were incubated with 80 mg nylon fibres/ml for 15 min at 37ºC. Then the incubated blood samples were analyzed again for TLC and DLC, respectively to give neutrophil index of blood samples. The percentage Neutrophil adhesion was calculated using the formula:

$$\text{Neutrophil adhesion \%} = \frac{NI_u - NI_t}{NI_u} X\ 100$$

Where, NIu is the neutrophil index of untreated blood samples and NIt is the neutrophil index of treated blood samples [16].

Statistical Analysis

All the datas are expressed as mean ± S.E.M (n = 6 mice / group). Graph pad prism 5 software was used for all statistical analysis. The statistical significance (*p*) was calculated by one-way ANOVA between the treated groups and the EAC control group. Followed by Dunnett's *post hoc* test of significance where, $p<0.05$, $p<0.01$ and $p<0.001$ considered being (*) significant, (**)

very significant and (***) highly significant, respectively.

RESULT AND DISCUSSION

Immunomodulatory activity of Chloroform extract of *Crateva magna*

The results as shown in Table 1 shows that, the immunomodulatory activity in terms of carbon clearance assay in mice for Chloroform extract of *Crateva magna* bark confirms that mice treated with doses of the extracts at 500 and 1000 mg/kg show increased rate of carbon clearance from blood (0.02971 ± 0.0012 respectively). While that of the standard drug Levamisole was (0.04921 ± 0.0016) as shown in figure 7.1.

The Haemagglutination titer is believed to be related with humoral mediated and cell mediated immunity. The Haemagglutination titer test results as presented in Table 1 and Figure 2, shows that treating mice with chloroform extracts at doses ranging from 250 – 1000 mg/kg shows much increase in immunomodulatory activity as is evident from haemagglutination values after incubation of serum with SRBCs (337.13 ± 0.0015 and 389.21 ± 0.0012 for chloroform extract of *Crateva magna* bark at 500 and 1000 mg/kg). When the reports are compared with that of the standard drug Levamisole (532.37 ± 0.0029), it is evident that the Chloroform extract is a close competitor for the standard drug, in terms of immunomodulatory activity.

For neutrophil adhesion model, incubation of blood with nylon fibers (NF) shows a decrease in neutrophil counts due to adhesion of neutrophils to the fibres. Various doses of chloroform extract of *Crateva magna* bark showed significant increase in the neutrophil adhesion 26.29 ± 0.07, 30.07 ± 0.20, 33.16 ± 0.18, with 250 mg/kg, 500 mg/kg and 1000mg/kg dose of chloroform extract of *Crateva magna* bark when compared to standard drug Levamisole (42.62 ± 0.67 with 50 mg/kg dose), as shown in Table 1 and Figure 3.

Again for the DTH response, the extracts showed significant decrease in mean foot pad thickness in dose dependent manner with time (0.28 ±0.02 for 24 hrs, and 0.24 ± 0.03 for 48 hrs with 250 mg/kg, 0.30 ± 0.07 for 24 hrs and 0.27 ± 0.04 for 48 hrs with 500 mg/kg and 0.33 ± 0.05 for 24 hrs and 0.30 ± 0.01 for 48 hrs with 1000 mg/kg doses of chloroform extract of *Crateva magna*) in mice, as compared with (0.46 ± 0.08 24 hrs and 0.40 ± 0.02 for 48 hrs respectively) the standard drug Levamisole at a dose of 50 mg/kg (Table 2; Figure 4)

Table 1: Carbon clearance assay, HA titer and Neutrophil Adhesion test of Chloroform extract of *Crateva magna*.

Treatment	Carbon clearance assay	Haemeagglutination (HA) titre (µl)	% Neutrophil Adhesion
Control	0.02114 ± 0.0021	158.21 ± 0.0029	17.13 ± 0.09
+ Std (Levamisole 50 mg/kg)	0.04921 ± 0.0016**	532.37 ± 0.0029*	42.62±0.67**
- Std (Cyclophosphamide 2.5mg/kg)	0.01764 ± 0.0029	58.65 ± 0.0019**	11.13±0.21**
CH 250	0.02132 ± 0.0015**	236.36 ± 0.0028 ***	26.29 ±0.07**
CH 500	0.02971 ± 0.0012	337.13 ± 0.0015	30.07± 0.20**
CH 1000	0.03837 ± 0.0218***	389.21 ± 0.0012**	33.16±0.18***

All values are expressed as mean ± SEM of six observations. P value * <0.05, **< 0.01, *** < 0.001.

CH 250- Chloroform extract of *Crateva magna* bark at 250 mg/kg body weight, CH 500- Chloroform extract of *Crateva magna* bark at 500 mg/kg body weight and CH 1000- Chloroform extract of *Crateva magna* bark at 1000 mg/kg body weight.

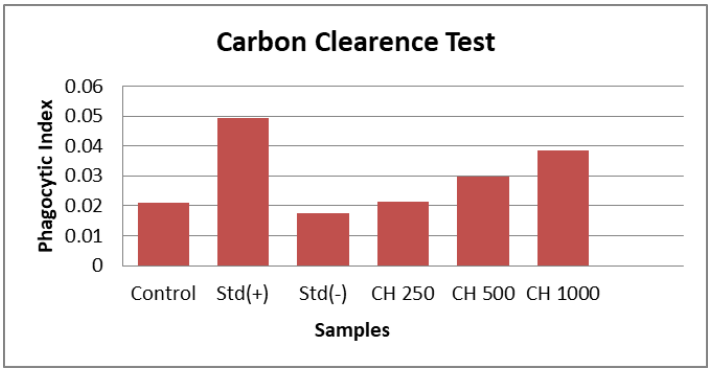

Fig. 1: Carbon clearance test for Chloroform extract of *Crateva magna* bark

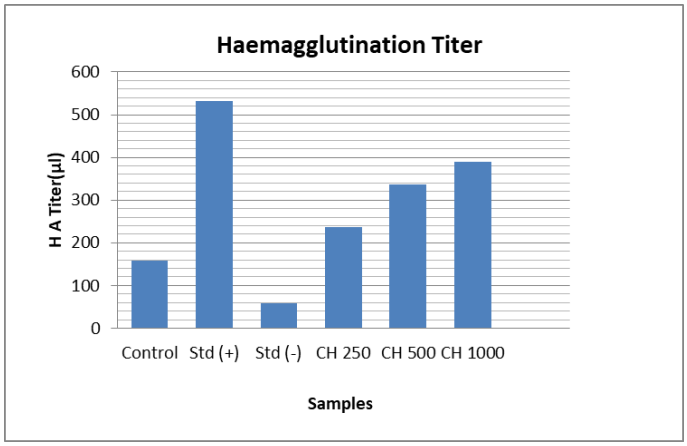

Fig. 2: Humoral antibody titer for Chloroform extract of *Crateva magna* bark

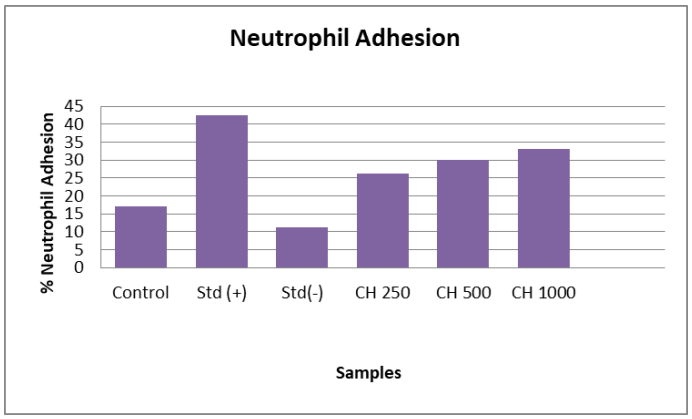

Fig. 3: Neutrophil adhesion (%) for Chloroform extract of *Crateva magna* bark

Table 2: Delayed type hypersensitivity reaction of Chloroform extract of *Crateva magna*

Treatment	Dose (mg/Kg)	Mean Right foot pad thickness (mm)	
		24 hrs	48 hrs
Control	-	0.15±0.05	0.15± 0.08
+ Std (Levamisole)	50	0.46 ± 0.08*	0.40 ± 0.02**
- Std (Cyclophosphamide)	2.5	0.13 ± 0.04**	0.13± 0.07*
CH 250	250	0.28 ±0.02	0.24 ± 0.03*
CH 500	500	0.30 ± 0.07**	0.27 ± 0.04*
CH 1000	1000	0.33 ± 0.05*	0.30 ± 0.01**

Statistical analysis was carried out employing the ANOVA.. *=P<0.05, **=P<0.01
CH 250- Chloroform extract of *Crateva magna* bark at 250 mg/kg body weight, CH 500- Chloroform extract of *Crateva magna* bark at 500 mg/kg body weight and CH 1000- Chloroform extract of *Crateva magna* bark at 1000 mg/kg body weight.

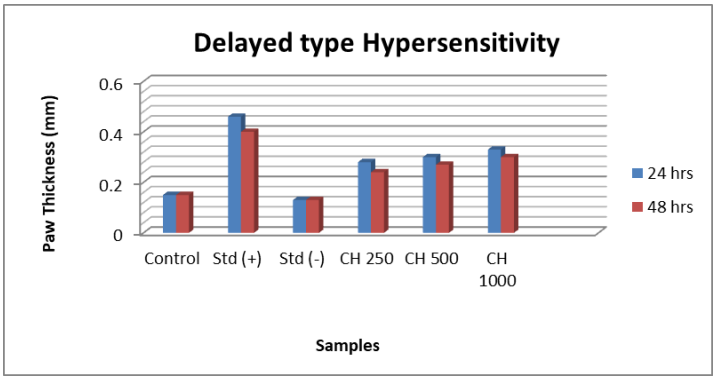

Fig. 4: Delayed type Hypersensitivity of Chloroform extract of *Crateva magna* bark

CONCLUSION

The results clearly indicate that the chloroform extract possess immune boosting properties in a dose dependent manner, and suggest usefulness in the disorder of immunological origin. However, mechanisms of immune-modulations are still to be investigated. The results moreover open scope for further exploration of the chloroform extract to isolate bioactive phyto-constituents with potent immunomodulatory activity.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research article.

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