

ORIGINAL ARTICLE

Immunomodulatory activity of Hot Aqueous Extract of *Anthocephalus cadamba* Leaves in Albino rats

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ABSTRACT

Present work has been designed to investigate immunomodulatory efficacy of hot aqueous extract (HAE) of *Anthocephalus cadamba* leaves. To study the effect of HAE of *Anthocephalus cadamba* leaves over cell mediated immunity of wistar albino rats, hypersensitivity (Type IV) model was being used using 1-chloro, 2, 4-dinitro chloro benzene (DNCB) as an allergen. Experimental animals were divided into four groups as control (group I), group II, group III and group IV. Measurement of skin thickness was done by using vernier caliper after sensitizing all animals with DNCB at different time intervals. Dose dependent enhanced cell mediated immune response was found. Significant increase ($p < .05$) in skin thickness at 24 hrs, 48 hrs and at 72 hrs with 500 mg/Kg b.wt of HAE was found. Rats fed with 500 mg/Kg b.wt of HAE exhibited significant enhancement in skin thickness by 42.85% at 24 hrs, 30.43% at 48 hrs and 18.75% at 72 hrs with respect to control (Group I) where as 250 mg/Kg b.wt of HAE showed increase in skin thickness by 23.8% at 24 hrs, 19.56% at 48 hrs and 12.5% at 72 hrs respectively.

Keywords: *Anthocephalus cadamba*, DNCB, Hot aqueous extract, cell mediated response

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INTRODUCTION

The immune system of the human is equipped to fight a wide array of potential pathogens. Host damage can occur directly by microbial factors or by host factors such as inflammatory responses or both [1]. For most host-microorganism interactions, apparent clinical damage/disease occurs at the extremes of the immune response. Therefore, modulation of host immune system to increase removal of infectious agents and reduce tissue damage due to inflammation is the fundamentally new approach for the cure of infectious disease [2]. All drugs which are used to modify immune response generally categorized as immunomodulators. In clinical perspective, immunomodulators can be classified as immunostimulants, immunoadjuvants and immunosuppressants [3]. Scientific concept of immunomodulation has been put forwarded, and it now appears to have some of the advantageous effects of Indian medicinal plants, proposed in Ayurveda by Charaka and Sushruta Samhita, may be due to these "immunomodulatory" effects [4]. Ayurveda termed 'Rasayanas' is dedicated to enhancement of body's resistance. Instead of directly neutralizing the infectious agents, Ayurvedic medicine boost body's natural resistance to disease causing agent. In practice, this is done by using various medicinal plant products called 'rasayanas' [5]. Several Indian medicinal plants which contain immunomodulatory activity have been identified and reviewed [3, 5].

The *Anthocephalus cadamba* is one of the important medicinal plants belonging to the *Rubiaceae* family. In traditional Indian system of medicine, it has been used to treat fever, uterine complaints, blood diseases, skin diseases, eye inflammation, anemia, diarrhea, leprosy, dysentery and stomatitis [6]. It has the largest number of phytochemicals and secondary metabolites (*viz.*, cadambagenic acid, cadamine, quinovic acid, β -sitosterol, cadambine, etc.) having various biological and pharmacological properties [7]. *Anthocephalus cadamba* has a wide spectrum of biological activities including anti-hepatotoxic activities [8], antioxidant [9], anti-inflammatory [10], anti-helminthic [11] and antimicrobial [12]. However, no

study to date has investigated the immunomodulatory role of *Anthocephalus cadamba*. Therefore an attempt has been made to investigate immunomodulating efficacy of this plants.

MATERIALS AND METHODS

Plant Material and extract preparation:

Leaves of *Anthocephalus cadamba* were procured from Vrindavan, Mathura (U.P) at the month of July 2015 and authenticated by Dr. A.S. Upadhye, Agharkar research institute, Pune with voucher deposition no.L-084 by. Authenticated fresh leaves were collected in mass, washed, dried under shade and grinded to obtain coarse powder. About 200-250 gm coarse powder of shaded dried leaves were subjected to hot aqueous extraction with triple distilled water in a soxhlet apparatus at 100°C for 2-3 hours. The extracted solution was evaporated in a rotary evaporator under controlled temperature and reduced pressure to get a dark brownish crystals (yield: 17-21%, w/v) and was stored at 4°C for further use.

Experimental animals:

Wistar albino rats of either sex weighing 60-80 gm were obtained from central animal house, GLA University, Mathura with GLAIPR/CPCSEA/IAEC/2014/Biotech02. Animals were provided with standard diet as prescribed by ICMR, water *ad libitum* and acclimatized to the environment at least one week before the start of experiment. Experiments were approved by "Institutional Animal Ethics Committee (IAEC)", Government of India, and research was conducted under their guidelines. 5 animals/test group/control were used.

Cell mediated immune response by DNCB hypersensitivity test:

This study was designed to assess the effect of HAE of *A. cadamba* on DNCB (1-chloro, 2, 4-dinitro chlorobenzene) sensitized albino rats by the method as described by [13]. Four groups having five rats in each were made. Group I served as control and was given triple distilled water. Group II, III and IV were fed orally with 125 mg/Kg b.wt, 250 mg/Kg b.wt and 500 mg/Kg b.wt of HAE of *A. cadamba* leaf extract respectively. Doses like 125 mg/Kg b.wt, 250 mg/Kg b.wt and 500 mg/Kg b.wt of HAE was found to be safe and non toxic [14].

Hairs from thigh of all albino rats of all groups were removed and cleaned properly. Animals of all groups were sensitized by 100µl of 0.2% DNCB on 7th day, 14th day and 17th day. DNCB challenge was done on 22nd day and measurement of thigh thickness was done using vernier caliper before challenge and after 24, 48 and 72 hrs post challenge. Sites of DNCB were examined for erythema, indurations and vesicle formation. With respect to control (group I) percentage change in skin thickness of animals of group II, III, and IV was calculated.

Statistical Analysis

Values are expressed as mean ± SEM. One way analysis of variance (ANOVA) using SPSS version 20.0 software and DMRT was used for data analysis and *p < .05 considered as significant.

RESULT AND DISCUSSION

Table 1. Mean values of skin thickness (mm) in rats of control and HAE fed groups at different time interval

Periods Hrs	Skin Thickness (mm)				Difference				Percentage increased as compared to control		
	Control	125 mg	250 mg	500 mg	Control	125mg	250mg	500mg	125mg	250mg	500mg
0	0.502 ±0.02	0.504 ±0.02	0.516 ±0.02	0.542 ±0.02	-	-	-	-	-	-	-
24	0.628 ±0.04	0.658 ±0.05	0.672 ±0.05	0.722 ±0.04	0.126	0.154	0.156	0.18	22.22	23.8	42.85*
48	0.594±0.05	0.61 ±0.05	0.626 ±0.04	0.662 ±0.04	0.092	0.106	0.11	0.12	15.21	19.56	30.43*
72	0.534±0.05	0.54 ±0.03	0.552 ±0.04	0.58 ±0.02	0.032	0.036	0.036	0.038	12.5	12.5	18.75*

The values represent the mean ± SEM of five animals in each groups. Statistical analysis through one way ANOVA followed by DMRT revealed that results are significant at *p < .05

Hypersensitivity test was performed using DNCB as an allergen and measured in terms of skin thickness. Mean value of skin thickness of rats of experimental (Group II, III and IV) and control groups was

calculated at 24, 48 and 72 hrs post challenge of DNCB. Among all the groups rats fed with 500 mg/Kg b.wt of HAE showed significant increase ($*p < .05$) in skin thickness as compared to control (Table no 1). Rats fed with 500 mg/Kg b.wt of HAE exhibited significant enhancement in skin thickness by 42.85% at 24 hrs, 30.43% at 48 hrs and 18.75% at 72 hrs with respect to control (Group I) where as 250 mg/Kg b.wt of HAE showed increase in skin thickness by 23.8% at 24 hrs, 19.56% at 48 hrs and 12.5% at 72 hrs respectively (Table no 1). Dose dependent increase in skin thickness was found with respect to control group (Fig. no 1). Significant increase ($*p < .05$) in skin thickness at 24 hrs, 48 hrs and at 72 hrs with 500 mg/Kg b.wt of HAE indicates increased cell mediated response. This study revealed the T-cell stimulating effect of HAE of *A. cadamba* leaves. Our findings on DTH are in affirmation with the finding of [14] suggested significant increase in total leukocytes count. [12] Suggested the presence of alkaloids, carbohydrates, tannins and phenolics compounds on HAE of *A. cadamba* leaves, which have been reported to have immunomodulatory activities [15].

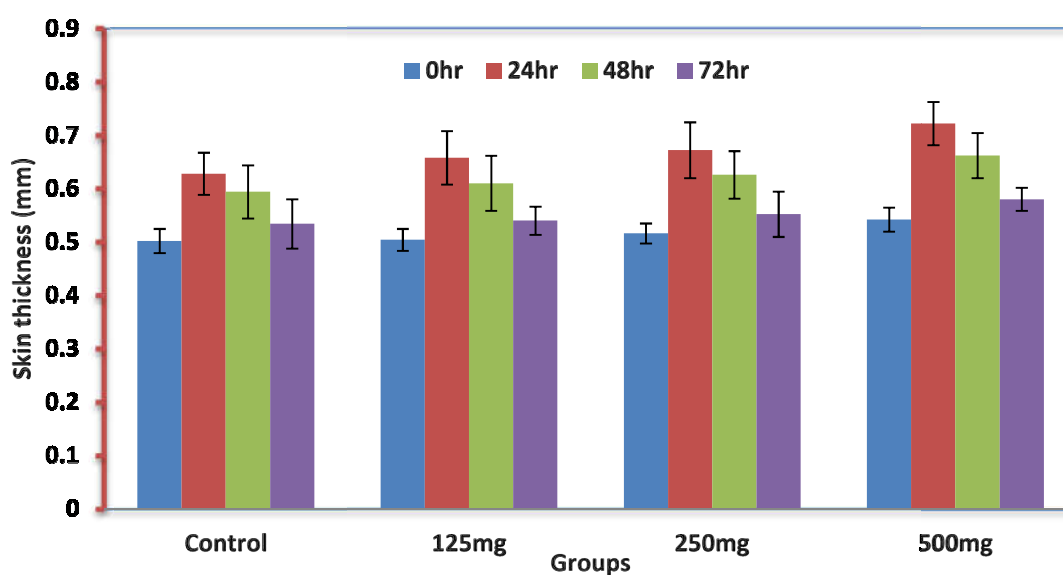


Figure 1- Skin thickness (mm) in rats of control and HAE fed groups

CONCLUSION

Present study concludes about enhanced cell mediated response and probable enhancement in immunomodulatory activity of aqueous extract of leaves of *A. cadamba* which needs to be further investigated as well as the cytokines responsible for this should be further studied.

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