

Evaluation of Immunomodulatory Effect of Aqueous Extract of *Bauhinia variegata* L. Leaves

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ABSTRACT

Natural products have the potential to modulate the immunity of human beings either by stimulating or depressing it and the approach was being used over time by medicinal practitioners to treat ailments. This present study was planned to ascertain the immunomodulating potential of one of the Indian traditional medicinal plants *Bauhinia variegata* L. The aqueous extract of powdered leaves of *Bauhinia variegata* L. was prepared by using the decoction method and the extract was then dried and made free from solvent. The extract of *Bauhinia variegata* L. (BVAE) was screened for immunomodulating activity by hemagglutination reaction for humoral and delayed-type hypersensitivity study for cellular immune responses. The sheep RBC (5×10^9 cells/mL) was used as antigen and levamisole was used as the standard drug in both studies. The extract of plant material (BVAE) showed a dose-dependent response in these studies. BVAE at 400 mg/kg dose among all the doses of plant material showed significantly ($p < 0.05$) high antibody titer 7.13 ± 0.17 in humoral immune response and potential delayed-type hypersensitivity response (0.96 ± 0.06) in the cellular immune study. Whereas, the standard drug levamisole showed the most significant ($p < 0.01$) higher antibody titer (7.87 ± 0.19) and delayed-type hypersensitivity response (1.24 ± 0.04). Though the standard drug showed a better response the plant material also showed the potential immunomodulatory effect in the present study. Hence, the findings of this study proposed the immunomodulatory effect of the aqueous extract of *Bauhinia variegata* L., and 400 mg/kg dose of the extract was found to be potent among other doses.

Keywords: *Bauhinia variegata*, Cellular immune response, Haemagglutination, Humoral study, Immunomodulation.

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INTRODUCTION

The immune system is a defense mechanism for all living organisms. Innate and adaptive, two types of immune systems are observed. The innate immune system is a natural non-specific immune mechanism initiated during the entry of any pathogens into the host body. Unlike the innate system, adaptive immunity is an antigen-specific immune mechanism that provides an immunologic memory to strengthen the defense system of the host.^{1,2} Further, adaptive immune systems are two types, humoral and cell-mediated immune responses which are facilitated by B and T lymphocytes, respectively.^{3,4} In healthy conditions, the homeostasis of the immune system is maintained but during pathological conditions cells and chemokines trigger the innate and/or adaptive immune system to control the disorders. Nevertheless, in several disease conditions, like viral infections, cancer, HIV, etc., the natural body defense system failed to

fulfill the desired purposes.⁵ In such conditions additional immunomodulators are required.

Immunomodulators are natural or synthetic agents which modulate the immune system either by stimulation or suppression to treat disorders.⁶ The adaptive immune system plays an important role in immunomodulation as it provides antigen-specific immunological memory which is found to be useful in the treatment process. Though, there are several immunomodulator drugs are available, natural immunomodulating drugs are found to be safe and have the potential for the reason of their synergistic effect.⁷ Additionally, the search for effective botanical immunomodulators is always an interest to the scientist.

India has age-old traditional healthcare systems which provide great knowledge about local natural products for the treatment of various diseases. Ayurveda, the ancient Indian medicinal system provides information about several natural immunomodulatory drugs under the Rasayana treatment process.⁸ *Bauhinia variegata* L., a traditional Indian plant is usually called "Raktakanchana".⁹ Previous studies reported anti-inflammatory, anticarcinogenic, cytotoxic antimicrobial, antioxidant and hepatoprotective



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activities of the plant.¹⁰⁻¹⁴ Authors of the present study have also reported their previous works on the antioxidant and anti-inflammatory activities of the plant.^{15,16} Hence, this study was designed to ascertain the immunomodulatory activity of the aqueous extract of leaves of *Bauhinia variegata* L., an Indian traditional medicinal plant.

MATERIALS AND METHODS

Chemicals and drugs

Analytical-grade materials were used in this experiment. Standard immunostimulant drug, Levamisole tablets "DICARIS" was procured from Encore Healthcare Pvt. Ltd, Paithan. 0.9% NaCl, was procured from MERCK Ltd, Mumbai. Ether for Anesthesia was procured from Qualigens Fine Chemicals, Mumbai. Carboxymethyl cellulose (CMC) was obtained from Loba Chemie Pvt. Ltd., Mumbai.

Collection of plant material

Leaves of *Bauhinia variegata* were collected from Paneer, Deralakatte, Karnataka, India and the identity of the plant was authenticated by Dr. Noeline J. Pinto, Head of Botany, Department St. Agnes College, Mangaluru. A specimen (no. 324 b) was submitted to the institute.

Preparation of aqueous extract

Collected leaves were dried under shade and powdered. The powdered leaves were extracted by boiling them with distilled water and then filtered. Four times the process was repeated. The resultant extract was concentrated in the water bath and further made into a dry extract. This aqueous extract (BVAE) was further used in the study to screen for the immunomodulatory effect of the plant material.

Pharmacological activity studies

Animals

Swiss albino mice of weight between 15-20g and of male and female both sex were obtained from KSHEMA (K. S. Hegde Medical Academy), Deralakatte Mangaluru. Acute toxicity and immunomodulatory activity studies of the plant drug were carried out by using these animals. Animals were acclimatized for one week in cages in 12 hr light and dark cycle at room temperature and $60 \pm 5\%$ relative humidity. Animals of the present study were fed with pellets which were supplied by Hindustan Lever Co., Mumbai, and water *ad libitum* during the period of the study. Experiments of the present study were conducted as per the institutional animal ethics committee guidelines, KSHEMA, Deralakatte, Mangaluru (KSHEMA /AEC/077/2008).

Acute toxicity study

The toxicity study of BVAE was carried out in adult female albino mice by the "up and down" method of OECD guidelines 425 and the test drug was found to be safe till a higher dose.¹⁷ Based on the toxicity study, 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, per oral doses of plant material (BVAE) were decided for the immunomodulatory activity studies.

Immunomodulatory effect of aqueous extract of *Bauhinia variegata* L. (BVAE)

The immunomodulatory activity of BVAE was evaluated by *in vivo* models, humoral study, and delayed-type hypersensitivity study models in mice.

Experimental antigen

The antigen of the present study sheep red blood cells/sheep RBC (SRBC) was collected as per the requirement freshly in the container containing Alsever's solution. The SRBC was centrifuged three times with normal saline (0.9% NaCl w/v) at 2000 rpm for 10 min at 20°C. Phosphate buffer saline (pH 7.4) was mixed with SRBC to adjust the final volume. 20 microliters of SRBC (5×10^9 cells/mL) were administered intraperitoneally (i.p) to animals for immunization and challenge as per the schedule.

Group design

For each study, five groups of animals (Swiss albino mice) were made and each group contains six animals ($n=6$) as follows:

Sensitized control group: Immunized animals of this group received only 1mL of 2% solution of CMC (vehicle).

BVAE 100, 200, 400 mg/kg group: Immunized animals of these groups received respectively 100 mg/kg, 200 mg/kg, 400 mg/kg b.w., p.o. dose of BVAE as a suspension in the vehicle.

Standard group: Immunized animals of this group were treated per day with 2.5 mg/kg b.w., p.o dose of suspension of standard drug Levamisole in the vehicle.

Humoral study: Hemagglutination reaction model

Animals of all groups were immunized with 0.2mL of 5×10^9 SRBC/mL SRBC by injecting intraperitoneally (i.p) on day 0. From day 1 to day 14 animals of the individual group as explained in the group study received oral treatment of respective drugs and blood was collected from those animals on the 15th day of the study to check primary humoral immune responses. On the same day, all animals were further received 0.2mL SRBC intraperitoneally (i.p) and kept under observation till day 21 of the study. To check secondary humoral immune responses blood was collected from animals of all groups on day 21. In the present study, the hemagglutination reaction method i.e., anti-SRBC antibody titer,

was used to measure humoral response as described by Nelson and Midenhall (1967).¹⁸

Cellular immune response study: Delayed type hypersensitivity (DTH) model

A delayed-type hypersensitivity model in the present study was carried out as described by Doherty, to evaluate the cellular immune response or protective immune response of BVAE.¹⁹ On day 0, 5×10^9 cells of SRBC/mL and saline respectively were injected subcutaneously (S.C.) in the footpad of the left hind paw and the right hind paw of animals of all groups. From day 1 to day 14 animals of the individual group as explained in the group study received oral treatment of respective drugs and on day 15, animals were challenged with SRBC. After 24 hr of the challenge, the paw thickness of animals was measured by using digital vernier calipers.

Statistical Analysis

All results were expressed as mean \pm S.E.M. ANOVA, followed by Dunnet's *t*-test was used to determine statistical significance and the results with *p*-value < 0.05 were considered statistically significant.

RESULTS

The acute oral toxicity study of aqueous extract of *Bauhinia variegata* L. leaves was carried out for up to 2000 mg/kg dose as per OECD guidelines. The test drug was found to be safe in the study up to a higher dose. Hence, 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, per oral dose of the plant drug were chosen for further studies. The immunomodulatory activity of BVAE was evaluated by humoral and cell-mediated immune response study models.

Humoral study

BVAE showed a dose-dependent immune response in the present study. The 400 mg/kg dose of BVAE showed a significant

($p < 0.05$) and the most potent humoral response (7.13 ± 0.17) among the test materials (Table 1 and Figure 1). Standard drug levamisole showed a significant ($p < 0.01$) effect (7.87 ± 0.19) in the study. BVAE at 100 and 200 mg/kg doses also showed humoral response but the result was found to be insignificant. Concerning the percentage of immunomodulatory effect, the standard drug showed the highest 37.83% effect (Table 1) but the response of the BVAE with 400 mg/kg dose was also found to be nearby (24.87%).

Cellular immune response study

As shown in Table 1, in the present study, the standard drug, levamisole showed a potent delayed-type hypersensitivity response ($p < 0.01$, 1.24 ± 0.04) with 58.97% immunomodulatory effect (Figure 2). A dose-dependent response for BVAE was observed in this study also and 400 mg/kg dose showed a higher response ($p < 0.05$, 0.96 ± 0.06) with 23.07% immunomodulatory effect among all the test materials (Table 1 and Figure 2).

DISCUSSION

Bauhinia variegata L. is a widely available Indian traditional medicinal plant. As we discussed in the introduction part of this article, the anti-inflammatory, antidiabetic, antioxidant, anti-carcinogenic, and hepatoprotective activity of the plant was reported earlier in several studies. The present study was designed to evaluate the immunomodulatory activity of the plant as there are no studies that have reported so far, the above-mentioned activity of the leaves of *Bauhinia variegata* L.

Adaptive immunity is a common phenomenon for the defense mechanism of the human body against antigens. The immune mechanism either triggers antibody development and/or apoptosis as a cell-mediated response to defend the body against foreign pathogens.^{20,21} The natural adaptive system can be modified and improved by immunomodulating agents. In this context, a natural product was evaluated for immunomodulating activity in this study.

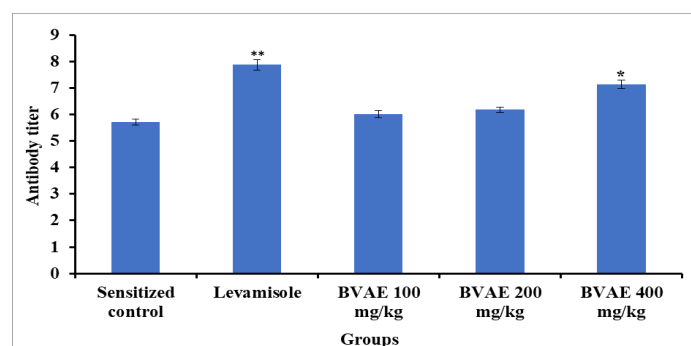


Figure 1: Immunomodulatory effect of aqueous extract of leaves of *Bauhinia variegata* L. by *in vivo* models for humoral immune response study. Values are mean \pm S.E.M for each group containing six animals ($n=6$); *indicates the *p*-value is < 0.05 and ** indicates the *p*-value < 0.01 in comparison with the sensitized control group. BVAE: aqueous extract of leaves of *Bauhinia variegata* L.

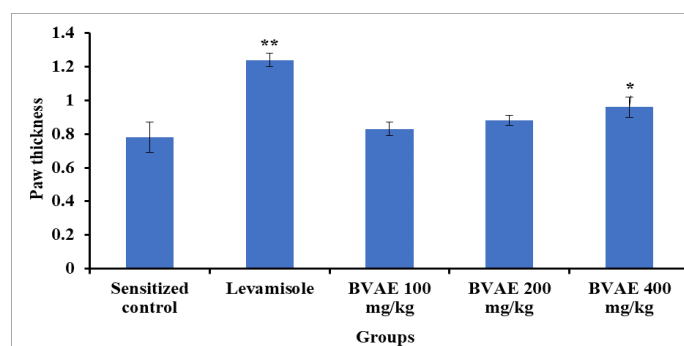


Figure 2: Immunomodulatory effect of aqueous extract of leaves of *Bauhinia variegata* L. by *in vivo* models for delayed-type hypersensitivity study. Values are mean \pm S.E.M for each group containing six animals ($n=6$); *indicates the *p*-value is < 0.05 and ** indicates the *p*-value < 0.01 in comparison with the sensitized control group. BVAE: aqueous extract of leaves of *Bauhinia variegata* L.

Table 1: Immunomodulatory activity of aqueous extract of leaves of *Bauhinia variegata* L. by *in vivo* models for humoral and cellular immune response study.

Group	Humoral study		Cellular immune response	
	Antibody titer	Immunomodulatory activity (%)	Paw thickness	Immunomodulatory activity (%)
Sensitized control	5.71±0.12	-	0.78±0.09	-
Levamisole	7.87±0.19	37.83**	1.24±0.04	58.97**
BVAE 100 mg/kg	6.02±0.14	5.42	0.83±0.04	6.41
BVAE 200 mg/kg	6.18±0.10	8.23	0.88±0.03	12.82
BVAE 400 mg/kg	7.13±0.17	24.87*	0.96±0.06	23.07*

Values are mean ± S.E.M for each group containing six animals ($n=6$); *indicates the p -value is <0.05 and ** indicates the p -value <0.01 in comparison with the sensitized control group. BVAE: aqueous extract of leaves of *Bauhinia variegata* L.

The formation of antigen-specific antibodies takes place in B-lymphocyte cells. This humoral immune response takes certain days to develop, hence the study period used to be kept for 21 days to assess the response of the test drugs.²² The humoral immune response of the test drugs is evaluated by the antigen-antibody interactions. In the *in vivo* immunomodulatory evaluation study, the haemagglutination reaction is being used for assessing antigen and antibody interactions and the titer value of the reaction is considered to be the humoral response.²³ In the present study, the aqueous extract of leaves of *Bauhinia variegata* L. (BVAE) at 400 mg/kg dose showed a 24.87% humoral immunomodulatory effect with the titer value of 7.13±0.17 and the result were found to be most potent among all the other doses of the test material.

The immune response of the test material in the humoral study was further corroborated by the protective immune response study. The protective or cellular immune response of the drug was assessed by a delayed-type hypersensitivity study. T lymphocytes are the marker for cellular immune response and the previous studies reported its correlation with B cells for humoral responses.²⁴ BVAE at 400 mg/kg dose showed a 23.07% immunomodulatory effect in the cell-mediated immune response study, whereas, levamisole showed a 58.97% response in the study. Hence the present study showed the immunomodulatory effect of the aqueous extract of leaves of *Bauhinia variegata* L.

CONCLUSION

The present study was planned to evaluate the immunomodulatory effect of leaves of *Bauhinia variegata*. The results of the study showed the potent effect of plant material in humoral and cellular immune response studies. Doses of aqueous extract of leaves of *Bauhinia variegata* were decided by performing an acute toxicity study as per the standard guideline and the test material with all those doses was used for further immunomodulatory activity studies. The test material showed a dose-dependent immunomodulatory response in studies and 400mg/kg dose of BVAE was found to be the most potent among all the test materials. Hence the finding suggested the immunomodulatory activity of leaves of *Bauhinia variegata* L. which also supported

the hypotheses of the present study. Nevertheless, further in-depth studies are required to be carried out to find the possible mechanism of action and phytoconstituent of the drug which is responsible for the above activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

b.w.: Body weight; p.o.: Per oral.

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