

# Immunomodulatory Effect of *Decalepis hamiltonii* Wight and Arn Roots Extract on Rodents

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## ABSTRACT

**Background:** *Decalepis hamiltonii* Wight and Arn (Asclepiadaceae) commonly known as 'nannari' is an Indian medicinal plant which is having ethno-medicinal uses as antioxidant, tonic and blood purifier. It is used in many Ayurvedic preparations like *Drakshadi churna* (general vitalizer), *Shatavari rasayana* (adaptogenic), and *Yeshtimadhu taila* (mild analgesic, rheumatism). Aim of the present study is to evaluate the immunomodulatory activity of *D. hamiltonii* roots aqueous extract in rodents. **Methods:** *D. hamiltonii* aqueous extract (DHAE) was prepared by cold maceration. Two doses of DHAE (100 and 200 mg/kg, *per os*) were used to evaluate immunomodulatory activity *in vivo* and *ex vivo* through assessments of hemagglutination antibody titers, hematological profiles (Hb, WBC, RBC), delayed-type hypersensitivity (DTH), neutrophil adhesion, and carbon clearance. **Results:** DHAE at both dose levels evoked significant dose-dependent increases in antibody titers and DTH reactions induced by SRBC, and potentiated percentage neutrophil adhesion to nylon fibers as well as phagocytosis of carbon particles *in situ*. **Conclusion:** The evidence collected here lends support to traditional claims behind the use of *D. hamiltonii* for medicinal purposes. The study also affirmed that DHAE imparts immunomodulatory activity. The DHAE not only potentiated non-specific immune responses, but also was effective in improving immune responses in cyclophosphamide-immunosuppressed hosts. The results here indicate that *D. hamiltonii* root extracts stimulates both cellular and humoral immune responses.

**Key words:** Nannari, Cyclophosphamide, Hemagglutination antibody titer, Neutrophil adhesion test, Carbon clearance assay.

## INTRODUCTION

Immune system dysfunction is responsible for various diseases including ulcerative colitis, arthritis, allergy, asthma, parasitic diseases, multiple sclerosis, cancer and infectious diseases.<sup>1</sup> Oxidative processes is interlinked with many pathological conditions including host immune immune system, with high oxidative stress usually breaking down the immune system.<sup>2</sup> Modulation of immune responses to alleviate such diseases has been of great interest for many years. Immunologic means of controlling disease has two objectives i.e. development of immunity by the stimulation of the immune system

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and suppression of undesired immune reaction. In this regard, the traditional medicines play an important role in strengthening or suppressing the host immune response. Thus, medicinal plants and their active components as a source of immunomodulatory agents are gaining importance.<sup>3,4</sup>

Many herbs such as *Azadirachta indica*,<sup>3</sup> *Centella asiatica*,<sup>5</sup> *Asparagus racemosus*,<sup>6</sup> *Phyllanthus debelis*<sup>7</sup> and *Chenopodium ambrosioides*<sup>4</sup> have been shown to alter immune functions and to possess a wide array of immunomodulatory effects. *Decalepis hamiltonii* Wight and Arn (Asclepiadaceae) is a mono-generic climbing shrub of Western India. It is commonly known as 'swallow root' and is used in traditional medicine as blood purifier, appetizer, and bacteriostatic and in many Ayurvedic preparations. The root of *D. hamiltonii* is rich in sterols, lupeol, quercetin, kaempferol, coumarin, rutin, 4-O-methyl-resorcyllaldehyde, a- and b- amyryns, and lupeol.<sup>8</sup> Each of these agents has been shown to good antioxidant activity *in vivo*. In addition, the plant has been reported to impart pharmacological effects and so act as an anti-oxidant<sup>9</sup> and a preventative against hepatic and gastric toxicities.<sup>10</sup> The studies reported herein were undertaken to investigate whether *D. hamiltonii* might also impart beneficial (or conversely, toxic) effects on the immune system of experimental animals.

## MATERIALS AND METHODS

### Chemicals

Cyclophosphamide was bought from Endoxane (Cadila Healthcare Ltd., Ahmedabad, India). Ascorbic acid, WBC and RBC diluting fluid and ferric chloride were obtained from Sd Fine-Chemicals Ltd. (Mumbai, India). Both carbon ink suspension and Leishman's stain were purchased from, respectively, Pelican AG (Mumbai) and Span Diagnostics Ltd., (Pelican AG, Surat, India). All chemicals used were of analytical grade.

### Plant material and extraction procedure

The roots of *D. hamiltonii* Wight and Arn were collected from Devaranadurga, (Tumkur, India). The plant was identified and authenticated by Prof. K. Siddappa, Department of Botany, Sree Siddaganga College of Arts, Science and Commerce (Tumkur) and a voucher specimen placed in the college herbarium (SSCYP11PC0003). The roots were washed thoroughly with tap-water, air-dried, and then powdered. *D. hamiltonii* root powder ( $\approx$  500 g) was then extracted with water using a cold maceration process for 21 days. The material that resulted (DHAE) was then concentrated to yield a dark brown residue (yield: 20.88% w/w).

### Animals

Healthy adult male albino mice (25-35 g) and Wistar rats (160-250 g) raised in animal facilities at Sree Siddaganga College of Pharmacy (Tumkur)-were used in these studies. All animals were housed in pathogen-free facilities maintained at  $25 \pm 3^\circ\text{C}$  with a 45-65% relative humidity and a 12 h light:dark cycle; each had *ad libitum* access to standard rodent chow and water. All procedures performed herein were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, under Ministry of Animal Welfare, Government of India, New Delhi. The Institutional Animal Ethics Committee (SSCYPT/IAEC Clear/76/2010-11) approved all experimental protocols.

### Effect of *D. hamiltonii* root extract on humoral immune response

#### Antigen and immunization

Sheep red blood cells (SRBC) were used as an antigenic material. SRBC were obtained from a local slaughterhouse. The SRBC were washed three times in pyrogen-free sterile normal saline by centrifugation at  $3000 \times g$  for 10 min each cycle. The washed SRBC were then adjusted to a 20% [v/v] concentration with normal saline.

#### Treatment protocol

A chronic treatment specific immune response model was used to assess effects of DHAE on humoral immunity. The Wistar rats were divided into six groups (I-VI; n=6/group): Group I=normal control; Group II=extract (DHAE) control; Group III=SRBC control; Group IV=cyclophosphamide (CYP) control; Group V: 100 mg DHAE/kg, and Group VI: 200 mg DHAE/kg. All groups were treated daily by gavage for 21 days; rats in Groups I, III and IV received vehicle (distilled water) at 2 ml/kg/d, rats in Group V received DHAE 100 mg/kg/d, and rats in Groups II and VI received DHAE 200 mg/kg/d.

To assess any impact of treatment on host humoral immunity, rats in Groups III - VI were immunized (Day 7) and challenged (Day 14) IP with 0.1 ml of the freshly prepared 20% [v/v] SRBC suspension.<sup>11</sup> On Days 9 and 16, rats in Group III were also treated (IP) with 1 ml saline/kg while rats in Groups IV, V, and VI received 50 mg CYP/kg (Table 1). On Days 7, 9, 14, and 16, rats in Groups I and II were IP-injected only with saline. On Days 14 and 21, blood samples were collected from the retro-orbital plexus of each rat for use in evaluating hemagglutination antibody (HA) titre.<sup>12</sup> Serum was

separated from blood by centrifugation at 10000 rpm for 10 min at 4°C and used for HA titer to determine the antibody levels. To determine HA titer 96 wells 'U' bottom microtitre plates, were used. Each well of well microtitre plate was initially filled with 25 µl of normal saline and than 25 µl of serum was mixed in the first well of the microtitre plate. Two rows i.e. 24 wells were used for the dilution of each serum sample. From each diluted serum sample 25 µl was removed from the first well and added to the next well to get twofold dilutions of the antibodies present in the anti- serum. Further twofold dilutions of this diluted serum were carried out till the last well of the second row, so that the antibody concentration of any of the dilutions is half of the previous dilution. 25 µl of 1% v/v SRBC was added to each well and the microtitre plates were incubated at 37°C for one h and then observed for hemagglutination. The highest dilution giving hemagglutination was taken as the antibody titre. The antibody titre was expressed in a graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significance. The HA titers obtained with Day 14 and Day 21 blood samples were considered reflective of the primary and secondary humoral immune responses, respectively.

### CYP-induced myelosuppression model

Rats were divided into five groups (n=6/group): Groups I (normal control) and III (CYP control) received vehicle (distilled water, 2 ml/kg); Group IV=100 mg DHAE/kg; Groups II and V, respectively, DHAE control and DHAE hi-dose (i.e., each 200 mg DHAE/kg). Rats received each treatment daily by gavage for 10 days.

After respective above oral treatments, every day rats in Groups I and II received normal saline (0.1 ml, i.p.) and those in Groups III-V (30 mg CYP/kg, IP) shown in Table 2. At the end of treatment schedule blood samples were collected from animals of all groups and used for the evaluation of haematological profile such as hemoglobin (Hb), WBC and RBC. Specifically, Hb was estimated by the acid hematin method, while WBC and RBC levels were estimated by hemocytometry.<sup>13</sup>

### Effect of *D. hamiltonii* root extract on cellular immune response

#### Delayed-type hypersensitivity

Delayed-type hypersensitivity (DTH) was assayed by the method of Ghaisas *et al.* (2009).<sup>12</sup> In brief, rats were divided into five groups (n=6): I=normal control; II=extract control; III=immunosuppressed control; IV=DHAE 100 mg/kg; and, V=DHAE 200 mg/kg. Rats in Groups I and III were treated with vehicle

(2 ml/kg/d). Rats in Groups II and V were treated with DHAE 200 mg/kg/d and those in Group IV with DHAE 100 mg/kg/d. all these treatment were given orally for 21 days. On Day 14 of the experiment-except for rats in Group II-all rats were IP challenged with 0.1 ml of the 20% SRBC suspension. Two h later, except for rats in Group I, all rats received an IP injection 50 mg CYP/kg. On Day 21 of the experiment, all rats received a subcutaneous injection of 0.1 ml SRBC suspension (1% [v/v]) into the sub-plantar region of their right hind paw; 0.1 ml saline was injected into the left hind paw. Twenty-four h later (on Day 22), footpad reactions were assessed via measures of paw volume with a digital plethysmometer (IITC life Sciences). All values were expressed as % difference between the right and left footpad volumes.

### Neutrophil adhesion

The neutrophil adhesion test was assessed to determine the effect of DHAE on migration of neutrophils towards the site of inflammation.<sup>14</sup> Rats were divided into three groups (n=6/group): Group I=distilled water 2 ml/kg/d, for 7 d (normal control); and, Groups II and III=DHAE at, respectively, 100 and 200 mg/kg/d, for 7 d (treatments). On Day 7, blood samples from all rats were collected from the retro-orbital plexus and subjected to total leukocyte (TLC) and differential leucocytes (DLC) count analyses. These measures were done both before and after incubation of samples with nylon fibers (80 mg/ml blood, 15 min, 37°C). The neutrophil index and percent neutrophil adhesion were calculated as: Neutrophil index=TLC X % neutrophils.

$$\text{Neutrophil adhesion (\%)} = \frac{N_{Iu} - N_{It}}{N_{Iu}} \cdot 100$$

Where, NI<sub>u</sub>=Neutrophil index of untreated blood sample (i.e., before incubation with nylon fibers), and NI<sub>t</sub>=Index of treated blood sample (i.e., after incubation).

### Carbon clearance

Carbon clearance *in situ* was evaluated to determine effect of DHAE on phagocytosis.<sup>12</sup> Mice were divided into three groups (n=6/group): Group I=distilled water 2 ml/kg/d by gavage, for 5 d (normal control); and, Groups II and III=DHAE 100 and 200 mg/kg/d, respectively, for 5 d. After 48 h (i.e. Day 7) of the experiment, all mice were injected in the tail-vein with dilute carbon ink (1:8, in saline) at 10 µl/g BW. Blood samples (25 µl) were taken from the retro-orbital plexus at 0 and 15 min post-injection. The recovered blood was lysed in 3 ml of 0.1% [w/v] sodium carbonate solution and the optical density of each sample then measured at 660 nm

**Table 1: Effect of *D. hamiltonii* on humoral immune response in CYP-immunosuppressed rats**

Groups	Treatment and dose			Hemagglutination antibody titer	
	For 21 days (per os, Kg <sup>-1</sup> )	Days 7 & 14 (0.1 ml, IP)	Days 9 & 16 (IP, Kg <sup>-1</sup> )	Primary (Day 14)	Secondary (Day 21)
Normal control	Vehicle (2 ml)	Saline	Saline (1 ml)	7.4 ± 0.24	15.8 ± 0.58
DHAE control	DHAE (200 mg)	Saline	Saline (1 ml)	9.5 ± 0.43	18.3 ± 0.56
SRBC control	Vehicle (2 ml)	SRBC	Saline (1 ml)	<sup>a</sup> 12.0 ± 0.45	<sup>a</sup> 19.8 ± 0.87
CYP control	Vehicle (2 ml)	SRBC	CYP (50 mg)	<sup>c</sup> 4.5 ± 0.67	<sup>a,c</sup> 5.3 ± 0.56
DHAE 100	DHAE (100 mg)	SRBC	CYP (50 mg)	<sup>b</sup> 9.8 ± 0.48	<sup>b</sup> 18.7 ± 0.33
DHAE 200	DHAE (200 mg)	SRBC	CYP (50 mg)	<sup>a,b</sup> 14.3 ± 0.49	<sup>a,b</sup> 21.67 ± 0.42

Values are expressed in mean (n=6/group) ± SEM.

<sup>a</sup>p<0.001 vs. normal control, <sup>b</sup>CYP control, or <sup>c</sup>SRBC control.

**Table 2: Effect of *D. hamiltonii* on hematological profile in CYP-immunosuppressed rats**

Group	Treatment/kg Oral IP	Hb (g%)	WBC (x 10 <sup>3</sup> /μl)	RBC (x 10 <sup>6</sup> /μl)
Normal control	Vehicle (2 ml)+Saline (1 ml)	9.37 ± 0.37	5.71 ± 0.57	8.67 ± 0.25
DHAE control	DHAE (200 mg)+Saline (1 ml)	<sup>a</sup> 13.63 ± 0.23	6.89 ± 0.32	<sup>a</sup> 6.50 ± 0.30
CYP control	Vehicle (2 ml)+CYP (50 mg)	<sup>b</sup> 7.70 ± 0.17	<sup>a</sup> 1.24 ± 0.07	<sup>a</sup> 4.91 ± 0.06
DHAE 100	DHAE (100 mg)+CYP (50 mg)	<sup>a,c</sup> 12.43 ± 0.20	<sup>a,c</sup> 9.25 ± 0.13	<sup>a,d</sup> 6.30 ± 0.25
DHAE 200	DHAE (200 mg)+CYP (50 mg)	<sup>a,c</sup> 14.87 ± 0.28	<sup>a,c</sup> 9.18 ± 0.26	<sup>c</sup> 8.87 ± 0.19

Values are expressed in mean (n=6/group) ± SEM.

<sup>a</sup>p<0.001 and <sup>b</sup>p<0.05 vs. normal control.

<sup>c</sup>p<0.001 and <sup>d</sup>p<0.05 vs. CYP control.

**Table 3: Effect of *D. hamiltonii* on cellular immune response in CYP-immunosuppressed rats**

Group	Treatment/kg Oral IP IP	% edema increase
Normal control	Vehicle (2 ml)+SRBC+Saline	127.0 ± 4.12
DHAE control	DHAE (200 mg)+Saline+CYP (50 mg)	<sup>a</sup> 218.7 ± 7.67
CYP control	Vehicle (2 ml)+SRBC+CYP (50 mg)	<sup>b</sup> 68.7 ± 11.24
DHAE 100	DHAE (100 mg)+SRBC+CYP (50 mg)	<sup>a,c</sup> 183.1 ± 16.37
DHAE 200	DHAE (200 mg)+SRBC+CYP (50 mg)	<sup>c</sup> 251.8 ± 17.90

Values are expressed in mean (n=6/group) ± SEM.

<sup>a</sup>p<0.001 and <sup>b</sup>p<0.05 vs. normal control.

<sup>c</sup>p<0.001 vs. CYP control.

**Table 4: Effect of *D. hamiltonii* on neutrophil (PMN) adhesion by cells from normal rats**

Group	Sample	TLC	% PMN	PMN index	% PMN adhesion	Phagocytic index (K)
Normal control	UB	6.0 ± 0.8	19.0 ± 2.5	119.4 ± 28.5	27.6 ± 1.8	11.6 ± 0.8
	NFTB	4.8 ± 0.8	13.8 ± 2.6	85.6 ± 22.0		
DHAE (100 mg/kg)	UB	6.6 ± 1.0	<sup>b</sup> 17.8 ± 2.0	<sup>b</sup> 122.2 ± 27.1	<sup>c</sup> 40.3 ± 4.5	<sup>c</sup> 32.2 ± 5.2
	NFTB	5.5 ± 0.9	11.5 ± 1.5	<sup>b</sup> 78.2 ± 20.5		
DHAE (200 mg/kg)	UB	<sup>b</sup> 7.2 ± 1.2	18.2 ± 1.0	<sup>b</sup> 131.1 ± 29.4	<sup>a</sup> 47.3 ± 1.9	<sup>a</sup> 46.1 ± 6.4
	NFTB	<sup>b</sup> 6.7 ± 1.2	<sup>b</sup> 14.3 ± 1.3	67.2 ± 13.0		

Values are expressed in mean ± SEM.

<sup>a</sup>p<0.001, <sup>b</sup>p<0.01, and <sup>c</sup>p<0.05 vs. normal control.

TLC=Total leukocyte count (x 10<sup>3</sup>, cells/mm<sup>3</sup>); UB=untreated blood; NFTB=nylon fiber treated blood.

in a UV spectrophotometer (LABINDIA UV 3000<sup>+</sup>). Phagocytic index (K) was calculated as:

$$K = \frac{\ln OD1 - \ln OD2}{t2 - t1}$$

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

### Statistical analysis

All data were expressed as mean  $\pm$  SEM. Parameters such as humoral immune response, cellular immune response, and hematological profile, were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison test. The neutrophil adhesion test and carbon clearance assay data were analyzed by one-way ANOVA followed by a Dunnett's multiple comparison test using Graph Pad Prism version 5.0, USA. A p-value <0.05 was considered significant.

## RESULTS

### Hemagglutination antibody titer

Administration of SRBC alone to the animals led to significant increases in primary (62.16%) and secondary (25.31%) HA titer values as compared to non-SRBC-injected controls (Table 1). In the case of CYP + SRBC hosts, the titres were significantly (62.5% and 73.23%, respectively) decreased compared to those in rats that received only the SRBC. Pre-treatment with DHAE at either dose led to significant increases in each titer value compared to those in their CYP immunosuppressed counterparts.

### Hematological profile by Hb, WBC and RBC estimations

Treatment with DHAE at 200 mg/kg produced statistically significant increases in Hb levels and decrease in RBC values, concurrent with an insignificant increase in WBC content (Table 3). Among CYP-only-treated animals, there were significant decreases in WBC, RBC, and Hb in the blood. Pre-treatment of CYP-treated hosts with DHAE at either test dose led to significant rebounds in Hb, WBC and RBC levels.

### Cellular immune response by delayed type of hypersensitivity reaction

There was a significantly ( $p < 0.05$ ) decrease in DTH reactions in the CYP control rats compared to that seen with the normal controls (Table 3). Pre-treatment with DHAE at either test dose significantly ( $p < 0.001$ )

increased these responses when compared against those due to CYP alone.

### Neutrophil adhesion

Adhesions of neutrophils to nylon fibers in control animal blood was 27.58 [ $\pm$  1.80] %. In comparison, pre-treatment with DHAE at 100 or 200 mg/kg increased neutrophil adhesion to, respectively, 40.27 [ $\pm$  4.47] and 47.33 [ $\pm$  1.91] ( $p < 0.05$  and  $p < 0.001$ , respectively) (Table 4).

### Carbon clearance

Changes in macrophage function were assessed via measures of their phagocytic ability *in situ*, specifically by the rate of carbon particle removal from the bloodstream. DHAE treatments resulted in stimulation of macrophage activity as evidenced by increased phagocytic indices (Table 4). The phagocytic index was significantly ( $p < 0.05$  and  $p < 0.001$ ) increased at the doses of 100 and 200 mg DHAE/kg, respectively when compared to the values obtained with the normal control hosts.

## DISCUSSION

The immune system is designed to protect the host from invading pathogens and to eliminate disease. Protection from infection and disease is provided by innate and the adaptive immune systems. The innate immune system is the first line of defense.<sup>15</sup> The major effectors of innate immunity are complement, granulocytes, monocytes/macrophages, natural killer cells, mast cells and basophils. An intact skin or mucosa is the first barrier to infection, when this barrier is broken the body protection is accomplished by lysozyme, which breaks the peptidoglycon cell wall from the antigen and split the product arising from compliment activation.<sup>15</sup> The adaptive immune system is put into action when the innate immune response is inadequate to cope with infection. The adaptive immune system is mobilized by cues from the innate response. The adaptive immune system has a number of characteristic which include the ability to respond to a variety of antigens, in a specific manner and to discriminate between foreign (non-self) and self antigens of the host. Adaptive immune system also responds to a previously encountered antigen in a learned way by initiating a vigorous memory response.<sup>16</sup> Immunomodulators are the substances that modify the immune response, and those that suppress or activate immunity are termed immunosuppressants or immunostimulants<sup>17</sup> respectively. Primary targets of immunostimulants are T-or B-lymphocytes or the complement system. Activation of macrophages is probably important

for a stimulating agent to remain in contact with the reactive cells. The second most important role is the stimulation of T-lymphocytes, which can be achieved, either directly or indirectly via macrophages.<sup>18</sup>

In the present study, an effort was made to exploit, for the first time the immunomodulatory potential of *Decalepis hamiltonii* Wight and Arn using various *in vivo* screening models in experimental animals. The scientific evidences collected here supported the traditional claims behind usage of the plant *Decalepis hamiltonii* Wight and Arn which is being used in India and abroad for medicinal purposes. The study affirms that aqueous extract of *Decalepis hamiltonii* Wight and Arn has an effective immunomodulatory activity. The DHAE, not only potentiate non-specific immune responses such as percentage neutrophil adhesion and phagocytic index, but also effective in improving specific immune responses such as humoral and cell-mediated immunity in cyclophosphamide induced immunosuppressed rats.

Antibody molecules, a product of B-lymphocytes and plasma cells, are central to humoral immune responses, IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins, etc.<sup>19</sup> The augmentation of the humoral immune response to SRBC by DHAE as evidenced by increase in the antibody titer in rats indicated the enhanced responsiveness of T- and B-lymphocyte subsets involved in the antibody synthesis. The high values of haemagglutinating antibody titer obtained in the DHAE indicated that immunostimulation was achieved through humoral immunity.

Delayed-type hypersensitivity (DTH) represents a type of cell-mediated immune response that exerts important immunopathological effect. When it activates TH cells encounter certain antigens such as SRBC. They secrete cytokines that induce a localized inflammatory reaction called DTH.<sup>20</sup> It comprises of two phases, an initial sensitization phase after the primary contact with SRBC antigen. Heightened DTH suggests activation of cellular immune system.<sup>21</sup> Therefore; SRBC-induced delayed-type hypersensitivity was used to assess effects of DHAE on cell-mediated immunity. The DTH response is a direct reflection of the status of a host's cell-mediated immune status of CMI.<sup>22</sup> Here, the DTH was significantly as a result of the 100 and 200 mg DHAE/kg regimens. As such, one could infer that these increases in the DTH response may have reflected a stimulatory effect of the DHAE on lymphocytes and accessory cell types required for expression of that reaction.<sup>23</sup>

Phagocytosis is the process by which certain body cells, collectively known as phagocytes, ingests and removes microorganisms, malignant cells, inorganic particles and tissue debris.<sup>24</sup> DHAE appeared to enhance the phagocytic function by exhibiting a dose-related increase in clearance rate of carbon by the cells of the reticulo-endothelium system.

## CONCLUSION

The present investigation suggests that aqueous extract of *Decalepis hamiltonii* Wight & Arn (DHAE) stimulates both cellular and humoral immune responses. The effectiveness of DHAE treated animals in overcoming the side effects of drug-induced myelosuppression provides sufficient evidence for balancing and adaptogenic efficacy.

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## CONFLICT OF INTEREST

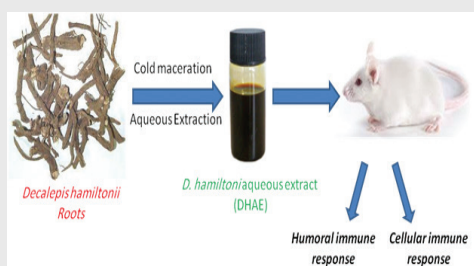
Authors declare that there is no conflict of interest for this article.

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## PICTORIAL ABSTRACT



## ABBREVIATIONS USED

**CYP:** Cyclophosphamide; **DHAE:** *D. hamiltonii* aqueous extract; **DLC:** Differential leucocytes count; **HA:** Hemagglutination antibody; **Hb:** Hemoglobin; **RBC:** Red blood cells; **SRBC:** Sheep red blood cells; **TLC:** Total leukocyte count; **WBC:** White blood cells.

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## SUMMARY

- *Decalepis hamiltonii* Wight and Arn (Asclepiadaceae) an Indian medicinal plant which is having ethno-medicinal uses as antioxidant, tonic and blood purifier.
- In the present study *D. hamiltonii* aqueous extract (DHAE) was evaluated for its immunomodulatory activity of in rodents.
- Two doses of DHAE (100 and 200 mg/kg, per os) were used to evaluate immunomodulatory activity *in vivo* and *ex vivo* method.
- DHAE at both dose levels evoked significant dose-dependent increases in antibody titers and DTH reactions induced by SRBC, and potentiated percentage neutrophil adhesion to nylon fibers as well as phagocytosis of carbon particles *in situ*.
- The evidence collected here lends support to traditional claims behind the use of *D. hamiltonii* for medicinal purposes. The study also affirmed that DHAE imparts immunomodulatory activity.