

**Immunomodulatory potentials of standardized dried fruit extract of *Aegle marmelos* in experimental system.****\*<sup>1</sup>Vijay A. Mohite, <sup>2</sup>R. Duraisami, <sup>1</sup>S. Sengottuvelu, <sup>1</sup>S. Haja Sherief**<sup>1</sup>Department of Pharmacology, <sup>2</sup>Department of Pharmacognosy, Nandha College of Pharmacy and Research Institute, Erode-638052, Tamilnadu, India.**Abstract**

To evaluate the Immunomodulatory potentials of standardized dried fruit extract of *Aegle marmelos* in experimental system. The immunomodulatory activities of standardized dried fruit extract from *Aegle marmelos*, namely SDEAM I (100 mg/kg), SDEAM II (200 mg/kg) and SDEAM III (400 mg/kg), were studied in mice for immune inflammation: delayed type hypersensitivity (DTH) and humoral antibody with 7 days pretreatment (compared with Cyclophosphamide) and 15 day pretreatment. In addition to this, the efficiency was evaluated by Plaque forming colony test and the haematological test (WBC Count). *Aegle marmelos* extract was found to significantly increase the circulating antibody titre and antibody forming cells as compared to control and cyclophosphamide. In fact, antibody forming cells were found to be stimulated much earlier and the maximum antibody titre was obtained. However, increased titre indicated that the *Aegle marmelos* shows immunological activity. Administration of *Aegle marmelos* was found to stimulate the phagocytosis of macrophages i.e. significant increased in number of plaques formation as compared to control. On the basis of the results obtained in the various experimental studies, concluded that the standardized dried extract of *Aegle marmelos* has the potential to stimulate cell-mediated and humoral immunity.

**Key Words**

*Aegle marmelos*; Delayed type hypersensitivity; Cell mediated immunity; Humoral immunity.

**Introduction**

The immune system is remarkably versatile defense system that has evolved to defend itself against this vast range of harmful agents. Deficiency of defence mechanism may involve specific immune functions – humoral immunity cell mediated immunity or both – or nonspecific mechanisms such as phagocytosis and complement,

which augment and act in conjunction with specific immune processes. As immune system plays central role of in the pathophysiology of many diseases suggesting a common target for therapeutic action (Dahanukar, 1999; Puri, 2003). Hence, drugs acting on the immune system are likely to influence many systems in the body. Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents but there

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are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system. Immunosuppression is a major drawback in conventional therapy of cancer such as radiation and chemotherapy. Both these method have severe side effect such as nausea, vomiting, alopecia and mucosal ulceration etc. Therefore a need arises for the development of newer immunomodulatory agents from natural source with more powerful activity and lesser side effects which act as effective substitute for chemical therapeutics. Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' in Ayurveda is based on related principles. Immunostimulation in a drug-induced immunosuppression model and immunosuppression in an experimental hyper- reactivity model by the same preparation can be said to be true immunomodulation (Patwardhan *et al.*, 1990). Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents. *Aegle marmelos*<sup>2</sup> is a fruit-bearing tree indigenous to dry forest on hills and plains of central and southern India, SriLanka, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia and Thailand. It belongs to the family Rutaceae, related to citrus. Plants were employed largely as analgesic, anti-inflammatory, antiviral, antimicrobial. The unripe

dried fruit is astringent, digestive, stomachic and used to cure diarrhea and dysentery. The ripe fruit is a good and simple cure for dyspepsia. Aqueous leaf extract of *Aegle marmelos* has preventive effect on isoprenaline (isoproterenol) induced myocardial infraction. The effect of constituents isolated from methanolic extract of root bark of Bael on spontaneous beating of cultured mouse myocardial cells was examined. The extract at a concentration of 100Hg/ml inhibited the beating rate by approximately 50%. Most of the plant parts of *Aegle marmelos* were screened for various activities. The crude petroleum ether, chloroform, ethanolic and aqueous extract shows presence of flavonoids, monoterpenoids, triterpenoids, glycosides and phenolic compounds in *Aegle marmelos* which was screened for antibacterial activity. The anti fungal activity of essential oils isolated from the leaves of Bael has been evaluated using spore germination assay. The cytoprotective activity of the extract of *Aegle marmelos* (AME) was evaluated in the Chinese hamster V79 cells in Vitro. G. J. Zlabinger *et al* (1994) has characterized the mode of action of coumarin as immunomodulatory and also Chaing LC *et al* (2003) shown that flavonoids, monoterpenoids, triterpenoids, glycosides and phenolic compounds shows immunomodulatory activities.

Therefore in the present study the effect of *Aegle marmelos* plant species on cell mediated and humoral immunity was evaluated.

## Materials and methods

### Plant Material

The standardized dried extract of *Aegle marmelos* was obtained from Amruta Herbal Pvt. Ltd., Indore, along with certificate of analysis.

### Chemicals and reagents

The chemicals used in the present study were Cyclophosphamide (Zyclus Cadilla Pvt. Ltd.), Sheep Red Blood Cells (SRBC) Collected from local slaughter house. All laboratory chemical used are analytical grade.

### Apparatus

The apparatus used in the study were V Bottom Plates (Tarson India Pvt. Ltd. Kolkata), Sodium Heparin BD Vacutainer (Relicare SRL Diagnostics, Satara).

### Experimental animals

Wistar albino rats of either sex weighing 125–150 g, and albino mice of either sex weighing 25–35 g, were used. They were fed standard diet and water ad libitum and housed in cages at room temperature ( $30\pm 2^\circ\text{C}$ ) with a 12 h light and dark cycle. Animal experiments were approved by the Institutional Animal Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India.

### Dosage schedule

The animals were divided into three groups of six animals each, for each test except for test 1 (Effect of test extract and cyclophosphamide on HA titer and DTH response using SRBCs as an antigen in mice-7 days pre-treatment.) which was carried out with four such groups. In case of Effect of

test extract and cyclophosphamide on HA titer and DTH response using SRBCs as an antigen in mice-7 days pre-treatment. Group I was administered with 1 % SCMC. Group II served as cyclophosphamide (50 mg/kg). In group III, group IV, group V mice were administered with SDEAM 100 mg/kg, SDEAM 200 mg/kg and 400 mg/kg respectively for 7 days. Whereas in case of Effect of test extract on HA titer and DTH response using SRBCs as an antigen in 15 day pretreatment and plaque forming colony test Group I was administered with 1 % SCMC, Group II was administered with SDEAM I 100 mg/kg and Group III was administered with SDEAM II 200 mg/kg, group IV was administered SDEAM III 400mg/kg.

### Effect of test extract and cyclophosphamide on HA titer and DTH response using SRBCs as an antigen in mice-7 days pre-treatment.

Animals were divided into seven groups of six animals each. Group I (control group) and group II (cyclophosphamide treated control) received the vehicle (1% SCMC, p.o.) for a period of 7 days. Groups III–VII were given the *Aegle marmelos* (50–800 mg/kg, p.o.) daily for 7 days. The animals of groups II–VII were injected with cyclophosphamide (50 mg/kg, p.o.) on the 4th, 5th and 6th day, 1 h after the administration of the respective treatment. The animals were immunised by injecting 0.1 ml of 20% of fresh sheep red blood cells suspension intraperitoneally on day 0. Blood samples were collected in microcentrifuge tubes from individual

animal by retro-orbital plexus on the 7th day and serum was separated. Antibody levels were determined by haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 x 1 volumes of normal saline in microtitration plate and to that were added 25 x 1 of 1% suspension of sheep red blood cells in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre. The thickness of the right hind footpad was measured using digital Vernier caliper on the 7th day. The mice were then challenged by injecting 20 x 1 of 1% SRBCs in right hind footpad and after 24 h of this challenge the foot thickness was measured again. The pre- and post-challenge difference in the thickness of footpad was expressed in mm and taken as a measure of DTH.

**Effect of test extract on HA titre and DTH response using SRBCs as an antigen in mice-15 days pre-treatment.**

Mice were divided into six groups, each group containing six mice. Group I represented the control group (1% SCMC, p.o.). Groups II–VI received the *Aegle marmelos* (50–800 mg/kg, p.o.). The pre-treatment time of 15 days was based on the method described by Sharma et al. (1996). Schedule for drug administration was 7 days prior to immunization (days -6, -5, -4, -3, -2, -1, 0) and 7 days after immunization (days +1, +2, +3,

+4, +5, +6, +7), Blood samples were collected in microcentrifuge tubes from individual animal by retro-orbital plexus on the 15th day and serum was separated. Antibody levels were determined by haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 x 1 volumes of normal saline in microtitration plate and to that were added 25 x 1 of 1% suspension of sheep red blood cells in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre. The thickness of the right hind footpad was measured using digital Vernier caliper on the 15th day. The mice were then challenged by injecting 20 x 1 of 1% SRBCs in right hind footpad and after 24 h of this challenge the foot thickness was measured again. The pre- and post-challenge difference in the thickness of footpad was expressed in mm and taken as a measure of DTH.

**Plaque Forming Colony Test**

Four days after immunization with SRBC and different treatments in mice, the PFC assay was performed using spleen cells according to the method of Jerne and Nordin (1963). Spleen cells collected from individual animals (0.1 mL;  $1 \times 10^6$  cells/0.1 mL), 0.4 mL of 0.5% “low melting point” agarose (GIBCO, Grand Island, NY, USA) in HBSS, and 50  $\mu$ L of a

suspension of 5% SRBC were added to test tubes at 37°C and poured onto microscope slides containing a bottom layer of 0.5% agarose in water. The slides were then incubated for 2 h at 37°C and 5% CO<sub>2</sub>. Guinea pig serum diluted 1:4 in HBSS was added to the slides and after another 40 min (37°C and 5% CO<sub>2</sub>) incubation the number of plaques was counted and values were expressed as PFC per 10<sup>6</sup> cells. For the preparation of guinea pig serum as the complement source, animals were anesthetized before cardiac puncture and blood samples were collected. Serum was obtained and previously absorbed with SRBC before being stored at -70°C.

#### **Statistical Analysis.**

All the results were expressed as mean ± standard error mean (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's *t*-test. *p*<0.05 was considered as statistically significant. The analysis was carried out using Graph pad software of version 4.

#### **Result**

##### **Effect of test extract SDEAM and cyclophosphamide on HA titre and DTH response using SRBCs as antigen in mice-7 days pretreatment.**

The Effect of test extract SDEAM and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in mice-7 days pretreatment was illustrated in Table 7. The Test extract SDEAM and cyclophosphamide actions exhibited remarkable differences in the humoral antibody titre parameter. It increased the HA titre while cyclophosphamide

treatment reduced the HA titre. However, there was increase in the DTH response in case of both the drugs. Administration of Cyclophosphamide resulted in decreased HA titre. The reduction was significant (*p* < 0.001) when compared to control animals (group I). When compared with cyclophosphamide treated animals (group II), animals receiving SDEAM treatment (groups III–V) showed dose dependent recovery in HA titre. However, significant increase in HA titre could be obtained at 200 (*p* < 0.001) and 400 mg/kg (*p* < 0.001) dose. A significant increase in paw edema was observed on day 8 after challenge on day 7 with SRBCs. Animals treated with cyclophosphamide showed significant (*p* < 0.001) DTH response was compared to control animals. Animal treated with SDEAM was found to increase the delayed type hypersensitivity reaction significantly (*p* < 0.001) as compared to control, but the result does not show dose dependent manner.

##### **Effect of test extract SDEAM on HA titre and DTH response using SRBCs as antigen in mice— 15 days pretreatment.**

All the animals of groups I–IV were sensitized on day 0 with SRBC. The control group received only vehicle from day -7 to +7. The humoral antibody titre value was found to be 46.5±1.848. The test extract SDEAM produced a dose dependent increase in the HA titre after incubation with SRBCs (Table 8). Administration of higher doses, i.e. 100, 200 and 400 mg/kg produced significant increase

in HA titre as evident from haemagglutination after incubation of serum with SRBCs. DTH was determined 24 h after the challenge. Higher doses of test extract SDEAM (200 and 400 mg/kg) showed statistically significant increase in mean paw thickness.

#### **Plaque forming cell test.**

The SRBC sensitized animals were administered with SDEAM for five days. The Spleen cells or peripheral blood lymphocytes, previously incubated with antigen, are mixed with sheep red blood cells (SRBC). After addition of complement and incubation, plaques (clear areas) caused by the lysis of SRBC appear in the otherwise cloudy layer. Effect was significantly compared to control group ( $P < 0.001$ ) and shown dose dependent increase.

#### **Discussion**

In the present investigation two schedules of pretreatment period, i.e. 7 days pretreatment and 15 days pretreatment were selected. The results obtained in the present studies showed that test extract SDEAM displays a significant immunostimulatory effects in relation to antigenic stimulation. Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation (Patwardhan *et al.*, 1990). A high degree of cell proliferation renders the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone marrow is the organ most affected during any

immunosuppression therapy with this class of drugs. Loss of stem cells and inability of the bone marrow to regenerate new blood cells results in thrombocytopenia and leucopenia (Agarwal *et al.*, 1999). Administration of the extract of *Aegle marmelos* was found to increase the total WBC count, which was lowered by cyclophosphamide, a cytotoxic drug. The results of the present study indicate that the test drug can stimulate the bone marrow activity. Antibody production to T-dependent antigen SRBC requires co-operation of T- and B-lymphocytes and macrophages. Cyclophosphamide has a particularly intense effect on short-lived lymphocytes known to include a great proportion of B-cells. The values of haemagglutinating antibody titre obtained in case of *Aegle marmelos* have indicated that immunostimulation was achieved through humoral immunity. In the delayed type hypersensitivity model, i.e. type-IV T lymphocytes and activated macrophage mediated reaction, sensitized animals, when challenged with 1% SRBC, resulted in a significant increase in paw edema when compared with right paw (receiving normal saline as control) and left paw edema (receiving SRBC) ( $P < 0.001$ ), establishing the validity of the model (Table 1). Cyclophosphamide 50 mg/kg is found to be the efficient potentiating dose of DTH as compared to other groups. Animals treated with cyclophosphamide 50 mg/kg showed maximum potentiation of DTH (because cyclophosphamide damaged short-lived suppressor T cells in

immune regulatory systems) as observed from an increase in mean foot pad thickness 24 h after challenge ( $P < 0.001$ ). SRBC Sensitized animals treated with SDEAM I, SDEAM II, SDEAM III and challenged with 1% SRBC, resulted in mild potentiation of DTH response ( $P < 0.001$ ). Thus, it can be observed that the extracts, act as potentiator of DTH but suppress, or favorably modulate, the inflammatory reaction (Arul V *et al.*, 2005). Thus, the immunostimulatory effect produced by dried extract of *Aegle marmelos* in cyclophosphamide-induced immunosuppression may be due to cell mediated and humoral antibody mediated activation of T and B cells. It can therefore be concluded that *Aegle marmelos* is a potential immunostimulant against cytotoxic drugs and can be used as a complimentary therapeutic agent. Identification of antibody producing cells is based on the ability of the secreted IgM antibody to fix complement and thereby lyses the indicator erythrocytes. Spleen cells or peripheral blood lymphocytes, previously incubated with antigen, are mixed with sheep red blood cells (SRBC). After addition of complement and incubation, plaques (clear areas) caused by the lysis of SRBC appear in the otherwise cloudy layer. Antibody forming cells can be detected by the appearance of plaques. The number of plaques obtained is proportional to the number of antibody producing lymphocytes in the cell population. *Aegle marmelos* extract was found to increase the circulating antibody titre and antibody forming cells. In fact, antibody forming cells shown

significant increased as compared to control group ( $P < 0.001$ ) and shown dose dependent increase. Phytochemical investigations on *Aegle marmelos* revealed the presence of different types of compounds. It contain Scopoletine (7-Hydroxy-6-methoxy coumarin), *Aegle marmelos* extract contains a triterpinoid, lupeol (E. Lambertini *et al.*, 2005) which is capable of inhibiting cell proliferation. It also contain inorganic trace elements such as Cu, Ni, Zn, K and Na where as Fe, Cr and V levels found in marginal levels (R. T. Narendhirakannan *et al.*, 2005). A new 7-geranyloxy coumarin[7-(2,6-dihydroxy-7-methoxy-7-methyl-3-octaenyloxy)coumarin] named marmenol (M. S. Ali *et al.*, 2004) has been isolated from the fruit extract of *Aegle marmelos*. In addition to marmenol, several known compounds, praealtin D, trans-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, cis and transcoumaroyltyramine, montanine and rutaretin have also been obtained from the extract. These observations indicate that various phytochemical like triterpinoid, coumarins have the capacity to modulate the immune response. There is a need for detailed investigation of the mechanism of modulation of immunoglobulin isotype and based on that, a possible therapeutic can be visualized.

### Conclusion

On the basis of the results obtained in the various experimental studies, concluded that the standardized dried extract of *Aegle marmelos* has the

potential to stimulate cell-mediated and humoral immunity.

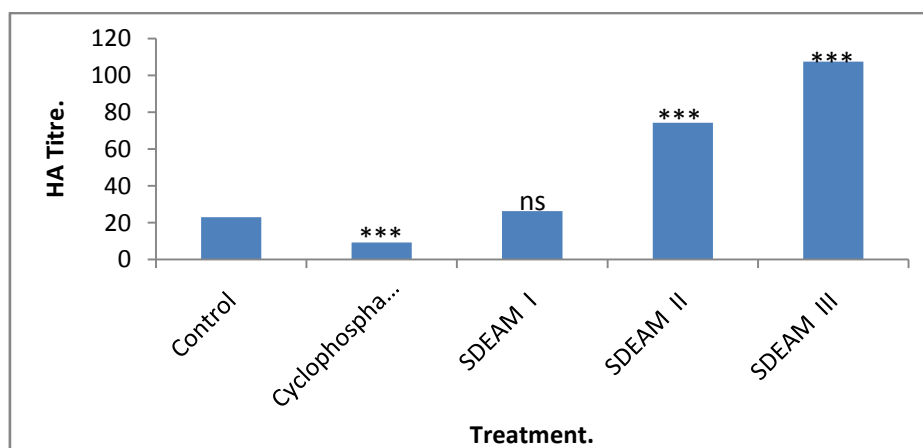
#### References:

1. Agarwal R. et al, Studies on immunomodulatory activity of *Withania somnifera* (Ashwagandha) extracts in experimental immune inflammation. *J Ethnopharmacol.* 1999; 67: 27–35.
2. Ali M. S., Perrez M. K. Marmenol: A 7-geranyloxycoumarin from the leaves of *Aegle marmelos* Corr. *Nat Prod Res.* 2004; 18: 141-146.
3. Ananthanarayan R, Paniker CKJ. *Textbook of Microbiology* Hyderabad, Orient Longman; 2003:275-76.
4. Arul V. et al. Mechanism of the contractile effect of the alcoholic extract of *Aegle marmelos* Corr. on isolated guinea pig ileum and tracheal chain. *Phytomedicine.* 2004, 679-183.
5. Arul V. et al Studies on anti-inflammatory, antipyretic, and analgesic properties of the leaves of *Aegle marmelos* Corr. *J Ethanopharmacol.* 2005; 96: 159-163.
6. Babita Madan, Balaram Ghosh. *Canscora decussata* promotes adhesion of neutrophils to human umbilical vein endothelial cells. *J Ethnopharmacol* 2002; 79: 229–235.
7. Babita Madan et al, *Curcuma longa* activates NF-kB and promotes adhesion of neutrophils to human umbilical vein endothelial cells. *J Ethnopharmacol* 2001; 75: 25–32.
8. Bafna A. R., Mishra S. H., Immunostimulatory effect of methanol extract of *Curculigo orchioides* on immunosuppressed mice *J Ethnopharmacol* 2006; 104: 1–4.
9. Bafna A. R., Mishra S. H., Protective effect of bioactive fraction of *Sphaeranthus indicus* Linn. Against cyclophosphamide induced suppression of humoral immunity in mice. *J Ethnopharmacol* 2006; 104: 426–429.
10. Balakrishnan N. et al Antibacterial activity of *Mimosa pudica*, *Aegle marmelos* and *Sida cordifolia*. *PHCOG MAG.* 2006; 198-199.
11. Carpentep L. Immunology and serology, 2nd edn., Philadelphia, 1965, 308.
12. Chauhan Agarwal M. et al Supression of fertility in mail albino rats following the administration of 50% ethanolic extract of *Aegle marmelos*. *Contraception.* (2007), 474-81.
13. Citavasu T. et al Influence of selected immunostimulant herbs against while sport syndrome virus (WSSV) infection in black tiger shrimp, *penaeus monodon* with reference to haematological, biochemical and immunological changes. *Fish Shellfish Immunol.* 2006; 372-84.
14. Coombs, R.R.A., Gell, P.G.P. Classification of allergic reactions responsible for clinical hypersensitivity and disease, In; *Clinical Aspects of Immunology.* Edited by Gell, P.G.H, Coombs, R.R.A.



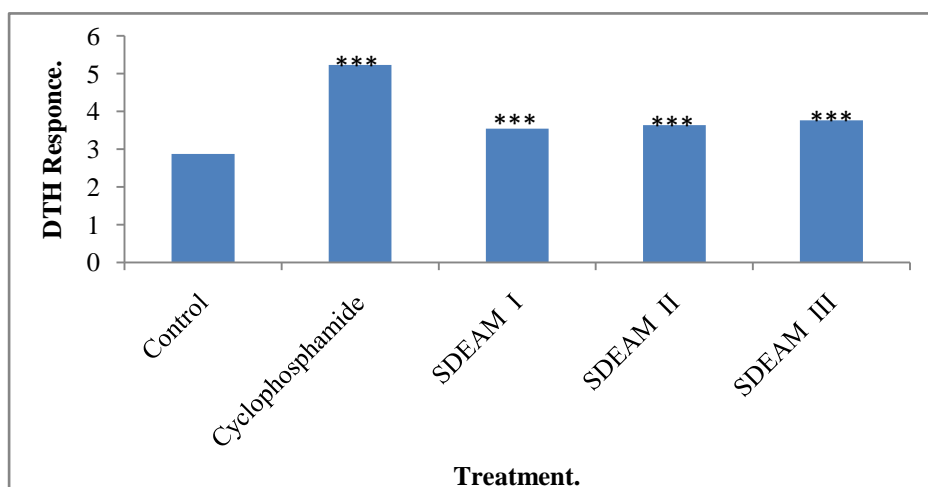
- and Lachmann, P.J., 3<sup>rd</sup> edn. Blackwell Scientific Publication, Oxford; 1975; 761.
15. Davis L., Kuttan G., Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol* 2000; 71: 193–200.
  16. Figueredo A *et al* Adjuvant therapy for stage II colon cancer after complete resection. *Cancer Prev Control* 1997; 1:379- 392.
  17. Geiger B. *et al* The effect of irradiation on the release of lymphocyte-activating factor (LAF). *Cell. Immunol.* 1973; 7: 177.
  18. Gerhard H., Vogel (Ed.), *Drug Discovery and Evaluation Pharmacological Assays*, Second Edition, Springer 2002; 788-820.
  19. Girija Kuttan. *J Ethnopharmacol* 2000; 72: 93–99.

**Fig. 1:** Effect of test extracts SDEAM and cyclophosphamide on HA titre.



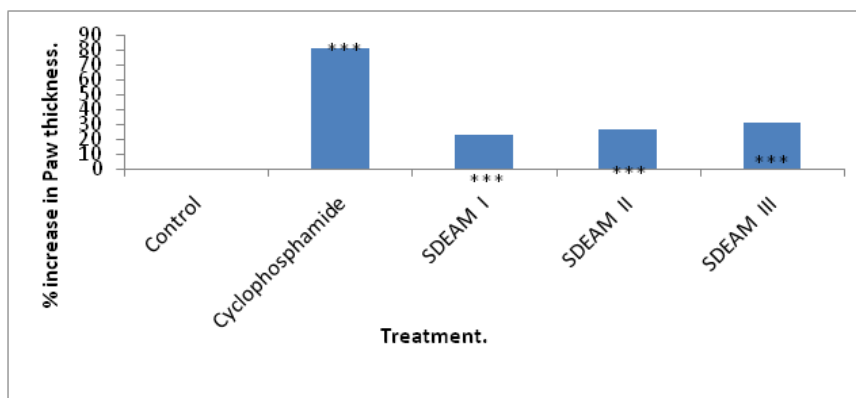
Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, V, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$ .

**Fig. 2:** Effect of test extracts SDEAM and cyclophosphamide on DTH Response.



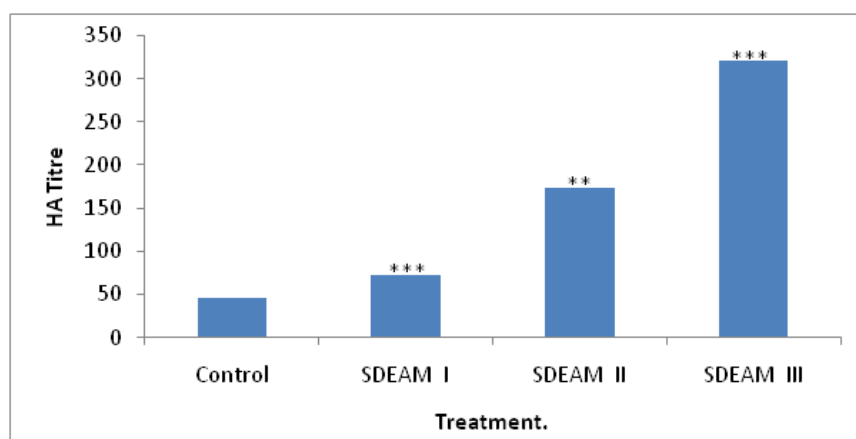
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**Fig. 3:** Effect of test extracts SDEAM and cyclophosphamide on DTH Response. Results are expressed as percentage increase in paw thickness.



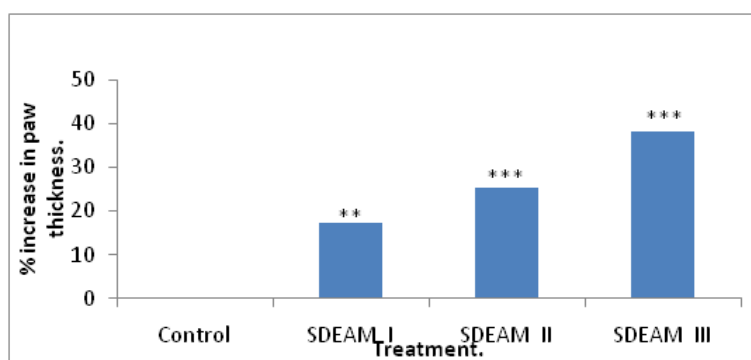
Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, V, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$ .

**Fig 4:** Effect of test extracts SDEAM on HA Titre.



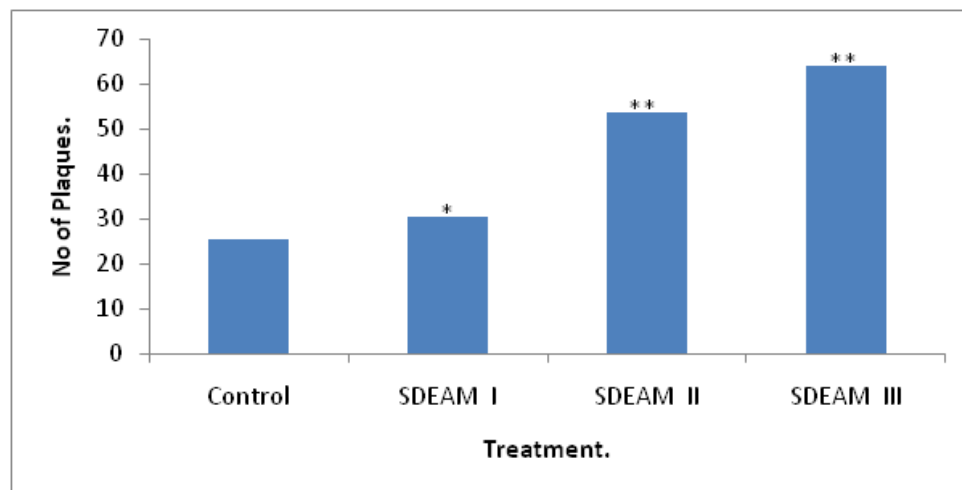
Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$ .

**Fig. 5:** Effect of test extracts SDEAM and cyclophosphamide on DTH Response. Results are expressed as percentage increase in paw volume.



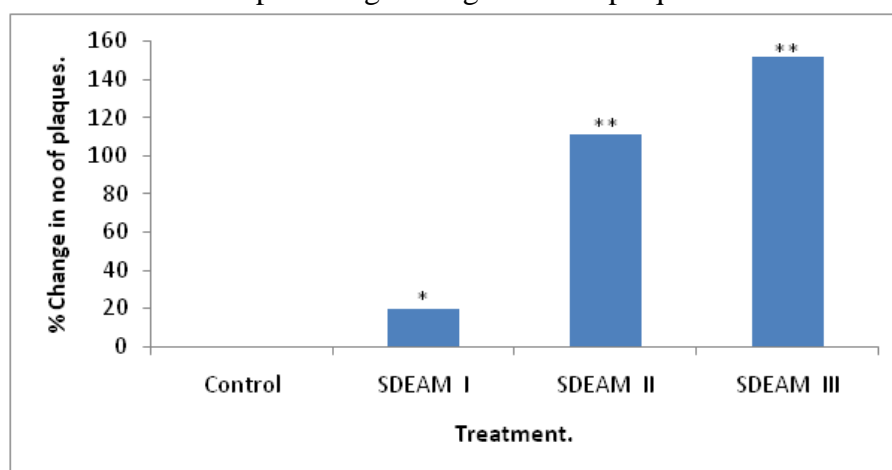
Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$ .

**Fig 6:** Effect of test extracts SDEAM on plaque forming cells.



Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$

**Fig 7:** Effect of test extracts SDEAM on plaque forming cells. Results are expressed as a percentage change in no of plaques.



Control: 1% Sodium carboxy methyl cellulose;  $n=6$  per group comparison of I with II, III, IV, n.s., Not significant, \*  $p < 0.05$ ., \*\*  $p < 0.01$ ., \*\*\*  $p < 0.001$ .

**Table 1:** Effect of test extract SDEAM and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in mice-7 days.

Group	Treatment	Dose mg/kg p.o. for 7 days	HA titre (Mean $\pm$ SEM.)	DTH response (mm) mean paw edema $\pm$ SEM.	Percentage Increase in Paw thickness.
I	Control	-	23.00 $\pm$ 0.91	2.87 $\pm$ 0.08	-
II	Cyclophosphamide	50	9.25 $\pm$ 0.85***	5.22 $\pm$ 0.08***	81.74
III	SDEAM I	100	26.25 $\pm$ 0.85 <sup>ns</sup>	3.545 $\pm$ 0.07***	23.30
IV	SDEAM II	200	74.25 $\pm$ 1.03***	3.63 $\pm$ 0.08***	26.43
V	SDEAM III	400	107.50 $\pm$ 1.19***	3.76 $\pm$ 0.05***	30.95

**Table 2:** Effect of test extract SDEAM on HA titre and DTH response using SRBCs as an antigen in mice-15 days pretreatment.

Group	Treatment	Dose mg/kg p.o. for 15 days	HA titre (Mean $\pm$ SEM.)	DTH response (mm) mean paw edema $\pm$ SEM.	Percentage Increase in Paw thickness
I	Control	-	46.50 $\pm$ 1.84	2.87 $\pm$ 0.08	-
II	SDEAM I	100	72.75 $\pm$ 1.88 <sup>***</sup>	3.37 $\pm$ 0.04 <sup>**</sup>	17.39
III	SDEAM II	200	174.75 $\pm$ 1.70 <sup>**</sup>	3.60 $\pm$ 0.09 <sup>***</sup>	25.21
IV	SDEAM III	400	321.75 $\pm$ 2.13 <sup>***</sup>	3.97 $\pm$ 0.08 <sup>***</sup>	38.26

Control: 1% Sodium carboxy methyl cellulose; *n*=6 per group comparison of I with II, III, IV, n.s., Not significant, \* *p* < 0.05., \*\* *p* < 0.01., \*\*\* *p* < 0.001.

**Table 3:** Effect of test extract SDEAM on Plaque forming cell test.

Group	Treatment	Dose mg/kg p.o. for 5 days	No of Plaque forming cells	% Change in no of plaques.
I	Control	-	25.50 $\pm$ 0.64	-
II	SDEAM I	100	30.50 $\pm$ 0.64 <sup>*</sup>	19.60
III	SDEAM II	200	54.00 $\pm$ 1.58 <sup>**</sup>	111.76
IV	SDEAM III	400	64.25 $\pm$ 1.10 <sup>**</sup>	151.96

*Conflict of Interest: Not Declared*

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