

## IMMUNOMODULATOR ACTIVITY OF MEGAEXT OF TRIAMRIT

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\*Corresponding Author: [rnishr@gmail.com](mailto:rnishr@gmail.com)**ABSTRACT**

The objective of study is to investigate the immunomodulatory activity of TriAmrit megaExt. Delayed-type hypersensitivity (DTH) response, Carbon clearance assay was determined in albino mice by *in vivo* experiments. The evaluation of immunomodulatory potential by oral administration of TriAmrit megaExt (250-500 mg/kg) evoked a significant increased carbon clearance, indicating the stimulation of reticulo-endothelial system and potentiated the delayed type hypersensitivity reaction induced by sheep red blood cells. The results obtained in this study indicate that TriAmrit possesses potential immunomodulatory activity and has therapeutic potential for the prevention of autoimmune diseases.

**Keywords:** TriAmrit, carbon clearance assay, delayed hypersensitivity test.

**INTRODUCTION**

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' is based on related principles<sup>1</sup>. Rasayana, listed as a class in the texts of traditional Indian medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. Besides, a number of medicinal plants as Rasayanas have been claimed to possess immunomodulatory activities.

Some of the Rasayana drugs as immunomodulatory agents such as *Withania somnifera*, *Tinospora cordifolia*, *Asparagus racemosus* and *Mangifera indica*<sup>2-5</sup> are well known for their traditional uses. Furthermore, medicinal plants used for immunomodulation can provide potential alternatives to conventional chemotherapies for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response. The use of plant products in the indigenous system of medicines as immunomodulators, indeed, can modulate the body's immune system, as a variety of plant derivatives such as polysaccharides, lectins, peptides, flavonoids

and tannins have been reported to modulate the immune system in various *in vivo* models<sup>6</sup>.

'TriAmrit' consists of the dried herbs of three medicinal plants, *Terminalia chebula* (combretaceae), *Allium sativum* (liliaceae) and *Tinospora cardifolia* (menispermaceae). These three medicinal plants are born out of Amrit (nectar) as per Ayurveda. It is used in the ayurvedic system of medicine in the treatment of variety of condition such as constipation headache, cardiovascular activity, antiageing, antiarthritis, hypoglycemic and hepatoprotective.

'In the present work 'TriAmrit' is evaluated for its immunomodulatory effect.

**Research envisaged (Justification- Aim- Objectives):**

In the literature we found some reports on the immunomodulatory properties of one or the other individual herb constituents of TriAmrit. However, there is no report on the mixture of these 3 herbs for pharmacological screening of such therapeutic activity like immunomodulatory. As there is synergistic effect or mutual potential of therapeutic action when such herbs of similar nature are mixed together. In the light of this, it was thought worthy to evaluate immunomodulatory activity of TriAmrit herbs and their correlation with

antioxidant activity. Such studies are important to substantiate the claims documented with regard to TriAmrit in ancient Ayurvedic texts.

### MATERIALS AND METHODS

**Animals:** Six-eight week old healthy, laboratory breed Swiss albino mice of either sex, weighing  $25 \pm 2$ g were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan lever Ltd. Kolkata, India, and water *ad libitum*.

**Plant material and Drugs:** The herbs of TriAmrit (*Allium sativum*, *Tinospora cardifolia* and *Terminalia chebula*) were collected from local market of sagar, dried in shade, coarsely powdered and all three powdered drugs were mixed in 1:1:1 w/w, to preparation of megaHerb. megaHerb was subjected to soxhlet successive extraction, using non polar to polar solvent (Pet.ether, Benzene, Chloroform, Ethyl acetate, 70% ethanol and water). All six extracts were concentrated by distilling the solvent and air dried. All six extract were mixed to prepare megaExtract (megaExt). The megaExt was subjected to qualitative phytochemical analysis for presence of various constituents like Alkaloids, Carbohydrate, Glycosides, Terpanoids, Protein and Amino acids, Phenolic and Tannins, Flavanoids, Oils and Fats, Saponins. A test solution of megaExt was prepared by dissolving it in acacia with distilled water for orally administration to animals. Levamisole in a dose of 25 mg/kg p.o. was used as a reference standard immunomodulator activity<sup>7</sup> for comparison in this study.

**Acute Toxicity:** The TriAmrit megaExt was screened for acute oral toxicity test according to OECD guideline No. 423<sup>8</sup>. Therefore 1/5 and 1/10<sup>th</sup> cut off dose for TriAmrit megaExt are 250 and 500 mg/kg body weight orally, were selected for *In vivo* study.

### EXPERIMENTAL/ METHOD

**Carbon clearance assay<sup>9</sup>:** Animals were divided in four groups of six mice each. Group I animals served as control (saline 10ml/kg b.w.p.o.), Group II animals were treated with levamisole (25 mg/kg b.w. p.o.), Group III animals were treated with test substances (250 mg/kg b.w.p.o.) and Group IV animals were treated with test substances (500mg/kg b.w. p.o.). All the animals were treated as above from day 0 to day 7. On 7<sup>th</sup> day of treatment animals of the

entire group received an intravenous injection of (0.3ml/30 gm of b.w.) of Indian ink dispersion (per warmed at 37°C). Blood samples were collected from retro orbital bleeding by using glass capillaries at an interval of 2 min and 10 min after the injection of ink dispersion. Blood samples were added to 4ml of 0.1% sodium carbonate solution to lyses the erythrocytes. Absorbance of these samples was measured at 675 nm using spectrophotometer, after 10 min of blood collection animals. The mean of phagocytic index was calculated of each group of animal.

**Delayed Type Hypersensitivity<sup>10</sup>:** On 7<sup>th</sup> day the thickness of right hind foot pad was measured using vernier caliper. The animals were then challenged by injecting  $0.5 \times 10^9$  SRBCs in right in foot pad. A foot pad thickness was measured again 24 hr. after the challenge. The difference between pre and post challenge foot pad thickness expressed in mm was taken as a measure of (DTH) and the mean value obtained for treatment groups were compared with that of control group. The TriAmrit megaExt was administered orally on day 0 and continued till day 7 of challenge. The data obtained was subjected to statistical analysis.

**Statistical analysis:** All the values are expresses as mean  $\pm$  SD and data was analyzed by one-way ANOVA, using Graphpad INSTAT. The post-hock analysis was carried out by Dunnet's multiple comparison tests to estimate the significance of difference between individual groups.

### RESULTS AND DISCUSSION

Qualitative phytochemical analysis of TriAmrit megaExt revealed that it contains Alkaloids, Carbohydrate, Glycosides, Terpanoids, Protein and Amino acids, Phenolic and Tannins, Flavanoids, Oils and Fats, Saponins. Acute toxicity studies with megaExt revealed that LD<sub>50</sub> is 250 mg/kg and 500 mg/kg body weight.

#### Carbon clearance assay

Effect of TriAmrit megaExt on the phagocytic activity by the carbon clearance test is shown in Table no. 1. The phagocytic activity of the reticulo-endothelial system is generally measured by the rate of removal of carbon particles from the blood stream. In carbon clearance test, TriAmrit megaExt treated all groups, exhibited significantly high phagocytic index. The phagocytic index of Dose I (250 mg/kg b.w) and Dose II (500 mg/kg b.w.)

Showed significant ( $p < 0.001$ ) increase in phagocytic index when compared to control group. This indicates stimulation of the reticuloendothelial system.

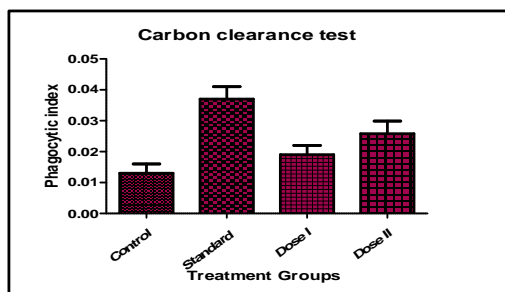
**Delayed Type Hypersensitivity Test**

Effect of megaExt on cell mediated immune response by DTH induced footpad oedema is shown in Table no. 2. Apart from humoral response there was significant increase in DTH response or cell mediated immunity indicated of increase mean paw edema value. TriAmrit megaExt showed increase in DTH in all treated group when compared with control group.

**Table 1: Effect of TriAmrit megaExt on Phagocytic activity by carbon clearance test**

Group	Dose (mg/kg)	Phagocytic index Mean±SD
I	10 ml/kg normal saline	0.013±0.003
II	Levamisole(25mg/kg)	0.037±0.004
III	250 mg TriAmrit	0.019±0.003***
IV	500 mg TriAmrit	0.0258±0.0041***

\*\*\* All values are mean ± SD,  $P < 0.001$  when compared to control group



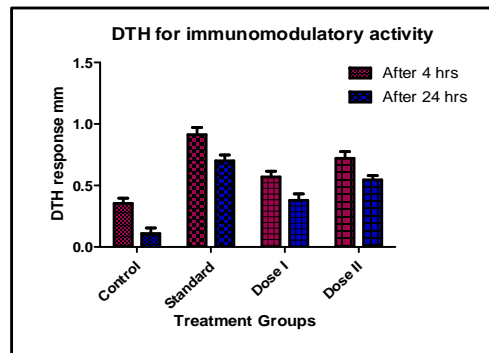
**Histogram showing – effect of TriAmrit megaExt in Carbon clearance test**

**Table 2: Effect of TriAmrit megaExt treatment in Delayed type hypersensitivity test by induced footpad oedema**

Group	Dose (mg/kg)	DTH response mm Mean±SD	
		4 hr.	24 hr.
I	10 ml/kg normal saline	0.355±0.042	0.11±0.0438
II	Levamisole (25 mg/kg)	0.913±0.059	0.701±0.047

III	250 mg TriAmrit	0.57±0.046	0.38±0.0521***
IV	500 mg TriAmrit	0.72±0.056	0.545±0.0361***

\*\*\* All values are mean ± SD,  $P < 0.001$  when compared to control group



**Histogram showing – effect of TriAmrit megaExt in Delayed type hypersensitivity test**

**DISCUSSION**

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions and if it results in an enhancement of immune reactions, it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effectors substances.

The results of TriAmrit megaHerb a potent immunostimulant, stimulating specific and non-specific immune mechanisms. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines).] DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase the vascular permeability, induce vasodilatation, macrophage accumulation, and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. When activated TH1 cells encounter certain antigens, viz. SRBCs. They secrete cytokines that induce a localized inflammatory reaction called delayed type hypersensitivity. DTH comprises of two phases, an initial sensitization phase after the primary contact with SRBC antigen. A subsequent exposure to the SRBCs antigen induces the effectors phase of the DTH response, where TH1 cells secrete a variety of cytokines that recruits

and activates macrophages and other non specific inflammatory mediators. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages. Therefore, increase in DTH reaction in mice in response to T cell dependent antigen revealed the stimulatory effect of mega extract on T cells<sup>11</sup>.

The role of phagocytosis is the removal of microorganisms and foreign bodies, dead or injured cells. The increase in the carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity. TriAmrit megaHerb appeared to enhance the phagocytic function by exhibiting a clearance rate of carbon by the cells of the reticulo endothelium system.

### CONCLUSION

In our present study we, conclude that TriAmrit megaExt has immunomodulatory activity. It could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. It is already reported that naturally occurring phenolic compounds have immunomodulatory activity.

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