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Research Article

# ADAPTOGENIC ACTIVITY OF MEGAEXT OF TRIAMRIT

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**ABSTRACT**

The objective of study is to investigate The Adaptogenic Activity of TriAmrit megaExtract. Swim endurance test and anoxic stress tolerance test in albino mice were determined by *in vivo* experiments. The evaluation of Adaptogenic Activity by oral administration of TriAmrit megaExt (250-500 mg/kg) showed significant increase in the swimming time (min) and also increases in anoxic tolerance time in physical and anoxic stress models. The results obtained in this study indicate that TriAmrit is good Adaptogenic agent.

**Keywords:** TriAmrit, Swim endurance test and Anoxic stress tolerance test.

## INTRODUCTION

Stress is a non-specific response of a body known to alter the physiological homeostasis of the organism resulting in a various endocrine and visceral responses. The immune system plays an important role in biological adaptation, contributing to the maintenance of homeostasis and to establishment of body integrity[1]. The term “stress” is used here in the classic sense as a state of threatened homeostasis. The general pharmacodynamic characteristics of an Adaptogenic substance .An adaptogen is almost non-toxic to the recipient; An adaptogen tends to be non-specific in its pharmacological properties and acts by increasing the resistance of the organism to a broad spectrum of adverse biological, chemical, and physical factors;; The effect of an adaptogen is as pronounced as deeper are pathologic changes in the organism[2].

Stressors can be broadly classified into two groups:

**External Stressors:** External stressors include adverse physical conditions (such as pain, uncomfortably hot or cold temperatures) or stressful psychological environments (such as poor working conditions or abusive relationships).

**Internal Stressors:** Internal stressors can also be physical (infections, inflammation) or psychological. An example of an internal psychological stressor is intense worry about a harmful event that may or may not occur. Internal psychological stressors are rare or absent in most animals except humans. Internal stressors can be classified as under:

Stressors may be of short term (acute) or long term(chronic).

***Acute Stress*:** Acute stress is the reaction to an immediate threat, commonly known as the *fight or flight* response. The threat can be any situation that is experienced, even subconsciously or falsely, as a danger.

***Chronic Stress:*** Frequently, however, modern life possesses on-going stressful situations that are not short-lived and the urge to act (to fight or to flee) is suppressed, then it will be chronic

TriAmrit consists of the dried herbs of three medicinal plants, Terminalia *chebula* (combretaceae), *Allium sativum* (liliaceae) and *Tinospora cardifolia* (menispermiacee).These three medicinal plants are born out of Amrit (nector) as per AyurvedaThe bulb *Allium sativum* belongs to family Alliaceae, perineal bulbous scapigerous herb. It is cultivated throughout India. It has great medicinal value; bulb used has anti diabetic, anti-cancer, anti

hypertensive, and lipidemic activity. The juice is effective in rheumatism, whooping cough, tuberculosis and has anti bacterial and rejuvenative actions. The fresh bulb is used as rejuvenative[3]. The plant contains proteins, amino acids, flavonoids, hydrocarbons, allin, sulphur amino acid, anthocyanins, kaempferal and carbohydrates[4]. The plant reported to have anti diabetic[5], anti-oxidant[6], and hepataprotective[7], anti stress[8] and anti lipidemic[9] effects In the present study ethanolic extract of *Allium sativum* was evaluated for its effects on adaptogenic activity.

Guduchi [*Tinospora cordifolia* (Willd.)[10] Miers is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae[11]. Guduchi is widely used in veterinary folk medicine/ ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties[12,13]. The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The root of this plant is known for its antistress, anti-leprotic and anti-malarial activities [14].

*T. chebula,* strengthens the different tissues of the body, prevents aging, and promotes health andimmunity[15]. It corrects constipation, cleanses and tonifies the gastrointestinal tract and also detoxifies the whole body and improves digestion and assimilation[16]. It exhibits antiviral, antibacterial, antifungal and anti allergic properties[17].

**Research envisaged (Justification- Aim-Objectives):** In the literature we found some reports on the Adaptogenic Activity 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 Adaptogenic. 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 Adaptogenic activity of Trikatu and their correlation with antioxidant activity. Such studies are important to substantiate the claims documented with regard to TriAmrit herbs in ancient Ayurvedic texts.

## MATERIALS AND METHODS

**Animals:** Six-eight week old healthy, laboratory breed Swiss albino mice of either sex, weighing 25±2g 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, All three herbs 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 etc.

**Acute Toxicity:** The TriAmrit megaExt was screened for acute oral toxicity test according to OECD guideline No. 423. Therefor 1/5 and 1/10$^{th}$ cut off dose for Trikatu megaExt are 250 and 500mg/kg body weight orally, were selected for *Invivo* study.

## EXPERIMENTAL/ METHOD

**Forced Swimming Stress:** 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 aqueous solution of water soluble power Ashwagandha (100mg/kg b.w. p.o.), Group III animals were treated with TriAmrit megaExt (250 mg/kg b.w.p.o.) and Group IV animals were treated with TriAmrit megaExt (500mg/kg b.w. p.o.) orally, using oral gauge, for 7 days. On the 8th day, the animals were allowed to swim till exhausted in a separate tanks filled with water. The end point was taken when the animals drowned "Swimming Time" for each animal. The mean swimming time in minutes for each group was calculated[18].

**Anoxic stress tolerance test:** 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 aqueous solution of water soluble power Ashwagandha (100mg/kg b.w. p.o.) , Group III

animals were treated with TriAmrit megaExt (250 mg/kg b.w.p.o.) and Group IV animals were treated with TriAmrit megaExt (500 mg/kg b.w.p.o.) orally, using oral gague, for 7 days. Conical flasks of 250 ml capacity were used for the study. These flasks were made airtight using rubber cork before beginning the experiment. Each animal was kept in the airtight vessel and time was noted using a stopwatch. The moment animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of "Anoxic stress tolerance". The mean time to convulsion was recorded and animal was removed at onset of convulsion[19].

**Statistical analysis:** All the values are expresses as mean ± SD and data was analyzed by one-way ANOVA, using Graph pad 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 of TriAmrit megaExt revealed that $LD_{50}$ is 2500mg/kg body weight.

**Swimming endurance test:** The TriAmrit megaExt at a Dose I (250 mg/kg/ b.w) and Dose II (500 mg/kg/ b.w.) showed significant ($p<0.001$) increase in the swimming time as compared to control.

**Anoxic stress tolerance test in mice:** The Anoxia tolerance test was determined by taking the appearance of convulsion as end point. The TriAmrit megaExt at a Dose I (250 mg/kg/ b.w) and Dose II (500 mg/kg/ b.w.) showed significant ($p<0.001$) increasing tolerance stress time in 7th day as compared with the control.

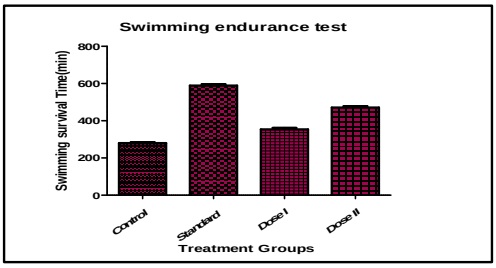


**Fig. 1: Histogram showing– effect of TriAmrit megaExt in swimming survival time**

**Table 1: Effect of TriAmrit megaExt on swimming endurance test**

| Group | Dose (mg/kg) | Swimming survival time (min) Mean ± SD |
|---|---|---|
| I – Control | 10 ml/kg normal saline | 279.5±5.009 |
| II – Control | Ashwagandha (100 mg/kg) | 588.83±7.13 |
| III – Dose I | 250 mg Tri Amrit | 353.66±7.55*** |
| IV – Dose II | 500 mg TriAmrit | 470.83±6.968*** |

**All values expressed, mean ± SD, test group vs. stress group *** p< 0.001, One way ANOVA**

**Table 2: Effect of TriAmrit megaExt on Anoxic tolerance test**

| Group | Dose (mg/kg) | Duration of anoxic stress tolerance (min) Mean±SD |
|---|---|---|
| I – Control | 10 ml/kg normal saline | 21.66±3.326 |
| II - Standard | Ashwagandha (100mg/kg) | 75.5±4.593 |
| III – Dose I | 250 mg TriAmrit | 36.5±3.619*** |
| IV– Dose II | 500 mg TriAmrit | 53.66±3.141*** |

**All values expressed, mean ± SD, test group vs. stress group *** p< 0.001, One way ANOVA**

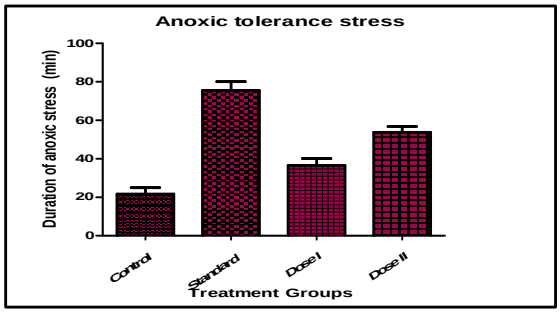


**Fig. 2: Histogram showing effect of TriAmrit megaExt in Anoxic stress tolerance test**

## DISCUSSION

Adaptogens are the substances meant to put the organs into a state of non-specific heightened resistance in order to resist stressor better and adapt to extraordinary challengers. They normalize body functions, strengthen systems and functions that are compromised by stress and have a protective effect against a wide variety of environmental and emotional stress. The forced swimming is the most widely used method for assessing the anti-stress property of a novel compound. This paradigm is based on the observation that animals when forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity. The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina with the end point being the moment when the mice could not swim further and started drowning. The increased swimming time has been observed in mice, pre-treated with TriAmrit megaExt with enhanced physical performance significantly longer than untreated (control) group and thus confirming its adaptogenic nature.

Anoxia is a very severe stressor. All the body functions including cellular respiration depend on oxygen supply to them. Any lack of this vital element plays havoc on all body mechanisms .Increase in adaptation during this stress by any drug could be considered as its major antistress effect.

## CONCLUSION

The results of the study shows that the TriAmrit megaExt significantly prolonged the meantime to convulsion, which therefore confirms its antistress property. Prolongation of meantime to convulsion could be as a result of its powerful antioxidant and free radical scavenging activities.

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