



## ANTI STRESS, ADAPTOGENIC ACTIVITY OF STANDARDIZED DRIED FRUIT EXTRACT OF AEGLE MARMELOS AGAINST DIVERSE STRESSORS

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### ABSTRACT

The Standardized dried aqueous extracts of Aegle marmelos (SDEAM) were evaluated for anti stress and adaptogenic activities using Swimming endurance and post-swimming motor function test, Cold swimming endurance test and forced swim test in rats. The extracts showed the presence of phenolics, flavonoids, carbohydrates and volatile oils in preliminary phytochemical screening. In present study the test extracts when subjected to forced swim model for adaptogenic activity in rats does not show an increase in serum cholesterol and serum triglyceride level, but the increase was not sustained on subsequent groups. It also increases the swimming endurance time significantly along with the post motar function like Rota rod falling time and spontaneous motar activity. The test extract also increases the cold swimming endurance time significantly. The test extracts could restrict the increase in the level of these markers during stress.

**Keywords:** Aegle marmelos, Anti stress, Adaptogenic, Serum cholesterol, Serum triglyceride.

### INTRODUCTION

The definition<sup>1</sup> of an adaptogen first proposed by Soviet scientists in the late 1950s, namely that an adaptogen is any substance that exerts effects on both sick and healthy individuals by 'correcting' any dysfunction(s) without producing unwanted side effects, was used as a point of departure. Traditional medicines are rich in non-specific anti-stress agents which are of increasing clinical significance. The action of an adaptogen has to be non-specific, that is to say, resistance to a wide variety of action of harmful factors, whether of a physical, chemical or a biological nature, has to increase. In other words, the action of an adaptogen has to be more intense as unfavorable changes occur in an organism, whereas the stressors external or environmental demands that placed on us is reverted by anti stress 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 infarction. 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%.

With the view to study the test drug for nonspecific anti-stress activity, tests involving diverse stressors were employed viz. swimming endurance and post-swimming motor function test, cold swimming endurance test, anoxia tolerance test.

### 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.

#### 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 ( $30\pm2^\circ\text{C}$ ) 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 (swimming endurance and post-swimming motor function test) which was carried out with four such groups. In case of swimming endurance and post swimming motor function test Group I was administered with distilled water and was not subjected to stress daily for 7 days. Group II served as stress control and received the vehicle only (distilled water) in the same volume (5 ml/kg) and subjected to stress. In group III and group IV mice were administered with SDEAM 200 mg/kg and 400 mg/kg respectively. Whereas in case of cold swimming endurance test and adaptogenic activity (Forced swim model) Group I was administered with distilled water, Group II was administered with SDEAM I 200mg/kg and Group III was administered with SDEAM II 400 mg/kg.

### EXPERIMENT

#### Swimming endurance and post-swimming motor function test <sup>3-5</sup>

The animals treated with SDEAM 100 mg/kg and 200 mg/kg were made to swim in a water tank (140x60x45 cm) maintained at room temperature ( $30\pm2^\circ\text{C}$ ) until they sank. This was recorded as the swimming time. The animals were removed and allowed to recover and dry for about 5 min. The animals were subsequently tested for muscle coordination on a Rota rod rotating at 15 rpm and the duration of stay on the rod was recorded. Immediately after that they were for spontaneous motor activity in a photoactometer for 10 min.

#### Cold swimming endurance test <sup>6</sup>

The animals treated with SDEAM 100 mg/kg and 200 mg/kg were made to swim in a water tank at  $20\pm2^\circ\text{C}$  until they sank (Bhattacharya, 1992).

#### Adaptogenic activity (Forced swim model) <sup>7</sup>

Albino rats of either sex (100–150 g) were divided in seven groups and administered the test extracts in similar manner as stated above. Stress was exerted by keeping rats in cylindrical vessels (length 48 cm and diameter 30 cm) filled with water to a height of 25 cm over period for two hours daily for seven days. Blood was collected from retro orbital plexus of each animal and the biochemical parameters such as SGPT, SGOT, serum glucose, cholesterol and triglycerides were determined.

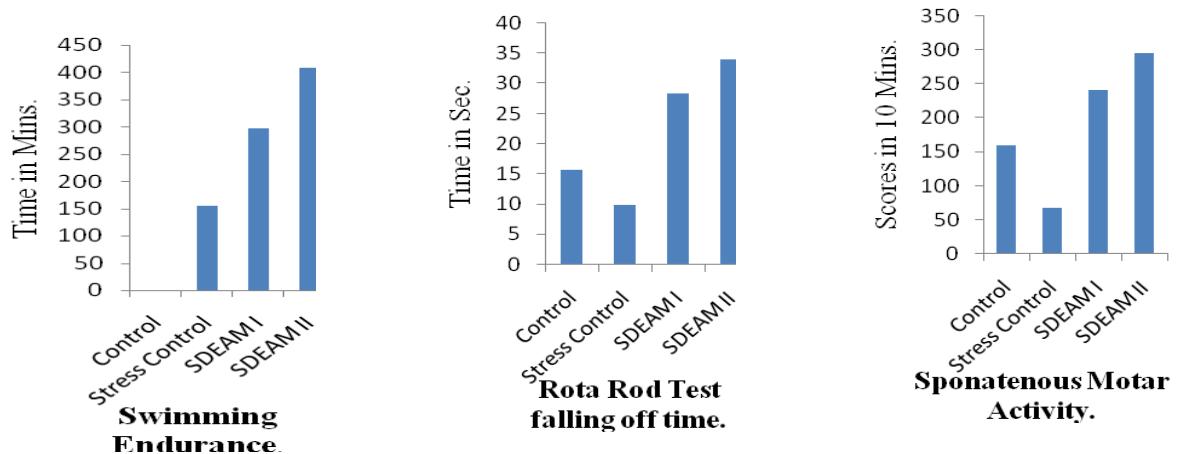


Fig. 1: Effect of Aegle marmelos on Swimming endurance and post-swimming motor function test

Table 1: Effect of Aegle marmelos on Swimming endurance and postswimming motor function test

Groups	Treatment	Dose Mg/Kg	Swimming Time In Mins	Rota-Rod Test Falling Off Time (Sec.)	Spontaneous Motor Activity
I	Control	-	-	15.66± 0.71	159.50±4.53
II	Stress Control	-	155.16±16.81	10.00±0.81*	67.66± 8.81**
III	SDEAM I	200	297.00±21.18**	28.33± 1.783**	241.00± 10.07**
IV	SDEAM II	400	408.83±11.70**	34.00±1.63**	295.83± 7.96**

n = 6, Values are mean±SEM, In case of Swimming endurance time \*p<0.01 v/s Stress Control, While in case of Rota rod falling off time and spontaneous motar activity \*\*p<0.01 v/s Control.

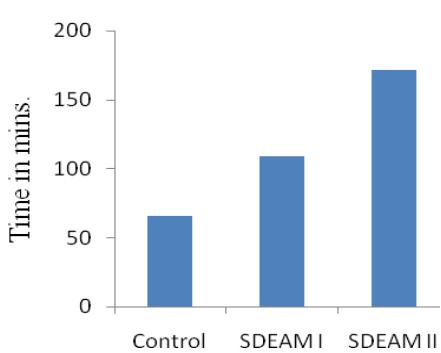


Fig. 2: Effect of Aegle marmelos on Cold swimming endurance test.

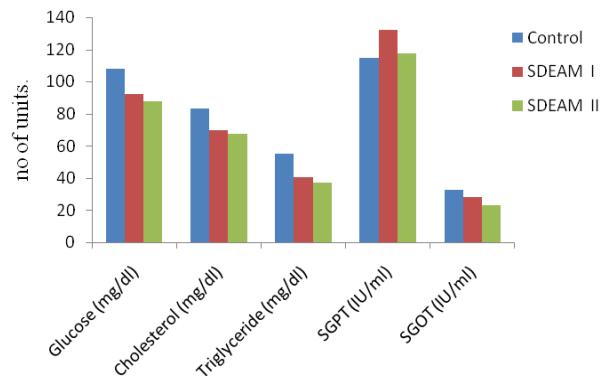


Fig. 3: Effect of Aegle marmelos on biochemical parametersin Adaptogenic activity. (Forced swim model).

Table 2: Effect of test extracts SDEAM on biochemical parameters in Adaptogenic activity

Group	Treatment	Dose mg/kg	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	SGPT (IU/ml)	SGOT (IU/ml)
I	Control	-	108.02±3.06	83.25±3.06	55.07±1.169	114.75±1.65	32.80±1.11
II	SDEAM I	200	91.95±0.74**	69.50±2.63*	40.24±0.77*	132.25±2.05**	27.97±1.62 ns
III	SDEAM II	400	87.62±0.29**	67.25±1.65**	37.12±1.54**	117.25±0.47 ns	22.80±0.30*

n=6 per group, Values are mean±SEM, comparison of I with II, III is done using Dunnet test. n.s., Not significant, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

#### 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.

#### RESULTS

##### Swimming endurance and post-swimming motor function test

It was observed that SDEAM I and SDEAM II induced a striking increase in the swimming endurance and post-swimming motor

function, however, the improvement by SDEAM I was less than by SDEAM II. SDEAM I and SDEAM II significantly increased the swimming time ( $P<0.01$ ) over stress control animals. The duration of stay on rota rod was significantly increased from  $15.667\pm0.7149$  in control group to  $28.333\pm1.783$  by SDEAM I ( $P<0.001$ ) and to  $34.000\pm1.633$  by SDEAM II ( $P<0.01$ ) as compared to stress control  $10.00\pm0.8165$  ( $P<0.01$ ). The photoactometer reading for spontaneous motor activity was  $159.50\pm4.537$  in control group and  $67.66\pm 8.819$  in stress control group while it was significantly increased to  $241.00\pm 10.073$  ( $P<0.001$ ) and  $295.83\pm 7.960$  ( $P<0.05$ ) in SDEAM I and SDEAM II treated groups, respectively (Table 1).

#### **Cold swimming endurance test**

SDEAM I and SDEAM II markedly increased the cold water swimming time of the test group by  $108.67 \pm 5.77$  and  $171.50 \pm 5.88$  over the control group  $65.50 \pm 4.639$  ( $P<0.01$ ).

#### **Adaptogenic activity (forced swim model).**

In the present study, on stress induced forced swimming model for Adaptogenic activity the values of biochemical parameters like glucose, cholesterol, triglycerides in the serum were found lower when compared with that of the values of control group.

#### **DISCUSSION**

Mice when forced to swim in a restricted space from which they cannot escape, become immobile after an initial period of vigorous activity, indicating the stress. The pretreatment with adaptogen increase swimming endurance in mice. Mice pretreated with SDEAM I 200 mg/kg and SDEAM II 400 mg/kg show significant improvement in the swimming time.

An increase in glucose level was observed, but the increase was not sustained on subsequent days. It may be due to suppression of glycogenesis, lipogenesis, increased glycogenolysis, lipolysis, and insulin resistant, which regulates blood glucose level<sup>8</sup>.

The mechanism by which stress raises serum cholesterol is likely to be due to enhanced activity of hypothalamus- hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids<sup>9</sup>. The change in serum triglyceride is possibly mediated via adrenal medullary secretions and through activation of sympathetic nervous system. In present study the test extracts when subjected to forced swim model for adaptogenic activity in rats showed an increase in serum cholesterol and serum triglyceride level in control group was observed, but the increase was not sustained on subsequent treated groups.

#### **CONCLUSION**

The studies indicate that the standardized dried extracts of Aegle marmelos possess a potential of significant antistress and adaptogenic activity, hence can be categorized as plant adaptogen. The results are encouraging to pursue further studies on the other

bioactivity guided fractionation of these extracts to isolate and characterize probable bio active molecules.

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