



PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 5 | Issue 1 | January-February 2014 | Pages 1859-1865

Original Research Article

ADAPTOGENIC ACTIVITY OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* FRUITS

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Received: 19-11-2013
Accepted: 27-12-2013

Revised: 23-12-2013
Available online: 01-01-2014

ABSTRACT

Emblca officinalis is a plant with diverse ethnical medicinal uses. The plant has been explored for diverse pharmacological actions, here it is planned to screen fruit extract for Adaptogenic activity. Wistar rats were subjected to acute physical stress (forced swimming endurance stress) to gauge the antistress potential of the extract. Stimulation of hypothalamus pituitary adrenal axis in stressful condition alters plasma glucose, triglyceride, cholesterol, SGPT and SGOT. There is also alteration in locomotor movement and blood cell counts. Pretreatment with extract significantly ameliorated the stress-induced variations in these biochemical levels and blood cell counts in acute stress model. The extract treated animals showed increase in swimming endurance time. Treatment groups also reverted back increase in liver, brain, kidney, adrenal gland weights and atrophy of spleen caused by swimming endurance stress model. The results indicate that Ethanolic extract of *Emblca officinalis* has significant Adaptogenic activity against variety of biochemical and physiological perturbations.

Key Words: Adaptogenic activity; Ethanolic extract; *Emblca officinalis*; Forced swimming endurance Stress.

INTRODUCTION

Stress basically is a reaction of mind and body against change in the homeostasis. The productive stress is called Eustress while harmful stress is called Distress. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions, stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). The stimuli, which produce GAS, are called the Stressors and range from physical to psychological factors including cold, heat, infection, toxins, major personal disappointment etc¹. In the stress-filled environment we live in, successful adaptation to stress is a prerequisite for survival. In the indigenous system of medicine, there are many herbal drugs and formulations recommended to enable one to withstand stress without altering the

physiological functions of the body. This, drug induced state of resistance against aversive stimuli is termed as adaptogenic activity and the drugs, named adaptogens. Stress alters the equilibrium of various hormones which have a significant impact on the immune response in general.

The status of immune system-immunosuppression versus immunopotentiality will depend upon the net effect of these changes. Stress and depression have been shown to affect immune system functioning, with both immunosuppression and immune activation². Correlations between depression and elevated susceptibility for infections or mortality rates have been observed and are associated with immune suppression³. The physiological reaction to stress involves alteration in the autonomic nervous system, the endocrine system and the immune system. The secretion of

Glucocorticoids is a classic endocrine response to stress. Stressful stimulation influences antigen-specific as well as nonspecific reactions⁴. Many herbs reported in ancient literature have potent antistress activity and their utilities in current scenario need to be unveiled. *Emblica officinalis* Gaertn., commonly known as amla (Hindi) and gooseberry (English), has been used in the traditional system of medicine to reduce fever, alleviate asthma, treat constipation and enhance digestion, strengthen the heart, benefit the eyes, enhance intellect and as a health tonic⁵. The fruits of *Emblica officinalis* have potent antioxidant activity due to the presence of tannoids, tannins, vitamin C and flavonoids⁶.

The pharmacological studies on *Emblica officinalis* fruit have revealed that it has good

- Antioxidant⁷
- Cytoprotective and immunomodulatory⁸
- Antidiabetic⁹
- Hypolipidemic¹⁰
- Antitussive¹¹
- Anticancer¹²
- Cardioprotective¹³
- Antiulcerogenic¹⁴ Antiepileptic and Hepatoprotective activity¹⁵.

The root contains ellagic acid and lupeol and bark contains leucodelphinidin. The seeds yield a fixed oil (16%) which is brownish-yellow in color. It has the following fatty acids: linolenic (8.8%), linoleic (44.0%), oleic (28.4%), stearic (2.15%), palmitic (3.0%) and myristic (1.0%). Since *Emblica officinalis* has a number of medicinal properties and is a potent antioxidant, the present study was undertaken to evaluate the potential usefulness of fresh fruits of *Emblica officinalis* for antistress and adaptogenic activity in experimental animals. *Withania somnifera*, an established ayurvedic herb used as an adaptogen is used as reference standard¹⁶.

MATERIALS AND METHODS

Plant material and extraction:

Fresh fruits of Amla were collected from the local market of Rangareddy District, Hyderabad and the botanical authentication was done by Dr. Ram Chandra Reddy, Head, Department of Botany, Osmania University and Hyderabad and voucher specimen no. MRCP/07 is lodged in our research laboratory for further reference. The fresh fruits were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20-mesh sieve. The dried, coarsely powdered plant material was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60°C for 24 hours. The solvent was evaporated under vacuum, which gave semisolid mass (yield: 26% w/w) with respect to the dried powder. Oral suspensions containing 100mg/ml, 200mg/ml and 400mg/ml of the ethanol extract of *Emblica*

officinalis were prepared in 1% w/v gum acacia and were used for the evaluation of Adaptogenic activity.

Animals

Swiss albino mice weighing 20-25 g and Albino Wistar rats weighing 150-250 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, approved the study.

Forced swimming endurance test (physical stress):

Rats of either sex (200-250g) were used for forced swim endurance stress. Group I rats received 0.1% gum acacia in saline; (vehicle control). Group II rats were treated with 0.1% gum acacia in saline and stress; (negative control). Group III, IV and V rats were treated with ethanolic extract at 100, 200 and 400 mg/kg, p.o. and stress. Group VI rats were treated with *Withania somnifera* (100 mg/kg, p.o.) and stress (positive control). The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37x37x30 cm), filled with water to a height of 25cm. Extracts were given to rats, once daily for period of 7 days. On 7th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal started drowning. The mean swimming time (swimming endurance time) for each group was calculated at the end of experimental period¹⁷. The change in locomotor activity was measured using photoactometer before and after induction of stress on 7th day. Then animals were killed and blood was collected by cardiac puncture to estimate biochemical parameters like serum glucose, triglycerides, cholesterol, SGPT, SGOT and blood cell count (RBC and WBC). The weights of organs such as liver, adrenals, spleen, brain and kidney were recorded after washing with alcohol.

Statistical analysis:

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post-hoc analysis was carried out by Dunnett's multiple comparison test to estimate the significance of difference between individual groups.

RESULTS

Effect of ethanol extract in forced swimming endurance stress:

The results of the study revealed that the extract possess antistress property as it significantly (P<0.05) increased the swimming time (Figure 1). Swimming endurance stress resulted in significant increase in adrenal gland, liver, brain and kidney weight with concomitant decrease in

spleen weight in stress control group, which was significantly reverted by *Embllica officinalis* pretreatment at 100 mg/kg, 200 mg/kg and 400 mg/kg (Table 1). Similarly stress induced elevated blood cell counts (RBC and WBC) were reduced by the ethanol extract in a dose dependant manner (Fig 2,3). Pretreatment of animals with *Embllica officinalis* at three doses

also significantly ($P<0.05$) restored back forced swimming stress induced alterations in plasma glucose, triglyceride, SGPT, SGOT and cholesterol (Table 2). The locomotor activity was significantly reduced in stress control animals. The treatment of animals with different doses ameliorated the stress induced reduction in the locomotor activity (Fig 4).

Table.1 Effect of ethanol extract of *Embllica officinalis* on Organ weights in forced swimming endurance stress in rats

Groups	Spleen (mg)	Liver (gms)	Adrenal Gland (mg)	Brain (gms)	Kidney (gms)
Control	340.01±2.25	4.68 ±1.58	2.4 ±0.57	1.13± 0.12	0.98 ± 0.24
Stress Control	198.24 ±1.58*	6.52±1.23*	3.4±1.46*	1.3 ± 0.10*	1.23 ± 0.22*
E.O extract 100mg/kg	280.2±2.54	4.58 ±1.69	2.8 ±1.65	1.31± 0.11	1.20± 0.24
E.O extract 200mg/kg	300.95±2.65**	4.37±2.58**	2.6*±1.58**	1.28 ±0.13	1.15 ± 0.26
E.O extract 400mg/kg	320.61±1.97**	4.28 ±3.35**	2.5±1.35**	1.26± 0.12**	1.06 ± 0.23**
W.Somnifera 100mg/kg	350.21±1.58**	4.20±2.54**	2.2±0.85**	0.97 ± .09**	1.21 ± 0.34**

The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 7 days. Control rats were given saline. Swimming stress was induced at the end of the treatment period. The animals were sacrificed, the organs were isolated from

the animals and the weights were noted. The results are expressed as mean±SEM, n=6 in each group. * $P<0.01$ significant as compared to control, ** $P<0.01$, significant as compared to stress control, statistical test employed is ANOVA followed by Dunnett's t test.

Table.2.Effect of ethanolic extract of *Embllica officinalis* on Biochemical parameters in Swimming Endurance Stress in rat

Groups	Glucose mg/dl	Cholesterol mg/dl	Triglycerides mg/dl	SGPT mg/dl	SGOT mg/dl
Control	79.03±0.086	85.18±0.03	89.41±3.63	63.22±2.85	28.18±2.69
Swimming Stress Control	104.8±0.083	100.00±0.01	184.62±1.89	95.87±5.02	58.63± 1.24
EO extract 100mg/kg p.o.	98.3±0.01*	72.59±0.01*	146.15±4.05*	89.42±2.64*	48.37±2.58*
EO extract 200 mg/kg p.o.	94.8±0.01**	65.9±0.06**	92.30±3.82**	72.56±3.98*	41.92±3.58*
EO extract 400mg/kg p.o.	91.2±0.0**	62.9±0.0**	88.46±1.5**	67.89±2.6**	38.29.3.25*
W. Somnifera 100mg/kg p.o.	87.4±0.01**	59.2±0.07**	84.62±3.59*	66.86±0.89*	36.42±0.78

The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 7 days. Control rats were given gum acacia. Swimming stress was induced at the end of treatment period. The animals were sacrificed; the blood collected and

the biochemical parameters were estimated. The values are expressed as mean±SEM, n=6 in each group. * $P<0.05$ significant as compared to control, ** $P<0.05$, significant as compared to stress control, statistical test employed is ANOVA followed by dunnett's t test.

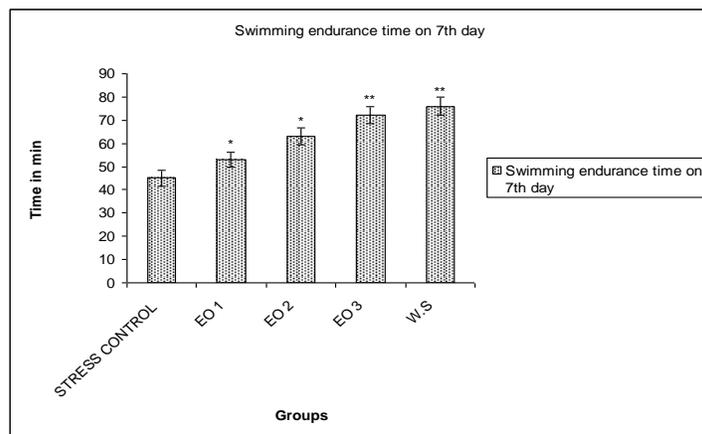


Fig.1.The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to stress control, **P<0.05, significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's t test.

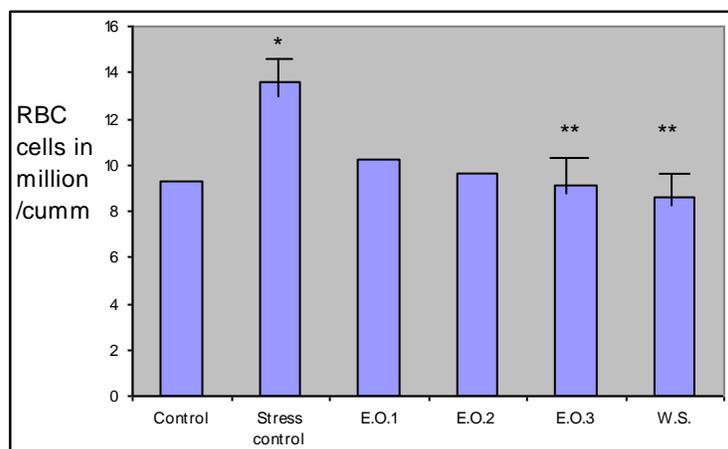


Fig.2.The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 7 days. Control rats were given saline. Swimming stress was induced at the end of treatment period. The animals were sacrificed; blood was collected for the estimation of cell counts. The values are expressed as mean±SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05 significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's t test.

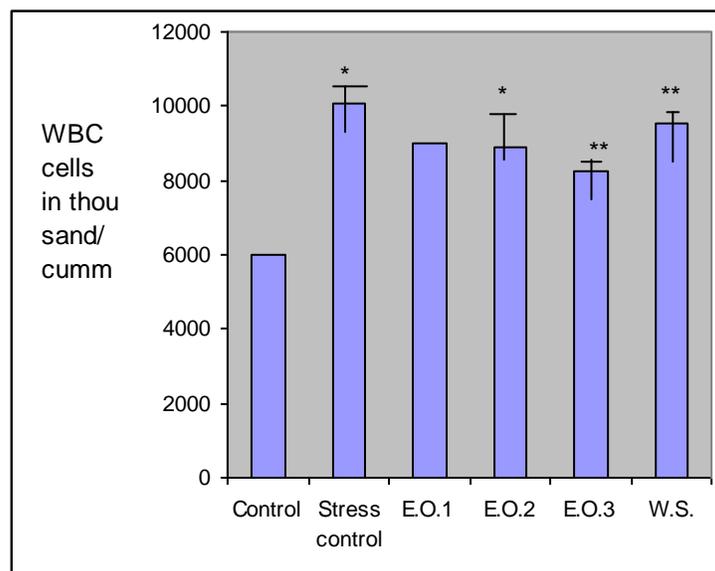


Fig.3. The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 7 days. Control rats were given saline. Swimming stress was induced at the end of treatment period. The animals were sacrificed; blood was collected for the estimation of cell counts. The values are expressed as mean \pm SEM, n=6 in each group. *P<0.05 significant as compared to control, **P<0.05 significant as compared to stress control, statistical test employed is ANOVA followed by dunnet's test.

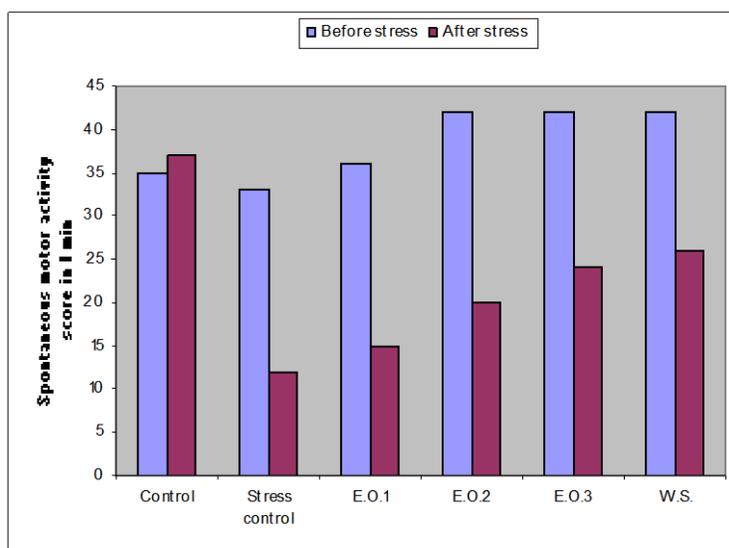


Fig.4. Effect of ethanol extract of *Embllica officinalis* on locomotor activity in rats. The rats were pretreated with *Withania somnifera* (100 mg/kg), EO (100, 200, 400 mg/kg, p.o.) once daily for 7 days. The change in locomotor activity was measured using photoactometer before and after induction of stress on 7th day. The values are expressed as mean \pm SEM, n=6 in each group.

DISCUSSION

Adaptogens are the substances meant to put the organisms into a state of non-specific heightened resistance in order to better resist stressor 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^{18, 19}. This paradigm is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity²⁰.

The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina with the end point being the moment when the rat could not swim further and started drowning²¹. However, increased swimming time has been observed in rat pre-treated with ethanolic extract and has enhanced the physical performance longer than untreated (control) group confirming their adaptogenic nature. In present study, the significant increase in blood glucose level was observed because; under stressful conditions adrenal cortex secretes cortisol in man and corticosterone in rats. Hyper secretion of cortisol helps in maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis²². Pretreatment with the *Emblica officinalis* as well as reference standard drug *Withania somnifera* significantly ($P < 0.05$) reduced the elevated glucose levels indicating their suppressant effect on hyper activity of adrenal cortex and maintained the homeostatic mechanism. The marked increase in serum cholesterol, triglycerides levels and also an increase in levels of serum marker enzymes SGPT, SGOT in stress induced animals is due to stimulation of hypothalamo-pituitary axis (HPA) and sympathetic system, resulting in, liberation of catecholamines and glucocorticosteroids, which inhibits the immune system at multiple sites like liver, kidney²³. *Emblica officinalis* as well as reference standard drug *Withania somnifera* significantly ($P < 0.05$) reduced the elevated serum cholesterol, triglycerides levels, which may be due to inhibition of stimulation of sympathetic nervous system. The increase in weight of adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals²². *Emblica officinalis* and *Withania somnifera* has significantly ($P < 0.01$) reduced the liver, brain, kidney and adrenal gland weight, this may be due to the reversal of the stress induced adrenomedullary response and hence decreased production of corticotropic hormone. The decrease in weight of spleen may be due to recruitment of lymphocytes to blood from spleen which results in squeezing of the spleen²⁴. The pretreatment with the *Emblica officinalis* and reference standard *Withania somnifera* significantly ($P < 0.01$) increased the spleen weight. This may be due to inhibition of recruitment of lymphocytes to blood from spleen. During stress, heart rate, blood pressure and blood flow rate increases. To meet these extra demands RBC and WBC counts will be increased. In the present study the extract has decreased the elevated levels of RBC and WBC in both swimming endurance and cold restraint stress models. This study has also shown that the extract prolonged mean time to convulsion, which therefore demonstrate antistress property.

The prolongation of mean time to convulsion could be attributed to its powerful anti-oxidant and free radical scavenging activities²⁵. A variety of biological activities including adaptogenic activity were reported with flavonoids, tannins and phenolic glycosides²². *Emblica officinalis* contains biologically active chemicals that include flavonoids, several phenolic compounds, tannins like gallic acid derivatives including epigallocatechin gallate²⁶. The antioxidant activity of tannoid active principles of *Emblica officinalis* consisting of emblicanin A, emblicanin B, punigluconin and pedunculagin have also been reported. The adaptogenic activity may be due to these constituents where as standard drug *Withania somnifera* an established adaptogenic drug too contains glycosides, steroids and flavonoids²².

CONFLICT OF INTEREST

Authors declare no conflict of interest

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HOW TO CITE THIS ARTICLE

N.Neelima, M. Sudhakar, Dr.B.V.S. Lakshmi*. (2014 January 1). Adaptogenic Activity of Ethanolic Extract of *Emblica Officinalis* Fruits. *PHARMANEST*, 5(1), 1859-1865. <http://www.pharmanest.net>