Research Article

Screening of adaptogenic potential of *Diascoria bulbifera* tubers

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Abstract

Objectives: The present research work was designed to screen adaptogenic and activity of methanolic extract of *Diascoria bulbifera* tubers (MEDBT) at different dose levels (100, 250 and 500 mg/kg) using different experimental animal models. **Material and methods:** In the present study adaptogenic activity was carried out against anoxia stress tolerance, swimming endurance and chemical induced stress models in rats. **Results:** In anoxia tolerance test, the mean time of appearing first convulsion in mice was taken as end point to determine the time of anoxia tolerance. In swimming endurance test, the mean time of swimming performance, percentage anti fatigue effect and swimming stress induced biochemical parameters such as serum cortisol, the weight of adrenal gland, adrenal gland ascorbic acid and cortisol level were recorded. The number of writhes were determined in acetic acid induced writhes and numbers of convulsions were determination in PTZ induced convulsions. **Conclusion:** The findings from the present study suggest that methanolic extract of *Diascoria bulbifera* tubers demonstrated adaptogenic effect.

***Key words*:** Adaptogenic, anoxia, convulsion, adrenal glands, serum cortisol

Introduction

Adaptability is probably the most distinct characteristic of life. Dr. Hans Seyle defined stress as the sum of all non specific responses of the body to any external stimuli acting up on it. Fundamentally, it is a physiological response towards external stimuli and the primary objective of such a response is to restore the normal process of life. Perhaps the single most important property of an adaptogen is its proven ability to combat stress in all forms (Selye, 1973).

In the present days, stress has become an integral part of human life (Ravindran et al., 2005). Stress is considered to be any condition which results in perturbation of the body's homeostasis (Emeny and Lawrence, 2007). If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened (Lakshmi and Sudhakar, 2009). Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, namely; diabetes, hypertension, immunosuppression, peptic ulcer, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous system, endocrine system, and metabolic system (Rai et al., 2003). Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely prescribed to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs (Umokoro and Ashorobi, 2005).

The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Various herbs like *Withania somnifera* (Bhattacharyaa and Muruganandam, 2003), *Emblica officinalis* (Nirmala et al., 1999), *Asparagus racemosus* (Garg and Gupta, 2010), *Ocimum sanctum* (Tapan et al., 2000), *Tribulus terrestris* (Shivakumar et al., 2006) and *Trigonella foenum- graecum* (Pawar and Shivakumar, 2001) are claimed to have adaptogenic effect and the ability to improve vital energy (Bhattacharyaa and Muruganandam, 2003).

Several poly herbal formulations namely Siotone (Bhattacharya et al., 2000), AVM (Shaik Azamathulla et al.,

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2006) and Geriforte have been reported to possess significant antistress properties. AVM is a poly herbal formulation possessing adaptogenic activity consists of various ingradients namely Root of *Withania somnifera*, Fruit of *Emblica officinalis*, Root of *Asparagus racemosus,* Tuber of *Diascoria bulbifera*, Powder of Trikatu, Leaves of *Ocimum sanctum* , Powder of Shilajit, Areal parts of *Tribulus terrestris* and Fruit of *Piper longum*. AVM a ploy herbal formulation, some of whose constituents like *Withania somnifera* (Bhattacharyaa and Muruganandam, 2003)*, Emblica officinalis* (Nirmala et al., 1999), *Asparagus racemosus* (Garg and Gupta, 2010)*, Ocimum sanctum* (Tapan et al., 2000) and *Tribulus terrestris* (Shivakumar et al., 2006) have earlier been reported to exhibit significant adaptogenic activity. However, the literature review reveals that an adaptogenic property of *Diascoria bilbifera* tubers has not been scientifically validated so far. Hence, the present study was undertaken to evaluate adaptogenic activity.

Materials and methods

Plant material

For this study, the tubers of *Diascoria bulbifera* were collected from the herbal garden of S D M College of Ayurveda, Udupi, Karnataka. The sample was identified and authenticated by Dr. M. B. Mulimani, Professor of Botany, S.B Arts and K.C.P. Science College, Vijaypur, Karnataka. The specimen was preserved in the herbarium of the HSK College of Pharmacy, Bagalkot- 587101.

Preparation of extract

Tubers of *Diascoria bulbifera* were cleaned, shade dried and coarse powdered. Then the powdered material was defated with pet ether for the removal of fatty material and then extracted with methanol using Soxhlet extraction method. Thereafter, the methanolic extract *Diascoria bulbifera* tubers (MEDBT) was concentrated using rotary flash evaporator and percentage yield (13.5%) recorded.. The obtained crude extract was stored in airtight container in refrigerator below 10 ^{0}C for further studies.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed on test extract for the detection of various phytoconstituents. Tests for the presence of common phytochemicals were performed by following literature reported methods (Trease and Evans, 1996).

Experimental animals

The albino mice 20 - 30 g and Wistar rats 150 - 200 g of either sex were used in the experimentation. The animals were procured from Sri Venkateshwara Enterprises, 4304, 13th main 2nd cross, Subramanyanagar, Bangalore-21 (237/CPCSEA). After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry condition as follows.

Room temperature: 27 ± 3$^{\circ c}$, Relative humidity: 65 ± 10%, 12 hr. light/dark cycle.

All the animals were fed with rodent pellet diet (VRK Nutritionals Industries, Pune, India) and water *ad libitium* under strict hygienic condition. Study protocol was approved from Institutional Animal Ethics Committee (IAEC) before initiation of the experiment. (Ref No. BLDEA's COP/IAEC/51 dated 29/07/2013)

Preparation of stock solution of methanolic extract of *Diascoria bulbifer* tubers

Appropriate concentration of stock solution of the test extract was prepared by suspending in 2% w/v of tween 80 in distilled water. This stock solution was administered orally at 1 ml/100 g body wt. of mice and 0.5 ml/100 g body wt. of rats.

Acute toxicity study (LD_{50}) (Prema Veeraraghavan, 2001)

The acute toxicity of methanolic extract of *Diascoria bulbifer* tubers was determined in female albino mice (20-30 g). The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method was employed for toxicity study. Based on the result of the study, 1/40th, 1/20th and 1/10th of LD_{50} cut off value, the screening doses of extract selected for adaptogenic and anxiolytic activities.

Evaluation models for antistress activity of methanolic extract of *Diascoria bulbifer* tubers

Anoxia stress tolerance test (Shivakumar et al., 2006, Pawar and Shivakumar, 2001)

Albino mice of either sex weighing 20 -30 g were selected and divided into five groups of six each.

1. Group I - Control, received vehicle only
2. Group II - Std. (*Withania somnifera*, 100 mg/kg, p.o.)
3. Group III - MEDBT (125 mg/kg, p.o.)
4. Group IV - MEDBT (250 mg/kg, p.o.)
5. Group V - MEDBT (500 mg/kg, p.o.)

Animals were treated as shown above for three weeks. At the end of 1st, 2nd and 3rd week i.e. on 7th, 14th, and 21st day 1 hr. after the treatment, stress was induced in all the groups of animals by placing each mouse individually in the hermetic vessel of 300 ml capacity to record anoxia stress tolerance time. The moment when the animal showed the first convulsions removed immediately from the vessel. The time duration of animal entry into the hermetic vessel and the appearance of the first convulsion was recorded as anoxic tolerance time. Appearance of convulsion is very

sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the same.

Swimming endurance test (Shivakumar et al., 2006, Pawar and Shivakumar, 2001)

Albino mice of either sex weighing 20 - 30 g divided into six groups of six animals each

1. Group I - Control, untreated
2. Group II - Stress control, received vehicle only
3. Group III- Std. (*Withania somnifera* 100 mg/kg, p.o.)
4. Group IV- MEDBT (125 mg/kg, p.o.)
5. Group V - MEDBT (250 mg/kg, p.o.)
6. Group VI- MEDBT (500 mg/kg, p.o.)

Treatment was given to mice for 7 days. On seventh day 1 hr. after treatment, all the mice (except normal control) were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 26 1^0C temperature. The end point was taken when the animals remained at the bottom of swimming tank for 10 sec. The mean swimming time for each group was calculated.

Post swimming antifatigue and motor coordination test

The animals were immediately taken out, dried with tissue paper and subsequently all the animals placed on digital rota rod (15 rpm) to monitor antifatigue and motor coordination effects.

Biochemical estimations and adrenal glands

The blood was collected (orbital sinus) from all the animals subjected to post swimming antifatigue effect for estimation of serum cortisol level. Then the animals were sacrificed for the removal of adrenal glands to record their weight. Then adrenal glands were used for the estimation of ascorbic acid and cortisol levels using standard procedures reported in the literature (Shivakumar et al., 2006, Pawar and Shivakumar, 2001).

Chemical induced stress (Patel et al., 2011**)**

PTZ (Pentylenetetrazol) induced convulsions

The mice of either sex weighing between 20 - 30g were randomly divided into five groups of six animals each.

1. Group I - Control, received vehicle only
2. Group II - Std. Diazepam (2 mg / kg, p.o.)
3. Group III- MEDBT 125 mg/kg (5000/40^{th} of LD 50 cut off value)
4. Group IV- MEDBT 250 mg/kg (5000/20^{th} of LD 50 cut off value)
5. Group V - MEDBT 500 mg/kg (5000/10^{th} of LD 50 cut off value)

Group I considered as normal control received only vehicle. The group II animals were administered with Diazepam (2 mg/kg, p.o.). The group III, IV and V animals were treated with test extract of different doses for a period of seven days. At the end of the seventh day, all the animals were injected with pentylenetetrazol (80 mg / kg, i.p.), one hour after oral administration of the drug. The onset of convulsions, severity of convulsions, rate of mortality and recovery of animals were recorded.

Statistical analysis

The data obtained from the above findings were subjected to statistical analysis following one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results using Graph pad prism software.

Results

Preliminary phytochemical screening

Results of the preliminary phytochemical investigation on methanolic extract of *Diascoria bulbifera* tubers reveal the presence of tannins, flavonoids, carbohydrates and alkaloids.

Acute toxicity study

In an acute toxicity studies, test extract of title plant did not cause any mortality (0/3 mice died) of the animals at dose of 2000 mg/kg, even at repeated dosing using 3 new mice. Hence, 5000 mg/kg was taken as LD_{50} cutoff value as per fixed dose method of OECD guideline number 423.

The doses selected for the evaluation of anti-stress and anxiolytic activities of the test extract were:

125 mg/kg - 1/40^{th} dose of LD 50 cut off value, 5000 mg/kg b.w.

250 mg/kg - 1/20^{th} dose of LD 50 cut off value, 5000 mg/kg b.w.

500 mg/kg - 1/10^{th} dose of LD 50 cut off value, 5000 mg/kg b.w.

Preparation of stock solution of methanolic tuber extract of *Diascoria bulbifera*

Appropriate concentration of stock solution of the test extract was prepared by suspending in 2% w/v of tween 80 in distilled water. This stock solution was administered orally at 1 ml/100 g body wt. of mice and 0.5 ml/100 g body wt. of rats.

Anoxia stress tolerance time in mice

In this model, the test extract demonstrated dose and duration dependent significant adaptogenic activity. This

was evident by observing subsequent increase in anoxic stress tolerance time at the end of 1st, 2nd and 3rd week of the study when compared to control group. Though, there was increase in anoxia stress tolerance time observed at dose of 125 mg/kg on 7th day but the results found statistically not significant. Antistress effect of 500 mg/kg dose of the extract was found to be less effective than that of standard drug, *Withania somnifera*. The results are tabulated in Table 1.

Table 1. Effect of MEDB on anoxia stress tolerance time in mice

Groups	Treatment	Duration of anoxia stress tolerance time (min)		
		7th Day	14th Day	21st Day
I	Control	25.6 ± 0.67	27.3 ± 1.78	31.6 ± 1.78
II	Std. (*Withania somnifera*) 100 mg/kg	50.2 ± 2.8***	54.0 ± 1.98***	57.6 ± 2.1***
III	MEDBT 125 mg/kg	30.4 ± 2.8ns	37.5 ± 2.1**	39.2 ± 1.7*
IV	MEDBT 250 mg/kg	33.4 ± 2.4*	38.3 ± 1.9**	41.5 ± 2.3**
V	MEDBT 500 mg/kg	39.0 ± 2.7***	43.2 ± 2.3***	45.3 ± 2.4***

The values are expressed as Mean ± SE, (n=6); *p < 0.05, ** p < 0.01, *** p < 0.001 as compared to control

Swimming endurance test in mice

Mice pretreated with graded doses (125, 250, 500 mg/kg) of the test extract for seven days, significantly increased the swimming performance time compared to the control group. The effect seen was dose-dependent and statistically significant. The percentage increase in the swimming time was found to be ranging from 18 to 43. The effect of MEDBT on swimming performance time at dose of 500 mg/kg was found closer to that of reference standard drug, *Withania somnifera*.

Post swimming antifatigue and motor coordination effect

Mice subjected to the motor coordination test followed by swimming stress exhibited significant fatigue and motor incoordination effect compared to control group. Animals pretreated for seven days with test extract demonstrated significant antifatigue and motor coordination effect in dose dependent manner when compared to stress control group. The antifatigue and motor coordination effect was found to be less potent as compared to standard. The results are tabulated in Table 2.

Swimming stress induced biochemical parameters in mice

Swimming stress resulted in increase in adrenal glands weight in association with depletion of adrenal contents namely ascorbic acid and cortisol, there was an elevated serum cortisol level also seen in swimming control as compared to normal control animals. Treatment with test extract of different doses prevented the adrenal hypertrophy. Increased serum cortisol level was reversed with treatment of MEDBT significantly. Furthermore, a significant increase in adrenal ascorbic acid and cortisol contents were observed in animals treated with different doses MEDB. The results are tabulated in table 3.

Chemical induced stress (PTZ Induced convulsions)

The MEDBT at graded doses significantly increased the onset of convulsions, percentage protection and decreased number of convulsions, rate of mortality in mice compared to control group, which was found to be dose dependent. The results are given in table 4.

Table 2. Effect of MEDBT on swimming endurance time and post swimming antifatigue effect in mice

Groups	Treatment	Swimming endurance time (min)	% increase in swimming time	Fall of time from rotarod (sec)	% antifatigue effect
I	Normal control	----	---	110 ± 4.25	--
II	Stress Control	222.2 ± 10.93	----	28.32±2.5	---
III	Std. (*Withania somnifera*) 100 mg/kg	411.2 ± 6.`71***	45.95	58.24±2.3***	51.72
IV	MEDBT 125 mg/kg	260.4 ± 2.15***	14.61	31.31±2.1ns	14.09
V	MEDBT 250 mg/kg	324 ± 18.86***	31.48	37.38±1.8**	26.56
VI	MEDBT 500 mg/kg	382.8 ± 4.14***	41.88	44.37±1.2***	37.30

The values are expressed as Mean SEM, (n=6); * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to control.

Table 3. Effect of MEDBT on swimming stress induced biochemical parameters and adrenal gland weight in mice

Groups	Treatment	Adrenal gland weight (mg/100 g b.w)	Serum cortisol (µg/dl)	Ascorbic acid (mg/100 g of adrenal wt.)	Cortisol (mg/100 g of adrenal wt.)
I	Normal control	7.25±0.62	23.56±0.58	280.2±0.25	3.73±0.45
II	Stress Control	15.65 ± 0.12	45.95±0.2	128.2 ± 2.3	1.05 ± 0.06
III	Std. (Withania somnifera) 100 mg/kg	10.20 ± 0.71***	25.25±0.6***	258.5 ± 2.3***	3.10 ± 0.03***
IV	MEDBT 125 mg/kg	13.4 ± 0.14***	34.96±0.4***	149.2±2.2***	1.33 ± 0.05***
V	MEDBT 250 mg/kg	12.2 ± 0.85***	29.30±0.8***	192.1±1.9***	1.87 ± 0.07***
VI	MEDBT 500 mg/kg	10.8 ± 0.13***	26.92±0.1***	224.2±1.3***	2.55 ± 0.01***

The values are expressed as Mean SEM, (n=6); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

Discussion

Since the introduction of adptogens, several plants that had once been used as tonics in the Ayurvedic medicine, have been investigated for their antistress property due to their adaptogenic and rejuvenating properties (Patel et al., 2011).

In the present study, MEDBT at different dose levels (125, 250 and 500 mg/kg) was investigated for antistress activity of against anoxic tolerance test, swimming endurance test and chemical induced stress animal models.

Anoxia is a very severe stressor. All the body functions including cellular respiration depends 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. The results of the anoxic tolerance test showed that MEDBT significantly delayed the latency of convulsions in experimental animals, which therefore confirm its antistress activity.

The swimming endurance test is most widely used physical stress model for the assessment of adaptogenic activity of a novel drug. 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 reduced stamina, fatigue and tiredness with the end point being the movement when the animal could not swim further and started drowning (McEwen 2001, Alexander et al., 2010, Sapolsky et al., 2006). The results of the swimming test demonstrated the marked increase in swimming time in mice, pretreated for seven days with test extract with enhanced physical performance and thus confirming its antistress property.

Motor coordination test was used to find out the spontaneous motor activity. Post swimming muscle coordination (anti-fatigue) was carried out using rota rod test, which determines an animal's ability to support its own body weight by holding on to the rotating rod. The loss of muscle grip is an indication of relaxation which is recorded by fall of time (Trivedi et al., 2011). The stress control animals showed an early fall from rota rod which interprets the reduced muscle coordination. The MEDBT showed increased the duration (sec) of stay on rota-rod in rats significantly as compared to stress control animals in a dose dependant fashion.

Stress induces adreno-medullary response resulted in greater release of ACTH, which stimulates adrenal medulla and cortex which leads to increase in the weight of adrenal glands (Bhattacharya 1994, Nocerino et al., 2000). Treatment with test extract of different doses prevented the adrenal hypertrophy. Increased serum cortisol level was reversed with treatment of MEDBT significantly. Furthermore, a significant increase in adrenal ascorbic acid and cortisol contents were observed in animals treated with different doses MEDBT.

Pentylenetetrazol has an inhibitory function of the GABAergic system in the brain (Corda et al., 1991). Gamma amino butyric acid (GABA) plays a major role in the central integration of the hypothalamic-pituitary adrenocortical (HPA) stress responses. GABAergic neurons in the bed nucleus of the stria terminalis, preoptic area, and hypothalamus can directly inhibit paraventricular nuclei outflow, and thereby, reduce adrenocorticotropic

Table 4. Effect of MEDBT on chemical induced stress (PTZ induced convulsions)

Group	Treatment	Onset of convulsion (Sec)	No of convulsions	Mortality (%)	Protection (%)
I	Control	08.23± 2.19	06.78± 3.22	100	00
II	Std. (Diazepam) 2 mg/kg	22. 23± 2.37***	00	00	100
III	MEDBT 125 mg/kg	10.28± 1.79*	06.21± 3.19**	90.29± 2.19**	25.35± 3.01*
IV	MEDBT 250 mg/kg	12.16± 2.10**	03.70±2.98***	73.37± 2.56**	45.53±2.98***
V	MEDBT 500 mg/kg	15.33± 1.98***	01.98±2.87***	47.67±3.13***	68.21±2.73***

The values are expressed as Mean SEM, (n=6); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.

hormone secretion. Thus, GABA produces a marked inhibitory effect on HPA axis activity (Giordano et al., 2006, Herman et al., 2006). Stress also causes a rapid decrease in GABA receptor binding in the central nervous system (Biggio et al., 1981). The adaptogenic activity of MEDBT against pentylenetetrazol is observed by increasing the latency for the onset of convulsions, mortality protection, and decreasing the duration of convulsions.

Conclusion

In conclusion, the findings from the present study suggest that methanolic extract of *Diascoria bulbifera* tubers demonstrated adaptogenic effect. However, the present study did not include the tests for establishing the exact mechanism of action.

Conflict of interest

The authors report no conflicts of interest.

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References

Alexander N, Shikov ON, Pozharitskay MN, Damien DHJ, Makarova VG, Raimo H. 2010. Adaptogenic effect of black and fermented leaves of *Bergenia crassifolia* L. in mice. Journal of Functional Foods, 2: 71-76.

Bhattacharya SK, Bhattacharya A, Chakrabarti A. 2000. Adaptogenic activity of siotone, a poly herbal formulation of ayurvedic rasayanas. Indian Journal of Experimental Biology, 38: 119-28.

Bhattacharya SK, Ghosal S. 1994. Experimental evaluation of antistress activity of a herbal formulation Zeetress. Indian Journal of Indigenous Medicine, 10: 1-8.

Bhattacharyaa SK, Muruganandam AV. 2003. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. Pharmacology, Biochemistry and Behavior, 75: 547-55.

Biggio G, Corda GM, Concas G, Demontis Z, Gessa GS. 1981. Rapid changes in GABA binding induced by stress in different areas of the rat brain. Brain Research, 229:363-9.

Corda MG, Orlandi M, Lecca D, Carboni G, Frau V. 1991. Pentylenetetrazole induced kindling in rats: effect of GABA function inhibitors. Pharmacology Biochemistry and Behavior , 40: 329-33.

Emeny RT, Lawrence AD. 2007. Psychoneuroimmunology. 4th ed., Elsevier, Inc.

Garg R, Gupta VB. 2010. Aadaptogenic activity of milk and aqueous decoction of *Asparagus racemosus* wild in acute and chronic stress paradigms in mice. Journal of Cell and Tissue Research, 10(2): 2281-286.

Giordano R, Pellegrino M, Picu A, Bonelli L, Balbo M, Berardelli R. 2006. Neuroregulation of the hypothalamus-pituitary-adrenal (HPA) axis in humans: Effects of GABA-, mineralocorticoid-, and GH-secretagogue-receptor modulation. Science World Journal, 6: 1-11.

Herman JP, Mueller NK, Figueiredo H. 2006. Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. Annals of the New York Academy of Sciences, 1018: 35-45.

Lakshmi BVS, Sudhakar M. 2009. Screening of *Psidium guajava* leaf extracts for antistress activity in different experimental animal models. Pharmacognosy Research, 1(6): 359-66.

McEwen BS. 2008. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. European Journal of Pharmacology, 583: 174-85.

Nikunj B Patel, Varsha J Galani, Bharatkumar G Patel. 2011. Antistress activity of *Argyreia speciosa* roots experimental animals. Journal of Ayurveda & Integrative Medicine, 2(3): 129-36.

Nirmala NR, Urmila MT, Sharadini AD. 1999. Adaptogenic properties of six *rasayana* herbs used in ayurvedic medicine. Phytotherapy Research, 13(4): 275-91.

Nocerino E, Amato M, Angelo A. 2000. The aphrodisiac and adaptogenic properties of ginseng. Fitoterapia, 71: 1-5.

Pawar VS, Shivakumar H. 2011. Antistress activity of *Trigonella foenum-graecum* (Linn.) seeds using swimming endurance and cold stress in rodents. Indian drugs, 48(2): 56-61.

Prema Veeraraghavan. 2001. Expert consultant, CPCSEA, OECD guideline No. 423.

Rai D, Gitika Bhatia G, Sen T, Palit G. 2003. Anti-stress effects of *Ginkgo biloba* and *Panax ginseng*: a comparative study. Journal of Pharmacological Sciences, 93: 458-64.

Ravindran R, Sheela Devi R, Samson J, Senthilvelan M. 2005. Noise-stress-induced brain neurotransmitter changes and the effect of *Ocimum sanctum* (Linn) treatment in albino rats. Journal of Pharmacological Sciences, 98: 354-60.

Sapolsky RM, Romero LM, Munck AU. 2006. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and pre- parative actions. Endocrine Review, 21: 55-89.

Selye H. The evaluation of the stress concept. American Scientist, 1973; 61.

Shaik Azamathulla, Amolkumar Hule, Suresh Ramnth Naik. 2006. Evaluation of adaptogenic activity profile of herbal formulation. Indian Journal of Experimental Biology, 44: 574-79.

Shivakumar H, Talha J, Prakash T. Rao N, Swamy BHMJ, Veeranagoud A. 2006. Adaptogenic activity of ethanolic extract of *Tribulus terrestris* Linn. Journal of Natural Remedies, 6: 87-95.

Tapan KM, Subhash CM, Saha BP, Pal M. 2000. Effect of *Ocimum sanctum* roots extract on swimming performance in mice. Phytotherapy Research, 14(2): 120-21.

Trease GE, Evans WC. 1996. Pharmacognosy, 3rd ed, Baillier Tindall, East Bourne: ELBS Publication.

Trivedi R, Sharma K. 2011. Hydroalcoholic extract of *Glycyrrhiza glabra* linn. attentuates chronic fatigue stress induced behavioral alterations in mice. International Journal of Pharmaceutical and Biological Archive, 2(3): 996–1001.

Umokoro S, Ashorobi RB. 2005. Antistress potential of aqueous seed extract of Aframomum melegueta. African Journal of Biomedical Research, 8: 119-21.