International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Suppl 4, 2010

Research Article

TO STUDY THE EFFECT OF *NIGELLA SATIVA* ON VARIOUS BIOCHEMICAL PARAMETERS ON STRESS INDUCED IN ALBINO RATS

ROSHAN S[1], ABDULLAH KHAN[1], TAZNEEM.B[2], SADATH ALI, [2*]

Department of Pharmacy, Singhania University, Rajasthan, Luqman college of Pharmacy, Gulbarga, Karnatak India
Email: roshansalfi@rediffmail.com

Received: 04 August 2010, Revised and Accepted: 13 September 2010

ABSTRACT

The present study was carried out to evaluate the effect of ethanolic extract of *Nigella sativa* (EENS) on swimming endurance and anoxia tolerance test in mice, cold induced stress and Immobilization in albino rats. The effect was assessed by swimming survival time and anoxia tolerance time, estimation of various biochemical parameters in cold, Immobilization stress like glucose, cholesterol, triglycerides and blood urea nitrogen (BUN), and by determining the weight of organ such as, liver, spleen, testes, adrenal gland, blood cell count (WBC) and also the differential count at a dose of 200mg/kg and 400 mg/kg body weight per oral. It was found that EENS significantly ($p<0.001$) increases swimming time and anoxia tolerance time. EENS showed significant ($p<0.001$) decrease in blood glucose, cholesterol, triglyceride and BUN and also decreased the weight of organs. It also showed a significant ($p<0.05$) decrease in weight of adrenal gland. A significant ($p<0.01$) decrease in WBC count, polymorphs and monocytes and decrease in lymphocytes ($p<0.05$) and eosinophils was observed, compared to control group. Thus the obtained results revealed that the *nigella sativa* has got a significant anti stress activity.

Keywords: Swimming, Anoxia, Cold stress, EENS, WBC, BUN

INTRODUCTION

The term stress is defined by Hans Selye as the sum of all the non-specific changes caused by function or damage and a state of threatened homeostasis. 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 etc[1-3]. Herbal medicines are rich in non-specific anti stress agents which are of increasing clinical significance, among them adaptogens are the plant derived biological active substances which increases the power of resistance against physical, chemical or biological noxious agents[4].

The *Nigella sativa* belongs to family Ranunculacae, commonly known as black cumin seed known to have a great medicinal importance possessing many medicinal properties particularly in Greco-Arab, Unani-Tibb and Ayurveda system of medicine, known as Habbat al-barakah in Arabic. The seeds have claimed to have several traditional medicinal properties [5]. Recently, the seeds have been reported to exhibit many pharmacological effects including immunomodulator[6], anticancer[7], anti-diabetic[8], anti hypertensive[9], hepatoprotective[10], anti-oxidant[11], anti-bacterial activity[12], anti-helminthic[12] and anti-inflammatory[13]. This study is designed to evaluate the anti stress activity *of Nigella sativa* seeds.

MATERIALS AND METHODS

The Ethanolic extract of Nigella *sativa* seeds **(**NAS C 0076**)** and Ethanolic extract of *Panax ginseng* (PAN-C00531) a gift sample obtained from Madhur Pharma, Bangalore,

The dried ethanolic extract was suspended in distill water using 1% Tween 80, used for pharmacological screening.

Experimental animals

Adult Swiss albino mice (20- 25g) and Wistar rats (150 -200g) of either sex were used for the study. The mice and rats were fed with standard pellet (Parnava Agro industries Ltd. Sangali, India) and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by IAEC. (No.CPCSEA/IAEC/PC-01/346)

Acute toxicity study [14]

The study was performed according to the acute toxic classic method (as per CPCSEA/OECD guidelines). Swiss albino mice were used for acute toxicity study. The animals were kept Fasting for overnight providing only water, after which the test drug extract dissolved in water was administered orally at the dose of 800 mg/kg and observed for 14 days. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24h (with special attention during the first 4h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, defecation) and central nervous system (ptosis, drowsiness, gait, tremors, convulsion). The toxicity study carried out as per the guidelines of AOT- 421 using albino mice. The extracts were found to be safe till 800mg/kg per oral. Hence the dose of 100 and 200mg/kg of EENS was selected for pharmacological screening.

Anoxia stress tolerance test [15]

Albino mice of either sex weighing between (20±2g) were divided into 4 groups of six each. Group I as control, Group II subjected to anoxia test and the oral administration of EENS at the dose 100 and 200 mg/kg p.o (per oral) and Group III subjected to anoxia test and the oral administration of EEPG at the dose 50 mg/kg p.o respectively for 21 days. Anoxia test was carried out on 7th, 14th and 21st day. One hour after the drug administration mice were placed in Hermetic vessel of 2000ml air capacity was used for this test. Each animal was kept in the hermetic vessel and the time to show the first sign of convulsion was noted, it was immediately removed from the vessel and resuscitated if needed. After one week of drug treatment the animals were once again exposed to the anoxia stress. Similarly the animals were also observed at the end of 2nd and 3rd week with the same treatment and the time duration for anoxia stress tolerance was noted. The data obtained were subjected to statistical analysis.

Swimming endurance test [16]

Swiss albino mice (20 ± 2 g) of either sex were randomly divided into 3 groups of 6 animals each consisting Group I as control, Group II swimming test and subjected to the oral administration of EENS at the dose 100 and 200 mg/kg, Group III subjected to anoxia test and the oral administration of EEPG at the dose 50 mg/kg p.o respectively for 21 days. Swimming test (36×36×30) was carried out

on 7th, 14th and 21st day. One hour after the drug administration mice were allowed to swim in cylindrical container filled with water maintained at 25 ± 2°C till they got exhausted and the moment they drowned head was considered as the endpoint. The time was noted and the data obtained were subjected to statistical analysis.

Cold stress

Albino rats (210-230gm) of either sex were divided in to 4 groups of 6 animals each. Group-1 served as control, Group-2 served as cold stress control, Group-3 cold stress induced and treated with EENS 100 and 200mg/kg p.o, and Group-4 cold stress induced and treated with EEPG 50mg/kg p.o. Cold Stress was induced in 2nd, 3rd and 4th groups in albino rats, by exposing animals to 4 ±1°C daily for 2 hrs for10 days[17]. On 11th day all the animals were sacrificed and blood was collected for estimation of biochemical parameters like, glucose, cholesterol, triglycerides, BUN[18], blood cell count[19] and weight of organs such as liver, spleen, testes and adrenal gland [20].

Immobilization stress

Albino rats (210-230gm) of either sex were divided into 4 groups of 6 animals each. Group -1 served as control, Group-2 served as immobilization stress control, Group-3 treated with EENS 100 and 200mg/kg and Group-4 treated with EEPG 50mg/kg. Immobilization stress was induced in 2nd, 3rd and 4th group animals by immobilizing the animals with head down in supine position, by fixing the animals to a board inclined position at an angle of 60° daily for 2 hrs for the duration of 10 days[21,22]. On 10th day, the animals were sacrificed and blood was collected for estimation of biochemical parameter like glucose, cholesterol, triglyceride, BUN[18], blood cell count[23] and weight of organs like liver, spleen, testes and adrenal gland[20].

Statistical analysis

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

RESULTS

The Anoxia tolerance test was determined by taking the appearance of convulsion as end point. EENS at the dose of 200mg/kg body weight has shown significant (p<0. 001) increasing tolerance stress time in 14th and 21st day as compared with the control (Table 1).

In swimming endurance test EENS at the dose of 400mg/kg body weight has shown significantly (p<0. 001) increased in the swimming time as compared to control (Table 2).

In cold stress induced, EENS has significantly (p<0.001) reduced the elevated levels of biochemical parameters glucose, cholesterol, BUN and triglyceride when compared with stress control group (Table 3). EENS has also reduced the blood cell count WBC's significantly (p<0.01), except lymphocytes and eosinophils (p<0.05) compared to stress control group (Table 4). Determination of weight of organs showed that EENS has significantly (p<0.01) reduced the weight of liver, spleen and testes, however it showed no effect on weight of adrenal gland (Table 5).

In immobilization stress induced, EENS has significantly (p<0.001) reduced glucose, cholesterol, BUN and triglyceride when compared with stress control group (Table 6). It has also reduced the blood cell count WBC's significantly (p<0.01) compared to stress control group (Table 7). Determination of weight of organs showed that the extract has significantly (p<0.01) reduced the weight of liver, spleen and testes, however it showed no effect on weight of adrenal gland (Table 8).

Table 1: Effect of *Nigella sativa* on anoxia tolerance test

Group	Mean duration of tolerance time (in min) Mean± SEM		
	1st week	2nd week	3rd week
Control	4.3 ± 0.91	7.2 ± 0.71	12.3 ±.05
ENS 200mg	3.2±0.78*	5.8 ±1.37***	8.4 ± 0.18**
ENS 400mg	3.9±0.78*	6.3 ±1.37***	10.6 ± 0.18**
EPG 100mg	3.8 ± 0.72	6.9. ± 0.98	11.4 ± 0.81

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to control.

Table 2: Effect of *Nigella sativa* on swimming endurance test

Group	Mean duration of swimming survival time (in min) Mean± SEM		
	1st week	2nd week	3rd week
Control	12.3 ±1.91	19.2 ±0.71	24.4 ±1.05
ENS 100mg	11.6± 0.78**	16.6 ±1.37**	19.3± 1.18***
ENS 200mg	12.2± 0.78*	18.3 ±1.07***	21.4 ± 0.18**
EPG 100 mg	11.2 ± 0.98	16.8 ± 0.98	22.6 ± 0.81

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to control

Table 3: Effect of *Nigella sativa* on biochemical parameter on cold stress induced in albino rats

Parameter	Control	Cold stress	ENS 100mg	ENS 200mg	EPG 100mg
Glucose mg/dL	80.24 ± 0.812	112.2 ±2.91	96.45 ±5.21***	84.35 ±4.21***	86.25 ± 3.11
Cholesterol mg/dL	40.21 ± 1.882	58.4 ±1.727	43.29 ±2.93***	40.29 ± 1.73***	44.54 ± 2.65
Triglyceride mg/dL	71.24 ± 0.712	105.2 ±2.64	83.14±1.95***	70.34 ± 0.65***	84.58 ± 1.65
BUN mg/mL	30.14 ± 0.512	53.41 ±2.41	39.18±1.22***	31.68 ± 0.92***	34.58 ± 1.87
Plasma cortisol (µg/100 ml)	13.04 ± 0.24	21.41±0.56*	18.63±0.59*	15.63±0.09**	13.97±0.38**

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control.

Table 4: Effect of *Nigella sativa* on weight of organ on cold stress induced in albino rat

Parameter	Control	Cold stress	ENS 200mg	ENS 400mg	EPG 100mg
Spleen mg/100g	3.27 ±0.11	2.591±0.12	3.48±0.29**	3.32±0.22**	3.38 ± 0.26
Testes mg/100g	1.681±0.06	1.121±0.02	1.421±0.29**	1.311±0.29**	1.431 ± 0.21
Liver g/100g	5.142±62.5	6.718±38.2	5.921±13.8***	5.221±11.8***	5.301 ± 10.8
Adrenal gland mg/100g	0.250±0.01	0.489±0.44	0.358±0.01*	0.283±0.01*	0.318 ± 0.01

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control

Table 5: Effect of *Nigella sativa* on blood cell count in cold stress induced in albino rats

Parameter	Normal	cold stress	ENS 200mg	ENS 400mg	EPG 100mg
WBC	4824 ± 41.02	6872 ± 143.7	5124 ± 64.2***	5013 ± 14.6***	5217 ± 48.21
Lymphocytes	48 ± 1.72	64 ± 1.09	56 ± 0.72**	52 ± 0.52**	59 ± 1.03
Monocytes	1.00 ± 0.0	3.25 ± 0.19	1.75 ± 0.50**	1.25 ± 0.50**	1.5 ± 0.76
Neutrophills	24 ± 0.40	35 ± 0.84	28 ± 0.50*	25 ± 0.40*	31 ± 0.22
Eosinophils	1.25 ± 0.4	4.50 ± 0.25	2.25 ± 0.25*	1.0 ± 0.25*	1.0 ± 0.25

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control

Table 6: Effect of *Nigella sativa* on biochemical parameter in immobilization stress induced in albino rats

Parameter	Normal	Immobilization stress	ENS 200 mg	ENS 400 mg	EEPG 100 mg
Glucose mg/dL	80.24 ± 0.812	118.2 ± 2.41	98.25 ± 5.21***	82.15±4.11***	87.15 ± 4.91
Cholesterol mg/dL	40.21 ± 1.882	62.4 ± 1.98	49.69 ± 2.03**	42.43 ± 1.69**	44.79 ± 1.93
Triglyceride mg/dL	71.24 ± 0.712	110.24 ± 2.94	88.94 ± 1.76**	73.64 ± 1.04**	83.84 ± 1.46
BUN mg/mL	30.14 ± 0.512	51.41 ± 2.61	43.98 ± 1.22**	32.08 ± 0.92**	43.78 ± 1.42
Plasma cortisol (µg/100 ml)	13.04 ± 0.24	21.41±0.56*	18.63±0.59*	15.63±0.09**	13.97±0.38**

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control

Table 7: Effect of *Nigella sativa* on weight of organ in immobilization stress induced in albino rats

Parameter	Normal	Immobilization stress	ENS 200mg	ENS 400mg	EPG 100mg
Spleen mg/100g	3.57 ± 0.11	2.891 ± 0.12	3.08 ± 0.29**	3.32 ± 0.19**	3.11 ± 0..21
Testes g/100g	1.681 ± 0.06	1.421 ± 0.02	1.341 ± 0.29**	1. 521 ± 0.16**	1.421 ± 0..03
Liver mg/100g	5.142 ± 62.5	6.818 ± 38.2	5.921 ± 13.8*	5.325 ± 11.8*	5.461 ± 19.76
Adrenal gland g/100g	0.250 ± 0.01	0.423 ±0.44	0.358 ± 0.01*	0.309 ± 0.41*	0.398 ± 0.22

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control

Table 8: Effect of *Nigella sativa* on blood cell count in immobilization stress induced in albino rats

Parameter	Normal	Immobilization stress	ENS 200mg	ENS 400mg	EPG 100mg
WBC	4824 ± 41.02	7540 ± 166.7	6124 ± 44.2***	5413 ± 24.6***	5817 ± 48.21
Lymphocytes	48 ± 1.72	71 ± 1.29	64 ± 0.72**	52 ± 0.52**	59 ± 1.03
Monocytes	1.00 ± 0.0	3.25 ± 0.19	1.75 ± 0.50**	1.25 ± 0.50**	1.5 ± 0.76
Neutrophills	24 ± 0.40	35 ± 0.84	28 ± 0.50*	25 ± 0.40*	31 ± 0.22
Eosinophils	1.25 ± 0.4	4.50 ± 0.25	2.25 ± 0.25*	1.0 ± 0.25*	1.0 ± 0.25*

The values are expressed as mean ± S.E.M, n=6.

Significance at *(P<0.05), **(P<0.01), ***(P<0.001), when compared to stress control

DISCUSSION

Animals when subjected to a period of stress produce characteristic changes in several hormones and parameters associated with the central nervous system and the hypothalamic-pituitary-adrenal axis (HPA). HPA changes include an increase in cortisol, a reduced sensitivity of the HPA to feedback down-regulation, and a disruption in the circadian rhythm of cortisol secretion. Central nervous system changes include the stress-induced depletion of catecholamine neuro transmitters such as nor epinephrine and dopamine. An acute increase in beta-endorphin levels is also observed under stressful conditions [24].

Rodents when forced to swim in a restricted space become immobile after an initial period of vigorous activity indicating the stress. Pretreatment with adaptogen increase swimming endurance in

mice[25]. Increase in total swimming time of EENS treated mice showed significant improvement in the swimming time. Cold stress typically increases total leukocyte count, eosinophils and basophils. Plant adaptogen are smooth prostressors which reduce the reactivity of host defense system. The mode of action of adaptogens is basically associated with stress system. Adaptogen increase the capacity of stress to respond to the external signals of activating and deactivating mediators of stress response subsequently[25,26]. The stress induced increase in total WBC count, which is decreased by EENS indicating its antistress and adaptogenic activity are similar to the changes produced by reference drug EEPG.

In response to stress ACTH is released which acts on adrenal cortex where by cortisol and corticosterone will be secreted. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases blood glucose level. The increased cortisol levels and increased blood glucose level are reversed by anti-stress agents [25-27].

During stress, blood glucose level increases, this is found to be significantly reduced by EENS which is an indication of its anti-stress activity[27].. EENS significantly reduced stress indused plasma cortisol level exhibiting anti-stress activity which is comparable to standard EEPG.

All the body functions, including cellular respiration depends on the oxygen supply. Any lack of vital element will play havoc on all body mechanisms and increase in adaptation during stress by any drug could be considered as its major anti stress effect. During stress adaptogens are capable of increasing succinate dehydrogenase[SDH] in the brain, decrease in brain neurotransmitters, i.e. norepinephrine(NE), dopamine(DA), serotonin(5-HT) and acetylcholine(ACh). Hence the observed drug effective in this model may be effective by the modulation of above mentioned neurotransmitters[28]. The extract of *nigella sativa* showed significant increase in anoxia tolerance time which is an indication of either resistance to it or reduction in cerebral oxygen consumption. Both these effect are useful to protect neuronal cell against oxidative stress, the enzyme is responsible for utilization and conservation of energy in the cellular system of the organism; which helps adaptive processes during stress.

The mechanism by which stress raises serum cholesterol, triglycerides and BUN levels in stress induced animals is due to the enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids[29]. EENS as well as the standard drug EEPG significantly reduced the elevated serum cholesterol, triglyceride and BUN levels which may be due to inhibition of stimulation of sympathetic nervous system.

The increase in 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 adrenals[29]. ENS and EPG has significantly reduced the liver and adrenal gland weight, this might be due to the reversal of stress induced adrenomedullary response and hence decrease production of corticotropic hormone. Pretreatment with ENS and EPG significantly increased the spleen weight. This might be due to the inhibition of recruitment of lymphocytes to blood from spleen.

Stress causes alteration in hematological parameters like increase in WBC and DLC counts, neutrophils[19,29]. ENS as well as the standard EPG significantly reduced the WBC, lymphocytes, eosinophils and monocyte counts in cold and immobilization stress.

A variety of biological activities including Anti-stress activity were reported with flavonoids, tannins and phenolic glycosides. *Nigella sativa* contains biologically active chemicals that include flavonoids, saponins, alkaloids, proteins, fixed oils and proteins[30,31]. The anti stress activity may be due to the presence of these constituents where as standard drug *Panax ginseng* an established adaptogenic drug too contains glycosides, steroids and flavoniods[32].

CONCLUSION

The results obtained from this study suggest that the Ethanolic extract of *Nigella sativa* has potential Anti-stress activity which is comparable to standard Ethanolic extract of *Panax Ginseng*.

REFERENCES

1. Selye. H. Experimental evidence supporting the conception of adaptation Energy. Amer J Physiol 1938;123:758.
2. Selye H. The evolution of the stress concept. American Scientist 1973;61:693-699.
3. Panossian A, Wikman G, Wagner H. Plant adaptogens III.* Earlier and more recent aspects and concepts on their mode of action. Phytomedicine 1999;6(4):287–300.
4. Ahmed G, Yusuf Amin KM, Khan NA, Tajuddin. The anti-stress activity of a gem containing Unani formulation against diverse stressors. J Ethanopharmacol 1998;59:187.
5. Kirtiar KR, Basu BD. Indian medicinal plants, Delhi India. sri satguru publication, 3rd revised and enlarged edition, Vol.I, 1983, pp. 11-24.
6. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Intl Immunopharmacology 2005;5:1749–1770.
7. Salomi NJ, Nair SC, Jayawardhanan KK, Varghese CD, Panikkar KR. Anti tumor principle from *Nigella sativa* seeds. Cancer letters 1992;63:41-46.
8. Bouchra M, Robert D, Moulay F, Bruno E, Lahcen M, Ali B, et al. *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol 2009;21:419-424.
9. Zaoui A, Cherrah Y, Lacaille-Dubois M, Settaf A, Amarouch H and Hassar M. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie 2000;55:379-382.
10. Mahmoud M, El-Abhar H and Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoniinfection in mice. J Ethnopharmacol 2002;79:1-11.
11. Burits M and Bucar F. Antioxidant activity of *Nigella sativa* L. essential oil. Phytotherapy Research 2000;14:323-328.
12. Agarwal R. Kharya MD, Shrivastava. Antimicrobial and anti helminitic activity of essential oil of *Nigella sativa*. Indain J experimental biology 1979;17:1264-1265.
13. Al-Ghamdi MS The anti-inflammatory,analgesic and antipyretic activity of *Nigella sativa.* J Ethnopharmacol 2001;76:45-48.
14. Nimbekar, Patil and Patki. Pharmacological evaluation of antistress and androgenic activity of polyherbal formulatiom AP 30000 containing P Ginseng. Indian drugs 2001:27-31.
15. Tomar VS. Effect of gerifort a herbal compound drugs on anoxia stress tolerance in animals. Indian Drugs. 1984 March; 233-235.
16. Nagaraja HS, Jeganathan PS. Forced Swimming stress induced alterations in ingestive behavior in rats. Indian J Physiol Pharmacol 2003;41(1):94-100.
17. Bhargava KP, Singh N. Anti stress activity of *Ocimum santium*. Ind J Exp biology 1981;73:443-451
18. Vinay Agarwal, Bhayesh Gupta, Ushan Singhal, Bajpal SK. Examination stress changes in serum cholesterol, triglycerides and total lipids. Ind J Physiol Pharmacol 1997;41(1):404-8.
19. Takano.T. Additive effect of nitrogen oxides and cold stress on circulating leukocyte counts in rats. Toxicology lett 1983;17:289-291.
20. Sandip, Sardesai R, Marjorie AE, Jolly FM.1993. Effect of stress on organ weight in rats. Ind J Physiol Pharmacol 1993;37:2:104-108.
21. Upadhya L, Shukla SS, Agrawal A, Dubey GP Changes in brain biogenic amines under influence of an indigenous drug, geriforte, following immobilization stress. Indian J Exp Biol 1988;26(11):911-912.
22. Shivprasad, Kharya and Rana. Antioxidant and Adaptogenic effect of an herbal preparation, Triphala. J Nat Remedies 2008;82-88

23. Charlie Esimone, Michael O, Adikwul U, Chukwuemeka NS, Festus BC, Charles OE, Michael U. Adikwu1, Chukwuemeka S. Nworu, Festus BC, Okoyeand Damian C. Dimegwu O. Adaptogenic potentials of *Camellia sinensis* leaves, *Garcinia kola* and *Kola nitida* seeds. Scientific Research and Essay 2007;2 (7): 232-237.
24. Gregory S, Kelly ND. *Rhodiola rosea* A Possible Plant Adaptogen. Alter Med Review 2001;(6)3: 291-296
25. Meera Sumanth, Mustafa SS. Antistress, Adaptogenic activity of Sida cordifolia roots in mice. Indian j Pharm Sci 2009;71(3):323-324.
26. Tache Y, Selye H. Shift in adenohypophyseal activity during chronic intermittent immobilization of rats. *Neuroendocrinol.* 1976;22:325–36.
27. Meera S, Mustafa SS. Antistress, adoptogenic and immunopotentiating activity of roots of *Boerhaavia diffusa* in mice. *Int J Pharmacol.* 2007;3:416–20
28. Deore SL, Khadabadi SS. Screening of antistress properties of *Chlorophytum borivilianum* tuber Pharmacologyonline 2009;1:320-328.
29. Lakshmi BVS, Sudhakar M. Adaptogenic Activity of Lagenaria: An Experimental study using acute stress models on rats. J Pharmacol Toxicol 2009;4(8):300-306.
30. Ali BH, Gerald Bluden. Pharmacoloical and toxicological properties of *Nigella sativa*. Phytotherapy Research 2003;17(4):299-305.
31. Mertfort I, Wray V, Barakat HH, Hussein SAM, Nawwar MAM, Willuhn G. Flavanol triglycosides from seeds of *Nigella sativa.* Phytochemistry 1997;46(2):359-363.
32. Bhattacharya SK, Muruganandam AV. Adaptogenic activity of Withania somnifera: an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75(3):547-555.