Effect Of Terminalia Catappa On Hypoxia And Chronic Cold Resistance Stress In Experimental Animals

Abdul Vahed Mohammad*[1], Roshan. S[2]
***Corresponding Author**: - Abdul Vahed Mohammad
Address: [1*,2]Department of Pharmacy, Mewar University,Chittorgarh, Rajasthan. India.,
E-mail- roshansalfi@hotmail.com

Abstract

Stress and stressor-related disorders are the global illnesses. The immune system is thought to be strengthened by adaptogenic substances due to their ability to restore normal body functions, which were otherwise imbalanced due to the stressful conditions. The aim of study anti stress potential ethanolic extract of *Terminalia catappa* L.(*T.catappa*) fruits on Anoxia tolerance test(hypoxia) in mice, chronic cold stress(CCS) in rats. The effect was assessed by hypoxia tolerance time and estimation of Glucose, Plasma cortisol, Cholesterol, BUN and TG biochemical parameter, blood count, weight of gland and organs in CCS a dose 300 and 500 mg/kg (p.o). The results revealed that compare with Gerifort and *T.catappa* shows significantly ($p<0.001$) increases anoxia time, decrease significantly ($p<0.001$) in blood glucose, cholesterol, TG, plasma cortisol and BUN levels and also decreased the weight of Liver, Spleen and adrenal gland weights. In WBC's, polymorphs and neutrophils count significant ($p<0.01$) decrease. Thus obtained results revealed that the *T.Catappa* have potential adaptogenic/ Anti-stress activity due to present of phenolic compounds, especially, having antioxidant molecules, such as kaempferol, terflavin, gentisic acid and quercetin,punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, cumarin a significant anti stress activity.

Key words: Hypoxia, CCS, *T.Catappa*, DLC

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Introduction

Stress is a circumstance of threatened homeostasis provoked by a psychological, environmental, or physiological stressor. The rapid development of science and technology, as well as economy and strong social competition, the nature of stress has changed dramatically.[1] Stressful events engender multiple neurochemical, neurotransmitter and hormonal alterations by mainly activating the sympathetic nervous system (SNS) and the hypothalamic-pituitary adrenal (HPA) axis. When stress stimuli are under control, the body responds to these in the physiological way. SNA and HPA axis are woken up to release chemical mediators to protect our body from stress. For instance, catecholamines are elevated to increase heart rate and blood pressure, which help us to fight or flight. This appropriate body reaction was called "allostasis" by Sterling and Eyer [2]. Hans Selye's theory of stress and general adaptation syndrome, which has 3 phases: alarm phase, phase of resistance, and phase of exhaustion.[3] as shown in figure 1

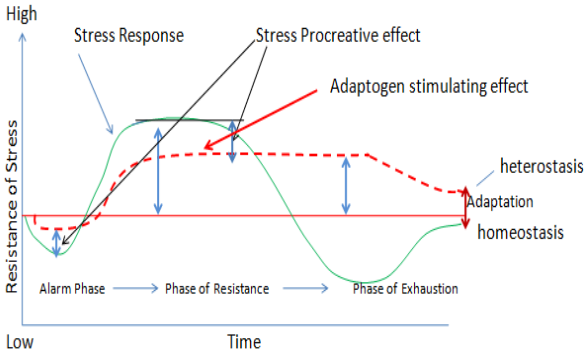

Figure1: Hans Selye's theory of stress and general adaptation syndrome

Terminalia catappa L.(*T. catappa*) of family Combretaceae, Its has many names are Indian almond, Malabar Almond and Tropical Almond, is a large deciduous tree and extensively planted in India, Philippines, Malaysia and Indonesia and Burma [4-5]. The *T. catappa* leaves, bark and fruits has been known as a folk medicine for antipyretic, liver problem related diseases, scabies, leprosy, diarrhea and CNS disorders [6-7]. Leaves, bark and fruits part of

T.catappa which contains the various phytoconstituents are kaempferol, terflavin, gentisic acid and quercetin, punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, cumaric, and its derivatives[5-8]. *T.catappa* plant has been investigated in various pharmacological studies as antioxidant, antidiabetic, anticancer, anti-inflammatory, antiviral, hepato-protective and antimicrobial activities[6-10]. Here, we investigated the adaptogenic or anti stress properties of the ethanolic extract *T. catappa* fruit on anoxia and cold stress in animals

Materials And Methods

Collection Of Plant Material:

Fresh fruit of *T.catappa* (Malabar Almond) family Combretaceae were procured from local vendor, Hyderabad, Telangana. Fruits are authenticated by authenticated by Dr. Rafiuddin Naser, Associated Professor, Department of Botany, Moulana Azad college of, Aurangabad, (M.S) (PCOG.H-217).

Preparation Of Extraction

The fresh fruit are shade dried for15 days, fruit material was powdered using mixer grinder and passed through sieve no 40. About The dried 150gm powder was subjected to soxhlet's apparatus extraction using ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator.

Photochemical Evaluation

Ethanolic Extracts of *T.catappa* (ETC) were subjected to different phytochemical tests to detect presence of phytoconstituents.[11]

Experimental Animals

Albino mice 20-25gm and adult rats (Wistar) of both sexes weighing 160–180 g were procured from Mahaveera enterprises, Hyderabad. Animals were accommodated in recommended laboratory environment at 25°C under 12 hr light-dark cycle. All the experimental animals had free access to chow and water *ad libitum*. The research protocol was approved by (HSKCP/IAEC, Clear/1/2020-12/R&D87)

Anoxia stress tolerance test

Albino mice of either sex weighing between (20±5gm) were divided into 4 groups of six animals each.

Group I as control, Group II subjected to anoxia test and oral administration of ETC at the dose 300 mg/kg mg/kg p.o ,Group III subjected to anoxia test and t oral administration of ETC at the dose 500 mg/kg mg/kg p.o , Group VI subjected to anoxia test and oral administration of gerifort syrup at the dose 2ml/kg p.o Group II, III, and IV treat with extract and gerifort for 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 5000ml 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 seen animal were immediately removed from the vessel and the time noted. 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 were noted.[12-13]

Chornic Cold Stress

Albino rats 160-180gms, male and female were cauterized in to five groups of six animals each.
Group-I Normal Group-II Chronic Cold stress [CCS], Group-III -CCS + ETC 300 mg/kg p.o for 10 days, Group-IV - CCS + ETC 500 mg/kg p.o for 10 days and Group-V - CCS + Gerifort 2ml/kg p.o respectively for 10 days.
Chronic Cold Stress was induced in 2nd, 3rd, 4th and 5th groups on animals, by exposing. 4 ±1°C temperature every day for 1 hrs for 10 days. On 10th day animals were sacrificed blood, organs and gland were collected. In blood estimation of Cholesterol, Glucose, TG, Plasma cortisol, BUN, WBC and DL cell count. Organ such as Liver, spleen, testes and adrenal gland weight noted [14-18].

Statistical Analysis

The results obtained from the pharmacological experiments were statistically analyzed using SPSS Version 19.0. The data is presented as mean of ± SEM. To compare the values and to establish the significance ANOVA was used and P value are reported as mean ± SEM. *a $p<0.001$, *b $p<0.01$ and *c $p<0.05$.

Results

Primilanary phytochemical study shows *P. granatum* peels extract presence of Alakloids,

Glycoside, Phytosteroids, Flavonoids, Terpinoids, Vitamins and Tannins.

Anoxia tolerance test in mice

The Anoxia tolerance test was determined by taking the appearance of convulsion as the end point. Control animals on 7th , 14th and 21 day increased anoxia tolerance time, when treatment with ETC at a dose of 500mg shows ($p<0.01$) significant at 14th day and 21st day on there was highly significant value ($p<0.001$), the extracts showed increase in tolerance stress time in 14th and 21st day compared to control and gerifort group (FigureA).

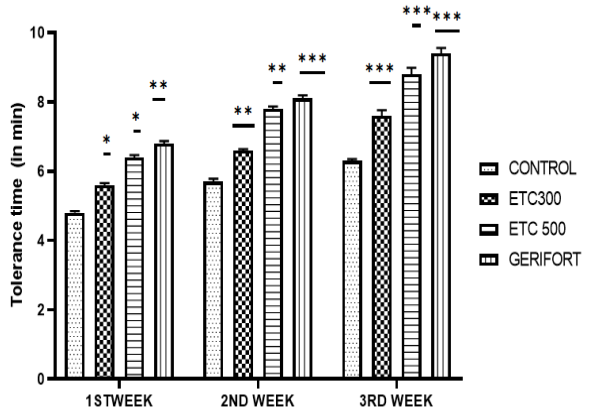

Figure A: effect of *T. catappa* on anoxia tolerance test

Biochemical Parameters of *T. catappa* on chronic cold stress [CCS] induced rats.

In CCS old stress induced significant increases the levels of biochemical parameters such as glucose, cholesterol, and BUN, triglyceride and plasma cortisol. Treatment with *T. catappa* at a dose of 300 and 500mg/kg showed significant ($p<0.001$) decrease in glucose, cholesterol, triglyceride BUN and plasma cortisol decrease, whereas gerifort has significantly ($p<0.05$, $p<0.01$, $p<0.001$) restored the elevated levels of biochemical parameters glucose, cholesterol, BUN, TG and plasma cortisol when compared with stress control group (figure B-F).

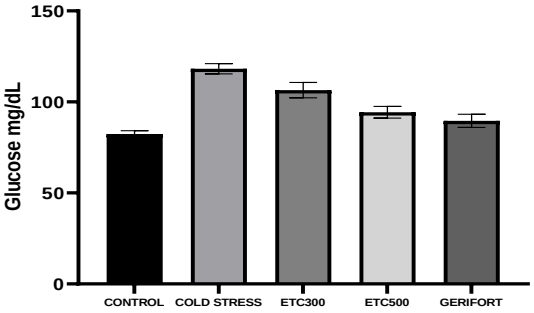

Figure B: Effect of *T. catappa* glucose on CCS

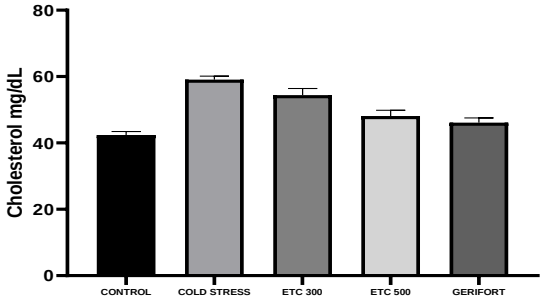

Figure C: Effect of *T. catappa* Cholesterol on CCS

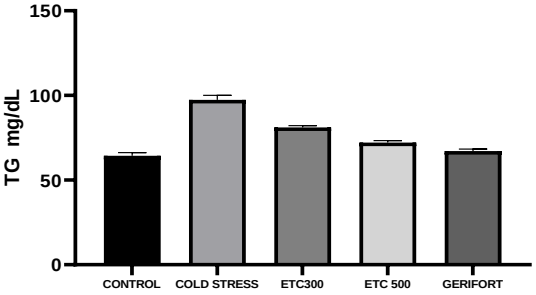

Figure D: Effect of *T. catappa* TG on CCS

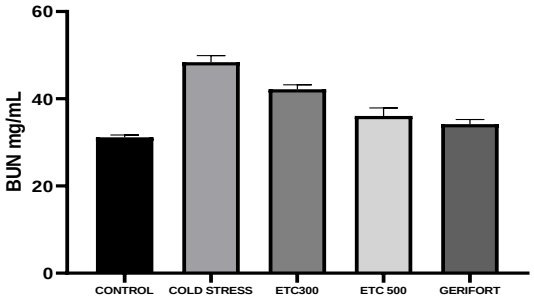

Figure E: Effect of *T. catappa* BUN on CCS

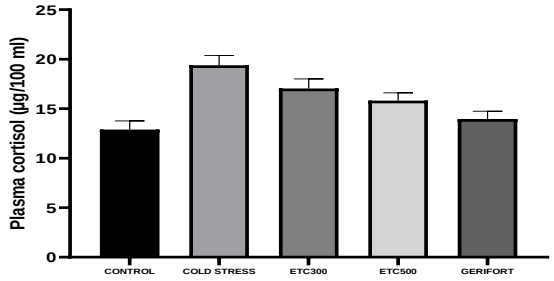

Figure F: Effect of *T. catappa* Plasma cortisol on CCS

CCS induced rats increased in liver, spleen and adrenal cortex Weights are observed, Whereas treatment of , *T. catappa* extract in a dose-dependent manner significantly ($p<0.01$) reduced in spleen, liver and adrenal gland as shown in (figure G- H). In hematological parameter, with an exception to eosinophils ($p<0.05$), all the other hematological parameter including the WBC's and differential blood cell count (DLC) were significantly ($p<0.01$) increased, compared to the control group. Whereas, treatment with *T. catappa* reduced the differential blood cell count and WBC's significantly ($p<0.01$) compared to stress control group (figure I-K).

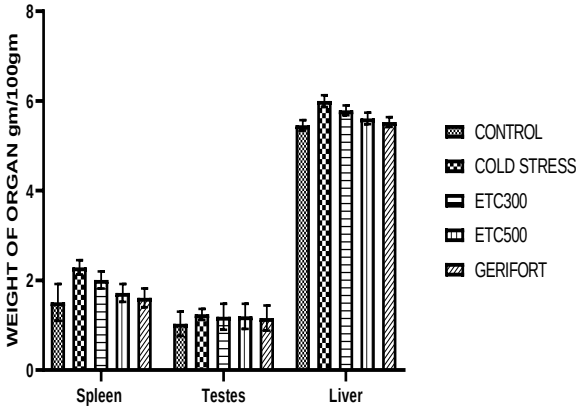

Figure G: Effect of *T. catappa* weight of organ on CCS

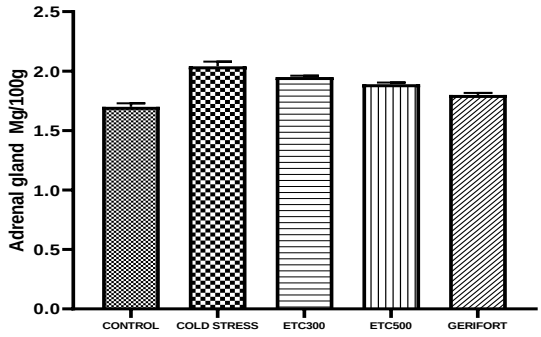

Figure H: Effect of *T. catappa* AG on CCS

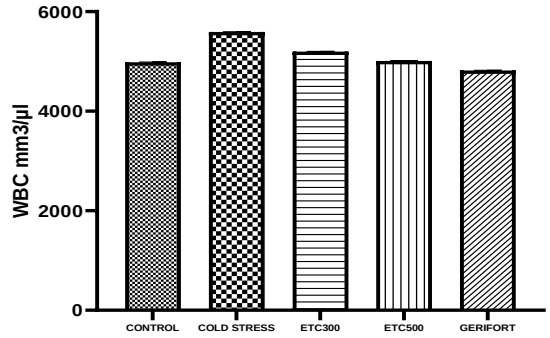

Figure I: Effect of *T. catappa* WBC count on CCS

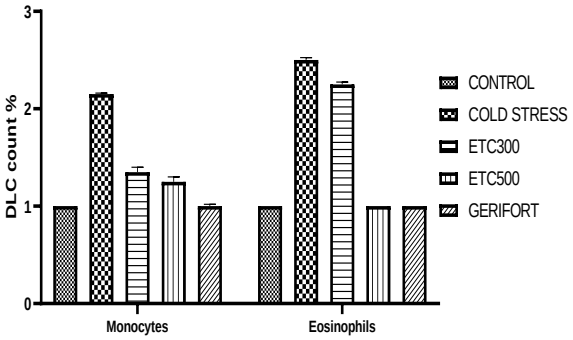

Figure J: Effect of *T. catappa* Monocytes and Eosinophilia count on CCS

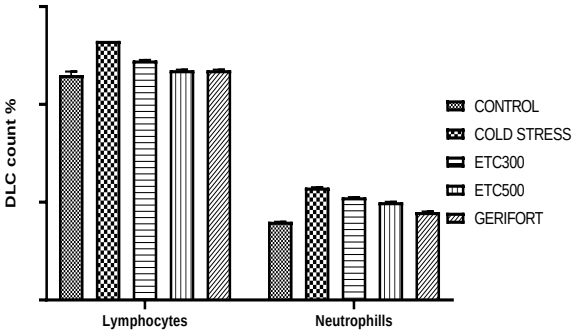

Figure K: Effect of *T. catappa* Lymphocytes and Neutrophils count on CCS

Discussion

Stress and stressor-related disorders are the global illnesses[1].The most common stressors could be physical (extreme environmental conditions, external force), emotional (tragedies, sadness, academic, etc.), and chemical stress (due to medicine, solvents, and exposure to the laboratory or industrial chemicals) [19]. Exposure to any of these stressors could readily imbalance the neuroendocrine systems of the body. The hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system are the two major systems involved in stress response. The sympathetic nervous system secretes catecholamines,

triggered mainly by a region in the brain stem (the lowest part of the brain) called the locus coeruleus. The hypothalamus is a major integrative center for receiving and converting messages from divergent centers to hormonal signals, via the regulation of pituitary gland and neural gland pathways [20].

The activation of this HPA mechanism results in corticotrophin hormone, adrenocorticotropin hormone (ACTH), β-endorphin, and glucocorticoids being secreted into the bloodstream. In a stressful condition ACTH is released and triggers the adrenals to increase the secretion of carticosteroids, epinephrine, and norepinephrine [21].

Anorexia is a critical stressor as it blocks the oxygen supply and halts the vital functions of the body primarily being the respiration though affects all the other vital functions [23]. *T.Catappa* extract significantly prolonged the meantime to convulsion as compared to the positive control group.

Release of ACTH under the CCS leads to synthesis and release of cortisol through the stimulation of adrenal cortex.[24]An increased cortisol levels in the plasma results in mobilization of fats and stored carbohydrates reserves thereby increasing the blood glucose levels, cholesterol, and triglyceride. Anti-stress agents could reverse the increased cortisol levels [25]. *T.Catappa* substantially decreased the elevated cortisol and blood glucose levels caused by stress. The standard drug Geriforte also provided similar results in this research. In stress-induced animals, the marked rise in serum cholesterol, triglycerides, and BUN levels is attributable to activation of the hypothalamic-pituitary axis (HPA) and sympathetic system, resulting in the release of catecholamines and glucocorticoids that suppress the immune system at multiple locations, such as the liver and kidneys [26]. The serum cholesterol, BUN, and triglycerides decreased with *T.Catappa* and the standard drug, which may be due to inhibition of the sympathetic nervous system. The increase in adrenal weight in stressed animals could be due to the stress-induced adrenomedullary response, which contributes to increased corticotrophic hormone production, leading to an increase in adrenal weight[27].

T.Catappa and Geriforte reduced the liver, adrenal gland weight. This increase in the liver and adrenal gland weight may occur due to the reversal of stress-induced adrenomedullary response and decreased production of the corticotropic hormone.

As a result of the draining of blood from the spleen to the lymphocytes, the spleen's size and weight will reduce. Pretreatment with *T. Catappa* and Geriforte increased the spleen weight. This may be due to the inhibition of recruitment of lymphocytes to blood from the spleen.

In stress spleen contracts and releases more amounts of lymphocytes, neutrophils, and eosinophils into circulations [28.] The increase in total WBC count is indicating a stressful state. Plant adaptogen is smooth stressors that reduce the reactivity of the host defense system. The mode of action of adaptogens is associated with the stress system. Adaptogen increase the capacity of stress to respond to the external signals of activating and subsequently deactivating mediators of the stress response [25].

Conclusion

The obtain phytochemical results from our study suggest that the Ethanolic extract of *T.catappa* fruit contain flavonoids like kaempferol, quercetin, punicalagin, punicalin, poly phenols are gallic acid,chebullic acid and ellagic acid, and other terfluvina A and B has potential Anti-stress activity and antioxidant molecules. The main active component in punicalagin, punicalin, quercetin and poly phenols a natural product of the group of secondary metabolites, several studies have shown that possesses a wide range of anti oxidant pharmacological activity. So we concluded the anti-stress activity due to these constituents' presence, whereas standard Geriforte, an established anti stress/adapt genic drug.

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Conflict Of Interest

We have no conflict of interest to declare

References

Landsbergis, P. A. (2003). The changing organization of work and the safety and health of working people: a commentary. J. Occup. Environ. Med. 45, 61–72. doi:

Sterling, P., and Eyer, J. (1988). ‘‘Allostasis: a new paradigm to explain arousal pathology,’’ in Handbook of Life Stress, Cognition and Health, eds S. Fisher and J. Reason (Oxford: John Wiley & Sons), 629–649.

Selye. H. Experimental evidence supporting the conception of adaptation Energy. Amer J Physiol 1938; 123:758.

Anjaria J, Parabia M, Dwivedi S. *Ethnovet heritage: Indian ethnoveterinary medicine, an overview.* 1st ed. Ahmedabad (India): Pathik Enterprise Publishers; 2002

Taganna JC, Quanico JP, Perono RM, Amor EC, Rivera WL. Tannin-rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in chromo bacterium violaceum and the QS-controlled bio film maturation and Las Astaphyolytic activity in Pseudomonas aeruginosa. J Ethno Pharmacol. 2011; 34(3):865-71.

Fan YM, Xu LZ, Gao J, Wang Y, Tang XH, Zhao XN. Phytochemical and antiinflammatory studies on Terminalia catappa. Fitoterapia. 2004;75(3-4):253-60

Naitik, P., Prakash, T., Kotresha, D., and Rao, N. R. (2012). Effect of *Terminalia catappa* on lipid profile in transplanted fibrosarcoma in rats. *Indian J. Pharmacol.* 44,390–392.Doi:10.4103/0253-613.96345

Roshan.S, N. Lal Mahammed ,Syed Nadeem, Shaik Aleem, Mohd Munawer Ahmed, Studies on Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of *Terminalia Catappa* Leaves Using In-vitro Models. American Journal of PharmTech Research 2015(5)3.

Hen, P. S., and Li, J. H. (2006). Chemopreventive effect of punicalagin, a novel tannin component isolated from *Terminalia catappa* on H-ras-transformed NIH3T3 cells. *Toxicol. Lett.* 163, 44–53. doi: 10.1016/j.toxlet.2005.09.026

Chen, P. S., Li, J. H., Liu, T. Y., and Lin, T. C. (2000). Folk medicine *Terminalia catappa* and its major tannin component, punicalagin, are effective against bleomycin-induced genotoxicity in Chinese hamster ovary cells. *Cancer Lett.* 52, 115–122.

Harborne JB: Photochemical Methods. A Guide to Modern Techniques of Plant Analysis.2nd edition, Chapman & Hall, London, 1973, p. 279.

Mohd Zubairali Anssari, Mohammed Fasiuddin, Syed Salman, Syed nazer Mohammed Imran, Mohd Toufeeq, Roshan S and N L Mohammed Pharmacological screening of polyherbal formulation for anti stress activity on albino rats. International Journal Of Pharmacological Research; 2015;May; 5;5: 125-128.

Ali, S., S. Roshan and A. Khan,. To study the effect of *Allium sativum* on swimming endurance, anoxia tolerance and cold stress. J. Global Pharma Technol., 2010.2: 27-32.

Roshan, S., A. Khan, B. Tazneem and S. Ali, to study the effect of *Nigella sativa* on various biochemical parameters on stress induced in albino rats. Int J Pharm Pharm Sci; 2010; Vol 2; Suppl 4: 185-189

Roshan, S., R.V. Savadi, B. Tazneem, S. Ali and A. Khan, 2010. Phytochemical investigation and effect of *Abutilon indicum* on various biochemical parameters on stress induced albino rats. Int. J. Curr. Pharmaceut. Rev. Res., 1: 17-26.

Roshan, S., B. Tazneem, A. Khan and S. Ali, 2010. To study the effect of *Allium sativum* on various biochemical parameters on stress induced in albino rats. Res. J. Pharmacol. Pharmacodyn., 2: 335-339.

Roshan, S., A. Khan, B. Tazneem and S. Ali, 2010. The effect of *Echinacea angustifolia* on various biochemical parameters in stressinducedrats. Pharmacologyonline, 2:542-550.

Hussain, S.M. and S. Roshan. Anti-stress activity of *Cissus quandanguluris*. Int. J. Pharm. Biosci., 201910: 2:132-139.

Roshan S, Hussain SM. Antistress activity of *olea europaea*. *Indian J Pharm Pharmacol* 2021;8(2):161-167.

Spasov AA, Wikman GK, Mandrikov VB, Mironova IA, Neumoin VV. A double-blind, placebo-controlled pilot study of the stimulating and adaptogenic effect of *Rhodiola rosea* SHR-5 extract on the fatigue of students caused by stress during an examination period with a repeated low-dose regimen. Phytomedicine. 2000;7(2):85–9.

Juvekar AR, Nachankar RS. Restraint stress induced changes and their modification by *Spirulina platensis* in albino rats: An experimental study. Acta Hort (ISHS) 2005; 680:49–55.

Biswas NM, Sengupta R, Roychaudhuri G, Chattopadhyay A, Sarkar M. Prevention of adrenocortical hyperactivity by dietary casein in rats exposed to forced swimming stress. Indian J Exp Biol. 2001; 39:178–80.

Negrao AB, Deuster PA, Gold PW, Singher A, Chrousos GP. Individual reactivity and physiology of the stress response. Neurology. 2000; 54:122–8.

Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab. 2006;291:E965–73.

Sadock BJ and VA Sadock. Major depression and bipolar disorder kalpan and sadocks synopsis of psychiatry. 9th edition. Lippincott Williams and Wilkins Philadelphia, pp: 534-590 ; 2003.

Tache Y, Seyl. Shift in adenohypophyseal activity during chronic intermittent immobilization of rats. Neuronendocrinol. 1976; 22: 325-336

Sen P,P Maiti, S Puri and A Ray. Mechanism of antistressactivity of Ocimum sanctum(Linn), eugenol and *Tinospora malabaria* in experimental animals. Indian J.Exp. Biol.30:1992, 592-596.

Schimmer BP and KL Parker. Adrenocortical steroids and their synthetic analogues. 11th Edn., McGraw-Hill Medical Publishing, New York, 2006, PP: 1655-1662.