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PHYTOREMEDIAL ROLE OF *WITHANIA SOMNIFERA* EXTRACT ON BIOCHEMICAL PARAMETERS IN LIVER OF EPINEPHRINE INDUCED STRESSED ALBINO MICE

SHIV SHANKER KUMAR[1], BHASKAR SHARMA[1] AND MOHAMMAD SUHAIL [2*]

1.Department of Biochemistry, C.M.J. University, Shillong, Meghalaya, India
2. Allahabad Institute of Medical Sciences & Management, Allahabad, U.P, India.

ABSTRACT

During our present study, we examined the *W. somnifera* extract effect on epinephrine induced stress. Forty Swiss albino mice were divided into three groups as non stressed group and stressed group and extract treated group .The stressed groups were exposed to 2 weeks and 4 weeks of chronic immobilization stress with epinephrine. At the end of the 2 and 4 weeks the mice were sacrificed and blood samples were collected through orbital sinus method. The blood samples of these groups were analyzed for selected biochemical parameters viz. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and Bilirubin which were found to be – significantly increased in the stressed group when compared to the non stressed Control. The present data indicate that epinephrine induced stress causes the significant alterations in the SGOT, SGPT and Bilirubin levels affecting the normal metabolism in liver. These elevations were found to be significantly reversed in W. somnifera extract treated group. Our findings have relevance as human beings are exposed to several stressors in routine life.

KEYWORDS: **Epinephrine, Stress, SGOT, SGPT and Bilirubin.**

MOHAMMAD SUHAIL
. **Allahabad Institute of Medical Sciences & Management, Allahabad, U.P, India.**

**Corresponding author*

INTRODUCTION

In the modern era, stress has become an integral part of human life [1]. It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress [2]. Stress is considered to be the condition which results in perturbation of the body's homeostasis [3]. If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened [4].Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous system (CNS), endocrine system, and metabolic system [5]. Drugs having antistress properties induce a state of nonspecific resistance against stressful conditions. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs [6]. Epinephrine has a wide range of clinical uses in medicine and surgery based on its actions on blood vessels, the heart, and bronchial muscle [7]. The most common uses of epinephrine hydrochloride are to relieve respiratory distress due to bronchospasm, to provide protective effect of such treatment and could develop to aggravated asthmatic reactions [8]. Between 1961 and 1966, a correlation between the widespread use of sympathomimetic bronchodilators to treat asthma and an increase in asthma mortality in 10 to 14 year old children in Great Britain was noted with concern, but the sudden asthma-related deaths were later attributed to the disease instead of the treatment [9]. For severe, acute asthma, nebulised or injectable epinephrine is still indicated [10]. Although the routine, long-term clinical use of epinephrine has been discontinued, it is still available as an over-the-counter agent (Primatenea and Bronkaida) for symptomatic relief of asthma [11]. Other respiratory disorders treated with epinephrine include viral croup, bronchitis, and emphysema [12].*Withania somnifera* Dunal (WS), commonly known as ashwagandha, has been used for centuries in Ayurvedic medicine to increase longevity and vitality. Western research supports its polypharmaceutical use, confirming antioxidant, anti-inflammatory, immune-modulating, and antistress properties in the whole plant extract and several separate constituents [13]. As an antioxidant, WS and active constituents sitoindosides VII-X and withaferin A (WA) have been proven to increase levels of endogenous superoxide dismutase, catalase, and ascorbic acid, while decreasing lipid peroxidation [14-17]. WS acts as an anti-inflammatory agent through inhibition of complement, lymphocyte proliferation and delayed-type hypersensitivity [18]. The actions of WS on the immune system are subtler than simply suppressing the immune/ inflammatory response. It modulates the immune response, increasing the expression of T-helper 1 (Th1) cytokines, as well as CD4 and CD8 counts, and natural killer (NK) cell activity [19-21]. Several studies also support its ability to increase circulating cortisol, decrease fatigue, increase physical performance, and decrease refractory depression in animals subjected to stress [22, 23]. *W. somnifera*, however, is often underutilized in the oncology arena, despite the fact that it shows direct antitumor and cancer preventive activity. Furthermore, WS has the potential to increase tumor sensitization to radiation and chemotherapy while reducing some of the most common side effects of these conventional therapies [22]. Present study was planned to evaluate liver function indicators like SGOT, SGPT and bilirubin levels during epinephrine induced stress followed by exploration of *W. somnifera* extract treatment effects.

MATERIALS AND METHODS

Chemical: Epinephrine, used to prepare stressed model was purchased from the Rohit laboratories (Hindustan) Pvt. Ltd. Surat, India. Commercially available kits for chemical analyses such as SGOT, SGPT and Bilirubin were used with the crest coral clinical system, Goa, India. Analytical grade ethanol was purchased from Merck Company.

Experimental model

Reared sexually matured 6-8 weeks old age group male and female Swiss Albino mice (Mus musculus) weighing 25-35gm b.w. in the animal house and used in the present study. The animals were housed at controlled environmental conditions 22±2ºC, relative humidity 50±10%, and 12h dark-light cycle. Animals were housed and allowed to free access to food and water. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Collection & Preparation of ethanolic extract

Alcoholic root extract of *W. somnifera* is administered after epinephrine exposure. Fresh root of *W. somnifera* was purchased from the local herbal store. The identity of the root was confirmed by a Botanist.It was soaked in 70% ethanol for 48 hrs, finally extracted with 5% absolute ethanol using soxhlet apparatus for 6 -8 hrs and the residue was concentrated and dried at 37ºC. The dose was calculated after LD_{50} estimation and finally made to 50mg/kg b.w.

Experimental protocol

Forty pathogen-free mice were randomly used during the present study. One group served as control and epinephrine was administered intramuscularly to other groups at the dose of 100nl/kg b.w. Epinephrine administered group was followed by an ethanolic extract of *Withania somnifera* administration @ 50 mg/kg. b.w. for two and four weeks. Animals were sacrificed after two weeks and four weeks of treatment and blood samples were collected by orbital sinus puncture method followed by serum collection.

Clinical analyses

Serum glutamate pyruvate transaminase and Serum glutamate pyruvate transaminase were performed using a standard kit (Coral) based on the methods of Reitman and Frankel (1957). Serum total bilirubin was evaluated using again the standard kit (Coral) based on the method of Jendrassik and Grofs (1938).

STATISTICAL ANALYSIS

Data from the experiments were presented as mean ± Standard deviation. Statistical analysis was done by using the Statistical Package for Social Science (SPSS) software for windows version 15 (SPSS Inc., Chicago, Illinois, USA). Paired T-test was done to see any difference between the paired groups. The level of significance was set at $p \leq 0.05$.

RESULTS

***Serum Glutamate Oxaloacetate Transaminase* (SGOT)**

In Control group of mice SGOT level was found to be 25.04 ± 4.5 IU/ml having the range of 18.59 to 30.91 IU/ml. After administration of epinephrine for two weeks (100nl/kg b.w) its level got raised to 32.15 ± 5.19 IU/ml with values ranging from 24.57 to 37.86. However, epinephrine administration for four weeks resulted in further elevation of SGOT level to 36.09 ± 4.53 (range 28.22 to 44.43) IU/ml. SGOT level in mice treated with the extract of *W. somnifera* decreased back to 26.53± 1.82 (range of 22.68 to 28.98) and 26.01 ± 3.22 (range 20.38 to 29.86) IU/ml after two weeks and four weeks respectively. These results are depicted by the box-plot in Figure 1.

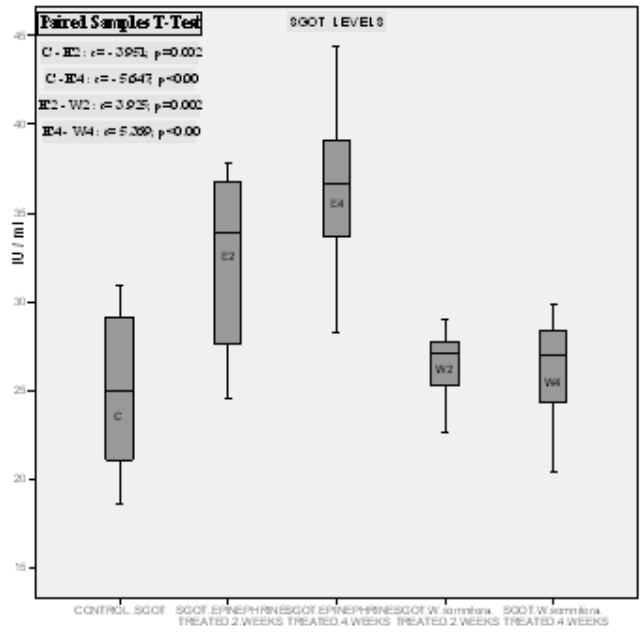

Figure 1
SGOT levels in control, epinephrine and W. somnifera treated mice

Serum glutamate pyruvate transaminase (SGPT)

In control mice group SGPT level was found to be 24.05 ± 5.09 having range values between 16.93 to 31.38 IU/ml which after two weeks administration of epinephrine got raised to 32.13±7.31 (range values 19.64 to 44.53) IU/ml and after four weeks administration it was 57.08±6.56 (range values 48.26 to 66.43) IU/ml. After two weeks treatment with the ethanolic extract of *W. somnifera* it was 28.33 ± 4.6 (ranging between 20.44 to 33.97) IU/ml and after four weeks 29.19 ± 4.34 (ranging between 22.20 to 34.84) IU/ml. These values are compared by the box-plot as evident in Figure 2.

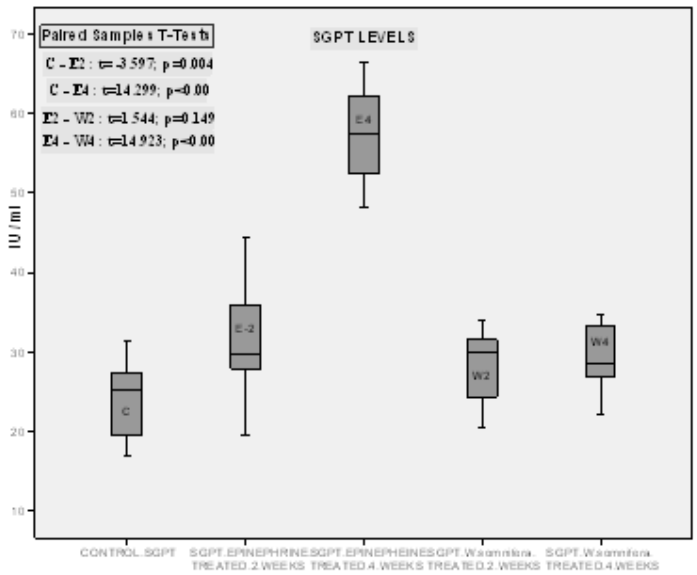

Figure 2
SGPT levels in control, epinephrine and W. somnifera treated mice

Bilirubin levels in various groups

Bilirubin level of control mice was observed to be 0.52 ± 0.08 mg/dl (values ranging between 0.42 to 0.67) which changed to 0.55 ± 0.06 (values ranged between 0.43± 0.65) after two weeks administration of epinephrine and 1.10 ± 0.07 (values ranged between 1.01 to 1.21) mg/dl after four weeks of epinephrine treatment. However, on treatment with the ethanolic extract of *W. somnifera* bilirubin level was found to be 0.90 ± 0.078 (values ranged between 0.77 to 1.02) after two weeks and 0.98 ± 0.138 mg/dl (range 0.83 to 1.23) after four of treatment. These variations are depicted by box-plot as shown in Figure 3.

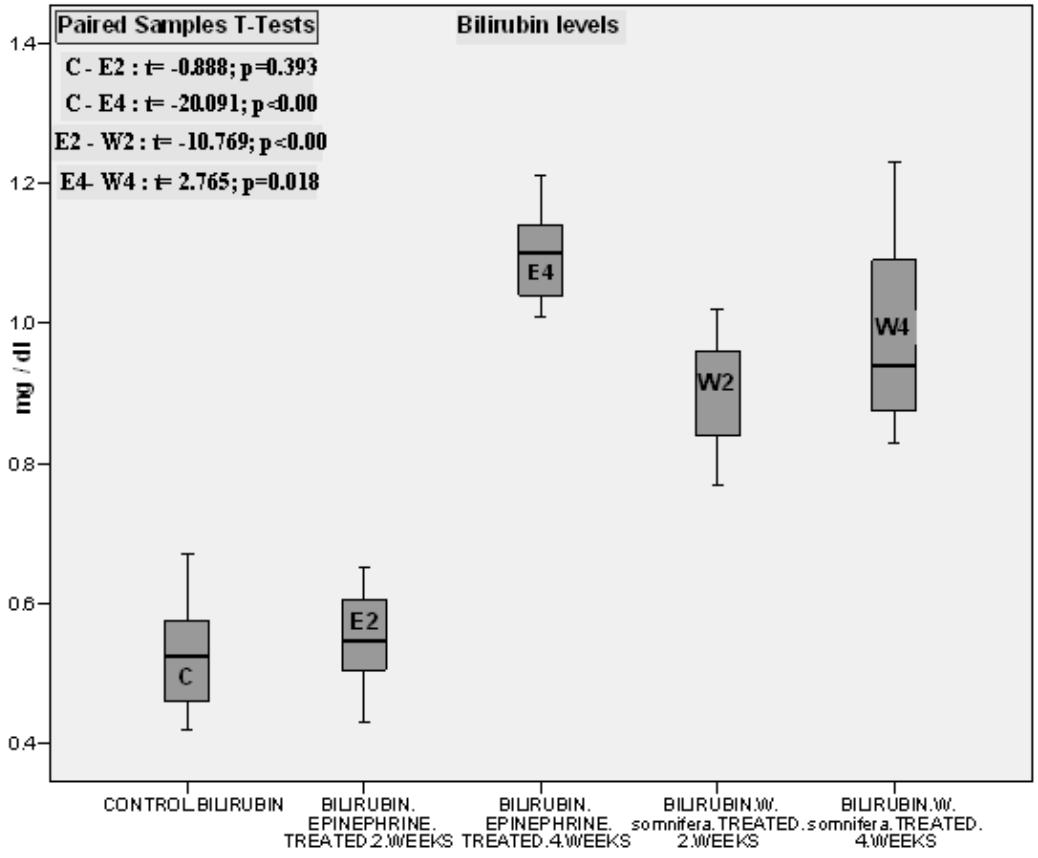

Figure 3
SGPT levels in control, epinephrine and W. somnifera treated mice

Table 1
Effect of the ethanolic extracts of Withania somnifera on liver function parameters

Parameter	Control	Epinephrine treated		*W.somnifera*		P^1-value (t^1-value)	P^2-value (t^2-value)	P^3-value (t^3-value)	P^4-value (t^4-value)
		Two weeks	Four weeks	Two weeks	Four weeks				
	[C]	[E2]	[E4]	[W2]	[W4]	[C-E2]	[C-E4]	[E2-W2]	[E4-W4]
SGOT(IU/ml)	25.04 ± 4.5	32.15 ± 5.19	36.09 ± 4.53	26.53 ± 1.82	26.01 ± 3.22	0.002 (-3.951)	<0.00 (-5.647)	0.002 (3.925)	<0.00 (5.369)
% change		28.39% ↑	44.13% ↑	17.48% ↓	29.93% ↓				
SGPT(IU/ml)	24.05 ± 5.09	32.13± 7.32	57.08 ± 6.56	28.33 ± 4.60	29.19 ± 4.34	0.004 (-3.597)	<0.00 (-14.299)	0.149 (1.544)	<0.00 (14.923)
% change		33.59% ↑	137.34 % ↑	11.83 % ↓	48.86 % ↓				
Bilirubin (mg/dl)	0.52 ± 0.08	0.55 ± 0.06	1.10 ± 0.07	0.90 ± 0.08	0.98 ± 0.14	0.393 (-0.888)	<0.00 (-20.019)	<0.00 (-10.769)	0.018 (2.765)
% change		5.77 % ↑	111.54 % ↑	63.64 % ↑	10.91 % ↓				

DISCUSSION

Our findings on the levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and bilirubin revealed alterations in mice treated with epinephrine and thereafter with the ethanolic extracts of *Withania somnifera.* There was 28.39 % and 44.13 % increase in SGOT levels in group treated with epinephrine for two weeks and four weeks respectively as compared to the control which got decreased to the extent of 17.48 % and 29.93 % when compared with their respective epinephrine treated groups on treatment with W. somnifera extract. The levels of SGPT showed 33.59 % and 137.34 % increase on epinephrine treatment for two and four weeks respectively which came down to the extent of 11.83 % and 48.86 % on treatment with the ethanolic extracts of *Withania somnifera.* The levels of bilirubin got increased to the extent of 5.77 % and 111.54 % in groups of mice treated for two and four weeks respectively. However, these groups on treatment with the ethanolic extracts of *Withania somnifera* showed recovery in the group of four weeks treatment.Acute and intense psychological stressors induce gastric ulceration and heart injury in rodents [26]. Several acute stressors also alter liver functions. Thus, stressors are different as restraint and forced exercise induce formation of autophagic vacuoles among several ultrastructural modifications [27]. This is associated with DNA oxidative damage [28], lipid peroxidation [29], protein oxidation [30] and ultimately the loss of hepatocyte integrity, as indicated by the rise in plasma transaminase activities [31]. All these alterations may be caused by catecholamines. Sustained elevation of plasma norepinephrine by means of miniosmotic pump implantation in peritoneal cavity causes hepatocyte injury and depresses liver function [32]. In recent years, it became clear that liver injury, caused by stress, is the consequence of an inflammatory response [33]. Both physical and psychological stressors elevate plasma IL-6 [34] and increase hepatic IL-6 expression [35].In the present study levels of SGOT, SGPT and bilirubin involved in liver function was significantly increased in epinephrine treated in stressed mice as compared to control group mice and after treatment with the ethanolic extract of *W. somnifera* their levels was significantly decreased as compared to epinephrine administered groups.The increased level of SGOT and SGPT enzymes in stress induced animals is due to the reason that stress increases hypatholamo-pitutaryoxis (HPA), and sympathetic system stimulation resulting in

liberation of catecholamine, glucocorticosteroids, which inhibit the immune system at multiple sites like liver, kidney [36], As stress is linked to many diseases, research on an effective anti-stress agent (adaptogen) from plants has gained importance

REFERENCE

1. Ravindran R., Sheela D. R., Samson J. and Senthilvelan M. Noise-Stress-Induced Brain Neurotransmitter Changes and the Effect of *Ocimum sanctum* (Linn) Treatment in Albino Rats, *Journal of Pharmacological Sciences*; 98: 354 – 360, 2005. http://www.mandalamuseum.com/Mandala&Ayurveda/PDF/tulsi_noisestress.pdf
   Verma N. and Khosa R .L. Effect of *Costus speciosus* and *Wedelia chinensis* on Brain Neurotransmitters and Enzyme Monoamine Oxidase Following Cold Immobilization Stress, *Journal of Pharmaceutical Sciences and Research*; 1(2): 22-25, 2009. http://jpsr.pharmainfo.in/Documents/Volumes/Vol1Issue2/pdfs/jpsr04.pdf
2. Khan N. M., Suresh J., Yadav K. S. H. and Ahuja J. Formulation and evaluation of antistress polyherbal capsule. Pelagia Research Library Der Pharmacia Sinica, 3 (2):177- 184, 2012.www.pelagiaresearchlibrary.com
3. Lakshmi B.V.S. and Sudhakar M. Screening of *Psidium guajava* Leaf Extracts for Antistress Activity in Different Experimental Animal Models, *Pharmacognosy Research*; 1(6): 359-366, 2009. http://www.phcogres.com/text.asp?2009/1/6/359/58016
4. Rai D., Gitika Bhatia G., Sen T. and Palit G. Anti-stress Effects of *Ginkgo biloba* and *Panax ginseng*: a comparative study, *Journal of Pharmacological Sciences*, 93: 458 – 464, 2003. https://www.jstage.jst.go.jp/article/jphs/93/4/93_4_458/_pdf
5. Umokoro S. and Ashorobi R. B. Antistress potential of aqueous seed extract of *Aframomum melegueta, African J. of Biomedical Research*; 8: 119-121, 2005. http://www.bioline.org.br/pdf?md05021
6. Laurence H., Coffin J., Jay L. Ankeney. and Edward M. B. Experimental Study and Clinical Use of Epinephrine for Treatment of Low Cardiac Output Syndrome. Circulation, 33: I-78-I-85, 1966. http://circ.ahajournals.org/content/33/4S1/I-78
7. Larsson K., Gronneberg R. and Hjemdahl P. Bronchodilatation and inhibition of allergen induced bronchoconstriction by circulating epinephrine in asthmatic subjects. J. Allergy Clin. Immunol; 75:586-593, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3989143
8. Martindale. The Extra Pharmacopoeia 28th ed. Reynolds, J.E.F.; Prasad, A.B, Eds. London: The Pharmaceutical Press; 1-6, 15-16, 1982.
9. Øymar K. and Halvorsen T. Emergency presentation and management of acute severe asthma in children. Scand J Trauma Resusc Emerg Med; 17:40, 2009. http://www.sjtrem.com/content/17/1/40
10. American Hospital Formulary Service (AHFS). Drug Information--88. McEvoy, G.K., Ed. Bethesda, MD: American Society of Hospital Pharmacists, Inc (1988).
11. Dougherty R.H. and Fahy J. V. Acute Exacerbations of Asthma: Epidemiology, Biology and the Exacerbation-Prone Phenotype. Clin Exp Allergy; 39(2): 193–202, 2009. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2730743/
12. Mishra L.C., Singh B.B., and Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev*; 5:334-346, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10956379
13. Bhatnagar M., Sisodia S.S., and Bhatnagar R. Antiulcer and antioxidant activity of *Asparagus racemosa* WILLD and *Withania somnifera* DUNAL in rats. *Ann N Y Acad Sci*; 1056:261-278, 2005.

http://www.ncbi.nlm.nih.gov/pubmed/16387694

14. Gupta S.K., Dua A., and Vohra B.P. *Withania somnifera* (ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. *Drug Metabol Drug Interact*; 19:211-222, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14682611
15. Bhattacharya A., Ghosal S. and Bhattacharya S.K. Anti oxidant effect of *Withania somnifera* glycowithanolides stress induced perturbations in chronic footshock of oxidative free radical scavenging enzy mes and lipid peroxidation in rat frontalcor tex and striatum. J Ethnopharmacol; 74(1):1-6, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11137343
16. Bhattacharya S.K., Satyan K.S. and Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. *Indian J Exp Biol*; 35:236-239, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9332168.
17. Rasool M. and Varalakshmi P. Immunomodulatory role of *Withania somnifera* root powder on experimental induced inflammation: An in vivo and in vitro study. Vascul Pharmacol; 44(6):406-410, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16713367
18. Khan B., Ahmad S.F., Bani S., Kaul A., Suri K.A., Satti N.K., Athar M. and Qazi G.N. Augmentation and proliferation of T lymphocytes and Th-1 cytokines by *Withania somnifera* in stressed mice. *Int Immunopharmacol*; 6:1394-1403, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16846833
19. Bani S., Gautam M., Sheikh F.A., Khan B., Satti N.K., Suri K.A., Qazi G.N. and Patwardhan B. Selective Th-1 up-regulating activity of *Withania somnifera* aqueous extract in an experimental system using flow cytometry. *J Ethnopharmacol*; 107:107- 115, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16603328
20. Davis L. and Kuttan G. Effect of *Withania somnifera* on cell mediated immune response in mice. *J Exp Clin Cancer Res*; 21:585-590, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12636106
21. Singh B., Chandan B.K. and Gupta D.K. Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dunn. (Part II). *Phytother Res*; 17:531-536, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12748992
22. Devi P.U. *Withania somnifera* Dunal (ashwagandha): potential plant source of a promising drug for cancer chemotherapy and radio sensitization. *Indian J Exp Biol*; 34:927- 932, 1996. http://www.ncbi.nlm.nih.gov/pubmed/9055640
23. Reitman S. and Frankel S. Determination of aspartat and alanine aminotransferase in blood serum and tissues. *Am. J. Clin. Path.*; 28: 56, 1957. http://www.ncbi.nlm.nih.gov/pubmed/13458125
24. Jendrassik, G. F. and Grofs B. M. Quantitative colorimetric determination of bilirubin in serum or plasma. *Clin.Chem. Acta.*; (27):79, 1938.
25. Konturek S.J., Brzozowski T., Konturek P.K., Majka J. and Dembinski A. Role of salivary glands and epidermal growth factor (EGF) in gastric secretion and mucosal integrity in rats exposed to stress. *Regul Pept*; 32: 203–215, 1991. http://www.ncbi.nlm.nih.gov/pubmed/2034822
26. Salas M., Tuchweber B. and Kourounakis P. Liver ultrastructure during acute stress. *Pathol Res Pract*; 167: 217–233, 1980. http://www.ncbi.nlm.nih.gov/pubmed/7433233
27. Adachi S., Kawamura K. and Takemoto K. Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. *Cancer Res*; 53: 4153–4155, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8364908

28. Kovacs P., Juranek I., Stankovicova T. and Svec P. Lipid peroxidation during acute stress. *Pharmazie*; 51: 51–53, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8999436
29. Liu J., Wang X., Shigenaga M.K., Yeo H.C., Mori A. and Ames B.N. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB J*; 10: 1532–1538, 1996.http://www.ncbi.nlm.nih.gov/pubmed/8940299
30. Fernández G., Mena M.P., Arnau A., Sánchez O., Soley M., and Ramírez I. Immobilization stress induces c-Fos accumulation in liver. *Cell Stress Chaperones*; 5: 306–312, 2000.http://www.ncbi.nlm.nih.gov/pmc/articles/PMC312860/
31. Wang P., Tait S.M. and Chaudry I.H. Sustained elevation of norepinephrine depresses hepatocellular function. *Biochim Biophys Acta*; 1535: 36–44, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11113629
32. Swain M.G. Stress and hepatic inflammation. *Am J Physiol Gastrointest Liver Physiol*; 279: G1135–1138, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11093934
33. Zhou D., Kusnecov A.W., Shurin M.R., DePaoli M. and Rabin B.S. Exposure to physical and psychological stressors elevates plasma interleukin 6: relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinolog*; 133:2523–2530, 1993.http://www.ncbi.nlm.nih.gov/pubmed/8243274
34. Kitamura H., Konno A., Morimatsu M., Jung B.D., Kimura K. and Saito M. Immobilization stress increases hepatic IL-6 expression in mice. *Biochem Biophys Res Commun*; 238: 707–711, 1997.http://www.ncbi.nlm.nih.gov/pubmed/9325153
35. Bharathi K.N., Sivaramaiah N., Nagarjuna C.G. and Gupta A. Evaluation of antistress, anxiolytic and hypnotic activity of vedic calm, a polyherbal formulation. Phcog Mag; 5:124-130, 2009. http://www.phcog.com/text.asp?2009/5/19/124/59796