International Journal of Experimental Research and Review (IJERR)

ISSN: 2455-4855 (Online) ***Original Article***
Received: 27th May, 2020; **Accepted:** 14th July, 2020; **Published:** 30th August, 2020

DOI: https://doi.org/10.52756/ijerr.2020.v22.005

Effects of cold stress, alprazolam and phytomedicine in combination with stress on blood glucose and haematogical parameter of the male albino rat

Piyasi Bhattacharjee

Department of Biology, Bansberia Girls High School, Bansberia, Hooghly-712502, West Bengal, India

Author's E-mail: piyasicyto@gmail.com

Abstract

The present study conducted to investigate the haematological changes and changes of blood glucose level in male albino rat due to cold stress. In this experiment normal 12:12 light dark phases were maintained for all the groups. Control group was kept at normal room temperature (22 ± 1). A (4°C), B in (0°C), C (4°C and 0.30 mg alprazolam / kg body weight /animal), D (0°C and 0.30 mg alprazolam/ kg body weight/ animal. E2 group was treated with (4°C and 1000 mg/kg body weight methanolic extract of *Withania somnifera* root extract /animal). F2 group was treated with (0°C and 1000 mg/kg body weight methanolic root extract of *Withania somnifera* / animal). The blood glucose level was significantly increased in stressed rats compared to the control animals. The results were also consistent with the exposure to the stress and chronic restraint stress. Action of Alprazolam over cold stress treated group significantly reduced the blood glucose level. Whereas methanolic root extract of *Withania somnifera* in low and high doses also showed significant effects to the control anxiety like effects on blood glucose level. Alprazolam + different stress treated groups in different experiment at conditions show significant changes in its haematological parameters in comparison to the stress treated group. Whereas herbal medicine (i.e., methanolic root extract of *Withania somnifera*) when applied to different stress treated group showed more significant result, compared to the Alprazolam+ different stress treated groups. The positive safe anti stress effects of the herbal plant medicine prove that the tribal medicines have the potentiality to act effectively and can be used as safe medicine for anti-stress purposes.

Keywords: ACTH, alprazolam corticotropin releasing factor, hypothalamic-pituitary-adrenal axis, *Withania somnifera*.

Introduction

Stress is the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis. In our daily lives, some stress prepares us to meet certain factors which have been linked with hypertension and atherosclerosis. Examination stress, unemployment stress etc., show various physiological changes in

response to increased hypothalamo-pituitary action, activation of pituitary-adrenal system and secretion of various hormones e.g., catecholamines, endorphins and encephalin etc. (Wright et al., 2010).The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and animals (Charles, 1971). Under stress, the paraventricular nucleus of the hypothalamus produces corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophyseal portal blood vessel system (Childs, 2008) CRF stimulates the anterior pituitary gland, causing release of andreno-corticotropic hormone (ACTH)into the blood stream (When stimulated by ACTH, the adrenal cortex synthesizes glucocorticoid hormones from the cholesterol precursor. Increased levels of glucocorticoid initiate metabolic effects that modulate the stress reaction (Dourish and Cooper, 1990). Cold stress related hypothermia may cause damage to various organ systems. There are very few studies on the effects of hypothermia on endocrine system. We therefore, investigated effects of cold induced hypothermia on adrenal and haematological functions and blood glucose level alterations in male albino rats. Hypothermia may be a consequence of environmental conditions, microbial infections and/or hypothyroidism. Although regulation of body temperature and individual adaptation to environmental climatic changes is well documented, little is known about mechanisms and pathological aspects of Hypothermia (Childs, 2008). Hypothermia may cause damage in various organs and systems in the body. However, most of the studies investigating the adverse effects of hypothermic conditions have focused on the central nervous system (Wang et al., 2009). It has been shown that hypothermia increases apoptotic cell death, a condition that is affected by duration of hypothermia (Jung et al., 2007; Lahiri et al., 2006; Michel et al., 2007). Thus, increased brain hypothermia may cause neurotoxicity directly.

Hyperthermia is one of the most frequent causes of pediatric complaints leading to hospital admission. Infant and child brain is susceptible to hyperthermia and may undergo various pathological conditions (Mishima et al., 2004; Mustafa, 2008). Increased levels of glucocorticoids initiate metabolic effects that modulate the stress reaction (Chan et al., 2002) have reported an increase in the HPA axis activity in diabetic patients. Also (Roy et al., 1993) have shown that this causes in types I and II diabetic patient elevated cortisol levels. (Chan et al., 2002) has suggested that the control of diabetes is influenced by the adreno-cortical function and patients with poorly controlled diabetes have a higher level of cortisol. There are limited studies on cold-induced alterations in endocrine functions and behavioural dysfunctions, particularly in infants and children. A few studies demonstrated adverse effects of hypothermia on the brain in rats (Nakai et al., 2001; Sessler et al., 2007). Hyperthermia may impair cognitive functions (Sharma et al., 2003) induce problems in coping and behaviour (Dourish and Cooper,, 1990) including motor functions(Sprague et al., 2003). Developing rats exposed to hypothermia have been shown to display signs of increased anxiety in the elevated-plus maze, but these changes were not associated with increased susceptibility to depression-like behaviour (Tomimatsu et al., 2003). Hypothermia is an important stress factor and known to increase blood cortisol levels (Trescher et al., 1997; Yager et al., 1999). This is expected since hypothalamo-pituitary-adrenocortical (HPA) axis is activated in response to stressors.

Methodology

Experimental schedule due to cold exposure on the activity of the male albino rat

Groups	Cold stress	No. of animals /cage	Dosage (mg/kg Bodyweight /animal)	Days of treatment	Date of Autopsy
Control	22^0C	6		1-14	15^{th} day
A	4^0C	6		1-14	15^{th} day
B	0^0C	6		1-14	15^{th} day
C	Stress (4^0C) + Alprazolam	6	0.30	1-14	15^{th} day
D	Stress (0^0C) + Alprazolam	6	0.30	1-14	15^{th} day
E1	Stress (4^0C) + Methanolic root extract of *Withania somnifera*	6	600	1-14	15^{th} day
E2	Stress (4^0C) + Methanolic root extract of *Withania somnifera*	6	1000	1-14	15^{th} day
F1	Stress(0^0C) + Methanolic root extract of *Withania somnifera*	6	600	1-14	15^{th} day
F2	Stress (0^0C) + Methanolic root extract of *Withania somnifera*	6	1000	1-14	15^{th} day

Haematological study

Estimation of Haemoglobin in blood

Hemoglobin concentration in blood was estimated using Shahli's haemoglobinometer. 0.1N hydrochloric acid was placed up to about 10 marks in the graduated mixing tube. Blood was taken in the blood pipette upto the 20 cu mm mark. The blood was discharged into the haemoglobinometer tube containing N/10 HCl. A little amount of blood was mixed with it and allowed to keep for 8-10 minutes. Then they formed acid haematin was diluted with water; mixed well with stirrer. The colour formed in the tube was compared with the colour of the standard. When an exact match was obtained, the reading was taken in gm/100 ml of blood.

Total Count of RBC and WBC

The total count of RBC and WBC were counted with the help of Neubauers' Haemocytometer.

Differential Count

A thin blood film was prepared and stained with Wright's stain. The stained film was dried and examined under high power of the microscope.

Different varieties of WBC cells were counted till 100 counts. The percentages of various white blood corpuscles were calculated.

Estimation of PCV, MCV and MCHC

The packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes. This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Since a tube is used, this can be calculated by measuring the lengths of the layers.

Mean Corpuscular Haemoglobin (MCH)

This is the average amount of haemoglobin in each red blood cell.

MCV (fl) = PCV as fraction/ number of red cells / L.

Mean Corpuscular Haemoglobin Concentration (MCHC)

This is the amount of haemoglobin in the circulating blood.

MCHC (g/dl) = Hb concentration (g/dl) / PCV as fraction

Estimation of blood glucose level

Principle

Glucose Oxidase (GOD) oxidizes Glucose to Gluconic Acid and Hydrogen peroxide. In the presence of enzyme peroxidase, released Hydrogen peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP)to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose in the sample.

(Glucose + O_2 + H_2O)	Glucose Oxidase	Gluconic Acid + H_2O_2
H_2O_2 + Phenol + 4-AAP	Peroxidase	Quinoneimine dye + H_20)

Blood glucose content was assayed by the GOD-POD end point colorimetric assay kit-supplied by Span Diagnostic Limited, Sachin, Baroda (Code : Old Code No: B0112, New Code No: 93 DP100-74).

Table 1. Reagents Composition.

Reagent No.	Reagent	Composition	Concentration	Normal Level
1	Glucose reagent	Phosphate Buffer Glucose oxidase Peroxidase 4 -AAT stabilizers	100Mm/L >15000 U/L > 1600 U/L, 0.28Mm/L qs	Fasting 65-110 mg/L Postprandial < 120 mg/dL
2	Glucose diluents	Phenol preservative	10 Mm/L qs	
3	Glucose standard	Dextrose preservative	100mg/dL qs	
4	Glucose Standard	Dextrose preservative	400 mg/ Dl qs	
Reagent 1, 3 and 4 were stored at 2-8° C and reagent 2 at room temperature				

Result

In experiment set (COLD STRESS TEST), the results show significant increase in blood glucose level, (in Table 2). In A, the level of blood glucose was (189 ± 1.19) and in B the level was (201 ± 1.02).

Haematological parameters showed significant changes in eosinophil, neutrophil ration of stress treated groups (A and B).

Other haematological parameters, such as haemoglobin content, total red blood corpuscles, differential count of WBC and Packed cell volumes were studied for all the control and experimental animals. No significant changes in all the haemoglobin parameters were observed in all the treated groups than the control groups.

Table 2. Effect of cold treatment as stress, some stress resistant drugs and phytomedicine in combination on the blood glucose level of male albino rat groups.

	Normal range	Mean ± S.E
Control	Fasting: 65-110mg/ L Post Prandial < 120 mg/L	125 ± 1.43
A		189 ± 1.19*
B		201 ± 1.02**
C		136 ± 1.07*
D		134 ± 1.92**
E1		127 ± 1.49#
E2		116 ± 1.39*
F1		129 ± 1.29#
F2		118 ± 1.32**

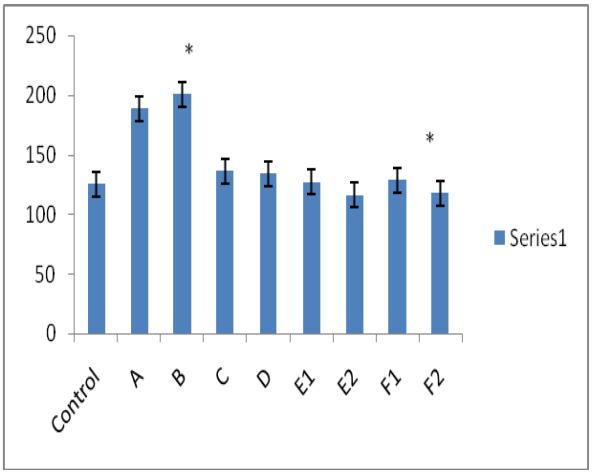

Fig. 1. Graphical representation to show effect of cold stress, some stress resistant drugs and phytomedicine in combination with stress on the blood glucose level of male albino rat.

Table 3. Effect of various (cold) stress and some anti-stress drugs and phytomedicine in combination with stress on the haematological changes of male albino rat.

GROUP	RBC COUNT (10/mm)	WBC COUNT (10/mm)	LYM (%)	MON (%)	NEU (%)
Control	8.57 ± 1.32	11.19 ± 1.94	84±3.34	1	14 ± 2.19
A	8.85 ± 0.98*	9.60 ± 1.35*	53±3.04*	0	10 ± 1.66*
B	9.37 ± 1.o5*	10.71 ± 2.09*	51±1.35*	0	8 ± 1.89*
C	8.33 ± 1.33#	8.51 ± 1.88#	81±2.18#	1	11 ± 1.44#
D	8.29 ±1.75#	10.30 ± 2.01*	77±2.445#	0	13 ± 1.65#
E1	8.41 ± 1.45#	9.62 ± 1.35#	80±1.92*	0	14 ± 1.35#
E2	8.57 ± 2.09*	9.31 ± 1.45#	83±1.17#	0	11 ± 1.65#
F1	8.41 ± 1.6#	9.30 ± 1.35#	85±2.88*	0	12 ± 2.72#
F2	8.49 ± 2.09*	9.16 ± 1.62	87±2.11*	0	16 ± 1.65*
*P<0.05; **P<0.01 ; # =non significance ; Values are mean ± SEM, n = 6.					

GROUP	EOS (%)	BAS (%)	Hb (gm %)	PCV (%)	MCV
Control	1	0	14.80 ± 1.35	45.68 ± 1.83	51.90 ± 2.45
A	1	0	15.00 ± 1.45#	44.78 ± 1.63#	51.49 ± 2.10
B	0	1	14.6 ± 2.09*	44.91 ± 1.49*	50.58 ± 2.38#
C	0	0	14.8 ± 1.50#	43.64 ± 2.09#	49.79 ± 2.31#
D	0	0	14.7 ± 2.22*	42.41 ± 2.39#	50.43 ± 1.45*
E1	0	0	13.8 ± 2.93#	43.21 ± 2.03*	51.23 ± 1.74*
E2	1	1	14.8 ± 1.45*	42.67 ± 2.77#	50.37 ± 1.86*
F1	0	0	15.5 ± 1.35#	45.20 ± 2.10#	50.62 ± 1.88#
F2	0	0	14.5 ± 3.14*	45.51 ± 2.90*	51.61 ± 2.66#
*P<0.05; **P<0.01 ; # =non significance ; Values are mean ± SEM, n = 6.					

Discussion

The stress response in individuals helps to meet the challenges. The situation may be due to the activation of the autonomic nervous system and hypothalamo-pituitary-adrenal (HPA) axis, and the —fight or flight response is the classical way of envisioning the behavioural and physiological response to a threat from a dangerous situation. Long-term exposure to stress can lead to serious health problems. Chronic stress disrupts nearly every system in the body. It can raise blood pressure, suppress the immune system, increase the risk of heart attack and stroke, contribute to infertility, and speed up the aging process. Long-term stress can even rewire the brain, leaving you more vulnerable to anxiety and depression. Modern medicine has provided several preventive and corrective methods of stress resistance but none of these are very safe and without any serious side effects. It is well known that the adrenal glands have a key-role in hormonal reactions to stress as they are involved both in the hypothalamic–pituitary–adrenocortical axis and the symphatho-adreno-medullary system. Adverse situations trigger responses on the adrenals, which result in an increase in glucocorticoid and/or catecholamine secretion. Under stressful conditions, cortisol provides the body with glucose by tapping into protein stores via gluconeogenesis in the liver. This energy can help an individual fight or flee a stressor. However, elevated cortisol over the long term consistently produces glucose, leading to increased blood sugar levels. The changes are the front-line endocrine mechanisms to defend the organism against the stressful conditions. In the control, stress and stress+antistress medicine/ stress +plant extract medicines within the albino rat some remarkable changes have been noticed. These results are quite authentic with regard to the action of medicine and extract of plants for anti depressant activity. Haematological parameters showed significant changes in eosinophil neutrophil ration of various stress treated groups. Other haematological parameters, such as haemoglobin content, total red blood corpuscles, differential count of WBC and Packed cell volumes were studied for all the control and experimental animals. No significant changes in all the haemoglobin parameters were observed in all the treatment groups than the control animals.

Biochemical parameters of blood serum showed different results. Group A, B showed a significant increase in the glucose level. Significant decrease in the glucose level showed in the E2 and F2 groups as compared to the control group.

In this context it can be concluded that 1000mg/kg body weight/animal crude methanolic root extract of *Withania somnifera* plant has anti-stress activity. It can be used as a safe anti-stress agent after further thorough investigation. Hence, the methanolic crude extracts of the *Withania somnifera* Linn. contains phytochemical compounds, active alkaloids in the form of withanoloids. The doses (1000mg/ kg body weight/animal) used in the investigation caused anti-depressant or anti stress activity. The positive safe anti stress effects of the herbal plant medicine prove that the tribal medicines have the potentiality to act effectively and can be used as safe medicine for anti-stress purposes.

Reference

Charles, H. (1971). The Biology and Psychology of Crowding in Man and Animal. *The Ohio J. of Sci*. 71: 65-72.

Childs, C. (2008). Human brain temperature: regulation, measurement and relationship with cerebral trauma: part 1. *Br. J. Neurosurge.* 22 : 486-496.

Dourish, C. T. and Cooper, S. J. (1990). Yawning elicited by systemic and interstitial injection of piribedil and apomorphine in the rat Pyschopharmacology. *Pyschol*. 199086: 175-181.

Gaoua, N., Racinais, S., Grantham, J. and El-Massioui F. (2011). Alterations in cognitive performance duringpassive hyperthermia are task dependent. *Int. J. Hyperthermia.* 27: 1-9.

Jung, A. and Schuppe, H. C. (2007). Influence of genital heat stress on semen quality in humans. *Andrologia.* 39: 203-215.

Lahiri, S. and Lloyd, B. (2006) The Effect of Stress and Corticotrophin on the Concentrations of Vitamin C in Blood and Tissues of the Rat. *Biochem. J*. 84: 432-439.

Chan, O., Inouye, K., Vranic, V. and Matthews, S. G. (2002). Hyperactivation of the hypothalamic-pituitary–adrenocortical axis in streptozotocin- diabetes in associated with reduced stress responsiveness and decreased pituitary and adrenal sensitivity. *Endocrinology.* 143 (5) : 1761– 1768.

Pavlik, A. and Aneja, I. S. (2007). Cerebral neurons and glial cell types inducing heat shock protein Hsp70 following heat stress in the rat. *Prog. Brain. Res.* 162: 417-431.

Michel, V., Peinnequin, A., Alonso, A., Buguet, A., Cespuglio, R. and Canini, F. (2007). Decreased heat tolerance is associated with hypothalamo-pituitary adreno-cortical axis impairment. *Neuroscience.* 147: 522-531.

Mishima, K., Ikeda, T., Yashikawa, T., Aoo, N., Egashira, N. and Xia, Y. X. (2004). Effects of hypothermia and hyperthermia and spatial learning deficits following neonatal hypoxic ischemic condition in rats. *Behav. Brain Res.* 151: 209-217.

Roy, M., Roy, A., Gallucci, W. T., Gollier, B., Young, K., Kami-laris, T. and Chrousos, G. P. (1993). The bovine corticotropin-releasing hormone stimulation test in type I diabetic patients and controls: suggestion of mild chronic hypercortisolism, *Metabolism.* 42 : 696–700.

Mustafa, S., Al-Bader, M. D., Elgazzar, A. H., Alshammeri, J., Gopinath, S. and Essam, H. (2008). Effect of hyperthermia on the function of thyroid gland. *Eur. J. Appl. Physiol.* 103: 285-288.

Nakai, A., Shibazaki, Y., Taniuchi, Y., Oya, A., Asakura, H. and Kuroda, S. (2001). Influence of mild hypothermia on delayed mitochondrial dysfunction after transient intrauterine ischemia in the immature rat brain. *Dev. Brain Res.* 128: 1-7.

Schobitz, B., Reul, J. M. and Holsboer, F. (1994). The role of the hypothalamic-pituitary-adrenocortical system during inflammatory conditions. *Crit. Rev.Neurobiol.* 8: 263-291.

Sessler, D. I. (2009). Thermoregulatory defense mechanisms. *Crit. Care Med.* 37: 7-10.

Sharma, H. S. and Hoopes, P. J. (2003). Hyperthermia induced pathophysiology of the central nervous system. *Int J Hyperthermia.* 19: 325-354.

Sprague, J. E., Banks, M. L., Cook, V. J. and Mills, E. M. (2003). Hypothalamic-pituitary-thyroid axis and sympathetic nervous system involvement in hyperthermia induced by 3,4 methyl enedioxy methamphetamine (Ecstasy).

J. Pharmacal. Exp. Ther. 305: 159-166.

Tomimatsu, T., Fukuda, H., Kanagawa, T., Mu, J., Kanzaki, T. and Murata, Y. (2003). Effects of hyperthermia onhypoxic-ischemic brain damage in the immature rat: its influence on caspase-3-like protease. *Am. J. Obstet. Gynecol.* 188: 768-773.

Trescher, W. H., Ishiwa, S. and Johnston, M. V. (1997). Brief posthypoxic- ischemic hypothermia markedly delays neonatal brain injury. *Brain. Dev.* 19: 326-38.

Wright, H. E., Selkirk, G. A. and McLellan, T. M. (2010). HPA and SAS responses to increasing core temperature during heat stress in trained and untrained males. *Eur. J. Appl. Physiol.* 108: 987-97.

Wang, J. S., Chen, S. M., Lee, S. P., Lee, S. D., Huang, C. Y. and Hsieh, C. C. (2009). Dehydro-epi-androsterone sulfate linked to physiologic response against hot spring immersion. *Steroids* 74: 945-949.

Yager, J. Y. and Asselin, J. (1999). The effect of pre hypoxic ischemic (HI) hypo and hyperthermia. *Dev. Brain Res.* 117: 139-143.