

## Effects of Cold Stress, Alprazolam and Phyto-Medicine in Combination with Stress on Endocrine Functions and Behaviour of the Male Albino Rat

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### Abstract

**Objective:** The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and animals. Under stress, the paraventricular nucleus of the hypothalamus produces corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophysial portal blood vessel system. CRF stimulates the anterior pituitary gland, causing release of adrenocorticotrophic hormone (ACTH) into the blood stream. When stimulated by ACTH, the adrenal cortex synthesizes glucocorticoid hormones from the cholesterol precursor. Increased levels of glucocorticoids initiate metabolic effects that modulate the stress reaction. Laboratory rats usually groom and lick in a stressful situation. It has been proposed that they groom to reduce stress and licking when the situation inducing the stress is over. **Method:** 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 (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). **Result:** the anxiety-like behaviour 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 anxiety like behaviour. Whereas methanolic root extract of *Withania somnifera* in low and high doses also showed significant effects to the control anxiety like behaviour. Alprazolam + different stress treated groups in different experiment at conditions show significant changes in its behavioural responses 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. **Conclusion:** The positive safe anti stress effects of the herbal plant medicine proves that the tribal medicines have the potentiality to act effectively and can be used as safe medicine for anti stress purposes.

**Keywords:** Hypothalamic-Pituitary-Adrenal (HPA) Axis; ACTH; CRF; *Withania Somnifera*; Alprazolam; Corticotropin releasing Factor.

### 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 encephalins etc.

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The hypothalamic-pituitary-adrenal (HPA) axis mediates the endocrine response to stress in humans and animals [1]. Under stress, the paraventricular nucleus of the hypothalamus produces corticotropin releasing factor (CRF), which is delivered to the anterior pituitary gland via the hypothalamic-hypophysial portal blood vessel system [2]. CRF

stimulates the anterior pituitary gland, causing release of andreno-corticotrop hormone (ACTH) into the blood stream [3] 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 [4].

Cold stress related hypothermia may cause damage to various organ systems. There are very few studies on the effects of hypothermia on the endocrine system. We therefore, investigated effects of exogenously induced hypothermia on adrenal and kidney functions and behavioural 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 [20].

Hypothermia may cause damage in various organs and systems in the body [19]. However, most of the studies investigating the adverse effects of hypothermic conditions have focused on the central nervous system [18]. It has been shown that hypothermia increases apoptotic cell death, a condition that is affected by duration of hypothermia [5,6,7]. Thus, increased brain hypothermia may cause neurotoxicity directly.

Hyperthermia is one of the most frequent causes of paediatric complaints leading to hospital admission. Infant and child brain is susceptible to hyperthermia and may undergo various

pathological conditions [8,9]. There are limited studies on cold-induced alterations in endocrine functions and behavioural dysfunctions, particularly in infants and children [3]. A few studies demonstrated adverse effects of hypothermia on the brain in rats [10-12]. Hyperthermia may impair cognitive functions [13], induce problems in coping and behaviour [11] including motor functions [14]. 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 [15]. Hypothermia is an important stress factor and known to increase blood cortisol levels [16]. This is expected since hypothalamo-pituitary-adrenocortical (HPA) axis is

activated in response to stressors such as cold stress [17].

Crowded animal populations often show a breakdown of normal social behaviour, with increased aggression and violence, aberrant sexual activity, improper parental care, abnormal states of activity, aggregation, or social withdrawal. A variety of stress related diseases and mortality patterns may ensue [21]. Laboratory rats usually groom and lick in a stressful situation [22]. It has been proposed that they groom to reduce stress [23] and licking when the situation inducing the stress is over [22]. In this study, we have examined effects of cold exposure-induced hypothermia on various endocrine functions and behaviours in albino male rats.

## Materials and Methods

Experimental Research Center (University Of Kalyani) and control group was housed at Standard pellet diet and water were provided *ad libitum*. The animals in each group were placed and exposed to cold stress together (n=6). Behaviour of the animals was monitored by using video recording throughout the experiments. The experiments were approved by the Animal Ethics Committee. 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 *Withaniasomnifera* root extract / animal). F2 group was treated with (0°C and 1000 mg/kg body weight methanolic root extract of *Withania somnifera* / animal).

### Mathematical study

Activity records of all the animals are shown in the graphical forms ( Fig. 1-6) Comparison of group mean suggested that differences in the control and various stress treated group may be related to differences in the activity level. Mean values of 15mnts and 30mnts for 1st and 2nd weeks for each group were demonstrated a small but significant difference (p is less than equal to 0.05). However, when tested on a weekly basis, this correlation was only significant during 2nd week, when differences in the activity level were most pronounced (p is less

### Experimental model

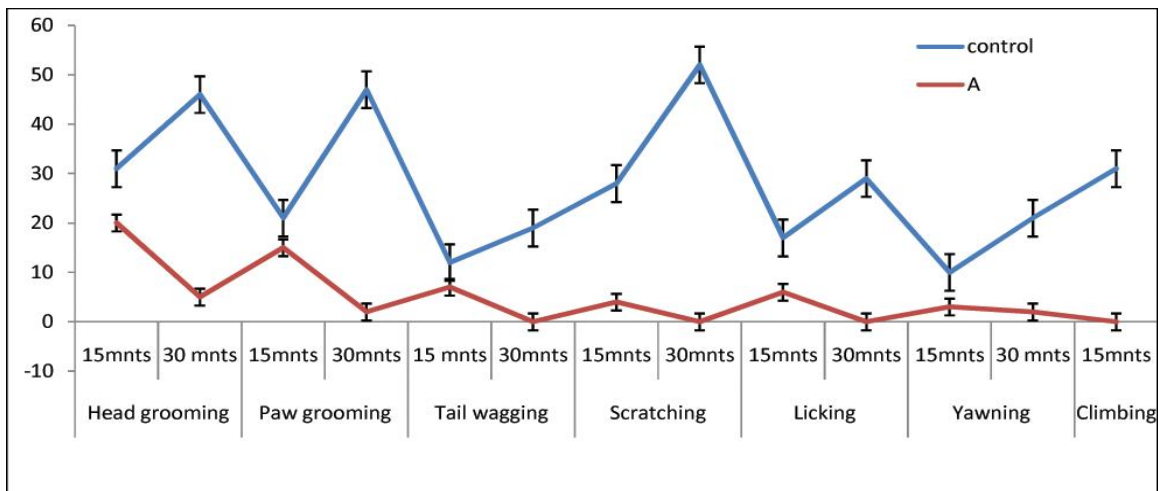
**Table 1:** Experimental schedule due to Cold Exposure in the albino rat

Groups	Cold stress	NO of animals /cage	Dosage (mg/kg Bodyweight /animal)	Days of treatment	Date of Autopsy
Control	22 ° c	6		1-14	15 <sup>th</sup> day
A	4° c	6		1-14	15 <sup>th</sup> day
B	0° c	6		1-14	15 <sup>th</sup> day
C	Stress+Alprazolam (4° c)	6	0.30	1-14	15 <sup>th</sup> day
D	Stress(0° c) + Alprazolam	6	0.30	1-14	15 <sup>th</sup> day
E2	Stress(4° c)+Methanolic root extract of <i>Withania somnifera</i>	6	1000	1-14	15 <sup>th</sup> day
F2	Stress(0° c) +Methanolic root extract of <i>Withania somnifera</i>	6	1000	1-14	15 <sup>th</sup> day

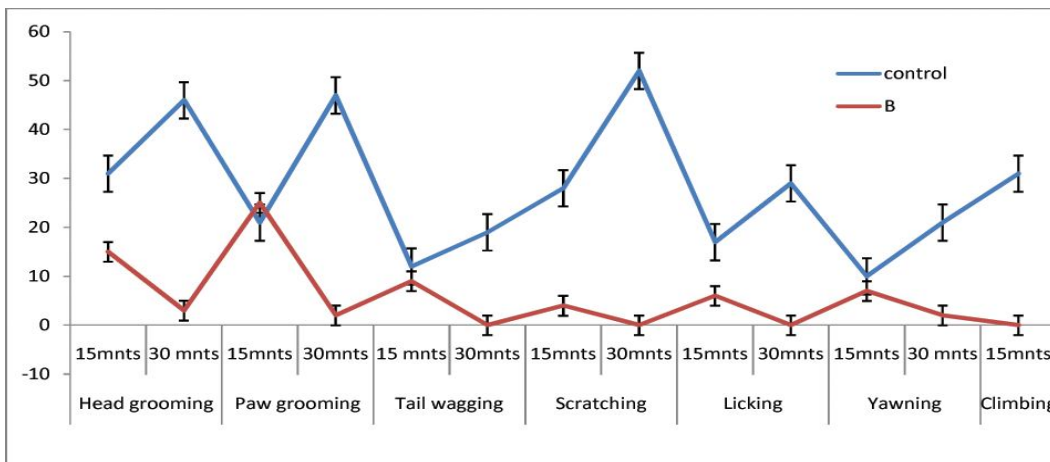
### Results

Relationship of major behavioural responses between the control and various Cold treated experimental groups of male albino rat

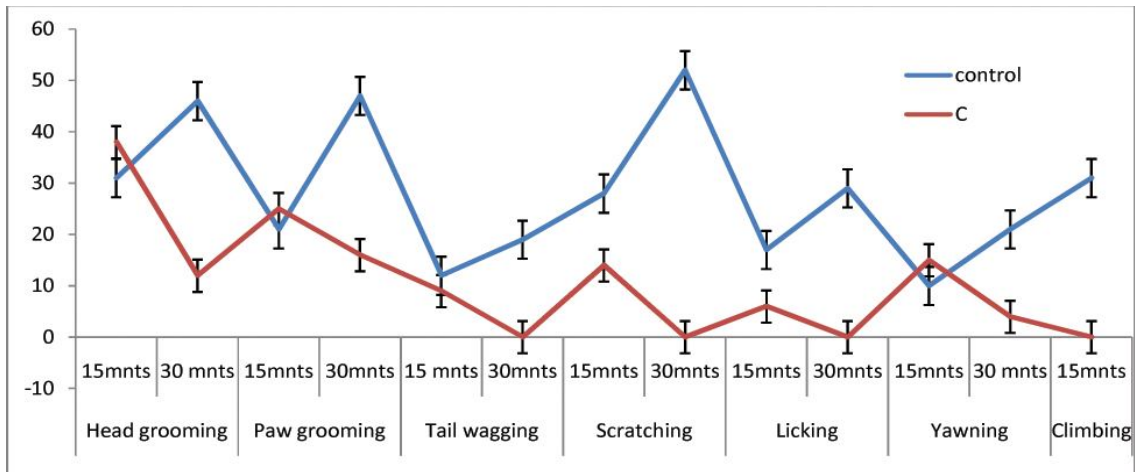
**Fig. 1:** Represents the relationship between the control and 4°C treated group of male albino rat



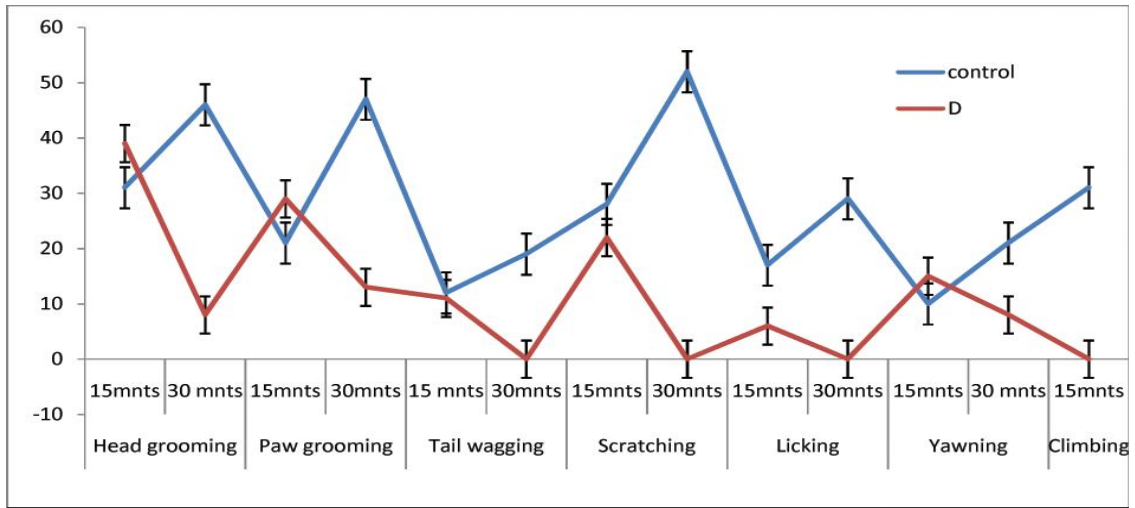
**Fig. 2:** Represents the relationship between the Control and 0°C treated group of male albino rat



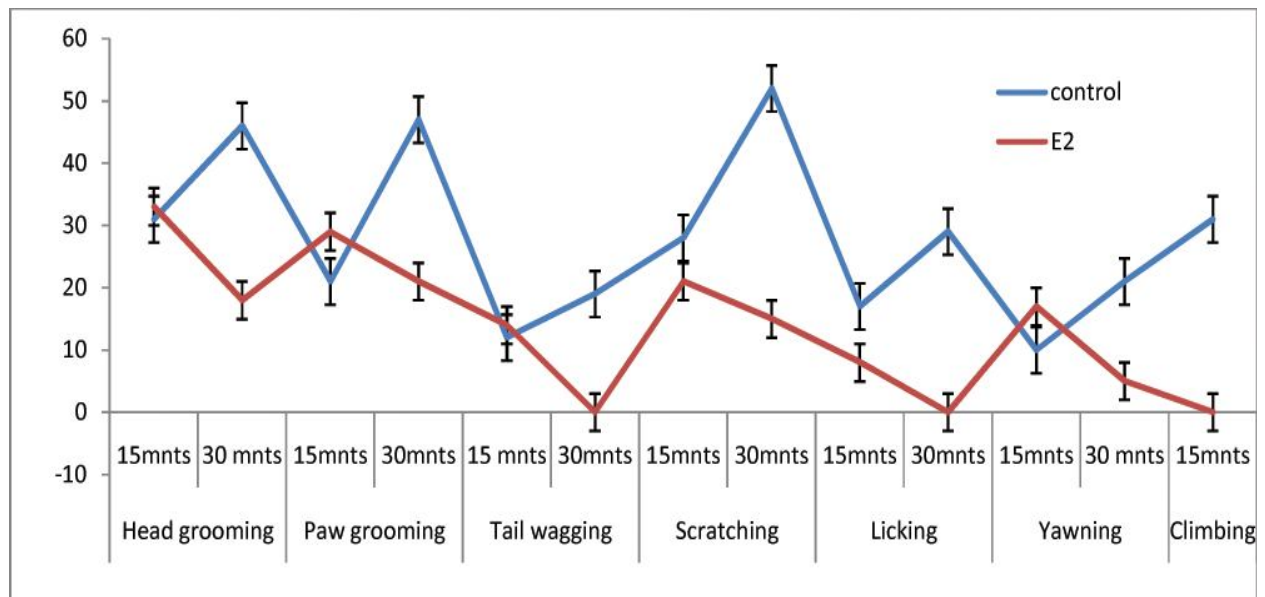
**Fig. 3:** Represents the relationship between the Control and 4°C + Alprazolam treated group of male albino rat



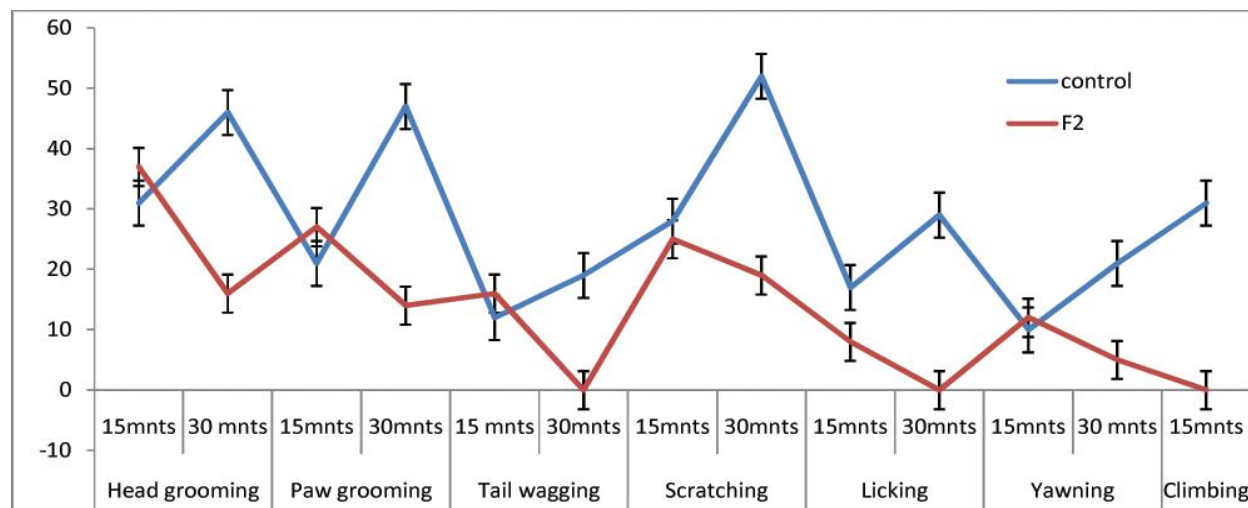
**Fig. 4:** Represents the relationship between the Control and 0°C + Alprazolam treated group of male albino rat



**Fig. 5:** Represents the relationship between the Control and Stress 4°C exposed+ Methanolic root extract of *Withania somnifera*. (High dose) treated group of male albino rat



**Fig. 6:** Represents the relationship between the control and Stress (4°C) exposed+ Methanolic root extract of *Withania somnifera*. (High dose) treated group of male albino rat



**Table 2:** Effect of Various Cold Stress (4°C And 0°C), Some Stress Resistant Drugs and Phytomedicine in Combination with Stress on The Serum Cortisol Level of Male Albino Rat

Group	Cortisol(mg/dl) Mean±SE
Control	8.23±0.35
A	8.54±0.28#
B	8.82±0.24*
C	8.41±0.32#
D	8.65±0.15#
E2	8.24±0.29#
F2	8.18±0.22*

than equal to 0.05). ANNOVA one way and Mann Whitney U test were performed in this experiment.

### Analysis

Serum corticosterone levels were significantly ( $P < 0.05$ ) reduced in both hyperthermia groups (Group A:  $8.54 \pm 0.28 \mu\text{g/dl}$ ) and Group B:  $8.82 \pm 0.24 \mu\text{g/dl}$ ) compared to control ( $8.23 \pm 0.35 \mu\text{g/dl}$ ). Serum levels of cortisol hormones significantly differ among the groups.

Progression time in the cold stress treated group was increased and anxiety test scores declined in animals exposed to 4°C and 0°C compared to the control values ( $P < 0.05$ ).

### Discussion

It was found that the anxiety-like behaviour 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 anxiety like behaviour. Whereas methanolic extract of *Withania somnifera* in low and high doses also showed significant effects to the control anxiety like behaviour. Previous studies also revealed that chronic stress or chronically elevated levels of glucocorticoid exert detrimental effects on the brain and behaviour. However, the present experimental data were inconsistent with previous studies that showed increased head grooming and paw cleaning and licking scores. Plasma ACTH concentration is usually elevated under the influence of all types of stressors applied, but quantitatively different. The most intense grooming, climbing licking and sniffing behaviours increase was provoked by the cold treated groups. These values remained enhanced after the animals were returned and maintained under the control conditions during a period equal to that of stress duration. Grooming, Climbing, yawning licking, stretching behaviour significantly lower down during cold stress treatment, during chronic treatment these activities stopped till the termination of the experiment. Alprazolam + different stress

treated groups in different experiment at conditions show significant changes in its behavioural responses 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. Repeated treatment with antidepressants (fluoxetine, desipramine, or imipramine, alprazolam) and herbal medicine is able to reverse the behavioural effects induced by stress in our daily life. The behavioural responses of the animals used in the present investigation showed more or less similar results when the stressed animals are treated with herbal medicine and some anti depressant medicines. Although these results are quite significant but to draw a generalised idea more research works on this aspect are needed to understand fully the effects of stress on the animal behaviour.

At the same time when some drugs eg, Alprazolam and the root extract of *Withania somnifera* were used in combination with various stressful conditions the situations were towards to control level. These results clearly indicate the satisfactory activity of the root extract of *Withania somnifera* in various stress exposed male albino rat. Although the detailed molecular mechanism of the action of this root extract has been clearly stated, the study emphasizes the importance of the tribal medicine as the useful sedative or nerve soothing agent. But to confirm the experimental results and to find out the mechanisms of actions of this tribal medicine, some more authentic works are required.

Now a days some medicines like Benzodiazepines, Alprazolam, require special precaution if used in children and in alcohol- or drug-dependent individuals. Particular care should be taken in pregnant or elderly patients, patients with substance abuse history, particularly alcohol dependence and patients with co morbid psychiatric disorders. Use of alprazolam should be avoided or carefully monitored by medical professionals in individuals with the following conditions: myasthenia gravis, acute narrow angle glaucoma, severe liver deficiencies. severe sleep apnoea, pre-existing respiratory depression, acute pulmonary insufficiency, chronic psychosis, hypersensitivity to alprazolam or other drugs in the benzodiazepine class, borderline personality disorder. Elderly individuals should be cautious in the use of alprazolam due to the possibility of increased susceptibility to side-effects, especially loss of coordination and drowsiness.

The positive safe anti stress effects of the herbal plant medicine proves that the tribal medicines have

the potentiality to act effectively and can be used as safe medicine for anti stress purposes.

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