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Effect of Drug Alprazolam on Restrained Stress Induced Alteration of Serum Cortisol and Antioxidant Vitamins (Vitamin C and E) in Male Albino Rats

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ABSTRACT

Introduction: Stress can cause harmful effects in the body that induce a wide range of biochemical and behavioural changes. As anti-stress drugs are routinely used to combat stress hence study is needed to assess the contraindication of these drugs in the physiological systems.

Aim: To investigate the effect of alprazolam on restrained stress induced alteration of serum cortisol, and antioxidant vitamin levels in male albino rats.

Materials and Methods: Adult male albino rats (body weight 175-225g) were divided into four groups of six animals in each. Group I (control), kept undisturbed in the metabolic cage throughout the 42 days experimental period. Group II (stress) rats were kept in a wire mesh restrainer for 6 hr/day for 42 days. Group III (stress+ withdrawal) rats were stressed for 21 days and withdrawal of stress for remaining 21 days (total 42 days). Group IV (stress + alprazolam) rats were only stressed for 21 days and treated with drug alprazolam (5mg/kg body weight, intraperitoneal) in continuation with stress for remaining 21 days (total period is 42 days). At the end of 42 days all the rats were sacrificed and serum cortisol, vitamin C and E levels were estimated.

Results: Group II (stressed) showed a significant increase in serum cortisol level with concomitant decrease of serum vitamin C and E levels. Group III (withdrawal) and Group IV (+alprazolam) rats showed significant reduction of serum cortisol along with subsequent increase of serum vitamin C and E concentrations.

Conclusion: Results indicate a possible antioxidant effect of alprazolam on restrained stress induced alteration of serum cortisol and antioxidant vitamin levels.

Keywords: Anti-anxiety agent, Harmful effects, Life threatening events

INTRODUCTION

Stress can be explained as any stimulus that creates an imbalance in the homeostasis processes [1]. Stress has an impact to induce alteration in various anatomical and physiological responses even leading to pathological conditions. Neurophysiological study has already reported the impact of long term stress on cellular function of the brain [2]. Stress has become an unavoidable entity in our lives. Stress produces life threatening events in early development and can result in long term effects on organ development and may lead to pathological conditions. Oxidative damage leads to mood and anxiety disorder and is a definite outcome of stress [3]. Many studies showed that serum cortisol levels are a reliable indicator of stress responses in animals [4,5]. Vitamin C is a well-known antioxidant required by all mammalian cells for proper functioning to control various biochemical reactions [6]. Vitamin E is a key lipid soluble antioxidant and the most effective chain breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation [7]. alprazolam is a benzodiazepine anti-anxiety agent that is frequently used for the treatment of generalized anxiety, panic attacks with or without agoraphobia, and depression in humans [8]. Hence, this study was aimed to assess the effect of drug alprazolam on restrained stress induced possible alteration on serum cortisol and antioxidant vitamin C and E level in male albino rats.

MATERIALS AND METHODS

The study was conducted during October-November 2015. All the experiment procedures followed were performed in accordance with the approval of the Institutional Animal Ethics Committee for the purpose of control and supervision of experiments on animals guidelines for the experimental studies.

Colony bred healthy adult male albino rats (Wister strain) weighting 175-225g was utilized from Central Animal Facility, Indian Institute of Science, Bengaluru, Karnataka for experiments. Wister rats fed with laboratory stock diet (Hindustan lever, Mumbai, India) and water ad libitum. Animals (n=24) were acclimatized for a week to the laboratory conditions at 22-24^{0}C and a 12 hour light:dark (circadian) cycle. Duration of the experimental protocol was 42 days in total for all the groups. All the animals were sacrificed by cervical dislocation and followed by decapitation at the end of the last dose after an overnight fast.

The acclimatized animals (n=24) were divided into four groups of six animals each and three animals were kept in each metabolic wire cage (60cmX30cmX20cm). Group I (untreated control) rats were healthy controls, kept undisturbed in the metabolic cage throughout the experimental period for 42 days. Group II (stress induced) rats were stressed in wire mesh restrainer for 6h/day for 42 days [9]. Group III (stress + withdrawal) rats were stressed for 21 days by keeping them inside mesh restrainer and withdrawal of stress for remaining 21 days by keeping animals in normal cages (total 42 days). Group IV (stress+alprazolam) rats were stressed for 21 days and treated with drug alprazolam (5mg/kg body weight, intraperitoneally) for remaining 21 days in continuation with stress i.e., total 42 days [10].

Induction of Stress

Rats were subjected to restrained stress in a wire mesh restrainer for 6 hours per day for 21 days. The wire mesh restrainer was made up of wooden bottom with stainless steel wire hinged to the base. The restrainer was 8cm (length) x 4cm (breadth) x 4cm (height) in size. A pad lock and latch helped to secure the rat in the restrainer [9].

Biochemical Parameters

The blood was collected from retro orbital plexus into the centrifuge tubes, kept at room temperature for about 2hr and centrifuged at 1500 x g for 15 min to collect serum. Serum was then used for the estimations of vitamin C level by Roe and Koether [11], vitamin E level by modified Baker and frank method [12] and cortisol level by the Enzyme-linked Immunosorbent Assay (ELISA) kit (DRG, USA) method [13].

STATISTICAL ANALYSIS

Data were expressed as mean±standard deviation of the mean. Statistical comparisons were performed by one-way image result for Analysis of variance (ANOVA), followed by post-hoc t-test and $p \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

RESULTS

Food Intake

There was a significant decrease in the final food intake ($p<0.05$) after 42 days of stress induced rats of group II when compared to untreated control group I. No significant changes in the final food intake were seen in stress withdrawal group (group III) and group IV (Stress +alprazolam) rats as compared to untreated control (group I) rats [Table/Fig-1].

Body Weight

All the rats in groups I, III and IV remained active and healthy with normal feeding behaviour. [Table/Fig-2] shows their mean body weight at the end of experiment. However, stress induced rats (group II) were found to be lethargic and their body weights gain (%) were less as compare to their respective controls. [Table/Fig-2] also showed that stress induced rats in group II had significant decrease in final body weight, as compared to their respective controls (14.78% vs 25.09%). However, administration of alprazolam for 21 days in rats under restrainers stress (group IV) or withdrawal of stress (group III) showed remarkable improvements of body weight gain (%) as compared to group II (stressed).

Group & Treatment	Initial Food intake (g/day)	Final Food intake (g/day)
Group I (Untreated control)	17.38 ± 0.45[a]	18.50± 0.39[a]
Group II (Stress induced)	17.17 ± 0.46[a]	15.46 ± 0.40[b]
Group III (Stress+ Withdrawal)	16.92 ± 0.56[a]	18.03 ± 0.52[a]
Group IV (Stress + alprazolam, 5mg/kg bwt)	18.29 ± 0.66[a]	19.32 ± 0.57[a]

[Table/Fig-1]: Effect of drug alprazolam on restrained stress induced alteration of food intake in male albino rats.
Each value is Mean ± SD of six observations in each group. In each column, values with different superscripts (a, b) were significantly different from each other ($p<0.05$). Post-hoc t-test analysis was used to test for differences among the means when ANOVA indicated a significant $p<0.05$.

Group & Treatment	Initial Body Weight (g)	Final Body Weight (g)	Percentage body weight gain (%)
Group I (Untreated control)	192.50±45.96[a]	242.50±47.38[a]	25.09±1.56[a]
Group II (Stress induced)	199.50±10.61[a]	227.00±14.24[b]	14.78±8.23[b]
Group III (Stress+ Withdrawal)	211.50±40.95[a]	266.50±38.95[c]	26.86±4.90[a]
Group IV (stress + alprazolam, 5mg/kg bwt)	195.67±12.78[a]	242.15±15.22[a]	24.95±3.41[a]

[Table/Fig-2]: Effect of drug alprazolam on restrained stress induced alteration of body weight in male albino rats.
Each value is Mean ± SD of six observations in each group. In each column, values with different superscripts (a, b, c) were significantly different from each other ($p<0.05$). Post-hoc t-test analysis was used to test for differences among the means when ANOVA indicated a significant $p<0.05$.

Serum Cortisol

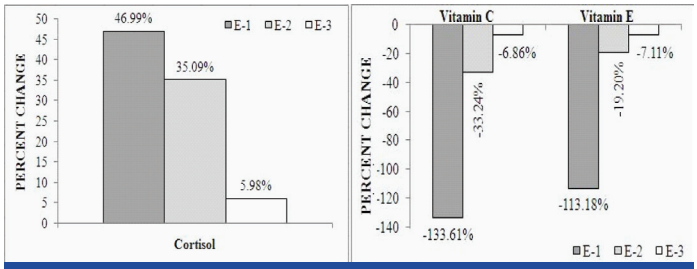
Our result in [Table/Fig-3], showed that in stress induced rats of group II the level of serum cortisol were significantly increased when compared to untreated control rats (group I). The percent change chart in [Table/Fig-4] showed 46.99% increased serum cortisol level as compared to group I. The stress withdrawal (group III) partially reversed this change by reducing the cortisol level. But administration of alprazolam for 21 days in a protocol of 21 days stress alone plus 21 days with treatment in group IV rats showed highly significant decrease in mean serum cortisol level as compared to only stress induced (group II) rats.

Antioxidant Vitamins

[Table/Fig-3] showed highly significant decrease in mean vitamin C and vitamin E level in stress induced rats of group II when compared to group I (untreated rats). The percent change chart in [Table/Fig-4] showed decreased serum vitamin C (-133.61%) and E (-133.18%) respectively as compared to group I. The stress withdrawal (group III) and alprazolam treated (group IV) rats showed remarkable improvement of both vitamin C and E levels as compared to the only stress group II rats.

Groups& Treatment	Serum Cortisol (ng/mL)	Serum Vitamin C (mg/mL)	Serum Vitamin E (µg/mL)
Group I (Untreated control)	78.61 ± 3.43[a]	71.11± 1.23[a]	17.63± 1.44[a]
Group II (Stress induced)	148.30 ± 6.98[b]	30.44± 1.44[b]	8.27 ± 1.57[b]
Group III (Stress+ Withdrawal)	121.10 ± 3.55[c]	53.37 ± 3.26[c]	14.79 ± 35[a]
Group IV (Stress + alprazolam, 5mg/kg bwt)	83.61 ± 4.19[a]	66.54 ± 3.84[a]	16.46 ± 1.64[a]

[Table/Fig-3]: Effect of drug alprazolam on restrained stress induced alteration of serum cortisol and antioxidant vitamins (vitamin C and E) in male albino rats.
Each value is Mean ± SD of six observations in each group. In each column, values with different superscripts (a, b, c, a) were significantly different from each other ($p<0.05$). Post-hoc t-test analysis was used to test for differences among the means when ANOVA indicated a significant $p<0.05$.



[Table/Fig-4]: Percent change chart of drug alprazolam on restrained stress induced alterations.
E-1, Group I (Untreated Control) vs Group II (stress induced); E-2, Group I vs Group III (stress+ Withdrawal); E-3, Group I vs Group IV (stress + alprazolam, 5mg/kg bwt).

DISCUSSION

Food Intake

Our observation indicates that restrained stress (chronic moderate stress) adversely affects food intake of the rats. Exposure to stressors with medium to severe for a long period of time caused decrease food intake and subsequently reduction of body weight in rat [14,15]. Although it was also noticed that the stress response may vary according to its type, duration, severity strains and gender of the experimental animals [15]. In some experimental studies severe stressors were found to have adverse effects on feeding behaviour [16]. Alprazolam was reported to reverse the dietary intake habit of animals exposed to mild stress [17,18].

Body Weight

Our observations indicate that restrained stress (chronic moderate stress) also adversely affects body weight of the rats. It may be due to low food consumption, stress induced hormonal imbalance and altered protein metabolism. The observed decrease in body weight could be due to the direct effect of stress on the food intake behaviour of the rats [19]. Stress might have increased the protein catabolism and hampered the utilization of food consumed during the stress period, thereby causing decrease in body weight. The treatment with drug alprazolam had cut down the percentage decrease in body weight of group IV rats in comparison to stress group of rats [20].

Serum Cortisol

Cortisol, a stress hormone, serves as a key controller for neurohumoral responses in turn leads to behavioural adaptations. Stressors in general induce sensory neuronal pathways projected to - diencephalic centers in the brain and evolve a response (in the form of behavioural, autonomic, endocrine and/or oxidative stress) [21]. Statistically significant increase in levels of serum cortisol after restrained stress suggests that hypothalamic-pituitary-adrenal (HPA) axis is activated. This feedback mechanism is extremely important for survival with stress [22]. Many studies showed that HPA axis is activated by new or unpredictable situations [20,22]. Statistically significant decrease in serum cortisol after administration of alprazolam in group IV rats may be due to reduction of stress response via HPA axis without involvement of internal tissues. The serotonergic system of the brain play a key role in autonomic, neuroendocrine and behavioural integration of the stress response and alprazolam probably influence it [23]. These observations were supported by the study on mice where alprazolam was found to be effective in ameliorating behavioural alterations due to immobilization and oxidative stress [8].

Antioxidant Vitamins

Stress may also damage the antioxidant defense system [24]. Our results from restrain stress induced lowering vitamin C and E depict that it definitely induced oxidative stress. Administration of alprazolam or withdrawal of stress improved both vitamin C and E which are the important markers of oxidative stress [25].

CONCLUSION

The results presented here led us to conclude that exposure to restrained stress resulted in increased level of serum cortisol and decreased antioxidant vitamin C and E levels in male albino rats. Treatment with alprazolam or withdrawal may probably neutralized restrained stress induced damage that lead to oxidant antioxidant balance and alter hypothalamic-pituitary- adrenal (HPA) axis.

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