Original Article

Effect of drug alprazolam on restrained stress induced alteration of serum cortisol and antioxidant vitamins (vitamin C and E) in male albino rats
ABSTRACT

**Background:** Stress can cause harmful effects in the body that induce a wide range of biochemical and behavioral changes. In this study we investigated the effect of Alprazolam, a commonly used antistress drug on restrained stress induced alteration of serum cortisol, and antioxidant vitamin levels in male albino rats.

**Material and Methods:** Adult male albino rats (b.wt. 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, i.p.) in continuation with stress for remaining 21 days (total period is 42 days). The end of 42 days treatment 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 reversal for all the three parameters studied towards near normal levels.

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

**Keywords:** Stress, Antioxidants, Alprazolam, Vitamin C, Vitamin E, Cortisol
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. The study of neurophysiology on effect of stress lasting weeks or months can impair cell communication in the brain’s region [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 is a definite outcome of stress that has been implicated in the pathogenesis of mood and anxiety disorder [3]. Many researchers have suggested 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
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. They acclimatized a week to the laboratory conditions at 22-24°C and a 12hr light:dark (circadian) cycle. All the animals were sacrificed at the end of the last dose after an overnight fast. 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.

**Study groups**

The acclimatized animals 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 6hr/day for 42 days [9]. 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 stressed for 21 days and treated with drug Alprazolam (5mg/kg body weight, i.p) [10]. In continuation with stress for remaining 21 days (total period is 42 days)

**Stress procedure**

Rats were subjected to restrained stress in a wire mesh restrainer for 6hrs per day for 21 days. The wire mesh restrainer had a wooden base and stainless steel wire restrainer hinged to the base. The restrainer having the dimension of 8cm (length) x 4cm (Breadth) x 4cm (Height) was used for the experiment. A pad lock and latch helped to secure the rat in the restrainer [9].

**Biochemical parameters**
The blood collected from retro orbital method. Blood was collected in centrifuge tubes, kept at room temperature for about 2hr and centrifuged at 1500xg for 15min 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 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 ANOVA, followed by post-hoc t-test and p≤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 1).

**Body weight**

All the rats in groups I, III and IV remained active and healthy with normal feeding behavior. Their mean body weight at the end of experiment is shown in Table 2. However, stress induced rats (group II) were found to be lethargic and their body weights decreased remarkably. Table 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).

**Serum cortisol**

Our result in Table 3, showed that in stress induced rats of group II the level of serum cortisol was significantly increased when compared to untreated control rats (group I). The percent change chart in Fig. 1 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 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 Fig. 1 showed decreased serum vitamin C (-133.16%) 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.

**DISCUSSION**

**Food intake**

Our observation indicates that restrained stress (chronic moderate stress) adversely affects food intake of the rats. Other studies have reported that chronic exposure to stressors of a certain severity decrease food intake and body weight of rat [14, 15]. However, the type, duration or
severity of stress and the different strains and gender of the experimental animals used may modify the responses to stress [15]. Models using more severe stressors have also shown effects on feeding behavior, in which the animals present decreased intake of food [16]. This effect is reversed by antidepressants drug Alprazolam. Previous studies also showed recovery in food intake in chronic mild stress [17].

**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 behavior of the rats [18]. 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.

**Serum cortisol**

Cortisol, the major stress hormone, serves as a key controller for neurohumoral responses which underlie behavioral adaptations. Stressors- whether they are physical or psychological activate afferent neural pathways within the central nervous system which project to diencephalic centers where they initiate a response [19]. This response may be behavioral, autonomic, endocrine and/or oxidative stress. Statistically significant increase in levels of serum cortisol after restrained stress suggests that hypothalamic-pituitary-adrenocortical (HPA) axis is activated. This feedback mechanism is extremely important for survival with stress [20]. Many studies showed that any situation which is new or unpredictable most likely activates the HPA axis [19, 20]. 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 behavioral integration of the stress response and Alprazolam probably influence it [21]. These observations were supported by the study on mice where Alprazolam was found to be effective in ameliorating behavioral alterations due to immobilization and oxidative stress [8].

**Antioxidant vitamins**

Stress may also impair the antioxidant defense system, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors [22]. 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.

**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 HPA axis.

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Table 1. Effect of drug alprazolam on restrained stress induced alteration of food intake in male albino rats

<table>
<thead>
<tr>
<th>Group &amp; Treatment</th>
<th>Initial Food intake (g/day)</th>
<th>Final Food intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong> (Untreated control)</td>
<td>17.38 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.50 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group II</strong> (Stress induced)</td>
<td>17.17 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.46 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group III</strong> (Stress+ Withdrawal)</td>
<td>16.92 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.03 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group IV</strong> (stress + Alprazolam, 5mg/kg bwt)</td>
<td>18.29 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.32 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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.
<table>
<thead>
<tr>
<th>Group &amp; Treatment</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Percentage body weight gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong> (Untreated control)</td>
<td>192.50±45.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242.50±47.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.09±1.56&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group II</strong> (Stress induced)</td>
<td>199.50±10.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>227.00±14.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.78±8.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group III</strong> (Stress+ Withdrawal)</td>
<td>211.50±40.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266.50±38.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.86±4.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group IV</strong> (stress + Alprazolam, 5mg/kg bwt)</td>
<td>195.67±12.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>242.15±15.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.95±3.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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.
Table 3. Effect of drug alprazolam on restrained stress induced alteration of serum cortisol and antioxidant vitamins (vitamin C and E) in male albino rats

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Serum Cortisol (ng/mL)</th>
<th>Serum Vitamin C (mg/mL)</th>
<th>Serum Vitamin E (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong> (Untreated control)</td>
<td>78.61 ± 3.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.11 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.63 ± 1.44&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Group II</strong> (Stress induced)</td>
<td>148.30 ± 6.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.44 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.27 ± 1.57&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Group III</strong> (Stress+ Withdrawal)</td>
<td>121.10 ± 3.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.37 ± 3.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.79 ± 35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Group IV</strong> (Stress + Alprazolam, 5mg/kg bwt)</td>
<td>83.61 ± 4.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.54 ± 3.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.46 ± 1.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is Mean ± SD of six observations in each group. In each column, values with different superscripts (a, b, c, d) 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.
Fig. 1: 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).