**Original article**

**Effect of acute stress on serum cortisol level in female Wistar rats**

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**Abstract**

**Aims:** Stress can be defined according to Psychoneuroimmunology experts as ‘environmental events (real or perceived) that perturb one’s psychological or physiological homeostasis or balance’. (1) Acute stress triggers release of several hormones in animals. Severity of discomfort experienced by the animal is directly proportional to an increase in glucocorticoid concentration. (2) The study was planned to estimate serum cortisol levels in Wistar female rats after exposure to acute stress.

**Methodology:** Randomly selected 6 Wistar female rats weighing 160-180 gm were used for the study. Animals were given stress by forced swim test and restraint test. Serum cortisol level was measured before and after the tests by ELISA.

**Results:** Analysis of the results was done by students paired ‘t’ test. After forced swim test and after restraint test serum cortisol levels were significantly more in female rats. Also serum cortisol levels were more after restraint test than forced swim test but no statistically significant difference was observed.

**Conclusions:** An increase in serum cortisol level was observed after acute stress.

**Key words:** Stress, Wistar rat, Forced swim test, Serum Cortisol, Restraint test

**Introduction**

The ability of an organism to respond and adapt to environmental and homeostatic challenges is very important for its continued survival. The first classification of the stress concept was derived from Bernard’s steady-state of the milieu intérieur. The sympathoadrenal system was identified as an internal regulator. (3) Stress was defined by Selye as ‘the nonspecific response of the body to any demand made upon it’. (4) Stress affects various systems of body such as immune system, cardiovascular system etc. Levels of catecholamines, adrenocorticotropic hormone and glucocorticoids are increased during stress response. (5) Normally adaptation of animal occurs by physiological and behavioral modifications to predictable situations. But when the situation is not predictable, changes in the endocrine status of an animal are obvious. (5) During stress, normal circadian pattern of hypothalamic-pituitary-adrenal (HPA) axis is altered. This has adverse effects on health. More is the intensity of stress more is the activation of the HPA axis. (6) Even a mild acute stressor is capable of increasing glucocorticoid (GC) levels in rats. (7) Irrespective of gender animal’s well being is affected by stress. (8,9) Serum cortisol concentrations have been used as an marker of stress in many studies. (8,9) Thus this study was planned to see effect of acute stress on serum cortisol level in female Wistar rats.

**Materials and methods**

Approval by institutional animal ethical committee was taken before initiation of the project. 6 adult female wistar rats were used.
Animals were exposed to forced swim test and restraint test with a gap of 15 days. Tests were carried out between 1 p.m. to 4 p.m.

Forced swim test: It is the tendency of the living being to escape or avoid a noxious stimuli/condition. If the animal is not able to escape the stressful stimuli or it feels threatened, the animal will show stress response. This principle is used for developing forced swimming model for inducing stress in laboratory animals. The glass cylinder was filled with water (25°C). Rat was placed in water filled cylinder and allowed to step on moving wheel for survival. Rat was judged to be immobile wherever it remained floating in water in a slightly upright position, making no movements except trying to keep its head above water surface. (8,2)

Restraint stress test: Animals were isolated in a restraint transparent tube for 4 hours. (8)

Blood was collected in all the animals before and after each test. The samples were taken at fixed timings. 2 ml of blood sample was collected by retro orbital puncture with all aseptic precautions in a plain vacutainer. It was allowed to clot for 30 minutes at room temperature. Serum was separated by centrifugation. Serum samples were stored at -20°C and were subjected for estimation of serum cortisol.

(2) The hormone was estimated by automated chemiluminescence immunoassay system Alpha Prime LS, France by using Cortisol ELISA kit (DRG,USA). This immunoassay kit allows for in vitro quantitative determination of endogenic cortisol concentration in serum. Competitive inhibition enzyme immunoassay technique has high sensitivity and excellent specificity for estimation of cortisol levels in rats.

**Observations and Results:**

Average weight of the female wistar rats was 169.17±10.22 gm.

**Table 1:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before forced swim test (n=6)</th>
<th>After forced swim test (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol (ng /ml) (Mean ±SD)</td>
<td>126.79±41.83</td>
<td>183.74±40.24</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

P < 0.05* -- Statistically significant

Table No. 1 shows statistically significant (P< 0.05*) increase in serum cortisol level after forced swim test in female Wistar rats.
Table 2:
Effect of acute restraint stress on serum cortisol in female Wistar rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before restraint stress (n=6)</th>
<th>After restraint stress (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol (ng/ml)</td>
<td>126.79±41.83</td>
<td>205.02±41.74</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>(Mean ±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P < 0.001**--Highly significant

Table No.2 shows statistically highly significant (P< 0.001**) increase in serum cortisol level after restraint stress in female Wister rats.

Table 3:
Comparison of serum cortisol levels after forced swim test and after Restraint stress in female rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>After forced swim test (n=6)</th>
<th>After restraint test (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol (ng/ml)</td>
<td>183.74±40.24</td>
<td>205.02±41.74</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>(Mean ±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P > 0.05 --Not significant

Table No.3 shows comparison of serum cortisol levels after forced swim test and after restraint stress in female rats. No statistically significant difference is observed.

Discussion:
This study showed effect of acute stress on serum cortisol level in female Wistar rats.

As shown in Table1 serum cortisol levels were significantly more after forced swim test in female rats. Contartese RVL et al (10) observed that HPA axis stimulation is directly proportional to duration of the activity, during acute forced swim test. In another study glucocorticoid levels were increased when rats were exposed to forced swim test till complete exhaustion. (11).Comparable results were also observed in male rats.(2)

Table 2 shows significant increase in serum cortisol levels after acute restraint stress in female rats. Renée Grimée et al. (12) observed fast and transient increase in plasma catecholamines and a slow but more sustained increase in plasma corticosterone (CORT) levels, in rats after immobilization for 5, 15 and 30 min. Rats immobilized for 15 min
demonstrated tremendous increase in plasma ACTH and plasma CORT levels. Similar findings have been observed by Young Ju Cho et al. (13) after exposing the rats for acute restraint stress for one and half hours. In another study 1 h immobilization stress (IS) was imposed to rats at the beginning of the dark period, i.e., when the animals started to be active. Serum concentration of corticosterone was also increased by 56% at the end of the IS and was close to baseline level 4 h later. (14) Table 3 shows comparison of increased serum cortisol levels amongst two tests. Serum cortisol levels are not increased significantly after comparison amongst forced swim test and restraint test in female rats. These observations have indicated that both the tests exhibited comparable amount of stress in female rats.

It has been also observed that animals exposed to any one type of stress also showed high levels of CRF. (15) Statistically significant increase in levels of serum cortisol after acute stress suggests that hypothalamic-pituitary-adrenocortical (HPA) axis is activated. This feedback mechanism is extremely important for survival with stress. (16) Many studies showed that any situation which is new or unpredictable most likely activates the HPA axis. (16,17,18)

**Conclusion:**

Our study showed effect of acute stress on serum cortisol level. Stress due to exhaustive physical activity or without any physical activity affects release of cortisol.

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**References:**