


\section*{INTRODUCTION}

The concentration of copper containing protein in plasma, ceruloplasmin was found to be altered in several situations. Stress also reportedly induced changes in its level in plasma.\textsuperscript{1,2} Reduced plasma ceruloplasmin level was associated with Wilson’s disease and Menke’s kinky hair disease. Ceruloplasmin also was known to prevent oxidative tissue damage.\textsuperscript{3,4} In women, overiectomy leads to several alterations in the psycho-physiological parameters, which

\section*{ABSTRACT}

\textbf{BACKGROUND:} Plasma ceruloplasmin, a copper containing protein, belongs to a class called acute phase proteins. Reduced level of ceruloplasmin was associated with Wilson’s disease and Menke’s kinky hair disease in man, primarily affecting copper metabolism. Stress was known to increase Ceruloplasmin. Several stress associated changes were commonly observed in women at menopause and also those who underwent overiectomy. Present experiment investigated the effect of estragen on ceruloplasmin level in acute stress. \textbf{AIMS:} To assess the estradiol induced changes in plasma ceruloplasmin concentration on exposure of the rats to acute stress. \textbf{SETTINGS AND DESIGN:} Acute stress was induced by forcing the rats to swim till exhaustion. The rats were overiectomised bilaterally to remove the primary source of sex hormones. And hormone replacement was done later. \textbf{MATERIAL AND METHODS:} Wistar albino female rats were used. Acute stress was induced before overiectomy, following recovery from surgery, and again after Estradiol Valerate injection (for 10 days) in same group of rats. The plasma ceruloplasmin was estimated immediately after stress during each stage -- that is preoperative control, stressed control, after overiectomy and then following treatment with Estradiol Valerate. \textbf{STATISTICAL ANALYSIS USED:} Paired sample T test was applied to analyze the findings. Results: We found lowest ceruloplasmin level after stress in overiectomised animals, while on substitution of estradiol the trend appeared to be reversed. \textbf{CONCLUSION:} The result suggested a direct effect of estrogen on hepatic ceruloplasmin production/release and this could account for some of the beneficial effects of hormone replacement therapy.

\textbf{KEY WORDS:} Ceruloplasmin, Estradiol valerate, Acute stress.
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are similar to changes occurring at the time of menopause. Their ability to cope with stress was remarkably compromised at the menopause, which are attributed to multiple causes. The present study was undertaken to elucidate the role of estrogen on ceruloplasmin level in acute stress induced by forced swimming in the Wistar rat model.

**MATERIAL AND METHODS**

Seven female Wistar albino rats (150-200 g) were selected from the inbred colony from the Central animal house of Kasturba Medical College, Mangalore. Selection was done after confirming that all of them have regular oestrous cycle by monitoring for not less than five cycles (by vaginal smear method). The clearance from the institutes’ Ethics Committee on Animal Experimentation was obtained prior to the study, which approved the protocol and the group size. The rats were individually housed in plastic cages under standard laboratory conditions. Rat feed pellets (HLL Ltd. India) and potable water were made available ad lib. The rats were first exposed to acute stress, then oophorectomised. After 10 days of recuperation period, they are exposed to acute stress. Further they were treated with hormone (for 10 days) and then exposed to acute stress.

**Surgical procedure**

Rats were anaesthetized using intraperitonial (ip) administration of Nembutal (40mg/kg wt. Sigma Chemicals, USA) and L-shaped incision was made on either sides of the abdominal wall and the ovaries were dissected and removed. The surgical wound was sutured and waterproof plaster was applied. Single dose (1 lakh units of Penicillin was injected to these rats to prevent infection, and allowed to recover from surgery.

Estradiol Valerate (Progynon Depot, German Remedies, India. 10mg/ml) was diluted in coconut oil (1ml in 2ml), and the mixture was injected subcutaneously to give a dosage equivalent of 50-microgram/kg-body weight of the rat, using insulin syringe and sub dermal needle, once daily for 10 days. The effect of estrogen injection was confirmed by appearance of oestrous type of vaginal smear. The vaginal smear was diestrous type following oophorectomy.

Rats were forced to swim (till exhaustion), which is a standard procedure of inducing stress. For this, water at room temperature, was filled in a large steel drum and the rats were (three or four at a time) made to swim. Water level was kept at about 1.5 ft. so that the rats wouldn’t reach the bottom, and thus they were forced to swim continuously. This was done only once in each period in the study (acute stress). Groups of 3 or 4 rats in the drum at a time pushed each other for survival. They were fatigued in about 30-40 minutes.

**Sample collection**

The blood samples were collected (Post stress) immediately after they were removed from the water by cardiac puncture under ether anaesthesia using 2ml disposable syringe.

At the end of experimental procedure, the animals were sacrificed by injecting lethal dose of anaesthesia.

**Estimation of ceruloplasmin by Ortho-**

**DISCUSSION**

Ceruloplasmin is a copper containing protein (α2 globulin) with a molecular weight of 180000. It has oxidative property and is capable of converting FeII to FeIII and also has been shown to decrease the free radical induced brain damage. In the present experiment, same group of rats have been tested for the stress induced plasma ceruloplasmin levels with intact ovaries and subsequently after ovariectomy, and that followed by estradiol valerate injection. In the stressed normal rats, the ceruloplasmin level was found to be lower, contradictory to the expected response. This could be because acute stress was induced in about less than an hour, probably there was increased removal of ceruloplasmin from the circulation during swimming exercise. However, higher ceruloplasmin concentration was observed on exposure to stress in estrogen replaced state, as compared to oophorectomised rats. Estrogen replacement resulted in highest plasma concentration than all the other states.

**RESULTS**

Serum ceruloplasmin (Table 1) level found in stressed rats with intact ovaries was about 98 mg/100ml, as against the Normal control, which was lower than control value. But on exposure to stress in oophorectomised rats, the level was very low (highly significant $P<0.001$). On hormone replacement and then exposure to stress, ceruloplasmin level found was significantly higher ($P<0.001$) when compared to all the other test conditions. The results were analysed by Paired sample T test (SPSS for windows version11). $P<0.05$ accepted as significant.

**Table 1: Serum Ceruloplasmin (mg/dl, Mean±SD) of group of rats (n=7) in control, stressed (N+Stress), oophorectomised and stressed (Ovx+Stress) and oophorectomised, estradiol treated and stressed (Ovx+E+Stress) conditions**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Control</th>
<th>N+Stress</th>
<th>Ovx+Stress</th>
<th>Ovx+E+Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>115 ± 10</td>
<td>98 ± 12</td>
<td>35 ± 8†</td>
<td>230 ± 15†</td>
</tr>
</tbody>
</table>

Values: mg per 100 ml. *$P<0.001$ when compared to control †$P<0.001$ when compared to N+Stress.