# COMPARATIVE EFFECTS OF ESTRADIOL, PROGESTERONE AND TESTOSTERONE ON THE LEVEL OF RAT SERUM CERULOPLASMIN

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*SUMMARY: Ceruloplasmin, a serum alpha-2-globulin with oxidase activity, plays an important role in the metabolism of iron. Following the treatment of animals with the daily doses o 1.6 mg/kg estradiol, 6 mg/kg progesterone and 8 mg/kg testosterone for 15 days, serum ceruloplasmin concentration was elevated 57, and 22 percent respectively. Administration of estradiol for 30, 45 or 60 days led to an increase in the enzyme activity by 92, 77 and 63 percent respectively. Animals receiving either progesterone or testosterone for longer period up to 60 days did not show any significant changes in their serum ceruloplasmin levels. The effects of these steroid hormones on ceruloplasmin activity have been discussed.*

*Key Words: Ceruloplasmin, estradiol, testosterone, progesterone.*

## INTRODUCTION

Contraceptive agents are consumed by millions of women throughout the world. These agents, mostly sex steroids, influence the activity of many enzymes including ceruloplasmin (1). Ceruloplasmin or ferroxidase (E.C., 1.16.3.1) is a blue-coloured metaloprotein which migrates electrophoretically as an alpha-2-globulin (2). Each molecule of ceruloplasmin contains eight atoms of copper four of which are in the cupric form (3). Normally ceruloplasmin accounts for 95% of total plasma copper and it acts as an oxidase agent and involves in the oxidation of ferrous to ferric ions (ferroxidase activity) to promote the binding of iron to apotransferrin (4, 5).

Liver is the major site of ceruloplasmin synthesis and many factors including toxic agents and sex steroids have significant influence on this enzyme synthesis and metabolism. It has already been reported that contraceptive agents such as ethinyl estradiol increase the enzyme concentration in the plasma (6, 18).

In order to evaluate the comparative effects of some sex steroids on serum ceruloplasmin level, three steroids, estradiol, progesterone and testosterone were chosen and their short and long term effects were investigated.

## MATERIALS AND METHODS

Adult male Wistar rats, weighing 150-175 gr were purchased from Pasteur Institute (Tehran, Iran) and maintained on a standard food and tap water. Pure injectable estradiol, progesterone and testosterone were obtained from Aboreyhan Chemical Company (Tehran, Iran). P-Phenylendiamine hydrochloride was obtained from Merck (Germany). All other chemicals used were reagent grade and obtained from Sigma company.

To study the effects of the hormones, indiceted doses of estradiol, progesterone and/or testosterone, dissolved in sesame oil, were injected intramuscularly alternating between right and left hind legs. Control animals were received sesame oil in the same manner as the experimentals. After 15, 30, 45 or 60 days of injection, rats were killed by decapitation and their blood were collected into precleaned centrifuge tubes. The serum, was then separated by centrifugation at 600 g for 10 min using a bench centrifuge, and kept refrigerated until needed.

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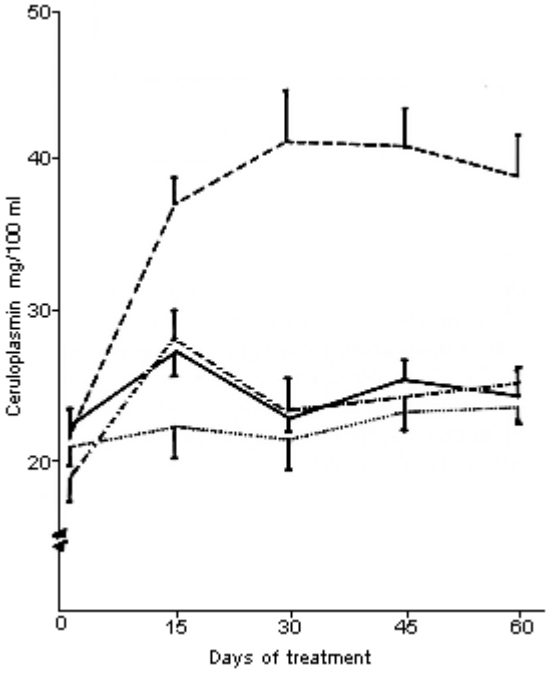


Figure 1: Changes in serum ceruloplasmin level following daily administration of estradiol (----), testosterone ( ——) and progestrone ( _._._. ), Compared with control (.....). The values are the mean ± SD indicated by verticle bars of at least 5 separate experiments. See text for hormone doses.

Ceruloplasmin levels were estimated on the basis of its oxidase activity using P-Phenylendiamine dihydrochloride as substrate and measuring the colour produced at the wawelength of 525 nm (7).

## RESULTS

To investigate the short term effects of estradiol, progesterone and testosterone on plasma levels of ceruloplasmin, male Wistar rats were treated with a single doses of the hormones. Animals were injected estradiol (0.8 and 1.6 mg/kg body weight), progesterone (4 and 6 mg/kg) and testosterone (4 and 8 mg/kg) and their bloods were collected 24 hours post-treatment. As shown in Table 1 no significant changes were observed after administration of different doses of these hormones in the period of 24 hours.

The long term effects of steroid hormones on plasma ceruloplasmin level were also examined, the results of which are shown in Figure 1. As shown in this Figure daily injections of estradiol (1.6 mg/kg) for 15 and/or 30 days increased the level of ceruloplasmin significantly ($p<0.001$). The enzyme did not show any further activation despite the continuation of the treatment for longer period of time up to 60 days. The effect of administration of testosterone and/or progesterone (8 mg/kg and 6 mg/kg respectively) on ceruloplasmin level was different from that of estradiol. Although injection of either of the hormone for 15 days led to an increase of about 25 percent in ceruloplasmin concentration but the enzyme activity returned to the control level, although the treatment continued up to 60 days.

## DISCUSSION

In the present study, it has been found that a single injection of either estradiol, progesterone or testosterone had no significant effect on the plasma level of ceruloplasmin after 24 hours. This finding is in accordance with the well known mechanism of action of steroid hormones in their target tissues (8, 9). There are also reports suggesting that progesterone administered to rats disappeared rapidly from the blood stream (10) and this could also be true for estradiol and testosterone.

The results obtained from long term administration of the hormones were different from what mentioned above. Estradiol, injected up to 30 days led to an increase in the concentration of ceruloplasmin in plasma but no further activation of the enzyme was observed despite the continuation of the treatment for up to 60 days. This result is in agreement with the previous reports that contraceptive

| Hormone | | Ceruplasmin mg/100 |
|---|---|---|
| Estradiol | Control | 20.3 ± 1.1 |
| | 0.8 mg/kg | 21.4 ± 0.8 |
| | 1.6 mg/kg | 21.1 ± 1.7 |
| Progestrone | Control | 18.5 ± 1.6 |
| | 4 mg/kg | 17.9 ± 2.8 |
| | 6 mg/kg | 19.5 ± 2.2 |
| Testosterone | Control | 23.8 ± 1.5 |
| | 4 mg/kg | 22.1 ± 1.2 |
| | 8 mg/kg | 22.8 ± 1.6 |

Table 1: The effect of steroid hormones on serum ceruloplasmin level after 24 hours. Each value represents mean ± SD of experiments.

agents containing estrogen hormones could increase serum levels of ceruloplasmin in four weeks of their consumption (11). Administration of ethinyl-erstradiol for two weeks or estradiol-benzoate for three to four weeks were also reported to increase the activity of ceruloplasmine in serum (6).

The exact mechanism by which estradiol increased ceruloplasmin level has not yet been fully elucidated. However, there are a bulk of evidence suggesting that estradiol could stimulate the synthesis of many enzymes in the liver (12-14). Further evidence suggests that this induction of enzyme synthesis by estradiol was inhibited by puromycine, a protein synthesis inhibitor (12) confirming the involment of protein synthesis in the mechanism of this hormone action.

Contraceptive agents have also been shown to increase serum copper concentration which in turn could induce ceruloplasmin synthesis (14). It has been shown, however, that if the concentration of copper rose above a critical level, the conversion of apoceruloplasmin to ceruloplasmin in the liver is inhibited (15). This observation may be an explanation to our finding that continuation of treatment by estradiol leads to a gradual reduction in ceruloplasmin activity. Another explanation to this finding might be that, due to the continuous effects of estradiol the liver is exhausted or a tolerance to the hormone may occur.

The difference in the effects of progesterone and/or testosterone on serum ceruloplasmin level might be explained on the basis of either their structural differences or it could arise from any differences in the mechanism of action of these hormones. It has been reported that receptors for estradiol and progesterone have different centrifugal properties (16). These two hormones have different effects on the cell cycle and the rate of RNA synthesis (17). However, much more studies should be done in order to elucidate the exact mechanism by which steroid hormones affect ceruloplasmin concentration.

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