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ANTITESTICULAR EFFECT OF COPPER CHLORIDE IN ALBINO RATS

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ABSTRACT — Copper chloride treatment adversely affects testicular activity in albino rats. To investigate its antitesticular effects mature (120 days) Wistar strain albino rats were treated intraperitoneally (i.p.) with copper chloride at doses of 1000, 2000 and 3000 μg/kg body weight/day for 26 days. Significant reduction of testicular and accessory sex organs (seminal vesicle, ventral prostate) weight, along with inhibition of testicular Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activity and reduction in plasma testosterone level, were observed at the doses of 2000 and 3000 μg/kg body weight/day. The degree of inhibition in all the parameters were increased with the increase of dosage. But no significant change was observed in the above parameters when the animals were treated with 1000 μg/kg body weight/day dose. This suggests that copper produces a suppressive influence on male reproductive activity, mainly on testicular weight and steroidogenesis and accessory sex organ weight in a dose-dependent manner.

KEY WORDS: Copper, Testis, Hydroxysteroid dehydrogenase, Testosterone, Rat

INTRODUCTION

Copper is an important biological trace element. In spite of its usefulness in normal metabolism, an abnormal rise in the plasma copper level can bring about adverse effects. Occupational exposure to copper can take place while working with algicides, fungicides, mordants, paints, alloys, catalysts, construction materials and electroplating materials (Plunkett, 1987). Copper toxicosis has been observed in farm animals, characterized by a decrease in body growth, microcytic hypochromic anemia, hemoglobinuria and jaundice (Hill, 1977). Observations on humans after copper poisoning in high doses show vomiting tendencies, diarrhea and other similar symptoms observed in animals, often leading to coma and death (Underwood, 1977).

It has been found that the testicular weight and motility of spermatozoa decreases, while the number of dead and defective spermatozoa increases following copper chloride treatment (Gabuchyyan, 1987). The level of testosterone in the male has been suggested to play a role in the severity of copper deficiency (Bedwal and Bahuguna, 1994). The role of copper in the sperm is unclear. Findings of different authors are contradictory, and some authors do not find any correlation between the seminal level of copper and amount of mobility of gametes (Skandhan, 1992). Thus, thorough knowledge on the toxic effect of copper on the testes is lacking.

The present study was undertaken to investigate the effect of copper chloride on testicular activity of Δ^5-3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD, which are important enzymes in testicular steroidogenesis, plasma testosterone level and testicular and accessory sex organ weight in Wistar strain rats

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after 26 days of treatment.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats weighing 150-160 g (120-140 days of age). They were maintained in a light (12L:12D) and temperature (28±20℃ ambient temp.) controlled animal house and given standard laboratory food and water *ad libitum*.

Copper chloride (assay 99%) was purchased from Sigma Chemical Co., St. Louis, MO, USA and dissolved in sterile distilled water. This test solution was prepared regularly on a day-to-day basis.

Thirty-two rats were used, divided into four equal groups, and one group of rats was injected intraperitoneally (i.p) with 1 ml physiological saline/kg body weight/day for 26 days and designated as control animals (Group I). The other three groups of animals were injected with either 1000 μg or 2000 μg or 3000 μg of copper chloride per 1 ml sterile distilled water/kg body weight/day for 26 days (Groups II, III and IV). All the rats were killed on the 27th day between 8.00 to 10.00 hr, 24 hr after the last injection, following protocols and ethical procedures. Blood samples for hormone assay were collected from the hepatic vein under light ether anesthesia. The heparinized plasma was separated from the cells by centrifugation. Plasma samples were stored at –20℃ until assayed.

The body weight of all the rats were recorded on the first day of injection (initial) and on the day of sacrifice (final). The testes and accessory sex organs were dissected out, trimmed of fat and weighed. The relative weights of organs were expressed per 100 g body weight.

Testicular Δ^5-3β-HSD was assayed by the method of Talalay (1962). Testis was homogenized in 20% spectroscopic grade glycerol containing 5 mM potassium phosphate and 1mM EDTA at a tissue concentration of 100 mg/ml of homogenizing mixture. It was centrifuged cold at 10000 rpm for 30 min. The supernatant (1 ml) was mixed with 100 μl sodium pyrophosphate buffer (pH 8.9) and 30 μg dehydroepiandrosterone, making the incubation volume 3 ml. Enzyme activity was measured after adding 0.5 μmol NAD^+ to the mixture in a spectrophotometer against a blank (without NAD^+). One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm. Testicular 17β-HSD was measured following the method of Jarabak *et al.*(1962). The same supernatant was mixed with 440 μmol sodium pyrophosphate buffer (pH 10.2) and 0.3 μmol testosterone to make the volume 3 ml. Enzyme activity was measured after adding 1.1 μmol NAD^+ to the mixture in a spectrophotometer against a blank (without NAD^+). One unit of enzyme activity was the same as the other enzyme.

Radioimmunoassay of testosterone was carried out following the method of Jacobs (1974) using a testosterone ^{125}I RIA Kit (ICN Biochemical Inc., Diagnostic Division, Costa Mesa, CA 92626, USA). Radioactivity was determined using the gamma counter (Model No. IC-4702, Electronic Corporation of India, Hyderabad, India). All samples were run in duplicate in a single assay to avoid interassay variation. The intraassay coefficient of variation was 6.5%.

Statistical analysis

For statistical analysis to test for differences between control and treated experimental groups, a multiple comparison test (Das, 1981) was used; $p < 0.05$ was considered to be significant.

RESULTS

The relative weight of testes, seminal vesicle and ventral prostate showed a significant decrease after treatment with copper chloride at the doses of 2000 μg/kg body weight (Table 1). No change in body weight was observed after copper chloride treatment for any of the above doses (Fig. 1).

The study showed that copper inhibits the activities of both Δ^5-3β-HSD and 17β-HSD, the important androgen biosynthetic enzymes of testes (Figs. 2 and 3). The inhibition is not statistically significant at the dose of 1000 μg/kg. With a further increase in dose the inhibition is significant, proving that a high dose of copper results in a deleterious effect on the testis.

Plasma level of testosterone was significantly depleted in rats at the doses of 2000 μg/kg/day and 3000 μg/kg/day. However, copper chloride at the dose of 1000 μg/kg/day did not exhibit a significant change when compared with the vehicle-treated control group (Fig. 4).

DISCUSSION

In our study no change in body weight was observed, but a decrease in mean testicular, ventral prostate and seminal vesicle weight after copper exposure was noted. These data correspond with the decrease in serum testosterone concentrations. As testosterone plays a major if not sole role in the maintenance of structural

integrity and functional activities of the accessory sex organs (Moor *et al* ., 1930), reduction in accessory sex organ weight is a reflection of decreased testosterone level in the blood.

There are reports that after inhalation of copper chloride fumes rats show decreased concentrations of plasma FSH, LH, Prolcatin and testosterone along with disfunction of virile gonads and disorders in spermatogenesis (Gabuchyyan, 1987). In our study we also found decreased serum testosterone along with reduced testicular steroidogenic enzymes activity (Δ^5-3β-HSD and 17β-HSD). Since these steroidogenic enzymes are

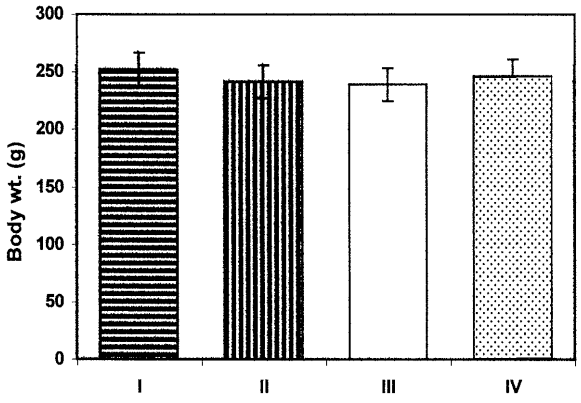

Fig. 1. Effect of copper chloride at the doses of I) 0 (vehicle-treated control group), II) 1000, III) 2000 and IV) 3000 μg/kg/day for 26 days on body weight in male rats.
Values are mean±SEM of 8 rats/group. No significant difference was observed from the corresponding control values with ANOVA followed by multiple two-tailed 't' test, where $p>0.05$.

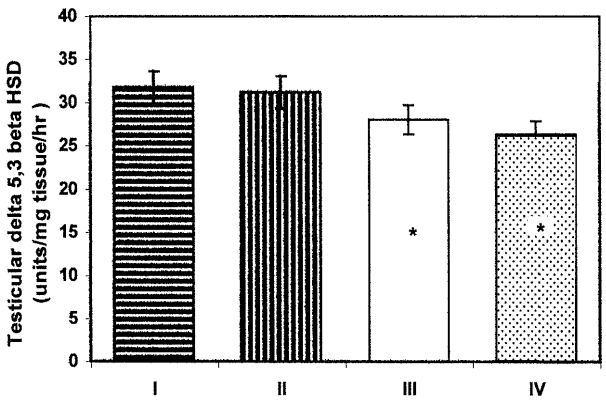

Fig. 2. Effect of copper chloride at the doses of I) 0 (vehicle-treated control group), II) 1000, III) 2000 and IV) 3000 μg/kg/day for 26 days on testicular Δ^5-3β-hydroxysteroid dehydrogenase (HSD) activity in male rats.
Values marked with asterisks are significantly different from corresponding control values (Values are mean±SEM of 8 rats/group). ANOVA followed by multiple comparison two-tailed 't' test, where *$p<0.05$.

Table 1. Effect of copper chloride at the doses of I) 0 (vehicle-treated control group), II) 1000, III) 2000 and IV) 3000 μg/kg/day for 26 days on testicular and accessory sex-organ weight in male rats.

Group	Dose of Treatment	Testicular Wt. (Pair) (mg% b. wt.)	Seminal Vesicle Wt. (with fluid) (mg% b. wt.)	Ventral Prostate Wt. (mg% b. wt.)
I	Control (Vehicle-Treated)	1478.61±32.93	472.62±22.84	227.29±16.78
II	Copper Chloride (1000 μg/kg body wt./day)	1434.65±36.75	450.87±29.38	212.42±12.79
III	Copper Chloride (2000 μg/kg body wt./day)	1274.89±39.19*	324.16±18.98*	158.42±13.69*
IV	Copper Chloride (3000 μg/kg body wt./day)	1248.39±36.16*	318.34±20.52 *	151.84±13.41*

Values marked with asterisks are significantly different from corresponding control values (Values are mean±SEM of 8 rats/group). ANOVA followed by multiple comparison two-tailed 't' test, where *$p<0.05$.

gonadotrophin-dependent (Murono and Payne, 1979), the decreased level of testosterone after copper treatment is, therefore, a result of decreased pituitary gonadotrophin secretion, as LH plays a major role in testicular androgenesis (Steinberger, 1971).

There may be three possible ways by which higher doses of copper decrease serum gonadotrophins and testosterone as well as prolactin level. First, copper may act at the pituitary receptors of LHRH, which control the release of gonadotrophins (Skandhan, 1992). As a result, pituitary secretion of FSH & LH may be decreased after copper treatment, which in turn may decrease the release of testosterone from testicular Leydig cells. Secondly, we have found that higher doses of copper cause increased adreno-cortical activity and elevated serum corticosterone level (communicated work). Increased corticosterone level may reduce serum gonadotrophin and testosterone levels (Philips *et al.*, 1989). Thirdly, testicular Δ^5-3β-HSD and 17β-HSD are gonadotrophin-dependent enzymes. We have confirmed from our results that higher doses of copper inhibit the activity of these enzymes, which in turn reduces steroidogenesis and serum testosterone level. At the same time, this reflects the decreased level of serum FSH and LH concentrations as well as prolactin level, since prolactin stimulates testicular Δ^5-3β-HSD (Hafiez *et al.*, 1971) and 17β-HSD (Musto *et al.*, 1972).

However, judging from this experiment, apart from the reduction of pituitary gonadotrophin and prolactin, a direct action of copper chloride on testicular steroidogenesis cannot be ruled out, and further studies are required to clarify these possibilities.

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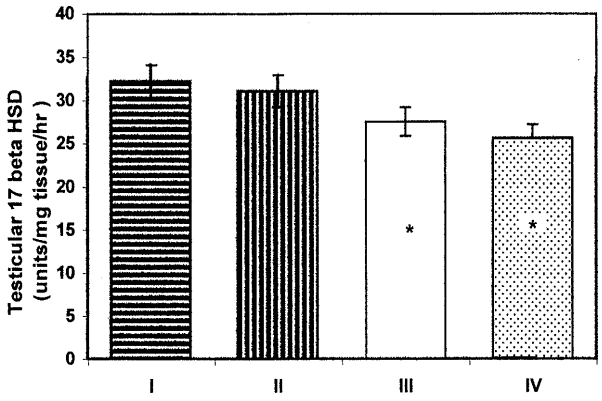

Fig. 3. Effect of copper chloride at the doses of I) 0 (vehicle-treated control group), II) 1000, III) 2000 and IV) 3000 μg/kg/day for 26 days on testicular 17β-hydroxysteroid dehydrogenase (HSD) activity in male rats.
Values marked with asterisks are significantly different from corresponding control values (Values are mean$\pm$SEM of 8 rats/group). ANOVA followed by multiple comparison two-tailed 't' test, where *$p<0.05$.

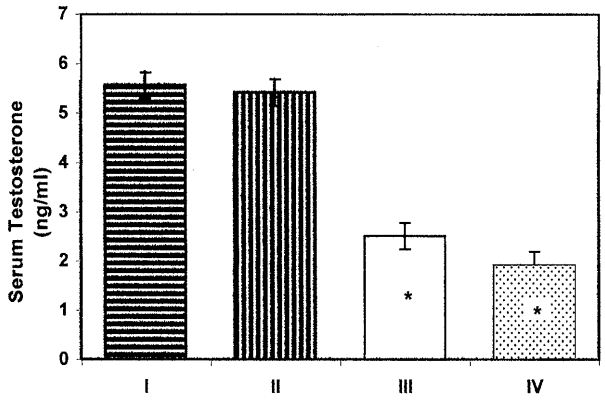

Fig. 4. Effect of copper chloride at the doses of I) 0 (vehicle-treated control group), II) 1000, III) 2000 and IV) 3000 μg/kg/day for 26 days on serum testosterone level in male rats.
Values marked with asterisks are significantly different from corresponding control values (Values are mean$\pm$SEM of 8 rats/group). ANOVA followed by multiple comparison two-tailed 't' test, where *$p<0.05$.

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