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The effects of copper toxicity on histopathological and morphometrical changes of the rat testes

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ABSTRACT

Objective: Exposure to environmental toxicants such as copper has been suggested to have adverse effects on male reproduction. Therefore, our aim in the present study was to investigate morphometrical changes of rat testes following long term consumption. **Methods:** Animals were divided into three experimental groups. Two different doses of copper sulfate were applied once a day for 8 weeks by gavage. The first treatment group received copper sulfate at a dose of 100 mg/kg (Cu100 group) and the second treatment group was given copper sulfate at a dose of 200 mg/kg (Cu200 group). Control animals received normal saline using the same method. Testes from five cases of 15 animals of each group were removed for histopathological examinations on days 14, 28 and 56. Morphometrically, seminiferous tubules diameter, spermatogonial cells nuclei diameter, sertoli cells nuclei diameter and epithelial height were measured in the experimental groups. Meiotic index and the percentage of spermatogenesis were also calculated.**Results:** The mean values of about mentioned morphometrical parameters in copper treated groups showed significant decrease on 14th day compared to the control group. Copper administration caused a significant damage to morphometrical parameters on 28th day compared to the day 14. Also, in some parameters further decreases were observed specially in the Cu200 group on 56th day such as the diameter of seminiferous tubules, spermatogonial and sertoli cells nuclei and epithelial height of germinal layer ($P<0.05$). **Conclusions:** The results show that exposure to copper has the deleterious effects on morphometrical structure of testes which are appeared as early as two weeks.

1. Introduction

Copper is an important biological trace element which is necessary for different metabolic functions and enzyme activities such as catalase, peroxidase, and cytochrome oxidase, and is essential for the utilization of iron[1,2]. Nevertheless, its over-exposure might produce wide adverse effects in different physiological systems. Usually, occupational exposure to copper may lead to copper toxicosis in the industrial workers[3]. In animals, long-term intake of copper compounds of different origin is the most common form of copper poisoning. It means that the animals are reared closed to industrial plants, and ingest copper from industrial deposits through feed or from air throughout their entire life[4]. Copper regulation is controlled mainly by the liver, where it can be mobilized into the circulation or excreted via the bile[5]. In chronic copper poisoning, copper is gradually deposited in the liver without producing any significant sign. When the hepatic copper storage capacity is exceeded, it may result in hepatocellular necrosis and consequently the liberation of copper from the liver into the blood stream produces hemolysis, jaundice, and renal insufficiency[6]. Thus, copper is a strong oxidant, in which it could bind to cell molecules during the high load[5]. Consequently, it may generate highly reactive hydroxyl radicals and then affect some cellular functions. Hence, dietary copper overload in rats has produced the lipid peroxidation of mitochondrial membranes[7].

Study on workers exposed to electric welding revealed an increase in semen concentration of copper along with lowering in sperm count, sperm viability and semen volume[8]. In adult male rats, long term ingestion of copper adversely affects fertility and testicular weight[9] and recently, deleterious effects of copper poisoning on sperm quality of rats has been investigated[10]. Pathological

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features of copper toxicosis specially in organs such as liver, kidney, spleen, lung and intestine have been well demonstrated in animals[4,11-13], but according to the authors' knowledge, there is a lack of information about the copper toxicity on histopathological and morphometrical changes of adult male rat testes. So, the present study was undertaken to thoroughly investigate morphometric parameters of rat testicular tissue following long term copper consumption.

2. Materials and methods

2.1. Animals

Forty-five Wistar albino male adult rats (200-240 g) were purchased from Razi Research Institute of Kerman, Iran and kept in the Center of Laboratory Animal Care at the Veterinary Faculty of Shahid Bahonar University of Kerman, Iran for one week before treatment. The rats were housed in groups of five per cage and maintained under standard laboratory conditions (12 h light: 12 h dark and 22±2 °C) during the experimental period. During the study, the animals received water and pellet food (Javaneh Khorasan Co., Iran) ad libitum. All investigations were conducted in accordance with the Guiding Principles for the Care and use of Research Animals and were approved by the Animal Ethics Committee at the Veterinary Faculty of Shahid Bahonar University of Kerman, Iran.

2.2. Experimental design

Animals were randomly allocated to either control (Con, n=15) or two treatment groups each containing fifteen animals. To monitor the short and long-term effects of copper on testicular structure, two different doses of copper sulfate were applied once a day for 56 consecutive days by gavage. The first treatment group received copper sulfate at a dose of 100 mg/kg in 0.2 cc (Cu100 group, n=15) and the second treatment group was given copper sulfate at a dose of 200 mg/kg in 0.2 cc (Cu200 group, n=15). Control animals received normal saline using the same volume and similar method. The dose of copper sulfate used in our experiment was according to the previous study for producing of copper poisoning in rats[10]. Animals from each experimental group were sacrificed upon diethyl ether anesthesia (May & Baker Ltd., Dagenham, England) by cervical dislocation on days 14, 28 and 56 after the beginning of copper sulfate consumption, respectively and left testes were removed for histopathological examinations.

2.3. Histhopatological and morphometrical examinations

All specimens were fixed in Bouin's solution, embedded in paraffin wax, sectioned with 5 μm thicknesses, stained with haematoxylin and eosin (H&E) and examined blindly by an expert pathologist under a light microscope. Morphometrically, the mean seminiferous tubule diameter and epithelial height were measured in each testis. The ten smallest, roundest tubules were identified and measured with an ocular micrometer under light microscopy. Mean diameter, in microns, was then determined for each group[14]. The epithelium height was obtained with the same tubules used to determined tubular diameters. The average diameter of the spermatogonia and sertoli cells nuclei were measured from 30 cells for each testis[15]. The other parameter was the percentage of spermatogenesis. For this purpose, two hundred seminiferous tubules were examined under light microscopy. The presence of spermatozoa within the seminiferous tubule was considered as the evidence of spermatogenesis. Lack of spermatozoa even in the presence of orderly progression of primary and secondary spermatocytes was not considered as the evidence of spermatogenesis for the purpose of this experimental study[14].

2.4. Statistical analysis

Data were subjected to analysis by SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). All data were tested for homogeneity of variances by *Levene* static test. When the variances were homogenous, the different morphometrical data between the control and copper-treated rats on days 14, 28 and 56 were separately analyzed by One-way ANOVA[16]. Results were expressed as mean±SEM and values were considered to be statistically significant at P<0.05.

3. Results

3.1. Histopathological observation

Figures 1, 2 and 3 illustrate sections from testes of animals in the control, Cu100 group on 28th day and Cu200 group on 56th day, respectively. The seminiferous tubules of the control rats showed normal morphology with the presence of spermatozoa in their lumens (Figure 1). The testes of copper treated groups were accompanied by various degrees of degenerative changes depending on the doses and duration of copper administration. These degenerative changes included destruction of seminiferous epithelium, significant depletion of the germinal layers with the presence of vacuoles in the seminiferous epithelium. On 28th day, the most of the seminiferous tubules were degenerated and a few of them seemed to be relatively normal (Figure 2). On 56th day, nearly all the seminiferous tubules were completely degenerated as only a single layer of sertoli cells and spermatogonia was present (Figure 3).

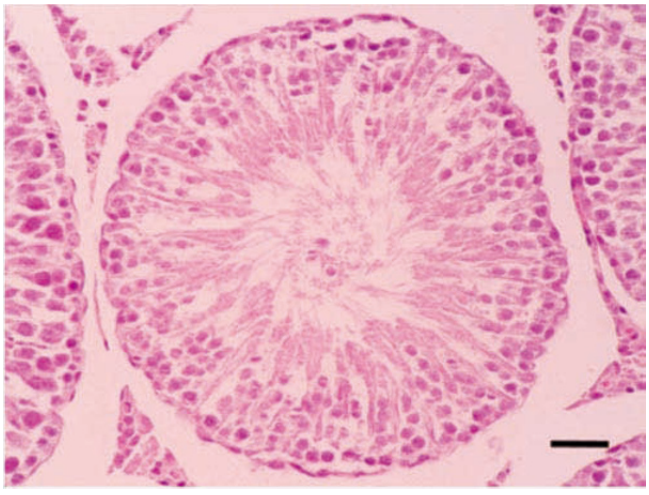

Figure 1. Control group. Photomicrograph showing normal seminiferous tubules morphology. H&E staining. Bar=30 μm.