The Toxicity of Copper on Serum Parameters Related to Renal Functions in Male Wistar Rats

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Abstract

**Background:** Copper (Cu) is one of the most important heavy metals. Due to Cu importance and its widespread use in medicine and industries, the study of its impact on human life and disorders of vital parameters would be necessary. This study has been investigated the toxicity of Cu on serum parameters related to renal function.

**Materials and Methods:** In this experimental study, 30 adult male Wistar rats were divided into 3 groups (N=10). Group I as control group received only 0.5 mL normal saline. Groups II and III received 0.5 and 1.5 mg/kg copper respectively as i.p. injection for 21 days. Then, blood samples were collected and levels of serum parameters related to renal function (sodium, potassium, urea, creatinine, protein) were measured. Data were statistically analyzed using SPSS-20 software (one way ANOVA, LSD).

**Results:** The results have shown that levels of urea, sodium and protein decreased and levels of creatinine and potassium increased as a result of Cu exposure in comparison to the control group. Significant effects were seen already at a dose and Cu toxicity on serum parameters related to renal function.

**Conclusion:** Cu i.p. injection in rats induces nephrotoxicity while increasing in copper levels with accompanying renal dysfunction may be an indication that copper mediates in oxidative-induced renal dysfunction. However, further study is needed to examine the exact correlation between copper and renal indices.

Keywords: Copper, Serum parameters, Toxicity, Renal function

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Introduction

Copper (Cu) is one of the most important heavy metals. Copper is an essential trace element capable of producing toxic effects in animals and man when ingested acutely or chronically in excess. Copper compounds have been widely used in industrial processes and agriculture [1]. As a result, elevated copper concentrations can be found in certain areas of the biosphere. Conversely, exposure to high levels of copper can result in a number of adverse health effects. Exposure of humans to copper occurs primarily from the consumption of food and drinking water [2]. Acute copper toxicity is generally associated with accidental ingestion; however, some members of the population may be more susceptible to the adverse effects of high copper intake due to genetic predisposition or disease [3]. In humans, the liver is the primary organ of copper-induced toxicity. Copper status has also been associated indirectly with a number of neurological disorders, including Alzheimer’s disease and prion diseases, including bovine spongiform encephalopathy [4]. Chronic copper exposure is increasingly recognized as a public health issue, its early effects remain largely unknown [5]. Multiple studies have shown copper nephrotoxicity after prolonged usage while increasing in copper level with accompanying renal dysfunction may be an indication that copper mediates in oxidative-induced renal dysfunction [6]. Intravascular haemolysis and a direct action of copper on the kidneys often lead to tubular necrosis. Renal proximal tubular accumulations of copper and tubular necrosis have been reported in Wilson's disease. Wilson's disease is one of a number of copper-related disorders where loss of copper homeostasis as a result of genetic, nutritional or environmental factors affects human health. Copper accumulated in the liver and extrahepatic organs such as the brain and cornea. So, the dietary intake of copper should be considered seriously in these patients. Due to copper importance and its widespread use in medicine and industries, the study of its impact on human life and disorders of vital parameters would be necessary [7].

According to the authors’ knowledge, there is a lack of information about the low dose copper toxicity on renal function. So, the present study was carried out to show whether copper intake could induce renal toxicity at in vivo condition.

Materials and Methods

**Chemicals:** In this experimental study, copper chloride was obtained from Acros Organics (New Jersey, USA). Kits for urea, creatinine, and protein estimation were purchased from the Ziest Chem Company (Tehran, Iran). All other reagents and chemicals were of analytical grade quality or higher purity.
Animals: 30 adult male Wistar rats (10 weeks old and initial body weight 200±50 g) were purchased from Shah-e-Kord University of Medical Sciences, Iran and kept in the Center of Laboratory Animal Care at Islamic Azad University of Falavarjan, Isfahan, Iran. The animals were housed in conventional conditions at a temperature of 25±1°C, with a relative humidity of 50±10% and a 12/12 h light/dark cycle. They were maintained on Ad libitum diet and water throughout the experimental period. The empirically protocols of this experimental study approved by department of physiology, university of Ishan and were performed according to guiding procedures in the care and use of animals, prepared by the Council of the American Physiological Society.

Experimental protocol: The rats were allocated randomly to 3 experimental groups of 10 animals each. Group I: as control group received 0.5 mL normal saline. Groups II and III received 0.5 and 1.5 mg/kg copper chloride (CuCl₂.2H₂O) as i.p. injection in single dose daily for 21 days. The treatment in all groups was lasted for 21 consecutive days. All animals were anesthetized 24 h after the administration of the final dose(s) and blood samples were withdrawn directly from their hearts. Serum samples were collected by centrifuging at 2000 rpm for 20 min. The serum samples were used in the present study to estimate the concentrations of parameters related to renal function such as urea, creatinine, potassium, sodium, and protein. Serum urea, creatinine, and protein concentrations were determined by routine laboratory methods and kites. Serum sodium and potassium were analyzed by flame photometer (corning 408).

Statistical analysis: SPSS-20 was used for data analysis. Results of all measurements are presented as mean±SE for 10 rats in each group. A one-way analysis of variance (one way ANOVA) was conducted to determine whether there were statistically significant (p<0.05) differences among the six experimental groups and further, the LSD multiple range post hoc test was performed to compare individual groups and to determine which means differed statistically significantly (p<0.05).

Results

Based on statistical analysis by SPSS-20 software (one way ANOVA, LSD) it was observed that the administration of copper 1.5 mg/kg, resulted in a significant increase of creatinine and potassium levels (0.62±0.04 and 4.79±0.06 respectively) (p<0.01 and p<0.05 respectively) in comparison to the control group. However, exposure to copper in a marked dose-dependent decreased serum protein levels compared with the one in the respective control group. Serum urea and sodium concentrations were not changed by any copper treatment. During the experiment a slight decrease in the body weight of copper treatment rats was noted. Significant effects were seen already at a dose and copper toxicity on serum parameters related to renal function (Table 1 and 2).

Discussion

The present study demonstrated that copper in dose dependent manner is renal toxicant and it changes serum parameter levels related to renal function. Serum levels of urea, sodium and protein decreased and levels of creatinine and potassium increased as a result of copper exposure in comparison to the control group. These observed changes are in accordance with other authors’ results [2, 8].

Copper is an essential element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor [8]. Acute renal failure due to tubular necrosis is characterized by oliguria, anuria, increased blood urea nitrogen concentrations, albuminuria and hematuria [9]. Also, Electrolyte and fluid imbalance may be expected to occur following vomiting and diarrhea and in association with acute renal failure [10, 11].

There are many reports indicating that copper excess is well correlated with renal dysfunction. Haywood et al. showed that rats received the supplemented diet with a copper content of 3 g/kg for up to 5 weeks had Kidney copper concentrations. Tubular necrosis and cellular pleomorphic was reported in these animals [12].

Serum protein concentration in copper treated animals was significantly lower than the control group. This finding agrees with the report by Cisternas et al. [13]. Decreasing in serum proteins following copper exposure may be due to many reasons. One of the most toxic effects of copper on kidney is proteinuria [14]. Moreover, liver damage induced by Cu caused serum protein decreasing [4]. It is believed that since the copper ion (Cu²⁺) is a strong oxidizing agent it will oxidize oxyhaemoglobin from the ferrous to the ferric form [4]. In this form, hemoglobin loses its oxygen-binding capacity resulting in methemoglobinemia and cyanosis. Furthermore, the restoration of hemoglobin to the ferrous
form depends on the transfer of electrons from NADH, NADPH and reduced glutathione [15]. Glucose-6-phosphate dehydrogenase, which has a major function in maintaining the NADPH concentration in the red cell, is inhibited by copper. NADPH is also necessary for maintaining the level of reduced glutathione, which in turn protects the red cell against the hemolytic effects of oxidizing substances. The inhibition of this enzyme by copper (Cu²⁺) would explain the hemolysis which is commonly observed in cases of acute copper poisoning. Intravascular hemolysis and a direct action of copper on the kidneys often lead to tubular necrosis [10, 15, 16]. Furthermore, copper caused weakness and weight loss by affecting the kidneys. Copper i.p. injection in Wistar male rats induces nephrotoxicity while increasing in copper levels with accompanying renal dysfunction may be an indication that copper mediates in oxidative-induced renal dysfunction [17].

In conclusion, our study clearly suggests that copper in dose-dependent manner by changing serum parameters related to renal function can induce renal toxicity. Since, Wilson's disease are more susceptible to copper toxicity, the importance of copper toxicity studies in these patients can be realized. However, further study is needed to examine the exact correlation between copper and renal toxicity caused by this element.

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Conflict of Interest
The authors declare no conflict of interest.

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