

Full Length Research Paper

Toxic Effects of Copper on Hematopoietic Progenitors and Circulating Blood Cells in Rats

Azza A. El-Masry

Department of Zoology, Faculty of Science, Alexandria University, Egypt
E-mail: ahssan555@yahoo.com

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This research was carried out to test the cytotoxic effects of copper in rats. Animals were divided into three groups, 5 animals each. Low dose of (2mg /kg) and high dose (4mg/kg) of copper sulphate were force-fed into the animal by a stomach tube daily for 3 weeks and the third group was used as control. At the end of each week three animals (one of each group) were randomly selected and sacrificed. Blood was collected and blood smears were made. The bone marrow was collected from the heads of long-bones and bone marrow smears were also prepared. It has been found that application of copper sulphate doses modulates the proliferation and differentiation of stem cell progenitors and erythrocytes. Several alterations were observed; they were time - and dose -dependent. Of these alterations predominant existence of giant proerythroblasts and promyeloblasts, marked increase of adipose cells, degeneration of proerythroblasts among the bone marrow cells. The most damage criteria in the blood erythrocytes were hypochromia, anisocytosis, fragmentation and burr-shaped erythrocytes. These observations suggest the cytotoxic effect of copper and that it is a modulatory factor of stem cell progenitors and blood erythrocytes.

Keywords: Copper toxicity- bone marrow- progenitors - Blood- Rats.

INTRODUCTION

While it is documented that copper is essential in certain metabolic activities, it is also documented that copper is also a potent cytotoxin when allowed to accumulate in excess of the cellular needs (Linder, 1991; Linder and Hazegh, 1996; Pena *et al.*, 1991). It is essential for a wide range of biochemical processes which are necessary for the maintenance of good health. Copper ions serve as important catalytic factors in redox for proteins that carry out fundamental biological functions that are required for growth and development. However, this redox property also contributes to its potential toxicity. Redox cycling between Cu^+ and Cu^{++} can generate the highly reactive oxygen species (ROS) including hydroxyl radicals (Halliwell and Gutteridge, 1984). These radicals are believed to be responsible for devastating cellular damages that include lipid peroxidation (Engle *et al.*, 2000), direct oxidation of protein and cleavage of DNA and RNA molecules. The action of (ROS) is the major contributing factor to the development of cancer (Keen, *et al.*, 1981; Halliwell and Gutteridge, 1990; Thornburg *et al.*, 1985), disease of nervous system (Kim *et al.*, 2005), cutaneous melanoma

and sarcoidosis (Vinceti *et al.*, 2005; Masel, 2005), hepatitis and cirrhosis (Keen *et al.*, 1981; Thornburg *et al.*, 1986; Meertens *et al.*, 2005).

The stem cells are continuously divided to form new cells. Some of the new cells remain unchanged and have a life long capacity for self renewal (pluripotential). Others have limited capacity for self renewal (unipotential), or progenitors, these become committed to form only one type of cell line (Messener, 1984; Gorin, 1986; Ploemasher and Born, 1988; Allickson, 2008). These cells, in both *in vivo* and *in vitro*, are continuously exposed to damaging protein and DNA molecules (Prchal and Prchal, 1994; Green, 1996). But damages can be greatly increased by exogenous toxic compounds, and copper is included (Dolle, *et al.*, 2000; Vijg, 2000; Ramaiah and Nahity, 2008; Gui *et al.*, 2009; Oliveira *et al.*, 2009). If the stem cells stop functioning because of drugs, radiation, infection, or other toxic events, they become unable to differentiate to any of the blood cells.

Deficiency of copper causes multiple ill symptoms in the animals, and so does the excess of copper cause toxicity. The focus of this work is to illustrate the effect of

Table 1. Number and percentage of animals in control and treated rat groups.

Tested period (weeks)	Initial number	Number and % of alterations *											
		(low dose) 2mg/ kg body weight						(high dose) 4mg/kg body weight					
		Blood			Bone Marrow			Blood			Bone Marrow		
		no	%	:	no	%	:	no	%	:	no	%	:
1	15	2	~14		1	7	--	3	20		2	~14	-
2	15	3	20		2	14	-	3	20		3	20	-
3	15	3	20		3	20	-	3	20		3	20	2
Total	45	8	~54		6	~41		9	60		8	~54	2

The % = number alterations \times 100

The initial number is the mean of 15 individual in each group

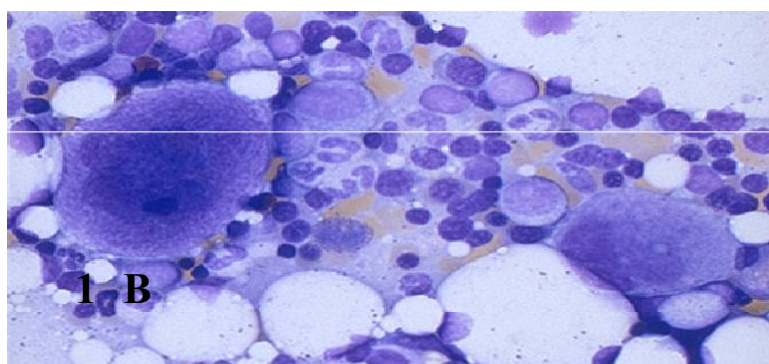
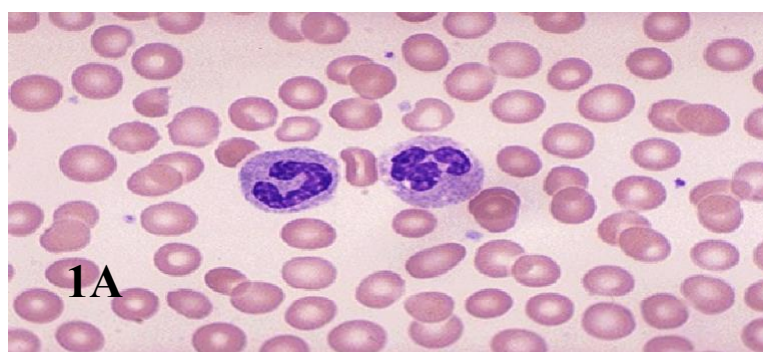


Figure 1. (1A): Blood smears from rats of the control group showing normal shaped erythrocytes (1000X). (1B): Bone marrow smears from the same group of rats of normal proerythroblasts and few amounts of adipose cells (1000X). Lieshmann – Giemsa stains.

Copper toxicity in bone marrow progenitors and the peripheral blood cells of rats.

MATERIALS AND METHODS

Fifteen immature local albinos Sprague Dawely rats weig-

hing about 65 ± 3.5 g were obtained from the animal experimental unit of Faculty of Agriculture, Alexandria University, Egypt at November 2011. They were housed in stainless steel cages under room temperature and air conditioning. They were fed commercial diet, crushed wheat and corn and left for a week to be acclimated. Then, they were divided into three groups, 5 animals

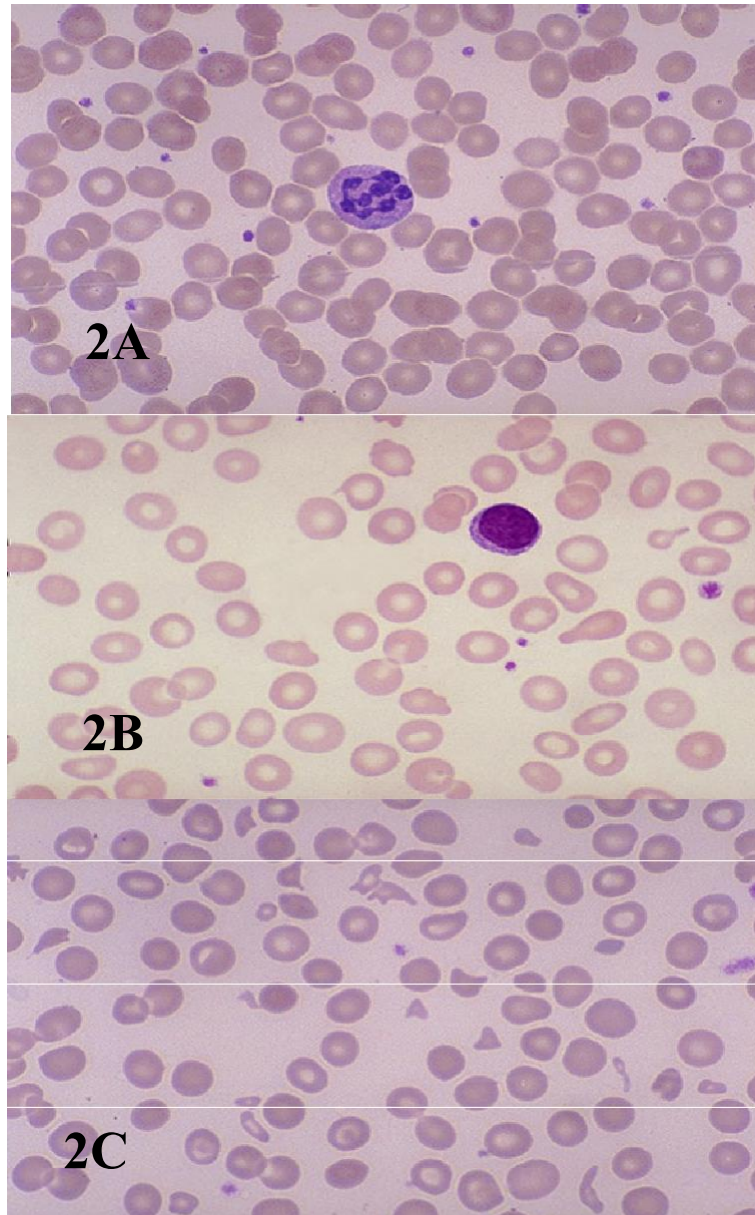


Figure 2. Blood smears from rats after daily administration of 4mg /kg body weight of CuSO₄ for three weeks. (1 A): Hypochromia (1 B): Burr-shaped erythrocytes. (1C): Poikilocytosis, anisocytosis and fragmentation of erythrocytes. Leshmann - Giemsa stains (1000 X).

each. Low dose of (2mg /kg) and high dose (4mg/kg) of copper sulphate were force-fed into the animal by a stomach tube daily for 3 weeks. The third group was used as control.

At the end of each week three animals (one of each group) were randomly selected and sacrificed. Blood was collected and blood smears were made. The bone marrow was collected from the heads of long-bones and bone marrow smears were also prepared. Blood and bone marrow smears were fixed in methyl alcohol and stained with Giemsa and Leshmann stains.

RESULTS

After the first week of administration, the animals seemed normal in all their biological activities. However, in the third week their activities declined, appeared ill; they lost some body hair and their eye white looked yellowish-red. At the end of the experiment one individual of the low-dosed group and two other individuals of high-dosed group dead, i.e., 2mg dose and 4 mg of CuSO₄ caused about 7 % and 14 % mortalities, respectively, (Table, 1).

Blood damages were recorded after the first week of

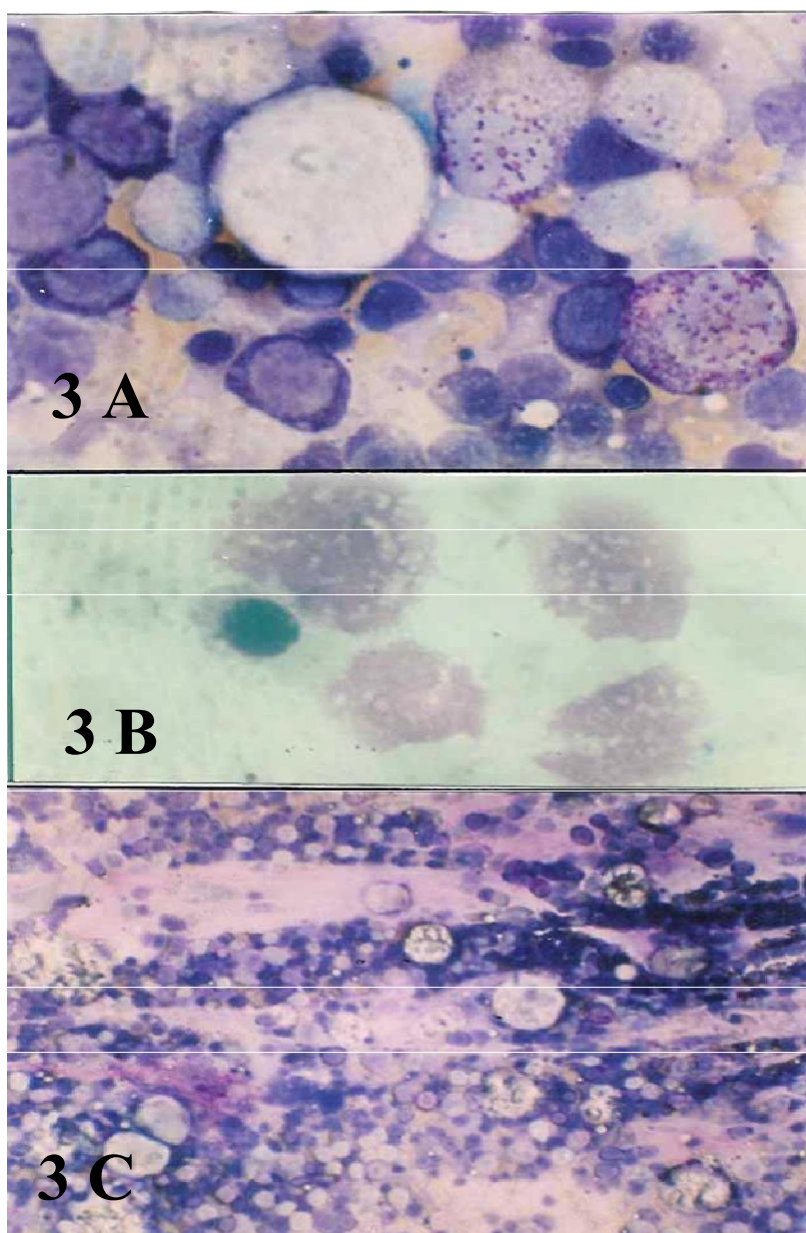


Figure 3. Bone marrow smears from rats after daily administering 4mg/kg body weight of CuSO₄ for three weeks. (2A): Giant proerythroblasts and absence of promyeloblasts (1000X). (2B): proerythroblasts suffering from vacuolar degeneration (1000X). (2C): Accumulation of large amount of adipose cells among the bone marrow cells (400 X). Lieshmann-Giemsa stains.

administration; they were about 14% and 20% of the tested animal's blood at low and high dose (respectively). The changes recorded were time/ dose dependent. However, they took place in faster rate than those of the bone marrow (Table, 1)

Alterations observed in the bone marrow were also time- and dose-dependent. The number of observed alterations was increased by increasing the time of exposure as well as by increasing the administered does. After the first week of exposure about 7% and 14% of the

tested animals showed number of alterations in animals given the low and high doses (respectively). As a total, about 40% and 54% of both low and high dosed animals showed bone marrow alterations (table, 1).

Microscopically, the blood smears examined showed repeated damages, including, hypochromia, burr-shaped cells, anisocytosis, and fragmentation of the red blood cells with predominant hypochromia (Figures 2A, 2B, and 2C) compared with blood smears from rats of the control group (Figure 1A).

Bone marrow changes were in the form of existence of giant proerythroblasts and promyeloblasts (Figure 3A) vacuolar degeneration (ghost cells) of the proerythroblasts (Figure 3B), and plenty of adipose cells in the bone marrow ground substance (Figure 3C) compared with bone marrow smears from the control group of rats which show normal proerythroblasts and few amounts of adipose cells (Figure 1B).

Predominance of giant proerythroblasts or degeneration of such cells may probably due to the mutant effect of excess copper toxicosis. Bhunya and Jena (1996) revealed the genotoxic potential of copper in chick *in vivo*. A reduction due to true loss of membrane components is consistent with earlier observations by LoBuglio *et al.* (1967) and Abramson *et al.* (1970).

In view of the current knowledge of red cell membrane asymmetry alterations induced by direct interaction between auto-antibodies and spectrin molecules are highly improbable (Lutz *et al.*, 1987; De Angelis *et al.*, 1996; Harvey, 2008). This can be considered suitable to reveal possible acquired lesions of red cell membrane proteins.

When copper accumulates in bone marrow, its toxicosis influences the iron metabolism, initially by causing a compensated hemolytic anemia, and later by interfering with re-utilization of iron from ferritin in the reticuloendothelial cells of the spleen (Theil and Calvert, 1978). Copper may interfere with iron absorption by binding to mucosal transferrin. Mobilization of iron from mucosal, reticuloendothelial and hepatic parenchymal cells may be affected through the action of ceruloplasmin. Copper may also participate in heme synthesis through cytochrome oxidase (Chan and Rennert, 1980). The results are the production of erythrocytes with shortage of hemoglobin which refers to hypochromic anemia.

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