African Journal of Biotechnology Vol. 11(22), pp. 6187-6191, 15 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4113
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effect of excess dietary copper on proliferation and differentiation of the proerythroblasts and erythrocytes in rats

Abu-Zinadah OA[1], Hussein HK[1, 2], Elshal MF[3, 4] and Elnaggar, MH[1, 5]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.
[3]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[4]Department of Molecular Biology, Genetic Engineering and Biotechnology Institute, Minuofia University, Minuofia, Egypt.
[5]Department of Zoology, Faculty of Science, Suez Canal University, Ismailia, Egypt.

Accepted 27 February, 2012

This research was carried out to test the cytotoxic effects of excess copper in rats. Animals were divided into three groups, each containing five animals. Low dose (2 mg/kg) and high dose (4 mg/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 the control. At the end of each week, three animals (one of each group) were randomly selected and sacrificed. Blood samples were 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 was found that the application of copper sulphate doses modulates the proliferation and differentiation of stem cell progenitors and erythrocytes. Several alterations were observed and these were time- and dose-dependent. Of these alterations, the predominant existence of giant pro-erythroblasts and pro-myeloblasts marked the increase of adipose cells and degeneration of pro-erythroblasts among the bone marrow cells. Also observed were hypochromia, anisocytosis, fragmentation and burr-shaped erythrocytes.

Key words: Environmental pollution, copper toxicity, stem cells, blood, rats.

INTRODUCTION

While the current knowledge 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 (Hawalliwell and Gutteridge, 1984). These radicals are believed to be responsible for initiating 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; Hawalliwell 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).

Generally, 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 (pluri-potential). 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).

*Corresponding author. E-mail: ahssan555@yahoo.com.

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

Tested period (week)	Initial nummber (control)	Low dose (2 mg/kg body weight)						High dose (4 mg/kg body weight)					
		Blood		Bone marrow		Mortality		Blood		Bone marrow		Mortality	
		N	%	N	%	N	%	N	%	N	%	N	%
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	14
Total	45	8	54	6	41	-	-	9	60	8	54	2	14

The % = number alterations × 100; the initial number is the mean of 15 individual in each group.

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. This study aims to illustrate the effect of copper toxicity in bone marrow progenitors and the peripheral blood cells of rats.

MATERIALS AND METHODS

Fifteen immature local albinos Sprague Dawley rats weighing about 65 ± 3.5 g (Vide No. 288, 2010) were obtained from the animal house, King Fahd for medical researches, King Abdul-Aziz University, Jeddah, in November 2010. 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 acclimatized. Then, they were divided into three groups, 5 animals each. Low dose (2 mg/kg) and high dose (4 mg/kg) of copper sulphate were force-fed into the animal by a stomach tube daily for 3 weeks (Abu-Zinadah and Hussein, 2010). The third group was not treated and served as the control.

At the end of each week, three animals (one from each group) were randomly selected and sacrificed. Blood samples were 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 Lieshmann stains.

RESULTS

Observations of the tested animals

After the first week of administration, the animals seemed normal in all their biological activities. However, in the third week, their activities declined; they appeared ill, lost some body hair, and their eye white looked yellowish-red. At the end of the experiment, one animal of the low-dosed group and two others of the high-dosed group died, that is, 2 mg dose and 4 mg of $CuSO_4$ caused about 7 and 14% mortalities, respectively compared with the control group with the initial number (15 animals without any mortality) (Table 1).

Blood and bone marrow examination

Blood damages were recorded after the first week of administration; they were about 14 and 20% of the tested animal's blood at low and high doses (respectively). The changes recorded were time- and 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 increased by increasing the time of exposure as well as increasing the administered dose. 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 (Figure 2a, b and c), compared with blood smears from rats of the control group (Figure 1a).

Bone marrow changes were in the form of existence of giant pro-erythroblasts and pro-myeloblasts (Figure 3a), vacuolar degeneration (ghost cells) of the pro-erythroblasts (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 pro-erythroblasts and few amounts of adipose cells (Figure 1b).

DISCUSSION

It is known that the hydroxyl radical which is produced from biochemical reaction of excess copper is believed to be responsible for devastating cellular damage including lipid peroxidation, direct oxidation of proteins and

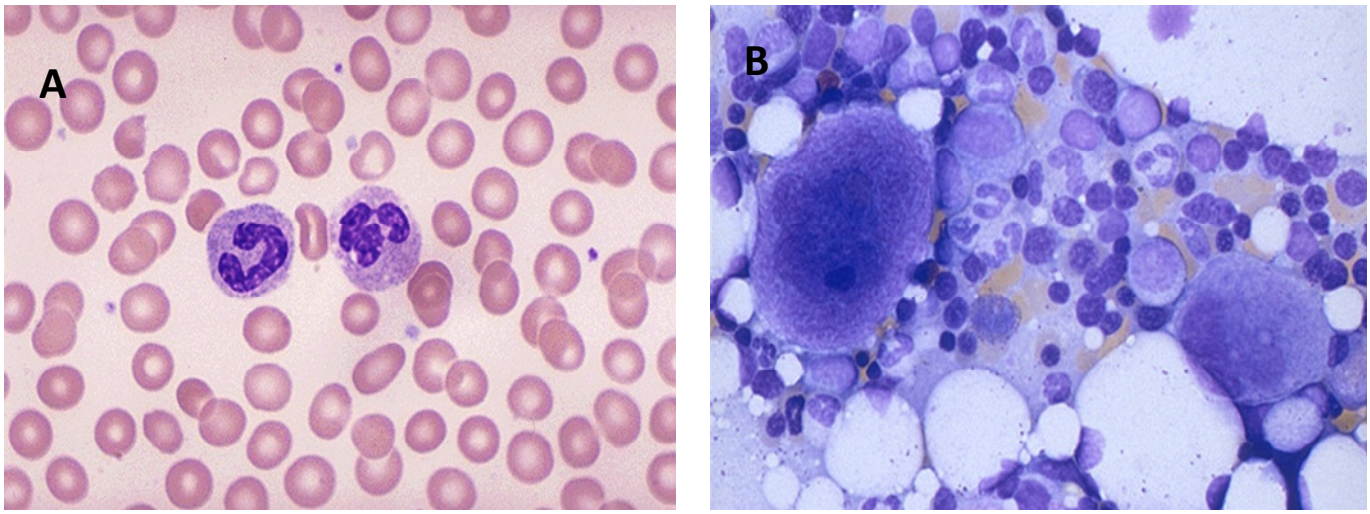

Figure 1. (a) Blood smears from rats of the control group showing normal shaped erythrocytes (1000×); (b) bone marrow smears from the same group of rats of normal proerythroblasts and few amounts of adipose cells (1000×). Lieshmann - Giemsa stains.

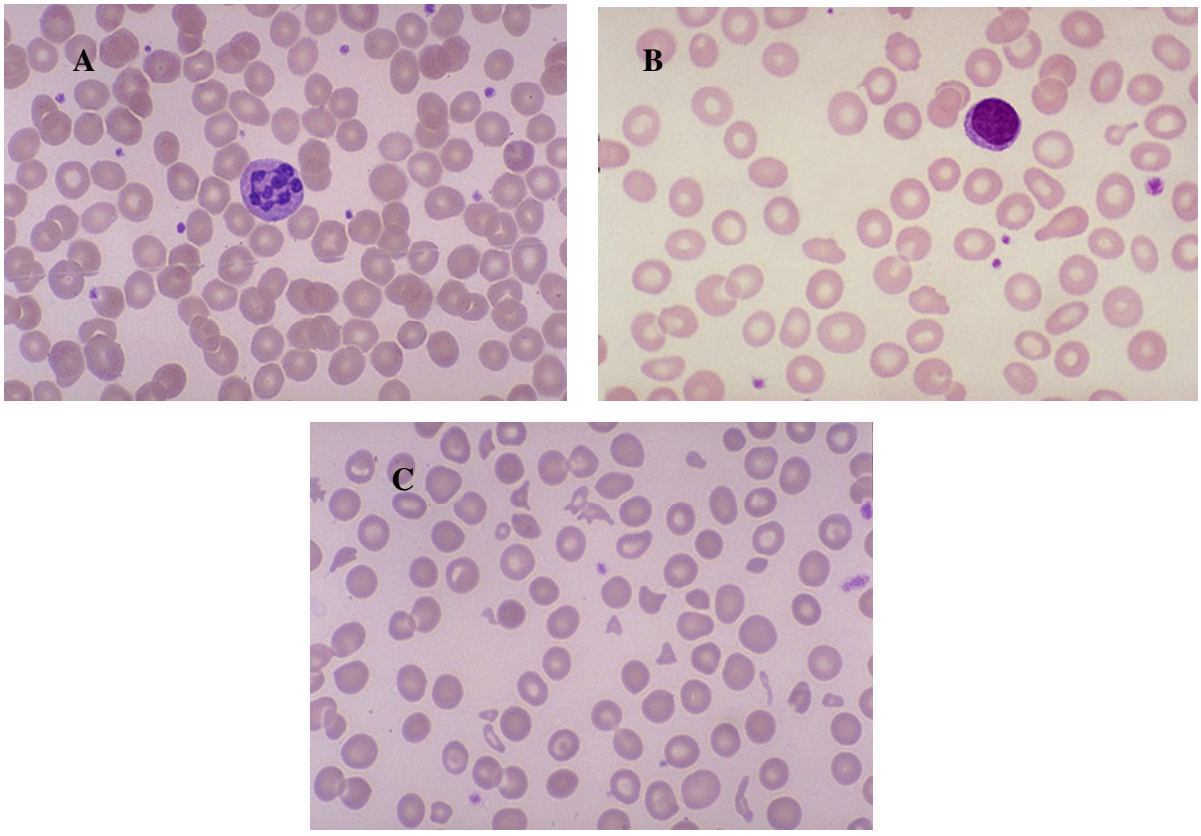

Figure 2. Blood smears from rats after daily administration of 4 mg/kg body weight of $CuSO_4$ for three weeks; a) hypochromia, b) burr-shaped erythrocytes, c) piokilocytosis, anisocytosis and fragmentation of erythrocytes. Lieshmann - Giemsa stains (1000 ×).

cleavage of DNA and RNA molecules (Hawalliwell and Gutterdge, 1990; Engle et al., 2000; Ramaiah and Nahity, 2007). The action of excess copper is considered a modulator factor of stem cells that alter and differentiate them into unpotential progenitor cells. Molecular defects of membrane proteins lead to perturbation of red cell

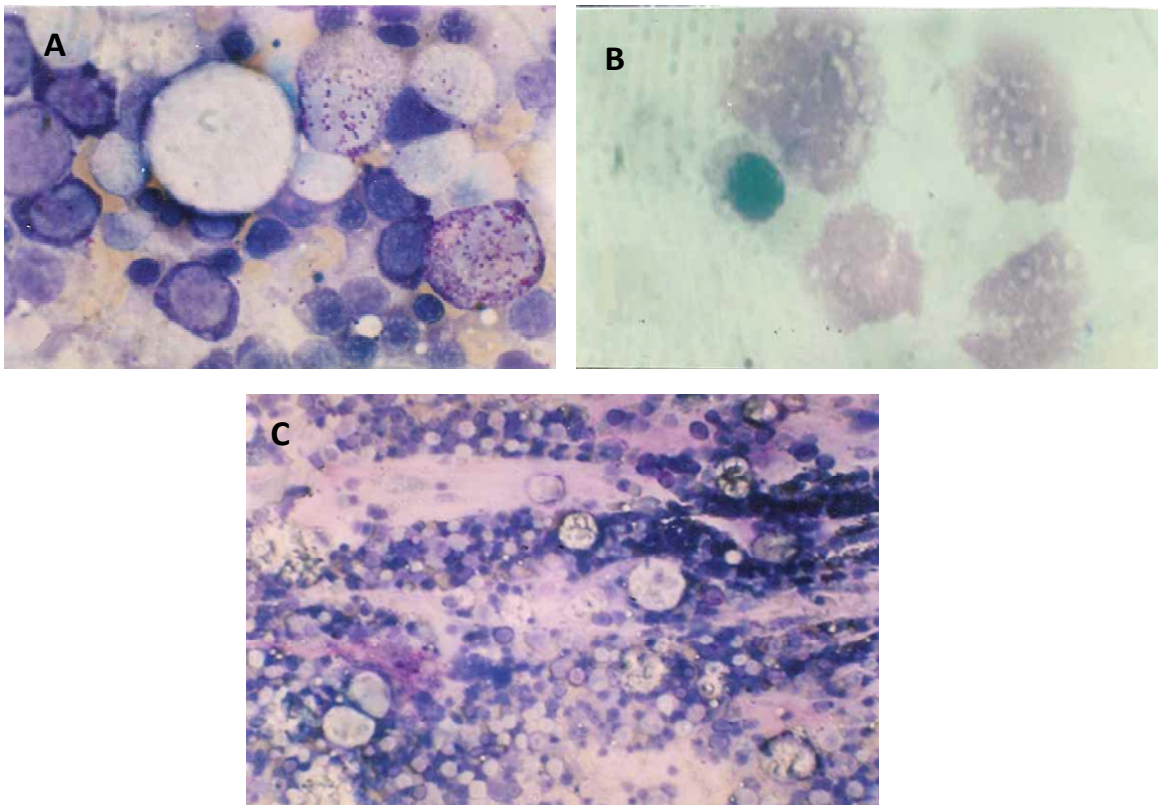

Figure 3. Bone marrow smears from rats after daily administrating 4 mg/kg body weight of $CuSO_4$ for three weeks; a) giant proerythroblasts and absence of promyeloblasts (1000×), b) proerythroblasts suffering from vacuolar degeneration (1000×), c) accumulation of large amount of adipose cells among the bone marrow cells (400×). Lieshmann-Giemsa stains.

shape and are associated with alterations of cellular flow properties which accelerate the removal of red cells from circulation (Mohandas and Chasis, 1993). Soli and Froslie (1977) and Miszta (1990) postulated a relation between chronic copper toxicity and haematocrit, total haemoglobin in plasma, methaemoglobin percentages in erythrocytes and plasma and the osmotic fragility of erythrocytes. They found a positive correlation between excess copper toxicities and the tested parameters. Roelofsen et al. (2004) also showed that extra-cellular copper substantially alters the intracellular protein expression. These studies considered copper as a modulator factor of haemolysis which increases osmotic fragility and erythrocyte spherical transformation occurring during red cell circulation. The presence of spherocytes and fragmented red cells in the peripheral blood film represents one of the principal red cell shape abnormalities (Palek and Jarolim, 1993).

Predominance of giant pro-erythroblasts or degeneration of such cells may probably be 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 for revealing possible acquired lesions of red cell membrane proteins.

When copper accumulates in bone marrow, its toxicity influences the iron metabolism initially by causing a compensated hemolytic anemia, and later by interfering with re-utilization of iron from ferritin in the reticulo-endothelial 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 (Abu-Zinadah and Hussein, 2010). Copper may also participate in heme synthesis through cytochrome oxidase (Chan and Rennert, 1980).

REFERENCES

Abu-Zinadah OA, Hussein HK (2010). The Potential of stem cells to correct the lost tissue mass in liver and kidney of rat after copper toxicity. Insight stem cell Res. 1(1): 1-5.

Abramson N, LoBuglio AF, Cotran RS (1970). The interaction between human monocytes and red cells: binding characteristics. J. Exp. Med. 132: 1191-1206.
Allickson JG (2008). Stem cells derived from cord blood. Principles of Regenerative Medicine 3rd ed., Norwalk, ed. Plenum Press, New York. pp. 238-257
Bhunya SP, Jena GB (1996). Clastogenic effect of copper sulphate in chick *in vivo* tested system. Mutat. Res. 367(2): 57-63.
Chan WY, Rennert OM (1980). The role of copper in iron metabolism. Ann. Clin. Lab. Sci. 10(4): 338-344.
De Angelis V, DeMatteis MC, Cozzi MR, Fiorin F, Pradella A, Steffan A, Vettore L (1996). Abnormalities of membrane protein composition in patients with autoimmune hemolytic anemia. Br. J. Hematol. 95: 273-277.
Dolle ME, Snyder WK, Gossen JA, Lohman PH, Vijg J (2000). Distinct spectra of somatic mutation accumulated with age in mouse heart and small intestine. Proc. Natl. Acad. Sci. USA. 97: 8403-8408.
Engle TE, Spears JW, Armstrong TA, Wright CL, Odle J (2000). Effects of dietary copper source and concentration on carcass characteristics and lipid cholesterol metabolism in growing and finishing steers. J. Anim. Sci. 78(4): 1053-1059.
Green AR (1996). Pathogensis of polycythemia vera. Lancet., 347: 844-845.
Goi S, Zhang Ta, Sun M, Wang H. Danli Y (2009). Effect of bone marrow stromal cell conditioned medium on the glutamate uptake of peroxide injured astrocytes. J. Clin. Neurosci. 16(9): 1205-1210.
Gorin NC (1986). Collection, manipulation and freezing of hemopoietic stem cells: A review. Clin. Hematol. 16:19- 48.
Harvey JW (2008). The Erythrocytes Physiology: Metabolism and Biochemical disorders. Clinical Biochemistry of domestic Animals (Sixth Edition) Hetwale Press, New York. pp. 173-240.
Hawalliwell B, Gutteridge JM (1984). Oxygen toixicity radicals, transition metals and diseases. Biochem. J. 219: 1-4.
Hawalliwell B, Gutteridge JM (1990). Role of free radicals and catalysitic metal ions in human disease: An overview. Method Enzmol. 186: 1-85.
Keen CL, Lonnerdal Bo, Fisher GL (1981). Age-related variations in hepatic, Iron, Copper, Zinc and Selenium concentrations in Beagles. Am. J. Vet. Res. 42(11): 1884 -1887.
Kim HJ, Kim JM, Park JH, Sung JJ, Kim A, Lee KW (2005). Pyruvate protects motor neurons expressing mutant superoxide dismutase 1 against copper toxicity. Neuro-Rep. 16(6): 585-589.
Linder MC (1991) Nutritional Biochemistry and Metabolism with Clinical Application . 2nd ed., P333, Appleton and Lange, Norwalk, ed. Plenum Press, New York.
Linder MC, Hazegh-Azam M (1996). Copper biochemistry and molecular biology. Am. J. Clin. Nutr. 63: 797-811.
LoBuglio AF, Cotran RS, Jandl JH (1967). Red cell coated with immunoglobulin G, binding and sphering by mononuclear cell in man. Science, 158: 1582-1584.
Lutz HV, Flepp R, Stammter P, Baccala R (1987) Red cell associated, naturally occurring anti-spectrin antibodies. Clin. Exp. Immunol. 67: 674-676.
Masel G (2005). Copper-induced cutaneous sarcoidosis. Aust. J. Dermatol. 46(1): 38-41.
Meartens NM, Bokhove CA, Vande Ingh TS (2005). Copper associated chronic hepatitis and cirrhosis in a European short hair cat. Vet. Pathol. 42(1): 97-100.
Messner HA (1984). Human stem cell in culture. Clin. Haematol. 13 : 393 .
Miszta H (1990). *In vitro* effect of copper on the stromal cells of bone marrow in rats. Toxicol. Ind. Health, 6(1): 33-39.
Mohandas N, Chasis JA (1993). Red blood cell deformability, membrane material properties and shape: regulation by trans-membrane skeletal and cytosolic protein and lipids. Semin. Hematol. 30: 171-192.
Oliveira JM, Sousa RA, Sousa RA, Kotobuki N, Tadokora M, Ohgushi, H (2009). The osteogenic differentiation of rat bone marrow stromal cells cultured with dexamethasone-loaded carboxymethyl chitosan (amidoamine) dendrimer nanoparticles. Biomaterials, 30(5): 804-813.
Palek J, Jarolim P (1993). Clinical expression and laboratory detection of red blood cell membrane protein mutations. Semin. Hematol. 30: 249-283 .
Pena MM, Lee J, Thiele DJ (1999). A delicate balance: Homeostatic control of copper uptake and distribution. J. Nutr. 129: 1251-1260.
Ploemasher RE, Borns HC (1988). Isolation of hemopoietic stem cell subsets from murine bone marrow: I. Radioprotective ability of purified cell suspensions deffering in the proportion of day-7 and day-12 CFV-S. Exp. Hematol. 16: p. 21.
Prchal JT, Prchal JF (1994). Evaluating and understanding of cellular defect in polycythemia vera; implications for its clinical diagnosis and mole. Pathophysiol. Blood, 83: 1-4.
Ramaiah SK, Nahity MB (2007). Blood and Bone marrow toxicity. Vet. Toxicol. 5: 277-288.
Roelofsen H, Balgobind R, Vonk RJ (2004) Proteomic analysis of copper metabolism in vitro model of Wilson disease using surface enhanced laser desorption/ionization time of flight-mass spectrometry. J. Cell Biochem. 93(4): 732-740.
Soli NE, Froslie A (1977). Chronic copper poisoning in sheep. I. The relationship of methemoglobinemia to Heinz body formation and hemolysis during the terminal crisis. Acta Pharmacol. Toxicol. 40(1): 169-177.
Theil EC, Calvert KT. (1978). The effect of copper excess in iron metabolism in sheep. Biochem. J. 170(1): 137-143.
Thornburg LP, Dennis GL, Olwin DB, Mclaughlin CD, Gulbas NK (1985). Copper Toxicosis in Dog: Part 2: the pathogenesis of copper-associated liver disease in dogs. Canine Practice, 12(5): 33-37.
Thornburg LP, Rottinghous G, Goge H (1986). Chronic liver disease associated with high hepatic copper concentration in a dog. J. Am. Vet. Med. Assoc. 188(10): 1190-1191.
Vijg J (2000). Somatic mutations and aging: a re-evaluation. Mutat. Res. 447: 117-135.
Vinceti M, Bassissi S, Malagoli C, pellacani G, Alber D, Bergomi M, Seidenari S (2005). Environmental exposure to trace elements and risk of cutaneous melanoma. J. Exp. Anal. Environ. Epidemiol. 15(5): 458-462.