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RESEARCH ARTICLE

# Effect of Sub Chronic Exposure of Copper on Some Hematological and Hepatological Parameters in Male Wistar Rats

**[1]Shruti Shukla*, [1]Pradeep Bhatnagar, [2]Priyankana Banerjee, [3]S J S Flora**
[1]Department of Life Sciences, The IIS University, Gurkul Marg, SFS, Mansarovar, Jaipur 302020 INDIA
[2]Department of Biochemistry, M S University, Pratapgunj, Vadodara 390002 INDIA
[3]Defence Research & Development Establishment, Jhansi Road, Gwalior 474002 INDIA

## Abstract

Copper is a naturally occurring element that is ubiquitously present in the environment. Copper, an essential trace element, is available through various food and water and is required for many biological functions. In the normal state, copper balance is tightly regulated. Copper enters the body through the digestive tract, is absorbed in the stomach and upper small intestine (Stern, *et al*, 2007), concentrated in the liver, and incorporated into cerulosplasmin or excreted into bile (Sass-Kortsak, 1965). Copper toxicity causes cellular damage in various tissues due to oxidative stress. (Valko *et al*, 2005). Copper toxicity is sometimes seen in people working in copper industry, or with people having a genetic disease, called Wilson's disease, an inherited disorder in which there is accumulation of copper in tissues due to defect in an enzyme responsible for its clearance, resulting in poor billiary excretion of copper. Normally, copper is available to humans through food and water. The present study was designed to evaluate the effect of copper sub chronically given to male Wistar rats, for a period of 16 weeks, administered orally as copper sulphate, once daily, at 1.0 mg per kg body weight. Hematological and hepatological variables in blood and liver were measured to see if this copper exposure resulted in any toxicity. It was observed that at the rate of 1 mg per kg body weight, when given for 16 weeks, copper did not induce any toxicity, histologically or in some blood and hepatological variables.

**Keywords**: Copper Exposure and toxicity, Histology, Hematological and Hepatological variables.

## Introduction

Copper is a unique essential trace mineral that is vitally important for both physical and mental health. Copper's essentiality as a unique trace element is due to its ability to act as an electron donor or acceptor as its oxidation state fluxes between $Cu^{+1}$(cuprous) and $Cu^{+2}$ (cupric). (Ralph and McArdle, 2001 (Tapiero *et al*, 2003). Copper participates in a wide variety of redox reactions in numerous biologic processes, including bones and connective tissue, energy production in the cells , immune response, the glandular system , reproductive system, (Angelova, *et al*, 2011) and nervous system (Wilson, 2011).

When copper in the soft tissues of the body increases, it causes copper toxicity. Unbound copper may circulate freely in the body, without sufficient binding proteins, where it may accumulate primarily in the liver, kidney, brain and other organs, causing oxidative stress. The freely available copper, both cupric and cuprous Cu ions participate in the formation of reactive oxygen species (ROS). In the presence of superoxide (*O2_) or reducing agents such as ascorbic acid or GSH, $Cu^{+2}$ can be reduced to $Cu^{+}$, which is capable of catalyzing the formation of hydroxyl radicals ($OH^{+}$) from hydrogen peroxide ($H_2O_2$) via the Haber-Weiss reaction (Bremner, 1998; Kadiiska *et al*, 1993).

$$*O_2^- + Cu^{+2} \longrightarrow O_2 + Cu^{+}$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{+2} + OH^{-} + OH^{*}$$

Being one of the most powerful oxidizing radical likely to arise in biological systems, this hydroxyl radical is capable of reacting with almost every biological molecule (Buettner, 1993)

The extent of oxidative changes caused by excess copper depends on the length and dosage of copper exposure, as well as the species. The symptoms of chronic Cu toxicosis are not constant and vary between species (Bremner, 1998).

Various scientists have given different dosage of copper to a number of species to evaluate the copper balance and see the extent of accumulation of copper in blood and soft organs as well as its toxic effects during the experimental period. Some of the experimental dosage range of copper in Wistar rats, per kg body weight with 90 days of copper exposure as seen in literature was 1.5 mg copper (Pal *et al*, 2013), 8 to 40 mg copper (Al- Naimi *et al,* 2010) 100-200 mg (Kumar *et al*, 2015).

*Corresponding Author : Email : shrutivs@gmail.com

Free Cu excess, rather than Cu deficiency, is more widespread in human population (Brewer and Althaus, 2008) primarily due to high intake of inorganic Cu in the form of mineral/vitamin supplements and Cu plumbing.

In the present study, Hematological parameters were assessed to see the immediate effect of copper exposure on blood indices. Since Liver is the primary organ of copper storage (Valko *et al*, 2005), liver function test were performed to reflect liver health.

## Material and Methods

### Animals

Healthy adult male Wistar rats (70-80 g) were obtained from and used for experiment at the K.B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat. They were housed in an air-conditioned room with constant access to a complete diet. The animal house temperature was maintained at 22 $\pm$ $1^0$C and photoperiodicity was 12 hr light and darkness. The Animal Use Committee of the KBIPER, Gandhinagar, India, approved the protocol for the experiments.

### Animals and treatment

Experiments were performed on healthy, male Wistar rats, of 4 weeks, weighing approximately 80-90 grams. Twelve rats were randomized into two groups of 6 animals each and were treated as follows for 16 weeks:

Group I: Control animal – These rats received normal diet and water for 16 weeks.

Group II: These rats received copper as copper sulfate, 1.0 mg/kg body weight, orally, once daily, for 16 weeks.

Body weight of all the animals was monitored weekly. All animals looked clinically healthy during the entire period of experiment of oral administration of copper sulfate.

After 16 weeks, the exposure was stopped and animals were sacrificed under light ether anesthesia, 48 h after the last dosing, to allow sufficient time for the complete absorption of the dose material. Blood was collected for hematological and clinical chemistry parameters, while liver was carefully removed, washed free of extraneous material with cold normal saline, blotted and stored at -70°C until use.

*Element analysis*

Copper concentration in blood and liver was measured after wet acid digestion using a conventional digestion system. Copper was measured in the Nitric acid and Perchloric acid digested tissue samples using Atomic Absorption spectrophotometry in AAS (Elico SL194 double beam) )(Parker *et al*, 1967)

### Histology

Blood smears were prepared immediately on dissection of rats, air dried, fixed by dipping in methanol, stained with fields staining, observed under a microscope on 1000x Oil immersion and appropriate images were taken to see the distribution of the cells.

Part of liver was stored in formal saline for 2-3 days and later fixed in paraffin. Required sections of 3-4 µm were prepared using a automated microtome. The slides were stained with Hematoxilin-Eosin (H & E) stain and observed microscopically on Olympus microscope fitted with Nikon Camera. Appropriate images were taken to identify the histoarchistructure of the tissue.

## Biochemical Assays

### *Clinical hematological variables*

White blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet (PLT) counts were measured on fully automatic cell counter (PCE 210 from ERMA).

### *Clinical Chemistry variables*

Serum Glutamate Oxaloacetate transaminase (SGOT/ AST), Serum Glutamate pyruvate transaminase (SGPT/ ALT) and Bilirubin (total, direct, indirect) were measured by fully automated biochemistry analyser (LWC 100 Auto Chemistry Analyser from LANDWIND).

### *Liver GOT and GPT*

10 % liver homogenates were prepared in 0.1 M phosphate buffer, pH 7.4 and used for estimating GOT and GPT by Enzopak Kits of Reckon Diagnostics. To conduct the decreasing kinetic reaction, 100 µl of homogenate was added to 1 ml of working reagent (provided in the Kit), mixed and incubated at Room Temperature for 1 minute, and absorbance was measured for 2 minutes at an interval of 30 seconds at 340 nm. GOT and GPT activity was measured as IU/L by the formula provided in the kit based on the millimolar extinction coreficient of NADH at 340 nm in a Jasco V – 530 spectrophotometer.

## Results and Discussion

Rats exposed to copper gained more weight as compared to rats on standard diet, as shown in Fig. 1.

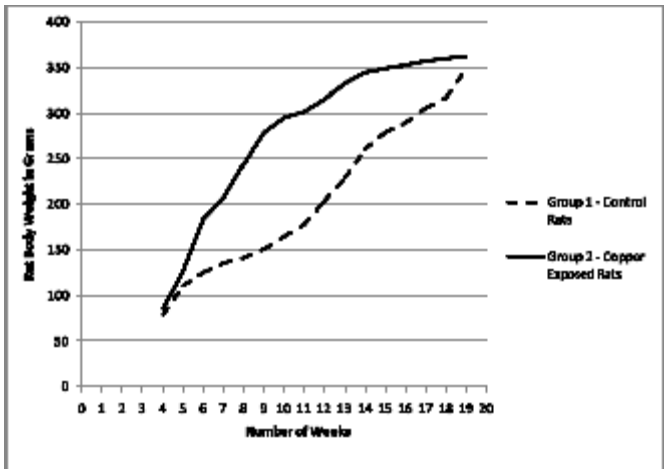


**Fig. 1. Weekly body weight of control and copper exposed rats for 16 weeks**

**Table 1. Effects of sub chronic copper exposure on copper concentration in blood and liver.**

| | Control rats | 1 mg/kg BW Copper exposed rats |
|---|---|---|
| **Blood** (µg/ml) | 22.15 ±0.05 | 22.20 ± 0.09 |
| **Liver** (µg/g wet liver) | 1.25 ± 0.27 | 1.31±0.53 |

Values are means ±SE, n=6; * P <0.05 compared to Normal animals as evaluated by Student's t test.

As seen in table 1, there is minimal changes w.r.t. the accumulation of copper in both blood and liver. At this level and time of exposure, copper homeostasis is finely managed by the body and hence has not resulted in its significant accumulation in either blood or liver.

**Table 2. Sub chronic exposure effects of copper on some hematological variables.**

| | Control rats | 1 mg/kg BW Copper exposed rats |
|---|---|---|
| WBC($10^3\mu l^{-1}$) | 8.93* ± 1.34 | 10.08* ± 1.32 |
| RBC($10^6\ \mu l^{-1}$) | 7.22 ± 0.23 | 7.24 ± 0.29 |
| Hb(g/dL) | 15.88 ± 0.59 | 15.98 ± 0.48 |
| HCT (%) | 44.10 ± 1.61 | 44.85 ± 1.75 |
| MCV(fL) | 61.0 ± 1.58 | 61.98 ± 3.7 |
| MCH(pg) | 21.95 ± 0.47 | 22.03 ± 1.06 |
| MCHC(g/dL) | 35.97 ± 0.55 | 35.58 ± 0.41 |
| PLT($10^4\ \mu l^{-1}$) | 59.53* ± 3.96 | 74.55* ± 0.66.66* |

Values are means ±SE, n=6; * P <0.05 compared to Normal animals as evaluated by Student's t test.

A significant difference was observed for WBC and Platelets count between Control and Test animals.

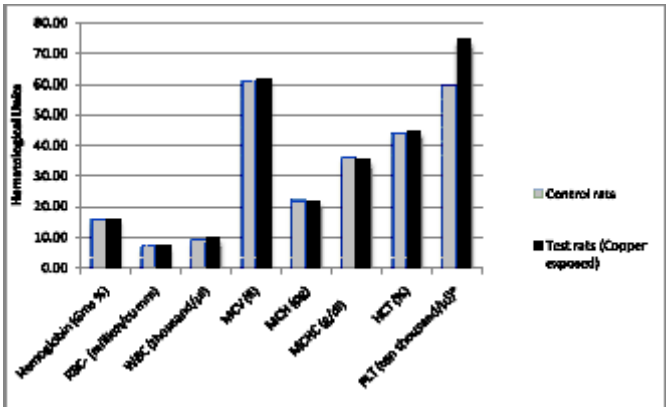


**Fig. 2. Hematological parameters of control and test (copper exposed) rats for 16 weeks**

In the hematological variables (Table 2, Fig.2), there is no significant change in blood parameters. At this stage of exposure, the intrinsic mechanism of scavenging free radicals may be sufficient enough for not causing any damage to hematological parameters. Ceruloplasmin, an acute reaction protein, binds to free copper in the blood, thereby aiding its transport and not allowing its accumulation in blood (Ranganathan *et al*, 2011). There is also availability of Glutathione in the blood, which helps in free radical metabolism, and is adequate enough at this stage to scavenge the free radicals generated by copper (Freedman *et al*, 1989).

**Table 3. Sub chronic exposure effects of copper on some hepatological variables in blood and liver**

| Parameter | Normal animal | Copper exposed rats |
|---|---|---|
| Serum GPT (ALT) IU/dL | 37.90* ± 15.32 | 19.24* ± 5.26* |
| GPT IU/mg liver tissue (ALT) | 103.83 ± 20.90 | 60.24 ± 8.82* |
| Serum GOT (AST) IU/dL | 20.05 ± 3.92 | 23.12 ± 8.02 |
| GOT IU/mg liver tissue (AST) | 76.48 ± 13.28 | 86.19 ± 9.35 |
| Total Bilirubin (mg/dL) | 0.72 ± 0.18 | 0.72 ± 0.15 |
| Bilirubin Direct (mg/dL) | 0.28 ± 0.10 | 0.27 ± 0.08 |
| Bilirubin Indirect (mg/dL) | 0.43 ± 0.15 | 0.45 ± 0.14 |
| SGOT/ SGPT Ratio | 0.64* ± 0.41 | 1.30* ± 0.61 |
| Liver GOT/GPT Ratio | 0.76* ± 0.20 | 1.47* ± 0.36 |

Values are means ±SE, n=6; * P <0.05 compared to Normal animals as evaluated by Student's t test

There is a significant rise in platelet count. High platelet levels do not necessarily signal any clinical problems, however, in our experiment, increased platelet count or thrombocytosis could be associated with elevation in stimulants of platelet production as part of the acute phase reaction due to continuous availability of dietary copper.

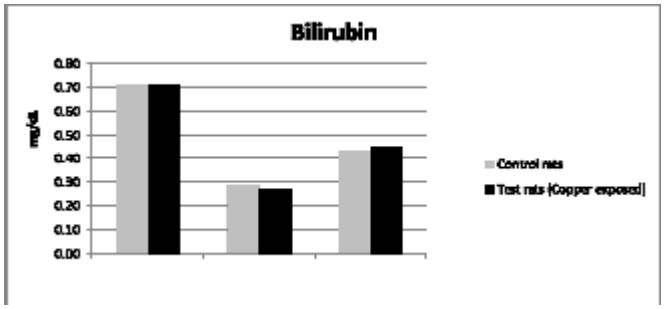


**Fig. 3. Bilirubin content in serum**

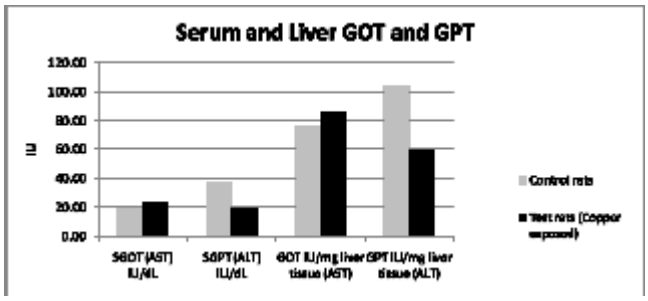


**Fig. 4. Blood and Liver Transaminase of control and test (copper exposed) rats.**

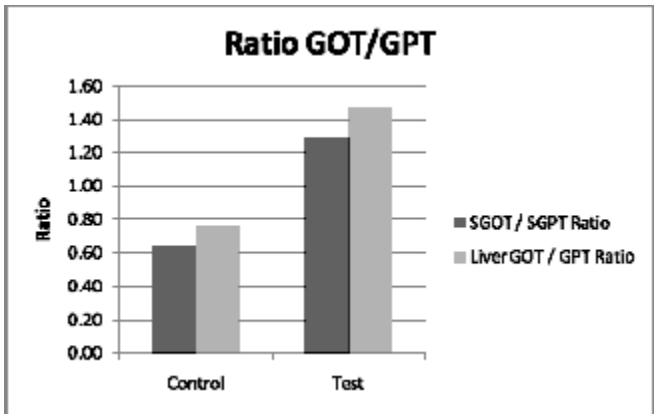


**Fig. 5. Ratio of Serum and Liver transaminase of control and test (copper exposed) rats**

Bilirubin content (total, conjugated and unconjugated) was not affected by 16 weeks of copper exposure (Fig. 3).

**GOT** (Glutamate-Oxaloacetate Transaminase) is normally present in a number of tissues such as heart, liver, muscle, brain and kidney. It is released into the blood stream whenever any of these tissues gets damaged. GOT, in both serum and liver is not significantly affected in the test group (Fig. 4, Table 3).

**GPT** (Glutamate-Pyruvate Transaminase) is normally present in large concentrations in the liver. In case of liver damage, it leaks out of the cell into the blood, hence level in the blood rises, thereby, serving as a specific indicator for liver injury. However, in this case, GPT, in both serum and liver has decreased significantly, thereby giving a positive response to copper exposure (Fig. 4). Higher or adequate copper intakes are known to decrease serum HDL and triglycerides (Chaudhari *et al*, 2014; Umoren, 1989; Lefevre *et al*, 1986) and this in turn may have caused a decrease in GPT levels (Fig. 5).

### Histology

Blood smears were prepared, air dried, fixed by dipping in methanol, stained with fields staining on 1000x Oil immersion, observed under a microscope and appropriate images were taken as shown in Fig. 6 (a) and (b).

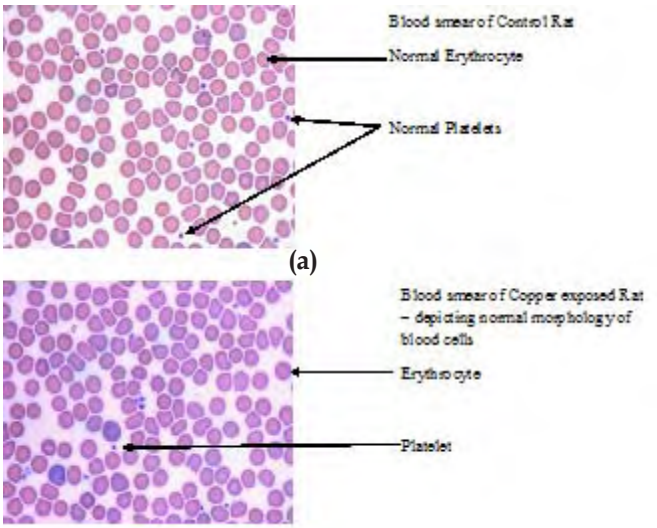


**Fig.6 (a) & (b). Blood smear of Control and Copper Exposed Rat**

Liver architecture of control rat at 400x magnification. Normal portal triad seen along with hepatocyte (Fig. 7(a)).

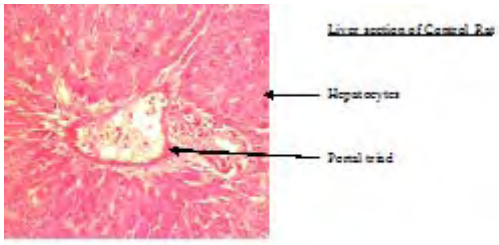


**Fig. 7(a). Liver Section of Control Rat**

Liver archistructure of copper exposed rat at 400x magnification. Normal portal triad along with hepatocyte seen. No signs of tissue damage identified (Fig. 7(b)).

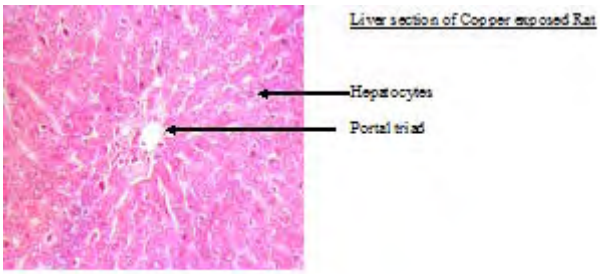


**Fig. 7(b). Liver Section of Copper Exposed Rat**

**Statistics:**

The results were presented as Mean ± SEM in both group at $P < 0.05$, with students' t test.

## Conclusion

Copper tolerance and adaptation, or toxicity, can develop under certain conditions, depending on factors such as species, genetics, age, and diet (Bremmer, 1979). Rats can adapt to prolonged exposure to copper (Fuentealba *et al*, 1989). Haywood (1985) showed that copper levels in liver and kidney rose until a period of 4-5 weeks and then started decreasing over a period of time, suggesting that Copper toxicosis in the rat can be a temporary phenomenon and adaptation occurs, facilitated probably by renal excretion, and the animals become tolerant.

This experiment showed that at the rate of 1 mg copper/ kg body weight, given orally as copper sulphate, for 16 weeks in young adult male Wistar rats, did not induce any abnormal changes in histology of blood and liver, hematological parameters as well as serum and liver transaminase levels. The homeostasis of copper seems to be managed finely at this stage and no toxicity is seen.

## Acknowledgements

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## References.

Al- Naimi, R. A., Al-Tayar, N. H. , Abdul-Hadi, D. (2010) Toxicopathological study due to effect of different doses of Copper Sulfate ingested in rat, Al-Anbar *J Vet Sci* **3 (2)** : 1-16.

Angelova M., S., Asenova, V., Nedkova , R., Koleva-Kolarova,(2011) Copper in the human organism *Trakia Journal of Sciences* **9(1)** : 88-98.

Bremner, I. (1998) Manifestations of copper excess. *Am J Clin Nutr* **67**:1069S–1073S

Bremner I. (1979) Copper toxicity studies using domestic and laboratory animals. In: Nriagu JO, ed. *Copper in the environment. Part II: health effects*. New York: John Wiley & Sons, 285 – 306.

Brewer, G.J., Althaus, J.(2008) How reliable and robust are current biomarkers for copper status? *Br J Nutr* **100(6)** : 1341– 1342.

Buettner, G.R. (1993) The Pecking Order of Free Radicals and Antioxidants: Lipid Peroxidation, á-Tocopherol and Ascorbate *Arch Biochem Biophys* **300 (2)**: 535 - 543

Chaudhari, S.Y., Galib, R., Iswajyoti, J.P., Prajapati P.K. (2014) Effect of *Tamra Bhasma* (Calcined Copper) on Ponderal and Biochemical Parameters *Toxicol Int* **21(2)**:156 –159.

Freedman, J.H., Maria, R.C., Peisach, J. (1989) The Role of Glutathione in Copper Metabolism and Toxicity *The Journal of Biological Chemistry* by The American Society for Biochemistry and Molecular Biology, Inc. **264(10)**: 5598-5605.

Fuentealba, I., Haywood, S., Foster, J. (1989) Cellular mechanisms of toxicity and tolerance in the copper-loaded rat. II. Pathogenesis of copper toxicity in the liver. *Exp Mol Pathol.* **50:** 26–37.

Haywood, S. (1985) Copper toxicosis and Tolerance in the Rat I – Changes in the copper content of Liver and Kidney. *J Pathol* **145:**149-158

Kadiiska, M.B., Hanna, P.M., Jordan, S.J., Mason, R.P., 1993. Electron spin resonance evidence for free radical generation in copper-treated vitamin E- and selenium-deficient rats: in vivo spin-trapping investigation. *Mol Pharmacol* **44 :** 222-227.

Kumar, V., Jayantee K., Misra, U.K., Bora, H.K. (2015) A study of dose response and organ susceptibility of copper toxicity in a rat model, *J Trace Elem Med Biol* **29**:269-274.

Lefevre, M., Keen, C.L., BO LÃ–Nnerdal, Hurley, L.S., Schneeman, B.O. (1986) Copper Deficiency-Induced Hypercholesterolemia: Effects on HDL Subfractions and Hepatic Lipoprotein Receptor Activity in the Rat. *J Nutr* 1735 - 1746.

Pal, A., Badyal, R.K., Vasishta, R.K., Verma, S.A., Thapa, B.R., Prasad R. (2013) Biochemical, Histological, and Memory Impairment Effects of Chronic Copper Toxicity: A Model for Non-Wilsonian Brain Copper Toxicosis in Wistar, *Rat Biol Trace Elem Res* **153(1):** 257-268.

Parker, M.M., Humoller, F.L., Mahler, D.J. (1967) Determination of copper zinc in biological materials *Clin Chem* **13:** 40–48.

Ralph, A., McArdle, H. J. (2001) Copper metabolism and requirements in the pregnant mother, her fetus, and children. New York International Copper Association

Ranganathan, P.N., Lu, Y., Jiang, L., Kim, C., Collins, J.F. (2011) Serum ceruloplasmin protein expression and activity increases in iron-deficient rats and is further enhanced by higher dietary copper intake. *Blood* **118:** 3146–3153.

Sass-Kortsak, A. (1965). Copper metabolism. *Advanc Clin Chem* **8**: 1 - 67.

Stern, B.R. et. al,(2007) Copper And Human Health: Biochemistry, Genetics, And Strategies for Modeling Dose-Response Relationships, *J Toxicol EnvironHealth,* Part B. **10:**157–222

Tapiero, H., Townsend, D.M., Tew, K.D. (2003) Trace elements in human physiology and pathology. *Copper Biomed Pharmacother* **57 (9)** : 386–398

Umoren, J. (1989) Serum total cholesterol and HDL-cholesterol levels as associated with copper and zinc intake in physically active and sedentary elderly men and women. *Adv Exp Med Biol* **258** : 171-81

Valko, M., Morris, H., Cronin, M.T. (2005) Metals, toxicity and oxidative stress. *Curr Med Chem* **12 (10) :** 1161–1208

Wilson, L., M.D., Copper Toxicity Syndrome (2011) The Center For Development (http://drlwilson.com/articles/copper_toxicity_syndrome)