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Prolonged administration of high doses of copper nicotinate to rats: Effect on biochemical and cellular constituents of blood and on copper level in serum, liver and muscle

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The effect of prolonged administration of high doses of copper nicotinate on biochemical and cellular constituents of blood and on copper level in serum, liver and muscle was studied in rats. Oral administration of copper nicotinate at doses of 0.8 or 4.0 mg/kg body weight (b. wt.) for 6 weeks did not affect GOT, creatinine or cholesterol in serum of rats. When given at 4.0 mg/kg b. wt. for 8 weeks copper nicotinate significantly (P <0.05) increased GOT, GPT, urea, creatinine and cholesterol in serum of rats. Minimal hematological changes were observed particularly when copper nicotinate was given at the high dose for 8 weeks. After administration of copper nicotinate at a dose of 0.8 and 4.0 mg/kg b. wt. copper level increased in the serum. The concentrat of copper in liver and muscle tissue initially increased through the first 2 weeks, but its level decreased within the next 2 weeks and remained almost at this level up to the 10th week. The present data indicate the safety of copper nicotinate complex when given at a high dose for 6-8 weeks. This was also confirmed by the high LD_{50} in mice (1104.17 and 128.33 mg/kg b. wt. after oral and intraperitoneal route respectively.

Key words: Copper nicotinate, copper complex, copper high doses, copper concentration, serum biochemical changes, hematological changes.

INTRODUCTION

Copper deficiency is extremely rare because the amount present in food is more than adequate to provide the needed body requirements (Velasco-Reynold et al., 2008). Copper is required for the function of several co-enzymes essential for different physiological functions (Lee et al., 1976; Linder, 1991). The daily copper requirement for individuals of more than 11 years is estimated to be 1.5-2.5 mg (Sandstead, 1982). However the tolerable upper intake level of copper is ranging from 5 to 10 mg elemental copper daily. A dose of elemental copper up to 15 mg / day for an adult can be considered as a safe supplemental dose. Above this dose some gastrointestinal side effects can occur (Araya et al., 2005). As a therapeutic dose, risk benefit ratio can be in favor of increasing the dose if treating a serious condition. Diet can be supplemented by copper in different forms such as copper sulfate or tribasic copper chloride. However, recently there is an increasing evidence for the use of copper complexes such as copper nicotinate and 2-methylthionicotinate in several clinical situations (El-Saadani et al., 1993; Dudova et al., 2002; Song et., 2003; El-Saadani, 2004; Salama et al., 2007). The effect of copper complexes on the health status requires investigations. This necessitates studying the effect of high doses of copper nicotinate on some serum and blood parameters and

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Table 1. Effect of copper nicotinate after 6 weeks of oral administration on serum biochemical constituents of rats (Mean ± SD, n=5).

	GOT I.U./l	GPT I.U./l	Urea mg/dl	Creatinine mg/dl	Cholesterol mg/dl
Control	131.0±17.1[a]	44.0±4.2[b]	18.3±1.2[b]	0.6±0.1[a]	59.0±4.6[a]
Copper nicotinate (0.8mg/kg)	120.6±7.5[a]	40.4±4.3[b]	16.4±1.4[ab]	0.5±0.2[a]	56.0±6.5[a]
Copper nicotinate (4.0 mg/kg)	134.0±3.8[a]	52.0±4.5[c]	16.60±1.1[b]	0.6±0.3[a]	55.0±7.8[a]

Means of different asterisks are significantly different at $P < 0.05$.

estimating copper level in serum, liver and muscles of rats. Moreover the acute LD_{50} was determined in mice.

MATERIALS AND METHODS

Acute LD_{50} of copper nicotinate in mice

Groups of 10 mice (25.0 ± 2 g b. wt.) were orally administered copper nicotinate in graded increased doses from 250 to 2000 mg/kg. b. wt. Another set of groups were injected copper nicotinate intraperitoneally in graded increased doses from 10 to 310 mg/kg b. wt. Mice were observed for morbidity or signs of discomfort. Mortality rate over 24 h was recorded in each group according to Gad and Weil (1982).

Effect of prolonged administration of copper nicotinate on serum constituents and blood profile of rats

Five groups of Sprague Dawley rats (250 ± 5 g b. wt.) each of five animals were reared under strict hygienic measures. The 1st group was kept as control receiving 2% acacia watery solution (suspending agent of the drug). Rats of the 2nd and 3rd group were given orally copper nicotinate daily at doses of 0.8 and 4.0 mg/kg b. wt. for the same period. Blood samples were collected every 2 weeks from the orbital plexus of veins in clean tubes with few drops of EDTA for complete blood picture. Another blood sample was taken for separation of clear serum. Serum transaminases (GOT and GPT) were determined according to Sherlock (1951) using comercial kits from Randox laboratories (United Kingdom). Urea, createnine and cholesterol concentrations were determined in serum according to (Abell et al., 1952).

Effect of prolonged administration of copper nicotinate in rats on serum, liver and muscle copper level

Two groups of Sprague Dawley rats each of five animals were given orally copper nicotinate daily at doses of either of 0.8 and 4.0 mg/kg b. wt. for 10 weeks. Another group was kept as control receiving equal volumes of 2% acacia watery solution (suspending agent of the drug). At the end of the experiment rats were sacrificed. Blood was collected, serum was separated and liver and muscle samples were taken for estimation of copper levels. Copper level was estimated in liver and muscle according to Mehmet and Akdeniz (2004). Serum copper was estimated colorimetrically according to Abe et al. (1989) using commercial kit from EliTech diagnostics (France).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Differences between control and treated groups were tested for significance using a one- way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to Snedecor and Cochran (1986).

RESULTS

Acute LD_{50} of copper nicotinate in mice

The LD_{50} for copper nicotinate was 1104.17 and 128.33 mg/kg after oral and intraperitoneal administration respectively. Oral doses of 500 mg/kg or less caused no fatalities and doses of 1750 mg/kg orally killed all animals. Doses of 40 mg/kg i.p. or less caused no mortalities and doses of 310 mg/kg i.p. killed all animals.

Effect of prolonged administration of copper nicotinate on serum constituents and blood profile of rats

Oral administration of copper nicotinate at doses of 0.8 or 4.0 mg/kg b. wt. for 6 weeks did not affect GOT, createnine or cholesterol in serum of rats. However GPT was increased and urea was decreased by the large dose only (Table 1). Oral administration of copper nicotinate at doses of 0.8 or 4.0 mg/kg b. wt. for 8 weeks significantly ($P < 0.05$) increased GOT, GPT, urea, creatinine, and cholesterol levels in the serum of rats (Table 2).

Oral administration of copper nicotinate at doses of 0.8 mg/kg b. wt. for 6 weeks decreased esinophiles and monocytes and increased lymphocytes counts but did not affect any of the other blood parameters. Oral administration of copper nicotinate at doses of 4.0 mg/kg b. wt. for 6 weeks resulted in decreased RBCS, WBCs, platelets, eosinophiles and monocytes but increased lymphocyte counts (Table 3). Oral administration of copper nicotinate at doses of 0.8 mg/kg b. wt. for 8 weeks resulted in decreased platelet counts, eosinophiles and monocytes and increased lymphocyte counts but did not affect any of the other blood parameters. Oral administration of copper nicotinate at doses of 4.0 mg/kg b. wt. for 8 weeks resulted in decreased RBCS, WBCs, platelets, esinophiles and monocytes but increased lymphocytes counts (Table 4).

Table 2. Effect of copper nicotinate on serum transaminases, urea, creatinine and cholesterol after oral administration for 8 weeks in rats (Mean ± SD, n=5). constituents.

	GOT I.U./l	GPT I.U./l	Urea mg/dl	Creatinine mg/dl	Cholesterol mg/dl
Control	115.0±19.4 [a]	35.0±4.5 [a]	17.0±1.6 [a]	0.6±0.3 [a]	50.0±2.8 [a]
Copper nicotinate (0.8 mg/kg)	121.0±21.9 [a]	42.4±2.9b	20.0±2.9 [ab]	0.7±0.1 [a]	65.0±2.1 [b]
Copper nicotinate (4.0 mg/kg)	151.0±22.5 [b]	42.0±1.9 [b]	21.2±2.3 [b]	0.8 ± 0.1 [a]	65.0±2.7 [b]

Means of different asterisks are significantly different at P < 0.05.

Table 3. Effect of copper nicotinate after oral administration for 6 weeks on blood picture in rats (Mean ± SD, n=5).

	RBCS X 10^6/mm^3	HBg/dl	WBCSX10^3/mm^3	PLT X10^3/mm^3	Eosino %	Seg %	Lymph %	Mono g/dl
Control	6.2±0.2 [b]	13.1±0.8 [a]	11.1±0.5[c]	620.0±59.6 [b]	6.0±1.2 [a]	13.0±4.3[a]	84.0±4.7 [a]	3.0±0.7 [b]
Copper nicotinate(0.8 mg/kg)	6.3±0.4 [b]	11.0±2.2 [a]	8.7±0.7 [b]	610.0±41.8 [b]	1.0±0.0 [b]	12.2±4.1[a]	91.0±1.4[b]	1.0±1.0 [a]
Copper nicotinate (4.0 mg/kg)	5.6±0.5 [a]	12.2±1.0 [a]	7.4±0.6 [a]	483.0±51.7 [a]	0.0	13.0±3.2[a]	92.0±2.5[b]	1.0±0.0 [a]

Means of different asterisks are significantly different at P < 0.05.

Table 4. Effect of copper nicotinate on blood picture after oral administration for 8 weeks in rats (Mean + SD, n=5).

	RBCS X10^6 /mm^3	HB g/dl	WBCSX10^3 /mm^3	PLT X10^3 /mm^3	Eosino %	Seg %	Lymph %	Mono g/dl
Control	6.3±0.4 [b]	14.2±0.8 [a]	17.9±4.3 [b]	600.0±67. [b]	3.0±1.2 [a]	15.6±3.3[a]	76.2±16.3 [a]	2.6±0.9 [a]
Copper nicotinate (0.8 mg/kg)	6.1±0.5 [b]	13.7±2.5 [a]	16.1±1.5[ab]	500.0±55.2 [a]	4.6±1.3 [a]	15.8±3.6[a]	87.0±1.4 [b]	1.4±0.5 [a]
Copper nicotinate (4.0 mg/kg)	5.4±0.3 [a]	13.4±0.7 [a]	13.2±0.8 [a]	430.0±23.2 [a]	2.2±1.3 [a]	16.6±3.4[a]	89.8±4.4 [b]	1.4±1.3 [a]

Means of different asterisks are significantly different at P < 0.05.

Effect of prolonged administration of copper nicotinate in rats on serum, liver and muscle copper level

The copper level in control non treated rats ranged from 192 ± 8.4 to 236.6 ± 27.6 µg/dl. After administration of copper nicotinate at dose of 0.8 and 4.0 mg/kg its serum level increased to a range from 278.2 ± 32.6 (P>0.05) to 340.0 ± 35.2 µg/dl and ranged from 350.0 ± 42.7 µg/dl to 408 ± 38.0 µg/dl after administration of the large dose (4.0 mg/kg b. wt.) (Figure 1).

The copper concentration in liver tissue of normal rats ranged from 37.2 ± 8.6 to 52. 3 ± 1.9 ppm. Liver of rats treated with copper nicotinate in a dose of 0.8 mg/kg b. wt. for 2 weeks was 57.3 ± 6.2 ppm. This level decreased to 22.3 ± 5.2 ppm within the next two weeks and remained almost at this level for up to the 10^{th} week. Liver of rats treated with copper nicotinate at a dose of 4.0 mg/kg for 2 weeks was 53.3 ± 2.1 ppm. This level decreased to41.0 ± 8.6 with no significant difference and remained almost at this level for up to the 10^{th} week (Figure 1).

Copper level in muscle of normal rats ranged from 21.0 ± 7.2 to 31.5 ± 6.9 ppm. Muscles of rats orally administered copper nicotinate at a dose of 0.8 mg/kg b. wt. was 35.5 ± 9.9 ppm after 2 weeks but decreased to27.5 ± 4.6 ppm after 6 weeks of administrations and remains almost at this level for up to the 10^{th} week (Figure 1).

DISCUSSION

The present data clearly demonstrate that oral administration of copper nicotinate at doses of 0.8 or 4.0 mg/kg for 6 weeks did not significantly alter liver enzymes, urea, creatinine and cholesterol. The mild increase in the GPT following the large dose (4.0 mg/kg) and the non significant change in GOT indicates little or no adverse effect on liver function when copper nicotinate was given for 6 weeks. Similar observations have been reported in human by Araya et al. (2005) who found that liver enzyme activities in individuals received a single daily dose of 10 mg Cu for 60 days, remained below the clinical cutoff value used to diagnose liver dysfunction. The authors concluded that the elevation in aminotransferases is transient and mild. Also, there were no significant alteration in li-

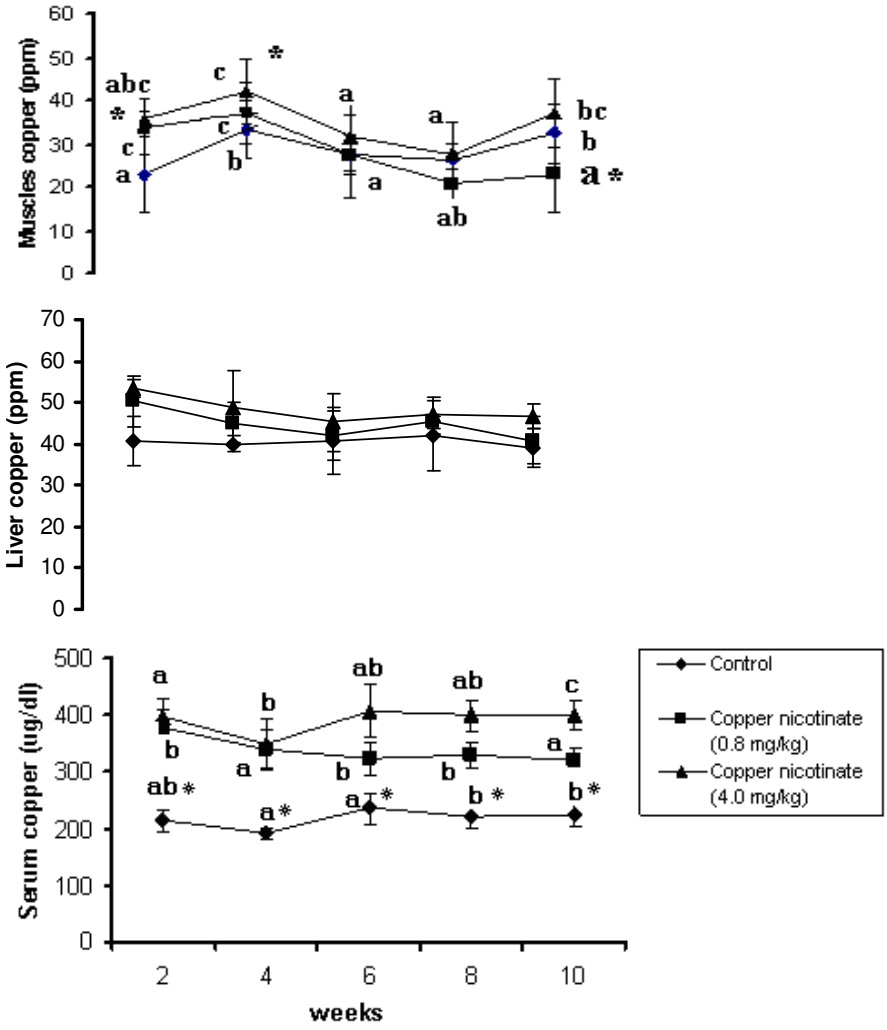

Figure 1. Serum, liver and muscles copper oral administration of copper nicotinate at 0, 0.8 and 4 mg/kg for 10 weeks in rats (mean + SD, n=5). A, b, c: values with different asterisk are significantly different compared to other weeks. ∗Significant at p>0.05 compared to other groups.

ver function after 6 weeks of copper intake at highest level of 7 mg/day in healthy adults (O'Connor et al., 2003).

In animal studies, it has been reported that administration of copper (II) complexes in rats caused a significant increase in superoxide dismutase activity without changing other biochemical parameters (alkaline phosphatase, GOT and GPT, blood urea, creatinine, total cholesterol, total protein and globulin) compared with the corresponding values of the normal control rat group (Abou-Seif et al., 2003). In chickens, copper toxicity is partially dependent on the copper salt formula, for example tribasic copper chloride is a safer product and more available to broilers than copper sulfate, and it is chemically less active than copper sulfate in promoting the oxidation of vitamin E in feed (Luo et al., 2005). The present data add additional evidence that copper nicotinate is safe for use at the used levels for at least 6 weeks. In this respect copper nicotinate has been proved to maintain liver function against the most frequent toxicities associating 5-fluro-uracil administration as indicated by significant reduction of serum bilirubin, transaminases and alkaline phosphatase (El-Saadani, 2004). Moreover, Cu(2)(DIPS)(4)L(2) complexes (L=diethylether, N,N-dimethylformamide) exhibited no toxicity when examined for anticonvulsant activity using the seizure produced by maximal electroshock, following oral administration to rats (Viossat et al., 2002). Cisternas et al. (2005) reported that supplementation with 1,200 ppm of Cu in rat food for 16 weeks induced mild light microscopy alterations in Cu-treated rats. The authors concluded that subchronic Cu loading in young rats induces early hepatic morphological changes, with enhancement in Kupffer cell-dependent respiratory burst

activity and NF-kappaB DNA binding, cellular responses that may prevent or alleviate the hepatotoxicity of the metal. Moreover the lack of significant effect of copper nicotinate administered for 6 weeks on cholesterol level is consistent with the findings of Rajendran et al. (2007) who reported that copper is less likely to play a role in the promotion of atherosclerosis than other elements.

In sheep drenched 3 mg/kg b. wt. copper daily for a week and an additional 3 mg/kg b. wt. every week until signs of toxicity appear, the onset of copper poisoning occurred between 42 and 55 d. AST activity increased only 14 days before the hemolytic crisis (Ortolani et al., 2003).

The present data indicate that the adverse effects of prolonged copper administration are dose-and duration-dependant since GOT, GPT, urea, creatinine and cholesterol as well as the cellular constituents of blood were significantly elevated after 8 weeks and the effects were prominent with the large dose. The increased lymphocyte count was similar to the finding of Turnlund, et al. (2004).

The copper level in control non treated rats ranged from 192±8.4 to 236.6±27.6 □g/dl. Several factors can affect serum copper including inflammation, infectious diseases and age (Coudray et al., 2005).

The present data showed a marked increase in serum copper of rats following oral administration of 0.8 and 4.0 mg/kg b. wt. The increase in serum copper is mostly due to increased hepatic synthesis and release of ceruplasmin (Linder, 1991) and is to some extent consistent with those of (Danks, 1988; Turnlund et al., 1999) who reported an elevated serum copper after administration of copper to a copper deficient person.

In contrast copper supplementation studies have shown that indexes of copper status do not tend to be influenced by additional dietary copper. For example copper status did not change in healthy infants supplemented with copper (Salmenpera et al., 1989) or adult men supplemented with 2 or 3 mg cu / day (Jones et al., 1997) or those supplemented with ≥6 mg/ day for 6- or 8-weeks (Kehoe et al., 2000). Moreover copper indexes did not change after feeding diets containing 7.8 mg Cu/day for 24 days (Turnlund et al., 1999). There were no changes in plasma copper level after a high copper intake for 5 months, although an increase in some other copper indexes was observed (Turnlund et al., 2004). This difference is probably due to dose difference, since the small doses used in our study is 0.8 mg/kg, 7-8 times the maximum doses in other studies (Turnlund, et al., 1999).

The copper concentration in liver tissue or muscle tissue was not significantly changed after oral administration of copper nicotinate at doses of 0.8 or 4.0 mg/kg for 6 or 8 weeks. It has been previously (Coudray et al., 2005) reported that plasma Cu level increased with age whereas liver and bone Cu levels and urinary Cu excretion remained unchanged. Homeostatic regulation of copper absorption and retention helped to minimize the amount of copper retained with high copper intake (Turnlund et al., 2004). Two mechanisms have been suggested to protect against copper toxicity; sequesteration in the metal binding metallothioneins or copper-translocating ATPase (Dameron and Harrison, 1998). The previously discussed mild changes correlate with the high oral LD_{50} (1104.17 mg/kg) of copper nicotinate in mice.

In conclusion, prolonged oral administration of a high dose of 4 mg/kg b.wt. of copper nicotinate for 8 weeks to rats causes only minimal hematological changes and minor serum biochemical alterations. The orally administered dose of copper nicotinate to rats approximately equals 7 to 8 times the maximum copper intake in adult man. These findings indicate the safety of copper nicotinate complex. Moreover, the mild adverse effects of copper in rats correlate with the high oral LD50 (1104.7 mg/kg) that reported in mice.

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