The antioxidant effect of different doses of chromium chloride supplementation in rats

Ibrahim A. I.; El- Ghannam, A. A.; Abeer A. Shalaby and Hoda I. Bahr
Department of Biochemistry, Faculty of Vet. Medicine, Suez Canal University

Abstract

The present work was done to elucidate the possible protective and antioxidant effect of low and high doses of chromium chloride supplementation in rats. The experiment was carried out on 63 adult male Sprague Dawley rats weighing 150-200 g. Rats were randomly divided into three equal groups each had 21 rats as follow; group I (control), group II (low dose) of chromium chloride (0.8 µg/ml drinking water) and group III (high dose) of chromium chloride (4 µg /ml drinking water). Blood samples were collected after 2, 4 & 6 weeks for assessment of erythrocytes antioxidants. Glutathione (GSH) content and the activity of superoxide dismutase (SOD) and catalase (CAT) as well as glutathione S-transferase (GST) were elevated by both doses of Cr than control during the whole period of experiment. Moreover, an increase in glutathione peroxidase (GSH-px) activity was observed after 4&6 weeks in chromium low dose (CLD)-treated group and during whole period of the experiment in chromium high dose (CHD)-treated group. It could be concluded that, administration of chromium at high dose (CHD) reveals the potent antioxidant. Key words: Chromium chloride, dose, antioxidant effect, rats.

Introduction

Chromium is a naturally occurring heavy metal found commonly in the environment as trivalent and hexavalent form *(Shrivastava et al, 2002)*. Commercially available trivalent chromium compounds are organic and inorganic. Chromium chloride (CrCl) is considered as an inorganic salt *(World Health Organization, 2009)*.

It has been known to be a micronutrient for mammals. The highest concentration of chromium was found in meat products, followed by oils and fats, bread nuts, miscellaneous cereals, fish, sugar, Brewer's yeast, mushrooms, carrots, potatoes, broccoli and spinach *(Eisenberg et al, 1998 and O'Connell, 2001 and Expert Group on Vitamins and Minerals, 2003)*. CrCl decreases tumor necrosis factor-α (TNF-α) secretion, oxidative stress and lipid peroxidation in both high glucose and H_2O_2 cultured U937 monocytic cells has antioxidant effect *(Jain and Kannan, 2001)*. Additionally, oral supplementation of CrCl (250 µg/kg body weight) increases lung glutathione activity in hyperlipemic rats (*Yanardag et al, 2005*). Additionally, the antioxidant effect of Cr can be observed through decreasing serum iron and total iron binding capacity. As Cr, Fe compete on B-site of transferrin (*Stearns, 2000*). It was suggested that higher doses of chromium reveal more potent anti-diabetic, anabolic and antioxidant effect (*Clodfelder et al, 2005; Sreejayan et al, 2008; Siripurkpong and Na-Bangchang,*

2009). Trivalent chromium is highly safe and has no genotoxic effect but has antioxidant and antiapoptotic activity *(Preuss et al, 2008 and Chen et al, 2010)*

Material and Methods

Sixty three adult male Sprague Dawley rats weighing 150-200 g were obtained from Research Center in Faculty of Veterinary Medicine (Zagazig University). They were housed in cages, fed on a balanced ration and water ad libitum and kept one week for accommodation before beginning of the experiment. Rats were randomly divided in to 3 equal groups each had 21 rats as follow; *Group I* was served as control, *Group II* received low dose of chromium chloride (0.8 µg/ml of drinking water) and *Group III* received high dose of chromium chloride (4 µg /ml of drinking water). Blood samples were collected from the medial canthus of the eye in control and other groups after 2, 4 and 6 weeks post administration and taken with anticoagulant (Heparin) to separate RBCs for determination of GSH content *(Beutler et al, 1963), SOD (Nishikimi et al, 1972),* CAT *(Aebi, 1984 and Fossati et al, 1980),* GST *(Habig et al, 1974)* and GSH-px activity *(Paglia and Valentine, 1967).* All data were subjected to statistical analysis by F-test and LSD *(Snedecor and Cochran, 1982).*

Results and Discussion

Table 1 shows a marked increase in erythrocytes GSH content after 4 and 6 weeks in chromium low dose-treated group and during whole period of the experiment in chromium high dose-treated group. This may be due to the possibility that, chromium chloride has anti-inflammatory properties where it inhibits TNF-α secretion which impairs GSH production *(Jain and Kannan, 2001 and Hinson et al, 2004).*

Table (1). Effect of different doses of chromium chloride on erythrocytes GSH content (mg/dL).

Duration / Group	2 weeks	4 weeks	6 weeks
Group I	36.94 ± 0.66^{aA}	35.66 ± 0.33^{aA}	34.83 ± 0.47^{aA}
Group II	40.43 ± 0.32^{bA}	55.45 ± 1.42^{dB}	66.94 ± 0.42^{dC}
Group III	51.25 ± 0.69^{dA}	61.44 ± 0.93^{eB}	74.97 ± 0.59^{eC}

Values represent mean ± SE. Different small letter in the same column means significant difference while same letters means no significance. Different capital letters in the same raw means significant difference. *Group I*= Rats served as control, *Group II*= Rats received low dose of chromium chloride (0.8 µg/ml of drinking water), *Group III*= Rats received high dose of chromium chloride (4 µg /ml of drinking water).

Additionally, Cr increases serum vitamin C by stimulation of L-ascorbic acid synthesis from L-gulonolactone in the liver of rats. Vitamin C scavenge superoxide and hydroxyl radicals, thus it protects GSH against consumption by H_2O_2 *(Chat-*

terjee et al, 1973; Krinsky, 1992 and Sahin et al 2003).

Table 2 reveals an increase in erythrocytes SOD, CAT, GST in both chromium low dose-treated group and chromium high dose- treated group than control during whole period of the experiment and in GSH-px activity after 4 and 6 weeks in low dose-treated group and during whole period of the experiment in chromium high dose-treated group. Clearly, chromium supplementation causes an increase in Cu and Zn level in blood that activates liver synthesis of Cu/ Zn- SOD

(Sahin et al, 1999; Pechova et al, 2002). Furthermore, chromium chloride could inhibit H_2O_2 that inhibit SOD activity

(Ceballos et al, 1992; Jain and Kannan, 2001; Hininger et al, 2007). There was an evidence that, Cr is able to increase catalase activity *(Atac et al, 2006).* This probably contributes to that, an increase in the activity of superoxide dismutase and quenching of superoxide radical might indirectly impart a protective effect on the activity of catalase and GSH-Px *(Ramachandran et al, 2004).* The increase in GST activity can be viewed on the host cell response in boosting up the GSH related conjugation system against the possible free radical mediated system. Since the concentration of GSH affect the activity of GSH-Px and GST *(Shanthi and Ramakrishnan, 1994; Kanna et al,* 2005). It is likely that, within cells SOD, GSH-px and CAT remove $O_2^{\cdot-}$ and H_2O_2 before they approach available promoters of Fenton chemistry for hydroxyl radical production *(Chattopadhyay et al, 2005).* Recently, *Chen et al, 2009* confirmed that Cr plays a potential regulatory role in Cu/Zn-SOD, CAT and GSH-px gene expression

Table (2). Effect of different doses of chromium chloride on erythrocytes enzymatic antioxidants in rats.

	SOD activity (U/ ml blood)			CAT activity (U/ml blood)			GSH-px activity (U/ ml blood)			GST activity (U/L blood)		
Duration / Group	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
Group I	232.4± 2.33^{aA}	229± 1.88^{aA}	226.1± 3.13^{aA}	593± 8.90^{aA}	579.5± 5.42^{aA}	545.7± 7.46^{aA}	42.2± 1.88^{aA}	39.5± 2.35^{aA}	38.6± 2.92^{aA}	350.3± 3.30^{aA}	347.1± 2.73^{aA}	343.1± 4.51^{aA}
Group II	260.4± 1.71^{cA}	291.9± 1.68^{dB}	329.8± 1.42^{dC}	650.4± 4.92^{cA}	706.3± 6.59^{dB}	828.9± 4.39^{dC}	44.5 ± 1.77^{aA}	54.8± 2.14^{cB}	68.4± 1.77^{dC}	382.4± 3.88^{cA}	423.4± 4.20^{dB}	476.8± 4.63^{dC}
Group III	277.9 ± 2.12^{dA}	312± 2.76^{cB}	364.5± 1.14^{cC}	762.7± 8.55^{dA}	835± 4.01^{cB}	910.2± 4.43^{cC}	51.4± 2.12^{cA}	62.5± 1.50^{dB}	77.8± 2.75^{cC}	410.2± 6.93^{dA}	450.3± 6.51^{cB}	492.9± 8.84^{cC}

Values represent mean ± SE. Different small letter in the same column means significant difference while same letters means no significance. Different capital letters in the same raw means significant difference. *Group I*= Rats served as control, *Group II*= Rats received low dose of chromium chloride (0.8 µg/ml of drinking water), *Group III*= Rats received high dose of chromium chloride (4 µg /ml of drinking water).

References

Aebi, H. (1984): Catalase in vitro. Methods Enzymol 105: 121-126.

Atac, I. A.; Peksela, A.; Yanardag, R.; Sokmen, B. B.; Doger, M. M and Bilen, Z. G. (2006): The effect of combined treatment with niacin and chromium (iii) chloride on the different tissues of hyperlipemic rats. Drug and chemical toxicology. 29 (40): 363-377.

Beutler, E.; Duran, O. and Kelly, B. (1963): Improved method for the determination of blood glutathione. J. of lab. And clinic. Med. 61: 882.

Ceballos-Picot, I.; Jean-Marc, T.; Nicole, A.; Pierre-Marie, S. and Thevenin, M. (1992): Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytess. Clin.Chem.38 (1): 66-70.

Chatterjee, G. C.; Roy, R. K.; Sasmal, N.; Banerjee, S. K. and Majumder, P. K. (1973): Effect of chromium and tungsten on L-ascorbic acid metabolism in rats and chicks. J. Nutr. 103:509–514.

Chattopadhyay, M. B.; Mukherjee, S.; Kulkarni, I.; Doloi, V.; Manika, K.; and Chatterjee, M. (2005): Proton-Induced X-ray Emission (PIXE) Analysis and DNA-chain Break study in rat hepatocarcinogenesis: A possible chemopreventive role by combined supplementation of vanadium and beta-carotene. Cancer Cell International. 5:16.

Chen, W. Y.; Chun-Jung, C.; Chia-Hsin, L. and Frank, M. C. (2010): Chromium attenuates high-fat diet-induced nonalcoholic fatty liver disease in KK/HlJ mice. Biochemical and Biophysical Research Communications 397: 459–464.

Chen, W. Y.; Chun-Jung, C.; Jiunn-Wang, L.; Frank, C. M. (2009): Chromium attenuates hepatic damage in a rat model of chronic cholestasis. Life Sciences. 12411: 9.

Clodfelder, B.J.; Gullick, B.M.; Lukaski, H.C.; Neggers, Y. and Vincent, J.B. (2005): Oral administration of the biomimetic [Cr3O $(O2CCH2CH3)_6(H2O)_3]+$ increases insulin sensitivity and improves blood plasma variables in healthy and type 2 diabetic rats. Journal of Biological Inorganic Chemistry 10:119–130.

Eisenberg, D.M.; Davis, R.B.; Ettner, S.L.; Appel, S.; Wilkey, S.; Van, R. M. and Kessler, R.C. (1998): Trends in alternative medicine use in the United States, 1990–1997. Results of a follow-up national survey. JAMA. 280:1569–1575.

Expert Group on Vitamins and Minerals (2003): Safe upper levels for vitamins and minerals. Part 3. Trace ele-

ments. Food Standards Agency of the United Kingdom, EVM, pp. 174–179.

Fossati, P.; Prencipe, L. and Berti, G. (1980): Use of 3.4 - dichloro-2–hydroxybenzene-sulfonic acid/4 - aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem. 26 (2):227-231.

Habig, W. and Pabst, M. and Jakoby, W. (1974): The first enzymatic step in mercapturic acid formationJ. Biol. Chem. 249: 7130- 7139.

Hininger, I.; Benaraba, R.; Mireille, O.; Faure, H.; Roussel, M. A. and Anderson, A. R. (2007): Safety of trivalent chromium complexes: No evidence for DNA damage in human HaCaT keratinocytes. Free Radical Biology & Medicine. 42: 1759–1765.

Hinson, J.A.; Reid, A.B.; Mc-Cullough, S.S. and James, L.P. (2004): Acetaminophen induced hepatotoxicity: role of metabolic activation, reactive oxygen/ nitrogen species, and mitochondrial permeability transition. Drug Metab. Rev. 36 (3–4): 805–822.

Jain, S.K. and Kannan, K. (2001): Chromium Chloride Inhibits Oxidative Stress and TNF-a Secretion Caused by Exposure to High Glucose in Cultured U937 Monocytes. Biochemical and Biophysical Research Communications 289: 687–691.

Kanna, P.; Saralaya, M.; Samanta, K. and Chatterjee, M. (2005): Vanadium inhibits DNA- protein cross-links and ameliorates surface level changes of aberrant crypt foci during 1, 2- dimethylhydrazine induced rat colon carcinogensis. Cell Biology and Toxicology. 21: 41-52.

Krinsky, N.I. (1992): Mechanisms of action of biological antioxidants. Proc. Soc. Exp. Biol. Med. 200-248.

Nishikimi, M.; Rao, N.A. and Yogi, K. (1972): The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research . Common. 46: 849– 854.

O'Connell, B.S. (2001): Select vitamins and minerals in the management of diabetes. Diabetes Spectrum.14:133–148.

Paglia, D. E. and Valentine, W. N. (1967): Studies on quantitative and qualitative characterization of erythrocytess glutathione peroxidase. J. Lab. Clin. Med. 70: 158-169.

Pechova, A.; Illek, J.; Sindelar, M. and Pavlata, L. (2002): Effects of chromium supplementation on growth rate and metabolism in fattening bulls. Acta Veterinaria Brno. 71: 535–541.

Preuss, G. H.; Echard, B.; Perricone, V. N.; Bagchi, D.; Yasmin, T. and Stohs, J. S. (2008): Comparing metabolic effects of six different commercial trivalent chromium compounds. Journal of Inorganic Biochemistry. 102: 1986–1990.

Ramachandran, B.; Ravia, K.; Narayananb, V.; Kandaswamyb, M. and Subramanian, S. (2004): Effect of macrocyclic binuclear oxovanadium complex on tissue defense system in streptozotocin-induced diabetic rats. Clinica. Chimica. Acta. 345: 141–150.

Sahin, K.; Sahin, N. and Kucuka, O. (2003): Effects of chromium and as-

corbic acid supplementation on growth, carcass traits, serum metabolites and antioxidant status of broiler chickens reared at a high ambient temperature. Nutr. Res. 23: 225-238.

Sahin, K.; Talat, G.; Sahin, N.; Ertas, O. N. and Erkal, N. (1999):The Effect of chromium added into basal diet on serum total protein, urea, triglyceride, cholesterol and serum and tissue chromium, zinc, copper levels in rabbits. Tr. J. of Veterinary and Animal Sciences. 23:109-113.

Shanthi, V.P. and Ramakrishnan, P. (1994): Mechanism of antioxidant effect of Bordetella pertussis extract. Indian J Biochem Biophys. 31: 398– 402.

Shrivastava, R.; Upreti, R.K.; Seth, P.K. and Chaturvedi, U.C. (2002): Effects of chromium on the immune system. FEMS Immunology and Medical Microbiology. 34:1-7.

Siripurkpong, P. and Na-Bangchang, K. (2009): Effects of niacin and chromium on the expression of ATP-binding cassette transporter A1 and apolipoprotein A-1 genes in HepG2 cells.Journal of Nutritional Biochemistry. 20: 261–268.

Snedecor, G. W. and Cochran, W.C. (1982): Statistical methods. 7th Edition, Iowa State Univ.Press, Iowa, pp.507.

Sreejayan, N.; Dong, F.; Machender, R.; Kandadi, Y. X. and Ren, J. (2008): Chromium Alleviates Glucose Intolerance, Insulin Resistance, and Hepatic ER Stress in Obese Mice. Obesity. 217 (6): 1331–1337.

Stearns, D M. (2000): Is chromium a trace essential metal? BioFactors. 11: 149-162.

World Health Organization (2009): Inorganic chromium (iii) compounds.Concise International Chemical Assessment Document 76, WHO.

Yanardag, R.; Peksel, A.; Yesilyaprak, B.; Doger, M. M and Atac, A. I. (2005): Effects of a combination of niacin and chromium (III)-chloride on the skin and lungs of hyperlipemic rats. 103 (3): 249-26

تاثير الجرعات المختلفة من كلوريد الكروميوم كمضاد للاكسدة في الفئران

أ.د/ إبراهيم عاشور إبراهيم،، أ.د/ عبد الرحيم احمد الغنام، د/ عبيرعبد الحميد شلبى ،
ط.ب/ هدى إبراهيم بحر حسونة.
قسم الكيمياء الحيوية- كلية الطب البيطري- جامعة قناة السويس.

الملخص العربى

تم اجراء العمل الحالي لدراسة التأثير العلاجي للجرعة المنخفضة والعالية لكلوريد الكروميوم في الفئران. أجريت الدراسة على ٦٣ فأر أبيض ذكر يزن١٥٠ –٢٠٠جم قسمت الى ثلاثة مجموعات كالتالى:- (مجموعة ضابطه – كلوريد الكروميوم (٠,٨ ميكروجم/ ميليلتر مِنْ ماء الشربِ)- كلوريد الكروميوم (٤ ميكروجم/ ميليلتر مِنْ ماء الشربِ). وجد زيادة فى محتوى الجلوتاثيون المختزل و نشاط الجلوتاثيون الاس الناقل و الكتاليز والسوبر اوكسيد ديسميوتيز في كرات الدم الحمراء طول فترة التجربة مع زيادة فى نشاط الجلوتاثيون البيرواكسيديز بعد ٤&٦ أسابيع (فى الجرعة المنخفضة) وطول فترة التجربة (فى الجرعة العالية). يُمْكِنُ أنْ يُستَنتجَ ان كلا من الجرعة المنخفضة و الجرعة العالية لكلوريد الكروميوم يلعبان دورا هاما كمضادات للاكسدة و لكن كان للجرعة العالية التأثير الأقوى معتمدا على الوقت و الجرعة.