

Chromium nanoparticles affect antioxidant activity, hormones and sperm parameters in heat-stressed male rats: a model of mammals

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Abstract

The main objective of this study was to assess the effects of nano-chromium (nCr) administration on plasma antioxidant activity, hormones and sperm quality and quantity in male rats exposed to heat stress. Twenty rats were randomly divided into four treatments with five replicates. Treatments were done 35 days included: C, control group received normal saline as gavage and remained at 21 °C; T1, rats exposed to higher temperature than optimum (35 °C) for 6 h/d considered as heat stress (HS); T2, received nano chromium (80 µg/kg body weight) as gavage and remained at 21 °C, and T3, exposed to HS and received nCr. Rats exposed to heat stress had higher antioxidant enzymes activity compared with the control group ($P < 0.05$). In stressed rats, nCr decreased superoxide dismutase, but had no effect on the activity of glutathione peroxidase. Heat stress resulted in decrease ($P < 0.05$) and administration of nCr resulted in increase ($P < 0.05$) in the levels of FSH and testosterone hormones as compared with control group, but had no effect on LH. Administration of nCr in stressed rats (T3) had no effect ($P > 0.05$) on insulin, cortisol and glucose levels as compared with rats in T1 group. Administration of nCr in stressed rats (T3) increased ($P > 0.05$) sperm concentration, viability and normal sperms, as compared with T1 and the control groups, but nCr had no effect on non-stressed rats (T2). It was concluded that nano-particle of chromium administration to stressed rats could ameliorate the negative effects of oxidative stress on antioxidant capacity, activities and sperm quality and quantity.

Key words: Cortisol; Chromium; Heat stress; Insulin; Rat

Introduction

Chromium (Cr) in the trivalent oxidation state is a trace mineral necessary for livestock animals in some conditions. This trace element functions as a cofactor for regulating the metabolism of carbohydrates, lipids and proteins via insulin function in humans and animals (Haldar *et al.*, 2009; Krzysik *et al.*, 2011). Cr deficiency in healthy humans and animals is unlikely to develop and dietary Cr supplement is usually unnecessary (Lukaski, 1999; Trumbo *et al.*, 2001). However, under stress conditions, chromium supplementation can alleviate the negative effects of heat stress on animal health and performance (Anderson, 1994; Sahin *et al.*, 2002). In the recent studies (McNamara & Valdez, 2005; Moeini *et al.*, 2011), the effects of organic and inorganic sources of Cr on immune response, performance, reproduction parameters and health in animals under heat stress or non-stress conditions were examined. Also, nanoparticles of chromium (nCr) supplementation had beneficial effects on performance parameters and resulting in increases in the muscle Cr concentration. In addition, it was reported that Cr absorption in rats enhanced when diet supplemented with nCr (Zha *et al.*, 2007; Lien *et al.*, 2009). One of the important factors that affect the absorption and utilization of dietary Cr supplement is particle size. When the dimension of particles is reduces to nanometers size, new biological properties of particle appear. In the literature, effect of nCr on reproductive parameters under stress condition still remained unclear. Therefore, the purpose of the current study was to evaluate the effects of nCr supplementation on the male reproductive parameters under heat stress condition.

Materials and methods

Rats used in this study were handled and cared in accordance with the guidelines on laboratory animals approved by the Pasteur institute of Iran, and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch.

Chemicals

Nano-chromium was prepared from American Elements Company (Los Angeles, USA). The Mastersizer particle size and zeta potential analyzer (Malvern Instruments, Malvern, UK) was used to determine the particle size of red elemental nano chromium. Its average particle size was 45 nm with purity of 99.9%. The nano-particles of chromium were suspended in normal saline with 1% stabilizer (sodium carboxymethyl cellulose), stirred for 15 minutes and then dispersed by ultrasonic vibration bath for 20 min. Fresh suspension was prepared before every use to avoid the aggregation of the particles.

Animals and treatments

Twenty male Wistar albino rats (120 g body weight) were obtained from the Pasteur Institute (Tehran, Iran). The rats were placed in polycarbonate plastic cages, fed a standard laboratory pelleted diet for rat and fresh water *ad libitum*. Rats were kept in a clean animal room with air-conditioner. The rats were quarantined for 7 days before starting the experiment. After additional a week for acclimatization to cages environment, rats were randomly divided into four treatments with five replicates. Treatment groups were: C, control group received normal saline as gavage and remained in 21 °C; T1, rats exposed to high temperature (35 °C) for 6 h/d in electrical chamber considered as heat stressed; T2, received nano chromium (80 µg/kg body weight) as gavage and remained in 21 °C, and T3, exposed to heat stress and received nCr. All groups were treated for 35 days and gavage of nCr was done each 48 hour.

Sample collection

Two days after final gavage and injection, feed was withdrawn and rats were fasted overnight with free access to fresh water. Animals were anesthetized with injection (ip) of ketamine-xylazine. Blood samples were collected into heparinized tubes from heart. Then tubes were centrifuged (2500 × g for 15 min) and the plasma as supernatant was collected in the clean tubes and kept at -80 °C for further analysis. Epididymis was removed from adhering tissues and immediately quantitative and qualitative measurements made.

Blood measurements

The plasma activity of superoxidase dismutase (SOD) and glutathione peroxidase (GPx) and levels of reduced glutathione (GSH), malondialdehyde (MDA), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and glucose levels were assayed using standard kits (Pars Azmoon, Tehran, Iran) and spectrophotometry (Shimadzu UV-260, Shimadzu Corp, Tokyo, Japan). Plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by using enzyme-linked immunosorbent assay (ELISA) using commercial kits for rat (Shibayagi Co., Gunma,

Japan) as described in the instructions provided with the kits. The plasma levels of cortisol and insulin were determined using commercially ELISA kits (Biocheck Inc., Foster City, CA, USA).

Sperm measurements

After removing, immediately the caudal epididymis of left testis was minced and the sperm cells extracted into 2 ml pre-warmed Ham's F10 (HamX1, Gibco, UK) culture medium with 10% fetal bovine serum (FBS, Gibco, UK) and kept at 37 °C and 5% CO₂ for 30 min to allow the sperm to swim into the medium and disperse based on the method of Krause (1995). Sperm parameters were evaluated in Royan Institute (Tehran, Iran) with a Computer Assisted Sperm Analyzer (CASA) according to the method of Krause (1995).

Statistical analysis

Kolmogorov-Smirnov test was used to evaluate the normality of obtained data using SAS Software. Thereafter, data was subjected to analysis of variance appropriate for completely randomized design using the general linear model procedures of SAS. Tukey test was used for mean comparison at $P < 0.05$.

Results

The effect of treatments on activities of SOD, GPx, MDA and reduced glutathione levels are shown in Table 1. Rats exposed to heat stress had higher SOD and GPx activities as compared with the control group ($P < 0.05$). In stressed rats, nCr administration (T3) decreased SOD, but had no effect on the activity of GPx. In non-stressed group (T2), there was no difference ($P > 0.05$) with the control group for SOD and GPx activities ($P < 0.05$). The highest MDA level was for heat stressed group and the lowest one was for nCr group ($P < 0.05$). Administration of nCr in stressed rats (T3) decreased ($P < 0.05$) MDA level compared with heat stressed group (T1). The highest level of reduced glutathione was for nCr (T2) and the lowest one was for T1 group ($P < 0.05$). The level of reduced glutathione in plasma of stressed rats received nCr (T3) was higher than those in heat stressed group (T1; $P < 0.05$).

Table 1. Effects of different treatments on the plasma antioxidant capacity and activity

Treatments	Superoxide dismutase IU/ml	Glutathione peroxidase IU/ml	Malondialdehyde $\mu\text{mol/ml}$	Reduced glutathione $\mu\text{mol/ml}$	ALT IU/l	AST IU/l	ALP IU/l
Control	3.17 ^c	2.07 ^b	4.10 ^c	11.4 ^b	26.1 ^b	29.7	42.5 ^b
nCr	2.68 ^c	2.19 ^b	3.51 ^c	13.62 ^a	23.9 ^b	31.6	39.1 ^b
Heat stress	5.72 ^a	3.35 ^a	8.69 ^a	7.06 ^d	42.6 ^a	36.8	66.3 ^a
nCr+HS	4.33 ^b	3.03 ^a	6.55 ^b	8.81 ^c	35.8 ^a	34.0	57.1 ^a
SEM*	0.192	0.173	0.189	0.490	2.09	2.00	2.39
P value	0.001	0.001	0.001	0.001	0.007	0.149	0.001

^{a, b, c, d} Means in column that possess different superscripts differ significantly ($P < 0.05$).

HS: exposed to heat stress, nCr: nano chromium *SEM: total standard error of means

The plasma levels of ALT, AST and ALP are presented in Table 1. Heat stress (T1) increased the levels of ALT and ALP, but had no effect on the level of AST. nCr administration had no effect on the plasma levels of these enzymes in non-stressed (T2) and stressed rats (T3).

Table 2 shows plasma levels of gonadotropins, testosterone, insulin, cortisol and glucose. Heat stress (T1) resulted in decrease ($P < 0.05$) and administration of nCr (T2) resulted in increase ($P < 0.05$) the levels of FSH and testosterone hormones as compared with the control group, but had no effect on LH. The highest measured insulin level was for rats received nCr (T2) and the lowest one was for T1 group ($P < 0.05$). Heat stress (T1) resulted in increase of blood glucose level as compared with the control group. The highest cortisol level was found in T1 group and the lowest level found in the control group. Administration of nCr in stressed rats (T3) had no effect ($P > 0.05$) on insulin, cortisol and glucose levels as compared with T1 group. The effect of different treatments on concentration, viability, motility and normal morphology of sperms are presented in Table 3. The Highest concentration of sperm was seen in the control group and the lowest one in T1 group ($P < 0.05$). nCr administration (T2) had had no effect ($P > 0.05$) on sperm concentration as there is no difference ($P > 0.05$) between nCr and the control group. In stressed rats, administration of nCr (T3) increased ($P > 0.05$) sperm concentration as compared with T1 group. Sperm viability decreased ($P < 0.05$) in the

stressed group (T1) compared with other treatments and nCr administration in stressed rat (T3) resulted in increase of sperm viability. Normal morphology of sperm was the highest in the control group and lowest in T1 group. nCr administration in stressed rats (T3) resulted in increase of normal sperms, but had no effect on non-stressed group.

Table 2. Effects of treatments on the levels of gonadotropin, testosterone and metabolism related hormones

Treatments	FSH ng/ml	LH ng/ml	Testosterone ng/ml	Insulin μU/ml	Glucose mmol/l	Cortisol μg/dl
Control	339 ^{ab}	36.7	1.36 ^{ab}	32.1 ^{ab}	3.66 ^c	42.3 ^b
nCr	397 ^a	39.4	1.69 ^a	34.4 ^a	3.90 ^{bc}	45.7 ^{ab}
Heat stress	288 ^b	33.4	1.20 ^b	26.1 ^b	5.09 ^a	54.5 ^a
nCr+HS	360 ^{ab}	32.7	1.56 ^{ab}	27.2 ^{ab}	4.88 ^{ab}	51.8 ^{ab}
SEM*	19.6	2.05	0.109	1.73	0.252	2.43
P value	0.028	0.159	0.049	0.027	0.090	0.026

^{a, b, c} Means in column that possess different superscripts differ significantly ($P < 0.05$).

HS: exposed to heat stress, nCr: nano chromium

* SEM: total standard error of means

Table 3. Effects of treatments on quantity, viability and morphology of sperms

Treatments	Count 10 ⁶ /ml	Viability %	Motility %	Normal sperm Morphology %
Control	28.9 ^a	82.3 ^a	80.2 ^b	88.3 ^a
nCr	27.1 ^{ab}	75.0 ^a	75.7 ^b	79.6 ^b
Heat stress	19.2 ^b	41.0 ^b	37.5 ^a	54.6 ^c
nCr+HS	29.8 ^a	74.6 ^a	74.0 ^b	76.3 ^b
SEM*	1.93	3.54	5.78	1.58
P value	0.017	0.001	0.002	0.001

^{a, b} Means in column that possess different superscripts differ significantly ($P < 0.05$).

HS: exposed to heat stress, nCr: nano chromium

* SEM: total standard error of means

Discussion

In the present study, activities of SOD and GPx increased in the plasma of rats exposed to heat stress. Under this condition which oxidative stress occurs, the defense mechanisms increase their activities to counteract the stress and antioxidant enzymes are essential for scavenging the reactive oxygen species. Our finding is in agreement with the study of Yang *et al.* (2010), who reported that heat stress induce antioxidant enzymes activities. In contrast, Hosseini-vashan *et al.* (2016) reported that heat stress reduced the activities of GPx and SOD in serum of broilers. The discrepancies exist among different studies concerning the effect of stress on SOD and GPx activities. It seems that SOD and GPx activities depend on exposure duration, intensity and protocol of heat stress.

Nano chromium administration in non-stressed rats (T2) had no effect, but in stressed rats (T3) decreased the activities of GPx and SOD. In line with our results, Mattagajasingh *et al.* (1995) and Dlugosz *et al.* (2012) reported that the activities of GPx and SOD significantly increased and MDA level decreased in the tissues of animals received chromium.

In this study, exposure of rats to heat stress (T1) resulted in increase of MDA and decrease of reduced glutathione. It was suggested that the acute exposure to high ambient temperatures could depress the activity of the mitochondrial respiratory chain (Yang *et al.*, 2010). This event leads to over-production of reactive oxygen species, which finally results in lipid peroxidation and oxidative stress. When stress is

overwhelming, the free radicals suppress antioxidant system, with consequently formed the malondialdehyde and consume GSH and finally damage to cell membrane. This finding are in agreement with many reports (Sahin *et al.*, 2002; Sahin *et al.*, 2005; Hosseini-vashan *et al.*, 2016) who speculated that heat stress resembled by decrease in antioxidant capacities and increase in MDA level of body organs. Rats in stressed group had lower level of reduced glutathione. Reduced glutathione is the electron donor and second substrate for GPx enzyme. This enzyme catalyzes the reduction of hydrogen peroxide to water and alcohol, using reduced GSH (Holmgren *et al.*, 2005).

Nano chromium administration (T2) increased the activities of GPx and SOD, increased GSH level and decreased MDA level. In line with our results, Berenjian *et al.* (2015) reported that MDA level significantly decreased in the tissues of groups fed nano chromium. The Previous studies showed that chromium could inhibit the increase in TNF- α and oxidative stress levels in cultured monocytes exposed to H₂O₂-treated monocytes and it related to the antioxidative effect of chromium. An interesting study showed that chromium supplementation results in a significant inhibition of oxidative stress (Jain & Kannan, 2001). It has been demonstrated that insulin metabolism influence on lipid peroxidation and chromium act as an antioxidant as this element has insulin topic effect (Preuss *et al.*, 1997). There was a decreased in serum MDA level of Japanese quail when chromium was supplemented in the diet in heat stress (Sahin *et al.*, 2002). In heat stress condition, it was reported that chromium supplementation decreased MDA level in the serum, liver and muscle of Japanese quail (Onderci *et al.*, 2005). A reduction in the serum MDA levels may be related to inhibition of epinephrine resulting from insulin topic effect of chromium which inhibits mobilization of lipid (Linder, 1991).

In this study heat stress (T1) increased the levels of ALT and ALP, but had no effect on the level of AST. In a study (Hosseini-vashan *et al.*, 2016), the serum activities of ALT, AST and ALP enhanced in broilers under heat stress. Chromium nanoparticles had no effect on the plasma levels of these enzymes in non-stressed (T2) and stressed rats (T3). In line with our study, Uyanik (2001) found no significant differences in serum ALT and AST levels of lambs received chromium in the diet.

The cause of the reduction in plasma FSH level in rats exposed to heat stress maybe high plasma corticosterone levels as seen in Table 2. High corticosterone can reduce plasma gonadotropin and steroids levels. The axis of HPA is activated in response to heat stress and an increase in blood cortisol levels occurs for adaptation to heat stress. In contrast to our result, plasma cortisol and adrenocorticotrophic hormone levels of heat stressed rats were found to be low (Wang *et al.*, 2015). In this study, nano chromium administration (T2) increased plasma gonadotropin and steroids levels. In line with our finding, Ernst and Bonde (1992) and also Marouani *et al.* (2015) reported an increase in FSH and LH serum levels in chromium supplemented rats. In our study, plasma glucose level increased in stressed rats (T1), and insulin decreased but cortisol increased. There are discrepancies about the effects of heat stress on blood glucose, a report showed an increase (Febbraio, 2001), decrease (Baumgard & Rhoads, 2013) or unchanged (Pearce *et al.* 2013). Differences in species, physiological status and heat protocols may be the reason for these discrepancies. An increase in plasma insulin in lactating cows exposed to heat stress was reported (Tao *et al.*, 2012).

In this study, nano chromium (T2) had no effect on insulin level in stressed and non-stressed rats. In line with our result, several studies (Matthews *et al.* 2003; Hung *et al.*, 2015) reported no effects of nano-chromium or other forms on plasma glucose and insulin. Also, Cr supplementation to the diet resulted in an increase in insulin sensitivity and glucose clearance (Matthews *et al.* 2001; Kim *et al.* 2004).

Heat stress (T1) decreased the quantity and quality of sperms through inducing the oxidative stress. Previous study demonstrated that oxidative stress impaired the motility, concentration, and morphology of sperms. The membrane of mammalian spermatozoa is substantially different from the somatic cells as it made from high levels of polyunsaturated fatty acids which are very sensitive to oxidative damages via lipid peroxidation (Khosrowbeygi & Zarghami, 2007). Thus sperms membranes are susceptible to attack of free radicals which results in decreased the sperm motility, presumably by a rapid loss in pool of intra-cellular ATP. When these events occur, axonemal damage appears which results in decrease of sperm viability and increase in morphological defects of sperm and loss of motility (Kao *et al.*, 2008).

In the present work, chromium administration (T2) resulted in increase the sperms parameters in non-stressed group. In the literature, study concerning effect of nano chromium on sperm quantity and quality was not found. Increase in FSH and insulin sensitivity maybe resulted in sperm quantity and quality.

It was concluded that nano-particle of chromium administration to stressed rats could ameliorate the negative effects of heat stress on antioxidant capacity and activities, also quantity and quality parameters of

sperm. Further studies are required to research the effect of nano chromium on fertilization capability of sperm and other reproductive parameters.

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