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Impact of Nickel Chloride on Wistar Rat Epididymis: A Biochemical Study

KEYWORDS: Epididymis, nickel chloride, lipid peroxidation, hydroperoxides, antioxidants

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ABSTRACT *Nickel, a ubiquitous environmental contaminant, poses a risk to human health. The present study evaluates the effect of nickel chloride as nickel on epididymides of Wistar rats. The rats were provided NiCl2 below LD 50 (250,500 and 1000 ppm) ad libtum for 2- and 4-weeks. Animals were sacrificed after 2- and 4-weeks under light ether anaesthesia. Organ viz. caput and cauda epididymides were excised, trimmed off of extraneous tissues, stored at -70 ˚ C until the assays were performed. A dose dependent increase (P < 0.05) in lipid peroxidation (MDA) and hydroperoxides concentration as well as catalase activity in caput and cauda epididymides was observed. However, total superoxide dismutase, Cu-Zn superoxide dismutase and Mn- superoxide dismutase activities decreased. Further, the total zinc decreased while total copper concentration increased in the epididymides at all doses. The results indicate that epididymides after NiCl2 intake are vulnerable to oxidative stress which could cause dysfunction and may impair fertility.*

Introduction

Industrialization and urbanization has enhanced the exposure of metals which is a major concern for human health. Nickel salts widely used in industries is one of the environmental pollutants (Venu Gopal and Luckey, 1978). Oral exposure of general public to nickel occurs either by (i) ingestion of aerosols or (ii) by indirect exposure – production or processing, the latter contributes to intake via food and drinking water (NTP 1996 a, b, c). Richter and Thies (1980) reported that in aerobic waters at environmental pH, the predominant form of nickel is hexahydrate Ni $(H_2O)_6^{\ 2+}$ ion which complexes with naturally occurring anions as OH^-, SO_4^{2-} and Cl^- to small degree. Ni ^{2} ions have the same radius as Mg^{+2} ions (0.69 and 0.66A° respectively) and similar ligand preferences i.e. for oxygen and nitrogen. Hence, Ni^{+2} can interfere with Mg^{+2} functions in enzymes of nucleic acid synthesis and repair (Beyersman and Hartwig, 2008).

Male reproductive organs are active physiologically and metabolically (Glover and Nicander, 1978). Epididymides has pro-oxidizing environment for the sperm as they are constantly exposed to intra- and extra- cellular production of reactive oxygen species (ROS) (Vernet et al. 2004). Futher, ROS are normally produced by spermatozoa (Aitken and Clarkson, 1987; de Lamirande et al. 1997) but it is controlled process as imbalance may lead to damage to DNA and other macromolecules (Agarwal et al. 2003). Spermatozoa membrane has high concentration of polyunsaturated acids which are vulnerable to free radical attack (Jones and Mann, 1973) and is therefore detrimental as it is associated with loss of motility and decreased capacity for sperm oocyte fusion (Aitken and Curry,2011). Study was therefore undertaken to evaluate the effect of $NiCl_2$ on Wistar rats epididymides assessing the generation of lipid peroxidation, hydroperoxides and potential effects of superoxide dismutase, catalase as well as zinc and copper.

Materials and Methods

Male Wistar rats (180-185g) were housed in polypropylene cages with stainless steel grills, maintained in a well ventilated animal room (12h:12h :: light: dark) and provided standard rat feed (Aashirwad Ltd., Chandigarh) and tap water *ad libitum*. Forty animals were randomly divided into 4 groups of 10 each: control (group1), group 2, group3 and group 4.

Nickel Chloride hexahydrate ($NiCl_2$. $6H_2O$) (CAS registry no. 7718-54-9, Hi-Media) was dissolved in distilled water. Control group animals were given distilled water (vehicle) *ad libitum* while groups 2, 3 and 4 animals were given $NiCl_2$ (250,500 and 1000 ppm) for a period of 2- and 4-weeks in drinking water. The doses were selected below LD_{50} – 112 mg Ni / kg b.wt. It was approved by the Departmental Animal Ethics Committee, Department of Zoology, University of Rajasthan, Jaipur, India. Animals were sacrificed after 2- and 4-weeks under light ether anaesthesia. Organ viz. caput and cauda epididymides were excised, trimmed off of extraneous tissues, stored at -70 ˚ C until the assays were performed.

Biochemical analysis

Estimation of lipid peroxidation (Okhawa et al. 1979), hydroperoxides (Jiang et al. 1992), total superoxide dismutase (Geller and Winge, 1984), Cu-Zn superoxide dismutase (Marklund and Marklund, 1974) and catalase (Sinha, 1972) were carried out spectrophotometrically using UV-VIS 911 GBC spectrophotometer (Australia). Biological trace elements- zinc and copper was estimated using GBC-902 double beam atomic absorption spectrophotometer (Australia).

Statistical Analysis: Data were expressed as mean ± SEM. One Way Analysis of Variance (ANOVA) was performed and if the test were found significant (P<.05) then post-hoc test (Duncan's test) was carried out using Systat software, Bangalore (www.systat.com).

Results

Lipid peroxidation (in terms of malondialdehyde) increased significantly (P<0.05) in caput and cauda epididymis at all dose levels after 2-and 4-weeks of nickel chloride intake. Similar increase in hydroperoxide generation and catalase activity was observed. However, total superoxide dismutase, Cu-Zn superoxide dismutase and Mn superoxide dismutase activities declined which was dose dependent. Biological trace element –zinc decreased significantly (P<0.05) after oral intake of nickel. Concurrently, the concentration of copper increased (P<0.05) in the epididymis at all dose levels (Tables 1 and 2).

Discussion

Extensive use of nickel in modern industries leads to environmental pollution. Coogan, Latta, Snow, and Costa (1989) reported that most of the intake of Ni and Ni compounds are via food and drinking water. Nausea, vomiting, abdominal pain, diarrhea, headache, shortness of breath and giddiness were reported from workers of an electroplating plant who drank water contaminated with nickel chloride and nickel sulfate (1.63g/L) (Sunderman Jr et al. 1988). Aboua, du

Pleiss,and Brooks (2009) studied *in vivo* effect of i.p. administration of organic hydroperoxides (t-butyl hydroperoxides and cumene hydroperoxides) on rat testes and epididymal sperm and reported increased lipid peroxidation and decreased sperm motility. Similar results were obtained leading to ROS generation (Kaur et al. 2006;Kumar et al. 2002) reflecting a correlation between high levels of ROS and sperm motility (Agrawal et al. 2003; Bilodeau et al. 2002).Lipid peroxidation results in the formation of aldehyde products (MDA) leading to generation of free radicals (Shi et al.1998). Similar induction of lipid peroxidation was observed in mice testes with multiple doses of nickel (Doreswamy et al. 2004) as well as rat erythrocytes, human platelets and lymphocytes (Chen et al. 1999; Chen and Lin 2001). Lipid peroxidation affects the membrane-bound proteins, most susceptible are those that have exposed tryptophan and cysteine residue in which the sulphydryl groups reacts with aldehydes and MDA leading to both intra-and inter- molecular cross links (Riley and Behrman,1991).

Huang et al. (1993) reported that nickel increased the level of oxidants in cells which is probably responsible for the ability of the ligand bound Ni^{2+} to be oxidized to Ni^{3+} , followed by the generation of ROS in the cell as Ni^{2+}/ Ni^{3+} oxidation/ reduction are not chemically favorable(Cotton and Wilkinson,1980).Chakrabarti and Bai (1999) reported that lipid peroxidation is caused by the induction of Fenton reaction generating hydroxyl radical. The production of superoxide anion, hydroxyl radical and singlet oxygen is probably from H_2O_2 reacting with Ni (II) complex of glycyl-glycyl-L-histidine (Inoue and Kawanishi, 1989). The study revealed an increase in lipid peroxidation (MDA) and hydroperoxide concentration in caput and cauda epididymides due to its effect on membrane proteins and subsequent generation of H_2O_2 as indicated by increased catalase activity.

Superoxide dismutase function as antilipoprotective defense system (Dacheux et al. 2003; Holland et al. 2003) as spermatozoa transits in the epididymides and dismutates superoxide anion to H_2O_2 and O_2 (Nehru and Anand, 2005).The decreased level of SOD in the present study reflects high level of ROS generation which overwhelmed the antioxidant capacity of total SOD, Cu-Zn SOD and Mn- SOD indicating susceptibility of epididymides to $NiCl_2$ at the selected doses. Several nickel compounds (NiS, Ni_3S_2, NiO and $NiCl_2$) have been shown to increase oxidation of 2' – 7 – dichlorofluorescin (DCFH) suggesting that nickel compounds increased the concentration of oxidants in cultured Hamster Ovary (CHO) cells as only strong oxidants such as H_2O_2 and organic hydroperoxides can oxidize DCFH (Huang et al. 1994). H_2O_2 acts as a suicide substrate at high concentration (> 100 μM) leading to an irreversible inactivation of catalase (Lardinois et al. 1996). In presence of H_2O_2 Nickel (II) ions produce oxidative damage in isolated DNA and chromatin (Llyod and Philips, 1999).Oxidative stress caused by generation of reactive oxygen species would lead to apoptosis effecting DNA of the sperm (Aitken and DeLuliis,2010)with subsequent release of DNA base adducts(Aitken et al. 2013). H_2O_2 can penetrate plasma membrane and cause protein oxidation (Ong et al. 2002). The increase in catalase activity in this present study is indicative of the fact that the H_2O_2 generated by $NiCl_2$ doses must have activated some toxic metabolic pathway leading to activation / inhibition of antioxidant enzymes taken into consideration. Further, there has been a concomitant decrease in organ zinc concentration at all dose levels after 2- and 4-weeks. Zinc has numerous functions which includes its association to finger domains, ~ 300 enzymes having catalytic and regulatory domains, membrane stabilization etc (Maret, 2009). The decrease can be due to (i) alteration of oxidant-antioxidant status as indicative of increased lipid peroxidation and catalase with decreased superoxide dismutase or (ii) hampering of metallothionein (MT)-trafficking protein which maintains release of labile zinc or(iii) nitrosative or oxidative stress depleting zinc due to its release from Zn^{2+} thiolate bonds (Berendji et al. 1997) The decreased zinc concentration would destabilize the membrane and other structures affecting the function of the cell. Copper with iron functions as catalyst for Fenton reactions and catalyzes the peroxidation of membrane lipids (Chan et al. 1982). The present study revealed an increase in copper concentration which exhibited dose and duration relationship and is indicative of $OH^{\cdot}$ formation leading to oxidative stress . Metallothionein may contribute Cu to key cellular copper level with decreased Cu-Zn SOD demonstrating impairment of copper homeostasis. Thus, the accumulation of lipid peroxidation product (MDA) and hydroperoxides would cause membrane instability, loss in membrane permeability with concomitant effect on antioxidant enzymes and biological trace elements zinc and copper leading to impairment of epididymal function which may affect fertility.

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TABLE 1 : Biochemical estimations of caput epididymides of Wistar rats after 2- and 4-weeks of $NiCl_2$ intake (Mean ± SEM)

	2 Weeks				4 Weeks			
	Control	250 ppm	500 ppm	1000 ppm	Control	250 ppm	500 ppm	1000 ppm
Lipid Peroxidation (mm MDA/mg)	4.622 ± 0.145	7.033 ± 0.533*	10.903 ± 0.291*	15.738 ± 0.548*	4.655 ± 0.019	8.303 ± 0.166*	16.138 ± 0.514*	25.683 ± 0.858*
Hydroperoxides (mm hydroperoxides/g)	133.093 ± 0.689	140.665 ± 1.861*	148.575 ± 0.959*	156.698 ± 1.838*	135.992 ± 0.469	143.337 ± 1.562*	151.688 ± 1.132*	166.830 ± 1.062*
Total Superoxide Dismutase (Units/mg protein/hr)	23.582 ± 1.025	19.155 ± 0.262*	12.135 ± 0.281*	8.938 ± 0.170*	21.203 ± 0.142	16.193 ± 0.748*	7.587 ± 0.245*	6.263 ± 0.269*
Cu-Zn Superoxide Dismutase (Units/mg protein/hr)	19.743 ± 0.121	15.133 ± 0.417*	10.430 ± 0.231*	7.320 ± 0.133*	19.825 ± 0.193	10.553 ± 0.125*	6.853 ± 0.267*	4.382 ± 0.084*
Mn Superoxide Dismutase (Units/mg protein/hr)	2.760 ± 0.117	2.113 ± 0.069*	1.634 ± 0.0875*	1.503 ± 0.0037*	2.277 ± 0.107	1.808 ± 0.0170*	0.800 ± 0.051*	0.598 ± 0.049*
Catalase (Kat.f)	0.142 ± 0.0013	0.178 ± 0.00071	0.214 ± 0.0019*	0.318 ± 0.024*	0.130 ± 0.0025	0.247 ± 0.0037*	0.323 ± 0.0041*	0.542 ± 0.0112*
Zinc (mg/g protein)	3.935 ± 0.0099	3.173 ± 0.0033*	2.342 ± 0.0114*	1.640 ± 0.0025*	4.758 ± 0.0074	2.557 ± 0.00211*	1.498 ± 0.0145*	1.140 ± 0.0257*
Copper (mg/g protein)	0.165 ± 0.0034	0.246 ± 0.0015*	0.346 ± 0.00050*	0.418 ± 0.00033*	0.231 ± 0.00033	0.314 ± 0.00051*	0.475 ± 0.00022*	0.533 ± 0.0010*

*** P < 0.05 Significant**
Note: Multiple comparisons of means were performed separately for 2 weeks and 4 weeks sub groups

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TABLE 2: Biochemical estimations of cauda epididymides of Wistar rats after 2- and 4-weeks of $NiCl_2$ intake (Mean ± SEM)

	2 Weeks				4 Weeks			
	Control	250 ppm	500 ppm	1000 ppm	Control	250 ppm	500 ppm	1000 ppm
Lipid Peroxidation (mm MDA/mg)	2.080± 0.028	7.787± 0.316*	9.305± 0.144*	11.607 ± 0.076*	2.175± 0.010	9.307± 0.428*	12.767± 1.122*	17.172± 0.202*
Hydroper-oxides (mm hydroperoxides/g)	137.738± 1.327	142.498± 0.953*	159.480± 0.228*	175.780± 0.982*	141.933± 0.412	155.167± 1.699*	168.775± 0.371*	189.580± 0.401*
Total Superox-ide Dismutase (Units/mg protein/ hr)	19.817± 0.039	14.492± 0.141*	13.487± 0.281*	8.990± 0.330*	18.452± 0.128	12.857± 0.313*	7.408± 0.233	4.010± 0.181*
Cu-Zn Superox-ide Dismutase (Units/mg protein/ hr)	16.720± 0.0132	12.182± 0.138*	10.247± 0.214*	7.180± 0.240*	16.143± 0.199	11.277± 0.462*	6.338± 0.230*	3.365± 0.090*
Mn Superox-ide Dismutase (Units/mg protein/ hr)	8.527 ± 0.090	2.247± 0.133*	1.870± 0.049*	1.607 ± 0.048*	8.112± 0.0855	1.785± 0.058*	1.472± 0.029*	0.833± 0.050*
Catalase (Kat.f)	0.173 ± 0.00098	0.235± 0.013*	0.356± 0.0083*	0.683± 0.0046*	0.187± 0.0017	0.293± 0.0095*	0.505± 0.0268*	1.875± 0.0441*
Zinc (mg/g protein)	4.383 ± 0.00333	3.828± 0.012*	3.257± 0.0033*	2.745± 0.0050*	4.712± 0.0030	3.500± 0.0057*	2.518± 0.0065*	1.755 ± 0.0056*
Copper (mg/g protein)	0.287± 0.000671	0.367± 0.00431*	0.461± 0.00296*	0.558± 0.00060*	0.337± 0.00030*	0.398± 0.00030*	0.532± 0.00115*	0.632± 0.00141*

*** P < 0.05 Significant**

Note: Multiple comparisons of means were performed separately for 2 weeks and 4 weeks sub groups

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