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Protective role of L-ascorbic acid on antioxidant defense system in erythrocytes of albino rats exposed to nickel sulfate

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Abstract

In this experimental study, we investigated whether L-ascorbic acid has any influence on the blood antioxidant defense system, lipid peroxidation and hematological parameters of the albino rats exposed to nickel sulfate($NiSO_4$).Twenty four adult rats were divided into four groups of six animals in each group. The control rats were untreated and comprised Group I. Group II rats were administered nickel sulfate (2.0 mg/100 g b.wt.; intraperitonially, i.p.). Group II rats were treated orally L-ascorbic acid (50 mg/100 g b.wt.) and Group IV rats were given both nickel sulfate and L-ascorbic acid simultaneously on alternate days until the tenth dose. The hematological parameters were assessed: red blood corpuscle counts, packed cell volume %, hemoglobin concentration, white blood corpuscle counts and platelets count decreased significantly and clotting time increased significantly in nickel treated rats. We also observed increase malondialdehyde (MDA) and decrease glutathione level (GSH) in erythrocytes of nickel treated rats. The activities of erythrocyte antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were significantly increased in rats treated with nickel sulfate. Simultaneously treatment of L-ascorbic acid exhibited a possible protective role on the toxic effect of nickel sulfate on the hematological values, erythrocyte MDA and GSH concentrations as well as antioxidant enzymatic defense system.

Introduction

Nickel is a shining, light-colored metal with high electrical and thermal conductivities. It is resistant to corrosion by air, water and alkalis but reacts with dilute oxidizing agents. A major use of nickel is as an alloying element for steel and cast iron, yielding alloys and steel with increased strength and resistance to corrosion and temperature. Nickel compounds are used in nickel–cadmium batteries, in electronic and computer equipments, and as constituent of pigments in the glass and ceramic industries (ATSDR 2003). Typical atmospheric nickel levels for human exposure range from about 5–35 ng/m^3at rural and urban sites, leading to a nickel uptake via inhalation of 0.1–0.7 μg/day, diet 100–300 μg/day and by drinking water 10 μg/l (US NIOSH/OSHA 1985). Globally millions of workers are exposed to nickel containing dust and fumes during welding, plating, grinding, mining, nickel refining, foundries, steel plants and other metal industries (ATSDR 2003).

After entering the body, nickel penetrates all organs and accumulates primarily in bone, liver, kidney (Das & Dasgupta 1998; ATSDR 2003) and excreted through bile and urine (Onkelinx *et al*. 1973). Nickel concentration in the urine of normal human subjects varies from 0.1 to 13.3 μg/l, whereas in the urine of welders, nickel has been found at a concentration of $>$ 188 μg/l (Scansetti *et al*. 1998). Studies by the National Institute of Occupational Health in the United States of America showed that following acute or intermediate duration exposure, the toxicity of different nickel compounds is related to its solubility, with soluble nickel sulfate being the most toxic and insoluble nickel oxide being the least toxic. The difference in the toxicity across compounds is probably due to ability of water-soluble nickel compounds to cross the cell membrane and interact with cytoplasmic protein (ATSDR 2003). It is well known that the toxicitiy due to nickel exposure by inhalation is much complicated by the fact that inhaled insoluble nickel compounds inside the lung for long times and give rise to high local concentration with corresponding severe irreversible effects (Dunnick *et al*. 1995; Costa & Klein, 1999). It is well established that rapidity of toxic effects, exerted by heavy metals is related to blood's transport function, with the blood distributing the metals to all the body parts. Evaluation of toxic effects of the metals is facilitated by results of the basic hematological assays (Brucka-Jastrz *et al*. 2005). Nickel induced alteration of hematological parameters were reported earlier (ATSDR 2003). The effect of certain inorganic and coordinated nickel compounds on the resistance to different destructive substances, rheological properties and functional activities of healthy human red blood cells (RBC) was investigated. It was shown that nickel compounds affect the erythrocytes membrane lipid bilayer, as well as membrane proteins to various extents depending on the type of compounds used (Tkeshelashvili *et al*. 1989; Vest *et al*. 2004). In general, the acceleration of erythrocyte ageing was observed to be more pronounced in young erythrocytes exposed with nickel compounds. The observed results also suggest that nickel compounds decrease water permeability across erythrocyte membranes. Nickel compound decrease erythrocyte thermostability, deformability, and the rate of oxygen release (Tkeshelashvili *et al*. 1989). It is well documented that heavy metal usually binds with the erythrocyte membranes and plasma albumin later stimulates metallothioneins and reactive oxygen species (ROS) and induces oxidative damage in erythrocyte and in various tissues, which results in loss of membrane function (Sarkar *et al*. 1998; Das *et al*. 2001). Apart from erythrocytes nickel can induce impairment and dysfunction of blood, cardiovascular system, detoxification pathways (colon, liver, kidney, skin), endocrine (hormonal), energy production pathways, enzymatic, gastro-intestinal, immune, nervous (central and peripheral), reproductive and urinary system (Das & Dasgupta 1997, 2000, 2002). Nickel induced severe liver and kidney damage by altering several marker enzymes and ascorbate-cholesterol metabolism (Das & Dasgupta 1998). One of the harmful effects of nickel action in the body is to induce formation of ROS and increase lipid peroxidation in the cells (Das *et al*. 2001). Free radicals and intermediate products of lipid peroxidation are capable of damaging the integrity and altering the function of biomembrane, which can lead to the development of many pathological process (Gutteridge 1993; Das *et al*. 2006). Erythrocytes are equipped with many defense systems representing their antioxidant capacity (Kurata *et al*. 1993). This protective system includes superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-Px) but their relative significance in H_2O_2 scavenging is not clear (Kurata *et al*. 1993; Nakababu *et al*. 2003).

Ascorbic acid is an important antioxidants which significantly decreases the adverse effect of reactive oxygen and nitrogen species that can cause oxidative damages to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis (Halliwell & Gutteridge 1986). Recently it was observed that the nickel-induced activation of hypoxia inducible genes greately depleted intra-cellular ascorbate levels. The supplementation of the media with ascorbate increased intracellular ascorbate and reversed both metal-induced stabilization of HIF-1 and HIF-1α gene expression (Salnikow *et al*. 2004). Additional supplementation of L-ascorbic acid was found to be beneficial in nickel induced alteration of testicular nucleic acid concentration, hepatic lipid peroxidation and histopathology of liver (Das

et al. 2001, 2006; Das & Das 2004). Several studies are underway to determine the effect of antioxidant supplementation following heavy metal exposure. The data suggests that antioxidants specifically ascorbic acid plays an important role in abating certain hazards of heavy metals (Nieboer & Nriagu 1988; Ercal *et al*. 2001).

The aim of our study was to investigate a possible protective influence of L-ascorbic acid on some hematological parameters, erythrocytes antioxidant defense system and lipid peroxidation in male albino rats treated with nickel sulfate.

Materials and methods

Adult (aged 60–70 days) laboratory bred male Wister strain rats (160 ± 5 g) fed with laboratory stock diet and water *ad libitum* for 7 days. The animals were kept in air-conditioned animal house maintaining 22–24 °C with relative humidity approximately 70%. The acclimatized animals were divided into four groups of six animals each. Group I served as untreated control. Group II rats were administered nickel sulfate (Sigma chemicals) in double-distilled water at a dose of 2.0 mg/100 g b.wt., intraperitonially (i.p.) on alternate days until the tenth dose (Bordes & Papillion 1983).

Group III rats were treated orally with L-ascorbic acid at a dose of 50 mg/100 g b.wt. (Hussain *et al*. 1992) and Group IV rats were given both nickel sulfate (2.0 mg/100 g b.wt., i.p.) and L-ascorbic acid (50 mg/100 g b.wt., orally) simultaneously on alternate days until tenth dose. The chances of nickel toxicity occurring by the oral route are remote because a large amount of nickel is required to produce a toxic effect by ingestion. In contrast to nickel salts administered orally, nickel salts administered intraperitoneally or subcutaneously are highly toxic (Nielsen *et al*. 1984). Hence nickel sulfate was administered intraperitoneally in this study. The entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure, as well as at the time of sacrifice, according to the Helsinki Declaration of 1964. After the treatment, the animals were sacrificed by decapitation always between 9:00 h and 11:00 h and fresh blood was immediately collected into heparinized test tubes. All the hematological parameters i.e. total red blood corpuscles (RBC) count, packed cell volume (PCV), total white blood corpuscles (WBC) count, total platelets count and clotting time were measured by using fully automated hematology analyzer (Sysmax K-4500) (Garcia-Mazano *et al*. 2001). The hemoglobin (Hb) concentration in the red cell lysates was determined by the cyanmethemoglobin method (Gowenlock 1996).

To determine antioxidant status of erythrocytes, blood samples were centrifuged at 3000 rpm for 15 min to remove the plasma and buffy coat (consisting leucocytes and platelets). The erythrocytes were washed three times in buffered saline (0.9% saline in 0.01 M phosphate buffer, pH 7.4), and the packed cells were suspended in equal volume of the buffered saline. Erythrocyte lipid peroxide (LPO) was measured as the production of malondialdehyde (MDA) which in combination with thiobarbituric acid (TBA) forms pink chromogen compound whose absorbance at 530 nm was recorded. The concentration of MDA was calculated using a standard curve obtained from the reaction between varying MDA concentration (0.1, 0.5, 2.0 and 3.0 nmol MDA/g hemoglobin) (Kei 1978). The activity of erythrocyte superoxide dismutase (SOD) was determined spectrophotometrically at 480 nm by the epinephrine method (Misra & Fridovich 1972) and it was expressed in units of enzyme activity per gram of hemoglobin (Unit/g Hb). Total erythrocyte glutathione (GSH) was estimated by the method of Beutler *et al*. 1963. Measurement of erythrocyte glutathione peroxidase (GSH-Px) was based on the following principle: GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured (Paglia & Valentine 1967). The erythrocyte GSH-Px activity was expressed as Unit/g Hb. The enzyme unit of GSH-Px was defined as the number of reduced NADP oxidized per minute at 37 °C and according to the Randox application procedure. Catalase (CAT) activity in hemolysate was assayed by the method of Aebi. From the hemolysate an aliquot of 10 μl was diluted with phosphate buffer (50 mmol/l, pH 7.4) to a final volume of 5.0 ml. CAT activity was measured by adding 1.0 ml H_2O_2 (30 mmol/l, Merck)

Table 1. Effect of nickel sulfate on hematological parameters in rats.

Parameters	Group I	Group II	Group III	Group IV
RBC (10^6cells/μl)	8.91 ± 1.41[a]	5.29 ± 1.12[b]	9.32 ± 1.56[a]	6.95 ± 1.45[c]
Hemoglobin (gm/dl)	18.90 ± 3.8[a]	13.89 ± 2.67[b]	19.07 ± 3.22[a]	16.44 ± 3.77[c]
PCV (%)	50.40 ± 5.31[a]	42.40 ± 2.71[b]	51.23 ± 4.78[a]	45.80 ± 4.32[b]
Clotting Time(min)	4.3 ± 0.98[a]	8.23 ± 2.21[b]	4.67 ± 1.08[a]	7.86 ± 1.05[b]
WBC (10^3cells/ μl)	9.89 ± 1.67[a]	4.80 ± 0.78[b]	10.12 ± 1.45[a]	6.82 ± 1.11[c]
Platelets(10^3cells/μl)	871.0 ± 34.4[a]	459.9 ± 45.2[b]	901.2 ± 37.9[a]	654.4 ± 67.8[c]

Treatment groups: I – untreated control; II – nickel sulfate; III – L-ascorbic acid; IV – nickel sulfate + L-ascorbic acid. RBC, red blood corpuscle; PCV, packed cell volume; WBC, white blood corpuscle. In each column, values with different superscripts (a, b, c) were significantly different from each other ($p < 0.05$).

to 2.0 ml diluted hemolysate and reading the decrease in absorbance at 240 nm between 20 and 30 s with a spectrophotometer against a blank consisting of 2.0 ml diluted hemolysate and 1.0 ml deionized water at 37 °C. Each sample was assayed twice, and the mean of the first order rate constant (k) was calculated. The final results corrected for the dilution and related to the Hb concentration, the enzyme activity was expressed as k/g Hb as described in the literature (Aebi 1974).

The mean ± SEM values were calculated for each group. For determining the significance of inter-group differences, each parameter was analyzed separately. A one way analysis of varience (ANOVA) was carried out at 1% Fisher's distribution to determine which of the groups differed among themselves. Duncan's multiple range test was applied with the level of significance fixed at $P < 0.05$ (Steel & Torrie 1960).

Results

Table 1 shows that intraperitoneal nickel sulfate administration resulted significant decrease of RBCs count, hematocrit value (PCV %) and Hb concentration in Group II rats when compared to untreated control (Group I). Group IV (nickel sulfate + L-ascorbic acid) also showed a significantly decrease RBCs count, PCV % and Hb concentration in comparison with Group I but when compared with Group II rats a significant increase of all above mentioned parameters were noticed. No significant alterations of any of those parameters were found in case of Group III (only L-ascorbic acid treated) rats when compared with untreated control (Group I) rats. Figure 1 shows the percent change decrease of RBCs count, PCV % and Hb concentration in Group IV (nickel sulfate + L-ascorbic acid) rats in comparison to the untreated control Group I. But when compared with Group II, receiving nickel sulfate alone, the decrease of RBCs count, PCV (%) and Hb concentration were found to be remarkably less [E-I vs. E-III]. Table 1 also depicts significant increase of clotting time (CT) followed by decrease of platelets count and WBCs count in Group II rats in comparison to untreated control (Group I). Incase of Group IV rats a significant increase in clotting time and decrease of platelets count and WBCs count were noticed when compared with Group I rats but when it was compared with Group II rats a significant rise in platelets count and WBCs count were found. No significant changes were found in any of these parameters in Group III rats when it was compared with untreated control (Group I). Figure 1 also shows the

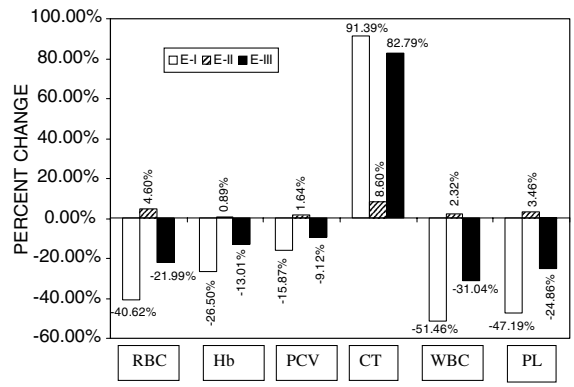

Figure 1. Percent change chart of various hematological parameters of nickel sulfate treated rats. RBC, red blood corpuscles; Hb, hemoglobin; PCV, packed cell volume; CT, clotting time; WBC, white blood corpuscles; PL, platelet. E I, Group I (control) vs. Group II (+$NiSO_4$); E II, Group I vs. Group III (+L-ascorbic acid); E III, Group I vs. Group IV ($NiSO_4$ + L-ascorbic acid).

Table 2. Effect of nickel sulfate (2.0 mg/100 g b.wt.; i.p) on erythrocyte antioxidant status in rats.

Parameters	Group I	Group II	Group III	Group IV
MDA (nmoles/g Hb)	250.8 ± 8.42[a]	348.6 ± 10.32[b]	261.4 ± 12.42[a]	300.8 ± 18.24[c]
GSH (μmoles/g Hb)	20.20 ± 32.48[a]	32.48 ± 2.82[b]	21.72 ± 1.54[a]	26.81 ± 2.82[c]
GSH-Px (units/g Hb)	74.82 ± 4.82[a]	191.45 ± 15.62[b]	80.43 ± 6.82[a]	133.84 ± 24.28[c]
SOD (units/g Hb)	1264 ± 42.19[a]	2104 ± 48.20[b]	1348 ± 28.22[a]	1609 ± 28.42[c]
CAT (units/g Hb)	1048 ± 20.89[a]	1384 ± 21.20[b]	988 ± 42.84[a]	1010 ± 18.81[a]

Treatment groups: I – untreated control; II – nickel sulfate; III – L-ascorbic acid; IV – nickel sulfate + L-ascorbic acid. MDA, malondialdehyde; GSH, glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase. In each column, values with different superscripts (a, b, c) were significantly different from each other ($p < 0.05$).

percent change increase of clotting time, decrease of platelets count and WBCs count in Group IV ($NiSO_4$ + L-ascorbic acid) rats when compared to untreated control (Group I). But when it was compared with Group II (only $NiSO_4$ treated) rats the increase of CT, decrease of platelets count and WBCs count were surprisingly less [E-I vs. E-III]. Rats receiving L-ascorbic acid alone (Group III) did not show any significant variation in the above parameters studied when compared with untreated control (Group I) [E-I vs. E-II].

In the red blood cells of the nickel sulfate treated rats (Group II), malondialdehyde (MDA), glutathione (GSH) levels and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were significantly increased when compared to untreated control (Group I) rats (Table 2). The simultaneous treatment with L-ascorbic acid (Group IV) partially reversed this change to near untreated control values (Table 2). Figure 2 shows the percent change increase of erythrocytes MDA and GSH contents and the activities of erythrocyte SOD, GSH-Px and CAT in Group IV ($NiSO_4$ + L-ascorbic acid) rats in comparison to Group I. But when compared with Group II receiving nickel sulfate only, the rise in erythrocyte MDA, GSH content and the activities of erythrocyte SOD, GSH-Px and CAT in Group IV rats were found to be amazingly less [E-I vs. E-III]. Even in case of erythrocyte CAT activity of Group IV rats the percent change shows a negative fall in comparison to Group I values [E-I vs. E-III]. Rats receiving L-ascorbic alone (Group III) did not show any significant variation in the antioxidant parameters studied above when compared with untreated control (Group I) [E-I vs. E-II].

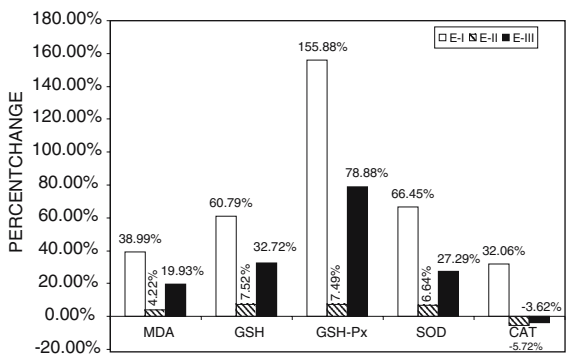

Figure 2. Percent change chart of antioxidant status in red cell lysates of nickel sulfate treated rats.MDA, malondialdehyde; GSH, glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase. E I, Group I (control) Vs. Group II (+ $NiSO_4$); E II, Group I vs. Group III (+ L-ascorbic acid); E III, Group I vs. Group IV ($NiSO_4$ + L-ascorbic acid).

Discussion

The results obtained in our present study show the treatment with nickel sulfate induces anemia (decrease RBC count, PCV % and Hb concentration) in rats (Table 1). It is interesting to note that nickel is thought to be indispensable to the hematopoietic process, due to its influence on iron absorption and metabolism, particularly when iron status is low (Nielsen 1984). But animal studies have shown that high dietary nickel may adversely affect hematopoiesis when in conjunction with marginal iron status (Nielsen 1984). A decrease hemoglobin concentration in rats exposed to 25 mg Ni/kg/day as nickel acetate in the diet for 6 wks has been reported (Whanger 1973). Low hematocrit levels were also observed in dogs after chronic dietary exposure to 62.5 mg/Ni/kg/day as nickel sulfate (Ambrose *et al.* 1976). Nickel implanted rats showed a significant decrease of red blood cells,

hemoglobin and hematocrit at the time of morbidity, including possible nickel induce anemia (Kalinih *et al*. 2005). It is also known that hematology assays provide information on bone marrow activity and on the status of other organs governing synthesis, function and destruction of components of the circulatory system. Disturbances in erythrocyte parameter often reflect an imbalance between the production and loss of red blood cells (Merchant & Mod 2004). In our study the decrease in RBCs count, PCV% and hemoglobin concentration may be due to non- regenerative anemia arise from nickel induced direct injury of hematopoietic stem cells resulting in decreased erythrocytes, leucocytes and platelets count. Effects of blood clotting mechanisms are assessed by both clotting time determination and platelets count. Decrease production or increased consumption of platelets may cause platelet counts to fall. It is well understood that test chemicals, which inhibit RBC and WBC formation also frequently, inhibit platelet formation (Merchant & Mod 2004). Alteration in WBC count of mice and decreased hematocrit in rats have been reported following administration of nickel chloride (USAT 1990). Probably nickel sulfate induced bone marrow activity and significantly decreased all the types of blood cells in present study. Simultaneous treatment of L-ascorbic acid decreased the toxic effects of nickel sulfate on hematological values and showed a protective role in anemia, leucopenia and thrombocytopenia (Table 1 and Figure 1). Ascorbic acid facilitates the rate of production of blood cells by stimulating bone marrow. It is also known that L-ascorbic acid promotes the production of RBCs in bone marrow and contributes to hemoglobin synthesis and even prevents blood clotting (Kanungo & Patnaik 1964).

Reactive oxygen species (ROS) are implicated as important pathologic mediators in many disorders. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of wide range of compounds (Halliwell & Gutteridge 1986; Shivarajashankara *et al*. 2001). Lipid peroxidation constitutes a complex chain reaction of free radicals, which leads to a degradation of polyunsaturated fatty acid in cell membrane (Halliwell & Gutteridge 1984). Present study reveals that treatment with nickel sulfate increase lipid peroxidation by increasing MDA concentration in erythrocytes of rats (Table 2, Figure 2). Increased erythrocyte MDA concentration of rats definitely accompanied by increased ROS formation (Ochi *et al*. 1988; Shi *et al*. 1999). As a consequence, enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked disturbances of antioxidant defense system occurred (Sarkar *et al*. 1995; Das & Das 2004; Doreswamy *et al*. 2004). It is well documented that nickel competes with calcium intracellularly and may cause membrane disruption via lipid peroxidation, ultimately leading to cell death (Kupferschmidt 2001). Simultaneous treatment with L-ascorbic acid was found to be effective in the prevention of oxidative damage in erythrocyte induced by nickel sulfate, which resulted in significantly lowering erythrocyte MDA concentration (Table 2, Figure 2). The present study reported increase in level of GSH and the activities of GSH-Px, SOD and CAT in the erythrocytes of rats treated with nickel sulfate (Table 1, Figure 2). Increased GSH levels and GSH-Px activity in erythrocyte may be attributed to adaptive response of the erythrocytes to the oxidant challenge due to nickel sulfate exposure. An increase in GSH-Px activity of rats exposed to high oxidant stress has already been reported (Edes *et al*. 1986). It is well known that SOD, CAT, GSH and GSH-Px play a vital role in the antioxidant defense mechanism of animal cells and tissues. In our study SOD activity in erythrocyte increased in response to nickel treatment. Since SOD is known to be an enzyme induced by its superoxide radical substrate, increase in its activities in the erythrocytes of nickel treated animals indicates an increased production of superoxide radical. The increase also might be involved in the elevated generation of OH^{-}.This increase in SOD activity also could be responsible for the observed lipid peroxidation (Kelle *et al*. 1999). Nickel induced increase of GSH-Px and CAT activities in erythrocytes may be explained by their influence on hydrogen peroxide as substrate, which is formed in the process of dismutation of superoxide anion radicals (Shaikh *et al*. 1999). This action is followed by increased reduction of oxidized glutathione by glutathione reductase to form GSH (Mates 2000). The simultaneous treatment with L-ascorbic acid brought erythrocyte GSH level near to the untreated control value confirming the protective role of L-ascorbic acid on nickel induced impairment of erythrocyte

antioxidant defense system. The simultaneous treatment with L-ascorbic acid also decreased erythrocyte GSH-Px, SOD and CAT activities indicating that L-ascorbic acid may eliminates the toxic effect of nickel sulfate on the activity of those enzymes. Ascorbic acid can be oxidized by most reactive oxygen and nitrogen species thought to play roles in tissue injury associated with various diseases. These species include superoxide, hydroxyl, peroxyl and nitroxide radicals, as well as non- radicals reactive species such as singlet oxygen, peroxynitrite and hypochlorate. By virtue of their scavenging activity, ascorbate inhibit lipid peroxidation, oxidative DNA damage and oxidative protein damage. It is also reported that L-ascorbic acid enters mitochondria of a cell in its oxidized form via GLUT-1 and protects mitochondria from oxidative injury. Since mitochondria contribute significantly to intracellular ROS, protection of the mitochondrial genome and membrane may be beneficial (Sagun *et al*. 2005). As nickel decreases the cellular transportation of ascorbic acid and deplete intracellular ascorbic acid resulting in intracellular hypoxia and metabolic dysfunction (Salnikow *et al*. 2004; Das *et al*. 2006) supplementation with L-ascorbic acid could reverse the nickel-induced alteration of erythrocyte antioxidant defense mechanism.

It can be concluded from present findings that nickel sulfate induced oxidative damage in erythrocytes or depression of the bone marrow leads to anemia or leucopenia and thrombocytopenia, loss of membrane function by elevating erythrocyte MDA level as well as alteration of GSH level or antioxidant enzymes like SOD, GSH-Px and CAT activities. But simultaneous treatment with L-ascorbic acid may protect against toxic influence of nickel on examined parameters in rat blood.

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