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PROTECTIVE EFFECT OF α-TOCOPHEROL AGAINST HEMATOTOXICITY, HEPATOTOXICITY AND NEPHROTOXICITY INDUCED BY NICKEL SULFATE IN MALE ALBINO RATS

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(Received on August 16, 2012)

Abstract : Among the chemical hazards, heavy metal like nickel (Ni) is considered to be a serious one. It induces severe liver and kidney damage by altering several marker enzymes and ascorbate-cholesterol metabolism. The objective of the study was to investigate the possible protective role of α-tocopherol on $NiSO_4$ (Ni II) exposed alteration of hematological parameters, markers of liver and kidney functions, hepatic and renal antioxidant defense system in male albino rats. We have studied the effects of α-tocopherol supplementation on nickel sulfate induced alteration of body weight, hematology, liver and kidney toxicity markers (SGOT, SGPT, total protein, urea, creatinine) and hepatic and renal antioxidant defense system of male albino rats. Nickel toxicity results in decreased body weight gain and relative liver and kidney weight. Nickel treatment also resulted in alteration of hematological parameters along with increased liver and kidney toxicity markers. Nickel sulfate administration significantly increased the level of lipid peroxides and decreased antioxidant enzyme activities in hepatic and renal tissue. Simultaneous treatment with á-tocopherol exhibited a possible protective role on the toxic effect of nickel on body and organ weights, hematological parameters, SGPT activity and improved tissue antioxidant defense system. α-tocopherol, may partially prevent nickel induced alteration of hematological and biochemical parameters as well as have amelioratic effects on nickel induced alteration of antioxidant status of liver and kidney.

Key words : α-tocopherol, antioxidant defense, hematology, nickel sulfate, SGOT/SGPT

INTRODUCTION

Nickel is a naturally occurring element, which can be found, in all environmental media. Nickel is released into environment through the extraction, processing and use

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of nickel compounds. After entering into the body nickel penetrates all organs, accumulating primarily in bone, liver and kidney and excreted in bile and urine (1). If nickel enters and accumulates in the tissue faster than the rate at which the body's detoxification pathways can dispose it, a gradual build up of toxins may occur.

It is interesting to note that nickel partially facilitates iron absorption from GIT (directly as ferric form) and helps hematopoiesis particularly when iron status is low in blood. But animal studies have shown that high dietary nickel may adversely affect hematopoiesis when in conjunction with marginal iron status (2). Nickel implanted rats showed a significant decrease of red blood cells, hemoglobin and haematocrit at the time of morbidity, including possible nickel induced anemia (3). Nickel induced severe liver and kidney damage by altering several marker enzymes like SGOT, SGPT and ascorbate-cholesterol metabolism has been reported earlier (4). Nickel sulfate administration to male albino rat significantly increased the lipid peroxide (LPO) level and simultaneously decreases the antioxidant enzyme activities (5).

A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins and polyunsaturated fatty acids (PUFA). Malondialdehyde (MDA) is one of the aldehydes produced during this attack from PUFA. MDA may be an indicator of oxidative stress, as its concentration in tissues and plasma increases after the influence of intensified free-radical processes (6). The most plausible mechanism operating *in vivo* is the generation of reactive oxygen species (ROS), which may initiates lipid peroxidation, oxidative damage to macromolecules such as protein, DNA and cell damage and death (7). Liver the major site of detoxification is the primary target of environmental and occupational toxicity (8).

The body is, however, not defenseless when facing free radicals. The antioxidant system enables transformation of reactive forms of oxygen into inactive and harmless compounds or molecules. Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are the most important antioxidant enzymes.

Among the non-enzymatic antioxidants, vitamin E is listed; its activity has been studied to a reasonable extent. α-tocopherol (Vitamin E) is a natural component of membrane lipid bilayer and thus helps to maintain membrane stability. The molecular and cellular effects of vitamin E have been explained either by acting as an antioxidant preventing damage to membranes or proteins and regulating their activity by specifically scavenging reactive oxygen species or by interacting or regulating specific enzymes and influencing cellular structures (9).

It has been observed that nickel sulfate induces increase in concentrations of lipid peroxidation products with concomitant decrease in α-tocopherol concentration in the liver (10). α-tocopherol acting in conjugation with glutathione peroxide (GSH-Px) could directly reduce phospholipids hydroperoxides within the membrane and lipoproteins to inhibit lipid peroxidation (11).

Therefore the aim of the study was to evaluate the effect of nickel sulfate on body and organ weight, hematological parameters as well as its effects on liver and kidney tissues. Further to investigate whether the antioxidant vitamin E (α-tocopherol) has any protective effects against nickel induced toxicity.

MATERIAL AND METHODS

Adult (aged 60 to 70 d) laboratory-bred male Wister strain rats (160±5g) were fed with laboratory stock diet (consists of 70% carbohydrate, 7% fat, 18% protein, 4% salt mixture and 1% vitamin mixture) and water *ad libitum* for 7 days. The animals were kept in an air conditioned animal house maintained at 22°C to 24°C with ~70% relative humidity. 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 ($NiSO_4$, $6H_2O$) (Sigma) in double–distilled water at a dose of 2.0 mg/100 g b.wt., (ip) on alternate days until the tenth dose (12). Group III rats were treated orally with á-tocopherol (13) at a dose of 10 mg/100 g. b.wt (im), and Group IV rats were given both nickel sulfate (2.0 mg/100 g. b.wt.; ip) and α-tocopherol simultaneously (10 mg/100 g.b.wt., im) on alternate days until the tenth dose. The dietary isocaloric status was maintained in each group by pair feeding technique. The entire animal experiments were performed according to the ethical guidelines suggested by ICMR and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India. After treatment, the animals were sacrificed by cervical decapitation between 9:00 h and 11:00 h.

Measurement of body and organ weights:

All animals were weighed by automatic balance on day 1 of the nickel sulfate treatment and the day of sacrifice. After decapitation the animals were dissected, liver and kidney were excised and weighed after washing in ice-cold saline to the nearest of 0.1 mg in a single pan electronic balance. The percentage of body weight gain, hepatosomatic index and relative kidney weight were calculated. The weights of the tissues were expressed after correction for difference in body weight i.e. relative body weight (per 100 gm body weight).

Determination of hematological parameters:

About 2 ml of blood were collected in commercial tubes containing about 40 µl of potassium salt of EDTA as anticoagulant and analyzed within 24 h by fully automated hematological cell counter (Sysmax K-4500 of Transasia Ltd.) (14). The parameters measured were red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV%), mean cell volume (MCV), platelet count. The values of the mean corpusular hemoglobin (MCH) and MCHC were calculated. Each sample was run in duplicate.

Biochemical determination of markers:

Serum free of hemolysis separated from blood cells as soon as possible after collection. 2 to 3 ml of blood samples were centrifuged at 3000 rpm for 15 min and later the following parameters were analyzed.

Assay of serum aspartate aminotranseferase activity (SAST/SGOT): The serum AST or

SGOT activity has been measured by modified UV (IFCC), kinetic assay using a commercial kit (Span diagnostics Ltd, India) according to manufacturer's protocol (15).

Assay of serum alanine aminotranseferase activity (SALT/SGOT): The serum ALT or SGPT activity has been measured by modified UV (IFCC), kinetic assay using a commercial kit (Span diagnostics Ltd, India) according to manufacturer's protocol (16).

Estimation of serum total protein: Total serum protein has been measured by modified Biuret method with end-point colorimetry according to manufacturer's protocol using semi automated analyzer (17).

Estimation of serum urea: Serum urea has been measured by urease, Berthelot end point assay using a commercial kit (Span diagnostics Ltd, India) according to the manufacturer's protocol (18).

Estimation of serum creatinine: Serum creatinine was determined by modified Jaffe's reaction (initial rate assay) using a commercial kit (Span diagnostics Ltd, India) according to the manufacturer's protocol (19).

Biochemical determination of oxidant stress/ antioxidant status of tissues:

The tissues of each side (liver, kidney) of each group were separately dissected out, washed in ice-cold saline, and weighed. Approximately 100 to 150 mg tissue was homogenized in 0.05 M Tris-Cl/1 mM EDTA (pH 7.0) buffer by Polytron (setting 7, 20 sec). The homogenate was centrifuged for 20 min at 3,000 × g at 40°C, and the supernatant was used for assaying antioxidant status.

1. Concentration of TBARS level: Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa et al (20). Lipid peroxide level was expressed in terms of µmoles TBA reactants/g protein and were estimated in terms of 2-thiobarbituric acid reactants, using 1,1',3,3', tetramethoxypropane as standard.

2. The glutathione level was measured by the method of Moron et al. (21). In this method 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) was reduced by -SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitro mercaptobenzoic acid anion released has intense yellow color and can be used to measure -SH groups at 412 nm. The unit was expressed as µmoles/g tissue weight.

3. The activity of superoxide dismutase: Total (Cu-Zn and Mn) superoxide dismutase activity was determined according to the method of Misra and Fridovich (22). The ability of superoxide dismutase to inhibit the auto oxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of epinephrine by 50%. Activity was expressed as units/mg of protein.

4. The activity of glutathione peroxidase: GSH-Px activity was determined using a modification of the method of Flohé and Günzler (23). The millimolar extinction

coefficient of 6.22 mM/cm was used to determine the activity of GSH-Px. Activity was expressed as units/mg of protein.

5. The activity of catalase: Catalase activity was determined by the method of Aebi (24). The molar extinction coefficient of 43.6 mM/cm was used to determine CAT activity. CAT activity was expressed as units/mg of protein.

6. Protein was determined by the method of Lowry (25).

Statistical analysis :

The data, obtained from all the control and experimental samples, has been subjected to statistical analysis. Mean±SD values were calculated for each group. To determine the significance of inter-group differences, we analyzed each parameter separately. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test by using statistical software (Stat Pac for Windows, Version 11.0). The level of significance was fixed at $P<0.05$.

RESULTS

Evaluation of body and organ weights:

No death was observed in any of the experimental groups. Table I shows that nickel sulfate treatment in Group II produced a significant decrease in final body weight as compared to untreated control (Group I). But simultaneous administration of α-tocopherol with nickel sulfate in Group IV rats, the decrease in final body weight improved remarkably. There was no significant difference in final body weight of nickel + α-tocopherol rats with control rats. When compared to respective initial body weight, control, α-tocopherol and nickel +

TABLE I: Changes in body and organ weight of male rats after nickel sulfate treatment alone or in supplementation with α-tocopherol.

Body / Tissue weight	*Untreated control*	*Nickel sulfate*	*α-tocopherol*	*Nickel sulfate + α-tocopherol*	*F-ratio & P value*
(i) Initial Body wt (g)	179.67±6.334	184.00±2.583	180.50±3.528	188.67±3.422*	F=5.678 P=.0056
(ii) Final Body wt (g)	247.83±12.776	167.67±4.154****	247.33±5.110^{++++}	221.00±10.89$^{***++++\$\$\$}$	F=104.784 P=0.0000
% of body wt gain [(i) vs. (ii)]	38.30±7.732	–8.67±3.309****	37.26±3.699^{++++}	16.98±4.790$^{****++++\$\$\$\$}$	F=108.896 P=0.0000
Absolute liver wt (g)	6.90±0.095	4.65±0.143****	6.92±0.136^{++++}	6.07±0.109$^{*+++\$}$	F=28.623 P=0.0000
Hepatosomatic index (g/100 g body wt)	2.75±0.051	2.77±0.049	2.796±0.078	2.79±0.180	F=0.204 P=0.8926
Relative Kidney wt (g/100 g body wt)	0.38±0.012	0.57±0.024****	0.37±0.017^{++++}	0.51±0.038$^{****++\$\$\$\$}$	F=94.821 P=0.0000

Group I – Control, Group II – Nickel treated, Group III - α-tocopherol treated, Group IV – Nickel sulfate + α-tocopherol. Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$); + depicts comparison with Group II ($^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$); $ depicts comparison with Group III ($^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$, $^{\$\$\$\$}P<0.0001$).

α-tocopherol rats showed a significant difference between initial and final body weight. A significant decrease in absolute liver weight was found in nickel treated rats in comparison with control groups although the hepatosomatic index of the nickel treated group was non-significant as compared with control. This may be indicative of more a proportional decrease in liver weight with decrease in respective body weight. Simultaneous treatment with α-tocopherol in Group IV rats had improved the absolute liver weight as well as hepatosomatic index up to the level of control group. There was also a significant increase in relative kidney weight in nickel treated group of rats compared to control group. In nickel + α-tocopherol treated rats, simultaneous α-tocopherol administration did not show any significant changes from Group II rats.

Changes in hematological parameters:

Table II shows that nickel sulfate administration resulted in significant decrease of RBC count, haematocrit value (PCV %) and Hb concentration in nickel treated group of rats when compared to untreated control. Nickel sulfate + α-tocopherol treated group also showed a significant decrease of RBC count, PCV % and Hb concentration in comparison with their control but when compared with nickel alone treated rats, a significant increase of

TABLE II: Changes in hematological parameters of male rats after nickel sulfate treatment alone or in supplementation with á-tocopherol.

Hematological parameters	*Untreated control*	*Nickel sulfate*	*α-tocopherol*	*Nickel sulfate + α-tocopherol*	*F-ratio & P value*
RBC (10^6 cell/μL)	8.46±0.39	5.59±0.39****	8.63±0.30^{++++}	7.03±0.34$^{****++++\$\$\$\$}$	F=94.886 P=0.0000
Hemoglobin (gm/dL)	18.07±0.27	12.39±0.64****	18.18±0.33^{++++}	14.99±0.49$^{****++++\$\$\$\$}$	F=221.83 P=0.0000
PCV (%)	49.38±0.96	41.74±0.86****	49.80±0.76^{++++}	45.96±0.44$^{****++++\$\$\$\$}$	F=137.914 P=0.0000
Clotting time (min)	4.21±1.14	7.61±0.28****	4.55±0.09^{++++}	7.23±0.27$^{****\$\$\$\$}$	F=51.387 P=0.0000
WBC (10^3 cell/ μL)	9.30±0.35	4.91±0.14****	9.31±0.22^{++++}	6.20±0.21$^{****++++\$\$\$\$}$	F=507.926 P=0.0000
Platelets (10^3 cells/μL)	866.75±24.87	523.33±27.89****	843.64±21.10^{++++}	566.67±21.86$^{****+\$\$\$\$}$	F=336.043 P=0.0000
MCV (fL)	58.93±2.79	76.52±5.50 ****	61.58±5.11 $^{+++}$	66.04±2.87 $^{*++}$	F=19.891 P=0.0000
MCH (pg)	21.64±1.26	22.37±0.87 ^{a}	21.18±0.66 ^{a}	21.19±1.10 ^{a}	F=1.882 P=0.1651
MCHC (g/dL)	36.65±0.75	32.55±2.32 ***	36.58±1.02 $^{+++}$	32.62±0.97 $^{***\$\$\$}$	F=16.032 P=0.0000

Group I – Control, Group II – Nickel treated, Group III – α-tocopherol treated, Group IV – Nickel sulfate + α-tocopherol. RBC, Red blood corpuscles; PCV, Packed cell volume; WBC, White blood corpuscle; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration. Each value is mean±SEM of six observations in each group. Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001); + depicts comparison with Group II ($^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001, $^{++++}$P<0.0001); $ depicts comparison with Group III($^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, $^{\$\$\$\$}$P<0.0001).

all the above mentioned parameters were noticed. No significant alterations of any of those parameters were found in case of only α-tocopherol treated rats when compared with untreated control rats.

Table II also depicts significant increase of clotting time (CT) followed by decrease of platelet count and WBC count in nickel treated rats in comparison to untreated control. In case of nickel + α-tocopherol treated rats a significant increase in clotting time and decrease of platelets count and WBC count were noticed when compared with control rats but when it was compared with nickel alone treated rats a significant rise in WBC count were found. No significant changes were found in any of these parameters in only α-tocopherol supplemented rats when it was compared with untreated control. In nickel treated rats, MCV was increased significantly when compared to control group of rats. This alteration was not rectified even after simultaneous supplementation with α-tocopherol. MCH and MCHC values remained statistically unchanged in all the groups.

Evaluation of biochemical changes:

Table III shows a very significant increase in serum total protein level in nickel treated rats in comparison with untreated control. No significant difference of serum total protein level was noticed in nickel sulfate + α-tocopherol treated rats when it was compared to nickel alone treatment. Hepatotoxicity was monitored by quantitative analysis of SGOT and SGPT activities, which were used as biochemical markers of liver damages. The activity of both SGOT and SGPT in rats after nickel treatment had increased significantly from that of control group (Table III). In nickel + α-tocopherol treated rats, the SGOT activity did not show any significant improvement from that of nickel alone treated rats. Whereas SGPT activity improved significantly in nickel + α-tocopherol treated rats when compared to nickel treatment alone.

TABLE III: Changes in SGOT and SGPT activities, serum total protein, urea and creatinine value of male rats after nickel sulfate treatment alone or in supplementation with á-tocopherol.

Treatment group	*Untreated control*	*Nickel sulfate*	*α-tocopherol*	*Nickel sulfate + α-tocopherol*	*F-ratio & P value*
SGOT (U/L)	276.483±14.208	330.117±7.837****	277.667±10.502^{++++}	316.800±9.045$^{****\$\$\$\$}$	F=39.393 P=0.0000
SGPT (U/L)	41.02±3.461	85.542±4.384****	36.767±2.147^{++++}	69.333±4.061$^{****++++\$\$\$\$}$	F=248.866 P=0.0000
Total protein (mg/dL)	7.30±0.245	11.77±1.029****	7.14±0.237^{++++}	10.19±0.302$^{****\$\$\$\$}$	F=96.83 P=0.0000
Urea (mg/dL)	42.280±1.049	48.193±0.429****	40.662±1.347^{*++++}	46.997±0.653$^{****\$\$\$\$}$	F=89.487 P=0.0000
Creatinine (mg/dL)	0.643±0.024	1.515±0.131****	0.632±0.045^{++++}	1.305±0.053$^{****+++\$\$\$\$}$	F=219.351 P=0.0000

Group I – Control, Group II – Nickel treated, Group III – α-tocopherol treated, Group IV – Nickel sulfate + α-tocopherol. Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001); + depicts comparison with Group II ($^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001, $^{++++}$P<0.0001); $ depicts comparison with Group III ($^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, $^{\$\$\$\$}$P<0.0001).

Table III also shows significant increases in serum total protein, urea and creatinine levels in nickel treated rats in comparison with untreated control. No significant difference was noticed in these three parameters in nickel sulfate + α-tocopherol supplemented rats when compared to nickel treatment alone.

Hepatic and renal oxidant stress/antioxidant status :

Table IV shows that nickel induced a significant increase in hepatic and renal lipid peroxide levels in comparison with their respective controls, whereas in nickel sulfate + α-tocopherol treated rats both hepatic and

TABLE IV : Changes in hepatic and renal antioxidant status of male rats after nickel sulphate treatment alone or in supplementation with á-tocopherol.

Tissue analyzed	*Treatment group*	*Untreated control*	*Nickel sulfate*	*α-tocopherol*	*Nickel sulfate + α-tocopherol*	*F-ratio & P value*
Liver	Lipid peroxide (µmoles TBA reactants/g protein)	4.02±0.16	$7.28\pm0.33^{****}$	$3.95\pm0.27^{++++}$	$4.27\pm0.25^{++++}$	F=229.32 P=0.0000
	Glutathione (µmoles/g tissue weight)	5.51±0.30	$3.52\pm0.17^{****}$	$5.28\pm0.15^{++++}$	$4.57\pm0.43^{***+++\$\$}$	F=58.848 P=0.0000
	GSH-Px (units/mg protein)	0.09±0.004	$0.05\pm0.002^{****}$	$0.09\pm0.003^{++++}$	$0.07\pm0.006^{****++++\$\$\$\$}$	F=135.387 P=0.0000
	SOD (units/mg protein)	63.67±2.51	$44.26\pm3.55^{****}$	$63.00\pm2.97^{++++}$	$55.26\pm2.97^{***+++\$\$}$	F=53.636 P=0.0000
	CAT (units/mg protein)	8.54±0.40	$4.84\pm0.53^{****}$	$8.51\pm0.48^{++++}$	$6.09\pm0.66^{****++\$\$\$\$}$	F=73.322 P=0.0000
Kidney	Lipid peroxide (µmoles TBA reactants/g protein)	4.59±0.63	$7.63\pm0.45^{****}$	$4.31\pm0.43^{++++}$	$5.37\pm0.48^{*++++\$\$}$	F=53.576 P=0.0000
	Glutathione (µmoles/g tissue weight)	5.09±0.41	$3.86\pm0.35^{***}$	$5.05\pm0.22^{+++}$	$4.41\pm0.54^{*++\$}$	F=13.02 P=0.0001
	GSH-Px (units/mg protein)	0.097±0.007	$0.038\pm0.009^{****}$	$0.099\pm0.004^{++++}$	$0.061\pm0.010^{****+++\$\$\$\$}$	F=87.162 P=0.0000
	SOD (units/mg protein)	120.89±9.01	$73.66\pm2.08^{****}$	$116.72\pm5.41^{++++}$	$95.30\pm8.84^{***+++\$\$\$}$	F=58.942 P=0.0000
	CAT (units/mg protein)	16.07±2.77	$8.83\pm1.05^{****}$	$15.34\pm0.85^{+++}$	$11.28\pm1.38^{***+\$}$	F=24.69 P=0.0000

Group I – Control, Group II – Nickel treated, Group III – α-tocopherol treated, Group IV – Nickel sulfate + α-tocopherol, SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; TBA, 2-thiobarbituric acids. Each value is mean±SEM of six observations in each group. In each column, values with different superscripts (a, b, c) were significantly different from each other (P<0.05). Each value is mean±SD of six observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tuky-Krammer test. The * depicts comparison with Group I (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001); + depicts comparison with Group II ($^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001, $^{++++}$P<0.0001); $ depicts comparison with Group III ($^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, $^{\$\$\$\$}$P<0.0001).

renal lipid peroxide level decreased significantly when compared with nickel treatment alone. The result shows that after nickel treatment, both the liver and kidney SOD, CAT, and GSH-Px activities were significantly suppressed in the experimental nickel treated rats in comparison with the control. In nickel sulfate + α-tocopherol combined treated rats an improvement of all antioxidant enzyme activities of liver and kidney were noticed in comparison with only nickel treatment. In case of liver and kidney glutathione concentration, a significant reduction of GSH was observed after nickel treatment but simultaneous treatment with á-tocopherol the GSH concentrations were found to be improved significantly in both liver and kidney. Rats of only α-tocopherol treatment did not show any significant variation in the parameters studied above when compared with their respective controls.

DISCUSSION

Nickel induced growth retardation and consistent reduction in body weight is already reported as it is found in our studies (26). Simultaneous treatment with α-tocopherol had shown a beneficial effect on the percentage decrease in body weight after nickel treatment. α-tocopherol induced restoration of growth rate has been reported in earlier studies (27). In the current study, administration of nickel sulfate also induced a significant decrease in absolute liver weight with a non-significant change in hepatosomatic index. The decreased absolute liver weight may be indicative to the degenerative effect of nickel sulfate on hepatic tissue, as it is a major site of metabolism. A significant increase in relative kidney weight after nickel sulfate treatment may be due to renal inflammatory response.

The results obtained in our present study show that the treatment with nickel sulfate induces anemia (decrease RBC count, PCV% and Hb concentration) in rats**.** Nickel implanted rats showed a significant decrease of red blood cells, hemoglobin and haematocrit at the time of morbidity (5). In our study the decrease in RBCs count, PCV% and hemoglobin concentration may be due to non-regenerative anemia arising from nickel induced direct injury of hematopoietic stem cells resulting in decreased erythrocyte, leucocyte and platelet count. Effects on blood hemostatic mechanisms are assessed by both clotting time determination and platelet count. Decreased production or increased consumption of platelets may lead to fall of platelet count. The leucopenia after nickel treatment may be attributed to the inhibition of white blood cell maturation, their release from tissue reservoir or occurrence of leucopoenia by an organism as a response to a stress caused by toxic compounds associated with allergic reaction (5, 27). A decrease in hemoglobin concentration may be due to increased rate of destruction or reduction in rate of formation of RBC. In addition, reduction in the blood parameters (RBC count, Hb concentration, PCV %) may be attributed to hyperactivity of bone marrow leading to production of RBC with impaired integrity that easily destructed in the circulation (5). Simultaneous treatment of α-tocopherol decreased the toxic effects of nickel sulfate on hematological values and also showed a protective role in anemia and leucopenia. An inverse correlation between increased lipid peroxidation and α-tocopherol

levels have been found in bone marrow of nickel chloride treated rats as well as in blood (28). Our study supports the previous observations which show a significant increase in RBC count, PCV% and Hb concentration after α-tocopherol supplementation in athletes who are exposed to exercise induce oxidative stress (28). This could be attributed to the protective effect of α-tocopherol.

Increased activity of both SGOT and SGPT after nickel treatment may be due to leakage of enzymes from liver cytosol into the blood stream giving an indication on the hepatotoxic effect of nickel (27). Following cell damage, the membranes become permeable and enzyme activity is found in the extra cellular fluid and serum, so the highest activity of alanine amino transferase was recorded in the serum. Similar results are published by Sindhu et al (29). Activity of SGPT was increased significantly following nickel treatment to normal rats. The improvement of SGPT activity towards control value in the rats simultaneously treated with α-tocopherol proved the hepatoprotective effect of α-tocopherol due to its antioxidant properties.

In our study, serum protein level had increased significantly after nickel sulfate treatment. This is in agreement with Gopal et al. who recorded that the exposure of *Caprinus carpio* to heavy metal salts (Cu and Ni) at lethal and sublethal concentrations induced an increase in total protein (30). Although most of the earlier studies showed a decrease in the level of the serum protein after metal treatment but in this study the increase in level of serum protein after nickel exposure may be due to the liberation of synthesized proteins as a result of cytolysis and to other pathological changes manifested in the liver tissue associated with progression of the toxicity condition (27).

A significant rise in serum urea and creatinine with nickel treatment in our study indicates that, synthesized proteins were liberated as a result of cytolysis and other pathological changes. It has been reported that low-level of oral exposure to soluble nickel either induces changes of glomerular permeability or enhances the normal age-related glomerular nephritis lesions in ageing rats (27). The hyperactivity of renal tubules as well as altered glomerular filtration altogether may have increased the serum urea and creatinine level in our study. Administration of α-tocopherol could not decrease serum urea and creatinine level when given simultaneously with nickel. A study by El-Demerdash et al. realized similar results like ours (31). Although α-tocopherol, as antioxidant, acted as a preventive measure in heavy metal induced lipid peroxidation in renal tissues, but in this study vitamin supplementation could not show any improvement in rise in serum urea and creatinine level which may be due to non-interference of these antioxidant vitamins on protein catabolism pathway as such.

The increased lipid peroxidation in the liver of nickel-treated rats suggests an increase in phospholipase activity during peroxidic decomposition of different sub organelle and plasma membrane lipids. These changes may be attributed to the effect of

nickel on the hepatic cells. Following cell damage, the membranes become permeable and enzyme activity are found in the extracellular fluid and serum (8, 27). Injury from nickel exposure may also be due to activation of Kupffer cells and a cascade of events involving several types of hepatic cells and a large number of inflammatory and cytotoxic mediators. In the nickel-treated rats, the decreased activities of hepatic SOD, CAT, and GSH-Px, suggests an interaction between the accumulated free radicals and the active amino acids of these enzymes. Nickel-induced decrease in glutathione levels may be due to its increased use in protecting the –SH-containing proteins from lipid peroxides. In Group IV rats a significant improvement in the activity of these antioxidant enzymes compared to Group II could be due to the relative ability of α-tocopherol to scavenge reactive oxygen species within the lipid region of the membrane. The α-tocopherol acting in conjugation with GSH-Px could directly reduce phospholipids hydroperoxides within the membrane and lipoproteins to inhibit lipid peroxidation (32).

The increase in lipid peroxidation resulted from renal cell injury caused by the induction of the Fenton reaction, generating hydroxyl radicals. In the nickel-treated experimental group, the decreased activities of SOD, CAT, and GSH-Px suggests an interaction between the accumulated free radicals and the active amino acids of these enzymes (5). Like the other metabolic tissues, nickel-induced decrease in glutathione levels in kidney seen here may be due to its increased use in protecting –SH-containing proteins from lipid peroxides (6). Studies also revealed that pretreatment with α-tocopherol reduced significantly the lipid peroxidation of renal cells and renal dysfunction induced by renal ischemia-reperfusion in rats (27). It has been found that, higher doses of vitamins are effective to protect oxidative renal damage. The protection is mediated partially by preventing the decline of renal antioxidant status (33).

It may be concluded from present findings that nickel induces oxidative damage in erythrocytes and other major metabolic tissues like liver and kidney. This results in disruption of overall hematology and liver and kidney function and also disrupts tissue antioxidant defense system. But simultaneous treatment with á-tocopherol may protect against toxic influence on above stated hematological and biochemical parameters, and as well as protect from heavy metal induced lipid peroxidation in hepatic and renal tissues.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the Director, Defence Institute of Physiology and Allied Sciences, New Delhi (DRDO, Ministry of Defence, Government of India) for providing financial assistance (*Ref.No. TC/292/TASK-116(KDS)/DIPAS/2006)*. The authors also acknowledge Dr. B. S. Patil, Dean, Al-Ameen Medical College, Bijapur, Karnataka, India for his constant encouragement during this study.

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