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Vitamins C and E Combination Potentially Prevented Oxidative Stress Mediated Liver Injury in Nickel Intoxicated Mice

[1]Faouzi Dahdouh, [1]Mohamed-Reda Djabar and [2]Zine Kechrid

[1]Laboratory of Toxicology, Department of Biology,
Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria
[2]Laboratory of Biochemistry and Applied Microbiology,
Department of Biochemistry, BadjiMokhtar University, Annaba, Algeria

Abstract: The hepatoprotective effect of vitamins C and E against nickel induced oxidative hepatotoxicity in mice was investigated. 64 mice were allocated in eight groups of eight animals each: control (tap drink water and a standard diet), vitamin C (Vit C: 1g /L of drinking water), vitamin E (VitE: 1g/kg of diet), VitC+ VitE (Vit(C+E)), nickel sulfate ($NiSO_4$: 2,7g /kg of diet), $NiSO_4$ + VitC, $NiSO_4$ + VitE and $NiSO_4$ + Vit (C + E); all groups were treated during four weeks. The results showed that nickel sulfate induced significant decrease in body weight along with significant increase in liver-body weight ratio when compared with untreated mice. Liver injury was indicated by the increased activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total bilirubin. Conversely, the serum levels of total proteins and albumin were significantly decreased in nickel treatment as compared to their controls. Liver oxidative indices appeared significantly decreased activities in enzymatic antioxidants like catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase and non enzymatic antioxidants like total sulfhydryl groups and reduced glutathione (GSH) levels. However, treatment with vitamins C and E in combination more significantly improved the altered biochemical and histopathological changes in the liver of Ni-intoxicated mice than the vitamins C and E treatment alone.It is concluded that nickel sulfate treatment causes hepatic oxidative damage in male mice, but simultaneous supplementation of vitamins C and E was found to be more efficient than either vitamin C or E in combating against such stresses.

Key words: Nickel · Vitamins C & E · Oxidative Stress · Hepatotoxicity · Mice

INTRODUCTION

Nickel is a widespread environnemental polluant able to produce undesirable effects and/or carcinogenicity in humans and animals [1]. Nickel is used in many industrial processes such as painting, manufatore of alloys, coins and bateries [2-3]. In the body, nickel can not be destroyed, but it accumulates especially in kidney [4-5] and liver [6-7]. Exposure to nickel occur through respiratory [8] and gastrointestinal systems in acute and chronic exposure [9]. The liver is the first organ exposed to internally absorbed nutrients and other xenobiotics. It's composed of highly active metabolic tissue containing huge complement of detoxification machinery system, since liver serves a major site of nickel acumulation and a target organ of nickel toxicity [10]. Several studies have reported that heavy metals including nickel may cause cell damage through reactive oxygen species (ROS) generation, lipid peroxidation and DNA alteration in liver [11] and kidney cells [12]. Cells can protect themselves against oxidative threat through a variety of defense scavenger systems, such as glutathione (GSH) and superoxide dismutase (SOD) [13]. Overall, these oxidative effects lead to depletion of glutathione and other endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and glutathione S-transferase (GST) [14]. The genotoxic effects of nickel might be prevented by some exogenous supplementation of antioxidant substances which play an important role against the adverse effects of reactive oxygen and nitrogen species [15]. Vitamins C and E are potential antioxidants and they exhibite a

Corresponding Author: Dahdouh Faouzi, Laboratory of Toxicology, Department of Biology, Faculty of sciences, Badji Mokhtar University, Annaba, 23000, Algeria. E-mail: faouzidahdouh@ymail.com.

synergitic interaction when they administred together [16-18], as well as both vitamins were administered as hepatoprotetive [19] and nephroprotective agents [20] over nickel toxicity in mice and rats [21-22]. this study was planned for screening the combined effect of both vitamins against nickel-induced liver dysfunction through studying some biochemical and pathological altration in nickel intoxicated mice.

MATERIALS AND METHODS

Adult Swiss albino mice (nearly 60 days old), weighing between 29 and 33 g were obtained from the Pastor Institute of Algeria. Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 70 % and room temperature of 22 ± 2°C. Food (Standard diet, supplemented by the ONAB, EL-Harouch, Algeria) and water available ad-libitum. After one week of acclimatization, animals were randomly selected into four groups of eight animals and were named:

- Group I (Control): Control animals fed a standard diet prepared as described previously [23] and received a tap water.
- Group II (Ni(II)): Nickel sulfate ($NiSO_4$, $6H_2O$) (Sigma) was administred in feed (2,7g Ni(II)/kg diet) [24].
- Group III (Vit C): Vitamin C (1g/L) was administred in drinking water [25].
- Group IV (Vit E): Vitamin E (1g/kg of diet) was administred in feed [26].
- Group V (Vit (C + E)): Vitamin E (1g/kg of diet) was administred in feed and Vitamin C (1g/L) was administred in drinking water.
- Group VI (Ni(II) + Vit C): Mice received nickel sulfate (2,7g Ni(II)/kg of diet) and vitamin C (1g/L) in their drinking water.
- Group VII (Ni(II) + Vit E): Mice received in feed nickel sulfate and vtamin E (1g/kg diet).
- Group VIII (Ni(II) + Vit (C+E)): Mice received nickel sulfate and combination of vitamin C and vitamin E.

The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution. The treatments of mice continued for a period of four weeks. At the end of the experiment, total body weight was recorded and animals were sacrificed by decapitation without anesthesia to avoid animals stress. At the time of sacrifice, blood was collected for biochemical serum evaluations. Liver was removed immediately and used for assaying glutathione level, antioxidant enzyme activities and to evaluate the liver histology.

Clinical Observations and Body Weight Measurement: Throughout the study, mice of control and treated groups were observed twice dialy (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and behavioural pattern. The body weight of each animal of all groups was measured at the beginning of the experiment and twice weekly during the exposure period using electronic analytical weighing balance.

Biochemical Analysis: Serum of biochemical paramateres (glucose, cholesterol, triglycerides, total lipids, total proteins and albumin) and the activity of liver marker enzymes (Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)) and lactate dehydrogenase (LDH) were assessed using Spinreact Laboratory Spain diagnostic kits and determined by enzymatic colorimetric method using spectrophotometer (Jenway 6505, Jenway LTD, UK).

Enzymatic, Non-Enzymatic Antioxidant and Marker Enzyme Estimations: Separate liver homogenates of experimental mice were used for the estimation of following enzymatic and non-enzymatic antioxidants:

- Glutathione peroxidase (GPx) activity was assayed exactly as described elsewhere [27]. The activity at 25 °C was expressed as µmol of GSH oxidized/min/g protein.
- Catalase (CAT) activity was measured as the decrease in H_2O_2 concentration by recording the absorbance at 240 nm [28].
- Reduced Glutathione (GSH): GSH level was estimated by the method as described previously [29].
- Total sulfhydryl groups (TSH) were measured by the method of Ellman [30].

Organ Histology: The liver samples were dissected and immediately fixed in bouin's solution for 24 h, processed by using a graded ethanol series and then embedded in paraffin. The paraffin sections were cut into 5µm thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed.

Statistical Analysis: Data are given as means ± SE. Statistical significance of the results obtained for various

comparisons was estimated by applying one way analysis of variance (ANOVA) followed by Protected Least Significant Difference Fisher's test (PLSD Fisher) and the level of significance was set at $p < 0.05$.

RESULTS

Clinical Signs of Toxicity and Mortality: Death was not noticed in any experimental groups during the treatment period. However, in nickel treated group, few clinical signs such as bluish discolouration of the skin (at the nasal, ear and genital regions), reduced activity, increasing weakness, hair loss and and reduced food and water intake were observed.

Effect of Treatments on Body Weight, Organ-Body Weight Ratio, Food Intake and Water Intake: As indicated in Table 1, the weekly and terminal mean body weight, water and pellet diet consumption in animals exposed to nickel were significantly decreased ($p < 0.01$) when compared with controls. In contrast, organ body weight ratio increased significantly ($p < 0.001$) in nickel treated group. All these changes induced by nickel sulfate intoxication were significantly ($p < 0.05$) reduced on simultaneous treatment of vitamin C and/or vitamin E when it was compared to Ni(II) alone and control groups. There was no significant difference between control mice and vitamin C and/or vitamin E treated.

Serum Biochemical Paramaters: As seen in Table 2, Ni (II) treatment caused a significant increase in the serum levels of glucose ($p< 0.01$), cholesterol ($p< 0.05$), triglycerides ($p<0.05$) and total lipids ($p<0.001$) along a significantly decrease in serum levels of total proteins ($p<0.001$) and albumin ($p<0.05$) when compared with control group. However, vitamin C and /or vitamin E along with nickel sulfate brought the mentionned biochemical paramaters to near control values. There was no significant difference was observed in vitamin treated groups as compared to control group.

Hepatic Enzyme Markers: As showed in Table 3, the activities of serum hepatospecific enzymes such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) were significantly ($P<0.01$) increased, when compared with control mice. The combined treatments (nickel + vitamin C and/or vitamin E) showed a significantly decrease in the enzyme activities; Ni(II) + Vit C ($p<0.05$), Ni(II) + VitE ($p<0.001$) and (NiII)+Vit(C+E) ($p<0.05$) as compared to nickel alone treated group. In vitamin C or vitamin E treated mice, the parameters analyzed were similar to control mice.

Hepatic Oxidative Stress Parameters: Table 4 illustrates the changes in antioxydant paramaters in liver of control and experimental mice. A significant depletion in reduced glutathione level (GSH) ($p < 0.001$), total sulfhydryl groups ($p < 0.01$) and the activity of catalase $p < 0.01$) and glutathione peroxidase (GPx) ($p < 0.05$) in liver of nickel treated mice when compared with controls. Treatment of vitamin C and /or vitamin E along with nickel sulfate increased the levels of enzymatic and non enzymatic antioxidants in liver when compared with nickel treated group. In vitamin C and/or vitamin E alone treatment, the measured antioxidants appreared a comparable values to controls.

Table 1: Body weight, organ weight, food and water intake in control and experimental mice.

	Body weight(g)				
Groups	Initial	Final	Food intake (g/kg body weight/day)	Water intake (ml/day)	Organ body weight ratio (%) liver
Control	30 ± 0.6	41 ± 1.11	6.62 ± 0.34	7.81 ± 0. 37	0.41±0.04
Ni(II)	29 ± 1.03	18 ± 0.13**	3.52 ± 0.15*	4.46 ± 0.17*	0.83±0.06***
Vit C	31 ± 0.71	38 ± 0.22	6.09 ± 0.26	7.06 ± 0.24	0.41±0.22
Vit E	33 ± 1.1	40 ± 0.17	5.86 ± 0.21	7.18 ± 0.11	0.38± 0.13
Vit(C+E)	29 ± 1.03	40 ± 0.23	6.12 ± 0.3	7.43 ± 0.08	0.40±0.18
Ni(II)+VitC	32 ± 0.9	26 ±1.21^{*+}	4.73 ± 1.2^{*+}	6.75 ± 0.55^{**+}	0.64 ± 0.10 $^{+}$
Ni(II)+VitE	33 ± 1.07	28 ± 0.73^{+}	4.91 ± 1.08^{**+}	6.83 ± 0.31^{*+}	0.60 ±0.24 $^{*+}$
Ni(II)+Vit(C+E)	31 ± 0.66	30 ± 0.45^{*+}	5.15 ± 0.66 $^{+}$	7.21 ± 0.38^{+}	0.57± 0.12^{*+}

Data are presented as means ± S.E.M, n = 8.

The * depicts comparison with control group ($^{**}p < 0.01$, $^{*}p < 0.05$).

The + depicts comparison with Ni(II) group ($^{+}P < 0.05$)

Table 2: Changes in serum biochemical paramaters in control and experimental mice.

Groups	Glucose(g/l)	Cholesterol (g/l)	Triglycerides (g/l)	Total lipids (g/l)	Total proteins (g/l)	Albumin (g/l)
Control	0.86± 0.04	0.41 ± 0.11	0.36 ± 0.03	2.09±0.04	6.01±0.19	2.85 ± 0.06
Ni(II)	1.71±0.14**	1.66±0.2**	1.62 ± 0.25*	3.8 ± 0.6 **	4.03±0.54**	1.08±0.1**
Vit C	0.85± 0.07	0.39± 0.02	0.36±0.06	2.08 ±0.05	5.92 ± 0.1	2.8 ± 0.08
Vit E	0.84± 0.02	0.40± 0.02	0.37±0.06	2.07 ±0.04	5.89 ±0.22	2.83 ± 0.07
Vit(C+E)	0.83± 0.03	0.41± 0.02	0.38±0.06	2.09±0.05	5.93 ± 0.13	2.82 ± 0.06
Ni(II)+VitC	1.2±0.02*++	0.83±0.04*+	1.11± 0.02*+	2.56±0.37++	5.06±0.24+	1.50 ± 0.10
Ni(II)+VitE	1.16±0.04*+	0.7±0.01*+	1.23 ±0.06*+	2.67±0.36*+	5.23±0.3**+	1.53 ± 0.10
Ni(II)+Vit(C+E)	1.05±0.03*	0.7±0.13**	0.93± 0.1*	2.32 ± 0.09	5.46 ± 0.44	1.94± 0.08

Data are presented as means ± S.E.M, n = 8.

The * depicts comparison with control group (*** p < 0.001, ** p < 0.01, * p < 0.05).

The + depicts comparison with Ni(II) group ++ p < 0.01, + P < 0.05)

Table 3: Changes in hepatic enzyme markers in control and experimental mice.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
Control	45.16 ± 1.81	47.39 ± 3.25	56.05 ± 3.81	103.21± 7.81
Ni(II)	86.9 ± 1.72**	78.71± 3.4**	72.30 ± 5.1**	166.1± 9.22**
Vit C	44.80 ± 1.67	46.34 ± 5.91	54.79 ± 4.34	104.12± 7.31
Vit E	43.60 ± 1.29	44.24 ± 2.23	55.42 ± 4.54	109.33 ± 7.17
Vit(C+E)	43.88 ± 2.4	46.15 ± 3.13	54.47 ± 3.18	108.05 ± 7.21
Ni(II)+VitC	54.49 ± 2.42*+	61.93±4.26*++	61.5 ±1.98*+	131.17± 8.11*+
Ni(II)+VitE	57.06± 3.79*+	58.90 ± 5.25*+	63.04 ±2.25++	122.22± 8.05**+
Ni(II)+Vit(C+E)	51.62 ± 3.12	53.97 ± 4.56	59.18 ±1.68	113.41± 6.75*

Data are presented as means ± S.E.M, n = 8.

The * depicts comparison with control group (** p < 0.01, * p < 0.05).

The + depicts comparison with Ni(II) group (++ p < 0.01, + P < 0.05)

Table 4: Changes in the levels of reduced glutathione (GSH), total sulfhydryl groups (TSH) and the activities of glutathione peroxidase (GPx) and catalase of control and experimental mice.

Groups	GSH(µg/mg)	TSH (µg/mg)	Catalase(µmol/mg)	GPx (µmol/mg)
Control	44.96 ± 2.82	12.33 ± 0.25	34.6 ± 2.54	4.48 ± 0.06
Ni(II)	28.41 ± 7.2** *	07.29 ± 0.65**	19.47± 5.40**	2.11 ± 0.07**
Vit C	43.46 ± 1.94	11.81± 0.43	33.1 ± 2.38	4.44 ± 0.03
Vit E	43.31 ± 2.87	11.07± 0.41	33.36 ± 2.63	4.46 ± 0.07
Vit(C+E)	42.34± 2.32	11.73± 0.54	34.08 ± 2.8	4 .47 ± 0.03
Ni(II)+VitC	34.79 ± 7.8*+	09.21± 0.27**+	24.88 ± 6.7*+	3.36± 0.07*+
Ni(II)+VitE	36.42 ± 3.6*++	09.32±0.42*+++	26.94 ±7.98*++	3. 33± 0.06*+
Ni(II)+Vit(C+E)	38.43 ± 2.82	09.40± 0.44*++	29.48 ± 1.73*++	3.68 ± 0.16+

Data are presented as means ± S.E.M, n = 8.

The * depicts comparison with control group (*** p < 0.001 ** p < 0.01, * p < 0.05).

The + depicts comparison with Ni(II) group (+++ p < 0.001, ++ p < 0.01, + P < 0.05).

Histological Results: The mentioned biochemical paramater alterations could be referred to the liver histological changes. The liver of control mice (Fig. 1A), vitamin C and/or vitamin E (Fig. 1C, 1D, 1E) treated mice showed a normal architecture of liver. Nickel exposure resulted in changes in liver architecture as indicated by necrosis of hepatic cells, congestion of central vein and vacuolar degeneration of hepatic cells (Fig. 1B). Nickel along with vitamin C and/or vitamin E treatment (Fig. 2F, 2G, 2H) showed normal hepatocytes with mild vacuolar degeneration of hepatic cells and congestion of central vein.

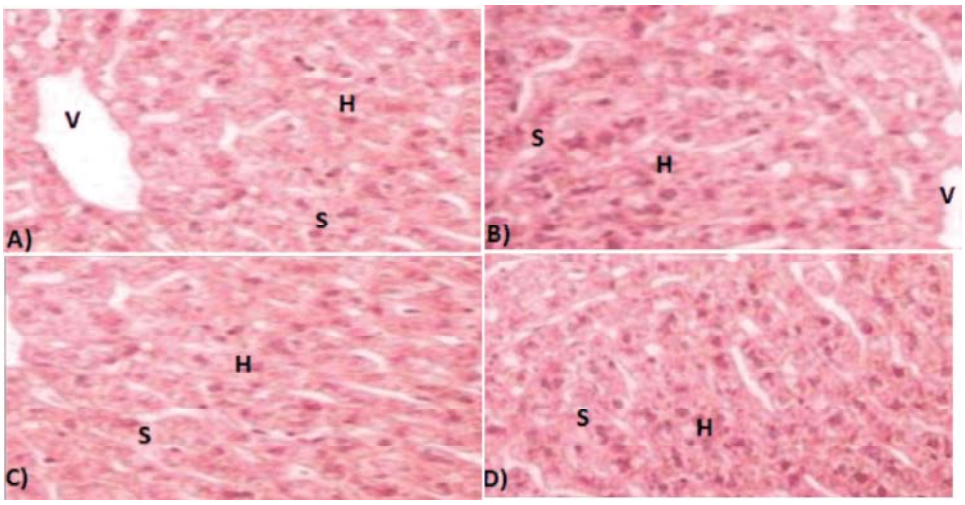

Fig. 1: Light micrograph of mouse liver from control mice (A), vitamin C (B), vitamin E (C) and vitamins C + E (D) treated mice, showing normal structure, central vein (V) hepatocytes (H) and sinusoids (S) H&E. X 280.

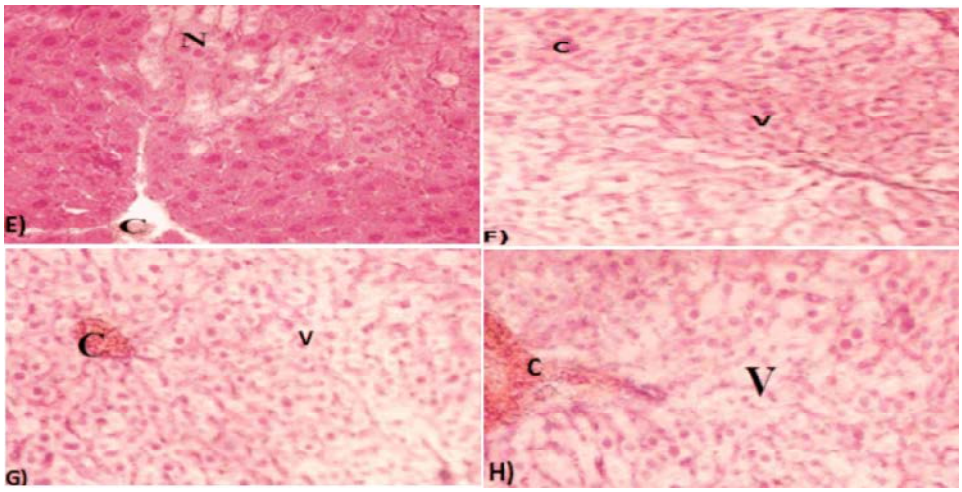

Fig. 2: Light micrograph of mouse liver in experimental groups:
E) Treated mouse with nickel sulfate showing necrosis of hepatic cells (N), congestion of central vein (C) and vacuolar degeneration of hepatic cells (V).
F): Mouse liver treated with nickel + vitamin C, G): Mouse liver treated with nickel + vitamin E,
H): Mouse liver treated with nickel + vitamins C + E, showing normal hepatocytes with mild vacuolar degeneration of hepatic cells and congestion of central vein. H&E. X 280.

DISCUSSION

Nickel is considered one of the most common toxic metals. Nickel intoxication occurred through consumption of contaminated water and food stuff [31]. Once it is absorbed from gastrointestinal tract, nickel bounds to erythrocytes and widely distributed initially to soft tissues such as liver [32] and kidney [33]. Therefore, we select the liver as a target organ to describe the preventive effects of vitamin C and/or vitamin E on nickel induced oxidative liver injury.

The results obtained in our present study show the treatment with nickel sulfate induces a remarkable decrease in body weight, food and water intake along with alterations of organ body weight ratio. All these symptoms of nickel toxicity in mice might be due to tissue damage and reduction in their functions [34-35]. Nickel induced morphological changes were attenuated by treatment with vitamins, which were in agreement with the results of Das *et al*. [36].

The rise in serum glucose may indicate a disruption of c arbohydrate metabolism resulting from enhanced

breakdown of liver glycogen, which is considered to be the main carbohydrate reserve of animals, possibly mediated by an increase of glucagon and concomitant hypo-insulinemia [37-38], mechanisms involved are not understood yet. In addition, hyperglycemia can cause increased production of free radicals via oxidation of glucose and non-enzymic protein glycation that may lead to disruption of cellular functions and oxidative damage to membranes which can be attenuated by vitamin C and/or vitamin E co-treatment [39]. As reported previously, both vitamins are biologically important in reducing toxic effects of toxic metals [40]. Vitamin C or ascorbic acid is defined as a free radical scavenger, able to mediate many intracellular enzymatic reactions. Whereas, the hypoglycemia observed in Ni(II) + Vit C treated mice might be due to the role of aldose reductase and ascorbic acid in reducing the blood glucose level [41]. The reduction of glucose level in Ni(II)+VitE treated group when compared with control group may be due to an attenuation of nickel induced oxidative stress and insulin resistance by α- tocopherol (vit E), thereby boosting insulin sensitivity [42]. Moreover, nickel treatment resulted in perturbations of carbohydrate and lipid metabolism as revealed by an increase in serum levels of total lipids, cholesterol and triglycerides. Similar changes in serum lipid profiles were observed in rats treated with nickel sulphate (20 mg/kg body weight) [43]. The increase in serum levels of cholesterol and triglycerides in mice may be due to changes in gene expression of some hepatic enzyme like HMG-CoA reductase (hydroxyl-methyl-glutaryl-CoA), which in turn depresses low density lipoprotein (LDL) -receptor gene expression [44-45]. On the other hand, the rise in serum triglycerides (TG) is possibly due to hypo-activity of lipoprotein lipase in blood vessels which breaks up TG [46]. Nickel treatment caused a significant decrease in serum total protein and albumin as compared to control mice. Interestingly, the reactive oxygen species (ROS) formation and lipid peroxidation due to nickel intoxication are able to induce liver injury leading to cellular structure alterations and somehow, the liver losses its function in synthesis of protein and carbohydrate molecules [47]. The activities of serum ALT, AST and ALP were significantly increased compared to their normal levels. This effect could be attributed to the hepatic damage resulting in increased release and leakage out of these enzymes from the liver cytosol into the blood stream which gives an indication on the hepatotoxic effect of this metal [48]. The liver was defined to be the first organ exposed to internally absorbed nutrients and other xenobiotic including heavy metals. Additionally, it is composed of highly active metabolic tissue containing huge complement of detoxification machinery system [49]. The most common findings in liver were fatty degenerative changes, necrosis of the parenchyma of hepatic lobule and a loss of normal architecture of the hepatocytes. These observations are in agreement with Rauen *et al.* [50]. In the combined treatments along with nickel sulfate, the histological alterations of liver have attenuated when compared with control normal group. In the current study, Ni(II) caused an oxidative stress by affecting the antioxidant defense system, like CAT, GPx and GSH due to ROS formation leading to lipid peroxidation and alterations of the antioxidant status of several tissues in rats and mice. The oxidative stress due to nickel intoxication was reported in some previous studies [51-52]. The sulfhydryl group and reduced glutathione interact and form a complex with nickel and thereby alter nickel distribution and excretion. It is in agreement with our findings, which showed that the levels of total sulfhydryl groups, reduced glutathione, were significantly depleted; it might also contribute to the development of nickel induced hepatic damage [53]. In addition, the decreased activity of hepatic catalase and GPx in nickel treated animals could be related to the interaction between the accumulated free radicals and the active amino acids of these enzymes or to the direct binding of the metal to the active sites of the enzyme [54]. In Ni(II)+ Vit C and /or Vit E treated groups, the significant improvement of the antioxidant parameters were noticed when compared with Ni(II) alone treated group. Thus, the observed normalization of GSH and TSH levels, GPx and catalase activities following vitamin C and/ or vitamin E treatment might be due to the decrease of lipid peroxidation (LPO) and the accumulation of oxygen reactive species (ROS) in cells [55].

In conclusion, our study has clearly demonstrated the protective effect of vitamin E and/or vitamin C on nickel induced liver oxidative injury, through alterations of liver biochemical markers and some major antioxidant parameters. On the other hand, our data showed that combined vitamins administration showed better protection than if given separately against nickel toxicity.

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Conflict of interest: All of the authors have declared that no competing interests exist.

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