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Preventive effect of zinc on nickel-induced oxidative liver injury in rats

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This study pertains to the potential ability of zinc, used as nutritional supplements, to alternate oxidative stress induced by nickel. Male rats were randomly divided into four groups of eight each. Group I served as the controls; group II received in their drinking water $ZnSO_4$ (227 mg/l); group III received $NiSO_4$ (2 mg/100b.w/day intraperitoneally); group IV was treated with $ZnSO_4$ and $NiSO_4$. The exposure of rats to nickel sulfate for 21 days resulted in a significant decrease in body weight gain and absolute liver weight, relative liver weight. Nickel treatment also produced oxidative liver injury characterized by increasing serum glucose concentration, glutamate-pyruvate transaminase (GPT), alanine aminotransferase (GOT) and alkaline phosphatase (ALP) activities. Meanwhile nickel supplementation decreased serum total protein and albumin in animals. In addition, liver glutathione level, catalase and glutathione peroxidase (GSH-Px) activities were diminished. The administration of zinc with nickel (Ni + Zn) corrective effects on Ni-induced oxidative stress in liver was observed. In conclusion, this study demonstrates that intraperitoneally injection with Ni caused reduction in enzymes activities in rat's liver and treatment with zinc offers a relative protection against nickel induced oxidative liver injury and lipid peroxidation probably due to its antioxidant proprieties.

Key words: Nickel, zinc, rats, oxidative stress, liver.

INTRODUCTION

The rapid development of science, industry, medicine, and agriculture has exposed man and his environment to number of exotic heavy metals. Nickel is the major components of the alloys employed in the plate and screw used for connecting bones in orthopaedic surgery and in the manufacture of artificial organs (Kocijan et al., 2004). However, excessive amounts of this transitional metal ion are toxic. Numerous authors have studied the impact of nickel on health. It can cause dermatitis to certain persons (Accominoti et al., 1998). Particle of nickel may cause some morphological transformations in numerous cellular systems and chromosomal aberrations (Coen et al., 2001). The salts of nickel as particles of nickel can be allergens and carcinogens in man while forming the oxygenated radicals (Lansdown, 1995). This cytotoxicity was investigated in numerous micro-organisms (Wu et al., 1994). Nickel was also found to be responsible on many sexual disorders (Chakroun et al., 2002). After entering the body,

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Abbreviations: GOT, Glutamate oxaloacetate transaminase; **GPT,** glutamate pyruvate transaminase; **ALP,** alkaline phosphatase; **GSH,** reduced glutathione; **GSH-Px,** glutathione peroxidase; **DTNB,** 5, 5'dithiobis-(2-nitrobenzoic acid; **ROS,** reactive oxygen species; **MT,** metalothionine.

nickel penetrates all organs and accumulates primarily in bone, liver, kidney and excreted through bile and urine (Kusal et al., 2007). Liver is the primary target for environmental and occupational toxicity and the major site for detoxification. Nickel induced severe liver and kidney damage by altering several marker enzymes and ascorbate-cholesterol metabolism. One of the harmful effects of nickel action in the body is to induce formation of reactive oxygen species (ROS) and increase lipid peroxidation in the cells (Sunderman et al., 1985). 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 processes (Kusal et al., 2007).

Zinc is ubiquitous in sub-cellular metabolism and is an essential component of catalytic site(s) of at least one enzyme in every enzyme classification (Coyle et al., 2002). Others have clearly demonstrated the hepatoprotective role of zinc under different toxic conditions (Cabre et al., 1999). Zinc is involved in stabilizing the cell membrane and prevents oxidative destruction caused by free radicals (Bettger and O' Dell, 1981; Ludwig and Chvapil, 1982) at least under certain conditions, may have antioxidant properties (Powell, 2000). It can protect against oxidative damage caused by certain xenobiotics (Fukino et al., 1986). In addition, zinc is also known for inducing methallothionein (MT) synthesis, a protein that is able to bind heavy metals and to scavenge hydroxyl radicals (Cousins and Hempe, 1990). The indications of biological antagonism between nickel and zinc have also been reported (Kasprzak et al., 1986). Nickel apparently affects zinc metabolism as evidenced by altered urinary excretion patterns (Clary, 1975) and organ distribution (Whanger, 1973). Therefore, the present study was designed to evaluate whether zinc supplementation could have a protective effect against nickel-induced oxidative liver injury in male albino rats.

MATERIALS AND METHODS

Chemicals

Zinc sulphate ($ZnSO_4$ $7H_2O$) and nickel sulphate ($NiSO_4 6H_2O$), 5, 5'dithiobis-(2-nitrobenzoic acid (DTNB) and reduced glutathione were purchased from sigma Chemical Co (St Louis, France) and all other chemicals used in the experiment were of analytical grade.

Animals

Thirty-two male albino (Wistar) rats of ten weeks of age with a body weight of 180-205 g were obtained from the Pasteur Institute (Algiers, Algeria). Animals were acclimated for two weeks for adaptation under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity of 40% and room temperature of 22 ± 2°C. Food (Standard diet, supplemented by the ONAB, EL-Harouch, Algeria) and water were provided *ad-libitum*.

Experimental design

Animals were randomly divided into four different groups of eight animals each. One served as normal control. The second group (Zn) was given zinc sulphate $ZnSO_4$ $7H_2O$ in drinking water at a dose level 227 mgZn/l, while the third group (Ni) was intraperitoneally given nickel sulphate ($NiSO_4 \cdot 6H_2O$) at a dose of 2 mg/100g b. w./day . Finally, the fourth group (Ni + Zn) was treated daily with both zinc sulphate and nickel sulphate as in group two and three. The treatment of all groups lasted for three consecutive weeks.

The dose of $NiSO_4$ $6H_2O$ and the period of treatment were selected on the basis of previous studies (Kusal et al., 2001), whereas $ZnSO_4$ $7H_2O$ dose was chosen based on clinical application and on results from previous studies (Sidhu et al., 2004). 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 rats continued for a period of three weeks. At the end of the experiment, total body weights were recorded and animals were sacrificed by decapitation without anesthesia to avoid animals stress. At the time of sacrifice, blood was transferred into ice cold centrifuged tubes. Tubes were then centrifuged for 10 min at 3000 rpm and serum was used for glucose, total protein, albumin, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) assays. Livers were removed immediately and one part of the lobe was processed immediately for assaying glutathione and antioxidant enzymes activities. The other lobe was used for light microscopic studies.

Analytical methods

Determination of biochemical parameters

Serum glucose level was estimated with a commercial kit (Spinreact, Spain, ref; 41011) and determined by enzymatic colorimetric method using spectrophotometer (Jenway 6505, Jenway LTD, UK). However, GOT, GPT and ALP activities were determined with commercial kits from Spinreact, Spain, refs: GOT-1001161, GPT-1001171 and ALP-1001131, respectively. Total protein and albumin concentrations were also measured using commercial kits (Spinreact, Spain, refs: total proteins-1001291 and albumin-1001020.

Tissue preparation

About 1 g of liver was homogenized in 2 ml of buffer solution of phosphate buffer saline 1:2 (w/v; 1 g tissue with 2 ml TBS, pH 7.4). Homogenates were centrifuged at 10000 x g for 15 min at 4°C and the resultant supernatant was used for the determination of reduced glutathione and protein levels in one hand and the estimation of catalase and GSH-Px activities in the other hand.

Estimation of reduced glutathione level (GSH)

Liver GSH content was estimated using a colorimetric technique, as mentioned by Ellman (1959) modified by Jollow et al. (1974), based on the development of yellow colour when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 ml of liver supernatant was added to 0.3 ml of 0.25% sulfosalycylic acid, and then tubes were centrifuged at 2500 x g for 15 min. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). The absorbance at 412 nm was recorded. Finally, total GSH content was expressed as n mol GSH/mg protein.

Determination of glutathione peroxidase (GSH-Px)

GSH-Px (E.C. 1.1.1.1.9) activity was measured by the procedure of Floche and Gunzler (1984). Supernatant obtained after centrifuging 5% liver homogenate at 15000 x g for 10 min followed by 10.000 x g for 30 min at 4°C was used for GPx assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M. pH 7.4). 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 H_2O_2 (1 mM) and 0.3 ml of liver supernatant. After incubition at 37°C for 15 min, the reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500 x g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Assay of catalase activity

The activity of catalase was determined according to the method of Aebi (1984). The reaction mixture (1 ml) that contained 0.78 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of liver supernatant, and 0.02 ml of H_2O_2 (0.5 M) was prepared. The reaction was started by adding H_2O_2 and decomposition was monitored by following the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated by using an extinction coefficient of 0.043 $mM^{-1}cm^{-1}$.

Protein determination

The protein content of tissues samples were measured by the method of Bradford (1976) by using bovine serum albumin as a standard.

Histological studies

For histological examination, livers was dissected and immediately fixed in bouin solution for 24 h, processed by using a graded ethanol series, and then embedded in paraffin. The paraffin sections were cut into 5 µm thick slices and stained with hematoxylin and eosin (Haoult, 1984) for light microscopic examination. The sections were then viewed and photographed.

Statistical analysis

Data are given as means ± SEM. Statistical significance of the results obtained for various comparisons was estimated by applying one way analysis of variance (ANOVA) followed by Student's t-test and the level of significance was set at $p < 0.05$.

RESULTS

Effect of treatment on body, absolute and relative liver weights

The body, absolute and relative liver weights of rats subjected to different treatments are shown in Table 1. In this experiment, it was observed that the control body weight gain and Zn-treated group have increased progressively during the study. However, in Ni-treated animals, the results showed obviously significant decrease ($p < 0.001$) in body weight gain as compared to the control group. In addition, a significant increase of Ni-treated group in absolute and relative weights was noticed at $p < 0.001$ and $p < 0.01$, respectively. However, zinc supplementation reversed these changes.

Effects of treatments on serum biochemical parameters

Compared to the controls, total protein and albumin levels in Ni-treated animals were significantly reduced ($p < 0.001$ and $p < 0.01$), but the combination of zinc with nickel produced a recovery in above mentioned biochemical variables (Table 2). In addition, the glucose concentration, GOT, GPT and ALP activities were significantly higher ($p < 0.001$) in nickel group than those of control group, indicating liver damage. However combined treatment of nickel and zinc markedly ameliorated these variations.

Effects of treatments on hepatic oxidative stress parameters

Figure 1 shows that after nickel treatment, the liver glutathione level, catalase and GSH-Px activities were significantly diminished ($p < 0.001$) in nickel experimental comparison with the control group. The simultaneous treatment with zinc partially reversed these changes to near untreated control values.

Histopathalogical results

The mentioned biochemical alteration could be referred to as the liver histological changes. In fact, liver of the control group had a regular histological structure with a characteristic pattern of hexagonal lobules separated by interlobular septa, traversed by portal veins (Figure 2A). In contrast, liver of nickel treated group had weak pathological alteration such as the presence of cellular debris within a central vein and cytological vacuolization (Figure 2C). In addition, no histological alterations were observed in the liver of Zn-treated group (Figure 2B) as compared to the control. However, the combination group of Ni-Zn showed prominent recovery in the form of the liver histo-architecture (Figure 2D), such as the reduced cytoplasmic vacuolization and the normal sinusoidal spaces.

DISCUSSION

In this experiment, body weight gain of nickel rats group was significantly depressed. This action of nickel may be mediated by alteration in zinc metabolism such as other heavy metals (Kuhnert et al., 1987). In fact heavy metals have been recognized as antimetabolite of zinc (Brozoska and Moniuszko-Jakoniuk, 2000). Disturbances in zinc function and metabolism may have serious consequences for health. This element plays an important role in growth, development and functioning of all living cells (Nishi, 1996; Sameeh et al., 2009). As a result, zinc supply significantly prevented the nickel induced decrease in body weight gain. In this experiment, nickel sulphate group animals showed also high level of glucose. The elevation in serum glucose is a common result of nickel toxicity and is usually linked with inhibition of insulin release from Langerhans'islets (Dormer et al., 1973; Kechrid et al., 2006; Djemli et al., 2012) or with a block of glucose utilization by cells even in the presence of elevated concentrations of insulin (Sunderman et al., 1976) or the high glycogen breakdown and new supply of glucose production from other non carbohydrate sources such as proteins (Cartana and Arola, 1992). However there is an amelioration of blood glucose concentration in

Table 1. Body weight gain, absolute and relative liver weights of control male rats, treated with zinc (zinc sulphate), nickel (nickel sulphate) and zinc coadministrated with nickel, after 3 weeks of treatment.

Parameter	Experimental groups (Mean ± SEM; n = 8)			
	Control	**Zn**	**Ni**	**Ni + Zn**
Initial body weight (g)	188 ± 2.4	191 ±3.1	190 ±3.4	189 ±3.1
Body weight gain (g)	53.2^{a} ±1.2	55.7^{a} ± 1.7	25.6^{b} ±1	37.6^{c} ±1.1
Absolute liver weight (g)	10.2^{a} ±0.1	10^{a} ±0.4	13.5^{b} ±0.3	10.4^{a} ±0.5
Relative liver weight (g)	2.90^{a} ±0.14	2.71^{a} ±0.16	3.56^{b} ± 0.08	2.78^{a} ± 0.14

a, b, c, Values within a horizontal line with different superscript letters were significantly different ($p < 0.05$). Values are mean ± SEM, n = number of animals.

Table 2. Changes of biochemical parameters of control male rats, treated with zinc (zinc sulphate), nickel (nickel sulphate) and zinc coadministrated with nickel, after 3 weeks of treatment.

Parameter	Experimental groups (Mean ± SEM; n = 8)			
	Control	**Zn**	**Ni**	**Ni + Zn**
Glucose (mg/100ml)	124^{a}±2.35	121^{a} ±2.66	190^{b}± 5.35	157^{c}± 2.76
Total protein (g/100ml)	8.8^{a}±0.2	8.6^{a}±0.3	6.6^{b}±0.1	8.4^{a}± 0.2
Albumin (g/100ml)	4.5^{a}±0.3	4.7^{a} ±0.2	3.1^{b}±0.2	4.3^{a}±0.1
GOT (U/L)	85^{a}±1.1	83^{a}±0.7	113^{b}±1.6	91^{c}±2.5
GPT (U/L)	39^{a}±1.6	40^{a}±2.7	72^{b}±2.4	60^{c}±3.4
ALP (U/L)	118^{a} ±3.8	116^{a}± 1.9	198^{b}±5.8	165^{a} ±1.8

a, b, c,Vvalues within a horizontal line with different superscript letters were significantly different ($p < 0.05$). Values are given as mean ± SEM, n = number of animals.

animals treated with both metals nickel and zinc. It is probably as a result of the glycaemia lowering effect of zinc sulphate by decreasing systemic glucose accumulation, diminishing nickel binding to biomolecules, improving insulin secretion and action (Song et al., 2006) and/or protects the enzymes and ATP involved in glucose metabolism against inactivation by nickel (Nielsen, 1980).

In the present study, significantly decrease in the total protein and albumin levels was found. These findings confirm the work of Sidhu et al. (2004), when both zinc and nickel were given together in drinking water. The decrease in these two biochemical parameters concentrations of Ni-treated rats might be due to changes in protein synthesis (Kusal et al., 2000; Dostal et al, 1989). The liver is the target organ of heavy metals toxicity and its cells spell out hepatic enzymes into blood, which are commonly used as biochemical indicator index of hepatocellular damage. In the present investigation, nickel intoxication caused a significant increase in the activities of GOT, GPT and ALP, probably due to hepatocyte membrane 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 (Gama and Eatmad, 2011). These results are consistent with previous findings by some research groups who had found an association between nickel toxicity and the increased oxidative stress of rats (Novelli et al., 1998; Al Hassan et al., 2010). Consequently, biochemical perturbations seem to be correlated with the liver histological alteration such as the presence of cellular debris within a central vein and a cytoplasmic vacuolization.

Previous histological studies on liver have documented Ni-induced changes characterized by dilated sinusoids, vacuolization and the appearance of hepatic cells with distorted nuclei (Ben Amara et al., 2010; Rabbani-Chadegani et al., 2011; Djemli et al., 2012). The combination treatment of zinc improved the histological alteration induced by nickel, which could be attributed to the antiradicals/ antioxidant and metal-chelating efficacy of this element. In addition, these findings are in good agreement with those obtained by other studies which postulated the beneficial role of zinc on histological and enzymatic changes of rats (Dhawan and Goel, 1992; Djemli et al., 2012). These reports emphasized the hepatoprotective efficacy of zinc under CCl4 induced liver injury, as zinc treatment helped in the maintaining the homeostasis through regulation of protein synthesis. Thus the supplementation of zinc had protected liver function from nickel intoxication as indicated by the significant restoration of serum total protein, albumin, GOT, GPT and alkaline phosphatase.

The diminution of glutathione level in nickel rats may be as a result of oxidative stress, which has been occurred, in nickel toxicity (Djemli et al., 2012). In other words the

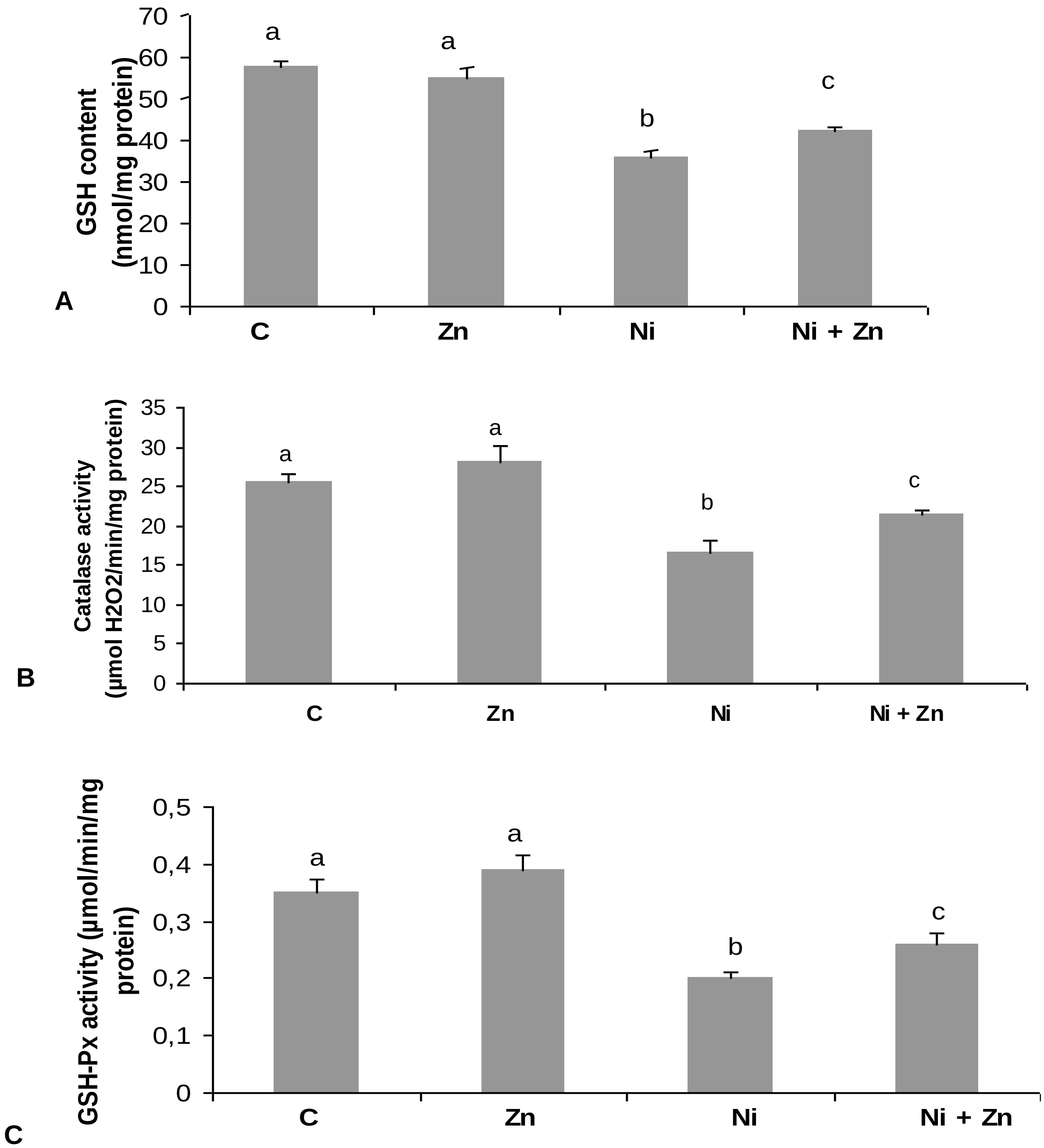

Figure 1. Values of glutathione, catalase and GSH-Px in liver of control and rats treated with zinc (zinc sulphate), nickel (nickel sulphate) and zinc coadministrated with nickel, after 3 weeks of treatment. a, b, c, Values with different superscript letters were significantly different ($p < 0.05$). Values are given as mean ± SEM for group of 8 animals each.

reduction of antioxidant production was due to the increased oxygen metabolites and the elevated free radicals, which cause a decrease in the activity of the anti-oxidant defense system (Gstraunthaler et al., 1983; Iscan et al., 2002) and several pathways have been proposed to show the depletion of GSH level in heavy metals toxicity (Mohandas, 2010). Firstly, the sulfhydryl group of cysteine moiety of glutathione has a high affinity of metals, forming thermo-dynamically stable mercaptide complexes with several metals (Aposhian, 1989). Secondly, GSH may be oxidized due to the interaction with the free radicals induced by nickel. These complexes are inert which can be excreted via the bile, and therefore GSH level could be consumed during Ni detoxification (Manna et al., 2008; Mohandas, 2010). In addition the decreased activity of hepatic CAT and GSH-Px in nickel treated animals,

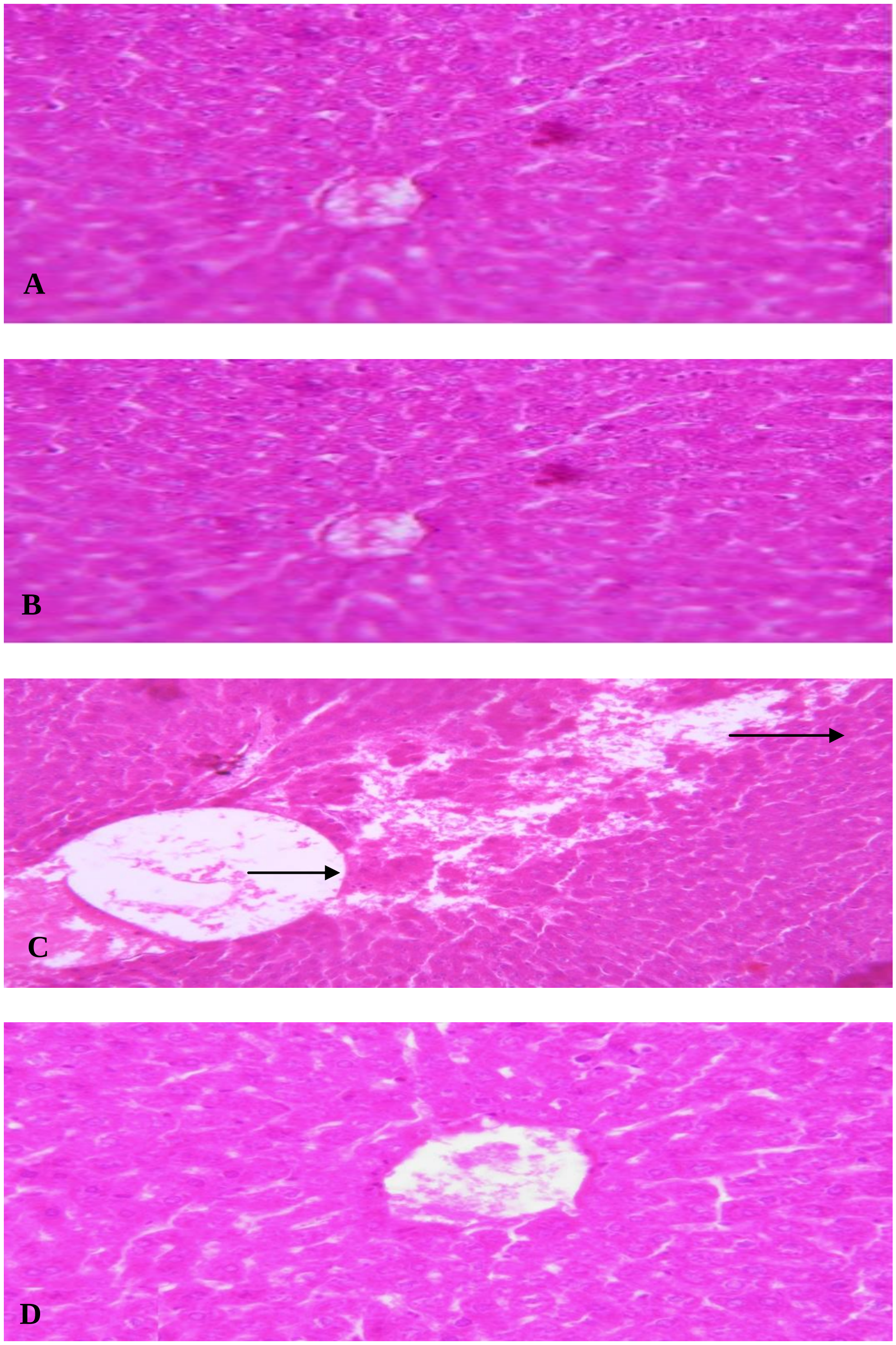

Figure 2. Effect of nickel (nickel sulphate) and zinc (zinc sulphate) coadministrated with nickel on histological damage in the liver. Control (A), treated with Zn (B), Ni (C) and Zn co-administrated with Ni (D). Optic microscopy: sections were stained using the haematoxylin-eosin method (400 x). Arrows: - indicate a presence of cellular debris within a central vein and- indicate cytoplasmic vacuolisation. Zn coadministrated with Ni maintained granular cytoplasm and normal hepatocytes.

suggests that there is an interaction between the accumulated free radicals and the active amino acids of this enzymes (Kusal et al., 2001). In Group III (nickel sulphate + zinc sulphate), the significant improvement of the glutathione level was noticed when compared with that of Group II. The observed normalization of GSH levels and GSH-Px and catalase activities following zinc treatment could be because of its property to induce metallothionein

(S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels and accumulation of oxygen reactive species (Seagrave et al., 1983; Cousins and Hempe, 1990).

Conclusion

In conclusion, this study demonstrates exposure to nickel provoked oxidative liver injury by inducing lipid peroxidation, which led to depletion of liver reduced glutathione, reduction in antioxidant enzyme activities and biochemical parameters variations of rats. However, zinc treatment could protect liver against nickel toxicity by increasing GSH level and the activities of antioxidant enzymes and ameliorated some biochemical parameters and approached them closer to their normal values.

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