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Effect of different dietary zinc levels on hepatic antioxidant and micronutrients indices under oxidative stress conditions

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Abstract

Dietary zinc (Zn) status exerts a powerful influence on the degree of oxidative damage caused by free radicals. We examined the effect of dietary Zn variations with oxidative stress (OS) treatment on antioxidant status, liver function, and status of vitamins in male Wistar rats. Oxidative stress was generated by intraperitoneal injections of tert-butyl hydroperoxide; and dietary Zn variations done were Zn deficient, normal, and excess, with 8, 30, and 60 mg Zn per kilogram diet, respectively. After 21-day dietary regimen, the animals were killed; and plasma aspartate aminotransferase, alanine aminotransferase, hepatic antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), Zn, reduced glutathione, lipid peroxidation (LPO), and hepatic riboflavin, nicotinic acid, and ascorbic acid estimations were done. The alanine aminotransferase and aspartate aminotransferase levels were elevated in rats with OS and Zn-deficient diet, which were restored to normal levels with excess dietary Zn. Hepatic antioxidant enzymes and reduced glutathione levels were significantly decreased with concomitant increase in LPO due to OS induction in animals with Zn-deficient diet. Corresponding enhanced enzyme activities, higher hepatic Zn, and lowered LPO were observed in animals with normal- and excess-Zn diet. A dose-dependent increase in hepatic nicotinic acid accumulation was observed as the dietary Zn level increased from deficient to excess; however, there was no influence on riboflavin and ascorbic acid status. The results suggest that Zn may have a therapeutic potential in treatment of oxidative liver damage along with enhanced nicotinic acid absorption.

1. Introduction

Zinc (Zn) is necessary for normal liver function; and vice versa, the liver plays a central role in Zn homeostasis. Consequently, liver diseases affect Zn levels, whereas Zn deficiency could participate in their pathogenesis [1] and reduced hepatic Zn levels have been correlated with impaired liver function and regeneration [2]. Zinc also plays an important role in the therapy for several liver diseases and has been shown to attenuate or protect against a variety of hepatotoxins such as carbon tetrachloride, bromobenzene, and several metals [3-5]. Zinc has a variety of effects on biological activities that might explain this hepatoprotective action. These are the following: (1) Zn stabilizes membranes and inhibits lipid peroxidation (LPO) [3,6,7]; (2) Zn induces hepatic metallothionein (MT), which is rich in SH groups and binds certain toxic metals such as Cu [3]; (3) Zn is required for P450 activity, which is important in toxic drug metabolism [8]; (4) Zn improves the protein synthesis function of liver [6]; and (5) the hepatoprotective action of Zn administration against oxidative stress (OS) was imposed through the regulation of the metabolism of superoxide dismutase (SOD), MT, reduced glutathione (GSH), and other antioxidants such as vitamins E and C [9-11].

The dietary Zn status exerts a powerful influence on the degree of injury caused by free radical–type, oxidative damage–producing agents. Several lines of indirect evidence also support this concept. Zinc inhibits oxidative damage to molecules, organelles, and cells in vitro and inhibits injury in rodents caused by CCl_4, endotoxin, or radiation [12]. However,

Authors' contribution to the study: RST and SGT were involved in data collection, data analysis, data interpretation, literature search, and manuscript preparation. KVT was involved in data collection. VVA was involved in study design, data analysis, data interpretation, literature search, manuscript preparation, and review of the manuscript.

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effect of Zn in oxidative injury due to tert-butyl hydroperoxide (t-BHP) has not been systematically investigated.

Our previous studies with erythrocytes, Caco-2 cells, hepatocytes, mice, and humans [13-20] have demonstrated effect of water-soluble vitamins on Zn bioavailability, indicating interactions between them. It was felt worthwhile to test if these interactions are bidirectional by study of dose response of Zn on vitamin status. Furthermore, these interactions may assume different directions under OS conditions. Hence, the effect of Zn supplementation on vitamin availability in the presence of OS was investigated.

The specific objectives were to study the effect of dietary Zn variations with OS treatment on (1) antioxidant status and liver function and (2) status of vitamins, that is, hepatic riboflavin (as flavin adenine dinucleotide [FAD]), nicotinic acid (NA) (as nicotinamide adenine dinucleotide [NAD]), and ascorbic acid (AA).

2. Materials and methods

2.1. Animals, diet, and experimental design

Weanling male Wistar rats weighing 50 ± 10 g were procured from the Animal Facility, Agharkar Research Institute, Pune. The animals were housed individually in polypropylene cages in the institute's animal house under hygienic conditions in a room maintained at 24°C ± 2°C and with a 12-hour light-dark cycle. During the study period of 3 weeks, the rats were fed modified AIN-93G diet prepared as per American Institute of Nutrition guidelines [21] containing casein as the source of protein, wheat bran as a source of fiber, and corn starch and sucrose as the sources of carbohydrates with only variation in the Zn content as described below. All of the rats were treated according to the experimental procedures approved by the Institutional Animal Ethics Committee.

The study design and grouping of animals for the experiment are shown in Fig. 1. The animals were randomly divided into 2 main groups, group (G) I and GII, which were with OS and without OS (OS control), respectively. Based on the diet given during the treatment, GI and GII animals were further divided into 3 subgroups, a, b, and c, depending on the dietary Zn levels, as follows: Zn marginally deficient, 8 mg Zn per kilogram diet (Zn D); Zn normal, 30 mg Zn per kilogram diet (Zn N); and Zn excess, 60 mg Zn per kilogram diet (Zn E), respectively. These levels were decided based on previous reports in which rats in Zn-deficient group were given 1 [22], 2.5 [23], 5 [24], and 10 mg Zn per kilogram diet [25]. In nutritionally adequate Zn diet group, Hosea et al [22] had given 30 mg Zn per kilogram diet, whereas excess Zn supplementation was performed by Zhou et al [26] by adding Zn sulfate to the liquid diet at concentration of 75 mg elemental Zn per liter. All rats were fed the treatment diets and distilled water ad libitum throughout the experimental period. Group I animals were given OS treatment on the fourth day of each week by intraperitoneal injection of 0.22 mmol/L per kilogram body weight of t-BHP. The given dose of t-BHP is reported to result in OS leading to acute hepatic damage in rats [27,28]. Animals from GII that served as non-OS controls were injected with saline on the same day. The diet and OS treatment schedule for GI and GII animals are shown in Fig. 2. A weekly record of the body weight changes and food intake of the rats for all the groups was maintained.

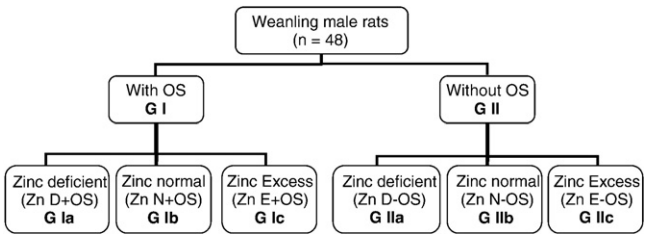

Fig. 1. Study design of dietary Zn variation experiment.

2.2. Tissue sampling

Twenty-one days after the initiation of the dietary regimen, the rats were deprived of food for 6 hours and then euthanized under light ether anesthesia. Blood was collected by cardiac puncture into tubes containing EDTA. Immediately after collecting the blood, the animals were dissected; and livers were excised and rinsed in phosphate-buffered saline. A small section of each liver was placed in 10% phosphate-buffered formalin for histopathologic analysis, and the remaining liver was processed for various biochemical estimations. The blood samples were centrifuged at 4000 rpm for 10 minutes. The plasma was separated, kept at 4°C, and processed on the same day for the estimation of liver marker enzymes, namely, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

2.3. Estimation of AST and ALT

The enzyme activities of AST and ALT were estimated using commercial kits (Ranbaxy Diagnostics, Gurgaon, India). A 0.1-mL fraction of plasma was mixed with 1 mL of substrate (aspartate and α-ketoglutarate for AST; alanine and α-ketoglutarate for ALT, in Tris buffer [pH 7.8] containing lactate dehdyrogenase, malate dehydrogenase, and NADH). After 1 minute of incubation, the change in absorbance at 340 nm was measured for 3 minutes. The rate of decrease in absorbance due to oxidation of NADH to NAD is proportional to AST and ALT activities. The mean

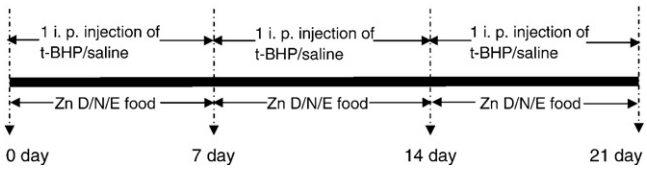

Fig. 2. Diet and OS treatment schedule for GI and GII rats during the experimental period.

absorbance change per minute was determined, and enzyme activities were calculated and expressed in terms of international units per liter of plasma at 37°C.

2.4. Hepatic antioxidant enzymes

2.4.1. Preparation of liver supernatant:

The liver samples were homogenized in 100 mmol/L potassium phosphate buffer (pH 7.5) containing 0.15 mol/L KCl with a Potter-Elvenjhem homogenizer to obtain 10% homogenate. Tissue homogenates were centrifuged at 10 000*g* for 30 minutes at 4°C, and the supernatant was used for the estimation of different enzymes and LPO.

2.4.2. Catalase assay

Catalase activity was determined by the method of Clairborne and Fridovich [29]. Decrease in absorbance at 240 nm was measured spectrophotometrically, and enzyme activity was expressed as nanomoles per liter of H_2O_2 decomposed per minute per milligram protein. The specific activity of catalase was calculated using the molar extinction coefficient for H_2O_2 as 43.6 L mol^{-1} cm^{-1} at 240 nm in the following equation:

$$\text{Specific activity (units per minute per milligram protein)} = \Delta A_{240nm}\ (1\ \text{minute}) \times 1000 / 43.6 \times \text{mg protein}$$

2.4.3. SOD assay

The activity of SOD in cells was estimated by using the method of Kono [30]. The rate of nitroblue tetrazolium (NBT) reduction was measured at 340 nm for 5 minutes using a spectrophotometer (Thermo Spectronic UV1, Thermo Fisher Scientific Inc., Waltham, USA). Percentage inhibition in the rate of NBT reduction was calculated, and 1 unit of enzyme was expressed as inverse of the amount of protein required to inhibit the reduction rate of NBT by 50%.

2.4.3. Glutathione peroxidase assay

Glutathione peroxidase (GPx) was assayed by the method of Mohandas et al [31]. The decrease in the absorbance due to NADPH oxidation was monitored spectrophotometrically at 340 nm. The nonenzymatic reaction rate was correspondingly assayed by replacing the cell lysate with phosphate buffer. The enzyme activity was expressed as nanomoles per liter NADPH oxidized per minute per milligram protein and was calculated using an extinction coefficient of 6.22 L $mmol^{-1}$ cm^{-1}.

2.4.4. Protein estimation

The protein concentration of the cell lysates was determined by the method of Lowry et al [32]. After incubation, sample absorbance was measured spectrophotometrically at 660 nm. The amount of protein in the samples was estimated by using bovine serum albumin as standard protein.

2.5. Estimation of GSH

Reduced GSH was estimated in the tissue homogenates of liver by using the method of Ellman [33]. Briefly, 0.25 mL of the tissue homogenate was added to 0.5 mL of precipitating buffer (5% trichloroacetic acid [TCA] in 1 mmol/L EDTA). After protein precipitation by TCA, the samples were centrifuged at 2000*g* for 10 minutes to obtain the supernatant. A 0.1-mL fraction of the supernatant was mixed with 2.5 mL 0.1 mol/L phosphate buffer (pH 8.0); and color was developed by adding 0.1 mL of 0.01% 5,5′-dithiobis(2-nitrobenzoic acid). Optical density of the yellow-colored complex was measured within 10 minutes at 412 nm against a blank sample without tissue homogenate. The GSH concentrations of samples were calculated using a molar extinction coefficient value of 14150 L mol^{-1} cm^{-1} and expressed as nanomoles per liter GSH per milligram protein.

2.6. Analysis of LPO

Malondialdehyde (MDA), the secondary product of LPO, was estimated in the liver samples using the colorimetric reaction of thiobarbituric acid. It gives an index of the extent of peroxidation of lipids. The estimation of LPO in liver was done by the method of Placer et al [34]. In brief, 0.5 mL of homogenate was mixed with 0.5 mL of 30% TCA and 0.1 mL of 0.1% thiobarbituric acid (Sigma, St Louis, MO). The tubes were placed in water bath at 80°C for 60 minutes. The tubes were then kept in ice-cold water for 10 minutes, followed by centrifugation at 3000 rpm for 15 minutes. The absorbance of supernatant was measured at 535 nm, and the concentration of MDA was calculated using a molar extinction coefficient value of 153000 L mol^{-1} cm^{-1}. The results were expressed as nanomoles per liter of MDA per milligram protein.

2.7. Estimation of vitamins

To analyze the effect of Zn on vitamin status, different water-soluble vitamins were estimated. A representative liver sample was processed for the estimation of AA, NAD as an indicator of liver NA status, and FAD as riboflavin status.

2.7.1. Hepatic NAD estimation

The estimation was done by the method of Levitas et al [35]. It involved alkali-ketone condensation of the nicotinamide nucleotides to highly fluorescent product that was measured by spectrofluorometry. An accurately weighed piece of liver was homogenized with ice-cold 10% TCA. To 0.2 mL of homogenate, 0.5 mL of ethylmethylketone and 0.2 mL of 5 N NaOH was added, mixed, and kept at room temperature for 5 minutes; 5 N HCl (0.3 mL) was then added, and the mixtures were vortexed and kept in boiling water bath for 5 minutes. After cooling the tubes, 1 mL of 20% KH_2PO_4 and 7 mL water were added to make up the volume up to 10 mL. Blanks were run with similar aliquots of homogenates, except that 0.3 mL 5N HCl was added in the beginning before adding ketone. Standards of 0.2 to 1 μg of NAD were

processed similarly. The fluorescence readings were taken using a spectrofluorometer (RF-5301PC, Shimadzu, Kyoto, Japan) at $\lambda_{excitation}$ = 365 nm and $\lambda_{emission}$ = 450 nm.

2.7.2. Hepatic FAD estimation

The estimation was done according to the method of Bessey et al [36]. For FAD analysis, 0.1 mL liver homogenate was taken; and volume was made up to 10 mL with distilled water. To this solution, 1 mL of 20% TCA was added, thoroughly mixed, and centrifuged at 5000 rpm for 20 minutes. From the supernatant, 2 mL aliquots were withdrawn to which 1 mL of 4 mol/L potassium phosphate was added and centrifuged at 3000 rpm. The fluorescence of riboflavin (F_1) was read in a spectrofluorometer (Shimadzu) at $\lambda_{excitation}$ = 530 nm and $\lambda_{emission}$ = 470 nm. Another 2-mL aliquot was incubated overnight at 37°C. It was treated similarly as F_1, and fluorescence was measured (F_2). The difference between F_2 and F_1 was noted; and from riboflavin standards (10-40 μg) that were processed similarly, the tissue FAD concentrations were determined.

2.7.3. Hepatic AA assay

The estimation was done by the method of Raghuramulu et al [37]. The dye 2,6-dichlorophenolindophenol gets reduced by AA to produce blue color that can be estimated colorimetrically. Ten percent liver homogenate (50 μL) was treated with equal volume of 6% metaphosphoric acid and 2.5 mL acetate buffer (pH 4.0). A sample blank, reagent blank (control), and standards were also run simultaneously. The dye solution (1 mL) was added in the control and sample tubes just before reading the absorbance at 520 nm. A standard curve was constructed; and based on the average slope value, the AA content was calculated. It was expressed as milligrams per 100 g wet weight for liver tissue.

2.8. Hepatic Zn estimation

Zinc concentrations in the liver samples were measured using atomic absorption spectrophotometer. Liver tissues were dried at 42°C for 24 hours. Weighed dried samples were transferred into tubes containing 4.5 mL of 30% nitric acid solution, and the tubes were heated at 60°C for 24 hours to obtain a clear solution. Zinc concentration in the samples was determined by flame AAS (AA 800; PerkinElmer, Shelton, USA). For analysis, Zn-specific cathode lamp with 213.9 nm wavelength and 0.7 nm monochromator slit width was used. Commercially available external standards of Zn (Merck, Whitehouse Station, USA) were used for calibration. In addition, rice flour samples from National Institute of Environmental Sciences, Japan, were similarly processed and used as reference biological standards for quality control. The hepatic Zn contents were expressed as micrograms Zn per gram liver.

2.9. Histopathologic studies

For histopathology, rat liver samples fixed in formalin were dehydrated in ascending grades of alcohol, cleared in benzene, and embedded in paraffin wax. The blocks were cut into 5- to 7-μm–thin sections, which were then double stained with hematoxylin and eosin. After staining, the sections were observed under light microscope and photographed.

2.10. Data presentation and statistical analysis:

Data were presented as mean ± SD. Effect of Zn supplementation and OS exposure in the treatment groups was assessed using 2-way analysis of variance (ANOVA). Student unpaired t test was used to further analyze differences between group pairs (ie, Zn-supplemented groups and OS exposure groups). To compare the effect of OS treatment, the subgroups from GI were compared with respective subgroups from GII, for example, GIa with GIIa. For comparison of Zn supplementation within GI and GII, the Zn D groups (GIa and GIIa) were compared with respective Zn N and Zn E groups. The level of significance was represented as $^{*}P < .05$, $^{\dagger}P < .01$, and $^{\ddagger}P < .001$.

3. Results

3.1. Effect of Zn supplementation on growth and AST and ALT levels

After 21 days of experimental period, the body weight and food intake records showed no significant difference in the different treatment groups and their subgroups. Slightly reduced weights (125.51 ± 3.29 g) and intake (148 ± 3.2) were observed in GIa animals with OS treatment as compared with animals with normal Zn diet in GIIb (129.45 ± 1.3 g; 152.3 ± 2), which may be due to consequences of marginal Zn deficiency and OS on growth.

As seen in Fig. 3, the ALT and AST levels were elevated in the rats treated with t-BHP and fed on Zn-deficient diet (GIa). These levels were restored to normal as the dietary Zn status changed to normal and excess states (GIb and GIc). Even in GIIa with Zn deficiency without OS, plasma ALT

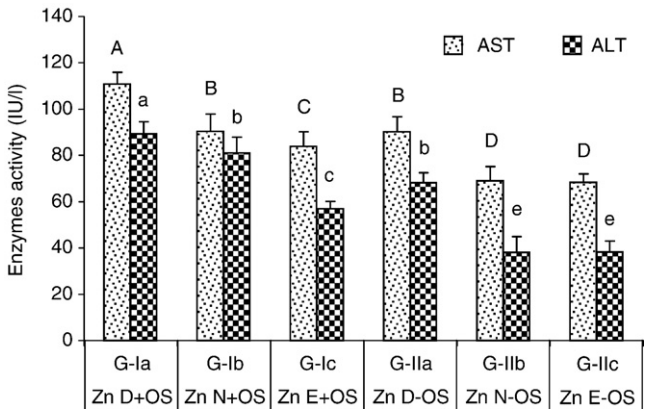

Fig. 3. Effect of dietary Zn variation and OS treatment on AST and ALT levels (results are means ± SD; without a common letter, significantly different; $P < .01$).

values were slightly elevated, indicating damage to some extent. These values declined toward normal with increased Zn content in the diet (GIIb and GIIc).

3.2. Zinc supplementation attenuated OS-induced hepatic antioxidant enzymes, GSH, and LPO status

The effect of Zn variation diet on hepatic primary antioxidant enzymes, Zn, GSH, and LPO levels are presented in Tables 1 and 2. The enzyme activities were significantly decreased by OS induction in GIa animals probably to overcome the OS, which concomitantly resulted in increased LPO and decreased GSH. Enhanced enzyme activities in response to increased dietary Zn were observed for GIb and GIc. Lower antioxidant enzymes and higher LPO even in the absence of externally induced OS in GIIa animals suggested that Zn deficiency itself may have resulted in some oxidative insult. Increase in dietary Zn level in GIb and GIc resulted in inhibition of LPO as against higher LPO in GIa.

3.3. The effect of dietary Zn variation on liver vitamin absorption

It was observed that, when dietary Zn level increased from deficient to excess, a dose-dependent increase in hepatic NAD accumulation was observed; also, the accumulation was more under OS compared with respective normal controls at each Zn level (Fig. 4A). However, as the Zn levels varied from deficient to excess level, liver FAD and AA concentrations were not significantly altered (Fig. 4B, C).

Table 1
Effect of dietary Zn variations on the hepatic antioxidant enzymes

Group	SOD, U/mg protein	Catalase, μmol/L H_2O_2 decomposed/ min/mg protein	GPx, nmol/L NADPH breakdown/ min/mg protein
GIa (Zn D + OS)	3.84 ± 0.55	39.15 ± 1.93 ^{a3}	132.65 ± 6.24 ^{a3}
GIb (Zn N + OS)	4.68 ± 1.11 ^{a1}	43.37 ± 3.38 ^{a3,b2}	137.32 ± 5.63 ^{a2}
GIc (Zn E + OS)	5.18 ± 0.26 ^{b3}	49.53 ± 2.42 ^{b3}	146.75 ± 5.36 ^{b3}
GIIa (Zn D − OS)	4.76. ± 1.12	47.52 ± 3.12	143.36 ± 8.97
GIIb (Zn N − OS)	6.17 ± 1.11 ^{b1}	49.41 ± 3.46	148.41 ± 4.45
GIIc (Zn E − OS)	6.02 ± 1.36 ^{b1}	50.92 ± 2.36 ^{b1}	148.89 ± 9.68
Effect of OS[a]	10.23‡	12.80‡	5.83*
Effect of Zn[a]	0.21 (NS)	13.61‡	3.29 (NS)
Interaction between Zn and OS[a]	0.78 (NS)	5.02*	2.85 (NS)

Values are mean ± SD for 8 rats in each group. NS indicates nonsignificant values.

[a] Results of 2-way ANOVA, as indicated by F value.

The level of significance was represented as follows:

* $P < .05$.
† $P < .01$.
‡ $P < .001$.

Intersubgroup comparison of OS treatment between GII and GIII subgroups (eg, GIa vs GIIa):

^{a1} $P < .05$.
^{a2} $P < .01$.
^{a3} $P < .001$.

Intragroup comparison of Zn variation (eg. GIa vs GIb and GIc):

^{b1} $P < .05$.
^{b2} $P < .01$.
^{b3} $P < .001$.

Table 2
Effect of dietary Zn variations on GSH, LPO, and Zn content in rats subjected to OS exposure

Group	LPO, nmol/L MDA per mg protein	GSH, nmol/L GSH/mg protein	Zn, μg/g liver
GIa (Zn D + OS)	7.29 ± 0.71 ^{a3}	18.77 ± 3.31	36.29 ± 1.95 ^{a2}
GIb (Zn N + OS)	6.59 ± 0.55 ^{a3,b3}	21.26 ± 1.91 ^{b1}	39.17 ± 2.38 ^{a2,b1}
GIc (Zn E + OS)	5.64 ± 1.29 ^{a3,b3}	23.55 ± 1.95 ^{b3}	42.05 ± 1.73 ^{b3}
GIIa (Zn D − OS)	6.05 ± 0.46	25.48 ± 1.33	40.79 ± 3.71
GIIb (Zn N − OS)	5.31 ± 0.96 ^{b3}	27.11 ± 2.42	43.67 ± 3.44
GIIc (Zn E − OS)	4.98 ± 2.13 ^{b3}	27.74 ± 2.92 ^{b1}	44.43 ± 3.88 ^{b1}
Effect of OS[a]	31.88 ‡	61.74 ‡	10.60 ‡
Effect of Zn[a]	15.25 ‡	8.47 ‡	2.98 *
Interaction between Zn and OS[a]	1.38 (NS)	2.42 (NS)	1.01(NS)

Values are mean ± SD for 8 rats in each group.

[a] Results of 2-way ANOVA, as indicated by F value.

The level of significance were represented as follows:

* $P < .05$.
† $P < .01$.
‡ $P < .001$.

Intersubgroup comparison of OS treatment between GII and GIII subgroups (eg, GIa vs GIIa):

^{a1} $P < .05$.
^{a2} $P < .01$.
^{a3} $P < .001$.

Intragroup comparison of Zn variation (eg, GIa vs GIb and GIc):

^{b1} $P < .05$.
^{b2} $P < .01$.
^{b3} $P < .001$.

3.4. Zinc supplementation attenuated OS-induced pathologic changes in the liver

The histopathologic images of liver samples from various treatment groups are shown in Fig. 5. In rats fed with Zn-deficient diet along with OS treatment, the liver sections showed distorted hepatic architecture with necrotic hepatocytes. In addition, marked dilation and congestion of blood vessels were also observed (Fig. 5A). With OS treatment, normal dietary Zn preserved hepatic architecture. However, focal areas of hepatocytes necrosis with few congested blood vessels were noticed (Fig. 5B). In excess Zn supplementation, although with OS exposure, liver sections showed preserved hepatic architecture and normal morphology of blood vessels; however, hepatocytes with mild necrosis were still present (Fig. 5C). In the group with Zn deficiency without OS treatment, the liver morphology was slightly distorted. Although preserved hepatic architecture and normal hepatocytes were present, some blood

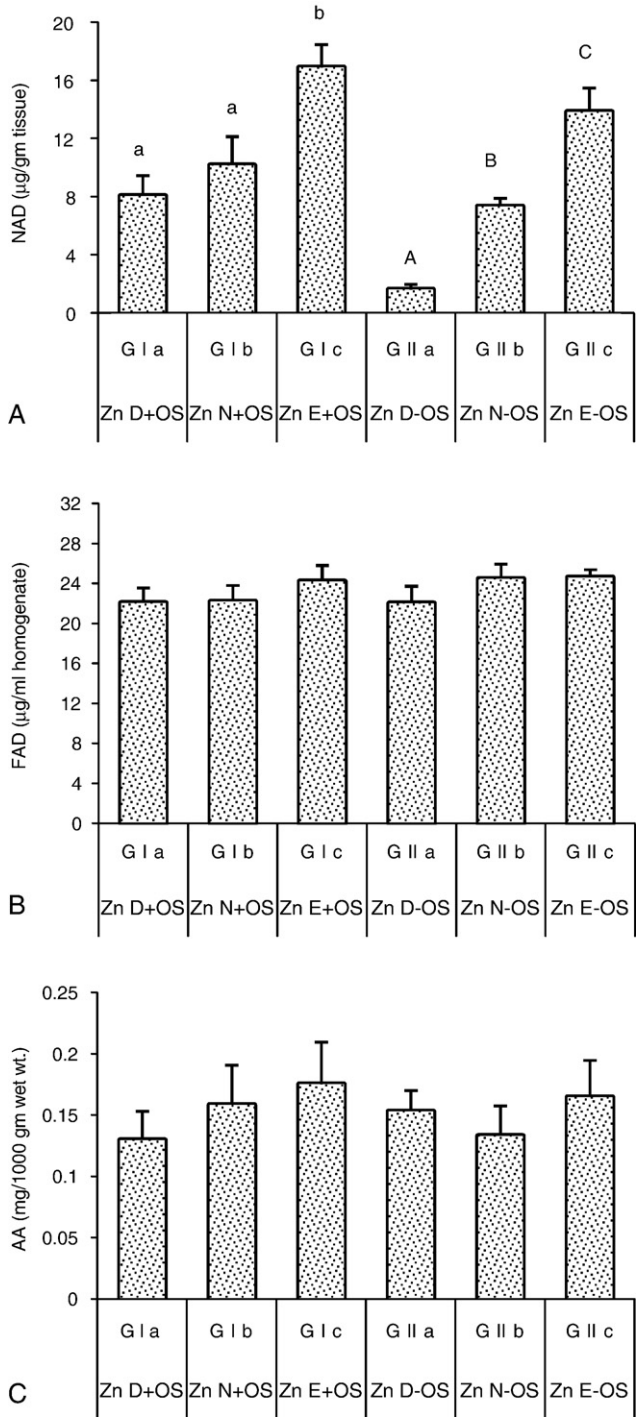

Fig. 4. A, Effect of Zn variation diet on NAD levels. B, Effect of Zn variation diet on FAD levels. C, Effect of Zn variation diet on AA level (results are means ± SD; without a common letter, significantly different; $P < .01$).

vessels were dilated and congested with cytoplasmic vacuoles in few of the hepatocytes (Fig. 5D). The GIIb animals with normal Zn and without OS treatment revealed clear hepatic lobules with polyhedral hepatocytes that were traversed by portal veins (Fig. 5E).

4. Discussion

Adequate Zn is critical for the optimal functioning of the oxidant defense system, which is supported by multiple lines of evidence showing that Zn deficiency is associated with an increased risk of tissue oxidative damage. Increased levels of lipid [38], protein, and DNA oxidation [39] have been described in different models of Zn deficiency. In the testes from rats fed Zn-deficient diets for 14 days, researchers had found several indicators of OS, including increased oxidation of cell constituents and alterations in selected components of the oxidant defense system [40]. The present design has incorporated dietary Zn variation as Zn-deficient, Zn-normal, and Zn-excess groups, each under normal and OS conditions in a single experiment. This design helped in evaluating the effect of dietary Zn variation along with OS treatment on liver function enzymes, antioxidant enzymes, and vitamin status.

In the present study, GI animals with OS treatment and Zn deficiency showed significant liver damage, that is, elevated plasma ALT value as compared with normal control group, which is in agreement with earlier reports. In the galactosamine-induced hepatotoxicity, the values for serum ALT were significantly higher ($P < .01$) in rats with low-Zn diet than animals with Zn-adequate diet [41]. Ishikawa et al [42] had reported elevated plasma AST activities by low-Zn diet in the growing rats. In other reports on hepatotoxicity studies, Zn supplementation has been demonstrated to improve liver dysfunction (as indicated by ALT activities) that was induced by specific factors like alcohol [43] and chlorpyrifos [44]. The present results imply the hepatoprotective effects of Zn under t-BHP–induced OS damage.

The antioxidant enzymes SOD, GPx, and catalase limit the effects of oxidant molecules by acting as free-radical scavengers. However, in excess burden of reactive oxygen species, cellular defense fails; and the level of these antioxidant enzymes decreases. In the present study, decrease in the activity of SOD in GIa animals was also observed. However, increased dietary Zn supplementation to GI animals significantly improved the levels SOD activities, which could be attributed to antioxidant property of Zn. The protective effects of Zn on Zn-dependent enzymes (erythrocyte SOD and plasma SOD) were observed by supplementation trials in human studies. In one study, these enzyme activities as well as plasma Zn concentration were significantly higher after than before supplementation [45]. The significance of the above experimental findings is underscored by the observation that marginal Zn intakes occur in many population groups and poor Zn nutriture has been correlated with an impaired oxidant defense system; for example, decreased SOD activities were found in subjects with low serum Zn concentrations (9.0-10.7 μmol/L) in a study of Korean populations [46].

Results indicated significant decrease in the activities of GPx and catalase in GI rats by t-BHP exposure. These

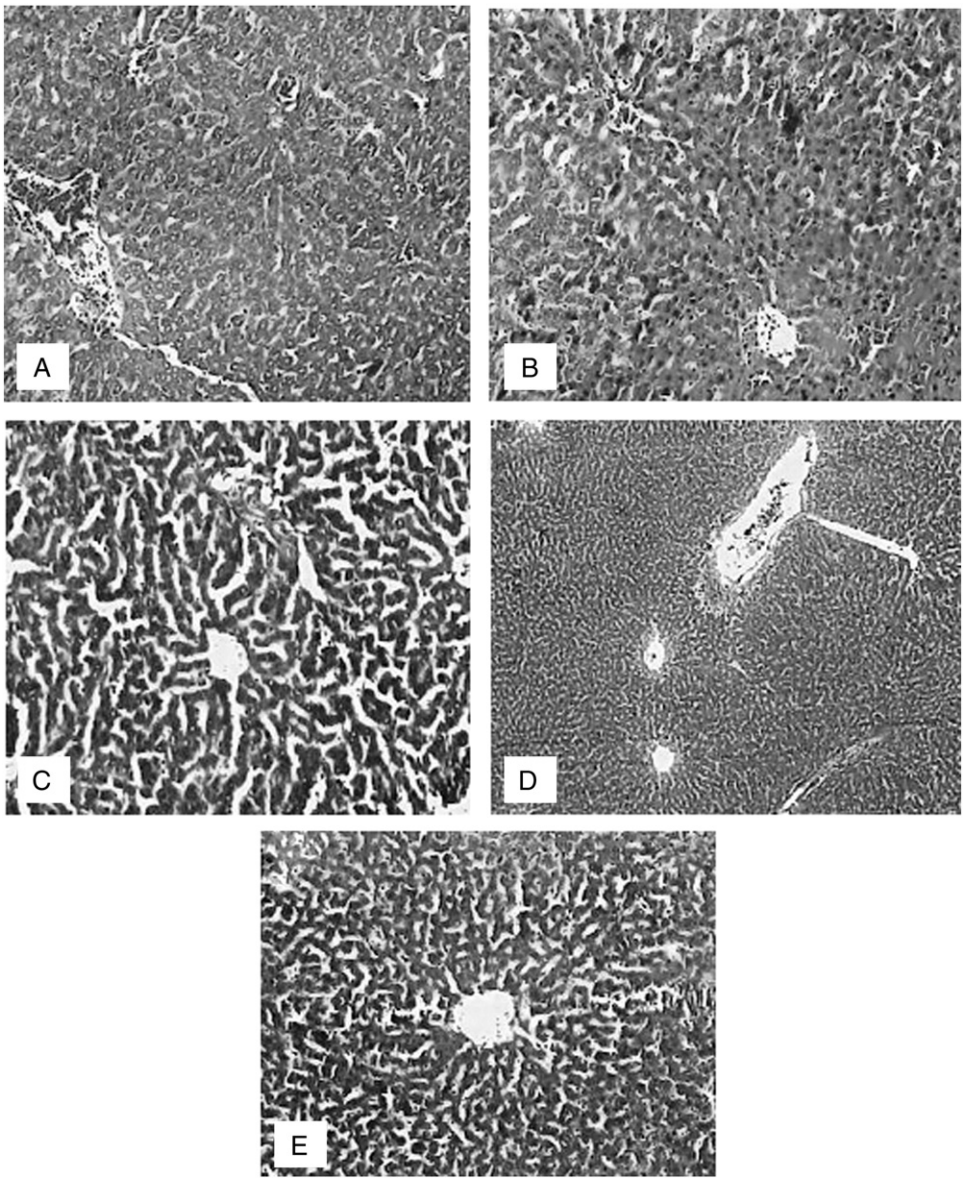

Fig. 5. Effect of dietary Zn variations on hepatic histoarchitecture in OS-induced and without–OS treatment groups. Light micrographs of rat liver from (A) Zn-deficient with OS group, (B) Zn-normal with OS group, (C) Zn-excess with OS group, (D) Zn-deficient without OS group, and (E) Zn-normal without OS group (magnification 100×).

activities were improved with increasing Zn supplementation in diet. As in GIIa rats, without OS exposure, still these enzyme levels were low, which were subsequently enhanced by increased dietary Zn. Amara et al [47] reported that Zn supplementation ($ZnCl_2$, 40 mg/L) in the Cd-exposed rats restored the activities of GPx, catalase, Cu-Zn SOD, and Mn SOD in the testes to the levels of the control group.

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and is mediated through the free-radical metabolites, affecting the antioxidants and ultimately the status of the cell. In present study, a significant increase in MDA adducts was observed in the OS-treated and Zn-deficient rats (GIa); and supplementation of Zn (GIb and GIc) resulted in decrease in the MDA levels. In a recent clinical trial, Zn supplementation in healthy individuals lowered the levels of OS-related LPO products [48]. Zinc supplementation in MT knockout mice reduced LPO and delayed fibrosis and cirrhosis induction [49]. Antioxidant effects of Zn supplementation in Tunisians with type 2 diabetes mellitus have been reported by Roussel et al [50]. Antioxidant and hepatoprotective effect observed in this Zn variation experiment is in agreement with many previous reports suggesting Zn as a beneficial agent during in vivo oxidative damage [51] and hepatotoxicity by CCl_4 [52].

In the present study, the observed increase in the LPO was concomitant with a remarkable decrease in GSH levels in

OS-treated rats. Zinc is believed to interact with GSH, affecting its antioxidant activity, although Zn and GSH interactions have not been completely delineated. Zinc deficiency in cultured HSCs caused GSH consumption and cellular activation, whereas GSH or Zn replenishment inhibited the actions of Zn deficiency [11]. Zinc supplementation in experimental animal studies either increased hepatic GSH levels [49] or did not alter them [53]. The levels of GSH in blood and liver were also found to correlate well with dietary Zn status [54]. In the present study, Zn supplementation effectively prevented OS-induced GSH decrease.

The results of vitamin status were suggestive of the micronutrient interactions, where synergistic effect of Zn supplementation on NA absorption was observed. There are reports on Zn affecting fat-soluble vitamins absorption, where Zn supplementation is useful for these vitamins' metabolism. The present results indicate for the first time the enhancing effects of Zn on liver water-soluble vitamin-NA absorption under OS. Vannucchi et al [55] had reported that, in NA-deficient rats, Zn repletion caused activation of NA metabolism, increasing the excretion of NA metabolites, emphasizing the role of Zn in the function of this vitamin. These interactions were further confirmed in alcoholic pellagra patients [56]. The researcher also indicated the possibility of obtaining therapeutic benefits by supplementing the diet with Zn for the treatment of these patients. Present results support these findings and also show the interactions during OS.

Zinc was reported to interact with cell membranes to stabilize them against various damaging effects, including those due to oxidative injuries [57]. In the current study, histopathologic observations indicated protective effect of Zn supplementation on hepatic histoarchitecture. There are various reports supporting protective effects of Zn on liver morphology with oxidative injury. Zhou et al [26] demonstrated a significant improvement in the ethanol-induced pathologic changes in the liver by Zn supplementation. Goel et al [58] showed that coadministration of Zn to chlorpyrifos-intoxicated rats resulted in marked improvement in the structure of the hepatocytes. Sidhu et al [59] have also made similar observations, where Zn supplementation improved the overall hepatic histoarchitecture in nickel-treated rats.

In conclusion, the present study demonstrated OS-induced oxidative liver injury prevention by dietary Zn supplementation. Results also indicated improvement in liver NA status by Zn. Data collectively suggest that Zn may have a therapeutic potential in treatment of oxidative liver damage along with enhanced NA absorption.

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