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Chronic Cadmium Toxicity in Rats: Treatment with Combined Administration of Vitamins, Amino Acids, Antioxidants and Essential Metals

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ABSTRACT

The present study was designed to investigate the protective effect of dietary nutrients against cadmium-induced hepatic and renal toxicities in male albino rats. The animals were randomly divided into eleven groups where the control group (Group I) received physiological saline (p.o.) and Group II was administered with cadmium chloride (2 mg/kg, s.c.) only. Groups III, IV, V, VI, VII, VIII and IX received 10 mg/kg dietary nutrients, i.e. thiamine, methionine, N-acetylcysteine (NAC), melatonin, zinc (Zn), vitamin E and cystine, respectively. Group X received a dose of 0.1 mg/kg of selenium (Se) and Group XI was administered with a combination of NAC, Zn, vitamin B_1 and melatonin. All treatments were administered orally for 21 days with a concomitant subcutaneous (*s.c.*) cadmium treatment. Cadmium-intoxicated rats exhibited hepatic and renal toxicities as indicated by elevation of lipid peroxidation and marked decline in superoxide dismutase, glutathione peroxidase, alkaline phosphatase activities and total protein. However, a combined treatment with dietary nutrients, i.e. methionine, zinc, vitamin B_1 and N-acetylcysteine ameliorated cadmium-induced lipid peroxidation and oxidative stress as they provoked the antioxidant defense system more significantly, when compared to each of them alone and hence is suggested as protective against cadmium-induced hepatic and renal toxicities.

Key words: dietary nutrients, lipid peroxidation, oxidative stress, antioxidant defense system, cadmium.

INTRODUCTION

Cadmium (Cd) is one of the most important environmental and occupational metallic toxicants and is widely dispersed in the environment. High level exposure to this toxic heavy metal is usually the result of environmental contamination from human activities. Exposure to Cd can cause both acute and chronic tissue injury and can damage various organs, including liver and kidney in human beings and experimental animals[1]. The generation of reactive oxygen species (ROS) followed by development of oxidative stress in the target organs is one of several mechanisms through which cadmium exerts its toxicity[2]. The scavenging potential of liver and kidney fluids is normally maintained by adequate levels of antioxidants[3]. Long term exposure to cadmium leads to an increase in lipid peroxidation and causes inhibition of superoxide dismutase (SOD) activity indicating oxidative damage in liver, kidney and testes[4,5]. The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system (AOS). This defense system includes the enzymes, glutathione peroxidase (GPx) and SOD, which normally protect against radical toxicity[6]. Protection against toxic actions of cadmium can be accomplished through AOS. Zinc, selenium, carotenoids and vitamins are among the important non-enzymatic AOS[7-10]. Similarly, dietary nutrients like melatonin (N-acetyl-5-methoxy tryptamine), N-acetylcysteine (NAC), thiamine and methionine act as strong antioxidants and increase the efficacy of the chelating agent in the treatment of cadmium intoxication[11]. It is known that chelators and antioxidants protect against potentially damaging effects of reactive oxygen and lipid peroxides[12].

The present study was conducted to investigate the effect of cadmium on glutathione peroxidase and superoxide dismutase activities in liver and kidney tissues of albino rats, with special reference to the possible beneficial role of dietary nutrients in individual and combinational studies in the prevention of cadmium-induced toxicity in rats.

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MATERIALS AND METHODS

I. *Animals*

Male Wistar albino rats weighing 200 ± 10 g were obtained from the Defense Research and Development Establishment (DRDE) animal facility. Ethical permission was obtained from the local ethic committee before the study. The rats were maintained in individual stainless steel cages under controlled conditions (23 ± 1°C, 12-h light-dark cycle, relative humidity of 50 ± 10%) and had access to a standard rodent laboratory diet and drinking water. They were divided into 11 groups of 5 rats each as follows:

Group I: Normal saline, orally (negative control)
Group II: 2 mg/kg $CdCl_2$, *s.c.* (positive control)
Group III: 2 mg/kg $CdCl_2$, *s.c.* + Thiamine (10 mg/kg, orally)
Group IV: 2 mg/kg $CdCl_2$, *s.c.* + Methionine (10 mg/kg, orally)
Group V: 2 mg/kg $CdCl_2$, *s.c.* + N-acetylcysteine (10 mg/kg, orally)
Group VI: 2 mg/kg $CdCl_2$, *s.c.* + α-melatonin (10 mg/kg, orally)
Group VII: 2 mg/kg $CdCl_2$, *s.c.* + Zinc (10 mg/kg, orally)
Group VIII: 2 mg/kg $CdCl_2$, *s.c.* + Vit-E (10 mg/kg, orally)
Group IX: 2 mg/kg $CdCl_2$, *s.c.* + Cystine (10 mg/kg, orally)
Group X: 2 mg/kg $CdCl_2$, *s.c.* + Se (0.1 mg/kg, orally)
Group XI: 2 mg/kg $CdCl_2$, *s.c.* + [Methionine + Zn + Vitamin B_1 + NAC] (10 mg/kg each, orally)

All applications were administered daily for 21 consecutive days. The animals of all groups were anaesthetized using chloroform and sacrificed after the last application. The kidney and liver were removed, washed in 0.25 M sucrose solution and weighed. A 10% tissue homogenate was prepared in 0.25 M sucrose in a glass homogenizer with motor driven Teflon pestle. The tissue homogenate was centrifuged at 10,000 ×g for 15 min. at 4ºC to remove the cell debris and the supernatant was collected for biochemical analysis, including lipid peroxidation, SOD, GPx, alkaline phosphatase and total protein.

II. *Cadmium Concentration*

After wet acid digestion in nitric acid and perchloric acid, the concentration of cadmium in blood, liver and kidneys were determined by atomic absorption spectrophotometry using a Perkin Elmer 5000 atomic absorption spectrophotometer[13].

III. *Lipid Peroxidation*

Tissue lipid peroxidation was measured by the method of Onkawa[14]. The tissue homogenate was incubated with 8.1% sodium dodecyl sulfate (SDS) (w/v) for 10 min followed by addition of 20% acetic acid (pH 3.5). The reaction mixture was incubated with 0.6% (w/v) thiobarbituric acid (TBA) for 1 h in a boiling water bath. The pink color chromogen formed was extracted in butanol/pyridine (15 : 1) solution and its absorbance was measured at 532 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56×10^5 M/cm.

IV. *Superoxide Dismutase*

Tissue superoxide dismutase was assayed by the method of Kakkar *et al*[15]. The reaction mixture contained 1.2 mL of (0.052 mM) sodium pyrophosphate buffer, 0.1 mL of (186 μM) phenazine methosulfate and 0.3 mL of nitro blue tetrazolium (300 μM). The reaction was initiated by adding 0.2 mL of NADH (780 μM) and stopped by the addition of 1 mL glacial acetic acid. The color intensity of the chromogen was measured at 560 nm and the activity was expressed as units/min/mg protein.

V. *Glutathione Peroxidase*

Glutathione (GPx) peroxidase activity was measured according to the procedure of Flohe and Gunzler[16]. The reaction mixture consisted of 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 mL of H_2O_2 (1 mM) and 0.3 mL of tissue homogenate was incubated for 15 min at 37°C. The reaction was stopped by the addition of 0.5 mL of TCA (5%). The mixture was centrifuged at 1500 ×g for 5 min, and 0.7 mL of DTNB (0.4 mg/mL) and 0.2 mL of phosphate buffer (0.1 M, pH 7.4) was added to the supernatant. After vortex, the absorbance was measured at 420 nm.

VI. *Alkaline Phosphatase*

The method of Halk[17] was used to estimate hepatic and renal alkaline phosphatase. A total of 0.5 mL of homogenate was reacted with 0.1 mL of Triton X-100 followed by the addition of 4 mL of alkaline buffer and incubation for 1 h at 37°C. The reaction was stopped by the addition of 0.5 mL of TCA (30%). The mixture was centrifuged. Two milliliters of supernatant was mixed with 6.6 mL of distilled water, 1 mL of 2% ammonium molybdate and 0.4 mL of amino naphthol sulfuric acid. The absorbance was measured at 620 nm and the phosphorus liberated was calculated.

VII. *Total Protein*

Protein content was estimated according to Lowry *et al*.[18] using bovine serum albumin as a standard. Protein reacts with folin ciocalteau reagent to give a purple blue color complex: the reaction of the alkaline copper with protein as in burette test and the reduction of

phosphomolybdate by tyrosine and tryptophan present in the protein. The color thus formed is proportional to the amount of protein, which was measured at a wavelength of 620 nm.

VIII. *Statistical Analysis*

The data are presented as mean ± S.E.M. value. The number of animals per group is stated in the figure legends. One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used to analyze mean differences between experimental groups for each parameter separately after ascertaining the homogeneity of variance between treatment groups by Bartlett's test.

RESULTS

I. *Effects of Cadmium and Dietary Nutrients on Liver, Kidney and Body Weight*

Cd-treated rats were found to be significantly higher in organ (liver and kidney) weights and significantly lower in body weights in comparison with the control rats. Treatment with combinational group (10 mg/kg) reversed the effects caused by Cd, as shown in Table 1 and 2. The concentration of Cd in the liver and kidney increased significantly ($p < 0.001$ and $p < 0.0001$, respectively) in animals exposed to Cd (Table 3).

II. *Effect of Dietary Nutrients on Cadmium-Induced Hepatotoxicity*

Evident from Figure 1, TBARS level increased significantly ($p < 0.001$; 173%) whereas the activity of superoxide dismutase ($p < 0.001$; 69%), glutathione peroxidase ($p < 0.001$; 90%), alkaline phosphatase ($p < 0.001$; 71%) and total protein content ($p < 0.001$; 65%) decreased significantly ($p < 0.001$) in the cadmium-treated group in comparison with the control rats.

Observed significant reduction in TBARS level in dietary nutrients treatment groups, i.e., Zn ($p < 0.001$; 63%), Se ($p < 0.001$; 54%), NAC ($p < 0.001$; 42%), Cyst ($p < 0.001$; 38%), Vitamin E ($p < 0.001$; 29%), Vitamin B_1($p < 0.001$; 61%), α-melatonin ($p < 0.001$; 60%), methionine ($p < 0.001$; 61%), combination ($p < 0.001$; 69%) and significant elevation in antioxidant enzyme activity in Zn (SOD: $p < 0.001$; 214%, GPx: $p < 0.001$; 104%, ALP: $p < 0.01$; 136%, total protein: 381%), Se (SOD: $p < 0.001$; 267%, GPx: $p < 0.05$; 110%, ALP: $p < 0.01$; 111%, total protein: $p < 0.001$; 355%), NAC (SOD: $p < 0.01$; 155%, GPx: 107%, ALP: 136%, total protein: $p < 0.001$; 249%), Cyst (SOD: $p < 0.001$; 211%, GPx: $p < 0.05$; 116%, ALP: $p < 0.001$; 132%, total protein: $p < 0.001$; 206%), Vitamin E (SOD: $p < 0.001$; 137%, GPx: $p < 0.001$; 129%, ALP: $p < 0.001$; 144%, total protein: $p < 0.001$; 189%), Vitamin B_1 (SOD: $p < 0.001$; 133%, GPx: 110%, ALP: 109%, total protein: $p < 0.001$; 180%), α-melatonin (SOD: $p < 0.001$; 132%, GPx:

Table 2. Organ weight of rats treated with different dietary nutrients

Treatment	Liver Weight	Weight (%)	Kidney Weight	Weight (%)
Control	2.54 ± 0.28	1.88	0.65 ± 0.02	0.48
Cd	3.82 ± 0.14^{m}	3.5	0.76 ± 0.06	0.69
Cd + NAC	2.54 ± 0.28^{o}	2.19	0.65 ± 0.03	0.49
Cd + Methionine	2.89 ± 0.29	2.24	0.62 ± 0.03	0.48
Cd + Zn	2.87 ± 0.10^{o}	2.14	0.62 ± 0.01^{z}	0.46
Cd + Se	3.10 ± 0.09	2.34	0.67 ± 0.03	0.50
Cd + α-Melatonin	2.96 ± 0.07	2.27	0.64 ± 0.01	0.49
Cd + Vit-B_1	2.89 ± 0.05^{o}	2.20	0.66 ± 0.02	0.50
Cd + Cyst	2.95 ± 0.03^{o}	2.23	0.68 ± 0.01	0.51
Cd + Vit-E	2.86 ± 0.13	2.16	0.62 ± 0.02	0.46
Cd + Methionine + Zn + Vit-B_1 + NAC	2.64 ± 0.07	1.98	0.62 ± 0.01^{z}	0.46

Results are shown as mean ± S.E.M (n = 5).
^{m} Significantly different from control group, $p < 0.05$
^{n} Significantly different from control group, $p < 0.001$
^{o} Significantly different from Cd-treated group, $p < 0.05$
Cyst: cystine

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Table 1. Body weight of rats treated with different dietary nutrients

Treatment	Initial Body Weight (g)	Final Body Weight (g)
Control	122 ± 4.06	135 ± 4.47
Cd	126 ± 2.92	109 ± 6.78[m]
Cd + NAC	121 ± 3.32	131 ± 6.63[o]
Cd + Methionine	121 ± 3.16	129 ± 2.35[o]
Cd + Zn	125 ± 6.96	134 ± 6.63[o]
Cd + Se	127 ± 3.74	132 ± 3.67[o]
Cd + α-Melatonin	122 ± 6.98	130 ± 3.32[o]
Cd + Vit-B_1	123 ± 2.55	131 ± 3.34[o]
Cd + Cyst	126 ± 2.54	132 ± 2.55[o]
Cd + Vit-E	124 ± 2.54	132 ± 3.39[o]
Cd + Methionine + Zn + Vit-B_1 + NAC	123 ± 2.00	133 ± 6.57[o]

Results are shown as mean ± S.E.M. (n = 5).
[m] Significantly different from control group, $p < 0.05$
[n] Significantly different from control group, $p < 0.001$.
[o] Significantly different from Cd-treated group, $p < 0.05$
Cyst: cystine

Table 3. Cadmium concentration in liver and kidney after cadmium alone and combined with dietary nutrients treated groups.

Treatment	Cadmium Content in Liver Tissue (μg/g)	Cadmium Content in Kidney Tissue (μg/g)
Control	0.02 ± 0.55	0.05 ± 0.02
Cd	2.52 ± 0.42[x]	2.73 ± 0.42[y]
Cd + NAC	1.51 ± 0.09[z]	2.49 ± 0.41
Cd + Methionine	1.85 ± 0.24	2.56 ± 0.53
Cd + Zn	2.02 ± 0.23	2.13 ± 0.10
Cd + Se	0.91 ± 0.39[z]	2.38 ± 0.02
Cd + α-Melatonin	1.44 ± 0.21[z]	0.37 ± 0.09[z]
Cd + Vit-B_1	1.68 ± 0.19	2.14 ± 0.21
Cd + Cyst	1.88 ± 0.17	1.90 ± 0.17
Cd + Vit-E	2.03 ± 0.26	1.84 ± 0.18
Cd + Methionine + Zn + Vit-B_1 + NAC	1.75 ± 0.18	1.65 ± 0.28

Results are shown as mean ± S.E.M. (n = 5).
[x] Significantly different from control group, $p < 0.001$
[y] Significantly different from control group, $p < 0.0001$
[z] Significantly different from Cd-treated group, $p < 0.05$
Cyst: cystine

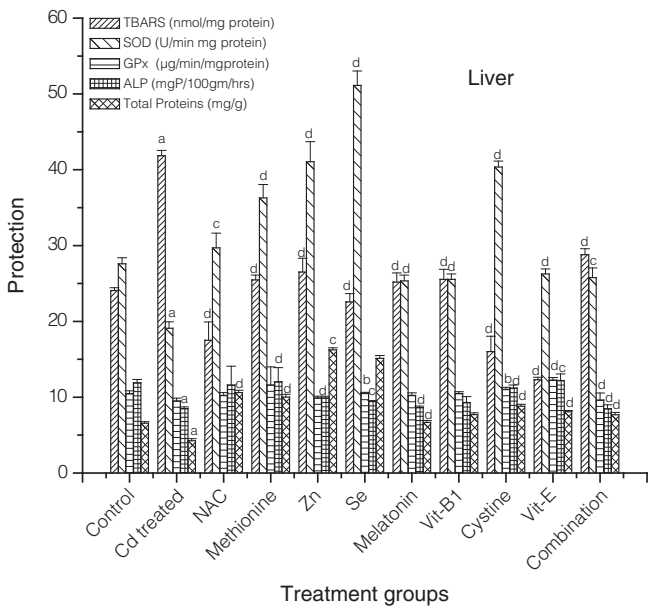

Figure 1. Protection against Cd-induced hepatotoxicity in terms of thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD), glutathione peroxidase (GPx), alkaline phosphatase (ALP) and total protein
Results are shown as mean ± S.E.M. (n = 5).
[a]Significantly different from control group, $p < 0.001$
[b]Significantly different from Cd-treated group, $p < 0.05$
[c]Significantly different from Cd-treated group, $p < 0.01$
[d]Significantly different from Cd-treated group, $p < 0.001$

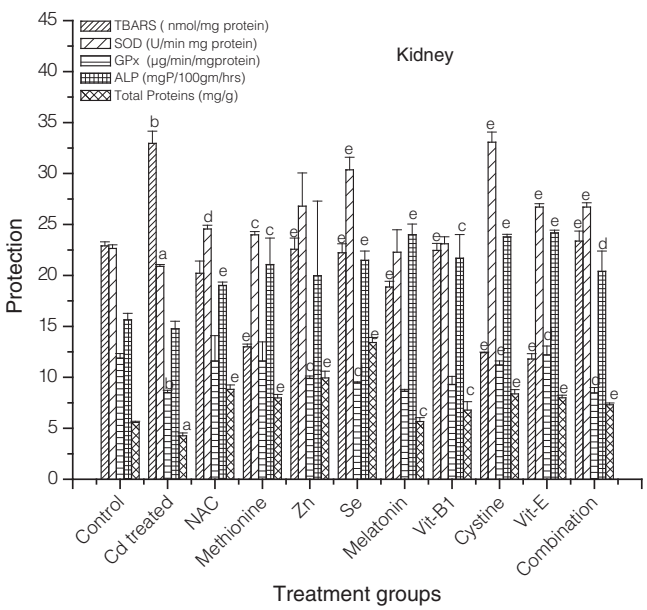

Figure 2. Protection against Cd-induced renal toxicity in terms of thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD), glutathione peroxidase (GPx), alkaline phosphatase (ALP) and total protein
Results are shown as mean ± S.E.M. (n = 5).
[a] Significantly different from control group, $p < 0.05$
[b] Significantly different from control group, $p < 0.001$.
[c] Significantly different from Cd-treated group, $p < 0.05$
[d] Significantly different from Cd-treated group, $p < 0.01$
[e] Significantly different from Cd-treated group, $p < 0.001$

108%, ALP: $p < 0.001$; 101%, total protein: $p < 0.001$; 156%), and methionine (SOD: $p < 0.001$; 190%, GPx: 122%, ALP: $p < 0.001$; 141%, total protein: $p < 0.001$; 235%) and combination (SOD: $p < 0.001$; 135%, GPx: $p < 0.001$; 101%, ALP: $p < 0.001$; 99%, TP: $p < 0.001$; 180%), demonstrated a well pronounced amelioration of cadmium-induced liver toxicity by the combinational group when compared to the individual dietary nutrients-treated groups.

III. *Effect of Dietary Nutrients on Cadmium-Induced Renal Toxicity*

Figure 2 reveals significant increase in TBARS level ($p < 0.001$; 144%) and significant decrease in the activity of superoxide dismutase ($p < 0.05$; 92%), glutathione peroxidase ($p < 0.001$; 71%), alkaline phosphatase (94%) and total protein content ($p < 0.05$; 76%) in animals treated with cadmium subcutaneously for 21 consecutive days when compared with the control group.

On the other hand, treatment with the dietary nutrients showed a significant decline in TBARS level, i.e., Zn ($p < 0.001$; 68%), Se ($p < 0.001$; 67%), NAC ($p < 0.001$; 61%), Cyst ($p < 0.001$; 37%), Vitamin E ($p < 0.001$; 35%), Vitamin B_1($p < 0.001$; 68%), α-melatonin ($p < 0.001$; 57%), methionine ($p < 0.001$; 39%), combination ($p < 0.001$; 69%) and significant increase in antioxidant enzyme activity in Zn (SOD: 128%, GPx: $p < 0.01$; 116%, ALP: 135%, total protein: $p < 0.001$; 233%), Se (SOD: $p < 0.001$; 145%, GPx: $p < 0.01$; 111%, ALP: $p < 0.001$; 145%, total protein: $p < 0.001$; 133%), NAC (SOD: $p < 0.01$; 117%, GPx: 136%, ALP: $p < 0.001$; 128%, total protein: $p < 0.001$; 207%), Cyst (SOD: $p < 0.001$; 158%, GPx: $p < 0.001$; 131%, ALP: $p < 0.001$; 161%, total protein: $p < 0.001$; 187%), Vit-E (SOD: $p < 0.001$; 127%, GPx: $p < 0.01$; 143%, ALP: $p < 0.001$; 163% , total protein: $p < 0.001$; 171%), Vit-B_1(SOD: 110%, GPx: 109%, ALP: $p < 0.05$; 146%, total protein: $p < 0.05$; 196%), α-melatonin (SOD: 106%, GPx: 101%, ALP: $p < 0.001$; 162%, total protein: $p < 0.05$; 159%), methionine (SOD: $p < 0.05$; 114%, GPx: 136%, ALP: $p < 0.05$; 142%, total protein: $p < 0.001$; 187%) and combination (SOD: $p < 0.001$; 135%, GPx: $p < 0.001$; 101%, ALP: $p < 0.001$; 99%, total protein: $p < 0.001$; 180%), illustrated the most effective alleviation of cadmium-induced toxicity in kidney by the combinational group as compared to the individual dietary nutrients-treated group of animals.

DISCUSSION

Cd is a ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in tissues. A significantly increased accumulation of Cd in liver and kidneys observed in animals treated with Cd is in accordance with several previous studies[9,19]. The present results have clearly demonstrated the ability of cadmium to induce oxidative stress as evidenced by increased lipid peroxidation and inhibition of SOD, GPx, ALP activity and total protein content in kidney and liver. Twenty one days of cadmium exposure at a dose of 2 mg/kg revealed clear signs of toxicity with reference to decreased body weight and increased organ (liver and kidney) weights (Table 2). These findings are in agreement with previous reports demonstrating that cadmium toxicity leads to abnormal body and organ weights[19] and induces oxidative stress in tissues by altering the antioxidant status[20]. In the present study, increase in SOD and GPx activities following cadmium treatment may be associated with overproduction of ROS or their accumulation due to dysfunction of the antioxidases-antioxidant system[21]. Lipid peroxidation is another manifestation of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics[22]. It has been proposed that the enhancement of lipid peroxidation by cadmium in rats is a consequence of a decrease of antioxidant enzymes[23].

The results from our experiments indicated that cadmium-induced oxidative stress in different organs could be prevented variably by different co-treatments. Thiamine, a water-soluble antioxidant has been reported earlier as a possible chelator for cadmium and lead[24]. Thiamine prevents oxidative damage to the cell membrane via direct interaction with free radicals. In the present study, the chemo-prophylactic potential of thiamine during cadmium exposure could be due to its metal-chelating and antioxidant properties. However, methionine and NAC treatments produced tissue-specific protection against oxidative damage in the liver and kidney of rats with more reactivity of methionine than NAC. This may be due to the fact that the former is readily taken up by the hepatocytes for the synthesis of glutathione, a low molecular mass antioxidant and thereby protect these organs from impending damage by free radicals. It has been reported that melatonin had an antioxidant effect as it quenches hydroxyl radicals, superoxide anion radicals, and singlet oxygen and peroxide radicals[25,26]. Melatonin, in spite of being a good free radical scavenger and SOD and GPx stimulator, surprisingly could not reverse the cadmium-induced effects in kidney tissue, while the appreciating results obtained in liver tissues are in agreement with the previous studies[25]. Cysteine is an important structural component of glutathione and thus could promote the detoxification of cadmium[26,27]. Zinc and copper are involved in cell and tissue growth[28]. Vitamin E stops free radical destruction throughout the body and helps to produce SOD[29]. The results suggest that dietary nutrients used in the present study have effective antioxidant properties and can well scavenge excess free radicals, which is consistent with the aforementioned studies.

Our results also provide direct evidence that combined treatment of Cd-exposed animals with Zn,

Vitamin B_1, NAC and methionine was more effective than treated with each of them alone in reversing Cd-induced changes in SOD ($p < 0.01$; $p < 0.001$), GPx ($p < 0.001$; $p < 0.01$), ALP ($p < 0.001$; $p < 0.01$) and total protein ($p < 0.001$; $p < 0.001$) levels at different 'p' values. However, further studies are necessary to determine the mechanisms involved in the synergistic role of dietary nutrients against Cd toxicity.

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