Effect of Alpha Lipoic Acid and Vitamin E on Heavy Metals Intoxication in Male Albino Rats

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Abstract: The present study was carried out to investigate the effect of some nutrients on Nutritional and Health status in copper and lead intoxicated male albino rats. Fifty male albino rats weighing (125±7 g) were used. The rats were divided into 5 groups (10 rats each). The first group received the basal diet only and served as control. The second and third groups received copper (copper sulfate in water at dose of 0.1 mg /kg body weight) with alpha lipoic acid (40 mg/kg body weight) and Vitamin E (20 mg/kg body weight) respectively. Fourth and fifth groups received lead (lead acetate in water at a dose of 0.2 mg/kg body weight) with alpha lipoic acid and Vitamin E respectively.. At the end of the experimental periods, blood samples were collected from Orbital plexus, the rats sacrificed, organs were weighed, and kept for metal determination. The obtained results revealed that alpha lipoic acid (ALA) and vitamin E could improved daily food intake, body weight gain and feed efficiency ratio; reduced copper and lead levels in serum and tissues as well as diminished ALT, AST, urea and creatinine levels in lead and copper intoxicated rats. Therefore, the current study recommends that alpha lipoic acid or Vit. E (or both of them), should be administered to minimize the toxic effects of lead and copper. [Journal of American Science 2010;6(8):56-63]. (ISSN: 1545-1003).

Key words: heavy metals intoxication - copper lead - rats - alpha lipoic acid- vitamin E - liver function kidney function.

1. Introduction

Heavy metals are widely distributed in the environment. Among of them is lead and copper (Walker et al., 1995). The most common heavy metals, implicated in acute and / or chronic intoxication can effect the development and the overall health causing depression, learning difficulties and neurological disorders. These metals may cause cell damage, impairment of enzymes, functions or alter genetic material (DNA), causing cancer or birth defects, when absorbed (David, 2001). Lead and copper have been recognized as highly toxic industrial and environmental pollutants representing a continuous hazard to biological organisms due to its carcinogenic potential in humans (Kokilavanti et al., 2005). Industry produces about 2.5 million tons of lead throughout world every year. Target organs affected by lead are bones, brain, blood, kidneys and thyroid gland (Occupational Safety and Health Information Center, 1999). Most studies have shown that copper (Cu) induced hepatotoxicity in rats, and reported that free radicals may play a role in cupper induced cell toxicity because of the powerful pro-oxidant action of its salts in vitro. They indicated that the resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates, resulting in wide ranging impairment in cellular function and integrity (Britton, 1996).

Antioxidant is a substance that prevents oxidation. In biological systems antioxidants can work in various ways, including catalytic removal of free radicals as scavengers of free radicals or in the form of proteins that minimize the availability of pro-oxidants such as metal ions. However, there are circumstances in which certain antioxidants can actually behave as pro-oxidants (halliwell et al., 1992; and halliwell, 1996).

Vitamin E is nature's major lipid soluble chain breaking antioxidant that is known to protect biological membranes and lipoproteins from oxidative stress. The main biological function of vit. E is its direct influencing of cellular responses to oxidative stress through modulation of signal transduction pathway (hsu and guo, 2002). Vit E primarily scavenges peroxyl radicals and is a major inhibitor of the free radical chain reaction of lipid peroxidation (Maxwell, 1995 and halliwell and gutteridge,1999).

Alpha lipoic acid (ALA) has the unique ability to regenerate several antioxidants such as vitamin E (Packer and Coleman, 1999). ALA has been classified as vitamin, which is a naturally occurring antioxidant (OU et al., 1995 and Femiano and Scully, 2002). It has been reported to be highly effective in improving the thiol capacity of the cell

and in reducing Lead induced oxidative stress; which suggested its possible role as a therapeutic intervention of lead poisoning in combination with a chelator (Gurer et al., 1998 and pandl, 2002).
Production of antioxidants and free radicals in the body is theoretically balanced. When conditions become favor free radical produced, a state known as oxidative stress occurs. Free radicals are directly cytotoxic. Prolonged oxidative stress can result in oxidative damage to tissues (Halliwell, 1994), specially, DNA, cellular protein and cellular lipids. It also affect cellular calcium metabolism and the cardiovascular system (Sally et al., 2003).
ALA and Vitamin E had been reported to have highly protective effect on lipid peroxidation. So, administration of ALA and Vitamin E. had significant protective effect on blood, liver and muscles against oxidative damage in diabetes (Naziroglu, 2001 and Pandl, 2002). Therefore, the aim of this work is to reduce or minimize the adverse effects of Cupper and Lead induced toxicity.

2. Material and Methods

Materials:

1) Animals:

Seventy adult male albino rates weighing (125 ± 7 g), were obtained from the laboratory Animal Colony, Ministry of Health and Population, Helwan, Cairo _ Egypt.

2) Metals and Drugs:

Lead & copper, Alpha Lipoic Acid & Vitamin E. were purchased from El Gomhoria Company for Drugs and Chemical Industries, Cairo, Egypt.

Methods:

1) Preparation of the basal diet:

Basal diet was prepared to meet (Table 1) to meet the rats nutrient requirements according to methods of Osfor (2003). It constituted of 10% protein, 3404 K. Calorie Energy, 5 % fiber, 7.5 fat, 0.5% vitamins and minerals mixture, 0.3 % salt (Sodium Chloride).

2) Experimental Design:

The present experiment was carried out on seventy male Albino rats (125 ± 7 g), that were housed in plastic cages at a room temperature maintained at 25 Cº. All rats were kept under normal healthy conditions and allowed to water and basal diet freely for one week before starting the experiment for acclimatization and then the rats were divided into main seven groups as follows:

Group - I: fed the basal diet only and served as control.

Group - II: fed the basal diet with copper in the drinking water at a dose of 0.1 mg /kg body weight.

Group - III: fed the basal diet with copper in the drinking water at a dose of 0.1 mg /kg body weight plus Alpha Lipoic acid (40 mg/kg body weight).

Group - IV: fed the basal diet with copper in the drinking water at a dose of 0.1 mg /kg body weight plus Vitamin E (at a dose of 20 mg/kg body weight).

Group - V: fed the basal diet with lead acetate in the drinking water at a dose of 0.2 mg/ kg body.

Group - VI: fed the basal diet with Lead acetate in the drinking water at a dose of 0.1 mg /kg body weight plus Alpha Lipoic acid (40 mg/kg body weight).

Group - VII: fed the basal diet with lead acetate in the drinking water at a dose of 0.1 mg /kg body weight plus vitamin E (at a dose of 20 mg/kg body weight).

3) Clinical observation:

The animals were observed daily throughout the experimental period. Complete physical examination was conducted weekly. Body weights were measured prior to offering the diet and recorded individually every week. Food consumption was calculated every other day to the nearest gram for each group of rats as the difference between the amounts of offered and residual food.

4) Clinical pathology:

Blood samples for clinical chemistry determination were obtained from the retro-orbital plexus of veins of all rats on the day before they were scheduled for euthanasia.

5) Clinical Chemistry:

Clotted blood samples were centrifuged and the serum was removed by aspiration for subsequent determination of the following:

a) Liver function tests: Glutamic Oxaloacetic Transaminase (GOT) "Unit/ dl", GlutamicPyruvic Transaminase (GPT) "Unit/ dl" were estimated according to the method of Pters et al., 1982

b) Renal function tests: Urea "mg/dl" and creatinine "mg/dl" were estimated according to the method of Sampson et al., 1980.

c) Lead and Copper Determination: Lead and Copper were determined in blood and tissues (liver and kidney), according to methods of Price, 1972, using the Atomic absorption spectrophotometer

using SPSS (2006).

6) Statistical analysis:

The obtained data were statistically analyzed

3. Results

Table (1): Composition of the basal diet:

Ingredients	Percentage
Sorghum	39
Corn yellow	31.6
Barley	8
Meat meal	8
Corn cobs	7.3
Vegetable oil	4
Lysin	0.3
Methionine	0.4
Di-calcium Phosphate	0.2
Lime stone	0.4
Sodium chloride	0.3
Vitamins and Minerals Mixture*	0.5
Calculated Nutrient Composition	
Crude protein	11.99
Energy	3404.2
Crude fiber	4.46
Ethrer extract	7.51
Lysine	0.71
Methionine	0.61
Calcium	0.45
Phosphorus	0.4

Vitamins and Minerals Mixture (g)

Copper sulphate (0.05), Ferric citrate (0.59), Zinc carbonate (0.053), Calcium carbonate (7.25), Calciumhydrogen phosphate (11.3), Di-sodium hydrogen phosphate (6.0), Potassium Iodide (0.003), Magnesium chloride (2.3), and Manganese sulphate (0.154).

Thiamine (0.3), Riboflavin (1.0), pyridoxine (0.2), Calcium carbonate (6.0), Nicotinicacid (20.0), Cyanocobalamine (0.005), Folic acid (0.2), Biotin (20.0), Inositol (60.0), Choline chloride (60.0), vitamin A (4000 IU), vitamin D (1000 IU), Vitamin E (30 IU) and Vitamin K (50 IU).

Table (2): Effect of ALA and Vit. E on food intake, body weight gain, food efficiency ratio, liver and kidney weights (g) in rats intoxicated with copper and lead:

Group	Food Intake (g)	Body weight gain (%)	Food Efficiency Ratio	Liver	Kidney
Group I	11.25 ± 0.73a	75.63 ± 4.92 a	1.44 ± 0.31 a	4.95 ± 0.50a	0.78 ± 0.10
Group II	6.18 ± 1.86b	18.77 ± 7.31 c	0.73 ± 0.03 b	2.96 ± 0.49c	0.72 ± 0.03
Group III	7.97 ± 1.63b	34.35 ±8.96 bc	0.83 ±0.04 b	3.26 ± 0.54bc	0.69 ± 0.13
Group IV	6.40 ±1.60b	21.29 ± 4.16 c	0.78 ± 0.03 b	3.94 ± 0.69bc	0.77 ± 0.13
Group V	6.72 ± 2.08b	31.79 ±16.86bc	0.85 ± 0.09 b	3.37 ± 1.04bc	0.76 ± 0.08
Group VI	10.45 ± 1.71a	53.3 ± 13.83 ab	0.99 ± 0.14 b	4.40 ± 0.42ab	0.77 ± 0.01
Group VII	10.87 ± 0.98a	58.82 ±18.86 a	1.05 ± 0.29 ab	4.75 ± 0.47a	0.83 ± 0.12

Means with different superscript are significantly different (P< 0.05)

Table (3): Effect of ALA and Vit. E on residues of copper and lead in serum and tissues of intoxicated rats:

Group	Copper			Lead		
	Serum	Liver	Kidney	Serum	Liver	Kidney
Group I	0.116±0.005c	0.67±0.19c	0.57±0.07c	0.403±0.045	0.305±0.24b	0.76±0.98b
Group II	0.435±0.052a	45.67±5.69a	2.10±0.18a	----------	----------	----------
Group III	----------	----------	----------	0.850±0.212	1.386±0.754a	3.73±0.54a
Group IV	0.247±0.13bc	39.19±4.22a	1.53±0.40b	----------	----------	----------
Group V	----------	----------	----------	0.524±0.302	0.207±0.04b	1.99±1.73ab
Group VI	0.34±0.098ab	29.71±7.15b	0.90±0.36c	----------	----------	----------
Group VII	----------	----------	----------	0.482±0.368	0.507±0.292b	2.12±0.48ab

Means with different superscript are significantly different (P< 0.05)

Table (4): Effect of ALA and Vit. E on liver and kidney functions (u / l) of intoxicated rats:

Group	Liver function tests		Kidney function tests	
	Alt	AST	Urea	Creatinine
Group I	47.00 ± 1.00c	6.33 ±0.57c	64.66 ± 1.52b	0.84 ± 0.05d
Group II	65.33 ± 3.05a	11.00 ± 1.00a	75.00 ± 5.00a	1.85 ± 0.05a
Group III	55.33 ± 3.51b	9.00 ± 1.00b	59.66 ± 1.52c	1.80 ± 0.10a
Group IV	44.66 ± 2.51c	8.00 ± 1.00bc	53.20 ± 2.30d	1.20 ± 0.10c
Group V	43.66 ± 1.52c	3.66 ± 1.52d	23.10 ± 1.85f	1.46 ± 0.15d
Group VI	41.66 ± 2.08c	3.00 ± 1.00d	46.00 ± 2.00c	1.13 ± 0.15c
Group VII	46.33 ± 1.52c	6.33 ± 0.57c	27.20 ± 2.55f	1.10 ± 0.10c

Means with different superscript are significantly different (P< 0.05)

4. Discussion

The goal of the present study was to investigate the effect of some nutrients such as alpha lipoic acid (ALA) and vitamin E (Vit. E), in" lead and copper- induced toxicity in rats. The following parameters were tested: daily food intake, body weight gain and feed efficiency ratio; levels of Cu and Pb in serum and tissues (liver and kidney), with some biochemical parameters.

Rats fed on diets containing Cupper and Lead, consumed statistically significant less food as compared to their control. Injection of Pbintoxicated rats with ALA acid or Vit. E. significantly improved food intake and body weight gain. In addition, injecting Cu- intoxicated rats with alpha lipoic acid or vit. E. and Pb- intoxicated rats with ALA had significantly improved feed efficiency ratio. Such findings might be attributed to ALA, which has two positive effects that influence weight loss. Firstly, it decreases food intake. Secondly, it increases energy expenditure. ALA exerts this effect by decreasing the levels of a key enzyme, AMP-activated protein kinase (AMPK) within the hypothalamus, whilst increasing AMPK activity within muscles.AMPK is a major regulator of cellular energy metabolism, which inhibits the synthesis of fatty acids when activated, whilst increasing both fat, and glucose metabolism, which will further enhances weight loss (Lee et al., 2005a and Lee et al., 2005b). Furthermore, ALA facilitates the production of energy in cells a nd enhances the effectiveness of other antioxidants, regenerating Vit. E. and supports healthy function of blood glucose within normal levels (Allergy Research Group, 2008).

In the present study,it was indicated that injecting Cu or Pb- intoxicated rats with ALA or Vito E significantly decreased liver enzymes levels when compared to control rat groups which received Cu or Pb only. On the protective roles of Vito E in Pb-induced damage is the preventing effects of lipid peroxidation and the inhibition of superoxide dismutase and catalase activities in liver (Chaurasia and Kar, 1997).

Sivaprasad et al (2004) tested the efficacies of lipoic acid against Pb-induced lipid peroxidation in rat liver. Rats had administered lead acetate (0.2 0/0) in drinking water for five weeks to induce toxicity and lipoic acid (25 mg /kg. body wt/day) was given during the sixth week. The authors reported that lead damage to the liver was evident through the reductions in hepatic enzymes alanine transaminase (-38%); aspartate transaminase (-42%), and lipoic acid completely ameliorated the Pb-induced oxidative damage.

The present study showed that injecting Pb or Cuintoxicated rats with ALA or Vito E significantly reduced urea and creatinine levels {as compared to the Pb- or Cuintoxicaed rats]. These findings were supported by those of (Hanafy and Soltan 2004), who reported that blood urea and creatinine levesl were significantly increased in

Norway rats when Pb and Cu were administered subcutaneously (at a dose of 0.5 mg/1 00 g body weight or as a mixture 0.25 mg/1 00 g) for 4 weeks. They also indicated that the exposure to Vito E (250 IU/1 00 g body weight) administered orally for 4 weeks and pretreatment for 7 days) minimized the higher levels of urea and creatinine in rat groups intoxicated with Cu and Pb. Pb is capable of inducing oxidative damage to brain, heart, , kidneys and reproductive organs. The mechanisms for Pb-induced oxidative stress include its effects on membranes, DNA, and antioxidant defense systems of cells (Ahamed et aI., 2005 ; Ahamed and Siddiqui, 2007). Recent epidemiological studies have reported that low-level of Pb exposure had an association with several disease outcomes such as hypertension, peripheral artery disease, kidney disease, neurodegenerative disease, and cognitive impairment (Ekong et aI., 2006; Menke et al., 2006). Acute exposure to lead acetate was shown to increase levels of urinary excretion. Progression to renal failure is significantly worsened by oxidative stress in chronic inflammatory kidney disease.

Vitamin E supplement may be beneficial in reducing and slowing progressive kidney diseases that are significantly accelerated by oxidative stress. Vitamin E therapy may also be effective in reducing cardiovascular disease associated with chronic renal failure and the uremia state. Vitamin E therapy is also considered as a mean of correcting plasma antioxidant status and attenuating the cardiovascular disease that accompanies kidney failure. The optimal dose for Vit. E that may be helpful in slowing renal failure in humans may lie between 300 to 700 IU/day (Fryer, 2000). Vit. E. protects against lipid peroxidation and copper toxicity and prevents the majority of metal-mediated damage both in vitro systems and in metalloaded animals (Gaetke and Chow, 2003 ; Valko et al., 2005).

ALA supplementation has been shown to enhance the uptake of creatinine within muscle cells (Burke et aI., 2003). This is likely to be due to ALA's ability to enhance the uptake of glucose within muscles. ALA exerts this effect via enhancing insulin sensitivity (Lee et aI., 2005a; Lee et aI., 2005b). Creatine has traditionally been combined with a high sugar drink, which increases insulin levels and helps to enhance creatine uptake by muscle fibers. Because ALA increases muscles' sensitivity to insulin, it further increases the uptake of creatine by the muscles. In vitro and animal studies suggested that lipoic acid supplementation might be a beneficial component in the treatment of heavy metal toxicity, particularly toxicity involving Pb and Cu (Packer et aI., 1995; Gurer et aI., 1999 ; Yamamoto et aI., 2001). Intraperitoneal injection of 25 mg/kg ALA given to Pb- intoxicated rats for seven days was reported to significantly alter oxidative stress induced by Pb toxicity (Gurer et aI., 1999).

The results of the present study indicated that injecting Cu- intoxicated rats with ALA significantly reduced serum copper. Moreover, whereas injecting these rats with Vito E significantly reduced liver copper. These results are supported by the findings of (Allergy Research Group, 2008), who reported that ALA is a powerful support for the detoxification processes of the liver since it reduced Pb- toxicity and excessive copper and increased cellular glutathione levels; and by the findings of (Packer et al. 1995). They indicated that ALA has been used for decades to protect the liver and to detoxify the body from heavy metal pollutants, further more concluded that ALA alone or together with Vito E is an effective treatment for lessening indices of oxidative damage and normalizing organ's functions.

ALA is a potent antioxidant (Kagan et aI., 1990; Midaoui et aI., 2003; Wollin and Jones, 2003). It was identified as an effective antidote against heavy metal induced toxicities (Anuradha and Varalakshmi., 1999; Gurer et al., 1999; Gale Group, 2006).

The present study proved that the injection of Cuintoxicated rats with either ALA or Vit. E. significantly reduced kidney copper. These findings agreed with those reported by (Hanafy and Soltan,2004), who showed that Cu-intoxication to Norway rats resulted in marked destruction and distortion of the renal tubule cells; while oral administration of Vit. E (250 lUll 00 g body weight) revealed no abnormal histological findings as compared to normal kidney (control group). In addition (Liu et al.,1992), suggested that the increased urinary excretion of copper was related to the manifestation of renal toxicity and the synthesis of metallothionen in the kidney.

The present study showed a favorable alteration in serum lead due to the injection of Pb-intoxicated rats with ALA or Vit. E, although this alteration was not statistically significant. The hematopoietic system is one of the target organs in lead toxicity. Lead has been shown to alter RBC membrane flexibility and to increase red blood cells fragility (RBC) leading to increased risk for hemolysis (Ahmed and Siddiqui, 2007). Vit. E has a known protective action in membrane stability and prevents membrane lipoproteins from oxidative damage (Packer, 1991). Alpha-tocopherol was shown to prevent RBC membrane damage in Pb- toxicity by lowering lipid peroxide levels and increasing superoxide dismutase and catalase activity (Chaurasia and Kar, 1997). Animal studies have reported that Vit. E effectively prevented lipoperoxide-related lead toxicity and was more effective than methionine r

vitamin C at decreasing lipoperoxidation in the liver and kidney of Pb-exposed rats when given at doses of 100 IU/kg body weight (Patra et aI., 2001).

Treating Pb-intoxicated rats with ALA or Vit. E significantly reduced liver lead as compared to the control. Autopsy studies ofPb- exposed humans indicated that liver tissue is the largest repository (33%) ofPb from among the soft tissues followed by kidney cortex and medulla. As environmental exposures to Pb have increased, the toxic effects of Pb on various organ systems in the body have been recognized (patrick, 2006). Rats received intragastrically 35 mg/kg of Pb2+ once a week or 70 mg /kg of Pb2+ twice a week for 7 weeks resulted in 16.8 or 32.4 mg Pb /dL blood, and caused inhibition of lipoprotein lipase in the context of arteriosclerosis (Skoczynska et aI., 1993).

Injecting Ph- intoxicated rats with ALA or Vit. E reduced kidney Pb as compared to the control rats, although this reduction was not statistically significant. Pb- intoxication had a profound effect on the structure and consequently on the function of the rat kidney (Hanafy and Soltan 2004), they also reported that four weeks of oral administration of Vit. E (250 IUIl 00 g body weight) did not show any abnormal histological findings as compared to normal kidney.

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