

## Study on the influence of L-lysine and zinc administration during exposure to lead and ethanol in rats

Maria Chichovska<sup>1</sup>, and Anguel Anguelov<sup>2\*</sup>

<sup>1</sup>Medical University, Plovdiv, Bulgaria

<sup>2</sup>Regional Veterinary Station, Plovdiv, Bulgaria

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### ABSTRACT

Influence of lysine and zinc administration on lead-sensitive biochemical parameters and the accumulation of lead during exposure to lead were investigated in rats. The lead exposure inhibited blood  $\delta$ -aminolevulinic acid dehydrogenase ( $\delta$ -ALAD) activity, increased blood zinc protoporphyrin (ZPP), urinary  $\delta$ -aminolevulinic acid ( $\delta$ -ALA), serum glutamic oxaloacetate transaminase (AST), serum pyruvic transaminase (ALT), blood and tissue lead level, and decreased blood and hepatic glutathione (GSH) contents. The simultaneous administration of lysine and zinc reduced tissue accumulation of lead and most of the lead-induced biochemical alterations. The depletion of endogenous calcium and magnesium owing to lead was also prevented by co-administration of lysine and zinc.

**Key words:** lead, intoxication, lysine, zinc, biochemical alterations, rats

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### Introduction

Alcoholism is one of the most predisposing factors in lead poisoning, especially in occupational workers in lead-based industries and related professionals who are habitual alcoholics (FLORA and DUBE, 1994). Individuals consuming more alcohol tend to have higher blood lead levels and to be more susceptible to the toxic manifestations of lead than normal drinkers and even those in occupations exposed to lead (BORTOLI et al., 1980; SHAPER et al., 1982; DALLY et al., 1989). Some early investigations have shown that the administration of lead produced more severe biochemical alterations for haematopoietic, hepatic, and neurotoxic effects in ethanol pre-exposed or concomitantly exposed animals (FLORA and TANDON, 1987; TANDON and FLORA, 1989; DHAWAN and FLORA, 1992). It

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\* Contact address:

Dr. Anguel Anguelov, Regional Veterinary Station, 1, Lule Burgas Street., 4002 Plovdiv, Bulgaria, Phone: 359 32 441-010; E-mail: aangel@abv.bg

has also observed that the ingestion of ethanol caused depletion of endogenous glutathione (GSH), calcium and magnesium in animals exposed to lead (DHAWAN and FLORA, 1992; FLORA et al., 1991). Ethanol administration has been reported to alter hepatic GSH metabolism and to cause GSH reduction attributable to its lowered synthesis (GONZALES et al., 1988; VIVA et al., 1980). The decrease in GSH concentration and increase in absorption of lead under the influence of ethanol (DHAWAN et al., 1989) seem to enhance the toxic effects of lead.

With a view to developing an efficient treatment of lead intoxication in people who are exposed to lead and who consume alcohol, some commonly used heavy metal chelators were investigated. It was found that they were more effective in rats pre-exposed to lead alone than in those co-exposed to lead and alcohol (DHAWAN et al., 1989). The therapeutic efficacy of N- (2 -mercaptopropionyl) glycine (TIOPRONIN), containing a sulfhydryl amino acid substitute and efficient antidote of inorganic and organic mercury toxicity (KITOH and TOSHIOKA, 1978; BASINGER et al., 1981; KHANDELWAL et al., 1988), was also more prominent in the former than in the latter (TANDON and FLORA, 1986).

The present study was intended to evaluate whether a normal chelator, such as an amino acid, e.g. lysine (an essential dietary nutrient), could reduce lead intoxication, particularly when modified by ethanol ingestion. Since the influence of zinc, a known antagonist of lead and reducer of the ethanol effect, might be beneficial (KULIKOWSKA et al., 1989; POON et al., 1989), it was supplemented with lysine administration in the experimental lead intoxication.

### **Materials and methods**

The trials were carried out on 70 male Wistar albino rats weighing  $150 \pm 10$  g maintained on standard pellet diet. Metal content of the feed, in ppm dry wt - copper - 10.0, cobalt - 5.0, iron - 70.0, manganese - 55.0, zinc - 45.0, and water ad libitum. The rats were divided into seven groups of 10 animals each and treated through gastric gavage 5 days a week, for 8 weeks as follows:

Group 1 - distilled water, 4 mL/kg (control)

Group 2 - lead in distilled water - 10 mg Pb/4 mL water/kg as lead acetate trihydratate (Fluka, Switzerland).

Group 3 - ethanol in distilled water - 1000 mg/4 mL water/kg. (as 98% ethanol for breakdown, Dimitrovgrad, Bulgaria)

Group 4 - lead as in step 2 and ethanol as in step 3

Group 5 - lead as in step 2, zinc in distilled water: 10 mg Zn/4 mL water/kg as zinc sulfate (Fluka, Switzerland) and lysine in distilled water: 25mg lysine/4 mL water/kg as L-lysine monochloride (Fluka, Switzerland)

Group 6 - ethanol as in step 4, and zinc and lysine as in step 5

Group 7 - lead as in step 2, ethanol as in step 3, zinc and lysine as in step 5

All mentioned doses are per 1 kg/body mass.

Thereafter, the animals were kept in metabolic cages (1/cage) for 24-h urine collection. All animals were sacrificed by decapitation; blood was collected in heparinized vials, and liver, kidney, and brain were removed.

Standard procedures were adopted to determine the activity of blood  $\delta$ -aminolevulinic acid dehydratase ( $\delta$ -ALAD) (BERLIN and SCHALLER, 1974), blood zinc protoporphyrin (ZPP) (GRANDJEAN, 1979), blood and liver GSH (JALLOW et al., 1974), urinary  $\delta$ -aminolevulinic acid ( $\delta$ -ALA) in urine (DAVIS et al., 1968), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) (REITMAN and FRANKEL, 1957). Accurately measured samples of blood, liver, kidney, and brain were digested completely in a mixture of concentrated nitric acid (Fluka, Switzerland) and perchloric acid (Fluka, Switzerland) 6:1, and carbon-free residue dissolved in 5 mL of 5% HNO<sub>3</sub>. The samples were read for concentrations of lead (283.3 nm), calcium (422.7 nm), and magnesium (285.2 nm) by atomic absorption spectrophotometer (Perkin Elmer 5000) using appropriate standards of each metal processed identically as sample.

The significance of mean of each parameter in seven groups (normal, lead, ethanol, lead+ethanol, lead+zinc and lysine, ethanol+zinc and lysine, lead+ethanol+zinc and lysine) was compared using two-way analysis of variance ANOVA (randomized block design). The independent variables considered were seven treatments in a single block and six replicates in seven different blocks (ZAR, 1984).

## Results

Exposure to lead for 8 weeks inhibited the activity of blood  $\delta$ -ALAD (Table 1.) and increased the blood ZPP level and urinary excretion of  $\delta$ -ALA, which were not influenced by co-exposure to ethanol (Table 1.) It also decreased blood and hepatic GSH concentration, while it increased AST and ALT activity (Table 1.), the effects of which, further enhanced on co-exposure to ethanol, was probably also attributable to such influences of ethanol alone (Table 1.) The simultaneous supplementation of lysine and zinc reduced these effects in animals exposed to lead alone, and in those exposed to lead and ethanol. The decreased blood and liver GSH levels resulting from ethanol action were also restored by the lysine and zinc administration (Table 1.) The administration of lead increased the lead levels in blood, liver, and brain, but more prominently in liver and kidney of animals co-exposed to lead and ethanol. The simultaneous administration of lysine and zinc reduced the uptake of lead in blood, liver and kidney in animals exposed to lead alone. The decrease in the uptake of lead owing to administration of lysine and zinc was also significant in liver, kidney, and, interestingly, in brain in animals co-exposed to lead and ethanol (Table 2.)

Table 1. Influence of simultaneous administration of zinc and lysine on lead or ethanol-sensitive biochemical parameters in rats exposed to lead, ethanol, or their combination<sup>a</sup>

Groups	Blood			Liver	Urine	Serum	
	$\delta$ -ALA-D /nmol PBG /hr/mg protein	ZPP $\mu$ g/g Hb	GSH $\mu$ mol/100 mL	GSH $\mu$ mol/g	ALA mg/100 mL	AST nmol hydro- zone/min	ALT nmol hydro- zone/min
Control	6.45 $\pm$ 0.33	1.71 $\pm$ 0.12	17.37 $\pm$ 0.65	6.75 $\pm$ 0.65	0.05 $\pm$ 0.01	9.28 $\pm$ 1.67	9.92 $\pm$ 2.37
Pb	1.81 $\pm$ 0.18 <sup>b</sup>	3.50 $\pm$ 0.15 <sup>b</sup>	12.68 $\pm$ 1.28 <sup>b</sup>	5.19 $\pm$ 0.98 <sup>b</sup>	0.43 $\pm$ 0.05 <sup>b</sup>	20.86 $\pm$ 3.34 <sup>b</sup>	15.77 $\pm$ 1.34
EtOH	5.84 $\pm$ 0.51 <sup>c</sup>	1.62 $\pm$ 0.15	9.73 $\pm$ 0.54 <sup>b</sup>	4.90 $\pm$ 1.02 <sup>b</sup>	0.07 $\pm$ 0.01	23.46 $\pm$ 3.89 <sup>b</sup>	25.43 $\pm$ 2.58 <sup>b</sup>
Pb+EtOH	1.50 $\pm$ 0.20	3.61 $\pm$ 0.24	9.47 $\pm$ 0.54 <sup>d</sup>	5.14 $\pm$ 0.56	0.48 $\pm$ 0.03 <sup>c</sup>	28.41 $\pm$ 4.11 <sup>d</sup>	38.79 $\pm$ 5.33 <sup>d</sup>
Pb+Zn+lys	2.31 $\pm$ 0.48 <sup>c</sup>	2.96 $\pm$ 0.23 <sup>d</sup>	22.33 $\pm$ 1.02 <sup>d</sup>	6.23 $\pm$ 0.21 <sup>c</sup>	0.34 $\pm$ 0.03 <sup>d</sup>	12.61 $\pm$ 2.47 <sup>d</sup>	16.22 $\pm$ 2.23
EtOH+Zn+ lys.	5.97 $\pm$ 0.36	1.41 $\pm$ 0.14	25.19 $\pm$ 0.59 <sup>f</sup>	6.71 $\pm$ 0.22 <sup>f</sup>	0.08 $\pm$ 0.03	23.75 $\pm$ 3.29	21.90 $\pm$ 5.76
Pb+EtOH+ Zn+lys.	2.05 $\pm$ 0.21 <sup>h</sup>	2.71 $\pm$ 0.17 <sup>g</sup>	27.17 $\pm$ 0.68 <sup>g</sup>	7.86 $\pm$ 0.35 <sup>g</sup>	0.31 $\pm$ 0.05 <sup>g</sup>	16.43 $\pm$ 2.26 <sup>g</sup>	20.82 $\pm$ 2.50 <sup>g</sup>

<sup>a</sup> Each value is mean  $\pm$  SD (ten values); <sup>b</sup>P<0.001 compared to control group; <sup>c</sup>P<0.05 compared to control group; <sup>d</sup>P<0.001 compared to Pb group; <sup>e</sup>P<0.05 compared to Pb group; <sup>f</sup>P<0.001 compared to EtOH group; <sup>g</sup>P<0.001 compared to Pb+EtOH group at 5% level of significance (ANOVA); <sup>h</sup>P<0.05 compared to Pb+EtOH group at 5% level of significance (ANOVA).

Table 2. Influence of simultaneous administration of zinc and lysine on lead levels in blood and tissues of rats exposed to lead, ethanol or their combination<sup>a</sup>

Groups	Blood $\mu$ g/100 mL	Liver	Kidney	Brain
		$\mu$ g/g fresh tissue		
Control	1.75 $\pm$ 0.20	0.45 $\pm$ 0.22	0.48 $\pm$ 0.15	0.12 $\pm$ 0.09
Pb	47.23 $\pm$ 6.23 <sup>b</sup>	20.66 $\pm$ 2.34 <sup>b</sup>	22.63 $\pm$ 2.57 <sup>b</sup>	0.37 $\pm$ 0.10 <sup>b</sup>
EtOH	2.08 $\pm$ 0.41	0.63 $\pm$ 0.15	0.42 $\pm$ 0.10	0.05 $\pm$ 0.01
Pb+EtOH	45.37 $\pm$ 4.10	24.17 $\pm$ 2.52 <sup>d</sup>	27.80 $\pm$ 3.05 <sup>c</sup>	0.47 $\pm$ 0.19
Pb+Zn+lys.	34.19 $\pm$ 4.24 <sup>c</sup>	17.63 $\pm$ 1.46 <sup>e</sup>	13.39 $\pm$ 2.97 <sup>c</sup>	0.31 $\pm$ 0.05
EtOH+Zn+lys.	1.84 $\pm$ 0.51	0.65 $\pm$ 0.24	0.50 $\pm$ 0.08	0.09 $\pm$ 0.05
Pb+EtOH+Zn+lys.	46.69 $\pm$ 5.95	21.12 $\pm$ 2.95 <sup>h</sup>	18.14 $\pm$ 2.20 <sup>f</sup>	0.28 $\pm$ 0.06 <sup>g</sup>

<sup>a</sup> Each value is mean  $\pm$  SD (ten values); <sup>b</sup>P<0.001 compared to control group; <sup>c</sup>P<0.001 compared to Pb group; <sup>d</sup>P<0.01 compared to Pb group; <sup>e</sup>P<0.05 compared to Pb group; <sup>f</sup>P<0.001 compared to Pb+EtOH group at 5% level of significance (ANOVA); <sup>g</sup>P<0.01 compared to Pb+EtOH group at 5% level of significance (ANOVA); <sup>h</sup>P<0.05 compared to Pb+EtOH group at 5% level of significance (ANOVA).

Table 3. Influence of simultaneous administration of zinc and lysine on calcium levels in blood and tissues of rats exposed to lead, ethanol or their combination<sup>a</sup>

Groups	Blood µg/100 mL	Liver	Kidney	Brain
		µg/g fresh tissue		
Control	5.51 ± 0.58	20.65 ± 2.60	25.20 ± 3.83	16.23 ± 1.67
Pb	3.78 ± 0.43 <sup>b</sup>	16.58 ± 1.98 <sup>c</sup>	19.96 ± 1.81 <sup>b</sup>	15.14 ± 1.81
EtOH	5.09 ± 0.31	17.22 ± 1.24 <sup>d</sup>	22.92 ± 1.95 <sup>d</sup>	14.33 ± 1.27 <sup>d</sup>
PB+EtOH	3.33 ± 0.29	15.04 ± 1.05	19.99 ± 1.45	13.76 ± 1.04
Pb+Zn+lys	4.97 ± 0.38 <sup>f</sup>	21.56 ± 3.02 <sup>e</sup>	27.67 ± 2.72 <sup>e</sup>	16.99 ± 1.04
EtOH+Zn+lys	5.35 ± 0.39	22.62 ± 2.50 <sup>g</sup>	25.10 ± 2.44	17.06 ± 1.37 <sup>h</sup>
Pb+EtOH+Zn+lys.	5.08 ± 0.85 <sup>j</sup>	22.93 ± 2.90 <sup>i</sup>	23.50 ± 2.35	17.32 ± 2.22 <sup>i</sup>

<sup>a</sup> Each value is mean ± SD (ten values); <sup>b</sup>P<0.001 compared to control group; <sup>c</sup>P<0.01 compared to control group; <sup>d</sup>P<0.05 compared to control group; <sup>e</sup>P<0.05 compared to Pb group; <sup>f</sup>P<0.05 compared to Pb group; <sup>g</sup>P<0.001 compared to EtOH group; <sup>h</sup>P<0.01 compared to EtOH group; <sup>i</sup>P<0.001 compared to Pb+EtOH at 5% level of significance (ANOVA); <sup>j</sup>P< compared to Pb+EtOH at 5% level of significance (ANOVA)

Table 4. Influence of simultaneous administration of zinc and lysine on magnesium levels in blood and tissues of rats exposed to lead, ethanol or their combination<sup>a</sup>

Groups	Blood µg/100 mL	Liver	Kidney	Brain
		µg/g fresh tissues		
Control	41.32 ± 2.78	165.86 ± 3.17	146.09 ± 1.51	133.51 ± 2.83
Pb	29.28 ± 2.25 <sup>b</sup>	170.47 ± 2.90	136.13 ± 3.49 <sup>b</sup>	117.66 ± 3.65 <sup>b</sup>
EtOH	42.57 ± 2.52	144.26 ± 2.62 <sup>b</sup>	130.97 ± 3.76 <sup>b</sup>	123.44 ± 7.18 <sup>c</sup>
Pb+EtOH	27.55 ± 3.22	134.03 ± 1.71 <sup>d</sup>	135.90 ± 3.39	117.09 ± 9.15
Pb+Zn+lys.	35.66 ± 2.38 <sup>d</sup>	161.63 ± 3.36 <sup>d</sup>	142.48 ± 2.84 <sup>e</sup>	124.65 ± 9.09
EtOH+Zn+lys.	42.74 ± 2.59	171.06 ± 6.62 <sup>f</sup>	147.75 ± 1.68 <sup>f</sup>	130.56 ± 5.65
Pb+EtOH+Zn+lys.	33.82 ± 2.78 <sup>h</sup>	163.36 ± 1.66 <sup>g</sup>	151.17 ± 7.78 <sup>g</sup>	128.55 ± 851 <sup>i</sup>

<sup>a</sup> Each value is mean ± SD (ten values); <sup>b</sup>P<0.001 compared to normal animal; <sup>c</sup>P<0.05 compared to normal group; <sup>d</sup>P<0.001 compared to Pb group; <sup>e</sup>P<0.01 compared to Pb group; <sup>f</sup>P<0.001 compared to EtOH group; <sup>g</sup>P<0.001 compared to Pb+EtOH group at 5% level of significance (ANOVA); <sup>h</sup>P<0.01 compared to Pb+EtOH group at 5% level of significance (ANOVA); <sup>i</sup>P<0.05 compared to Pb+EtOH at 5% level of significance (ANOVA).

Exposure to lead caused a depletion of endogenous calcium from blood, liver, and kidney, and of magnesium from blood, kidney, and brain, which were uninfluenced by co-exposure to ethanol, except the hepatic magnesium, which decreased. The ingestion of

ethanol alone also caused a decrease in levels of calcium and magnesium in liver, kidney, and brain. However, the decrease in levels of calcium and magnesium in various tissues resulting from exposure to lead, ethanol, or their combination, was prevented by the simultaneous administration of lysine and zinc (Tables 3. and 4.)

## Discussion

Inhibition in blood  $\delta$ -ALAD activity and increase in blood ZPP level and the urinary  $\delta$ -ALA excretion are specific manifestations of lead intoxication (ANGUELOV et al. 2002; ANGUELOV et al., 2003; ANGUELOV and CHICHOVSKA, 2004) which seem intensive to ethanol co-exposure (FLORA and TANDON, 1987; MAHAFFEY et al., 1974). Alcohol in the blood of alcoholics depresses  $\delta$ -ALAD activity also (BERITIC et al., 1977). Lead can interact with proteins, particularly those with sulfhydryl groups (GOERING, 1993). It is known that -SH groups, as well as a specific conformational form, are necessary for enzyme activity (SHERMIN, 1976). An example of this occurrence is the inhibition of the  $\delta$ -aminolevulinic acid dehydratase in the heme-synthesis pathway.

ALA-D is an octameric metalloenzyme that contains zinc in the active site (SIMON, 1995). The active site for Zn binding contains two cysteine residues. There is competitive inhibition between Pb and Zn with the ratio of the affinity of lead to zinc at the metal-binding site being about 25:1 for the 1-1 ALA-D (SIMON, 1995).

However, the decrease in blood and liver GSH levels and the increase in AST and ALT activities caused by lead as well as ethanol intoxication are indicative of hepatic peroxidative injury (DILUZIO, 1966).

The mechanism by which lysine ameliorate lead toxicity is unknown. However, it has been reported that 0.23% L-lysine supplementation of chickens' diet increased hepatic glutathione concentration (HSU, 1981). The formation of porphobilinogen from two molecules of 5-aminolevulinic acid by  $\delta$ -ALAD requires the presence of reduced glutathione (GSH) for its activation (GIBSON et al., 1955).

The conversion of ethanol into acetaldehyde and the subsequent binding of the latter to GSH and the lead-GSH interactions may be responsible for GSH decrease (DHAWAN and FLORA, 1992; VIVA et al., 1980; HSU, 1981). The impaired GSH synthesis under the influence of ethanol and the increased requirement of GSH for lead detoxication may also be a factor in the increase in GSH concentration in liver (GONZALES et al., 1988; HSU, 1981). The accumulation of lead in blood and tissues upon lead administration and its enhanced uptake, particularly in the hepatic and kidney tissues in animals co-exposed to lead and ethanol, are to be expected in view of their increased susceptibility and the predisposing condition attributable to ethanol. The depletion of endogenous calcium and magnesium from blood and tissues upon administration of lead or ethanol may be the result of their displacement by lead, or their altered metabolism under alcoholism (DHAWAN et al., 1989; FLORA et al., 1991; LEICHTER, 1986).

The simultaneous administration of lysine as an essential dietary requirement, and of zinc, a well known lead and ethanol antagonist, reduced the lead - or the ethanol-induced biochemical alterations, the tissues' accumulation of lead, and the depletion of endogenous essential elements, viz. calcium and magnesium, in animals exposed to lead alone or co-exposed to lead and ethanol (KULIKOWSKA et al., 1989; POON et al., 1989). CERKLEWSKI and FORBES (1976) reported that zinc decreased the absorption of lead. There are at least two possibilities that may explain the zinc effect on lead absorption: a zinc-lead complex of low solubility was formed, and lead and zinc competed for binding sites on a metallothionein-like protein in the intestine. TERRI and DONALDSON (1984) suggested that lysine increased an effective protein level in liver. Perhaps the most interesting finding is the prevention of lead accumulation in the brain by the administration of lysine and zinc and suggests the usefulness of dietary supplementation of lysine and zinc salt as a preventive measure against lead intoxication among alcohol-consuming occupational workers.

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### **SAŽETAK**

Istražen je utjecaj davanja L-lizina i cinka na biokemijske pokazatelje specifične za nakupljanje olova u štakora izloženih olovu. Izloženost olovu zakočila je aktivnost dehidrogenaze  $\delta$ -aminolevulininske kiseline ( $\delta$ -ALAD) u krvi, povećala razinu cinkova protoporfirina (CPP) u krvi,  $\delta$ -aminolevulininske kiseline ( $\delta$ -ALA) u mokraći, aktivnost glutamin oksalacetatne transaminaze u serumu, piruvat transaminaze u serumu, razinu olova u krvi i tkivu, a smanjila je sadržaj glutatona u krvi i jetri. Istodobno davanje lizina i cinka smanjilo je nakupljanje olova u tkivu i većinu biokemijskih promjena uzrokovanih olovom. Davanje lizina i cinka spriječilo je smanjivanje razine endogenog kalcija i magnezija prouzročene olovom.

**Cljučne riječi:** olovo, intoksikacija, lizin, cink, biokemijske promjene, štakor

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