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Influence of zinc–saccharide complexes on some haematological parameters in rats

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The effects of three recently synthesized zinc–saccharide complexes (zinc–fructose, zinc–galactose and zinc–glucose) on blood δ-aminolevulinic acid dehydratase (ALAD) activity, glutathione (GSH), zinc–protoporphyrin (ZPP) and urinary δ-aminolevulinic acid (ALA) levels have been investigated to ascertain the utility of these complexes as zinc supplements and as preventive agents against lead intoxication in rats.

Keywords: ALAD activity, haematological parameters, urinary ALA levels, zinc–saccharide complexes, ZPP levels

Introduction

Zinc is generally considered to be a relatively non-toxic metal; an essential element for the growth of life, even when in conditions of excess as compared with other metals. There is considerable evidence that zinc deficiency in humans is a serious world-wide problem and outweighs the potential problem of zinc excess. Alcoholism, malabsorption, sickle cell anaemia, chronic renal disease and other chronic debilitating diseases are known to be predisposing factors for zinc deficiency in humans. The relationship between dietary zinc and lead, and its possible clinical significance has been well documented. When dietary zinc is increased above requirement levels, it reduces apparent lead absorption in rats; this is reflected in a reduction both in tissue lead, and in its biochemical effects (Cerklewski & Forbes 1976, Flora *et al.* 1982, 1991). Despite the strength of these observations, it is also possible that lead and zinc are competitive at other tissue sites, which would account for at least part of the protective effect of zinc on lead toxicity. There are reports indicating that zinc has reversed lead inhibition of the lead-sensitive, zinc-dependent enzyme, δ-aminolevulinic acid dehydratase (ALAD) (Davis & Avram 1980, Trevisan *et al.* 1980).

We report here the effects of zinc–fructose (Zn–Fru), zinc–galactose (Zn–Gal) and zinc–glucose (Zn–Glc) complexes on blood ALAD activity, zinc–protoporphyrin (ZPP), glutathione (GSH) and urinary δ-aminolevulinic acid (ALA) levels in mice in the context of nutritional supplementation.

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Experimental

Chemicals

The zinc–saccharide complexes, namely Zn–Fru, Zn–Gal and Zn–Glc were synthesized in our laboratory, as reported earlier (Bandwar *et al.* 1996), using either $ZnCl_2$ or $[NEt_4]_2[ZnCl_2Br_2]$ and sodium salts of saccharides generated *in situ* in methanol. ALA was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were purchased from either Sigma Chemical Company, E. Merck (Darmstadt, Germany) or BDH (Mumbai, India).

Animals and treatment

Effect of zinc–saccharide complexes on ALAD activity, ZPP and GSH levels in male rats. Male albino rats, of Wistar strain, weighing approximately 100 g were employed in the study. They were maintained on sterile dust-free rice husk and given standard pellet diet (Lipton's India Ltd., Mumbai; metal contents of diet, in μg g^{-1} dry weight: Cu, 10.0; Mn, 55.0; Co, 5.0; Fe, 70.0; and Zn 45.0) and water *ad libitum*.

The rats were divided into six groups of three rats each and were given a single oral dose of 50 or 500 mg kg^{-1} body weight of one of the zinc–saccharide complexes. Three rats were given an equal amount of vehicle (4 ml kg^{-1}, orally) through the same route and served as a normal control group. Zinc–saccharide complexes were dissolved in distilled water and the pH was adjusted to 6.4.

Blood was collected in heparinized tubes from orbital plexus at Days 1, 3 and 7 following zinc–saccharide complex administration.

Effect of zinc–saccharide complexes on lead-poisoned male rats. A separate set of rats were divided into five groups of five rats each and treated as below for seven consecutive days:

Group 1: normal saline, 4 ml kg^{-1}, orally through gastric gavage (control);
Group 2: 50 mg kg^{-1} body weight (b.w.) lead (as lead acetate), orally through gastric gavage;
Group 3: lead as in Group 2 + 50 mg kg^{-1} b.w. Zn–Fru, intraperitoneally (i.p.);
Group 4: lead as in Group 2 + 50 mg kg^{-1} b.w. Zn–Gal, i.p.;
Group 5: lead as in Group 2 + 50 mg kg^{-1} b.w. Zn–Glc, i.p.

On completion of the seven day treatment, the animals were kept in metabolic cages (one per cage) for the collection of 24 h urine. Blood was collected in heparinized tubes, after which the animals were decapitated under light ether anaesthesia. Liver and kidneys were also removed.

Biochemical assays

The activity of blood ALAD was determined following the procedure of Berlin & Schaller (1974). The level of blood GSH was measured as described by Ellman (1959). Blood ZPP level was determined using an haematofluorometer (model ZP haematofluorometer, Aviv, Lakewood, NJ, USA). Urinary ALA level was determined using a dual ion-exchange chromatographic procedure (Davis *et al.* 1968).

Metal estimation

Concentrations of lead (Table 1) and zinc (Table 2) in blood, liver and kidneys were determined using the procedure of Yeager *et al.* (1971) and Parker *et al.* (1967), respectively, following wet acid digestion with nitric acid. Briefly, the digested samples were treated with 5 ml ammonium citrate buffer. To this, 1 ml potassium cyanide was added. The lead was complexed with 2% ammonium tetramethylene dithiocarbamate, extracted in 4 ml methylisobutylketone and read at 283.3 nm using an atomic absorption spectrometer (Varian Techtron, Australia). Recoveries were based on standard carried through the digestion procedure, and were 91 ± 8% for the liver, 92 ± 8% for kidney, and 90 ± 8% for blood. For each analysis, blank and standards were carried out in 0.7% nitric acid and calibration was done at the end of each sample run.

Table 1. Influence of simultaneous supplementation of zinc–saccharide complexes during lead administration on blood and tissue lead concentration in rats

Group	Blood (μg dl^{-1})	Liver (μg g^{-1})	Kidneys (μg g^{-1})
Normal animals[#]	2.01 ± 0.13	0.63 ± 0.03	0.57 ± 0.04
Lead	14.10 ± 2.00*	3.40 ± 0.45*	2.84 ± 0.37*
Lead + Zn–Fru	6.80 ± 0.91[a]	1.99 ± 0.30[a]	1.09 ± 0.10[a]
Lead + Zn–Gal	8.69 ± 0.79[a]	2.13 ± 0.24[a]	1.19 ± 0.08[a]
Lead + Zn–Glc	7.30 ± 0.98[a]	1.65 ± 0.16[a]	1.19 ± 0.13[a]

Values are mean ± SE; $n = 5$.

[#]No exposure to lead and/or any Zn–saccharide complex.

*$P < 0.05$ compared with normal animals.

[a]$P < 0.05$ compared with lead-treated rats.

Table 2. Influence of simultaneous supplementation of zinc–saccharide complexes during lead administration on blood and tissue zinc concentration in rats

Group	Blood (μg l^{-1})	Liver (μg g^{-1})	Kidneys (μg g^{-1})
Normal animals[#]	71.8 ± 8.15	35.49 ± 1.17	24.79 ± 1.40
Lead	56.3 ± 2.34	33.12 ± 1.00	24.29 ± 0.40
Lead + Zn–Fru	105.1 ± 7.24[a]	38.02 ± 0.66	27.03 ± 1.76
Lead + Zn–Gal	79.1 ± 4.63[a]	36.67 ± 0.41	27.67 ± 1.02
Lead + Zn–Glc	78.6 ± 3.85[a]	36.01 ± 0.97	27.56 ± 0.68[a]

[#]No exposure to lead and/or any Zn–saccharide complex.

Values are mean ± SE; $n = 5$.

[a]$P < 0.05$ compared with lead-treated rats.

Results and discussion

Effect of zinc–saccharide complexes on ALAD activity, GSH and ZPP levels

The administered dose of the three zinc–saccharide complexes, namely Zn–Fru, Zn–Gal and Zn–Glc did not indicate any sign of toxicity and had no influence on the body weight gain. The dose dependent effect of these complexes (50 and 500 mg kg^{-1}) on the various haematological parameters are summarized in Tables 3 (ALAD activity), 4 (ZPP level) and 5 (GSH level). The ALAD activity was found to be almost unaffected over the first three days and increased only marginally up to the seventh day. Zinc metabolism has been shown to be homeostatically controlled (Miller 1976); when zinc absorption is high, zinc associates with low molecular weight chelate, but when zinc absorption is low, it associates with a metallothionein (MT)-like protein. As reported earlier (Bandwar *et al.* 1996), the zinc–saccharide interactions were found to be absent under acidic conditions; consequently, in the highly acidic environment of the stomach, they are expected to dissociate, to release the zinc ion. No effect on the ALAD activity in the first three days, in spite of the presence of free zinc, could therefore be attributed to the chelation of dissociated zinc immediately after absorption into blood. This is in agreement with the observed increase in the liver MT only, as reported earlier (Bandwar *et al.* 1996). However, a marginal increase in ALAD activity thereafter could be attributed to the slow release of zinc from zinc-binding proteins. This was supported by a concomitant increase in the ZPP level over the same time period (Table 4). The ZPP level was also found to be sensitive to the concentration of zinc and showed increased levels in rats administered with a higher dose of zinc–saccharide complexes. GSH is an efficient chelator of heavy metals, such as Pb, Zn, Cd, etc., and its synthesis is triggered by the presence of heavy metals in the cell. Thus, the cellular concentration of GSH acts as an indicator of the presence of these heavy metals. As is evident from Table 5, the administration of the three zinc–saccharide complexes increased the GSH level over a period of seven days and no significant differences were observed between them.

Effect of zinc–saccharide complexes on lead-poisoned male rats

The effects of 50 mg kg^{-1} doses of lead and combined lead plus zinc–saccharide complexes on the ALAD activity, urinary ALA and GSH levels are shown in Figure 1. The administration of lead drastically decreased the activity of ALAD from the normal level. However, when the administration of lead was supplemented with the zinc–saccharide complexes, a significant restoration of the ALAD activity was observed. It is well known that increased dietary intake of zinc can reduce lead absorption and restore altered lead-induced biochemical variables (Cerklewski & Forbes 1976). Table 1 shows that in presence of zinc–saccharide complexes, the absorption of lead was significantly reduced in blood, liver and kidneys. The Zn–Fru complex was much better absorbed than Zn–Gal and Zn–Glc complexes (Table 2) and consequently showed a more pronounced effect on reduced lead absorption in these tissues. Lead–zinc interactions have been observed at absorptive as well as enzymatic sites (Willoughby *et al.* 1976, Flora *et al.* 1982). Protective effects of zinc against plumbism could be attributed to a decrease in lead absorption in the gastrointestinal tract where zinc and lead compete for similar binding sites on an MT-like transport protein (Flora *et al.* 1991). Another protein which may be involved in lead–zinc interaction is a low molecular weight chelating protein (Richard & Cousin 1975). *In vivo* and *in vitro* studies have shown that addition of zinc can activate ALAD and prevent inhibition by lead (Finelli *et al.* 1975, Flora *et al.* 1989). Thus, in the present case, the increase in the activity of ALAD in lead-poisoned rats supplemented with the zinc–saccharide complexes indicated that the activity was significantly restored to some extent, although below the normal level, and hence higher doses of zinc–saccharide complexes were required. When compared together, the Zn–Fru and Zn–Glc complexes exhibited a better restoration of ALAD activity than did the Zn–Gal complex, suggesting that the first two were better absorbed than the latter. The rejuvenation of ALAD activity was also evident from the decreased level of urinary ALA excretion upon zinc–saccharide complex administration. The Zn–Glc complex was found to be most effective in reducing the urinary ALA excretion to almost the normal level, as compared with the other two complexes (Figure 1). The concentration of GSH was also found to increase on lead exposure. This is in agreement with the fact that GSH has been shown to overcome lead-induced blood ALAD inhibition both *in vitro* and *in vivo* (Nakao *et al.* 1968). The supplementation of the zinc–saccharide complexes further increased the levels of GSH in accordance with the total concentration of lead and zinc. Therefore, the reversal of blood ALAD

Table 3. Dose-dependent effect of three zinc-saccharide complexes on blood ALAD activity (n mol per min per ml erythrocytes) in male albino rats[a]

Group	Dose (mg kg^{-1})	Day 1	Day 3	Day 7
Normal animals[#]		5.82 ± 0.45		
Zn–Fru	50	4.05 ± 0.49	4.54 ± 0.74	5.55 ± 0.31
	500	2.01 ± 0.22	3.27 ± 1.39	5.01 ± 0.14
Zn–Gal	50	4.59 ± 0.56	3.53 ± 0.51	5.71 ± 0.26
	500	4.96 ± 0.56	2.98 ± 0.39	5.78 ± 0.37
Zn–Glc	50	4.58 ± 0.42	4.19 ± 0.91	5.71 ± 0.25
	500	4.89 ± 0.45	4.95 ± 0.81	4.61 ± 0.22

[#]No exposure to Zn–saccharide complexes.
Values are mean ± SE of three rats; normal animal values for Days 1, 3 and 7 were pooled ($n = 9$).
[a]$P < 0.05$ compared with control.

Table 4. Dose-dependent effect of three zinc–saccharide complexes on blood ZPP levels (μg g^{-1}) in male albino rats[a]

Group	Dose (mg kg^{-1})	Day 1	Day 3	Day 7
Normal animals[#]		0.58 ± 0.04		
Zn–Fru	50	0.60 ± 0.04	0.43 ± 0.03	0.43 ± 0.03
	500	0.62 ± 0.09	0.76 ± 0.03	0.90 ± 0.05
Zn–Gal	50	0.46 ± 0.03	0.60 ± 0.05	0.60 ± 0.03
	500	0.58 ± 0.06	0.80 ± 0.05	0.76 ± 0.03
Zn–Glc	50	0.66 ± 0.08	0.43 ± 0.03	0.50 ± 0.10
	500	0.54 ± 0.05	0.60 ± 0.05	0.86 ± 0.03

[#]No exposure to Zn–saccharide complexes.
Values are mean ± SE of three rats; normal animal values for Days 1, 3 and 7 were pooled ($n = 9$).
[a]$P < 0.05$ compared with control.

Table 5. Dose-dependent effect of three zinc–saccharide complexes on blood GSH levels (μmol ml^{-1}) in male albino rats[a]

Group	Dose (mg kg^{-1})	Day 1	Day 3	Day 7
Normal animals[#]		8.23 ± 0.99		
Zn–Fru	50	5.21 ± 0.48	4.74 ± 0.67	8.67 ± 0.34
	500	7.62 ± 0.16	4.57 ± 0.35	7.40 ± 0.61
Zn–Gal	50	6.05 ± 0.16	5.17 ± 0.45	8.35 ± 0.37
	500	4.03 ± 0.68	5.55 ± 0.13	8.51 ± 0.99
Zn–Glc	50	5.78 ± 0.45	5.92 ± 0.12	9.07 ± 0.16
	500	6.74 ± 0.82	5.74 ± 0.52	8.55 ± 0.55

[#]No exposure to Zn–saccharide complexes.
Values are mean ± SE of three rats; normal animal values for Days 1, 3 and 7 were pooled ($n = 9$).
[a]$P < 0.05$ compared with control.

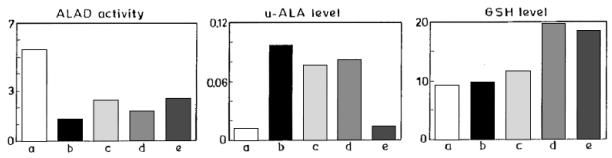

Figure 1. Effect of zinc–saccharide complexes, at a dose of 50 mg kg^{-1} given in addition to a 50 mg kg^{-1} dose of lead, on ALAD activity (n mol per min per ml erythrocytes), urinary ALA (mg per 100 ml urine) and GSH (μmol ml^{-1}) levels in male albino rats. (a) normal; (b) lead; (c) lead + Zn–Fru; (d) lead + Zn–Gal; and (e) lead + Zn–Glc.

inhibition may be regulated by two factors: (i) availability of thiol groups; and (ii) removal of lead from the binding sites.

The present studies indicate, therefore, that the three zinc–saccharide complexes, namely Zn–Fru, Zn–Gal and Zn–Glc could act as potential dietary supplements and as preventive agents against lead intoxication.

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References

Bandwar RP, Hidalgo J, Giralt M, Rao CP. 1996 Metal–saccharide chemistry and biology: saccharide complexes of zinc and their effect on metallothionein synthesis in mice. *Carbohydr Res* **284**, 73–84.

Berlin A, Schaller KH. 1974 European standardized method for the determination of δ-aminolevulinic acid dehydratase activity in blood. *Z Klin Chem Biochem* **12**, 389–390.

Cerklewski FL, Forbes RM. 1976 Influence of dietary zinc on lead toxicity in the rat. *J Nutr* **106**, 689–696.

Davis JR, Avram MJ. 1980 Correlation of the physicochemical properties of metal ions with their activation and inhibition of human erythrocytic δ-aminolevulinic acid dehydratase EC 4.2.1.24 *in vitro*. *Toxicol Appl Pharmacol* **55**, 281–290.

Davis JR, Abrahams RH, Fishbein WI, Fabrega EA. 1968 Urinary δ-aminolevulinic acid (ALA) levels in lead poisoning. II. Correlation of ALA values with clinical findings in 250 children with suspected lead ingestion. *Arch Environ Health* **17**, 164–171.

Ellman GL. 1959 Tissue sulphydryl groups. *Arch Biochem Biophys* **82**, 70–77.

Finelli VN, Klauder DS, Karaffa MA, Petering HG. 1975 Interaction of zinc and lead on δ-aminolevulinate dehydratase. *Biochem Biophys Res Commun* **65**, 303–311.

Flora SJS, Jain VK, Behari JR, Tandon SK. 1982 Protective role of trace metals in lead intoxication. *Toxicol Letts* **13**, 51–56.

Flora SJS, Singh S, Tandon SK. 1989 Thiamine and zinc in prevention or therapy of lead intoxication. *J Internat Med Res* **17**, 68–75.

Flora SJS, Kumar D, Das Gupta S. 1991. Interaction of zinc, methionine or their combination with lead at gastrointestinal or post-absorptive level in rats. *Pharmacol Toxicol* **68**, 3–7

Miller WJ. 1976 Absorption, tissue distribution, endogenous excretion and homeostatic control of zinc in ruminant. *Amer J Clin Nutr* **22**, 1523–1537.

Nakao K, Wada O, Lano Y. 1968 δ-aminolevulinic acid dehydratase activity in erythrocytes for the evaluation of lead poisoning. *Clin Chem Acta* **19**, 319–325.

Parker MM, Humoller FL, Mahler DJ. 1967 Determination of copper and zinc in biological material. *Clin Chem* **13**, 40–48.

Richard MP, Cousin RJ. 1975 Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. *Biochem Biophys Res Commun* **64**, 1215–1223.

Trevisan A, Gori GP, Zangirolami A, *et al*. 1980 Site of action of metals on the aminolevulinic acid dehydratase EC 4.2.1.24 of human erythrocytes. *Enzyme* **25**, 33–36.

Willoughby RA, McDonald E, McSherry BJ. 1976 Lead poisoning and the interaction between lead and zinc in the foal. *Can J Com Med* **36**, 348–359.

Yeager DW, Cholak J, Henderson EW. 1971 Determination of lead in biological and related material by atomic absorption spectrophotometry. *Environ Sci Technol* **5**, 1020–1022.