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THE INTERACTION OF MERCURY WITH HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN ALBINO MICE

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ABSTRACT

Mice were divided into control and treated groups of 20 mice each, and were given distilled water (control) or distilled water containing 0.002, 2.0, and 20 ppm of mercury . Three mice from each group were sacrificed after 30, 60, and 90 days of treatment. The results showed that there were no significant differences in the hematological parameters compared to the control group at any of the given concentrations, except the hemoglobin concentration at 20 ppm of mercury for 30 days .

Erythrocytic δ-ALAD activity was significantly decreased by mercury treatment . Brain ATPase was significantly inhibited while liver ATPase was not . Mercury did not inhibit brain AChE but it inhibited liver AChE at 2.0 and 20 ppm after 30 days of treatment .

INTRODUCTION

Mercury pollution is of great concern because it is highly toxic, persistent, and can undergo food chain amplification (kihlstrom et al. 1961). The major effects of chronic exposure are on the central nerv us system (CNS) in addition to hematological changes (Friberg and vostal, 1972). Within tissues, mercury may bind to SH-containing proteins including enzyme system of the microsomes and mitochondria, producing various types of injury which might lead tc death.

δ- Aminolevulinic acid dehydratase (Porphobilinogen synthase; 5-amino levulinate hydrolase, E.C.4.2.1.24) catalyzes the conversion of two molecules of δ-aminolevulinic acid to porphobilinogen which is the immediate precursor to porphyrins (Wu et al., 1974). This enzyme is expected to be one of the targets in exposed tissues.

The present study was undertaken to study the in vivo interaction of different concentrations of mercury with some hematological and biochemical parameters in albino mice.

MATERIALS AND METHODS

TREATMENT OF ANIMALS

Female albino mice, Mus musculus, one month old, each weighing 20-25gm were used as experimental animals throughout the present study. They were fed on a commercial standard diet. Mice were divided into control and treated groups of 20 mice each, and given distilled water (control) or distilled water containing 0.002, 2 and 20 ppm of mercury as mercuric acetate beginning at day 1 of gestation up to day 90.

Three mice from each group were sacrificed by decapitation after 30, 60, and 90 days of treatment. the blood was collected from each animal in citrated tube and used freshly for delta-aminolevulinic acid dehydratase (δ-ALAD) assay and hematological study. Animals were dissected and brains and Livers, were kept at -20° c for biochemical measurements.

SAMPLE PREPARATION

Whole blood:

The blood samples were collected from each animal in 5 c.c. citrated tube containig 250 ul of 3.8 % sodium citrate. The blood was mixed carefully to avoid the formation of foams, then used freshly for measuring delta-aminolevulinic acid dehydratase activity(δ-ALAD), white blood cells (WBC) count, red blood cells (RBC) count, hemoglobin (HGB) concentrations, hematocrite (HCT) values, and mean cell volume (MCV). Hematological parameters were measured by using Hemacomp 5 instrument.

Brain and Liver tissues:

Brains and livers were obtained from dissected animals, homogenized at 1:5 (W/V) in ice-cooled 0.1 M Tris-HCl buffer at pH 7.0 and then centrifuged at 6000 rpm for 10 min. using Beckman L5 -75 Ultracentrifuge type 40 rotor. Pellets were discarded and supernatants were recentrifuged at 17,000 rpm for 30 min. Resultant pellets were suspended in the same buffer and kept at-4c° until use.

BIOCHEMICAL PARAMETERS

ATPase and AChE activities in both control and treated mice were determined in both brain and liver tissues. ATPase activity was determined according to Koch (1969) . AChE activity was assayed according to Ellman et al. (1961). The erythrocyte δ-ALAD activity was assayed according to Joseph et al. (1971).

Protein concentration was determined by the method of Lowry et al.(1951), using crystalline bovine serum albumine as a standard. Standard curve for inorganic phosphate, using sodium phosphate dibasic, was done in order to calculate ATPase units.

Significant differences between two means were calculated with student's t-test. Differences were considered to be significant and highly significant when the corresponding levels of probability were 0.05 and 0.01,respectively.

RESULTS AND DISCUSSION

1- Effects of mercury on blood parameters

The data in tables 1 , 2 , and 3 show no significant differences in the measured parameters compared to the control groups at any of the given concentrations, except the hemoglobin concentration which was found to be significantly different in the mice treated with 20 ppm of mercury for 30 days.

Table (1): Interaction of Mercury with Blood Parameters After 30 Days of Gestation in Albino Mice .

Mercury conc. (ppm)	WBC's 10^3 cell/c.c.	RBC's 10^6 cell/c.c.	HGB gm/dL	HCT %	MCV Micron/RBC
0.00	5.23 ± 1.83	7.66 ± 0.01	12.80 ± 0.53	34.13 ± 2.35	46.67 ± 1.15
0.002	6.20 ± 3.65	7.12 ± 0.63	12.97 ± 0.83	36.13 ± 2.48	48.33 ± 2.08
2.00	4.07 ± 1.02	7.07 ± 0.27	13.03 ± 0.58	33.63 ± 1.46	47.67 ± 1.53
20.00	6.45 ± 0.78	7.34 ± 0.16	14.13 ± 0.38*	35.65 ± 0.78	48.67 ± 0.58

Each value is the mean ± SD of 9 replicates .

* Significantly different from the control by t-test at $P \leq 0.05$

Table (2): Interaction of Mercury with Blood Parameters After 60 Days of Gestation in Albino Mice .

Mercury conc.	WBC's 10^3 cell/c.c.	RBC's 10^6 cell/c.c.	HGB gm/dL	HCT %	MCV Micron/RBC
0.00	5.80 ± 2.43	7.65 ± .51	12.83 ± .57	36.00 ± 1.91	47.00 ± 2.00
2 ppb	6.93 ± 1.78	7.20 ± .18	13.17 ± .55	36.53 ± 1.33	50.33 ± 1.15
2 ppm	5.60 ± 2.00	6.80 ± .77	12.17 ± 1.70	33.67 ± 4.45	49.33 ± 0.58
20 ppm	4.50 ± .66	6.89 ± .71	12.70 ± .62	35.77 ± 2.42	51.67 ± 3.06

Each value is the mean ±SD of 9 replicates .

Table (3): In vivo Interaction of Mercury with Albino Mice Blood Parameters After 90 Days of Gestation .

Mercury conc.	WBC's 10^3 cell/c.c.	RBC's 10^6 cell/c.c.	HGB gm/dL	HCT %	MCV Micron/RBC
0.0	6.67 ± 1.70	7.47 ± 0.73	13.10 ± 1.57	34.87 ± 4.88	46.67 ± 2.08
2.0 ppb	5.77 ± 1.60	7.38 ± 0.87	13.10 ± 1.40	34.57 ± 3.40	47.00 ± 1.00
2.0 ppm	6.43 ± 1.76	7.47 ± 0.47	12.77 ± 1.17	33.23 ± 3.79	44.33 ± 2.52
20.0 ppm	7.13 ± 0.65	7.24 ± 0.47	13.36 ± 0.47	33.47 ± 2.51	46.67 ± 1.53

Each value is the mean ± SD of 9 replicates.

Table (4): Interaction of different concentrations of Mercury with δ-ALAD, ATPase, and AChE after 30 days of Gestation.

Mercury conc.	Blood @ δ-ALAD	ATP-ase μ mole Pi / mg protien / hr		AChE Δ OD_{412} / mg protien / hr	
		Brain	Liver	Brain	Liver
control	104.19 ± 4.64	61.55 ± 6.30	11.88 ± .21	24.56 ± 0.97	44.11 ± 1.36
2 ppb	101.51 ± 9.80	56.24 ± 8.71	11.82 ± .58	26.72 ± 4.16	43.47 ± 4.30
2 ppm	99.21 ± 6.13	46.84 ± 3.18*	11.04 ± .35	26.41 ± 0.94	33.24 ± 3.0*
20 ppm	45.35 ± 8.62**	47.91 ± 2.89*	11.56 ± 2.40	22.04 ± 2.37	31.18 ± 1.20**

@ Enzyme Unit = $OD_{60} - OD_0 \times \frac{100}{HCT} \times \frac{\text{Blood dilution}}{\text{Blood vol.} \times 60 \text{ min}} \times \frac{2}{0.036}$

* Significantly different from the control by t-test at $P \leq 0.05$

** Highly significant ($P \leq 0.01$)

Each value is the mean ±SD of 9 replicates .

Table (5): Interaction of different concentrations of mercury with δ-ALAD, ATP-ase, and ChE after 60 days of Gestation .

Mercury conc.	Blood @ δ-ALAD	ATP-ase μ mole Pi / mg protien / hr		AChE Δ OD_{412} / mg protien / hr	
		Brain	Liver	Brain	Liver
control	65.11 ± 7.30	44.44 ± 1.24	10.73 ± 0.46	30.50 ± 3.61	26.88 ± 1.65
2 ppb	49.87 ± 9.56	41.75 ± 1.15	10.33 ± 0.87	23.96 ± 5.00	27.94 ± 2.13
2 ppm	37.83 ± 8.14*	42.33 ± 5.46	10.69 ± 0.65	28.35 ± 3.8	26.89 ± 2.34
20 ppm	31.53 ± 8.66**	35.37 ± 3.62*	10.69 ± 0.60	29.24 ± 1.16	26.05 ± 4.87

@ Enzyme Unit = $OD_{60} - OD_0 \times \frac{100}{HCT} \times \frac{\text{Blood dilution}}{\text{Blood vol.} \times 60 \text{ min}} \times \frac{2}{0.036}$

Each value is the mean ± SD of 9 replicates.

* Significantly different from the control by t-test at $P \leq 0.05$

** Highly significant ($P \leq 0.01$)

Table (6): Interaction of different concentrations of Mercury with δ-ALAD, ATP-ase, and ChE after 90 days of Gestation .

Mercury conc.	Blood @ δ-ALAD	ATP-ase μ mole Pi / mg Protein / hr		AChE Δ OD_{412} / mg Protein / hr	
		Brain	Liver	Brain	Liver
0.00	84.88 ± 10.91	54.49 ± 0.72	8.39 ± 1.18	30.01 ± 2.68	30.31 ± 0.80
2.0 ppb	65.60 ± 13.12	56.01 ± 1.66	10.80 ± 1.91	28.65 ± 1.61	23.82 ± 2.67
2.0 ppm	35.05 ± 2.96**	54.93 ± 3.25	11.59 ± 1.80	26.13 ± 1.70	28.75 ± 1.29
20.0 ppm	35.03 ± 2.21**	49.88 ± 2.26*	10.93 ± 1.03	27.25 ± 1.55	31.46 ± 0.51

@ Enzyme Unit = $OD_{60} - OD_0 \times \frac{100}{HCT} \times \frac{\text{Blood dilution}}{\text{Blood vol.} \times 60 \text{ min}} \times \frac{2}{0.036}$

* Significantly different from the control by t-test at $P \leq 0.05$

** Highly significant ($P \leq 0.01$)

Each value is the mean ± SD of 9 replicates .

2- Interaction of mercury with δ-ALAD, ATPase, and AChE activities

Tables 4, 5 and 6 summarize the activities of δ-ALAD, ATPase, and AChE in the control and treated groups. Erythrocytic δ-ALAD activity is significantly decreased by mercury treatment. Mercury treatment of 2 ppm caused 4.8, 41.9, and 58.7 % inhibition after 30, 60, and 90 days of gestation, respectively, while the teatment by 20 ppm caused 56.4%, 51.6, and 58.7 % of inhibition at the same time intervals .

In 1976 WHO reported that the CNS is the major site of toxicity after exposure to elemental mercury, especially after chronic exposure; therefore, of the CNS enzymes, ATPase and AChE were studied as targets for mercuric toxicity. Tables 4 , 5, and 6 present the in vivo interaction of mercury with brain and liver ATPase activities at different time intervals of gestation. Inhibition of brain ATPase was observed at 30 days up to 90 days of gestation. On the other hand, mercury had no effect on liver ATPase .

Brain AChE shows no significant inhibition by mercury after 30, 60, and 90 days of gestation as shown in tables 4 , 5 , and 6 . However, a significant inhibition of liver AChE activity, at 2 and 20 ppm of mercury, after 30 days of treatment was observed (Table 4) . Liver AChE inhibition was recovered after 60 days of mercury treatment, which may be attributed to the allosterical binding of mercury to the enzyme binding site (Table 5) .

Mortalities within treated groups were recorded after 90 days of exposure. The mortality percentage caused by mercury treatments were 0 , 6.7 , 6.7, and 13.3 % at 0., 0 , 2 ppb , 2 ppm, and 20 ppm of mercury, respectively .

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الملخص العربي

تم دراسة تأثير عنصر الزئبق على نفران التجارب ، وقسمت الفئران الى ٤ مجاميع ،وكل مجموعه تحتوى على ٣٠ فأر . وكانت المعاملات كالتالى : كنترول (بدون زئبق) ٠٫٢٥ ، ٠٫٢٥ ، ٢٠ جزء فى المليون . وتم ذبح ٣ حيوانات من كل مجموعه فى الفترات التاليه : ـ ٣٠ ، ٦٠ ، ٩٠ يوم من تاريخ المعامله . وأوضحت النتائج أنه لا يوجد فروق معنويه فى قياسات الدم المختلفه بين الفئران المعامله وغير المعامله ماعدا الهيموجلوبين فقد أنخفض تركيزه فى الفئران المعامله بـ ٢٠ جزء فى المليون من الزئبق لمدة ٣٠ يوم . وأظهرت النتائج انخفاض واضح فى نشاط انزيم الدلتا أمينو ليفيولينيك أسيد ديهيدراتيز فى الأفراد المعامله . كما حدث أنخفاض فى انزيم الادينوسين ترای فوسفاتيز فى الدم ولم يحدث فى الكبد . وقد ثبط نشاط انزيم الاسيتايل كولين استريز فى كبد الحيوانات المعامله بتركيزى ٢ ، ٢٠ جزء فى المليون لمدة ٣٠ يوم .