Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(9):159-168

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of Anti Dote Dimercaprol (Bal) on the Heavy Metal Intoxication and Reversal Effects in Biochemical Constituents, on Direct Exposure to *Heterometrous fulvipes*

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ABSTRACT

Heavy metals exposure in animals can lead to profound effects in growth and development. It is necessary that heavy metal toxicity be well documented and adequate precaution should be taken in mother and fetus to decrease its detrimental effects. An experimental study was performed with viviparous animal Heterometrous fulvipes to access the cumulative effects on Bio chemical parameters on glycogen, glucose proteins, TNPS, and protein metabolism on chronic heavy metals exposure. Heavy metals disturb all most all functions in which proteins are involved and all most every protein in the body is a potential target. Heavy metals primarily cause biochemical lesions and affects in altering carbohydrate, protein and lipid metabolism. Chronic heavy metal exposure resulted in decrease in hepato pancreatic weight, hepato-somatic index and embryonic length with subsequent reduction and length and weight of the embryos.

Keywords: Heavy metals; Toxicity; Biochemical; Metals exposure

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INTRODUCTION

The toxic sequelae of heavy metal action on tissues result in primary biochemical lesion whereby a critical enzyme or metabolic process is inhibited. Cell membrane is known to contain sulfhydryl groups that are essential to the normal permeability and transport of materials. The same sulfhydryl groups are known to have a very high affinity for mercury, lead and other heavy metals. Almost all proteins contain sulfhydryl groups that are metal reactive. As the sulfhydryl groups are important in most protein functions, heavy metals can disturb almost all functions in which Proteins are involved. Thus, almost every protein in the body is a potential target. In other words, heavy metals are potent but non-specific enzyme poisons. Dimercaprol is a compound used in the treatment of mercury intoxication, however with low therapeutic efficacy. It is assumed that Dimercaprol acts by reactivating target sulfhydryl-containing proteins. The inhibitory effect of mercuric chloride treatment (3 days with 2.3 or 4.6 mg/kg $HgCl_2$, sc) in mice on cerebral, renal and hepatic σ-aminolevulinate dehydratase (ALA-D) activity, and a possible reversal of the effect of mercury by Dimercaprol (0.25 mmol/kg, 24 hr after the last mercury injection). Mercuric chloride did not inhibit cerebral ALA-D at the doses injected [1]. Dimercaprol calcium edetate pencil- amine, prednisone EDTA. (monocalcium ethylene demine tetra acetic acid) are the common chelating agents which may be used in treating cases of acute and chronic heavy metal poisoning. These heavy metal antagonists (chelating agents) prevent or reverse toxic effects and enhance the excretion of the metals. In lead poisoned children receiving 5 day courses of 1000 mg of Calcium disodium EDTA per square meter of surface area per day, given intramuscularly both blood lead and Plasma zinc concentrations were reduced rapidly [2]. Studies with the antidote 2, 3-dimer captopropane-1-sulphonate indicated that treatment of lead poisoned children with this agent results in increased urinary loss of lead

and a decline of lead in blood [3]. Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals. Chelating agents are capable of binding to toxic metal ions to form complex structures which are easily excreted from the body removing them from intracellular or extracellular spaces. 2,3-Dimercaprol has long been the mainstay of chelation therapy for lead or arsenic poisoning, however its serious side effects have led researchers to develop less toxic analogues [4]. Although dimercaprol is contraindicated in organic mercury exposures, meso-2,3-dimercaptosuccinic acid and sodium 2,3-dimercapto-1 -propanesulfonate may be used to chelate alt species of mercury. Recent evidence suggests that their efficacy in organic mercury poisoning is uncertain [5]. Dimercaprol, calcium disodium edetate, 1-10-phenantroline and 2,2′-dipyridyl were injected intraperitoneally in adult albino rats in doses ranging from 10 to 1500 mg per kg body weight. Ten animals were injected intracerebrally. At various survival times (2 minutes to several hours) their effects on the staining pattern of heavy metals as revealed by the sulfide silver method of Timm were determined. EDTA was virtually ineffective while dimercaprol, phenantroline and dipyridyl reduced the staining in many regions of the brain [6]. The primary site of mercury-induced injury is the kidney due to the uptake of Hg(2+)-conjugated organic anions in the proximal tubule, primarily across the organic anion transporter 1 (Oat1) at the basolateral membrane [7]. The ability of N-acetyl cysteine to enhance mercury excretion and its wide availability in clinical use indicate that it may be an ideal therapeutic agent against methylmercury poisoning [8]. It is thus clear that chelating agents like BAL serve as antidote by reducing the toxic effects of the heavy metals. It is, therefore, tempting to examine whether the effects of the toxic metals can be reversed or nullified if BAL is administered at the time of heavy metal exposure. It is all the more important to examine whether the antidotes can provide safety and protection to the fetus in viviparous systems. When the mother is exposed to heavy metals during the gestation period. Hence, an attempt is made here to investigate the impact of the antidote dimercaproI, on the toxic effects of mercury and lead on the biochemical constituents in the maternal tissues and the embryos during gestation period of the scorpion *H. fulvipes*.

MATERIALS AND METHODS

Four sets of the gravid females one set each month during October December February and April were isolated from the main stock. Each set was divided into five batches. One batch was administered a sub lethal dose of mercuric chloride and another batch received a sub lethal dose of lead acetate. The third batch of scorpions received a sub lethal dose of mercury as administered earlier to batch one along with dimercaprol 0.01 mg per gram body weight of scorpion. Batch four received the same quantity of dimercaprol along with the sub lethal dose of lead. Fifth batch of gravid females received distilled water and served as controls. The scorpions were sacrificed on the third day and the biochemical constituents like glycogen glucose, proteins TNPs and lipids, were determined in the maternal tissues and embryos in order to evaluate the antidote effect of dimercaprol on the impact of lead and mercury. The biochemical constituents, glycogen, glucose, proteins, TNPS and lipids were estimated.

Methods

Estimation of carbohydrates

Glucose and glycogen content of the hepatopancreas and glycogen content of the whole embryos were determined using the method of Kemp and Kits methods. Glucose content of the haemolymph was estimated using the Folin Malmros Micro procedure.

Estimation of glucose content in the hepatopancreas

50 mg of tissue was homogenized in 5 ml of 80% methanol and was centrifuged. To the supernatant, 10 mg of charcoal powder was added and the methanol was removed completely keeping the test tubes in warm water bath. To the residual aqueous solution, 10% TCA was added to bring the total volume to 5 ml and centrifuged sulphuric acid was added and the mixture was heated in a boiling water bath for exactly 6.50 minutes and subsequently cooled under running tap water. The colour developed was read at 520 mμ against a blank containing 2 ml of TCA and 6 ml of concentrated sulphuric acid.

Estimation of glycogen in hepatopancreas and pedipalpal muscle of maternal animal, and the embryos

To the tissue residue, remaining after the extraction of glucose with methanol, 5 ml of 10% TCA was added. Glycogen was extracted by heating the mixture at 100°C for 15 minutes. The solution was cooled under running tap water and the total volume was made upto 5 ml with 10% TCA to compensate for evaporation and then centrifuged. To 2 ml of the supernatant, 6 ml of concentrated sulphuric acid was added. The mixture was heated in a boiling water bath for 6.50 minutes and cooled. The colour developed was read at 520 mμ against a blank containing 2 ml of 10% TCA and 6 ml of concentrated sulphuric acid.

Estimation of glucose content in the haemolymph

To 0.1 ml of the haemolymph, 10 ml of 10% tungstic acid was added and centrifuged after 15 minutes at 4000 rpm for 5 minutes. To 1 ml of the supernatant, 1 ml of tungstic acid and 1 ml of Potassium fericyanide solution were added. The reaction mixture was kept in boiling water bath for 15 seconds and 1 ml of sodium cyanide buffer was added. The test tubes were covered with marbles and heated in boiling water bath for 15 minutes. The contents were cooled to 25-35°C and to the mixture; 2 ml of ferric dupanol reagent and 6 ml of distilled water were added. The colour was read at 640 mμ against a blank after 10 minutes. The carbohydrate contents were calculated from a standard graph using glucose as a standard. Monopan Electrical Balance (Owa Labor: USA) was used for weighing the maternal tissues and embryos, and colorimetric readings were taken.

Estimation of proteins

The tissue and haemolymph were homogenised in 5 ml of 5% TCA and centrifuged. The precipitate was dissolved in 1N sodiumhydroxide. To 0.2 ml of the protein solution, 5 ml of carbonate copper solution was added and allowed to stand for ten minutes. 0.5 ml of diluted Folin reagent (1N) was added to the above solution and after 30 minutes 0.D was read at 540 mμ. Protein contents of the samples were calculated from a standard graph plotted using Bovine serum albumin.

Estimation of TNPS

The tissues and haemolymph were homogenised in 5.0% TCA and centrifuged. To 0.30 ml of the supernatant, 1.5 ml of Ninhydrin reagent was added and the solution was kept in boiling water bath for 5.5 minutes. The reaction mixture was made upto 10 ml using glass distilled water. O.D. was read at 570 mμ. The TNPS of the samples was calculated from the standard graph using tyrosine. Colorimetric readings were taken using Bausch and Lomb Colorimeter (Spectronic-20). All the estimation was carried out between 9 AM and 12 PM to avoid possible influence of diurnal variation in *H. fulvipes*.

The tissues were homogenize in the chloroform methanol mixture (2:1 v/v) and were kept for 10 minutes. The reaction mixtures were filtered through a filter paper, prewashed with the solvent mixture into a glass stoppered test tube. The residues were re extracted twice with same solvent mixture and the pooled supernatants were washed with 0.20 ml of 0.90% sodium hydroxide by through mixing. The test tubes were stoppered and were kept in the refrigerator overnight. The mixture becomes biphasic containing lipids in the lower chloroform phase. The upper methanol phase containing the non – lipid contaminant is removed as completely as possible. The lower phase with the lipids was evaporated to dryness under vacuum. The dried residues were immediately treated with 3.0 ml of 2.0% potassium dichromate in 96% sulphuric acid and the reaction mixtures were kept in boiling water bah for 15 minutes. The contents were subsequently cooled under running tap water and 4.5 ml of distilled water was added carefully along the sides of the test tube and mixed thoroughly. The contents were once again cooled under running water and the intensity of the color developed was read at 580 mμ against a blank containing 3.0 ml of 2% chromic acid. Lipid contents of the samples were calculated from a standard graph plotted using cholesterol.

RESULTS

Effect of dimercaprol on the glycogen content of the maternal tissues and embryos of *H. fulvipes* exposed to mercury and lead during the gestation Period. As could be noted in Figures 1-3 and Table 1, administration of sub lethal doses of mercury and lead to the maternal animal lowered the glycogen content significantly in the hepatopancreas and the pedipa1pal muscle of the mother and the embryos throughout the gestation period Administration of the antidote, BAL did not bring a significant effect by way of reversal of the impact of the heavy metals in all cases during the gestation period, though indications are there.

Effect of dimercaprol on the glucose content of hepatopancreas and haemolymph of *H. fulvipes* exposed to mercury and lead during the gestation period. The glucose content of the hepatopancreas was depressed significantly by the single sublethal dose of both mercury and lead during different months (Figures 1-3).

Table 1: Levels of glycogen in heapatopancreas, pedipalpal muscle and embryo of *H. fulvipes* treated with mercury, mercury + dimercaprol, lead, lead + dimercaprol during different months of gestation N=8; b_p <0.01; c_p <0.001

	GLYCOGEN (ug/100 mg wet. Wt.)			
	HEPATOPANCREAS			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	140.40 ± 6.80	167.05 ± 5.52	170.92 ± 5.84	117.28 ± 1.09
MERCURY	129.91 ± 4.81b	145.82 ± 6.07c	142.63 ± 5.93c	83.33 ± 3.60c
Hg + DIMERCAPROL	138.87 ± 4.79a	146.15 ± 6.07*	143.26 ± 5.78*	84.037 ± 3.53*
LEAD	116.17 ± 4.73c	135.99 ± 3.36c	135.90 ± 3.38c	90.62 ± 3.42c
LEAD + DIMERCAPROL	116.56 ± 4.74*	134.02 ± 3.90*	140.65 ± 5.06a	91.02 ± 3.35*
	GLYCOGEN (ug/100 mg wet. Wt.)			
	PEDIPALPAL MUSCLE			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	134.48 ± 19.62	156.66 ± 14.47	156.6 ± 7.67	138.08 ± 21.5
MERCURY	110.18 ± 10.96b	108.33 ± 9.62c	112.03 ± 18.48c	79.62 ± 17.17c
Hg + DIMERCAPROL	113.30 ± 11.07*	109.71 ± 9.08*	112.25 ± 18.66*	80.87 ± 18.61*
LEAD	101.84 ± 17.47b	109.25 ± 9.96c	101.84 ± 17.47c	72.18 ± 14.17c
LEAD + DIMERCAPROL	113.02 ± 17.66a	109.40 ± 9.98*	103.14 ± 17.53*	72.43 ± 14.19*
	GLYCOGEN (ug/100 mg wet. Wt.)			
	EMBRYO			
TREATMENT	OCTOBER	DECEMBER	FEBRUARY	APRIL
CONTROL	1.33 ± 0.03	2.72 ± 0.08	5.72 ± 0.17	42.03 ± 0.43
MERCURY	1.23 ± 0.03c	2.35 ± 0.12c	4.96 ± 0.28c	40.16 ± 0.81c
Hg + DIMERCAPROL	1.24 ± 0.04*	2.38 ± 0.11*	4.97 ± 0.31*	40.25 ± 0.83*
LEAD	1.21 ± 0.03c	2.35 ± 0.11c	4.90 ± 0.29c	38.67 ± 0.65c
LEAD + DIMERCAPROL	1.22 ± 0.03*	2.44 ± 0.15*	4.81 ± 0.36*	38.83 ± 0.61*

Note: * Insignificant

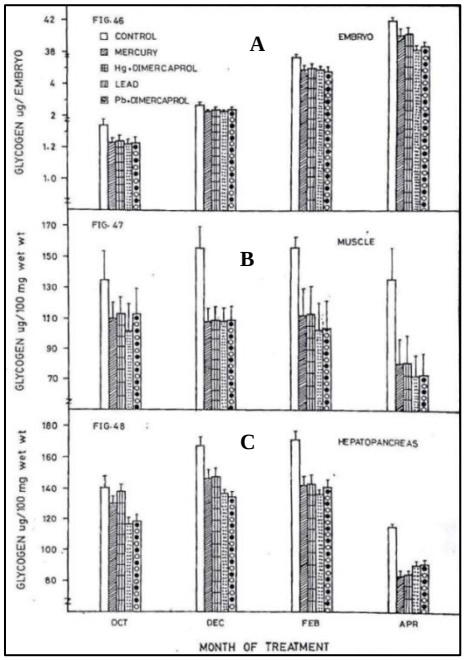

Figure 1: Glycogen levels vs. month of treatment (a-embryo; b-muscle; c-hepatopancreas)

Effect of dimercaprol on the glycogen content of the embryos (Figure 1) pedi pal pal muscle (Figure 2) and hepatopancreas (Figure 3) of *H. fulvipes* exposed to mercury and lead during different months of gestation. Gestation period administration of dimercaproI along with the same dose of mercury or lead did not significantly alter the glucose levels on the third day of administration of BAL by way of nullifying the effect of the heavy metals (Table 2 and Figure 3).

Table 2: Levels of glucose in heapatopancreas and heamolymph of *H. fulvipes* treated with, mercury, mercury + dimercaprol, lead, Lead + dimercaprol during different months of gestation N = 8. cp <0.001

	Glucose (mg/100 mg wet weight)			
	HEAPATOPENCREAS			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	1.32 ± 1.15	74.80 ± 2.81	60.17 ± 3.66	75.44 ± 2.88
MERCURY	116.42 ± 2.34C	61.60 ± 3.32C	49.07 ± 3.19C	56.33 ± 2.11C
Hg + DIMERCAPROL	116.95 ± 2.40*	62.18 ± 3.18*	49.14 ± 1.13*	56.73 ± 2.05*
LEAD	112.51 ± 2.09C	59.81 ± 3.74C	48.45 ± 1.76C	54.76 ± 2.10C
LEAD + DIMERCAPROL	112.48 ± 1.92*	65.51 ± 3.76*	48.77 ±1.82*	55.25 ± 2.14*
	Glucose (mg/100 mg wet weight)			
	HEAMOLYMPH			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	14.02 ± 0.49	12.37 ± 0.57	12.72 ± 0.57	13.05 ± 0.55
MERCURY	15.91 ± 0.57C	14.56 ± 0.56C	15.23 ± 0.73C	14.93 ± 0.74C
Hg + DIMERCAPROL	13.83 ± 0.51C	12.26 ± 0.55C	12.62 ± 0.57C	13.05 ± 0.54C
LEAD	16.18 ± 0.71C	14.93 ± 0.60C	15.51 ± 0.72C	15.40 ± 0.75C
LEAD + DIMERCAPROL	13.89 ± 0.51C	12.30 ± 0.56C	12.71 ± 0.48C	13.02 ± 0.64C

Note: * Insignificant

Glucose levels of haemolymph were elevated by both mercury and lead administered at different months of gestation. Dimercaprol administered along with mercury or lead significantly reversed the xhypoglycemic effect of the heavy metals and brought back the glucose levels to the control levels (Table 2 and Figure 2) as evidenced by the glucose levels determined on third day. Effect of dimercaprol on the TNPS content of maternal tissues and embryos of *H fulvipes* exposed to mercury and lead during the gestation period. The total Ninhydrin Positive substances of the hepatopancreas haemolymph and maternal tissues pedipalpal muscle were elevated by the sub lethal dose of mercury administered in the present study at all times during the gestation period (Figure 2).

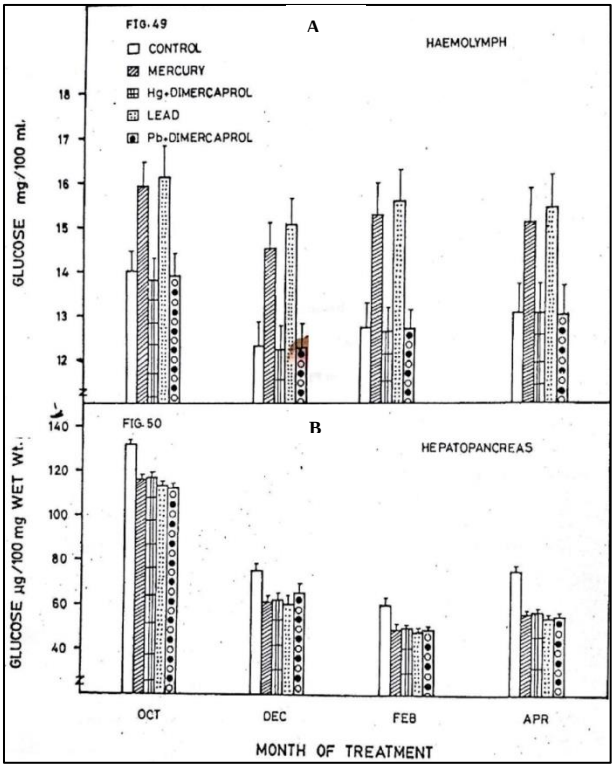

Figure 2: Glucose levels vs. month of treatment (a- haemolymph; b- hepatopancreas)

Effect of dimercaprol on the glucose content of the haemolymph (Figure 2) and glucose content lymph of hepatopancreas of *H. fulvipes* exposed to mercury and lead during different months of gestation. Dimercaprol along with mercury has reversed this effect by lowering the TNPS level (Table 3 and Figure 3).

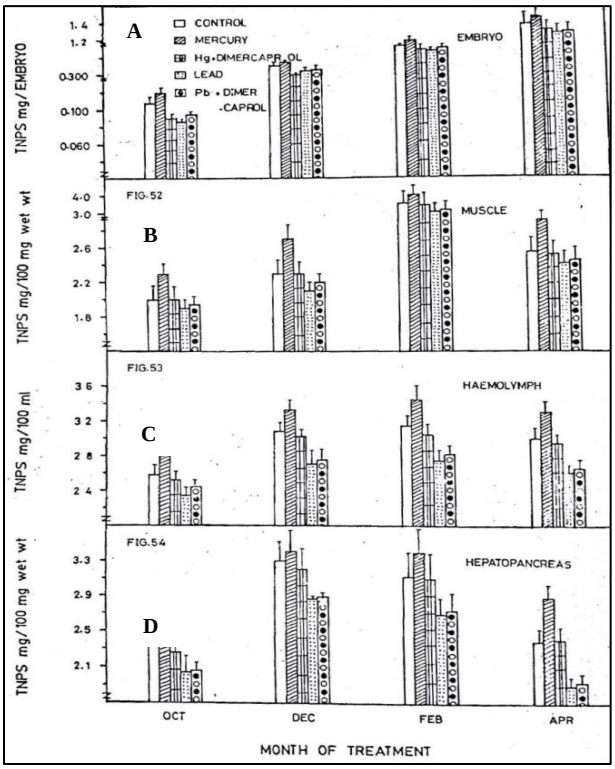

Figure 3: TNPS vs. month of treatment (a – embryo; b - muscle; c - haemolymph; d – hepatopancreas)

Administration of sub lethal doses of mercury to the maternal animal during different stages of gestation elevated the TNPS level of the embryos similar to the response noticed in other maternal tissues. Lead on the contrary, had an opposite effect. Application of BAL along with the sub lethal doses of heavy metals resulted in an antidote effect in the embryos by reversing the effects of mercury and lead as was noticed in the maternal tissues (Table 3). The sub lethal dose of lead on the contrary, depressed the TNPS content in the three tissues of maternal animal during the different months of the gestation period. Administration of BAL together with sub lethal dose of lead tended to elevate the TNPS level, though insignificant in all maternal tissues indicating an antidote effect (Table 4 and Figure 3). Effect of dimercaprol on the TNPS content of the embryo pedipapal muscle, haemolymph gestation. Effect of dimercaprol on the protein content of maternal tissues and embryos of *H. fulvipes* exposed to mercury and lead during gestation period. The depressant effect of mercury and lead on the protein content of maternal tissues (hepatopancreas, haemo lymph and muscle) and embryos is clearly indicated in the Figure 4 and Table 4.

Table 3: Levels on TNPS in heapatopancreas, pedipalpal muscle, heamolymph and embryo of *H. fulvipes* treated with mercury, mercury + dimercaprol, lead, lead + dimercaprol during different months of gestation N=8. ap <0.05; bp <0.01; cp <0.001

	TNPS (mg/100 mg wet weight)			
	HEAPATOPENCREAS			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	2.32 ± 0.13	3.30 ± 0.22	3.18 ± 0.28	2.40 ± 0.14
MERCURY	2.55 ± 0.13b	3.42 ± 0.13b	3.40 ± 0.28b	2.94 ± 0.15b
Hg + DIMERCAPROL	2.26 ± 0.12c	3.21 ± 0.22a	3.14 ± 0.28*	2.43 ± 0.14c
LEAD	2.05 ± 0.19b	2.85 ± 0.10c	2.72 ± 0.19c	1.92 ± 0.10c
LEAD + DIMERCAPROL	2.07 ± 0.19*	2.90 ± 0.10*	2.73 ± 0.19*	1.96 ± 0.11*
	TNPS (mg/100 mg wet weight)			
	PEDIPALPAL MUSCLE			
TREATMENT	OCTOBER	DECEMBER	FEBRUARY	APRIL
CONTROL	2.08 ± 0.16	2.32 ± 0.17	3.52 ± 0.15	2.57 ± 0.17
MERCURY	2.32 ± 0.13b	2.71 ± 0.16c	4.09 ± 0.11c	2.90 ± 0.10c
Hg + DIMERCAPROL	2.04 ± 0.16b	2.29 ± 0.15a	3.49 ± 0.14c	2.51 ± 0.17c
LEAD	1.93 ± 0.10a	2.16 ± 0.10a	3.06 ± 0.10c	2.40 ± 0.14a
LEAD + DIMERCAPROL	1.95 ± 0.10*	2.20 ± -0.10*	3.10 ± 0.10*	2.45 ± 0.14*

	TNPS (mg/100 mg wet weight)			
	HEAMOLYMPTH			
TREATMENT	OCTOBER	DECEMBER	FEBRUARY	APRIL
CONTROL	25.99 ± 1.24	30.90 ± 1.02	31.72 ± 1.29	30.17 ± 1.14
MERCURY	28.72 ± 1.21c	33.25 ± 1.19c	34.79 ± 1.65a	33.11 ± 1.24c
Hg + DIMERCAPROL	25.21 ± 1.13c	30.28 ± 0.88c	30.78 ± 1.21c	30.00 ± 1.09c
LEAD	23.64 ± 1.01c	27.16 ± 1.58c	28.00 ± 1.15c	26.14 ± 1.14c
LEAD + DIMERCAPROL	24.41 ± 0.96*	27.63 ± 1.47*	28.33 ± 1.13*	27.00 ± 1.08*
	TNPS (mg/100 mg wet weight)			
	EMBRYO			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	0.11 ± 0.008	0.40 ± 0.03	0.60 ± 0.01	1.36 ± 0.11
MERCURY	0.120 ± 0.006c	0.44 ± 0.02b	1.20 ± 0.02c	1.44 ± 0.12*
Hg + DIMERCAPROL	0.090 ± 0.006c	0.30 ± 0.02c	0.55 ± 0.01c	1.31 ± 0.10a
LEAD	0.081 ± 0.004c	0.34 ± 0.03c	0.54 ± 0.04b	1.27 ± 0.11*
LEAD + DIMERCAPROL	0.091 ± 0.004a	0.38 ± 0.03a	0.59 ± 0.04*	1.29 ± 0.11*

Note: * Insignificant

Table 4: Levels of proteins in heapatopancreas, pedipalpal muscle, heamolymph and embryo of *H. fulvipes* treated with mercury, mercury + dimercaprol, lead, lead + dimercaprol during different months of gestation n=8; ap <0.05; bp <0.01; cp <0.001

	Protein (mg/100 mg wet weight)			
	HEAPATOPANCREAS			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	18.37 ± 0.50	16.41 ± 0.36	14.72 ± 0.35	17.52 ± 0.46
MERCURY	14.20 ± 0.56c	14.46 ± 0.71c	13.00 ± 0.54c	16.01 ± 0.46c
Hg + DIMERCAPROL	14.40 ± 0.56*	14.70 ± 0.71*	13.10 ± 0.56*	16.15 ± 0.47*
LEAD	13.32 ± 0.68c	13.68 ± 0.75c	11.78 ± 0.62c	15.78 ± 0.75c
LEAD + DIMERCAPROL	13.56 ± 0.66*	13.80 ± 0.73*	12.00 ± 0.67*	15.90 ± 0.76*
	Protein (mg/100 mg wet weight)			
	PEDIPALPAL MUSCLE			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	12.55 ± 0.66	12.00 ± 0.61	13.27 ± 0.47	13.61 ± 0.45
MERCURY	11.36 ± 0.82a	10.52 ± 0.47c	11.45 ± 0.54c	12.20 ± 0.48c
Hg + DIMERCAPROL	11.70 ± 0.81*	10.73 ± 0.44*	11.65 ± 0.58*	12.37 ± 0.48*
LEAD	11.23 ± 0.65c	10.10 ± 0.40c	11.25 ± 0.61c	12.01 ± 0.42c
LEAD + DIMERCAPROL	11.52 ± 0.62*	10.65 ± 0.48b	11.40 ± 0.61*	12.11 ± 0.38*
	Protein (mg/100 mg wet weight)			
	HEAMOLYMPH			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	8.31 ± 0.21	7.96 ± 0.24	5.04 ± 0.26	3.90 ± 0.18
MERCURY	7.30 ± 0.26c	6.64 ± 0.31c	4.54 ± 0.21c	3.61 ± 0.23b
Hg + DIMERCAPROL	7.35 ± 0.25*	6.65 ± 0.31*	4.54 ± 0.21*	3.66 ± 0.23*
LEAD	7.12 ± 0.29c	6.54 ± 0.27c	4.47 ± 0.22c	3.59 ± 0.22b
LEAD + DIMERCAPROL	7.12 ± 0.28*	6.55 ± 0.27*	4.49 ± 0.21*	3.36 ± 0.25*
	Protein (mg/100 mg wet weight)			
	EMBRYO			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	0.06 ± 0.01	0.11 ± 0.04	0.31 ± 0.09	2.33 ± 0.62
MERCURY	0.06 ± 0.01*	0.11 ± 0.20*	0.27 ± 0.09*	2.13 ± 0.64a
Hg + DIMERCAPROL	0.06 ± 0.01*	0.11 ± 0.01*	0.27 ± 0.09*	2.14 ± 0.64a
LEAD	0.06 ± 0.01*	0.12 ± 0.02a	0.26 ± 0.09*	1.99 ± 0.50a
LEAD + DIMERCAPROL	0.06 ± 0.01*	0.11 ± 0.01*	0.26 ± 0.09*	1.94 ± 0.51*

Note: * Insignificant

Estimation of proteins on the third day after the administration of BAL simultaneously with the heavy metals, showed no statistically significant effect in embryos and the materna1 tissues though indications for the detoxifying effect of the antidote do exist. Effect of dimercapro1 on the lipid content of maternal tissues and the embryos of H. fulvi pes exposed to mercury and lead during gestation period. When the maternal animal was exposed to sub lethal doses of mercury or lead the lipid content was depressed on the third day of administration in the hepatopancreas

muscle, haemolymph and embryos. When the antidote, BAL was administered along with the sub lethal effect of dimercaprol on the protein content of the embryo, pedipal muscle, haemolymph and hepatopancreas (Figure 4) of *H. fulvipes* exposed to mercury and lead during different months of gestation. A dose of heavy metals to the maternal animal, the antidote effect was indicated in all the tissues with the trends showing elevation of lipids, reversing the depressant action of the heavy metals (Table 5 and Figure 5).

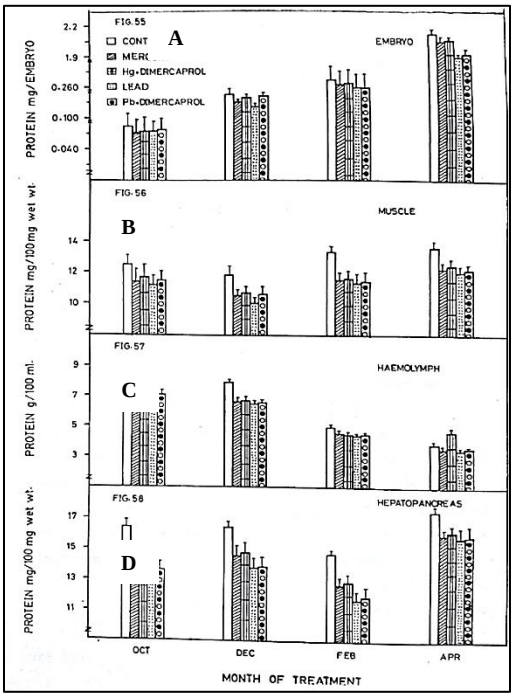

Figure 4: Protein vs. month of treatment (a – embryo; b - muscle; c - haemolymph; d – hepatopancreas)

Table 5: Levels of lipid in hepatopancreas, pedipalpal muscle, heamolymph and embryo of *Hiatus fulvipes* with mercury, mercury + dimercaprol, lead, lead + dimercaprol during different months of gestation; n=8. ap <0.05; bp <0.01; cp <0.001

	LIPID (mg/100 mg wet weight)			
	HEAPATOPANCREAS			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	28.52 ± 0.95	25.01 ± 0.72	22.00 ± 0.62	18.19 ± 0.46
MERCURY	27.16 ± 0.98b	22.69 ± 0.81c	19.17 ± 0.53c	16.59 ± 0.32c
Hg + DIMERCAPROL	27.45 ± 0.83*	24.50 ± 0.86a	21.03 ± 0.43c	17.52 ± 0.34b
LEAD	24.87 ± 0.80c	24.00 ± 0.84c	18.01 ± 0.51c	15.62 ± 0.65c
LEAD + DIMERCAPROL	25.15 ± 0.85*	24.60 ± 0.98*	18.29 ± 0.40*	17.64 ± 0.29c
	LIPID (mg/100 mg wet weight)			
	PEDIPALPAL MUSCLE			
TREATMENT	OCTOBER	DECEMBER	FEBRUARY	APRIL
CONTROL	1.33 ± 0.55	1.25 ± 0.02	1.22 ± 0.03	1.08 ± 0.04
MERCURY	1.23 ± 0.07b	1.13 ± 0.04c	1.12 ± 0.04c	1.02 ± 0.05b
Hg + DIMERCAPROL	1.31 ± 0.07a	1.20 ± 0.05*	1.19 ± 0.03b	1.04 ± 0.04*
LEAD	1.21 ± 0.07b	1.12 ± 0.03c	1.18 ± 0.02a	0.97 ± 0.05b
LEAD + DIMERCAPROL	1.28 ± 0.07*	1.16 ± 0.03a	1.20 ± 0.03*	1.05 ± 0.03b
	LIPID (mg/100 mg wet weight)			
	HEAMOLYMPH			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	421.00 ± 12.52	418.37 ± 10.71	385.62 ± 10.43	366.25 ± 8.11
MERCURY	373.12 ± 7.59c	370.12 ± 8.65c	352.50 ± 10.26c	315.62 ± 5.18c
Hg + DIMERCAPROL	383.12 ± 8.19a	376.25 ± 7.00*	368.75 ± 8.20b	326.00 ± 6.54c
LEAD	358.75 ± 8.13c	348.12 ± 6.81c	335.62 ± 8.50c	303.00 ± 4.24c

LEAD + DIMERCAPROL	370.00 ± 6.49b	358.75 ± 5.84b	350.00 ± 8.16b	310.62 ± 4.79b
	LIPID (mg/100 mg wet weight)			
	EMBRYO			
TREATMENT	**OCTOBER**	**DECEMBER**	**FEBRUARY**	**APRIL**
CONTROL	0.11 ± 0.007	0.27 ± 0.01	2.64 ± 0.03	3.26 ± 0.09
MERCURY	0.012 ± 0.004a	0.24 ± 0.01c	2.31 ± 0.04c	2.95 ± 0.12c
Hg + DIMERCAPROL	0.014 ± 0.004*	0.25 ± 0.01*	2.35 ± 0.05*	3.01 ± 0.11*
LEAD	0.013 ± 0.006b	0.23 ± 0.01c	2.09 ± 0.03c	2.84 ± 0.13c
LEAD + DIMERCAPROL	0.013 ± 0.07*	0.24 ± 0.01*	2.10 ± 0.03*	2.86 ± 0.13*

Note: * Insignificant

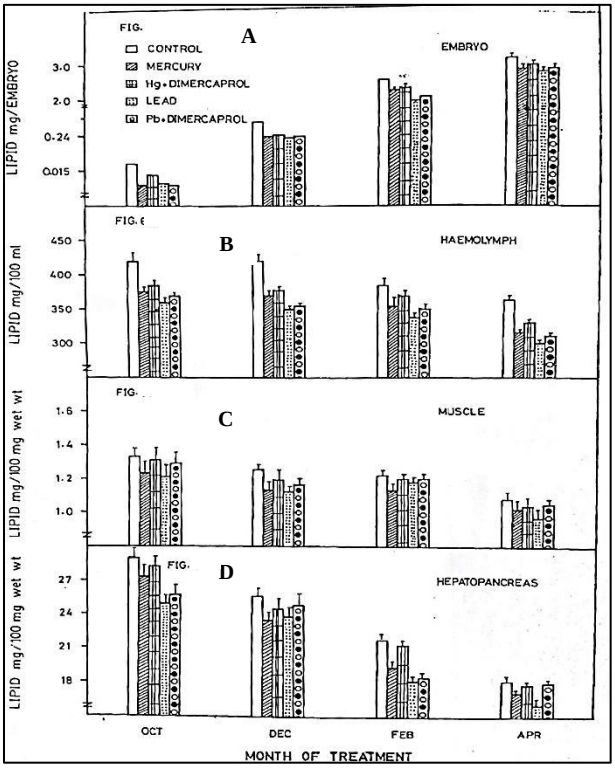

Figure 5: Protein vs. month of treatment (a – embryo; b - muscle; c - haemolymph; d – hepatopancreas)

DISCUSSION AND CONCLUSION

The results obtained in the present study reveal that both mercury and lead administered to the maternal animal, *H. fulvipes* in sub lethal doses bring about marked changes in the biochemical constituents in the different tissues of mother, and the embryos throughout the gestation period Glycogen, proteins and lipids were depressed in all the tissues by both the metals. TNPS was elevated only by mercury and not by lead. While these biochemical changes induced by lead and mercury could be viewed as a consequence of toxic manifestations of the heavy metals, reversal of these effects and restoration of control levels by any agents can be deemed an effective antidote action. Dimercaprol, administered to the maternal animal along with the heavy metals brought about complete reversal to the control levels, of glucose and TNPS, while the trends of recovery were indicated with reference to other constituents in other tissues thus exerting an antidotal action. The efficacy of chelation therapy in the very young can differ significantly from that in adults [9]. Chelation therapy is the preferred medical treatment for reducing the toxic effects of metals. Chelating agents are capable of binding to toxic metal ions to form complex structures which are easily excreted from the body removing them from intracellular or extracellular spaces. 2,3-Dimercaprol has long been the mainstay of chelation therapy for lead or arsenic poisoning [10]. Historical adverse outcomes with chelators, lessons learned in the art of using them, and successes using chelation to ameliorate renal, cardiovascular, and neurological conditions highlight the need for renewed attention to simple, safe, inexpensive interventions that offer potential to stem the tide of debilitating, expensive chronic disease [11].

Pharmacological investigation revealed that dimercaprol (British anti lewisite) would provide protection against the toxic effects of heavy metals. Dimercaprol has been proved to be such more effective when given as soon as possible after exposure to metal [12]. Dimercaprol, injected along with heavy metals in the present study exerted a clear cut effect by way of reversal of the toxic effects of the heavy metals with reference to glucose and TNPS and indicated biochemical reversal with reference to other constituents both in maternal tissues and embryos. It could hence be suggested that dimercaprol can be used to revert the biochemical lesions induced by heavy metals and achieve safety not only to the maternal animal but also to the fetus during gestation period; sulfhydral groups have a very high affinity for mercury lead and other heavy metals. Almost all proteins contain sulfhydryI groups that are metal reactive. Hence, every protein in the body is a Potential target Heavy metals are thus potent but nonspecific enzyme poisons.

REFERENCES

[1] T Emanuelli; JB Rocha; ME Pereira; LO Porciuncula; VM Morsch; AF Martins; DO Souza. *Basic Clin Pharmacol Toxicol*. **1996**, 79(3), 136-143.
[2] DJ Thomas; J Chisolm. *J Pharmacol Exp Ther*. **1986**, 239(3), 829-835.
[3] JJ Chisolm; DJ Thomas. *J Pharmacol Exp Ther*. **1985**, 235(3), 665-669.
[4] SJ Flora; V Pachauri. *Int J Environ Res Public Health*. **2010**, 7(7), 2745-2788.
[5] CR Baum. *Curr Opin Pediatr*. **1999**, 11(3), 265-268.
[6] K Fredens; G Danscher. *Histochem Cell Biol*. **1973**, 37(4), 321-331.
[7] H María; MS Trebucobich; TR Stoyanoff; AA Chevalier; AM Torres. *Toxicol Res*. **2015**, 4(5), 1324.
[8] D Joshi; DK Mittal, S Shukla; AK Srivastav; SK Srivastav. *Cell Biochem*. **2014**, 32(4), 351-360.
[9] M Blanusa; VM Varnai; M Piasek; K Kostial. *Curr Med Chem*. **2005**, 12(23), 2771-2794.
[10] SJ Flora; V Pachauri. *Int J Environ Res Public Health*. **2010**, 7(7), 2745-2788.
[11] ME Sears. *Sci World J*. **2013**, 2013, 219840.
[12] BR Santhanakrishnan; TP Bai. *Indian Pediatr*. **1978**, 15(6), 469-472.