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RESEARCH ARTICLE

STUDY OF THE EFFECTS OF MERCURY AND LEAD ON CARBOHYDRATE METABOLISM OF MATERNAL AND EMBRYONIC TISSUES USING AN ALTERNATE ANIMAL MODEL: *HETEROMETROUSFULVIPES*

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ABSTRACT

Heavy metal exposure in animals can lead to profound effect on growth, development and biochemical constituents. It is necessary that the 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 effect of chronic heavy metals exposure. Chronic heavy metal exposure resulted in decrease in biochemical constituents of carbohydrates, proteins and lipids. There was also significant decrease in hepato-pancreatic weight, hepato -somatic index and embryonic length and weight in these animals.

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INTRODUCTION

Heavy metals are naturally occurring inorganic elements which are present in very small amounts in the living tissues but are important for the vital processes of life (Kazi *et al*., 2008). Some metals (e.g. magnesium) are known as macro-metals and are found in high amount in the body tissues, therefore they are also called macronutrients (Simsek, 2007). Mercury (Hg), Chromium (Cr), Nickel (Ni) and Zinc (Zn) are most naturally occurring whereas lead (Pb), Cadmium (Cd), Copper (Cu) and Arsenic (As) are the direct consequence of human environmental pollution (Chen *et al*., 2016). Heavy metals are involved in a range of physiological processes such as prosthetic groups of many proteins, water balance, cofactors of many enzymes etc. (Fraga, 2005). Heavy metals are known to affect the reproduction and development of organism. They are also believed to have an impact in the biochemical constituents and bring about the decrease in carbohydrate, proteins and lipids.

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They are linked with the many visible changes in the animal models that can range from the decrease in hepato-pancreatic weight to hepato-somatic index (Rao, 2017). The exposure of heavy metals, in particular Pb, Cd, As, and methylmercury (MeHg) directly interfere with brain development and results in cognitive impairment. Several studies have reported that the imbalance of some essential metals might adversely affect pancreatic islet and cause development of diabetes (Chen, 2009). The exact mechanism of their toxicity is still unknown but their synergistic effect is well defined (Karri, 2016). The human health risk of heavy metals exposure is a public health problem. In a more recent study, investigators of environmental mercury exposure found that for every 1,000 pounds of mercury release, there is a 3.7% increase in autism rates of school age children living near coal fired power plants (Palmer *et al*., 2008). Severe zinc deficiency, allow copper to reach toxic levels in membranes leading to lipid peroxidation and cell damage (Geier, 2007). Uncontrolled pollution and industrialization might be a potential source to expose human population against toxic metals such as lead (Pb), nickel (Ni), cadmium (Cd) and arsenic (As). Some of the toxic metals are implicated to disrupt the glucose uptake and alter the related

molecular mechanism in glucose regulation (Kazi *et al*., 2009; Serdar *et al*., 2009). In case of Zn deficiency and increased exposure to toxic metals such as lead (Pb), body will use Pb instead of Zn (Duruibe, 2007). In fish they results in the production of lipid radicals with lipid degradation (Plaskett, 2005). It is very common for metal concentrations in the hepatopancreas to be higher than those in other tissues because of the heavy involvement of this organ in detoxification. Carbohydrates are considered to be the first among the organic nutrients to be depleted and degraded in response to stress conditions imposed on animals. Literature reveals that exposure of animals to heavy metals affects the carbohydrate metabolism. Exposure of *Channa punctatus* to chromium resulted in hyperglycemia and hyperlactemia (Sastry, 1984). Kulkarni and Utkar studying the effect of copper sulphate on biochemical composition of *Viviparous bengalensis*, found marked decrease in haemolymph glucose and tissue glycogen levels (Kulkarni, 1983). *Rana cynophlictis*, exposed to mercury and cadmium exhibited alterations of glycogen content in the tissues (Dudgall, 1986). Mercury and cadmium produced marked depletion in rainbow trout, of glycogen in both liver and muscle (Lowe-Jinde, 1984). The freshwater mussel, Parreysiarugosa subjected to mercuric chloride revealed changes in the levels of glycogen (Ravindra Reddy, 1987). Mercury induced depletion of glycogen and elevated free sugars in the liver and muscle of fish, Sarotherodonmossambicus (Ramalingam, 1988).

Glycogen depletion by sub-lethal concentration of mercuric chloride in the liver muscle of *Channa punctatus* was explained in terms of reduction in gluconeogenesis. Sastry and Rao stated that exposure of fish to mercury resulted in impaired carbohydrates metabolism and there was a trend in favor of protein utilization (Sastry , 1982). A study of the effects of mercury and lead on biochemical constituents of maternal animal and the consequential effects on the developing embryos of *H.fulvipes* was, therefore, considered highly profitable. To begin with, changes in carbohydrates in maternal tissues and embryos induced by the heavy metal treatment were investigated by determining the glycogen and glucose contents of the maternal hepatopancreas, glucose content of the maternal hepatopancreas, glucose content of the maternal haemolymph and glycogen content of the whole embryos of control and treated animals.

MATERIALS AND METHODS

Sample groups were distributed based on the exposure to mercury and lead: group I (controls), group II (mercury), and group III (lead). Monthly samples were administered successively at the intervals of one month for studying the effects of mercury and lead on the carbohydrate metabolism on groups I, II and III. Carbohydrates (glycogen and glucose) were estimated in the maternal tissues (hepato-panceas, pedipalpal muscle and haemolymph), and the whole embryos in samples drawn from group I, II, and III. Samples drawn every month from August to April received one sub-lethal dose each month. Therefore, August month samples represent the effect of single dose; September samples two doses, and in the similar order samples taken on the month of April represented the effects of nine sub-lethal doses of the heavy metals.

Estimation of Carbohydrates

Estimation of glucose content in the hepatopancreas

50 mg of tissue was homogenized in 5 ml of 80% methanol, and 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 5ml and then it was added with centrifuged sulphuric acid. This mixture was heated in a boiling water bath for exactly 6.50 minutes and subsequently cooled under running tap water. The color developed was read at 520 ml 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

5 ml of 10% TCA was added to the residual tissue, remained after the extraction of glucose with methanol. Glycogen was extracted by heating the mixture at100 degree Celsius for 15 minutes. The solution was then cooled under running tap water and the total volume was estimated up to 5 ml with 10% TCA to compensate for evaporation and then centrifuged again. To this 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 color developed was read at 520 ml 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 this 1 ml of the supernatant, 1 ml of tungstic acid and 1ml of Potassium ferricyanide solution were added again. 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 degree Celcius and to the mixture;2 ml of ferric dupanol reagent and 6 ml of saline were added. The color was read at 640 ml against a blank after 10 minutes. The carbohydrate contents were calculated from a standard graph using glucose as a standard.

RESULTS

Effect of mercury and lead on the glycogen and glucose content of hepatopancreas of *H.fulvipes* during the gestation period

The effect of the mercury and lead had a clear cut depressant action on the glycogen and glucose content of hepato-pancrease of *H. fulvives* during the gestation period (Tables 1 and 2, Fig. 18 and 19). The glycogen content of hepatopancreas was depleted in direct proportion to the number of doses of both mercury and lead throughout the gestation period. Maximum depletion in animals treated up to April being 21.73% for mercury and 28.03% for lead. Similar effects, leading to depletion of glucose content of hepatopancreas proportional to the number of doses, were observed. While a single dose of mercury and lead depleted glucose by 5.46% and 9.48% (statistically not significant) respectively, nine doses of mercury and lead produced highly significant reduction to the tune of 25.55% for the former and 27.63% for the later.

Effects on the glucose content of the maternal haemolymph

Hyperglycemic effect of the heavy metals, mercury and lead is indicated in Table 3 and Fig.20.

Table 1: Effect of mercury (Hg) and lead (pb) on the glycogen content of the hepatopancreas of *H.fulvipes* during the gestation period

MONTH OF TREATMENT	Glycogen (µg/100 mg wet wt.)		
	CONTROL	EXPERIMENTAL	PERCENT depletion
AUG		Hg 120.83±3.29 * (8)	4.96
	127.14±4.97 (7)	Pb 110.30±2.50b (8)	13.24
SEP		Hg 126.00±4.01a (8)	7.65
	136.45±3.90 (7)	Pb 115.65±2.50c (8)	15.24
OCT	140.38±6.80 (7)	Hg 129.914.81±4.81* (8)	7.45
		Pb 116.17±4.73b (8)	17.24
NOV	154.14±4.54 (7)	Hg 137.07±5.51a (8)	11.07
		Pb 120.83±3.21c (8)	21.61
DEC	167.06±5.55 (7)	Hg 145.82±6.07a (8)	12.71
		Pb 135.99±3.36c (8)	18.59
JAN	202.10±4.44 (7)	Hg 168.33±3.69a (8)	16.71
		Pb 160.01±5.29c (8)	20.82
FEB	170.80±5.86 (7)	Hg 142.63±5.93b (8)	16.49
		Pb 135.90±3.38c (8)	20.43
MAR	156.12±3.896 (7)	Hg 132.26±3.73c (8)	15.28
		Pb 120.83±3.29c (8)	22.60
APR.	115.79±2.67 (7)	Hg 083.33±3.60c (8)	21.73
		Pb 090.62±3.43c (8)	28.03

Values represent mean ± S.E. with Number of observations (N) given in parentheses.
a_p<0.05; b_p<0.01; c_p<0.001; * insignificant

Table 2: Effectof mercury (Hg) and lead (pb) on the glucose content of the Hepatopancreas of *H.fulvipes* during the gestation period.

MONTH OF TREATMENT	Glycogen (µg/100 mg wet wt.)		
	CONTROL	EXPERIMENTAL	PERCENT depletion
AUG	63.45±2.68	Hg 59.98±1.68*	5.46
		Pb 135. 29±2.37*	9.48
SEP		Hg 135.28±2.37b	9.19
	148.98±3.72	Pb 131.42±1.55c	11.78
OCT		Hg 116.42±2.34c	11.67
	131.81±2.68	Pb 112.51±2.09c	14.64
NOV		Hg 73.27±2.78b	13.56
	84.77±1.94	Pb 71.05±1.44c	16.18
DEC		Hg 61.60±3.32b	17.95
	75.085±2.84	Pb 59.81±3.74b	20.33
JAN		Hg 31.72±1.74b	18.60
	38.97±1.93	Pb 30.81±1.35b	20.94
FEB		Hg 49.07±3.19a	18.57
	60.26±3.67	Pb 48.42±1.05b	19.64
MAR		Hg 36.51±1.72c	25.64
	49.10±1.97	Pb 35.31±1.62c	28.08
APR.		Hg 56.33±2.11c	25.55
	75.67±2.86	Pb 54.76±2.10c	27.63

Values represent mean ± S.E. with number of observations (N) =8.
a_p<0.05; b_p<0.01; c_p<0.001; * insignificant

Table 3. Effect of mercury (Hg) and lead (pb) on the glucose content of the hemolymph of *H. fulvipes* during the gestation period

MONTH OF TREATMENT	Glucose (mg/100 ml.)		
	CONTROL	EXPERIMENTAL	PERCENT elevation
AUG	15.36±0.66	Hg 18.78±0.87b	22.26
		Pb 19.17±0.92b	24.80
SEP		Hg 18.32±0.89b	21.42
	15.50±0.69	Pb 19.43±0.86b	25.35
OCT		Hg 15.91±0.57a	13.25
	14.05±0.50	Pb 16.18±0.71a	15.16
		Hg 16.05±0.58b	20.13
NOV	13.36±0.60	Pb 15.50±0.73a	16.01
		Hg 14.56±0.56a	17.23
DEC	12.42±0.57	Pb 14.94±0.60b	20.21
		Hg 13.21±0.99a	19.22
JAN	11.08±0.63	Pb 13.56±0.98a	22.38
		Hg 15.23±0.73a	19.26
FEB	12.77±0.58	Pb 15.52±0.72b	21.53
		Hg 21.37±0.92a	13.24
MAR	18.87±0.88	Pb 22.27±0.80b	18.01
		Hg 14.93±0.74a	14.32
APR.	13.06±0.66	Pb 15.28±0.75a	16.99

Values represent mean ± S.E. With Number of observations (N) =8.
a_p<0.05;b_p<0.01 ; *insignificant

Table 4. Effect of mercury (Hg) and lead (pb) on the glycogen content of the pedipalpal muscle of *H. fulvipes* during the gestation period

MONTH OF TREATMENT	Glycogen (µg/100 mg wet wt.)		
	CONTROL	EXPERIMENTAL	PERCENT elevation
AUG	109.25±18.62	Hg 93.51±14.04*	14.40
		Pb 84.25±9.18*	22.87
SEP		Hg 93.51±12.27a	26.28
	126.85±6.58	Pb 91.66±15.70a	27.74
		Hg 110.18±10.96a	15.74
OCT	130.55±19.65	Pb 101.84±17.47a	21.99
		Hg 117.58±10.71a	23.02
NOV	152.77±12.41	Pb 124.99±20.04b	18.18
		Hg 108.33±9.62b	29.51
DEC	153.70±10.46	Pb 109.25±9.96b	28.91
		Hg 122.21±13.01a	24.57
JAN	162.02±11.85	Pb 123.14±24.79a	23.80
		Hg 112.03±18.48a	29.24
FEB	158.33±9.62	Pb 101.84±17.41b	35.67
		Hg 117.58±10.71b	33.15
MAR	175.91±13.64	Pb 116.63±35.78a	33.69
		Hg 79.62±17.17a	41.49
APR.	136.11±21.50	Pb 72.18±14.17a	46.96

Values represent mean ± S.E. with Number of observations (N) =9.
a_p<0.05;b_p<0.01 ; *insignificant

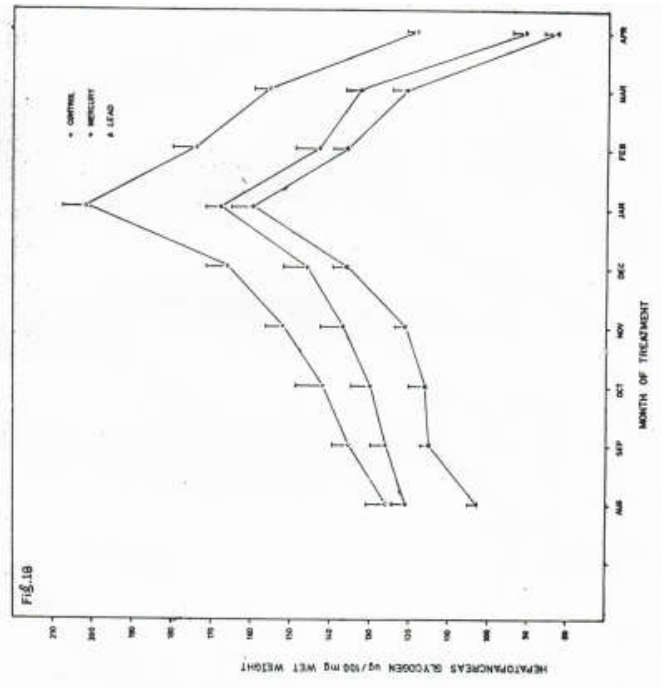
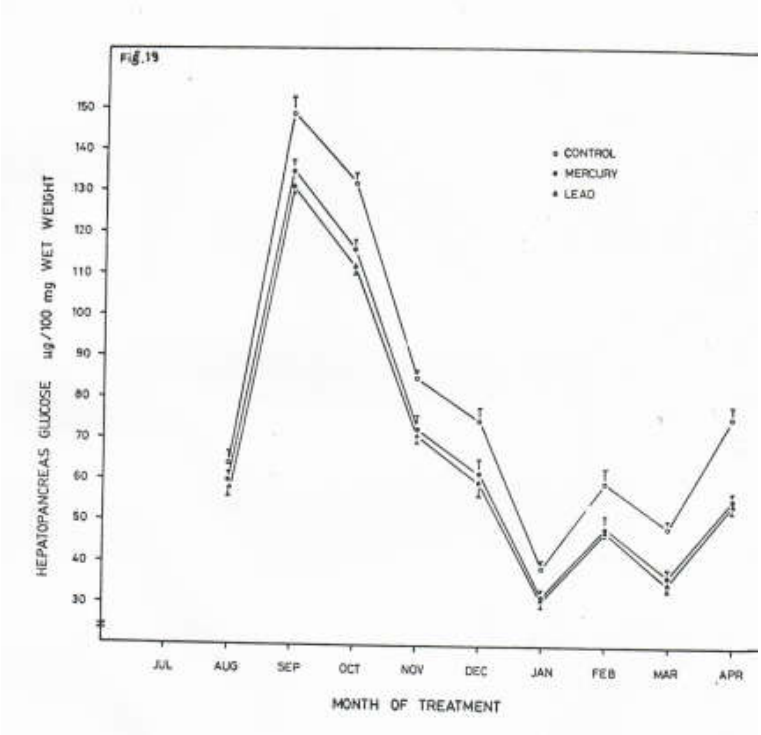
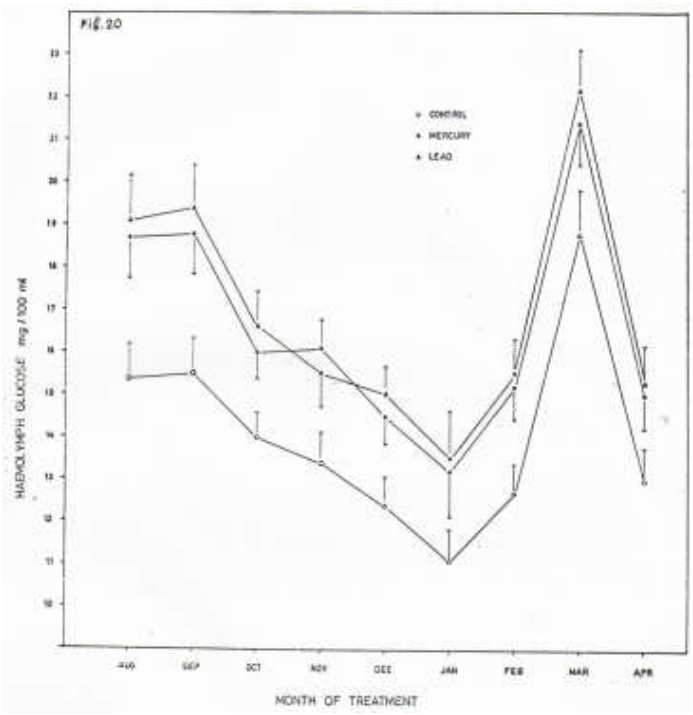


Table 5. Effect of maternal treatment with mercury (Hg) and lead (pb) on the glycogen content of the embryos of *H.fulvipes*during the gestation period

MONTH OF TREATMENT	Glycogen (µg/Embryo)		
	CONTROL	EXPERIMENTAL	PERCENT elevation
AUG	1.11±0.06	Hg 0.98±0.07*	11.17
		Pb 0.95±0.07*	14.14
SEP		Hg 1.03±0.08*	8.24
	1.12±0.06	Pb 1.01±0.08*	9.84
OCT		Hg 1.23±0.03a	9.55
	1.30±0.04	Pb 1.21±0.03a	10.44
NOV		Hg 1.25±0.03a	13.02
	1.44±0.08	Pb 1.24±0.04a	14.06
DEC		Hg 2.35±0.12*	15.12
	2.70±0.08	Pb 2.30±0.11*	16.89
JAN		Hg 2.87±0.15a	12.53
	3.28±0.12	Pb 2.78±0.11b	15.36
FEB		Hg 4.96±0.28a	13.80
	5.75±0.17	Pb 4.90±0.29a	14.81
MAR		Hg 7.93±0.24a	6.67
	8.49±0.30	Pb 7.79±0.24a	8.29
APR.		Hg 40.16±0.81a	4.54
	42.00±0.42	Pb 38.67±0.65c	8.08

Values represent mean ± S.E. with Number of observations (N) =8.
a_p<0.05;b_p<0.01; c_p<0.001; *insignificant





The elevation in haemolymph glucose levels did not reflect any dose dependent depletion of glycogen content of maternal tissues.

Effects on the muscle glycogen

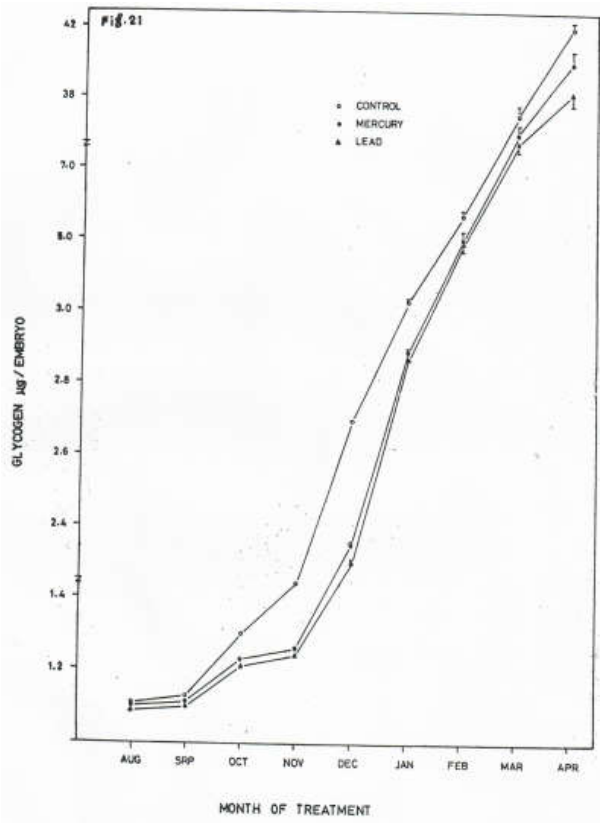
The glycogen content of the pedipalpal muscle of *H. fulvipes* depleted under the impact of the heavy metals, mercury and lead (Table 4). The dose dependent effect is obvious in the near consistent increase in the percent depletion with the increase in the number of doses of the metals administered.

Effect on the glycogen content of the embryos

The glycogen content of the embryos both in the control animals and experimental animals treated with mercury and lead showed a continuous increase with advancement in development from September to May, with a spurt during the last month of gestation. Exposure of the maternal animals to the heavy metals depleted the accumulation of glycogen in the embryos though the depletion was not always statistically significant (Table 5; Fig.21).

DISCUSSION

Major portion of energy requirements of animals is provided by carbohydrates and glycogen, as they are the readily available source of energy. Carbohydrates are often considered to be the first among organic nutrients to be depleted and degraded in response to stress conditions imposed on the animals. Carbohydrates are important metabolites that provide the animal with the energy required for performing different processes. It is obvious that heavy metals have a profound influence on carbohydrate metabolism of organisms.



Heavy metals exerting stress on animals influence carbohydrate metabolism and often deplete the carbohydrate reserve. In the present study, the depletion of glycogen in the hepatopancreas and the pedipalpal muscle of *H. fulvipes* in a dose and duration dependent fashion in response to sub-lethal concentrations of mercury and lead might be explained in terms of utilization of energy induced by the heavy metal stress. The possibility of the glycogen drain resulting in the depletion can also be a result of suppression ofgluconeogenesis known to be induced by metals. The facts that there is reduction in glucose parallel to glycogen in the hepatopancreas suggest either excessive utilization of glucose for energy release or quick drain into the haemolymph or both. Hyperglycemic condition induced by the toxicity of mercury and lead in *H. fulvipes* is a natural consequence of glycogenolysis in tissues. Enhanced utilization of carbohydrate for yielding energy is reflected by lack of dose dependent elevation in blood sugar level parallel to the tissue glycogen.

The impact of heavy metals on carbohydrate metabolism in the maternal tissues of *H. fulvipes as* assessed by the depletion of carbohydrates is reflected in the embryos with depletion of the glycogen content. If the same condition occurs in *H.fulvipes*under heavy metal stress, the depletion in carbohydrate content of maternal animals would affect the embryonic development during the gestation period as the embryos draw their nourishment from the mother from development. The failure of accumulation of normal level of carbohydrates in the embryos of heavy metal treated scorpions is perhaps indicative of the enhanced energy demands of the mother and/or embryonic tissues under stress conditions imposed by the sub lethal concentrations of both mercury and lead.The possible impairment of carbohydrate metabolism induced by sub-lethal doses of mercury and lead, when the maternal animals were exposed, might at least partially account for the gravimetric changes induced in the embryos by the heavy metals.

Conclusion

Heavy metals cause the depletion of glucose and glycogen in the hepatopancreas and the pedipalpal muscle of *H. fulvipes* in a dose and duration dependent fashion in response to sub-lethal concentrations of mercury and lead. There was also significant decrease in the glycogen and glucose possibly due to suppression of gluconeogenesis induced by these heavy metals. Whether the heavy metals similarly affect the other biochemical constituents like proteins and lipids contributing additively to the observed effects of mercury and lead on the embryonic development in *H. fulvipes* deserves to be investigated.

REFERENCES

Kazi, T.G., Afridi, H.I., Kazi, N., Jamali, M.K., Arain, M.B., Jalbani, N., Kandhro, G.A. 2008. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. *Biol Trace Elem Res*. 122(1):1–18. doi: 10.1007/s12011-007-8062-y. [PubMed] [Cross Ref] [Ref list]

Simsek, A., Aykut, O. 2007. Evaluation of the microelement profile of turkish hazelnut (corylusavellana L.) varieties for human nutrition and health. *Int J Food SciNutr*.; 58(8):677–688. doi: 10.1080/09637480701403202. [PubMed] [Cross Ref] [Ref list]

Chen, M., Lu, W., Hou, Z., Zhang, Y., Jiang X, Wu J.Heavy metal pollution in soil associated with a large scale cyanidation gold mining region in south east of Jilin , China. *Environ Sc pollute Res int*.2016;1. Doi: 10.1007/s11356-016-7968-3.

Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med*. 2005; 26(4–5):235–244. [PubMed] [Ref list]

Rao MVR, Acharya Y,Bala S, Paramban S. The study of heavy metals in abnormal growth and development using an Alternate animal model Heterometrous fulvipes.*Int. J. life sciences Res.* 2017; 3:800-807.

Chen YW. 2009.Heavy metals, islet function and diabetes development. *Islets*. ;1(3):169–176. doi: 10.4161/isl.1.3.9262. [PubMed] [Cross Ref] [Ref list]

Karri V, Kumar V. 2016. Heavy metals (Pb Cd, As, and Me Hg) are risk factors for cognitive dysfunction. A general review of metal mixture mechanism in brain. Environ. *ToxicolPharmacol*. 26, 48:203-213. Doi:10.1016/j.etap 2016.09.016

Palmer R, Blanchard S, Wood R. 2008. Proximity to point sources of environmental mercury release as a predictor of autism prevalence. *Health Place*. [PubMed]

Geier D, Geier M. 2007. A prospective study of mercury toxicity biomarkers in autism spectrum disorders. *J Toxicol Environ Health A*. 70:1723–1730. [PubMed] [Ref list]

Kazi TG, Jalbani N, Kazi N, Arain MB, Jamali MK, Afridi HI, Kandhro GA, Sarfraz RA, Shah AQ, Ansari R. 2009. Estimation of toxic metals in scalp hair samples of chronic kidney patients. *Biol Trace Elem Res*., 127(1):16–27. doi: 10.1007/s12011-008-8222-8.[PubMed] [Cross Ref] [Ref list]

Serdar MA, Bakir F, Hasimi A, Celik T, Akin O, Kenar L, Aykut O, Yildirimkaya M. 2009. Trace and toxic element patterns in nonsmoker patients with noninsulin-dependent diabetes mellitus, impaired glucose tolerance, and fasting glucose. Int J Diabetes Dev Ctries. 29(1):35–40. doi:

10.4103/0973-3930.50713.[PMC free article] [PubMed] [Cross Ref] [Ref list

Duruibe J. Heavy metal pollution and human biotoxic effects. *Int J Phys Sci*. 2007;2(5):112–118. [Ref list]

Plaskett, D., Potter, IC. 2005. Heavy Metal the Iowa State Univ. Press. Ames. Teleost from Cockburn Sound, Western Australia. Australian J. Marine Freshwater Res.30: 607-616.

Sastry, K V, Sunitha. 1984. Chronic toxic effects of Chromium in Channa punctatus. *J.Environ. Biol*., (5) 1; 47-52

Kulkarni, A.B, Utkar UN. 1983. Effect of molluscide on the on the biochemical composition of the tissues in the fresh water prosobranch snail viviparousbengalensis (Lamark).J. Environ.Biol.4:215-220.

Dudgall, CF, Patil M.S. 1986. Effect of Cadmium and mercury in Liver, muscle and kidney of the frog Rana cyanophlyctis. *Environ Eco*., 4 (2); 255-258

Lowe-Jinde L, Niimi AJ. 1984. Short term and long term effects of Cadmium on Glycogen reserves and liver size in rainbow trout Samogairdineri.*Arch Environ contamToxicol*. 13 (6); 759-764.

Ravindra Reddy *et al*. 1987. Effect of mercuric chloride on carbohydrate metabolism of mantle, foot and gills of fresh eater mussel Parreysiarugosa. *J. Emerson Biol*., 7 (4):225-230 .

Ramalingam, K. 1988. Toxic effects of DDT, Melathion and Mercury on the tissue carbohydrate metabolism of Sarotherodonmossambicus (Peters). *ProcIndAcadSci*. 97 (5); 443-448.

Sastry KV, Rao DR. 1982. Mercuric chloride induced alterations in the intestinal transport of glucose in the fresh water murrelChanna punctatus. *Int., J. Environ Stud*. 30:265-267.

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