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Study of Heavy Metals in Abnormal Growth and Development using an Alternate Animal Model: *Heterometrus fulvipes*

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ABSTRACT

Background: Heavy metals exposure in animals can lead to profound effects on growth and development. There have been incidences of various teratogenic effects in the past due to heavy metals exposure from various sources. The present study was conducted to access the effect of chronic heavy metals exposure in animal models.

Methods: An experimental prospective study was performed with viviparous animal *Heterometrus fulvipes* to access the cumulative effect of chronic heavy metals exposure. *H. fulvipes* was exposed to mercury and lead; and the effects monitored and documented in different times.

Results: Chronic heavy metal exposure had considerable effects in the mother and fetus of *H. fulvipes*. The effects in mother were represented by the loss of body weight and decrease in hepato-pancreatic weight and hepato-somatic index. Chronic exposure in fetus resulted in decrement in the embryonic length with subsequent reduction in the length and weight of embryos. These studies and results of heavy metals in animal have proven the harmful effects of chronic heavy metal exposure with multitude of questions. The question of particular concern would be how well animal teratology studies will predict the human hazard.

Conclusion: It is necessary that the heavy metal toxicity be well documented in humans, and adequate precaution should be taken in the mother and fetus to decrease its detrimental effects in the long run. The primary area of focus could be on the prevention of the birth defects induced by maternal exposure to heavy metals during pregnancy, as well as early prevention of teratogenic effects.

Key-words- *Heterometrus fulvipes*, Heavy Metals, Hepato-somatic indices, Morphometry

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INTRODUCTION

Heavy metals are known to affect the reproduction and development of organisms. Heavy metals have different sources: Mercury (Hg), Chromium (Cr), Nickle (Ni) and Zinc (Zn) are mostly naturally occurring, whereas lead (Pb), Cadmium (Cd), Copper (Cu) and Arsenic (As) are the direct consequence of human environmental pollution [1].

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The human health risk of heavy metals exposure is a public health problem. The exposure of heavy metals, in particular Pb, Cd, As, and Methyl mercury (MeHg) directly interfere with brain development and results in cognitive impairment. The exact mechanism of their toxicity is still unknown but their synergistic effect is well defined [2].

Much information has been accumulated on the toxicity of the mercury and lead to humans. There are many reported incidences of heavy metals poisoning due to potential contamination, for instance arsenic and lead, leading to lower cognitive scores in children [3-5]. Lead and mercury toxicity are linked with the use of nutritional contamination, ayurvedic supplements and skin creams [6,7]. Seafood is one potential source of mercury, and it has been recommended for limiting its use during the pregnancy [8]. Heavy metals toxicity is implicated in

magnification of estrogen dependent disease in female, including cancer of breast, endometrium, and pregnancy related complications like abortions, still births and pre-term deliveries [9].

Mercury is a very toxic metal and there is no acceptable level of mercury for animal exposure. Mercury can induce damage in development of human at any period. It is a potential neurotoxic, nephrotoxic and immunotoxic. The prenatal exposure of methyl mercury, placenta being a very ineffective barrier, can result in negative fetal impact to central nervous system maturation [10,11]. Pregnant wistar rats showed that mercury is capable of crossing the placenta, accumulating in fetal organs notably highest concentration in kidney, succeeded liver and brain [12]. Similar to mercury, lead exposures in rats have a profound effect in their memory and overall cognition [13]. Exposure of rats to high levels of lead has been associated with an increased frequency of abortion and premature birth in experimental animals.

It is evident that most of the studies on the effects of heavy metals on the maternal animal with extended influence on the embryonic development are confined to mammals and fishes. Little information is available on other viviparous forms, particularly the invertebrates, where viviparity and long gestation period are known to exist. Identifying a good viviparous model for evaluating the effect of heavy metals, drugs and other xenobiotics on fetuses, when mother is exposed or treated is of paramount importance. The scorpion *Heterometrus fulvipes* with its long gestation period is an ideal organism amongst invertebrates for a study of how the heavy metals affect the embryonic development when maternal animals are treated. An attempt is, there for, made to study the effect of heavy metals on the maternal animal and the embryonic development of the viviparous invertebrate *H. fulvipes*. The embryonic development depends upon the maternal conditions as the mother provides the nourishment and environment to the embryo.

Hence, it is likely that the effect of heavy metal on the mother influence the embryonic development. To further validate the extent of the influence, a study of the effects of mercury and lead on embryonic development as reflected by the changes in the morphological, morphometric and gravimetric parameters of the embryos is undertaken. Parturition is the pride of motherhood. A set of physiological changes occur inside the body of mother during pregnancy and parturition is the outcome of successful embryonic development. The effect of heavy metal on embryonic development, if any, would also reflect in the parturition. Therefore, a study to access and document the effects of the heavy metals on parturition after the administration of cumulative long term doses were performed and documented.

MATERIALS AND METHODS

An experimental prospective study was conducted in the department of Zoology, Nagarjuna University, Guntur during April 2011 to May 2013 with *H. fulvipes* by using mercuric chloride and lead acetate to represent mercury and lead respectively for studying the effect of these heavy metals on the maternal and embryonic development.

The toxicity of mercury and lead was determined by injecting different concentrations of the heavy metals dissolved in distilled water into batches of test animals through the arthrodial membrane in pedipalp between the hand and the brachium. Control specimens were given corresponding doses of distilled water. Mortality was recorded after 24 hours, for each of the concentration administered. LD_{50} value was calculated using Finney's probit method (Finney, 1964), and 1/3 LD_{50} (mercury-0.042 mg/g body weight and lead-0.58 mg/g body weight) to be administered in the present study [14].

Gravid females collected during latter half of the July were stocked in the laboratory in individual jars and maintained feeding regularly with cockroaches. 1/3 LD_{50} value of mercury and leadwere administered to the maternal animal for monthly administration of sub-lethal doses of mercury in successive doses during the gestation period. The stock was divided into three groups. Group I received the sub lethal dose of mercury, group II the sub lethal dose of lead and Group III, as a control, received the corresponding dose of solvent (Distilled Water).

After a month (mid-September), a batch of gravid females was drawn from each group for studying the effects of first doses of the heavy metals on the maternal animal, on the embryonic development and on the parturition. Rest of the animals in the three groups were administered a second dose of the corresponding heavy metals by mid- September. After taking samples from the three groups for experimental purposes to evaluate the impact of second dose by mid-October, the remaining ones were administered a third dose. Similarly, the fourth, fifth, sixth, seventh, eighth and ninth doses were administered by the middle of November, December, January, February, March and April respectively after drawing samples every month for experimental purpose.

Gravid females drawn each month from the three groups were individually weighed and the weight of the hepato- pancreas of each animal was noted using a monopan electrical balance (OWALABOR). Hepato-somatic indices (HIS) were calculated using the following formula:

$$\text{HIS} = \frac{\text{Weight of the Hepato-pancrease}}{\text{Weight of the animal}} \times 100$$

Samples of gravid females drawn each month from the groups I, II and III were sacrificed to examine cumulative effects of successive monthly doses of mercury and lead on the time course of development and on the morphological, morphometric and gravimetric aspects of developing embryos, using the corresponding group III samples (distilled water treated animals) as controls. Now to study

the morphological features of the embryos, the maternal the diverticula containing embryos were fixed in Bouins fixative and preserved in 70% alcohol. Photographs of the diverticula chosen at random were taken before isolating the embryo to provide information on the size and shape of the diverticula which are also known to change in accordance with the development of the embryos. The embryos in the diverticula were isolated and photographs were taken for studying the morphological features of the embryo for studying the morphometry, the length of the embryo during the early month of gestation was measured with the help of an ocular micrometer, in the later months, lengths were measured using dividers, from the anterior margin of prosoma to the tip of the metasoma. The average length of the embryos in an animal was considered as the length of the embryo of that animal. The values of lengths obtained from a number of animals observed. For gravimetric studies, the embryos were weighed together with the diverticular membrane (excluding appendix and oviduct). The average weight of the embryo in an animal was considered as the weight of the embryo in that animal. The values given in the data represent the means of such values obtained from a number of animals examined. Monopan electrical balance (OWALABOR) was used for weighing the embryos.

To study the effect of mercury and lead on parturition, the scorpions separated from each group every month and maintained in the laboratory for delivery of young ones. They were kept under observation till the end of June as parturition in this species of scorpion always occurs during animals were dissected in scorpion Ringer's solution and May and June under normal conditions. The occurrence of parturition, if otherwise was noted. When the parturition occurred, the condition of the pulley (newborn young ones) as reflected by its morphological features, length and weight was noted. The pullies of those animals that received different doses of mercury, and lead were compared with those of the corresponding controls that received distilled water.

RESULTS

Effect of sub lethal doses of mercury and lead on the maternal animal during the gestation period

Sub lethal doses of mercury and lead administered to the maternal animal at monthly intervals from August to April induced changes in maternal body weight, weight of the hepato-pancreas and hepato-somatic index (Tables 1-3; Fig. 1 to Fig. 3). The reduction in the body weight was statistically significant only after administration of fifth dose of mercury and fourth dose of lead. A reduction of 25.33% and 24.36% of body weight was recorded following administration of ninth dose in the month of April for mercury and lead respectively.

The weight of hepato-pancreas also has decreased gradually in a dose dependent fashion leading to 13.77% and 16.43% of depletion in April in response to mercury and lead respectively, through the depletion was statistically not significant (Table 2 & Fig. 2).

Table 1: Effect of mercury (Hg) and lead (Pb) on the maternal body weight of *H. fulvipes* during the gestation period

Month Treatment	Control Group	Experimental group	Percent depletion
AUG.	7.287±0.166	Hg 7.246±0. 210*	0.47
		Pb 7.170±0. 174*	1.49
SEP.	7.510±0.170	Hg 7.360±0. 170*	1.99
		Pb 7.259±0. 141*	3.34
OCT.	7.260±0.249	Hg 7.105±0.140*	2.13
		Pb 6.900±0. 152*	4.95
NOV.	7.500±0.228	Hg 7.000±0.235*	6.66
		Pb 6.802±0.166[a]	9.30
DEC.	7.580±0.200	Hg 6.800±0.103[b]	10.29
		Pb 6.600±0.142[c]	12.92
JAN.	7.560±0.196	Hg 6.440±0.119[b]	14.81
		Pb 6.400±0.103[c]	15.34
FEB.	7.800±0.182	Hg 5.960±0.136[c]	23.59
		Pb 5.850±0. 151[c]	25.00
MAR.	8.01±0.280	Hg 6.050±0.136[b]	24.47
		Pb 5.856±0.152[c]	26.89
APR.	8.21±0.339	Hg 6.130±0.148[b]	25.33
		Pb 6.210±0.176[c]	24.36

a_p<0.05; b_p< 0.01; c_p< 0.001; *Insignificant

Values represent mean±S.E with number of observations (N)= 10, Body Weight (g)

Table 2: Effect of mercury (Hg) and lead (Pb) on the weight of the hepatopancreas of *H. fulvipes* during the gestation period

Month of treatment	Control	Experimental	Percent depletion
AUG.	1.574±0.123	Hg 1.450±0.114*	7.87
		Pb 1.401±0.113*	10.99
SEP.	1.859±0.148	Hg 1.755±0.135*	5.59
		Pb 1. 680±0.123*	9.62
OCT.	1.773±0.123	Hg 1.654±0.123*	6.71
		Pb 1.600±0.117*	9.75
NOV.	1.700±0.135	Hg 1.566±0.107*	7.88
		Pb 1.509±0.117*	11.23
DEC.	1.558±0.106	Hg 1.462±0.113*	6.16
		Pb 1.390±0.100*	10.78
JAN.	1.385±0.100	Hg 1.267±0.128*	8.51
		Pb 1.200±0.135*	11.19
FEB.	1.379±0.096	Hg 1.222±0.134*	11.38
		Pb 1.200±0.130*	12.98
MAR.	1.561±0.120	Hg 1.351±0.137*	13.45
		Pb 1.309±0.140*	16.14
APR.	1.539±0.122	Hg 1.327±0.134*	13.77
		Pb 1.286±0.133*	16.43

***Insignificant, Values represent mean± S.E. with number of observations (N) = 10**
Weight of hepato-pancreas (g)

Table 3: Effect of mercury (Hg) and lead on the hepatosomatic index of *H. fulvipes* during the gestation period

Month of Treatment	Control Group (8)	Experimental group (10)	Percent change
AUG.	20.98±0.14	Hg 19.39±0.19^{c}	-7.57
		Pb 18.08±0.21^{c}	-13.81
SEP.	22.85±1.09	Hg 21.83±0.96*	-4.50
		Pb 20.62±0.45^{a}	-9.79
OCT.	24.87±0.23	Hg 23.00±1.68*	-7.51
		Pb 22.07±0.75^{b}	-11.25
NOV.	23.86±0.21	Hg 24.22±1.37*	+1.52
		Pb 22.55±0.25^{c}	-3.44
DEC.	21.16±1.13	Hg 22.75±1.62*	+7.51
		Pb 20.16±0.54*	-4.72
JAN.	20.54±0.49	Hg 21.18±0.75*	+3.11
		Pb 19.12±0.12^{b}	-6.88
FEB.	22.12±0.30	Hg 20.17±0.59^{a}	-11.10
		Pb 19.85±0.53^{b}	-12.51
MAR.	21.36±0.46	Hg 19.52±0.17^{c}	-8.59
		Pb 19.12±0.12^{c}	-12.85
APR.	20.00±0.66	Hg 18.80±0.20^{a}	-6.00
		Pb 18.49±0.16^{b}	-7.50

a_p<0.05 ;b_p< 0.01; c $_p$< 0.001; *Insignificant
Values represent mean ± S.E. with number of observations (N) given in parentheses
Hepato-somatic index

Table 4: Effect of maternal treatment with mercury (Hg) and lead (Pb) on the embryonic length during the gestation period of *H. fulvipes*

Month of Treatment	Control Group (9)	Experimental group (10)	Percent change
AUG.	1.25±0.09	Hg 1.23±0.11*	1.99
		Pb1.21±0.11*	3.58
SEP.	2.90±0.10	Hg 2.72±0.15*	6.20
		Pb2.65±0.09*	8.62
OCT.	3.15±0.15	Hg 2.83±0.11*	10.15
		Pb 2.78±0.13*	11.74
NOV.	4.42±0.15	Hg 3.50±0.15c	20.85
		Pb 3.30±0.12c	25.37
DEC.	4.83±0.16	Hg 4.00±0.12c	17.18
		Pb 3.80±0.15c	21.32
JAN.	7.01±0.20	Hg 6.05±0.12c	13.70
		Pb 5.70±0.13c	18.69
FEB.	13.00±0.22	Hg 8.58±0.15c	34.00
		Pb 8.04±0.18c	38.15
MAR.	15.50±0.26	Hg 12.09±0.22c	22.03
		Pb 11.20±0.28c	27.77
APR.	16.50±0.33	Hg14.00±0.21c	15.15
		Pb 13.10±0.21c	20.60

c_p< 0.001;* Insignificant
Values represent mean ± S.E. with number of observations (N) given in parentheses
Hepato-somatic index

Table 5: Effect of maternal treatment with mercury (Hg) and lead (Pb) on the embryonic weight during the gestation period of *H. fulvipes*

Month of Treatment	Control Group (8)	Experimental group	Percent depletion
AUG.	1.5±0.18	Hg 1.4±0.15*(8)	6.45
		Pb 1.3±0.13*(10)	7.85
SEP.	2.5±0.08	Hg 2.2±0.11a(8)	13.08
		Pb 2.1±0.14a (10)	18.25
OCT.	3.5±0.13	Hg 3.0±0.10b (10)	13.38
		Pb 2.8±0.11c (10)	20.56
NOV.	6.1±0.25	Hg 4.9±0.20c(8)	19.88
		Pb 4.7±0.18c(10)	23.72
DEC.	13.1±0.10	Hg 11.3±0.29c(10)	13.19
		Pb 11.0±0.40c (10)	15.55
JAN.	17.4±0.36	Hg 15.5±0.46b (8)	11.26
		Pb 14.0±0.23c (10)	19.85
FEB.	32.4±0.75	Hg 24.0±0.23c(8)	26.12
		Pb 22.0±0.47c(10)	32.28
MAR.	55.6±0.82	Hg 39.3±0.27c (8)	29.28
		Pb 39.0±0.24c(10)	29.91
APR.	65.5±0.50	Hg 40.6±0.42c (8)	37.93
		Pb 40.8±0.49c (10)	37.64

ap<0.05 ;bp< 0.01; cp< 0.001; * Insignificant
Values represent mean ± S.E. with number of observations (N) given in parentheses

Effect of mercury on pullies and parturition

Maternal animals that received a single dose of mercury in August delivered young ones normally as those that received second dose in September without any differences in the duration of gestation period and length of the pullies. However, a significant reduction in weight of the baby scorpion was evident. Those scorpions that received three, four, five, six, seven, and eight doses of mercury by October, November, December, January, February and March respectively, also delivered young ones normally after the completion of gestation period along with the controls. However, the size of the pullies as reflected by their length and weight was significantly reduced, the percent depletion being proportional to the number of doses of mercury up to April failed to deliver young ones. On examination in July (a month beyond the time of normal delivery), it was found that all the young ones were dead (Table 6).

Table 6: Effect of maternal treatment with mercury (Hg) and lead (Pb) during the gestation period on the length of pullies of *H. fulvipes*

Month of Treatment	Control Group (10)	Experimental group	Percent depletion
AUG.	16.7±0.10	Hg 16.4±0.12* (10)	1.675
		Pb 16.3±0.10^{a} (10)	2.189
SEP.	17.1±0.19	Hg 16.7±0.10*(10)	2.214
		Pb 16.6±0.14^{a}(10)	3.263
OCT.	16.8±0.16	Hg 16.1±0.14^{a} (10)	4.223
		Pb 15.9±0.14^{b} (10)	5.413
NOV.	16.6±0.10	Hg 15.4± 0.20^{b}(10)	7.151
		Pb 15.5±0.19^{c} (10)	6.851
DEC.	16.8±0.14	Hg 15.2±0.19^{c} (10)	9.846
		Pb 14.9±0.19^{c}(8)	11.621
JAN.	16.4±0.12	Hg 14.4±0.13^{c}(7)	12.621
		Pb 14.0±0.17^{c}(4)	14.805
FEB.	16.5±0.07	Hg 14.0±0.15^{c}(5)	15.254
		Pb 13.5±0.17^{c}(2)	18.280
MAR.	16.8±0.08	Hg 14.0±0.17	17.010
		Pb Parturition did not occur	
APR.	16.7±0.10	Hg Parturition did not occur	
		Pb parturition did not occur	

ap< 0.05; bp< 0.01; cp< 0.001; * Insignificant

Values represent mean ± S.E. with number of observations (N) given in parentheses. Length of pullies (mm)

The hepato-somatic indices following the administration of lead were significantly lowered throughout the gestation period. Mercury exerted relatively lesser influence, the depletion during November, December and January being resulted in general, cumulative effect of mercury also resulted in the reduction of the indices (Table 3 & Fig. 3).

Effect of sublethal doses of mercury and lead on the embryonic development during the gestation period:

Effect on morphological differentiation

Examination of diverticula and the embryos contained within at any time during the gestation period, both in the controls and the experimental animals treated with sub lethal doses of mercury and lead from August to April, revealed no differences in the sequence of morphological differentiation as could be noted (Fig. 4 to Fig. 12), except for the difference in the size, with the sequence and time course of embryonic development remaining the same in both controls and experimental animals, the gestation period appears to remain unaltered.

Effect of mercury and lead on the embryonic length and weight

The administration of sub lethal doses of either mercury or lead to the maternal animal during the gestation period from August to April resulted in reduction in embryonic length exhibiting a cumulative effect (Table 4). The decrease in length tended to increase gradually with an insignificant effect up to October and highly significant effect beyond. Sub lethal doses of mercury and lead administered to the pregnant females during the gestation period caused a very significant reduction also in the weight of embryos. Gradually increasing percentage of depletion in the embryonic weights up to a maximum of about 38% from August to April reflected a dose dependent effect for both the metals (Table 5).

Administration of sub lethal concentrations of lead to pregnant scorpions during the gestation period also had comparable effects. All females that received a single dose or two or three or four up to seven doses delivered young ones without any difference in the duration of gestation along with the controls. The pullies were, however, significantly lesser in length and weight compared to those of the controls. A cumulative effect of lead was evident as reflected by the gradually increasing percentage of reduction in lengths and weights of the pullies in accordance with the increase in the number of doses administered (Table 6). In females that received more than seven doses of lead, parturition did not occur. The long term cumulative effect of lead was fetocidal as evidenced by the dead young ones within the diverticula when examined during July, a month beyond the time off normal parturition.

DISCUSSION

The result obtained in the present study reveals that there is a well recognizable impact of the heavy metals, mercury and lead, on the maternal animal resulting in the loss of body weight and weight of hepato-pancreas and hepato-somatic index. The decrease in the body weight and the weight of the hepato-pancreas of *H. fulvipes*, following the administration of the heavy metals can be explained in terms of altered metabolic levels and increased utilization of reserve stores by the animal for its maintenance and for the maintenance of the developing embryos in the face of the excessive energy demands under the toxic stress of the heavy metals. The decline in the hepato-somatic indices together with decrease in the weight of the hepato-pancreas of the animals treated with mercury and lead suggests a preferential utilization of liver components.

In *H. fulvipes* treatment with cumulative doses of mercury and lead significantly reduced the length and weight of the embryos. The effect of cumulative doses of heavy metals on embryonic development is reflected in a new born also resulting in significantly reduced lengths and weights of the young ones. The observed reduction might possibly be due to the lowered availability of nutrients owing to the increased utilization of reserves by the mother under heavy metal toxicity, or decrease in the organic reserves of the embryos due to the direct effect of heavy metals or due to competitive utilization by the mother and the embryo under heavy metal stress condition. Failure of parturition observed in the present study in animals that received eight doses of mercury and seven doses of lead could be attributed to the cumulative effect of the repeated doses of heavy metals. It may also be suggested that the duration of stress was inadequate to prevent parturition during the eight doses of mercury and seven doses of lead.

The study of result of heavy metals in animal has definitely raised a multitude of questions. The question of particular concern would be that how well animal teratology studies will predict the human hazard. The focus should be on the prevention of the birth defects induced by maternal exposure to heavy metals during pregnancy.

CONCLUSIONS

There is a significant effect of heavy metals in mother and fetus. In view of the fact that the loss of weight under the heavy metal stress is suggested to be due to the excess utilization of reserve stores, if it is felt desirable that a detailed investigation on the biochemical constituents of animals exposed to heavy metals is carried out. It will also help to have an insight into the extent to which the impact of heavy metals on the biochemical constituents of the maternal animal extends into the embryonic development holding responsibility for the observed effects of the heavy metals on the embryos of *H. fulvipes*. Therefore, it is pertinent that this information should be reflected to humans and more research should be conducted to explore the effect of heavy metals in growth and developments.

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