Combined Effect of Heavy Metals on Longevity of *Drosophila Melanogaster*

V. Shakunthala[1], Shalini H.K[2]

Assistant Professor, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-6, Karnataka, India

Abstract: ***Heavy metals have been considered as one of the key environmental toxicants with a wide range of health effects on humans. The bulk of information available today is mainly focused on the single toxin studies with only few studies enumerating the synergistic, additive or protective effects of these heavy metals. Drosophila has great potential as a model system for studying toxic effects because they have metallothioneins similar to those of mammals. In current study, Drosophila was used as a model to investigate the effects of two prominent heavy metals- Lead (Pb) and Zinc (Zn) with respect to the longevity of the D. melanogaster. Larval feeding method was employed throughout the developmental period of the experimental subjects to administer the compounds singly or in combination while maintaining multiple replicates in order to minimize experimental error. Subsequently, number of adults survived was recorded. Our results show that developmental exposure of sub lethal concentrations of lead acetate and zinc chloride provokes disturbances in lifespan of the flies.***

Keywords: *Drosophila melanogaster*, longevity, zinc chloride, Lead acetate, heavy metal, synergistic

1. Introduction

Heavy metals are essential elements of many fundamental biological processes but may also possess a threat to the integrity of the organisms if their homeostasis is not tightly regulated. Heavy metals have recently come to the forefront as dangerous substances and are considered as serious chemical health for living organisms (Gossel and Bricker 1991). Trace levels of some metals are very essential for the metabolic activity and good health (Eisler, 1981) but when their bio-concentrations exceed the safe level, they act as metabolic inhibitors besides disrupting other physiological, biochemical and behavioral aspects (Vallee and Wacker, 1970). Heavy metals form a major causative group with cognitive and neurological implications of exposure. Excess accumulation of few heavy metals like Copper and Zinc which are otherwise of nutritional and physiological importance and non-essential toxic metals like Lead and Mercury is detrimental (Hapke *et al.*, 1987; Yepiscoposyan *et al.*, 2006). *Drosophila* has great potential as a model system for studying toxic effects because they have metallothioneins similar to those of mammals (Silar et al., 1990). *Drosophila* also has a lot of similarities in the developmental pathways, signaling pathways as well as some protein-coding pathways, say for example the Notch signaling pathway in the embryonic development of the nervous system in fruit flies and humans are similar (Engel et al., 2012). The ability to detect particular toxic substances may be important to insect survival. Taste and smell guided behaviour in most insects provide a means for food localization, oviposition site selection, and selection of reproductive partners. Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry have concluded in policy statements that lead at low doses is a serious threat to the central nervous systems of infants and children (Herbert and Needleman 1990).

2. Materials and Methods

For Doses we followed methods followed by the Stafny and Shakunthala, 2015

Group 1, Control group: Agar media (Delcour media) – for egg collection, Wheat cream agar media.
Concentration group:

Group 2: Lead acetate:
LT: 1.605mM (LT": Lead treated)
LT2: 2.407mM

Group 3: Zinc chloride:
ZT1: 7.115mM (ZT: Zinc treated)
ZT: 10.672mM

Group 4: Combined effect:
ST1= LT1 + ZT1 (ST = synergistic treatment)
ST2= LT2 +ZT2

Longevity:
Equal aged flies were collected to analyze longevity. Ten replicate vials (2.5 cm_9.5 cm) containing 6ml of food medium were maintained for each group. The pair of flies (male + female) was transferred to vials immediately after emergence. These pairs were then transferred to vials containing fresh food media for control group and mixed with different dose of either zinc or lead or both to treated groups. Flies were transferred to vials with fresh media every two days once until death and recorded the date of death of males and females separately. The data recorded for each treatment groups separately.

Statistical Analysis: ANOVA is applied for comparison of both treated and control groups

3. Result

Figure 1 depicts the effect of lead on the life expectancy of *D. melanogaster*. Longevity of flies greatly reduced in LT2 group compared to control. No significant difference in between sexes. Control group flies lived for 56 days 10 days reduction in LT1 group ie 40 days in case of LT2 group. Survival rate is much more reduced in Zinc treated groups. The comparison between the control and the treated groups is significant. Almost 50% reduction in the control and the treated group. In case of the synergistic group (fig 4) almost 50% reduction in ST1whereas, 60% reduction in ST2 groups. In all the three treated groups no significant difference observed between sexes. Both sexes equally affected. The total number of days flies lived in lead treated groups in significant F= 20.023(table1). Similarly zinc and combined effect with F value 20.023. Compared to lead treated group zinc treated group lived less almost 50% reduction in their life expectancy (fig 1, 2&3). The flies treated with both heavy metals showed less life expectancy. i.e., more than 50% reduction. That in sex ratio is not altered. There is a dose dependent reduction in the life expectancy and combined effect has more severe compared to individual effect. Males lived longer than compared to females in many groups.

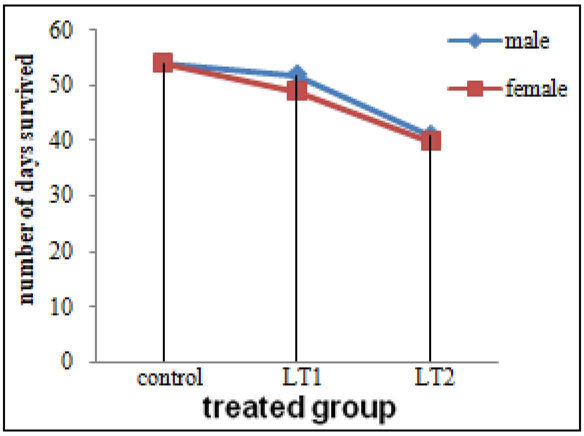

Figure 1: Survival curve of lngevity of *D. melanogaster* showing the number of days survived in control and Lead treated groups

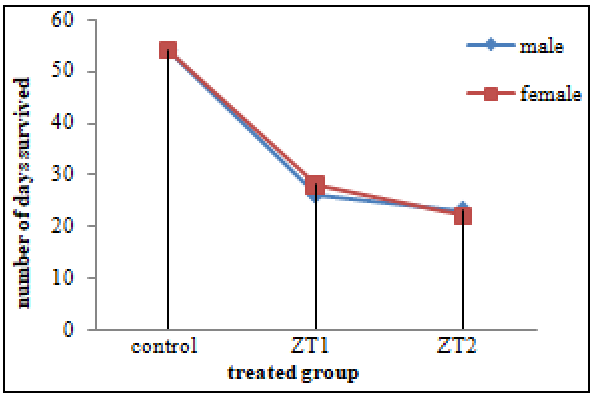

Figure 2: Lngevity of *D. melanogaster* showing the number of days survived in control and zinc chloride treated groups.

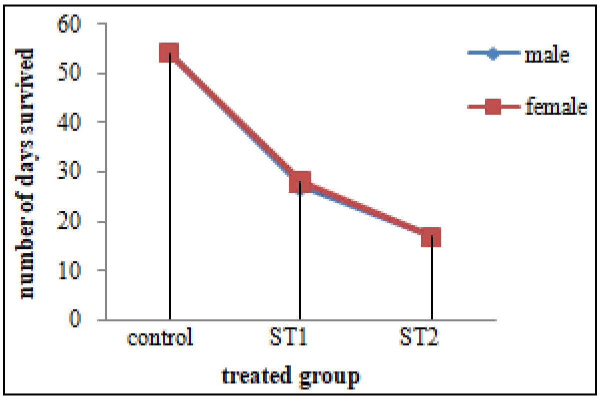

Figure 3: Longevity of *D. melanogaster* showing the number of days survived in control and zinc chloride + Lead treated groups

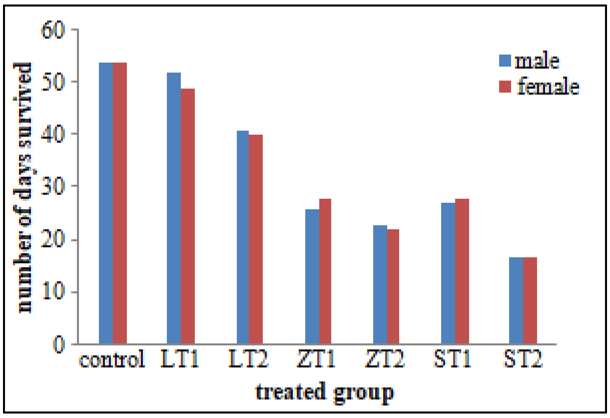

Figure 4: Longevity of *D. melanogaster* showing the number of days survived in control, lead, zinc chloride and synergetic treated groups

4. Discussion

Our results reveal that the combined effect of heavy metals lead and zinc intoxication on fitness of *Drosophila melanogaster*. The *Drosophila* life cycle provides wonderful opportunities to reveal the possible tradeoffs between its lifespan traits with respect to metal toxicity. As hypothesized, our result shows that the developmental exposure of excess lead acetate and zinc chloride together in *Drosophila melanogaster* hamper its fitness by reducing the longevity of flies. But lead seemed to show compensation for the longevity caused by zinc.

Response to metals is dose dependent. Increase in the concentration of metals increases the mortality rate of the flies. One of the reasons could be higher concentrations of metals can interfere with the necessary enzymes or hormone production. But determining the exact threshold is a difficult task as it can differ depending on the organism. Environmental conditions, genetic constituent and chemical properties of the compound (Bonneton et al., 1996)

For the above result the longevity is more in case of control flies compared to heavy metal treated flies. Heavy metals are naturally found in soils and rock formations but may also occur in fertilizers and pesticides. As a result of which may cause heavy metal contamination in fruit and interfere with *Drosophila* survival and development (He et al., 2005; Massadeh et al,.2005). In *D. melanogaster*, there are a variety of metal homeostatic mechanism, as well as oxygen defense mechanisms that intercept their extremely toxic byproduct, the hydroxyl radical (Hilliker and Phillips 1990). For instance, the antioxidant enzyme, superoxide dismutase (SOD)-1, plays a critical role in defense against heavy metal toxicity, where RNA 1- induced silencing of SOD1 significantly shortens life span of adult flies on iron-supplemented medium (Bahadorani et al,. 2001). The combined effect of heavy metals has severe effect on the survivability compared to individual effect.

Lead is less affected than the zinc in the subleathal concentration. Further, *Drosophila melanogaster* exposure to lead did not produce any apparent effect on male and female survival however, a dose- dependent delay in larval maturation was observed (Cohn et al,. 1992). 0.5-1% zinc caused reduction in growth, anemia and poor reduction in rats. .Longevity is affected at most by excess dietary lead and zinc in the form of lead and zinc chloride. Significant extension of longevity refers to a proportional increase in both mean and maximum lifespan (Arking et al.,2002). The extended longevity phenotypes comes at a cost of decreased fitness to other important environmental factors, and ability to respond to stress plays a major role in determining longevity (Kuether and Arking, 1999). Further, several studies also report there is a positive association of longevity with stress-related life history traits, environmental stressors (starvation, desiccation and cold) and metabolic profile, pollution. In the present study the heavy metal pollution may cause the stress as there is a reduction in the lifespan of the heavy metal treated flies ie., zinc and lead and the combined effect. Interestingly there is no significant difference in the male and female lifespan. In contrast to our study Deepashree et al 2017 have showed that there is an extension of the lifespan in selected lines of LLS flies and both sexes differ in their life history traits in response to the extended longevity. The combined effect of lead and zinc has showed much more reduction in the longevity compared to the individual effect. According to Fauad et al. 2005, heavymetals damage the intestine and reproductive tissues, there histopathological study on the German cockroach have revealed that the alimentary canal, gonads and the abdominal tissue showed evident signs of damage. Further, they opines that these signs suggested irreversible metablic alterations and disorders in the mitotic and meiotic pathways. This suggests that the reduction in the fertility and fecundity, viability and longevity is due to damage in the reproductive tissue. The chronic treatment of the heavy metal also impairs the spermeogenesis and oogenesis.

There is dose dependent survivability effect observed in the heavy metal treated groups. Heavy metal has no effect on the sex ratios. More than one heavy metal combined together causes more damage compared to single heavy metal effect.

5. Acknowledgements

We are grateful to the chairmen Department of Studies in Zoology for their support and PSFS financial support by the University of Mysore.

References

[1] Arking D.E. A. Krebsova, M. Macek Sr.M. Macek Jr. A. Arking S. Mian, L Fried,A Hamosh, S. Dey, I Mcintosh and H.C. Dietz, 2002. Association of human aging with a functional variant of Klotho. Proceedings of the National Acacemy of Sciences 99(2):856-861

[2] Bonneton F. and M. Wegnez 1995. Developmental variability of metallothonein Mtn gene expression in the species of the Drosophila melanogaster subgroup. Developmental genetics 16(3):253-263.

[3] Bahadorani S and A.J. Hilliker, 2009. Biological and behavioral effects of heavy metals in *Drosophila melanogaster* adults and larvae, Journal of. instect behaviour 22(5): 399-411

[4] Deepashree S. S.R. Ramesh, and T. Shivanandappa 2017. Lifehistory traits of an extended longevity phenotype of Drosophila melanogaster. Current Aging Science. 10(3):224-238.

[5] Eisler, R. 1981. Trace metal concentration in marine organisms. Pergagamon press, New York :687.

[6] Engel G.L, A.Delwig, M.D. Rand.2012. The effects of methylmercury on Notch signaling during embryonic neural development in *Drosophila melanogaster.* Toxicolology In Vitro., 26(3): 485-92

[7] Fouad A..Al-Momani and A.M. Masadeh 2005. Effect of different heavy-metal concentrations on *Drosophila melanogaster* larval growth and development. Biological Trace Element Research 1 (3):271-277.

[8] Gossel, T.A., and J.D. Bricker, 1990. Principles of clinical Toxicology. 2nd Edition Raven Press New York 107-109 ().

[9] Hapke H., M.P. Laura and R. Lothar 2010. The essential Toxin: Impact of Zinc on Human Health. International journal of Environment Esearch and Public Health 7: 1342-1365.

[10] Hilliker A.J., and Phillips J.P. 1990. Gemetic Analysis of Oxygen defense mechanisms in D. melanogaster Advances in Genetics 38: 43-71.

[11] Kuether K. and R. Arking 1999. Drosophila selected for extended longevity are more sensitive to heat shock. Age 22(4):175-180.

[12] Olakkaran S, Antony A, Purayil A. K , Kumbar S. T., Gurushankara H P. Lead modulated Heme synthesis inducing oxidative stress mediated Genotoxicity in *Drosophila melanogaster. Sci. Total Envir.* 634, 628–639 (2018)

[13] Silar P, L. Théodore , R. Mokdad , N. E. Erraïss , A. Cadic , M. Wegnez . 1990. Metallothinein Mto gene of *Drosophila melanogaster* structure and regulation *J. mol. Biol.*, 215(2), 217-224

[14] Stafny M. Dsouza and V. Shakunthala, 2015. Combined effect of Lead acetate and Zinc chloride on the Fitness of *Drosophila melanogaster.* International Journal of Advanced Research 3(3):1054-1064.

[15] Vallee, B.L., W.E.C. Wacker, 1970. The proteins 2nd Ed. Vol.5 Nurath.H Edition New York Academic Press.

[16] Yang X, Y. Feng Z.I. He, P.J. Stoffella. 2005. Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. Journal of Trace Elements in Medicine and Biology 18(4): 339-353.

[17] Yepiskoposyan H, D.Egli, T.Fergestad , A. Selvaraj, C. Treiber, G. Multhaup, O.Georgiev W. Schaffner 2006. Transciptome response to heavy metal stress in *Drosophila* reveals a new zinc transporter that confers resistance to zinc. Nucleic Acid research 34(17): 4866-487

Supplementary resource

Groups	Mean±Std. Error	F ratio	Sig
control male[a]	54.8000±3.47946	20.023	*b×f
control female[b]	56.0000±2.99629		*b×g,h,i,j
Lt1 male[c]	52.4000±2.32475		*b×k,l,m,n
Lt1 female[d]	49.8000±1.96525		*c.×g,h,i,j,k,l,m,n
Lt2 Male[e]	41.6000±2.10396		*d×g,h,i,j,l,mn
Lt 2 female[f]	40.6000±1.23108		*e×g,i,j,k,n
Zt1 male[g]	26.2000±4.76515		*f×a,b,c,d,e,i,j,m,n
Zt1 female[h]	28.6000±3.42604		*g×a,b,c,d,e
Zt2 male[i]	23.4000±3.91067		*h×a,b,c,d
Zt2 female[j]	20.0000±1.19257		*i×a,b,c,d,e,f
St1 male[k]	27.2000±3.93503		*j×a,b,c,d,e,f
St1 female[l]	28.6000±3.71244		*k×a,b,c,d
St2 male[m]	19.0000±3.11627		*l×a,b,c,d,e,f
St2 female[n]	17.4000±3.46474		*m×a,b,c,d,e,f
			*n×a,b,c,d,e,f

Table: Shows the survivability of the flies (days) in different treatment groups along with ANOVA and Post hoc test (*P<0.05)