Effects of Lead Acetate on Light Protein of *Drosophila melanoigaster*

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Abstract Lead is supposed to be an important poisonous waste, which could contaminate the environment, therefore, insects could be influenced easily by the lead. *Drosophila melanogaster*, was studied at 48 hours post treatment, under the effects of lead acetate, in different concentrations of 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg. It was observed that, under the effects of lead abnormalities, and deformity were developed in the larvae of flies. Thus these flies could present a useful module for the quick transmission of the environmental hazards due to lead contamination, which exerts a specific physiological and morphological effect on these flies.

Keywords: Effects, Lead acetate, Proteins *D. melanogaster*

INTRODUCTION

Lead is a heavy industrial metal, which contaminates environment food, water, urban soil and air. The exposure of possible toxic effects of this metal is the issue of urgent concern for the biological life. Its toxic potential against insects remains to be well-known [1]. Lead has been found to have a definite cytogenetic effect [2-11]. Some studies have been accepted out on natural populations of D. melanogaster in respect of effects of heavy, metals, it has been established that contamination with heavy metals (Zinc, Lead etc.) can induce the effects on feeding behavior of some diptera, their structural and functional modifications and malformations [12, 13]. Investigations on Drosophila melanogaster indicated abnormalities due to the effect on meiotic nondisjunction [9]. However, sufficient data on the action of heavy metals and lead is limited available on the group of insects such as Drosophila melanogaster, those are widely distributed species of the family tephritidae.

Drosophila is belonging to the family Drosophilidae, also called "fruit flies" and less frequently called pomace flies, vinegar flies, or wine flies, *Drosophila* feed mostly on unripe or ripe fruit. As it breed rapid, and put down a lot of eggs. *Drosophila melanogaster*, is being used extensively in research as a model organisms [14]. The *Drosophila melanogaster* natural life is about 30 days at 29 °C (84 °F). The developmental process in *Drosophila melanogaster* varies with temperature. The developmental period from egg to adult take 7 days, at 28 °C (82 °F). Under ideal conditions, the development time at 25 °C (77 °F) is 8.5 days, the eggs, which are about 0.5 millimetres long, hatch after 12–15 hours (at 25 °C (77 °F). The eggs, which are about 0.5 millimetres long, hatch after 12–15 hours (at 25 °C (77 °F) [15, 16].

Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soils and foods [17] lead inhibits the activity of enzymes that are dependant on the presence of free sulphydryl groups (SH). The clearest manifestation of these effects is the disturbance on the biosynthesis of heme, which in humans is accompanied by abnormalities in porphyrin metabolism [18]. Lead acetate is used as a topical astringent and is found to be a renal carcinogen in rats [19-24]. In the Syrian harnster lead induces neoplastic changes in the bronchio-alveolar area [25, 26]. It also produces infertility in mice [27] and reduces the reproductive ability of rats [28-30]. In *Drosophila melanogaster* lead induces enzymatic alterations in esterase and triose phosphate isomerase [31] and affects non disjunction [32].

Electrophoresis is being broadly used for categorization of proteins and peptides for the diagnostic and/ or preparative unification of organic macromolecules [33]. The process of electrophoresis first used by [34] for the separation of proteins has found many dimensions in analyzing and separating macromolecules. These techniques, wheather alone or in combination, have proved to be very useful for proteins and peptides and the complex proteome analysis, [35].

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MATERIAL AND METHODS

The *Drosophila melanogaster* flies were reared in wide mouth bottles containing usual medium. The eggs of these flies were collected from fermenting fresh baker's yeast supplemented with sucrose [36]. After removing the parental flies, the egg were left for their development. After three days, the 72 hours larvae were collected. Soon after that, the larvae were transferred to small bottles (10 larvae/bottle). Prepared three bottles with 3 grams bananas, mixing with lead acetate as diet purpose for each concentration, as, 0.125 mg, 0.25 mg, 0.5 mg, 01 mg and 02 mg and three bottles were left untreated as control. Put the 10 larvae in each bottle for 48 hours. After that the mortality of larvae was noted in the each bottle. the live larvae were transferred in separate bottles on pure feed of bananas for pupation and adults emergence.

The determination of lead acetate on protein of *Drosophila melanogaster* larvae were studied with lead acetate kept for 48 hours exposure. Thereafter, crushing and homogenizing of the treated and untreated larvae was made.

1. Preparation of Solutions:

   i. Acrylamide-Bisacrylamide solution(30.0:0.8)

   ii. 1.5 M Tris-HCl buffer

   iii. 10% Sodium dodecyl sulfate

   iv. 10% Ammonium per sulfate

   v. Sample diluting buffer (SDB)

   vi. Reservoir Buffer

   vii. Staining solution: (Bromophenol blue and 0.2% Comassic blue)

   viii. Destaining solution

2. Reagent and Chemicals:

   i. Acrylamide (Fluka)

   ii. N,N,Methylene bisacrylamide (Fluka)

   iii. Tris (hydroxymethyl) aminomethane (Fluka

   iv. HCL (Merck)

   v. Sodium dodocylsulfate (Fluka)

   vi. Ammonium persulfate (Merck)

   vii. Glycine (Fluka)

   viii. TEMED (Merck)

   ix. Bromophenol blue (Merck)

3. Preparation of Gel:

Component volumes (ml) per mold volume of solution components: 10 ml

6%

i. H_2O (Deionized water) 5.3

ii. 30% acrylamide mix 2.0

iii. 1.5 M Tris (pH. 8.8) 2.5

iv. 10% SDS 0.1

v. 10% ammonium persulfate 0.1

vi. TEMED 0.008

vii. Gel Casting

In the process of electrophoresis, the capillary tubes of electrophoresis were cleaned by water and ethanol then dried it by air. The lower mouth of capillaries were covered by rubber stopper. 10 ml resolving gel was prepared with above ingredient. The mix solution was filled in capillaries tube, then added the 0.1 ml ammonium sulphate and 0.008 ml TEMED in capillaries, then left it for 3-4 hours for polymerization, after that 200 µl. (micro litre) sample was added and then Bromophenol solution was added. After 30-40 min. the mouth of above and lower part of capillaries were exposed with Reservior Buffer solution in the electrophoresis tank for one day under 110 volt. After that gel were exposed to coomassi blue solution for 2 hours, after colorization of Gel, It was kept in the de-staining solution for removing the excess color on the Gel then the bands of proteins were observed. After this process the length and bands on Gel was measured for Rf determination. Egg albumin was also run simultaneously, for the comparison.

RESULTS

The effect of lead acetate on proteins of *Drosophila melanogaster* is shown in Table **1**. While Egg albumin was used as a reference protein. The rf. of Egg albumin was found as 0.04. As compared the rf. with

Table 1: Protein Remarks on *Drosophila melanogaster*

Protein	Rf	Egg Albumin	*Drosophila melanogasr* Untreated Control	*Drosophila melanogastr* Treated
I	0.61		-	+
II	0.64		+	-
III	0.73		+	-
IV	0.76		-	+
V	0.78		+	-
VI	0.86		-	+
VII	0.87		+	-
VIII	0.93		+	-
IX	0.94		-	+

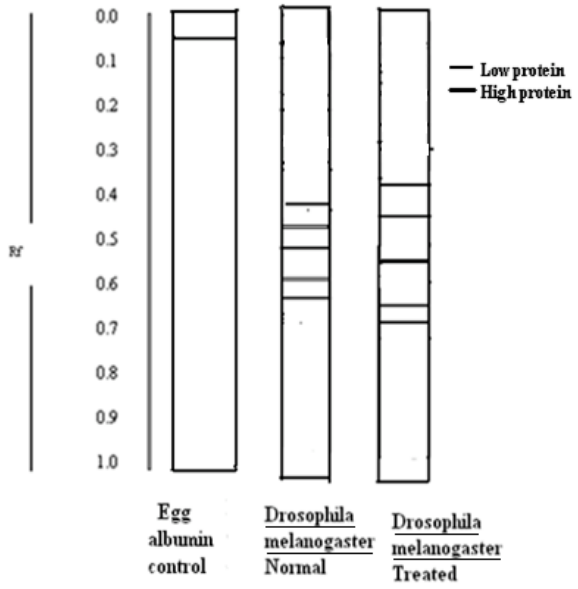

Figure 1: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Drosophila melanogaster.*

Drosophila melanogaster (untreated), the values were found to be 0.64, 0.73, 0.78, 0.87, 0.93. The rf proteins of *Drosophila melanogaster* (treated) showed the values as 0.50, 0.61,0.76,0.86,0.94, respectively.

DISCUSSION

Protein IV (rf 0.14) is found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumim, while corresponding protein in *Drosophila melanogaster* untreated is absent, that suggest that protein IV is affected at small extend.

Protein VI (rf. 0.23) was found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated *Drosophila.melanogaster*, was absent. That suggest that the protein VI was affected at some extend.

Protein IX (rf 0.42) is found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein,in the untreated *Drosophila melanogaster*, was absent at the same Rf. This suggests that the protein IX was changed with some alteration in the treated insect.

Protein X (rf 0.43) is found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated *Drosophila melanogaster*, was absent at the same Rf. This suggests that the protein X is changed with some alteration in the untreated insect. That suggest that the protein X was affected at a large extend.

Protein XI (rf 0.61) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated *Drosophila.melanogaster*, was absent at the same Rf. That suggest that the protein XI was affected at a low extend.

Protein XII (rf 0.64) was found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated *Drosophila. melanogaster*, was absent at the same Rf. This suggests that the protein XII was affected with some alteration in the untreated insect.

Protein XIII (rf 0.73) was found in *Drosophila melanogaster* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated *Drosophila. melanogaster*, was absent at the same Rf. This suggests that the protein XIII was affected with some alteration in the untreated insect.

Protein XIV (rf 0.76) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Drosophila melanogaster*, was absent at the same Rf. This suggests that the protein XIV was affected with some alteration in the treated insect.

Protein XVI (rf 0.86) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein,in the untreated *Drosophila melanogaster*, was absent at the same rf. This suggests that the protein XVI was affected with some extension in the treated insect.

Protein XVIII (rf 0.93) were found in *Drosophila melanogaster*, (untreated) that is seems to be lighter than the egg albumin, while corresponding protein,in the treated ones, were absent at the same Rf. This suggests that the protein XVIII were affected with some extension in the untreated insect.

Protein XIX (rf 0.94) was found in *Drosophila melanogaster* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Drosophila melanogaster,* was absent at the same Rf. This suggests that the protein XIX was affected with some extension in the treated insect.

Drosophila melanogaster treated with different doses of lead acetate viz. 0.125 mg., 0.25 mg., 0.5 mg., 1.0 mg and 2.0 mg resulted deformities. Nukhet *et al.*, indicated cellular damage in processes of lead exposed to PC-12 cells [37]. After lead exposure the N-acetyleysteine (NAC), glutathione (GSH), glutathione disulfide (GSSG) and malondialdehyde (MDA), were found effected after treated to various doses of lead acetate, these results could be correlated with the present findings with the presence of affected proteins in the lead treated insects. Corey and Galvao, indicated that, lead is a pollutant heavy metal [38], which can be absorbed by the digestive system in a 10%, Roy, indicated that when lead incorporated by cells [39], it produces free radicals, H_2O_2 and ·OH. Friedberg *et al.*, found free radicals can also produce simple breaks in the DNA chains these results resembled with present finding [40]. that exposure of lead produced the abnormal morphological effects in the larvae and the adults emerged therefrom. Chandrik *et al.*, reported newly hatched nymphs of an Indian short horned grasshopper *Oxya fuscovittata* (Marschall) Orthoptera: Acrididae were fed on foods treated with three sub lethal concentrations of CdCl i.e. 2 25 ppm in oat or dose 1 (d1), 50 ppm in oat or dose2 (d2) and 100 ppm in oat or dose3 (d3) until they reached on adult stage for a complete generation [41]. Growth was measured in terms of specific growth rate (SGR), average daily growth (ADG), percent weight gain (PWG) and Growth rate (GR). They observed that growth retardation occurred significantly with the increase of doses in both sexes. Adult life period found reduced in both sexes however, in females a significant difference was found only with higher doses (d2 and d3). Lower survival was in d3 was observed. These adverse effects of heavy metals on diptera are in the line with the present findings.

Kalajdzic *et al.*, found morphological changes in wild Drosophila species that found over almost all of Europe, under the effects of lead [42]. The effects of lead on inversion polymorphism were studied by cytological analysis of gene arrangements on all of the five acrocentric chromosomes, as well as by cytological analysis of karyotypes on all of the four autosomes. The frequencies of particular gene arrangements on the four autosomes changed significantly in the samples maintained on medium not supplemented with lead. The frequencies of some gene arrangements on all of the five acrocentric chromosomes changed significantly in the flies maintained on media supplemented with lead. The length of exposure to different lead concentrations results in a significant change in the frequency of a few gene arrangements on two autosomes. Their results showed that different concentrations of lead, and exposure period caused affects on chromosome. the effects on the DNA

configuration and chromosome cause effects on morphology and the physiology of the affected organism, in this way presently the obtaining of altered protein bands, deform larvae, pupae and deform adults are in the line with the previous findings.

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