Determination of Lead Acetate Effects on Heavy Weight Protein of *Musca domestica*

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Abstract: So many insects are influenced easily by many pollutants; therefore, the influence of lead (as lead acetate) on *Musca domestic*a was studied, lead is considered to be an important toxic waste which could contaminate the environment, such as soil, air and water, therefore, insects could be influenced by the lead. *Musca domestica*, was studied at 48 hours post treatment, under the effects of lead acetate, in different concentrations. Thus the lead is found to exert a definite specific physiological and morphological effect on these flies, 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. The purpose of the present work was to determin the effects of lead on proteins as a major indicator of physiological features alongwith morphology features of larvae of Diptera flies.

Keywords: Effects, lead acetate, *M. domestica.*

INTRODUCTION

Lead is considered as an environmental pollutant, which exert the possible hazardous effects of this heavy metal, the detection of its presence in the environment is therefore a matter of urgent concern. Although, many studies have been carried out in relation with the biological effects of lead, its toxic potential against insects remains to be established. Though [1-8b, 9, 10] have reported cytological effects of this metal leading to expected morphological abnormalities. Some studies have been carried out on natural populations of *Musca domestica* 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 flies. Their morphological abnormalities, functional modifications and malformations could be induced under heavy metal actions [11, 12]. [8a] indicated that induced abnormalities could be obtained due to the effect on meiotic nondisjunction Rizwan *et al.* [13, 14] have reported morphological effects of this metal on insects and suggested to use these insects as indicators of the presence of this metal in the environment. It lives feeding on garbage, specially where sanitary conditions are unsatisfactory [15-17]. Since it is found all over the world, therefore, it could act as a model for the detection system of pollution with this heavy metal.

Among the heavy metals, lead, has been shown to be widely distributed in the atmosphere, water, soils and foods [18]. 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 [19]. Lead acetate is used as a topical astringent and is found to be a renal carcinogen in rats [20-25]. In the Syrian harnster lead induces neoplastic changes in the bronchio-alveolar area [26, 27]. It also produces infertility in mice [28] and reduces the reproductive ability of rats [29-31]. In *Drosophila melanogaster* lead induces enzymatic alterations in esterase and triose phosphate isomerase [32] and affects non disjunction [8a]. However, information about the mutagenic effects of lead salts in humans who are occupationally exposed to them and information obtained from *in vitro* studies are contradictor [33].

Electrophoresis is being broadly used for categorization of proteins and peptides for the diagnostic and/ or preparative unification of organic macromolecules [34]. The process of electrophoresis first used by [35] 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 [36].

MATERIALS AND METHODS

For obtaining an initial wild house flies culture, a usual larval media for *Musca domestica*, was prepared in an wide plain pot by mixing wheat bran with milk and

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Solution	Prepration
i) Acrylamide-Bisacrylamide solution (30.0:0.8)	Dissolve 30 gm acrylamideand0.8 gm bisacrylamide in deionized water.Make up the volume to 100 ml. Filter through Whatman no.1.
ii) 1.5 M Tris-HCl buffer:	Tris 18.2.0 gm,dissolve in 80 ml and adjust the pH of this solution to 8.8 using 0.1M HCl. Make up the volume to 100 ml with deionized water.
iii) 10% Sodium dodecyl sulfate:	Dissolve 1 gm SDS in 9 ml water and make the volume up to 10 ml with deionized water.
iv) 10% Ammonium per sulfate:	Dissolve 1 gm APS in 1ml water and make the volume up to 10 ml with deionized water.
v) Sample diluting buffer (SDB):	Dissolve 6.25 ml of 1M Tris-HCl pH 6.8 (Solution C), 2 gm SDS, 5 ml 2-mercaptoethonol and 10 ml glycerol together. Make volume up to 100 ml with deionized water.
vi) Reservoir Buffer:	Dissolve o.9 gm Tris, 3.6 gm Glycine and 1.0 gm SDS in 500 ml deionized water. Make up to 1liter.
vii) Staining solution:(Bromophenol Blue and 0.2% Comassic blue).	Dissolve 0.5 gm Coomassie blue in 18.75 ml acetic acid and 12 ml methanol, Make volume upto 2.50 ml with
viii) Destaining solution	Mix 10 ml Acetic acid and 30 ml methanol. Make up the volume to 100 ml.

Figure 1: Preparation of solutions.

sugar in a ratio of 10: 3: 3 respectively, some amount of water added to make it a uniform mixture in a texture of horse dung and put it in an open place for wondering.

House flies freely to visit thereon. Thereafter, the pots were brought into the laboratory and kept for 2 to 3 days in a cage for egg hatching. Housefly larvae were reared under aseptic conditions on a basic diet with amended procedure described by [37]. Larvae took around 6 days to become full grown, full grown larvae were removed from the medium and allowed to pupate in separate covered glass bottles at 29-30 °C. After having established a good supply of desired aged Insects, early 3rd instar larvae were drawn out and treated in the batches of 10 each in separate culture bottles, supplies with 3 grams bananas mixed with desired amount of lead acetate. Doses of lead acetate were used as, 0.25 mg, 0.5 mg, and 1.0 mg. A batch of untreated three bottles was kept as control. larvae were exposed in each bottle for 48 hours. After that mortality of larvae in each bottle was observed. Surviving larvae were kept in separate bottles on lead free banana diet up to emergence. During that period effects of lead acetate on pupation and adults of under test insect was observed.

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

Reagents and Chemicals	Brand
N,N,Methylene bisacrylamide	Fluka
Acrylamide	Fluka
Tris (hydroxymethyl) aminomethane	Fluka
Ammonium persulfate	Merck
Sodium dodocylsulfate	Fluka
HCL	Merck
Glycine	Fluka
Bromophenol blue	Merck
TEMED	Merck

Figure 2: Reagent and chemicals are shown in Figures **1** and **2** respectively.

Preparation of Gel

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 200µl. (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 destaining solution for removing the excess color on

Table 1: Values of Various Proteins Observed in Lead Acetate Treated and Untreated *Musca domestica* Larvae

Protein	Rf	Egg Albumin control	*Musca domestica* Normal (untreated)	*Musca domestica* (treated)
	0.03		+	-
	0.04	+	-	-
	0.28		-	+
	0.45		-	+
	0.46		+	-
	0.61		-	+

Protein: + = present; - = absent; Rf= Relative flow.

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 compariso.

RESULTS

The relative flow (rf) of the protein of *Musca domestica* (untreated) were found to be 0.03, 0.08, 0.18, 0.27, 0.35, on the other hand the relative flow (rf) of Protein of *Musca domestica* (treated) indicated the values as 0.08, 0.18, 0.28, 0.35 and 0.45, respectively.

As shown in (Table **1**) Protein rf. 0.03, 0.04, and 0.46, have not been observed in treated *Musca domestica*. While protein relative flow (rf) 0.28, 0.45 and 0.61, have been dectected as altered in *Musca domestica*.

Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Musca domestica* shown in (Figure **3**).

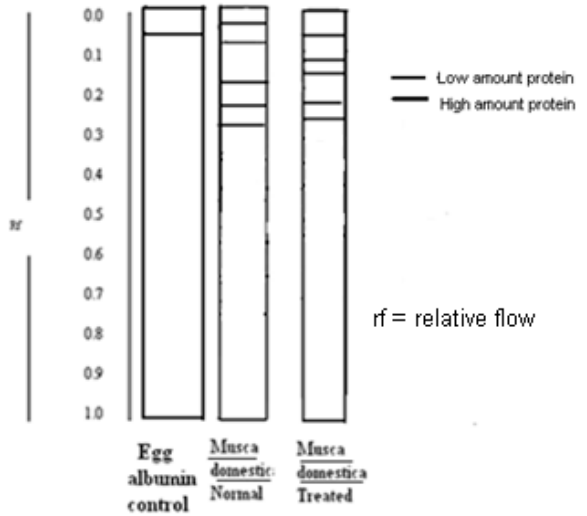

Figure 3: Electrophoratic expression of various proteins flow as compared to egg albumin in treated and untreated *Musca domestica.*

DISCUSSION

Protein I (rf 0.03) is found in Musca domestica (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated Musca domestica, is absent at the same rf. This suggests that the protein (rf 0.03) was affected with some alteration in insect. Protein (rf 0.28) was found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Bactrocera dorsalis*, was absent at the same rf. This suggests that the protein (rf 0.28) was affected with some extend. Protein (rf 0.45) is found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the untreated *Musca domestica*, was absent at the same rf. This suggests that the protein (rf 0.45) is changed with some alteration in the treated insect. Protein (rf 0.46) was found in *Musca domestica* (untreated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Musca domestica*, was absent at the same rf. That suggest that the protein (0.46) was affected at a low extend. Protein (rf 0.61) was found in *Musca domestica* (treated) that is seems to be lighter than the egg albumin, while corresponding protein, in the treated *Musca domestica*, was absent at the same rf. That suggest that the protein (0.61) was affected at a low extend.

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. [38], indicated cellular damage in processes of lead exposed to PC-12 cells. [39], reported that heavy metal compounds divergence is evidently a extensive observable fact in invertebrates. After lead exposure the Nacetyleysteine (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 [40], indicated that, lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10% [41], indicated that when lead incorporated by cells, it produces free radicals, H2O2 and ·OH [42], found free radicals can also produce simple breaks in the DNA chains these results resembled with present finding that exposure of lead produced the abnormal morphological effects in the larvae and the adults emerged therefrom [43] 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. 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 d3 was observed. These adverse effects of heavy metals on diptera are in the line with the present findings. [44] found morphological changes in wild Drosophila species that found over almost all of Europe, under the effects of lead. 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.

The present observation showed that lead acetate induced there effects mostly on the morphology and development of the under test insects. On the other hand, the differences due to increase in concentration of lead acetate influenced various changes as compared to control.

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