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Research Article

EFFECT OF LEAD ACETATE ON THE DEVELOPMENT OF *CHRYSOMYA MEGACEPHALA* (DIPTERA: CALLIPHORIDAE) AND IMPLICATIONS FOR ESTIMATING POSTMORTEM INTERVAL

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A B S T R A C T

The present study was conducted to investigate the lead acetate impact on the larval development of *Chrysomya megacephala*. Larvae of *Chrysomya megacephala* reared on rat carcass treated with different concentrations of lead acetate; Half lethal, Lethal and Twice Lethal by intraperitoneal injection. It was also observed that the concentrations of lead acetate in the immature stages of *C. megacephala* were lower than their concentrations in the visceral organs and blood of lead acetate dosed rats. The development rate of larvae between treated group and control group varied significantly. Development took longer time in the presence of high lead acetate concentration compared to control. Mortality results indicated greater mortality among the larvae with increased lead acetate concentration as compared to control. An estimate of the postmortem interval based on the normal development of *C. megacephala* could have an error of upto 8-80 hours.

INTRODUCTION

Lead does not have any known biological use but is widely used in the industries (Mielke *et al.,* 1999; Gaw *et al.,* 2006). Exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing young and infants being more sensitive than adults (Raymond *et al*.,2011). Lead can cause the following human diseases: renal failure, hypertension, anemia, disorders of genital organs, nervous system disorders (Ahamed and Siddiqui, 2007). Lead has a tendency to accumulate in bones and concentrate in the bloodstream during hemopoietic processes, thus affecting not only the condition of mature organisms, but also the development of embryos. Lead can affect the genital organs of foetuses (Apostoli and Catalani, 2011).

Lead has been found to have a definite cytogenetic effect (Tachi & Nishime, 1975; Michailova, 1987a&b; Short, 1990; Wilson, 1995; Watson, 1999; Walter, 2000; Porter, 2002; Ramel, 2003 and Margim 2005). Ions of lead bind with ferments of cells responsible for metamorphosis of insects, damaging mitochondrial cristae, thus decreasing synthesis of ATP. Heavy metals are accumulated in plants and transported through trophic chains, maintained by the soil-adsorption complex (Bessonova *et al*., 2015).

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Larvae of many synanthropic diptera at the stage of puparium dwell in the litter and upper layers of the soil. Therefore, they can be a mediator in the processes of migration and accumulation of pollutants (Shulman *et al.,* 2017). Besides, blowfly larvae (Calliphoridae) fulfil an important ecological function in the decomposition of animal remains (Shulman *et al.,* 2017). They are used extensively in forensic entomology, predominantly to establish a minimum time since death, or a minimum post-mortem interval, using the larval parameters as a "biological clock" (Donovan *et al.,* 2006). Blow flies are among the first insects to discover and colonize animal and human remains (Greenberg, 1991; Donovan *et al.,* 2006). The objective of this study is to evaluate the effect of lead acetate larval development *Chrysomya megacephala and* implications for estimating postmortem interval.

MATERIAL AND METHOD

Stock colony was populated from the wild type specimens (*Chrysomya megacephala)* collected with the help of sweeping net from Punjabi University Campus, Patiala (29°49' and 30°47' north latitude, 75°58' and 76°54' east longitude).

Female Sprague-Dawley laboratory rats (100-110 g) were used for experiments that were previously exposed to in different concentrations: Half lethal, Lethal and Twice Lethal by intraperitoneal injection (Institutional Animal Ethical Committee, vide letter no. 107/99/CPCSEA-2010-25). Newly hatched larvae (250-300) were obtained from these laboratory stock colonies and allowed to feed upon the rat carcasses LA1 (Half Lethal), LA2 (Lethal) and LA3 (Twice Lethal). Controls

were also maintained to compare with the treated group in order to study the effect of Lead acetate only leaving the other factors unaltered (food, temperature humidity etc.). Time of hatching was noted and subsequently development time was noted for each larval instar (1st, 2nd and 3rd), postfeeding larvae, pupa and time of emergence of adults. 10 larvae were randomly collected at 8 hour intervals each. Two replicates were done and the results combined for analysis.

Data Analysis

Data were analyzed using Arithmetic Mean, Standard deviation, Analysis of Variance (ANOVA), Pearson correlation coefficients and Chi square test. Graphs and tables were prepared using Microsoft Excel 2007.

RESULT

Effects of Lead Acetate on Larval Length and width

The development length data shows (Fig.1) that larvae in the control set attained maximum length at 64 hours i.e. 15.92 ± 0.27 mm. Larvae in the LA1 group attain mean maximum length earlier than other treated groups i.e. 15.67 ± 0.75 mm in 72 hours, while the maximum length for the larvae from higher dose in LA2 and LA3 was recorded at 88 (15.52± 0.10 mm) and 104 hours (14.8 ± 0.86 mm). There are significant differences in the lengths of the larvae feeding upon different doses of Lead acetate and the time required to reach the maximum length (F= 86.139; p=0.000).

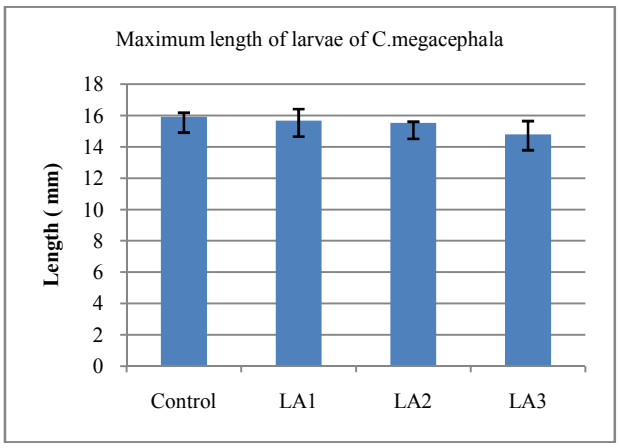

Fig 1 Bar graph showing maximum mean length of larvae of *C. megacephala* feeding upon different dosages of Lead acetate treated rats antemortem.

The development width data shows (Fig.2) that mean maximum width of control set was 3.75 ± 0.41 at 64 hours. The maximum width for the larvae feeding upon the treated group LA1, LA2 and LA3 was recorded at 72 (3.7± 0.16), at 88 (3.45 ± 0.045) and at 104 (3.25 ± 0.01) hours respectively. A decrease in mean larval width is observed in all of the treatment sets. There are significant differences in widths of the larvae feeding upon the different doses of Lead acetate and the time required to reach the maximum width (F=4.124; p=0.000) .

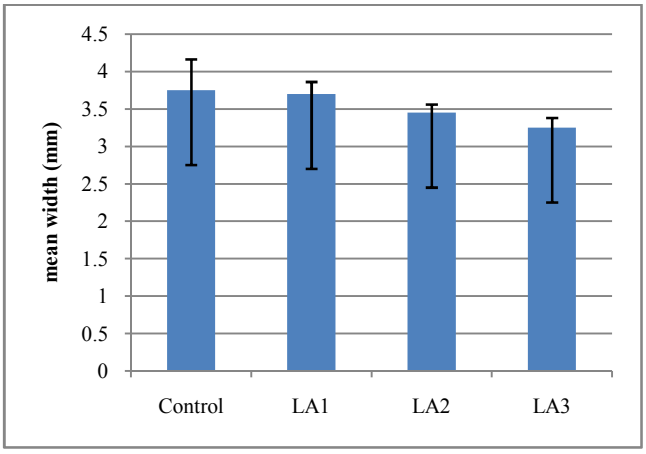

Fig 2 Bar graph showing mean maximum width of larvae of *C. megacephala* , feeding upon different dosages of Lead acetate treated rats antemortem**.**

Effects of Lead acetate on larval,pupal and adult weight

The development curve generated from the larval weight data is illustrated in Fig.3. The curve shows that mean maximum weight of larvae, feeding upon control is 70.25 ± 2.46 mg and is attained in 64 hours. The maximum weight for the larvae from treated group LA1, LA2 and LA3 is 68.6± 2.14 mg, 65.15 ± 1.24 mg and 60.5 ±1.36 mg recorded at 72, 88 and 104 hours respectively. There are significant differences in the weights of the larvae feeding upon the different doses of Lead acetate and the time required to reach the maximum weight (F= 61.136 ; p=0.001).

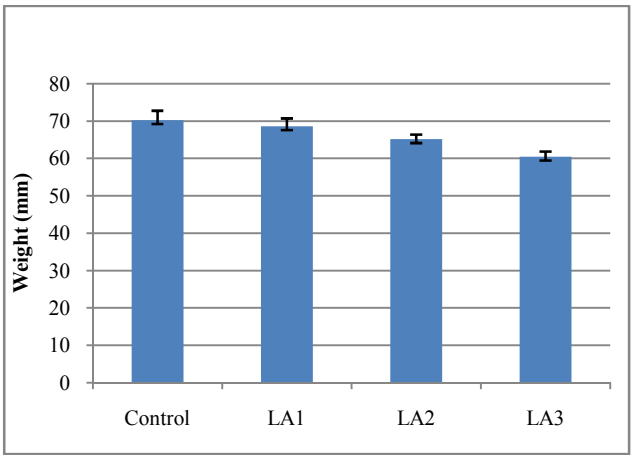

Fig 3 Bar graph showing maximum weight of larva of *C. megacephala* feeding upon different dosages of lead acetate treated rats antemortem.

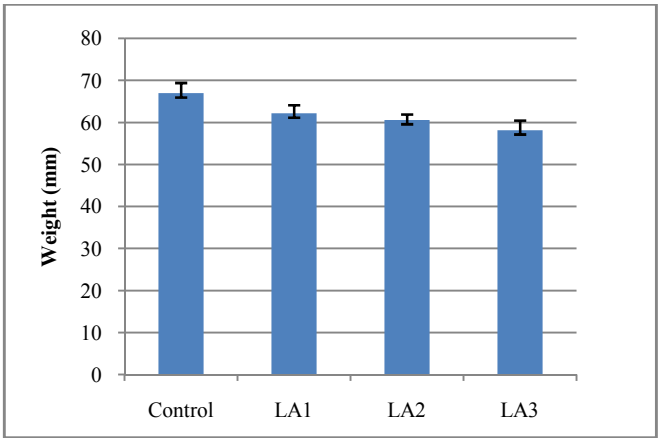

Fig 4 Bar graph showing maximum pupal weight of *C. megacephala* feeding upon different dosages of Lead acetate treated rats antemortem.

The maximum pupal weight (66.98 ± 2.46 mg) was observed in the control, while, minimum pupal weight (58.17 ± 2.29 mg) was observed in the LA3 treatment. The maximum adult weight was observed in LA1 and LA2 i.e. 69.16 ± 1.99 mg and 60.06 ± 1.34. The maximum adult weight (61.74 ± 1.45 mg) was observed in the control, while, Minimum adult weight (49.5 ± 1.21 mg) was observed in the LA3.

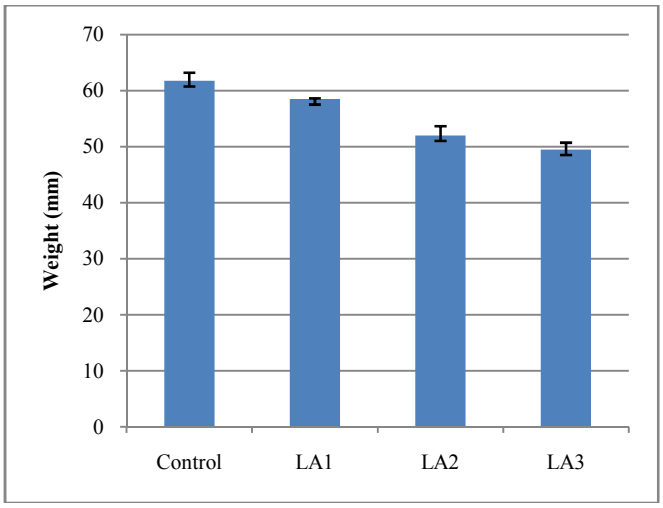

Fig 5 Bar graph showing maximum adult weight of *C. megacephala* feeding upon different dosages of Lead acetate treated rats antemortem.

Effects of lead Acetate on Larval, Pupal Mortality

Results of Pearson's Chi square analysis suggest that the number of deaths within each generation of experimental flies (in larval and pupal stages) is dependent upon treatment group. In the control group there were no significant differences (X^2 = [p =0.23,df= 3,]= 2.63), but with increase in the concentration of Lead acetate larval and pupal mortality was increased i.e. LA1 (X^2 = [p =0.563,df= 3,]= 1.34), LA2 (X^2 = [p =0. 673,df= 3,]= 0741) and LA3 (X^2 = [p =0.653,df= 3,]= 2.84).

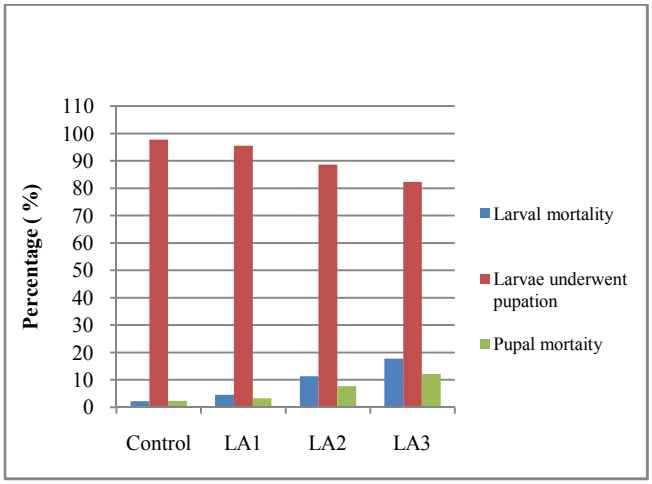

Fig 6 Bar graph showing larval mortality, pupation success and pupal mortality of *C. megacephala,* feeding upon different dosages of Lead acetate treated rats antemortem.

Larval mortalit.y was highest in LA3 (17.72%), whilst lowest mortality was in the control group (2.27 %). With increasing dosage of Lead acetate, the larval mortality was higher. Similarly, highest pupal mortality was observed in LA3 (12.15%). The highest percentage of successfully emergent adult flies was observed for colonies reared on control group (97.67%), while the lowest percentage was observed in the LA3 87.84 %). Results of Chi square analysis was conducted to evaluate differences among the four treatments (Control, Half Lethal, Lethal and Twice Lethal) on median total mortality. The mortality increased correspondingly, as the dosage of Lead acetate was increased (Pearson correlation coefficient (R) between total mortality and dosage of lead acetate = 0.984.

Duration of Development of C. megacephala

The presence of Lead acetate in decomposing tissues was a deterrent to development and this caused a prolongation of time needed for development for all the immature stages. (Table. 1). The total time taken to complete development of control group is earlier than treated group i.e. 184± 2.73 hours, while LA1, LA2 and LA3 completed total development in 192± 2.42, 232± 1.27 hours and 264 ± 7.23 hours respectively. The durations of development were statistically different in various dosages (F= 50, 619.34,p=0.000).

Pearson correlation coefficients (R) between time spent by the larvae in each developmental stage and dosage of lead acetate illustrated. Data indicates that there was positive correlation observed between all the dosages of Lead acetate and total duration of development.

DISCUSSION

Chrysomya megacephala is a dominant fly in the forensic field. Larvae of *Chrysomya megacephala* can serve in the estimation of postmortem interval and also be used in qualitative identification of drugs or toxins (Lord, 1990), and it is one of the dominant carrion flies in India (Singh and Bharti, 2000). Their preference for a fresh corpse gives members of this species a high priority at crime scenes, whenever they are encountered (Smith,1986). Morphometric parameters and biological features are widely used by forensic entomologists to establish the minimum time elapsed since death (Bharti, 2009; Chen *et al*., 2011).

After examining the results related to the morphological changes in larva, it has been observed that generally the higher the Lead acetate concentration, the smaller the larval size. It means that the average length and width in the examined samples compared to the control group has been reduced (Figure 1, 2). The observations are similar to the Safaee *et al*. (2014) who studied effects of lead on the development of *Drosophila melanogaster*.

Table 1 Showing the Mean (±SD) duration of development of larvae of *C. megacephala,* feeding upon different dosages of Lead acetate treated rats antemortem.

Dosage of lead acetate	Duration of first instar(hours)	Duration of second instar(hours)	Duration of third instar (hours)	Duration of post feeding stage (hours)	Duration of pupal stage(hours)	Duration of total development (hours)
Control	8±0.009	16± 0.05	40± 1.04	24± 0.08	96± 0.02	184 ± 2.73
LA1	8± 0.07	24± 0.21	40± 0.62	32± 0.31	88± 1.45	192 ± 2.42
LA2	16± 0.05	24± 0.11	48± 0.21	40± 0.11	104± 1.5	232 ± 1.27
LA3	16± 0.12	32± 3.56	56± 0.27	48± 6.5	112± 1.16	264 ± 7.23
Analysis of variance	**F= 59,406.09 P= 0.00 Df= 3**	**F= 12,851.70 P= 0.00 Df= 3**	**F= 67,923.01 P= 0.00 Df= 3**	**F= 8,591.54 P= 0.00 Df= 3**	**F= 67,412.59 P= 0.00 Df= 3**	**F= 50,619.34 P= 0.00 Df= 3**

The results show that with increasing the lead concentration, the average length and width of the larvae is significantly reduced. Obviously, a small larva will become a small pupa, further will affect weight. Present result showed significant differences in the weights of the larvae and pupa. Control larvae attained maximum weight as compared to the treated group (Fig 3,4,5). The size reduction of adults is probably caused by the negative effect of lead on the production of growth hormones, enzymes functions, metabolic genes and their expression (Al-momani and Massadeh, 2005).

As a result, the negative effects of Lead acetate decrease the survival potential of larvae and showed higher mortality (Fig.6). Parke *et al*. (1991) have shown that after the exposure of lead acetate the death rate increased with the increase in the dose of the chemical compound. Present results are also in agreement with Ferhat *et al.* (2010) who found that the larvae of *Calliphora vicina*, had significantly higher mortality when exposed to cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni) and chromium (Cr). Also Safaee *et al*., (2014) found that the negative effects of lead decrease the survival potential of larvae and thereby decreasing the rate of larva transforming to pupae.

Lead after being swallowed induces its toxic effects on the body of larva and decreases its growth, delaying the development of the insect (Al-momani and Massadeh, 2005; Roeterdink *et al*., 2004). In the present study, similar results were observed. The increased concentrations of Lead acetate increases the developmental period (Table 1). Duration of development was increased in Lead acetate treated group as compared to control group. Higher concentration of Lead acetate reduced the ability of ATP synthesis that affect the metamorphosis as Malecka *et al.,* 2001 observed in their experiment that Lead can reduce the cristae space of mitochondria which can result in the reduction of oxidative phosphorylation and ATP synthesis that when the larvae have reached the pupation stage, due to the existing problems, they need more time to pupate (Safaee *et al.*, 2014).

CONCULSION

All developmental stages of *Chrysomya megacephala* except adults are reliable for toxicological analysis of Lead acetate. There was a significant increase in mean larval growth period between lead acetate treatment groups and control group. Duration of development was increased in lead acetate treated group as compared to control group. The time of death on the basis of insect evidence is generally determined by estimating the age of the maggots from a dead body. Since Lead acetate alters the rate of development in *Chrysomya megacephala*. There are chances of miscalculation of PMI if the presence of Lead acetate is not taken into consideration. For example there could be wrong estimation of PMI by up to 8-80 hours if age of larvae is determined on the basis of its length ignoring the effect of lead acetate. Hence the investigator must take into consideration the presence of chemical in the larval food that may affect the rate of development.

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