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Original Article

Impact of Methamphetamine on the Development of the Forensically Important Species *Lucilia sericata* (Diptera: Calliphoridae)

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Abstract

Background: Drugs or poisons can affect the larvae's developmental period in cadavers, leading to variations in the post-mortem interval (PMI) calculation. One of the most misused psychostimulant drugs in Asia and North America, Methamphetamine (MA), is commonly involved in forensic entomotoxicological situations. This study investigated the impact of various MA concentrations on the developmental rate, morphology, and behavior of *Lucilia sericata*.

Methods: *Lucilia sericata* was maintained at 28 °C and 50±10% humidity in the Calliphoridae breeding laboratory at the School of Public Health, Tehran University of Medical Sciences. Chicken liver substrates with varying concentrations of MA (0, 45, 90, and 180 ng/mg) were used to produce *L. sericata* eggs. Ten samples were randomly selected from each treatment group for morphological analysis, including weight and size measurements, at each sampling stage (every 12 hours up to 300 hours). With a significance level of $p< 0.05$, the General Linear Model (GLM) Mixed Model ANOVA was used to compare the data.

Results: The development time of *L. sericata* was shown to be changed by Methamphetamine concentrations, which decreased by 24 hours at 90 and 180 ng/mg. Compared to the control group, larvae and pupae lengths decreased by 1.22 and 0.7 mm, respectively, at 180 ng/mg. Additionally, the weight of the larvae and pupae in the concentration mentioned above decreased by 7.52 and 7 mg, respectively, in comparison to the control group.

Conclusion: The PMI is estimated incorrectly if the presence of Methamphetamine in the corpse is disregarded.

Keywords: Entomotoxicology; *Lucilia sericata*; Methamphetamine; Post-mortem; Drug testing

Introduction

In the field of forensic entomotoxicology, researchers examine how drugs or toxins affect the developmental rate at which necrophagous insects colonize dead bodies and how to use these insects as substitute matrices in cases where the corpse is severely decomposed or skeletonized (1). According to Goff et al. (2) and George et al. (3), chemicals have an impact on these insects' developmental cycle through food intake or food chains that are transmitted by necrophagous insects. This reduces the accuracy of PMI estimation and previous studies have shown that in the larval stage of *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae), codeine appeared to accelerate the larva's development, whereas morphine appeared to retard the larva's development during this time (4, 5). The great differences between the effects of various drugs on the development of the same flies are demonstrated by these in-

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vestigations. Therefore, the application of drug effects on fly development is crucial to the estimation of PMI (6). Methamphetamine was developed early in the 20th century from its parent drug, amphetamine, and was used originally in nasal decongestants and bronchial inhalers. Methamphetamine causes increased activity and talkativeness, decreased appetite, and a pleasurable sense of well-being or euphoria (7). Meth is the slang word for methamphetamine (MA), which is frequently abused in social contexts such as bars and parties (8, 9). Methamphetamine produces stimulant effects on the central nervous and cardiovascular systems and is more lipid soluble than amphetamine. It also crosses the blood-brain barrier with ease (9, 10). Moreover, prolonged misuse of MA can lead to fatal consequences as well as severe physical and mental illnesses (11). Since 1999, the number of deaths linked to MA abuse has quadrupled, and the number of deaths from MA abuse has been continuously increasing in North America and Asia, according to the 2018 United Nations Office on Drugs and Crime (UNODC) Report (12). As a result, by offering national mortality data, forensic entomotoxicological studies frequently expand on research on MA-related deaths. Using RIA (Radio Immune Assay) as the analytical method, Goff et al. (13) first observed the effects of MA on *Parasarcophaga ruficornis* (Fabricius, 1794) (Diptera: Sarcophagidae) and demonstrated that this compound accelerated the larvae's rate of development. To investigate the impact of MA on the rate of development, Mullany et al. (14) focused on *Calliphora stygia* (Fabricius, 1781) (Diptera: Calliphoridae), and they found that MA increased the size of all life stages and accelerated larval development. Magni and colleagues (15) also discovered that while MA did not affect the rate of larval development, the entire developmental period in *Calliphora vomitoria* (Linnaeus, 1758) (Diptera: Calliphoridae) was observed to be longer for those reared on livers treated with MA. Goff et al. (13) reiterated that a given drug may have varying effects on the development of different fly species at different times. The average length of larvae exposed to methamphetamine concentration was greater than that of the control group, and the development of *Aldrichina grahami* (Diptera: Calliphoridae) species until pupation was statistically slower than that of the latter. Additionally, pupae exposed to the highest concentration of methamphetamine had an average weight that was noticeably lower than that of the control group (16). The fly species *L. sericata* is of forensic importance as it is frequently discovered on human remains in Asia and Europe in the early stages of decomposition (5). In English, *L. sericata* (Diptera: Calliphoridae) is commonly known as green bottle flies. Adult flies are metallic green and rarely exhibit a coppery or yellowish sheen. Females lay their eggs either within a mass or near a food source. After going through three stages of feeding and molting in the soil, the larvae seek a suitable location to pupate. Whereas development is rapid during hot weather, it can take months during unfavorable seasons (17). In the larval stage, overwintering occurs frequently. Even though they can also breed in excrement, carcasses are the most common habitat in which they develop. This species inhabits various geographical regions and is mainly active during the warm temperate seasons (17). The impact of stimulants belonging to the family Amphetamine (Methamphetamine) on the various biological stages of *L. sericata* in forensic medicine and PMI calculations has not been studied anywhere in the world until now. According to Bailey and Shaw (18) and Chaturvedi et al. (19), the human lethal dose was used to determine the MA spiking concentrations. The primary objective of this study was to examine how MA influenced the development rate, morphology (including the length and weight of larvae and pupae), and survival of *L. sericata*. Additionally, the study investigates the behavior of these organisms, alongside their morphology and life cycle.

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Materials and Methods

Lucilia sericata breeding and food preparation

A temperature and humidity-controlled environment (28 °C and 50±10% RH) was used for the culture of *L. sericata* flies in the Calliphoridae breeding laboratory at the School of Public Health, Tehran University of Medical Sciences. In this investigation, 98% of the methamphetamine used was supplied by the Tehran Province Forensic Medicine Organization, and fresh chicken liver was used to feed *L. sericata* larvae. The eggs of *L. sericata* were placed on chicken liver substrates that had been spiked with varying amounts of MA in this investigation (0, 45, 90, and 180 ng/mg, simply referred to as C, T1, T2, and T3, respectively).

The steps involved in making minced chicken liver were as follows: To make sure the mince was uniformly homogenized, the chicken liver was first blended for 20 minutes in four different blenders. The mince was divided into four equal groups of 1000 g each, and each group was further split into two 500 g subgroups after dissolving three different MA dosages (45, 90, and 180 ng/mg) in 10 cc of saline solution, each subgroup was again blended for 20 minutes using four blenders. The control mice received only saline solution treatment (16). Colonies of *L. sericata* were maintained in insect cages measuring 30 cm on each side. The insectarium was equipped with natural light, maintained a temperature of approximately 28 °C, and had a relative humidity of 50±10%. According to studies by Wang et al. (16), Zhu et al. (20), and Bugelli et al. (21), 150 g of fresh chicken liver was provided as a medium for egg collection. To ensure an adequate supply of eggs for the experiment, they were collected within a three-hour window.

Once collected, the eggs were divided into four groups, and one of the three MA concentrations was added to each group's chicken liver mince (16).

Morphological analyses

Collecting larvae and measuring length and weight

Ten larvae were randomly selected from each treatment group for morphological analysis, which included weight and size measurements at each sampling stage every 12 hours, up to 300 hours.

From the first larval stage (L1) to pupae, sampling was conducted. Before testing, the samples were stored in a refrigerator at 6 °C and treated with 75% ethanol after being submerged in hot water (over 80 °C) for 30 seconds. According to Bugelli et al. (21), the processed samples can be used for up to two weeks. Fresh food was added to the petri dish and larvae containers every 12 hours during the sampling process.

Using a stereomicroscope, the number of terminal respiratory pores was counted to determine larval age (22). Subsequently, digital scales and calipers were utilized to measure the characteristics of both the larvae and pupae.

Pupa and adult emergence times were reported for every treatment, including the weight and length of the measured samples and the average values for each group.

Both the weight and the length of the larvae and pupa were measured using a caliper and a Mettler Toledo electric scale with a sensitivity (readability) of 0.00001 g.

Statistical Analysis

Following each sampling, the growth period, weight, and body length of *L. sericata* larvae and pupae were noted. SPSS version 24.0 software was used to perform analyses for each treatment.

Data were compared between methamphetamine-treated and control groups in terms of larval weight and length using General Linear Model (GLM) Mixed Model ANOVA (Repeated Measure ANOVA) at a significance limit of $P< 0.05$.

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Statistical analysis was conducted using mean values. GraphPad Prism version 8.0.2 was also utilized to create all three-dimensional graphs.

Results

Effects of Methamphetamine on the behavior of *Lucilia sericata*

The treated larvae with MA were more mobile and appeared to be more active during the first sampling period (the first 12 hours). In addition to the control group's lower larval activity, some of the eggs in that group did not hatch. Larvae in all three treatment groups grew faster than those in the control group when sampling was done at 72 hours. Samples of the pre-pupa stage were observed in the treated group after 120 hours of testing, and most larvae of the three treatment groups had developed into pupae in 132 hours; in contrast, all of the larvae in the control group were in the pre-pupa stage.

Whereas the adult flies in the control group were observed after 284 hours of sampling, the adult flies of *L. sericata* were visible in the cages of treatment groups 2 and 3 at 260 hours of sampling. Interestingly, the adults in treatment groups 2 and 3 drank a lot of water (their water container was half empty in less than 12 hours) and remained very withdrawn and isolated, sticking to the cage walls. There was no flying at all. Methamphetamine's effect on the flies in treatment groups 2 and 3 was evident in their behavior, as they became incredibly irregular and nervous when placed inside the cage with water, sugar, and liver. They also left their cage nets extremely filthy. Following 300 hours from the beginning of the study, a considerable number of adult flies were observed in the control group's cage and a smaller number in the treatment 1 (45 ng/mg) cage; however, all of the adult flies were found in the treatment 2 and 3 cages. After 348 hours, the control group produced fewer offspring than the treatments; additionally, treatments 2 and 3's water and sugar containers were empty, but the control group continued to behave normally.

Effects of Methamphetamine on larval length of *Lucilia sericata*

The larval lengths of the three treatment groups and the control group were analyzed at various sampling times. The results indicate that both Methamphetamine and the interaction with time have a significant effect on larval length, with a p-value of 0.000 for their interaction effects. There was notable variation in larval length across different sampling times, and the significance level was established at p= 0.000. Table 1 shows the comparison of the groups and the methamphetamine intervention effect, along with the computation of the significance level (Sig= p-value) for each concentration compared to the others. An asterisk denotes a significant difference between the groups when the p-value is less than 0.05. Table 1 indicates that all treatments' p-values for larvae length within the control group were less than 0.05, so the differences were statistically significant. Additionally, there was a significant difference between the treatment 3 group (180 ng/mg) and the other two treatment groups (45 and 90 ng/mg), as well as the control group. A significant reduction in larval length was observed at all MA concentrations (45, 90, and 180 ng/mg). Specifically, at 180 ng/mg, the larval length decreased by approximately 1.22 mm in comparison to the control group (Fig. 1).

Effects of methamphetamine on pupal length of *Lucilia sericata*

The lengths of the pupae at various sampling times did not differ significantly, with p-values of 0.555 and 0.398. However, there was a significant difference (p= 0.006) among the three treatment groups, indicating differences between the groups and the effects of the methamphetamine intervention. Table 2 presents the analyses conducted using Tukey's HSD (honestly significant difference) test, which allowed for a precise determination of the treatment differences. Treatment group 3 (180 ng/mg) was the only group that showed a significant difference from the control group. Overall, the

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average length of the pupae decreased across all concentrations analyzed (45, 90, and 180 ng/mg). This demonstrates the differences in pupae lengths among the three treatment groups and the control group at two sampling intervals (132 and 248 hours), as illustrated in Fig. 2.

Effects of methamphetamine on the weight of *Lucilia sericata* larvae

Weight differences among larvae at various sampling times were statistically significant (p= 0.000). The weights of the larvae across the different treatment groups showed significant variation, particularly regarding the effects of the methamphetamine intervention and the differences among groups (p= 0.000). Table 3 presents a further analysis of these differences using the Tukey HSD test. The table indicates that for all treatments involving the control group, the significance level was below 0.05. This suggests a significant difference between the average weights of treated and control larvae. Additionally, treatments with dosages of 45, 90, and 180 ng/mg resulted in a notable decrease in larval weight (Fig. 3).

Effects of methamphetamine on the pupal weight of *Lucilia sericata*

In the study done on the pupae of the three treatment groups and the control group at various times, a significant difference was observed between the pupae's weight at different sampling times. In an analysis of the groups' differences as well as the methamphetamine intervention effects, a significance level of 0.000 indicates that there was a significant difference between the three treatment and control groups. To further closely evaluate the differences between the treatments and the control, Tukey's HSD test was used (Table 4). The data make this evident: although there was not a significant distinction across the treatment groups, the significance level of all the treatments with the control group was less than 0.05. There was a statistically significant difference in the pupae's average weight between the treatment and control groups. In this study, the pupa weight decreased at the 45, 90, and 180 ng/mg treatment levels. Additionally, Figure 4 illustrates the variation in pupae weight between the treatment and control groups at two different sampling times (132 and 248 hours).

Effects of methamphetamine on the life cycle of *Lucilia sericata*

Our findings indicate that larvae fed on chicken liver containing the substance MA at concentrations of 90 and 180 ng/mg developed more rapidly into the pupal and adult stages compared to the control group. Figure 5 illustrates each biological stage of *L. sericata*, starting with the egg and progressing through the three larval stages, as well as the prepupal and pupal stages. In comparison to the control group, all developmental stages showed accelerated progression after 24 hours. Consequently, the estimated time of death for these concentrations may be inaccurate by 24 hours (see Fig. 6).

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Table 1. The results of Tukey's HSD test show the difference between the treatment groups and the control of *Lucilia sericata* exposed to methamphetamine, considering its effect on the larval length

Methamphetamine concentrations	Methamphetamine concentrations	Mean difference	Sig.
Control (**0** ng/mg)	45	.646*	.003
	90	.787*	.001
	180	1.227*	.000
45 ng/mg	Control	-.646*	.003
	90	.141	.499
	180	.582*	.008
90 ng/mg	Control	-.787*	.001
	45	-.141	.499
	180	.441*	.039
180 ng/mg	Control	-1.227*	.000
	45	-.582*	.008
	90	-.441*	.039

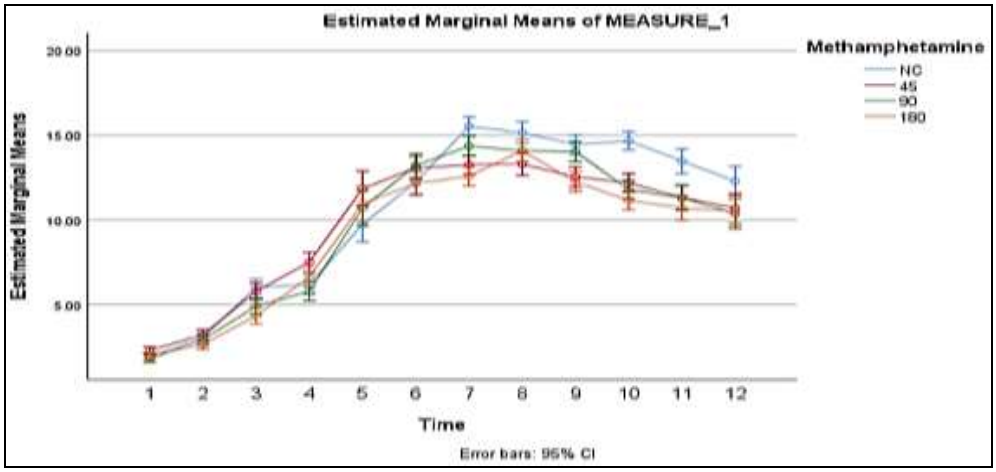

Fig. 1. The difference between the length of *Lucilia sericata* larvae (mm) in the treatment and control groups at different sampling times (Day)

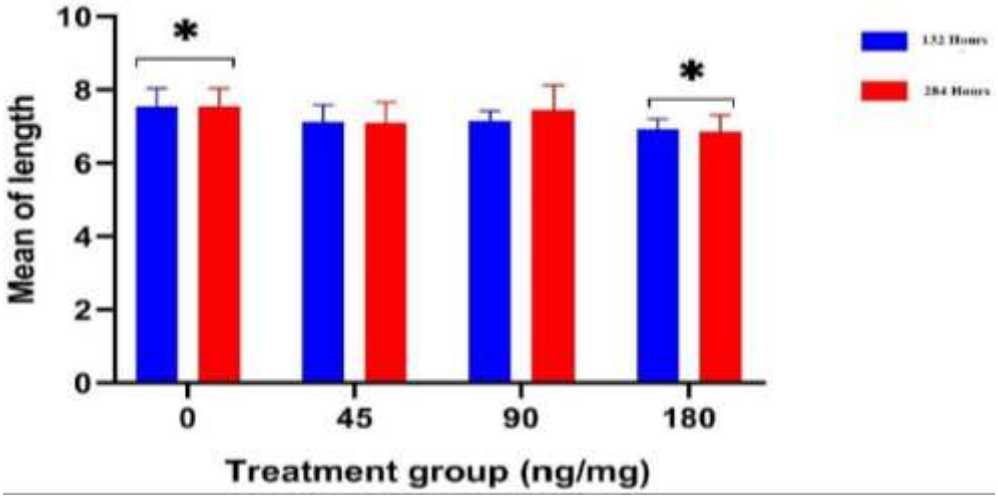

Fig. 2. The difference between the body length of *Lucilia sericata* pupae (mm) in treatment and control groups at two sampling times, 132 and 248 hours after exposure to methamphetamine

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Table 2. Tukey's HSD test results to determine the difference between the treatment groups and the control of *Lucilia sericata*, considering its effect on the pupal length

Methamphetamine concentrations	Methamphetamine concentrations	Mean difference	Sig.
Control (**0** ng/mg)	45	.4350	.096
	90	2500	.522
	180	.6650*	.004
45 ng/mg	Control	-.4350	.096
	90	-.1850	.740
	180	.2300	.590
90 ng/mg	Control	-.2500	.522
	45	.1850	.740
	180	.4150	.121
180 ng/mg	Control	.6650*	.004
	45	-.2300	.590
	90	-.4150	.121

Table 3. The results of Tukey's HSD test to more accurately examine the difference in the weight of *Lucilia sericata* larvae (mg) in the treatment and control groups

Methamphetamine concentrations	Methamphetamine concentrations	Mean difference	Sig.
Control (**0** ng/mg)	45	5.201*	.001
	90	4.238*	.004
	180	7.527*	.000
45 ng/mg	Control	-5.201*	.001
	90	-.962	.491
	180	2.326	.101
90 ng/mg	Control	-4.238*	.004
	45	.962	.491
	180	3.289*	.023
180 ng/mg	Control	-7.527*	.000
	45	-2.326	.101
	90	-3.289*	.023

Table 4. The results of Tukey's HSD (honestly significant difference) test to check the difference in the weight of the pupae (mg) between the treatment and control groups

Methamphetamine concentrations	Methamphetamine concentrations	Mean difference	Sig.
Control (**0** ng/mg)	45	6.45*	.000
	90	5.40*	.001
	180	7*	.000
45 ng/mg	Control	-6.45*	.000
	90	-1.05	.835
	180	.55	.971
90 ng/mg	Control	-5.40*	.001
	45	1.05	.835
	180	1.60	.581
180 ng/mg	Control	-7.00*	.000
	45	-.55	.971
	90	-1.60	.581

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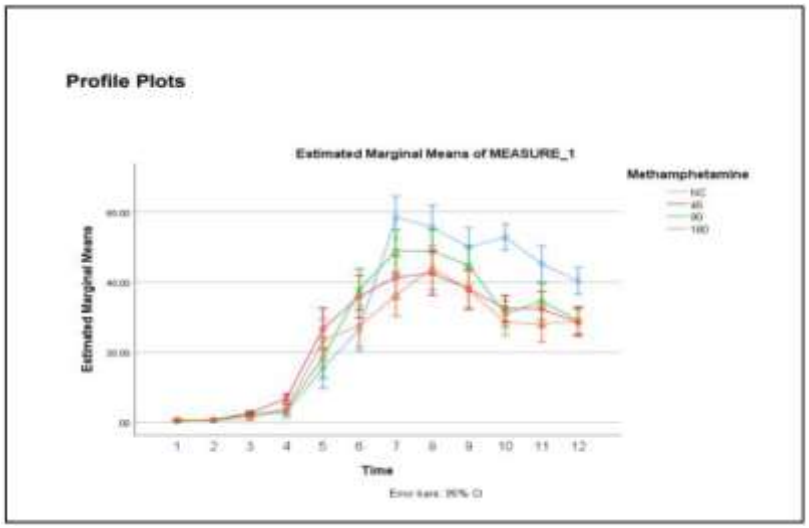

Fig. 3. The difference between the weight of *Lucilia sericata* larvae (mg) in the treatment and control groups at different sampling times (Day)

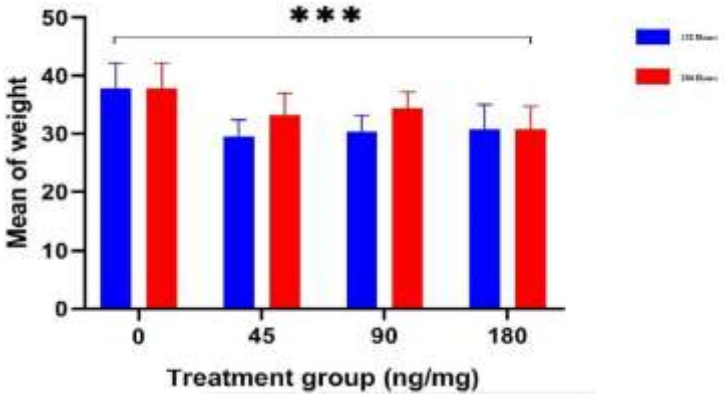

Fig. 4. The difference between the weight of *Lucilia sericata* pupae (mg) between the treatment and control groups at two times, 132 and 248 hours after exposure to Methamphetamine

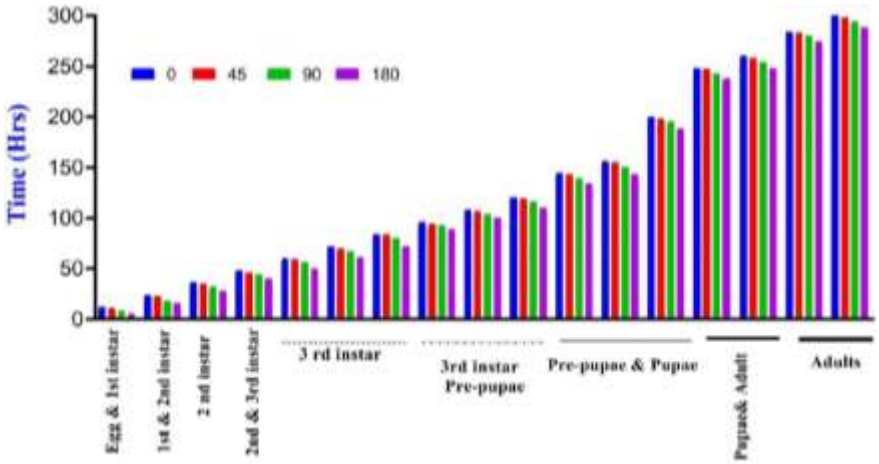

Fig. 5. The effects of different concentrations of methamphetamine (three treatment groups and the control group) on the life cycle of *Lucilia sericata* at different sampling hours (from 12 hours to 300 hours after exposure to methamphetamine)

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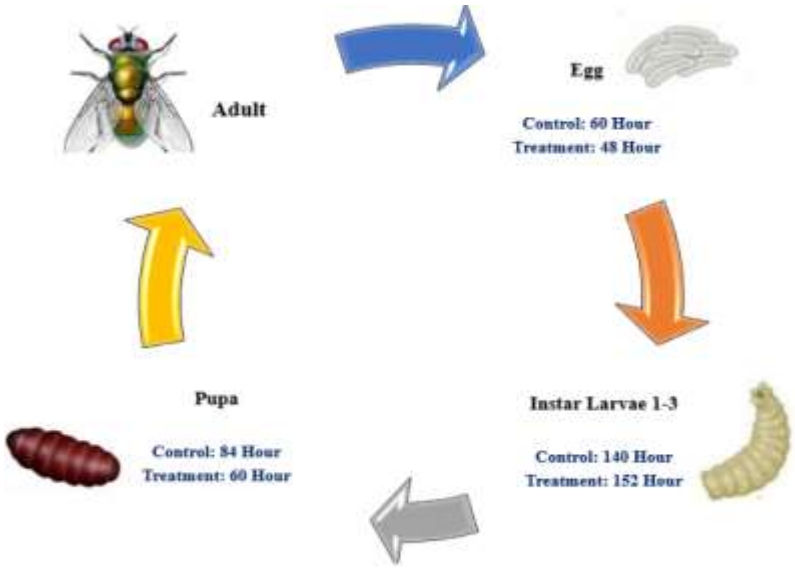

Fig. 6. The life cycle of *Lucilia sericata* in the control and treated with Methamphetamine (90–180 ng/mg) groups

Discussion

Entomotoxicology is the process of identifying drugs and other chemical components in necrophagous insects that have consumed decaying body tissues by using analytical and toxicological techniques (15). One of the newest subfields of forensic entomology is entomotoxicology, which leverages useful data from specimens of immature insects to determine the drugs and poisons that the deceased person ingested before their death. Determining the time passed since death and the impact of different drugs and poisons on the growth period of insects that feed on dead bodies is one of the primary objectives of forensic entomology (23). The growth patterns of necrophage species and potentially the precision of postmortem estimates can be impacted by certain toxins and drugs. Failing to consider the conditions that could impact the growth of necrophagous insects can result in a violation of justice since an accurate PMI estimate can be a crucial component of criminal investigations. One of the main causes of mortality worldwide, suicide, is also regarded as a public health issue. The annual number of suicide deaths is thought to exceed 700,000. Regrettably, it is estimated that 14–20% of suicide deaths (110,000–168,000 deaths) result from self-poisoning with pesticides and illegal drugs, including methamphetamine (24). Even though several studies have been carried out regarding the impact of drugs of abuse on the rate of growth and development of Diptera, the review of sources indicates that there are only four entomotoxicology studies worldwide that examine the effects of methamphetamine on necrophagic flies (13–16). There hasn't been any published research on methamphetamine's impact on *L. sericata* so far. The effect of Methamphetamine on the life cycle of the *L. sericata* fly was examined in this study. Methamphetamine concentrations changed the fly's growth time, according to the findings of our investigation. Therefore, the pupal stage started approximately 24 hours earlier with dosages of 90 and 180 ng/mg. To accurately estimate the time of death, this error should be considered in account. Additionally, the three doses used in this study, 45, 90, and 180 ng/ mg, significantly decreased the weight and length of the larvae and pupae. At the 180 ng/mg concentration, the larvae and pupae's lengths were,

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respectively, reduced by 1.22 and 0.7 mm in comparison to the control group. Additionally, in comparison to the control group, the weight of the larvae and pupae dropped by 7.52 and 7 mg, respectively, in the concentration mentioned above.

Radioimmunoassay (RIA) was used in the 1992 investigation by Goff et al. (25) to examine the effects of methamphetamine on *Sarcophaga ruficornis* growth rate. Additionally, they noticed that the larval and pupal durations were shorter as an analytical technique. Similarly, there was a reduction in the growth period from the larval to the adult stage. The calculation of the actual time of death was generally changed by variations in the growth rate up to 18 hours in the larval stage and up to 48 hours in the pupa, which was similar to our findings (13). Changes in the developmental course and growth rate were observed in another study on *Calliphora stygia*, where the larval and pupal stages completed up to 44 hours faster (14). The results of this investigation aligned with the findings of our own research as well. In a different study on the *Aldrichina grahami* species, the average length of larvae exposed to methamphetamine concentration was higher than that of the control group, and the growth to pupal age was significantly delayed (16). According to Wang et al. (16), the pupae exposed to the highest dose of methamphetamine had an average weight that was noticeably lower when compared to the control group. In terms of growth and development and larval length, the results of this study did not agree with our results; however, in our investigation, the weight of treated larvae and pupae significantly decreased. Thus, it may be stated that methamphetamine's effects on many species remain constant. Methamphetamine's action as a stimulant for weight loss and fat burning in humans, which increases energy and decreases the need for rest, is thought to be responsible for the weight reduction of larvae and pupae. Naturally, this energy is false, and methamphetamine users experience a high level of internal metabolism. As a result, metabolism accelerates, fats burn more quickly, and growth and development occur more quickly (26, 27). All of this information should be considered to account for a more realistic assessment of PMI employing necrophagous larvae, as some investigations have revealed that different species, and even individuals within the same species, respond differently to certain poisons and drugs (28). Different drugs have varying effects on *L. sericata*. The effect of Cocaine on *L. sericata* larvae was investigated by Introna et al. (29). They found that the larvae fed in the nasal cavity of cocaine users were longer than control larvae by more than 8 mm (28). According to Hecht et al. (30), a study on *L. sericata* revealed that larvae exposed to methadone had an increase in length and a decrease in growth and development speed of roughly 15 hours. Additionally, the effects of Tramadol on *L. sericata* were observed as resulting in a considerable increase in the length of larvae as well as the species' rate of growth and development (31). About the physicochemical properties of the drugs (or poison), the physiological characteristics of insects, and the pharmacokinetic behavior in both humans and larvae, all of this information demonstrates that the response of individuals within the same species is entirely different and specific (4). Consequently, special attention should be given to the impact of drugs including methadone, tramadol, heroin, methamphetamine, as well as tranquilizers, on the life cycle of insects to estimate the time of death by looking at the life cycle of insects. Because the pupae had no eating or activity, no significant difference in pupae length was found at different collecting periods in each treatment concentration ($p= 0.55$). However, at varying concentrations, the treatment group's pupae had shorter average lengths than the control group. Additionally, the treatment group's pupae weighed much less than the control groups, making them lighter. Pupae's ability to burn fat is linked to its ability to reduce weight at different concen-

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trations, as we have already stated. Adult treatment groups 2 and 3 (90 and 180 ng/mg) were much more active than the control group, as the results of this indicated. Occasionally, they became attached to the cage walls and refused to fly. The effects of methamphetamine on the brain and the rise in monoamine neurotransmitter levels may be the cause of all of these behaviors (32, 27). The changed eating patterns of *L. sericata* adults, which included a strong desire to consume sugar and water, were another interesting observation of this study. Methamphetamine causes abnormalities in eating patterns and an increase in the desire to consume sweets, as well as disrupting the electrolyte balance in humans (27, 26).

Conclusions

The field of entomotoxicology has gained popularity due to findings that drugs accumulated by various fly species can influence larval development and behavior. This is crucial from a forensic perspective because a variety of fly species are capable of determining a deceased person's PMI; however, since drugs can change these flies' developmental patterns, the PMI can be changed as well. An inaccurate PMI estimate results if the corpse's methamphetamine content is disregarded. The impact of drug usage on the development of flies, including *L. seicata*, is regarded as an essential element in estimating PMI. Future research will concentrate on how MA affects flies developed under different temperatures, providing an improved understanding of the *L. sericata* development data.

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Ethical considerations

Research ethics approval ID: IR.TUMS.SPH.REC.1399.027IR.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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