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Assessment of Post Mortem Interval, (PMI) from Forensic Entomotoxicological Studies of Larvae and Flies

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Abstract

Current research involving studies of the effects of drugs on fly's development has also allowed better estimations of Postmortem Intervals (PMI) or cause of death. Insects' specimen collected from decomposing or decomposed bodies enable forensic entomologists to estimate the minimum Post-Mortem Interval (PMI) or cause of death. Drugs within a corpse may affect the development rate of insects that feed on them. This study consequently points out the possibilities of analyzing insects, larvae and pupae or empty pupa cases when suitable tissues or other source of information's are absent. This study investigated the effects of commonly abused drugs on growth rates of insects. These results can be used by the forensic entomologist in forensic entomology for the postmortem interval (PMI) estimation or cause of death of decomposed bodies or tissues in medico criminal investigations.

Keywords: Drugs; Insects; Decomposed bodies; Forensic entomology; Postmortem interval (PMI); Medico-criminal investigations

Introduction

Forensic Entomotoxicology includes the study of the effects of drugs on the development rate of carrion-feeding insects [1], and the use of these as alternative sample in the absence of other tissues. Most forensic Entomotoxicological studies have concentrated on commonly prescribed drugs. Insect specimens collected from decomposing bodies enable forensic entomologists to estimate the minimum post-mortem interval (PMI). The most common application of entomological evidence in forensic medicine [2] is the estimation of the time of death, i.e., of the decomposing interval [3-7]. There are additional applications which include determination of the place of death, cause of death or detection of an ante mortem trauma [8-15].

Insects: a resource for investigating drug consumption

The accurate estimation of the postmortem interval is extremely critical to the successful completion of death investigations. At the present time, there are several methods available for estimating the postmortem interval. Forensic entomology is the well-researched method of determining the time since death in the later postmortem interval. In forensic entomology, the arthropod evidence associated with the corpse is most often used to estimate the elapsed time since death, or postmortem interval. At present, there are two approaches available for estimating the postmortem interval using insect evidence, and the application of either one largely depends on the state of decomposition of the corpse at the time of discovery by humans. The first method involves the analysis of the pattern of colonization of the carrion by successive waves of insects and other arthropods. The second method relies on the development of immature flies that are deposited on the carrion shortly after death. The use of either approach will also depend on factors such as season, climate, and location of the corpse and treatment of the corpse.

Furthermore, insects may serve as important alternative species for toxicological analysis in cases where human samples are not available for this purpose. Several publications have described the detection of toxic and controlled substances through analyses of arthropods [16-20] including different drugs. Drugs within a corpse may affect the development rate of insects [21] that feed on them For examples drugs and poisons like Morphine [14], heroin [17], Opiates [22,23], cocaine [16], barbiturates [7], clomipramine, amitryptiline [19], nortriptyline, levomepromezine and tioridazine [5,6], Diazepam [24], hydrocortisone, Sodium methohexital [25], Methadone [26], methamphetamine [27], phencyclidine [28] and Malathion [29] are commonly involved in cases where forensic entomology is used. Normal life cycle of fly is shown in table 1.

Commonly used techniques for sample detection

As for drug related deaths, gas chromatography and mass spectrometry can be performed on the fly larvae [30] to test for the presence of phencyclidine, cocaine, heroin, amitryptiline, and methamphetamine. Drugs such as amitryptiline and nortriptyline can also be detected by extracting material from the fly and beetle pupae casings, and beetle droppings using acids or bases. Selective determination of morphine in the larvae of Calliphora stygia (Fabricius) (Diptera: Calliphoridae) using acidic potassium permanganate chemiluminescence detection coupled with flow injection analysis and High-Performance Liquid Chromatography (HPLC). Presence of amitryptiline and nortriptyline could be detected in larvae from all colonies fed on tissues from the rabbits receiving amitryptiline using high-performance liquid chromatography (HPLC) [31].

Calliphora Vicina larvae reared on artificial foodstuffs concentrations of amitryptiline and nortriptyline, alone and in various combinations, were harvested at various stages of development and analyzed for drug content by high-pressure liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS. Presence of amitryptiline and nortriptyline could be detected in larvae from all colonies fed on tissues from the rabbits receiving amitryptiline using high-performance liquid chromatography (HPLC).

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Larvae of Chrysomya albiceps (Weidemann) and Chrysomya putoria (Weidemann) (Diptera: Calliphoridae) were reared on tissues from rabbits administered twice the lethal dosage of diazepam in order to study the effects of this drug on the development of these two species. The presence of diazepam could be detected through gas chromatography-mass spectrometer (GC-MS) in all rabbit samples and in almost all Diptera samples. Examples of some detected drugs with Insects show below in figure 1 (a-h).

Complicating Factors for the determination of PMI

One of the disadvantages of using forensic entomology is that the growth rate of the maggots can be affected by changes in the temperature, Geographic location may change developmental rates, indoor or outdoor exposure, sun or shade, time of day and season, humidity, and rain, hogon [32]. Temperature is an extremely crucial factor for the utilization of this method because flies won't be able to lay eggs below 40 degrees, nor do they seek the body after sundown. Another complicating factor is the presence of foreign substances in the decomposing body such as drugs and toxins can affect the growth rate of feeding maggots, information that can be crucial in estimating the PMI [28]. Drugs and poisons; Cocaine speeds up development, heroin down, Methamphetamine speeds up development but greatly increases maggot mortality. Bioaccumulation, Presence/absence of clothing, Changes with substrate (sand vs. soil. vs. concrete, significant errors introduced up to 29 hours. Entomologists will have to take each of these variables into consideration in order to give a more accurate estimate of PMI.

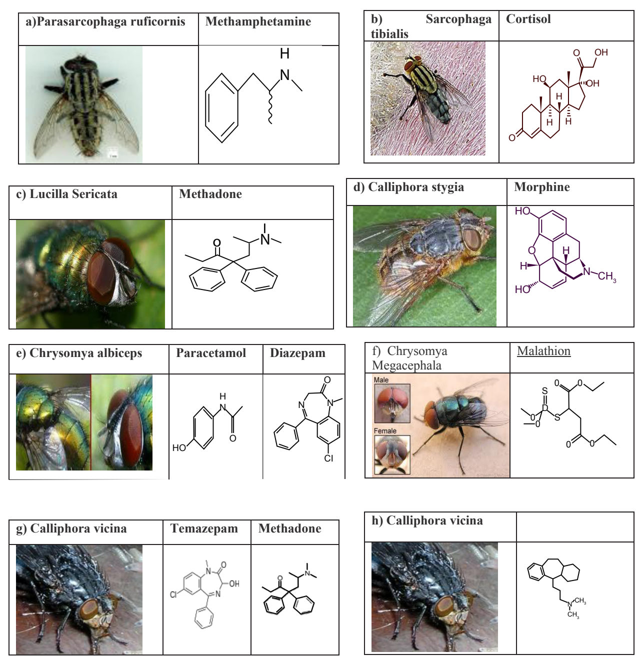

Figure 1: a), b), c), d), e), f), g), h) shows examples of some detected drugs with Insects.

Effects of drugs on Insect's growth rate

Flies are the most commonly used insect in Entomotoxicology. Some effects of drugs on these flies depend on the concentration of the drugs while others simply depend on its presence. Drugs can have a variety of effects on development rates of flies. Substances like Cocaine, Heroin, Morphine, methamphetamine, Methylene Dioxymethamphetamine, Triazolam, Oxazepam, Chloripriamine, Barbiturates, Malathion, Nortriptyline and Amitryptiline, and Paracetamol are commonly encountered in cases where forensic Entomotoxicology is used.

Various studies have shown that ante mortem use of various drugs and toxins affect maggot development rate, manifesting into an inaccurate PMI estimation based on insect development. Errors of up to 29 h can occur in PMI estimates with heroin containing tissues based on development of the fly *Boettcherisca peregrina*. Similar results were reported for methamphetamine and amitryptiline. Errors of up to 24 h can occur in estimates with heroin on *Lucilia Sericata*.

Cocaine and methamphetamine accelerate the rate of fly development. Cocaine causes larvae to "develop more rapidly 36 to 76 hours after hatching". The amount of methamphetamine, on the other hand, affects the rate of pupal development. A lethal dose of methamphetamine increases larval development through approximately the first two days and afterwards the rate drops if exposure remains at the median lethal dosage. However, closer examination of the effects of heroin on fly development has shown that it actually speeds up larval growth and then decreases the development rate of the pupal stage. The differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 29 h and estimates based on pupal development by 18 to 38 h.

Barbiturates were found to increase the length of the larval stage of the fly, which will ultimately cause an increase in the time it takes to reach the stage of pupation. Blowfly larval development is slightly impacted by paracetamol if present in the rearing foodstuff particularly during days 2-4 of development. *Chrysomya Megacephala* larvae from control group developed more rapidly than larvae feeding on tissue containing Malathion. The time required for adult emergence was significantly greater for Malathion treated colony which was 10 days compared to 7 days in control colony.

Life Cycle Stage	Timing	Description	Observations
Eggs	1 day	1-2 mm	Located especially around the body's natural orifices, such as the nose, eyes, ears, anus, penis, vagina, and in any wounds.
Larvae —1st Instar	1.8 days	2-5 mm	
Larvae — 2nd Instar	2.5 days	10-11 mm	
Larvae — 3rd Instar	4-5 days	14-17 mm	
Pre pupae	8-12 days	11-12 mm	Larvae become restless and start to move away from the body, crop organ is gradually emptied of blood, and internal features are gradually obscured by the larvae's enlarged body
Pupae	14-18 days	14-18 mm Darkens with age	Presence of empty puparia an indication that the person in question has been dead approximately 20 days.
Adult flies	Emerges from pupa cases after 4-7 days	New generation	Small adult flies

Table 1: show Normal Life cycle stages of fly.

The presence of Malathion in the carcasses delayed initial oviposition by 1 to 3 days and prolonged the pupation period by 2 to 3 days. Through the analysis of specific cases, it was revealed that toxins present in a person's body upon death can confound postmortem interval estimations. Presence of the organophosphate Malathion in the man's system delayed oviposition for a few days.

Discussion and Conclusion

This review by the authors is primarily aimed to orient the enlightened researchers and investigators in the field of forensic science that forensic Entomotoxicology involving studies on drugs and toxins effects on growth phase of insect development are to be seriously taken more frequently in the forensic investigation of crime scenes in order to get an accurate estimate of time since death especially from the remains of decomposed bodies. PMI is defined as the time from death to discovery of the corpse; it is the most familiar use of entomological evidence in criminal investigations [33].

Barbiturates were found to increase the length of the larval stage of the fly, which will ultimately cause an increase in the time it takes to reach the stage of pupation. Morphine and heroin were both believed to slow down the rate of fly development (Calliphora stygia, *Protophormia terraenovae* and *Calliphora vicina* (Diptera: *Calliphoridae*), *Lucilia Sericata* (Diptera: *Calliphoridae*). Research of *Lucilia Sericata* (Diptera: Calliphoridae), reared on various concentrations of morphine injected meat, found higher concentrations of morphine in shed pupal casings than in adults. Sadler et al was able to detect trimipramine, trazodone, and temazepam, in the larvae of *Calliphora vicina*, but was unable to detect the drugs in the pupae.

Cocaine and methamphetamine also accelerate the rate of fly development (*Parasarcophaga ruficornis* (Diptera: Sarcophagidae). Some effects depend on the concentration of the toxin while others simply depend on its presence. Through the study of *Chrysomya Megacephala* and blowflies (Diptera: *Calliphoridae*) and *Calliphora vicina*, Malathion has been found to be actually increasing the overall timing of development from egg to adult. These studies prove that, Forensic Entomologists will have to take each of these variables into consideration apart from the normal time taken for the development of insect in its life cycle in order to give a more accurate estimate of PMI.

There are two primary ways to estimate the PMI of human remains using entomological evidence. Insect succession of arthropod species found on a body provides one method of determining the post mortem interval (PMI) [34]. Insects arrive at decomposing remains in predictable, successive waves based on the stage of decomposition [35,36]. The other method utilizes the degree of development of the oldest maggots feeding on the corpse, from which one can determine a close approximation of the time since death. Insects often lay eggs within minutes or hours after death [37], thus providing a developmental reference. The use of either approach will also depend on factors such as season, climate, and location of the corpse and treatment of the corpse. Furthermore, their rate of progress can be affected by a number of factors, including humidity, temperature, the presence or absence of clothing, burial and depth of burial [38].

The duration of the decay process depended on climatic conditions and reflected yearly temperature changes. Corpses in summer and rainy season decayed at much faster rate than those in winter and spring. Warmer temperature in summer speeded up succession while low temperatures in winter retarded succession by slowing down the development of dipterous larvae. Higher temperature of the corpse due to maggot activity also leads to quick decay. Chrysomya megacephala and Chrysomya rufifacies were the two Calliphorids, which were found in all the seasons of the year. Various problems were experienced that affected the estimate of post mortem interval from entomological evidence.

This review by the authors is primarily aimed to orient the enlightened researchers and investigators in the field of forensic science that forensic Entomotoxicology involving studies on drugs effects on growth phase of insect development are to be seriously taken more frequently in the forensic investigation of crime scenes in order to get an accurate estimate of time since death especially from the remains of decomposed bodies.

More research work in the field of forensic Entomotoxicology can also help to generate more interest. After that, forensic Entomotoxicology will be a more respectable field with a wider acceptance as evidence in court. This study demonstrates again the importance and need of considering the possible effects of drugs in tissues on insect growth rates when estimating the postmortem interval using entomological techniques.

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