Review article:

Forensic Review of Entomology Toxicology: The Use of Insects for Toxin Detection in The Case of Human Death

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Abstract:

Determining the cause of death is an important factor in the forensic investigation process. Some aspects of death will be difficult to find if the condition of the body has suffered severe damage when found. Corpses due to drug abuse found in hidden places are often difficult to know with certainty the cause of death. Insects that eat the corpse tissue, then xenobiotics such as drugs and other toxic substances will be transferred to the larval body's metabolic system so that it is easily detected with sophisticated equipment. Techniques such as immunoassays, High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) have been used routinely to detect xenobiotics from the body, larvae, and insects. The study of insects as alternative toxicological samples is known as forensic entomotoxicology

Keywords: Calliphoridae, Dermestidae, Drug use, Pesticide, Suicide

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INTRODUCTION

Forensic investigation

One of the most important factors in investigating crimes of death is the cause of death. These aspects will be difficult to ascertain when the body had been badly damaged when it was founded¹. Case finding the bodies are often found in cases of death due to drug abuse in hidden places². When a toxicological samples such as tissues, body fluids, and internal organs have been degraded or no longer available, the alternative is to use serangga³⁻⁵ investigation. Insects are closely related to forensic science for the use of cadaver as a food source⁶. When eating corpse network, xenobiotics such as drugs and other toxic substances will be transferred to the metabolic system larva⁷. Insects as toxicological samples because the number of corpses that are infected, and the pupa stage will not change for the long time⁷. The study of the use of insects as an alternative toxicology sample known as entomotoxicology⁸. Deaths due to drug abuse, "insecticides, pesticides, and other poisons are common cases in the world.

It was reported that around 450,000 people died worldwide as a result of drug abuse in 2015⁹. It is estimated that nearly 800,000 people die each year from suicide, of which 20% are deaths from swallowing pesticides¹⁰. Many deaths occur due to substance abuse, and in some cases, new dead bodies are discovered after a few weeks to 11 months¹¹. Entomotoxicology will "be a useful tool in solving cases of poisoning" when a corpse is found in advanced decay. "Detection of drugs and poisons in insects (larvae, and other arthropods) is further detected by several techniques." A technique like immune-assays^{12,13}, "High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS)^{12,14,15,16}, and "Gas Chromatography-Mass Spectrometry (GC-MS)^{8,17}, have been used routinely for detecting xenobiotics from bodies, larvae and insects.

History of Forensic Toxicology Entomology

Entomotoxicology relatively recent history. The first report on drug use in the field of phenobarbital was published in 1980. A 22-year-old woman

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was found in the early stages of skeletonized, no fluids or tissues were found for further decomposition. The larvae are used as samples for toxicological analysis, and phenobarbital found in the detection result. Since then, a lot of drugs and toxic substances such as amitriptyline, Propoxyphene, Acetaminophen¹⁸, Steroid¹⁹, Trazodone, Trimipramine and Temaze-pam¹⁴, benzodiazepine, barbiturates, and Meprobamate¹⁵, Methamphetamine¹⁶, Meth-amphetamine²⁰, Clamepamber, Brom, Clampine and Opioid^{13,22}, Nordiazepam²³, Phencyclidine²⁴, Codeine, Insecticide and Pesticide^{25,26}, Mercury²⁷, etc. have been detected in various tissues and insect larvae.

Insects as toxicological samples

After someone dies their body undergoes a process of decay which consists of five stages: fresh, swollen, rotten, and become a skeleton. Insects are generally the first visitors to a corpse, which can lay eggs in a carcass within hours of death. Insects happen to experience several changes from one stage of life to another (metamorphosis): First, eggs are laid in large numbers on corpses; maggots emerged from eggs, ate corpses and experienced three larvae instars; On the third instar, adult maggots move from food sources to cocoons at the appropriate pupa site (usually on the ground); Adult flies appear after empty pupae and pupa pockets are sometimes seen on body clothing²⁹. The pupa skin serves as the final step for toxicological samples if the body is found at the skeletonized stage. Medicines which accumulate in the cuticle of the larva during growth will suppress the skin of the pupa.

Entomotoxicological organic materials that can be analyzed are larvae, pupae, adult insects, pupa shells, exuviae (beetle skin remnants), beetle feces, fly predators, and spoilage bacteria³⁰. The most frequently analyzed toxicological insects are flies (Diptera) and beetles (Coleoptera). The most studied insect species are *Calliphoravicina* and *Luciliasericata*³¹.

METHODS

Sample collection and the storage of the collection

Good collection and storage of samples are essential for more accurate estimates of postmortem (PMI) estimates. Samples were collected from various parts of the corpse because drug redistribution caused variability in drug concentration³. Various methods of collecting and storing samples and effects on larval length have been studied to determine which media

are most suitable. Experiments³² showed that larval specimens were carried out in two ways: directly put into preservatives (10% formalin, 80% ethanol, and 95% ethanol); or hot water to kill 80°C and 100°C larvae for 1, 30, 60, and 90 seconds and then soaked in preservatives. The larvae were turned off at 80°C with an immersion time of 30 seconds found to have minimal changes regarding the initial length before being killed, and therefore this method was found to be more suitable. Ethanol(80%) in beetle larvae provides a large variation in the length of beetle larvae observed³³. Therefore, beetle larvae must be measured while alive³⁴. Various methods of killing and storing samples and effects on larval length have been studied to determine which media are most suitable.

For toxicological analysis, the specimen is preserved at -4 °C, and the analysis is carried out in the same manner as human tissue or liquid³⁰. It is recommended that specimen storage be carried out under dry conditions at -20 °C to ensure drug stability and also to reduce drug extraction from the matrix when submerged in alcohol³.

Sample Extraction

The extraction of xenobiotic material from insect samples provides several advantages compared to

human tissue^{12,35}. Sampling is easier and faster, and the emulsion does not interfere with the results of the analysis, which sometimes occurs with human tissue²³. Several extraction techniques such as liquids extraction and solid-phase extraction are used to extract various poisons

and drugs according to the chemical features of the material to be detected^{36,37}. Solid-phase extraction is known to provide the best purification of organic toxins from liquid extracts of insect specimens³⁰. The various types of samples and the systematic processes for which the analysis is prepared is illustrated in Figure 1

Figure 1. The layout of insect analysis for toxicological analysis³⁸

Toxicological Analysis

Different animal models (rabbits, mice)³⁹, and meat substrate^{11,12} with medicine, as well as toxic substances are used to simulate the process of death, spoilage, and drug discovery from larvae on dead carcasses. Successful detection and discovery of toxins from insect specimen depend on the extraction technique and the effectiveness of the used technique. Figure 1 above shows the stages of systematic analysis of different chemical compounds in entomotoxicological specimens which are of interest to the analyst. Some insect species for which drugs and toxic substances can be detected are shown in Table 1.

Qualitative Analysis

In the absence of specimens such as tissue, blood, or urine for toxicological analysis, insects can reveal the possibility of exposure to drugs that poison the related deaths. For example, amphetamines and alcohol were detected from larvae in corpses that were thought to be one month old¹⁷. Several research articles have evidence that identification of the drug may be from insects themselves^{18,21}. In forensic entomotoxicology, immunoassay techniques have been mainly used to detect morphine drugs from various substrates and specimens¹³. By using this technique, the distribution of morphine in various parts of the insect's body (hemolymph, cuticle, and larval integument) was detected⁴⁵. This technique has also been useful in the qualitative and quantitative analysis of estimates of other drugs and pesticides⁴³.

For confirmation testing of compounds in the sample, the chromatography technique coupled with Mass Spectrometry is considered the gold standard⁴⁵. This technique is more selective, sensitive, accurate, reproducible, and requires only a few samples. In forensic analyses of drug abuse cases, this technique has been applied to detect various drugs on different matrix samples⁴⁶.

Quantitative Analysis

The toxin concentration will be reduced in insects compared to the substrate where the insects at⁴⁷. Drug concentration in the larvae decreases in

the post-meal stage because there is no active absorption of xenobiotics from food¹⁶. Larvae are also effective at removing drugs and toxic substances from the body and get lower when the maggots become adults. But for certain types of drugs (Antidepressants), they contain drugs in higher concentrations

Table 1. Drugs and other toxic substances detected from several insect species

Insect species	Insect genus	The detected drug	The detection technique
Calliphora Vicina	Calliphoridae	Amitriptyline, Temazepam	GC-MS ⁸
Dermestes Frisco	Dermestidae	Morphine	Immunoassay ⁴⁰
Lucilia Sericata	Calliphoridae	Morphine	Immunoassay ⁴⁰
Chrysomya Albiceps	Calliphoridae	Diazepam	GC-MS ⁴¹
Protophormia Terraenovae	Calliphoridae	Morphine	Immunoassay ⁴²
Musca domestica	Muscidae	Parathion	HPLC ⁴³
Calliphora Stygia	Calliphoridae	Morphine	HPLC ⁴⁴
Chrysomya Megacephala	Calliphoridae	Malathion	GC ⁸

(bioaccumulation)¹³. Some quantities of the drug are known to accumulate in the puparium cuticle and because the drug can be identified from empty puparial cases⁵.

The technique used must be sensitive enough to detect low concentrations such as drugs in larvae, insects, and puparial cases. The immunoassay technique cannot measure morphine drug substances from larvae⁴⁰. Similarly, the HPLC technique cannot measure drugs from larvae that given minced meat⁴⁴. "Drug quantification is only possible from eating larvae in minced meat with morphine above 2500 ng/g. Quantification of low-level drugs is best done using LC-MS and GC-MS techniques. Using this technique, drugs measured from different parts of the body give varying levels of concentration of drugs in the liver, heart, lungs, blood, brain, urine and skin¹⁶. The highest drug concentration in the liver is related to xenobiotic metabolism occurring in the liver, therefore a careful interpretation of quantitative results requires high conscientiousness.

1.1. Correlation Analysis

The correlation between drug concentrations in the substrate and insects is still controversial. Factors such as drug pharmacokinetics, metabolism, drug redistribution, drug accumulation, larva feeding activity, and several other factors that can influence correlation studies are not known yet. Extensive studies on 29 human bodies that were

thought to have died of poisoning were analyzed, and correlation could not be concluded because there was no reproducibility¹⁵ In contrast, several studies have shown that the correlation between pesticides in the substrate and larval samples⁸.

In most of these experiments, larvae are fed minced meat without allowing the drug to undergo a natural metabolic process. Therefore, the findings and results may differ from the results of actual life poisoning cases. So far, few studies have looked into metabolism, drug redistribution, accumulation, and excretion mechanisms in insects. So, until all the right factors are available, vigilance needs to be taken to interpret the results of the correlation analysis⁸.

CONCLUSION

Insect and larvae of toxicological specimens can be relied upon especially when the material for toxicological analysis of the victim's body material is no longer available. In addition to the qualitative role, the possibility of a quantitative role can also be carried out if the pharmacokinetics of the drug in insects are known.

SUGGESTION

Until now, the absorption-metabolism-elimination-accumulation of drugs and other toxic substances in insects has not been fully understood^{3,20,48}. The pharmacokinetics of drugs in insects depends on

the species, their stage of development, and feeding activity as well¹⁴ The results of the analysis are also influenced by factors such as drug stability, temperature, and humidity¹⁵.

Thus, the main weakness of entomotoxicology is the correlation of detected drug concentrations. To overcome these limitations, it is recommended the development of standard protocols which must include models of organisms (e.g. available insect species), standard matrices for feeding substrates, setting sample sizes, and using sophisticated analytical methods³¹. This will help in achieving comparative results between various studies.

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Authors' contribution

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