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REVIEW ARTICLE

Perspective of Entomotoxicology in Forensic Investigations: A Critical Review

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ABSTRACT

CONTEXT: A Forensic Entomotoxicology is a contemporary technique which makes use of insects as an alternative samples for forensic investigation. In case of recovery of degraded cadaver, in which body fluids and tissues are vanished, the toxicological studies of insects aid in forensic investigation to some extent in such legitimate cases. The drugs consumed by the corpse and further ingested by the insects feeding in the corpse affect the life cycle and development of the insects. Various analytical methods are employed for proper identification, detection, as well as quantitative analysis of the corpse. This paper summarizes the definition, applications, analytical techniques and the future perspective of forensic Entomotoxicology.

KEY MESSAGE: This article discusses the importance of insects as the toxicological evidence in forensic investigation.

KEYWORDS | forensic entomology, Entomotoxicology, insects, corpse, putrefaction, PMI

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INTRODUCTION

Criminal investigation involves diverse factors. Although ascertaining cause of death is one of the crucial factors. It incorporates the discernment amid natural, accidental, suicidal and homicidal death. There are numerous practices in a criminal investigation to resolve the root of death. The united collaboration professionals of distinctive curriculum for instance crime scene technicians, death investigators, anthropologists, entomologists, forensic pathologists and many other frontline professionals are key requirement for competent death inspection. The death scene should be thoroughly handled to bring out the facts which are helpful to re-establish the incidents that ascertain integrity of proclamation by witnesses. The delayed recovery of cadaver under suspicious conditions makes the death investigation complex in concluding the cause and manner of death. All the dermis and vitals are severely putrefied in disintegrated and skeletonized corpse. Under such conditions the toxicological samples are neither available nor useful for analysis. In such situations the exclusive and mere approach to find out the cause and time since death is entomology.[4] The study of insects and arthropods is interpreted as Entomology which is evolved from to Greek words entomon and logos meaning insect and word or reason respectively. When the study of entomological evidences like insects and their arthropod counterparts

populating on decaying remains is enforced to legitimate proceedings is described as Forensic Entomology or Medicolegal or Medicocriminal entomology.[3] Insects are extensive constituent of kingdom *Animalia* which are plausibly diverse and remarkably prosperous entities on earth. These are significantly versatile class of approximately 1 million species presently well recognized. Insects have successfully oppressed around whole credible environment and the immense mass of insects have ambiguous link to humans or are valuable to us. Toxicology is the field that amalgamates the assorted features of biology, chemistry and medicines that targets the detrimental consequences of chemicals, drugs and other substances on human body. Toxicology is further extended to clinical toxicology (therapeutic approach), analytic toxicology (laboratory testing and analysis) and forensic toxicology (for provision of justice in legitimate matters in both ante mortem and post mortem medico-legal cases. Entomotoxicology is described as the sampling of insects and arthropods (flies and beetles) that feed on cadaver as a positive substitute for testing of toxins and drugs. Entomotoxicology is composite of distinctive concepts of ancient Greek words entomon (insect), toxikos (poisonous) and logous (subject matter) which means study of xenobiotics affecting insects. The insects are utilized as redundant source for drug identification when traditional source like blood, urine, vitals are not feasible. Entomotoxicology investigates the presence of drugs in corpse at death time. The positive identification of insects is the key constituent for death investigation. Data like nature, growth rate, developmental records and geographical assortment is utilized.[9] Forensic Entomotoxicology is utilized for detecting xenobiotics and post mortem interval (PMI). Numerous arthropod species primarily Diptera and Coleoptera (flies, beetles and their larvae) are the first visitors to the cadaver. These species feed, reside and propagate on and in the cadaver according to the stages of decaying. By utilizing the information of developmental stage, time since death or post mortem interval is computed. PMI is the approximate value of time since death which is known as Colonization interval for forensic entomology analysis goals.[6] Various techniques like GC-MS, LC-MS, HPLC and immunoassays have been used in routine xenobiotic detection.

HISTORY

The insect importance of facilitating decay and assisting usual organic matter depletion has been studied in preceding centuries. It was initiated in the 13th century in China when the first medicolegal case was reported and solved with the help of entomology. In 1767 decomposition by insects was studied. In 18th and 19th centuries in French, buried bodies consumption was witnessed and PMI calculations were initiated. Further studies include utilization of forensic entomology in famous Buck Ruxton Case in 1935. In 20th century, new species of discovery, their life cycle study was conducted. Now new trend to describe life cycles of forensic insects to rule out cases of murders and assaults, negligence, deceiving was fruitfully achieved, but it still needs further research. It is the historical background of forensic entomology. The history of Entomotoxicology dates back to 1980 when a 22-year-old female cadaver was recovered in skeletonized stage. The cadaver was recovered 14 days after her last sight. No toxicological sample was available for analysis, thence the fly larvae were utilized as substitute for toxicological samples and phenobarbital was successfully detected. Afterwards numerous toxins, drugs, narcotics, for instance, Benzodiazepines, Morphine, Amitriptyline, Nortriptyline[1], Acetaminophen, Cocaine[2], Malathion, Bromazepam, Diazepam, Nordiazepam[5], Temazepam, Propoxyphene, Trazodone, Methylphenidate, Levopromazine, Nicotine, Fluoxetine, Barbiturates and Meprobromate, Clomipramine[8], Trimipramine, Opiates And Opioids, Phencyclidine, Codeine, insecticides and pesticides for instance Terbufos (OPs), metal toxins, for instance, lead, mercury, Aluminum phosphide, and alcoholic

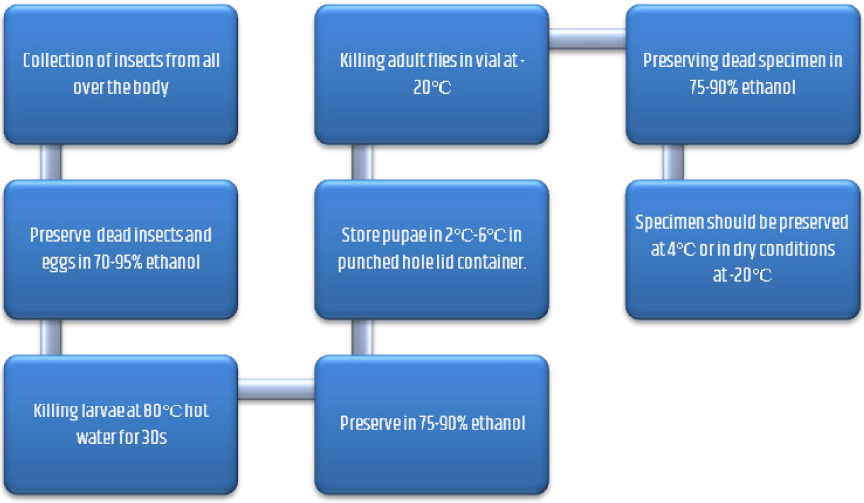

Figure 2: Sample collection and Preservation protocol

beverages.[10]

Insects as Toxicological Sample

The progression of decomposition commences right after the death of an individual. The decomposition proceeds in five stages: fresh, bloated, decay, post-decay, and skeletal. Putrefying remnants caters an interim micro-territory extending dynamically altering food source to numerous organisms such as bacteria, fungi and vertebrate scavengers. Insects are the foremost invitees to invade the cadaver onset putrefaction. They were intrigued to the body fluids from natural orifices and blood from wounds immediately within minutes. The majorly encountered insects are flies and beetles which belong to Dipteran families (Calliphoridae, Sarcophagidae) and Coleopteran (Histeridea) respectively that construct a compound food web within the corpse. Arthropods are the key constituent of the kingdom Animalia insects as predominant taxa in terrestrial surroundings worldwide. These insects were fascinated due to the strong odor from corpse. Flies arrive before beetles to the cadaver and feed on liquid oozing out of fresh cadaver but unable to feed on fresh cadaver's tissues. The prevalent flies are blow flies despite of this other insect flies, beetles and arthropods are also present. Blowflies provide the most accurate estimate of time since death. Beetles prevails at extreme stage of decomposition. The materials for entomo-toxicological investigation are larvae, pupae, adult insects, puparial cases, beetle fecal material, cast beetle skin, and fly predators. Some of the species that are investigated thoroughly on which toxins have been recovered auspiciously. The most recovered insect species are Calliphora vicina and Lucilica sericata. About 63 papers relevant to Entomotoxicology were reported to be published in the year 2016. Approximately 73 research papers were known to be published during 1980-2018. The authors have tried to include all articles till 2020.[10]

METHOD

Sample Collection and Preservation

For the decisive and authentic assessment of PMI assorted procedures for collection and preservation of the entomo-toxicological evidences have been devised. Sample collection and preservation of insect specimens is the integral constituent of toxicological analysis. Because of the redistribution of drugs, sample is collected from different area of cadaver.[10] The samples were collected mainly from the

key organ (liver), muscles, and head area as well as in the environment surrounding the cadaver. Skin surfaces can also be considered as sampling site where no key organ is present. The investigators must sample the specimen by keeping in mind that the source of the insects can be divergent from the cadaver. There are numerous standards and guidelines published for entomological sampling in forensic Entomotoxicology. Sampling can be done in diverse developmental stages at systematic lacuna of time. Subsequently the preservation is initiated on the completion of sampling procedure. In one experiment, specimen samples are rinsed with normal water froze at -68°F to -39.2°F. The organic samples were rinsed to remove human fluids, crushed and heated at 650°C for 24 hours. In other experiment, samples collected were placed in hot water (780°C) for 30s and stored in 75% ethanol 60°C for at least 2 weeks. In another experiment, larvae were directly stored in preservative (10% formalin, 80% ethanol and 95% ethanol) or hot water killed at 80°C and 100°C for 1s, 30s, 60s, 90s and stored in preservative. 80% ethanol was considered as best preservative medium. But it is not applicable to beetle larvae thence it is suggested that beetle larvae should be measured alive.[7] Keeping in mind the aforementioned experiments, a summarized method for sampling and preservation of insects is depicted in figure 1.

Sample Extraction

The removal of xenobiotic material from the entomological specimen is advantageous than human tissues. Sampling procedure is effortless and no interference in analysis is encountered unlike the human samples. Quantification was achieved in larvae sample but not in human tissue sample was explained in an experiment. Extraction procedure of insects is similar to human tissue samples. Diversified extraction protocols for instance solid phase extraction and liquid-liquid extraction are utilized for extraction of different drugs and toxins. Amidst aforementioned protocols solid phase extraction administers utmost organic decomposition from aqueous concentrate of entomological exhibits.

Toxicological Analysis

Numerous animal models and substrates had been utilized by diversified researchers for identification, quantification of toxins from insects and larvae. The extraction protocol and its efficiency affect the successful detection and redemption of drugs from insect specimens. There is a systematic analysis layout of diversified chemical compounds in entomo-toxicological samples of interest.[10] Analysis can be further categorized as qualitative and quantitative.

Qualitative Analysis

Insects can serve as the sample for the detection of any drug of toxin presence in the cadaver. There are numerous research articles that have proved the successful drugs for instance, cocaine, methamphetamines, Malathion, nicotine by using GC-MS. Many analytical techniques such as HPLC, GC, GC-MS, LC-MS, LC-MS/MS, GC-MS/MS, immunoassays re used for qualitative analysis of insect sample for toxin detection because of the accuracy, sensitivity, selectivity, and reproducibility.

Quantitative Analysis

In addition to determine the kind of drug present, the quantitation of drugs is also considered as a crucial aspect of forensic entomo-toxicological analysis. It was found that the drug concentration is less in insects and larvae when compared to substrate. If the substrate was treated with high doses than the concentration of drug is also increased in the insects and larvae. Also there is occurrence of drug elimination when the maggots mature, which in turn results in reduction of drug concentration exempting antidepressants whose concentration is high in post feeding stages because of the bioaccumulation. Immunoassays and HPLC techniques are not capable to quantify low levels of drugs, thence LC-MS and GC-MS techniques are utilized for best output. By utilizing these hyphenated

techniques quantification of diversified body parts of humans and animal models by numerous researchers. Quantification of diversified concentration levels of drugs in liver, heart, lungs, blood, brain, urine and skin have been reported. However liver is considered as the utmost vital in reference to other vitals as the metabolism of numerous xenobiotics occurs there. Thence, attentive perception of quantitative output should be executed.

Determination of Post Mortem Interval (PMI)

The prevailing usage of insects in forensic entomology is to assist the determination of PMI. Flies are utilized to evaluate the PMI in legitimate proceedings, it is achieved through collection of immature larvae, pupa and insects from the body which aids the identification of insects well as the size and stage.[10] There are numerous measures to be considered for estimation of PMI. For instance, stages of succession of arthropod species on carrion which varies corresponding to geographical location, age dependent changes in the intestinal contents as insect life cycles acts as precise clocks which initiates immediately after death (it is reportedly efficient method for PMI estimation), on stage invasion as entomological protocols is statistically reliable, developmental pattern of blowfly larvae as age is utilized to estimate minimum time since death, weight, length, width of larvae, isomegalen/isomorphen diagrams, fly eggs. Insects in gut content, simulation model and many more. PMI calculation is affected by the climatic conditions, seasons, geographic region, substrate type, location and position of body, altitude, latitude, cause of death, inter and intra specific competition and larval migration amid others. The growth rate of insect larvae vary from one species to another species, thence estimation of PMI through age of larvae can be accomplished by thoroughly studying life cycles of insects. In forensic Entomotoxicology in addition to PMI detection of presence of toxins, xenobiotics is also considerate. As xenobiotics affect the growth rate of insects thence insects are beneficial for forensic entomo-toxicological studies. For instance presence of dimethoate enlarges the life cycle duration of calliphoridae flies as dimethoate lengthens the feeding, post feeding and pupal stages of development of blowflies. Whereas malathion decreases the growth rate and alters the PMI estimation by 36 hr and 28 hrs.[1] lead toxicity in low concentration accelerates the development of immature L. cuprina whereas at high concentration delay the developmental rate.[6] As the toxins alters the growth rate efficiently, therefore wrong estimation of PMI can culminate if the type of species, their succession pattern, and their life cycle knowledge is not considered.

Future Perspective

The research and studies conducted on forensic Entomotoxicology is restricted. More research needs to be done on various aspects of this field, establishing the link between the insects and drugs including their interaction, insect metabolism etc. Moreover, bioaccumulation studies can be done. For analyzing the insects and detecting the drug samples from the corpse, proper data or reference library should be constructed, digital databases should be made depicting the morphology of insects, geographical location as well as the toxicological aspects for unchallenging detection of such evidences. Presently, DNA based identification, digital protocols are of great interest in forensic scenario, these applications can also be employed in the future trends of forensic entomo-toxicological investigation.

CONCLUSION

Forensic Entomotoxicology is captivating and exhilarating discipline. The preeminent intent of the study is the utilization of insects such as flies and arthropods for determination of cause of death such as the deceased was intoxicated before death or not. It can also be beneficial for determination of the manner of death for instance natural, accidental,

suicidal, homicidal or other unidentified manner. Amidst aforementioned details the utmost crucial factors are to determine cause of death and estimate PMI. It is useful when there is complete putrefaction of the cadaver but for precise estimation of PMI all important measures should be considered for instance climatic conditions because in high temperature and humidity decomposition process fastens and vice versa. It is beneficial tool for toxicological analysis but it is time consuming, needs thorough knowledge about insect species, their growth rate and succession pattern. Pharmacokinetics of drugs in insects is not thoroughly known. There is a drawback of interpretation of detected drug concentrations. There are not standard protocols established for analysis. This field is still emerging and needs more research for its proper beneficial usage.

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