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COMPARATIVE DEVELOPMENTAL EFFECTS OF TRAMADOL HYDROCHLORIDE AND CYPERMETHRIN ON *CHRYSOMYA ALBICEPS* (WEID.) (DIPTERA: CALLIPHORIDAE) REARED ON RABBIT CARRIONS

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ABSTRACT

This study compares effects of cypermethrin (a pyrethroid pesticide) and tramadol hydrochloride (an opioid) on the development of *Chrysomya albiceps* (Diptera: Calliphoridae) reared on intoxicated rabbits (*Orctylagus cunicunus*) carrions (injected intra-veinously with 4ml of the toxins, being a dosage always witnessed in drug abusive cases. The larvae body lengths were measured with a pair of compass and a transparent meter rule while the body weights were measured with Mettler Toledo weighing balance with sensitivity of 0.000 -1g. The mean maximum length and weight (Mean±SD): cypermethrin (06.77±0.03mm and 0.0056±0.0001g), tramadol (14.02±0.12 mm and 0.0123±0.0002g), and the control (10.50± 0.16mm and 0.0095±0.0001g) were reached at 96 hours after eggs were laid. However, the mean total developmental (from egg to adult *C. albiceps*) period was highest at 380.28±1.35hrs (15.8 days), 330.05±1.50 hours (13.8 days) and 281.2±1.20 hours (11.7 days) respectively for cypermethrin, tramadol hydrochloride and controlled groups. The results showed Post Mortem Interval (PMI) estimation errors of about 4 and 2 days respectively, when cypermethrin (pesticide) and tramadol hydrochloride (opioid) toxins used are compared with the control group of *C. albiceps*. Therefore, caution is required in the interpretation and application of insects' data in forensic entomology when drugs and toxins are the suspected cause of death.

Keywords: Entomotoxicology, Post Mortem Interval**,** *Chrysomya albiceps,* opioid, forensic entomology, carrions

INTRODUCTION

Some 2500 years ago, the Egyptians embalmed deceased individuals in order to protect organisms from insects and decay (Monthei, 2009). Maggots were even referenced in the book of Dead, Chapter 154 "That my body will not become prey to maggots". Insects that have been associated with the disease bodies were neglected until 13th Century that these insects were used in the solving of criminal issues. Sung Tz 'u in 1247 wrote a book, "The Washing Away of Wrongs, a training manual for deaths scene investigators". He was the first person to mention of a case involving forensic entomology. Entomotoxicology, a relatively new branch of forensic entomology, deals with the use of insects in detecting drugs and other toxins in decomposing tissues (Gautam, *et al*., 2013). This also examines the application of analytical techniques to carrion feeding insects in order to identify drugs and toxins present in any intoxicated tissues. These insects, as they feed on corpses, ingest, incorporate, and accumulate drugs and metabolites in the cadaver into their own body. The drugs and toxins are locked in the cuticle of the larvae or empty pupal cases; therefore, they are useful sources for toxicological analysis. Their use as an alternative matrix for drug detection is well-documented and recommended when conventional matrices such as blood, urine, and tissues are not available (Gautam, *et al*., 2013).

The pharmacokinetics of drugs in insects depends on species, developmental stage, and feeding activity (Gautam, *et al*., 2013). Apart from necrophagous species, bioaccumulations have also been observed in parasitoids, predators, and omnivorous species (Dayananda and Kiran, 2013). In entomotoxicological investigations, necrophagous species belonging to Coleoptera (beetles) and Diptera (flies) are highly recommended as they are the first to colonize a corpse and these species are commonly encountered in crime scenes. Calliphoridae, also known as the blowflies (order: Diptera) are predominant and forensically important insects as they are generally the first arthropods to locate and oviposit on a corpse appearing within minutes following death (Gosselin, *et al*., 2011). Blowflies detect carcases primarily through the odor of decaying tissues. Other insects include sarcophagidae (flesh flies, order: Diptera) which generally arrive after the blowflies and muscidae (houseflies, Order: Diptera). Therefore, the knowledge of local insect assemblages and their growth rate as well as population dynamics is important for application of entomotoxicology for forensic purposes.

Different drugs and toxins show different reactions on the insects developmental rates, while some toxins like opioids accelerate the larval growth, others like the pesticides retards the larval growth (Lamia *et al*., 2011; Goff and Lord, 1991). Tramadol is a centrally acting analgesic used for the treatment of moderate to severe pains and has become the most prescribed opioid worldwide (Shipton, 2000). Tramadol in experimental and chemical trails exhibited a good analgesic efficacy and potency compared to codeine. Using forensic materials, Clarkson *et al*., 2004, reported that tramadol might be significant contributor to lethal intoxication when taken in excess with other drugs that depress central nervous functions, such as analgesic, muscle relaxants and ante depressants. Cypermethrin, a pyrethroid compound is widely used due to its high insecticidal potential and slow resistance in pest (Aggarwal, *et al*., 2015). It is considered less toxic for human use because of poor dermal absorption, rapid metabolism, less tissue accumulation, and environmental persistence (Das and Parajuli, 2006). Cases of accidental pyrethroid poisoning at work places have been reported (Ray and Fry, 2006), but poisoning with suicidal intention is extremely rare (Bradberry and Cage, 2005). Increased over-the-counter availability of these insecticides is likely to increase the prevalence of their toxicity. Furthermore, resemblance of cypermethrin toxicity to organophosphate poisoning poses a diagnostic dilemma in the emergency department (Walters, *et al*., 2009). The rabbit (*Orctylagus cunicunus*) has been and continues to be used in laboratory research such as in toxicological and forensic sciences (Goff *et al.,* 1999; Hedouim *et al.,*1999).

This study compares effects of cypermethrin (a pyrethroid pesticide) and tramadol (an opioid) on the development pattern of *Chrysomyia albiceps* (Diptera: Calliphoridae) from intoxicated rabbit (*Orctylagus cunicunus*) carrions through the assessment of the larvae body weight, body length and the developmental period.

MATERIALS AND METHOD

The experimental site

The study was carried out at the Biology Departmental farm of the College of Education Warri, Warri South Local Government Area, Delta State, Nigeria. The study spanned from January 9 to March 7, 2016 in an open fallow plot of the farm. The school is located in Warri on Latitude 5.5432 and Longitude 5.7382, and 7.1m, Altitude of 10.5, bearing of 61.31ᴼC (information time; 09:38:31(GMT), date; 09/01/2016, provider; GPS). It has a tropical climate characterized by two distinct seasons; the wet season occurs between April and October with a break in August. The dry season lasts from November to April with a cold harmattan between December and January. Warri ranges from 32ᴼC to 37ᴼC at an altitude of 21m, with mean annual rainfall of 2673.8 mm. The natural vegetation is rainforest, in some areas; the forest is rich in timber tree, as well as fruit trees (Egborge 1994).

The farm lies east of a botanical farm and southeast by a plantation orchard and surrounded by other research crop plots. Grasses, wildflowers, herbs, and weeds cover the field. Measurement of the area approximated 50 × 150 m. This size of land is to reduce overlapping olfactory cues.

Experimental animals

Twelve rabbits (*Orctylagus cunicunus*) with mean weight of 2.14 ± 0.18 kg purchased from Ogbuwangue market, along N.P.A express road, Warri in Warri South Local Government Area of Delta State, Nigeria were used for this experiment.

The killing methods and the experimental layout

The rabbits received toxins of cypermethrin and tramadol hydrochloride through intra muscular injection. The twelve rabbits were grouped into three groups of four rabbits each. A group of four rabbits was injected each with 4ml of cypermethrin (pyrethroid pesticide) intra veinously. Another group of four rabbits was injected with 4ml of tramadol injection (opioid) in a similar fashion. These dosages are similar to those commonly encountered in suicidal and abusive cases involving cypermethrin and tramadol hydrochloride. The last group of four rabbits was kept as control experiment (was killed by cervical dislocation).

Each carrion group was labelled accordingly, placed in thrash bags, and transported to the study area. The rabbit carrions were guarded against vertebrate scavengers with wire mesh that permits entrance of all forms of arthropod. The mesh was used to form cylindrical cages of heights and widths of 30 and 20cm respectively supported by iron rods for each rabbit carrion. An inter carrion distance of at least 20m was maintained to minimize interruption of flies from adjacent colonies (Mahat *et al*., 2012).

Arthropods sampling and data collection.

Sampling of *Chrysomya albiceps* larvae for the entomotoxicological studies was carried out in accordance with the morphological aspects of the larval instars of *Chrysomya albiceps* as described (Queiroz *et al*., 1997; Abdalla and Mohamed, 2014). Second instar larvae were collected from each decaying group and was bred in transparent plastic containers with depth of 15 cm and width diameter of 11.5 cm at 25 ᴼC. Breeding containers were covered with muslin cloth and firmly held by rubber bands to permit ventilation and to prevent the escape of the insects. Breeding substrate include sawdust and part of the decaying carrion-remains to feed the immature insects. The second instar larvae were reared until adult stage. Times for the emergence of all the stages of the insects were recorded accordingly.

Measurement of body Length and body weight

Measurement of larvae body length and weight was carried at regular intervals of 6-12 h. Larvae were randomly sampled from each rabbit carrion from the rearing unit and demobilized in the boiled water according to the method of Adams and Hall (2003). The lengths and weights were measured and mean values recorded for each carrion group. Mean values were used for statistical analysis. Portions of sampled larvae from each carrion were allowed to complete their development cycle. This was to ensure that time estimation was documented for all carrion groups. Times for pupation and adult emergence were recorded for each carrion. This was followed by group mean developmental period. Mettle Toledo electrical weighing balance with sensitivity (of readability) of 0.001g -1g was used to measure the weight of the larvae while the length of the fly larvae from the second instar stage were obtained by a pair of compass and read on transparent metre rule.

Statistical Analysis

Basic Microsoft Excel and SPSS (Norusis 2005) were used to analysed the data collected.

RESULTS

Effects of tramadol hydrochloride and cypermethrin on the body length of the larvae of *Chrysomya albiceps*

The mean length of the larvae sampled from tramadol hydrochloride, cypermethrin killed carrions, and the control groups are presented in Fig.1. The results showed that tramadol hydrochloride and cypermethrin have different effects on the mean body length of the larvae of the *C. albiceps*. The tramadol hydrochloride treated colonies attained highest increment in length while the colonies from the cypermethrin recorded the least body length compared with the control colonies at the same time interval. While the tramadol group increased in larval body length from 02.00 mm at 24 hours to 14.02 mm at 96 hours, cypermethrin increased from 02.00 mm to 06.05 mm, with the control increasing from 02.00 mm to 10.50 mm at the same period. In a similar vein, mean larval body lengths decreased to 11.01 mm and 6.43 mm respectively for tramadol hydrochloride and cypermethrin treated colonies at 132 hours whereas control colonies decreased to 8.00 mm during the same period.

Effects of tramadol hydrochloride and cypermethrin on the body weight of the larvae of *Chrysomya albiceps*.

The mean weight of the larvae sampled from the tramadol hydrochloride, cypermethrin and the control (no toxin) colonies of carrions are represented in Fig.2. Larvae (*C. albiceps*) groups from tramadol hydrochloride showed the highest increase from 0.003 g at 24 hours to 0.012 g at 96 hours with cypermethrin recorded weight movement from 0.003 g to 0.0056 g at the same time compared to the control group from 0.003 g to 0.0095 g at the same time. However, there was observed decrease in mean larval body weights after 132 hours to 0.006 g and tramadol hydrochloride and 0.0051 g in the cypermethrin treated groups respectively, whereas at the same time, control group decreased to 0.005 g.

Effects of tramadol hydrochloride and cypermethrin on the mean developmental period of *C. albiceps*.

Results of the effects of cypermethrin and tramadol hydrochloride poisons on the developmental periods of the *C. albiceps* are presented in Fig. 3. The appearance of eggs and larvae from both the treated groups and the control ones were observed at of 6.25 and 24 hours post death respectively. Pupal and adult stages from cypermethrin-poisoned carrions comparably recorded the longest periods of 205.2 and 380.2 hours compared to 185.2 and 330.3 hours for tramadol-poisoned carrions while control carrions recorded 170.2 and 281.2 hours respectively. From these values, it is clear that; development from egg to adult of *C. albiceps* recorded three values 15.84, 13.76, and 11.72 days respectively for cypermethrin, opioid and controlled-grouped carrions.

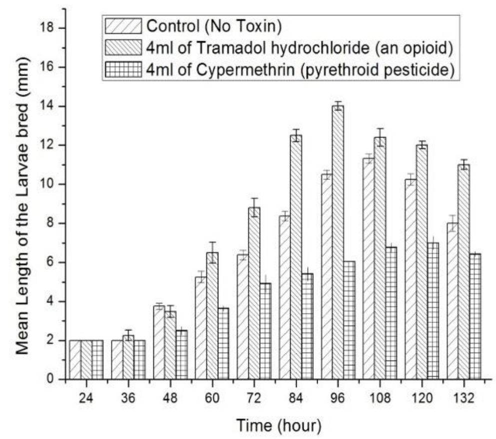

Fig. 1. Mean body length of the larvae *Chrysomya albiceps* bred from carrions groups with time of development

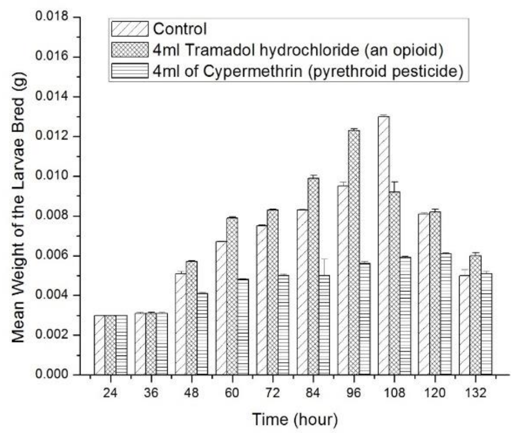

Fig. 2. Mean weight of the larvae of *Chrysomyia albiceps* bred from carrions with time of development.

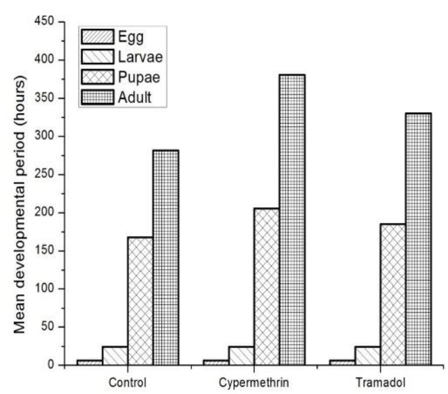

Fig.3. Mean developmental periods of *Chrysomya albiceps* reared on carrions intoxicated with tramadol hydrochloride, cypermethrin and control ones

DISCUSSION

Beyond the use of insect data in forensic entomology, entomotoxicology, which is one of the newest aspects of forensic entomology, is using valuable information from drugs and toxins intoxicated by insects in the course of decomposition process to ascertain cause and time since death. Since one of the core purposes of forensic entomology is to determine Post Mortem Interval (PMI), among others, the varying causes of death must be determined and paired with an insect template unique to it. This requires that data be collected from intentional and accidental cases relating to suicide and homicide. The effects of various drugs and toxins to carrion-feeding insects have been investigated, and this area of study is still expanding (Monthei, 2009). Tracqui *et al*., (2004) examined 29 necropsies in which various organic compounds (including benzodiazepines, barbiturates, antidepressants, phenothiazine, opiates, cannabinoids, meprobamate, digoxin, and nefopam) were detected in arthropod larvae sampled from human corpses.

The results of this present work revealed that, the larval body length, weight of *C. albiceps* together with their developmental periods were influenced by both the presence and type of toxin. Larvae from tramadol hydrochloride (opioid) treated group of carrions attained larger sizes and weights compared to the group treated with cypermethrin (a pyrethroid pesticide) with a length and weight difference of about 7.97 mm and 0.0064 g respectively in favour of the opioid at the same period of 96 hours after death. Whereas length and weight difference between tramadol treated group and control is 3.52 mm and 0.0025 g in favour of opioid at 96 hours after death. It is evidently clear that in comparism with the control group killed by cervical dislocation, the larvae from tramadol killed group gained body length and weight better than cypermethrin and control groups. Therefore, the two toxins used had two different effects on the larvae of *C. albiceps*. While the opioid accelerated the development of the larvae and other stages of *C. albiceps*, the pesticide retarded its development compared to the control-grouped insect. This observation is in line with those of earlier researchers such as, Introna, *et al*., (2001), who noted that *Lucilia sericata* larvae that fed in the nasal cavity of a cocaine abuser grew over 8 mm longer than larvae of the same generation found elsewhere on the body. According to Bourel, *et al*., (1999), the overall effects of morphine appear to be dose dependent as the larvae feeding on the rabbit that received the greatest dosage were the slowest to develop. Based on results from this study, between hours 91 and 165, estimations of larval age based on total length can be significantly in error if the presence of morphine in tissues is not considered. The error can be as great as 24 h for *Lucilia sericata* larvae measuring from 8 to 14 mm total length. Research involving insects and opiates indicate that they act as a feeding stimulant in some species (Kavaliers, *et al*., 1987, Goff *et al*., 1999). Goff, *et al*., (1989) had colonies of *Boettcherisca peregrine* feed on tissues containing heroin (as morphine) and showed differences in larval development rates sufficient to alter postmortem interval estimate by up to 29 hours. Goff and Lord (1994) reviewed various studies in entomotoxicology and concluded that entomotoxicological testing was essential to accurate forensic entomology conclusions. Data indicating the presence of drugs allow for corrections to the data in cases when drugs affect insect development.

The result of the developmental period of *C. albiceps* from egg to adult under similar conditions with the exception of toxins revealed that, the presence of the toxins altered the Post Mortem Interval by about 4 days comparing cypermethrin treated with control and about 2 days respectively for cypermethrin and opioid, and opioid and control. This implied that, a PMI estimated without considering the resultant effect of the drugs or toxins could lead to estimation error with a concomitant error in the insect data presented before a panel or a jurist. From these results and others obtained by researchers, it is hoped, that, caution is always required both at the implementation and at applications of forensic entomological data. This is with a view to avoiding the pitfall of the PMI estimation errors, which could be inevitable when toxins are involved in the death.

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REFERENCES

Abdalla, S.B. and Mohamed, A.R. (2014). Morphological and taxonomical characteristics of Chrysomya putoria (Dipteria: Calliphoridae). *Sudan Journal of Science*, 6(1):35-40.

Adams, Z.J. and Halls, M.J. (2003). Methods used in the killing and presservation of blowfly larvae and their effects on the PMI length. *Forensic Science International.* 138:50-61.

Aggarwal, P., Jamshed, N., Ekka, M. and Imran, A. (2015). Suicidal poisoning with cypermethrin: A clinical dilemma in the emergency department. *J Emerg Trauma Shock*. 8(2): 123–125.

Berenbaum, M.R. (1995). Bugs in the systems: Insects and Their Impacts on Human Affairs*,* Addisson Wesley. and Castner, eds. CRC Press, Boca Raton.

Bradberry, S.M, and Cage, S.A, (2005). Proudfoot AT, Vale JA. Poisoning due to pyrethroids. *Toxicol Rev.*, 24:93–106.

Clarkson, C.A., Hobischak, N.R. and Anderson, G.S. (2004). A comparison of the development rate of *protophormia terraenovae* (Robineau-Desvoidy) raised under constant and fluctuating temperature regime. *Canadian Society of Forensic Sciences Journal*, 37:95-101.

Das, R.N, and Parajuli, S. (2006). Cypermethrin poisoning and anti-cholinergic medication- a case report. *Internet J. Med Update*, 1:42–44.

Dayananda, R. and J. Kiran, J. (2013). Entomotoxicology. *International Journal of Medical Toxicology and Forensic Medicine,* 3(2):71-74.

Egborge, A.B.M. (1994). Water pollution in Nigeria :Biodiversity and chemistry of Warri River, Ben Miller Books Nigeria Limited. ISBN 0-999-768, pp. 275-288.

Gautam, L., Pathak, R., Danlami, H.S., and Cole, M.D. (2013) : Entomotoxicology: Alternative Matrices for Forensic Toxicology. *International Journal of Medical Toxicology and Forensic Medicine,* 3 (2). www.forensicmag.com.

Goff, M.L. and Lord, W.D. (1991). Entomotoxicology. *American Journal of Forensic Medicine and Pathology,* 15:51-57.

Goff, M.L. and Lord, W.D. (1994) Entomotoxicology: A new area of forensic investigation. *American Journal of Forensic Medicine and Pathology***,** 15:51-57

Goff, M.L., Brown, W.A., Hewadi Karana, K.A. and Omori, A.I. (1999) Effect of heroin in the decomposing tissue on the development of *Boettecherisca peregrine* (Diptera: Sarcophagidae) and the implications of the effect on the estimation of the post-mortem interval using arthropod development patterns. *Journal of Forensic Sciences*, 36:537-542.

Goff, M.L., Miller, M.L., Parlson, D., Richard, E. and Omori, A.I. (1997). Effects of 3, 4-methylenedioxymethamphetamine in the developing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and detection of drugs in the PMI blood, liver, tissue, larvae and puparia. *Forensic Science Journal,* 42:276-280.

Goff, M.L., Omori, A.I. and Godbrod, J.R. (1989). Effect of cocaine in tissues on the development rate of *Boettcherisca peregrine* (Diptera: Sarcophagidae). *Journal of Medical Entomology*, 26:91-93.

Gosselin, M., Wille, S.M.R. Fernandez, M. dle Mar R. Fazio, V.Di M. Samyn, G. De Boreck, and Bourel, B. (2011). Entomotoxicology, experimental set-up and interpretation for forensic toxicologists. *Forensic Science International*, 208:1-9.

Hedouim, V., Bourel, B., Martin-Bouyer, L., Becart, A., Tournel, G., Deveaux, M. and Gosset, A. (1999). Determination of drugs levels in the larvae of *L. sericata* (Diptera: *Calliphoridae*) in reared rabbits carcasses containing morphine. *Journal of Forensic Science*, 44: 351-353.

Introna, Francesco; Campobasso, Carlo P.; Goff, Madison L. (2001). "Entomotoxicology". *Forensic Science International*, 120: 42–47.

Lamia, M.E., Zeinab, A.E. and Hassan, M.M. (2011). Effects of Tramadol on the Development of *L.sericata* and the Detection of the Drug Concentration in the Post-mortem Rabbit Tissues and Larvae. *Journal of Entomology*, 8:353-365.

Mahat, N.A., Zafarana, Z. and Jayaprakash, P.T. (2009). The influence of rain and Malathion on the oviposition and development of the blowfly *L. sericata* (Diptera: Calliphoridae) ingesting rabbits carcasses in Kelatain, Malaysia. *Forensic Science international*, 192:19-28.

Monthei D.R. (2009). Entomotoxicological and Thermal Factors Affecting the Development of
Forensically Important Flies. Dissertation submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy In Entomology,122pp.

Norusis, M.J. (2005). SPSS 13.0. Guide to Data Analysis. 1st edition, Upper Saddle River, Practice Hall, New Jersey, ISBN: 013186538.

Shipton, E.A. (2000). Tramadol: Present and Future. Anaesth. Intensive Cares*,* 28: 363-374.

Tracqui, A., Keyseri-Tracqui, C., Kintz, P. and Lides, B. (2004) entomotoxicology for the forensic toxicologists: Much ado about nothing. *International Journal of Legal Medicine,* 118:194-196.

Ray, D.E and Fry, J.R. (2006). An assessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol Ther*., 111:174–193.

Queiroz, C.M.M., Mello, P.R. and Lima, M.M. (1997). Morphological aspects of the larval instar of Chrysomya albiceps (Dipteria, Calliphoridae) reared in the laboratory. *Mem Inst. Oswaldo Cruz, Rio de Janeiro,* 92(2):187-196.

Walters, J.K, Boswell, L.E, Green, M.K, Heumann, M.A, Karam, L.E. and Morrissey, B.F. (2009). Pyrethrin and pyrethroid illnesses in the Pacific Northwest: A five-year Review. *Public Health Rep.*, 124:149–159.