Egyptian Journal of Forensic Sciences

ORIGINAL ARTICLE Open Access

Impact of using *Sarcophaga* (*Liopygia*) *argyrostoma* (Robineau-Desvoidy, 1830) as a toxicological sample in detecting clonazepam for forensic investigation

Fatma Mohamed Afifi, Eman Alaaeldin Abdelfattah and Ghada Mohamed El-Bassiony*

Abstract

Background: Sarcophagidae along with Calliphoridae (superfamily Oestroidea) were known as important colonizers of cadavers and could be used to estimate the postmortem interval (PMI), through tracking the flies colonizing pattern and/or monitoring their growth rates on cadavers. Many previous researches discussed the impact of toxins in decomposing corpses, on the developmental stages of insects, which would affect the accuracy of PMI estimation. Clonazepam belongs to the benzodiazepines, and it is one of the most routinely used drugs to control humans' seizures. The current study aimed to investigate the effect of clonazepam on the developmental stages of *Sarcophaga argyrostoma*, one of the most widespread Sarcophagidae in Giza Governorate. Also, we investigate the ability of these developmental stages to detect the drug.

Results: The current study used different concentrations of clonazepam (25, 50, and 100 mg/ml) as an in vitro application of the flesh fly *S. argyrostoma*. The drug has affected significantly the morphological measurements (weight, length, and width) of the different developmental stages, especially in the highest concentration (100 mg/ml). Quantitative and qualitative analyses were applied, by using high-performance liquid chromatography-mass spectrometry (HPLC–MS) and protein carbonyls amount respectively, to detect clonazepam in the second, early third, and late third larval instars and prepupa of *S. argyrostoma*. The results showed that the relationship between the concentration of the drug and its detection in the same developing instar is interdependent, using HPLC–MS. However, the drug faded from instar to the following one and transformed to its metabolite form. Measuring the protein carbonyls amount (OD/mg protein/min) revealed an elevation in the macromolecules damage, compared to the control groups, in almost all treated groups.

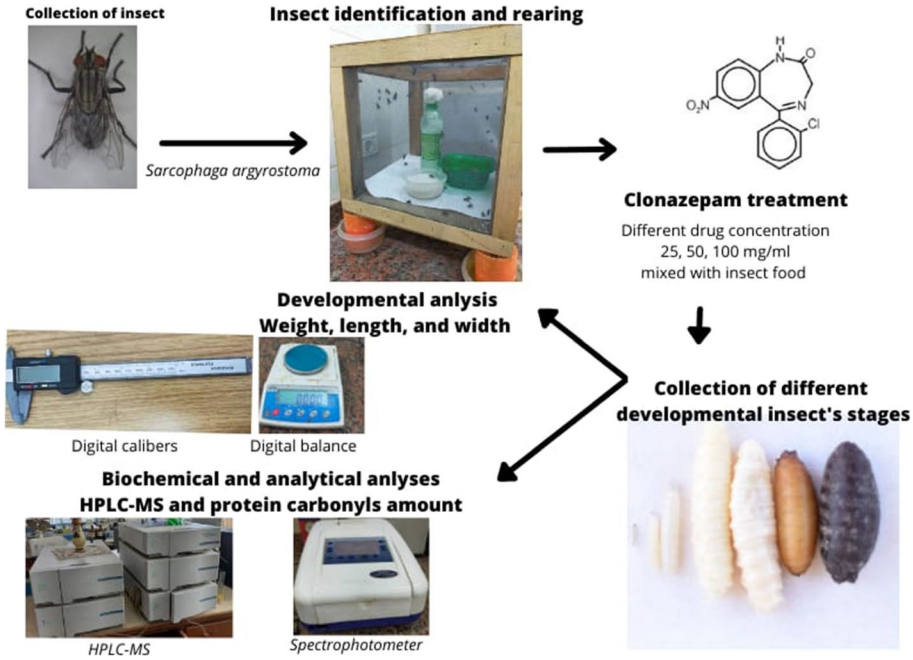
Conclusions: The current data suggested that clonazepam has oxidative damage in *S. argyrostoma*. While HPLC–MS was efficient in measuring the concentration of the drug in the insect, protein carbonyls analysis was a time- and cost-saving method and could be used to detect the drug in insects qualitatively.

Keywords: Forensic entomology, Development, Entomotoxicology, Sarcophagidae, Enzodiazepines

*Correspondence: ghada@sci.cu.edu.eg

Department of Entomology, Faculty of Science, Cairo University, Cairo 12613, Egypt

Graphical abstract



Background

The terminology of forensic entomotoxicology was firstly announced in 1991 (Pounder 1991). Medicolegal forensic entomotoxicology referred to the science of evaluating toxins/drugs in insects that fed on corpses. Insect could play a role as alternative toxicological sample (Kintz et al. 1990; Nolte et al. 1992; Gosselin et al. 2011). They could help in detecting crimes, especially in homicides' mysteries where the corpses were in an advanced stage of decomposition or the human samples are limited in their application.

Many studies were dealing with the usefulness and sensitivity of insects as an alternative toxicological sample in case of partial or complete decomposition of a dead body (Kintz et al. 1990; Goff and Lord 1994; Introna et al. 2001; de Carvalho 2009; Goff 2009; Waghmare et al. 2015; Chophi et al. 2019; Boulkenafet et al. 2020). Dipteran larvae were successfully used to detect cocaine, amphetamine, and alcohol in corpses that were in advanced stage of decomposition, which were dead a month ago or even reached the skeletonized stage (Nolte et al. 1992 and Campobasso et al. 2004). Entomotoxicology garnered little attention in Egypt despite its importance in determining the possibility of toxins exposure in poisoning-related abuses (El-Bassiony 2020). Sarcophagid flies were among the initial colonizers of corpses in Egypt, and the growth patterns of their larvae could be used to estimate PMI (Aly et al. 2013, 2017). Also, they could be useful as alternative toxicological samples (Gosselin et al. 2011; Badenhorst and Villet, 2018). Yet, the lack of information about the drug's interaction inside the insect's body impedes its use in forensic investigations. The colonization period of forensically important species could differ due to several factors including the presence of toxins in the dead body; these might mislead the PMI estimation (Campobasso et al. 2001; Salimi et al. 2018).

Clonazepam (5-(2-chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepin-2-one) belongs to benzodiazepines which are used to treat panic attacks and may sometimes cause addiction even in therapeutic doses for short periods. This drug is considered as one of the 69 gamma-aminobutyric acid (GABA) modulator compounds, which can affect the GABA receptor-ionophore complex. Concurrent use of benzodiazepines and opioids may lead to sedation, respiratory depression, coma, and death due to respiratory arrest (Stark et al. 1987; Woods 1987; Funderburk et al. 1978). Benzodiazepines were linked to an increased risk of being diagnosed with Alzheimer's disease (de Gage et al. 2014) and could be used for criminal purposes. Also, they are involved in intoxication cases around the world (de Aguiar França et al. 2015). According to the National Institute on Drug Abuse statistics, the death number from benzodiazepines only increased around 4.3-fold in 2015 than in 2002 (Rates 2017). Benzodiazepines were investigated lately in two postmortem forensic samples (Groth et al. 2022). The

authors collected human and insect's larvae (unknown larvae), and they found that several benzodiazepines could be detected in human samples as well as in the larvae.

Several methods have been used in toxicological analyses of insect specimens. These methods include analytical analyses such as gas chromatography (GC), radioimmunoassay (RIA), gas chromatography/mass spectrometry (GCMS), liquid chromatography-tandem mass spectrometry (LC–MS-MS), enzyme-linked immunosorbent assay (ELISA), ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/QTOF-MS), and HPLC–MS (Elian 2003; Wood et al. 2003; Campobasso et al. 2004; Souza et al. 2011; Boulkenafet et al. 2020). However, biochemical analysis that includes macromolecules damage such as protein carbonyls, lipid peroxide, and DNA single-strand breaks, which are used as biomarkers in entomotoxicology, was rarely used (Azam et al. 2015).

The present work investigated the impact of using different concentrations of clonazepam in the larval foodstuff, on the development of *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830) (Diptera: Sacrophagidae), in terms of larval body weight, length, or width. Additionally, the study aimed to detect the drug in the treated groups of larvae, using HPLC–MS and protein carbonyl analyses.

Methods

Maintaining stock colony

Sarcophaga argyrostoma was collected on minced buffalo meat from gardens of the Cairo University, identified based on morphological characterization by Prof. Dr. Magdy Shaaban due to Pape (1996), and reared in the Medical Entomology Lab, Entomology Department, Faculty of Science, Cairo University, Egypt, for three generations before the experiment. Adults had continual access to water and granulated sugar in 45 l × 45 w × 45 h wooden cages with mesh gauze on three sides and a wooden side with a round hole covered with a cotton cloth that allows a hand to change the water and sugar periodically. Females were allowed to oviposit on fresh minced buffalo meat, and the larvae were reared on the same food source. The experiment was maintained under laboratory conditions in July 2019 (14 L: 10 days h cycle at 30 ± 4 °C and 60% ± 5% RH).

Application of clonazepam on minced buffalo meat

Clonazepam (C1277) Sigma-Aldrich was dissolved in purified distilled water at three concentrations: (1) mg/ml, 2 (mg/ml), and 4 (mg/ml). Each concentration was mixed with minced buffalo meat. The levels of clonazepam in the three meat treatments were 25 mg/ml, 50 mg/ml, and 100 mg/ml. Each concentration was added to a group of three plate replicates, with 80 g meat in each plate. Meat mixed with distilled water served as the control treatment.

A group of 80 larvae (first instar) of *S. argyrostoma* was reared on 80 g of each clonazepam-minced buffalo meat concentration (25 mg/ml, 50 mg/ml, and 100 mg/ml) in a 100 ml plastic container covered with fabric mesh. Each treated or control group was replicated three times. Ten larvae from the different developmental instars (second, early, and late third and prepupa) were collected once every 12 h for 2 days and then every 24 h until pupation. They were identified by using ZOM.03.ZB45B1 Stereo Zoom Binocular, killed by immersing in hot water (90 °C) for 10 s, dried on tissue paper, morphologically measured, preserved in 70% alcohol, and stored at − 20 °C until used for both analytical and biochemical analyses.

Morphological, analytical, and biochemical data analyses

The morphological data (weight, width, and length), of each experimental group, were manually evaluated using a digital caliper (mitutoyo digital vernier) and digital balance (RADWAG, WTB 200). Ten larvae were measured and weighed for each tested replicate.

Clonazepam concentration inside the insect was determined using HPLC–MS analysis, according to the methodology of Rojas et al. (2017). Briefly, each experimental sample composed of the mixing up of the three replicates of insects to form a one pool sample which was homogenized in ice-cold phosphate buffer, pH to 7.0, and then centrifuged at 2000 × g for 10 min at 4 °C, and the supernatant was taken. Samples for HPLC–MS analysis are prepared using ethanol extraction system, where each cartridge C-18 is hardened with 2 ml of methanol and 2 ml of distilled water. Elution of the analyte is collected using 1 ml of 1:1 methanol/distilled water, then mixture added in a chromatography vial, and then 20 µl of the extracted sample was injected into an HPLC–MS DAD Agilent Technologies 1100 series®, using a column RP-18 endcapped (5 µm), 150 mm 4.6 (Purospher® STAR), with a mobile phase which contained 40% v/v acetonitrile and 60% v/v buffer K2HPO4/KH2PO4, and adjusted to pH 8.5 with triethylamine. The flow of this mobile phase was run at 1 ml/min. The analysis runs at wavelength 245 nm for 11 min.

The biochemical analysis, which includes quantitatively determination of protein oxidative damage in form of protein carbonyls amount, was determined according to Levine et al. (1990), with minor modifications. Briefly, experimental samples were homogenized in ice-cold phosphate buffer, pH to 7.0, and then, they were centrifuged at 2000 × g for 10 min at 4 °C. For each experimental sample, 800 µL of supernatant was mixed with 200 µl

2, 4-dinitrophenyl hydrazine (DNPH) then incubated for 30 min at room temperature, precipitated with 10% trichloroacetic acid (TCA), and left for 10 min at 4 °C. The samples were centrifuged at 5000 × g for 7 min at 4 °C. The pellet was washed four times with an ethanol/ethyl acetate (1:1) mixture and redissolved in 1 ml of sodium phosphate buffer, pH to 6.8. At the last step, the absorbance was measured at 366 nm, and the rate of protein carbonyls concentration was expressed as OD/mg protein. The total protein concentration of samples was determined spectrophotometrically according to the method of Bradford (1976).

Statistical analysis

Nonparametric analysis was performed, on all experimental groups, using a Kruskal–Wallis revealed test, with "p-value < 0.05"). The correlation and regression analysis among different clonazepam concentrations and current results was performed based on Pearson's correlation equation. Hierarchical cluster analysis (HACA) dendrogram analysis was calculated for clonazepam concentration and insect's developmental instars using Ward's method. A generalized estimated equation (GEE) was accomplished to establish the interaction between different variables. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

Results

A generalized estimated equation (GEE) showed that the different concentrations of clonazepam (25, 50, and 100 mg/ml) influenced significantly the morphological (weight, length, width), the analytical (HPLC–MS), and the biochemical measures of different developmental stages (2nd, early 3rd, and late 3rd instars and prepupa) of *S. argyrostoma* (Table 1).

Applying a nonparametric test (Kruskal–Wallis) on the data clarified significant differences in the morphology of the larval and prepupal stages of the treated groups of *S. argyrostoma* in comparison with the control group, especially within the weight of 2nd instar and length of prepupa stage ($\chi^2 = 9.61$, 9.79; $df = 3$, 3; and p-value < 0.05, < 0.05, respectively). The higher two concentrations of clonazepam (50 and 100 mg/ml) elevated the weight (mg), the length (mm), and the width (mm) significantly in both the late third instar and prepupa (Fig. 1a, b, and c).

Clonazepam was analytically measured (mg/ml) by HPLC–MS, in different treated groups of *S. argyrostoma* (Fig. 2). In all stages, there were significant increases of clonazepam concentration inside insects in respect to their control samples, especially in the 50 mg/ml and 100 mg/ml clonazepam-treated groups ($\chi^2 = 9.667$, 9.425, 9.495, 9.367; $df = 3$; p-value < 0.05).

Table 1 Testing the interaction of different concentrations of clonazepam (25, 50, or 100 mg/ml) on different developmental stages (second, early third, and late third larval instars and prepupa) of *Sarcophaga argyrostoma*, in terms of morphological measurements and, analytical and biochemical analyses, using generalized estimating equation (GEE)

Categorical variables	Chi-square (χ^2)	*df*	[a]QIC	*p*-value
Intercept				
Weight	3341.4	1	49.1	< 0.0001
Length	20,950.1	1	46.1	< 0.0001
Width	10,909.1	1	33.9	< 0.0001
HPLC	15,815.8	1	32.5	< 0.0001
PC	91,213.3	1	32.03	< 0.0001
Effect of different insect developmental stages				
Weight	5066.4	3	49.1	< 0.0001
Length	5753.3	3	46.1	< 0.0001
Width	2173.4	3	33.9	< 0.0001
HPLC	6253.7	3	32.5	< 0.0001
PC	2679.1	3	32.03	< 0.0001
Effect of different clonazepam concentrations				
Weight	4.01	3	49.1	< 0.0001
Length	133.4	3	46.1	< 0.0001
Width	55.7	3	33.9	< 0.0001
HPLC	2128.4	3	32.5	< 0.0001
PC	2498.7	3	32.03	< 0.0001
Effect of interaction between different insect developmental stages and clonazepam concentration				
Weight	38.6	9	49.1	< 0.0001
Length	308.4	9	46.1	< 0.0001
Width	43.11	9	33.9	< 0.0001
HPLC	1770.1	9	32.5	< 0.0001
PC	6135.9	9	32.03	< 0.0001

[a] QIC is the quasi-likelihood under the independence model criterion and is a derivation of Akaike's information criterion for GEE

The biochemical analysis of the macromolecules damage in *S. argyrostoma* showed that the protein carbonyls amount (OD/mg protein/min) elevated significantly for all developmental stages in comparison with the control groups, especially in the 100 mg/ml clonazepam-treated group (10.458; 10.495; 10.421; 10.274; $df = 3$; p-value < 0.05, respectively), with only one exception which was the second instar in the 25 mg/ml clonazepam-treated group (Fig. 3).

The current results showed an almost positive correlation (weak to strong) between clonazepam concentration and the tested parameters, morphological, analytical, and biochemical, with a linear predicted equation and R2 values ranging from 0.02 to 0.98, ($p < 0.05$) (Table 2).

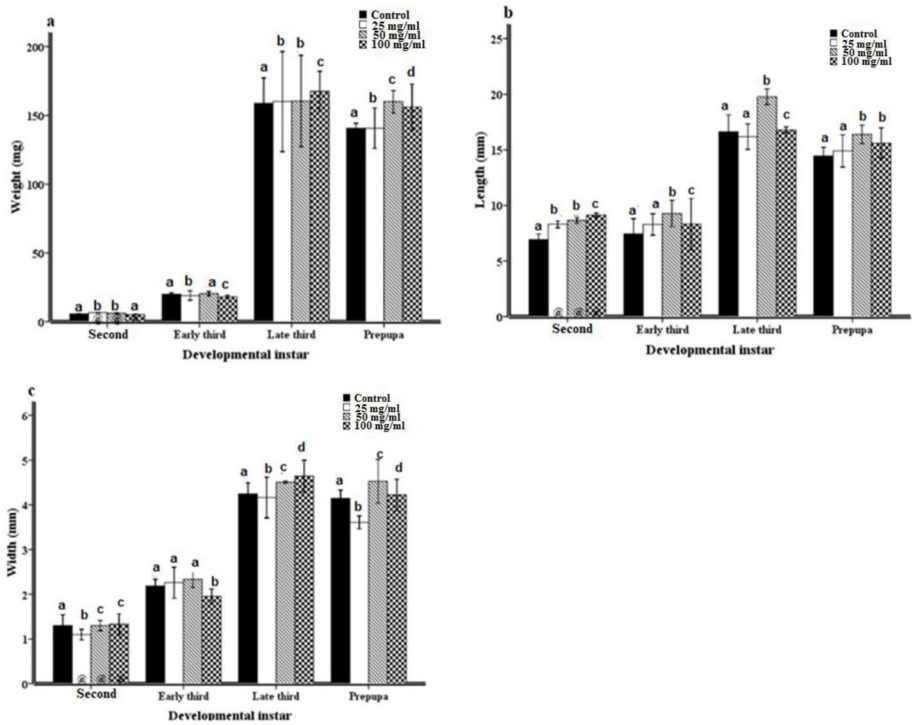


Fig. 1 Impact of different concentrations of clonazepam (25, 50, or 100 mg/ml) on the morphological measurements of larval and prepupal stages of *Sarcophaga argyrostoma*: **a** weight (mg), **b** length (mm), and **c** width (mm). Data expressed as median and standard deviation (SD). Median values marked with different small letters were significantly different ($p < 0.05$) regarding to clonazepam concentration. @ expressed the missing data which simulated using a Monte Carlo concept (Roth and Switzer, 1995)

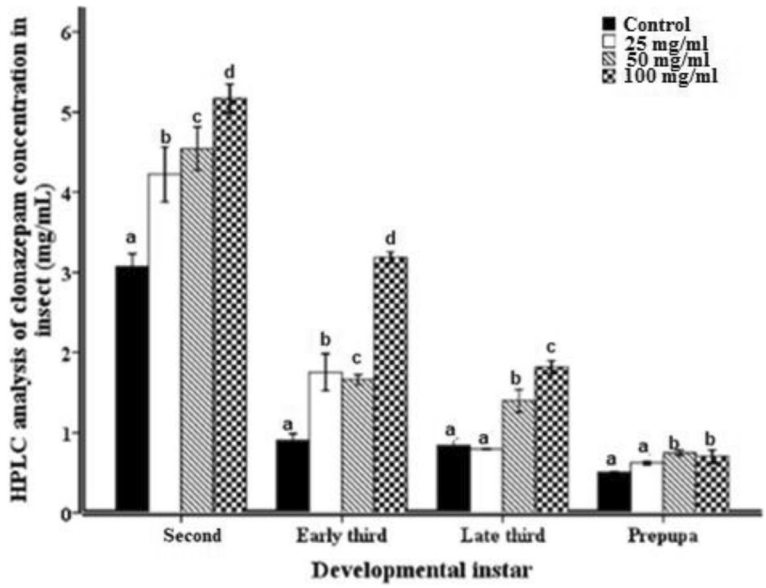


Fig. 2 Detection of clonazepam concentration in larval and prepupal tissues of *Sarcophaga argyrostoma*, which fed on different concentrations of clonazepam (25, 50, or 100 mg/ml), using HPLC. Data expressed as median and standard deviation (SD). Median values marked with different small letters were significantly different ($p < 0.05$) regarding to clonazepam concentration

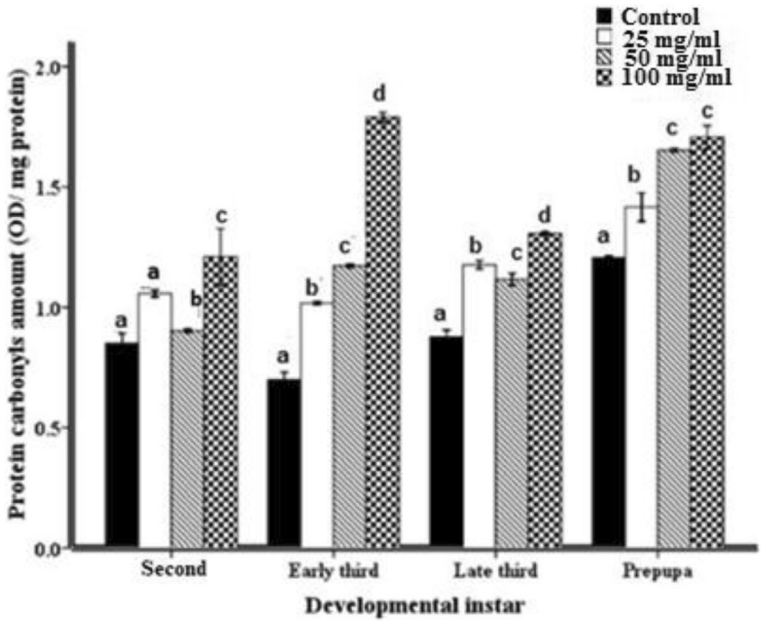


Fig. 3 Detection of protein carbonyle amount (OD/mg protein) in larval and prepupal tissues of *Sarcophaga argyrostoma*, which fed on different concentrations of clonazepam (25, 50, or 100 mg/ml). Data expressed as median and standard deviation (SD). Median values marked with different small letters were significantly different ($p < 0.05$) regarding to clonazepam concentration

Table 2 A linear predicted equations and R^2 values clarified the effect of different concentrations of clonazepam (25, 50, or 100 mg/ml), with respect to control samples, on different developmental stages (second, early third, and late third larval instars and prepupa) of *Sarcophaga argyrostoma*

		Stage	r^a	*p-value*	R^2	Equation	Type of equation
Morphological measurements	Weight	2nd stage	−0.34	< 0.05	0.23	$y = -0.007 \times +6.25$	
		Early3rd stage	−0.29	< 0.05	0.12	$y = -0.015 \times +20.6$	
		Late 3rd stage	0.15	< 0.05	0.02	$y = 0.089 \times +157.8$	
		Prepupa	0.59	< 0.05	0.29	$y = 0.17 \times +141$	Linear equation for prediction
	Length	2nd stage	0.91	< 0.01	0.74	$y = 0.01 \times +7.39$	
		Early3rd stage	0.31	< 0.05	0.05	$y = 0.008 \times +7.9$	
		Late 3rd stage	0.28	< 0.05	0.02	$y = 0.006 \times +17.1$	
		Prepupa	0.51	< 0.05	0.17	$y = 0.012 \times +14.7$	
	Width	2nd stage	0.21	< 0.05	0.06	$y = 0.001 \times +1.21$	
		Early3rd stage	−0.33	< 0.05	0.18	$y = -0.002 \times +2.29$	
		Late 3rd stage	0.58	< 0.01	0.34	$y = 0.004 \times +4.18$	
		Prepupa	0.32	< 0.05	0.08	$y = 0.003 \times +3.91$	
Analytical analysis	HPLC analysis of clonazepam concentration	2nd stage	0.913	< 0.01	0.91	$y = 0.019 \times +3.41$	
		Early3rd stage	0.954	< 0.01	0.94	$y = 0.021 \times +0.93$	
		Late 3rd stage	0.939	< 0.01	0.87	$y = 0.011 \times +0.73$	
		Prepupa	0.720	< 0.01	0.81	$y = 0.0019 \times +0.56$	
Biochemical analysis	Protein carbonyls amount	2nd stage	0.766	< 0.01	0.57	$y = 0.0031 \times +0.87$	
		Early3rd stage	0.994	< 0.01	0.98	$y = 0.011 \times +0.70$	
		Late 3rd stage	0.866	< 0.01	0.74	$y = 0.0037 \times +0.95$	
		Prepupa	0.906	< 0.01	0.81	$y = 0.0049 \times +1.28$	

[a] Pearson correlation coefficient (two-tailed test)

The clustering of all insect developmental stages revealed that there was a separate cluster group between control, 25, and 100 mg/ml clonazepam-treated groups in weight and length parameters (Fig. 4a–b). However, there was a separate cluster between control, 50, and 100 mg/ml clonazepam-treated groups in width parameter (Fig. 4c). Both HPLC–MS and protein carbonyls analyses revealed an obvious dissimilarity pattern between the control and 100 mg/ml clonazepam-treated group (Fig. 4d–e). There was a similarity pattern between the second and early third insects, also between late third and prepupal stages in all morphological data (weight, length, and width) (Fig. 5a–c); however, the treated second instar and the prepupa showed a unique pattern in case of HPLC–MS and protein carbonyls analyses (Fig. 5d–e).

Discussion

Many references discussed the feasibility of using Calliphoridae as toxicological indicators in the forensic investigations; however, little were found concerning Sarcophagidae. Dipteran maggots have been collected from fresh, putrefied, early decayed, and advanced decayed cadavers (Matuszewski 2021). Moreover, these maggots have proved their importance as toxicological samples in the absent, burnt, or badly decomposed corpses (Dayananda & Kiran 2013; Bugelli et al. 2017). Insect evidence can help in PMI estimation through analyzing the insect's cadaver succession pattern, to determine the initial colonizers, and also through studying the size and developmental stages of the carrion insects, to help in determining their age, and consequently estimating PMI in criminal or non-criminal death scenes (Bala & Sharma 2016). The geographical location, the climate condition, and the diverse within the insect's populations would greatly affect the maggot's developmental data. So, we need a reference developmental catalogue for each forensically important species (Owings et al. 2014; El-Bassiony 2020). Furthermore, the knowledge about the carrion insect's ability to detect toxins in large vertebrate cadavers will help the medicolegal investigations.

We found slight, if any, impact of clonazepam on weight, length, or width when we used *f*-test to analyze the changes in the morphological measurements

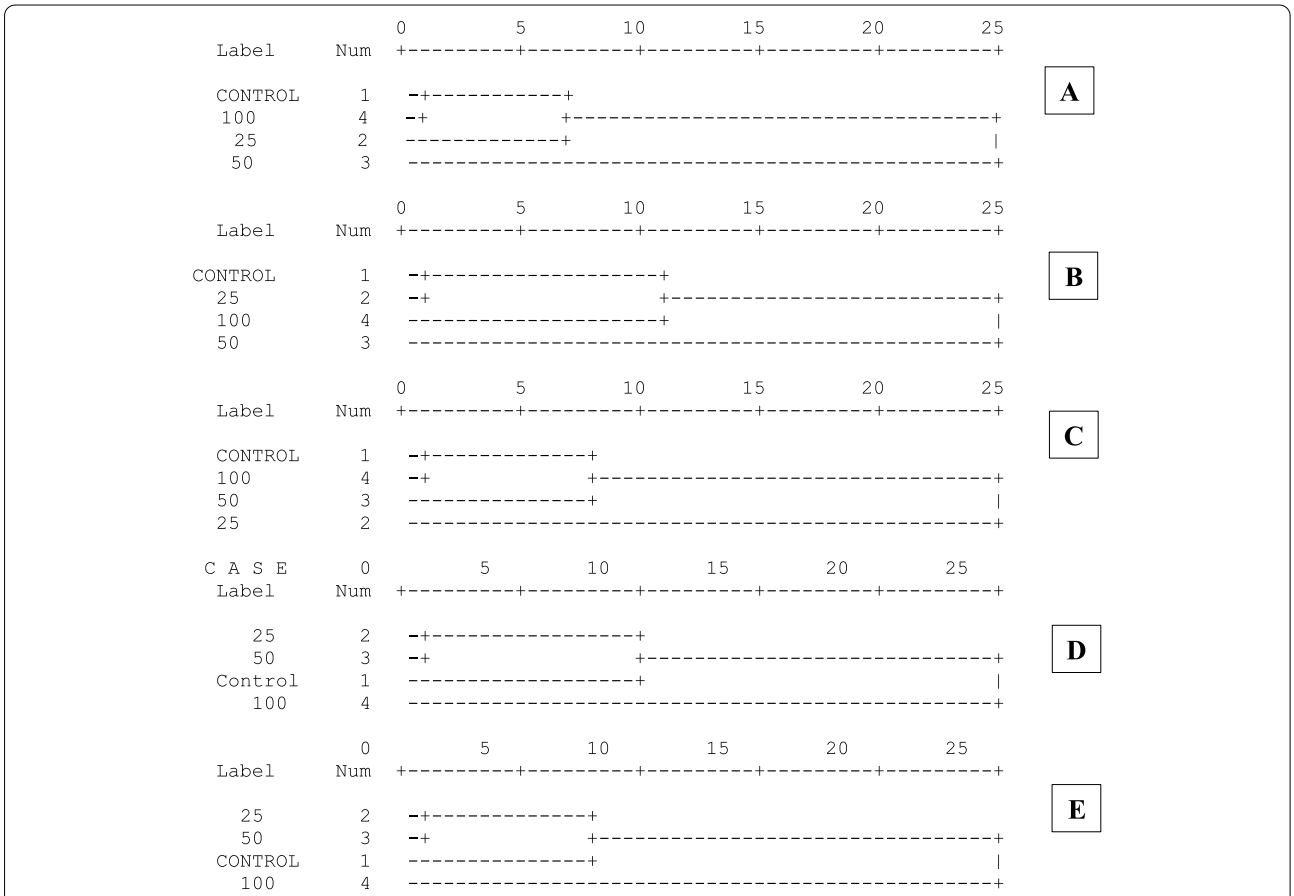


Fig. 4 Dendrogram of the cluster analysis (using Ward's method) to evaluate the similarity between different clonazepam concentrations (25, 50, or 100 mg/ml), which has been fed to the developmental stages (second, early third, and late third larval instars and prepupa) of *Sarcophaga argyrostoma*, and the (**A**) weight (mg), **B** length (mm), **C** width (mm), **D** concentration of clonazepam (mg/ml) in insect's tissues using HPLC–MS, and (**E**) protein carbonyls amount in insect's tissues using (OD/ mg protein/ min)

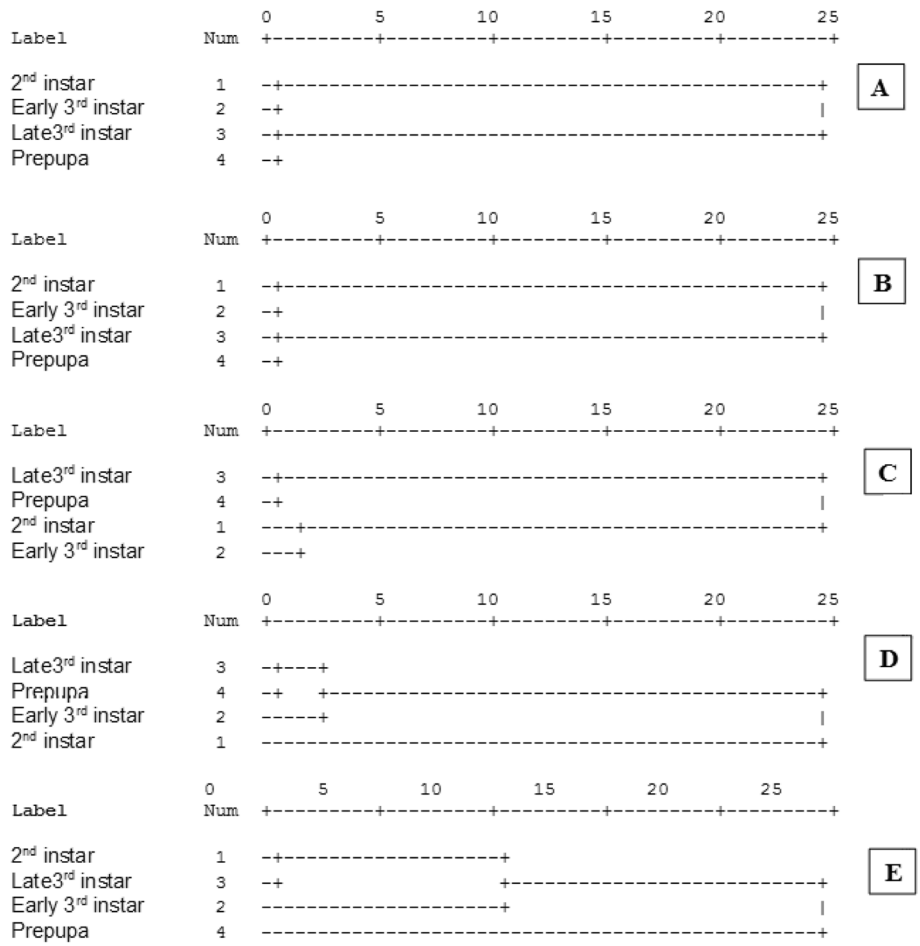


Fig. 5 Dendrogram of the cluster analysis (using Ward's method) to evaluate the similarity effect between the developmental stages (second, early third, and late third larval instars and prepupa) of *Sarcophaga argyrostoma*, which fed on different clonazepam concentrations (25, 50, or 100 mg/ml), and the (**A**) weight (mg), **B** length (mm), **C** width (mm), **D** concentration of clonazepam (mg/ml) in insect's tissues using HPLC–MS, and (**E**) protein carbonyls amount in insect's tissues using (OD/mg protein/min)

of treated *S. argyrostoma*. However, using the Kruskal–Wallis test in the current study declared that the drug has significant effects on all morphological measurements in larvae and prepupae of the treated *S. argyrostoma*. *Chrysomya albiceps* (Wiedemann 1819) (Diptera: Calliphoridae), and *Chrysomya putoria* (Wiedemann 1818) larvae, fed on diazepam mixed tissues and had a developmental rate more than control larvae; also, there was a significant increase of adult emergence time than the control one (Carvalho et al. 2001). In the present study, Clonazepam concentration 100 mg/ml resulted in significant ($p < 0.05$) increases in weight and width of the late third larval stage with levels being about 0.1 and 0.2 times compared to their control, respectively (Fig. 1). Our results agreed with Carvalho et al. (2001) who recorded a significant positive correlation between the diazepam drug concentration and the weight parameters of *C. albiceps* and *C. putoria* larvae. Similarly, different categorical drugs have increased the development of flies, such as cocaine (de Carvalho et al. 2012), methamphetamine (Goff et al. 1997), and ethylene glycol (Essarras et al. 2018).

Measuring clonazepam by HPLC–MS in the current study indicated that the drug faded gradually from a stage to the following one. This might indicate that the drug transformed to its metabolite form, 7-acetamidoclonazepam (Sjö et al. 1975). On the other hand, clonazepam concentration elevated significantly, especially, in the 50 mg/ml and 100 mg/ml treated groups. It became evident that HPLC–MS could measure clonazepam in *S. argyrostoma* larval and prepupal tissues. Benzodiazepine, oxazepam, was detected analytically by LC–MS-MS in larvae and puparia of *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae), after rearing on beef heart containing 1 pg/g nordiazepam (Wood et al. 2003). Also, Campobasso et al. (2004) measured

antidepressant drugs such as levomepromazine and thioridazine in both human tissues and blowfly larvae using GC analysis. Moreover, amphetamine drug was found in the Calliphoridae and Sarcophagidae larvae, which were collected from a suicide corpse using GC/MS analysis (Definis-Gojanović et al. 2007). Salimi et al. (2018) assessed morphine quantitatively, using HPLC–MS and TLC, from *C. albiceps*, where the higher doses were detected in feeding and post-feeding larvae.

The current results reveal the higher sensitivity of the protein carbonyls analysis in detecting clonazepam. Our current study reported bioaccumulation of the drug and its metabolites, which increased the protein carbonyls amount from an instar to the next instar (Fig. 3). The metabolites of some addictive drugs and toxins could be detected in both feeding and post-feeding larvae (Gosselin et al. 2010). Also, Boulkenafet et al. (2020) found carbamazepine and clobazam and their metabolites (carbamazepine epoxy, oxazepam, desmethylclobazam) in the larvae of *C. albiceps*, *Lucilia sericata* (Meigen, 1826) (Diptera:Calliphoridae), and *Lucilia silvarum* (Meigen 1826) fed on rabbit which was injected with the two drugs. This might indicate that benzodiazepines affect insects as well as humans.

Conclusions

A carrion-related dipteran database is important because of its role in decomposing most terrestrial carrion. Little work was made on sarcophagid flies related to their role in detecting toxins in forensic investigations. Feeding the larvae of *S. argyrostoma* on minced meat mixed with high concentration (50 & 100 mg/ml) of clonazepam accelerated the larval growth rate and might cause a difference in the PMI estimation for crime/forensic investigations. Current work revealed the ability of *S. argyrostoma* to accumulate clonazepam and the possibility of using this insect to reveal the drug presence in poisoning-related abuses. Also, HPLC–MS proved its efficiency in detecting the drug in *S. argyrostoma* maggots. Likewise, protein carbonyls analysis was used, for the first time, as a new technique to detect the drug and proved itself as cost-saving method. Insects could tell a lot about a corpse that was completely decayed or dislocated from its death seen.

Abbreviations

HPLC–MS: High-performance liquid chromatography-mass spectrometry; GABA: Gamma-aminobutyric acid; GC: Gas chromatography; RIA: Radioimmunoassay; GCMS: Gas chromatography/mass spectrometry; LC–MS-MS: Liquid chromatography-tandem mass spectrometry; ELISA: Enzyme-linked immunosorbent assay; UQTOF-MS: Ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; DNPH: 2, 4-Dinitrophenyl hydrazine; TCA: Trichloroacetic acid; HACA: Hierarchical cluster analysis; GEE: Generalized estimated equation; TLC: Thin-layer chromatography.

Acknowledgements

The authors would like to express their gratitude to Prof. Dr. Magdy Shaaban who identified the insect's specie, and to Dr. Mahitab E. El Daly who reviewed the English language.

Authors' contributions

FMA, this paper is a part of A's M.Sc. thesis; A, investigation, methodology, writing (original draft preparation), and funding acquisition; EAA, investigation, methodology, and writing (original draft preparation). GMEB, conceptualization, visualization, supervision, writing (review and editing), and funding acquisition. The authors read and approved the final manuscript.

Funding

This research was supported by research services funds, Faculty of Science, Cairo University, to the Medical and Forensic Entomology Lab supervised by Prof. Dr. Ghada Mohamed El-Bassiony.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the then corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals that require ethical approval.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Received: 9 November 2021 Accepted: 14 August 2022
Published online: 31 August 2022

References

Aly SM, Wen J, Wang X, Cai J, Liu Q, Zhong M (2013) Identification of forensically important arthropods on exposed remains during summer season in northeastern Egypt. J Cent South Univ (med Sci) 38:1–6

Aly MZ, Osman K, Galal FH, Ali GH (2017) Comparative study on outdoor and indoor forensic insects encountered on rabbit corpses in Upper Egypt. IOSR J Pharm Biol Sci 12:41–54

Azam I, Afsheen S, Zia A, Javed M, Saeed R, Sarwar MK, Munir B (2015) Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. BioMed Res Int. Article ID: 942751. doi:https://doi.org/10.1155/2015/942751

Badenhorst R, Villet MH (2018) The uses of Chrysomya megacephala (Fabricius, 1794)(Diptera: Calliphoridae) in forensic entomology. Forensic Sci Res 3:2–15

Bala M, Sharma A (2016) Review of some recent techniques of age determination of blow flies having forensic implications. Egypt J Forensic Sci 6:203–208

Boulkenafet F, Dob Y, Karroui R, Al-Khalifa M, Boumrah Y, Toumi M, Mashaly A (2020) Detection of benzodiazepines in decomposing rabbit tissues and certain necrophagic dipteran species of forensic importance. Saudi J Biol Sci 27:1691–1698

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bugelli V, Papi L, Fornaro S, Stefanelli F, Chericoni S, Giusiani M, Vanin S, Campobasso CP (2017) Entomotoxicology in burnt bodies: a case of maternal filicide-suicide by fire. Int J Legal Med 131:1299–1306

Campobasso CP, Di Vella G, Introna F (2001) Factors affecting decomposition and Diptera colonization. Forensic Sci Int 120:18–27

Campobasso CP, Gherardi M, Caligara M, Sironi L, Introna F (2004) Drug analysis in blowfly larvae and in human tissues: a comparative study. Int J Legal Med 118:210–214

Carvalho LM, Linhares AX, Trigo JR (2001) Determination of drug levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. Forensic Sci Int 120:140–144
Chophi R, Sharma S, Sharma S, Singh R (2019) Forensic entomotoxicology: current concepts, trends and challenges. J Forensic Leg Med 67:28–36
Dayananda R, Kiran J (2013) Entomotoxicology. Int J Med Toxicol Forensic Med 3:71–74
de Aguiar FJ, Brandao M, Sodré FF, Caldas ED (2015) Simultaneous determination of prescription drugs, cocaine, aldicarb and metabolites in larvae from decomposed corpses by LC–MS–MS after solid–liquid extraction with low temperature partitioning. Forensic Toxicol 33:93–103
de Carvalho LML (2009) Toxicology and forensic entomology. In Current concepts in forensic entomology (pp. 163–178). Springer, Dordrecht
de Carvalho LML, Linhares AX, Palhares FAB (2012) The effect of cocaine on the development rate of immatures and adults of Chrysomya albiceps and Chrysomya putoria (Diptera: Calliphoridae) and its importance to postmortem interval estimate. Forensic Sci Int 220:27–32
de Gage SB, Moride Y, Ducruet T, Kurth T et al (2014) Benzodiazepine use and risk of Alzheimer's disease: case-control study. BMJ 349:g5205. https://doi.org/10.1136/bmj.g5205
Definis-Gojanović M, Sutlović D, Britvić D, Kokan B (2007) Drug analysis in necrophagous flies and human tissues. Arh Hig Rada Toksikol 58:313–316
El-Bassiony GM (2020) Blowflies (Diptera: Calliphoridae) as forensic indicators in Egypt with special reference to the development data of Lucilia Cuprina (Wiedemann). Sumerianz J Biotechnol 3:43–51
Elian AA (2003) ELISA detection of clonazepam and 7-aminoclonazepam in whole blood and urine. Forensic Sci Int 134:54–56
Essarras A, Pazzi M, Dadour IR, Magni PA (2018) The effect of antifreeze (ethylene glycol) on the survival and the life cycle of two species of necrophagous blowflies (Diptera: Calliphoridae). Sci Justice 58(2):85–89
Funderburk FR, Allen RP, Wagman AM (1978) Residual effects of ethanol and chlordiazepoxide treatments for alcohol withdrawal. J Ner Men Dis 166:195–203
Goff ML (2009) Early post-mortem changes and stages of decomposition in exposed cadavers. Exp Appl Acarol 49:21–36
Goff ML, Lord WD (1994) Entomotoxicology. A new area for forensic investigation. Am J Forensic Med Pathol 15:51–57
Goff ML, Miller ML, Paulson JD, Lord WD, Richards E, Omori AI (1997) Effects of 3, 4-methylenedioxymethamphetamine in decomposing tissues on the development of Parasarcophaga ruficornis (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae, and puparia. J Forensic Sci 42:276–280
Gosselin M, Fernandez MDMR, Wille SM, Samyn N, De Boeck G, Bourel B (2010) Quantification of methadone and its metabolite 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine in third instar larvae of Lucilia sericata (Diptera: Calliphoridae) using liquid chromatography-tandem mass spectrometry. J Anal Toxicol 34:374–380
Gosselin M, Wille SM, Fernandez MDMR, Di Fazio V, Samyn N, De Boeck G, Bourel B (2011) Entomotoxicology, experimental set-up and interpretation for forensic toxicologists. Forensic Sci Int 208:1–9
Groth O, Franz S, Fels H et al (2022) Unexpected results found in larvae samples from two postmortem forensic cases. Forensic Toxicol 40:144–155. https://doi.org/10.1007/s11419-021-00601-x
Introna F, Campobasso CP, Goff ML (2001) Entomotoxicology. Forensic Sci Int 120:42–47
Kintz P, Godelar B, Tracqui A, Mangin P, Lugnier AA, Chaumont AJ (1990) Fly larvae: a new toxicological method of investigation in forensic medicine. J Forensic Sci 35:204–207
Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG et al (1990) [49] Determination of carbonyl content in oxidatively modified proteins. Meth Enzymol 186:464–478
Matuszewski S (2021) Post-mortem interval estimation based on insect evidence: current challenges. Insects 12:314. https://doi.org/10.3390/insects12040314
Nolte KB, Pinder RD, Lord WD (1992) Insect larvae used to detect cocaine poisoning in a decomposed body. J Forensic Sci 37:1179–1185
Owings CG, Spiegelman C, Tarone AM, Tomberlin JK (2014) Developmental variation among Cochliomyia macellaria Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas. USA Int J Leg Med 128:709–717
Pape T (1996) Catalogue of the Sarcophagidae of the world (Insecta; Diptera). Mem Entomol Int 8:1–558
Pounder DJ (1991) Forensic entomo-toxicology. J Forensic Sci Soc 31:469–472
Rates OD (2017) National institute on drug abuse. Bethesda, MD
Rojas SA, Duffau BE, Escobar M (2017) Analysis of clonazepam in oral fluid by SPE-C18 and HPLC-UV-DAD. J Anal Pharm Res 5(5):00152. https://doi.org/10.15406/japlr.2017.05.00152
Salimi M, Rassi Y, Chatrabgoun O, Kamali A, Oshaghi MA, Shiri-Ghaleh V et al (2018) Toxicological analysis of insects on the corpse: a valuable source of information in forensic investigations. J Arthropod Borne Dis 12:219–231
Sjö O, Hvidberg EF, Naestoft J, Lund M (1975) Pharmacokinetics and side-effects of clonazepam and its 7-amino-metabolite in man. Eur J Clin Pharmacol 8:249–254
Souza CM, Thyssen PJ, Linhares AX (2011) Effect of nandrolone decanoate on the development of three species of Chrysomya (Diptera: Calliphoridae), flies of forensic importance in Brazil. J Med Entomol 48:111–117. https://doi.org/10.1603/me09291
Stark C, Sykes R, Mullin P (1987) Temazepam abuse [letter]. Lancet. ii:802–3
Waghmare PB, Chikhalkar BG, & Nanandkar SD (2015) Establishing identity and cause of death in mutilated and un identifiable corpses: a challenging task for medico legal expert. J Forensic Biomed 06(01) https://doi.org/10.4172/2090-2697.1000120
Wood M, Laloup M, Pien K, Samyn N, Morris M, Maes RAA et al (2003) Development of a rapid and sensitive method for the quantitation of benzodiazepines in Calliphora vicina larvae and puparia by LC-MS-MS. J Anal Toxicol 27:505–512
Woods JH, Katz JL, Winger G (1987) Abuse liability of benzodiazepines. Pharmacol Rev 39:251–413

Publisher's Note