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Fly Maggots as Bio-Indicators of Warfarin Poisoning in Rabbits as An Animal Model

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ABSTRACT

Arthropods collected off the corpse or at a crime scene can be used to determine the presence of toxins in a body at the death time. This study was planned and objected at examining the possible use of fly larvae as indicators for detecting rabbit poisoning with Warfarin rodenticide. For this, Warfarin was examined in blood samples of an intoxicated rabbit carcass and maggots that were collected off such remain using the High-Performance Liquid Chromatography. The blood of the intoxicated rabbit was positive**.** The maggots showed two different results: (1) Positive that may refer to the feeding of larvae upon the rabbit liver which retains the toxin for 90 days post-mortem and (2) Negative that may be due to that this toxin is highly degraded after 18 hours of ingestion while the maggots started to invade the carcass after 48 hours of death. The controls of both rabbit and maggots were negative. Our results confirmed the reliability of entomological specimens for qualitative analyses of the toxins.

INTRODUCTION

In Forensic entomology, Entomotoxicology is defined as the analysis of toxins in arthropods (mainly flies and beetles) that feed on carrions. By using arthropods off the corpse or at a crime scene, investigators can determine whether toxins were present in a body at the death time. This technique is a major advance in forensic science. Previously, such determinations were impossible in the case of severely decomposed bodies devoid of intoxicated tissue and body fluids.

The potential use of blowflies larvae as indicators for detecting toxins and drugs in decomposing carcasses have been widely demonstrated (Nolte *et al.*, 1992). The analysis of intoxicated larvae can contribute to the qualitative identification of drugs in post-mortem tissues.

The insect life cycle stage that feeds on the cadaver is a potential reservoir of undigested flesh from the corpse. Because, in some circumstances, the flesh from the corpse can retain some types of drugs that had been consumed by the victim before death and which may even have been the cause of death, these drugs may be recovered by analyzing the insects (Introna *et al.*, 1990). Several researchers (Gunatilake and Goff, 1989; Nolte *et al.*, 1992; Miller *et al.*, 1994; Sadler *et al.*, 1997; Bourel *et al.*, 1999 and Hédouin *et al.*, 2001**)** detected several toxins and drugs in different species of fly larvae that fed on intoxicated corpses. However, there are no previous studies on the detection of Warfarin rodenticide in maggots that fed on the intoxicated rabbits.

An analysis of blood or liver samples is the best way to confirm the rabbit's exposure to rat poison and can also determine the specific product ingested. High-performed Liquid Chromatography (HPLC) seems to be the most effective method for the determination of anticoagulant rodenticides (Warfarin as an example) in the animal liver (Fauconnet *et al.*, 1997).

This study was planned and objected at examining the possible use of fly larvae as indicators for detecting Warfarin poisoning that was the cause of rabbit death.

MATERIALS AND METHODS

Experimental Animals:

Two European rabbits (*Oryctolagus curicullus* Linnaeus, 1758) in a weight of 1.5 Kg each were used. One was asphyxiated via hanging "Control" and the other was poisoned by an oral administration of Warfarin rodenticide at a rate of 50 mg per Kg "Intoxicated" following the method described by Abd El-Gawad *et al.* (2019). The animals were handled and treated following the guiding principles of The Research Ethics Committee (REC) for animal subject research at the Faculty of Science, Ain Shams University, Cairo, Egypt, that approved the experiments.

Sampling of Fly Larvae:

Dipteran larvae were collected off the Warfarin-intoxicated and control rabbit carcasses. After collection, larvae were killed with boiling water and then kept freezing at 4°C to avoid any physical and/or chemical changes then were ground and centrifuged at 2000 Xg for 5 minutes to be ready for analyses.

Blood Sampling of Rabbit Carcasses:

By using a sterile scalpel, an incision was made in the abdominal region of the control and intoxicated rabbit carcasses and about 100 mL blood samples were taken into heparinized vacutainers. The blood samples were immediately placed on ice, centrifuged at 2000 Xg at room temperature for 5 minutes and the separated plasma was kept frozen until analysis to detect the toxin.

Chromatographic Analysis:

Warfarin was detected in post-feeding larvae and post-mortem blood of rabbit carcasses using two different methods **(Table 1)** by High-Performance Liquid Chromatography (HPLC Agilent 1100 series), using Quaternary Pump with G1311A auto sampler and diode array detector. Besides, a standard curve for Warfarin(in distilled water) was prepared for comparison.

Table 1: Chromatographic methods for post-feeding maggots and post-mortem blood samples of rabbits.

Column	Maggots	Blood samples
	µBanda pack C18	
Mobile phase	Methanol (A) and phosphoric acid (B), (A:70: B:30 isocratic elution)	Methanol (A) and 2% acetic acid in dist. water (B), (A:23: B:77 isocratic elution)
Flow rate	1.5 ml/min.	
Wavelength	280 nm.	

RESULTS

The results of Chromatographic analysis **(**Table 2) showed that the post-mortem blood samples of the Warfarin-intoxicated rabbit were positive for the toxin (Fig. 1), The maggots that were collected off the intoxicated rabbit carcass showed two different results (Fig. 2) which were: (1) Positive (Sample 1) and (2) Negative (Sample 2), while the controls of both rabbit blood and maggots were negative.

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Table 2: Warfarin qualification in samples of postmortem rabbit blood and post-feeding maggots on rabbit carcasses through HPLC analysis.

Samples	Maggots		Blood	
	Retention Time (min)	**+/-ve**	**Retention Time (min)**	**+/-ve**
Standard	7.707, 8.017		2.938	
Tested	7.430, 8.064	+ve	2.584, 2.775, 2.929, 6.576	+ve
	4.006, 6.626	**-ve**		
Control	5.774	**-ve**	3.846, 4.299, 6.276, 6.759	**-ve**

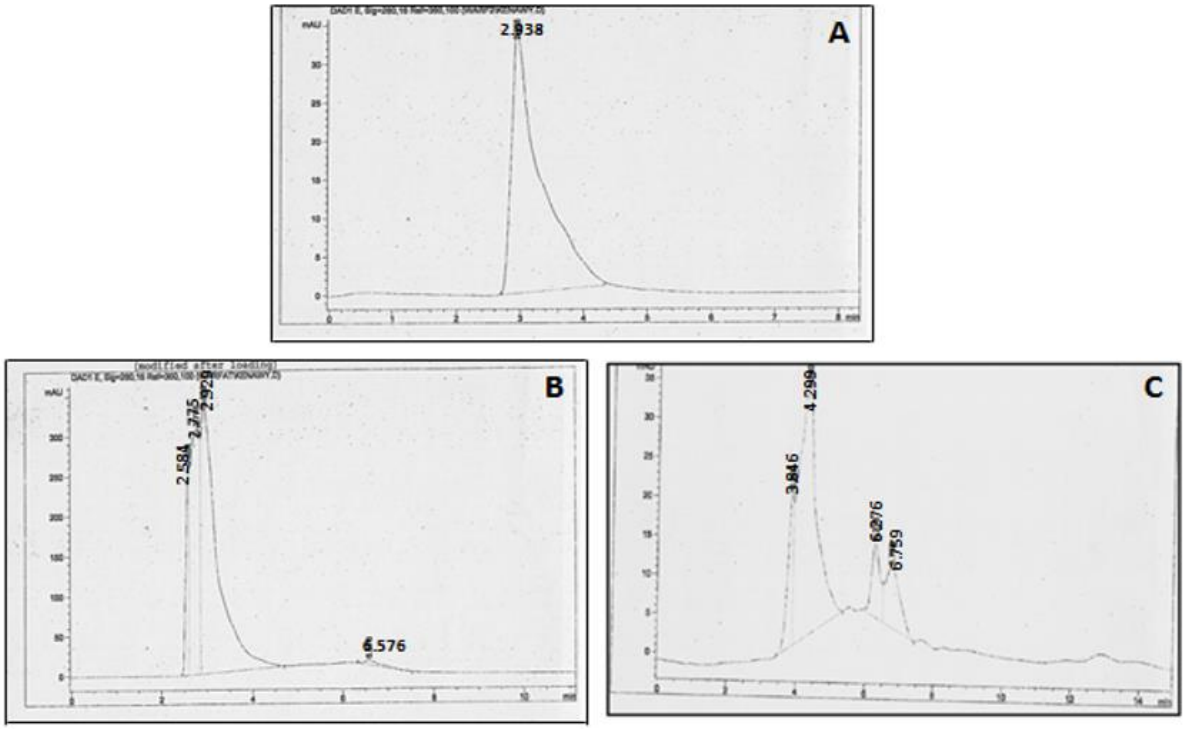

Fig. 1: HPLC Chromatogram of (A) Standard Warfarin, (B) Warfarin-intoxicated postmortem blood sample, and (C) Control postmortem blood sample.

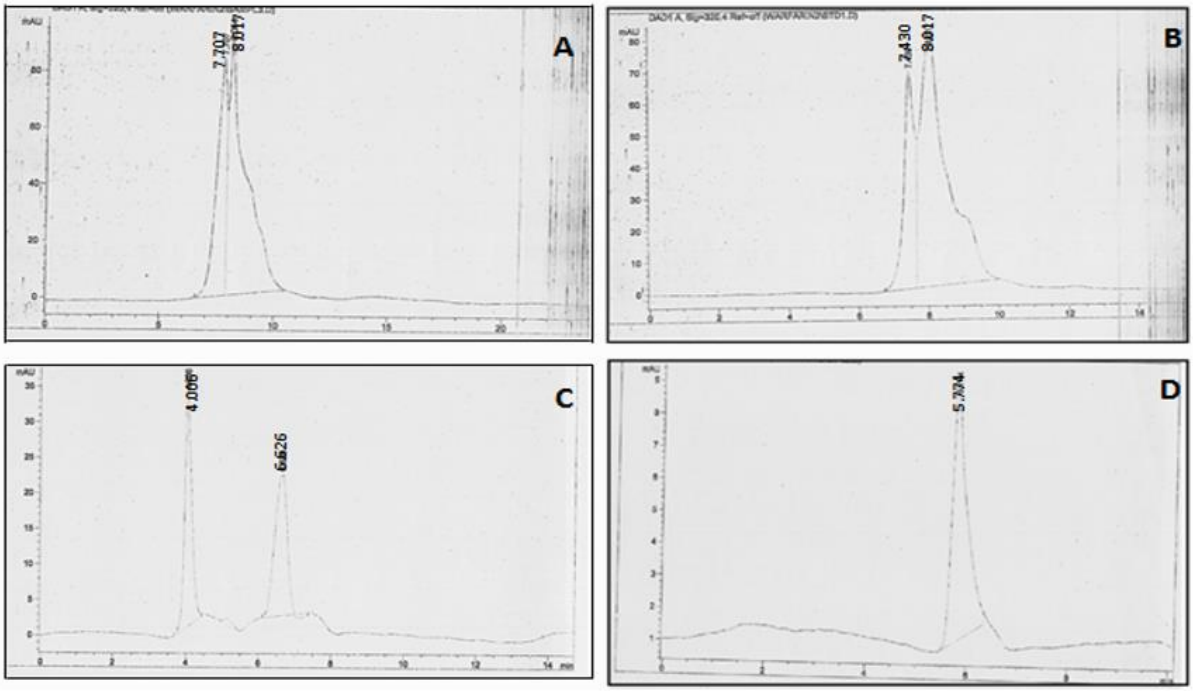

Fig. 2: HPLC Chromatogram of (A) Standard Warfarin, (B) Warfarin-intoxicated post-feeding maggots (1st sample), (C) Warfarin-intoxicated post-feeding maggots (2nd sample), and (D) Control maggots.

DISCUSSION

Research on Entomotoxicology has been directed towards elucidating the effects of drugs of use and abuse on the development of fly larvae (mainly blowflies). Necrophagous insects are valuable tools for post-mortem interval (PMI) estimation or for determining the cause of death. Like other toxins and drugs (Carvalho *et al.*, 2001; Musshoff and Madea, 2001; Arnaldos *et al.*, 2005; El-Samad *et al.*, 2011 and de Carvalho *et al.*, 2012), Warfarin affects in accelerating the development of dipteran larvae (Abdel-Gawadet *et al.*, 2018). Several studies (Bjornsson *et al.*, 1977; Wong *et al.*, 1977; Fauconnet *et al.*, 1997 and Chua *et al.*, 2012) analyzed Warfarin and other anticoagulant rodenticides in human and animal tissues.

The potential use of insects as alternative samples for the detection of drugs and toxins has been well documented. Campobossa *et al.* (2004) detected drugs in *Lucilia sericata* feeding on human tissues and confirmed the reliability of entomological specimens for qualitative analyses. Definis-Gojanovi *et al.* (2007) indicated that the toxicological analysis of human tissues, including blood, and the maggots flesh (Sarcophagidae and Calliphoridae) showed the presence of Amphetamine in all samples. El-Samad *et al.* (2011) detected Tramadol in *Lucilia sericata* that were reared on treated rabbit carcasses. Despite these studies and as far as we are aware, no available studies on the analysis of Warfarin in dipteran maggots.

The present study detected Warfarin in the maggots and the post-mortem blood of the rabbit carcasses. The positivity of maggots to the toxin (sample 1) may refer to the feeding of larvae upon the post-mortem liver which retains the toxin for 90 days after death (Bullard *et al.*, 1976). The negative detection of Warfarin in dipteran larvae (sample 2) may be due to that this toxin is highly degraded after 18 hours of ingestion (Pyrola, 1968) while the maggots started to invade the carcasses after 48 hours of death.

Our results confirmed the reliability of entomological specimens for qualitative analyses of the toxins in agreement with previous observations of Bjornsson *et al.* (1977), Wong *et al.* (1977), Fauconnet *et al.*

(1997), Chua *et al.* (2012) and Peyrovi and Hadjmohammadi (2015).

Conclusion

The detection of Warfarin in fly maggots that fed on intoxicated-rabbit carcass indicates the possible use of such maggots as bio-indicators for poisoning with toxins or drugs that may be the cause of death.

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ARABIC SUMMARY

يرقات الذباب كمؤشرات حيوية للتسمم بالورفارين في الارانب كنموذج حيواني

ألاء عبد الجواد* ، محمد أ قناوي، روضه م بدوى، ومرح م عبد البر
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من الممكن استخدام المفصليات التي يتم جمعها من على الجثث أو في مسرح الجريمة ، لتحديد وجود السموم في الجسم في وقت الوفاة. استهدفت هذه الدراسة احتمالية استخدام يرقات الذباب كمؤشرات للكشف عن تسمم الأرانب بمبيد القوارض الوارفارين ولذلك تم فحص الوارفارين في عينات الدم من جثة ارنب مسمم وفى اليرقات التي تم جمعها من على الجثة باستخدام الكروماتوغرافيا السائلة عالية الأداء. أظهرت النتائج إيجابية الدم لوجود السم بينما أظهرت اليرقات نتيجتين مختلفتين: (1) إيجابية والتى قد تشير إلى اغتذاء اليرقات على كبد الأرانب التي تحتفظ بالسم لمدة 90 يومًا بعد الإماتة و (2) سلبية وقد تكون بسبب أن هذا السم يتلاشي بشدة بعد 18 ساعة من الابتلاع فى حين تبدأ الديدان في غزو الجثث بعد 48 ساعة من الموت. كانت ضوابط كل من الأرنب واليرقات سلبية. وقد أكدت النتائج موثوقية العينات الحشرية للتحليلات النوعية للمواد السامة.