The Use of Green Metal Fly Chrysomya Albiceps and the Leather Beetle Dermestes Maculactus in Detecting the Cause of Death Resulting from the Use of Tramadol

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Abstract
The study was conducted in Al-Nahrain sub-district of Al-Dhuluiya district, south of Salah Al-Din Governorate, for the period from 10/13/2021 to 4/5/2022. During the study, 600 larvae of each of the green Adenium fly Chrysomya albiceps and Dermestes maculactus are used, to implement the experiment through Feeding these larvae on the bodies of rabbits treated with 2 ml of Tramadol hydrochloride at a concentration of 100 mg, for 24 hours. D. maculactus leather beetle larvae fed on the carcass of rabbits treated with Tramadol 8.03 mg / ml, and in the extract of the bodies of the larvae of green metallic flies Ch.albiceps, the drug concentration was 13.58 mg / ml, and the drug retention period in the control group is 3.46 minutes, while it was in tissue extract In rabbits, 3.50 minutes, and in the extracts of leather beetle and green metallic fly larvae bodies, 3.41 and 3.50 minutes, respectively.
Keywork: Al-Nahrain; Chrysomya albiceps ; Tramadol hydrochloride ; Forensic medical

1. Introduction

Forensic medical entomology is one of the most important fields of forensic entomology that deals with criminal cases, including knowledge of the post-mortem period (Huntington and Hall; 2019). Insects were used in criminal investigations decades ago, specifically in the thirteenth century AD, when Chinese investigators used blue flies in Disclosure of the circumstances of the murder of a Chinese farmer in the rice fields, despite the perpetrator's attempt to hide the features of the crime, but the flies were attracted to the remnants of blood on the killing tool (the sickle), forcing the criminal to confess his crime (Al-Mallah ; 2016). The evidence provided by insects on or near the bodies of the victims is useful in revealing the circumstances of the crime, such as identifying the cause of death resulting from the use of narcotics or toxins (salami et al ;2018). One of the most common causes of death around the world is drug overdoses, and Tramadol is one of the opiates that cause more than 47,500 deaths each year. This drug has serious effects as it can cross the blood-brain barrier, causing many health problems (Hedouin et al; 2001). The leather beetle D. maculactus of the Coleoptera order is one of the insects associated with human and animal corpses and one of the important factors in identifying the cause of death, such as the use of drugs in excess or with lethal doses with the aim of committing suicide (Noelia et al ; 2020).and the green metal fly Chrysomya albiceps of the family Calliphoridae is one of the most important and most widely used types of insects in forensic medicine, as the larvae of this insect consume the decomposing tissues of the corpse, which in turn gives important information about the circumstances of the crime (Al-Qasim,2021). The current study aimed to use the larvae of leather beetles D. maculactus and Ch.albiceps green metal flies in detecting tramadol concentrations in tissues of laboratory animals and considering it one of the causes of death resulting from drug abuse.

2. Materials and working methods:

Whole insects were collected from Al-Sayer village, belonging to Al-Nahrain district, south of Salah Al-Din Governorate, for the period from 10/13/2021 to 4/5/2022 at a temperature of 29°C and a relative humidity of 60%. In book No. (16) on 3/17/2022 and as shown in the table below

NO	Scientific term	Common term
1	Chry Chrysomya albiceps	green metal fly
2	Dermestes maculactus	Leather beetle

2-1. Breeding the insect samples used in the study

After acquiring adults all of the green metallic flies Ch. albiceps and leather beetles D. maculaetus, using a fishing net, a piece of decomposing flesh was placed inside it, and then each one was reared separately according to the appropriate conditions to obtain the larvae and use them in the study

2-2. Breeding of the green metal fly Chrysomyma albiceps

10adult males and females of the Avi insect were placed in plastic containers with perforated lids with dimensions of 15 x 15 x 15 cm. The floor was spread with sawdust from wood to maintain moisture. A small water bowl was placed inside the container. The adults fed 100 grams of decomposed sheep

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liver. 6 blocks of eggs were obtained. After 24 hours of feeding at a temperature of 30-32C, a relative humidity of 60%, and a 12-hour lighting period, the number of eggs per mass ranged from 175-200eggs, while some eggs were single. The adults died 36 hours after laying the eggs, while the eggs hatched in the same conditions after The first larval age is only 24 hours, after which it turns into the second age, which took 48 hours, to turn into the third larval age, which took 8 days to turn into the pupal stage, which took 10 days, and then the exit of the adult 14 hours **(**Al-Shihabi et al ;2019 **).**

Picture (1) A bowl and food for raising Chrysomyma albiceps larvae.

Picture (2) Third instar larvae of flies Ch. albiceps used in the experiment.

2-3. breeding of leather beetles D. maculactus

10 complete males and females of the insect were placed in a plastic container with perforated lid with dimensions of 15 x 15 x 10 cm. The floor was spread with sawdust. The adults were fed 100 grams of dry chicken liver. The adults laid their eggs individually by 17 eggs per day at a temperature ranging from 32 -30 ° C, relative humidity 60%, and a 12-hour light period. The eggs hatched after 3 days of laying in the same conditions. After hatching, the larvae were reared in a dark period of 20 hours, as it required 17 days to complete their growth to reach the last larval age to transform after that to the virgin, which took 3 days until the emergence of adults (Al-Qasim et al, 2021).

Picture (3) larvae of leather beetles D. maculactus (the researcher).

2-4 Breeding the samples on the treated medium

According to the method of. (Hedouin et al; 2001). in breeding insects. During this experiment, four male field rabbits were used, their weights ranged from 1800-2000 g. Three of them were given a dose of 2 ml of tramadol intravenously, while the fourth male was given 2 ml of distilled water. After dosing the males, the vital changes that occurred to them before the merciful slaughter were recorded, such as dilatation and exophthalmos of the eyes, exposure of the teeth, increased heart rate and hair erection, as they were slaughtered by a sharp instrument (knife) an hour after dosing, then the liver, heart and part of the muscles were extracted for the purpose of feeding the experimental insects on them. and conduct the study. 100 third instar larvae of leather beetles D. maculactus with a total weight of 8 g and 100 larvae of Ch. albiceps green metal fly were transferred and after starvation for 48 hours were placed in plastic containers of dimensions 15 x 15 x 10 cm in three replicates and fed on 50 g of tissue. The treatment (liver, heart and a section of muscles) for 24 hours killed the larvae and rinsed with distilled water to remove the plankton in it. Then it was placed in a 15 ml tube after education, then placed in the refrigerator for 48 hours. The larvae were transferred to the laboratory for the purpose of study.

2-5. Preparation of the extracts of the bodies of the insect larvae under study

According to the method of. (Al-Shihabi et al; 2019) the larvae of each of the beetles and flies were taken separately and dried. The larvae were ground with liquid nitrogen (196-) using a ceramic mortar until the samples became in the form of a fine powder. After grinding, 2 grams of each powder were weighed and placed in a test tube. Capacity of 15 ml the sample was mixed for one minute, then 1 ml of distilled water was added to it and 1 ml of the resulting solution was taken and placed in a 10 ml test tube with 0.5 ml of sodium hydroxide added to it, then 5 ml of ethyl acetate was added to the sample and the mixture was centrifuged. At 2000 rpm for 10 minutes, the precipitate was kept and ground again with liquid nitrogen, then 1 ml of methanol was added to it, and the solution was ready for use.

2-6. Chemical analysis of treated larval body extract

The chemical analysis of the extract of larval bodies fed on food treated with tramadol was carried out. The HPLC chromatograph was injected with 50 PPm of tramadol hydrochloride using 0.01ml reagent and UV visible spectrophotometer at a wavelength of 280 nm. The column was 4.6×C18,5u,250 and the mobile phase It is methanol in a ratio of water (20:80) that contains triethylamine, where the mobile phase flow rate was 1.0 m/min, and the retention period of tramadol was 3.46 minutes.

3. Results and Discussion

The larvae of flies and beetles are considered as alternative clues to find medicines and toxins in the event that tissues and some of the victim's body fluids cannot be obtained, which are used to reveal the causes of death or the circumstances of the crime. Leather D. macuclactus, the green metal fly Ch.albiceps used in the implementation of the experiment, where the highest concentration of the drug was 52.43 mg / ml in the tissues of rabbits treated with a dose of 2 ml of tramadol with a concentration of 100%, while the lowest concentration of the drug was recorded at 0% mg / ml in the control group while ingesting the drug concentration in the extract of leather beetle larvae and green metallic mollusks 8.03, 13.58 mg / ml, respectively as shown in Table (1) the concentrations of the drug in the extracts of tissues of experimental insects and animals after 24 hours of treatment

NO	Type of transaction	Tissue	Concentration
1	Tramadol	Fly tissue	13.58
2	Tramadol	Beetle tissue	8.03
3	Tramadol	Rabbit tissue	52.43
4	Control	Rabbit tissue	0

Through the results, it was found that the average concentration of tramadol in the extract of the bodies of the larvae of metallic flies Ch.albiceps was higher than its concentration in the extract of the bodies of the larvae of leather beetles D. maculactus and the reason is due to the nature of nutrition, as fly larvae prefer feeding on fluids and decomposing tissues of the carcass, which allows them to retain a greater amount of fluids, as these fluids contain medicines and chemicals, unlike leather beetle larvae whose late feeding is on dry tissues and skin (Thompson et al ; 2020). The reason may be due to the metabolism that occurs in the bodies of the larvae, which in turn leads to the disposal of drugs, and this differs from one insect to another (Huntington and Hall,2019), as it was found during the process of rearing larvae on the treated tissues that the metabolism process in the larvae of leather beetles D. maculactus was Much more than the larvae of the green metal fly Ch.albiceps, which makes the process of disposal of drug residues in beetle larvae higher than that of the larvae of flies. The results of the current study agreed with the results of the study of (As-Samad et al ;2011). with the difference in the type of insect, as it was used during the study Lucilia green glass fly larvae. sericata in the detection of tramadol in Lucilia sericata fly larvae extract treated with concentrations (2200,1100,550) mg/kg of tramadol. µg/g, while the concentration of tramadol in larval tissues was 5.60 µg/g The results of the study also showed that the average concentration of tramadol in the extract of the bodies of the larvae of metallic flies Ch.albiceps was higher than that of the extract of the bodies of the larvae of the leather beetle D. maculactus. The reason may be due to the nature of the feeding, as the larvae of flies prefer feeding on fluids and decomposing tissues, which allows them to retain a larger
amount in contrast to the larvae of leather beetles, whose feeding is delayed on dry tissue and skin
The results are also in line with the results of the study (Salami et al ;2018). used during the study of the larvae of the green metal fly Chrysomya albiceps and the larvae of hairy ruff beetles Creophilus maxillosus fed on rabbit carcasses treated with concentrations (50, 25, 12.5 mg / ml) to detect the presence of the drug heroin. The presence of the drug in rabbit tissue for all concentrations except for the concentration 12.5 mg / ml, where the highest concentration of heroin was 210 ng / g in the extract of the bodies of the larvae of the green metal fly Ch. albiceps fed on carcasses of rabbits treated with the lethal dose of 50 mg/ml, while the lowest concentration was 0% ng/g in extract of C. maxillosus larvae bodies fed on rabbits carcasses treated with a concentration of 12.5 mg/ml of heroin. The results of the study were similar to the results of the study of (Al-Qasim ; 2021). as it was revealed during the study the effect of tramadol on the larvae of the green metal fly Ch. albiceps fed on rabbit tissue treated with a concentration of 38.8 mg / ml of tramadol and the results of the study showed that the highest concentration of the drug was 63.86 mcg / g in rabbit liver and the lowest concentration in the control group was 0%, while the concentration of tramadol in larval tissues was 29.62 mcg / g. The results of the current study also with the results of the study of (Noelia et al ;2020). with the difference in the type of insect and the drug, where the larvae of the green glass fly Lucilia cuprina were used during the detection of heroin drug in meat treated with concentrations (500, 1000, 2500, 5000, 10000 ng / microliter) for the same drug The results of the study revealed the presence of the drug in the larvae treated with concentrations (5000,10000) ng/microliter, where the highest concentration was 96% in the tissues of fly larvae and the lowest concentration was 0% in the control group. The color scheme in Figure (2) shows that the retention period of tramadol in the standard sample of the drug lasted for 3.46 minutes after injecting 50 ppm of the original substance into a high-tech HPLC device, while Figure (3) showed the period of drug retention in rabbit tissue extract treated with the same treatment. The property is under the same conditions, as it lasted 3.50 minutes, as shown below

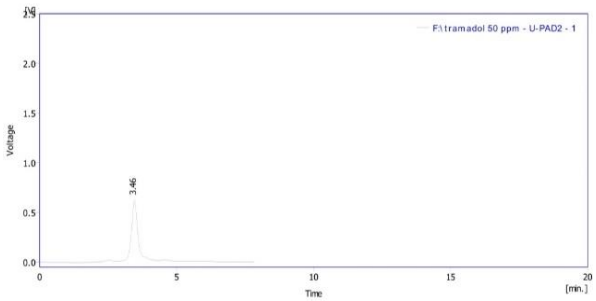

Figure (2) The holding period of pure tramadol

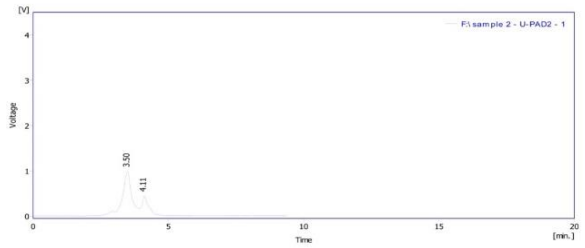

Figure (3) The period of tramadol retention in treated rabbit tissue extract

The color chart (4) shows the retention period of the drug for *D. maculactus* skin beetle larvae extracted on the carcasses of rabbits treated with the lethal dose of tramadol, and the retention period lasted for 3.41 minutes, which is close to the original sample, while Figure (5) showed the retention period of tramadol for the body extract of fly larvae. The green mineral fed on the treated tissues, which lasted 3.50 minutes, and the retention period was the same as that of rabbit tissue extract with a difference in area.

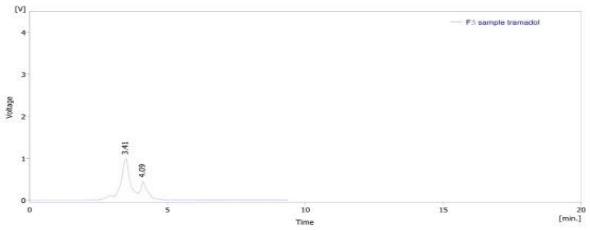

Figure (4) The retention period of tramadol for extracting the bodies of skin beetle larvae

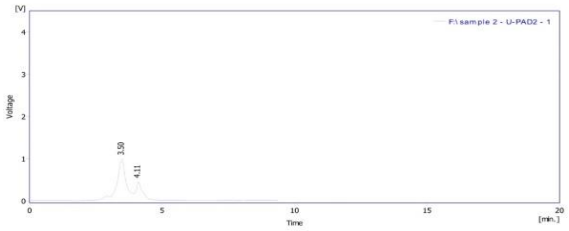

Figure (5) Tramadol retention period for extracting bodies of green metal fly larvae

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