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Effects of sildenafil citrate and diazepam on developmental stages of forensically important fly: *Chrysomya albiceps* (Diptera: Calliphoridae)

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Abstract
The blowfly *Chrysomya albiceps* is a member of Calliphoridae that feed on the carrion, garbage and feces. *Chrysomya albiceps* are most commonly associated with corposes. *Chrysomya albiceps* was reared on three groups of rabbit tissues administered (group Sildenafil citrate (SC or Viagra) an over dose of 10.26 mg/kg and group Diazepam an over dose of 4.06 mg/kg each of drugs dissolved in distilled water, by intra-peritoneal injection twice daily for one week. The control group was reared on rabbits injected with Distilled water. Residue of Sildenafil citrate and Residue Diazepam concentrations from both treated and control groups in some tissues of rabbit and third larval stage, pupae, adult *Chrysomya albiceps* were analyzed by high performance liquid chromatography (HPLC). Concentrations of this drugs in larval stage, pupae, adult were lower than those found in tissues rabbit. The developmental time for total durations life stage in treated carcasses were the longest at than the control. The life cycle *Chrysomya albiceps* increased gradually by adaptive for the two treated groups and decrease gradually by adaptive for control group in F 2 and F3. The durations of stages that fed on control carcasses was less time than diazepam, sildenafil respectively. These results indicate that (SC) and Diazepam retard larval development.

Keywords: *Chrysomya albiceps*, diazepam, larvae, sildenafil citrate

Introduction
The blowfly *Chrysomya albiceps* (green fly), is a member of the family Calliphoridae, that feed on the carrion, garbage and feces (Whitworth, 2006) [33]. *C. albiceps* is found throughout the world, it is one of the first insects to arrive at corpses, and appears on carcasses within minutes of death (Yassin and Mohamed 2015) [34].

Adult females of *C. albiceps* lay clusters of up to 150 eggs at a time, on the host or carcass then hatch to larvae and the 3rd instar larvae leave the host or carrion and burrow into the soil or substrate surrounding it and then develop to Pupae that emergence to adult and they can be multiple generations per year (Anderson, 2000; Tarone and Foran, 2006 and Strikewise, 2007) [3, 30, 27].

C. albiceps plays an important role in forensic medicine because the period of insect colonization can be used to determine the post-mortem interval (PMI) aiding law enforcement in their investigations (Rueda *et al*., 2010) [23], for determination of the Post Mortem Interval, there are three key areas of biological variation: time of oviposition,; developmental time and temperature, thus it is essential to collect accurate data about determination of maggots age (Schoenly *et al*., 2005) [24].

Insect development data has a wide range of uses, including: agricultural pests, disease vectors, medical interventions and medicolegal interpretation, entomotoxicology is new and important branch of forensic entomology because the toxicology and entomology interact in several ways: first, in the chemical control of pests of agriculture and agents causing myiasis in cattle and other animals (Mahon *et al*., 1993); second, in the effects of environmental pollution (chemical compounds) on growth and survival of beneficial insects (Simkiss, 1993); and third, in the recently emerging field of forensic entomotoxicology (Goff and Lord, 1994) [20]. Recent developments in forensic entomotoxicology have taken two paths, the first when a body is in the later stages of decomposition there are often no tissues remaining that are normally sampled for toxicological analysis in these cases, insects may be used as alternative sources of material for analyses (Beyer, 1980 and Nolte *et al*., 1992) [4, 20], the second area for consideration has been the effects of drugs and/or toxins in decomposing tissues on the rates of development of insect larvae feeding on those tissues. These effects can alter the times required for development and introduce an error into the estimation of post mortem intervals using entomological techniques (Goff, 1991) [9]. So that, forensic entomology is based on the analysis of the insects, which sequentially colonized a corpse as decomposition progresses.

Anderson and Van Laerhoven (1996) [2] added that the Forensic entomology is now an integral part of a death investigation when estimating the time since death beyond 72h, forensic entomology is considered the most accurate method for estimating the elapsed time since death, particularly when more than 3 days have elapsed, so that knowledge of the distribution, biology, ecology and behavior of insects found at a crime scene can provide information on when, where and how the crime was committed.

Therefore, *C. albiceps* plays an important role in PMI determination and it is one of the dominant flies of forensic importance in Egypt (Tantawi *et al*., 1996) [29], performed a multiple study on this fly but studies on the effects of drug abuse on the developmental rate of *C. albiceps* are still poorly understood. The objective of this thesis is to study the effects of the principal substances of drug abuse

(Sildenafil and Diazepam) on: Developmental rates of the *C. albiceps*. To determine the concentration of these drugs in each developmental stages of *C. albiceps* and to evaluate the relationship between the concentration of drugs in the tissues and stages against the initial dosage.

Materials and Methods

Experimental 1gypt. The animals were housed in suitable iron cages for two week in the insects laboratory of Zoology Department, faculty of science in Al-Azhar university (Assiut) for acclimation at 30±4ºC, under a 12:12h light – dark cycle and the animals food is a vegetable and fresh tap water. All animals were received care in compliance with the standard in situation's criteria for the care and use of experimental animals. Rabbits were keept under similar environmental conditions of temperature, illumination, acoustic noise, and ventilation, and received the same diet during the course of the experiment. Cleaning and changing water and food was done for all animals twice daily.

Doses determination

Sildenafil citrate (100 mg) and Diazepam (3 mg) (Zydol ® SR Tabs; Grünenthal Ltd, UK) were provided by pharmacist Ahmed Noqrashy (Dier Mawas city in AL Menia Governorate in Egypt.

Preparation of over dose

The dosage for human is 100 mg/day, according to the severity of the condition, the dose for human was converted to equivalent dose for rabbits according to Paget and Barnes (1964). The rabbits dose Sildenafil citrate was calculated as 5.13 mg/Kg body weight. An over dose of 10.26 mg/kg and the rabbits dose Diazepam was calculated as 2.03 mg/kg body weight. An over dose of 4.06 mg/kg rabbit. Sildenafil citrate and Diazepam were ground in a dry-sterilized porcelain mortar with pestle and dissolved in distilled water.

Group one (control)

Rabbits of this group were administrated normal distilled water through intraperitoneal injection (IP) twice a daily for 1 week and were killed by cervical dislocation.

Group (Sildenafil citrate (SC or Viagra)

Rabbits of this group were administrated Sildenafil through intra-peritoneal injection (IP) in adose of 10.26 mg/kg body weight twice a daily for 1 week until the death of each individual.

Group three (Diazepam)

Rabbits of this group were administrated Diazepam through intraperitoneal injection (IP) in a dose of 4.06 mg/kg body weight twice a daily for 1 week until the death of each individual.

Sampling of tissues for toxicological analysis

Blood sampling

From both treated and control groups, blood samples were withdrawn from the retro-orbital sinus of the eye of each rabbit using a sterile heparinized micro- hematocrit capillary tube and samples of 2 ml were collected into heparinised vacutainers at zero time, 12h and 24h after the drug administration. The blood samples were, centrifuged at 10000x g at room temperature for 10 min, the separated plasma was then stored at -80°C until analysis to determine the drug concentration.

Tissue preparation

After whole rabbits' death from both treated and control groups the samples of liver, kidney, and muscles were taken by cutting on the ventral side. 1.0 g of each solid tissue was dissected out over a stainless-steel plate cooled with ice. After weighting, the solid tissue were rinsed in phosphate buffer, wrapped in a piece of aluminum foil, sprayed with ethylene chloride and immediately frozen at -80°C till analysis. Three samples per tissue per carcass were taken.

Adult flies' collection

Rotten chicken viscera was used as bait for collecting adult stage of the *C. albiceps* in open area using hand catch by net, this was performed at March 2018, beginning in mornings and continued until a sufficient number of specimens for the colonization process had been captured according to Hall (1995) [11]. The samples were caught from different places including gardens and around livestock, at the campus of Al-Azhar university, Assiut and were sent to the laboratory of Zoology Department, faculty of science, in appropriately labeled tubes.

laboratory design

The experimental study was performed from April to June 2018. The parental adult insects 30 flies (20 females and 10 males). Identification and taxonomic determinations were made by using current keys (Whitworth, 2006) [33], and by medical Entomologists in Cairo University and insect collection of Ministry of Agriculture, Dokki, Giza, Egypt, in order to identify the live specimens, adults were anesthetized by chloroform for a short time and were examined by binuclear dissecting light microscope. All insects were identified to the minimum of the family level. All efforts were made to identify Diptera to the species level as they were considered of forensic importance. After identification, the adults were transferred to new cages (40 × 40 × 40 cm^3) and the cages were placed 1m from each other and the flies were maintained in the laboratory under controlled conditions of mean temperature of 30±4°C, daily light /dark period of 12:12 h and relative humidity of 60±10%.Thermometer and hygrometer was used to record daily temperature and the relative humidity (RH%). A container of water putted under each cages to maintain a sufficiently high humidity and the cages were protected with an external net curtain to avoid the entry of other insect species according to Zumpt (1965) [35]; James (1974) [14] and Whitworth (2006) [33].

After the death of the rabbit's cadaver or carcass (a whole body) were transferred to a plastic box of 25×10×15 cm^3 and labeled with date of death and putted in the cages, boxes were examined daily to observe the stages of decomposition of each carcass.

The adult flies were fed on the carcass of rabbits that were divided into four equal groups. Group (one) was used as a control group (did not receive any treatment). Group (two) received the over dose of sildenafil and Group (three) received the over dose of Diazepam, that were evaluated to three continuous generations and replicate of three cages for each food to F1, F2 and F3 generations. Carbohydrate-rich source were put 20 ml (30% sucrose) in petri dishes (10×10 cm) contains cotton as well as and amount of soil in the rearing box. Measuring the time required for eggs hatching,

total larval stages development, pupation, adults emergence to eggs laying and total time for eggs to adults had been done. Eggs hatching were cheked every three hours and every twelve hours for larvae, pupae and adults, then count cluster and number of eggs by binuclear dissecting light microscope, count first, second and third instar larvae, pupae, adults male and female and the emergence adults were placed in new cages and provided with essential food according to Spiller (1996) [25].

Sampling of larvae pupae and adult for toxicological analysis

20 of third feeding instars larvae, pupae and adult were randomly collected from each exposed rabbit carcasses of treated and control groups and then were rinsed in phosphate buffer, wrapped in a piece of aluminum foil and immediately frozen at -80°C till toxicological analysis (Gagliano-Candela and Aventaggiato, 2001) [8].

Toxicological analysis

Samples extraction

From both treated and control groups, solid tissue (liver, kidney and muscle), 0.1g were homogenized for 30 sec and diluted with 1 mL of double distilled water. Extraction was accomplished with liquid-liquid extraction. Briefly, in 10 mL tube, 0.5 of serum or 10 mL of tissue suspension was alkalinized with 500 mL of sodium hydroxide 0.5 mol L^{-1}. Samples were extracted with 5 mL of ethyl acetate and centrifuged at 2000xg for 10 mm. The organic layer was evaporated under a gentle stream of nitrogen. The residue was redissolved in 1 mL methanol and an aliquot 10 µL was used for injection.

Sample analysis and HPLC conditions

The concentrations of the three drugs from both treated and control groups were analyzed by high performance liquid chromatography (HPLC. Agilent 1260 series) with UV-Visible spectrophotometric detector at 218 nm. The separation was carried out using Eclipse Plus C18 column (4.6 mm x 100 mm), The mobile phase consisted of 0.1 tri-floro-acetic acid in water: acetonitrile: Methanol (70:25:5 v/v) at a flow rate 1 ml/min. The injection volume was 20 µl for each of the sample solutions. The column temperature was maintained at 40 °C. the drugs retention time was 11.16 min. The extraction recovery was estimated by comparing the slope of the standard curves of extracted standards with that for unextracted standards.

Statistical analysis

Statistical analysis were performed using the program SPSS for windows, version 16.0. Using the mean (average ± standard Error). And Excel program for windows to make statistical figures (Norusis, 2005) [21]. Data were analyzed statistically by using one-way Analysis of Variance (ANOVA) to compare and determine the data obtained from the effects of drugs dosage on the life cycle of blowfly *C. albiceps* development. A linear regression was used to evaluate the relationship between the concentration of drugs in the tissues and larvae, pupae and adult against the initial dosage. Different letters within a column indicate significant differences ($P < 0.05$).

a. Non-significant.
b. Significant.
c. High significant
d. Highly significant**.**

Results

Entomotoxicological studies

Results are presented in Table (1), indicated to significant differences to the all blood and tissue samples from the rabbits receiving dosages of sildenafil and diazepam were positive for this drug. While, all samples from the control rabbit were negative. The concentrations of sildenafil and diazepam are significantly decreased in blood with the passing of time after the initial tramadol dose. The sildenafil concentrations were detected in blood and the liver content of rabbit recording 36.6 and 31.2 µg/ml, respectively and the diazepam concentrations were detected in blood and the liver content of rabbit recording 28.3 and 25.8 µg/ml, respectively. For the lethal doses, tissues could be in the following arrangement according to their sildenafil and diazepam concentrations; blood> liver> kidney> muscles and the concentrations of drugs in various organs significantly decreased with the initial dosage (Table. 1). the toxicological analysis (Table 1) showed that sildenafil and diazepam transferred from administered animals to the feeding larvae, pupae and adult of *C. albiceps* so that, concentrations of tramadol, sildenafil and diazepam in the feeding third instar larvae, pupae and adult of *C. albiceps* were significantly lower than those detected in the rabbit tissues and this result indicated to the concentrations of sildenafil concentrations were detected in larvae, pupae and adult recording 27.1, 13.9 and 7.88 µg/g, respectively and the diazepam concentrations were detected in larvae, pupae and adult recording 15.3, 9.7 and 5.8 µg/g, respectively.

Table 1: Residues of sildenafil and diazepam in blood, postmortem tissues of rabbits and in third instar larvae, pupae and adult of *C. albiceps*.

Tissues	Control			Sildenafil			Diazepam		
Blood (ug/ml)	Zero h	12 h	24 h	Zero h	12 h	24 h	Zero h	12 h	24 h
	0	0	0	49.8	40.05	36.6	40.7	32.2	28.3
Liver (ug/g)	0			31.2			25.8		
Kidney (ug/g)	0			30.01			21.3		
Muscle (ug/g)	0			28.05			18.6		
Third Instar Larvae (ug/g)	0			27.1			15.3		
Pupae (ug/g)	0			13.9			9.7		
Adult (ug/g)	0			7.88			5.8		

Biological study

Laboratory rearing

Adults of the blowfly *Chrysomya albiceps* were collected from the garden in Al- Azhar university (Assiut) and transferred to rearing laboratory for three generations F1 (First generation), F2 (Second generation) and F3 (Third generation) and three replicates of culture. The life cycle of *C. albiceps* is shown (Fig.1 A, B, C and D). The duration of the developmental stages of male and female *C. albiceps* that fed on three different carcasses are given (Table 2). The mean time required for egg hatching for the three generations in control, sildenafil and diazepam were 15.2±0.27h, 23.6±0.74h and 20.7±1.06h respectively. The mean time for larval development for the three generations control, sildenafil and diazepam were 105.7±1.9h, 120.4±0.44h and 112.5±1.9h, respectively. The mean time for pupae development for the three generations in control,

sildenafil and diazepam were 119.5±0.37h, 131.0±0.00h and 125.3±0.57h, respectively. The mean time for emergence from pupae to adults laying eggs for the three generations in control, sildenafil and diazepam were 73.2±1.4h, 77± 0.57h and 73.2±0.74h, respectively, and total time from ovipositon eggs to adults eclosion and eggs laying again in the three generations for control, sildenafil and diazepam were 313. 6±2.1h, 352±3.7h and 326.6±2.4h, respectively.

Number of batches, eggs, first, second and third instar larvae, pupae and adults yielded in F1, F2 and F3 generations for the three carcasses are shown in (Table 3). The average number of female parents emerged per eggs batch for the three generations in control, tramadol, sildenafil and diazepam were 2.7±0.6, 1.2±0.4 and 1.65±0.3 respectively. The average number of eggs for the three generations for the three carcasses were 389.7±80, 130.6±46 and 194.4±33.2 respectively. The average number of first instar larvae for the three generations for the three carcasses were 363.8±79, 117.8±42 and 184.2±33 respectively. Average number of pupae for the three generations for the three carcasses were 304.8±74, 100.3±34 and 151.9±34 respectively. Total average mortality rate during the three generations for the four the treatment carcasses were 20.6%, 27.9% and 32.7% respectively. The results indicated that the developmental time for total durations life stage in sildenafil and diazepam carcasses were the longest at than the control carcasses respectively.

The durations increased gradually by adaptive for the two treated groups and decrease gradually by adaptive for control group in F 2 and F3. The durations of stages that fed on control carcasses was the shorter time than diazepam and sildenafil and the average number of female parents emerged per eggs batch for the three generations their parents fed on three groups were found higher in flies which feeding on the control carcass than the other two carcasses,

Fig 1: The life cycle of C. *albiceps*: (A) eggs, (B) larvae, (C) pupae and (D) adults.

Table 1: Effects of different drugs on the duration time of life cycle spans for *C. albiceps* from eggs to adults during three generations under laboratory condition

Generation		Cages	Adults Male Female	Egg hatched period (h) Mean ± SEM	Larval stage period (h) Mean ± SEM	Pupal stage period (h) Mean ± SEM	emergency to egg laying period (h) mean ± SEM	Total Duration from eggs laying to eggs laying again (h)
F1	Control	Cage 1	10 20	15	119	121	69	317.9±2.3[a]
		Cage 2	10 20	14 15±0.57[a]	112 110.3 ±5.5[a]	120 120.6±0.33[a]	68 72±3.5[a]	
		Cage 3	10 20	16	100	121	79	
	Sildenafil	Cage 1	10 20	22	120	-----	----	345.3±2.8[b]
		Cage 2	10 20	20 21±0.57[b]	120 119.3±0.66[a]	128 128±0.00[c]	77 77±0.00[b]	
		Cage 3	10 20	21	118	-----	-----	
	Diazepam	Cage 1	10 20	16	114	121	71	322.9±2.1[a]
		Cage 2	10 20	19 18.3±1.2[c]	115 111.6±3.2[a]	122 121±1.4[d]	70 72±1.5[a]	
		Cage 3	10 20	20	106	120	75	
F2	Control	Cage 1	10 20	16	104	120	70	311.6±1.5[a]
		Cage 2	10 20	15 15.3±0.3[a]	104 103.3±0.33[a]	119 119±0.57[a]	76 70±2.6 [a]	
		Cage 3	10 20	15	103	118	76	
	Sildenafil	Cage 1	10 20	----	----	-----	-----	350±2.6[c]
		Cage 2	10 20	24 24±0.00[b]	120 120±0.00[c]	131 131±0.00[c]	75 75±0.00[a]	
		Cage 3	10 20	----	----	-----	-----	
	Diazepam	Cage 1	10 20	22	111	126	73	323.2±2[d]
		Cage 2	10 20	23 21±1.5[c]	113 112.6±0.88[a]	124 125.6±0.88[d]	72 73.3±1.4[a]	
		Cage 3	10 20	18	114	127	75	
F3	Control	Cage 1	10 20	16	104	120	70	311.2±1.3[a]
		Cage 2	10 20	16 15.3±0.66[a]	104 103.3±0.33[a]	119 119±0.57[a]	76 73.6±1.6[a]	
		Cage 3	10 20	14	102	118	75	
	Sildenafil	Cage 1	10 20	----	----	-----	-----	358±2.4[c]
		Cage 2	10 20	26 26±0.00[b]	122 122±0.00[c]	131 131±0.00[c]	79 79±0.00[c]	
		Cage 3	10 20	----	----	-----	-----	
	Diazepam	Cage 1	10 20	27	112	126	73	330.5±2.2[d]
		Cage 2	10 20	20 23±2.08[b]	113 113.3±1.2[d]	124 125.6±0.86[d]	74 74.3±0.88[a]	
		Cage 3	10 20	22	115	127	76	
Average F1, F2 and F3	Control	9 cages	90 180	15.2±0.27a	105.7±1.9a	119.5±0.37a	73.2±1.4a	313. 6±2.1[a]
	Sildenafil	9 cages	90 180	23.6±0.74b	120.4±0.44b	131.0±0.00c	77± 0.57c	352±3.7[b]
	Diazepam	9 cages	90 180	20.7±1.06c	112.5±1.9a	125.3±0.57d	73.2±0.74a	326.6±2.4[a]

Data are mean ± SE. Different letters within a column indicate significant differences (P <0.05).
Non-Significant. (b) Significant. (c) High Significant. **(**d**)** Highly Significant.

Table 2: Effects of different drugs on the number of eggs, batches, larvae, pupae and adults of *C. albiceps* during three generations (from eggs to adults).

Generations		Cages	Eggs Batch No.	Eggs No.	Larval stage			Pupae	Adults		Total male and female
					First instar	Second instar	Third instar		Male	Female	
F1	Control	Cage 1	2	245	238	232	215	192	62	84	146
		Cage 2	3	475	436	421	400	372	120	143	263
		Cage 3	2	175	152	124	108	100	95	133	228
	Average	1, 2 and 3	2.3±0.33a	298.3 ±90a	275.3±89 a	259 ±84 a	241±154 a	221.3±35.8a	92.3±18.7 a	120±18.2 a	212.3±34.6a
	Sildenafil	Cage 1	1	112	100	96	83	141	-	-	-
		Cage 2	4	454	400	390	345	500	187	231	418
		Cage 3	1	90	86	81	80	28	-	-	-
	Average	1, 2 and 3	2 ±1 a	218.6±118 b	195.3±102 b	189±100 b	169.3±87.8 b	162±85 b	62.3±0.00 a	77±0.00c	139.3±0.00 a
	Diazepam	Cage 1	4	314	300	287	281	332	130	195	325
		Cage 2	2	276	270	261	244	82	33	45	78
		Cage 3	1	195	182	164	159	75	25	44	69
	Average	1, 2 and 3	2.3±0.88 a	261±35 a	250.6±35 a	237.3±37.4 a	228 ±36.1 a	163±142.2 b	62.6±33.7 a	94.8±50 a	157.3±83.8 a
F2	Control	Cage 1	2	200	195	192	187	185	75	97	172
		Cage 2	3	673	549	521	500	472	139	242	381
		Cage 3	2	90	85	74	70	62	58	70	128
	Average	1, 2 and 3	2. 6±0.33a	321±178.8 a	276.3±89.1 a	262.3±84.4 a	252.3±84.8 a	239.6±79.8 a	90.6±24.6 a	136.3±53.2 a	227±28.04 a
	Sildenafil	Cage 1	-	-	-	-	-	-	-	-	-
		Cage 2	3	345	325	311	293	285	101	152	253
		Cage 3	-	-	-	-	-	-	-	-	-
	Average	1, 2 and 3	1±0.00 b	115±0.00c	108.3±0.00 c	103.6±0.00 c	97.6±0.00 c	95.3±0.00 c	33.6±0.00 c	50.6±0.00 c	84.3±0.00 c
	Diazepam	Cage 1	2	284	273	262	233	217	97	105	202
		Cage 2	1	90	85	78	65	61	25	33	58
		Cage 3	1	110	95	90	81	74	33	39	72
	Average	1, 2 and 3	1.33±0.33 a	161.3±61 c	151±61 c	143.3±59.4 c	126.3±53d	117.3±49c	51.6±22.7 c	59±23.06 c	110.6±45.8 c
F3	Control	Cage 1	5	720	691	677	665	655	285	332	617
		Cage 2	4	450	420	375	314	300	134	142	276
		Cage 3	3	480	455	435	422	403	195	201	396
	Average	1, 2 and 3	4±0.57 a	550±85.1 a	522±85 a	495.6±92 a	467±103.7 a	452.6±105.9 a	205±43.5 a	225±55.8 a	429.6±99.3 a
	Sildenafil	Cage 1	-	-	-	-	-	-	-	-	-
		Cage 2	2	175	150	130	112	104	42	53	95
		Cage 3	-	-	-	-	-	-	-	-	-
	Average	1, 2 and 3	0.6±0.00 b	58.3±0.00 b	50±0.00 b	43.3±0.00 b	37.3±0.00 b	34.6±0.00 b	14±0.00 b	17.6±0.00 b	31.6±0.00 b
	Diazepam	Cage 1	2	284	273	262	233	217	90	102	192
		Cage 2	1	90	85	78	65	61	25	29	54
		Cage 3	1	110	95	90	81	74	29	38	67
	Average	1, 2 and 3	1.33±0.33a	161±61.8c	151±61.6 c	143.3±59.4 c	126.3±53.4 c	117.3±49 c	48±21 c	56.3±22.9 c	104.3±43.9 c
Average F1, F2 and F3	Control	9 Cages	2.7±0.6a	389.7±80 a	363.8±79 a	344.8±75 a	320.1±73 a	304.8±74 a	129.3±37 a	160.5±32.5 a	289.8±70 a 79.4%
	Sildenafil	9 Cages	1.2±0.4a	130.6±46c	117.8±42 c	111.9±42 c	101.4±38 c	100.3±34 c	36.6±14 c	48.4±17 c	85±31.06 c 72.1%
	Diazepam	9 Cages	1.65±0.3a	194.4±33.2 c	184.2±33d	174.6±31 d	160.2±33 d	151.9±34 d	54.06±4.3 c	70.03±32 c	124.06±1 d 67.3%

Data are mean ± SE. Different letters within a column indicate significant differences (P<0.05).
(a) Non-Significant. (b) Significant. (c) High significant. (d) Highly significant.

Discussion

Entomotoxicological studies

Insects could act as reliable alternative specimens for toxicological analysis when body fluids and tissues are absent or not valid for analysis (Chen *et al*., 2004 and Amendt *et al*., 2011) [6, 1].

Few literature deals with the potential toxicological value of necrophagous insect larvae (Mergaoui *et al*., 2007) [19]. Regarding postmortem sildenafil and diazepam concentration in rabbit tissues, the present results showed that the sildenafil concentrations were detected in blood and the liver content of rabbit recording 36.6 µg/ml and 31.2 µg/g, respectively and the diazepam concentrations were detected in blood and the liver content of rabbit recording 28.3 µg/ml and 25.8 µg/g, respectively. This is in agreement with the work of El-Samad *et al*. (2011) [7], who detected tramadol by (HPLC) in various organs of experimentally injected rabbits, including the liver. sildenafil and diazepam excretion is mainly through the renal. On the other side, the present study revealed that the concentration of the toxicological analysis showed that sildenafil and diazepam in larvae, pupae and adult of *C. albiceps* were significantly lower than those detected in the rabbit tissues and this result indicated to the concentrations of sildenafil and diazepam were detected in larvae recording 27.1and 15.3 µg/g, respectively. While in pupae recording, 13.9 and 9.7 µg/g respectively and in adult recording, 7.88 and 5.8 µg/g, respectively, which is comparable to postmortem rabbits' tissue concentrations. This is supported by the work of Introna *et al*. (1990) [13] who reported that the concentrations of morphine in the larvae of C. vicina reared on decomposing liver tissues of humans that were deceased and the cause of their death was morphine poisoning were strongly correlated to post mortem tissue concentrations.

It was postulated that the metabolism and elimination of various drugs by larvae vary significantly throughout larval developmental stages with a decrease in drug concentrations in post-feeding larvae suggesting that actively feeding and fully developed larvae only should be used as a sample for toxicological analysis (Hedouin *et al*., 2001) [12].

In present study, the higher concentration of sildenafil and diazepam in tissues was found in the blood, following the

liver, kidney contains lower concentration and this result suggests that there is a high excretion.

Moreover, the recorded results showed higher drugs concentration in the blood which is inconsistent with a study carried by Juzwin *et al*. (2000) [15]. Levine *et al*. (1997) [18] found that the heart blood concentrations were the lowest. In the present study, sildenafil and diazepam concentration in kidney was approximately two times lower than for blood, this may be attributed to the tramadol destruction by liver and kidney.

Variability in the concentration of drugs has been found in muscle tissue (Langford *et al*, 1998) [17]. This may interpret the lowest drug distribution in muscles for our results.

These studies found that metabolism and elimination of drugs by larvae vary considerably throughout larval development with a clear decrease in drug concentrations measured in post feeding larvae and during pupariation. The decrease in drug concentrations suggested that only larvae actively feeding on a corpse and fully developed should be sampled for toxicological analysis because they represent the best source of drug or toxin residue.

These results are consistent with those recorded in the larvae of *L. sericata* (Hedouin *et al*., 1999) [12] containing morphine. On the other hand, Introna *et al*. (1990) [13] found that the concentrations of morphine in the larvae of *C. vicina* were quite similar to those in human liver tissues used as a food source.

Biological study

The development of the *C. albiceps* can assist with determination of the post-mortem interval and thus can be use as tool to help solve crime. The main focus of this work was to develop reliable data for growth on different drugs. Deeper knowledge is needed for its life cycle, and reproductive, since this information can support the mass rearing of larvae under laboratory conditions (Grassberger and Reiter, 2001) [10].

Effect of different drugs on the duration of developmental stages of *C. albiceps*

Results of this research show highly significant different for mortality in larval stage, survival adults' percentage in comparison of the three drugs. On the other hand, important differences were observed for the various biological stages because each stage presented a different period for development.

On another side, the total average duration time from eggs laying to eggs laying again in the three generation for control, sildenafil and diazepam carcasses were 313.041 h, 352 h and 326.6 respectively. On the other hand, the results show that the duration time is shorter in the flies that feeding on control comparing with the fly which feeding on sildenafil and diazepam and the total duration of life stages for control reduced gradually by adaptive for diets in F 2 and F3 and increases for treated groups.

This results agree with Wallman and Day (2006) [32] reported that the food type is an essential factor on growth and development of green flies, can cause a various duration time of larval stages in comparison to the diets traditionally used and agree with Spiller (1966) [26], reported that the effect of different kinds of food on larval development of *L. sericata* is considerable.

Although insect remains represent the main samples available for analyses so that sildenafil and diazepam could alter the development *C. albiceps* and the larval growth and or on the duration of insect development stage. In the present work the larvae of the treated group of animals developed slowly than the control larvae. This indicates that the presence of sildenafil and diazepam in the tissue of treated rabbits retard growth of C. albiceps during larval period and this in agree with the result of Kharbouche *et al.* (2007) [16] in which codeine or its metabolites stimulate the growth of *L. sericata* during the larval period. Also Carvalho *et al.* (2001) observed that *C. albiceps* larvae reared on rabbit tissues containing diazepam developed more rapidly than larvae from control colonies. On the other side was late in the total time for pupariation and adult emergence was noticed in injected group compared to controls. This contrast with Carvalho *et al.* (2001) that deals with the effects of diazepam in fly tissues that there was a bioaccumulation since the presence of the drug had a significant impact on larval growth, pupariation, adult emergence and mortality. It means that the drug affects the fly development from the larval stages until the total mortality of adults. There were faster development of both calliphorids led on tissues containing diazepam when related to control. Studying the effects of heroin on development of sacrophagidae (*B. pergrina*) fed on intoxicated rabbit tissues Goff *et al*. (1991) [9] observed that maggots grow at rates significantly faster. So, the effect of heroin alter postmortem in terval estimates based on larval development. Also, Kharbouche *et al.*, (2007) [16] showed that the larvae reared on homogenized tissues of pig liver (250 gm) spiked with 20 ml Na Cl solution (0.9%) containing lethal dose of codeine (30 mg/kg), were observed 29 h before the control group. Also, this study demonstrated the differences observed in the rates of development were sufficient to alter postmortem interval estimates based on the larval development.

The present study showed that presence of this drugs in rabbits' tissues late the larval feeding growth rate and retarded the total development. Present results were in accordance with Goff (1991) [9], who conducted a study for heroin effect on the development of *B. pergrina*. They found more rapid development of maggots in all treated colonies until maximum size was attained. On the other hand, in this study, the number of eggs batch, eggs number, first, second and third instar larvae, pupae and adults of *C. albiceps* during three generations showed that, total average numbers of three generations that fed on control carcasses from eggs to adults were found higher in flies which feeding on the control than the other treated groups, and total developmental stages (offspring) increasing gradually by adaptive for control carcasses in F 2 and F3 but decreasing gradually by adaptive for sildenafil and diazepam carcasses in F 2 and F3. These are disparity between the present results and other studies can be mainly explained by two factors; the different diets that used and the characterizations of the local populations that studied.

Conclusion

The present work showed that Sildenafil and Diazepam caused alter rate of developmental stages of *C. albiceps* that could affect PMI estimation and HPLC analysis are considerable value in this study. Additional studies using different species of Calliphoridae and Sarcophagidae are needed as there may be different responses to the drug**.**

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