EFFECT OF CODEINE PHOSPHATE ON DEVELOPMENTAL STAGES OF FORENSICALLY IMPORTANT CALLIPHORIDE FLY : CHRYSOMYA ALBICEPS

BY

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ABSTRACT

Insects can be used as alternative specimens for toxicological analysis when conventional post-mortem samples are not available as drugs that can be detected in severely decomposed tissues of a corpse may still be found in the insects that did feed on the corpse. Several studies illustrate the great potential importance of entomotoxicology for providing additional information on cause of death. This study was aimed to study the effect of intoxication with codeine phosphate in decomposed bodies on the development of carrion flies and on the evaluation of postmortem interval (PMI). The experiment was performed during summer season from last of June to the mid of July. Four rabbits, two control and two injected with lethal dose of codeine phosphate were used. Each one was placed in a cardboard box, floored with muddy soil and protected with a metal cage. Different developmental stages of Chrysomya albiceps were collected and studied for both biological and morphological changes using dissecting light microscopy. There were morphological changes in the form of disfiguring of segments, loss of colouration and abnormalities in the shape of both anterior and posterior spiracles in the larvae, while the adult flies show rudimentary wings, abnormal bands on the undersurface of the abdomen,fading of normal colour to complete loss of it. Also there were biological changes in the form of acceleration in the development during life cycle and incomplete emerge of some adult flies from their pupae in the ingected group. These acceleration of development lead to bias in estimation of postmortem interval up to 24h when estimation based on larval development and 48h when estimation based on pupal development. This work should provide data for the use of morphological and biological changes that occur in Chysomya albiceps as indicator for death from lethal dose of codeine phosphate and to estimate the post mortem interval.

INTRODUCTION

Forensic entomology is the study of the insects associated with a dead body, primarily to determine time since death and is very valuable tool in homicide investi-

gations worldwide (Anderson, 2001). Insects are the first witnesses to a crime, the high proportion of blowflies reflects the rapidity and accuracy with which these flies will locate dead bodies often within only minutes after death (Hall, 1995; Faucheree et al., 1999; Anderson, 2002). The attraction of a large numbers of adult blowflies occur as a result of odour of decay, which are mainly due to bacterial action on dead tissues. Thus the major stimulus to the adult flies is an olfactory rather than a visual one (Gill, 1982; Hall, 2002). They are of great forensic importance as they are sometimes the only way to estimate PMI (Chittars et al., 2005). However, the information provided by these insects, particularly during their immature stages, may be distorted by the presence of toxic substances in the corpse.

They can affect the developmental time of the larvae feeding on the cadaver, that can affect the estimation of the PMI (Bourel et al.,1999; Estrada et al., 2006; Fremdt et al., 2007).

The first documented forensic entomology case is reported by the Chinese lawyer and death investigator Sung Tzu in the 13th century in the medicological text His Yuan Lu (Muknight, 1981; Benecke, 2001).

Entemotoxicology is a relatively new emerging branch of forensic entomology exploits flesh feeding insects as alternative toxicological specimens because they bioaccumulate drugs and toxins (Gunn et al., 2006).

Codeine is a psychoactive alkaloid obtained from the opium (papaver somniferum). It is especially used in therapeutic for its analgesic and antitussive properties (Maurer et al., 2006). Codeine is often abused as a substitute of heroine, and can be found in fatal cases as a result of drug intoxication after accidental or criminal administration (Kintz et al., 1991; Jensen and Hansen, 1993).

The objective of the study was to investigate the effect of codeine phosphate on the development of necrophagous flies after exposure of the carrions to the lethal dose and its effect on the estimation of the PMI.

MATERIAL AND METHODS

1- Codeine phosphate powder was obtained from Knoll, USA.
2- Laboratory spoon, different size of jars, bottle of alchol (Ethanol 70 %).
3- Distilled water (for dissolving codeine phosphate and for control rabbits).

Methods:-

Time of study:-

Daily weather data (mean of maximum

and minimum temperature and relative humidity) were aquired from the Egyptian Meteorological Authority, Assiut station which occurs within the University Campus during a period of 28 days in summer from 20/6/2007 to 17/7/2007, the maximum temperature was varied from 42 to 45 at shade.

Experimental design:-

Four male domestic rabbits weighing 3.5-4 kg each were used. They were divided into two groups two rabbits on each. The first group of two rabbits injected with the lethal dose of codeine phosphate; 28 mg/kg body weight, via an ear vein calculated according to paget's formula (Paget and Barnes, 1964), and the other two rabbits used as (control) were injected with distilled water. The rabbits were mechanically sacrified by cervical dislocation, and were subsequently placed in cardboard boxes floored with mudy soil and protected with metal cages with a 10 meter distance between boxes of the two study groups.

Arthropod sampling and data collection:-

During the 28 days period of the study, samples were collected twice daily for the first 8 days, then once daily till the end of study. All insect forms (larvae, pupae and adults) were collected in the following order: First, adult flies flying over or landing on the carcasses by hand net. Second, the larvae and pupae which were found on or in the specimen collected with a forceps. Finally, the larvae and pupae which were under the specimen and in the soil till the depth of 5 cm.

Laboratory work:

The collected flies were killed by ethyl acetate and preserved in numbered and dated vials containing 70 % alcohol for further identification according to Conteno et al. (2002).

The collected larvae and pupae were divided into three groups:-

In the first group 10 larvae were randomly collected from the carcasses and were copiously washed with deionized water, dried with a filter paper and then frozen at -20°C for approximately 5 minutes before being weighted. The mean weight of each sample of these 10 larvae was used to establish the larval growth curve (fresh weight/time).

The second group was killed in hot water and placed in vials containing 70 % alcohol for further identification.

The larvae of the third group were kept alive for breeding till the adult stage, they were reared in the lab, by transferring into dry jars containing small pieces of fresh cow liver on a layer of saw dust. The jars were closed with gauze to allow proper ventilation. All jars were labeled indicat-

ing hour, date and environmental data according to Tantawi et al., (1996). The time in hours required for pupariation, emergence and longevity was recorded. Identification of adults and larvae were carried out according to specific keys (Greenberg, 1971; Shaumar et al., 1989; Wells et al., 1999).

RESULTS

The adult specimens collected during the study were identified as individuals of Chrysomya albiceps (Dipteria: callphoridae). This species was the only fly evidence associated to the carcasses either injected or control.

As regard the effect of codeine phosphate on development of larvae of chrysomya albiceps, it was observed that the presence of drug was significant for insect development. The acceleration in the larval growth was observed as regard the weight and development time. The larvae that developed on rabbits ingected with codeine weighted significantly more than the controls. The larval growth was faster when compared to controls in almost all hours of observation (Tables 1 and 2 and Diagram 1). The total time of development of larvae in the injected group was 72 hours, compared to that of controls which was 96 hours (Table 5). Some abnormal morphological findings were observed in association with the larval growth acceleration, in the form of more pigmented scales on dorsal surface on each segment (Fig. 1b) compared to the control (Fig. 1a), and some larvae shows much smaller in size, deformed and depigmented segment (Fig. 1c), rudimentary mouth hooks (Fig. 2b) compared to the control (Fig. 2a), absence of anal protuberance (Fig. 3b) compared to the control (Fig. 3a).

As regards the effect of codeine phosphate on development of pupae of chrysomya albiceps, there was acceleration in the total time of pupariation. It took 120 hours in the injected group to obtain pupae, compared to 168 hours in controls (Tables 3 and 5). It was noticed that the cases of pupae was harder and more pigmented in the treated group compared to controls (Figs. 8 and 9).

As regard the effect of codeine phosphate on adult emergence, significant acceleration of adult emergence was noticed in the injected group compared to controls; which took 168 and 192 hours respectively (Tables 4 and 5). Some developmental abnormalities were observed in the adults of the injected group, including pale colour (Fig. 5), small size, less apparent abdominal strips, hypopigmented lower abdominal surface (Fig. 6b), shrunken rudinmentary wings (Fig. 7b). It also observed that there was arrest of adult emergence in some flies of the injected group,

which was not observed in control group (Fig. 9).

All the above mentioned findings were based upon observation in open carcasses and similar findings were detected in developmental sequence of collected larvae reared in the laboratory (Table 6).

Finally, it was demonstrated that difference observed in the rate of development were sufficient to alter postmortem interval estimates based on larval development by up to 24 hours and estimates based on puparial development by up to 48 hours.

DISCUSSION

Although insect remains represent the main samples available for analyses after months or years, only a few references deal with the potential toxicological interest of such samples (Bourel et al., 2001). A drug or toxin can be detected in the larvae when its rate of absorption exceeds the rate of elimination, but it is not yet known exactly how larvae bioaccumulate or eliminate drugs, and how these affect larval development (Introna et al., 2001).

To evaluate whether codeine phosphate could alter the development of chrysomya albiceps and, therefore, bias the estimation of PMI, entomological methods based on the larval growth and or on the duration of insect development stage were used. Also to evaluate its effects on the morphological changes of the different stages.

In the present work the larvae of the treated group of animals developed faster than the control larvae. This indicates that the presence of codeine in the tissue of treated rabbits stimulate growth of chrysomya albiceps during larval period and this agree with the result of Kharbouche et al. (2007) 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.

In the present study there was acceleration in the total time for pupariation and also acceleration of adult emergence was noticed in injected group compared to controls.

This agree with Carvalho et al. (2001) that deals with the effects of diazepam in fly tissues and verified 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 calliphor-

ids led on tissues containing diazepam when related to control.

Previous workers ,Goff et al.(1992),Goff and Lord (1994), Miller et al.(1994) and Bourel et al.(1999) using in their experiments methamphetamine, amitriptyline, cocaine and morphine, showed that these drugs can alter the rate of development of some diptera flies. All these results were in agreement with the present results.

Studying the effects of heroin on development of sacrophagidae (B. pergrina) fed on intoxicated rabbit tissues Goff et al. (1991) observed that maggots grow at rates significantly faster from 18 to 96 h. So the effect of heroin alter postmortem interval estimates based on larval development by up to 29h and estimates based on puparial development by 18 to 38h.

Regarding the effects of methamphetamine on the development patterns of P. ruficornis, Goff et al. (1992) illustrated substantial analogies with the studies carried out on heroin by Goff et al. (1991) and cocaine Goff et al. (1989). These studies were in agree with the results of the present study, that it was demonstrated that differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 24h and estimates based on puparial development by up to 48h.

Also in (2007) Kharbouche et al. 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.

Insects are "cold blooded", so their development is extremely temperature dependant. Their metabolic rate is increased with increased temperature, which results in a faster rate of development, so that the duration of development decreased in a linear manner with increased temperature (Anderson, 1998). These agree with our results as the temperature at the time of experiment was very high up to 42-45 °C.

Moreover, the aggressive feeding behaviour of second and third instar larval of C. albiceps on local carrion breeding larvae could reset the postmortem insect clock by clearing a corpse of all earlier arrives (Grassberger et al., 2003). These results explain the dominance of C. albiceps in the present study.

As regards the abnormal morphological findings that were observed in larvae, pupae and adults in the present study no previous studies were reported to compare with them. However, these abnormal morphological findings which occurred in different stages of C. albiceps

as a result of lethal dose of codeine phosphate indicate that the drug leads to congenital anomalies so further studies must be considered.

Also this study demonstrated again the necessity of considering the possible effects of drugs in tissues on insect growth rates when estimating the postmortem interval using entomological techniques, as the differences observed in the rates of development were sufficient to alter postmortem interval estimates based on the larval development by up to 24 h and estimates based on pupal development by 48h.

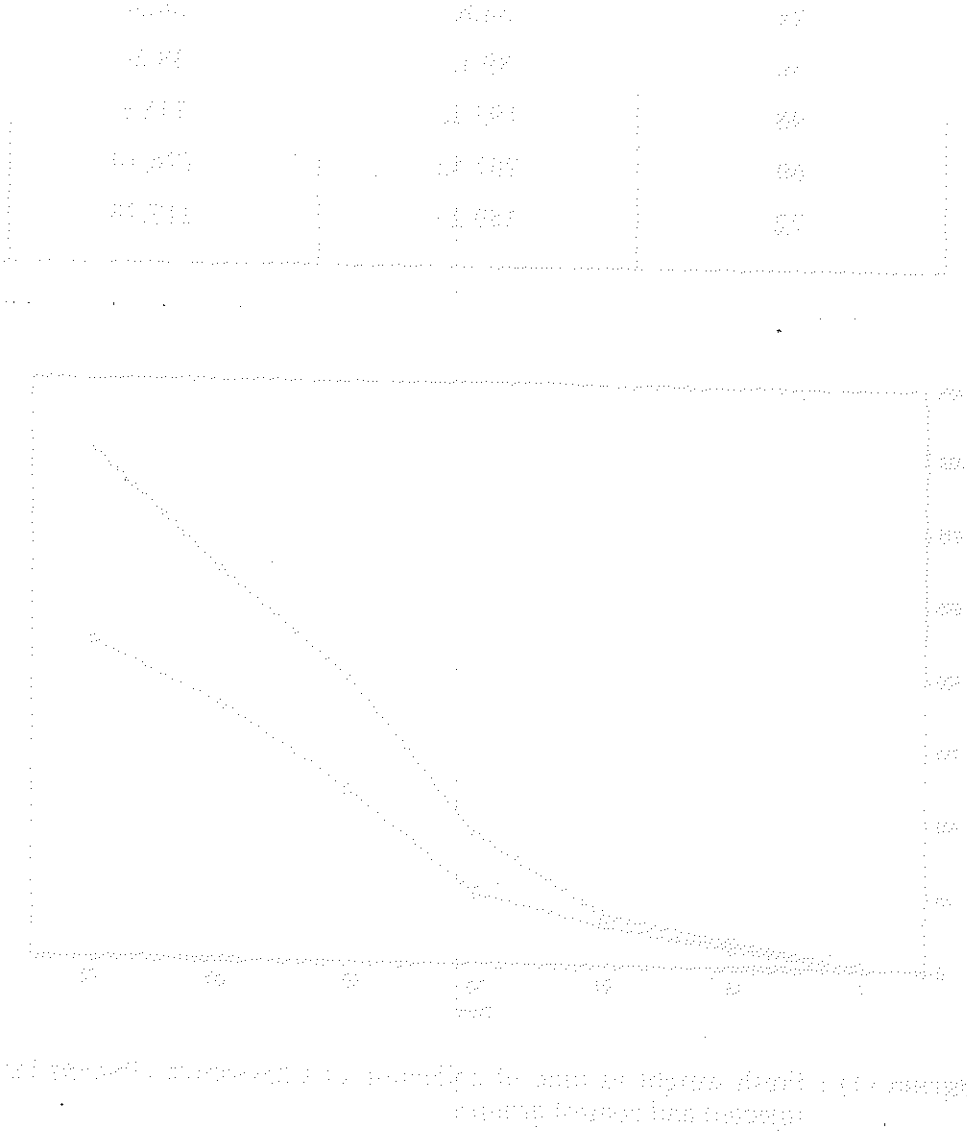

Table (1) : Larvae weight (mg) means of Chrysomya albiceps releted to time of collection in injected and control groups.

Time (hours)	Injected Groups	Control Groups
1	1	1
12	16.88	12.84
24	34.81	26.98
36	89.12	48.28
48	195.23	118.5
60	267.53	176.14
72	349.23	217.78

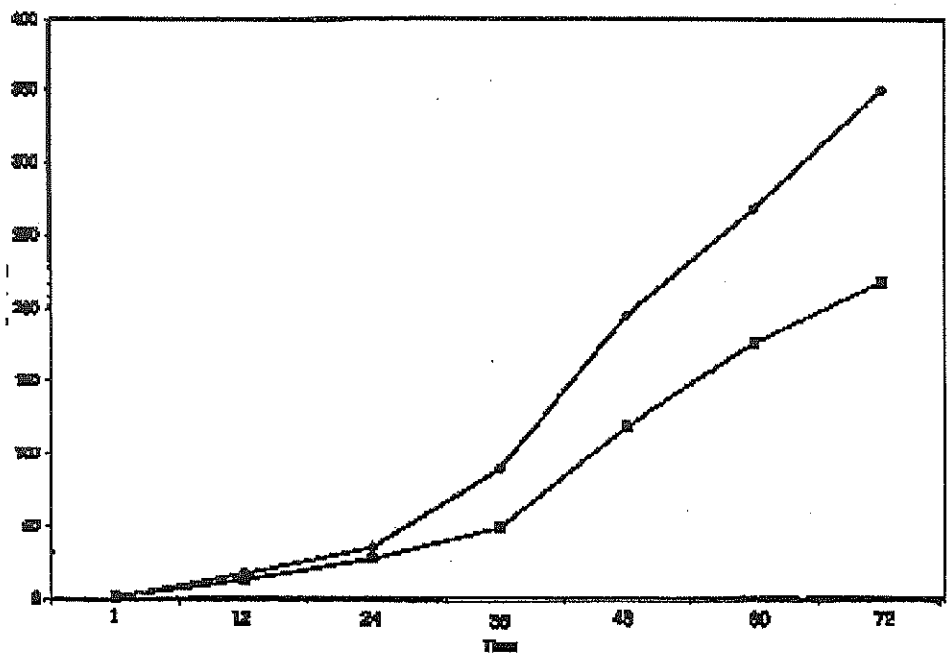

Diagram (1) : Fresh weight vs time of collection of Chrysomya albiceps larvae in injected and control groups.

Table (2): Effect of codeine phosphate on the development of larvae of *Chrysomya albiceps* in injected rabbit compared to control

Date	Injected Rabbit					Control Rabbit				
	Number		Colour	Average length	Abnormal Findings	Number		Color	average length	Abnormal findings
	specimen	Rearing jars				specimen	Rearing jars			
23-6-2007	Large number of living larvae (hundreds)		More pigmented scales	9-12 mm	-More pigmented scales on dorsal surface of each segment -Rudimentary mouth hooks -Poorly-developed anterior spiracles -Ill-developed oral protuberance	Fewer number of living larvae (hundreds)		Less pigmented (dirty white)	8-12mm	No abnormal findings
24-6-2007	Hundreds but less than previous			11-12 mm		Thousands, more than previous			10-11mm	
25-6-2007	Less number	100% of larvae start to pupate				Still high number	Only 50% of larvae start to pupate			
26-6-2007	Less number					Still high number	More pupae			
27-6-2007	No larvae (only pupae)					Few larvae				

Table (3): Effect of codeine phosphate on the development of pupae of *Chrysomya albiceps* in injected rabbit compared to control

Day	Injected Rabbit					Control Rabbit				
	Pre-Pupal Stage			Pupal Stage		Pre-Pupal Stage			Pupal Stage	
	No.	Length	Characters	No. in Specimen	No. in rearing jars	No.	Length	Characters	No. in Specimen	No. in rearing jars
23-6-2007	Just 2	5-6 mm	Harder more pigmented cases			3	6-8 mm	Softer less pigmented cases		
24-6-2007	Much more					*Less No				
25-6-2007				** Much more number					Less No	
26-6-2007				Tens (60-70)	More No.				Tens (20-30)	Less No.
27-6-2007				Fewer number of pupae Many empty puparial cases	Few No.				More number of pupae Few empty puparial cases	More No.
28-6-2007				Fewer than previous days					Many pupae still exist	

* The number of larvae higher than pre-pupal stage
** Number of pupae, much higher than number of larvae

Table (4) Effect of codeine phosphate on the development of imago of *Chrysomya albiceps* in injected rabbit compared to control

Date	Injected Rabbit				Control Rabbit			
	in specimen	In rearing jars			in specimen	In rearing jars		
	Number	No.	Length	Abnormalities	Number	No.	Length	Abnormalities
26-6-2007		First fly appeared from larvae collected on 23-6		Paler in colour Smaller in size Less apparent abd. Strips Shrunken rudimentary wings Orange eyes Partial exit of the adult (heads & legs) Hypopigmented lower abd. surface	No emerging adult	No emerging adult		No abnormal findings
27-6-2007	Many coloured flies (tens) around the rabbit & in the cage	One fly from reared larvae collected on 23-6	7-8 mm		No emerging adult	No emerging adult		
28-6-2007	Hundreds of flies	Many flies from reared larvae collected on 23-6 and pupae on 27-6	4-5 mm		Few No. (tens)	Few No. from larvae collected on 23&24-6	8 mm	
29-6-2007 To 30-6-2007	Few flies (4-6)	Many flies from reared larvae (tens) collected on 24&25-6	4-5 mm		High No. (tens)	High No. (tens)		

Table (5): Development time of different stages of *Chrysomya albiceps* under the effect of codeine phosphate compared to control (in open air)

Stage	Injected		Control	
	Days of development	Total duration of development (hrs)	Days of development	Total duration of development (hrs)
Larvae	3 days (d_0-d_3)	72 hrs	4 days (d_0-d_4)	96 hrs
Pre-pupal stage	4 days (d_0-d_4)	96 hrs	5 days (d_0-d_5)	120 hrs
Pupae	5 days (d_0-d_5)	120 hrs	7 days (d_0-d_7)	168 hrs
Imago (adult)	7 days (d_0-d_7) 8 days (d_0-d_8)	168 hrs 192 hrs	8 days (d_0-d_8) 9 days (d_0-d_9)	192 hrs 216 hrs

Table (6): Developmental sequence of collected larvae of *Chrysomya albiceps* under the effect of codeine phosphate compared to control (in rearing jars)

Date of Larvae collection	Injected							Control						
	Date of Pupation			Date of Adult emergence				Date of Pupation			Date of Adult emergence			
	25/6	26	27	28	26	27	28	29	25	26	27	28	29	29	1/7
23	+				+	+									
24		+					+	+		+				+	
25			+				+	+			+			+	.
26			+				+					+			+
27												+			+

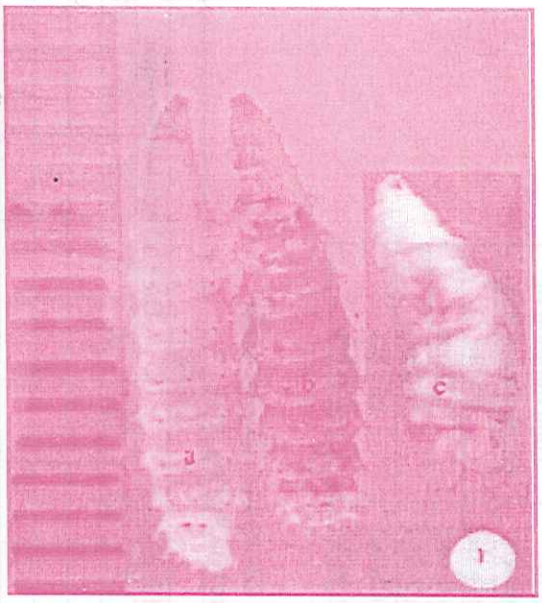

Fig. (1) : (a) Normal larvae of C.albiceps of the control rabbit.
(b) Larva collected from the carcass of the injected rabbit (slightly smaller in size, darker in colour, more pigmented in the darsal surface), and more darker spots on the dorsal surface of each segment).
(c) Larva of the injected rabbit (much smaller in size, deformed segments and depigmented, complete absence of the normal arrangement of the tubercles in each segment).

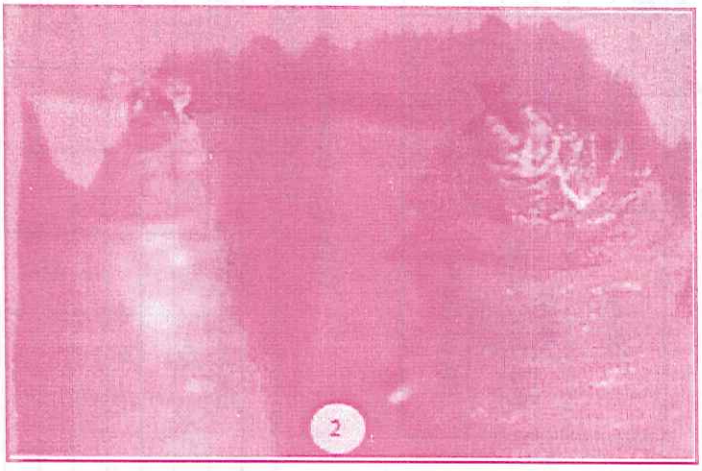

Fig. (2) : Two larvae normal control one to the left (a) and injected one to the right (b) showing: ill developed head with ill developed mouth hooks.

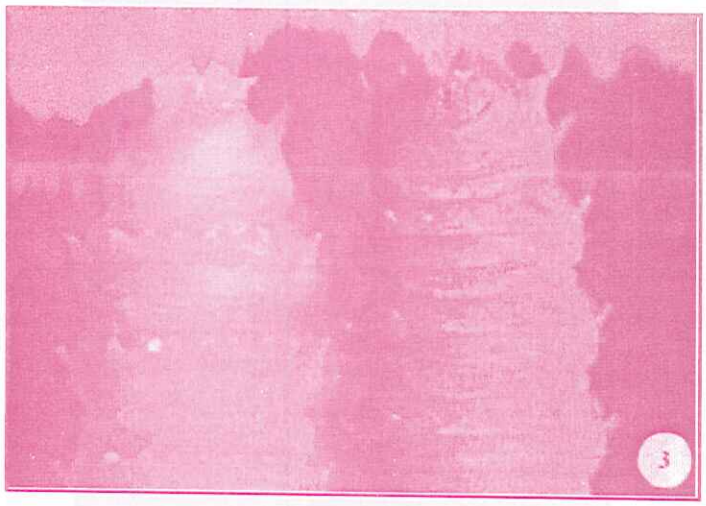

Fig. (3) : Posterior end of two larvae normal one to the left (a) showing presence of the anal protuberance and injected (darker) one to the right (b) showing absence of the anal protuberance.

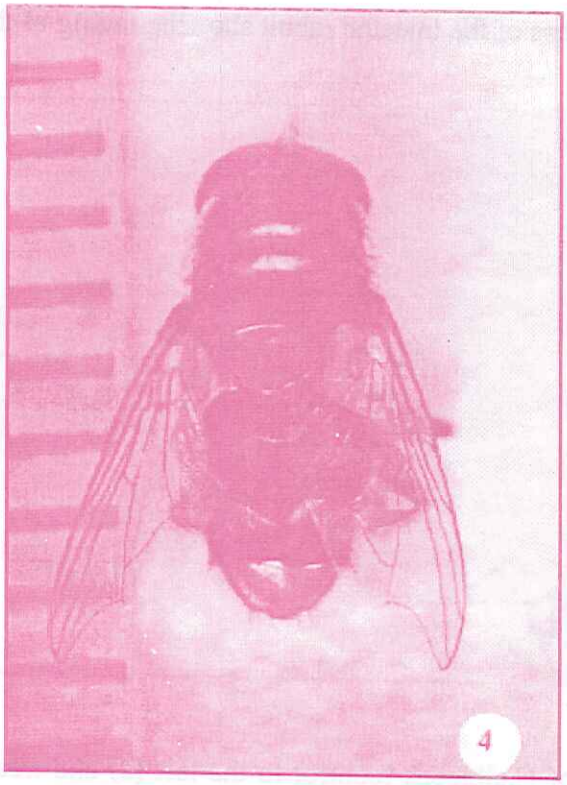

Fig. (4) : Normal adult Chysomya albiceps.

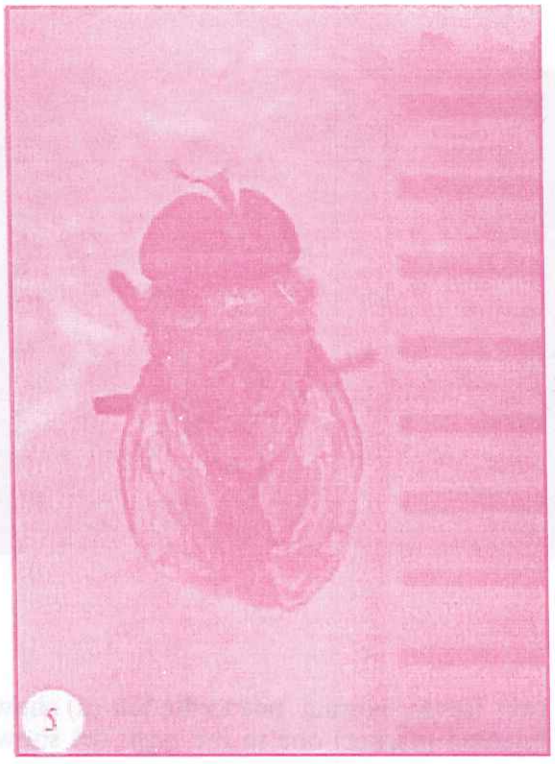

Fig. (5) : Adult C.albiceps of the injected rabbit showing fading of the normal green colour.

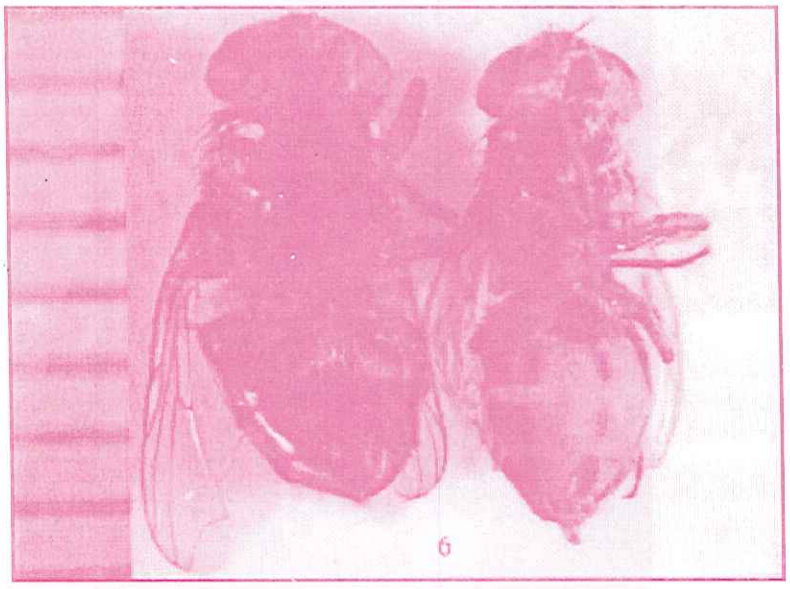

Fig. (6) : Two adults of C.albiceps (control to the left (a) and treated to the right (b) showing that the treated one had less width and depigmented patches in the ventral surface of the abdomen.

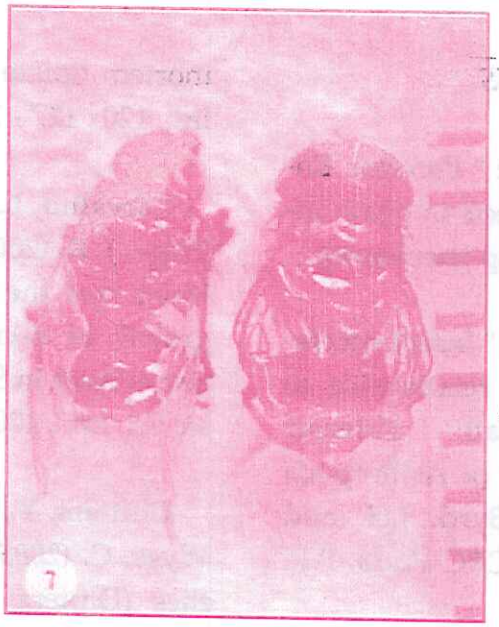

Fig. (7) : Two abnormal flies of C.albiceps of the injected rabbit showing in the left one (a) depigmented eyes and abnormal depigmentes wings and in the right one (b) shrinked wings.

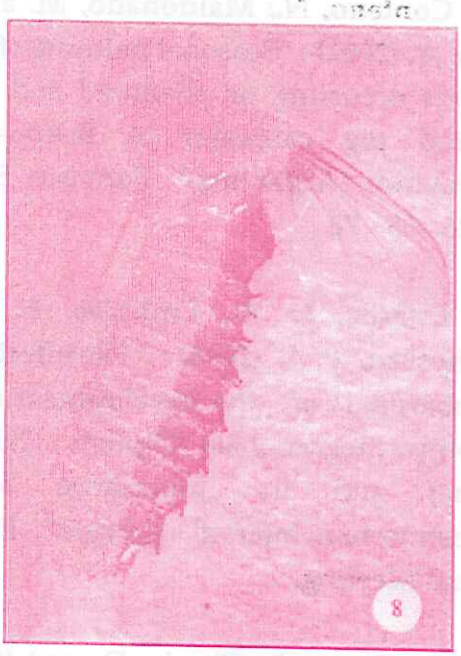

Fig. (8) : Partial exit of the adult fly from puparial case of control rabbit.

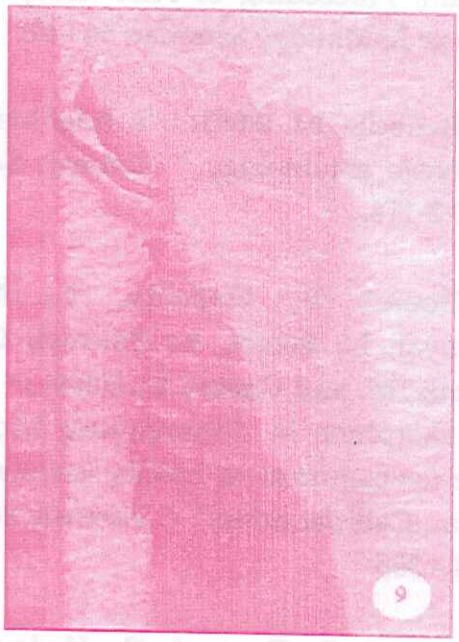

Fig. (9) : Partial exit of the adult borne dead fly from the puparial case of treated rabbit showing marked deformities.

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تأثير الكودايين فوسفات على مراحل نمو ذبابة كريزوماليا ألبيسبس ذات الأهمية الطبية الشرعية

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تستخدم الحشرات كعينات بديلة فى حالة تحاليل السموم عندما تكون العينات التقليدية مثل البول والدم غير متيسر الحصول عليها بعد الوفاة وهذا لأن الأدوية والسموم الموجودة فى الأجسام المتحللة من الممكن إيجادها فى الحشرات التى تتغذى على الجثة. كما أن هناك دراسات عديدة أظهرت علم السموم المتعلق بالحشرات فى إضافة معلومات عن سبب الوفاة. وتهدف هذه الدراسة إلى معرفة تأثير الجرعة المميتة من الكودايين فوسفات فى الأجسام المتحللة على تطور الحشرات التى تتواجد على الجثة وتقييم تأثيرها على فترة مابعد الوفاة. وقد تمت هذه الدراسة فى فترة الصيف مابين ٢٠ يونيو إلى ١٧ يوليو سنة ٢٠٠٧. وقد أجريت هذه التجربة على أربعة أرانب أوزانهم تتراوح بين ٣ر٥ - ٤ كجم قسمت إلى مجموعتين كل منها تحتوى على أرنبين مجموعة ضابطة وتم حقنها بماء مقطر والأخرى تم حقنها عن طريق وريد الأذن بالجرعة القاتلة من الكودايين فوسفات المذاب فى الماء المقطر. وبعد فصل فقرات العنق تم وضع كل أرنب فى كرتونة مفروشة بتربة طينية وتم وضع قفص معدنى عليها لحمايتها وتم وضعها على سطح مبنى كلية الطب البشرى جامعة أسيوط. وتم تجميع مراحل التطور المختلفة لذبابة الكريز ومايا ألبيسبس وفحصها لمعرفة التغيرات البيولوجية والمورفولوجية باستخدام الميكروسكوب الضوئى الدقيق.

وقد أظهرت الدراسة وجود تغيرات مورفولوجية على شكل تشوه فى أجزاء البطن وفقدان فى اللون واختلال فى شكل الفوهات التنفسية الأمامية والخلفية فى اليرقات. بينما فى الذبابة الكاملة يوجد ضمور فى الأجنحة واختلال فى نطاق السطح السفلى للبطن وبهتان فى اللون حتى تمام الاختفاء. كذلك يوجد تغيرات بيولوجية على هيئة سرعة فى التطور أثناء دورة الحياة وبعض الذباب لم يستطيع الخروج من العذراء. وهذه السرعة فى التطور من الممكن أن تؤدى إلى خطأ فى حساب فترة مابعد الوفاة يصل إلى ٢٤ ساعة فى حالة حسابها من تطور اليرقات و ٤٨ ساعة عندما يكون حسابها يعتمد على تطور العذراء.

ومن هذا يثبت هذا البحث أن إستخدام التغيرات المورفولوجية والبيولوجية التى تحدث فى ذبابة الكريز ومايا ألبيسبس من الممكن أن تستخدم كدليل على الوفاة من الجرعة المميتة من الكودايين فوسفات وفى حساب فترة مابعد الوفاة.